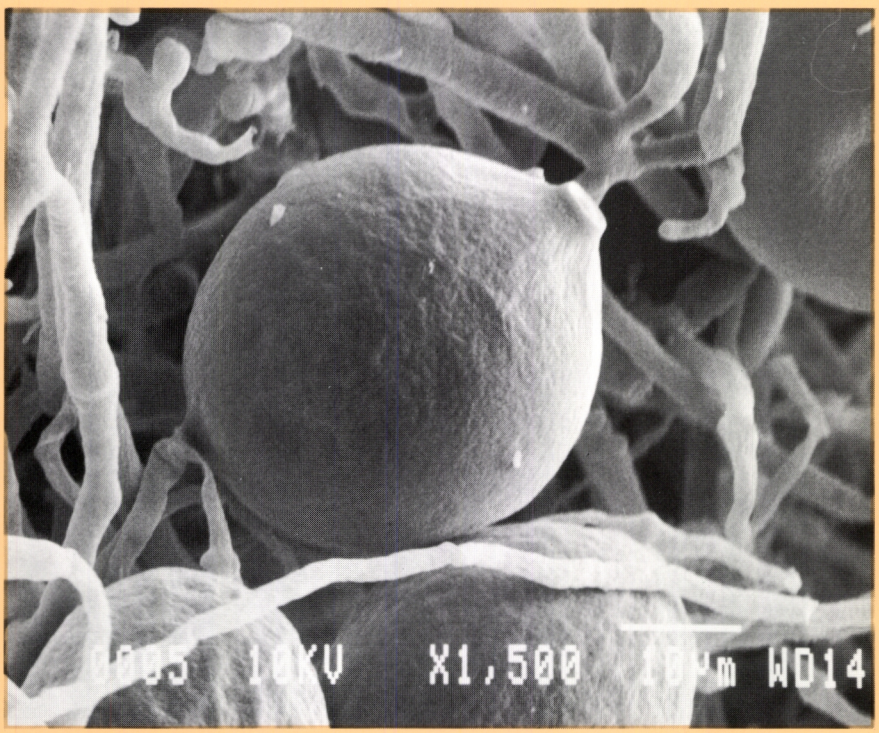
METSÄNTUTKIMUSLAITOKSEN TIEDONANTOJA 618, 1996
The Finnish Forest Research Institute, Research Papers 618

Root dieback and stem lesions in Finnish forest nurseries

Arja Lilja



METLA

METSÄNTUTKIMUSLAITOKSEN TIEDONANTOJA 618, 1996
The Finnish Forest Research Institute, Research Papers 618

Root dieback and stem lesions in Finnish forest nurseries

Arja Lilja

Academic Dissertation in Plant Pathology

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Viikki B_3, on Friday 13th December 1996, at 12 o´clock noon.

Arja Lilja 1996. Root dieback and stem lesions in Finnish forest nurseries.

The Finnish Forest Research Institute. Research Papers 618, 1996. 43+56 pages.
Metsäntutkimuslaitoksen tiedonantoja 618, 1996. 43+56 s.
ISBN 951-40-1538-X, ISSN 0358-4283

Key words: *Pinus sylvestris*, *Picea abies*, *Betula pendula*, uninucleate *Rhizoctonia*, *Pythium*, *Phytophthora undulata*, *Phytophthora cactorum*

Publisher: The Finnish Forest Research Institute
Metsäntutkimuslaitos

Correspondence: The Finnish Forest Research Institute, Vantaa Research Centre, P. O. Box 18, FIN-01301 Vantaa, Finland.
Tel. +358-9-857 051. Fax +358-9-8572 575.

Metsäntutkimuslaitos, Vantaan tutkimuskeskus, PL 18, 01301 Vantaa.
Puhelin (09) 857 051. Telekopio (09) 8572 575.

Layout: Anne Turunen
Research Forest Service
Tutkimusmetsäpalvelut

NORDIC ENVIRONMENTAL LABEL
411 011
Recyclable product with low emissions during production
Hakapaino Oy, Helsinki 1996

Acknowledgements

Most of this work has been carried out at the Vantaa Research Centre, Finnish Forest Research Institute which has provided me and my coworkers with good working facilities. The atmosphere in the 'old' Department of Forest Protection created by Timo Kurkela, Kari Korhonen, Sakari Lilja, Anna-Maija Hallaksela, Ari Hietala, Heikki Nuorteva, Katriina Lipponen, Michael Müller, Jarkko Hantula, Matti Kaivos, Brita Aarnio, Rauni Kantola, Kerttu Rainio, Sonja Sarsila, Maarit Niemi and Ritva Vanhanen, has made it easy to work and cooperate. Without my colleagues' assistance and support this thesis would never have been finished.

