Project title: Detection and decontamination of *Phytophthora* spp., including those of statutory significance, from commercial HONS nurseries.

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Headline

- Slow sand filters can decontaminate water of *Phytophthora* species. A number of disinfectants have proved effective against the same species contaminating surfaces and organic matter.

Background and expected deliverables

Every year, significant economic losses to hardy nursery stock are attributed to infection by various *Phytophthora* species. Those causing root rot symptoms such as *P. cinnamomi*, *P. cryptogea*, *P. cactorum* and *P. nicotianae* are particularly prevalent. A newly described species *Phytophthora ramorum*, 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. It has also been found on a number of tree species. To date, there have been over 450 confirmed outbreaks of *P. ramorum* on nurseries in England and Wales and legislation 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, utilising currently available techniques (PCR and ELISA).

- An evaluation of the effectiveness of slow sand filtration for the removal of different *Phytophthora* species from water sources on the nursery.

- Determination of the most effective disinfectant/chemical treatments for the 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.

Summary of the project and main conclusions

Effectiveness of slow sand filters

In the first year of the project, two sets of slow sand filters (SSF) were constructed. The first was used at the Central Science Laboratory (CSL) for the tests using quarantine *Phytophthora* species *P. ramorum* and *P. kernoviae*. The second was used at Stockbridge Technology Centre (STC) for tests using indigenous species. These included *P. cactorum*, *P. citrophthora* (ex Ceanothus) and two isolates of *P. nicotianae* (ex Cordyline and Poinsettia).

In both sets of filters, a flow rate of water (400 ml per minute) was established and over a period of two years, different rates and methods of Phytophthora inoculation experimented with to validate their effectiveness.

A temporary failure in the *P. ramorum* filters occurred due to a break in the biologically active layer but this was easily rectified. However, when working correctly, both filters successfully removed the introduced *Phytophthora* species from the system.

The filters tested against the indigenous *Phytophthora* species were tested outdoors over a wide range of temperatures and worked effectively throughout.

Efficacy of disinfectants

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.

The efficacy of the disinfectants was tested initially on contaminated inert surfaces and organic substrates, before later testing them on contaminated compost/soil, Mypex, leaves and water. These were chosen to represent the typical sources of contamination that occur on a commercial nursery.

Contaminated inert surfaces:

The disinfectants were tested against each of the *Phytophthora* species on contaminated cellophane squares. This provided an inert surface on which the *Phytophthora* species could grow, whilst not affecting the activity of the disinfectant. With the exception of Hortisept, which was not effective on *P. cactorum*, all disinfectants were effective against all *Phytophthora* species. However, the exposure time required to ensure complete decontamination

varied depending on the species and exposure temperature (see science section).

- 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.

- Overall, IMS (70%) proved to be the most effective disinfectant.

Organic substrates:

As most of the disinfectants performed well against the different *Phytophthora* species on an inert surface, they were assessed in the presence of an organic substrate. Contaminated cellophane was laid below the surface of compost and disinfectant added to wet the compost to a depth just below the cellophane.

The Unifect G was largely unaffected and compared to cellophane alone, required a similar exposure time to ensure complete decontamination. However, the IMS (70%) was reduced in effectiveness and required a longer exposure time, whilst all the other disinfectants failed to decontaminate the cellophane in the times tested. It is likely that the presence of compost in the test system reduced the efficacy of the disinfectants by expending some of the disinfectant activity before it reached the fungal growth on the cellophane.

Contaminated compost/soil:

The disinfectants were tested for their effectiveness at controlling *P. ramorum* and *P. kernoviae* in compost, when applied either at 1 litre/m^2 or as a drench to completely saturate the compost.

None of the disinfectants achieved complete decontamination at the 1 litre/m^2 rate, but all except Hortisept and Menno Florades achieved full decontamination when the compost was saturated. Bleach also worked in this case, but required longer exposure.

Most of these disinfectants seem to be effective, but only if applied at a level sufficient to counter the effect of the organic matter on the disinfectant's activity.

Contaminated Mypex matting:

The disinfectants were tested on Mypex, which was infected by rubbing contaminated compost into the weave of the matting.

IMS (70%) and Unifect G were the most effective and only Hortisept did not effectively decontaminate the Mypex matting. Some of the disinfectants required longer exposure times to ensure effective decontamination, confirming that most disinfectants are effective in the presence of organic matter provided sufficient quantities are applied.

Infected leaves:

The disinfectants were generally less successful at controlling infected leaf material. When tested against established *P. ramorum* infection on rhododendron, camellia and viburnum leaves, only Unifect G achieved effective decontamination of all plant species and only after an exposure of 24 hours. Menno Florades and bleach decontaminated some plant species (but not all) after 24 hours exposure.

Efficacy of disinfectants in water:

Both hydrogen peroxide (as Jet 5) and sodium hypochlorite were extremely effective at decontaminating water containing Phytophthora spores after only 5 minutes of exposure.

Financial benefits

It is too early to predict the likely financial outcome from this project. However, *Phytophthora* spp., are aggressive pathogens and can cause significant economic losses, especially in wet seasons. The occurrence of the new quarantine species, *P. ramorum* and *P. kernoviae*, has added another dimension to the potential for economic damage and therefore any action that can be taken to minimise risk is potentially very valuable.

Action Points for Growers

- Check plants for suspicious symptoms of Phytophthora infection on roots, foliage and stems.

- Where suspicious symptoms are found, submit a sample of the material to a reputable diagnostic laboratory or 'Plant Clinic' for identification purposes.

- If buying-in plants, regularly establish a temporary 'quarantine' or 'holding' area well away from other susceptible plants. Check the plants before more widespread release onto the site.

- Consider the risk of water-borne dissemination of *Phytophthora* species on your nursery. If there is a risk of leaf debris or other plant material entering reservoirs, holding tanks etc then consider routine water monitoring for the pathogen.

- Ensure all holding tanks are covered to prevent contamination and, where appropriate, consider some form of disinfection treatment such as slow sand filtration prior to use (see HDC Grower Guide).

- Because *Phytophthora* species prefer wet conditions, take measures to minimise standing water around the site by improving drainage.

- Maintain a high level of nursery hygiene and use disinfectants where appropriate to further reduce the risk of pathogen dissemination.

Science Section - HNS 134

Detection and decontamination of *Phytophthora* spp., including those of statutory significance, from commercial HONS nurseries.

Introduction

Significant economic losses in HONS are 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* 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 measures are having a major impact on the HONS industry, resulting in the destruction of large numbers of plants.

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 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 will be evaluated under commercial conditions.

