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# Observations of *Phytophthora* spp. in water recirculation systems in commercial hardy ornamental nursery stock

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**Abstract**

Samples of water and sediment were taken from drains, reservoirs and wells from four commercial hardy ornamental nurseries with water recirculation systems. The samples were taken on seven different dates throughout a single year from August 1994 to July 1995. The samples were screened for *Phytophthora* species using five different methods: direct plating, three bait tests (using lupin seedlings, apples and Rhododendron leaves) and a DAS-ELISA (*double-antibody sandwich enzyme-linked immunosorbent-assay*) with two antisera. In the nurseries with old water recirculation systems, *Phytophthora* species were detected in the drains and in the reservoirs. In the nursery with a new recirculation system, the pathogens were only present in the drains. None of the water samples from wells in any of the nurseries were contaminated. *Phytophthora* species were present in the water as well as in the sediment samples from drains and reservoirs. They were detected in the water recirculation systems irrespective of the season. The number of isolates increased about sevenfold between late summer and spring. At least 12 different *Phytophthora* species were identified: some isolates were previously unrecorded species. The epidemiology of the pathogens in outdoor water recirculation systems as well as the importance of the results for commercial nurseries is discussed.

*Abbreviations:* DAS-ELISA – double-antibody sandwich enzyme-linked-immunosorbent-assay; HNS – hardy ornamental nursery stock.

## Introduction

The production of hardy ornamental nursery stock (HNS) is a profitable part of the horticultural industry in Germany (Anonymus, 2000). To enable the selling and planting of HNS all the year round, plants are grown outdoors in containers in special areas (stands). Unlike nurseries producing 'bare-rooted' plants growing in the soil, the surplus water from irrigation and natural rain is collected from container stands *via* drains and/or special drainage systems and stored in special reservoirs. In German horticultural practice, these reservoirs can have capacities of up to 6000 $m^3$. It is estimated, that about 35% of the water used for irrigation can be recovered and run back to such a reservoir (Hanselmann, 1991), thus significantly reducing demand for and costs of water. Furthermore, recycling of the surplus and drainage water prevents nutrients and pesticides from the stands entering the groundwater.

Experiments with herbaceous ornamentals, vegetables and HNS plants in glasshouses and in model systems under controlled conditions have shown that

plant pathogenic organisms like bacteria, fungi and viruses can be spread in contaminated water (Wohanka, 1999). One of the most important pathogens spread by water is the genus *Phytophthora*. Many species of this genus cause root rot, stem rot, twig and/or leaf blight in container HNS plants. Losses can be up to 100% within one year. *Phytophthora* spp. are well adapted to live in water and to spread from plant to plant *via* motile zoospores. Contaminated recycled water is a major means of dissemination for *Phytophthora* spp. (McIntosh, 1966; Whiteside and Oswalt, 1973; Shokes and McCarter, 1979; Ali-Sthayeh and MacDonald, 1991; Lutz and Menge, 1991; MacDonald et al., 1994; Pettitt et al., 1998; Oudemans, 1999), but there has been little detailed information published on the occurrence and epidemiology of these pathogens in full scale water recirculation systems on commercial nurseries.

This paper presents detailed observations on the occurrence of *Phytophthora* spp. in the water recirculation systems of four different commercial HNS nurseries throughout a single year. These observations are discussed in the context of the epidemiology of these pathogens in commercial systems and the possibility for developing non-chemical control strategies.

## Materials and methods

### *Nurseries*

Four nurseries with container cultivation systems typical of Germany were selected and identified as nurseries 1–4 (Table 1). They produced a wide range of the usual HNS species, e.g., *Chamaecyparis* spp., *Taxus* spp. and *Rhododendron* spp. Once potted in containers, these plants usually remained on the container stands for at least one year before sale.

Prior to this study, nurseries 1 and 3 had not reported problems with *Phytophthora*. However, nursery 2 had recognized significant problems with *Phytophthora* for many years, whilst in nursery 4, *Phytophthora* had been observed on *Chamaecyparis obtusa* in 1994 and 1995.

