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The results and conclusions in this report are based on an investigation conducted over a 3 year period (it is a final report). 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.

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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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Grower Summary

Headline

- Both slow sand filtration and disinfectant treatment control a range of *Phytophthora* species, providing a robust strategy for effective management of *Phytophthora* diseases on commercial nurseries.
- Using a leaf bait diagnostic combined with a lateral flow device (LFD) test could provide a quick and easy method to determine whether *Phytophthora* species are present on a nursery.

Background and expected deliverables

Every year, significant economic losses to hardy nursery stock are attributed to infection by various *Phytophthora* species. Those species that cause root rot symptoms, such as *P. cinnamomi*, *P. cryptogea*, *P. cactorum* and *P. nicotianae*, are particularly prevalent. Two newly described species *Phytophthora ramorum and P. kernoviae*, are currently the most significant quarantine pathogens in the UK. The disease caused by *P. ramorum*, known as sudden oak death in the USA, has affected a wide range of ornamentals in the UK including Viburnum, Rhododendron, Pieris, Camellia, Kalmia and Syringa spp. It has also been found on a number of tree species. To date, there have been over 510 confirmed outbreaks of *P. ramorum* and four of *P. kernoviae* on nurseries in England and Wales and legislative measures are resulting in the destruction of large numbers of plants.

There are three main aims to this project:

1. To evaluate techniques for improved detection of *Phytophthora* species on nurseries.
2. To investigate the effectiveness of slow sand filtration for the removal of different *Phytophthora* species from water sources (including those of quarantine significance).
3. To test the effectiveness of disinfectant/chemical treatments for the decontamination of irrigation equipment, standing areas, Danish trolleys and other equipment.

The project is expected to deliver:

- A validation of baiting techniques for detection of a wide range of *Phytophthora* spp. of significance to nursery stock, from water sources, Danish trolleys, soil/compost and other potential risk sites on nurseries.
- An increased speed and accuracy of detection and identification of *Phytophthora* species utilising currently available techniques (PCR and ELISA).

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- An evaluation of the effectiveness of slow sand filtration for the removal of different *Phytophthora* species from water sources on the nursery.
- An assessment of disinfectant/chemical treatments for effective decontamination of irrigation equipment, standing areas and other equipment found on nurseries.
- An evaluation of the effectiveness and applicability of refined disinfection technologies under commercial conditions.
- A simple robust strategy for effective control of *Phytophthora* species on the nursery

Summary of the project and main conclusions

This project was co-funded by HDC and the Defra Plant Health Division project PH0 320. All work on *P. ramorum* and *P. kernoviae*, including the work on slow sand filters, was carried out under license (PHL251C/5574 (02/2007) amended (10/2007)).

1) *Evaluation of techniques for improved detection of* Phytophthora *species on nurseries*

Two baits (autoclaved rhododendron leaves – see Figure 1 - and a selective antibiotic agar) were compared to determine how effective they were at detecting of a range of *Phytophthora* species (*P. ramorum*, *P. kernoviae*, *P. cryptogea*, *P. ilicis*, *P. cactorum* (*P. nicotianae*) and *P. cinnamomi* from water. All the *Phytophthora* species tested, regardless of the spore type, were detected using the rhododendron leaf bait, however the level of detection was dependent on the species tested. The selective agar bait was surprisingly much less sensitive. The rhododendron leaf bait was most effective in water contaminated by *P. ramorum* or *P. cryptogea* and least effective with *P. ilicis*.

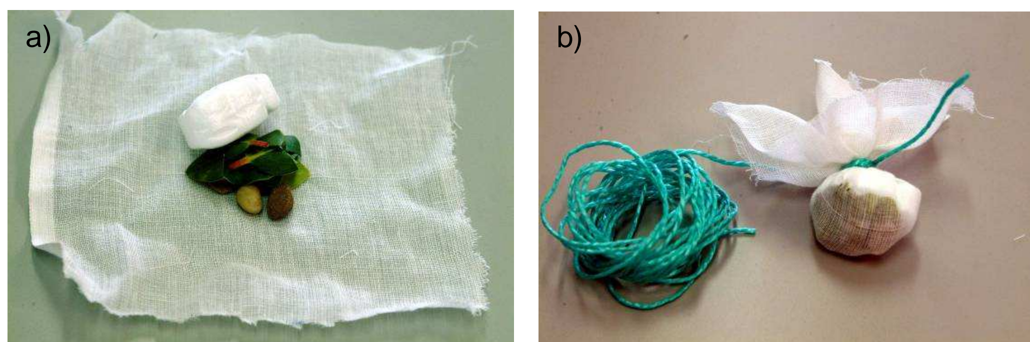

Figure 1. Construction of rhododendron bait a) showing bait contents and b) completed bait.

Three methods were tested to detect *Phytophthora* species from bait material; direct plating onto agar, a genus specific lateral flow device (LFD) and TaqMan PCR analysis. Direct plating had the advantage that all the species present were likely to be detected, however it

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can take up to 7 days to make a full species identification. The LFD detected all *Phytophthora* species tested. Results from the LFD are rapid (in minutes), easy to interpret and can be used on site. Results from the use of TaqMan PCR were obtained within the day and the *Phytophthora* could be identified to species level, provided the appropriate primers and probes are available.

Using the leaf bait combined with the LFD test could provide a quick and easy method to determine whether *Phytophthora* species are present on a nursery. Technology is now available that allows DNA trapped on the LFD to be analysed by TaqMan enabling species identification to be carried out should it be required. Growers interested in adopting this technology should contact their diagnostic clinic for more information.

2) *Investigation of the effectiveness of slow sand filtration for the removal of Phytophthora species from water sources.*

Two sets of slow sand filters (SSF) were constructed (See Figure 2), one to evaluate the potential elimination of the quarantine *Phytophthora* species *P. ramorum* and *P. kernoviae* from water and the second for tests using indigenous *Phytophthora* species. The species tested included *P. cactorum*, *P. citrophthora* (ex Ceanothus), two isolates of *P. nicotianae* (ex Cordyline and Poinsettia) and a range of different, but unidentified, species isolated from citrus, begonia, fuchsia and pansy.

Figure 2. Slow sand filters held at CSL (left) and STC (right)

A flow rate of water 400 ml per minute was established for both sets of filters and, over a period of two years, different spore loads and methods for the introduction of inoculum into the system were used to monitor their effectiveness.

When working correctly, both filters successfully removed the introduced *Phytophthora* species from the system. The filters challenged with the indigenous *Phytophthora* species were tested outdoors and worked effectively over a wide range of temperatures. The filters

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challenged with the quarantine pathogens were effective when installed in a quarantine glasshouse facility.

Any breaks in the biologically active layer of the filters (top few centimeters) i.e. through cleaning or addition of disinfectant, resulted in failures of the filtration system. The activity of the filter recovered once they had re-primed themselves.

3) *Testing the effectiveness of disinfectant/chemical treatments for the decontamination of irrigation equipment, standing areas, Danish trolleys and other equipment.*

The efficacy of six disinfectants was tested against a range of *Phytophthora* species - *P. ramorum, P. kernoviae*, *P. cactorum*, *P. ilicis*, *P. cinnamomi*, *P. cryptogea* and *P. nicotianae*. The disinfectants were chosen from different chemical categories including oxidising agent, cationic surfactant, reducing agent, organic acid, alcohol and halogen/halogen releasing compounds. The full list was 'Jet 5', 'Hortisept', 'Unifect G', 'Menno Florades', Industrial Methylated Spirits (IMS) and bleach. Disinfectant efficacy was initially tested on contaminated inert surfaces and organic substrates, before later testing them on contaminated compost/soil, Mypex, leaves and water. These media were chosen to represent the typical sources of contamination that occur on a commercial nursery.

Contaminated cellophane squares were used to provide an inert surface for the *Phytophthora* species to grow on, whilst not affecting the activity of the disinfectant. On this surface IMS (70%) was the most effective disinfectant. The exposure time required for decontamination varied depending on the species and temperature.

- In general, at temperatures between 10 and 20°C, less time was required to achieve complete decontamination and *P. ramorum* required longer periods than other species.

The addition of organic matter to the test system altered the efficacy of the disinfectants. 'Unifect G' remained effective and, compared to cellophane alone, required a similar exposure time to ensure complete decontamination. IMS (70%) required a longer exposure time for equivalent efficacy. All the other disinfectants failed to decontaminate the cellophane over the time period tested.

The disinfectants, applied at 1 litre/m^2 or as a drench, were tested for efficacy against compost contaminated by *P. ramorum* or *P. kernoviae*. Saturation of compost with disinfectant gave full decontamination, for all but 'Hortisept' and 'Menno Florades'. No disinfectant worked at 1 litre/m^2.

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IMS (70%) and 'Unifect G' were the most effective disinfectants on contaminated Mypex matting, only 'Hortisept' did not effectively decontaminate the matting. The exposure times required varied between disinfectants.

