European Journal of Plant Pathology **104:** 529–535, 1998.

Pathogenicity and genetic variation of *Phytophthora cactorum* from silver birch and strawberry

Arja Lilja[1], Reijo Karjalainen[2], Päivi Parikka[2], Kari Kammiovirta[3] and Heikki Nuorteva[1]
[1] *Finnish Forest Research Institute, Vantaa Research Centre, P.O. Box 18, FIN-01301 Vantaa, Finland;* [2] *Agricultural Research Centre, Institute of Plant Protection, FIN-31600 Jokioinen, Finland;* [3] *University of Helsinki, Department of Plant Biology, Plant Pathology Section, P.O. Box 28, FIN-00014 Helsinki, Finland*

Accepted 10 March 1998

Key words: Betula pendula, Fragaria × *ananassa,* stem lesions, crown rot

Abstract

Phytophthora cactorum strains isolated from necrotic stem lesions on *Betula pendula* seedlings or from *Fragaria* × *ananassa* plants suffering from crown rot were pathogenic to their host plants. Only isolates from birch caused clear lesions on non-wounded bark of birch. *P. cactorum* isolates from birch were not detrimental to strawberry. Random Amplified Polymorphic DNA (RAPD) analysis revealed variation within *P. cactorum*, isolates from silver birch having different banding patterns than those from strawberry. UPGMA analysis clustered isolates from silver birch and strawberry plants into separate groups. The data show that the recent outbreak in Finland of *P. cactorum* in birch could not be caused by the import of strawberry plants affected by crown rot.

Introduction

Birches are economically the most important deciduous tree species in three of the Nordic countries; Finland, Sweden and Norway (Frivold and Mielikäinen, 1991). In Finland, the number of silver birch (*Betula pendula* Roth) seedlings delivered annually for planting has increased from 3 to 17 million seedlings from 1980 to 1996, with a maximum of 23 million in 1992 (Anonymous, 1997). In addition, 1 to 5 million downy birch (*B. pubescens* Ehrh.) seedlings have been produced annually throughout the 1990's for planting (Anonymous, 1997). Increased production in forest nurseries has revealed serious diseases, of which stem lesions and cankers are among the most serious (Kurkela, 1974; Petäistö, 1983). Several fungi are known to cause stem lesions, including *Alternaria alternata* (Fr.) Keissl., *Botrytis cinerea* Pers. ex Nocca & Balb., *Fusarium avenaceum* (Corda ex Fr.) Sacc, *Godronia multispora* J. W. Groves and *Phytophthora cactorum* (Leb. & Cohn) Schr (Petäistö, 1983; Lilja and Hietala, 1994; Lilja et al., 1996). In Finland the last mentioned was isolated from birch for the first time in 1991 (Lilja and Hietala, 1994). Since then it has been isolated from 20–80% of the diseased seedlings sampled in three forest nurseries (Lilja et al., 1996).

In Europe, strawberry crown rot, caused by *P. cactorum*, was first reported from Germany in 1952 (Deutschmann, 1954), but has since occurred in many other European countries. In Finland *P. cactorum* has caused crop losses in strawberry (*Fragaria* × *ananassa* Duch.) fields since 1990 (Parikka, 1991). One year later, it was isolated from necrotic stem lesions on silver birch seedlings (Lilja and Hietala, 1994). Due to this close succession of events, it was suspected that strawberry plants affected by crown rot served as the source of infection of *P. cactorum* in birch.

In this study we tested the pathogenicity of *P. cactorum* isolates from birch and strawberry and investigated their genetic similarity by means of RAPD markers.

