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Alien Species in Finnish Nurseries, *Phytophthora* spp.

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Abstract – International trade has increased the risk of pest spread. The climatic change will also improve the establishment of introduced species into new geographical areas. Since the beginning of 1990's *Phytophthora cactorum* has caused losses in agriculture on strawberries and caused stem lesions on silver birch seedlings in forest nurseries in Finland. *P. ramorum* was found in Finland for the first time in spring 2004 on marketed *Rhododendron* spp. plants originating from other EU member states. In August 2004 the pathogen was also found in one Finnish nursery on German *Rhododendron catawbiense* plants and on several other Finnish *Rhododendron* spp. cultivars. Most common microbes isolated from the lesions on the Finnish *Rhododendron* leaves collected in 2005 were *Pestalotiopsis* sp., *P. cactorum*, *P. inflata* and *P. ramorum*. In pathogenicity trials *P. inflata* was capable to infect most host plants used in tests including *Fragaria* x *ananassa*, *Betula pendula*, *Alnus glutinosa*, *A. incana*, *Picea abies* and *Vaccinium vitis-idea*. *P. ramorum* caused also stem lesions on birch and alder, but was less pathogenic than *P. inflata. Pinus sylvestris* was resistant to both *P. ramorum* and *P. inflata.*

***Phytophthora cactorum / P. ramorum / P. inflata /* hosts / pathogenicity**

Kivonat – Behurcolt fajok a finnországi csemetekertekben, *Phytophthora* spp. A nemzetközi kereskedelem növeli a károsítók terjedésének kockázatát. A klímaváltozás elősegíti a behurcolt fajok megtelepedését az új földrajzi területeken. A *Phytophthora cactorum* Finnországban az 1990-es évek elejétől a földiepren mezőgazdasági károkat, erdészeti csemetekertekben, a bibircses nyír magoncokon pedig szárnekrózist okoz. A *P. ramorum*-ot Finnországban először 2004 tavaszán találtuk meg, más EU tagállamokból származó kereskedelmi *Rhododendron* növényeken. 2004 augusztusában a kórokozót egy finn csemetekertben is megtaláltuk németországi származású *Rhododendron catawbiense* növényeken és több más, finnországi *Rhododendron* fajtán. A 2005-ben begyűjtött finnországi *Rhododendron* levelek nekrózisaiból leggyakrabban a *Pestalotiopsis* sp., *P. cactorum*, *P. inflata* és a *P. ramorum* mikroorganizmusokat izoláltuk. A patogenitási vizsgálatok során a *P. inflata* képes volt megfertőzni a legtöbb tesztnövényt, köztük a *Fragaria* x *ananassa*, *Betula pendula*, *Alnus glutinosa*, *A. incana*, *Picea abies* és *Vaccinium vitis-idea* fajokat. A *P. ramorum* is szárnekrózist okozott a nyíren és az égeren, de a *P. inflata*-nál kevéssbé bizonyult patogénnek. A *Pinus silvestris* mind a *P. ramorum* mind a *P. inflata* fajokkal szemben ellenálló volt.