Only 'Unifect G' was effective against established *P. ramorum* infection on Rhododendron, Camellia and Viburnum leaves and then only after an exposure of 24 hours. 'Menno Florades' and bleach decontaminated some of the plant species also after 24 hours exposure.

Both hydrogen peroxide (as 'Jet 5') and sodium hypochlorite were extremely effective at decontaminating water containing *P. ramorum* spores after only 5 minutes of exposure. Chlorine dioxide (as 'Sanogene') was effective within 5 minutes following exposure to a concentration of 500 ppm or 1 hour at 50 ppm but it was not effective at any of the exposure times tested at 5ppm.

Disinfectant use on commercial nurseries:
'Horticide' (equivalent to 'Unifect G') and 'Jet 5' were tested at two nurseries. However, a lack of consistent infection over the sites made comparisons difficult. Disinfectants were tested on standing areas (gravel and Mypex), roads (gravel) and in soil. As *Phytophthora* species were not detected on equipment (trolleys etc) these were not included in the testing. A lack of consistent isolation of *Phytophthora* species pre-treatment and control samples at both sites made evaluation of the disinfectants difficult, however 'Horticide' appeared to be the more effective of the two disinfectants.

Financial benefits

Phytophthora species of statutory significance –
Results from this project will not prevent losses incurred to nurseries through the inadvertent introduction of quarantine *Phytophthora* species on infected bought-in plants. However, the information generated, providing growers take precautionary measures, will go a considerable way to minimising secondary spread around the nursery. The prevention of secondary spread should help minimise the imposition of a protracted quarantine status, which could, and has been, financially crippling on some HONS nurseries.

Indigenous *Phytophthora* species -
The damage caused by indigenous *Phytophthora* species is less obvious and growers or their advisers do not always determine the primary cause of plant death accurately. In dry years losses are likely to be relatively low (ca. <1-5%) and are generally tolerated as 'natural wastage'. However, in wet seasons, especially in standing areas where drainage is poor,

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losses can be much more significant (>5-10%). The utilisation of the information in this report and adoption of the measures described could have a considerable impact on the survival and subsequent carry-over of *Phytophthora* species on the nursery.

Action points for growers

These action points have been identified as key components of a robust strategy to minimise the risk of *Phytophthora* infection on HONS nurseries.

- Take care when buying in plants, as they may be a primary source of *Phytophthora* infection on a nursery.

- Ensure familiarity with the range of species susceptible to the quarantine *Phytophthora* species and check plants regularly for suspicious symptoms.

- On receipt of new stock check health of foliage and roots. Get plants checked at a reputable diagnostic laboratory or Plant Clinic if there is evidence of wilting, necrosis or dieback or general poor vigour.

- Establish a 'quarantine' or holding area for new stock away from the main production unit. Hold plants for as long as possible to ensure they are free from all pests and pathogens before general nursery release.
    - Run-off water from this area should not contaminate other areas of the nursery and leaf debris should be swept up regularly and disposed of.

- *Phytophthora* species prefer wet conditions, so measures should be taken to minimise standing water around the site by improving drainage.

- For some *Phytophthora* species, especially those of quarantine significance, there is a risk of pathogen dissemination through contaminated leaf litter and other debris entering uncovered mains water holding tanks or reservoirs used to collect run-off water. Where possible tanks should be covered to prevent contamination.

- Where water bodies are too large to cover, routine monitoring for *Pythium* and *Phytophthora* species should be carried out. Some diagnostic laboratories offer such services.
    - Consider the installation of a slow sand filter or similar technology for effective water disinfection.

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- Alternative technologies (e.g. UV, ozone, pasteurization) are available however their use can be more costly and, in some cases, less effective due to variable water quality.
- Growers should seek professional advice before investing in expensive disinfection technology.
- For more information on slow sand filtration technique used in this project please talk to specialists with knowledge of the system e.g. STC Ltd. Also, make sure you have a copy of the HDC grower guide.

- Maintain a high level of nursery hygiene and, where appropriate, use disinfectants to further reduce the risk of pathogen dissemination. To ensure greatest disinfectant activity remove as much organic matter as possible. Data generated in this project suggests that disinfectants based on glutaraldehyde (e.g. 'Horticide') are most likely to be effective; though further validation is required. With the transfer of responsibility of the Biocides Directive to the HSE it will be important to keep up to date with changes any in legislation.

- Where indigenous *Phytophthora* species occur apply appropriate fungicide treatments (consult a BASIS qualified advisor or plant pathologist for specialist guidance). Where *P. ramorum* or *P. kernoviae* have been identified specific quarantine measures should be followed.

- So, by considering the various potential routes of entry of *Phytophthora* species, by having a better understanding of the life-cycle, survival and dispersal mechanisms of the pathogen and by taking appropriate and, in most cases, a precautionary approach to its control on the nursery, it should be possible to develop a robust and sustainable disease control strategy (see Figure 3). This will significantly reduce the risk of pathogen introduction and, most importantly, delay or halt subsequent dissemination around the nursery and beyond. Crucially, measures taken for the control of indigenous Phytophthora species are likely to be equally effective against the quarantine Phytophthora species.

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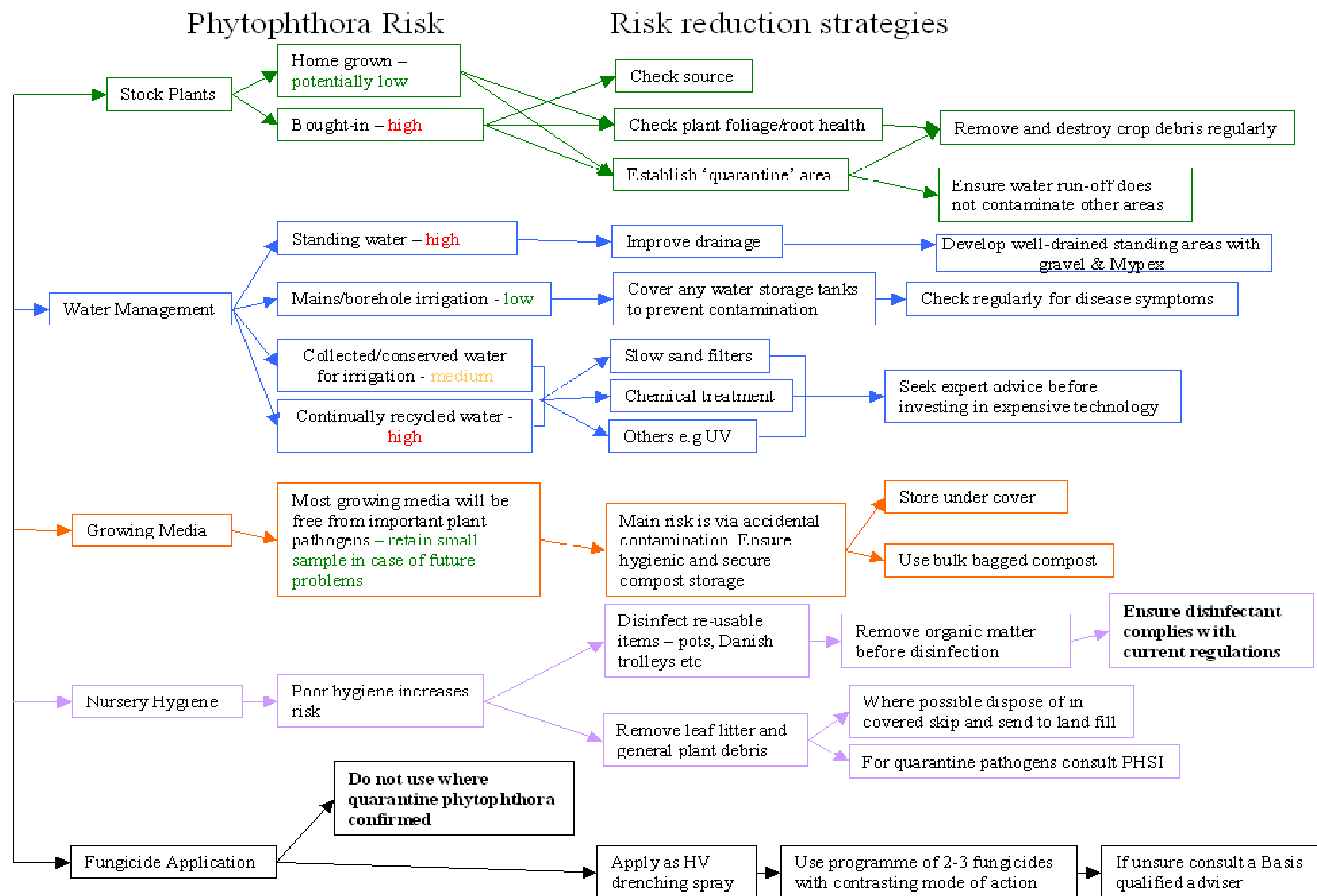

Figure 5. Schematic of disease management strategy developed to minimise the risk from *Phytophthora* species on HONS nurseries.

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Science Section

This project was co-funded by DEFRA Plant Health Division project PH0 320). All work on *P. ramorum* and *P. kernoviae*, including the work on slow sand filters, was carried out under license (PHL251C/5574 (02/2007) amended (10/2007)).