Table 1. Identities and sources of *Phytophthora cactorum* isolates

	Collection code	Host	Geographical origin	Source
P. cactorum	PH2, PH3, PH4, PH5, PH8, PH10, PH11, PH14, PH15, PH18, PH20, PH21, PH22, PH23, PH24	*Betula pedula*	Finland	A. Lilja
P. cactorum	S3, S5, S6, S7, S9, S10, S13, S14, S15, S19	*Fragaria* × *ananassa*	Finland	P. Parikka
P. cactorum	145(H)	*Fragaria* × *ananassa*	England	D. Harris
P. cactorum	TAM(1)	*Fragaria* × *ananassa*	Scotland	D. Kennedy
P. cactorum	S21	*Fragaria* × *ananassa*	Finland	J. Tegel
P. cactorum	CH09, CH12, CH15, CH19	*Fragaria* × *ananassa*	Sweden	C. Olsson
P. cactorum	CH17	*Fragaria* × *ananassa*	Estonia	C. Olsson
P. cactorum	A1, W1	*Fragaria* × *ananassa*	Germany	S. Werres

Materials and methods

Fungi used in this study

P. cactorum isolates from birch were isolated from stem lesions on silver birch seedlings grown in Finnish forest nurseries (Lilja et al., 1996). *P. cactorum* isolates from strawberry were obtained from plants affected by crown rot in Finland, Sweden, Estonia, Germany, England and Scotland (Hantula et al., 1997). More detailed information about the *Phytophthora* isolates is given in Table 1.

Pathogenicity test 1: 1-year-old silver birch seedlings

In the first experiment, 1-year-old, nursery-grown silver birch seedlings (seed orchard SV363, JR-1) from cold storage (− 4 °C) were thawed and transplanted into pots (5 l) containing fertilized, low-humidified Sphagnum peat (Finn peat M6, Kekkilä Corp., Finland). Two weeks after transplantation 10 non-wounded and 10 wounded seedlings were inoculated with each of three *P. cactorum* isolates (PH10, PH11 and S3) or a sterile PDA block (control). Each seedling received one inoculation. The wound was made prior to inoculation by removing with a scalpel a 3 mm × 3 mm piece of periderm 5 cm above ground level. A 3 mm × 3 mm agar block from a 1-week-old *P. cactorum* culture on potato dextrose agar (PDA 39 g, Difco $l\text{-}^{1}$) or a block from sterile PDA was placed on the wound or on sound bark and secured with parafilm (Parafilm M, Amer. Nat. Can.). PDA cultures of *P. cactorum* isolates were kept for two days in a refrigerator before inoculation to stimulate zoospore release (Erwin and Ribeiro, 1996). The plants were arranged in a completely randomized design in a greenhouse with a temperature range of 19–22 °C. On 1.9.1995, stem lesion margins were drawn with lumocolor pencils on overhead film (PPC, Canon) and the size of the lesions on the drawings were measured with an image analyser Leica Q500MC. The data were analysed using ANOVA and the mean differences were tested with Duncan's multiple-range test (Anonymous, 1990).

Pathogenicity test 2: 2-month-old silver birch seedlings

The silver birch seedlings were produced according to standard nursery practice at Tyllilä nursery. On 15.5.1995, 400 seedlings representing two seed origins, Joutsa (seed collection stand M694) and Savon koivu (seed orchard SV378), were transplanted into pots (0.5 l) containing the same kind of peat as above. Two seedlings were grown in each pot and one of them was wounded just before inoculation. On 21. 6.1995, each of four *P. cactorum* isolates (PH18, PH20, S3 and S13) or a sterile PDA-block (control) were tested on 40 wounded and 40 non-wounded seedlings. 20 wounded and 20 non-wounded seedlings represented one seed origin in each treatment. Wounding and inoculations were performed as described above. The pots were arranged in a completely randomized design in a greenhouse with a temperature range of 19–22 °C. Seedling health was evaluated every second week on a disease severity scale of 1 to 4: 1) no lesion or lesion < 9 mm^2, 2) lesion > 9 mm^2, but not spread over half

of the stem diameter, 3) lesion spread over half of the stem diameter, 4) lesion girdled the stem. Seedlings belonging to category four were nearly dead or dead. The last assessment was on 15.9.1995. The disease index (DI) of the seedlings was calculated from the frequency (*f*) of the different disease ratings (1–4):

$$\mathrm{DI} : (1\times f_1 + 2\times f_2 + 3\times f_3 + 4\times f_4)/\mathrm{N} \quad (1)$$

Kruskal-Wallis one-way analysis of variance and a non-parametric test were used for comparing the treatments (Anonymous, 1990).