***Phytophthora cactorum* / *P. ramorum* / *P. inflata* / gazdák / pathogenitás**

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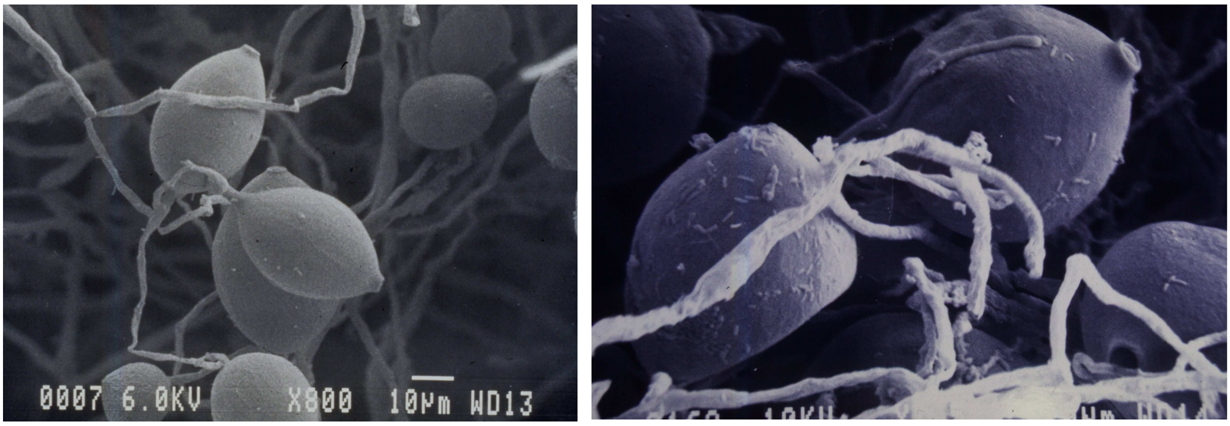
Phytophthora cactorum

Phytophthora cactorum (Lebert and Cohn) J. Schröt is a pathogen which is transported with plant material as latent infections and can also survive in soil and plant debris. It is an economically important soil-borne pathogen of many herbaceous and woody species (Erwin and Ribeiro 1996). In forestry *P. cactorum* is known to cause root rot and stem cankers on many tree species (Erwin and Ribeiro 1996). In Sweden it was present in soil together with other *Phytophthora* species in stands where oaks (*Quercus robur* L.) showed tree crown defoliation (Jönsson et al. 2003). In inoculations it caused significant dieback of fine root and necrotic lesions on coarser root of oak seedlings (Jönsson, 2004). In Finland *P. cactorum* was isolated for the first time in 1990 from strawberry (*Fragaria* x *ananassa* Duch.) plants suffering from crown rot (Parikka 1991). A year later it was isolated from necrotic stem lesions on silver birch (*Betula pendula* Roth.) seedlings growing in forest nurseries (Lilja et al. 1996; Hantula et al. 1997, 2000). Since then this imported pathogen has caused crop losses in strawberry fields mainly as an agent of crown rot and increased culling of seedlings in forest nurseries.

Phythophthora cactorum and different host species

According to our observations the morphology of *P. cactorum* isolates from strawberry and birch differ from each other (*Figue 1a, b*).



(a) *(b)*

Figure 1. Sporangia of Phytophthora cactorum isolated from strawberry (a) and silver birch (b).

We carried out microscopic measurements and found several statistically significant differences in the sizes of the oogonia, oospores and sporangia (Hantula et al. 2000). However, the individual variation in morphological characteristics was evident, the microscopic examinations are insufficient for the identification of intraspecies genetic groups or host specificity among our isolates (Stamps et al. 1990). Both the random amplified microsatellite (RAMS) and Random Amplified Polymorphic DNA (RAPD) analysis showed substantial genetic variation among isolates of *P. cactorum*. However, Finnish isolates from birch and European crown rot isolates, including Finnish isolates, from strawberry formed own clusters in cluster analyses (Hantula et al. 1997, 2000, Lilja et al. 1998). Similarly in amplified fragment length polymorphism (AFLP) analysis 16 of 23 crown rot isolates from Europe, Japan, Australia and New Zealand were identical (Eikemo et al. 2004). However, the isolates from strawberry from different states or from different host in the USA or from strawberry from German or Canada, were polymorphic and formed 42 unique AFLP profiles (Huang et al. 2004). We also found high genetic variation within the North American population of *P. cactorum* on strawberry fruits (Hantula et al. 2000). These results might suggest that the origin of *P. cactorum* is in the USA and that differences found in *P. cactorum* in Europe, Oceania, Asia and Africa are simply subsets of variation occurring in North America, which has been exported from there to other parts of the world (Hantula et al. 2000, Eikemo et al. 2004, Huang et al. 2004).