Introduction

Significant economic losses in HONS are regularly attributed to infection by various indigenous *Phytophthora* species e.g. *P. cinnamomi*, *P. cryptogea*, *P. cactorum*, *P. nicotianae*, on an annual basis; their overall severity depending on the prevailing climatic factors. *Phytophthora ramorum* (Werres *et al*. 2001), a newly described species, is currently the most significant quarantine pathogen in the UK. The disease, known as sudden oak death in the USA, has affected a wide range of ornamentals in the UK including Viburnum, Rhododendron, Pieris, Camellia, Kalmia and Syringa spp. and has also been found on a number of tree species. To date, there have been over 450 confirmed outbreaks of *P. ramorum* and 4 outbreaks of *P. kernoviae* on nurseries in England and Wales. Emergency UK and EC measures have been introduced with the specific aim to prevent spread of the disease. The legislation requires destruction of all plants within a 2 m radius of a diseased plant and holding all susceptible plants within a 10 m radius, plus any remaining plants from the same consignment as the diseased plants, for a period of 3 months without application of fungicides active against oomycetes, for further assessment. These statutory measures are undoubtedly having a major impact on the HONS industry, resulting in the destruction of large numbers of plants and threatening the future viability of some businesses.

This project aims to evaluate techniques for improved detection of *Phytophthora* species on nurseries, to investigate the effectiveness of slow sand filtration for the removal of different *Phytophthora* species from water sources (including those of quarantine significance) and to test the effectiveness of disinfectant/chemical treatments for the decontamination of irrigation equipment, standing areas, Danish trolleys and other equipment. The effectiveness and applicability of the refined technologies have been evaluated under commercial conditions.

Materials and methods

1. Baiting *Phytophthora* species from water

In the first year of the project the effectiveness of two bait types, (i) autoclaved rhododendron leaves and (ii) a ‘fishing bait’ containing selective agar, were tested for the detection of *Phytophthora* species from water. Baits were tested against five *Phytophthora* species (two quarantine species: *P. ramorum* and *P. kernoviae*; and three indigenous species: *P. cactorum*, *P. ilicis* and *P. cryptogea*). Where possible, baits were tested against both

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zoospore and sporangial suspensions. During these experiments spores of *P. cinnamomi* and *P. nicotianae* could not be produced, however during the course of the final year of the project spores of *P. cinnamomi* were produced and tested against the rhododendron leaf bait.

1.1. Bait production

For each test, 30 young healthy leaves were pinched from a rhododendron 'Cunninghams White' plant, cut into four, and autoclaved at 110°C for 10 min. Baits were constructed by wrapping eight autoclaved leaf sections together with a piece of polystyrene packing and two small pieces of sterile gravel (≈6 g) in a piece of muslin [approx 9cm^2 (Figure 1a)]. The addition of polystyrene and gravel ensured that the bait floated just below the surface of the water. The contents of the muslin bag were secured with string (Figure 1b); the string also allowed easy retrieval of the bait.

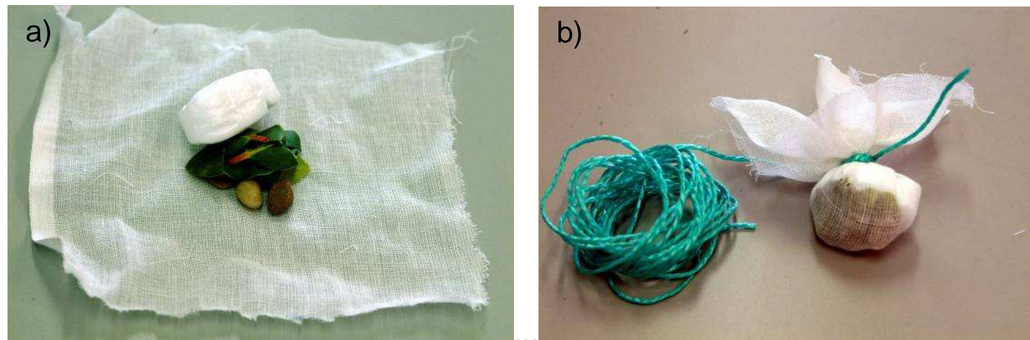

Figure 1. Construction of rhododendron bait a) showing bait contents and b) completed bait.

1.2. *P. cinnamomi* inoculum production

A plug of *Phytophthora cinnamomi* (IMI 335525) (obtained from the CABI fungal collection) was grown on 10% V-8 agar at room temperature, under day light bulbs (12h light/12h dark regime) until the colony reached the edge of the agar plate. The agar plate was flooded with 20 mL of sterile distilled water (SDW) and incubated at 20°C for 4-5 days. The resulting sporangia were removed from the agar surface using a sterile plastic rod and sporangial counts carried out using a haemocytometer.

1.3. Bait testing

Bait tests were set up in 1 L plastic microwave tubs. Four replicate tubs were set up containing 500 mL water with 0.01, 0.1, 1 or 10 spores mL^{-1} (5, 50, 500 or 5000 spores total). Tubs containing 500 mL distilled water only were used as controls. A single bait was placed into each tub and left at room temperature for two days. Baits were removed after two days and the presence of *P. cinnamomi* determined either by direct plating of leaf

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sections onto a *Phytophthora* selective agar or through the use of a *Phytophthora* genus specific lateral flow device.

1.4. Comparison of methods used for the detection of *P. cinnamomi* from bait material.

1.4.1. Direct plating onto *Phytophthora* selective agar

Four leaf sections were selected at random and plated directly onto $PARP_5H$ agar (Appendix I). After seven days incubation at room temperature the $PARP_5H$ plates were examined for the presence of *Phytophthora* species. For each spore concentration and bait type, the number of leaf sections/agar pieces showing growth of *P. cinnamomi* was recorded and the percentage baits infected calculated.

1.4.2. Lateral flow device (LFD)

Two leaf sections were selected at random for testing with the *Phytophthora* (genus specific) CSL Pocket Diagnostic LFD. Extraction from leaves and the test itself were carried out according to the manufacturer's instructions.

2. Slow sand filters (SSFs)

In the first year of the project, six SSFs were constructed, two for use with *P. ramorum* and *P. kernoviae* [held in a quarantine glasshouse at CSL (Figure 2)] and four for use with indigenous *Phytophthora* species [located outdoors at STC (Figure 2)].

Figure 2. Slow sand filters held at CSL (left) and STC (right)

Testing the effectiveness of the SSFs for the removal of *Phytophthora* species from contaminated water was started in 2005 and continued until November 2007.

2.1. Removal of *P. ramorum* and *P. kernoviae*.

Over the first two years of the project SSFs were challenged in two different ways, firstly by one-off challenges with high concentrations of spores added to the filter headwater

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(sporangia, zoospores or chlamydospores for *P. ramorum,* and sporangia or zoospores for *P. kernoviae*). Spore suspensions ranged in concentration from 10^4 to 10^6 spores/litre. The second method involved the addition of leaf material infected by *P. ramorum* or *P. kernoviae* to the headwater to provide continuous low-level release of inoculum. During the third year of the project SSFs continued to be challenged using infected leaf material.

2.1.1. Production of infected leaf material

Detached leaves of Rhododendron 'Cunningham's White' and Magnolia 'Grandiflora' were wounded by a single stab on the adaxial (upper) surface and a 5 mm agar plug, taken from the leading edge of a seven day-old colony of either *P. ramorum* or *P. kernoviae* placed over the wound. Leaves were placed in a moist chamber and incubated at room temperature (20˚C) for between 7 and 14 days. Fresh infected leaf material was added to the SSF headwater every four to eight weeks.

2.1.2. Sampling SSFs

Following the introduction of infected leaf material to the system, Rhododendron leaf baits were placed in the SSF headwater every one to two months to ensure the continued presence of *P. ramorum* or *P. kernoviae*. Baits were removed from the headwater after two days and plated onto $PARP_5H$. To determine whether the filters had effectively removed *P. ramorum* and *P. kernoviae* from the water, Rhododendron leaf baits were placed in the post filtration collection tank monthly. In general, baits were left in the tank for 5 days before being plated onto $PARP_5H$ agar. To ensure that *P. ramorum* and *P. kernoviae* were not passing through the filters at levels below the limit of detection of the leaf baits water was collected from the filter outlet every few months and tested using a newly developed TaqMan PCR technique (PHD R+D project PH0414) for quantifying *P. ramorum* and *P. kernoviae* in water samples.

2.1.3. Effect of cleaning on the ability of SSF to remove *P. ramorum* and *P. kernoviae* from water.

After running for 21 months the SSF were cleaned by removing the top 5 mm of the filter bed. Following cleaning, Rhododendron baits were placed in the collection tank. In general, baits were replaced daily between days 1 and 8. In addition baits were also examined at 30, 52 and 68 days after cleaning, these baits were removed from the collection tank after 22, 5 and 5 days respectively. Leaf baits were plated onto $PARP_5H$ agar and examined for the growth of *P. ramorum* and *P. kernoviae* after seven days. Water samples were also collected from both the headwater and collection tank to determine the presence of *P. ramorum* and *P. kernoviae* DNA.