Materials and Methods

1. Efficacy of disinfectants.

1.1. Standing areas and equipment

The efficacy of six disinfectants (Table 1) was tested against a range of *Phytophthora* species - *P. ramorum* (CSL ref cc47), *P. kernoviae* (CSL ref cc95), *P. cactorum* (CSL ref 2151), *P. ilicis* (CSL ref 2195), *P. cinnamomi*, *P. cryptogea* (CSL ref 1708) and *P. nicotianae* (CSL ref cc1001). The activity of the disinfectants was examined in/on a range of different substrates – cellophane (inert surface), compost/soil, Mypex, and infected leaf material. The choice of disinfectants selected for the study was made based on their classification and included one example from each of the following: oxidising agent, cationic surfactant, reducing agent, halogen/halogen releasing compounds, organic acid and alcohol.

1.1.1. Contaminated surfaces (cellophane)

Initial tests to determine the overall efficacy of the disinfectant against each of the *Phytophthora* species were carried out on cellophane squares; these provided a surface on which *Phytophthora* species could grow while not affecting the activity of the disinfectant. The disinfectants were tested over a range of temperatures (0, 5, 10, 15, 20 and 30°C) and exposure times (5, 10, 15, 20, 30, 60 and 120 minutes).

Cellophane discs (90mm diameter) were boiled in distilled water for 20 minutes to remove any plasticisers present, autoclaved at 121°C for 15 minutes and placed onto 10% V-8 agar (Appendix I). For isolates of *P. ramorum*, *P. kernoviae* and *P. cactorum*, cellophane discs were inoculated by spread plating 100µl sporangial suspension containing 10^4 sporangia/mL over the cellophane surface. Sporangial suspensions were produced by growing the individual *Phytophthora* species on 10% V-8 agar 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 detached using a sterile plastic rod. Sporangial concentrations were calculated using a haemocytometer and

Table 1. Disinfectants selected for study.

Disinfectant	Class*	Subclass*	Dilution and application rate (if specified)			Comments (on class)*
			Equipment	Hard surfaces	Matting	
Jet 5	Oxidising agent	Peroxide	0.4 %	0.8 %	0.8 % at 0.5-1 litre/ m^2	Broad spectrum, effective in cold
Hortisept	Cationic surfactant	Quaternary ammonium compound	0.8 %	0.8 %	0.8 %	Spectrum varied, may be less active in presence of organic matter
Unifect G	Reducing agent	Aldehyde	4 %	4 %	4 %	Broad spectrum – need long contact time, action temperature dependant
Sodium Hypochlorite	Halogens/ halogen releasing compounds	Active chlorine	10 %	10 %	10 %	Broad spectrum (activity slower against spores), effective at cool temperatures, may be corrosive
Menno Florades	Organic acid	Aromatic acid	1 %	1 % at 0.2 litre/m^2	1 % at 0.2 litre/m^2	Good activity against viruses, fungi and bacteria does not control algae
IMS	Alcohol		70 %	70 %	70 %	

*Information taken from HDC factsheet 15/05 – Use of chemical disinfectants in protected ornamental production.

adjusted as required. As sporangia of *P. ilicis, P. nicotianae*, *P. cinnamomi* and *P. cryptogea* could not be readily produced, cellophane discs were inoculated using a mycelial plug placed in the centre of each disc.

The inoculated cellophane discs were incubated at 20°C, under day light bulbs (12h light/12h dark regime) for 10 days. Cellophanes were then examined under a microscope to identify the fungal structures present and then cut into squares (1cm x 1cm).

One hour prior to the start of each test, disinfectants were diluted to the manufacturers recommended rate (Table 1) and 30 mL of each placed at 0, 5, 10, 15, 20 or 30°C to equilibrate. Phytophthora-contaminated cellophane squares (still on V-8) were also placed at each temperature to equilibrate before the start each test.

For each individual disinfectant and *Phytophthora* species, at each test temperature, seven contaminated cellophane squares were removed from the V-8 agar, and placed in each of three 10cm x 10cm replicate plastic weigh boats, and 10 mL of disinfectant added; this gave an application rate equivalent to 1 litre/m^2. One cellophane square was removed from each of the three replicate tests after 5, 10, 15, 20, 30, 60 and 120 minutes and plated onto 10% V-8 agar plates. Plates were incubated at 20°C for 5 days and the effectiveness of the disinfectant determined based on the level of growth from the cellophane squares. For each test, 10 mL of sterile distilled water (SDW) was used as a control treatment.

1.1.2. Cellophanes in soil

Soil (John Innes No 3) was sterilised by autoclaving at 121°C for 15 min on two consecutive days. Approximately 80 g of the sterile soil was placed in a 1 litre plastic container and 24 cellophane squares (contaminated as described in section 1.1.1) placed, contaminated side up, on the soil surface. A further 80 g of sterile soil was then evenly distributed over the cellophanes.

Each disinfectant was diluted according to the rate shown in Table 1 and 40 mL applied evenly over the soil surface. This volume of liquid was sufficient to penetrate to the lower level of the container, ensuring complete soaking of the cellophane. A control container was established using 40ml of water.

Three replicate cellophane squares were removed from the soil after 2, 5, 10, 15, 20, 30, 60 and 120 minutes, blot dried to remove excess soil and disinfectant and plated onto 10% V-8 agar. Plates were incubated at 20°C for 5 days and the effectiveness of the disinfectant determined based on the growth of *Phytophthora* from the cellophane squares.

1.1.3. Contaminated soil

Soil (John Innes No 3) was sterilised as described in section 1.1.2 and 400g inoculated with 30ml of a 10^5 sporangia/mL spore suspension of *P. ramorum* or *P. kernoviae,* (produced as described in section 1.1.1). Inoculated soils were incubated at 20°C for 7-14 days under a 12 h day 12h night light regime.

Contaminated soil (~80 g) was added to weigh boats (10 cm x 10 cm); this was sufficient to form a soil layer approximately 1 cm thick. To this, disinfectant diluted and applied at a rate equivalent to that used for matting (Table 1), was applied evenly across the surface of the soil. Three replicate tests were set up for each *Phytophthora* species and disinfectant tested. For the control tests, an equivalent volume of water replaced the disinfectant. Soil sub samples (1g) were transferred from each test and plated onto 10 % V-8 agar after 5, 15 and 30 min, 1, 2, 3 and 20 hrs. Plates were incubated at 20°C for 7 days then assessed for growth of *Phytophthora* species.

This test was repeated using sufficient disinfectant (~70 mL) to fully saturate the soil.