Pathogenicity test 3: strawberries

Each of four *P. cactorum* isolates (PH18, PH20, S3 and S13) or a sterile PDA block were tested on 24 plants of cv Jonsok, a cultivar susceptible to crown rot (Parikka, 1991). Runner plants were cut and rooted in limed, low humidified Sphagnum peat (800 g dolomite limestone, 50 g fine ground limestone, 50 g super phosphate, and 130 g peat fertiliser l^{-1} peat). After 40 days, the plants were removed from the rooting substrate and the roots were washed with tapwater. The crowns of the plants were wounded near to ground level with a sharp stick. The 2 mm deep and 2 mm wide wound was covered with fungal mycelium from 3-week-old PDA cultures kept for two days in a refrigerator before inoculation. The inocula were secured with parafilm. After inoculation the plants were planted in pots (0.5 l) containing the same kind of fertilized peat as above and grown in a greenhouse with a temperature range of 22–24 °C. A replicate consisted of four plants and the six replicates of all five treatments were arranged in a randomized design.

The condition of the plants was classified just before inoculation, as well as one, two, and three weeks later on a disease severity scale of 1 to 4: 1) good, all leaves green, 2) moderate, 0–1 leaves brown, 3) weak, >3 leaves brown, 4) nearly dead or dead, all leaves brown. The disease index of the runner plants was calculated from the frequency (*f*) of different disease rates (1–4) as before. Kruskal-Wallis one-way analysis of variance and a non-parametric test were used for comparing the treatments (Anonymous, 1990).

RAPD analysis

DNA was isolated as described by Hantula et al. (1997). The amplification conditions were modified from those of Williams et al. (1990) as follows: 50 μl reactions were used, including 25 ng of genomic DNA, 200 μM of each dNTP, 200 nM of primer, and 5 μl 10 × polymerase buffer [200 mM $(NH_4)_2SO_4$, 750 mM Tris-HCl, pH 9.0, 1% (w/v) Tween, 25 mM $MgCl_2$] to give a final Mg concentration in the PCR mixture 5.0 mM according to suggestions by D. Howland, G. Arnan and R. Oliver from the University of East Anglia, U.K. (personal communication), and 0.6 U of Red Hot polymerase (Advanced Biotechnologies Ltd., UK). Pre-mixtures (without DNA) of each reaction were prepared to minimize the risk of contamination. Amplification was performed in an MJ Research Programmable Thermal Controller programmed for 40 cycles of 1 min at 94 °C, 1 min at 40 °C and 2 min at 72 °C. Amplification products were electrophoresed in 1.4% agarose gel with 1 × TAE (40 mm Tris-Acetate, pH 8.0, 1 mm EDTA) and stained with ethidium bromide. Amplifications were run twice for each isolate and only reproducible bands were scored.

The primers were selected from Operon Kit B (Operon Technologies Inc., Alameda, California, USA). All twenty primers were tested, and the three best (OPB-6: TGCTCTGCCC; OPB-11: GTAGACCCGT and OPB-12: CCTTGACGCA) were selected for further study. The statistical analysis of the RAPD data, dissimilarity coefficients (DIST) and clustering analysis was based on the NTSYS program (Rohlf, 1992).

Results

All the *P. cactorum* isolates from birch induced typical symptoms within two weeks after inoculation of non-wounded as well as wounded bark in both experiments, whereas the control seedlings remained healthy in both cases.

Pathogenicity test 1

Only *P. cactorum* from birch caused lesions on the non-wounded bark of 1-year-old seedlings. Isolates PH10 and PH11 from birch infected four and three out of ten seedlings respectively on non-wounded bark. The average lesion size was 38 mm^2 (SD = 90) and 19 mm^2 (SD = 29), respectively. These isolates produced more and much larger lesions on wounded bark. The size of the wound-initiated lesions were 198 mm^2 (SD = 45) and 273 mm^2 (SD = 42), respectively. These lesions were much larger ($P < 0.01$) than those caused by *P. cactorum* isolate S3 from strawberry (107 mm^2, SD = 22) (Figure 1).