2.1.4. Effect of disinfectant on the ability of SSF to remove *P. ramorum* from water.

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'Jet 5' was added to the headwater of the *P. ramorum* SSF to give an indication of how the running of the filter would be affected should disinfectant inadvertently enter the filter. The disinfectant was added to the headwater to give a final headwater concentration of 0.4% (102 mL disinfectant in 25.5 L water). The pH of the headwater and outlet water was monitored hourly to indicate the progress of the disinfectant through the system. Fresh inoculum and Rhododendron leaf baits were added to the filter headwater and collection tank respectively seven hours after the addition of disinfectant. In general, the baits were replaced daily between 3 and 11 days after the addition of Jet 5. Two further baits were assessed at day 18 and 25; these were left in the collection tank for seven days. Leaf baits were plated onto $PARP_5H$ agar and examined for the growth of *P. ramorum* after seven days. Water samples were also collected from both the headwater and collection tank to determine the presence of *P. ramorum* DNA.

2.2. Removal of indigenous *Phytophthora* species from water using SSF

Tests on the indigenous *Phytophthora* species were carried out using leaf material infected with *P. cactorum*, *P. citrophthora* (ex Ceanothus), two isolates of *P. nicotianae* (ex Cordyline and Poinsettia) and a range of species isolated from citrus, Begonia, Fuchsia and Pansy. Tests carried out to determine the effectiveness of the filters were similar to those used with *P. ramorum* and *P. kernoviae,* the main exception being that water samples were not tested using TaqMan but filtered through Nitrocellulose membrane filters (3μm), the filter papers were re-suspended, aliquots of the filtrate plated onto PARP5H and any *Phytophthora* species counted.

3. Efficacy of water treatment chemicals against *P. ramorum*.

Three chemicals were tested for efficacy in water contaminated with *P. ramorum* spores - hydrogen peroxide (as 'Jet 5'), sodium hypochlorite and chlorine dioxide (as 'Sanogene').

A sporangial suspension of *P. ramorum* was produced by growing an agar plug taken from the culture collection on 10% V-8 agar (Annex I) at 20°C, under day light bulbs (12h light/12h dark regime) until the colonies reached the edge of the agar plates. The agar plates were flooded with 5 mL of sterile distilled water (SDW) and the sporangia removed from the agar surface using a sterile plastic rod. Fresh 10% V-8 agar plates were inoculated with 100 μL of the resulting sporangial suspension and incubated under the previously described temperature and light regime for 3 days. Plates were flooded with 10 mL SDW, sporangia removed from the agar surface using sterile plastic rods and the sporangial concentration adjusted to give a final spore concentration of 10^4 sporangia/mL. For each treatment, 10 mL of the sporangial suspension was pipetted into three replicate sterile universal containers, sufficient disinfectant was added to each universal to give a final concentration of 0.4% and 10% for hydrogen peroxide and sodium hypochlorite respectively and, 5, 50 and 500 ppm for

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chlorine dioxide. In the control tests the chemical treatment was replaced by SDW. After 5, 15, 30, 45, 60, 90 and 120 minutes, 100 µL of the disinfectant/spore suspension was removed from each replicate test, spread-plated onto 10% V-8 agar and incubated at 20°C. After 24 h incubation 100 sporangia from each plate were assessed for germination. Spores were classed as germinated when the germ tube was longer than the spore.

4. Use of disinfectant on HONS nurseries

Two HONS nurseries were chosen to examine the effectiveness of selected disinfectants on site. The nurseries had differing *Phytophthora* histories and working practices:

Nursery 1

Pre-treatment testing indicated the presence of various *Phytophthora* species in most areas of the site, with *P. ramorum* found at two locations, 1) a gravel standing area and 2) a soil at the edge of a Mypex standing area.

Nursery 2

This nursery has previously had problems related to indigenous *Phytophthora* root rots and still reports problems in terms of plants dying or failing to thrive.

Two disinfectants were tested at each nursery, 'Horticide' (equivalent to 'Unifect G') and 'Jet 5'. The disinfectants were applied at 4 and 0.8% respectively and in three application volumes 0.5, 1 and 2 L per 50 cm^{-2}. At nursery 1 three areas were treated, a soil standing area, a gravel standing area and a gravel road. At nursery 2 a soil area was treated with soil samples taken at depths down to 40 cm. Samples from both sites were taken pre-treatment and 1, 7, 14 and 30 days post treatment. Presence of *Phytophthora* species was determined in the samples using Rhododendron leaf baits.

Additional swab and soil samples were taken from nursery 1 these included trolleys, trailers, tractor wheels, crates and pallets.

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Results and discussion

1. Baiting *Phytophthora* species from water

In year 1 of the project, bait testing of five *Phytophthora* species was undertaken, this testing was subsequently extended to include *P. cinnamomi*. The Rhododendron leaf bait detected *P. cinnamomi* down to a level of 10 sporangia/L (Figure 3). Comparison of this result with those from other sporangial suspensions showed that the level of detection was equivalent to that with *P. ramorum*, but higher than that with *P. kernoviae*, *P. cactorum* and *P. ilicis*. As discussed in the first year report the level of detection achieved from a sporangial suspension was higher than from a zoospore suspension. Therefore it is likely that had a zoospore suspension of *P. cinnamomi* been used, the detection limit of the leaf bait would be lower than seen here due to sporangia releasing zoospores into the water thus increasing the actual spore concentration.

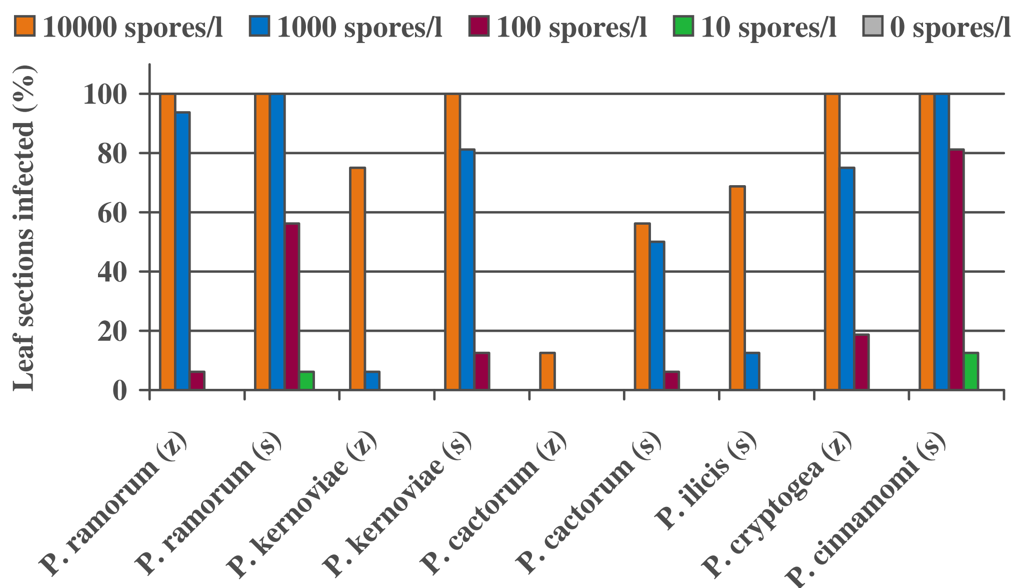

Figure 3. Detection of *Phytophthora* species from water using a Rhododendron leaf baits. *Phytophthora* isolation from leaf sections was on $PARP_5H$ agar. Zoospore suspensions (z); sporangial suspensions (s).

2. Slow sand filters (SSFs)

2.1. Removal of *P. ramorum* and *P. kernoviae.*

The CSL SSF ran successfully for an initial period of 21 months. At the outset, the filters were challenged with high spore loads (10^4-10^7 spores per litre of headwater – Tables 1 and 2) approximately once a month. For *P. ramorum*, the filter was challenged with three spore types - zoospores, sporangia and chlamydospores, whereas the *P. kernoviae* filter was challenged with zoospores and sporangia (*P. kernoviae* does not produce chlamydospores). Following an initial failure in the *P. ramorum* filter (see first year report) both *P. ramorum* and

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P. kernoviae were effectively removed by the SSF from water throughout the duration of the experiment. The failure was shown to be due to a break in the schmutzdecke (the biologically active layer) at the surface of the filter bed caused by the inflow to the filter being too close to the surface of the sand, hence disrupting the surface layer.

Table 1. Effectiveness of slow sand filtration at removing *Phytophthora ramorum* from contaminated water.

Date	Level and type of inoculum added to headwater	Recovery of *P. ramorum*	
		Headwater	Filter outlet
20/04/07		+	
03/05/07	Infected leaves		-
18/06/07			-
20/08/07	Infected leaves		
24/08/07			-
27/09/07	Infected leaves	+	-

+/- indicates whether sample tested positive or negative for *P. ramorum*; grey area indicates no test carried out.