1.1.4. Contaminated Mypex matting

Initial tests using sporangial suspensions to contaminate Mypex sheeting proved to be ineffective, as a result Mypex was contaminated by rubbing pre-contaminated soils in to the cross-weaving of the sheets. Soil was

contaminated with *P. ramorum*, *P. kernoviae*, *P. cactorum* and *P. ilicis* as described in section 1.1.3 before being rubbed vigorously into the weave of the Mypex. For the other species tested, soil was inoculated with 50 mycelial plugs taken from the leading edge of seven day old colonies grown on V-8 agar. Loose soil was removed from the Mypex matting, and the matting placed in a plastic microwave box containing damp blue roll (tissue) in order to keep the Mypex moist until required. Just prior to each experiment, the Mypex was cut into 1cm x 1cm square pieces.

For each individual *Phytophthora* species and disinfectant, seven Mypex squares were placed into 3 individual 10 x 10cm plastic weigh boats and 10 mL of disinfectant (equivalent to a rate of 1 litre/m^2) added to each. Disinfectants were diluted according to the manufacturer's recommendations (Table 1). After 5, 10, 15, 20, 30, 60 and 120 minutes, one Mypex square was removed from each replicate test, blot dried to remove excess disinfectant, plated onto 10% V-8 agar and incubated at 20°C. In control tests, disinfectant was replaced by 10 mL SDW. The effectiveness of the disinfectants was determined by assessing the growth of the individual *Phytophthora* species from the Mypex pieces after 7 days incubation.

1.1.5. Infected leaves

The effectiveness of disinfectants on contaminated leaf material was established using a detached leaves of Rhododendron 'Cunningham's White', Camellia japonica and Viburnum tinus.

For each disinfectant and leaf type, 12 leaves of were placed in moist chambers and 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 *P. ramorum*, was placed over the wound. Leaves were incubated at room temperature (20˚C) for 21 days.

For each disinfectant and leaf type, four leaves were placed in each of three 10 x10cm plastic weigh boats, adaxial surface down, and 10 mL of

disinfectant (diluted according to the manufacturers instructions), added to each weigh boat. In control tests disinfectant was replaced by 10 mL SDW. After 30, 60, 120 minutes and 24 hours, one leaf was removed from each replicate test and a cross section of the leaf cut through the point of heaviest infection. The cut section of the leaf was blot dried, plated onto 10% V-8 agar and incubated at 20°C. Growth of *Phytophthora* species from the cut leaf section was assessed after seven days.

No sample was taken after 24 hours when testing IMS due to full evaporation taking place after 2 hours.

1.2. Efficacy of disinfectants in water

Two chemicals were tested for efficacy in water contaminated with *Phytophthora* spores, hydrogen peroxide (as Jet 5) and sodium hypochlorite. A third chemical, chlorine dioxide will also be tested.

A sporangial suspension of *P. ramorum* was produced as described in section 1.1.1 and the concentration adjusted to give a final spore concentration of 10^4 sporangia/mL. For each disinfectant, 10 mL of the sporangial suspension was pipetted into three replicate sterile universals, sufficient disinfectant was then added to each universal to give a final concentration of 0.4% and 10% of hydrogen peroxide (as Jet 5) and sodium hypochlorite respectively. In the control test the disinfectant 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 three days 100 sporangia from each plate were assessed for germination.

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 1)] and four for use with indigenous *Phytophthora* species [located outdoors at STC (Figure 1)].

Figure 1. Slow sand filters held at CSL (left) and STC (right)

Testing the effectiveness of the SSF for the removal of *Phytophthora* species from contaminated water was started in 2005 and has continued during 2006.

2.1. Removal of *P. ramorum* and *P. kernoviae* from water using SSFs.

SSFs were challenged in two different ways, firstly one-off challenges with high concentrations of spores added to the filter headwater [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 rhododendron and magnolia leaves infected with *P. ramorum* or *P. kernoviae* to the headwater to provide continuous low-level release of inoculum.

2.1.1. Inoculum production

2.1.1.1. Production of sporangial and zoospore suspension

Sporangial suspensions were produced as described in section 1.1.1 and sporangial levels adjusted as required.

Where zoospores were required, the three-day-old V-8 agar plates containing sporangia were flooded with 15-20 mL SDW, chilled at -20°C for 5 min and then returned to 20°C for 1 h. Plates were then checked for zoospore release and the spore suspension filtered through Whatman No 113V filters (retention size >30 μm) to remove any spent or full sporangia. Zoospore counts were carried out using a haemocytometer and spores levels adjusted as appropriate.

2.1.1.2. Production of chlamydospore suspensions

Cellophane discs were prepared and inoculated as described in section 1.1.1, and incubated at 20°C in the dark for 14 days. Mycelia and chlamydospores were removed from the cellophane using the back of a scalpel blade, then suspended in approximately 25 mL of SDW and blended for 30 seconds with a hand blender. To separate the chlamydospores from the mycelial fragments, the suspension was centrifuged at 500g for 2.5 min, the supernatant containing the mycelial fragments removed and discarded, and the pellet containing the chlamydospores resuspended in SDW. Chlamydospore counts were carried out using a haemocytometer and spore levels adjusted as appropriate.

2.1.1.3. 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

Immediately following the addition of spores to the SSF, a 250 mL water sample was taken from the headwater. In addition water samples (1 litre) were taken from the SSF outlet two hours after the addition of spores and then hourly for the next six hours. All water samples were filtered through Durapore® membrane filters (5μm) and the filter papers inverted onto a selective agar ($PARP_5H$: Appendix I). Plates were incubated at room temperature for seven days and any growth of *Phytophthora* species recorded.

Where SSF were challenged with infected leaf material, rhododendron leaf baits were placed in the SSF headwater every 14-28 days to confirm the

presence of *P. ramorum* or *P. kernoviae* in the headwater. Baits were removed from the headwater after two days and plated onto $PARP_5H$.

For both methods of inoculation, rhododendron leaf baits were placed in the post filtration collection tank to detect the presence of *P. ramorum* or *P. kernoviae* and hence potential failures in the filters. Baits were changed every 7-14 days and plated onto $PARP_5H$ agar.

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

Tests on the indigenous *Phytophthora* species were carried out on *P. cactorum*, *P. citrophthora* (ex Ceanothus) and two isolates of *P. nicotianae* (ex Cordyline and Poinsettia). Tests carried out were similar to those for *P. ramorum* and *P. kernoviae.*

Results and Discussion

1. Efficacy of disinfectants.

The efficacy of six disinfectants (Table 1) was tested against seven *Phytophthora* species - *P. ramorum, P. kernoviae*, *P. cactorum*, *P. ilicis*, *P. cinnamomi*, *P. cryptogea* and *P. nicotianae*, for activity on a range of different substrates.

