Project title:	Baiting and diagnostic techniques for monitoring *Phytophthora* spp. and *Pythium* spp. in irrigation water on ornamental nurseries
Project number:	HNS/PO 188
Project leader:	Erika F. Wedgwood, ADAS
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

GROWER SUMMARY

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.

SCIENCE SECTION

Introduction

Laboratory testing of nursery water

There is a risk that irrigation water collected from roofs and growing areas is contaminated with species of plant pathogenic *Phytophthora* and *Pythium*. These water moulds are likely to be found in nursery run-off water throughout the year, probably as swimming spores (zoospores). Researchers in Germany, the USA and UK have identified at least ten species of *Phytophthora* in nursery run-off including *P. cactorum*, *P. cryptogea* and *P. citricola* in nursery run-off. Plant clinic samples show that *Phytophthora* and *Pythium* frequently cause rotted roots in ornamental plants without causing obvious foliar wilt or dieback. Zoospores can thus be released without growers being aware that there are infected plants on the nursery. Some growers send bottles of water from their irrigation tanks for laboratory checking for plant pathogens. However, the sampling technique, sample numbers, and sampling intervals are at the discretion of the grower. Frequent tests are needed to allow a reasonably quick response to any failure in water treatment. *Phytophthora* zoospore concentrations as high as 100 / litre of water can be found in nursery bed effluent. A badly contaminated reservoir might have 30 spores per litre of water. *Pythium* spore counts can be 200 to 300 per litre in reservoirs. Detection of *Pythium* and *Phytophthora* spores in samples by filtering and culturing is down to 3 spores in a litre of water (Tim Pettitt, *pers. comm.*).

Some growers no longer send in water samples after having received only negative results. However, zoospore release may be more likely under particular conditions and information is lacking on target sampling dates. Water sampling using sample bottles is a “lucky dip”, particularly when zoospores are at a low concentration, as the probability of catching zoospores is not high. In an alternative sampling procedure, leaf bait bags are floated just below the water surface for a few days. These leaves become infected by the zoospores and develop lesions, but the volume of water “sampled” (i.e. distance to the bait from which zoospores swim) and hence whether more than one bait per reservoir is needed is not known. Laboratory results are returned as colony forming units/litre (cfu/L) of sampled water and/or % of bait leaves infected following membrane filtration-dilution plating. A quantitative immunodiagnostic test (zoospore trapping) has been developed in which viable spores are filtered out and germinated and this can be more sensitive for *Pythium*, but is not routinely used (Pettitt *et al*., 2002). It is considered that 1 cfu/L of a plant pathogenic *Pythium* or *Phytophthora* species in irrigation water is of concern.

Use of baits and in situ diagnostic tests

Plant tissue (fruit, leaves or seedlings) can be used to assay for *Phytophthora* spp. and *Pythium* spp. in water, with researchers selecting particular material based on availability and the success in detecting pathogen species. Information is lacking on bait selection for use in detecting different *Phytophthora* and *Pythium* root rotting species. Preliminary investigations under HNS 181 (Wedgwood 2010) showed that growers can prepare their own leaf baits out of materials on the nursery (pebbles, polystyrene chips and leaves wrapped in horticultural fleece). More research is needed to give confidence in the use of baits, and to understand more about how placement and other factors affect infestation. Research carried out to compare different diagnostic techniques for *Pythium* and *Phytophthora* species in water samples (Pettitt *et al*, 2002) also showed the higher the zoospore concentration in water the greater proportion of rhododendron leaf discs baits became infested, with 6000 zoospores/L giving 100% infestation.

Infection of bait tissue by either *Phytophthora* and/or *Pythium* can be confirmed using a commercially available lateral flow device (LFD) costing £5.98 plus VAT (in November 2011) per pathogen. This is less than the c. £65 laboratory test and enables growers to know immediately whether or not *Phytophthora* and/or *Pythium* are present and water is safe to use. It is also possible for growers to send the LFD test strip for a PDplus test which samples the DNA and can identify a number of *Phytophthora* species, including *P. ramorum*, *P. kernoviae* and *P. cactorum*, to species level. After verification in HNS 181, *P. cinnamomi* and *P. citricola* will also be included in the PDplus procedure. UK Plant Health Inspectors use Rhododendron and Pieris leaves as baits in rainfall traps for the detection of airborne *P. ramorum* and *P. kernoviae*. In HNS 181, both Nordmann Fir needles and Ceanothus leaves were found to be suitable as baits for *P. cinnamomi* and *P. citricola*.

Knowledge gap of zoospore behaviour in relation to baits

It is not known whether the zoospores, cysts or resting spores are mainly near the water surface where baits are usually placed. It is possible that they circulate in the water column with light or temperature (as do related algae). At one nursery in the USA, water contained more *Phytophthora* species when collected 1 m below rather than at the surface, and if true elsewhere this could affect test sampling. It is possible that cysts and resting spores are stimulated to germinate by bait tissue exudates. Damaged leaves are said to be infected more than intact ones by *P. ramorum*, but it is not known if this information can be utilised for root pathogens. In HNS 181 larger lesions of water-moulds developed in leaf baits along the line where leaves had become folded, possibly due to release of exudates resulting in a

higher zoospore location and infestation success.

In the laboratory, *Phytophthora* and *Pythium* sporangia are stimulated to release zoospores by cold shock below 6°C. There may be peak periods of zoospore release by plants in winter which growers should be aware of so monitoring can be increased. A greater understanding of zoospore behaviour would allow more confidence in bait testing. It is possible that with information gained on zoospore behaviour, and further research, a method (e.g. absorbent mats or mass-bait trapping) could be developed for collecting or killing zoospores. Such control methods could be used when *Pythium* or *Phytophthora* propagules are detected in water storage tanks.

Future irrigation water costs and potential shortages are encouraging growers to collect and reuse irrigation water, either for field, container or hydroponically grown crops. Whether or not the water is treated by one of various means, growers would benefit from the ability to have a rapid check that the water being used on plants was tested free of *Phytophthora* spp. and *Pythium* spp..

Some nursery stock nursery reservoirs were experimentally bait-tested in 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* spp. and *Pythium* spp in irrigation water.

Information will be sought on: the selection of a plant material type and quantity that will bait-out zoospores successfully and be readily available to growers, and to determine if the position of the bait in the reservoir affects zoospore trapping success. 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. The assistance of some growers will then be sought to test out bait construction and testing and the clarity of an accompanying instruction sheet on the techniques, with workshops then run to encourage wider uptake.

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 for detecting *Phytophthora* spp. and *Pythium* spp. 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.

Materials and Methods

Plant tissue baits

All work was carried out with bait bags made of a square of horticultural fleece, containing a polystyrene packing piece and heat-sterilised stones (quartz or flint so that they were not absorptive). With 6-7g of stones, a floating depth of the plant material inside the bag of around 35 mm below the water surface was obtained. The fleece was clean (cut off and used directly from the roll). Only the stones were sterilised as the other materials had not come into contact with soil or water (which could otherwise have contaminated them with water-mould resting spores). As in HNS 181, the fleece corners were gathered up to form a bag, and the neck secured with polypropylene twine which was also then used to suspend the bag so that it could be readily retrieved from the water. The fleece size was increased from 200 mm x 200 mm to 280 mm x 280 mm after the preliminary tests because this gave a wider gap between the stone weight and the float for the plant material so that water was able to pass around the latter more freely.

All bait material was examined before use to ensure it had no wounds or spotting that could have introduced contamination into the test. None of the plants had had any fungicide application for at least two months. Tables 1 and 2 summarise the focus of each of ten experiments (test type), the type and quantity of the plant material used as bait, how many replicate bags of baits were used in each test, the way the plant material was cut to obtain the specified number of bait pieces in each bait bag and the immersion period of the baits in the zoospore infested water.