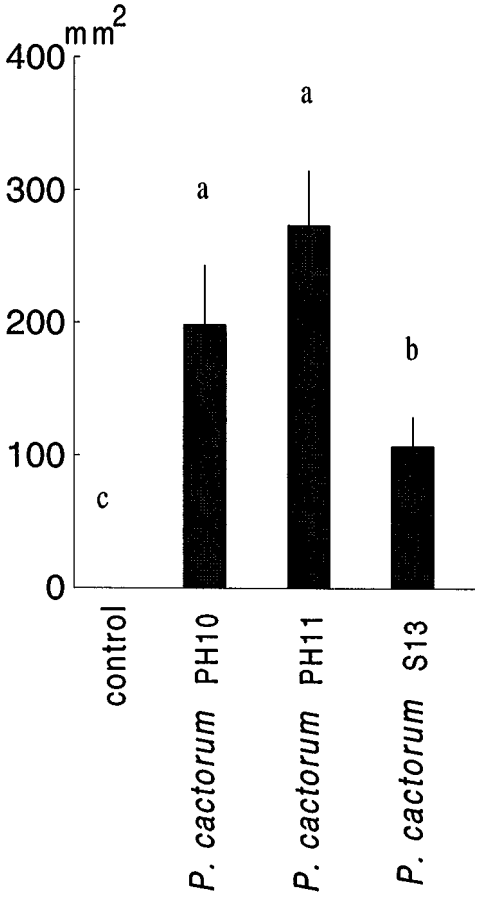

Figure 1. The size of lesions on silver birch seedlings three months after inoculation with *Phytophthora cactorum* isolates PH10 and PH11 from stem lesions on silver birch and *P. cactorum* S13 from strawberry plants suffering from crown rot. Inoculations were made on wounded bark of 1-year-old seedlings. The same letter above the columns indicates the size of lesions does not differ statistically ($P > 0.05$). Bars represent the standard error.

Pathogenicity test 2

The seed origin did not effect infection ($P > 0.05$), which made it possible to combine the data from the two origins. The isolates from birch occasionally infected the non-wounded bark of 2-month-old birches, whereas the isolates from strawberry were non-pathogenic. 14% of the lesions produced by the birch isolates were so large that they girdled the stem, whereas the strawberry isolates resulted in 10% infection and the lesions were so small that the disease index did not differ from that of the healthy control plants ($P < 0.05$). (Figure 2). As in the first test, the occurrence and incidence of the disease increased dramatically when the inoculations were performed on wounded bark. All the isolates from both birch and

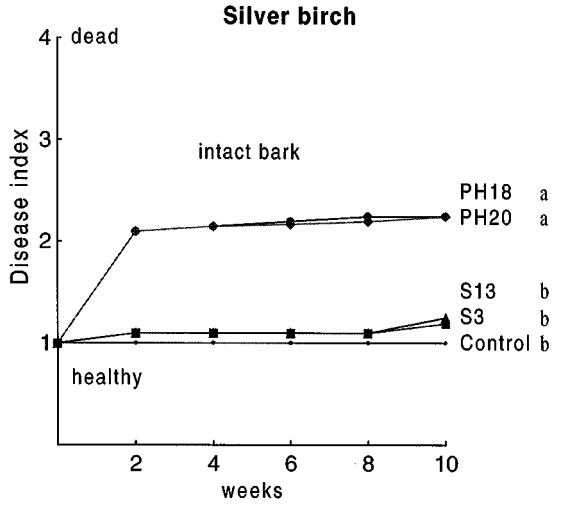

Figure 2. The condition of young silver birch seedlings 10 weeks after inoculation with *Phytophthora cactorum* isolates from stem lesions on silver birch (PH18 and PH20) and strawberry plants suffering from crown rot (S3 and S13). Inoculations were made on intact bark of 2-month-old seedlings. Curves followed by the same letter indicates that the health condition of the seedlings does not differ statistically ($P > 0.05$) at the last assessment.

strawberry caused large lesions, most of which girdled the stem. Their disease index was very similar, although not completely the same because one of the strawberry isolates had a somewhat lower index than the isolates from birch (Figure 3).