Following the initial high spore load challenges, the inoculation method used was altered to one of low level, constant inoculum by placing infected leaf material in the filter headwater (Tables 1 and 2). Once again, no *P. ramorum* or *P. kernoviae* was detected in any of the baits taken from the filter outflow. To check that *P. ramorum* and *P. kernoviae* were not passing through the filters at levels below the limit of detection of the leaf baits water was collected from the filter outlet and tested using the newly developed TaqMan PCR technique which allows low level quantitative detection of *P. ramorum* and *P. kernoviae* in water. During normal running of the filters *P. ramorum* or *P. kernoviae* DNA was detected in the filter headwater, however DNA of either species was not detected in any of the water samples collected from the SSF outlet.

Table 2. Effectiveness of slow sand filtration at removing *Phytophthora kernoviae* from contaminated water.

Date	Level and type of inoculum added to headwater (HW)	Recovery of *P. kernoviae*	
		Headwater	Filter outlet
20/04/07		+	
03/05/07	Infected leaves		-
18/06/07			-
20/08/07	Infected leaves		
24/08/07			-
27/09/07	Infected leaves	+	-

+/- indicates whether sample tested positive or negative for *P. kernoviae*; grey area indicates no test carried out.

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2.1.1. Effect of cleaning on the ability of SSF to remove *P. ramorum* and *P. kernoviae* from water.

After running for 21 months the SSF were cleaned by removing the top 5 mm of the filter bed, in doing this some of the schmutzdecke, the biologically active layer, was removed. The results of an intensive period of water baiting in the days following the cleaning of the filters are shown in Table 3. Neither *P. ramorum* nor *P. kernoviae* were recovered from the filtered water in the days immediately following cleaning, however a bait removed from the collection tank on day 30 (i.e. left in the collection tank for 22 days) was positive for *P. kernoviae,* indicating this filter had failed. Due to the length of time the bait remained in the collection tank it is not possible to determine when the filter failed but it is likely that this bait had detected a low level failure not detected by the earlier baiting. Baiting carried out after 52 and 68 days indicated that neither species was present in the filtered water (on both occasions baits were left in the collection tank for five days). This was confirmed when no *P. ramorum* or *P. kernoviae* DNA was detected in water samples collected 68 days after cleaning. These results suggest that disruption of the filter active layer reduces their efficacy, however once the biologically active layer has recovered (after about two weeks) both *Phytophthora* species were once again removed from the system.

Table 3. Effect of cleaning SSF on the efficacy of a SSF to remove *P. ramorum* or *P. kernoviae* from water.

Time after cleaning (Days)	Leaf baiting of *P. ramorum* SSF		Leaf baiting of *P. kernoviae* SSF	
	Headwater	Collection tank	Headwater	Collection tank
0	Filter cleaned		Filter cleaned	
1*				
2		-		-
5		-		-
6		-		-
7	+	-	+	-
8		-		-
30		-		+
52		-		-
68		-		-

*fresh inoculum added; +/- indicates whether sample tested positive or negative for a *Phytophthora* species; grey area indicates no test carried out.

2.1.2. Effect of disinfectant on the ability of SSF to remove *P. ramorum* from water.

The addition of 'Jet 5' to the headwater of the *P. ramorum* SSF had the immediate effect of changing the headwater pH from 7 to 5. Continued monitoring of the headwater showed that within 2h the pH had returned to 7, indicating that the disinfectant had passed from the headwater into the filter bed. Over an 8h monitoring period (the time normally taken for water to pass through the filter) the pH of the filter outlet water did not change. Two possible explanations for this are (1) the disinfectant had not yet passed through the filter or (2) the

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disinfectant had been diluted within the filter to such an extent that it no longer affected the pH of the water.

Results from the baiting and PCR analyses of filtered water are shown in Table 4. Samples taken three and four days after the addition of 'Jet 5' showed that *P. ramorum* could be recovered from the filtered water, indicating that the disinfectant had had a detrimental effect on the ability of the SSF to remove *P. ramorum* from water; this was probably a result of a breakdown in the biologically activity of the schmutzdecke caused by the disinfectant. *P. ramorum* was not detected in water sampled following the five and six day samplings, however it was recovered at the nine day baiting. The nine day bait had been left in the system for three days, rather than one day as with the previous baits, this suggests that by day nine the level of *P. ramorum* in the system was lower than that immediately after the addition of 'Jet 5' but had still not been completely removed. Positive baits were also found at days 17 and 27 after the addition of disinfectant (both baits left in the system for seven days) which suggested that *P. ramorum* was still present in the system post filtration. However, a 1 litre water sample taken direct from the filter outlet on day 23 (and then baited for seven days) was negative for *P. ramorum*, this result suggests that the filters had removed *P. ramorum* from the water and that baiting the collection tank was picking up residual contamination remaining from the initial failure after the addition of disinfectant. Baiting of the collection tank after 37 and 47 days was negative for *P. ramorum*, indicating that *P. ramorum* had been removed from the whole system.

Table 4. Effectiveness of slow sand filtration at removing *P. ramorum* following the addition of 'Jet 5' to the headwater.

Time after addition of 'Jet 5' to headwater (days)	Leaf baiting		PCR analysis	
	Headwater	Collection tank	Headwater	Collection tank
0*	+		+	-
3		+		
4		+		
5		-	+	+
6		-		
9		+		
10	+	-		
17		+		
27	+	+		
37		-		
47		-		

*fresh inoculum added (7 h after addition of disinfectant); +/- indicates whether sample tested positive or negative for *P. ramorum*; grey area indicates no test carried out.

2.2. Removal of indigenous *Phytophthora* species

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The indigenous *Phytophthora* species used to contaminate the SSF headwaters were *P. cactorum*, *P. citrophthora* (ex Ceanothus), two isolates of *P. nicotianae* (ex Cordyline and Poinsettia) and a range of unidentified *Phytophthora* species isolated from citrus, Begonia, Fuchsia and Pansy (Table 5). The filters were challenged using infected leaf material to provide continuous low-level inoculum. No failures in the filters were seen throughout the course of the monitoring, although a *Phytophthora* was detected from filter 4 on 21st November 2007. Interestingly, this *Phytophthora* species did not conform to the species that was originally introduced via the headwater though its presence in the collection tank cannot be fully explained at this time.

Table 5. Effectiveness of slow sand filtration at removing indigenous *Phytophthora* species from contaminated water.

Date	SSF No.	*Phytophthora* species inoculated	Detection of *Phytophthora* spp*	
			Headwater**	SSF outlet or filtrate
21/08/07	1	*P. cactorum*	-/-	-
	2	PC4524 *Phytophthora* sp ex. citrus	+/+	-
12/09/07	1	PC4110 *P. nicotianae* ex. Cordyline	-/+	-
	2	CC114 *Phytophthora* sp ex. Begonia	-/+	-
	3	CC115 *Phytophthora* sp ex.Fuchsia	-/+	-
	4	PC3976 *P. nicotianae* ex. Poinsettia	-/+	-
15/10/07	1	*P. cactorum*	-/-	-
	2	CC115 *Phytophthora* sp ex.Fuchsia	-/-	-
	3	PC4324 *P. citrophthora* ex. Ceanothus	+/-	-
	4	PC4524 *Phytophthora* sp ex. citrus	+/+	-
30/10/07	1	*P. cactorum*	-/-	-
	2	CC114 *Phytophthora* sp ex. Begonia	-/-	-
	3	PC4110 *P. nicotianae* ex. Cordyline	-/-	-
	4	PC3976 *P. nicotianae* ex. Poinsettia	-/-	-
21/11/07	1	PC4775 *Phytophthora* sp ex. Pansy	+/-	-
	2	CC114 *Phytophthora* sp ex. Begonia	+/-	-
	3	CC115 *Phytophthora* sp ex.Fuchsia	+/+	-
	4	PC4524 *Phytophthora* sp ex. citrus	+/+	+#

* +/- indicates whether sample tested positive or negative for a *Phytophthora* species;

** headwater samples were tested by two methods:- 1. leaf bait in headwater and 2. filtering and plating of headwater (the + or – indicates whether the method gave a positive or negative result for a *Phytophthora* species;

the specific *Phytophthora* species isolated and reported here was not the same as that introduced into the top of the filter, and this result cannot be fully explained.

3. Efficacy of water treatment chemicals against *P. ramorum*.

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Three chemicals were tested to determine their efficacy in decontaminating water contaminated by *P. ramorum* spores - 0.4% 'Jet 5', 10% bleach and chlorine dioxide (as 'Sanogene') at 5, 50 and 500 ppm. Both 'Jet 5' and bleach were extremely effective at decontaminating water of *P. ramorum* after a 5 minute exposure (Table 6). Chlorine dioxide did not decontaminate water of *P. ramorum* when used at 5 ppm, however it was effective at 50 ppm following an exposure time of 60 min and at 500 ppm within 5 minutes.

Table 6. Effectiveness of water treatment chemicals for the decontamination of water contaminated by *P. ramorum*.