1.1. Standing areas and equipment

1.1.1. Contaminated surfaces (cellophanes)

To determine the overall efficacy of the disinfectant against each of *Phytophthora* species initial tests were carried out on cellophane squares over a range of temperatures and exposure times (Tables 2-7). Of the disinfectants tested, 70% IMS (Table 2) proved to be the most effective against contaminated cellophane squares, with only limited growth of *P. cryptogea* occurring following a 5 min exposure. With the exception of Hortisept, which was not effective on cellophanes contaminated with *P. cactorum* (Table 6), all disinfectants were effective against all *Phytophthora* species. However, the exposure time required to ensure the cellophanes were decontaminated varied depending on the species and the exposure temperature. To ensure complete decontamination of all species using bleach, a minimum exposure time of 60 minutes was required (Table 3), although the required time was reduced for some species. Treatment with Unifect G (Table 4), Jet 5 (Table 5) and Hortisept (species other than *P. cactorum* – Table 6) required an exposure time of 30 min to ensure the cellophanes were decontaminated, irrespective of temperature. In general, the required time was reduced if the contamination was caused by species other than *P. ramorum* or the temperature was between 10 and 20°C. An exposure time of 60 minutes was also required to ensure complete decontamination of all species using Menno Florades, provided the temperature was above 10°C. Below 10°C, *P. cryptogea* required an exposure of 120 min to prevent growth and *P. ramorum* was not controlled.

Table 2. Efficacy of 70% IMS against *P. ramorum*, *P. kernoviae*, *P. cactorum*, *P. ilicis*, *P. nicotianae*, *P. cinnamomi* and *P. cryptogea* over a range of temperatures.

Phytophthora species	Temperature	Time exposed to disinfectant (min)							Control
	(ºC)	5	10	15	20	30	60	120	
P. ramorum	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. kernoviae	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cactorum	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. ilicis	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. nicotianae	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cinnamomi	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cryptogea	0	---	---	---	---	---	---	---	+++
	5	+--	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	+--	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++

+ indicates growth and - indicates no growth of the *Phytophthora* species in the test. The number of + or – for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur.

Table 3. Efficacy of 10% bleach against *P. ramorum*, *P. kernoviae*, *P. cactorum*, *P. ilicis*, *P. nicotianae*, *P. cinnamomi* and *P. cryptogea* over a range of temperatures.

Phytophthora species	Temperature	Time exposed to disinfectant (min)							Control
	(ºC)	5	10	15	20	30	60	120	
P. ramorum	0	+++	+++	++-	---	---	---	---	+++
	5	+++	+++	+++	---	---	---	---	+++
	10	+++	+++	+++	---	---	---	---	+++
	15	+++	+++	+++	+--	---	---	---	+++
	20	+++	+++	+++	+++	---	---	---	+++
	30	+++	+++	+++	+++	---	---	---	+++
P. kernoviae	0	+++	+--	---	---	---	---	---	+++
	5	+++	---	---	---	---	---	---	+++
	10	+++	+++	+++	+++	+--	---	---	+++
	15	+++	+++	+++	+++	+--	---	---	+++
	20	+++	---	---	---	---	---	---	+++
	30	+++	+++	+++	+++	---	---	---	+++
P. cactorum	0	---	---	---	---	---	---	---	+++
	5	+++	+++	++-	---	---	---	---	+++
	10	+++	+++	+++	+++	---	---	---	+++
	15	+++	+++	+++	+++	+--	---	---	+++
	20	+++	+++	+++	+++	+++	---	---	+++
	30	+++	+++	+++	+++	+++	---	---	+++
P. ilicis	0	+++	+++	---	---	---	---	---	+++
	5	+++	++-	---	---	---	---	---	+++
	10	+++	+++	---	---	---	---	---	+++
	15	+++	++-	---	---	---	---	---	+++
	20	+++	++-	---	---	---	---	---	+++
	30	+++	+--	---	---	---	---	---	+++
P. nicotianae	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cinnamomi	0	+++	+++	+++	+++	+++	---	---	+++
	5	+++	+++	+++	+++	+--	---	---	+++
	10	+++	+++	+++	+++	---	---	---	+++
	15	+++	+++	+++	+--	---	---	---	+++
	20	+++	+++	+++	++-	---	---	---	+++
	30	+++	+++	+++	+++	---	---	---	+++
P. cryptogea	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	++-	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++

+ indicates growth and - indicates no growth of the *Phytophthora* species in the test. The number of + or – for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur.

Table 4. Efficacy of Unifect G against *P. ramorum*, *P. kernoviae*, *P. cactorum*, *P. ilicis*, *P. nicotianae*, *P. cinnamomi* and *P. cryptogea* over a range of temperatures.

Phytophthora species	Temperature	Time exposed to disinfectant (min)							Control
	(ºC)	5	10	15	20	30	60	120	
P. ramorum	0	+++	+++	+++	+++	---	---	---	+++
	5	+++	+++	+++	+++	---	---	---	+++
	10	+++	+++	+++	+++	---	---	---	+++
	15	+++	+++	+++	+++	---	---	---	+++
	20	+++	+++	+++	+++	---	---	---	+++
	30	+++	+++	+++	+++	---	---	---	+++
P. kernoviae	0	+++	+++	+++	---	---	---	---	+++
	5	+++	+++	+++	++-	---	---	---	+++
	10	+++	+++	+++	+--	---	---	---	+++
	15	+++	+++	+++	---	---	---	---	+++
	20	+++	+++	+++	---	---	---	---	+++
	30	+++	+++	---	---	---	---	---	+++
P. cactorum	0	+--	+--	---	---	---	---	---	+++
	5	+++	++-	+--	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	+--	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. ilicis	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. nicotianae	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cinnamomi	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cryptogea	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++

+ indicates growth and - indicates no growth of the *Phytophthora* species in the test. The number of + or – for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur.

Table 5. Efficacy of Jet 5 against *P. ramorum*, *P. kernoviae*, *P. cactorum*, *P. ilicis*, *P. nicotianae*, *P. cinnamomi* and *P. cryptogea* over a range of temperatures.

