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Key staff:	Erika Wedgwood
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Industry Representative:	Roger Ward, Golden Grove Nursery
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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

- A survey of 17 nurseries found that all the nurseries had lost plants to root rots, attributed to infection from a number of *Phytophthora* and *Pythium* species.

- Root and stem tissue sampling and water bait tests can be useful disease monitoring tools, when used in combination with on-site *Phytophthora* and *Pythium* diagnostic test kits.

Background and expected deliverables

Conifer growers have reported conifer root rot and die-back problems for many years. Species of *Phytophthora,* and to a lesser extent species of *Pythium,* are recognised causes of conifer root rot and die back.

It has recently become possible for plant samples to be diagnosed on-site by lateral flow devices (LFDs) for both *Phytophthora* spp. and *Pythium* spp.. LFDs could, for example, allow suspect recently-arrived plants to be tested before moving them into the growing area, or allow the testing of any plants developing symptoms so that surrounding plants could be treated the same day before infection developed further.

There is also potential to test water sources, through the use of leaf baits, for spores of *Phytophthora* spp. or *Pythium* spp. thereby avoiding the infection of plants through use of contaminated irrigation water. Baits could be tested on-site by LFD (HNS 134) so saving time compared with sending them for laboratory confirmation.

Once an LFD test has confirmed *Phytophthora,* it then can be tested using the PDplus service by Forsite Diagnostics Ltd to determine species identity. This could show whether plants arriving from different sources or those kept within particular locations were infected with the same *Phytophthora* species and so potentially allow tracking back to the source.

Yew is one conifer that can be infected by *P. ramorum* (HDC Factsheet 19/03) and species such as *P. kernoviae* and *P. cactorum* could also be simultaneously checked for using the PDplus testing of DNA in an LFD. This project will help validate the use of PDplus on LFDs

for an extended range of pathogen species to include the important root pathogens *P. cinnamomi* and *P. citricola*.

The plant clinics at the RHS and Fera (Food and Environment Research Agency) hold information from several years of hardy nursery stock disease and pest diagnosis. Examination of this data could raise awareness of specific diseases associated with certain hosts. In addition, there has been concern about the spread of *P. cinnamomi and P. nicotianae* northwards from the Continent and the current prevalence of these species could be determined from the plant clinic records.

Once more information is available on the main diseases causing root rot and dieback in conifers, research and development work can be directed to provide suitable cultural, microbiological and fungicidal control measures.

Summary of the project

Occurrence of root rots

A survey of 17 nurseries was conducted, of which responses were received from 14. All the nurseries had lost plants to root rots, attributed to *Phytophthora* or *Pythium* infection, with some total losses of *Taxus*, and cultivars of *Juniperus* and *Chamaecyparis*. *Araucaria* plants were also lost in large numbers (Table GS 1). Most growers said that certain *Chamaecyparis* cultivars were particularly susceptible to root rot, in particular cv. 'Ellwoodii' and those cultivars with blue foliage. A number of *Juniperus* cultivars were also named as more susceptible particularly if overwatered. *Abies, Cedrus, Cryptomeria, Cupressus, Larix, Picea, Pinus* and *Thuja* were reported to rarely suffer from root rotting. On five nurseries visited in summer 2010, a number of conifers were only starting to show foliar wilt yet had severe root rot. Information on growing conditions and control measures employed on the nurseries was recorded (which are detailed within the full report) and has provided the basis of action points to reduce risk of root rot and improve plant quality.

Table GS 1: Average percentage annual losses to possible root rot of conifers at any production stage between 2005 and 2010 reported from nurseries situated on the eastern side of England (1E to 7E), and the western side of Britain, including Scotland (1W to 7W)

Nurseries	Conifer origins*	*Abies*	*Araucaria*	*Cedrus*	*Chamaecyparis*	*Cryptomeria*	*Cupressocyparis*	*Cupressus*	*Juniperus*	*Larix*	*Picea*	*Pinus*	*Taxus*	*Thuja*
1E	H	X	30	X	3	0	0	0	3	X	X	10	5	0
2E	B	X	X	X	10	0	0	5	2	0	0	0	0	0
3E	H	0	100	X	50	X	0	0	1	X	0	0	10	0
4E	B	80	20	0	15	0	0	0	0	0	0	0	0	0
5E	H	0	0	0	20	0	0	0	0	0	0	0	10	0
6E	HB	X	10	2	75	5	20	2	10	20	2	20	50	0
7E	HB	0	X	0	100	0	s	0	0	X	0	0	50	0
1W	HB	0	s	0	s	0	2	0	10	0	0	0	b	0
2W	B	0	s	0	60	0	0	0	s	s	0	0	30	0
3W	H	10	0	0	10	0	0	0	0	0	10	15	0	0
4W	H	0	50	0	50	s	0	0	s	0	0	0	s	0
5W	HB	0	5	0	7	0	0	0	0	0	0	0	5	s
6W	HB	0	X	0	40	0	s	0	s	X	X	X	5	s
7W	B	0	12	X	X	X	X	X	0	0	0	0	10	X

* H = nursery principally home-producing conifers, B = principally growing-on bought-in conifers
X = conifer not grown at this nursery. Losses to root rot not quantified s = some, b = bad.

It is interesting to note that *Taxus* also dominated the records obtained from the RHS plant clinic of members' conifers. Information was also gained from the plant clinic records on the species of *Phytophthora* and *Pythium* which had been identified from the stems and roots of a range of conifer species submitted by the public. No greater recent prevalence was seen of the *Phytophthora* species *P. cinnamomi* and *P. nicotianae*, although area expansion had been considered a potential outcome of global warming as these species are favoured by warmer climates.

Laboratory testing (using the Polymerase Chain Reaction technique) of conifers collected from seven nursery sites (Table GS 2), and also the conifers received from RHS members at RHS Wisley, showed a wide range of *Phytophthora* and *Pythium* species involved in root rots and stem die-back.

Table GS 2: Conifer species from different nursery sites and species of *Phytophthora* and *Pythium* identified using PCR of stem or root tissue. Where more than one plant of the same family was sampled at a nursery this is shown as a separate record

Plant host	Site	***Phytophthora*** **in stems**	***Pythium*** **in stems**	***Phytophthora*** **in roots**	***Pythium*** **in roots**
Abies (Fir)	1W			*P. cryptogea*	
Araucaria (Monkey Puzzle)	1E			*P. citricola* complex	
	1E			*P. citricola* complex, *P.* sp. *Salixsoil*	
Chamaecyparis (Lawson cypress)	2E	Sp. closest to *P. gonapodyides*	*P. sylvaticum*		*Pythium* sp.
	4E			*P.* sp. *Salixsoil*	
	4E	*P. cactorum*			*P. vexans*
	4E	*P. cryptogea*		*P. cryptogea*	
	5E			*P. quercina /P.* sp. *Ohioensis*	
	5E			*Phytophthora* sp.,*P. quercina /P.* sp. *Ohioensis*	
	5E		*P. intermedium*	*P. quercina /P.* sp. *Ohioensis*	*P. monospermum / P. attrantheridium*
	5E			*P. cinnamomi*	
	1W				Sp. closest to *P. diclinum* and *P. lutarium*
	2W			*P. quercina /P.* sp. *Ohioensis*	
	2W				*P. sylvaticum, Pythium* sp., *P. vexans*
Cupressocyparis (Leyland cypress)	4E			*Phytophthora* sp. closest to *P. heveae*	*P. vexans, P. intermedium*
Juniperus (Juniper)	1E			*P. gonapodyides*	
	2E			*P. gonapodyides*	
	3E	*P. austrocedrae*	*P. attrantheridium*	*P. gonapodyides*	
	4E	*P. austrocedrae*		*P. gonapodyides*	
	4E	*P. gonapodyides*			*P. attrantheridium*
	1W			*P. gonapodyides*	
Pinus (Pine)	2E	*P. cactorum*		*P. cactorum*	
Taxus (Yew)	1E				*P. irregulare*
	3E			*P. cinnamomi*	
	1W	*P. cinnamomi*			

Phytophthora gonapodyides, usually considered a weak pathogen, occurred on Juniper roots at all five sites where this host was sampled. Other *Phytophthora* species found on roots included *P. cinnamomi*, *P. citricola* complex, *P. cryptogea*, *Phytophthora* sp. *Salixsoil* and *P. quercina* / *Phytophthora* sp*. Ohioensis. Pythium* species recorded on roots included *P. attrantheridium, P. intermedium*, *P. irregulare*, *P. sylvaticum, P. vexans* and *P. sylvaticum*.

From the nursery visits, as well as causing die-back through root infection, direct foliar or stem infection caused by *Phytophthora* and *Pythium* species was principally identified as the cause of localised foliage death, including the record of non-indigenous *Phytophthora austrocedrae* on *Juniperus*. In addition to *P. austrocedrae*, the *Phytophthora* species *P. cactorum*, *P. cinnamomi* and *P. gonapodyides* were isolated from stems with foliar wilt without evidence that they were also in the roots and had spread upwards. *Pythium* species *P. attrantheridium*, *P. intermedium* and *P. sylvaticum* were identified in samples from stems at the location of foliage browning. From questionnaire replies and nursery visits, diebacks and leaf blights by pathogens such as *Keithia* on *Thuja* and *Pestalotiopsis* on *Juniperus* were only of intermittent concern and usually treated with a foliar applied fungicide.

Use of Lateral Flow Device kits and leaf bait tests

LFDs were used on nursery sites and shown to be easy to use with both stem and root tissue. The LFDs that recorded a positive reading (Figure GS 1) were sent to RHS Wisley for testing (by PCR) to determine the species of *Phytophthora* and *Pythium* present and to compare the results with the number of times the LFD results were positive. The positive detections of *Phytophthora* in roots and stems by LFD were confirmed by PCR. However, *Pythium* was detected in more roots and stems by LFD than by PCR. This difference in detection may have been because the exact same tissue sample could not be used within both (i.e. LFD and PCR) tests.

Figure GS 1: Lateral flow devices showing intensity of positive test (T) line as indexed 0 to 4 (darkest). The control (C) line indicates that the LFD is working properly.

The use of LFDs allows recently arrived plants to be tested by growers before moving them into the growing area, or any plants developing symptoms within a particular growing area to be tested and the surrounding plants treated the same day before the disease develops any further. The cost of £8.30 + VAT per kit means a large cost saving on laboratory fees which are usually £60 + VAT, plus the additional time and expense of packaging and postage of plants.

Leaf baits have been used for detecting *Phytophthora* and *Pythium* in irrigation water sources. In HNS 134 bait leaves were bound in a muslin square to form a bundle (Figure GS 2) which was kept floating just below the water surface by using a combination of stones and a polystyrene foam packing piece. This work identified *Abies nordmanniana* (Nordmann Fir) as a good source of bait leaf material (10-20 one-year old needles per bait). An alternative to muslin (which would not normally be found on a nursery), horticultural fleece, was also discovered to be an effective alternative.

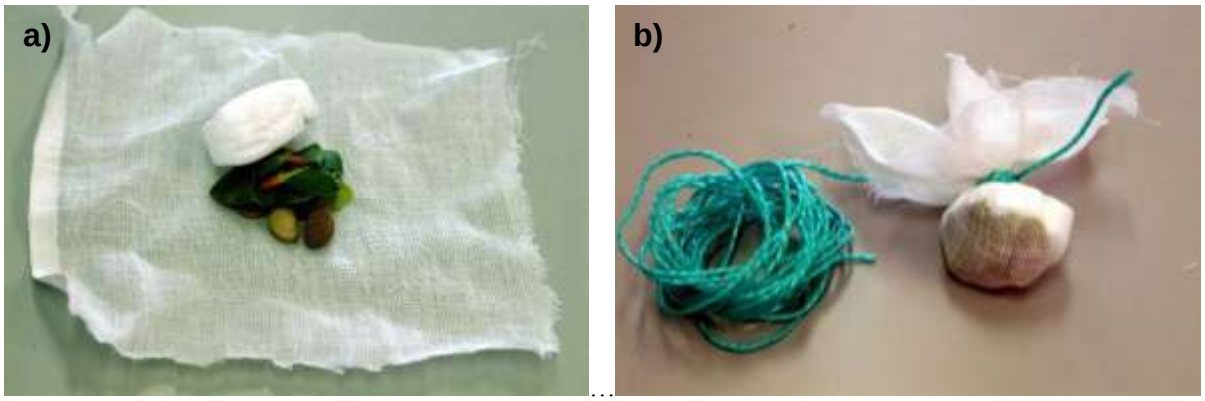

Figure GS 2: Construction of rhododendron bait a) showing bait contents and b) completed bait.

Assembled bait bags can be placed into a water source and retrieved after three days. Each bait bag after blotting (to remove excess water) can then be placed in a grip seal bag and mailed first class to a laboratory for testing. It was also shown that baits can be tested by LFD so that on-the-spot confirmation of water contamination by *Phytophthora* or *Pythium* is possible.

Once an LFD test has confirmed *Phytophthora,* it can then be tested further using the PDplus service by Forsite Diagnostics Ltd to determine the identity of some species if this is required. This can show whether plants from different sources had the same *Phytophthora* spp. and so allow tracking back to the likely source. The use of PDplus was confirmed to be

a practical option for growers for the detection of *P. cactorum*. The number of samples was not sufficient to be able to validate the detection of *P. cinnamomi* and *P. citricola*. No *P. ramorum* or *P. kernoviae* were detected on nurseries, but PDplus has been extensively tested and can be used for these notifiable species.

In addition to *P. cinnamomi*, *P. citricola* and *P. cactorum* confirmed in conifers from sampled nurseries using PCR directly on tissue (rather than via DNA deposited in a LFD), other *Phytophthora* species were present that cannot currently be identified via PDplus. These included *P. austrocedrae*, *P. cryptogea*, *P. gonapodyides*, *P. quercina* and *Phytophthora* sp. *Salixsoil*. In addition, conifers with dieback and root rot from RHS members showed another four species (*P. hibernalis*, *P. megasperma*, *P. plurivora* and *P. syringae*). Therefore, growers sending positive *Phytophthora* LFDs could receive a negative identification match from PDplus and so the current benefit of this additional test with the wider range of *Phytophthora* species is more limited.

Application of test results

Control measures are simplified if they are aimed at *Phytophthora* or *Pythium* rather than fungal pathogens in general as cultural, biological and chemical control measures can be focused on these water-moulds. The information from this survey can also be utilised to focus the attention of future research (e.g. to determine pathogenicity of *Phytophthora* and *Pythium* species) and could also be used by manufacturers seeking to develop detection and control products.

Financial benefits

Annual losses of 10% of plants to root rot are not unusual for many conifer cultivars, with susceptible species frequently reported to have 50% losses. It should be possible if growers carry out the action points to put procedures in place to make significant reductions in these losses.

The use of nursery-made leaf baits in irrigation water and on-site detection by LFDs of *Phytophthora* or *Pythium* will not only save the cost of laboratory testing, but allow rapid action to be taken to prevent further use of contaminated water on the crop. The ease of testing may encourage more regular monitoring to ensure that water-treatment procedures are working correctly.

The use of LFDs on nurseries will allow rapid on-site diagnosis of *Phytophthora* or *Pythium*

infection. This will mean that there can be swift follow-up action to treat or dispose of affected plants and to search for and remove the source of the infection. Detection and treatment of these pathogens at an early stage in conifer production will prevent infected plants being potted-on and taking up time and space while dieback and root rot progresses to finally make mature plants unmarketable.

Information given in the report will increase awareness on nurseries of the species and cultivars requiring special care. *Phytophthora* and *Pythium* species not previously widely known to be associated with conifer dieback or root rots have been identified and reported to growers.

Information from this work will be applicable to monitoring *Phytophthora* spp. and *Pythium* spp. in crop sectors beyond the conifer and hardy nursery stock sector.

Action points for growers

Plant inspection

- Check bought-in plants for root rotting on arrival and again within a couple of months, paying particular attention to *Araucaria, Chamaecyparis*, *Juniperus* and *Taxus.*
- Check plants regularly for root rot. By the time the plants start to feel “soft” and look dull an attack by *Phytophthora* or *Pythium* can be well advanced. It would seem that foliage symptoms from root rot can develop in the summer within a couple of months of plants appearing healthy.
- Remove diseased plants as soon as they are seen and put then straight in a skip.
- Ideally, plants around affected ones should be inspected for root rot and considered for disposal as well.
- Keep new stock apart from other older stock, if possible
- Label plant batches with their source to aid any tracking-back of infection.
- Be alert for unusual situations of foliar dieback or root rotting and, if present, determine whether a non-indigenous pathogen might be involved.

Growing conditions and disease control

- Regulate watering to plants to prevent the growing-media remaining saturated; consider the use of evapo-transpiration sensors to allow automation of the process.
- Consider the use of more open growing-media to reduce the risk of roots sitting wet.
- Conifers such as *Araucaria, Juniperus* and *Taxus* are particularly affected by root rot if overwatered and so these should be kept where they can be given a lower irrigation frequency and may benefit from protection from rain.
- Avoid autumn potting as the growing medium is more likely to be overwatered as plant growth slows at this time of year.

Use of LFDs

- Test roots and / or stem tissue with LFDs to show whether or not *Phytophthora* or *Pythium* is present to permit either rapid and appropriate chemical treatment or destruction of affected plants to prevent further disease spread.
- When potting-on, plants with poor roots should be checked for disease and ideally rejected.
- Request the full Final Report for information on diagnostic kits available to growers (Appendix 4), and instructions on how to use LFDs (Appendix 3).

Testing irrigation water

- Ensure that clean irrigation water is used. Check water treatment equipment regularly and service it as recommended.
- Consider the use of leaf baits coupled with LFD kits for on-site detection of *Phytophthora* and *Pythium* in collected run-off and irrigation water.

SCIENCE SECTION

Introduction

A number of root rot and die-back diseases can affect conifers, but information is lacking on the specific cause, incidence and relative importance of different conifer diseases in the UK and what control measures are being used. Several *Phytophthora* species including *P. cinnamomi* and *P. cactorum* can attack conifers, either causing wilting and dieback via the roots or by direct infection of the foliage causing patches of browning. Many species of *Pythium* can also cause root and stem base rots of hardy nursery stock. Diseases caused by both *Pythium* and *Phytophthora* are generally favoured by wet soil conditions. Both genera are commonly detected in water intended for irrigation which has been roof-collected or includes run-off from beds (Pettitt *et al.,* 2002).

Foliage die-back of hardy nursery stock can also occur due to direct foliar infection and also following root infection by species other than *Phytophthora* and *Pythium*. Symptoms such as conifer browning can result from an interaction between nutrient application and growing conditions (HNS 148) or from pest damage (HNS 151).

In order to determine the current extent of *Phytophthora* and *Pythium* root rots and die-back diseases on UK conifer nurseries, and strategies used to manage the diseases, three lines of investigation were used. Growers were surveyed by post and telephone, plant clinic records for conifer problems were examined and a number of growers were visited to sample diseased conifers. Additionally, the use of lateral flow device (LFD) kits for the detection of *Phytophthora* and *Pythium* using an antibody-based method was demonstrated to growers and a baiting technique was developed to sample irrigation water on nurseries for *Phytophthora* and *Pythium* which then allowed the use of an LFD on the bait material.

Plant and bait samples tested with LFD detection kits were also further examined by PCR testing to see what range of species of *Phytophthora* and *Pythium* were present in conifers and irrigation water on different nurseries.

It is possible for growers to send an LFD that has tested positive for *Phytophthora* spp. for a further test (PDplus Forsite Diagnostics) to record whether *P. cactorum, P. ramorum* or *P. kernoviae* is present. This test was recently expanded to include two more species of relevance to conifer growers - *P. cinnamomi* and *P. citricola*. To assist with verification of the

expanded test range, a number of the devices used with conifers were sent away for DNA testing using real-time PCR and the results matched against the PCR results obtained by testing plant material directly.

Materials and methods

Survey of nurseries growing conifers

A survey form was produced in consultation with the industry representative (Appendix 1). The aim was to assess the extent of conifer losses to *Phytophthora* root rot and other causes of die-back on UK nurseries and to identify common factors which may influence the likelihood of various infections developing. Information on the survey and the form was e-mailed by the HDC in April 2010 to all growers recorded as having an interest in conifers. The survey was noted in the HDC News No. 160 (February 2010) promoted in the HDC Weekly News e-mail and in the ADAS Technical Notes for Nursery Stock. Growers were invited to complete a survey, whether or not they had seen disease on their conifers. Growers were requested to contact Erika Wedgwood for a survey form and then either e-mail their replies, return a hard copy, or complete the questionnaire via telephone. Only a few growers responded to the survey initially and so nurseries were telephoned using contact details obtained by web searches (in particular the Conifer Growers Association) and more grower participation was achieved. All information from growers, together with results from plant clinic databases, were to be used to highlight situations of higher disease risk, to recommend ways of reducing disease incidence, and to help target future work on control measures. Information was received on the understanding that nursery details would not be included in the report.

Plant sampling and disease testing on nurseries

Five growers were sought to utilise LFDs for on-site diagnosis of *Phytophthora* and *Pythium* in plants and irrigation water. LFD use was to be followed by the laboratory identification of these pathogens to species level using molecular techniques, including utilising the PDplus service on positive LFDs to speedily identify the pathogen/s present from a range of possible *Phytophthora* species. The PDplus service if used by nurseries would cost £25 per LFD, but the tests as part of this research were carried out in conjunction with Forsite Diagnostics to assist with validation of the test for an extended range of *Phytophthora* species. The use of these two testing procedures were investigated as a quicker and cheaper alternative for growers than sending plants to a plant clinic for visual assessment and pathogen isolation from tissue for identification.

Five visits were carried out between June and August 2010 to nurseries who had reported problems with root rot and dieback. The nurseries were all on the eastern side of the UK and so the results were supplemented by plants sent in for diagnosis by two nurseries on the other side of the country. The nurseries participated in the sampling with agreement that their identities would not be given in this report.

The plants sampled were determined by the main areas of concern on each nursery, the grower or site manager pointing out beds with dying plants. This mainly involved a mixture of species and principally focused on 3 L pots. Roots and stems of the plants were examined and if symptoms (root rot or stem staining, respectively) were seen then LFD tests were carried out for both *Pythium* and *Phytophthora* (detailed below). Plant testing was carried out using up to eight LFDs per pathogen per site visited (two *Pythium* and two *Phytophthora* LFDs/plant on each of four plants per nursery). Information on potting-on dates, and the incidence and severity of symptoms was recorded (Appendix 2). At each of the sites the grower or site manager watched and assisted while at least one plant was sampled and the LFD kits demonstrated. They then carried out the testing themselves under supervision. Each grower was left three unused *Phytophthora* LFDs with extraction buffer and pipettes together with the sampling instructions produced for this project (Appendix 3) to utilise on future potentially infected plants.

The LFDs used were “Pocket Diagnostics” (produced by Forsite Diagnostics Ltd, a private company set up to market the kits developed at the Central Science Laboratory at York). The LFDs were supplied at a reduced price to the project as an in-kind contribution to the work. Individual kits would retail to nurseries at £8.30 + VAT. Tests using the same antibody technology are also sold by Biobest, but the indicator paper is on a dipstick rather than within a plastic sheath. Information on the diagnostic kits available to growers (which include tests for diseases other than *Pythium* and *Phytophthora*) was provided to growers in ADAS Technical Notes in September 2010 and is reproduced in Appendix 4.

Directions for tissue sampling and use of LFD kits

The detailed directions on how to sample the tissue are given in Appendix 3 and were compiled from the instructions supplied with the LFD kits. Care was taken on nurseries that cross-contamination of samples did not occur, ensuring that hands were washed between samples. Each plant was allocated an identification code and a recording sheet was completed (Appendix 2) for each sample to determine the extent of the symptoms as the stems and roots were sampled. LFDs were also marked with the ID code, and the speed of appearance and strength of any positive test line on the LFD was recorded on the sheet.

Photographs were taken of some plants to supplement records.

Stem samples were cut using a sterile (dipped in 90% alcohol and set alight) pruning knife. Care was taken not to allow growing-media contamination. The sampled stem face was covered straight away with masking tape to avoid cross-contamination that might be picked up when the plant was sent for PCR. If there was no obvious leading edge of tissue death the stem was sampled from 10 mm to about 50 mm above soil level, taking care not to get growing-media in the cut. The bark was removed and discarded to obtain the stained wood underneath. Sampling was from only one side of the stem as the other side was left to be tested by laboratory PCR. Slithers of wood were taken and collected in a small weighing boat and then cut into smaller pieces, using sterile scissors, which were then placed in the buffer bottle for extraction and use with the LFDs. Frequently there was only a small amount of stained tissue available and so about half the buffer was poured away (leaving more than enough for the 6 drops required for the two LFD wells) before adding the tissue so that the extract would be sufficiently concentrated for the test.

A record was made of the approximate % of the roots brown in the original plug and the outer growing-media to be able to assess whether infection may have already been established on the plug at potting-on or come in through the pot drainage holes. Browning roots of a range of sizes were sampled taking material from within the original root ball as well as from the roots which had grown out into the growing-media after potting on. About 10-20 root pieces about 30 mm long were taken, particularly from the margin with the brown rot. The final amount was a “large pinch” which would e.g. fill the lid of a plastic milk bottle. Tap water was sloshed over the roots to remove the majority of the growing medium (or the roots were placed in a clean tube and shaken in the water). The roots were then torn apart into shorter sections using clean fingers and placed into the labelled buffer bottle. The bottle was shaken vigorously for 60 seconds so that the ball-bearings inside smashed open the tissue. The supplied pipette was used to suck up liquid without too much debris and three drops without air bubbles were placed into the well on the ID-labelled LFD kit. The indicator strip results were then recorded within 10 minutes of adding the drops.