### *Container stand and water recirculation systems*

The container areas were sloped for better runoff. The stands were lined with (poly-)sheeting to prevent seepage. This lining was covered with a layer of sand or gravel. Above that, there was another layer, usually a black 'Mypex' mat. The surplus water, from irrigation and natural rain, flows from the container stands into drains which are on or under the soil surface. These drains often become filled with sediment or water or both. From there, the water flows through pipes or open channels into reservoirs. If there is insufficient water in the reservoirs, e.g., during the summer months, water was added to the reservoirs from wells that are fed from groundwater.

*Table 1*. Size and age of the container production areas and reservoirs, and the perceived *Phytophthora* disease problems on the four nurseries assessed (data provided by the nurserymen)

| | Nursery | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Age (years) of the container area with water recirculation | 3 | 3–12 | 8–10 | 2–7 |
| Size (ha) of the container area with water recirculation | 3.5 | 8.0 | 15.0 | 1.6 |
| Age (years) of the reservoir | 3 | >20 | Reservoir a and b: >20 | >20 |
| Size ($m^3$) of the reservoir | 6000 | 1000 | Reservoir a and b: 2500 each | 1000 |
| Problems with *Phytophthora* spp. in the container cultures | No | Yes | No | Yes[1] |

[1]Only in 1994/95.

### *Samples and sampling*

On seven dates between August 1994 and July 1995, one sample was taken from the drains, near the container stands, from the reservoirs and from the wells. From nurseries 2–4 sediment and water was also taken from drains and the reservoirs (Table 1). A sample consisting of 3 l of water and/or sediment was transported in plastic containers and stored overnight at +4 °C.

### *Detection methods*

For DAS-ELISA, one antiserum had been produced against *Phytophthora cactorum*, the other against *P. cinnamomi*. Both antisera were specific for the genus *Phytophthora* but not for individual species.

For direct plating, a selective medium (NVP) based on vegetable-oatmeal agar and amended with 50 ppm Nystatin (SIGMA N-3503), 100 ppm Vancomycin (SIGMA V-2002) and 10 ppm Pentachlornitrobenzene (PCNB; SIGMA P-3395) was used

(McCain et al., 1967). The water samples were passed through filter pads (Schleicher & Schuell No. 400 114) using an apparatus for sterile filtration (Satorius, Göttingen) and a vacuum pump. The filter pads were placed on the surface of NVP-Agar in Petri dishes. After three days, the pads were removed and the incubation continued. Organic material like leaves or needles were collected from the sediment samples, surface sterilized and placed on the selective medium. All Petri dishes were incubated at +20 °C in the dark to allow *Phytophthora* to grow out.

The standard procedures for the three bait tests (using lupin seedlings, apples and Rhododendron leaves) and the DAS-ELISA are described by Themann and Werres (1997).

The standard procedure to identify the *Phytophthora* spp. are described by Werres et al. (2001a).

## Results

*Occurrence of* Phytophthora *spp.*

*In the nurseries and in different parts of the recirculation system*. *Phytophthora* spp. were never detected in the wells, but were detected in the water recirculation systems of all four nurseries (Table 2a). There was great variation between nurseries and between different sampling sites on individual nurseries. In nursery 1, the detection rate was 29–50%, in nursery 3 it was 29–86% and in nurseries 2 and 4 it was 83–100%.

In most cases, detection rates were higher in samples from drains than from reservoirs (Table 2a), but the incidence of *Phytophthora* in the drains of a single nursery could vary greatly. In nursery 1, all samples from the first drain were free of *Phytophthora* spp., whereas half the samples of the second drain were contaminated with these pathogens. In nursery 3, contamination in terms of detection rate in the drains varied from 29% to 86%.

*In reservoirs*. *Phytophthora* spp. were detected in the water as well as in the sediment samples (Table 2b), but detection rates were much more variable in sediments (17–100%) than in water samples (43–86%).