I am most grateful to Reijo Karjalainen who encouraged me to write this doctoral thesis and Heikki Smolander for his support. I would like to express my warm thanks to Ritva Vanhanen for her skillfull assistance and friendship during all these years. I also wish to thank all other coauthors, Sakari Lilja, Marja Poteri, Ziren Li, Ari Hietala, Robin Sen, Reijo Karjalainen, Risto Rikala, Riitta Heinonen, Jarkko Hantula and Päivi Parikka.

Part of this work has been done at the Plant Pathology Section, Department of Plant Biology, University of Helsinki, and I am grateful to Kari Kammiovirta for his assistance.

I wish to express my gratitude to all the nursery managers for their help over the years.

Kari Korhonen and Anna-Maija Hallaksela read this work. Their proposals and changes in the text were gratefully observed.

This thesis was reviewed by Dr. Mauritz Vestberg from the Agricultural Research Centre and Dr. Elna Stenström from Swedish University of Agricultural Sciences. Their positive and constructive comments on this work are acknowledged.

I also thank John Derome who revised the English and Anne Turunen who was responsible for the layout.

And finally, I thank my husband Sakari and daughters Hanna and Katja for their existence and support.

Vantaa
November 1996

Arja Lilja

Contents

Abstract

Two diseases, root dieback of conifer seedlings and stem lesions on birch caused by *Phytophthora cactorum,* have occurred in Finnish forest nurseries during the last ten years. The root dieback disease was first recorded in 1985 and since then it has caused economical losses in some nurseries. The production of birch seedlings has increased since 1980. At the same time a number of problems have appeared, of which stem lesions and cankers are among the most serious.

Surveys of micro-organisms showed that fungi are always present in diseased seedlings. The most pathogenic fungus isolated from roots of diseased conifer seedlings was uninucleate *Rhizoctonia* sp., and the strongest pathogen found in stem lesions on birch was *Phytophthora cactorum*. Both these fungi are new findings in our nurseries.

The uninucleate *Rhizoctonia* fruited in laboratory conditions, and its basidial characteristics placed this pathogen in the genus *Ceratobasidium*. The uninucleate strains anastomosed readily with each other producing a killing reaction, but did not anastomose with standard testers of binucleate *Rhizoctonia* (anastomosis groups AG-A to AG-S), nor with *R. alpina* and *R. quercus*. In a RAPD-PCR analysis, the uninucleate isolates had different banding patterns from the binucleate *Rhizoctonia*s. UPGMA analysis clustered the uninucleate isolates together at a similarity greater than 75 %, while the binucleate isolates used formed distinctly separate clusters and their similarity with the uninucleate *Rhizoctonia* sp. was 10-25 %. Hyphal anastomosis and DNA data suggest that the uninucleate *Rhizoctonia* sp. is a homogeneous group and distinct from the tested binucleate *Rhizoctonia*s.

In pathogenicity tests, uninucleate *Rhizoctonia* isolates reduced the root system development of Scots-pine and Norway-spruce seedlings, resulting in death or stunted growth depending on the age of the seedlings. *Pythium* species caused damping-off, but the only Pythiaceae damaging older seedlings were *P. ultimum* var. *ultimum* and *Phytophthora undulata*. A hypothesis is presented according to which primary infection by uninucleate *Rhizoctonia* results in a high moisture content in the growth medium because the stunted roots of infected seedlings cannot take up available water. Wet conditions favour Pythiaceous fungi and promote secondary attack by them.

The pathogenic fungi *Phytophthora cactorum*, *Fusarium avenaceum* and *Godronia* sp. were isolated from stem lesions of silver birch seedlings sampled from three nurseries. The percentage of seedlings infected by *P. cactorum* varied from 20 to 80. Inoculations of wounds or leaf scars on birch stems with *P. cactorum* resulted in necrotic lesions identical to those occurring naturally on birch seedlings in nurseries. Most of the 2-month-

old silver birch seedlings inoculated in spring died or broke already during the same summer. Seedlings inoculated at the age of 1year, were still alive after the first summer. However, after the cessation of lesion enlargement during winter, further host invasion during the following summer resulted in breakage of the plants.