The same root or stem sample was used for both LFD tests per plant i.e. one buffer bottle for roots and another for stems and the drops of the extract placed in turn on the *Pythium* and *Phytophthora* LFDs. The buffer bottle used for each sample was kept on cool blocks for transport and then frozen straight away at ADAS to have as a back-up reference. The LFDs were returned to their individual foil packets and stored in a box at room temperature at ADAS Boxworth. When all the LFDs were together the strength of each blue test line was

compared and given an index to be able to check whether those with a faint line might not be picked up as positive by either the PCR tests carried out at RHS Wisley, or in particular by the PDplus tests at Forsite Diagnostics which use the indicator strip from within a LFD.

Any branches with dieback that did not produce a positive result with the LFD kits were examined in the laboratory, and given damp chamber incubation if there was no obvious fungal growth or other damage symptom on the fresh sample. Isolations were sometimes carried out, but fruiting bodies for identification were more likely to be produced on the host tissue.

PCR testing

The sampled plants were returned to their pots and taken away for next-day delivery to the laboratory at RHS Wisley for PCR testing (a maximum of 38 tests). The plant material was tested using nested PCR analysis of roots and stems (separately) for the identification of as many species of *Phytophthora* and *Pythium* species as have reference DNA sequences available. More sequences are available for *Phytophthora* than *Pythium* because less research has been carried out on the latter (Geoff Denton, pers. comm.). Sampling of stem bases and root tips followed the same procedure as used for on-site LFD tests. Sampled plants were kept in cold storage by the RHS in case further samples were required.

The sample was ground using liquid nitrogen in a pestle and mortar. DNA was extracted using DNeasy plant mini kits (QIAGEN) following the protocol provided. Any additional plant products inhibitory to PCR were removed by passing through PVPP in a chromatography column. A nested PCR was carried out in pure Taq Ready-To-Go PCR beads (Amersham Biosciences) using the primers and cycles described by Cooke *et al.* (2000). A gel was run to see if a 900 bp band was produced, confirming the presence of an organism in the Peronosporales or Pythiales. If a 900 bp band was seen then it was isolated using QIAquick gel extraction kit (QIAGEN) following the manufacturers protocol. The 900 bp product was sequenced (John Innes Genome Laboratory). Two readings were created and aligned together (Lasergene Software) and then compared to the online database (Genank, NCBI).

PDplus verification

Once PCR test results giving identification to species level were returned by the RHS then eleven LFDs were selected to send to Forsite Diagnostics for PDplus testing. This testing was carried out for the HDC free of charge. These had been used on plants which had been found by the PCR tests to be infected in the roots or stems with *Phytophthora cinnamomi*, *P. citricola* or *P. cactorum*. This was aimed at validating the use of PDplus on LFDs to test for

P. cinnamomi and *P. citricola*. The identity of *Pythium* and *Phytophthora* species as determined by PCR at RHS was not disclosed until after the PDplus had been run.

Monitoring irrigation water using leaf baits

Determining the type of bait material to be used

Leaf baits have been used for detecting *Pythium* and *Phytophthora* commonly using young growth of *Rhododendron ponticum* (Pettit *et al*, 2002 and reported in HNS 134) or cedar leaves (Dance *et al.*, 1975). Some baits appear to attract certain species of zoospores better than others, but results are variable. Fruit and seeds have also been used as baits (Singleton *et al.*, 1993 and Erwin and Ribero, 1996), but if to be used on the nursery would require to be bought specially. In some methods the leaf pieces are cleaned using a 75% ethanol wipe or autoclaved for 10 mins to remove any other micro-organisms. If leaves are to be collected on a nursery then plants that are likely to have *Phytophthora* root rot and so liable to upwards splash onto the foliage e.g. *Chamaecyparis,* or to have the risk of *Phytophthora ramorum* e.g. rhododendron should be avoided as baits. In HNS 134 the leaves were bound in a muslin square to form a bundle which was kept floating just below the water surface by using a combination of stones and a polystyrene foam packing piece.

For the current research, two conifers *Picea abies* (Norway Spruce) and *Abies nordmanniana* (Nordmann Fir), that might provide leaves all year and be found on a conifer nursery were selected for testing and collected from a garden in comparison with rhododendron. The rhododendron was from a containerised plant in a polytunnel at ADAS Boxworth. Microwave treatment was investigated as a replacement for heating by autoclaving as a microwave would probably be available on nurseries. The effect of this heat treatment on the effectiveness of the bait was checked by leaving some leaves fresh. Alcohol wipes were not tested as a way to surface sterilise as although the surfactants and ethanol might be effective it was feared that they might also kill or not attract zoospores. It was also not thought feasible for growers to wipe every leaf on both sides before using them in the bait.

Sterile universal tubes were partially filled with sterile pond water with two 7 mm diameter agar discs of a common root rot, *Phytophthora cinnamomi,* at the bottom of each tube taken from cultures which had been flooded a week before with sterile distilled water to stimulate sporangia production. Sporangia were expected to release zoospores which would swim to infect leaf material floated on the water surface. Four replications were carried out with and without the leaves having been microwaved at 800W for 2 minutes (plus 30 second stand) together with a jug of water so that the leaves remained moist and flexible. Leaf material

used, in separate tubes, was 8 mm x 8mm sections (with no natural edge) of 1 year old rhododendron leaf cv. Cunningham's White, one 20 mm long *Abies nordmanniana* needle and two 10 mm long *Picea abies* needles.

The tubes were placed in the dark at room temperature for 3 days, then leaves were assessed for browning that could be infection. The leaves were then blotted on sterile filter paper and cultured in the dark on P5ARP agar selective for *Phytophthora* for 4 days (Singleton *et al.,* 1993). The plates were re-assessed 4 weeks later as this pathogen grows relatively slowly on agar.

Bait bag use on nurseries

An alternative to muslin (which would not normally be found on a nursery), horticultural fleece, was also used to make bait bags when on nurseries to see if the pathogens would still pass through the weave. Before using horticultural fleece to hold the baits it was examined under a microscope to measure the size of the pores and the gaps between threads within the main weave. Experiments were also carried out with the fleece to determine the required weight of stones and number of polystyrene pieces needed to give the correct floatation at a position near the water surface where water-mould zoospores have been suggested to collect (Tim Pettitt, pers. comm.).

At the second nursery, *Ceanothus* leaves were brought from a garden to be tested as baits in comparison with Nordmann Fir needles. This plant was available on this nursery and could be purchased and grown in any soil type (in contrast to rhododendron) if not stocked by other nurseries. The suitability of this evergreen shrub as a bait had previously been recognised (T. Pettitt pers. comm.). Different baits can attract different species of zoospore (Erwin and Ribero, 1996). A check was made of the upper leaves from the garden bush of *Ceanothus* used in the tests to confirm that there was no *Pythium* or *Phytophthora* on the visibly healthy leaves. Leaves were floated in both sterile rain water and sterile rain water for three days and then blotted and plated out on P5ARP agar (which favours the growth of Phycomycetes i.e. water-moulds).

Bait bags were made at each of the three nurseries which had a reservoir containing untreated water. Leaves were picked the same morning and the stems wrapped in wet tissue for transport to the nursery. At the first site only five Nordmann Fir needles were used per bait. For subsequent baits, 10-20 one-year old Nordmann Fir needles or 8-12 leaves of a large-leaved cultivar of *Ceanothus* were used per bait (the number depending on leaf size) in order to provide sufficient material to use for both the LFD test and PCR testing. The

instructions for the LFD kits state that the equivalent of 25 mm² of leaf material is required for the test. The leaves were not surface sterilised before use.

The assembly of the bait bags was demonstrated to the grower or site manager and then the baits placed into the reservoir held by a slack length of twine suspended from a fixture so that the bag would remain floating if the water level dropped. Bait bags were placed 30 cm apart in the first two sites' open reservoirs, and in separate post-slow sand filter collection tanks at the third site.

The grower was asked to retrieve the bags after three days in the water, blot them gently to remove excess water and place each one intact into a grip seal bag before mailing them first class post in a padded envelope to the RHS for testing.

It should be possible for growers to use LFD test kits themselves on the baits in the same way as they were shown for the plant stems and roots, but for the current research the leaves were also required for PCR testing. When they were received by the RHS they were examined for any darkening or water-soaking of the tissue which could have indicated infection by *Pythium* or *Phytophthora*. Using sterile technique, the leaves and needles were then slit up the midrib so that both tests received material from the petiole as the base was broken tissue which might produce exudates and attract zoospores. If there was a visible lesion this was shared where possible between the PCR and LFD tests. The leaf material from each bait was placed in one buffer bottle, shaken and then samples taken for both the *Pythium* and *Phytophthora* devices. As a cross-check for experimental purposes both fresh leaf material and used buffer from the same bait bag were taken for comparative PCR testing. The RHS froze surplus leaves from the second nursery left with baits, later also testing these leaves by PCR. Buffer bottles were frozen in case any further DNA testing of them was subsequently required. Records were made of the strength of any colour reaction at the Test position on the LFD strip and the strips stored before being returned to ADAS. The remaining leaf material was kept for PCR testing using the same procedures as for the whole-plant samples.

Results

1. Nursery survey

Out of 26 nurseries contacted, fourteen growers completed the survey fully, with seven nurseries in the eastern half of England (east of a line northwards from the Isle of Wight), six in the west and one in Scotland. Many of these growers continued discussions about their problems with root rots and the different growing methods they had tried beyond the formality of the survey form. Three more growers gave some information on root rots, a further four said they had no problems or had stopped growing conifers. One grower did not wish to participate because he did not think any new information would be gained. The remainder of nursery managers/owners were unavailable on the phone or did not return the survey forms they requested.

Sources of plants material and disease prevalence

Of the six nurseries with 3 ha or more (up to 8 ha) of conifers, four principally produced their own material (usually only buying-in if they required a particular cultivar), one nursery had significantly increased the proportion bought-in, and the sixth nursery only bought-in (mainly 70 mm modules). The two nurseries with 1 to 2 ha only bought-in, and of the six remaining growers with under a hectare all but one was a home-producer and the others had a mixture of own-grown and bought-in (principally buying plants to supplement the range in their on-site shops).

The majority of nurseries having both home-grown and bought-in material did not notice root rot in plants from either source in particular. The growers surveyed who bought plants principally did so from UK nurseries, usually mainly keeping to a few growers known by them. Growers producing their own material of various conifers reported some losses, as did those solely buying-in (Table 3).

Table 3: The average % annual losses possible to root rot of conifers at any production stage between 2005 and 2010 reported from nurseries situated on the eastern side of England (1E to 8E), and the western side of Britain including Scotland (1W to 6W)

Nurseries (coded)	Conifer origins*	*Abies*	*Araucaria*	*Cedrus*	*Chamaecyparis*	*Cryptomeria*	*Cupressocyparis*	*Cupressus*	*Juniperus*	*Larix*	*Picea*	*Pinus*	*Taxus*	*Thuja*
1E	H	X	30	X	3	0	0	0	3	X	X	10	5	0
2E	B	X	X	X	10	0	0	5	2	0	0	0	0	0
3E	H	0	100	X	50	X	0	0	1	X	0	0	10	0
4E	B	80	20	0	15	0	0	0	0	0	0	0	0	0
5E	H	0	0	0	20	0	0	0	0	0	0	0	10	0
6E	HB	X	10	2	75	5	20	2	10	20	2	20	50	0
7E	HB	0	X	0	100	0	s	0	0	X	0	0	50	0
1W	HB	0	s	0	s	0	2	0	10	0	0	0	b	0
2W	B	0	s	0	60	0	0	0	s	s	0	0	30	0
3W	H	10	0	0	10	0	0	0	0	0	10	15	0	0
4W	H	0	50	0	50	s	0	0	s	0	0	0	s	0
5W	HB	0	5	0	7	0	0	0	0	0	0	0	5	s
6W	HB	0	X	0	40	0	s	0	s	X	X	X	5	s
7W	B	0	12	X	X	X	X	X	0	0	0	0	10	X

* H = nursery principally home producing conifers, B = principally growing-on bought-in conifers
X = conifer not grown at this nursery. Losses to root rot not quantified s = some, b = bad

Pot drainage

A few growers reported the greatest losses in plants when outdoors. Plants in 2 L or 3 L pots were more often seen to have root rot than modules or liners (to 1L) which tended to be kept in polytunnels. Plants in 7 L or 10 L pots were less likely to be seen with die-back, although most were outdoors. The majority of growers had stopped growing *Taxus* in the ground or in pots outdoors and now grew them in polytunnels because of earlier losses (with 50% and 100% losses reported at two nurseries). *Juniperus* were also highlighted as requiring less water than other conifers otherwise they tended to get root rot and, some nurseries set them apart from more water-demanding species such as *Chamaecyparis* and *Thuja*. One nursery had started to grow *Araucaria* in peat with high bark content (50%) instead of the more usual 15-20% and root rot had not been seen since using this more open growing-media. Another nursery had had no losses to root rot since starting to use 10 L Airpots which stopped the roots spiralling and air-pruned the roots and created fibrous roots.

Six of the growers surveyed used a 80:20 peat : bark mix for liners and finals, two using equal proportions of peat, bark and perlite to give a freer draining mix for propagation of cuttings. Two growers used 70% peat, with the remainder as bark, or bark plus 20% coir. One grower used 60% peat in liners, but only 50% peat in finals together with a proprietary mix containing bark and 10% green waste. Four growers used mainly peat, with 5-10% grit, loam, or bark. The two nurseries using only a bit of grit reported above average (60% and up to 100%) losses in their *Chamaecyparis*, but 75% loss was also seen at a nursery with 20% bark use. One grower attributed an incidence of *Phytophthora* root rot to having used contaminated loam.

In general, all nurseries had incidences of root rot and dieback (particularly of *Chamaecyparis, Juniperus,* and *Taxus* and of *Araucaria* when grown) and although open growing-media should be less favourable to the water-mould zoospores which swim to infect roots it cannot be known without carrying out replicated trials using known water volumes, and the same plant material with the same inoculum levels and pathogen species whether the incidence and severity of root rot was lower than it would otherwise have been at sites using a lower proportion of peat. All growers were aware that overwatering was likely to increase the probability of root rot occurring.

Irrigation

All the nurseries used automatic overhead sprinkler irrigation across the majority of their containers, some topping-up pots that needed it by hosepipe. A disadvantage of this can be that lower branches can be splashed by growing-media containing water-mould spores and cause direct infection and die-back of the foliage. Drippers were sometimes used on pots of over 10 L, particularly where foliage otherwise would impede water entering the growing-media. One nursery had tried to use drippers more widely, but found them unreliable. The use of an evapo-transpiration sensor had been found beneficial at one nursery as this altered the irrigation according to the weather and was particularly useful at times of the year such as September and October when the water demand of plants can be variable.

Half the 14 nurseries surveyed used only mains water (without further treatment), with another nursery taking a quarter of the supply from a borehole as well. One nursery used borehole water which passed through a slow sand filter before storage in enclosed tanks. Another nursery used river water untreated. Three nurseries collected roof and bed run-off water. This was treated until recently at one site by chlorination to under 2 ppm, but it is now held in a 5.5 million gallon reservoir for a long time before passing through iris beds. Since

then previously severe losses of *Erica carnea* to root rot have not occurred. Water samples are sent for water-mould colony counts. At the second nursery using re-circulated water, chlorination and copper ionisation was used on the recycled water. The third site carried out no treatment of the recycled water, but drew water from the top of the reservoir, thereby hoping to avoid picking up resting spores which were thought likely to have sunk to the bottom and with the anticipation that any zoospores swimming in the water would be short lived. Water samples were initially taken at the third site twice a year, but as they were always negative, samples have not been taken recently. None of the other surveyed nurseries have sent their irrigation water to a laboratory to check for the presence of *Pythium* or *Phytophthora*, and no nurseries had used bait-tests for *Phycomycetes* on site.

Root rot and die-back species susceptibility

The conifers most frequently reported to have root rot were *Chamaecyparis* (on 100% of nurseries growing it), *Araucaria* Monkey Puzzle (on 82%), *Taxus* (on 78%), *Juniperus* (on 64%) and *Cupressocyparis* (on 38%), with the first three conifers having losses of 10% or more on at least half of the nurseries. The other principle conifer species could at times be found with root rot (as visible by foliar die-back) in a few plants, and this was usually attributed to water-logged growing conditions (Table 4).

Ten of the nurseries did not know what was causing root rot, another five growers suspected *Phytophthora*. *Juniperus, Chamaecyparis* and *Araucaria* were said to be the most commonly affected of all conifer species by *Phytophthora* at one nursery. Two nurseries reported having *Rhizoctonia* in plants, one having it in propagation material. A single nursery had had black root rot (*Thielaviopsis basicola*). There were no reports of *Fusarium* root rot. None of the growers had used a Lateral Flow Device to test roots or stems to see whether or not *Phytophthora* or *Pythium* species were present (although one had seen LFDs in use by PHSI to test for *P. ramorum*). One grower had once sent irrigation water for laboratory testing and been told there was *Pythium* present, but he did not know if this had included species likely to be pathogenic.

Table 4: The number of nurseries stocking each conifer and the proportion reporting root rot, showing the number of nurseries with 10% or more plant loss at one of more stages of production for each conifer type

Nurseries sites	***Abies***	***Araucaria***	***Cedrus***	***Chamaecyparis***	***Cryptomeria***	***Cupressocyparis***	***Cupressus***	***Juniperus***	***Larix***	***Picea***	***Pinus***	***Taxus***	***Thuja***
No. with root rot	2	9	2	13	2	5	2	9	3	3	4	11	2
No. with ≥10% loss	2	6	0	10	0	1	0	2	1	1	3	7	0
No. of nurseries	11	11	10	13	12	13	13	14	10	12	13	14	12

The figures shown in Table 4 rely on growers being aware that their plants have root rot. Nursery visits to some sites showed that plants could have over 50% rotted roots (in particular seen in *Chamaecyparis*) and have no foliar symptoms. On some nursery visits more plants with dieback (probably caused by root rot) tended to be found than had been mentioned in the surveys. Nurseries did not appear to write down and keep records of the numbers of plants of the different cultivars lost throughout the production stages.

Most growers identified some varieties of *Chamaecyparis lawsoniana* (Lawson cypress) as particularly susceptible, with 50 – 100% losses possible (Table 5).

Table 5: *Chamaecyparis* species and cultivars reported by nurseries to have above average incidence of root rot, with the number of nurseries reporting each

Cultivar	**No. of nurseries reporting high loss**	**Cultivar**	**No. of nurseries reporting high loss**
Blue Gown	1	Columnaris	2
Blue Surprise	2	Ellwoodii	5
Pelts Blue	3	Ellwoods Gold	4
Pembury Blue	5	Green Pillar	2
Columnaris Glauca	5	Pottenii	2
Blue cultivars	6	Silver Threads	2
		Snow White	1

Propagation losses were high for five *Chamaecyparis* cultivars at one of the smaller nurseries, but since buying plants in there have been no problems. One grower said that plants become more susceptible at the end of summer with the shorter days of autumn when there is a dramatic fall in water uptake and so care is needed not to overwater. He had stopped growing the more susceptible cultivars. Pembury Blue is one of the cultivars with thick roots, without a fine network and these seemed more susceptible to root rot. Other cultivars with fine, vigorous roots such as Pottenii are able to survive if watering is cut back to hold back root rotting. This difference in root structure and thus susceptibility was supported by another grower who said that the roots of Ellwoodii types and blue cultivars tend to be weaker rooted than green or gold cultivars. One nursery attributed losses to potting-on in autumn. In particular, root-balled material of Pelts Blue was lost because the roots did not grow out.

Several growers had large losses with blue *Chamaecyparis* cultivars, with 60% loss reported by a supplier of field-grown stock. Columnaris was also often reported as highly susceptible to root rot; one grower had 5% loss of Ellwoodii, but 50% loss of Columnaris.

There was variation between nurseries of when root rot and dieback losses were greatest, with some losing potted-on cuttings, others having losses of around 5-10% at each stage (effectively each year) of production and slightly more losing this proportion principally in 2 L or 3 L pots (when plants were more than two to three years old).

Ellwoodii tip burn (probably caused by over-potting and trace element imbalance) which could be confused with splash spread *Phytophthora* (or when pots fall over and become infected through foliage resting on contaminated ground-cover matting) would not cause the plant death reported by growers.

Araucaria was reported with yellowing of the newer growth (visible in seedlings) which then became dark brown with whole branches dying back. The dieback could be sudden. When the remaining visibly healthy plants were moved into 1 L pots the plants then often died. 30% loss of 3 L containers had occurred at one site, and other nurseries reported total losses of 50% or 100%. One grower had reduced watering on affected *Araucaria* and found the plants could grow away from the disease. Generally, losses were seen even with growing-media with a high proportion of bark. One grower said the plants would prefer to grow in pure sand. Another grower changed from using loam growing-media to peat, having had high losses in 1 L pots, such that from 500 seedlings only 350 plants were left after two years. *Araucaria* plants are grown from seed (whereas most conifers of specific cultivars are propagated via

rooting cuttings or grafting onto rootstocks). One grower thought that the mycorrhiza which would normally be present in native soils and be able to suppress/compete with root pathogens were absent in growing-media. He had seen healthier root production in containers of seed grown *Pinus* found to have mycorrhiza.

There is a wide range of *Juniperus* species, some native to countries with dry mountain slopes and so possibly less adapted to growing in wet growing-media. Nurseries reported problems of root rot with particular cultivars (Table 6). At a nursery in the west of England, prostrate-growing Junipers were most likely to get die-back, with dead patches of leaves in the plants. This penetrated the stem and progressed and could not be pruned out making about 10% of 3 L pots unsaleable. At one nursery, as soon as the branch trails touched the ground they were seen to start to die back, with symptoms becoming visible in the winter.

Table 6: *Juniperus* species and cultivars reported by nurseries to have an above average incidence of root rot, with the number of different nurseries reporting each

Species and cultivar	No. of nurseries reporting high loss	Species and cultivar	No. of nurseries reporting high loss
J. conferta (Shore Juniper)	1	*J. procumbens* Nana (Bonin Island Juniper)	1
J. communis	1	*J. repandra*	1
J. communis var. *depressa aurea*	1	*J. horizontalis* Prince of Wales	1
J. horizontalis (Creeping Juniper)	1	*Juniperus squamata* Blue Carpet	1

Losses of *Taxus* were high, particularly if grown outdoors (Table 3). One grower reported 20 -50% losses, with seed raised *T. baccata* worse than cultivars from cuttings. Plants had a slow death, surviving with a large number of dead roots. Root rot could be reduced by putting drainage in the bottom of pots, otherwise because they were slow growing and thus had a low water demand they could sit too wet. *T. baccata* Standishii was noted as susceptible by two growers, with one grower losing 30% of *T. baccata* Standishii after planting-out 30 – 40 cm tall plants into the field before lifting at 60 – 90 cm. Problems were noted on several nurseries with plants that had been dug up for potting from the field. On one nursery field-grown *Taxus* were potted into 10 L containers and initially grew away, but

half of the 1000 died in the subsequent year. Most plants died in the summer, but some had already started to die by the spring.

One grower in the west of England had been losing 40% of his container-grown *Taxus*, but this has now been reduced to 5% by keeping the plants under cover. Another reported healthy root growth from affected plants after they were left to dry out prior to disposal and that the plants appeared to be growing well after re-potting. One grower thought that the plants became more susceptible to root rot if they did not have sufficient nitrogen in the pots. Several growers mentioned their susceptibility to vine weevil damage (many using the insecticide Suscon Green (chlorpyriphos) at potting against vine weevil). It is possible that root damage by feeding could aid infection by *Phytophthora* and *Pythium. Taxus baccata* Fastigiata aureomarginata was said to be particularly susceptible to root rot.

A number of nurseries noted root rot problems with conifers other than *Taxus, Araucaria, Chamaecyparis* and *Juniperus* (Table 7). There was said to be a range of susceptibility in *Pinus* with *P. sylvestris* particularly prone (e.g. 15-20% losses at one nursery) and *P. strobus* even more badly affected. Cultivars are grafted onto *P. sylvestris* and so they become affected if the rootstock is infected with 10% loss not unusual. Although infection is of the roots, not at the graft union, more losses are found in some cultivars while *P. mugo* on *P. sylvestris* will seldom die.

Table 7: Conifers reported by nurseries to have above average incidence of root rot, showing the number of different nurseries reporting each host

Species and cultivar	**No. of nurseries reporting high loss**	**Species**	**No. of nurseries reporting high loss**
Abies procera / nobilis	1	*Picea omorika*	1
Cryptomeria japonica	1	*Pinus radiata*	1
X Cupressocyparis Wilma	1	*Pinus strobus*	1
Thuja occidentalis	1	*Pinus sylvestris*	1

Some nurseries in addition to reporting conifers with low root rot incidence (Table 3) also mentioned cultivars of susceptible species in which fewer pots than average (or none) usually became affected (Table 8).