Pathogenicity test 3

Both of the strawberry isolates of *P. cactorum* were detrimental to strawberry plants, the isolate S13 being more pathogenic than isolate S3 ($P < 0.05$) (Figure 4). The *P. cactorum* isolates from birch were not pathogenic to strawberry plants (Figure 4).

RAPD analysis

Genetic variation in *P. cactorum* was examined by RAPD-PCR analysis using three primers. The RAPD profiles of all the strawberry isolates were very uniform (Figure 5). In contrast, *P. cactorum* isolates from silver birch were polymorphic with all the primers tested (Figure 5). UPGMA clustering analysis confirmed that the genetic variability in *P. cactorum* isolates from silver birch was higher than that in the isolates from strawberry (Figure 6).

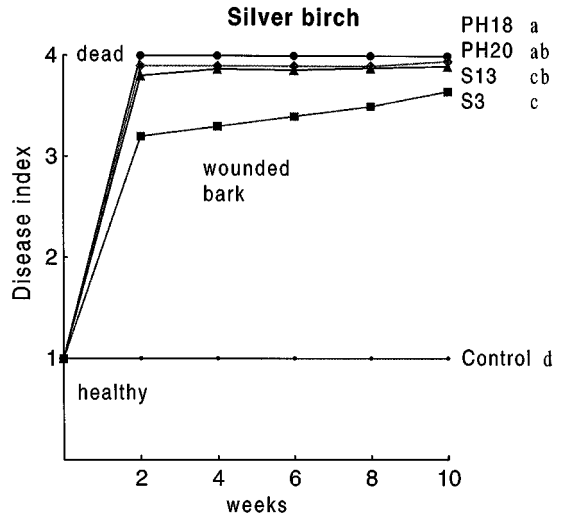

Figure 3. The condition of young silver birch seedlings 10 weeks after inoculation with *Phytophthora cactorum* isolates from stem lesions on silver birch (PH18 and PH20) and strawberry plants suffering from crown rot (S3 and S13). Inoculations were made on wounded bark of 2-month-old seedlings. Curves followed by the same letter indicates that the health condition of the seedlings does not differ statistically ($P > 0.05$) at the last assessment.

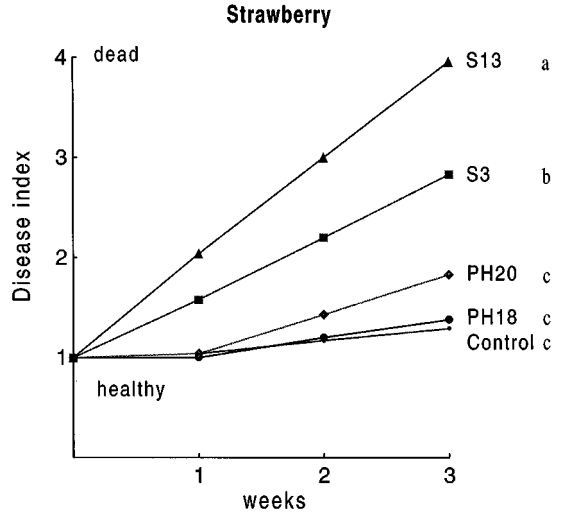

Figure 4. The condition of strawberry plants 3 weeks after inoculation with *Phytophthora cactorum* isolates from strawberry plants suffering from crown rot (S3 and S13) and stem lesions on silver birch (PH18 and PH20). Inoculations were made on wounds. Curves followed by the same letter indicates that the health condition of the plants does not differ statistically ($P > 0.05$) at the last assessment.