According to Seemüller and Schmidle (1979) leather rot of strawberry fruits is caused by different strain of *P. cactorum*, since in general leather rot pathogens are not able to cause crown rot, but crown rot pathogens can infect fruits. Most isolates from other hosts are also incapable to infect strawberry (Hantula et al. 1996, 2000, Eikemo et al. 2004). In our studies the isolates from strawberry infected birch only via wounds and the isolates from birch did not infect strawberry seedlings at all suggesting that there are differences in host specificities (Hantula et al. 1997, Lilja et al. 1998, Eikemo et al. 2004). This is in accordance with the observation of Seemüller and Schmidle (1979) who showed that *P. cactorum* isolates from strawberry and apple differ in their capability to cause symptoms on strawberry and apple. On *Alnus* the percentage of successful wound inoculations with a birch isolate was 40 and most lesions were small compared to those on the same size birch seedlings (Hantula et al. 1997). Based on the pathogenicity experiments done so far we conclude that *P. cactorum* strains cause more serious symptoms on the plants from which they are derived than on other plants, although variations exist between different host plants. Thus, strains tend to be more virulent on their hosts than on non-host plants.

We have also monitored the effect of *P. cactorum* infection on the development of container-grown, silver birch seedlings in nursery and after out-planting (Lilja et al. 1996, Lilja et al. unpublished). A PCR- based pathogen detection system, developed by us, was used to confirm the presence of *P. cactorum* in lesions after out-planting (Lilja et. al. 2006). In spring 1999 we collected diseased and healthy silver birch seedlings from a nursery field. Each seedling was assessed using a scale of 1 to 4 where: 1= no lesion , 2 = lesion < 5 mm^2, 3 = lesion > 5 mm^2, but not covering over half of the stem diameter, and 4 = lesion spread over half of the stem diameter, but not girdling the stem. On 4 May, after storage at + 4°C, the seedlings were out-planted. In the nursery stem lesions affected the height growth of birch seedlings, the shoot height of seedlings was related to the disease severity. Asymptomatic seedlings were taller than the diseased birches and the shortest were those with stem lesions covering over half of their stem diameter (*Figure 2*). After out-planting the stem lesions did not affect significantly on the mortality or the number of leader shoot changes. The height growth of seedlings in the reforestation increased with the disease rating. Growth of seedlings with stem lesions covering over half of their stem diameter grew more than healthy control seedlings or seedlings with smaller stem lesions (*Figure 3*). Thus the differences in shoot heights present in the nursery between diseased and apparently healthy seedlings were reduced, but not totally disappeared, after seven growing seasons in the field (*Figure 3*). However the mortality of seedlings increased with increased disease severity (*Figure 4*).