Exposure time (min)	Sporangial germination (%)					
	Control	'Jet 5' 0.4%	Bleach 10%	Chlorine dioxide (ppm)		
				5	50	500
5		0	0	94	1.3	0
15		0	0	93	1.3	0
30		0	0	92	0.3	0
45		0	0	93	0.3	0
60		0	0	93	0	0
90		0	0	86	0	0
120	100	0	0	90	0	0

4. Use of disinfectant on HONS nurseries

A number of swab and soil samples were taken from trolleys, trailers, tractor wheels, crates and pallets at nursery 1. These were all negative for the presence of *Phytophthora* species.

Swab and soil samples taken from standing areas and roads at nursery 1 in November 2007 indicated the presence of *Phytophthora* species in most areas of the site, with *P. ramorum* found at two locations, 1) a gravel standing area and 2) soil at the edge of a Mypex standing area. However, the pre-treatment sampling carried out in February 2008 showed that although *Phytophthora* species were present, none of these were *P. ramorum* (Table 7). Of the three test areas on nursery 1, only the gravel standing area gave consistent isolation of *Phytophthora* species from both the control and pre- treatment disinfectant samples (Table 7). In this area the results indicate that the 0.5 and 1.0 L 50 cm^{-2} treatments were effective by the end of the isolation period, although *Phytophthora* species were isolated from the 2.0 L 50 cm^{-2} treatment samples taken. Due to the sporadic incidence of *Phytophthora* species in each test area it is difficult to determine the true effectiveness of the disinfectants, however there is a suggestion that 'Horticide' was more effective than 'Jet 5'; which is in line with the *in vitro* disinfectant results obtained in year 2 of this project.

Pre-treatment soil sampling at nursery 2 at depths of 10, 20 and 40 cm indicated that the majority of *Phytophthora* species were found in the top 20 cm of soil, although they were occasionally isolated from the samples taken at 40 cm (Table 8). As found at nursery 1, a

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lack of consistent isolation of *Phytophthora* species in both the pre-treatment and control samples made it difficult to fully evaluate the effectiveness of the two disinfectants, however, as was the case at nursery 1, 'Horticide' appeared to be more effective than Jet 5. It should be noted that it is not recommended to use either disinfectant in the presence of high organic content and this is presumed to be because the product will be inactivated quickly and hence be less effective against the target pathogens in such situations.

Table 7. The effect of disinfectant application to different substrates on the growth of *Phytophthora* species at Nursery 1.

Test Area (Substrate tested)	Treatment	Application rate (L/50 cm^2)	Timing of sample*				
			Pre	Post (days)			
				1	7	14	30
Edge of Mypex	Control		-	-	-	-	-
standing area	'Horticide'	0.5	-	-	-	-	-
(Soil)#		1.0	-	-	-	-	-
		2.0	+	-	-	-	-
	'Jet 5'	0.5	-	-	-	-	-
		1.0	-	-	-	-	-
		2.0	-	-	-	-	-
Standing area	Control		+	-	+	+	+
(Gravel)#	'Horticide'	0.5	+	-	+	-	-
		1.0	+	-	-	-	-
		2.0	+	-	-	+	+
	'Jet 5'	0.5	+	+	+	+	+
		1.0	+	-	+	+	+
		2.0	+	-	+	-	+
Road	Control		+	-	-	+	+
(Gravel)	'Horticide'	0.5	+	-	+	+	-
		1.0	-	-	-	+	+
		2.0	-	-	-	-	-
	'Jet 5'	0.5	-	-	+	+	-
		1.0	+	-	+	+	-
		2.0	+	-	+	+	+

* + or – indicates the presence or absence of a *Phytophthora* species in the sample; # areas which tested positive for *P. ramorum* in November 2008

Table 8. The effect of disinfectant application on the growth of *Phytophthora* species at Nursery 2.

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Treatment and application rate	Soil depth (cm)	Timing of sample*				
		Pre	Post (days)			
			1	7	14	30
Control	10	-	+	-	-	-
	20	-	-	++	-	+
	40	-	+	-	-	-
'Horticide' (0.5 L/50 cm^2)	10	++	+++	+	-	-
	20	+	+	+	-	-
	40	-	-	-	-	-
'Horticide' (1.0 L/50 cm^2)	10	++	-	-	+	-
	20	+	+	+	-	-
	40	-	+	-	-	-
'Horticide' (2.0 L/50 cm^2)	10	-	-	-	-	-
	20	-	+	-	+	-
	40	-	-	-	-	-
'Jet 5' (0.5 L/50 cm^2)	10	+	++	++	-	+
	20	+	+	+	+++	+
	40	-	+	-	-	-
'Jet 5' (1.0 L/50 cm^2)	10	+	+	+	+	+
	20	+	+	+	-	+
	40	-	-	-	-	-
'Jet 5' (2.0 L/50 cm^2)	10	-	+	++	+	++
	20	-	+	+	+	+++
	40	-	+	-	-	-

* – indicates the absence of a *Phytophthora* species in all three reps, the number of + (1 to 3) indicates the number of reps with *Phytophthora* species present.

5. Development of a robust strategy to minimise risk.

The development of a robust nursery strategy to minimise the risk from *Phytophthora* species relies heavily on the growers overall perception and understanding of the pathogens biology, life-cycle (Figure 4) and control options and, in many cases, the nursery will require some support to ensure the majority of risks have been considered and hopefully addressed. Unfortunately <u>all</u> risks cannot be accounted for and sporadic outbreaks of Phytophthora infection are likely to occur and growers are encouraged to have a contingency plan to ensure appropriate action is taken should the pathogen be confirmed on the nursery. The precise measures required will depend on the species of *Phytophthora* found.

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Naturally, it is important to have a good understanding of the organism itself and what conditions it prefers so that these can be avoided where possible and counter measures taken as appropriate. This then allows an effective, nursery-focused, disease management strategy to be developed to help prevent crop loss due to this important pathogen group.

Pathogen Biology (see life-cycle Figure 4): *Phytophthora* species prefer an aqueous environment and do not fare well under drier conditions. They generally spread via motile spores (zoospores) in water films, e.g. on the leaf surface or in the soil, or in water bodies, e.g. ponds or reservoirs, and this is a primary means of pathogen dispersal and disease spread. Some species infect aerially and have an air-borne spore (sporangia) dispersal phase (similar to the potato blight pathogen). Species to be aware of in this regard are *P. citrophthora* on Ceanothus, *P. illicis* on Ilex and the quarantine species *P. ramorum* and *P. kernoviae* on various ornamental hosts, but particularly Viburnum, Rhododendron & Camellia. In these species leaf litter is likely to be an important means of disease spread around the nursery especially if there is an opportunity for the leaves to contaminate water supplies used for irrigation. Other species are root-infecting pathogens e.g. *P. cinnamomi, P. cactorum, P. nicotianae & P. cryptogea* and here infection and spread tends to be more localised and slower; though various nursery practices can impact on this significantly. In all cases there is a significant risk of spread via water, including stored supplies used for irrigation and it is important to consider and address this risk as a priority action (see below).

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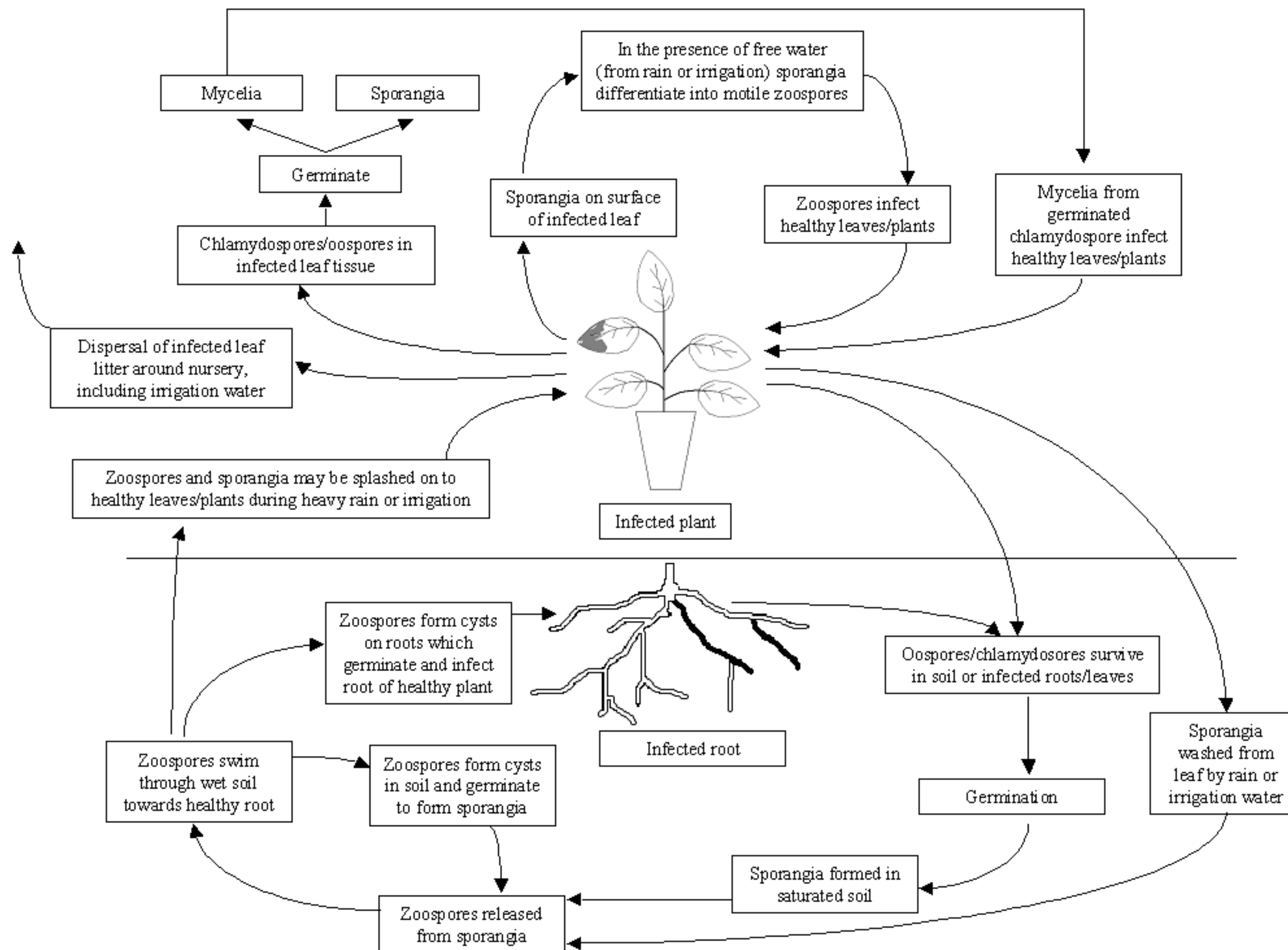