Phytophthora species	Temperature	Time exposed to disinfectant (min)							Control
	(ºC)	5	10	15	20	30	60	120	
P. ramorum	0	+++	+++	+++	++-	---	---	---	+++
	5	+++	+++	+++	---	---	---	---	+++
	10	+++	+++	+++	+--	---	---	---	+++
	15	+++	+++	+++	---	---	---	---	+++
	20	+++	+++	+++	---	---	---	---	+++
	30	+++	+++	+++	---	---	---	---	+++
P. kernoviae	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cactorum	0	---	---	---	---	---	---	---	+++
	5	+--	---	---	---	---	---	---	+++
	10	+--	---	---	---	---	---	---	+++
	15	+--	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. ilicis	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. nicotianae	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cinnamomi	0	+++	---	---	---	---	---	---	+++
	5	+++	---	---	---	---	---	---	+++
	10	++-	---	---	---	---	---	---	+++
	15	+++	+--	---	---	---	---	---	+++
	20	++-	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cryptogea	0	+++	+--	---	---	---	---	---	+++
	5	+++	---	---	---	---	---	---	+++
	10	+++	++-	+--	+--	---	---	---	+++
	15	+++	---	---	---	---	---	---	+++
	20	+++	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++

+ indicates growth and - indicates no growth of the *Phytophthora* species in the test. The number of + or – for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur.

Table 6. Efficacy of Hortisept against *P. ramorum*, *P. kernoviae*, *P. cactorum*, *P. ilicis*, *P. nicotianae*, *P. cinnamomi* and *P. cryptogea* over a range of temperatures.

Phytophthora species	Temperature	Time exposed to disinfectant (min)							Control
	(ºC)	5	10	15	20	30	60	120	
P. ramorum	0	+++	+++	+++	+++	---	---	---	+++
	5	+++	+++	+++	+++	---	---	---	+++
	10	+++	+++	+++	---	---	---	---	+++
	15	+++	+++	+++	---	---	---	---	+++
	20	+++	+++	+++	---	---	---	---	+++
	30	+++	+++	+++	++-	---	---	---	+++
P. kernoviae	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cactorum	0	+++	+++	+++	+++	+++	+++	+++	+++
	5	+++	+++	+++	+++	+++	+++	+++	+++
	10	+++	+++	+++	+++	+++	+++	+++	+++
	15	+++	+++	+++	+++	+++	+++	+++	+++
	20	+++	+++	+++	+++	+++	+++	+++	+++
	30	+++	+++	+++	+++	+++	+++	+++	+++
P. ilicis	0	++-	---	---	---	---	---	---	+++
	5	+--	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	+++	+++	---	---	---	---	---	+++
	30	+++	+--	---	---	---	---	---	+++
P. nicotianae	0	+--	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	+--	---	---	---	---	---	---	+++
P. cinnamomi	0	---	---	---	---	---	---	---	+++
	5	---	---	---	---	---	---	---	+++
	10	---	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	---	---	---	---	---	---	---	+++
P. cryptogea	0	+++	+++	+--	---	---	---	---	+++
	5	+++	+++	+++	---	---	---	---	+++
	10	+++	+++	+++	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	++-	---	---	---	---	---	---	+++

+ indicates growth and - indicates no growth of the *Phytophthora* species in the test. The number of + or – for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur.

Table 7. Efficacy of Menno Florades against *P. ramorum*, *P. kernoviae*, *P. cactorum*, *P. ilicis*, *P. nicotianae*, *P. cinnamomi* and *P. cryptogea* over a range of temperatures.

Phytophthora species	Temperature	Time exposed to disinfectant (min)							Control
	(ºC)	5	10	15	20	30	60	120	
P. ramorum	0	+++	+++	+++	+++	+++	+++	+++	+++
	5	+++	+++	+++	+++	+++	+++	+++	+++
	10	+++	+++	+++	+++	---	---	---	+++
	15	+++	+++	+++	+--	---	---	---	+++
	20	+++	+++	+++	+++	+++	---	---	+++
	30	+++	+++	+++	+++	+++	---	---	+++
P. kernoviae	0	+++	++-	---	---	---	---	---	+++
	5	+++	+--	+--	---	---	---	---	+++
	10	+++	+++	+++	---	---	---	---	+++
	15	+++	---	---	---	---	---	---	+++
	20	+++	---	---	---	---	---	---	+++
	30	+++	---	---	---	---	---	---	+++
P. cactorum	0	+++	---	---	---	---	---	---	+++
	5	+++	+++	+++	---	---	---	---	+++
	10	+++	+++	---	---	---	---	---	+++
	15	+++	+++	---	---	---	---	---	+++
	20	+++	+++	---	---	---	---	---	+++
	30	+++	+++	---	---	---	---	---	+++
P. ilicis	0	+++	---	---	---	---	---	---	+++
	5	+++	++-	+--	---	---	---	---	+++
	10	+++	+--	---	---	---	---	---	+++
	15	+++	---	---	---	---	---	---	+++
	20	+++	+++	+++	++-	---	---	---	+++
	30	+++	+--	+--	---	---	---	---	+++
P. nicotianae	0	++-	---	---	---	---	---	---	+++
	5	++-	+--	---	---	---	---	---	+++
	10	++-	---	---	---	---	---	---	+++
	15	---	---	---	---	---	---	---	+++
	20	---	---	---	---	---	---	---	+++
	30	+--	---	+--	---	---	---	---	+++
P. cinnamomi	0	+++	+++	+++	+++	+++	---	---	+++
	5	+++	+++	+++	+++	---	---	---	+++
	10	+++	+++	+++	+++	++-	---	---	+++
	15	+++	+++	+++	+++	---	---	---	+++
	20	+++	+++	+++	+++	+++	---	---	+++
	30	+++	+++	+++	+++	+++	---	---	+++
P. cryptogea	0	+++	+++	+++	+++	+++	+++	---	+++
	5	+++	+++	+++	+++	+++	+++	---	+++
	10	+++	+++	+++	+++	+++	+++	---	+++
	15	+++	+++	+++	+++	++-	---	---	+++
	20	+++	+++	+++	+++	---	---	---	+++
	30	+++	+++	+++	+++	---	---	---	+++

+ indicates growth and - indicates no growth of the *Phytophthora* species in the test. The number of + or – for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur.

1.1.2. Cellophane squares in compost

As the majority of the disinfectants performed well against the different *Phytophthora* species growing on an inert surface, the next stage of the testing was to determine if the performance of the disinfectants altered in the presence of an organic substrate such as compost. To test this contaminated cellophane discs were placed below the surface of sterile compost and sufficient disinfectant added to ensure that it wetted the compost to a point below the cellophanes.

The presence of compost in the test rendered treatments with bleach, Jet 5, Hortisept and Menno Florades ineffective over the exposure times tested. Only Unifect G and IMS were effective under these conditions. The efficacy of Unifect G appeared unaffected by the presence of compost in the test system, with an exposure time of 30 min required to prevent growth of the *Phytophthora* species from the cellophane disc (Table 8). Presence of compost in the test system reduced the effectiveness of 70% IMS, in that a longer exposure time (15 min) was required to ensure that cellophanes were decontaminated; the exception to this was cellophane squares contaminated with *P. nicotianae* where an exposure time in excess of 60 min was required.