Table 1. Plant bait materials, size and number of pieces per bag, and time left in water with either *Pythium*, *Phytophthora* or uninoculated

Experiment	Test type	Bait types used in bait bags	No. replicate bags with (+) or without (-) zoospores		Plant material and preparation	No. baits per bait bag	Time immersed
			+	-			
1	Phytophthora Bait type. Bait amount. +/-Wounds.	Nordmann	1	1	intact leaf	5	64 h
		Nordmann	1	1	one leaf torn in two	10	
		Ceanothus	1	1	intact leaf	2	
		Ceanothus	1	1	one leaf torn in two	4	
		Rhododendron	1	1	half a leaf torn in two	2	
		Rhododendron	1	1	two thirds of a leaf	1	
2	Phytophthora Bait type. Bait amount.	Apple	1	1	cut squares 5p sized	3	65 h
		Carrot	1	1	cut squares 5p sized	3	
		Rhododendron	1	1	one leaf into halves	2	
		Rhododendron	1	1	two leaves into four	8	
		Rhododendron	1	1	two quartered leaves	8	
		Rhododendron	1	1	two quartered leaves (indoors)	8	
		Ceanothus	1	1	one leaf in half	2	
		Ceanothus	1	1	four leaves in half	8	
3	Phytophthora Bait type. Bait size.	Apple	10	0	cut with No.3 borer	8	64 h
		Carrot	10	0	cut with No.3 borer	8	
		Nordmann	10	0	leaf split across midrib	8	
		Ceanothus	10	0	one leaf torn in two	8	
		Rhododendron	10	0	one leaf cut into eight	8	
4	Phytophthora Eden lab. check-count.	N/a	N/a	N/a	N/a	N/a	N/a
5	Pythium Bait type.	Apple	10	0	cut with No.3 borer	8	70 h
		Carrot	10	0	cut with No.3 borer	8	
		Nordmann	10	0	leaf split across midrib	8	
		Ceanothus	10	0	one leaf torn in two	8	
		Rhododendron	10	0	one leaf cut into eight	8	
6	Pythium	N/a	N/a	N/a	N/a	N/a	3 to

	zoospore survival.	5 h

Table 2. Carrot and apple bait tests with number of pieces and time left in water with either *Pythium*, *Phytophthora* or uninoculated

Experimental	Test type	Bait types used in bait bags	No. replicate bags with (+) or without (-) zoospores (+)	(-)	Plant material and preparation	No. baits per bait bag	Time immersed
7	Pythium LFD detection on increasing numbers baits (10 ml bottles)	Carrot	1 rep of each bait "conc."	0	cut with No.3 borer, not put in bag	8	48 h
8	Phytophthora LFD detection on increasing numbers baits (10 ml bottles)	Apple cv. Golden Delicious	1 rep of each bait "conc."	0	cut with No.3 borer, not put in bag	8	48 h
9	Phytophthora bait sensitivity to decreasing zoospore conc.	Apple cv. Golden Delicious	10 reps 25, 125 & 250 spores /L	10 reps 0 spores /L	cut with No.3 borer	8	46 h
9b	Phytophthora LFD sensitivity to baits from low conc.	Apple cv. Golden Delicious	4 reps 25, 125 & 250 spores /L	4 reps 0 spores /L	cut with No.3 borer	8	46 h
10	Pythium bait sensitivity to decreasing zoospore conc.	Apple cv. Golden Delicious	10 reps 25, 125 & 250 spores /L	10 reps 0 spores /L	cut with No.3 borer	8	70 h
10b	Pythium LFD sensitivity to baits from lower conc.	Apple cv. Golden Delicious	4 reps 25, 125 & 250 spores /L	4 reps 0 spores /L	cut with No.3 borer	8	70 h

The plant material initially used as baits was selected using various information sources. In HNS 181, Ceanothus leaves (as used in routine water tests at the Eden Project, Tim Pettitt pers. comm.) and Nordmann Fir needles had been shown to pick up both *Pythium* and *Phytophthora* from irrigation water tanks (Wedgwood, 2011). Work in HNS 134 on *Phytophthora ramorum* detection in water had used eight pieces of Rhododendron cv.

Cunningham's White in bait bags (Jennings, 2007). The potential spread of *P. ramorum* across Britain could, however, make obtaining a healthy rhododendron leaf less certain in future (particularly as the pathogen can have a symptomless phase). Lists of bait material used in various researches (Singleton *et al*., 1993; Erwin & Ribeiro, 1996) were also consulted to find material that had been used with a number of hosts. Apple flesh, in particular cv. Golden Delicious was one of the plant material types often used in laboratory tests for the detection of *Pythium* and *Phytophthora* root roots (usually by placing roots in slits in the whole apple). Carrot has occasionally been used in the laboratory for baiting of *Thielaviopsis basicola* from rotted roots and *Pythium* from soil. Carrots are usually available in most households and relatively cheap and so this bait material was introduced in later tests. However, carrot roots are susceptible to attack by some *Pythium* species, especially *P. violae* and *P. sulcatum* causes of carrot cavity spot, infected via root hairs in the growing crop. Details of the materials used are given in Table 3.

Table 3. Details of the plant material selected from to use as baits

Plant material type	Variety	Source
Rhododendron leaf	Cunningham's White	Potted plants in polytunnel
Ceanothus leaf	*C. thyrsiflorus* var. *repens*	Potted plant held outside
Nordmann Fir needle	unknown	Christmas tree plantation
Apple internal flesh	Golden Delicious	Retail store
Carrot internal flesh	unknown	Retail store

The Rhododendron cultivar Cunningham's White (kept in pots a polytunnel to reduce the possibility of airborne or soil splash *Phytophthora* spp. contamination) and the evergreen *Ceanothus thyrsiflorus* var *repens* (kept in a pot on concrete to avoid splash-up of soil-borne *Pythium*) were used as baits. The apple and carrot were purchased just before each batch of experiments. The apple was cv. Golden Delicious, but the carrot cultivar/s were not labelled by the suppliers.

Phytophthora and Pythium inoculum production

The Oomycete pathogens used in this work were all zoospore producing (Table 4). Originally *Pythium irregulare* was to be used, but the culture from the ADAS collection was found to be contaminated and although new isolates were obtained from the Royal Horticultural Society they could not be stimulated to produce zoospores (isolates of this species are known to have variable zoospore production).

Table 4. Oomycete isolates used for the water inoculation in laboratory experiments in 2012

Species	Reference number	Year isolated	Host	Plant tissue

Phytophthora cryptogea	E556 (ex T. Pettitt)	-	-	-
Pythium sp.	BX 10/60a (ADAS)	2010	Gerbera	Root

P. cryptogea was confirmed by molecular testing (polymerase chain reaction, (PCR)).
The *Pythium* species was either *P. diclinum, P. lutarium, P. dissotocum* or *P. coloratum* (Fera PCR testing reported that the DNA sequence data used matched 100% to these four different species and these require morphological characters examination for their separation).

All agars and culture solutions were produced according to standard mycological recipes, such as given in Erwin & Ribeiro (1996).

P. cryptogea was grown on potato dextrose agar (PDA) in the dark at 20°C and squares of mycelium floated in clarified V8 broth to produce mycelial mats. The culture solution was then rinsed from the mats and they were left in sterile soil water overnight to produce zoospores. Synchronised release of zoospores was obtained by refrigeration for an hour followed by another hour when they were held at room temperature.

Pythium sp. was grown on V8 agar in the dark at 20°C and squares of mycelium (10 mm x 10 mm) incubated in sterile rain water overnight at 20°C to produce zoospores.

The suspensions of zoospores in water were counted under the microscope using a haemocytometer, just before they were required for the test. The volume of zoospore infested water required to achieve the target spore count in the test container of water was then calculated. In the principal tests 10 replicates of each concentration were prepared; an extra sample of the target zoospore concentration was sent to a laboratory carrying out water tests for growers, in order to see how the two estimations of zoospore numbers compared.

Bait bags were added to the zoospore suspensions within half an hour. The period of bait immersion varied between tests (Tables 1 and 2) and in the main experiments was carried out under diurnal lighting (out of direct sunlight) within an air-conditioned room at 20°C. The weighted bait bags were pushed down in the water straight after deployment to wet the fleece to ensure they were submerged at the start of the incubation (as with no movement in the containers it could take a period for the water to enter the pores in the fleece).

When bait bags were removed from the water they were kept separate from each other.

Baits that were to be cultured on agar were opened in a laminar flow cabinet to ensure sterile conditions, and equipment was sterilised between handling each bag. When tests were carried out with the lateral flow devices (LFDs), care was taken to avoid cross-contamination, but the procedure was not carried out in a laminar flow cabinet.

When isolation was required, the bait pieces were gently blotted and placed on an agar selective to water-moulds (P5ARP growth medium), with one piece per plate. The number of bait pieces per bag with either *Pythium* or *Phytophthora* (depending on which was used in the test) was recorded and notes made of any contaminants (as fast growing species such as *Mucor* can overgrow water-moulds). The morphology of the colonies, and microscope examination (to confirm the presence of non-septate hyphae typical of Oomycetes) was carried out with reference to the original cultures and also diagrams of *Phytophthora* (Waterhouse, 1970) and *Pythium* (Van der Plaats-Niterink, 1981) in order to identify isolates. Plates were assessed after five days, with further examination a week later. To be able to examine sporangia during diagnosis it was necessary to cut out sections of mycelium from colonies and float them in sterile rain or soil water for up to three days to stimulate sporangial production. Growth of coralloid mycelium confirmed *P. cryptogea*; plates with identical colonies were classed as *P. cryptogea*.

The LFDs were used according to the instruction sheet prepared for growers (Appendix 1), with all the baits from one bag being put in one buffer bottle and shaken for around a minute until colouration appeared in the liquid to show that the tissue had been broken down by the ball-bearings. Drops were taken from the bottle to use on both a *Pythium* and a *Phytophthora* LFD. The results were always read after 10 minutes, with the strength of the blue test line scored using a 1-5 index using a standard reference picture of intensity (5 = very strong) as this might give information about the extent of infestation of the baits which could in turn be related to the number of zoospores colonising the pieces. All used LFDs were labelled and retained in their foil packets.

Preliminary laboratory experiments

Experiment 1. P. cryptogea - bait type, amount and need for wounding.