Discussion

This study shows that *P. cactorum* isolates from silver birch and strawberry are pathogenically and genetically separate. The isolates from birch were highly pathogenic to birch, causing necrotic lesions on wounded and non-wounded bark, whereas the isolates from strawberry were only pathogenic to wounded bark. The isolates from strawberry were detrimental to strawberry plants, whereas the isolates from birch did not infect strawberry. In the RAPD-PCR analysis, the RAPD markers and dendrogram analysis placed the isolates in host-specific groups.

The differentiation in *P. cactorum* according to host preference, found here and in previous studies (van der Scheer, 1971; Seemüller and Schmidle, 1979; Cooke et al., 1996; Hantula et al., 1997), is an interesting observation. Selection forces driven by a host plant appear to be most likely factor contributing to the population differentiation of *P. cactorum* into host-specific subgroups. As regards the time scale, if we assume that the initial founder population has been small, differentiation into separate host-specific groups has probably taken place relatively rapidly. The fact that strawberry is propagated by vegetative means is consistent with this idea. Contaminated runner plants form an inoculum source in regions where they are planted. The high genetic homogeneity of all the strawberry isolates studied by Cooke et al. (1996), Hantula et al. (1997) and those investigated in the present study indicate a narrow founder population, suggesting that in Europe this crown rot pathogen has been dispersed by man, and it might be caused by a single clone.

Although *P. cactorum* is thought to be a species that can be identified on the basis of morphological key characteristics (Waterhouse, 1963; Stamps et al., 1990), the finer resolution of the molecular markers such as RAPD's here and Random Amplified Microsatellites (RAMS) (Hantula et al., 1997) revealed variation among *P. cactorum* isolates, most of the variation correlating with the original host plants. Subspecific groups have also been found using RAPD analysis: the *P. cactorum* isolates from apple clustered separately from the strawberry isolates, but the raspberry isolates had affinities with both the strawberry and apple clusters (Cooke et al., 1996). Oudemans and Coffey (1991) were able to separate morphologically different isolates from strawberry into two electrophoretic groups by isozyme analysis. RAPD markers may also allow further differentiation of isolates from strawberry when a large number of *P. cac-*