*Range of Phytophthora species*

*Number of isolates*. At least 12 different *Phytophthora* species and some isolates of previously unrecorded species were isolated from the reservoirs of nurseries 2–4 (Table 3).

Most of the *Phytophthora* isolates belonged to group VI according to the keys of Kröber (1985), Stamps et al. (1990) and Erwin and Ribeiro (1996). This consists of *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. drechsleri*, *P. gonapodyides*, *P. richardiae* and *P. undulata*. Two species belonged to group III

*Table 2a*. Occurrence of *Phytophthora* spp. in different parts of the water recirculation systems of the four nurseries ($n$ = number of samples)

| Nursery | Maximum percentages of samples ($n$) with *Phytopthora* spp. | | | | | |
|---|---|---|---|---|---|---|
| | Drain | | | Reservoir | | Well |
| | a | b | c | a | b | |
| 1 | 0 (*7*) | 50 (*6*) | — | 29 (*7*) | — | 0 (*4*) |
| 2 | 100 (*7*) | 100 (*6*) | — | 86 (*7*) | — | 0 (*4*) |
| 3 | 71 (*7*) | 29 (*7*) | 86 (*7*) | 57 (*7*) | 50 (*6*) | 0 (*4*) |
| 4 | 83 (*6*) | — | — | 100 (*6*) | — | 0 (*4*) |

a, b, c = Different sampling sites (drain or reservoir).
— = Drain or reservoir either non-existent or not sampled.

*Table 2b*. Occurrence of *Phytophthora* spp. in water recirculation systems – in water and sediment from reservoirs

| Nursery | Maximum percentages of samples ($n$) with *Phytopthora* spp. | | | |
|---|---|---|---|---|
| | Water | | Sediment | |
| | Reservoir a | Reservoir b | Reservoir a | Reservoir b |
| 1 | 29 (*7*) | — | —[1] | — |
| 2 | 86 (*7*) | — | 57 (*7*) | — |
| 3 | 43 (*7*) | 50 (*6*) | 57 (*7*) | 17 (*6*) |
| 4 | 67 (*6*) | — | 100 (*6*) | — |

— = Reservoir b in nurseries 1, 2, 4 non-existent.
[1]Sediment in the reservoir of nursery 1 non-existent.

*Table 2c*. Occurrence of *Phytophthora* spp. in water recirculation systems – at different seasons (total of all samples and nurseries)

| Sampling date | $n$ | Maximum percentage of samples with *Phytophthora* spp. | Maximum number of *Phytophthora* isolates in the samples |
|---|---|---|---|
| 24 August | *11* | 64 | 19 |
| 24 October | *17* | 82 | 23 |
| 28 November | *17* | 59 | 73 |
| 06 February | *16* | 87 | 109 |
| 27 March | *17* | 88 | 146 |
| 15 May | *17* | 59 | 76 |
| 11 July | *17* | 76 | 116 |

*Table 3*. Phytophthora species and number of isolates in the reservoirs[1]

| Phytophthora species | Water and sediment | | | Over a year[1] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Water $n = 33$ | Sediment $n = 26$ | Total $n = 59$ | 29 Aug. $n = 11$ | 24 Oct. $n = 17$ | 28 Nov. $n = 17$ | 06 Feb. $n = 16$ | 27 Mar. $n = 17$ | 15 May $n = 17$ | 11 Jul. $n = 17$ |
| *P. cactorum* | 6 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| *P. cambivora*[2] | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| *P. cinnamomi* | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| *P. citricola* | 10 | 0 | 10 | 1 | 1 | 0 | 0 | 0 | 0 | 8 |
| *P. citrophthora* | 6 | 1 | 7 | 0 | 0 | 1 | 0 | 0 | 0 | 6 |
| *P. cryptogea*[2] | 15 | 26 | 41 | 0 | 2 | 1 | 18 | 4 | 8 | 8 |
| *P. drechsleri*[2] | 17 | 14 | 31 | 5 | 3 | 0 | 4 | 3 | 4 | 12 |
| *P. gonapodyides*[2] | 57 | 43 | 100 | 2 | 5 | 20 | 14 | 37 | 0 | 22 |
| *P. ramorum* | 8 | 1 | 9 | 0 | 0 | 2 | 1 | 6 | 0 | 0 |
| *P. richardiae*[2] | 2 | 3 | 5 | 0 | 0 | 2 | 2 | 0 | 1 | 0 |
| *P. syringae* | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| *P. undulata*[2] | 1 | 6 | 7 | 0 | 0 | 1 | 3 | 3 | 0 | 0 |
| *Phytophthora* spp. | 12 | 10 | 22 | 2 | 0 | 6 | 3 | 11 | 0 | 0 |
| Total | 141 | 107 | 248 | 10 | 11 | 35 | 45 | 64 | 16 | 67 |