Preliminary work started on 17 July 2012 using distilled water without and with *P. cryptogea* zoospores. Two bait bags of each of the selected leaf baits Ceanothus, Rhododendron and Nordmann Fir were made (Table 1), one to go in the inoculated water, the other in the uninoculated water. There was no replication. All the leaves were rinsed in freshly distilled

water before use. For each bait type, half the bags had leaves which were torn in half to see if wounded leaves were more attractive to zoospores.

The experiment was carried out in natural daylight and ambient temperatures outside (temperature range 10°C to 19°C) against the north side of a building. Zoospores were added to give 900 spores per inoculated 750 ml glass jar (1200 per litre). Baits bags were then immersed 30 mm below the water surface for 3 nights.

Experiment 2. P. cryptogea - bait type and amount.

Work was done with *P. cryptogea* jars containing 1000 spores per L (750 spores per inoculated 750 ml glass jar) on 24 July 2012 using Rhododendron and Ceanothus leaf baits with up to 8 pieces per bait bag, as shown in Table 1. Additionally, apple and carrot pieces were also tested. As previously, duplicate bags were prepared for placement in the inoculated and uninoculated water. There was no replication of the different compositions of baits in bags. Work was carried out in the laboratory with water in the jars at 20°C, except for one rhododendron bait bag jar which was placed outside to allow comparison with Experiment 1.

Main laboratory experiments

Experiments 3 (P. cryptogea) and Experiment 5 (Pythium sp.) - high spore concentration bait selection.

These and all future experiments were carried out on shelves in an air-conditioned room at 20°C, as it was thought that temperature fluctuations outside with the small water volume could differ more than those in a reservoir. The water volume used was increased to 1 L and translucent polypropylene (HDPE) bottles used, with lids resting over the bottle necks during the running of the experiments. Ten replicate bottles each containing one bait bag were used, with treatments randomised within each replicate block (two blocks per shelf on each of five shelves). As no uninoculated water was baited, samples of the plant material used were placed directly onto agar plates to check for any natural infection or contamination with *Pythium* or *Phytophthora*.

The preliminary tests with *Phytophthora* found a lot of secondary fungal growth on the agar plates and so after the leaf pieces were cut to size they were surface sterilised with 75% ethanol before being rinsed twice in sterile distilled water. There was no need to surface sterilise the apple and carrot as, after washing them under the tap, their outer surfaces were removed and inner tissue used for the baits.

After the preliminary tests, the bait pieces were made smaller (to increase the amount of edge tissue which may be favoured by the zoospores) and used a greater number of pieces to get a slightly larger surface area. Each bait bag thus contained eight pieces of freshly wounded or cut material, aiming to produce a similar surface area of 25 mm x 25 mm (as given in manufacturer's instructions for leaves for the LFD test). This required sections taken from one Rhododendron leaf, four large Ceanothus leaves cut in half and eight Nordmann Fir largest needles from current season growth to provide sufficient material for all 10 replicates (Table 1). For the apple and carrot pieces, a sterilised No. 3 corer (6 mm wide) was cut through a 7 mm thick cross-section of internal flesh to produce a plug weighing around 1.4 g.

With each of eight bait pieces plated-out onto agar per treatment bottle, a total of 400 plates for each of the *Pythium* and *Phytophthora* experiments were examined over a period of two to three months. The number of bait pieces infested per bag was recorded. Where white/colourless fungal mycelium grew from the plate, because water-moulds seldom sporulate on agar, agar with mycelium was placed in sterile rain water floats in order to confirm the presence of *Pythium* or *Phytophthora* sporangia. Growth of either coralloid mycelium or pear-shaped sporangia confirmed *P. cryptogea*. The *Pythium* sp. used in the work produced filamentous sporangia. The infestation of bait pieces producing growth on agar of identical colony morphology was then able to be identified.

For Experiments 3 (on 7 August 2012) and 5 (on 28 August 2012), to improve the results from the preliminary tests the spore concentration used was increased. In work on bait methods for detection of *Pythium* and *Phytophthora* zoospores in water (Pettit *et al.*, 2002) 6000 spores per litre were required to give good (10 out of 10) Rhododendron bait infestation. This concentration was achieved for *Phytophthora* for Experiment 3 (although zoospores of *Phytophthora* had been produced overnight rather than after chilling just before required), but only 4800 spores per litre was available for Pythium. Freshly made distilled water was used in the 1 L bottles. The haemocytometer zoospore counts were checked by laboratory culture tests at the Eden Project commercial testing laboratory.

Experiment 4. P. cryptogea - zoospore check count.

The results from the Eden laboratory for Experiment 3 did not match the spore concentration calculated from use of the haemocytometer prior to making the dilutions for the bait bottles at ADAS Boxworth. To investigate this difference in spore counts between the laboratories, a volume of *P. cryptogea* spores was produced at a concentration of 5000 spores / L without baits being added. This water sample was sent to the Eden laboratory on 21 August 2012

(as before, by “next day delivery”) to determine the concentration by filtration and isolation. The pH and temperature of various types of water were checked in case there were significant differences between the rain water zoospore production float dishes and the 1 L bottles of distilled water that might cause either zoospore encystment or rupturing.

Experiment 6. Pythium sp. - water type and zoospore survival.

This experiment, on 10 December 2012, sought to determine whether zoospores were affected by the water they were suspended in for the laboratory experiments. This could also have relevance on nurseries to the baiting of different sources of irrigation water. Distilled water was used in previous experiments, rather than tap water, because it did not contain the chlorine which might affect zoospore behaviour, such as causing encystment. It was possible that the lack of ions in distilled water might affect the osmotic balance of zoospores, causing them to swell and rupture. Tap water can be made “safe” for fish using a proprietary hydrosulfite salts product e.g. “Prime” intended for aquariums that removes chlorine and chloramine and it was possible that this could be utilised in the experiments to improve zoospore counts. If tap water does affect zoospores then this would also be useful to know, as some nurseries mix reservoir and tap water for irrigation. Rain water had not been used previously because it would have required autoclaving to remove micro-organisms and the breakdown products could possibly affect the zoospores or the baits.

A five-replicate observation experiment was thus carried out to record the behaviour of zoospores, in particular any encystment, bursting or changes in movement frequency in the different water sources shown in Table 5. An inverted microscope was used to examine zoospores in a 25-chambered (5x5) transparent dish which allowed all the treatments to be adjacent (randomised within each replicate column of five chambers), so removing any differences in environmental conditions. Each chamber held 2 ml of spore suspension which had been drawn off from 500 ml of the specified water type to which 1 ml from the (concentrated) float culture had been added. Observations were started after three hours, counting the number of active and encysted zoospores. Observations were made in replicate order and were complete after two hours.

Table 5. Water types used to determine any affect on *Pythium* sp. zoospore behaviour (Experiment 6)

Water used in test	**Comments**
Sterile rain water (autoclaved)	Collected in a water butt from a glasshouse roof
Distilled water	Freshly made

Tap water (stood)	Stood for 7 days to reduce chlorine
Tap water (fresh)	Freshly drawn from the tap
Tap water (treated with “Prime”)	Freshly drawn & treated with 2 x 0.5 ml drops / 4L

The experiment was repeated. The first experiment (on 6 December 2012) used around 130 to 230 *Pythium* sp. zoospores per chamber; the second (on 7 December 2012) used around 300 to 400 *Pythium* sp. zoospores per chamber.

Experiment 7 (Pythium sp.) and Experiment 8 (P. cryptogea) - LFD sensitivity.

It was not known whether apple and carrot bait materials would be able to be utilised with lateral flow devices (LFDs) as previous experience of their use had been with plant stems and fibrous roots. It was possible that the sugar in the apple could prevent the buffer solution being draw across the indicator paper of the LFD, and that the carrot might produce an orange stain that obscured the test window indication. It was also not known whether the LFDs might show different strengths of positive indication with varying amounts of infested bait pieces. The LFDs were Pocket Diagnostic kits for *Pythium* spp and for *Phytophthora* spp. manufactured in the UK by Forsite Diagnostics, Sand Hutton, York, YO41 1LZ.

To obtain different bait strengths, a standard zoospore suspension was made up to have 10 spores in 10 ml of distilled water (i.e.1000 per L) and 1 bait plug was put in each small glass bottle with the 10 zoospores (estimated by dilution) of either *Pythium* or *Phytophthora* sp. and left in diurnal lighting at room temperature of around 20 °C. The bait plugs were left for 4 days (10-14 August), by which time it was expected that all the zoospores should have come into contact with the bait material. Varying numbers (1, 3, 5 or 8) of infested baits were then mixed with fresh uninfected baits (7, 5, 3 or 0) to create 4 bait strengths (Table 6). The buffer bottles were shaken vigorously for a standard 90 seconds before drawing off liquid to use on the relevant LFD. The test line was assessed after 10 minutes and intensity recorded using a 0 to 5 Index (negative to strong positive).

Table 6. Combinations of infested and uninfested bait plugs used to create four strengths of *Pythium* sp. and *P. cryptogea* infected tissue to test by LFD

Experiment	Bait type and pathogen	No. infested bait plugs	No. uninfested bait plugs used	No. bait plugs put into buffer for LFD test
7	Carrot and	1	7	8
	Pythium sp.	3	5	8
		5	3	8
		8	0	8

8	Apple and	1	7	8
	P. cryptogea	3	5	8
		5	3	8
		8	0	8

Experiment 9 (P. cryptogea) and Experiment 10 (Pythium sp.) - bait and LFD sensitivity.