Table 8: Conifer cultivars specifically reported by growers to have a lower than average incidence of root rot, with the number of different growers reporting each

Species and cultivar	**No. of nurseries reporting low loss**	**Species and cultivar**	**No. of nurseries reporting low loss**
Abies nordmanniana Golden Spreader	1	*Juniperus communis* Hibernica (Common Juniper)	1
Chamaecyparis lawsoniana Yvonne	1	*Juniperus horizontalis*	1
Cryptomeria (Japanese cedar) *vilmoriniana*	1	*Juniperus* Carbury Gold	1
Cryptomeria sp.	1	J*uniperus communis* Green Carpet	1
Cupressus macrocarpa (Monterey Cypress) Goldcrest	1	*Thuja occidententalis* (White cedar)	1
X Cupressocyparis leylandii	1	*Thuja plicata atrovirens*	2
X Cupressocyparis leylandii Gold Rider	1		
X Cupressocyparis Wilma	1		

It was reported by one grower that there were two seasons of disease symptom expression, with one in early to mid spring following the effects of a wet (not necessarily cold) winter. The other peak of visible symptoms occurred in early autumn, following the effects of drought stress in summer. In summer, problems can develop because there can be plants in wet pockets as the newly potted plants become overwatered because they are not taking up much water. In October the plants slow down growth as the days get shorter and the pots don't dry out enough. These two periods of loss were also reported at another nursery and also related to pots sitting wet in autumn or occurring before growth started in spring. Two other growers reported plants showing most rotting in autumn/winter, although one said that plants could show root rot throughout the year. The season was more important than whether or not the plants had recently been potted, or required repotting.

Dieback not associated with root rot, including needle spots and blights

Four growers did not record any problems with foliar diseases such as the blights *Phomopsis, Pestalotiopsis, Kabatina* and *Keithia* or with *Botrytis.* Others reported some instances of foliage being killed, but the impression gained from the survey was that damage did not appear to usually be of major concern. The identification of diseases was made by the growers themselves.

Botrytis causing needle browning (and usually found on a wide variety of hosts particularly in propagation, in closely spaced plants or where it can first colonise moribund tissue) (Peace, 1962) has occurred at one nursery on *X Cupressocyparis* Wilma and *Chamaecyparis lawsoniana* at all stages of production. It also occurred on another nursery in autumn/winter on finals of *Chamaecyparis* Boulevard and *Chamaecyparis picetera* outdoors, with the same hosts affected at another nursery on liners indoors in September possibly because they were pot-tight (which would have given good infection conditions).

Keithia was reported by four nurseries. It had caused leaf damage and dieback on *Thuja plicata* and *Thuja atrovirens* and received an Octave (prochloraz) spray. One grower was aware that the disease had come in on purchased stock. Thuja blight was not a problem at one nursery, although they had seen scorch on *T. plicata* and the cultivar Spiral Emerald in March, this was attributed to wind damage and the tips then dropped off leaving a healthy plant. *Keithia thujina* has been renamed *Didymascella thujina* (Sinclair *et al.,* 1987).

Pestalotiopsis was also reported by four growers, two with it on *Juniperus,* one grower saying that the disease had been fatal to his plants as it worked its way back into the stem from the foliage. One nursery sprays against this disease, but another grower shreds any plants that get the disease as he had found it hard to control by fungicide use such as Octave. Tip dieback leading to total death had occurred on *Juniperus procumbens* Nana and the *Juniperus horizontalis* Prince of Wales. *Pestalotiopsis funereal* usually causes needles to die from the tips (with black spore bodies forming under the epidermis), but sometimes browning of entire branches occurs following primary damage by another agent (Sinclair *et al.,* 1987).

Phomopsis had been seen by three growers causing dieback on their *Juniperus*, specifically on blue junipers at one nursery. The same species, *P. juniperovora*, is recorded as also attacking *Cupressus,* while another species *P. occulta* is known to kill the tops of *Picea* seedlings. Black pustular fruiting bodies are produced (Peace, 1962). *Phoma* was reported

causing dieback on *Juniperus* by one grower who was aware this conifer was a host to various foliar other pathogens.

At one nursery, *Chamaecyparis lawsoniana* Ellwoodii used to get tip burn (which was shown by research to be caused by over-potting and effects related to trace elements in the growing medium). The grower halved boron, doubled copper and changed from 0.13% to 1% molybdenum in his growing-media and has not had tip loss problems since. One grower used to lose the tips on his conifers a couple of years after potting-on, but this was stopped by changing his source of grit to one that wasn't alkaline. Another grower also reported twig dieback on *Chamaecyparis,* but did not know the cause. Tip dieback was also reported on *X Cupressocyparis leylandii*.

Samples of brown foliage when seen on nurseries were collected, with material taken from *Abies amabilis, Picea brewariana, Juniperus* Old Gold and *Chamaecyparis* Obtusa cv. Karamachiba and *Chamaecyparis* Minima Aurea. The *Abies* tip death had black flecks on the leaves, and closer examination suggested this might be frass. A tortix moth caterpillar was then found on a fresh dying shoot. The Minima Aurea had fans with scattered brown patches (although its roots were infected by *Pythium diclinum* / *lutarium* this was not thought to be the cause of the browning). *Pestalotiopsis* spores were found bursting out of the leaves. The *Juniperus* was amongst 15% of a batch of 100 plants with dieback developing from where they had been trimmed to get branching. A positive *Phytophthora* LFD test was made on the dying stem, which was subsequently identified as having *P*. a*ustrocedrae*. *P. austrocedrae* has previously been isolated from cypress tree roots, but direct stem infection was shown to be possible through wounds (Greslebin and Hansen, 2010). The grower notified the presence of this non-native pathogen to the Plant Health Inspectors. Although tissue was cultured from *P. brewariana* and *Chamaecyparis* Obtusa various different fungi were isolated, none consistently, suggesting that the fungi (which included *Fusarium* spp.*)* were probably secondary colonisers.

A sample of the upper 100 mm of *Taxus fastigiata* Aurea was collected after *Pythium* was found in the roots. The tops are used for cuttings and because the cultivar was yellow it was not so easy to see that the plant was infected (in contrast to neighbouring green cultivars with yellowing tops). Cultures of the stem were made on selective agar for *Pythium*, but the pathogen was not detected suggesting that in this instance cuttings taken away from the plant base could be healthy. Tissue was cultured from dying and (to see if there was non-symptomatic spread) apparently healthy Juniper stems of a plant affected by *P. austrocedrae*. *Phytophthora* was not able to be isolated from either stem, but *P.*

austrocedrae has proved difficult to isolate from plants at Fera and RHS laboratories (J. Walker & G. Denton pers. communications).

Chemical and biological control measures

There was very little use of fungicides on nurseries, with seven never using them. One grower stated that he does not use chemicals because they are only fungistatic (only holding back diseases, not curing them). Two nurseries only used the foliar fungicide Octave (prochloraz), and then either only as needed, or on certain cultivars. One grower used an alternating programme of Octave, Repulse (chlorothalonil) and Rovral (iprodione) at 10-14 day intervals during propagation. Five growers had used Aliette 80W (fosetyl-aluminium) as a growing-media drench, but two had not done so in recent years, one only used it if a problem developed or and another sometimes used it on *Chamaecyparis* liners as this conifer was known to be susceptible to root rot. Only one nursery used Aliette 80 four or five times a year on outdoor finals and also Filex/Proplant (propamocarb hydrochloride) once or twice in the same situation. Another grower used Filex/Proplant twice a year on *Araucaria*, but rarely on other conifers. One grower used Aliette 80 on the sandbed in the propagation house if a root rot problem had arisen.

Of the growers who had used fungicides at some time, one had also tried a bio stimulant, Wormcast Tea, a few years ago, but found no difference in the plants. This grower also added mycorrhiza to the growing-media of pine and spruce, but not to other conifers that naturally picked up mycorrhiza.

Only three other growers used bio stimulants. Compost Tea (a brewed mix containing various beneficial micro-organisms) is used fortnightly on propagation material and liners and finals indoors at one nursery, while another used to use it when they were using chlorinated water. Agrilan Revive (a product containing beneficial microbes used diluted as a growing-media drench) was also used by the latter nursery when problems arose such as damping-off in Sequoia seedlings and was said to be very effective. The third product used by this nursery (for example giving good powdery mildew control on evergreen *Clematis*) was Serenade ASO (*Bacillus subtilis*) which is registered as a pesticide. Trianum (*Trichoderma harzianum*) had been tried incorporated in growing-media at another nursery, but was not still used.

Only one nursery used Hortiphyte (potassium phosphite liquid fertiliser) as a growing-media application on indoor containers, with some spray application outdoors mainly to *Taxus.*

Growers were asked about their disinfection of propagation areas and standing beds and, as with fungicide use, the predominant finding was that this was done infrequently. Three growers disinfected beds annually, and another nursery sometimes gave a second treatment. Five growers only used disinfectant (most using Jet 5 containing peroxyacetic acid) if there had been a root rot problem in the previous crop. Three nurseries no longer disinfected beds. Only one grower had never used disinfectant, believing that Jet 5 makes resting spores more resilient. He could use steam sterilisation in the propagation house, but had not done this since using iris beds for irrigation water cleaning.

2. The selection and use of baits for sampling irrigation water for Phycomycetes

Bait selection

The laboratory test was set up on the 25 May and the leaves assessed for browning on the 28 May, before plating out. The three day's immersion was the same as to be used in the field bait placements. The Rhododendron leaves showed brown lesions on the cut edges and the needles had darkening of the tips and petiole bases. Fewer leaves (4 Rhododendron, 2 Fir and 2 Spruce) had lesions than the number subsequently shown to be infected by *Phytophthora* (Table 9) showing that examination alone by growers on-site would not be sufficient to determine infection. Two of the Rhododendron leaves and one of the fir needles with a lesion produced fungal growth that did not grow well on the P5ARP, and was possibly *Fusarium* and *Cladosporium*.

The P5ARP plates with infected leaves showed the same knobbly hyphae typical or the morphology of *P. cinnamomi.* No further leaves were found to be infected after a further 28 days incubation compared with an assessment after 4 days.

Table 9: Isolation of *P. cinnamomi* from three species of bait leaves, used fresh or microwaved, after floating in water artificially inoculated with the water mould for three days

Leaf treatment before use as bait tissue	**No. of leaves from which *Phytophthora* was isolated**			**% leaves with *Phytophthora***
	Rhododendron 4 leaves / treatment	**Nordmann 4 leaves / treatment**	**Abies 8 leaves / treatment**	
Fresh	2	4	6	75
Microwaved	1	3	1	31
% leaves with *Phytophthora*	38	88	43	

The highest proportion of leaves infected was for Nordmann Fir, and there was greater infection success using fresh leaves compared with the other treatments. Fresh (unsterilized) Nordmann Fir needles were thus selected for subsequent use in the field with baits.

Ceanothus leaves were used in addition after the first nursery visit to see if different species of water-mould would be caught. These were easier to use in baits than the rhododendron leaves as they were able to fit into the bait bag without cutting. The laboratory check for any pre-existing water-moulds on or in the *Ceanothus* leaves showed none, with only a colony of *Fusarium* sp. growing out from a leaf incubated in the sterile distilled water.

3. Preparation and nursery placement of bait bags

White horticultural fleece was proposed as an alternative to muslin for holding the bait leaves. When a section of fleece was examined under the microscope the pores were 70 µm and the main area of multi weave still had gaps of 30 µm within it. Zoospores of *Pythium* and *Phytophthora* are about 10 µm and so would be able to pass through the fleece. A square of 15 – 20 mm was used for the bait bags, although a bigger area could be used and then excess trimmed off after tying up the bag so that it would not affect floatation.

Small stones sterilised by standing in boiling water were used as weights with a preference for quartz or flint pebbles so that they would be less likely to absorb pathogens than a sedimentary stone. Polystyrene foam packaging pieces for floats were selected to have sealed rather than crumbly outer surfaces to reduce the risk of pre-use contamination.

Experiments were carried out so the bags floated with the leaves no deeper than 50 mm below the water surface and this required 15 – 20 g of stones and two foam pieces. To construct the bait bags; first stones, leaves, then foam pieces were piled onto the centre of the fleece square and the corners gathered up and bound with twine to give a sealed neck. The twine had an approx 2 m length left after knotting. These bags were made up at each site to so that the leaves would be fresh when placed into the water and so any wounding caused by the bagging process would not have become contaminated. Where possible, the bags were briefly pushed under water once in place so that the fleece became wetted and settled into the correct floating position as soon as possible after placement.

In the open reservoir at Nursery 1E there was a rope going into the water that the bags were knotted to so that they were floating away from the slope of the edge. The reservoir stored

rain water topped up from the mains at times and was pumped up and acidified and chlorinated before being stored for 1-2 days before use. Nursery 2E also had an open reservoir and the grower placed the baits floating free in the water. At this site the reservoir water was collected from rainfall and bed run-off via a reed bed and used without further treatment at times of high water demand. The grower also carried out his own bait test of the water the next month using *Ceanothus* from the nursery and the *Phytophthora* LFD kits left for him and obtained a positive test reaction. At the third site with non-mains irrigation water (Nursery 4E) the baits were suspended over the side of the metal water storage tanks. Borehole water taken within 15 m of a river (on land subject to occasional flooding) was passed through a slow sand filter before being stored in a number of sealed metal tanks ready for use without further treatment.

4. LFD and PCR testing of leaf baits retrieved from water reservoirs

Full results of the LFD results and PCR identification of species from the bait tests are given in Appendix 5. At two nurseries (2E and 4E) *Phytophthora* was detected from both the Nordmann Fir and *Ceanothus* baits used with LFDs and confirmed by PCR testing of another sub-sample of each of the leaves. *Pythium* was also detected by LFD test on a *Ceanothus* leaf from Nursery 2E, but not found by PCR of another sub-sample from the bait, however *Pythium* was confirmed in a third sub-sample of Nordmann Fir leaves from Nursery 2E and also when the extraction buffer and macerated leaves of the first Fir sub-sample was tested. *Pythium* was detected by PCR at Nursery 1E (both of Fir leaves and of the buffer plus macerated leaves from which the LFD drops were taken), but there was only an Index 1 very faint detection of *Pythium* on the LFD. An Index 1 detection of *Pythium* was also made from Nursery 4E, but not confirmed by PCR testing.

Phytophthora sp. *Salixsoil* was found in baits and in *Taxus* and *Chamaecyparis* at Nursery 4E, but there was no match between the *Pythium* & *Phytophthora* spp. found in the baits (Appendix 5) and those isolated from plants at the other two nurseries (Table 9). Some *Pythium* species were not, however, able to be identified by PCR as DNA reference sequences are not available and so there could have been a match between the baits and the plants for this species at Nursery 2E (Table 10).

Table 10: Identification of *Pythium* and *Phytophthora* species by PCR of leaf baits placed in irrigation water in June and July 2010 at two nursery sites and whether or not the same species were identified by PCR testing of roots or stems of plants taken from the same nursery on the same day

Site	***Pythium* species in baits from irrigation water**	**Same *Pythium* spp. in baits as in plants from that site?**	***Phytophthora* species in baits from irrigation water**	**Same *Phytophthora* spp. in baits as in plants from that site?**
1E	*Pythium* sp. either *P. catenulatum* or *P. torulosum*	No	No	n.a.
2E	*Pythium* sp.	Possibly - *Pythium* sp.	*Phytophthora* sp. *Salixsoil*	No
	Pythium sp. either *P. catenulatum* or *P. torulosum*	No	*Phytophthora* sp. closest to *cryptogea*	No
	Pythium sp. closest to *heterothallicum* and *glomeratum*			
4E	No.	n.a.	*Phytophthora* sp. *Salixsoil*	Yes

1E from an open reservoir on the nursery site
2E was from an open reservoir at a different site from that of the plant samples
4E from enclosed water collection tanks after sand filtration

At Nursery 1E, Nordmann Fir needles were placed in both a muslin and a fleece bait bag The leaves in both bags were found (by PCR of the tissue) to have the same water-mould species present, a *Pythium* sp. either *P. catenulatum* or *P. torulosum.* The same *Pythium* sp. was identified by PCR testing of the extraction buffer with macerated leaves used for the LFDs, showing that *Pythium* was likely to have been in the drops used on the LFD and so should have given a positive reaction. No *Phytophthora* was detected by PCR, although there had (as for *Pythium*) been a faint detection on the LFDs.

At Nursery 2E, *Phytophthora* sp. *Salixsoil* was detected initially by PCR from both Nordmann (in fleece and muslin) and *Ceanothus* leaves. When the buffer used for the LFD tests was used for PCR testing this confirmed the same species from the *Ceanothus*. Additional PCR

tests were carried out using a reserve set of leaves. The second set of PCR leaf tests showed only the *Ceanothus* to have *Phytophthora* sp. *Salixsoil*. A *Phytophthora* sp. closest to *cryptogea* was detected instead from one Nordmann bait. *Phytophthora* sp. *Salixsoil* was consistently detected in both host baits from Nursery 4E.
Phytophthora sp. *Salixsoil* was detected by PCR of the Nordmann Fir and *Ceanothus* leaves from Nursery 2E, backing-up the Index 2 positive reaction on *Phytophthora* LFDs from both leaf types. The same *Phytophthora* sp. was confirmed to have been present in the leaf extraction buffer made for the LFDs.

Both *Pythium catenulatum* and *P. torulosum* have been isolated from plants, the latter including conifers, while the pathogenicity of *P. heterothallicum* is not known as it has only been recorded as a soil saprophyte (Van der Plaats-Niterink, 1981).

When sampling material, whether by LFD, PCR or traditional tissue isolation, there is a risk that the sample will not be fully representative. The variability in the *Phytophthora* results at Nursery 2E (Appendix 5) is likely to be because no sample of leaf or buffer extract included all the material in the bait bag. It is likely that there were multiple Oomycetes on the leaves or needles and their detection depended on whether their location was included in that sample. Even though several leaves were broken up in the buffer for the LFD test they may not all have been sampled in the three drops of buffer extract used in the LFD well. At Nursery 2E, two out of three tests gave positive LFDs for *Phytophthora* compared with three out of three PCR tests. One out of three LFDs using Nursery 2E baits were positive for *Pythium* spp., but it was not detected by PCR, while conversely there were two positive PCR tests when the same baits had not given positive LFDs. This sample variability makes it difficult to draw firm conclusions about the reliability of the LFD tests by comparing with the positive or negative PCR test, although in general a good level of reliability was shown.

5. The detection of *Pythium* and *Phytophthora* from conifer stems and roots using LFDs and the identification of species of using PCR

The majority of conifer samples collected in summer 2010 from the five nurseries visited and two nurseries which sent in plants were sent to the RHS Wisley for PCR testing straight after carrying out LFD tests. The PCR DNA fingerprinting allowed the confirmation, or otherwise, of positive and negative *Pythium* and *Phytophthora* LFDs (Appendix 6) and additionally gave identification to species level (where DNA sequences are available) of these water-moulds (Tables 11 and 12).

Comparison of LFD and PCR detection of Pythium spp. and Phytophthora spp. from plants

When stems did not show staining under the bark, or if there was no obvious rotting on roots, then no LFD test was carried out (marked as n.t. in Appendix 6). Plants were only sent to RHS Wisley for PCR testing if they had produced a positive test line on one of the LFDs, and the test was then usually only carried out on the tissue (stem or root) that had produced a positive LFD reaction for either *Pythium* or *Phytophthora*.

An LFD index was used to record the strength of blue test line where 0 = no line, 1 = very very faint indefinite line which was counted as a negative result (as marked in the table), 2 = faint blue but definite line, counted as positive; 3 = obvious blue line, clearly positive, 4 = strong dark blue line (as strong as the control line), obvious positive (Figure 1). After storage for seven months the indexes had not changed from the field records of the test line and so the kits would be suitable for use by growers as evidence of earlier results.

There was discussion with growers about how distinct a blue Test line needed to be to be confident that there was a positive result. Advice from Forsite was that any colour change should be counted as a reaction. Where a device appeared to show a shadow line (Index 1) in some lights it was decided in this investigation to count this as a negative. Comparison of the results for this LFD index with the RHS PCR results on the same plant gave both negatives and positives, as was true for much stronger LFD indices. However, of three LFDs later sent for PDplus which had showed negative for *Phytophthora* (Table 14), the pathogen was actually detected on one (sample 2E.3). This plant was, however, not typical as the *Pinus* stem had pink staining on the older tissue, but no leading edge, and so the area sampled may have had too little pathogen present for the LFD test to show positive, but sufficient pathogen DNA for the PCR test. Although LFDs may be unable to detect the pathogens in recently infected and probably symptomless tissue before the mycelium has multiplied, this tissue would not normally be sampled unless there was a hypersensitive reaction causing necrosis.

Figure 1: Lateral flow devices showing intensity of Test line as indexed 0 to 4 (darkest).

i) *Root samples* (Appendix 6)

The investigations show that there should be good grower confidence in the root test LFD results for *Phytophthora. Phytophthora* was detected by LFDs in the roots from 19 plants and all but three shown positive by *Phytophthora* LFDs were also positive by PCR (84% matched). Of the 18 positive PCR results for *Phytophthora*, all but one (*citricola* complex) was also detected by the LFD tests on the roots.

Pythium was detected by LFD in the roots from 19 plants, but when the plants were tested using PCR 14 of these were not recorded as having *Pythium* (26% matched). The large sample area available for roots meant that the tissue taken for the LFD and PCR test could have been from different areas of the pot where the water-mould species present could have been different. In addition, because of the relatively large amount of material required for the LFD test, some totally rotted root lengths were included as well as the leading edge of recent infection mainly only used where possible for the PCR tests. *Pythium* can be found as a secondary coloniser of already weakened tissue and so it is possible that this accounts for the discrepancy.

Of the eight plants recorded by PCR as having *Pythium* all but one was also detected by the LFDs, and the roots which were recorded as negative by the LFD had actually given a very faint positive reaction. This suggests that (as given in the kit instructions) growers should try to avoid sampling roots that have been dead for some time, but concentrate on where the infection is actively damaging healthy tissue and then good results will be obtained for the

primary pathogen. However, all *Pythium* species will be contributing to root decay and could cause different levels of this on different cultivars.

ii) Stem samples (Appendix 6)

Stems tested with the *Phytophthora* LFDs produced seven positives, and all but two were confirmed by the PCR tests. The two negative PCR results could be because the limited amount of infected stem tissue was taken for the LFD tests and not then available for PCR. The location of the stem lesion had been easy to locate for PCR (G. Denton, pers. comm.) as the bark had already been cut back during nursery inspection. Of the eight stems producing positive *Phytophthora* PCR tests, two did not give positive LFDs. PCR testing can "multiply-up" tiny amounts of DNA to be able to confirm pathogen presence and this may have been the reason the LFD kits did not pick up the pathogen as the LFD test requires relatively large amounts of tissue. In general the match was good for *Phytophthora.*

Seven stems had positive *Pythium* LFD tests, but five of these were not detected by the PCR test. Only two stems gave positive PCR tests and one was not detected by the LFD and a LFD was not used on the other one. The recording of *Pythium* by the LFDs of stem tissue, but not PCR, may be because (as with the roots) of the inclusion of a greater proportion of decaying tissue in the test. With stem samples there is a limited amount of tissue available at the leading edge (where the pathogen has usually moved into new tissue but has not killed it and so the tissue remains unstained) on a relatively small surface area. The PCR tests used a relatively even ratio of white to brown tissue, and tried not to include the bark to avoid any micro-organisms sitting on the surface. The LFD test used more brown tissue to be able to have enough tissue for the test and because the work was mainly done in the field it was not possible to remove all the bark. Further PCR v LFD test investigation with and without the bark, and with and without decayed stem would need to be carried out to see whether or not growers need to be more selective in the tissue taken if a *Pythium* test is being carried out.

It is possible that the PCR was not able to detect certain *Pythium* species present. There are fewer DNA fingerprints for *Pythium* species than *Phytophthora*, but it was expected that *Pythium* spp. would be picked up by the PCR technique used. It would be possible in further work to utilise the frozen extraction buffer plus macerated leaves kept after LFD testing to send for PCR testing in order to gain a closer match between the material tested for PCR and that used for the LFDs. However, even with this the exact same tissue would not be tested, and variability in results was shown to some extent in the testing of buffers kept after the bait testing (Appendix 5).

Identification of Pythium spp. and Phytophthora spp. from different conifer species using PCR testing of stained stem and rotted root tissue

A range of *Pythium* and *Phytophthora* species were identified from the conifers, with several same species in conifers from both eastern (Table 11) and western Britain (Table 12).