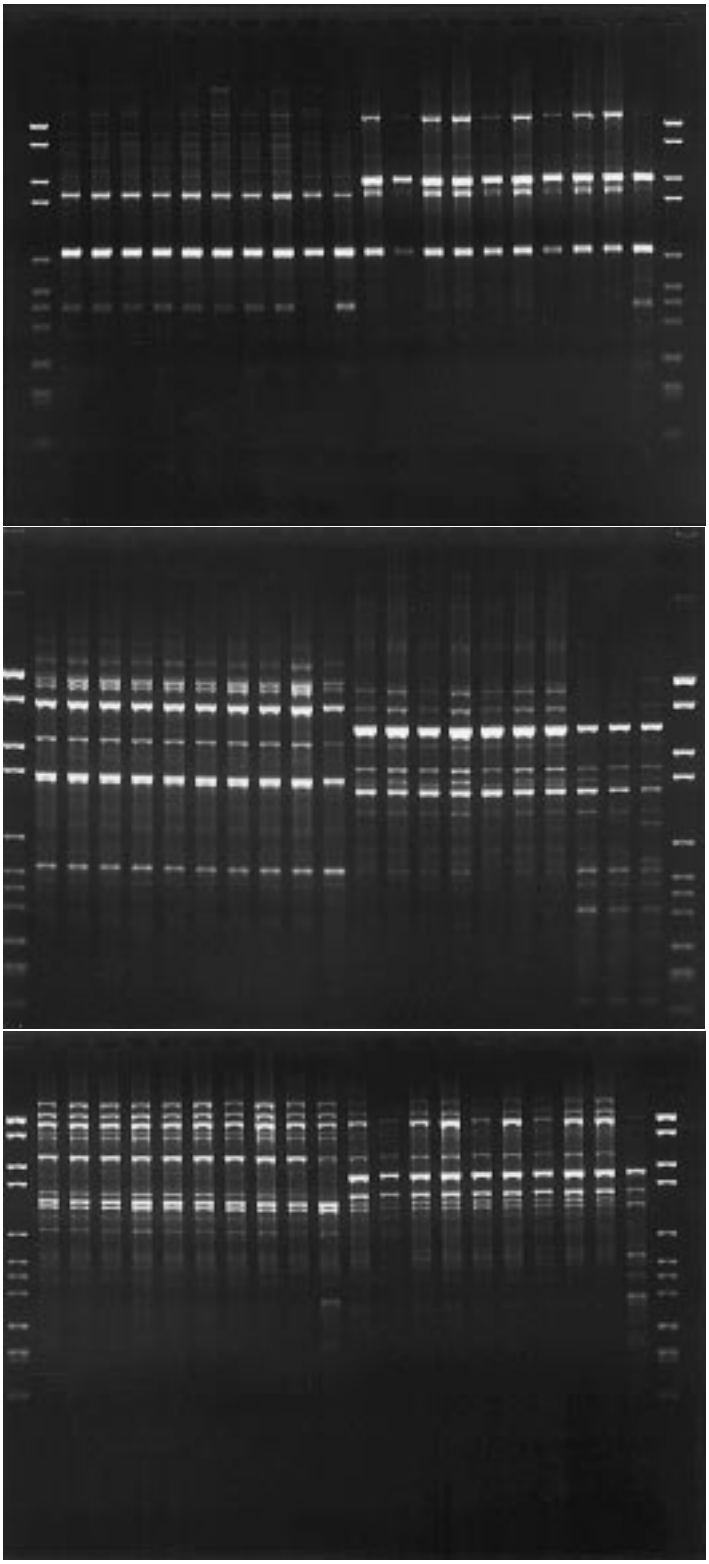

Figure 5. Analysis of *Phytophthora cactorum* isolates from strawberry and silver birch by RAPD-PCR method. a, b and c: lane a and v = molecular weight markers (MW, Boehringer VI, Germany) are 2176, 1766, 1230, 1033, 653, 517, 473, 394, 298, 234, 220 and 154 bp, lanes b-k, isolates from strawberry = S3, S6, S9, S13, S15, S19, TAM(1), CH09, CH12, A1, lanes l-u, isolates from birch = PH2, PH3, PH4, PH5, PH8, PH10, PH11, PH14, PH20, PH21. (a, b and c) Primers OPB-6: TGCTCTGCCC; OPB-11: GTAGACCCGT and OPB-12: CCTTGACGCA.

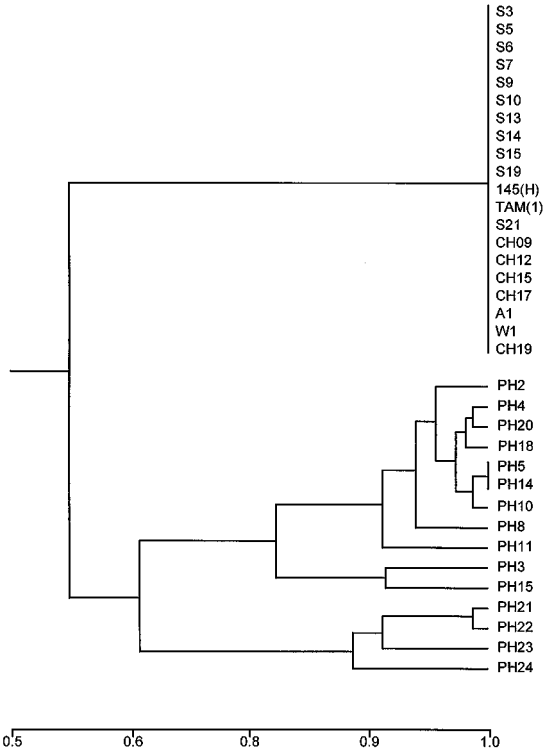

Figure 6. UPGMA dendrogram of the relationships between *Phytophthora cactorum* isolates based on similarity (DICE) coefficients using SAHN computer program (NTSYS-pc, Rohlf, 1989). Strawberry isolates (S3-S19, 145(H), TAM(1), CH09-CH19, A1, W1) form a separate group from the silver birch isolates (PH2-PH24).

torum isolates from both fruits and crowns originating from different parts of the world are tested.