These experiments used sterilised rain water. The baits were left in place for 2 nights (Tuesday pm to Thursday am), rather than 3, as 48 hours had been used in other work (Jennings, 2007) and the shorter time might be preferable for use on nurseries. It was also likely that there would be faster decay of apple baits in water than for leaf pieces.

A range of spore concentrations was used (Table 7) to see if this affected the proportion of plugs affected. The concentrations chosen were closer to what might be present in a nursery reservoir. Both pathogens were tested using apple bait bags with each spore concentration (and the uninoculated) replicated ten times. On retrieval, these baits were plated out onto agar as in previous experiments. In addition to these 10 replicates, another 4 replicates were set up with the same range of spore dilutions in order to test the sensitivity of the LFDs to baits infested by this relatively low range of zoospore concentrations. It was anticipated that the LFDs might show a decreasing test band intensity the lower the spore concentration baited.

Table 7. Zoospore concentrations used of *P. cryptogea* and *Pythium* sp. (Experiments 9 and 10)

Zoospore concentration	**Baits per bag**
0 spores / L	8 apple plugs (Golden Delicious)
25 spores / L	8 apple plugs (Golden Delicious)
125 spores / L	8 apple plugs (Golden Delicious)
250 spores / L	8 apple plugs (Golden Delicious)

Reservoir water sampling and nursery testing of bait bags

In preparation for carrying out baiting experiments in nursery reservoirs, 1L water samples were taken at two nurseries (“A” and “B”) in August 2012 in order to select a reservoir for use in 2013 to examine bait positioning and any seasonality of zoospore catches. Samples were taken from four locations at nursery “A” (reservoir, lagoon, pond, ditch) and from four locations at nursery “B” (Site 1 reservoir, Site 1 bed run-off, Site 2 reservoir, Site 2 bed run-off). Samples were taken from run-off in order to assess if there was *Pythium* spp. and *Phytophthora* spp. present on the nurseries. Bed run-off would be expected to have a higher pathogen concentration than in the reservoirs and so they would be more easily detected.

The Nursery “B” site 1 reservoir was selected on the basis of the results of the August 2012 water samples. This was a butyl-lined open reservoir (about 15 m x 70 m) with a reed bed filtration area across the width at one end where water from the beds entered. Water was taken out from the centre of the reservoir via a slow sand filter. Excess water drained out by an overflow at the opposite end to the reed bed. In January 2013, bait bags were used to investigate whether there might be differences in the zoospore populations at different sides of the reservoir and at different depths. Water samples were taken to send to the Eden laboratory and baits placed on tethered strings at two positions around the edge of the reservoir. An extra 1 L water sample was taken at the outfall end to return to ADAS Boxworth laboratory for baiting. This was baited in the same way as at the Eden laboratory – with Ceanothus leaves floating freely in the water. Apple bait bags with eight pieces (as using in the final laboratory experiments) were tested. Ceanothus leaf baits bags (four leaves torn in half per bag) were used in addition as these had been used previously in reservoirs before to bait water-moulds (in HNS 181), in order to compare with the apple bait bags in this initial reservoir experiment.

Both bait bag types were floated at two depths. This was done by using 7g and 10 g of stones inside to give respectively shallow (30 mm) and deeper (250 mm) floatation below the water surface. These were tied to the bank at the reed bed (R1) and the outfall (R2) end of the reservoir so they could float within a water surface area of about 0.75 x 0.75 m. All these bags were to be checked for water-mould infestation by isolation without surface sterilisation on P5ARP agar plates (as for the laboratory experiments in 2012) at ADAS Boxworth. An extra bait bag of each type was also floated at the shallow depth at the outfall end to test with an LFD. The bags were set out, with the participation of the nursery staff, on Tuesday 29 January 2013 and retrieved by the staff on the 31 January (after 48 hours). A logger was also floated in the water to record the temperature. On retrieval each bait bag was placed in its own bag and posted the same day to ADAS Boxworth, together with the logger. The bags were received at Boxworth on Monday 4 February and isolations and LFD testing were carried out (6 days from immersion). The bait pieces were isolated onto individual plates. Some Ceanothus leaves direct from the bush were also isolated from to check if they had any water-mould infestation. These leaves were dipped in 75% ethanol to surface sterilise them, in the same way as those used in the bait bags. For the two extra bait bags, a single LFD buffer bottle was used for all eight pieces of apple and another bottle for the eight leaf bait pieces. The same apple or leaf bait containing buffer bottle with was then used to provide extract drops to test on both a *Pythium* and a *Phytophthora* LFD.

In February, the Nursery “B” site 1 reservoir was baited by the grower at the same locations and depths. The grower produced his own bait bags using a locally purchased Golden Delicious apple and the stones, fleece, floats and string supplied by ADAS. Instructions on how to make the baits (Appendix 1) and a demonstration bait bag were supplied by ADAS. Only apple baits were used this month. An additional shallow floating bait bag was used at each location so that these could be tested on site by the grower using LFDs immediately on retrieval of the baits. Instructions on how to test the baits with the LFDs were provided to the grower (Appendix 1), together with LFDs from the same batch as those to be used at Boxworth. The remaining shallow and deep bait bags from each location were returned to ADAS Boxworth for LFD testing. In addition, an apple bait bag was also placed in water collected from the outflow from the slow sand filter and stood in the nursery office (as there was nowhere *in situ* that the bait could be left). This bait bag was retrieved from the water and posted to ADAS at the same time as the reservoir bait bags. The baits in the reservoir and post-sand filter were placed on 26 February 2013. At the same time 1 L water samples were taken from these three locations and posted to the Eden laboratory. The baits used for the grower LFD tests were retrieved after 48 hours on Thursday 28 February. The used LFDs were returned to ADAS to confirm the grower’s reading. The remaining baits were left in place until Monday 4 March (6 days immersion) and then posted to ADAS together with a temperature logger from the reservoir. The baits were received and tested using LFDs at ADAS on 6 March. A 0 to 5 test line intensity index was recorded for each LFD. Based on the results of the January reservoir experiment, no isolations were carried out in February.

Materials were sent for apple bait bags to a further nursery, “C”. The grower made the bags with freshly cut apple pieces before placing them for 48 h on 26 February 2013 in water which had come in to a slow sand filter from glasshouse roofs. The bags were weighted to float at the same shallow and deep depths, (30 mm) and (250 mm), as at the other nursery.

Further baiting will be carried out in 2013, with deployment planned for every other month until November to obtain a seasonal record of infestation, and to compare zoospore bait trapping results (as given by LFD use) with those of colony forming units and Pythium and Phytophthora identification from water samples.

Results

Table 8 summarises the results of the ten laboratory experiments carried out in 2012. The full results of each experiment are then presented and discussed.

Table 8. Summary of laboratory tests evaluating the effect of bait type, water type and zoospore concentration on the detection of *Pythium* sp. and *P. cryptogea* in water – 2012. Detection was assessed as the proportion of baits with growth from baits on selective agar and by LFD tests

Experiment	Date	Pathogen	Test Type	Spores/L	Size of container	Baits	Total % infected of the number of bags or individual baits per experiment
1	17.07.12	*P.cryptogea* E556	Baiting and culture	1200	750 ml	Mixed	Zero
2	24.07.12	*P.cryptogea* E556	Baiting and culture	1000	750 ml	Mixed	Zero
3	07.08.12	*P.cryptogea* E556	Baiting and culture	6000	1 L	Mixed	24% bags. 5.5% baits.
4	21.08.12	*P.cryptogea* E556	Baiting and culture	5000*	1 L	Not Baited	Not Baited
5	28.08.12	*Pythium* BX10/60A	Baiting and culture	4800 **	1 L	Mixed	56% bags. 13.3% baits.
6	6.12.12 & 7.12.12	*Pythium* BX10/60A	Zoospore observ-ation	up to 400, 000	2 ml	N/a	N/a
7	10.12.12	*Pythium* BX10/60A	Baiting and LFD	1000	10 ml	Carrot	very faint +ve for 1, 3 & 5 infested plugs per bottle
8	11.12.12	*P.cryptogea* E556	Baiting and LFD	1000	10 ml	Apple	Zero
9	12.12.12	*P.cryptogea* E556	Baiting and culture	0 - 250	1 L	Apple	2% bags. 0.2% baits.
9b	12.12.12	*P.cryptogea* E556	Baiting and LFD	0 - 250	1 L	Apple	One faint +ve
10	18.12.12	*Pythium* BX10/60A	Baiting and culture	0 - 250	1 L	Apple	28% bags 5.8% baits
10 b	18.12.12	*Pythium* BX10/60A	Baiting and LFD	0 - 250	1 L	Apple	Two faint +ve

*Experiment 4; Eden laboratory culture tests indicated 67 – 187 cfu/L

**Experiment 5; Eden laboratory culture tests indicated 47 spores/L

Experiment 1. P. cryptogea - bait type, amount and need for wounding

No Phytophthora was recovered from any of the bait material after four days on agar. Rhododendron leaf pieces were too large and curved to sit down on the agar so smaller pieces were to be used in future.