Table 11: Identification of species of *Pythium* and *Phytophthora* using PCR of stem or root tissue (or isolates taken from the host tissue) from conifers sampled from nurseries in the eastern side of Britain between June and August 2010

Plant host	**Code**	***Pythium* in stems**	***Phytophthora* in stems**	***Pythium* in roots**	***Phytophthora* in roots**
Araucaria	1E.1	No PCR test	No PCR test	Negative	*P. citricola* complex
Araucaria seedling	1E.4	No PCR test	No PCR test	Negative	*P. citricola* complex, *P.* sp. *Salixsoil*
Juniperus	1E.3	No PCR test	No PCR test	Negative	*P. gonapodyides*
Taxus	1E.2	No PCR test	No PCR test	*P. irregulare*	Negative
Chamaecyparis	2E.1	*P. sylvaticum*	Sp. close to *P. gonapodyides*	*Pythium* sp.	Negative
Juniperus	2E.2	Negative	Negative	Negative	*P. gonapodyides*
Pinus	2E.3	Negative	*P. cactorum*	Negative	*P. cactorum*
Juniperus	3E.2	*P. attrantheridium*	*P. austrocedrae*	Negative	*P. gonapodyides*
Taxus	3E.1	No PCR test	No PCR test	Negative	*P. cinnamomi*
Chamaecyparis	4E.1	Negative	Negative	Negative	*P.* sp. *Salixsoil*
Chamaecyparis	4E.2	Negative	*P. cactorum*	*P. vexans*	Negative
Chamaecyparis	4E.5	Negative	*P. cryptogea*	Negative	*P. cryptogea*
Cupressocyparis	4E.4	No PCR test	No PCR test	*P. vexans, P. intermedium*	*P.* sp. close to *P. heveae*
Juniperus	4E.3	Negative	*P. austrocedrae*	Negative	*P. gonapodyides*
Juniperus	4E.7	Negative	*P. austrocedrae*	*P. attrantheridium*	Negative
Chamaecyparis	5E.1	No PCR test	No PCR test	Negative	*P. quercina / P.* sp. *Ohioensis*
Chamaecyparis	5E.2	No PCR test	No PCR test	Negative	*P.* sp., *P. quercina / P.* sp. *Ohioensis*
Chamaecyparis	5E.3	Negative	Negative	No PCR test	Not PCR tested
Chamaecyparis	5E.4	*P. intermedium*	Negative	*P. mono-spermum / P. attrantheridium*	*P. quercina / P.* sp. *Ohioensis*
Chamaecyparis	5E.5	Not PCR tested	Not PCR tested	Negative	*P. cinnamomi*

Table 12: Identification of species of *Pythium* and *Phytophthora* using PCR of stem or root tissue (or isolates taken from the host tissue) from conifers received from nurseries in the western side of Britain between June and August 2010

Plant host	Code	*Pythium* in stems	*Phytophthora* in stems	*Pythium* in roots	*Phytophthora* in roots
Abies	1W.6	Negative	Negative	Negative	*P. cryptogea*
Chamaecyparis	1W.5	No PCR test	No PCR test	*Pythium* species closest to *P. diclinum* and *P. lutarium*	Negative
Juniperus	1W.4	Negative	Negative	Negative	*P. gonapodyides*
Taxus	1W.1	Negative	*P. cinnamomi*	Not PCR tested	No PCR test
Chamaecyparis	2W.1	No PCR test	No PCR test	Negative	*P. quercina* / *P.* sp. *Ohioensis*
Chamaecyparis	2W.2	No PCR test	No PCR test	*P. sylvaticum*, *Pythium* sp., *P. vexans*	Negative

Pythium species *P. intermedium, P. irregulare, P. sylvaticum, P. monospermum* and *P. vexans* have all previously been recorded as being pathogens of a variety of plants as well as being present in soil. *P. diclinum* has been isolated from rice plants. *P. attrantheridium* and *P. lutarium* are not listed in the *Pythium* Monograph (Van der Plaats-Niterink, 1981).

There was little consistent isolation of water-mould species from particular conifer species across different nurseries (Table 13). The exception to this was for *Phytophthora gonapodyides* which was only isolated from Juniper roots and found at five nurseries. This *Phytophthora* species, together with *P.* sp. *Salixsoil* are believed to be both weak pathogens and involved in litter breakdown (Brasier and Jung, no date). Two nurseries had *Phytophthora austrocedrae* on stems, a species not thought to be endemic to the UK and thus reported by the growers to the PHSI. It as been reported in Patagonia (South America) causing necrotic lesions in the bole of *Austrocedrus chilensis* (Cupressaceae) trees on the Andean foothills (Greslebin & Hansen, 2010).

Phytophthora cinnamomi was only detected in *Taxus* (at two nurseries) and one *Chamaecyparis* although this is the species commonly attributed as causing conifer root rot and dieback (Evans, 1979). *P. quercina* has previously been reported from oaks (Brasier and Jung, no date), but *Phytophthora quercina* / *P.* sp. *Ohioensis* was found in the roots of *Chamaecyparis* at two nurseries. The PCR results in the current work are not in doubt (G.

Denton pers. comm.) and the result might be explained at Nursery 5E as oak trees were around the site and the compost heap from which growing-media was re-used was under their canopy. Nursery 2W only buys-in conifers, as rooted cuttings, and it is possible that these could have come from Nursery 5E.

Phytophthora citricola was only isolated from the roots of *Araucaria. P. citricola* has been identified together with *P. cambivora,* and *P. gonapodyides* causing aerial bark lesions on beech in southern England. How inoculum of these soil pathogens becomes aerial is not known (the sporangia do not detach like *P. infestans)* (Brasier and Jung, no date).

P. austrocedrae, P. cinnamomi, Phytophthora cryptogea and *Phytophthora cactorum* were all isolated from stems, although the latter two were also present in the roots of the same plants and so might have spread upwards.

Three *Pythium* species (*P. sylvaticum,* P. *intermedium* and *P. attrantheridium*) were found in stained stems and not the roots of the same plants.

A mixture of *Pythium* species were present in rotted roots, with no particular species occurring more frequently. Many of the plants with *Pythium* in the roots produced negative results for *Phytophthora* and this increases the probability that the *Pythium* species detected were the cause of the root rot seen, rather than entering roots already damaged by *Phytophthora*.

The wilt index allocated to each plant gave a general impression of the severity of symptoms with 1 = bits of foliage wilted or brown, 2 = some branches wilted or brown, 3 = more than 60% of the foliage wilted (or feeling soft) or brown and dry. Records were also made of the extent of rotting of the original plug or liner rootball and on the extent of rotting of the roots which had grown since potting. Most pots were 3 L and had been potted-on a year before, in spring 2009. The percentage rot seen on removing the pot (before breaking apart the rootball) was probably the information that many growers would use to assess their plants, rather than also breaking into the rootball. However, in a number of samples (e.g. 2E.2, 5E.1) the roots at the pot centre were badly rotted and likely to provide a source of infection for the more outer roots even though the plant had apparently “grown-away”.

Nursery 1W makes some purchases from nursery 5E, but the pathogens isolated from plants differed. Nursery 4E makes some purchases from 1E. Both nurseries had *Phytophthora gonapodyides* on *Juniperus* in common with the other two sites. No matching species

isolations were found between 4E and a second source - nursery 5E. Plant sources were not otherwise divulged by growers.

Table 13: Conifers of each species from different sites listed together in order, to show any similarities in the species of *Pythium* and *Phytophthora* identified in each by PCR of stem or root tissue.

Plant host	**Site**	***Pythium* in stems**	***Phytophthora* in stems**	***Pythium* in roots**	***Phytophthora* in roots**
Abies	1W				*P. cryptogea*
Araucaria	1E				*P. citricola* complex
	1E				*P. citricola* complex, *P.* sp. *Salixsoil*
Chamaecyparis	2E	*P. sylvaticum*	Species closest to *P. gonapodyides*	*Pythium* sp.	
	4E				*P.* sp. *Salixsoil*
	4E		*P. cactorum*	*P. vexans*	
	4E		*P. cryptogea*		*P. cryptogea*
	5E				*P. quercina* / *P.* sp. *Ohioensis*
	5E				*Phytophthora* sp., *P. quercina* / *P.* sp. *Ohioensis*
	5E	*P. intermedium*		*P. monospermum* / *P. attrantheridium*	*P. quercina* / *P.* sp. *Ohioensis*
	5E				*P. cinnamomi*
	1W			*Pythium* sp. closest to *P. diclinum* and *P. lutarium*	
	2W				*P. quercina* / *P.* sp. *Ohioensis*
	2W			*P. sylvaticum, Pythium* sp., *P. vexans*	
Cupressocyparis	4E			*P. vexans, P. intermedium*	*Phytophthora* sp. closest to *P. heveae*
Juniperus	1E				*P. gonapodyides*
	2E				*P. gonapodyides*
	3E	*P. attrantheridium*	*P. austrocedrae*		*P. gonapodyides*
	4E		*P. austrocedrae*		*P. gonapodyides*
	4E		*P. gonapodyides*	*P. attrantheridium*	
	1W				*P. gonapodyides*
Pinus	2E		*P. cactorum*		*P. cactorum*
Taxus	1E			*P. irregulare*	
	3E				*P. cinnamomi*
	1W		*P. cinnamomi*		

6. Verification of PDplus on LFDs by comparison with PCR testing of the same plant

All the LFDs from plants which had *Phytophthora cactorum, P. cinnamomi and P. citricola* complex by their PCR test were sent for PDplus testing to verify the test for these species. LFDs from *P. cryptogea* infected plants were also sent to see if the PDplus would mis-identify them (as this species is not offered for identification by PDplus). The eleven LFDs utilised are marked in bold print in Appendix 6. Some negative (0 Index) LFDs were also sent for PDplus because the PCR of that plant had been positive (Table 14).

Table 14: Verification of the use of PDplus for the diagnosis of certain *Phytophthora* spp. by the testing of plant material using both a *Phytophthora* LFD and PCR

Sample code	**Host plant**	**Tissue sampled**	**LFD Test line index (0-4)**	**RHS PCR result for *Phytophthora* spp.**	**PDplus result for *P.cactorum* *P. cinnamomi*, *P. citricola***
1E.1	*Araucaria*	root	0	*P. citricola* complex	Negative for all
2E.3	*Pinus*	stem	0	*P. cactorum*	Positive for *P. cactorum*
2E.3	*Pinus*	root	4	*P. cactorum*	Positive for *P. cactorum*
4E.2	*Chamaecyparis*	stem	4	*P. cactorum*	Positive for *P. cactorum*
4E.2	*Chamaecyparis*	root	4	Negative	Positive for *P. cactorum*
4E.5	*Chamaecyparis*	stem	4	*P. cryptogea*	Negative for all
4E.5	*Chamaecyparis*	root	4	*P. cryptogea*	Negative for all
1W.1	*Taxus*	stem	0	*P. cinnamomi*	Negative for all
1W.6	*Abies*	root	4	*P. cryptogea*	Negative for all
3E.1	*Taxus*	root	2	*P. cinnamomi*	Failed
5E.5	*Chamaecyparis*	root	3	*P. cinnamomi*	Negative for all

Conifers were examined and tested from nurseries from June to August 2010. The LFDs were stored in their foil sleeves at room temperature in the dark until January 2011 and then sent to Forsite for immediate PDplus using the indicator strip. The LFDs work by antibody reaction (ELISA), but the DNA of the pathogen will also be present for detection by PDplus.

P. cactorum: The PDplus test was confirmed to be robust for this pathogen. It confirmed all 3 positive PCR results for *P. cactorum*. Two had strong LFDs, the other (2E.3) gave a result even with a negative LFD. In addition, *P. cactorum* was found in a strong LFD (4E.2) where root samples taken by RHS had given a negative PCR test.

P. cinnamomi: this was found by PCR but was not confirmed by PDplus in the LFD from *Taxus* roots for sample 1W.1. However, there may have been no *P. cinnamomi* in the LFD submitted for PDplus as that from the positive Pythium test was mistakenly sent, but should still have been suitable for the PDplus if *Phytophthora* was present in the tissue tested.

In the other Yew root sample (3E.1) PDplus did not pick up the *P. cinnamomi*. The result was shown as 'failed'. According to Paul Meakin (Forsite Diagnostics) this means that the control reactions carried out as part of the testing did not detect any DNA, either host plant or fungal. Therefore, it cannot be said whether the result was a positive or a negative; it is simply a null result. Forsite Diagnostics see this with coniferous samples fairly frequently, and it may be due to inhibitors in the sample. The LFD result for this sample was also very weak, which did not help.

P. citricola complex: This was not detected by PDplus, but the LFD submitted was negative and so there was probably no DNA to test. The PCR sample which gave a positive result for the conifer for *P. citricola* would have been taken from a different root.

The assays for *P. cinnamomi* and *P. citricola* complex have not been developed as thoroughly as that for *P. cactorum*, but Forsite have seen correct identification of these pathogens in other samples (P. Meakin, pers. comm.).

P. cryptogea: Three samples (4E.5 roots and stems, 1W.6) had strong LFDs test lines for *Phytophthora* and PCR identified the presence of *P. cryptogea* in samples taken from the same plant and no other *Phytophthora* species. PDplus was not expected to identify *P. cryptogea* and did not confuse it with any of the other species intended for verification.

6. Conifer nursery sampling visits

All the nurseries visited for sampling plants and water for *Pythium* and *Phytophthora* and using and demonstrating LFDs and water baits were on the eastern side of England. Records were made of the incidence and severity of root rot and dieback to provide examples of symptoms to match against subsequent identifications of *Pythium* and *Phytophthora.*

Plants were also tested on-site by LFD by the grower at one western nursery site and then posted to the RHS and another western site sent plants to ADAS Boxworth for testing. The western samples are not described here, but the results are summarised in Table 12.

i) Nursery 1E

Nursery 1E produced a wide range of home-propagated conifer species. There was irrigation by chlorinated reservoir water (which includes run-off), mainly overhead, of container-grown plants in both polytunnels and outdoor beds. Most liners were grown in 100% peat, while finals were given 10% bark. The visit was made in early June.

Araucaria (Monkey Puzzle) was grown from seed in 50 mm² modules in a polytunnel (sample 1E.4). Of the 40 one year old seedlings per tray, around three to five (at random positions) had yellowing tops which progressed to plant death. Root browning was not severe (about 30% of roots, some others being naturally brown with healthy cores) and there was no stem base internal staining. Two *Phytophthora* species were detected by PCR, *P. citricola* and *P.* sp*. Salixsoil.* The latter species was first identified in the UK in the 1970s on the soil around roots of *Salix* and *Viburnum* after flooding and it is suggested to be a weak pathogen and secondary coloniser (Brasier and Jung, undated), but further information is not available. 3 L *Araucaria* (3 year old) in another tunnel were sampled (sample 1E.1). Plants were growing in a bark growing-media and so free-draining. *P. citricola* was again identified by PCR test. *P. citricola* was probably the cause of the partially dead branches and yellowing tops in 20% of the 100 plants still present. There was no internal stem staining. About half the roots were brown, but in many the centres were white and healthy. For both the seedling and 3 L samples *Pythium* was detected in the roots by the LFD, but not *Phytophthora.* That *Phytophthora* was not present in the roots sampled was supported by the PDplus result for the 3 L plant.

Over 60% of the plants of 1 L *Juniperus conferta* standing outdoors in a 3 x 5 m area had bleached then bronzing branches. The roots of sample 1E.3 were almost totally rotted throughout the pot, which they totally filled. There was no internal stem staining. The LFD was positive for *Phytophthora* in the roots and PCR positive results showed that *P. gonapodyides* was present in the roots. In the absence of any other water mould pathogen it was presumed that *P. gonapodyides* had caused the rot. This species is often reported as a saprophyte on twigs or on debris floating in water (Erwin & Riberio, 1996), but it is also suggested to be a weak pathogen (Brasier and Jung, undated). Other root pathogens such as *Thielaviopsis* were not, however checked for.

The grower had noticed that young growth of 10% of plants on a 10 m x 10 m outdoor standing area of 3 L *Taxus baccata* was wilting in hot weather although the roots did not look rotted. The whole plant also looked bronzed. When examined there were distinct brown/white areas on about 7% of the roots visible on removing the pot. However, on breaking the root mass apart, about 30% of roots which had grown from the 1 L pot had rotted after filling the 3 L pot. The original liner roots were principally healthy. There was no stem staining and the roots tested negative by LFD. However, PCR detected *Pythium irregulare* (possibly because the PCR test was better able to detect the small amount of the water-mould present). *P. irregulare* is a pathogen closely related to the plant pathogen *Pythium ultimum* (Smith *et al,* 1988).

ii) Nursery 2E

Nursery 2E was also visited in June and had batches of conifers in beds sections of about 4 x 5 m with overhead watering from the mains. Only three batches of plants with dieback were found. Plants were all bought-in and had beengiven a batch label with their source and arrival date. Plants of *Chamaecyparis* Ellwoodii had been bought from France in April 2008 as 90 mm liners and were potted-on into 3 L in the nursery's standard 80:20 peat:bark mix. About 50 out of 300 plants had over 60% of branches totally brown, and some pots had already been thrown away. The first dieback symptoms had been noticed six months previously. On removing the pot 100% of roots were rotted and this was seen to be the same throughout the pot. Stem staining was not found on-site, but the stem was sampled at the RHS and *Pythium sylvaticum* and a species close to *Phytophthora gonapodyides* were detected. Surprisingly, given the amount of root rotting, only a very faint line was seen on both *Pythium* and *Phytophthora* LFDs, and only a *Pythium* sp. found by PCR. Either the *P. gonapodyides* was more aggressive than usually accepted (Brasier and Jung, undated) or *P. sylvaticum* (a known pathogen) was in the roots as well as the stems, or the *Pythium* species

for which no DNA reference sequence exists was the cause of the total root rot. The roots were not checked for the additional presence of other pathogens, such as *Fusarium*.

Sample 2E.2, *Juniper squamata* Blue Carpet, was one of 5% of plants with a 70 mm section of branch brown towards the stem base at the fork. The foliage otherwise looked healthy. The plants arrived in March 2009 from the Netherlands, when they were potted-on into 3 L. By June 2010, on removing the pot 25% of roots were seen to be brown and easily broke in pieces. On breaking open the pot 100% of roots were rotted. Pinky coloured stem staining (which was not necessarily caused by disease) was seen from the stem base extending up 30 mm to the dead branch and samples of this stem staining were taken for LFD testing supplemented with pieces of the dead branch. The grower carried out the LFD tests and got strong positive lines for *Pythium* and *Phytophthora* for the stem and for *Phytophthora* only on the roots. The RHS detected nothing by PCR, but probably only sampled the stem (not the foliage). However, *Phytophthora gonapodyides* was found on the roots by PCR. It was possible that the *P. gonapodyides* had caused the aerial infection, as in beech is has been hypothesised that it collects in water held in branch forks and then infects the bark via shoots or wounds (Brasier and Jung, undated). The leading stem of plant would have been pruned to create a splayed habit and the pathogen may have established on the wounded tissue at this point.

Pinus sylvestris had been purchased from Belgium in March 2009 as bare-root when they would have been two years old and 0.5 m tall, and potted into 3 L. The plants had started dying, and this was attributed to poor new root formation, with 50% of the plants lost by June 2010. The pots had then been stood in trays off the ground-cover matting to try to improve pot drainage. Sample 2E.2 was a wilted (blue coloured) plant next to a totally dead one. There was pinkish colouring under the bark. It was difficult to get a good quantity of stem tissue for the LFDs and the bark had to be included (it should be discarded so that surface contaminants are not included). Although the LFDs were negative for the stem the PCR test found *Phytophthora cactorum* (a well known pathogen). The roots were 100% brown, with only 70% root fill of the pot, and gave positive LFDs for *Pythium* and *Phytophthora. Phytophthora cactorum* was identified in the roots by PCR. This species and *Phytophthora cryptogea* in a sample from another nursery (4E.5) were the only two instances where the same pathogen was found in both the roots and stem of a plant, probably spreading internally to the stem base from the roots (Table 11).

iii) Nursery 3E

In August, two samples were taken from nursery 3E from the outdoor standing area which held a wide range of species and cultivars within batches of 100 – 200 plants propagated on site from October to December under polythene with bottom heat. Potted plants were stood on pea gravel on plastic and irrigated overhead with mains water stored in an uncovered tank. The gravel did not receive disinfectant. 20% of the 200 1.5 L pots of four year old *Taxus fastigiata aurea* (sample 3E.1) from cuttings scattered throughout the batch were wilting over the whole plant, and this had first been noticed by the grower one to two months earlier. 60% of the roots were brown on removing the pot (which 75% filled the pot showing that there had been healthy growth until recently). The central liner rootball was 80% brown and the new rooting was 50% affected. There was no stem staining, but the roots gave a distinct positive with both *Pythium* and *Phytophthora* LFDs. The PCR testing did not find roots with *Pythium*, but *Phytophthora cinnamomi* was confirmed. This pathogen is associated with sudden death of cork oak in Portugal, and is believed to produce a toxin (Brasier and Jung, undated). The grower had recently changed his propagation practice from taking cuttings from field-grown stock plants, to keeping the tops from potted plants "stopped" to encourage branching.

The second sample (3E.2) was obtained from a 1.5 L pot of *Juniperus* Old Gold. 15% of the 100 plants of this cultivar had a branch which was wilting and dying, with other branches on the same plant looking healthy. The dead foliage was where the main branch was pruned with secateurs to get branching. Stem staining was seen only on the dying branch, not below on the main stem or side branches. Both *Pythium* and *Phytophthora* LFDs were positive, with the reaction being very strong for the latter. PCR identified the species as *Pythium attrantheridium* and *Phytophthora austrocedrae*, the later not known to be native to the UK, and reported by the grower to the PHSI after the PCR results were received from the RHS. When further samples were taken by the inspector for official diagnosis *P. austrocedrae* was not able to be isolated and so no further action was taken, although a further inspection may be made (Justine Walker, PHSI, pers. comm.).

iv) Nursery 4E

Nursery 4E was visited in July 2010 and had only a few visibly affected plants with die-back within each batch e.g. in *Chamaecyparis* cultivars Sulphur Spire, Ellwoods Pillar and Karamachiba, *Thuja* Micky and *Juniperus* Blue Arrow. The plants *of x Cupressocyparis* Gold River and *Juniperus* Gold Coast had larger numbers of plants affected and these could have

picked up disease spreading for contamination of the ground by the river when it flooded on that side of the nursery after heavy snowfall melted.

The three *Chamaecyparis* cultivars in 3 L pots in the same shade area from Nursery 4E (which buys-in material only) all had different pathogens isolated (Table 11) which might be an indication that they came from different suppliers, because, for example, the *Chamaecyparis* cultivars sampled at Nursery 5E which grows its own stock had the same root colonising water moulds throughout its cultivars. Specimen 4E.1 cultivar Karamachiba was among 10 out of 48 two year old plants that were a paler yellow than normal and had dieback on sections of branches (which also had an unidentified pycnidial fungus). Half the roots were rotted throughout the pot (which had 50% root fill), with dark brown roots at the base of the stem and the cortex sloughing off. There was internal cinnamon coloured stem staining from 15 mm above the pot surface for 15 mm. The stem had been potted below the growing-media surface and mosses and liverworts were packed around the stem keeping it moist. *Pythium* was detected in stem tissue by LFD but not by PCR, and similarly in the roots. *Phytophthora* was detected by LFD and *P.* sp. *Salixsoil* by PCR in the roots.

Only two out of 100 *Chamaecyparis* Ellwoods Pillar were starting to wilt (feeling softer than healthy plants). All the roots of an affected plant (sample 4E.2) were rotted and there was stem staining to 10 mm above the pot surface. *Phytophthora* was detected by LFD from stem tissue, and both *Pythium* and *Phytophthora* in the roots. *Phytophthora cactorum* (a known conifer root pathogen) was found in the stem with *Pythium vexans* (which has been isolated from a wide host range, Van der Plaats-Niterink, 1981). The wilting of the plants had not been observed two months previously in nursery monitoring, and this was likely to be because of the warmer weather since then. A similar incidence and manifestation of wilting was seen with *Chamaecyparis* Sulphur Spire (sample 4E.5) which had stem staining from the growing-media surface to 30 mm up the stem and a positive *Phytophthora* LFD. However, this time PCR identified another known conifer root rot, *Phytophthora cryptogea*. This species was also confirmed in the roots (which were 100% rotted, with 75% pot fill). Both *Pythium* and *Phytophthora* were found using the LFDs.

Nearly all the 3 L plants of the 300 X *Cupressocyparis* Gold River stood on beds backing onto the river at nursery 4E were wilting, with foliage drooping. The foliage felt soft, but there was no browning. The nursery had only recently noticed symptoms. They had been bought-in and potted-on in autumn 2009. The sample (4E.4) had only 30% or roots brown (mainly around the pot base and so possibly the pathogen entry point) on removing the pot with nearly 100% pot fill by roots. However, half of the roots in the original root ball were rotted

and most of the newer roots were dead. The root cortex was not soft. There was no stem staining. The roots gave a strong LFD test line for both *Pythium* and *Phytophthora*. Two pathogens, *Pythium vexans* and *Pythium intermedium* were detected in the roots by PCR, together with a *Phytophthora* that could not be fully identified, but had a DNA sequence closest to *Phytophthora heveae* (a pathogen of rubber trees (Waterhouse, 1970)). This area was flooded by the river in December 2009 and this could have brought water moulds with it.

A *Thuja occidentalis* Micky (sample 4E.6) was one of four plants among 48 that was looking a paler green with a few bits of foliage brown near the centre of the plant. All the roots around the pot edge were brown, with the cortex of roots around the pot base sloughing off (a symptom usually associated with *Pythium* infection). There was good root fill and none of the original root ball was rotted, but half the new roots were rotted. There was no stem staining. A positive LFD for root *Pythium* was found, but the PCR test was negative. This could have resulted from different areas of the roots being sampled for the different tests. The plant had not been checked for the presence of other root pathogens before it was sent to the RHS.

