Grower Summary

HNS 181

Survey, detection and diagnosis of *Phytophthora* root rot and other causes of die-back in conifers

Final 2011

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Project Number:	HNS 181
Project Title:	Survey, detection and diagnosis of *Phytophthora* root rot and other causes of die-back in conifers
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Report:	Final report, March 2011
Publication Date:	1st August 2011
Previous report/(s):	-
Start Date:	1 April 2010
End Date:	31 March 2011
Project Cost (total project cost):	£28,583 (£ 30,083)

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.

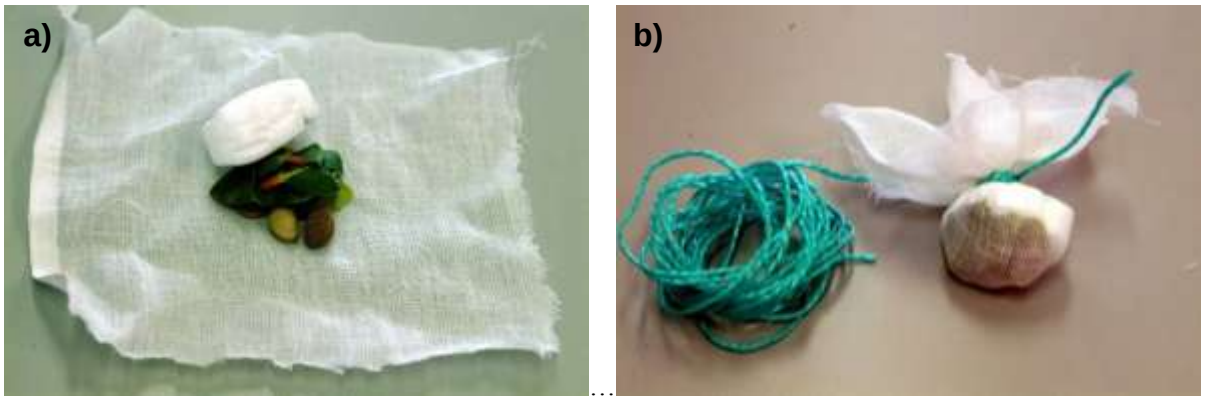

Figure GS 2: Construction of rhododendron bait a) showing bait contents and b) completed bait.

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.