The hypothesised reason for differences in the efficacy of the disinfectants on infected cellophane squares in compost compared with those in direct contact with the disinfectant is that the organic matter compromised the oxidising/reducing activity of the disinfectants rendering then ineffective against the *Phytophthora* species present.

1.1.3. Contaminated compost/soil

The aim of this set of experiments was to establish how effective the disinfectants were in decontaminating compost/soil which was heavily contaminated with either *P. ramorum* or *P. kernoviae*.

Two experiments were set up one in which disinfectants were applied at a rate equivalent to 1 litre/m^2 and the other where the soils were completely

saturated with the disinfectant. None of the disinfectants applied at 1 litre/m^2 were effective in decontamination the compost of either *P. ramorum* or *P. kernoviae* from compost (results not shown). However, when compost was saturated with the disinfectant, only Hortisept and Menno Florades failed to work (Table 9). IMS, Unifect G and Jet 5 were the most effective, decontaminating compost of *P. ramorum* or *P. kernoviae* after a 5 min exposure time. Saturation of compost with 10% bleach also decontaminated the compost of *P. ramorum* and *P. kernoviae*, but was only effective after a 1h exposure time. Using the two different application rates has shown that most of the disinfectants tested were able to decontaminate compost of *P. ramorum* or *P. kernoviae* provided that they were applied at a level sufficient to overcome the effect of the organic matter on the disinfectant's activity.

Table 8. Efficacy of disinfectants against *Phytophthora* contaminated cellophane discs in soil.

Disinfectant	*Phytophthora* species tested	Time exposed to disinfectant (min)								Control
		2	5	10	15	20	30	60	120	
IMS	*P. ramorum*	+++	---	---	---	---	---	---	---	+++
	P. kernoviae	+++	+++	---	---	---	---	---	---	+++
	P. cactorum	+++	++-	+--	---	---	---	---	---	+++
	P. ilicis		---	---	---	---	---	---	---	+++
	P. nicotianae	++-	+--	+--	++-	+--	+--	++-		+++
	P. cinnamomi	+++	++-	---	---	---	---	---	---	+++
	P. cryptogea	+++	+++	+--	---	---	---	---	---	+++
Bleach	*P. ramorum*	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. kernoviae	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cactorum	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. ilicis	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. nicotianae	+++	+--	---	+--	++-	---	++-	---	+++
	P. cinnamomi	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cryptogea	+++	+++	+++	+++	+++	+++	+++	+++	+++
Unifect G	*P. ramorum*	+++	+++	++-	---	---	---	---	---	+++
	P. kernoviae	+++	+++	+--	---	---	---	---	---	+++
	P. cactorum	+++	+++	+++	---	---	---	---	---	+++
	P. ilicis		---	---	---	---	---	---	---	+++
	P. nicotianae	---	---	---	---	---	---	---	---	+++
	P. cinnamomi		---	---	---	---	---	---	---	+++
	P. cryptogea	+++	+++	+++	+++	+++	---	---	---	+++
Jet 5	*P. ramorum*	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. kernoviae	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cactorum	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. ilicis	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. nicotianae	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cinnamomi	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cryptogea	+++	+++	+++	+++	+++	+++	+++	+++	+++
Hortisept	*P. ramorum*	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. kernoviae	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cactorum	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. ilicis	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. nicotianae	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cinnamomi	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cryptogea	+++	+++	+++	+++	+++	+++	+++	+++	+++
Menno Florades	*P. ramorum*	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. kernoviae	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cactorum	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. ilicis	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. nicotianae	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cinnamomi	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cryptogea	+++	+++	+++	+++	+++	+++	+++	+++	+++

+ indicates growth and - indicates no growth of that *Phytophthora* species in the test. The number of +'s or –'s for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur.

These experiments are currently being repeated using infected peat and clay soil types.

Table 9. Decontamination of *P. ramorum* and *P. kernoviae* infested compost following saturation of compost with disinfectant.

Disinfectant	*Phytophthora* species tested	Exposure time to disinfectant (hr-min)							Control
		0-5	0-15	0-30	1-0	2-0	3-0	20-0	
IMS	*P. ramorum*	---	---	---	---	---	---	---	+++
	P. kernoviae	---	---	---	---	---	---	---	+++
Bleach	*P. ramorum*	+++	+++	+++	---	---	---	---	+++
	P. kernoviae	+++	+++	+++	---	---	---	---	+++
Unifect G	*P. ramorum*	---	---	---	---	---	---	---	+++
	P. kernoviae	---	---	---	---	---	---	---	+++
Jet 5	*P. ramorum*	---	---	---	---	---	---	---	+++
	P. kernoviae	---	---	---	---	---	---	---	+++
Hortisept*	*P. ramorum*	+++	+++	+++	+++	+++	+++	+++	+++
	P. kernoviae	+++	+++	+++	+++	+++	+++	+++	+++
Menno Florades	*P. ramorum*	+++	+++	+++	+++	+++	+++	+++	+++
	P. kernoviae	+++	+++	+++	+++	+++	+++	+++	+++

+ indicates growth and - indicates no growth of the *Phytophthora* species in the test. The number of + or – for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur.

*Product breaks down after 5h

1.1.4. Contaminated Mypex matting

Under the test conditions used, only Hortisept did not effectively decontaminate the Mypex matting (Table 10). IMS and Unifect G were once again the most effective disinfectants with no growth of any of the *Phytophthora* species occurring after an exposure time of 5 min. Bleach, Jet 5 and Menno Florades required exposure times of 1, 2 and 20 hrs respectively to ensure that all the *Phytophthora* species were effectively controlled. These results once again show that most of the disinfectants tested are effective in the presence of organic matter, provided sufficient disinfectant is applied to overcome the effects of the organic matter.

1.1.5. Infected leaves

To indicate whether the disinfectants could effectively decontaminate infected leaf material, detached leaves of rhododendron, camellia and viburnum were infected with *P. ramorum* and then treated with disinfectant.

Only Unifect G effectively decontaminated all leaf types, and then only after an exposure time of 24 hrs (Table 11). Treating leaves with Menno Florades decontaminated rhododendron and viburnum leaves while treatment with bleach only decontaminated rhododendron leaves, again only after an exposure time of 24 hrs. IMS and Jet 5 did not decontaminate any of the leaf types under the conditions used in these tests.

1.2. Efficacy of disinfectants in water

Two chemicals were tested for efficacy in decontaminating water contaminated with *Phytophthora* spores - hydrogen peroxide (as Jet 5) and sodium hypochlorite. A third chemical, chlorine dioxide will also be tested. Both chemicals tested to date were extremely effective in decontaminating water of *Phytophthora* after only 5 min exposure time.