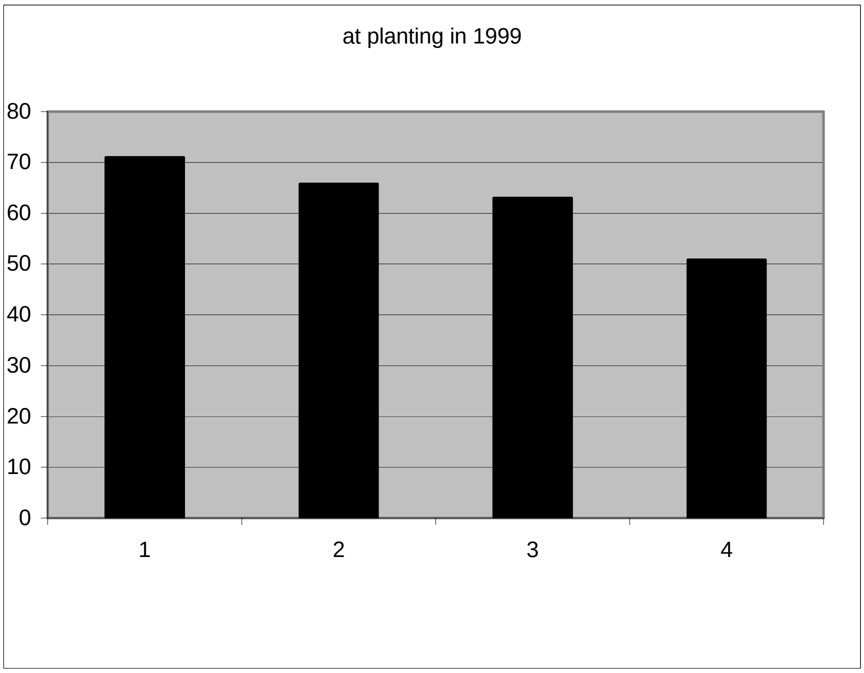


Figure 2. The height of silver birch seedlings before planting.
The health condition of seedlings was assessed using a scale of 1 to 4 where:
1= no lesion , 2 = lesion < 5 mm^2, 3 = lesion > 5 mm^2, but not covering over half of the stem diameter, and 4 = lesion spread over half of the stem diameter, but not girdling the stem.
Seedlings with lesions were infected with Phytophthora cactorum.
The number of seedlings in each disease severity category was 120

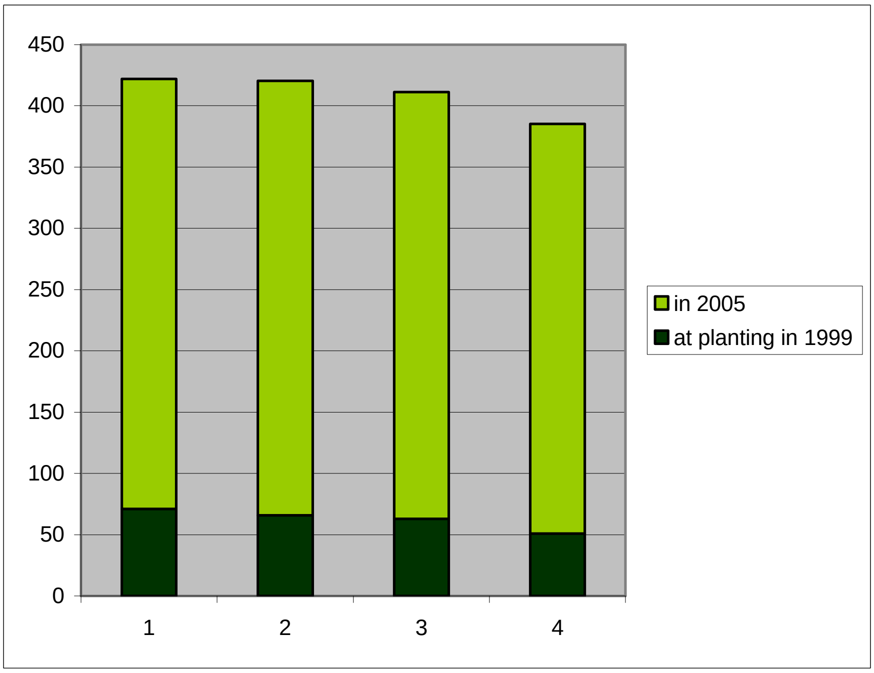


Figure 3. The height of silver birches seven years after out-planting.
The health condition of seedlings before planting was assessed using a scale of 1 to 4 where:
1= no lesion , 2 = lesion < 5 mm^2, 3 = lesion > 5 mm^2, but not covering over half of the stem diameter, and 4 = lesion spread over half of the stem diameter, but not girdling the stem.
Seedlings with lesions were infected with Phytophthora cactorum.
The number of seedlings in each disease severity category was 120