In Finland, *P. cactorum* was isolated for the first time in 1990 from strawberry plants showing leather- and crown rot symptoms. One year later it was found in necrotic lesions on silver birch, and in summer 1995 it was isolated from lesions of common alder. *P. cactorum* isolates from silver birch and strawberry were highly pathogenic to their host plants. Isolates from strawberry caused necrotic lesions on silver birch, but isolates from birch were not pathogenic to strawberry.

Genetic comparison of *P. cactorum* isolates from different host plants and different countries with other *Phytophthora* species belonging to Waterhouse's Group I showed that *P. cactorum* isolates form a unique group, different from other species, with narrow pored papilla and paragynous antheridia. Within *P. cactorum*, strain differences were found using the Random Amplified Microsatellites (RAMS) technique. A dendrogram based on RAMS-variation separated isolates from silver birch, beech and all other hosts (red raspberry, strawberry, apple, rhododendron and horse-chestnut). This suggests that *P. cactorum* isolates infecting strawberry or silver birch are genetically different and their migrations to Finland have taken place separately.

List of publications

This thesis is based on the following articles, which in the text will be referred to by their Roman numerals.

I Lilja, A., Lilja, S., Poteri, M. & Ziren, L. 1992. Conifer seedling root fungi and root dieback in Finnish nurseries. Scandinavian Journal of Forest Research 7: 547-556.

II Lilja, A. 1994. The occurrence and pathogenicity of uni- and binucleate *Rhizoctonia* and Pythiaceae fungi among conifer seedlings in Finnish forest nurseries. European Journal of Forest Pathology 24: 181-192.

III Hietala, A., Sen, R. & Lilja, A. 1994. Anamorphic and telemorphic characteristics of a uninucleate *Rhizoctonia* sp. isolated from the roots of nursery grown conifer seedlings. Mycological Research 98: 1044-1050.

IV Lilja, A., Hietala, A. M. & Karjalainen, R. 1996. Identification of a uninucleate *Rhizoctonia* sp. by pathogenicity and hyphal anastomosis and RAPD analysis. Plant Pathology 45: 000-000 (In Press).

V Lilja, A., Rikala, R., Hietala, A. & Heinonen, R. 1996. Stem lesions on *Betula pendula* seedlings in Finnish forest nurseries and the pathogenicity of *Phytophthora cactorum*. European Journal of Forest Pathology 26: 89-96.

VI Hantula, J., Lilja, A. & Parikka, P. 1996. Genetic variation and host specificity of *Phytophthora cactorum* isolated in Europe. Mycological Research. 100: 000-000 (In Press).

1 Introduction

Nursery technology and cultural practices have changed during the last twenty years in Finland. Containerized seedling production has to a great extent replaced bare-rooted seedling production. Since the beginning of the 1980's the proportion of containerized production increased from 29 % to 80 % (Anonymous 1980, 1995, Rikala 1993). Greenhouses where the temperature can be regulated, selected growth substrate, irrigation and fertilization accelerate seedling growth, but they may also favour the development of many biotic diseases (Landis 1984, 1989). Abiotic stress caused by environmental conditions or injury can also predispose a large number of rapidly growing seedlings to fungal attack (Landis 1984, 1989). Of course there is a relationship between the presence of the pathogen, host and suitable conditions for the pathogen, but in general the risk of diseases is always high in nurseries (Landis 1989) and a correct diagnosis of diseases is needed to guarantee the production of healthy seedlings that will survive after transplantation in the forest or on abandoned agricultural land.

Damping-off is the first disease that can affect seeds, germinants and young seedlings of all tree species (Hanioja 1969, Lilja 1979, Lilja, S. 1980, Lilja et al. 1995). Grey mould is another omnivorous pathogen in dense growing units (Lilja, S. 1980). Snow blights and Lophodermium needle cast were among the first diseases which were controlled with fungicides in Finnish nurseries (Jamalainen 1956a,b, 1961). Scleroderris canker may also cause considerable losses in Scots pine (*Pinus sylvestris* L.) in forest nurseries (Kurkela 1967, Kurkela and Lilja, S. 1983). Pine twist rust can be a problem in some places were aspen trees grow in the immediate vicinity of nurseries (Kurkela 1973a, Kurkela and Lilja, S. 1983). Root dieback has occurred on Scots pine and Norway spruce (*Picea abies* L. Karst.) during the last ten years (Jalkanen 1985, Lilja et al. 1988). Birch rust epidemics occur regularly in late summer and fungicide spraying is obligatory every year (Lilja, S. 1973, 1986). Stem lesions are another problem with birch seedlings (Petäistö 1983).