Figure 4. Generalised life cycle for foliar (top) and root borne (bottom) *Phytophthora* species responsible for disease in hardy nursery stock. Foliar pathogens include *P. ramorum*, *P. kernoviae, P. citrophthora* and *P. ilicis*, whereas root and crown rot pathogens include *P. cinnamomi*, *P. cryptogea*, *P. cactorum*, *P. nicotianae*, *P. cambivora* and *P. citricola*. Root and crown rot pathogens are known to produce leaf blights on the lower leaves, whereas foliar pathogens have not been shown to cause root rots even though part of their life cycle may occur in the soil.

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Buying-in plants: Early symptoms of *Phytophthora* infection can be difficult to detect on the nursery and it is quite easy to disseminate the pathogen unwittingly by the mere action of moving apparently healthy looking plants around the site. It is imperative therefore that stock is only bought-in from reputable sources and preferably with some knowledge that the crop has had an effective fungicide programme to protect against such important pathogens. It is good nursery practice to have a designated holding or 'quarantine' area, well away from other plants, to give time to check plants for pest and disease problems in advance of their wider dissemination on the nursery itself. Some growers use the services of diagnostic laboratories to provide a routine 'due diligence' check on the overall health of newly introduced stock. This can be very advantageous in future 'dispute' situations. Once the plants have been thoroughly checked they can then be released more widely on the nursery in the knowledge that the risk of pest & disease introduction is minimal.

Water management: As indicated above, *Phytophthora* species thrive in an aqueous environment. This is largely due to the production of motile ('swimming') zoospores that enable the pathogen to be disseminated readily in such an environment. It is therefore important to consider water management on the nursery very carefully, not in relation to the risk of diseases caused by these pathogens, but also in terms of water conservation to minimise the impact of rising mains water costs. However there is the danger that an ill-conceived conservation strategy could increase the risk of disease. For growers who continue to rely on mains water the risk from *Phytophthora* is low providing it is not stored in 'buffer' tanks on the nursery. However, as soon as water is stored there is the possibility of secondary contamination, which increases the risk of pathogen dissemination considerably. Thus it is imperative that all mains water storage tanks are covered and that the covers are checked regularly to ensure they remain intact. Floating covers are unsuitable for this purpose.

Where water is collected and conserved for use on the nursery i.e. for irrigation purposes the risk of dissemination of oomycete fungi such as *Phytophthora* and *Pythium* species is significantly increased. An extremely important component of a disease control strategy on the nursery therefore is to ensure that this risk is minimised. This project has demonstrated the potential benefits from simple technology such as slow sand filtration, which could provide a cost-effective solution to allow growers to further conserve and re-use water without increasing the risk of *Phytophthora* infection. Where water is stored in reservoirs etc it is not always practical to cover these to prevent contamination and, in any case, water collected from roofs of glasshouse / other buildings and hard-standing areas is likely to be contaminated before it enters the reservoir. The introduction of a biological filtration system downstream of the reservoir combined with a series of well-covered holding tanks would provide a clean and safe water supply for irrigation purposes. It is recommended that the

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treatment process be monitored regularly to ensure the biological filtration process is working effectively. Diagnostic laboratories can quantify propagule levels of *Phytophthora* & *Pythium* species in water samples and these should be taken pre- and post-filtration.

Where water is continually being recycled, including pre-dispatch irrigation systems, probably provides the greatest potential for *Phytophthora* dissemination and it is advisable to pay particular attention here.

Finally, for growers constrained by space, chemical treatment of water may be a preferred option and this project has demonstrated that water bodies can be decontaminated from pathogens like *P. ramorum* using chemical intervention e.g. chlorine dioxide. For other water disinfection systems, e.g. UV, it is very important to have a good understanding of their likely efficacy in relation to fluctuating water quality before incurring considerable expenditure on such systems.

Growing media management: Whilst it was not a component of the current project, management of the growing medium is also very important to avoid undue risk. In general, most growing media will be free from important plant pathogens on receipt though it is recommended, for a variety of reasons, that nurseries practice 'due diligence' and retain a small (ca. 1kg) sample of each batch of fresh compost as delivered for reference purposes. In the event of crop problems developing this can be an important reference sample for nutrient, pest and disease analysis etc. Once the compost has been used and crops marketed successfully the retained samples can be discarded. This will become increasingly important as green waste or similar compost is included in the mix.

The greatest risk with growing media is accidental post-delivery contamination, especially if the medium is delivered in bulk. Here, undercover storage is eminently preferable to minimise risk. Consideration also needs to be given to contamination of media by wheels of vehicles, other machinery and even personal footwear. In most cases, the risk can be reduced significantly through supply and use of bulk bagged compost though consideration still needs to be given to nursery practices to minimise risk.

Nursery equipment and general hygiene practice: *Phytophthora* species are able to survive in soil and other growing media once contaminated. Therefore, general nursery hygiene is of considerable importance to reduce the risk of spreading disease, especially during the more vulnerable periods of propagation such as potting-on. Where pots and other equipment are re-used they must be effectively cleaned to remove the bulk of organic matter prior to disinfection. Evidence from this work, whilst limited, tends to suggest that products based on glutaraldehyde e.g. 'Horticide' are more effective. Given the recent transfer of

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responsibility for biocides (including disinfectants used on nurseries) to the Health & Safety Directorate it will be important for growers to ensure they are aware of their legal responsibilities prior to use of any such products on the nursery. Understandably, there has been concern about the risk of disease spread on Danish trolleys their movement between nurseries. Whilst the current work has not been able to demonstrate a particular risk in this area, it is advisable to hose them down prior to use (in a designated quarantine area) to remove the bulk of the organic matter and, if practicable, to apply an appropriate surface disinfectant.

Standing areas/beds: As *Phytophthora* species can survive for long periods in soil and other growing media there is a considerable risk of carry-over between crops. The ability to effectively disinfectant standing areas and the soil beneath would be of considerable benefit. The work conducted in this project, whilst limited, has demonstrated some of the difficulties associated with the challenge, and shown that the choice and depth of penetration of the disinfectant is important. This work suggests that glutaraldehyde based products such as 'Horticide' are probably the disinfectants of choice, though further work in this area could potentially identify others that are equally effective. As pointed out earlier, there is a requirement for 'duty of care' on behalf of the user to ensure the specific use complies with current regulations regarding biocides and it is important to note that their regulation is now controlled by the Biocides Directive and the Health & Safety Executive in the UK. It is also possible that the new REACH legislation could impact on this area in time.

Leaf litter and general plant debris: This is particularly pertinent to the *Phytophthora* species that infect leaf and shoot tissues e.g. *P. ramorum, P. kernoviae, P. citrophthora* and *P. ilicis*, as infected leaf litter and other plant debris is likely to blow around the nursery to further contaminate reservoirs, uncovered water-holding tanks and crop standing areas. Therefore, it is very important that leaf and other plant debris are removed to prevent wind-blown dissemination around the nursery.

Fungicide application: There is a wide range of fungicides that can be used on HNS crops for the control of oomycete fungi, including *Phytophthora* species and these have been evaluated in a separate HDC-funded project (Ref HNS123/123a) at CSL. The range of products available will depend on whether the crop to be treated is outdoors or protected and, due to the need for operator safety data the choice of products for use under protection is likely to be more limited compared to outdoor crops.