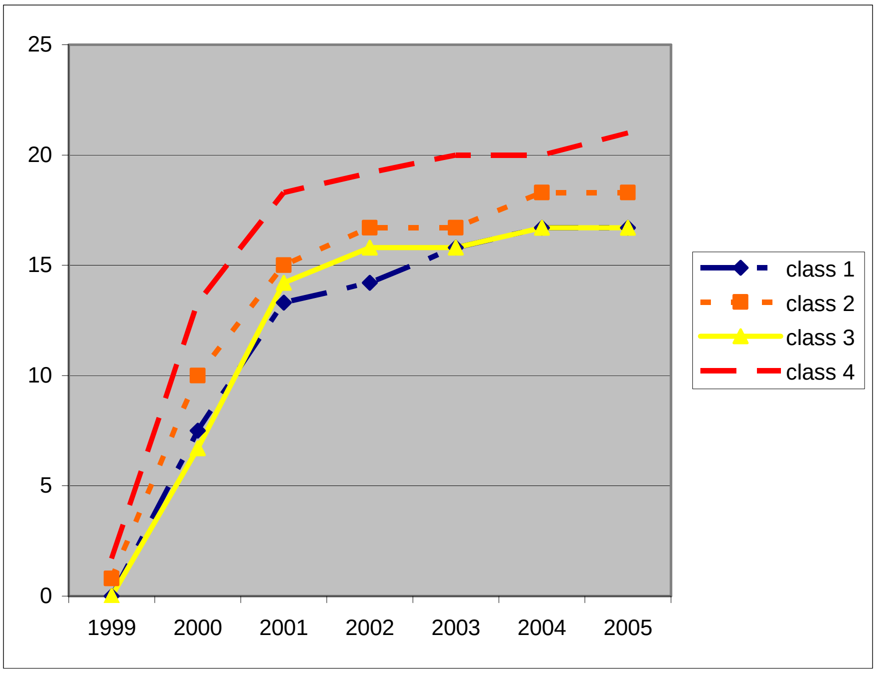


Figure 4. The mortality of seedlings after outplanting.
The health condition of seedlings before planting was assessed using a scale of 1 to 4 where: 1= no lesion , 2 = lesion < 5 mm^2, 3 = lesion > 5 mm^2, but not covering over half of the stem diameter, and 4 = lesion spread over half of the stem diameter, but not girdling the stem. Seedlings with lesions were infected with Phytophthora cactorum. The number of seedlings in each disease severity category was 120

Phytophthora ramorum

Phytophthora associated with a twig blight disease on rhododendron (*Rhododendron* sp.) and viburnum (*Viburnum* sp.) in Germany and the Netherlands was described as a new species, *Phytophthora ramorum* (Werres, de Cock & Man in't Veld) in 2001 (Werres et al. 2001). Later the same species was found to be responsible for the Sudden Oak Death disease (SOD) of oaks (*Quercus* spp.) and tanoaks (*Lithocarpus densiflorus* Hook. & Arn., Rehd) in California, USA (Rizzo et al. 2002). The spread of *P. ramorum* in North America has been very rapid. The disease was first discovered on tanoaks near Mill Valley in 1995. Since then it has spread throughout coastal counties around the San Franscisco Bay area where many tanoaks, coast live oak (*Q. agrifolia* Née), and California black oaks (*Q. kelloggii* Newb.) have been killed (Rizzo et al. 2002, Davidson et al. 2002, 2005). It has now spread to Washington and Oregon (Davidson et al. 2005, Hansen et al. 2003, Rizzo et al. 2005). In Europe *P. ramorum* was first found and identified in Germany and the Netherlands (Werrs et al. 2001). Later it was proved to be present in many other countries, but in Europe there has not been such epidemic as the SOD in the western North America. *P. ramorum* has mainly occurred as a cause of leaf, twig and shoot blight on different ornamental hosts in nurseries and gardens in Belgium, Finland, Denmark, France, Italy, Ireland, Norway, Poland, the UK, Slovenia, Spain, Sweden and Switzerland (Delatour et al. 2002, Moralejo and Werres 2002, Orlikowski and Szkuta 2002, De Merlier et al. 2003, Beales et al. 2004a, b, Heiniger et al. 2004, Zerjav et al. 2004, Lilja et al.2007). More recent isolations have been done in Estonia (Hanso, personal communication). In general the native, European oak species such as common oak (*Q. robur*) or sessile oak [*Q. petraea* (Matt.) Liebl] are more resistant than American oaks, although in inoculations individual trees have shown different susceptibility

(Denman et. al. 2005). In some countries finds locate also in mature trees as American southern red oak (*Q. falcata* Michx.) (Brasier et al. 2004). Other infected trees in the UK and the Netherlands have grown in large gardens and the source of infection has been rhododendrons.