Plants of *Juniperus virginata* Blue Arrow in the shade area (two out of 40) were greyer, and softer indicating wilting. There were 25 spaces on the bed where plants had been removed, possibly on the post-winter clear-up two months previously. One plant only had the lower four branches wilted. On sample 4E.7 a ring of brown twigs occurred 40 mm up the stem. The stem inside was unstained up until this point, but ringed around brown at the position of the dead branches. Strong positive LFD results were shown for *Pythium* and *Phytophthora*. However as only 5-10% of the roots were rotted on the outside of the root ball, and none of the more inner roots were rotted LFDs were not carried out on the roots. PCR testing of the stem found the non-indigenous *Phytophthora austrocedrae. Pythium attrantheridium* was present on the roots but did not seem to have been associated with much damage. The plants had been bought-in as 70 mm pots in 2009. All the arriving plants of different species are kept in a separate area and no problems in any species were observed in the batches which were currently awaiting re-potting.

The final plants examined were in an area for 7 L and 15 L pots so that they could be given more water. 30% of the 7 L *Juniperus* Gold Coast plants in a 10 m x 4 m area were not healthy. They were scattered throughout the area, but with a concentration of affected plants at the front of the bed which was at the bottom of the bed drainage slope. A sudden deterioration had been seen in the plants after Christmas 2009. The plants were now 5 years old, having been bought-in as 3 L plants, and had been potted-on nine months before the

nursery visit. Most plants had some whole branches which were yellowing or dead, with some plants wilting overall or totally dead. Plants that had been alive three months ago were now dead. The nursery supervisor noted that the symptoms were not typical of root rotted plants on the nursery because the affected foliage did not feel soft and “greasy”. The plant selected for testing (sample 4E.3) had one dying branch and the others looking pale. The plant had splayed, horizontal, branches and it was seen that there were little cups (probably needle sockets) at the junction of the branches which were soft/corky. There was stem staining at the branch junction, 10 mm above the growing-media surface and the staining did not extend up the dying branch. The stem sample gave LFDs positive for both *Pythium* and *Phytophthora*. The roots were only about 10% rotted on removing the pot because of two patches of much-branched roots near the top of the pot. There was 90% pot fill by roots; healthy plants having 100% fill. The central roots appeared totally healthy, but half of the new growth beyond the centre was brown. The LFD tests showed both *Pythium* and *Phytophthora* in the roots. The PCR tests did not find any *Pythium* spp., but the stem was affected by the notifiable pathogen *Phytophthora austrocedrae* and the roots by the usually weak pathogen *Phytophthora gonapodyides*. As the plants had been on the nursery for five years and had not shown any symptoms when in the 3 L pots it is likely that *Phytophthora austrocedrae* infection was acquired on the nursery, either recently such as in the flood water, or that conditions such as the heavy and persisting snow cover in December 2009 followed by water-logging gave the pathogen the right conditions to develop. The results from the PCR testing were not available until October and the grower was visited again and advised of the implications of the results and agreed to notify his local PHSI office. PHSI officers later removed samples but because it was not found possible to isolate any *P. austrocedrae* no further action was taken (Justin Walker, PHSI).

v) Nursery 5E

At nursery 5E samples were taken of different *Chamaecyparis* cultivars in 3 L posts within a 10 m length of standing area receiving overhead mains irrigation. Growing-media from unsold plants was piled under mature oak trees and the growing-media re-used in potting plants. *Chamaecyparis* cultivars had been on the same standing bed for several years because the stock was arranged alphabetically with about 30 plants of each cultivar. Samples 5E.1 (Columnaris glauca), 5E.2 (Blue Surprise), 5E.5 (Pelts Blue) did not show any obvious wilting, but the grower said that the plants recently had not been doing well e.g. they were a bit “leggy”. They were all produced on the nursery from cuttings taken from field-grown mother plants. 5E.1 & 5 were two years old; 5E.2 was nearly four years old.

The roots of *Chamaecyparis* cv. Columnaris glauca (sample 5E.1) visible on removing the pot were thick roots mainly around the pot base and only 5% affected by rot. The central roots were similarly little rotted, but the 75% of the roots which three-quarters filled the pot were brown with the cortex sloughing off (a symptom called “stringy root” usually associated with *Pythium*). There was no stem staining, but the roots gave strong LFD tests for both *Pythium* and *Phytophthora*. One plant in the batch was bluer with wilting and this had all roots totally brown and soft. *Pythium* was not identified by PCR, but a *Phytophthora* which was closest to *P. quercina* and *P. sp.* Ohioensis was identified. *P. quercina* is associated with oak decline sites in central and northern Europe and has been confirmed to cause extensive fine root damage (Brasier and Jung, undated). The presence of this pathogen may have been because of contamination of the growing-medium by contact with oaks.

The same absence of stem staining and positive LFD and PCR results for the roots were also found for cv. Blue Surprise (sample 5E.2), but in these plants potted in spring 2008 there had only been 50% pot fill by roots and nearly all the roots were rotted (only 80% rot visible on removing the pot) and it was surprising that there was not any dieback. Although cv. Pelts Blue (sample 5E.5) also had no stem staining and was positive for *Pythium* and *Phytophthora* in LFD tests on the roots, and 100% root rot (75% on removing the pot) throughout, these roots did not have a rotted cortex. These roots were found to contain only *P. cinnamomi* by PCR.

The final sample at nursery 5E was from a separate area of 3 L, 5 L and 7.5 L home-produced pots (some 20 years old) of *Chamaecyparis* Obtusa. The plants had whole long branches or smaller branches (fans) totally dead and a golden brown, 50 mm and 250 mm up from the growing-media surface, with a sharp demarcation to green foliage lower down the branch. This cultivar is pruned to produce a spiralling shape, but the fans themselves had not been trimmed. This differed from the darker brown dead foliage around the stem base, which may have died through shading. The symptoms had been noticed in the last couple of years. On removing the pot 50% of a 3 L plant’s roots were dead, being principally alive on one side of the pot where there was a greater distance between the off-centre-planted root ball and the pot side. The dead roots looked like they had been allowed to dry out. Once inside the ring of outer roots only a few, about 5%, of roots were rotted in the central and mid area, however there were few roots present. There was a blood-red colouration under the bark, but this was also seen on a healthy branch. The stem gave a strong positive to *Pythium* with the LFD, and the pathogen *P. intermedium* was identified by PCR. *Pythium* was also found in the roots using a LFD, and a *Pythium* similar to *P. monospermum* and *P. attrantheridium* (the former a known root pathogen) recorded.

Phytophthora was also found in the roots using an LFD. A *Phytophthora* which was closest to *P. quercina* and *P. sp.* Ohioensis was identified in the roots by PCR.

Four seed trays of 4 week old *Chamaecyparis* cv. Penberry Blue (5E.3) in the propagation glasshouse had 25% of cuttings with brown foliage on small foliage lengths (sometimes on one side of the cutting), or just brown tips on some leaves,. The cuttings had rooted and the roots appeared healthy and so were not tested. The foliage produced a positive *Pythium* LFD result, but the PCR test was negative.

7. Survey of plant clinic record for conifers

Plant clinics at Fera, York (Table 15) and at the RHS Wisley (Tables 16 & 17) were able to provide information on conifer samples received with dieback and root rot.

Only *Abies* spp. and *Picea abies* were found to have been submitted to Fera. This was probably because samples were principally submitted by the Forestry Commission nurseries (Paul Beales pers. comm.). Ornamental conifer growers, or their advisors, did not appear to send plants for diagnosis of any problems. The survey of conifer growers carried out as part of this research confirmed that plants with root rots or die-back had not been sent to any clinics for diagnosis prior to the current work.

Table 15: Fera Plant Clinic results for all conifers received from 2004 to 2009

Sample type logged by Fera	Date received	Host	Diagnosis
Plant(s)/Tree(s)	02-Feb-04	*Abies*	No pathogen detected
Culture/Isolate(s)	26-May-04	*Abies nordmanniana*	*Sydowia polyspora*
Leaf/Leaves,Twig/branch	30-Jul-04	*Picea abies*	No pathogen detected
Leaf/Leaves	17-Aug-04	*Abies*	*Botryotinia fuckeliana**
Leaf/Leaves,Twig/branch	02-Sep-04	*Picea abies*	No pathogen detected
Leaf/Leaves,Stem(s)/Shoot(s)	15-Nov-04	*Abies*	No pathogen detected
Leaf/Leaves,Stem(s)/Shoot(s)	19-Nov-04	*Abies*	No pathogen detected
Leaf/Leaves,Stem(s)/Shoot(s)	19-Nov-04	*Abies*	No pathogen detected
Leaf/Leaves,Stem(s)/Shoot(s)	19-Nov-04	*Abies*	No pathogen detected
Leaf/Leaves	25-Jul-05	*Abies*	No pathogen detected
Leaf/Leaves	02-Aug-05	*Picea abies*	*Botryotinia fuckeliana*
Leaf/Leaves,Twig/branch	19-Aug-05	*Picea abies*	No pathogen detected
Leaf/Leaves	26-Aug-05	*Abies*	No pathogen detected
Leaf/Leaves	16-Sep-05	*Abies fraseri*	No pathogen detected
Leaf/Leaves	16-Sep-05	*Picea abies*	No pathogen detected
Leaf/Leaves	16-Sep-05	*Picea abies*	No pathogen detected
Leaf/Leaves,Twig/branch	09-Dec-05	*Abies nordmanniana*	*Fusarium* spp.
Plant(s)/Tree(s)	09-Feb-06	*Abies*	*Phytophthora* sp.
Plant(s)/Tree(s)	09-Feb-06	*Abies*	*Phytophthora* sp.
Plant(s)/Tree(s),Soil	22-Mar-06	*Abies nordmanniana*	No pathogen detected
Plant(s)/Tree(s)	29-Aug-06	*Picea abies*	No pathogen detected
Leaf/Leaves	22-Sep-06	*Abies concolor var. concolor*	No pathogen detected
Unknown	21-Dec-06	*Abies nordmanniana*	No pathogen detected
Leaf/Leaves	15-Feb-07	*Picea abies*	No pathogen detected
Plant(s)/Tree(s)	12-Jun-07	*Abies*	No pathogen detected
Stem(s)/Shoot(s)	17-Jul-07	*Abies nordmanniana*	No pathogen detected
Twig/branch	12-Nov-07	*Abies koreana*	No pathogen detected
Leaf/Leaves	08-Aug-08	*Abies*	No pathogen detected
Leaf/Leaves	11-Sep-08	*Abies koreana*	*Sydowia polyspora*
Leaf/Leaves	11-Sep-08	*Abies balsamea*	*Sydowia polyspora*
Plant(s)/Tree(s)	30-Oct-08	*Picea abies*	*Camarosporium strobilina* *Rhizosphaera kalkhoffii*
Bark	09-Dec-08	*Picea abies*	No pathogen detected
Plant(s)/Tree(s)	03-Jul-09	*Abies nordmanniana*	*Pucciniastrum epilobii*
Twig/branch	21-Aug-09	*Picea abies*	No pathogen detected

**Botryotinia fuckeliana* is the official name (sexual stage) for grey mould, although present in the field and known as *Botrytis cinerea*, the asexual stage (Smith *et al.*, 1988).

Sydowia polyspora is associated with current season needle necrosis (CSNN) on fir (*Abies* spp.) and has been recorded in Europe and North America causing chlorotic spots on needles within a month of bud break, with needles then becoming necrotic and being shed. It affects the marketability of Christmas trees and was initially thought to be a physiological disorder. *Kabatina abietis* was then believed to have been found but molecular diagnosis corrected this to *Hormonema dematiodes,* and as by tradition a disease is named after its sexual stage it is now called *S. polyspora* (Talgø *et al.,* (2010).

A greater range of conifers were sampled by the RHS plant clinic than by Fera (Table 16). The plants sent to the RHS clinic would include plants which had been planted-out and so the water-moulds present would have included species which could have colonized the plants from garden soil as well as species present in the roots of plants when RHS members purchased them.

Table 16: Summary of RHS Plant Clinic diagnoses for members' conifers showing the number of samples with root rot / dieback submitted for ten years to 2009 when PCR testing was available for diagnosis of the Oomycete (water moulds) species present

		Number of samples with Oomycetes present		
Host	**Number of samples**	**Stems only**	**Stems and roots**	**Roots only**
Abies	1	0	0	1
Araucaria	1	0	0	1
Cedrus	3	1[a]	0	2
Chamaecyparis	11	1[b]	0	10
Cupressus	3	0	0	3
Cupressocyparis leylandii	8	0	0	8
Juniperus	13	0	1	12
Larix	1	0	1	0
Taxus	161	2[c]	17	141
Thuja	8	0	1	7

[a] *Pythium diclinum* [b] *Pythium* sp. [c] *Phytophthora* spp. *P. citrophthora, P. cinnamomi* and *P. cryptogea*

The full details of the species found in the tissue shown in Table 16 are presented in Appendix 7. The majority of plants had water-moulds only in the roots. Some of these could have been secondary colonizers of tissue damaged by pathogens or other causes. A small number of plants only had water-moulds in the stems (see Table 16 footer for species). All the RHS PCR isolations directly from stems remove and discard the bark to remove any surface contamination. *P. cinnamomi* (found in 8 *Taxus* stems) and *P. cryptogea* (found in 3 *Taxus* stems) both produce zoospores that spread readily in water. *Phytophthora* species *P. citricola* and *P. plurivora* were each found twice inside *Taxus* stems and *P. citrophthora* and *P. gonapodyides* once each. Notifiable *Phytophthora austrocedrae* was found in stem only of the *Juniperus* sample, with *Pythium* spp. present in the roots of the same plant.

Water-moulds were present in both stems and roots in 11% of the *Taxus* plants with Oomycetes present. Eight of these 12 joint incidences were for *P. cinnamomi;* one for *P. cryptogea* and another for *Pythium intermedium*, otherwise different species were isolated from the stems and roots of the same plants. It is possible for infection to spread internally between the stem base and roots or to splash up onto foliage from the infested growing-media.

A large number of different water-mould species were found in or on roots of the principal host species received at the RHS clinic (Table 17). For some species of *Pythium* no exactly matching DNA sequence for that species was available and the species with the closest sequences were recorded instead. The number of samples is too small, for hosts other than *Taxus,* to be able to conclude what water-mould species are more likely than others to colonise a particular host. In *Taxus,* out of the 10 *Phytophthora* species identified *P. cinnamomi, P. cryptogea, P. citricola* and *P. plurivora* (in order of incidence) dominated. There were 17 *Pythium* species identified (or closely identified) from *Taxus* roots and *P. intermedium* dominated, followed by *P. attrantheridium, P. plurivora* and *P. sylvaticum.*

Table 17: Water mould species present in roots and their frequency in the RHS members' clinic samples shown in Table 16 in each of five hosts. Information was taken from samples with root rot / dieback submitted for ten years to 2009 when PCR testing was available for diagnosis of the Oomycete (water moulds) species present

	Conifer host				
	Chamae-cyparis	***Cupresso-cyparis***	***Juniperus***	***Taxus***	***Thuja***
Phytophthora					
P. austrocedrae	0	0	1	0	0
P. cactorum	0	0	0	1	0
P. cinnamomi	2	0	2	47	0
P. citricola	0	0	1	16	0
P. citrophthora	0	0	0	1	1
P. cryptogea	1	1	1	23	1
P. gonapodyides	0	1	0	6	0
P. hibernalis	0	0	0	0	1
P. megasperma	1	0	0	1	0
P. plurivora	0	0	0	10	0
P. syringae	0	0	0	2	0
Phytophthora sp.	0	0	0	7	0
Pythium					
P. amasculinum	0	0	0	1	1
P. anandrum	0	0	1	0	0
P. attrantheridium	0	0	1	11	1
P. diclinum	1	0	0	4	0
P. heterothallicum	1	1	0	1	0
P. intermedium	1	2	5	37	2
P. macrosporium	1	0	0	0	0
P. montanum	0	0	0	1	0
P. oligandrum	0	0	0	1	0
P. perplexum	0	0	0	2	0
P. plurivora	0	2	2	10	0
P. rostratum	0	0	0	1	0
P. sylvaticum	0	1	0	8	1
P. ultimum	0	0	0	1	0
P. vanterpoolii	0	0	0	1	0
P. vexans	1	1	0	0	0
Pythium sp.	2	2	2	26	2
P. conidiophorum / salpingophorum	0	0	0	1	0
P. cylindrosporium / regulare / cryptoirregulare	0	0	0	1	0
P. dissocotocum / lutarium	0	0	0	1	0
P. glomeratum / heterothallicum	0	0	0	7	0
P. vexans / ucubitacearum				0	1
P. montanum / arbonicum	0	1	0	0	0
P. sterilum / litorale	0	1	0	0	0
P. torulosum / folliculosum	0	1	0	0	0

The *Phytophthora austrocedrae* was detected in the stem of a *Juniperus* plant received from an RHS member in June 2009 and notified (as with any detections of *P. ramorum* and any other non-native pathogen) to the PHSI. The original source of this plant was not traced back to the supplier and so it is not known if it came from one of the two nurseries shown to have it in *Juniperus* from visits for the current project.

Appendix 7 shows many more *Phytophthora* spp. than *Pythium* spp. in records from PCR testing of conifers by the RHS clinic pre-September 2007. According to G. Denton (pers. comm.) these results mirror the general data across other non-coniferous plant species sent to the RHS plant clinic. A contributory factor to there being less *Pythium* spp records may be due to Hemp seed baiting only starting in late 2006. This predominantly recovers *Pythium* and the increase in records of *Pythium* after this date could be a delayed effect of initiating this new baiting method. In addition, the records may have noted the predominant problem, so some early records of *Pythium* may have been reduced due to co-infection with *Phytophthora* or another, better known, serious pathogen.

The receipt of 161 *Taxus* samples (Table 16) is a fair representation of the number of enquires to the RHS, not for example the result of a request for specimens of this species. Yews account for around 20% of the RHS yearly *Phytophthora* tests. They are a common garden plant due to their use for topiaries and hedges, and as they an important plant for structure in a garden RHS members raise concerns when they start dying (G. Denton pers. comm.). As these plants will often have been planted-out before any dieback was seen by gardeners there is no information to say how many may have carried pathogenic species of *Phytophthora* and/or *Pythium* with them from nurseries rather than become infected in the garden. From the HDC survey, growers report *Taxus* root rot mainly in outdoor containers and so plants placed in poorly draining gardens may be particularly susceptible to water-mould infection.

Discussion

Nursery detection of Phytophthora and Pythium by LFDs and water baiting

Government evaluation was made of LFDs for the rapid diagnosis of *Phytophthora* species at the point of inspection before their use by the PHSI in situations where *P. ramorum* or *P. kernoviae* were suspected (Lane *et al.*, 2007). LFD results compared highly favourably with identification by isolation and real-time PCR and were considered particularly valuable as a primary screen for selecting samples for laboratory testing to determine species identification.

In general, confidence was gained during the current project in the ability of the LFDs to record the presence of *Pythium* or *Phytophthora* species. Of 18 detections of *Phytophthora* in roots by PCR; all but one was picked up by the LFD test on same plant. All 8 *Pythium* positives by PCR of roots were picked up by the LFD tests. A number of plants with negative LFDs were sent for PCR and the same negative result usually obtained. Some additional detections of *Pythium* in roots were made by LFD. Similar correlation was achieved with the smaller number of stem samples tested. Their ease and speed of use was demonstrated. These LFDs will be most accurate when the area of disease progress can be identified and sampled to reduce the chance of secondary colonisers or saprophytes being picked up instead of the primary pathogen. At the moment the PDplus test is only for some *Phytophthora* species, not all those possible on conifers, and not for any other Oomycete families. The nature of inhibitors in samples from coniferous species said to sometimes be causing neither host nor pathogen DNA to be detected in LFDs using PCR (P. Meakin, pers. comm.) should be investigated to see if they can be removed.

Principally zoospore-producing *Pythium* species are likely to be detected in run-off water using baits because they swim to infect the baits. Mycelium and oospores for all *Pythium* spp. can be collected by water sampling as well as zoospores, but the chance of catching zoospores is less likely in the small volume taken than likely in baits as the latter may be able to attract zoospores from a large volume around them. There is a knowledge gap for in pathogen distribution in water reservoirs and baiting techniques. There is *ad. hoc*. information on bait materials (Erwin & Riberio, 1996), but how to gain optimum Oomycete attraction such as the best bait floating depth, retrieval interval, and the seasonal and weather influences on success is more sketchy (T. Pettitt, pers. comm.). Before PCR testing any *Pythium* species identification required a mycologist with specialisation in that subject. Although PCR can now assist in species identification, the database of sequences for *Pythium* species is not as complete as that for *Phytophthora* species (G. Denton, pers.

comm.) and more work is required. Information on *Pythium* is available online (Van der Plaats-Niterink, 1981). Koch's postulate testing to confirm which *Pythium* species are primary pathogens on conifers is required as much current pathogenicity determination is based only on the detection of the species in affected plants.

There is the additional potential for growers to use LFDs to test their own leaf baits from irrigation water. However, based on the small number of samples taken there would be concern if non-pathogenic *Pythium* species were present and so gave positive readings on the LFD. However, saprophytes can also be counted in water samples taken for plating-out and so follow-up species identification by the laboratory is required as an additional service. It could be said that, particularly if the sample is taken after water treatment, if any water-mould is present it shows that the treatment is not functioning properly and there is a potential risk from water-borne infection.

There is a possibility that unsterilized visibly healthy leaves used in baits could have splash or air-dispersed water-moulds on them. In most of England (where *P. ramorum* and *P. kernoviae* are infrequent) *Phytophthora* is unlikely to be present. There may be a greater chance of *Pythium* species being present, particularly if leaves are taken lower down where there might be soil-splash. However, it is likely that for the LFD testing that there would need to be high contamination on the leaves for detection (given that 25 mm² of usually visibly infected leaf material is required for a reaction). Less water-mould material would, however be required for PCR as the technique multiplies up the DNA by a chain reaction to a quantity sufficient for identification by sequencing. Further investigation to test surface sterilants on bait leaves, using materials likely to be available to nurseries, would be worthwhile, in order to find a treatment that did not reduce the attractiveness of the bait.

Control of Phytophthora and Pythium by host resistance, microbial and chemical means

Growers consistently reported that some species and cultivars were more susceptible to root rot than others. This might be because of differing morphology or the differing production of chemicals such as tannins. There is circumstantial evidence for the latter as when making isolations from conifer roots it is necessary to remove the tannins by keeping the roots in running water overnight as otherwise the Phycomycetes are held within the roots. Another cause of resistance could be the microbial flora around the roots, which certainly in *Pinus* includes mycorrhiza, although naturally occurring soil fungi such as *Trichoderma* spp. and *Gliocladium* spp. (as used in biological control products) could also be involved. There is some evidence that a lack of mycorrhiza has checked conifer growth (Peace, 1962) and their

benefits are the subject of current crops research (Andrews and Andrews, 2009). Future research on conifers could utilise work on microbial interactions in other crops. There is also the potential, as well as being able to identify micro-organisms in plants by PCR, to exploit new molecular techniques (QPCR) which can identify and quantify micro-organisms in growing-media. This technique could be used to investigate the root ecosystems of plants producing healthier roots in the presence of pathogens.

The existence of cultivar resistance to soil-borne Phycomycetes is recognised, with the testing of new pea varieties by planting in oospore infected soil by NIAB in the UK to produce resistance ratings to downy mildew. Genetic resistance to *Phytophthora infestans* exists in potatoes and variety rankings produced. With continuing widespread development of molecular techniques it is possible that greater understanding of the resistance mechanisms to root pathogens will become known, with the potential of breeding for resistance. It is not know what appears to make blue cultivars of *Chamaecyparis* more susceptible than other forms. Further research could investigate the existence of more resistant or tolerant rootstocks to *Phytophthora* and *Pythium* that might be able to be used in the propagation of susceptible cultivars.

There was little use of disinfectants or fungicides on nurseries against *Pythium* and *Phytophthora*. It is possible that more use of such materials early in the life of the plant could prevent infection and/or stop it developing. It was likely in many instances that the 3 L pots examined at nurseries had been infected when liners, and quite possibly not long after being sown, or rooted. Use of disinfectants only when a problem has become obvious (possibly in older stock) is likely to miss the early build-up of the disease. After an outbreak of *Pythium* or *Phytophthora* root rot, nurseries should clear away any crop debris and disinfectant drench the affected areas of ground-cover to reduce the risk of infection in new crops. Up-to-date information is needed on the efficacy of available disinfectant products, and efforts are needed to ensure that good products do not become lost to the industry through the implementation of the Biocides Directive.

Fungicides drenches are best used as protectant treatments, rather than expecting much curative action. Options include Filex or Proplant (propamocarb hydrochloride), Aliette (fosetyl-aluminium), Previcur Energy (under a SOLA) contains both the former active ingredients, Subdue and SL567A. The latter two products contain metalaxyl-M and can have a curative effect. Fungicide drenches are, however, limited to a certain number of applications. Nurseries should consider restricting fungicide use to particular cultivars or specific situations on the nursery where there have been problems, but they should not be

used as an alternative to good hygiene measures. Subdue or Filex/Proplant can be incorporated into growing-media at potting, and nurseries may find their use to be particularly worthwhile if the conifer species (such as *Taxus*) or cultivar (such as *Chamaecyparis* Ellwoodii) has been susceptible to root rot in the past. A new fungicide with fertiliser product as granules for incorporation in growing-media with eight months slow release has been tested on *Chamaecyparis* infected with *Phytophthora cinnamomi* and could prove useful and economic at least on more susceptible valuable hosts such as *Taxus* and *Araucaria* (E. Wedgwood, commercial trials, 2007-9). Potato blight fungicides are being investigated for use against leaf and stem infection by *P. ramorum* and *P. kernoviae*, and investigations are also required to determine their efficacy against *Phytophthora* root rots to obtain Specific Off Label Approvals to permit use on ornamentals (www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/phytophthora/research.cfm).