2. Efficacy of 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] and four for use with indigenous *Phytophthora* species [held outdoors at STC]. The validation of this technology for the decontamination of *Phytophthora* species from water has continued in the second year of the project.

Table 10. Effectiveness of disinfectants for decontamination of *Phytophthora* contaminated Mypex matting

Disinfectant	*Phytophthora* species tested	Exposure time to disinfectant (hr-min)								Control
		0-5	0-10	0-15	0-20	0-30	1-0	2-0	20-0	
IMS	*P. ramorum*	---	---	---	---	---	---	---	---	+++
	P. kernoviae	---	---	---	---	---	---	---	---	+++
	P. cactorum	---	---	---	---	---	---	---	---	+++
	P. cinnamomi	---	---	---	---	---	---	---	---	+++
	P. cryptogea	---	---	---	---	---	---	---	---	+++
Bleach	*P. ramorum*	+++	+++	+++	+++	+++	---	---	---	+++
	P. kernoviae	+++	+++	+++	+++	+++	---	---	---	+++
	P. cactorum	+++	+++	+++	+++	+++	---	---	---	+++
	P. cinnamomi	+++	+++	+++	+++	++-	---	---	---	+++
	P. cryptogea	+++	+++	+++	+++	+--	---	---	---	+++
Unifect G	*P. ramorum*	---	---	---	---	---	---	---	---	+++
	P. kernoviae	---	---	---	---	---	---	---	---	+++
	P. cactorum	---	---	---	---	---	---	---	---	+++
	P. cinnamomi	---	---	---	---	---	---	---	---	+++
	P. cryptogea	---	---	---	---	---	---	---	---	+++
Jet 5	*P. ramorum*	+++	+++	+++	+++	---	---	---	---	+++
	P. kernoviae	+++	+++	+++	++-	---	---	---	---	+++
	P. cactorum	+++	+++	++-	---	---	---	---	---	+++
	P. cinnamomi	+++	+++	+++	+++	+++	+--	---	---	+++
	P. cryptogea	+++	+++	+++	+++	+++	---	---	---	+++
Hortisept*	*P. ramorum*	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. kernoviae	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cactorum	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cinnamomi	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P. cryptogea	+++	+++	+++	+++	+++	+++	+++	+++	+++
Menno Florades	*P. ramorum*	+++	+++	+++	+++	+++	+++	+++	---	+++
	P. kernoviae	+++	+++	+++	+++	+++	+++	+++	---	+++
	P. cactorum	+++	+++	+++	+++	+++	+++	+++	---	+++
	P. cinnamomi	+++	+++	+++	+++	+++	+++	+++	---	+++
	P. cryptogea	+++	+++	+++	+++	+++	+++	+++	---	+++

+ indicates growth and - indicates no growth of the *Phytophthora* species in the test. The number of + or – for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur. *Product breaks down after 5h.

Table 11. Effectiveness of disinfectants for decontamination of detached leaf material (rhododendron, camellia and viburnum) infected by *P. ramorum*.

Disinfectant	Infected leaf type	Exposure time to disinfectant (hr-min)				Control
		0-30	1-0	2-0	24-0	
IMS*	Rhododendron	+++	++-	+--		+++
	Camellia	++-	++-	++-		+++
	Viburnum	+++	+--	++-		---
Bleach	Rhododendron	+++	+++	+++	---	++-
	Camellia	+++	++-	++-	++-	+++
	Viburnum	+++	+++	+++	++-	+++
Unifect G	Rhododendron	+++	++-	+++	---	+++
	Camellia	+++	+++	+++	---	+++
	Viburnum	++-	++-	+--	---	+++
Jet 5	Rhododendron	++-	++-	++-	++-	+++
	Camellia	+++	+++	+++	++-	+++
	Viburnum	+++	+++	+++	+--	+--
Menno Florades	Rhododendron	+++	+++	+++	---	+++
	Camellia	++-	+++	+++	+--	+++
	Viburnum	+++	+++	+--	---	+++

+ indicates growth and - indicates no growth of that *Phytophthora* species in the test. The number of +'s or –'s for an individual test indicates the number of replicates where growth of the *Phytophthora* species either did or did not occur.

* Tests were not carried out for IMS after 24 hr exposure as it had evaporated from the leaves by this time

2.1. Removal of *P. ramorum* and *P. kernoviae* from water using SSF

Initially the SSFs were challenged with high spore loads (10^4-10^7 spores per litre of headwater – Tables 12 and 13) 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 only challenged with zoospores and sporangia (*P. kernoviae* does not produce chlamydospores). Following an initial failure in the *P. ramorum* filter (Table 12) both *P. ramorum* and *P. kernoviae* were effectively removed by the SSF from the 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.

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 12 and 13). Once again, no *P. ramorum* or *P. kernoviae* was detected in any of the baits taken from the filter outflow. It is now planned to use a newly developed quantitative TaqMan PCR technique to check that *P. ramorum* or *P. kernoviae* are not passing through the filters at levels below the limit of detection of the leaf baits currently being used.

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

The indigenous *Phytophthora* species used to contaminate the SSF headwaters were *P. cactorum*, *P. citrophthora* (ex Ceanothus) and two isolates of *P. nicotianae* (ex Cordyline and Poinsettia) (Table 14). To date, the SSFs have been challenged with high-level contamination of the headwaters (Table 14) and, as with the quarantine *Phytophthora* species, none of the indigenous *Phytophthora* species introduced at the top of the filters have been detected in any of the samples collected at the filter outlet. It is now planned to introduce infected leaf material to the top of these filters to provide continuous low-level inoculum.

The SSFs used for testing the indigenous *Phytophthora* species are located outdoors and as a result have been operating over a wide range of temperature (0.6-26°C, Table 14). A lack of failures in the filters to date indicates they work effectively over this temperature range.