Experiment 2. P. cryptogea - bait type and amount

This repeat of Experiment 1, but using more (a total of eight) bait pieces did not produce any positive isolations of *P. cryptogea.* There were a lot of secondary non-target fungi (probably *Fusarium* spp. and one *Pythium* sp.) on the *Ceanothus* baits on the agar plates. As a result of the failure to detect *P. cryptogea* in these preliminary experiments, a higher zoospore concentration was used in subsequent work.

Experiment 3. P. cryptogea - high spore concentration bait selection

A mixture of fungi was recovered from the baits after plating onto selective agar. *Fusarium* was predominant on the apple, carrot and Rhododendron plates, generally starting as white mycelium similar to that recorded for *Phytophthora* and *Pythium* spp.. The presence of contaminant fungi would not matter with LFD testing as this selects for either *Phytophthora* spp. or *Pythium* spp. (depending on the device used). In total, the number of baits confirmed to be infested by Phytophthora (with morphology checking of the isolations including taking samples of mycelium to floats) was relatively few (Figure 1).

Apple pieces were most frequently infested by *Phytophthora* (16 out of 80 pieces), but only 7 out of 10 bags were positive, and within these there were commonly only 3 or fewer out of 8 pieces infested (maximum 4 pieces positive per bag). None of the Nordmann Fir needles became infested. In total across all bait types, out of 50 bags used there were 12 with *Phytophthora* i.e. 26% became infested (Table 9).

Although 6000 zoospores were added to each bottle the laboratory water filtration and plate test (Eden laboratory) found zero colony forming units (spores or mycelial fragments). This result suggests no viable spores were present in the sample when it was tested by the water filtration and plate test. Nursery water samples also arrive by post and using the same methods, and *Phytophthora* and *Pythium* species are commonly detected so these procedures do not account for the lack of spores. It was possible that the air-gap left in the top of the bottle caused a pressure change/physical impact which caused the spores to burst. Normally any knocking or other chemical or physical shock can cause the zoospores

to encyst, however encysted spores are filtered and cultured from water in the same way as un-encysted zoospores.

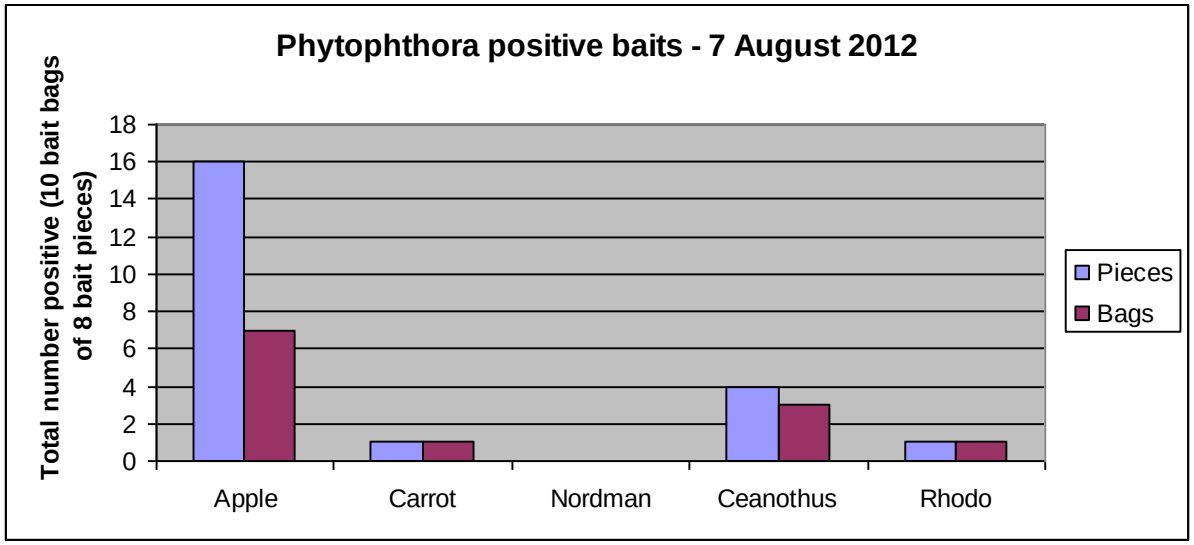

Figure 1: Detection of *P. cryptogea* by tissue baits showing the total number of bait pieces and the total number of bait bags from which *Phytophthora* was isolated (Experiment 3)

Experiment 4. P. cryptogea - zoospore check-count

It was noted the zoospores were starting to encyst on the haemocytometer slide before making up the spore suspension to send to the Eden laboratory. Two methods were used by this laboratory to count the spores with results of only 67 or 187 colony forming units per litre, for a suspension originally calculated to contain 5000 spores/L. When 10 Ceanothus leaf baits were added to the zoospore suspension, there was 100% infestation recorded by both direct examination and by agar plating of the leaves. No tissue baiting of the original suspension was done by ADAS in this experiment.

The Eden laboratory also confirmed the culture being used was *Phytophthora cryptogea.* A *Pythium* LFD on the agar culture in the ADAS laboratory had given a strong positive for *Pythium*. However, mycologists Tim Pettitt (Eden Project laboratory) and Charles Lane (Fera) have found in the past that if agar cultures are tested in this way the reaction of the LFD test kit can be wrong (Tim Pettitt, pers. comm.). This means that LFD tests cannot be used to confirm in the laboratory the identity of mycelium growing onto agar from baits.

Measurements on water used in the laboratory experiments showed the sterile rain water was pH 7.4 and the distilled water pH 5.9. The bottle water temperature was 18°C and the spore suspension in the float dishes 22°C. Neither of the waters used in the experiment gave an EC reading. These water pH and temperatures were not considered to have an adverse effect sufficient to kill zoospores (Tim Pettitt, pers. comm.).

Experiment 5. Pythium sp. - high spore concentration bait selection

For *Pythium* sp., many of the agar isolation plates grew white colonies which were initially thought to be *Pythium* when examined without the aid of a microscope. Out of 80 plates this growth was seen on 78 apple, 72 carrot, 66 Ceanothus, 46 Rhododendron and 27 Nordmann plates. However, when the bait isolation plates were flooded with sterile rain water and each of the agar plates examined under an inverted microscope for zoospore-producing swollen hyphae and the absence of septa in young hyphae, then fewer were confirmed as *Pythium* (Table 10 and Figure 2).

Carrot pieces were most commonly infested by *Pythium* (26 out of 80 pieces), with 9 out of 10 bags positive, and within these 1 to 5 out of 8 pieces were positive per bag). In total, out of 50 bags used there were 28 from which *Pythium* grew i.e. 56% were infested (Figure 2).

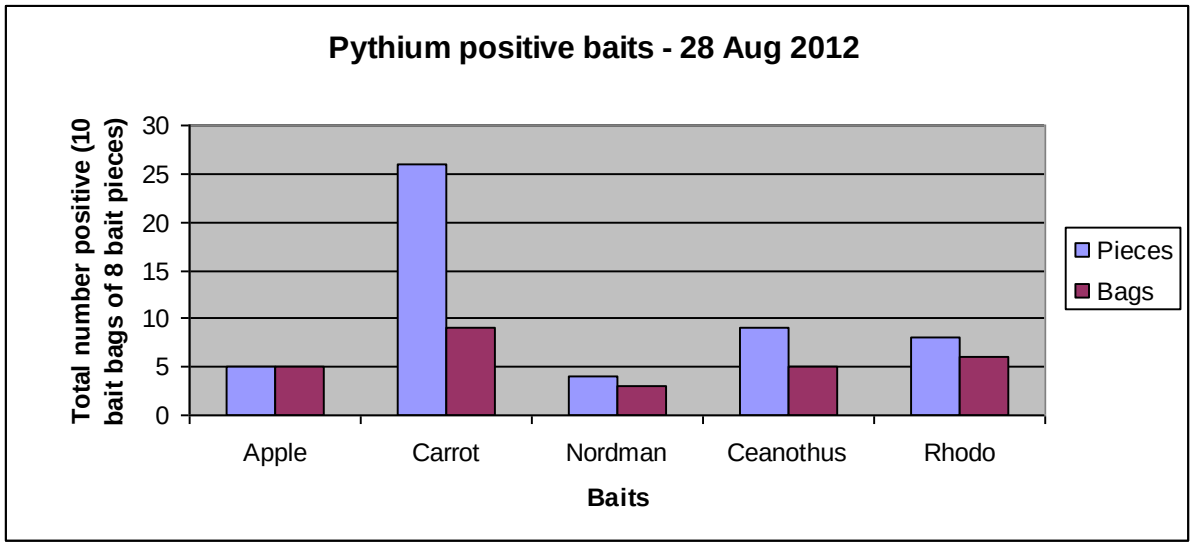

Figure 2: Detection of *Pythium* sp. by tissue baits showing the total number of bait pieces and the total number of bait bags from which *Pythium* was isolated (Experiment 5)

There were 4800 spores in each L bottle based on laboratory calculations on the infestation day, but after postage to the Eden laboratory only 47 colony forming units were counted. The Eden laboratory obtained 100% Ceanothus bait infestation at this low water infestation level, compared with 11% of bait pieces recorded by ADAS using the zoospore suspension before a sample of it was posted to the Eden laboratory. The reason for this discrepancy was unknown, but Experiment 6 examined the water type used as a potential influence.