Phytophthora and Pythium pathogenicity

There is information available on the pathogenicity of *Phytophthora* species (Table 18), with conifers infected by a number of different species (Table 19). A synopsis of *Phytophthora* species including host range and geographic distribution is available via the internet with hyperlinking to databases (Cline et al., 2008) and gives *Phytophthora austrocedrae* as found in Argentina and absent from the United States of America.

Table 18: Information on pathogenicity of species recorded from conifers within this report (Based on Erwin & Riberio, 1996)

***Phytophthora* species**	**Pathogenicity**	**Symptoms**
P. cactorum	Infects over 200 spp.	Root & collar rot, leaf blights
P. cinnamomi	Infects over 1000 hosts	Root rot of woody spp.
P. citricola	Infects over 50 woody hosts	Root rot & trunk canker
P. citrophthora	Infects citrus & other hosts	Crown & root rot
P. cryptogea	Infects many plant types	Root rot
P. hibernalis	Infects citrus & a few hosts	Root rot & airborne leaf blight
P. gonapodyides	Infects beech / saprophyte	Root rot & bark lesions
P. megasperma	Infects over 40 hosts	Root rot & blight
P. syringae	Infects over 19 woody hosts	Root rot & dieback

Table 19: Conifer hosts listed by Erwin & Riberio (1996) that are susceptible to more than one species of *Phytophthora*

Conifer host	*Phytophthora* species	Source of information
Chamaecyparis lawsoniana	*P. citricola*	NL
	P. eriugena	Ireland
	P. hibernalis	UK
	P. lateralis	USA
Pinus spp.	*P. boehmeriae*	Australia
	P. cactorum	UK
	P. cinnamomi	USA / NZ
	P. citricola	USA
	P. citrophthora	Argentina
	P. cryptogea	Australia
	P. hevae	Australia
	P. parasitica	USA

A vast amount of technical information on plant pathogenic *Phytophthora* species including host range, morphology plus ITS fingerprinting is available on CPC Datasheets www.phytid.org (free to view) from PhytID (CAB International).

There is much less information available on the pathogenicity of *Pythium* spp., with a key text still being Van der Paats-Niterink (1981). Several species were identified from root rots or foliar blights in the current research. Further research is required to gain more information on the virulence of *Pythium* species, including the enlargement of the DNA sequence database for this Oomycete.

Foliage blights and spots

Leaf blights and spots were rarely seen on the nursery visits or reported as other than a sporadic problem by growers during this survey. Nevertheless, although dieback (most frequently seen as an overall wilt) was almost exclusively attributable to root rotting or

infection spreading up into the stem there are large numbers of foliar fungi including rusts that can affect conifers (Peace, 1962 and Sinclair *et al.*, 1987, www.ecoflora.co.uk). High numbers of foliar diseases have been recorded in the UK for conifers grown as plantation conifers such as *Abies* (Fir) (24 pathogen species), *Larix* (Larch) (34 pathogen species), *Picea* (Spruce) (44 pathogen species) and *Pinus* (Pine) (54 pathogen species) (Beatrice Henricot, RHS, pers. comm.). Several fungi produce spore bearing structures which are just visible as dark specks with the naked eye. Confusingly, there is, however, also a range of fungi that are secondary colonisers on the tissue killed by other pathogens or pests (including on necrosis following wilting caused by root loss) (Ellis and Ellis, 1985). Some pathogenic fungi may also cause secondary infestations such as *Phomopsis* on *Chamaecyparis* after frost damage (Peace, 1962).

Foliar blights causing needle necrosis include Diplodia tip blight of pines, Phomopsis blight of junipers and cedars, and Hypoderma and Lophodermium needle casts of pines (Conway and Olson http://osufacts.okstate.edu) with *Lophodermium pinastri* by far the commonest fungus on pine needles in Britain (Peace, 1962). Further information on conifer diseases is available elsewhere e.g. Sinclair *et al*., (1987), with images and factsheets from a variety of sources available via internet search engines.

Tip burn has been a problem in *Chamaecyparis* and work under HNS 148 suggested it to be physiological and reduced by shading the plants. Conifer browning was researched by the RHS in HDC project HNS 151 and linked to trimming in autumn and the presence of the conifer aphid in May, although an unidentified fungal problem probably also exacerbates the damage.

Non-indigenous diseases causing leaf blight in trees

Phytophthora ramorum infects more than 100 different plant species from 16 different plant families, including several species of conifers. In the USA, where it was first reported in the mid 1990's on oaks and called Sudden Oak Death, hosts include Douglas-fir (*Pseudostuga menziesii*), Fir (*Abies* spp.) and Pacific Yew (*Taxus brevifolia*) are recognised (Anon, 2006). In the UK it was first found in 2002, with trees with bleeding bark cankers (especially Fagaceae e.g. beech and oak) being found in close proximity to evergreen rhododendrons with sporulating *P. ramorum* (Anon, 2005). In 2009 it was found in South-west England causing leaf blight to mature Japanese larch (*Larix kaempferi*) trees where it sporulates profusely and risks widespread infection of plants on the ground below http://www.forestry.gov.uk/forestry/INFD-8EJKP4. In 2010, infected larch was found in Wales and one small site in western Scotland, as well as in Northern Ireland, The Isle of

Man and the Republic of Ireland. In March 2011 it was confirmed on a European larch (*Larix decidua*) in Cornwall and in April 2011 it was confirmed in a small Sitka spruce (*Picea sitchensis*).

Chamaecyparis lawsoniana, Pseudostuga menziesii, Tsuga heterophyllia and *Picea sitchensis* have been shown in laboratory tests on stem sections to be more susceptible to *P. ramorum* than *Taxus baccata,* whereas *Pinus nigra* appears resistant (Brasier and Jung, no date). Implications for the HNS industry of this disease have been given in HDC Factsheet 19/03 (2011).

In October 2003, *Phytophthora kernoviae* was found in south-west England causing a bleeding canker on a mature beech. In November 2007, it was found on *Vaccinium myrtillus* and has since been found aerially infecting other ornamental trees and shrubs (principally Magnoliaceae) causing dieback. Up to February 2009 the only conifer attacked was Giant Sequoia (*Sequoiadendron giganteum*) (Cupressaceae) but research is ongoing on the host range of *P. kernoviae*. A vast amount of information on *P. ramorum* and *P. kernoviae* is available from web searches on www.fera.defra.gov.uk and www.forestry.gov.uk.

Red band needle blight, also known as Dothistroma needle blight (*Dothistroma septosporum*), is one of the most important diseases of *Pinus* species in the world. The red banding is not always evident and the needles may have overall brownish colouration instead. As the disease is of serious plant health concern, growers of nursery trees in Britain are required to report suspected outbreaks to the relevant Plant Health authority in order to minimise the risk of further spread. The disease has increased dramatically in the northern hemisphere since the late 1990s, including in Britain where it has severely affected plantations of Corsican and lodgepole pine; there is now a moratorium on planting these very susceptible species in public forests managed by the Forestry Commission. In summer 2010 the disease was confirmed on Scots Pine seedlings on several forest nurseries. On one nursery alone the value of stock destroyed in 2010 was over £120,000 (Brown and Weber, 2008; O'Neill, 2011).

Phytophthora lateralis, a particularly virulent pathogen, was found in November 2010 on one now dead *Chamaecyparis lawsoniana* tree in Balloch Park in Scotland, with dying yew trees also being tested for infection; the disease is known to kill Pacific Yew, *Taxus brevifolia* (www.bbc.co.uk/newsuk-scotland-1187981). The disease is known from North America but has recently been found in France and the Netherlands. Information on this root rot is available at www.eppo.org/QUARANTINE/Alert_List/fungi/PHYLTLA_a.htm .

In the current research, although the growers with *P. austrocedrae* in *Juniperus* notified, and were visited by, PHSI, the Fera plant clinic could not detect this species in the samples they took and so no action further was taken. Further inspections are likely, however (Justine Walker, PHSI Inspector, pers. comm.). The containment measures for non-indigenous *Phytophthora,* or other species, required by PHSI are the same as for *P. ramorum* and *P. kernoviae*. This requires plant destruction within 2 m of the affected material. Plants to a distance of 10 m beyond this area (to allow for splash dispersal) would not be able to be moved for three months and only released if free from the disease after this time. The contaminated area would need to be cleared of debris and disinfected, and any tools and footwear moved out of this area would also require disinfection (Justine Walker, pers. comm.). PHSI inspectors use LFDs to check tissue for *Phytophthora* spp. and then remove samples for PCR testing to identify the species involved. Growers worried about potential *Phytophthora* infections of their plants are also able to utilise LFDs, possibly followed up by the identification of some species (including *P. ramorum* and *P. kernoviae*) by PDplus testing of the LFDs.

Conclusions

Occurrence of root rots

- The majority of nurseries surveyed reported losses of conifers throughout the production process, although most Phytophthora or Pythium root rot and dieback was noticed in 3 L pots when conifers were generally three years old
- Nurseries propagating their own material had some root rot, and nurseries buying-in had some instances of root rot developed after potting-on but it was not clear when infection had occurred
- Araucaria, Chamaecyparis, Juniperus and Taxus had the greatest incidence of Phytophthora or Pythium root rot on surveyed nurseries, with 50% to 100% losses possible in Araucaria and Chamaecyparis and 20% to 50% in Taxus
- Differences in susceptibility were noted between cultivars of Chamaecyparis (with blue cultivars having greatest losses) and Juniperus
- Moving Taxus under cover minimised losses to Phytophthora and Pythium root rots, probably because this allowed the roots to be kept drier. Araucaria and Juniperus roots also benefited from reduced watering
- Re-potting in autumn or other periods of slow root growth, and over-potting, increased the likelihood of root rot

- Abies, Cedrus, Cryptomeria, Cupressus, Larix, Picea, Pinus and Thuja rarely had root rotting
- There was minimal use of Phytophthora and Pythium control products, either pesticides or disinfectants, and use of microbial products was rare
- Conifer dieback from root rot was more widespread than incidences of foliar blight from pathogens such as Keithia and Pestalotiopsis

Detection of Phytophthora and Pythium

- LFD kits were shown to be easy to use on nurseries to determine the presence of Phytophthora and Pythium in rotting roots and stained stems
- Positive Phytophthora LFDs matched well with PCR testing on the same plants, confirming the value of these LFD tests. Pythium was more frequently detected by the LFDs possibly because bark and older dead root material was included in the LFDs which could have had saprophytic or secondary colonisation by Pythium rather than primary pathogenic infection
- Test lines on LFDs whether faint or strong indicating the presence of Pythium or Phytophthora were confirmed in several plants by the PCR tests, but because the destructive sampling for each test could not use the same tissue there cannot be direct comparison between the LFD and PCR tests

Phytophthora and Pythium species present in conifers on nurseries

- Phytophthora species found on roots were P. cinnamomi, P. citricola complex, P. cryptogea, Phytophthora sp. Salixsoil and P. quercina / Phytophthora sp. Ohioensis.
- Pythium species on roots included P. attrantheridium, P. intermedium, P. irregulare, P. sylvaticum, P. vexans and P. sylvaticum
- Phytophthora species P. austrocedrae (a non-indigenous pathogen), P. cactorum, P. cinnamomi and P. gonapodyides were isolated from stained stems with foliar wilt.
- Pythium species P. attrantheridium, P. intermedium and P. sylvaticum were identified in samples from stems at the location of foliage browning
- PDplus testing of LFDs corresponded with PCR results for Phytophthora cactorum, but the technique does not currently extend to all the other Phytophthora species detected in plants and so PDplus may be of limited value to growers

Bait tests for Phytophthora and Pythium in water on nurseries

- Baits using Ceanothus leaves or Nordmann Fir needles wrapped in horticultural fleece were developed and shown to be able to trap Pythium and Phytophthora species in irrigation water, with the leaves being able to be tested using LFDs

- Pythium species were most commonly retrieved from leaf baits used in irrigation water although the same species were not retrieved from plants, possibly because these baited species were not pathogenic to the conifers tested.
- Phytophthora sp. Salixsoil and P. cryptogea were retrieved in baits and found in conifer roots in this survey

Methods for growers

- Simple, reliable methods (leaf baits tested with LFDs) are now available for growers to test irrigation in order to asses any potential disease risk from using this water on plants
- LFD testing of plants with suspect roots during the production process would allow decisions to be made about the treatment or disposal of infected plants and so save plants having to be thrown away later on in production when root rot had become more extensive

Acknowledgements

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Glossary

LFD – An abbreviation for Lateral Flow Device, which utilises genus-specific monoclonal antibodies to identify pathogens to genus level (e.g. *Phytophthora*) but not to species. The reaction takes place within a membrane held for convenience in a plastic window.

Mycorrhiza – Fungi which exist in intimate association with the active feeding roots of most trees and shrubs and the plants can benefit from these fungi being efficient nutrient absorbers. In endotrophic mycorrhiza the fungal hyphae grow between and mainly within the root cells, whereas in ectotrophic mycorrhiza the hyphae clothe the root with a mycelial mantle and penetrate only between the root cells.

Oomycetes – These comprise two orders of water moulds (fungus-like micro organisms). The order Pythiales includes the family Pythiaceae containing *Phytophthora* and *Pythium*. The order Peronsporales includes Peronosporaceae, a family including downy mildews.

PCR – An abbreviation for Polymerase Chain Reaction which is part of a technique whereby DNA of both living and dead organisms can be bulked up from miniscule amounts available in a sample until there is enough to match against databases of DNA fingerprints to identify species

Saprophytic – Micro organism colonisation of plant material, frequently on tissue damaged by other organisms and contributing to decay, without having caused primary damage (parasitism).

References

Andrews, M. and Andrews, M.E. (2009). Positive plant microbial interactions in relation to plant performance and ecosystem function. Aspects of Applied Biology 98.

Anon (2005). *Phytophthora ramorum.* A Practical Guide for the Nursery Stock and Garden Centre Industry. Defra.
www.fera.defra.gov.uk/plants/publications/documents/factsheets/pramnurs.pdf

Anon (2006). A Christmas Tree Grower's Guide to Sudden Oak Death (*Phytophthora ramorum*). California Oak Mortality Task Force. www.suddenoakdeath.org

Anon (2008). Is feeding by the conifer aphid *Cinara cupressivora* causing the browning seen in conifer hedges. Horticultural Development Company report for HNS 151.

Brasier, C. and Jung, T. (undated). Chapter 1, Recent developments in *Phytophthora* diseases of trees and natural ecosystems in Europe. In; http://www.forestry.gov.uk/pdf/Phytophthora_Diseases_Chapter01.pdf/%24FILE/Phytophthora_Diseases_Chapter01.pdf

Brickell, C. The Royal Horticultural Society A-Z Encyclopedia of Garden Plants. Dorling Kindersley.

Brown, B. and Weber, J. (2008). Red band needle blight of conifers in Britain. Forestry Commission FCRN002. www.forestry.gov.uk/pdf/fcm002.pdf/$FILE/fcin049.pdf

CAB International, Wallingford. PhytID – Identification of Plant Pathogenic *Phytophthora* Species by ITS Fingerprinting. www.phytid.org

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G. and Brasier, C.M. (2000). A molecular phylogeny of *Phytophthora* and related Oomycetes. Fungal Genetics and Biology 30:17-32.

Cline, E.T., Farr, D.F. and Rossman, A.Y. (2008). A synopsis of *Phytophthora* with accurate scientific names, host range, and geographic distribution. Online. Plant Health Progress 10.1094/PHP-2008-0318-01-RS. www.plantmanagementnetwork.org/pub/php/review/2008/phytophthora/

Conway, K.E. and Olson, B. (undated). Common Disease of conifers in Oklahoma. Oklahoma Cooperative Extension Service EPP-7618. http://osufacts.okstate.edu

Dance, M.H., Newhook, F.J., and Cole, J.S. (1975). Bioassay of *Phytophthora* spp. in soil. Plant Disease Reporter 59: 523-527.

Ellis, M and Ellis, J.P. (1985). Microfungi on Land Plants. An Identification Handbook. Croom Helm.

Erwin, D.C. and Ribeiro, O.K. (1996). Phytophthora Diseases Worldwide. APS Press.

Evans, S. G. (1979). *Phytophthora* foot rot of hardy nursery stock. ADAS Leaflet 625, Ministry of Agriculture, Fisheries and Food.

Greslebin, A.G. and Hansen, E. M. (2010). Pathogenicity of *Phytophthora austrocedrae* on *Austrocedrus chilensis* and its relation with *mal del ciprés* in Patagonia. Plant Pathology 59: 604-612.

HDC Factsheet 19/03, (2011 revised). Sudden Oak Death/Ramorum Dieback implications for the HNS industry. Horticultural Development Company.

HDC Factsheet 16/04, (2004). Control of *Phytophthora, Pythium* and *Rhizoctonia* in container-grown hardy ornamentals. Horticultural Development Company.

HNS 123a, (2006) Chemical control of *Phytophthora ramorum* causing foliar disease in outdoor hardy nursery stock. Horticultural Development Company.

HNS 134, (2005). Detection and decontamination of *Phytophthora* spp., including those of statutory significance, from commercial HONS nurseries. Horticultural Development Company.

HNS 148, (2007). A desk study to determine the possible causes of tip burn in conifers Horticultural Development Company.

HNS 151, (2008) Feeding by the conifer aphid Cinara cupressivora causing the browning seen in conifer hedges Horticultural Development Company.

Lane, C.R., Hobden, E., Walker, L., Barton, V. C., Inman, A.J., Hughes, K.J.D., Swan, H., Colyer A. and Barker I. (2007). Evaluation of a rapid diagnostic field test kit for identification of *Phytophthora* species, including *P. ramorum* and *P. kernoviae* at the point of inspection. Plant Pathology 56: 828-835.

O'Neill, T. Red band needle blight (2011). Horticultural Development Company report for HNS 184.

Pettitt, T.R., Wakeham, A.J., Wainwright, M.F. and White, J.G. (2002). Comparison of serological, culture, and bait methods for detection of *Pythium* and *Phytophthora* zoospores in water. Plant Pathology 51: 720-727.

Peace, T. R., (1962). Pathology of Trees and Shrubs with special reference to Britain. Clarendon Press.

Sinclair, W. A., Lyon, H.H. and Johnson, W.T. (1987). Diseases of Trees and Shrubs. Cornell University Press.

Singleton, L.L., Mihail, J.D. and Rush, C.M. (1993). Methods for Research on soil borne Phytopathogenic Fungi. APS Press

Smith, I.M., Dunez, J., Lelliott, R.A., Phillips, D.H. and Archer, S. A. (1988). European Handbook of Plant Diseases. Blackwell Scientific Publications.

Steer, W., (undated). *Phytophthora* diseases, Chapter 1 consulted on-line www.forestry.gov.uk/pdfPhytophthora_Diseases_Chapter01.pdf/%24FILE/Phytophthora_Disease_Chapter01.pdf

Talgø, V., Chastagner, G., Thomsen, I. M., Cech, T., Riley, K., Lange, K., Klemsdal, S.S. and Stensvand, A. (2010). *Sydowia polyspora* associated with current season needle necrosis (CSNN) on true fir (*Abies* spp.). Fungal Biology 114: 545-554. www.elsevier.com/locate/funbio

Van der Plaats-Niterink, A. J. (1981). Monograph of the Genus *Pythium*. Studies in Mycology No. 21. Centraalbureau voor Schimmelcultures, Baarn. (Free online) http://www.cbs.knaw.nl/publications/1021/content_files/content.htm)

Waterhouse, G. M. (1970). The Genus *Phytophthora* de Bary. Diagnoses (or descriptions) and figures from the original papers. Second Edition. Commonwealth Mycological Institute.

Information on the lifecycle of Phytophthora cinnamomi is available at http://www.cals.ncsu.edu/course/pp728/cinnamomi/p_cinnamomi.htm

Information of fungi and hosts is available from the The Ecological Flora of the British Isles. www.ecoflora.co.uk

APPENDICES

Appendix 1 Survey Form

HDC SURVEY OF CONIFER ROOT ROT AND DIE-BACK INCIDENCE & HOW GROWING CONDITIONS MAY AFFECT THIS. HNS 181

You do not need to fill in your contact details, but please as a minimum record your County. Are you willing to be contacted about your answers **Yes / No** (please delete).

Business name	
Postcode	Phone number/s
Contact name	E-mail
Preferred contact days and times	

Area of outside growing beds	Area of protected growing beds	Area of open ground

Thinking back over the last five years, please answer as many questions as possible for the different stages in conifer production. If you don't have details then answer yes (Y) or no (N). You may print-off and complete this form by hand. Please feel free to add margin notes. If you wish, please contact Erika Wedgwood on 01954 268 231at ADAS Boxworth (Mon-Fri).

1.0 The scale of the problem

Plant stage	**% mean annual crop loss** to root rot for each conifer. Mark X if not grown.					
	Abies	*Araucaria*	*Cedrus*	*Chamae-cyparis*	*Crypto-meria*	*Cupresso-cyparis*
Propagation						
Liners indoor						
Finals indoor						
Liners outdoor						
Finals outdoor						

Plant stage	**% mean annual crop loss** to root rot for each conifer. Mark X if not grown.					
	Cupressus	*Juniperus*	*Larix*	*Picea*	*Pinus*	*Taxus*
Propagation						
Liners indoor						
Finals indoor						
Liners outdoor						
Finals outdoor						

Plant stage	**% mean annual crop loss** to root rot for each conifer. Mark X if not grown.					
	Thuja	Other (specify)				
Propagation						
Liners indoor						
Finals indoor						
Liners outdoor						
Finals outdoor						

Name three **cultivars** in which fewer pots than average become affected (if any).		
Name three **cultivars** in which above average numbers of pots become affected (if any).		

Plant stage	For your six species worst affected by root rot (specify) note whether symptoms were first seen in **home produced (H)** or **bought-in (B)** material.					
	1	2	3	4	5	6
Propagation						
Liners indoor						
Finals indoor						
Liners outdoor						
Finals outdoor						

	Record the **names** of the most commonly affected hosts for each disease. State **months** when the highest % of pots suffer from each **root rot**.					
	Phyto-phthora	*Rhiz-octonia*	*Thiela-viopsis*	*Fusarium*	*Pythium*	Other (specify)
Commonest host						
Propagation						
Liners indoor						
Finals indoor						
Liners outdoor						
Finals outdoor						

Do more plants develop root rot, or show more severe rotting, at certain **crop stages**. Y/N		
Not long after potting-on	When plants need potting-on	At any other time (specify)

Have you used Lateral Flow Devices (**LFDs**) to detect root pathogens Yes/No		
Never	As needed	Other, or comments

	Record the **names** of the most commonly affected hosts. Indicate **months** when the highest % of pots have **die-back**.					
	Phomopsis	*Pestalo-tiopsis*	*Kabatina*	*Botrytis*	*Mycosph-aerella*	*Keithia*
Commonest host						
Propagation						
Liners indoor						
Finals indoor						
Liners outdoor						
Finals outdoor						

2.0 Practices which may affect disease incidence

2.1 Irrigation

Plant stage	Record the **source/s** of irrigation water. State Yes or No at each crop stage.					
	Bore hole	River	Roof	Mains	Recycled from beds	Other (specify)
Propagation						
Liners indoor						
Finals indoor						
Liners outdoor						
Finals outdoor						

Name the water sources	Record the water **treatment** method(s) used on your nursery. State Yes/No.					
	Slow sand filter	Chlorin-ation	UV sterilise	Copper ionisation	Other (specify)	None

Plant stage	Record your usual **method** of irrigation.					
	Hosepipe/ by hand	Overhead automatic	Capillary sand bed	Ebb and flow	Drip	Other (specify)
Propagation						
Liners indoor						
Finals indoor						
Liners outdoor						
Finals outdoor						

Water sample source	Record the **frequency** and type of disease monitoring in water.			
	Weekly	Monthly	Other (specify)	Monitoring technique

2.2 Growing-media

Plant stage	Record the **%** composition of growing-media and if own or proprietary mix.					
	% Peat	% Bark	% Other (specify)	% Other (specify)	Own mix	Proprietary
Propagation						
Liners indoor						
Finals indoor						
Liners outdoor						
Finals outdoor						

2.3 Crop protection

Plant stage	**Mean number of times** plant protection products are on average. incorporated/applied to a crop as it passes through each production stage.				
	Aliette 80W	Filex / Proplant	Subdue	Other (specify)	Other (specify)
Propagation					
Liners indoor					
Finals indoor					
Liners outdoor					
Finals outdoor					

Plant stage	**Mean number of times** bio-stimulant products are on average. incorporated/applied to a crop as it passes through each production stage.				
	Agrilan Revive	Compost Tea	Trianum	Other (specify)	Other (specify)
Propagation					
Liners indoor					
Finals indoor					
Liners outdoor					
Finals outdoor					

Plant stage	**Number of times** disinfectant product is applied to beds/ benches per year.			
	Jet 5	Virkon	Other (specify)	Other (specify)
Propagation				
Liners indoor				
Finals indoor				
Liners outdoor				
Finals outdoor				

3.0 Comments

Please feel free to add any further comments by way of observations and background information e.g. differences between years or changes in growing practice

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Thank you for your time

Please e-mail this form back to Erika.wedgwood@adas.co.uk, or post it to
Dr Erika Wedgwood, ADAS Boxworth, Battlegate Road, Boxworth, Cambridge, CB23 4NN.