Table 12. Effectiveness of slow sand filtration for the removal of *Phytophthora ramorum* from contaminated water

Date	External temp (°C)	Inoculum (spores/litre headwater)	Sample time (Hours after introduction of *P. ramorum* to headwater)								Outlet bait
			0*	2	3	4	5	6	7	8	
31/01/06	18.7	$1x10^4$ (zoospores)	✓	x	x	x	x	x	x	x	x
16/02/06											x
28/02/06	18.8	$1x10^5$ (zoospores)	✓	x	x	x	x	x	x	x	✓#
09/03/06											✓#
21/03/06											✓#
31/03/06											x
12/04/06	19.9	$4.5x10^6$ (zoospores)	✓	x	x	x	x	x	x	x	x
06/06/06		$1x10^4$ (sporangia)	✓	x	x	x	x	x	x	x	x
06/07/06		$1.5x10^5$ (chlamydospores)	✓	x	x	x	x	x	x	x	x
02/08/06											x
25/09/06		$9x10^6$ (sporangia)	✓	x	x	x	x	x	x	x	x
09/10/06											x

07/11/06	Infected leaves		x
27/11/06	Infected leaves		x
15/12/06	Infected leaves		x
02/02/07		✓	
05/02/07	Infected leaves	✓	x
09/03/07	Infected leaves		x
20/04/07		✓	
03/05/07	Infected leaves		x

*sample taken from SSF headwater; ✓ indicates sample positive for *P. ramorum*, x indicates sample negative for *P. ramorum.*

#failure in filter due to break in schmutzdecke.

Table 13. Effectiveness of slow sand filtration for the removal of *Phytophthora kernoviae* from contaminated water

Date	External temp (°C)	Inoculum (spores/litre headwater)	Sample time (Hours after introduction of *P. kernoviae* to headwater)								Outlet bait
			0*	2	3	4	5	6	7	8	
31/01/06	18.7	$1x10^4$ (zoospores)	✓	x	x	x	x	x	x	x	x

16/02/06											x
28/02/06	18.8	$1x10^5$ (zoospores)	✓	x	x	x	x	x	x	x	x
09/03/06											x
21/03/06											x
31/03/06											x
12/04/06	19.9	$3.5x10^6$ (zoospores)	✓	x	x	x	x	x	x	x	x
06/06/06		1x104 (sporangia)									x
06/07/06											x
02/08/06											x
25/09/06		$9x10^5$ (sporangia)	✓	x	x	x	x	x	x	x	x
09/10/06											x
07/11/06		Infected leaves									x
27/11/06		Infected leaves									x
15/12/06		Infected leaves									x
02/02/07			✓								
05/02/0		Infected leaves	✓								x

7			
09/03/07	Infected leaves		x
7			
20/04/07		✓	
7			
03/05/07	Infected leaves		x
7			

*sample taken from SSF headwater, ✓ indicates sample positive for *P. kernoviae*, x indicates sample negative for *P. kernoviae*

Table 14. Effectiveness of slow sand filtration for the removal of indigenous *Phytophthora* species from contaminated water

Phytophthora species	Date	External Temp min-max (°C)	Zoospore concentration (spores/litre headwater)	*Phytophthora* counts (colony forming units/L headwater) at each sample time (hrs after inoculum addition).								
				0.5*	2	3	4	5	6	7	8	8*
P. cactorum	02/02/06	2.0-3.0	$1.3x10^7$	225	0	0	0	0	0	0	0	0
	14/03/06	0.6-6.5	$8.6x10^6$	713	0	0	0	0	0	0	0	0
	04/05/06	6.0-23.0	$1.35x10^7$	625	0	0	0	0	0	0	0	
	07/06/06	10.4-24.7	$7.5x10^6$	550	0	0	0	0	0	0	0	413
	05/07/06	13.0-26.0	$2.35x10^7$	1388	0	0	0	0	0	0	0	200
	02/08/06	14.0-17.5	$3.0x10^6$	125	0	0	0	0	0	0	0	163
	01/09/06	15.4-22.2	$7.5x10^6$	688	0	0	0	0	0	0	0	113
	05/10/06	12.0-16.5	$2.5x10^6$	475	0	0	0	0	0	0	0	0
	2/11/06	0.5-8.5	$2.1x10^7$	925	$0^{\#}$	0	0	0	0	0	$0^{\#}$	25
	17/1/07	3.1-8.2	$5.29x10^7$	163	0	0	0	0	0	0	0	338
Phytophthora cocktail	03/10/06	11.9-17.5	$1.2x10^9$	763	0	0	0	0	0	0	0	0

	26/10/06	10.0-14.4	6.3 x10^7	TMTC	0	0	0	0	0	0	0	463
P. nicotianae	29/11/06	4.7-11.9	2.9 x10^7	TMTC	0	0	0	0	0	0	0	0
ex Cordyline	17/01/07	3.1-8.2	5.17 x10^7	TMTC	0	0	0	0	0	0	0	138
	26/10/06	10.0-14.4	6.6 x10^7	TMTC	0	0	0	0	0	0	0	531
P. nicotianae	29/11/06	4.7-11.9	3.4 x10^7	0	0	0	0	0	0	0	0	25
ex Poinsettia	17/01/07	3.1-8.2	7.72 x10^7	950	0	0	0	0	0	0	0	38
P. citrophthora	15/11/06	8.2-15.0	4.1 x10^8	TMTC	0	0	0	0	0	0	0	325
	13/12/06	6.9-12.1	2.55 x10^7	TMTC	0	0	0	0	0	0	0	713

* Sample taken from SSF headwater; # *Phytophthora*-like mycelium found, but not consistent with the *Phytophthora* sp. introduced to the SSF; TMTC - Too many colonies to count

Conclusions

- All the disinfectants tested (Jet 5, Hortisept, Unifect G, Menno Florades, Industrial Methylated Spirits (IMS) and bleach) effectively decontaminated an inert surface of *Phytophthora* species, in the absence of organic matter. For individual disinfectants the exposure time required for decontamination varied depending on the *Phytophthora* species and to a lesser extent the ambient temperature.
- Disinfectant activity was inactivated in the presence of organic matter, with the exception of Hortisept. This effect could be overcome provided sufficient disinfectant was applied.
- Only Unifect G was effective in decontamination of infected leaves of all three hosts tested (rhododendron, camellia and viburnum). Menno Florades only decontaminated rhododendron and viburnum leaves, while a treatment with bleach only decontaminated rhododendron leaves.
- Water contaminated was successfully decontaminated of *P. ramorum* following a 5 min exposure to either Jet 5 or a 10% bleach solution.
- Continued testing of slow sand filters has indicated that they are highly effective in removal of a broad range of *Phytophthora* species from contaminated water.

Future work

Work in the third year of the project will focus on in-field testing of the detection and disinfection strategies developed in the first two years. From this work a simple robust strategy will be devised comprising a nursery audit to determine the presence/absence of different *Phytophthora* species on nurseries. At the same time, it will provide recommendations for effective disinfection of the pathogens in different commercial situations.

Evaluation of the effectiveness of slow sand filtration for the removal of different *Phytophthora* species, including those of quarantine significance, from water sources on the nursery will continue.

References

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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.

Appendix I

10 % V-8 agar

V8 juice	200 mL
$CaCO_3$	2 g
Agar N^o3	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.

$PARP_5H$ agar (Jeffers and Martin, 1986)

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}