In conclusion, we have shown on the basis of pathogenicity tests and DNA marker analysis that *P. cactorum* has evolved into genetically distinct populations on silver birch and strawberry. The recent outbreak in Finland of *P. cactorum* in birch could not be caused by the import of strawberry plants affected by crown rot. Furthermore, we have demonstrated that our *P. cactorum* isolates from strawberry are genetically very homogeneous, perhaps representing a single clonal genotype that may reflect the extensive migration of isolates via propagation material transported throughout Europe.

References

Anonymous (1990) BMDP statistical software manual. Vol 1. Univ. Calif. Press. Berkeley

Anonymous (1997) Statistical Yearbook of Forestry. The Finnish Forest Research Instutute. STV Agriculture and Forestry 1997: 4. 348 p

Cooke DEI; Kennedy DM, Guy DC, Russell J, Unkles SE and Duncan JM (1996) Relatedness of Group I species of *Phytophthora* as assessed by randomly amplified polymorphic DNA (RAPD's) and sequences of ribosomal DNA. Mycol Res 100: 297–303

Deutschmann F (1954) Eine Wurzelfäule an Erdbeeren, hervorgerufen durch *Phytophthora cactorum* (Leb. et Cohn) Schroet. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 6: 7–9

Erwin DC and Ribeiro O.K. 1996. *Phytophthora* diseases wordlwide. APS, St. Paul Minnesota

Frivold LH and Mielikäinen K (1991) The silviculture of birch in Scandinavia. In: Lorrain-Smith R and Worrell R (eds) The Commercial Potential of Birch in Scotland (pp 55–65) The Forestry Industry Committee of Great Britain, Edinburgh

Hantula J, Lilja A and Parikka P (1997) Genetic variation and host specificity of *Phytophthora cactorum* isolated from Europe. Mycol Res 101: 565–572

Kurkela T (1974) *Godronia multispora* Groves (Helotiales) and its pathogenicity to *Betula verrucosa* Ehr. and *B. pubescens* Ehr. Karstenia 14: 33–45

Lilja A and Hietala A (1994) *Phytophthora cactorum* and a novel type *Rhizoctonia* sp. as forest nursery pathogens. In: Perin R and Sutherland JR (eds) Diseases and Insects in Forest Nurseries (pp 59–64) Dijon (France), October 3–10, 1993. (Les Colloques, n°68) INRA, Paris

Lilja A, Rikala R. Hietala A and Heinonen R (1996) Stem lesions on *Betula pendula* seedlings in Finnish forest nurseries and the pathogenicity of *Phytophthora cactorum*. Eur J For Path 26: 89–96

Oudemans P and Coffey MD (1991) Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. Mycol Res 95: 19–30

Parikka P (1991) *Phytophthora cactorum* on strawberry in Finland. Nord Jordbruksfor 73: 121.

Petäistö RL (1983) Stem spotting of birch (*Betula pendula*) in nurseries. Folia For 544: 1–9

Rohlf FJ (1992) NTSYS-pc. Numerical taxonomy and multivariate analysis system. Exeter Publishing, Ltd., Setauket, NY

Seemüller E and Schmidle A (1979) Einfluß der Herkunft von *Phytophthora cactorum*-Isolaten auf ihre Virulenz an Apfelrinde, Erdbeerrhizomen und Erbeerfrüchten. Phytopath Z 94: 218–225

Van der Scheer, HATH (1971). Isolation of *Phytophthora cactorum* from soil in orchards and strawberry fields and differences in pathogenicity to apple. Netherl J Plant Path 77: 65–72

Stamps DJ, Waterhouse GM, Newhook FJ and Hall GS (1990) Revised key to the species of *Phytophthora*. Mycol Pap (CMI) 162: 1–28

Waterhouse GM (1963) Key to species of *Phytophthora* de Bary. Mycol Pap (CMI) 92: 1–22

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531–5