The information you provide will be held for the purposes of this research and it will not be disclosed to third parties. The report to HDC will not identify individual nurseries.

Appendix 2 Nursery Sample Recording Form

NURSERY RECORD OF CONIFER ROOT ROT AND DIE-BACK

Plant number	Site	Species	Date sampled	Code

Record of affected crop

Cultivar | **Pot size (L)**

Location | **Area of this crop bed (m x m)**

% plants of this species with dieback/wilt in this area

Severity of wilting/browning (index 1 = bits, 2 = branches, 3 = >60%

Home-produced when | **or** **Bought-in when**

Date any symptoms first seen

Potting on date | **Plant age now (months)**

Root rot seen when; just potted = 1, growing = 2, potting-on due = 3

% of outermost root area affected on removing pot

% of mid/outer roots affected

% of central/original roots affected | **% pot filled by roots**

Stem staining under bark (if present, approx spread in cm up stem)

LFD stem +/- Pythium | **LFD roots +/- Pythium**

LFD stem +/- Phytophthora | **LFD roots +/- Phytophthora**

Appendix 3 Instructions for using Pocket Diagnostic Lateral Flow Device

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

Step 1; Plant material selection

- Refer to the Keycard in the kit for any specific guidance on sampling.
- Where possible, select areas of leaf, stem, root, fruit or flowers which show the effects (symptoms) of disease. For best results include material where healthy and diseased zones meet (the leading edge).
- Do not use completely dead plant material.
- If testing roots, remove soil and other debris by washing in clean water (this will remove most fungi living saprophytically on the surface not causing disease).
- Root tips are usually good sample area (as there is often a leading edge).
- With large roots take slices of the outer tissues for testing (recommended for *Pythium*). Cut into small pieces or crush before putting in the bottle.
- Remove slivers of outer bark on stems to sample the inner bark. Ensure that the conducting tissue of the stem under the bark is also sampled – this can be expected to be stained brown in diseased tissue.
- When sampling a large plant, it is advisable to take tissue from several locations on the plant. However, consider whether separate root and shoot samples may be needed if there may be more than one disease present.
- Do not use large amounts of tissue;
    - Leaf material: approximately 0.2 g (about 25 mm^2, the diameter of a 2p coin). Thick cuticle leaves should be crushed or scored before putting in the buffer
    - Hard stem material: 0.2 g (soft stems no more than 5 mm length).
    - Roots: 0.2 g
- Break up large pieces before adding to the buffer bottle. Cutting woody or fleshy material finely into small pieces (softer material will be broken by shaking with the ball bearings in the buffer bottle)
- Ensure that knife blades used to sample, and fingers or scissors used to break up the material are not contaminated by contact with other samples. Wash and dry them or use an alcohol wipe, or use disposable knives and gloves

Step 2; Extraction in buffer

- Unscrew the extraction bottle lid and add the plant material pieces. Replace the lid tightly. Take precautions to avoid cross contamination of samples by carryover of plant material on hands or cutting tools.
    - *Phytophthora*: Shake the bottle vigorously for 20-30 seconds for fine roots and soft samples such as leaves, flowers and fruit. At least 60 seconds will be needed if the material is hard or woody. Shake until the extraction buffer is no longer colourless.
    - *Pythium*: Shake the bottle firmly for 60 seconds.
- The buffer should start to become green or brown as the tissue is broken down. If this does not happen the plant pieces may have been too big, or the shaking not vigorous enough. Beware of over-doing the tissue breakdown because too concentrated a green stain will make the blue line/s on the LFD hard to read.
- Grasping the bottle during shaking will normally warm it to above 10°C to enable the process to work.

Step 3; using the LFD

- If the test is being performed in conditions below 10°C then warm the packaged LFD before opening.
- Remove the test device from its foil packing and place on a level surface with the viewing window upwards. DO NOT TOUCH THE VIEWING WINDOW.
- The test can be carried out with the device held horizontally in the hand and this is recommended if the temperature is below 10°C.
- Each test device can be used once only. Foil packs should be kept sealed until required. Once the foil pack is opened, shelf life is not guaranteed.
- Allow the plant debris a few seconds to settle in the extraction bottle.
- Remove the lid from the extraction bottle and draw some of the liquid into the pipette from above the debris.
- Gently squeeze 2 or 3 drops of the sample liquid into the sample well of the test device. Blue dye will start to flow across the sample strip. Take care not to flood the sample well.

Step 4: Examining the results

- After about 30 seconds blue dye will appear in the viewing window as liquid flows along the test device.
- A blue line (the Control line) will appear next to the letter 'C' on the device. This line confirms the test is working properly.
- If the test is positive, a second blue line, the Test line (next to the letter 'T'), will appear.
- The lines can appear in *Pythium* and *Phytophthora* kits within 3 – 4 minutes of adding the sample to the device, but may take up to 10 minutes
- If no blue dye becomes visible in the viewing window after 30 seconds, a third drop of sample can be added to the sample well. Using too much sample will cause the test to run incorrectly.
- If the test still runs very slowly tap the device gently to remove any air bubbles.
- If too much debris has been added with the sample liquid the test will run slowly. It may be necessary to use a new device with clearer liquid from the extraction bottle.
- Read the result within 10 minutes of adding the sample to the device. Ignore any changes which happen after 10 minutes.
- Where comparison of the strength of the line between samples is being sought for research purposes the LFD should be placed against a similar coloured background and read under a good light.
- Note that disease symptoms can be caused by a mixed infection, and that further testing for other causes of disease might be necessary.
- Use the label on the reverse of the device to write details such as sample identification, date and result.
- After use, the test devices can be stored for long durations with only slight loss of results if kept dry and out of the light.

Step 5: Interpretation of the results

- A positive result indicates that the plant material sampled contains the fungus under test. Note that disease symptoms can be caused by a mixed infection, and that further testing for other causes of disease might be necessary.
- Under some circumstances, laboratory confirmation of an on-site test result may be necessary. If tests show positive for a notifiable or quarantine pathogen, the customer is responsible for complying with the local regulations on reporting to the official plant health inspection authorities.

- A negative result indicates that the target pathogen was not detected in the test sample. As with all diagnostic testing, a negative result does not confirm that the plant is free from the fungus under test.
- A faint or absent line may indicate a low concentration of the pathogen, uneven distribution in the host, or recent infection.

Problems with the readings

- Faint test lines are caused by either low pathogen concentration; uneven distribution; too small a sample; sample not broken up enough; or sample not shaken long enough. If in doubt, repeat with a new device using a fresh sample, or repeat in a few days.
- 'T' line visible, but no 'C' line may be due to a high level of pathogen in the sample, preventing test from working properly. Dilute sample 1 in 10 and 1 in 100 with fresh buffer and retest with new device.
- No 'T' line, no 'C' line can occur when too much sample material is added. Retest with a new device.

Appendix 4 Information provided by ADAS to growers on diagnostic kits

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Bedding and Pot Plant Notes ADAS

September 2010 No. 252 (Editor J. England, copyright ADAS)

Bedding, patio and pot plant species are prone to a wide range of diseases caused by fungal pathogens, bacteria and viruses, many of which cannot be correctly identified without further tests. Accurate diagnosis of the causes of diseases allows affected plants to be segregated, disposed of or for treatments to be applied as appropriate. It is important that viruses are identified early to allow insect vectors to be controlled to prevent further spread of the disease through the crop.

The options available for pest and disease identification are to send samples to a plant clinic or to use mobile test kits such as the Pocket Diagnostic™ kits produced by Forsite Diagnostics, based in York, and the Flashkits produced by the Belgian company, Biobest. Such tests are specific for individual pathogens as listed in the table below. Further tests by a plant clinic or the Forsite PDplus™ service (*Phytophthora* diseases only*)* will be needed if identification of the pathogen species involved in an infection is required.

Biobest Flashkits and Forsite Pocket Diagnostic™ kits each have slightly different designs:

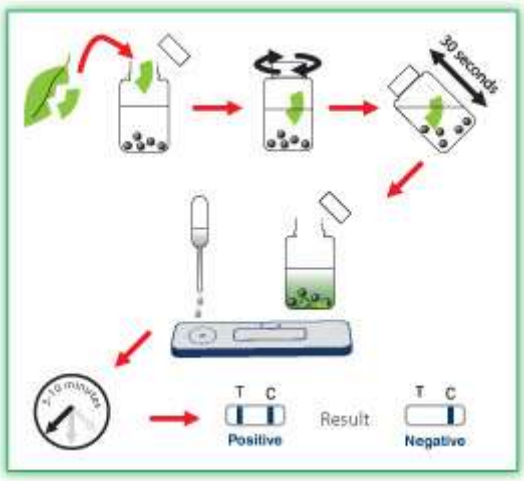
Biobest Flashkits

Biobest Flashkits are easy to use (see images below). A plant sample with symptoms is crushed in the extraction bag provided, the appropriate detection strip is inserted and after a few minutes a clear indication of the result is given, with two lines developing to indicate a positive result. Biobest Flashkits are immuno-chromatography-based kits which use the antigen-antibody-reaction to detect the presence of a specific pathogen. Biobest kits should be stored at between 4°C and 6°C, under dark and dry conditions. Temperatures greater than 37°C and below 0°C should be avoided.

http://www.biobest.be

Forsite Pocket Diagnostic™ kits

For Forsite Pocket Diagnostic™ tests a section of leaf (approximately 3 cm x 4 cm) with symptoms is placed into the bottle supplied containing buffer solution and ball bearings and shaken for 30 seconds (see image below). The plant sap extract obtained is applied to the lateral flow device (LFD).



www.pocketdiagnostic.com/quickstart_guide

The LFD uses technology similar to that used in home pregnancy test kits. Drops of buffer solution containing the extracted plant sap is deposited onto the LFD and then flows across a membrane which contains monoclonal antibodies which recognise specific mycelial proteins. Samples are left for up to 10 minutes, depending on which kit is being used. The results are easily interpreted: two coloured lines develop to indicate a positive result (see image below).

Pocket Diagnostic™ kits should be stored at room temperature, not refrigerated or frozen.

Forsite PDplus™ service

Whilst the Forsite Pocket Diagnostic™ kits will diagnose *Phytophthora* pathogens in general, Forsite Diagnostics have also developed their PDplus™ service whereby molecular characterisation of a target pathogen is carried out using PCR (polymerase chain reaction) techniques to give a precise identification of the pathogen. This service is currently available for *Phytophthora* and is limited to four species: *P. ramorum*, *P. kernoviae*, *P. fragariae* and *P. cactorum*.

For this service, the customer carries out a Pocket Diagnostic™ test which is then sent to Forsite Diagnostics who analyse DNA from the positive LFD to confirm the identity of the pathogen. Results are provided within 48 hours.

Activity range

Diagnostic test kits are available to test for a wide range of pathogens as shown in the table below:

Pathogen		Forsite Diagnostics	Biobest
Erwinia amylovora		✓	
Xanthomonas campestris pv. *pelargonii* (syn. *Xanthomonas hortorum* pv. *pelargonii*)	Xcp	✓	✓
Acidovorax avanae spp. citrulli	Aac		✓
Clavibacter ssp. michiganensis	Cmm		✓
Ralstonia solanacearum	Rs	✓	✓
Botrytis		✓	
Rhizoctonia *		✓	
Pythium		✓	
Phytophthora		✓	
Arabis mosaic virus	ArMV		✓
Calibrachoa mottle virus	CbMV		✓
Cucumber mosaic virus	CMV	✓	✓
Cymbidium mosaic virus	CyMV	✓	✓
Hosta virus X	HVX		✓
Impatiens necrotic spot virus	INSV	✓	✓
Odontoglossum ringspot virus	ORSV	✓	✓
Pepino mosaic virus	PepMV		✓
Potato virus Y	PVY		✓
Tobacco mosaic virus	TMV		✓
Tomato mosaic virus	ToMV	✓	✓
Tomato spotted wilt virus	TSWV	✓	✓

*currently unavailable

UK distributors

Forsite and Biobest products are distributed within the UK by Agralan Ltd, Forsite Diagnostics and Aquaculture Ltd:

	Email:	Website
Agralan Ltd	sales@agralan.co.uk	www.agralan.co.uk
Forsite Diagnostics Ltd	info@forsitediagnostics.com	www.forsitediagnostics.com
Aquaculture Ltd	callie@aquacult.com	www.aquaculture-hydroponics.co.uk

The Forsite LFDs for *Pythium* and *Phytophthora* are currently being utilised by ADAS as part of HDC project HNS 181. They are also used by ADAS Horticulture Consultants to provide initial diagnoses of plant diseases. Please contact your local ADAS Horticulture Consultant for further information.

Appendix 5 Nursery bait placement in water reservoirs; results of *Pythium* and *Phytophthora* LFD and PCR testing

(The same (domestic garden) source of *Ceanothus* and Nordmann Fir leaves were used without surface sterilisation for baits at each site LFD Test line strength is given where 1 = very very faint band (negative) to 4 strong sharp line (positive) and may relate to the quantity of water-mould material present to give an antibody/antigen reaction).

Appendix 5.1 Nursery 1E Baits placed 3 June 2010 using five needles each (the leaf area which would be required for the LFD test).

Sample code	Date of LFD test	Lesion visible	*Pythium* LFD +/-	*Phyto-phthora* LFD +/-	*Pythium* PCR +/-	PCR identification	*Phyto-phthora* PCR +/-	PCR identification
Bait 1 Nordmann Fir needles	11.06.10	N	1-	1-	+	*Pythium catenulatum* or *torulosum*	-	Not applicable
Bait 2 Nordmann Fir needles	11.06.10	Y, but likely a wound	1-	1-	+	*Pythium catenulatum* or *torulosum*	-	Not applicable
Bait 1 Nordmann in LFD buffer		n.a.	n.a.	n.a.	+	*Pythium catenulatum* or *torulosum*	-	Not applicable
Bait 2 Nordmann in LFD buffer		n.a.	n.a.	n.a.	+	*Pythium catenulatum* or *torulosum*	-	Not applicable

One bait was wrapped in muslin, the other in fleece, but this was not noted at sampling.
Needles for LFD were not those used in the needle PCR, but the LFD tested needles were in the buffer used for PCR

Appendix 5.2 Nursery 2E Baits placed 15 June 2010. 12 *Ceanothus*, and 20 needles per bait.

Sample code	Date of LFD test	Lesion visible	*Pythium* LFD +/-	*Phytophthora* LFD +/-	*Pythium* PCR +/-	PCR identification	*Phytophthora* PCR +/-	PCR identification
Bait 1 Nordmann Muslin	22.06.10	Y a few	0-	2+	-	Not applicable	+	*Phytophthora* sp. *Salixsoil*
Bait 2 Nordmann Fleece	22.06.10	Y?*	1-	0-	-	Not applicable	+	*Phytophthora* sp. *Salixsoil*
Bait 3 *Ceanothus* Fleece	22.06.10	Y#	2+	4+	-	Not applicable	+	*Phytophthora* sp. *Salixsoil*
Bait 1 Nordmann in LFD buffer			n.a.	n.a.	+	poor PCR *Pythium* sp	-	Not applicable
Bait 2 Nordmann in LFD buffer			n.a.	n.a.	+	*Pythium catenulatum* or *torulosum*	-	Not applicable
Bait 3 *Ceanothus* in LFD buffer			n.a.	n.a.	-	Not applicable	+	*Phytophthora* sp. *Salixsoil*
Bait 1 Nordmann 2nd sample			n.a.	n.a.	+	*Pythium* sp closest to *heterothallicum* and *glomeratum*	-	Not applicable
Bait 2 Nordmann 2nd sample			n.a.	n.a.	+	poor *Pythium* sp.	+	*Phytophthora* sp. closest to *cryptogea*
Bait 3 *Ceanothus* 2nd sample			n.a.	n.a.	-	Not applicable	+	*Phytophthora* sp. *Salixsoil*
Bait 4 *Ceanothus* grower test	August 10		Not tested	+	Not tested	Not applicable	Not tested	Not applicable

Y?* Nordmann bait in fleece – A few lesion were seen on the needles but they are all centred round wound sites (supporting negative LFDs)
Y# *Ceanothus* bait in fleece – These leaves were found to have a larger amount of lesions/staining present. It was noticeable that they weren't just associated with the edges, wound sites or veins of the leaves, suggesting definite lesion and not general rotting.

Leaves for LFD were not those used in the leaf PCR, but the LFD leaves were in the buffer used for PCR.

Appendix 5.3 Nursery 4E Baits placed 15 July 2010. 12 *Ceanothus*, and 20 needles per bait.

Sample code	Date of LFD test	Lesion visible	*Pythium* LFD +/-	*Phytophthora* LFD +/-	*Pythium* PCR +/-	PCR identification	*Phytophthora* PCR +/-	PCR identification
Bait 1 Nordmann Fleece	22.07.10	N*	1-	2+	-	Not applicable	+	*Phytophthora* sp. *Salixsoil*
Bait 2 *Ceanothus* Fleece	22.07.10	Y#	0-	2+	-	Not applicable	+	*Phytophthora* sp. *Salixsoil*
Bait 1 Nordmann in LFD buffer		n.a.	n.a.	n.a.	-	Not applicable	+	*Phytophthora* sp. *Salixsoil*
Bait 2 *Ceanothus* in LFD buffer		n.a.	n.a.	n.a.	-	Not applicable	+	*Phytophthora* sp. *Salixsoil*

Both Phytophthora sp. Salixsoil from leaves and lateral flow device
N* Nordmann bait in fleece - There was discolouration of some of the needles, although not in distinct lesions, that would indicate infections.
Y# *Ceanothus* bait in fleece - There were clear lesion present on the leaves, particularly noticeable on and around the veins.

Leaves for LFD were not those used in the leaf PCR, but the LFD leaves were in the buffer used for PCR.

Appendix 6 Assessments, LFD and PCR tests of conifer samples obtained from seven nurseries from June to August 2010

The wilt index allocated to each plant gave a general impression of the severity of symptoms with 1 = bits of foliage wilted or brown, 2 = some branches wilted or brown, 3 = more than 60% of the foliage wilted (or feeling soft) or brown and dry. Some foliage was examined in the laboratory in addition to the LFD tests. Whether or not there was stem staining and/or and root rot is recorded. No LFD test was carried out (marked as n.t. in Appendix tables 6.1 to 6.7) on stems which did not show staining under the bark, or on roots if there was no obvious rotting.

An LFD index was used to record the strength of blue test line where 0 = no line, 1 = very very faint indefinite line was counted as a negative, 2 = faint blue but definite line counted as positive, 3 = obvious blue line were positive, 4 = strong dark blue line (as strong as control line) obvious positive. Plants were sent for PCR testing if they produced a positive test line on one of the LFDs.

The selection of LFDs sent for PDplus testing to match against the PCR tests carried out directly on the plants by the RHS are marked in bold in the tables.

Appendix 6.1 Nursery 1E results of wilting, staining and root rot and LFD and PCR tests carried out on samples collected on 3rd June 2010

	Foliage		Stem under bark					Root browning and rotting				
Sample code	**Wilt / die-back**	**Lab check done**	**Stem stain seen**	***Pythium* LFD**	***Phyto-phthora* LFD**	***Pythium* PCR**	***Phyto-phthora* PCR**	**Root rot seen**	***Pythium* LFD +/-**	***Phyto-phthora* LFD +/-**	***Pythium* PCR +/-**	***Phyto-phthora* PCR +/-**
1E.1	2	n	n	n.t.	n.t.	n.t.	n.t.	y	4+	**0-**	-	+
1E.2	3	n	n	n.t.	n.t.	n.t.	n.t.	y	2+	1-	+	-
1E.3	3	n	n	n.t.	n.t.	n.t.	n.t.	y	1-	4+	-	+
1E.4	2	n	n	n.t.	n.t.	n.t.	n.t.	y	2+	0-	-	-
1E.4 extra	2	n	n	n.t.	n.t.	n.t.	n.t.	y	n.t.	n.t.	-	+
1E.5	3	n	y	0-	0-	n.t.	n.t.	y	1-	2+	n.t.	n.t.

Sample 1 = *Araucaria*, 2 = *Taxus baccata*, 3 = *Juniperus conferta,* 4 = *Araucaria,* 5= *Chamaecyparis lawsoniana* cv.. Snow White.

Appendix 6.2 Nursery 2E results of wilting, staining and root rot and LFD and PCR tests carried out on samples collected on 15th June 2010

	Foliage		Stem under bark					Root browning and rotting				
Sample code	**Wilt / die-back**	**Lab check done**	**Stem stain seen**	***Pythium* LFD**	***Phyto-phthora* LFD**	***Pythium* PCR**	***Phyto-phthora* PCR**	**Root rot seen**	***Pythium LFD***	***Phyto-phthora* LFD**	***Pythium* PCR**	***Phyto-phthora* PCR**
2E.1	3	n	n	n.t	n.t.	+	+	y	1-	2+	+	-
2E.2	1	n	y	2+	4+	-	-	y	2+	3+	-	+
2E.3	3	n	y	0-	**0-**	-	+	y	2+	**4+**	-	+
2E.4	3	n	n.t.	n.t.	n.t.	n.t	n.t	y	n.t.	3+	n.t.	n.t.