[1]Total of the four nurseries; $n$ = number of samples.
[2]Some of these isolates were only similar to a known species. They differed, for example, in size of a propagule from the original description given in the keys of Kröber (1985), Stamps et al. (1990) and Erwin and Ribeiro (1996).

(*P. citricola* and *P. syringae*). One belonged to group II (*P. citrophthora*) and another to group I (*P. cactorum*). Fourteen isolates of the *Phytophthora* species that could not be classified to species level belonged to group V or VI according to Stamps et al. (1990). Eight isolates could not be categorized into a known group. The isolation frequency of single *Phytophthora* species was highly variable (Table 3). The lowest isolation rate was for *P. syringae* (two isolates) and the highest for *P. gonapodyides* (100 isolates).

*Isolates in water and sediment of the reservoirs*. Nearly all of the *Phytophthora* spp. detected in the reservoirs were present in the water but not always in the sediment (Table 3). *P. cactorum*, *P. cambivora*, *P. citricola* and *P. syringae* were isolated only from water while *P. cinnamomi* occurred only in the sediment. This indicates the range of *Phytophthora* species present was greater in water than in sediment. The number of isolates in water and sediment varied with the *Phytophthora* species (Table 3). For example, isolates of *P. cryptogea* were isolated mainly from sediment samples, while more isolates of *P. gonapodyides* were present in water than in sediments.

*Occurrence of* Phytophthora *species with season*

*Phytophthora* species were detected during all seasons (Table 3). Fewest species were isolated in August, October and May (four species including the unknown ones), whilst most were found in November (eight species including the unknown ones), February (seven species including the unknown ones) and July (seven species). Some species were only isolated on one sampling date (*P. cactorum*, *P. cambivora*, *P. cinnamomi*). Others were present on nearly all sampling dates (e.g. *P. cryptogea*, *P. gonapodyides*).

The number (total of all samples from the drains and the reservoirs; Table 2c) of *Phytophthora* isolates increased from 19 to 146 between August 1994 and March 1995. In the reservoirs, the number rose from 10 to 64, then it decreased dramatically until May before increasing again to near the maximum by July (Table 3).

There was no clear interaction between season, *Phytophthora* species and number of isolates per species in the reservoirs (Table 3) For example, in November and in March, more then 50% of the isolates detected belonged to *P. gonapodyides*. On the other hand, most *P. drechsleri* isolates were detected in July, and most *P. cryptogea* in February. The unknown *Phytophthora* species were detected mainly in March. Furthermore, there was no clear correlation between the maximum number of samples with *Phytophthora* spp. and the number of isolates detected (Table 2c). For example in August and October there were high detection rates but only a low number of isolates. However, between February and July the number

of samples with *Phytophthora* was correlated with the number of isolates.

## Discussion

The results confirm the results of studies from other countries that *Phytophthora* spp. are established in commercial HNS nurseries (McIntosh, 1966; Thomson and Allen, 1974; Shokes and McCarter, 1979; Ali-Sthayeh and MacDonald, 1991; MacDonald et al., 1994). In the reservoirs under investigation, *Phytophthora* spp. became established within three years under the climate and cultivation conditions of Central Europe (Germany). Infected plants appear to be the primary and most important source for water contamination in commercial nurseries. Similar results were obtained in simulation systems with HNS in the United Kingdom (Pettitt et al., 1998).