Experiment 6. Pythium sp, - water type and zoospore survival

There were no apparent differences in zoospores present and the proportion encysted between the different water samples (Table 13) following two separate observation experiments. There was no evidence of zoospores having burst in the distilled water, or encystment resulting from immersion in tap water. In all the water types the zoospores had encysted within three hours, leaving only around 10 to 20% still motile in the first run, and fewer (around 2%) in the second run which had a higher total count added to the counting chambers initially. Results for individual 2 ml chambers and means are given in Appendix 2. The numbers of zoospores plus cysts counted after 3-5 hours in various types of water were within the range expected from the original haemocytometer counts of zoospores.

Table 13. *Pythium* sp. zoospore survival and encystment three to five hours after adding to various types of water (Experiment 6) for tests on 6 and 7 December 2012

	6 December 2012			7 December 2012		
Water type	**Mean number zoo-spores**	**Mean number cysts**	**Total count**	**Mean number zoo-spores**	**Mean number cysts**	**Total count**
Sterile rain water	30	106	136	9	342	351
Distilled water	21	174	195	6	354	360
Stood tap water	21	158	179	8	324	332
Fresh tap water	25	174	199	6	342	348
'Primed' fresh tap water	28	155	183	8	312	320

Experiment 7 (Pythium sp.) and Experiment 8 (P. cryptogea) - LFD sensitivity

These tests used a different ratio of infected: uninfected baits. There were few positive LFDs across the two pathogen tests, and the indications were only faint. It was not known whether the zoospores did not infest the baits, or if the LFDs were not able to detect the infestation. In theory, where the maximum eight infested baits were used then there could have been infestation at 80 points by the zoospores across all the baits (10 spores x 8 baits), which would have been expected to have been sufficient to be detected by the LFD.

Experiment 9 (P. cryptogea) and Experiment 10 (Pythium sp.) - bait and LFD sensitivity

There was little detection of *Phytophthora* by isolation, with only one apple bait becoming infested (Table 11). Only one *Phytophthora* LFD (for 125 spores/L) was positive, (faintly = Index 1). A higher number of baits and bait bags were infested by *Pythium* sp. (Table 12), with most in the bottles with 250 spores / L (with 60 % of bags infested). Bait infestation in the 125 and 25 spores / L was similar, (with 40% of bags infested). The LFD results were not

very good, however, with faint test lines (Index 1) for *Pythium* sp. from baits taken from two of the 125 spores/L bottles.

Table 9: Laboratory baiting Experiment 3 (6000 *Phytophthora* zoospores/ L) on 7 August 2012 showing % of bait pieces and bait bags infected

Bait tissue	% positive baits (out of 8 per bait bag) as determined by isolation onto agar										Mean % infected baits per bag	% of reps with an infected bait bag
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10		
Apple	25	37.5	0	0	12.5	50	25	0	37.5	12.5	20	70
Carrot	0	0	0	0	12.5	0	0	0	0	0	1	10
Nordmann fir	0	0	0	0	0	0	0	0	0	0	0	0
Ceanothus	25	0	0	12.5	0	0	0	12.5	0	0	5	30
Rhododendron	0	12.5	0	0	0	0	0	0	0	0	1	10

Table 10: Laboratory baiting Experiment 5 (4800 *Pythium* zoospores/ L) on 28 August 2012 showing % of bait pieces and bait bags infected

Bait tissue	% positive baits (out of 8 per bait bag) as determined by isolation onto agar										Mean % infected baits per bag	% of reps with an infected bait bag
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10		
Apple	12.5	12.5	0	12.5	12.5	0	0	12.5	0	0	6	50
Carrot	50	37.5	12.5	0	37.5	25.0	62.5	25.0	25.0	50.0	33	90
Nordmann fir	0	0	0	0	0	0	0	25.0	12.5	25.0	5	30
Ceanothus	0	12.5	37.5	0	0	25.0	25.0	0	12.5	0	11	50
Rhododendron	12.5	12.5	25.0	12.5	0	0	25.0	0	12.5	0	10	60

Table 11: Laboratory Experiment 9 (0 to 250 *Phytophthora* zoospores / L) on 12 December 2012 showing % of bait pieces & bait bags infected

Zoospores per litre (per litre bottle)	Bait	% positive baits (out of 8 per bait bag) determined by isolation										Mean % infected baits per bag	% of reps with an infected bait bag
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10		
0	Apple	0	0	0	0	0	0	0	0	0	0	0	0
25	Apple	0	0	0	0	0	0	0	0	0	0	0	0
125	Apple	0	0	0	0	0	0	0	12.5	0	0	1	10
250	Apple	0	0	0	0	0	0	0	0	0	0	0	0

Table 12: Laboratory Experiment 10 (0 to 250 Pythium zoospores / L) on 18 December 2012 showing proportion of bait pieces & bags infected

Zoospores per litre (per litre bottle)	Bait	% positive baits (out of 8 per bait bag) determined by isolation										Mean % infected baits per bag	% of reps with an infected bait bag
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10		
0	Apple	0	0	0	0	0	0	0	0	0	0	0	0
25	Apple	0	0	0	0	0	12.5	0	12.5	25.0	37.5	9	40
125	Apple	0	12.5	50.0	0	12.5	0	0	12.5	0	0	9	40
250	Apple	37.5	37.5	0	25.0	50.0	0	0	0	25.0	12.5	19	60

Reservoir water sampling

One nursery, ("A"), had a clean reservoir and lagoon in August 2012 with nil *Pythium* or *Phytophthora* detected, although *Pythium* spp. were present in the 1 L water samples from a pond and ditch on the nursery as shown by both baiting with Ceanothus leaves and by agar plate tests. At the second nursery, ("B"), isolates of *Pythium* were obtained from water in one of the two reservoirs and in the runoff from both sets of beds collected in the reservoirs (Appendix 3 and Table 14). Nursery "B" Site 1 reservoir was used for bait testing in 2013.

Table 14. 1 Litre water sample results for two nurseries in August 2012

Water sample source	% Bait pieces infested after adding to the water sample	Colony forming units (cfu l^{-1}) filtered from sample
Nursery "A" reservoir	0	0
Nursery "A" lagoon	0	0
Nursery "A" pond	100*	200
Nursery "A" ditch	100*	450
Nursery "B" site 1 reservoir	80*	80
Nursery "B" site 1 runoff	70*	113
Nursery "B" site 2 reservoir	0	0
Nursery "B" site 2 runoff	90	900

* a mixture of fast and slow *Pythium* species and non-pathogenic *Saprolegnia* sp.

Nursery reservoir bait bag experiments

Colony counts of the water samples are not yet available from the Eden Project laboratory. However, the 1 L water samples from the Site 1 reservoir in January have been reported to contain colonies that look like *Pythium* and *Phytophthora,* and Ceanothus leaf baits added to the water all became infested by these species.

The day the baits were set out at Nursery "B" on 29 January 2013 at the reed bed (R1) and outflow (R2) locations was unusually mild for the time of year with an air temperature of 13°C and a water temperature of 6°C. Oomycetes were isolated at ADAS from the baits, showing the presence of zoospores in the water this early in the year. They were identified as species of *Pythium* or *Phytophthora* on the basis of colony morphology on P5ARP agar and zoospore producing structures in the water floats (Table 15). Positive LFDs for both *Pythium* and *Phytophthora* spp. were obtained from both the reed bed and outfall position baits placed for 48 h in the reservoir in January, with infection of the shallow baits of both the

Ceanothus leaves and the apple pieces (Table 16). Both bait isolation and the LFDs showed *Pythium* and *Phytophthora* zoospores had been present at the outfall. It is possible that a higher LFD index relates to a greater quantity of the pathogen present; a greater amount of water-mould tissue could be expected to be present following further bait colonisation during the post-retrieval incubation period.

No *Pythium* spp. or *Phytophthora* spp. was isolated from the Ceanothus leaves which were tested without immersion, and so this confirms that those water-moulds detected after being in the reservoir were from the reservoir. Of the five Ceanothus leaves which were placed in the January water sample taken back to ADAS Boxworth, three leaves baited out *Pythium* spp. and one leaf had *Phytophthora* spp. (as determined by agar culture and water float).