Sample 1 = *Chamaecyparis lawsoniana* cv.. Ellwoodii, 2 = *Juniperus squamata* cv.. Blue Carpet, 3 = *Pinus sylvestris*, 4 =*Taxus baccata*

Appendix 6.3 Nursery 3E results of wilting, staining and root rot and LFD and PCR tests carried out on samples collected on 9th August 2010

	Foliage		Stem under bark					Root browning and rotting				
Sample code	**Wilt / die-back**	**Lab check**	**Stem stain seen**	***Pythium* LFD +/-**	***Phyto-phthora* LFD +/-**	***Pythium* PCR +/-**	***Phyto-phthora* PCR +/-**	**Root rot seen**	***Pythium* LFD +/-**	***Phyto-phthora* LFD +/-**	***Pythium* PCR +/-**	***Phyto-phthora* PCR +/-**
3E.1	3	y	n	n.t.	n.t.	n.t	n.t	y	2+	**2+**	-	+
3E.2	2	n	y	2+	4+	+*	+	y	1-	4+	-	+

Sample 1 = *Taxus fastigiata aurea* (no *Pythium* isolated from upper stem which would be used for cuttings) 2 = *Juniperus* cv.. Old Gold (staining only on trimmed branch, not below)

Appendix 6.4 Nursery 4E results of wilting, staining and root rot and LFD and PCR tests carried out on samples collected on 15th July 2010

Sample code	Foliage		Stem under bark					Root browning and rotting				
	Wilt / die-back	Lab check	Stem stain seen	*Pythium* LFD +/-	*Phyto-phthora* LFD +/-	*Pythium* PCR +/-	*Phyto-phthora* PCR +/-	Root rot seen	*Pythium* LFD +/-	*Phyto-phthora* LFD +/-	*Pythium* PCR +/-	*Phyto-phthora* PCR +/-
4E.1	1	y	y	3+	0-	-	-	y	3+	3+	-	+
4E.2	3	n	y	1-	**4+**	-	+	y	4+	**4+**	+	-
4E.3	2	n	y	4+	4+	-	+	y	4+	2+	-	+
4E.4	3	n	n	n.t.	n.t.	n.t	n.t.	y	3+	4+	+	+
4E.5	3	n	y	1-	**4+**	-	+	y	4+	**4+**	-	+
4E.6	3	n	n.t.	n.t.	n.t.	n.t	n.t.	y	4+	0-	-	-
4E.7	3	n	y	4+	4+	-	+	n	n.t.	n.t	+	-

Sample 1 = *Chamaecyparis obtusa* cv.. Karamachiba (also had a pycnidial leaf spot), 2 = *Chamaecyparis lawsoniana* cv.. Ellwoods Pillar, 3 = *Juniperus* cv.. Gold Coast, 4 = *Cupressocyparis x Cupressocyparis* cv.. Gold River, 5 = *Chamaecyparis lawsoniana* cv.. Sulphur Spire, 6 = *Thuja occidentalis* cv.. Micky, 7 = *Juniperus virginata* cv.. Blue Arrow (only brown around the ring of twigs, not below 40 mm)

Appendix 6.5 Nursery 1W results of wilting, staining and root rot and LFD and PCR tests carried out on samples collected on 11th August 2010

Sample code	Foliage		Stem under bark					Root browning and rotting				
	Wilt / die-back	Lab check	Stem stain seen	*Pythium* LFD +/-	*Phyto-phthora* LFD +/-	*Pythium* PCR +/-	*Phyto-phthora* PCR +/-	Root rot seen	*Pythium* LFD +/-	*Phyto-phthora* LFD +/-	*Pythium* PCR +/-	*Phyto-phthora* PCR +/-
1W.1	2		y	**2+**	0-	-	+	n	n.t.	n.t.	n.t.	n.t.
1W.2	3		n	n.t.	n.t.	n.t.	n.t.	y	0+	1-	-	-
1W.3	2		y	0-	0-	n.t.	n.t.	n	n.t.	n.t.	n.t.	n.t.
1W.4	2		dead	n.t.	0-	-	-	y	3+	4+	-	+
1W.6	1		n	n.t.	n.t.	n.t.	n.t.	y	1-	2+	+	-
1W.6	3		y	0-	4+	-	-	y	No kit left	**4+**	-	+

Sample 1 = *Taxus standishii*, 2 = *Picea breweriana,* 3 = *Abies* cv.. Pigelmee, 4 = *Juniperus communis* cv.. Compressa 5 = *Chamaecyparis lawsoniana Minima aurea*, 6 = *Abies amabilis* cv.. Spreading Star

Appendix 6.6 Nursery 5E results of wilting, staining and root rot and LFD and PCR tests carried out on samples collected on 18th August 2010

	Foliage		Stem under bark					Root browning and rotting				
Sample code	**Wilt / die-back**	**Lab check**	**Stem stain seen**	***Pythium* LFD +/-**	***Phyto-phthora* LFD +/-**	***Pythium* PCR +/-**	***Phyto-phthora* PCR +/-**	**Root rot seen**	***Pythium* LFD +/-**	***Phyto-phthora* LFD +/-**	***Pythium* PCR +/-**	***Phyto-phthora* PCR +/-**
5E.1	0	n	n	n.t.	n.t	n.t	n.t	y	4+	4+	-	+
5E.2	0	n	n	n.t.	n.t.	n.t	n.t	y	4+	4+	-	+
5E.3	2	n	leaf	3+	0-	-	-	n	n.t	n.t.	n.t.	n.t.
5E.4	1	y	y	4+	0-	+	-	y	3+	4+	+*	+
5E.5	0	n	n	n.t.	n.t.	n.t.	n.t.	y	4+	**3+**	-	+

Sample 1 = *Chamaecyparis lawsoniana columnaris glauca* 3L (Zero wilt but the grower recognised the plants as less vigorous)
Sample 2 = *Chamaecyparis lawsoniana* cv.. Blue Surprise 3L (Zero wilt but the grower recognised the plants as less vigorous)
Sample 3 = *Chamaecyparis lawsoniana* cv.. Penberry Blue (2 leafy stem cuttings not long rooted with a partially brown wilted fan of foliage and clean roots so no root was assessed).
Sample 4 = *Chamaecyparis lawsoniana* cv.. Obtusa 3L (sample from base of brown wilted fan of foliage)
Sample 5 = *Chamaecyparis lawsoniana* cv.. Petts Blue 3L (Zero wilt but the grower recognised the plants as less vigorous)

Appendix 6.7 Nursery 2W results of wilting, staining and root rot and LFD and PCR tests carried out on samples collected on 27th August 2010

	Foliage		Stem under bark					Root browning and rotting				
Sample code	**Wilt / die-back**	**Lab check**	**Stem stain seen**	***Pythium* LFD +/-**	**Phyto-phthora LFD +/-**	***Pythium* PCR +/-**	***Phyto-phthora* PCR +/-**	**Root rot seen**	***Pythium* LFD +/-**	***Phyto-phthora* LFD +/-**	***Pythium* PCR +/-**	***Phyto-phthora* PCR +/-**
2W.1	3	n	n.t.	n.t.	n.t.	n.t.	n.t.	y	4+	2+	-	+
2W.2	3	n	n.t.	n.a.	n.a.	n.t.	n.t.	y	2+	3+	+	-

Sample 1 = *Chamaecyparis lawsoniana* cv.. Columnaris, 2 = *Chamaecyparis lawsoniana* cv.. Snow White

Appendix 7.1 RHS plant clinic records for conifer root rot and dieback from 2007 to 2009 (provided by G. Denton)
Species identification by the RHS using PCR testing of plant material and also following extraction using apple and hemp seed baits

Cultivar	Received	GridReference	Sample taken from stems	Sample taken from roots
Abies	18-Sep-07	TQ377953		*Phytophthora citrophthora*
Araucaria araucana	23-Jul-99	TQ015657		*Phytophthora cryptogea*
Cedrus	01-Oct-08	TQ065583	*Pythium diclinum*	
Cedrus libani	25-Mar-09	SU262708		*Pythium intermedium*
Cedrus libani	29-Apr-09	TQ211495		*Pythium dissotocum / lutarium*
Chamaecyparis	21-Aug-00	TQ145556		*Phytophthora cryptogea*
Chamaecyparis	13-Apr-07	SP719053		*Pythium intermedium*
Chamaecyparis	25-May-07	SU687959		*Phytophthora cinnamomi*
Chamaecyparis	05-Jul-07	SU995590		*Phytophthora megasperma*
Chamaecyparis	05-Oct-07	TQ006592		*Phytophthora cryptogea* *Pythium diclinum*
Chamaecyparis	19-Dec-07	TQ244735		*Pythium heterothallicum*
Chamaecyparis	01-Aug-08	SJ364784		*Pythium* species
Chamaecyparis lawsoniana	01-May-07	SN289362		*Pythium macrosporum* *Pythium intermedium* *Pythium vexans*
Chamaecyparis lawsoniana	06-Mar-09	SU807535		*Pythium* species
Chamaecyparis lawsoniana	06-Mar-09	SU807535	*Pythium* species	
Chamaecyparis lawsoniana	22-Jan-09	TQ040618		*Pythium* species
Cupressus	14-Apr-08	TQ136561		*Pythium vanterpoolii*
Cupressus	14-May-08	SU971534		*Pythium intermedium*
Cupressus	24-Jun-08	TQ294716		*Pythium sylvaticum* *Phytophthora gonapodyides*
x Cupressocyparis leylandii	01-May-07	SN289362		*Pythium vexans*
x Cupressocyparis leylandii	22-Nov-07	TQ217620		*Pythium heterothallicum*
x Cupressocyparis leylandii	15-Jul-08	SP678222		*Phytophthora gonapodyides* *Pythium* species
x Cupressocyparis leylandii	13-Aug-08	SE664474		*Pythium montanum / carbonicum* *Pythium sterilum / litorale*
x Cupressocyparis leylandii	18-Aug-08	SU573668		*Pythium intermedium*

x Cupressocyparis leylandii	17-Feb-09	SU879210		*Pythium intermedium*
x Cupressocyparis leylandii	03-Sep-09	SU993677		*Phytophthora cryptogea*
x Cupressocyparis leylandii	09-Nov-09	SU948420		*Pythium sylvaticum* *Pythium torulosum / folliculosum* *Pythium* species
Juniperus	02-May-07	TQ065583		*Pythium* species
Juniperus	13-Jun-07	SE382057		*Phytophthora plurivora*
Juniperus	10-Aug-07	SU175688		*Phytophthora plurivora*
Juniperus	16-Aug-07	TQ036556		*Pythium intermedium*
Juniperus	02-Jul-08	TG498041		*Pythium intermedium*
Juniperus	15-Jul-08	SE250449		*Pythium intermedium*
Juniperus	02-Jun-09	SN953058	*Phytophthora austrocedrae* *Pythium* species	*Pythium intermedium* *Pythium* species *Pythium anandrum*
Juniperus communis	21-Jul-99	TQ318659		*Phytophthora cinnamomi*
Juniperus communis	22-May-07	TQ036556		*Phytophthora citricola*
Juniperus communis	22-May-07	TQ036556		*Phytophthora cinnamomi*
Juniperus sabina	07-Aug-08	SP289328		*Pythium intermedium*
Juniperus squamata	01-May-07	SN289362		*Pythium attrantheridium*
Juniperus x pfitzeriana	12-Aug-09	TQ607486		*Phytophthora cryptogea*
Larix	13-Aug-09	SU700819	*Phytophthora plurivora* *Pythium dissoticum / lutarium*	*Pythium intermedium*
Thuja	01-Mar-07	TQ231623		*Pythium sylvaticum*
Thuja	21-May-07	TQ443886		*Pythium* species
Thuja	15-Oct-03	TQ100696		*Phytophthora cryptogea*
Thuja	22-Apr-08	SU872598	*Pythium* species *Pythium attrantheridium*	*Pythium vexans / cucurbitacearum* *Pythium attrantheridium*
Thuja	04-Nov-08	TL188195		*Pythium* species *Pythium amasculinum* *Pythium intermedium*
Thuja	29-Jul-09	SP958201		*Pythium intermedium*
Thuja plicata	28-Oct-05	SU398842		*Phytophthora citrophthora*
Thuja plicata	01-Nov-04	TL771010		*Phytophthora hibernalis*

Appendix 7.2 RHS plant clinic records conifer root rot and dieback for *Taxus* from 2007 to 2009 (provided by G. Denton)

Cultivar	Received	GridReference	Sample taken from stems	Sample taken from roots
Taxus baccata	14-Jan-99	SK356104		*Phytophthora citricola*
Taxus baccata	05-Oct-99	SP706285		*Phytophthora cinnamomi*
Taxus baccata	13-Jul-00	TQ142649		*Phytophthora cinnamomi*
Taxus baccata	08-Aug-00	SP506105		*Phytophthora cinnamomi*
Taxus baccata	25-Aug-00	TQ065583		*Phytophthora cinnamomi*
Taxus baccata	31-Oct-00	NY141215		*Phytophthora cryptogea*
Taxus baccata	12-Feb-01	TQ390227		*Phytophthora citricola*
Taxus baccata	05-Nov-03	TQ694991	*Phytophthora cryptogea*	*Phytophthora cryptogea*
Taxus baccata	01-Dec-03	SU476615		*Phytophthora citrophthora*
Taxus baccata	13-Feb-04	NZ032802		*Phytophthora citricola*
Taxus baccata	23-Mar-04	TQ083651		*Phytophthora cinnamomi*
Taxus baccata	15-Apr-04	ST810771		*Phytophthora citricola*
Taxus baccata	27-May-04	TQ460373		*Pythium intermedium*
Taxus baccata	02-Jun-04	TQ137650		*Phytophthora citricola*
Taxus baccata	07-Jun-04	TQ334857		*Phytophthora cinnamomi*
Taxus baccata	20-Sep-04	ST665142		*Phytophthora syringae*
Taxus baccata	14-Oct-04	SP068034		*Phytophthora citricola*
Taxus baccata	18-Oct-04	SK139354		*Phytophthora cinnamomi*
Taxus baccata	18-Oct-04	TQ523161		*Phytophthora plurivora*
Taxus baccata	22-Oct-04	SN005127		*Phytophthora cinnamomi*
Taxus baccata	22-Oct-04	TQ428236		*Phytophthora citricola*
Taxus baccata	11-Nov-04	SJ554629		*Phytophthora cryptogea*
Taxus baccata	13-Dec-04	SP162179		*Phytophthora citrophthora*
Taxus baccata	14-Dec-04	SK139354		*Phytophthora cinnamomi*
Taxus baccata	14-Dec-04	SK139354		*Phytophthora cryptogea*
Taxus baccata	14-Dec-04	SK139354		*Phytophthora cinnamomi*
Taxus baccata	21-Jan-05	SJ402660		*Phytophthora cinnamomi*
Taxus baccata	10-Feb-05	SU941654		*Phytophthora cinnamomi*
Taxus baccata	04-Apr-05	SJ614535	*Phytophthora cinnamomi*	*Phytophthora citricola*
Taxus baccata	27-Apr-05	TR029578		*Phytophthora cinnamomi*
Taxus baccata	28-Jul-05	TM280987	*Phytophthora gonapodyides*	*Phytophthora citricola*

Taxus baccata	06-Jan-06	SD528423		*Pythium* species
Taxus baccata	21-Mar-06	SO939220		*Phytophthora cinnamomi*
Taxus baccata	29-Mar-06	TQ692534		*Phytophthora cinnamomi*
Taxus baccata	21-Apr-06	TQ065583		*Phytophthora cinnamomi*
Taxus baccata	15-May-06	SP061289	*Pythium* sp.	*Phytophthora cinnamomi*
Taxus baccata	16-Jun-06	TQ843375	*Phytophthora cinnamomi*	*Phytophthora cinnamomi*
Taxus baccata	06-Sep-06	SK341426		*Phytophthora citricola*
Taxus baccata	02-Jan-07	TQ255757		*Phytophthora cryptogea*
Taxus baccata	17-Jan-07	TQ684087		*Phytophthora cinnamomi*
Taxus baccata	30-Jan-07	TF878242	*Phytophthora citrophthora*	
Taxus baccata	31-Jan-07	SJ278806		*Pythium* species
Taxus baccata	22-Feb-07	TF664145	*Phytophthora cinnamomi*	*Phytophthora cinnamomi*
Taxus baccata	23-Feb-07	SU900315		*Phytophthora citricola*
Taxus baccata	01-Mar-07	SU994893		*Pythium* species
Taxus baccata	12-Apr-07	TQ345140		*Phytophthora plurivora*
Taxus baccata	12-Apr-07	TQ345140		*Pythium intermedium*
Taxus baccata	27-Apr-07	TR179543	*Phytophthora cinnamomi*	*Phytophthora cinnamomi*
Taxus baccata	01-May-07	TQ065583		*Pythium montanum*
Taxus baccata	22-May-07	SU965351		*Phytophthora gonapodyides*
Taxus baccata	21-Jun-07	SU878205	*Pythium attrantheridium* *Phytophthora citricola*	*Pythium sylvaticum*
Taxus baccata	25-Jun-07	TQ037633		*Phytophthora cryptogea*
Taxus baccata	16-Jul-07	SU900315		*Phytophthora cinnamomi*
Taxus baccata	18-Jul-07	TQ632162		*Phytophthora citricola*
Taxus baccata	18-Jul-07	TQ065583		*Phytophthora cinnamomi*
Taxus baccata	18-Jul-07	TQ007269		*Phytophthora citrophthora*
Taxus baccata	21-Aug-07	TM382642	*Phytophthora plurivora*	*Phytophthora citricola*
Taxus baccata	03-Sep-07	ST180799		*Phytophthora cinnamomi*
Taxus baccata	03-Oct-07	TQ007598	*Phytophthora citricola* *Phytophthora cinnamomi*	*Pythium intermedium*
Taxus baccata	19-Oct-07	TL431398		*Phytophthora cryptogea*
Taxus baccata	31-Oct-07	TQ065583		*Phytophthora cinnamomi* *Pythium intermedium*
Taxus baccata	06-Nov-07	SU784444		*Pythium intermedium*
Taxus baccata	06-Nov-07	ST883792		*Phytophthora gonapodyides* *Pythium* species

Taxus baccata	07-Nov-07	TQ065583		*Phytophthora cryptogea* *Pythium diclinum*
Taxus baccata	12-Nov-07	TQ1017647		*Pythium ultimum*
Taxus baccata	13-Nov-07	TL118730		*Phytophthora cryptogea* *Pythium intermedium*
Taxus baccata	13-Nov-07	TQ639319		*Phytophthora cinnamomi*
Taxus baccata	19-Nov-07	SJ891576		*Pythium intermedium*
Taxus baccata	04-Dec-07	SU990909		*Pythium* species *Pythium intermedium*
Taxus baccata	03-Jan-08	SP325655	*Phytophthora* species *Pythium* species	*Pythium intermedium*
Taxus baccata	03-Jan-08	SP325655	*Phytophthora cryptogea*	*Pythium intermedium*
Taxus baccata	15-Jan-08	SP031661		*Phytophthora cryptogea* *Pythium* species *Phytophthora cinnamomi*
Taxus baccata	15-Jan-08	SP031661		*Phytophthora cryptogea* *Pythium* species
Taxus baccata	15-Jan-08	TQ065583		*Pythium sylvaticum*
Taxus baccata	15-Jan-08	TQ065583		*Pythium sylvaticum*
Taxus baccata	23-Jan-08	SU680221		*Phytophthora citricola* *Pythium sylvaticum*
Taxus baccata	01-Feb-08	TQ830408		*Phytophthora citricola*
Taxus baccata	14-Feb-08	SU344333		*Pythium oligandrum* *Pythium* species
Taxus baccata	14-Feb-08	SU344333		*Pythium amasculinum*
Taxus baccata	15-Feb-08	SO956208		*Phytophthora cinnamomi* *Phytophthora plurivora*
Taxus baccata	15-Feb-08	SO956208		*Pythium intermedium* *Pythium* species
Taxus baccata	25-Feb-08	SP318673		*Phytophthora cryptogea* *Phytophthora* species
Taxus baccata	07-Mar-08	SP635677		*Pythium* species
Taxus baccata	11-Mar-08	SU738791		*Phytophthora megasperma* *Pythium intermedium*
Taxus baccata	11-Mar-08	SU738791		*Phytophthora cryptogea* *Phytophthora cryptogea* *Pythium perplexum*

				Pythium intermedium
Taxus baccata	04-Mar-08	SO442157		*Pythium attrantheridium*
Taxus baccata	07-Apr-08	SK568107		*Phytophthora cryptogea*
Taxus baccata	17-Apr-08	SP482148		*Pythium vanterpoolii* *Pythium intermedium*
Taxus baccata	21-Apr-08	ST886978	*Phytophthora cinnamomi* *Phytophthora cryptogea*	
Taxus baccata	22-Apr-08	TM216637	*Phytophthora cinnamomi*	*Phytophthora cinnamomi* *Pythium intermedium*
Taxus baccata	22-Apr-08	TM216637	*Phytophthora plurivora* *Phytophthora cinnamomi* *Pythium intermedium*	*Phytophthora cinnamomi* *Pythium intermedium*
Taxus baccata	30-Apr-08	TQ377953		*Pythium intermedium*
Taxus baccata	27-May-08	SO907762		*Pythium intermedium* *Pythium* species
Taxus baccata	29-May-08	SJ403607		*Phytophthora plurivora* *Pythium intermedium*
Taxus baccata	29-May-08	TQ218780		*Pythium glomeratum / heterothallicum*
Taxus baccata	15-Jul-08	TL318293		*Pythium perplexum*
Taxus baccata	21-Jul-08	TL003439		*Phytophthora gonapodyides* *Pythium* species
Taxus baccata	22-Jul-08	SK232316		*Phytophthora cinnamomi* *Pythium* species
Taxus baccata	07-Aug-08	SU813843		*Phytophthora cryptogea*
Taxus baccata	13-Aug-08	TQ099133		*Phytophthora cryptogea* *Phytophthora cactorum*
Taxus baccata	27-Aug-08	TQ495393		*Phytophthora gonapodyides*
Taxus baccata	03-Sep-08	SP243741		*Pythium intermedium*
Taxus baccata	05-Sep-08	SK322094		
Taxus baccata	29-Sep-08	TL457020		*Phytophthora* species *Pythium intermedium*
Taxus baccata	30-Sep-08	SU385633		*Phytophthora* species *Pythium heterothallicum*
Taxus baccata	14-Oct-08	SU785771		*Phytophthora gonapodyides* *Pythium irregulare*
Taxus baccata	14-Oct-08	SK044727		*Phytophthora cryptogea* *Pythium intermedium*
Taxus baccata	16-Oct-08	TQ099133		*Pythium* species

Taxus baccata	24-Oct-08	SU958379		*Pythium intermedium* *Phytophthora cinnamomi* *Pythium attrantheridium*
Taxus baccata	03-Nov-08	Ireland		*Pythium cylindrosporum / regulare / cryptoirregulare* *Pythium intermedium* *Pythium diclinum* *Pythium dissotocum / lutarium*
Taxus baccata	14-Nov-08	TQ499218		*Phytophthora cinnamomi* *Pythium attrantheridium*
Taxus baccata	19-Nov-08	SU938848		*Pythium attrantheridium* *Phytophthora* species *Pythium intermedium*
Taxus baccata	20-Nov-08	TQ254807		*Pythium intermedium* *Phytophthora plurivora*
Taxus baccata	27-Nov-08	SP528141		*Pythium* species *Pythium intermedium*
Taxus baccata	27-Nov-08	TV556982	*Phytophthora cinnamomi* *Pythium* species	*Phytophthora cinnamomi*
Taxus baccata	22-Dec-08	TQ300905		*Phytophthora cinnamomi* *Pythium* species
Taxus baccata	22-Dec-08	TQ300905		*Pythium intermedium* *Pythium glomeratum / heterothallicum*
Taxus baccata	22-Dec-08	TR054399		*Pythium rostratum* *Pythium attrantheridium*
Taxus baccata	29-Dec-08	Ireland		*Pythium heterothallicum* *Phytophthora cinnamomi*
Taxus baccata	02-Jan-09	TQ741261		*Pythium* species *Phytophthora cryptogea*
Taxus baccata	05-Jan-09	TQ104396		*Pythium perplexum* *Phytophthora cryptogea*
Taxus baccata	14-Jan-09	SX470737		*Pythium* species *Phytophthora cinnamomi*
Taxus baccata	14-Jan-09	SU989639		*Pythium intermedium* *Pythium sylvaticum*
Taxus baccata	15-Jan-09	SK927627		*Phytophthora cinnamomi* *Pythium attrantheridium*
Taxus baccata	27-Jan-09	SJ501735	*Phytophthora cinnamomi*	*Pythium sylvaticum* *Phytophthora cinnamomi*

				Pythium intermedium
Taxus baccata	30-Jan-09	SU757832		*Pythium* species
				Phytophthora species
Taxus baccata	06-Feb-09	TF206822	*Phytophthora cinnamomi*	*Phytophthora cinnamomi*
				Phytophthora plurivora
Taxus baccata	25-Feb-09	TQ277779		*Pythium intermedium*
Taxus baccata	03-Mar-09	NX757753		*Pythium sylvaticum*
Taxus baccata	05-Mar-09	SP419270		*Pythium sylvaticum*
				Pythium ultimum
				Phytophthora species
Taxus baccata	06-Mar-09	TM460991		*Phytophthora cinnamomi*
				Pythium intermedium
Taxus baccata	26-Mar-09	SP201822		*Pythium glomeratum / heteterothallicum*
				Phytophthora plurivora
Taxus baccata	26-Mar-09	SP201822		*Pythium perplexum*
Taxus baccata	24-Apr-09	TR015438		*Pythium intermedium*
				Phytophthora plurivora
Taxus baccata	24-Apr-09	TR015438		*Pythium diclinum*
Taxus baccata	29-Apr-09	SP215597		*Phytophthora cinnamomi*
				Phytophthora cryptogea
Taxus baccata	14-May-09	SP865407		*Phytophthora plurivora*
				Pythium intermedium
Taxus baccata	11-Jun-09	SO865355		*Pythium* species
				Phytophthora plurivora
Taxus baccata	11-Jun-09	SU643454		*Pythium attrantheridium*
Taxus baccata	08-Jun-09	Ireland		*Phytophthora syringae*
Taxus baccata	22-Jul-09	TQ711150		*Phytophthora cinnamomi*
Taxus baccata	29-Jul-09	SE507577		*Pythium* species
				Pythium attrantheridium
Taxus baccata	07-Aug-09	SJ956286		*Pythium attrantheridium*
				Pythium glomeratum / heterothallicum
Taxus baccata	21-Aug-09	SK614022		*Phytophthora cryptogea*
				Pythium species
				Pythium attrantheridium
Taxus baccata	26-Aug-09	TQ276781		*Pythium conidiophorum / salpingophorum / tracheiphilum*
Taxus baccata	25-Sep-09	SU974619		*Phytophthora cinnamomi*
Taxus baccata	25-Sep-09	SU974619		*Pythium* species

Taxus baccata	07-Oct-09	SO691183	*Phytophthora* species *Pythium* species *Pythium intermedium*
Taxus baccata	08-Oct-09	TQ526506	*Phytophthora* species *Pythium glomeratum / heterothallicum*
Taxus baccata	19-Oct-09	TQ005450	*Phytophthora citricola*
Taxus baccata	06-Nov-09	TQ278952	*Pythium intermedium*
Taxus baccata	06-Nov-09	TQ278952	*Pythium irregulare* *Pythium intermedium*
Taxus baccata	24-Nov-09	SU930331	*Pythium intermedium* *Pythium irregulare*
Taxus baccata	24-Nov-09	SU930331	*Phytophthora cinnamomi* *Pythium irregulare*
Taxus baccata	30-Nov-09	SP584014	*Phytophthora cinnamomi* *Pythium diclinum*
Taxus baccata	15-Jan-10	TM011125	*Pythium* species
Taxus baccata	24-Feb-10	TL618652	*Phytophthora gonapodyides*
Taxus baccata	12-Mar-10	SK710090	*Phytophthora cinnamomi*
Taxus baccata	12-Mar-10	ST546423	*Pythium* species *Pythium attrantheridium*
Taxus baccata 'Fastigiata'	23-Nov-09	N/A	*Pythium torulosum / folliculosum* *Pythium glomeratum / heterothallicum*
Taxus baccata 'Fastigiata'	17-Dec-09	SJ891594	*Phytophthora cryptogea* *Pythium glomeratum / heterothallicum*

Descriptions in Tables	Meaning
Phytophthora species	Unable to identify species either due to poor sequence quality or not matching any in database
Phytophthora citricola	This is a complex so although labelled P. citricola later research may create new species
Pythium species	Unable to identify species either due to poor sequence quality or not matching any in database
Pythium glomeratum / heterothallicum	Some species are difficult to separate by PCR so have been labelled with more than one species