*Phytophthora* propagules were not detected in any of the wells. At all four nurseries the wells contained groundwater which had no direct connection to the water recirculation systems. Furthermore, the underground water had passed through different layers of sand and other materials. Filtration with sand filters is one of the most successful methods of removing *Phytophthora* from contaminated water (Wohanka, 1999).

A wide range of *Phytophthora* species was detected in the water recirculation systems. Most of these pathogens, especially *P. cambivora* and *P. syringae*, have a wide host range which mainly includes trees and shrubs. The predominant *Phytophthora* species were *P. gonapodyides*, *P. cryptogea* and *P. drechsleri*. *P. gonapodyides* is an 'aquatic' species very often isolated from water (Hallett and Dick, 1981; Ali-Shtayeh and MacDonald, 1991). *P. cryptogea* and *P. drechsleri* are better known as important pathogens on ornamentals in Germany (Werres et al., 2001a). In addition, *Phytophthora* species, such as the isolates similar to *P. richardiae*, *P. gonapodyides* and *P. undulata*, not found previously in German nurseries were detected. Nine isolates belong to a new species, *P. ramorum*, recently isolated from diseased *Rhododendron* and *Viburnum* (Werres et al., 2001b).

There was not always a clear correlation between the optimum temperature of a species and the season when it was detected. For example *P. drechsleri* which prefers high temperatures was detected on all sampling dates except in November. That may be due to the very moderate temperatures in the winter months 1994/1995 and the warm summer in both years. These unusual climate conditions could be one reason why *Phytophthora* species which prefer higher temperatures have become established during the last decade in Central Europe (Brasier and Scott, 1994). Furthermore, the reservoirs themselves enable the survival and development of *Phytophthora* species sensitive to extremes of high or low temperature. Due to the depth (2–4 m) of the reservoirs, neither the water nor the sediment freezes over in winter. In addition, the organic material in the sediment and the anaerobic conditions at the base of the reservoirs are known to favour survival and development of *Oomycetes* (Old et al., 1984; Ostrofsky et al., 1977).

There was a dramatic increase in *Phytophthora* isolates from autumn to spring. During this period, irrigation and refill of the reservoirs is very rare, so there was no dilution of the water by non-contaminated groundwater. In addition, there were usually no applications of fungicides specific to *Oomycetes* over the period from late autumn to early spring.

For *Phytophthora* spp. no general threshold of number of propagules required for infection can be fixed (Bruck and Kenerly, 1983; Lutz and Menge, 1991). Nevertheless any propagules in the water recirculation systems of nurseries are potentially dangerous and results reported here show that their numbers can build up at strategic points in a water recirculation system through the year. Control of *Phytophthora* in water recirculation systems has long been discussed (Wohanka and Helle, 1997; Jamart and van Laere, 1998; van Os et al., 1998; Wilson et al., 1998). To prevent contamination of the storage water *via* infected organic material in new recirculation systems it would be preferable to filter surplus water before it reaches the reservoir rather than after withdrawing it from the reservoir for irrigation. Furthermore, *Rhododendron* or other hosts for *Phytophthora* spp. should not be planted around the water reservoirs, because the fallen leaves and other plant debris provide a means for *Phytophthora* to increase the contamination through colonization and sporulation.

In summary, a wide range of *Phytophthora* spp. were present in the water recirculation systems of HNS nurseries throughout the year, although the groundwater in the wells was not contaminated. Large open reservoirs allow the survival of *Phytophthora* propagules during both winter and summer. *Phytophthora* is transported in surplus water through drains into the reservoirs where it also becomes established in the sediment. The most likely source of this contamination of the

water on these commercial nurseries, appears to be the presence of infected plants on the container beds.

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