Fungicide application is likely to form an integral, and very important, component of any disease management strategy and nurseries should annually review their fungicide programmes to ensure they are using products to their full efficacy. At outbreak sites where

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quarantine *Phytophthora* species, such as *P. ramorum* and *P. kernoviae* have been confirmed, Defra regulations under statutory notice prohibit certain fungicide applications within specified areas of the nursery and on suspect stocks. In all other situations there is a need to routinely protect susceptible crops from both air-borne (e.g. *P. citrophthora* on Ceanothus) and soil-borne (e.g. *P. cinnamomi* on Chamaecyparis and other susceptible species) *Phytophthora* species. As the method of fungicide application is not currently a statutory condition of approval it is advisable that oomycete fungicides to be applied as HV drenching sprays to target both the foliage and root zone. It is important to ensure crop safety prior to the widespread use of any fungicide in this way. Growers requiring more specific information on fungicide programmes to suit their own particular nursery situations should consult a BASIS qualified adviser. Finally, due to the risk of selecting insensitive strains in the pathogen population it is recommended that an alternating programme of 2-3 different fungicides from contrasting mode of action groups be used where possible.

Disposal of infested/infested plants and other materials: Where *Phytophthora* species occur and cause crop loss on a nursery it is important to dispose of any infected material in an appropriate manner to prevent infection of subsequent crops. The most effective action is to place the material in a covered skip and quickly dispose of it at a suitable land-fill facility. However, in situations where this is not practical, it may be necessary to investigate alternative disposal routes. These would need to be considered on a case-by-case basis taking due regard of the relative risks in each case. Naturally, where quarantine *Phytophthora* species have been confirmed on the nursery it will continue to be necessary to take appropriate advice and instruction from the Plant Health & Seeds Inspectorate (PHSI).

So, by considering the various potential routes of entry of *Phytophthora* species, by having a better understanding of the life-cycle, survival and dispersal mechanisms of the pathogen and by taking appropriate and, in most cases, a precautionary approach to its control on the nursery, it should be possible to develop a robust and sustainable disease control strategy. This will significantly reduce the risk of pathogen introduction and, most importantly, delay or halt subsequent dissemination around the nursery and beyond. Crucially, measures taken for the control of indigenous *Phytophthora* species are likely to be equally effective against the quarantine *Phytophthora* species.

A summary of the above strategy is presented in Figure 5.

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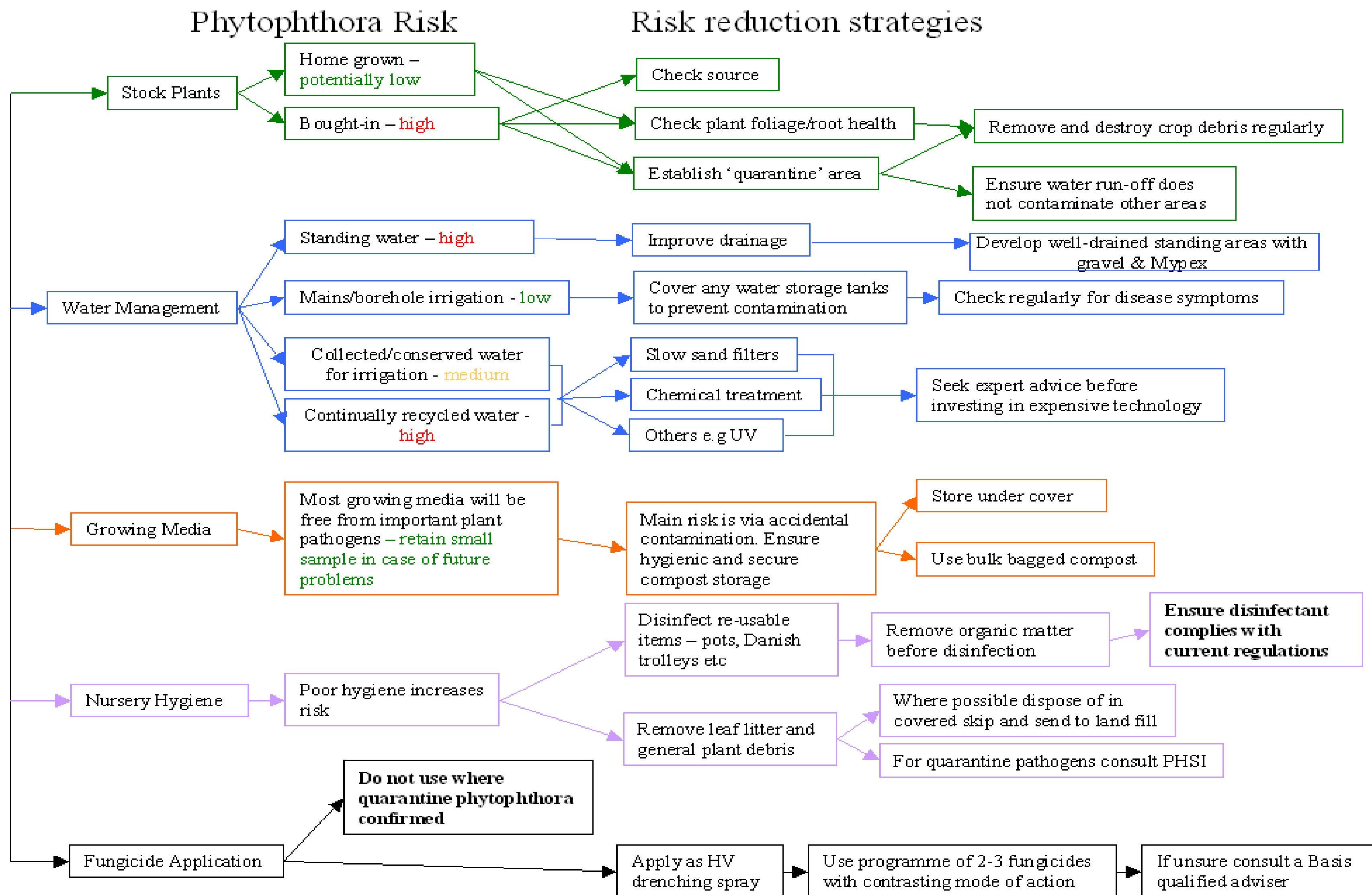

Figure 5. Schematic of disease management strategy developed to minimise the risk from *Phytophthora* species on HONS nurseries.

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Conclusions

- The rhododendron leaf bait has proved to be a useful tool for use on nurseries for the detection of *Phytophthora* species and diagnostic laboratories should adopt this technique as a routine for improved detection of both indigenous and quarantine *Phytophthora* species.
- Testing of slow sand filters indicated that they were highly effective at removing all the *Phytophthora* species examined from contaminated water and this provides an excellent and cost-effective method of reducing the risk of pathogen survival and dissemination on HNS nurseries.
- The efficacy of the filters was reduced when the 'schmutzdecke', the biologically active layer, was in some way disrupted e.g. pitting of the layer, cleaning of the filter or addition of disinfectant. The effectiveness of the filter was regained once the biologically active layer had recovered.
- Water could be effectively decontaminated of *P. ramorum* following exposure to chemical treatments.
- Disinfection of standing areas was partially effective using glutaraldehyde based products e.g. 'Horticide' and, subject to ensuring 'due diligence' in their use in specific situations this could help in further reducing survival and re-infection of *Phytophthora* spp. on nurseries.
- Precautionary measures taken as part of an overall disease control strategy for the control of indigenous *Phytophthora* species are likely to be equally effective against quarantine *Phytophthora* species.

Technology transfer

HDC News No. 127 (October 2006) p8 – New bait test pinpoints sources of phytophthora.
HDC News No. 128 (November 2006) p19-21 – Making ready to control ramorum.
The work will be featured one further HDC News article and will be presented at HDC events and other conferences as appropriate.

References

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HNS 123. Control of *Phytophthora ramorum* in nursery stocks (COPRINS). Final Report 2005

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Werres SR, Marwitz R, Man in't Veld W A, Cock A W A M, de. Bonants P J M, Weerdt M, de Themann K, Ilieva E, Baayen RP, 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. *Mycological Research* **105**, 1155-1165.

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Appendices

$PARP_5H$ agar

(Jeffers SN, Martin SB, 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease* 70, 1038-1043.)

Cornmeal Agar (CMA) 17 g/L

All amendments were either suspended or dissolved in 10 ml SDW and added to CMA after it had been autoclaved and cooled to 50˚C in a water bath.

Pimaricin	5 mg
Sodium ampicillin	250 mg
Rifampicin	10 mg dissolved in 1ml DMSO
PCNB	100 mg
Hymexazol	50 mgL^{-1}

10 % V-8 agar

V8 juice	200 mL
$CaCO_3$	2 g
Agar N°3	40 g
0.1M KOH	50 mL (0.280 g in 50 mL distilled water)
Distilled water	1750 mL

Autoclave at 121˚C for 15 min.

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