Table 15. Isolation of species of *Pythium* spp. and *Phytophthora* spp. from apple and Ceanothus baits immersed for 48 hours in the Site 1 reservoir at Nursery "B" in January 2013 and the number of bait pieces infested per bag of eight

Deployment positions (and code) for baits	**Bait type**	**Number of bait pieces with *Pythium* spp.**	**Number of bait pieces with *Phytophthora* spp.**
R1 reed bed, Deeper bait	Apple	2	0
R1 reed bed, Shallower bait	Apple	4	0
R2 outfall, Deeper bait	Apple	3	0
R2 outfall, Shallower bait	Apple	3	1
R1 reed bed, Deeper bait	Ceanothus	1	0
R1 reed bed, Shallower bait	Ceanothus	1	0
R2 outfall, Deeper bait	Ceanothus	5	0
R2 outfall, Shallower bait	Ceanothus	3	0

Table 16. LFD tests carried out six days after baits were added in the Site 1 reservoir on 29 January 2013 at Nursery "B". Assessed as Index 1 = positive, but faint test to Index 5 = Test line as intense blue as the Control line

Deployment positions (and code) for baits	**Bait type**	***Pythium* LFD Index**	***Phytophthora* LFD Index**
R2 outfall, Shallower bait	Apple	4	4
R2 outfall, Shallower bait	Ceanothus	4	2

The longer (7 day) bait placement period (Tuesday 26 February to Monday 4 March 2013) with the baits tested in the laboratory on Wednesday 6 March 2013 showed positive LFD tests for both *Pythium* and *Phytophthora* at both locations and both depths (Table 17).

LFD testing of apple baits on-site by the grower after bag retrieval from the shallow depths at the reed bed and outfall locations in February 2013 gave negative results for *Pythium* and *Phytophthora*. It was not known if this resulted from the shorter (two day) bait placement period in the reservoir or the more immediate use of the LFD on retrieval compared with the tests carried out at ADAS five days later (of longer immersed baits).

Table 17. LFD tests carried out seven days after baits were added in the Site 1 reservoir on 26 February 2013 at Nursery "B" and in water from a slow sand filter on the nursery. Assessed as Index 1 = positive, but faint test to Index 5 = Test line as intense blue as the Control line

Deployment positions (and code) for apple baits	***Pythium* LFD Index**	***Phytophthora* LFD Index**
F after slow sand filter	2	2
R1 reed bed, Deeper bait	1	3
R1 reed bed, Shallower bait	1	4
R2 outfall, Deeper bait	1	1
R2 outfall, Shallower bait	1	2

At a third nursery "C", two apple baits placed in the water before the slow sand filter on 26 February, retrieved on the 28 February and put in the post and then incubated at ADAS until Monday 4 March 2013 were positive for both pathogens (Table 18).

Table 18. LFD tests carried out six days after baits were added before the slow sand filter on 26 February 2013 at Nursery "C" and in water from the slow sand filter. Assessed as Index 1 = positive, but faint test line to Index 5 = Test line as intense blue as the Control line

Deployment positions (and code) for apple baits	***Pythium* LFD Index**	***Phytophthora* LFD Index**
B1 Deeper bait	2	4
B2 Shallower bait	2	3

It was noted, however that at neither nursery did the deeper float sink much below the depth of the shallow bait bag. The weighting of bags will need to be adjusted.

From the baits tested so far, *Pythium* might have been more abundant than *Phytophthora* in January (based on isolations). In February, *Phytophthora* was either present at higher concentrations, or is trapped more efficiently than *Pythium* spp; or *Phytophthora* LFDs give a stronger test line than *Pythium* LFDs and the quantity of each water-mould required to give this line strength differs. For growers, however, any positive line warns there is infestation.

Discussion

The discrepancy between the spore concentrations determined from haemocytometer spore counts with calculation for the dilution factor, and the results from standard water tests (filtration followed by culturing to produce colony forming unit counts) was not solved. It was possible that the zoospores encysted on the sides of the HDPE bottles used. Growers generally send samples for testing in drinking water bottles made of PET (T. Pettitt, pers. comm.) rather than HDPE bottles. Encystment will reduce the number of motile zoospores available to infest the bait material. It was noted that in the zoospore observation test the number of spores was close to that sought by dilution, however in the absence of bait material, most zoospores had encysted within 3 hours. It was also hypothesised that as the baits were unable to move in the water in the laboratory bottles that the zoospores were not flushed through the bags. Movement of the bait bags and the water circulation in reservoirs is likely to mean that the bait comes into contact with more zoospores than when placed in a fixed 1 L volume. This could mean that significant bait infestation arises in water samples with relatively low spores per litre sample. Infestation of bait bags was shown from a reservoir and pre-slow sand filter, and when results from the water samples taken at the same time as the baits were placed become available than this will show the colony forming unit concentration at which the baits were able to become infested and then give positive LFD results.

The aim of this project was to produce a “generic” bait bag that can be used to trap a wide range of zoosporic species. Results on the species present in the reservoir water are not available yet and so it is not know what the range of, and the commonest, species were. It is possible to have LFDs tested by molecular methods for the presence of particular *Phytophthora* species, although this is unlikely to affect the control measures that will be used on plants against them (other than if non-indigenous species such as *Phytophthora ramorum* and *Phytophthora kernoviae* are detected).

It is possible that bait bags could be made that hold more than one type of plant material. The amount of material in the bag is dictated by the LFD test. The manufacturer’s instruction’s for direct sampling of infected plants is to take approximately 0.2g (equivalent to a piece of typical leaf tissue 25 mm square). It might be worth testing baits containing both apple and carrot pieces as it seemed in the laboratory tests that carrot was infested more frequently than apple by *Pythium* sp., whereas *Phytophthora* was found more frequently on apple. However, if the strength of the LFD test line depends on the number of bait pieces that are positive, then substitution by a bait type less favoured in general could reduce the

chance of a positive indication. If a nursery specialises in a particular range of crops then baits could be developed by the grower or advisor by performing a “choice test” in the reservoir that might be more attractive by Oomycete pathogen species tending to be found more frequently on those hosts. For example, including surface sterilised leaves of the species grown in one bait bag and Ceanothus in the other. Ceanothus gave a good trapping rate (trapping both pathogens in around half the bait bags).

Conclusions

Although the laboratory bait infestations were not always successful, sufficient information was gained to achieve the objective to identify plant material baits which have the greatest sensitivity for zoospore detection. Nordmann Fir needles were least frequently infested (both by water-moulds and other fungi). Although Rhododendron and Ceanothus leaves were shown to trap both *P. cryptogea* and *Pythium* sp., the possibility of leaves carrying splash-borne *Phytophthora* and *Pythium* species meant that apple (which trapped more *Phytophthora* than *Pythium*) and carrot (which trapped more *Pythium* than *Phytophthora*) were selected for further testing. *Pythium* infestation was, however, seen across all bait types. Internal apple flesh, with eight pieces (7 mm x 7 mm) per bait bag was the final design. These bait bags were utilised on two nurseries in early 2013 and gave catches of both *Pythium* and *Phytophthora*.

The second objective, to examine the sensitivity of lateral flow devices for detecting *Phytophthora* spp. and *Pythium* spp. to different quantities of infested bait material was partially achieved. Laboratory tests with either different amounts of infested baits per test or a range of spore concentrations to see the strength of positive indication of the test line on a lateral flow device (LFD) were not completely successful. However, LFDs for both pathogen groups were successfully used on bait bags retrieved from irrigation water at nurseries after leaving the retrieved baits for three to four days to incubate before testing.

Work has started on Objective 3 to determine the optimum number of bait bags, quantity of bait material and placement positions in reservoirs to maximise detection and on Objective 4 to determine whether there are any seasonal / weather related influences on zoospore release to use as guidance to maximise detection.

Objective 5, to provide step-by-step instructions for nursery staff on bait use has been achieved, and utilised by growers on two nurseries. Demonstrations of the baiting and LFD techniques used to other growers will be arranged during 2013.

Knowledge and Technology Transfer

- Three nurseries have participated in this project, with two growers preparing and setting their own bait bags in their irrigation water. One grower also utilised the baits with LFDs. Instruction sheets have been prepared for the construction of bait bags on the nursery and for the use of LFDs to test baits.

- Information of zoospore behaviour was presented to HDC panel members at the Oomycete workshops at Stoneleigh on 1 October 2012. Two workshops are to be planned for growers in 2013 together with Tim Pettitt.

Glossary

Technical terms have been explained within the text.

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Appendices

Appendix 1: Instructions for using Pocket Diagnostic Lateral Flow Device

Store test kits at room temperature (up to 40°C), not refrigerated or frozen.

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, or add to the bottle (see below) and squash the apple a little with a washed item e.g. biro.

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 buffer bottle per bait bag will be used for both the Pythium and Phytophthora tests.
- Label the bottle with the sample identity if there is more then one sample.
- Shake the bottle firmly 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 bottle during shaking will normally warm it to above 10°C to enable the process to work.

Step 3; using the LFD

- If the test is being performed in conditions below 10°C then warm the packaged lateral flow device (LFD) before opening.
- Remove the test device from its foil packing just before it is needed. DO NOT TOUCH THE VIEWING WINDOW.
- Label the back of the device with the sample identification and date. One Pythium and one Phytophthora device will be needed per buffer bottle.
- 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 2 large or 3 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 pale blue dye will appear in the viewing window as liquid flows along the test device.
- If no pale blue dye becomes visible in the viewing window after 30 seconds, another drop of sample can be added to the sample well. Using too much liquid 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.

Contd.