It is not known how *P. ramorum* originally entered Europe or North America, but the mating type, morphology, growth characters and population distribution suggests that separate introductions into Europe and into North America may have occurred from a third unknown location (Brasier 2003, Ivors et al. 2004, Rizzo et al. 2005, Werres et al. 2005). It is, however, probable that imported, infected ornamentals have been the main source of the pathogen.

In Finland *P. ramorum* was found for the first time in spring 2004 on marketed rhododendron plants originating from other EU member states. In August 2004 the pathogen was also found in one Finnish nursery on rhododendrons (*Rhododendron catawbiense* Michx) and on several other cultivars produced in Finland by micropropagation. Most common microbes isolated from the lesions on the leaves of a Finnish cultivar 'Elvira' were in 2005 *Pestalotiopsis*, *P. cactorum*, *P. inflata* and *P. ramorum* (Lilja et al. 2007).

Phythophthora ramorum and different host species

P. ramorum spreads mostly aerially and it has not been shown to infect roots. It has many hosts in different plant families: Aceraceae, Anacardiaceae, Betulaceae, Caprifoliaceae, Ericaceae, Fagaceae, Hippocastanaceae, Lauraceae, Oleaceae, Pinaceae, Pittosporaceae, Primulaceae, Rhamnaceae, Rosaceae, Taxaceae, Taxodiaceae and Theaceae and thus the disease expressions differ depending on hosts (Knight 2002, Hong 2003, Henricot - Prior 2004, Hüberli et al. 2004, 2005, Lane et al. 2004, Denman et al. 2005). On woody shurbs and other understory hosts, which can serve as a source of inoculum for trees, *P. ramorum* mainly causes leaf lesions or/and twig blight. In larger trees, symptoms vary between tree species. Leaf lesions occur always first at the tip or edges of the leaves. Bark infections cause cankers with tarry or rusty colored exudations. The leaves of infected oak trees may turn brown over a short period, but death may take one or more years (Garbelotto et al. 2001). On tanoak the pathogen affects both bark and leaves and death can be rapid. On the stem base the infections cause wilting and death eg. on viburnum (Werres et al. 2001). On conifers, *P. ramorum* causes needle blight and dieback of young shoots. Myrtlewood tree/ Bay laurel (*Umbellaria californica* Matt.) harbors the pathogen without suffering serious damage itself, while supporting abundant production of spores and thus caused the epidemic in California (Davidson et al. 2005, Maloney et al. 2005).

In pathogenicity tests run by us *P. ramorum* caused stem lesions on silver birch and common alder (*Alnus glutinosa* (L.) Gaertner), but was less pathogenic than *P. inflata.* Scots pine (*Pinus sylvestris* L.) was resistant to *P. ramorum.*

Phytophthora inflata

Phytophthora inflata (Caroselli & Tucker) was described by Caroselli and Tucker (1949) as a pathogen causing cankers on elms (*Ulmus american* L. and *U. fulva* Michaux) in the USA. Later it has been reported from rotten roots of nursery plants of elder (*Sambucus tenuifolium* L.) and common lilac (*Syringa vulgaris* L.) in the UK (Hall et al. 1992). Within surveys for *P. ramorum*, *P. inflata* was isolated from rhododendrons (*R. ponticum* L.) with wilting foliage and blackened shoot tips in a nursery in Scotland (Schlenzig 2005). Later the same pathogen was recovered from single plants of lingonberry (*Vaccinium vitis-idaea* L.) and salal (*Gaultheria shalon* L.), both with leaf lesions and dieback symptoms, from another nursery in

Scotland (Schlenzig 2005). The results by Schlenzig (2005) and Testa et al. (2005), that *P. inflata* was found in surveys for *P. ramorum,* were in accordance with our experience. We also isolated *P. inflata* from the cultivars known to be infected with *P. ramorum* (Lilja et al. 2007).

In pathogenicity trials *P. inflata* was capable to infect most host plants used in tests including strawberry, silver birch, common alder, grey alder [*Alnus incana* (L.) Moench], Norway spruce (*Picea abies* L. Karst.) and lingonberry. Scots pine was resistant to *P. inflata*.

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