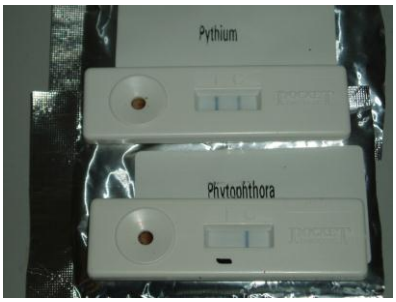
Instructions for using Pocket Diagnostic Lateral Flow Device contd.

Step 4: Examining the results

- A blue 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 blue 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 good lighting.
- 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 which happen after 10 minutes.
- 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 artificial light.
- After use, the test devices should be returned to the foil packet with the silica gel packet provided. They can be stored for long durations with only slight loss of results if kept dry and out of the light.

Step 5: Interpretation of the results

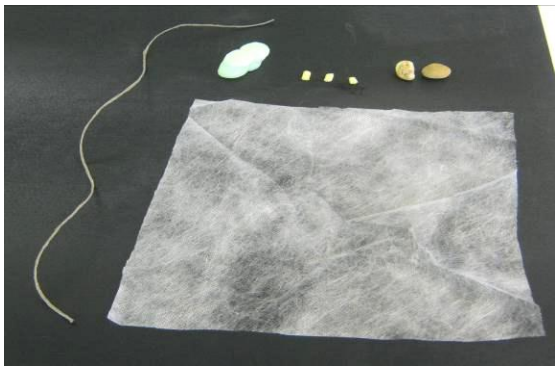
- A positive result indicates that the plant material sampled contains the fungus under test.
- Under some circumstances, laboratory confirmation of an on-site test result may be necessary.
- A negative result indicates that the target pathogen was not detected in the test sample. As with all diagnostic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



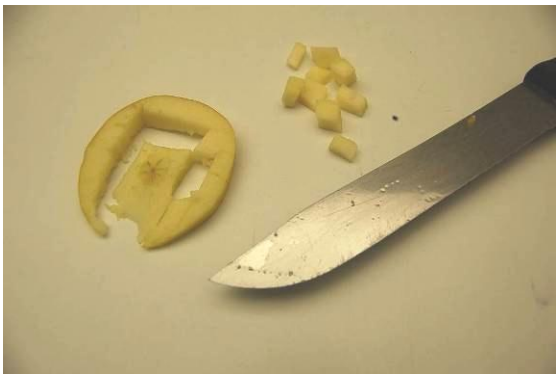
Problems with the readings

- 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 long enough. If in doubt, repeat with a new device using a fresh sample, or repeat in a few days.
- '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 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.

Pythium & Phytophthora Water Baiting: Instruction Sheet for Growers



1. Components needed for the bait bag; 7-10 g boiled stones, polystyrene, apple pieces, string, fleece (28 x 28 cm).



2. Cut a slice of Golden Delicious apple 7mm thick. Cut out eight squares approx 7 x 7 mm 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.

Picture of bait in reservoir to be taken and inserted here once final set-up is decided in 2013.

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

5. Untie the collected bag. With washed hands, place the apple pieces in the buffer bottle. Shake buffer bottle vigorously for at least 1 min until the buffer becomes coloured by the apple.

6. Draw up apple solution from the buffer bottle and pipette 2-3 drops into the well on the LFD test device. The C line should show blue. If a blue T line then appears within 10 mins the test is positive.

Appendix 2: *Pythium* sp. zoospore survival and encystment after three to five hours* in various types of water (Experiment 6) December 2012

6 December 2012 experiment

Block & Plot	Treatment	Zoospores	Encysted
A1	1	29	120
B2	1	25	60
C3	1	35	100
D4	1	32	150
E5	1	29	100
A2	2	28	200
B3	2	9	130
C4	2	20	180
D5	2	26	190
E1	2	21	170
A3	3	25	210
B1	3	7	110
C5	3	22	150
D2	3	25	200
E4	3	25	120
A4	4	24	160
B5	4	21	190
C1	4	24	195
D3	4	30	150
E2	4	23	150
A5	5	39	100
B4	5	20	200
C2	5	32	100
D1	5	20	175
E3	5	29	200

Treatment	Water type	Mean Zoospore count	Mean Encysted count	Total count
1	sterile rain water	30	106	136
2	distilled water	21	174	195
3	stood tap water	21	158	179
4	fresh tap water	25	174	199
5	'primed' fresh tap water	28	155	183

* counts took two hours progressing from cell A1 to E5 (with treatments 1 to 5 randomised in each block A to E)

Appendix 2 contd.: *Pythium* sp. zoospore survival and encystment after three to five hours* in various types of water (Experiment 6) December 2012

7 December 2012 experiment

Block & Plot	Treatment	Zoospores	Encysted
A1	1	7	450
B2	1	6	350
C3	1	11	300
D4	1	10	310
E5	1	10	300
A2	2	4	400
B3	2	5	300
C4	2	7	320
D5	2	6	450
E1	2	10	300
A3	3	8	300
B1	3	8	400
C5	3	7	310
D2	3	8	300
E4	3	9	310
A4	4	6	300
B5	4	7	300
C1	4	3	400
D3	4	8	310
E2	4	4	400
A5	5	9	320
B4	5	10	320
C2	5	8	300
D1	5	6	300
E3	5	8	320

Treatment	Water type	Mean Zoospore	Mean Encysted	Total count
1	sterile rain water	9	342	351
2	distilled water	6	354	360
3	stood tap water	8	324	332
4	fresh tap water	6	342	348
5	'Primed' fresh tap water	8	312	320

* counts took two hours progressing from cell A1 to E5 (with treatments 1 to 5 randomised in each block A to E)

Appendix 3: Results from water sampling in August 2012 at various locations at "Nursery A".

Sample 12/C219 collected 9/8/12, processed 10/8/12				
RESULTS OF TESTS ON SAMPLES COLLECTED FROM "NURSERY A" IN WEEK 32, 2012:				
Water sample	Sample 1 Reservoir	Sample 2 Lagoon	Sample 3 Pond	Sample 4 Ditch
Bacteria (cfu l^{-1}) on PDA	-	-	-	-
TOTAL FUNGUS(cfu l^{-1})	1000	2480	6080	4320
Trichoderma spp.	0	1040	880	90
Fusarium spp.	0	0	160	20
Tests for Pythium and Phytophthora				
Bait tests (% infected)	0 (3*)	0 (3*)	100**	100**
Plate tests (cfu l^{-1})	0 (10*)	0 (40*)	200**	450**
Immunodiagnostic tests (viable spores l^{-1})	not requested	not requested	not requested	not requested

* *Mortierella* sp. = not a plant pathogen, often seen in water samples – a group of fungi that grow on *Pythium/Phytophthora* selective media.

** A mixture of fast and slow-growing *Pythium* species (some plant pathogens) and *Saprolegnia* sp. (not a plant pathogen).

Comments:
Samples from the reservoir and lagoon look relatively clean. The high *Trichoderma* count from the lagoon was of some interest as some *Trichoderma*s can be effective biocontrol agents. Samples from the pond and the ditch contained relatively high oomycete counts, with large numbers of *Pythium* propagules present – as stated above, examples of these have been sub-cultured for full identification and possible use in the HDC baiting project if they turn out to be horticultural plant pathogens (and prolific zoospore producers).

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Appendix 3: Results from water sampling in reservoirs and bed runoffs in August 2012 at "Nursery B". The LG reservoir was utilised for bait testing in 2013.

Sample 12/C222 collected 21/8/12, processed 22/8/12				
RESULTS OF TESTS ON SAMPLES COLLECTED FROM "NURSERY B" IN WEEK 34, 2012:				
Water sample	Site 1 Res. (used for baiting in 2013)	Site 1 Runoff	Site 2 Res.	Site 2 Runoff
Bacteria (cfu l^{-1}) on PDA	1.79×10^4	7.34×10^4	4.78×10^4	1.92×10^5
TOTAL FUNGUS(cfu l^{-1})	3627	4907	1013	6800
Trichoderma spp.	14	173	0	550
Fusarium spp.	133	0	0	50
Tests for Pythium and Phytophthora				
Bait tests (% infected)	80*	70*	0 (20**)	90*
Plate tests (cfu l^{-1})	80*	113*	0	900*
Immunodiagnostic tests (viable spores l^{-1})	-	-	-	-

- A mixture of fast and slow-growing *Pythium* species (some possible plant pathogens) and *Saprolegnia* sp. (not a plant pathogen).

** *Saprolegnia* sp only.

Comments:
Isolates of fast-growing *Pythium* sp. were taken from Site 1 Res, Site 1 Runoff and Site 2 Runoff samples for identification to species. The Site 2 Res. Sample looks comparatively clean but the Site 1 Res sample has a high concentration of both oomycetes (some possible pathogens) and *Fusarium* spp. (a mixture of members of the '*Roseum*' group {most frequently pathogens of cereals} and *F. oxysporum* a potential pathogen to a wide range of plant species). The Site 2 Runoff sample also contained a small quantity of compost and root debris – some of this was plated out onto selective agar and fast-growing *Pythium* spp. were isolated and subcultured for identification to species.

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