1.1 Root dieback

In the Nordic Countries, the problem of root rot of conifer seedlings was first recorded in Norway, and Galaaen and Venn (1979) named the disease 'root dieback'. In 1985, it was diagnosed for the first time in a Finnish nursery (Jalkanen 1985). It has been estimated that this disease results in

an approx. 4 % decrease in forest nursery production in Norway and in Sweden (Venn et al. 1986, Beyer-Ericson et al. 1991). In Finland too, the economical losses have been temporarily high in some nurseries.

The symptoms of the disease on containerized or bare-rooted seedlings of both Scots pine and Norway spruce are needle discoloration, partial death of the root system and stunted growth. Several explanations for this type of root rot were considered, including fungi, nursery routines, pesticides, temperature, excess of water etc. (Jalkanen 1985, Venn 1985a). However, the occurrence of damaged seedlings in patches scattered over the seedling beds is one of the features suggesting that pathogens are involved (Venn 1985a, Venn et al. 1986).

The first studies concentrated on the whole mycoflora present in the roots of diseased seedlings. Common soil fungi from the genera *Pythium*, *Fusarium, Rhizoctonia* and *Cylindrocarpon* were represented among the micro-organisms isolated in Finland and Norway (Galaaen and Venn 1979, Venn 1983, Venn 1985a, b, Lilja et al. 1988). In Sweden, *Rhizoctonia* spp. were not isolated but, in addition to *Fusarium* and *Pythium, Cylindrocarpon destructans* (Zins.) Scholt. was associated with root death of containerized Scots pine (Beyer-Ericson et al. 1991). Unestam et al. (1989) even chose *C. destructans* as a model pathogen to study the possible associations between environmental conditions and root dieback. In Norway, a series of studies was carried out on the disease development and host response in primary roots of young Norway-spruce seedlings inoculated with *Pythium dimorphum* J. W. Hendrix & W. A. Campbell (Sharma et al. 1993, 1995, Børja et al. 1995).

1.2 Stem rots and cankers

The urgent need to decrease agricultural production has favoured, and will continue to favour afforestation of agricultural land. Arable land area afforested in 1993 amounted to 17 688 hectares (Anonymous 1995), and silver (*Betula pendula* Roth) or pubescent birch (*B. pubescens* Ehrh.) plantations have, in general, been rather successful (Hytönen 1995). Almost 100 000 ha of forest land have been planted with silver birch (Frivold and Mielikäinen 1991). The number of birch seedlings delivered for planting has also increased from 3 million to 19 million seedlings during 1980-1994 (Anonymous 1981, 1995). In connection with this increased production, problems have appeared, of which stem lesions and cankers have been among the most serious (Petäistö 1983, Lilja 1996). Both silver and pubescent birch seedlings have suffered from the disease and lesions have also recently occurred on common alder (*Alnus glutinosa* (L.) Gaertner) seedlings (Lilja et al. 1996). Several fungi such as *Botrytis*, *Fusarium* and

Godronia are known to cause such lesions in birch (Kurkela 1974, Petäistö 1983, Lilja and Hietala 1994a,b). Many other fungal species from the genus *Phytophthora* (Hamm and Hansen 1991, Chastagner et al. 1995), *Fusarium* (Morgan 1983, Viljoen et al. 1994) and *Phomopsis* (Sinclair et al. 1987) are also known to cause stem lesions and cankers in forest nurseries.

1.3 Pathogenic fungi associated with root dieback and stem cankers

1.3.1 *Cylindrocarpon*

Cylidrocarpon species can be roughly subdivided into two ecological groups: 1) those having an affinity for plant roots, and 2) those which are facultative parasites (Domsch et al. 1980). Several species of *Cylindrocarpon* have been isolated from tree seedling roots of both diseased and apparently healthy seedlings (Vaartaja 1967, James et al. 1994). Some reports have showed that *Cylindrocarpon* spp. might be pathogic to many tree species under conditions unfavourable for the host (Salt 1979, Kessler 1988). *C. derstructans*, which has also been isolated from root dieback seedlings (Venn 1983, 1985b, Galaaen and Venn 1979, Unestam et al. 1989) is an opportunist, the relationship between stress and the disease being obvious (Dahm and Strzelczyk 1987, Chakravarty and Unestam 1987, Unestam et al. 1989). In Brittish Columbia the corky root disease was believed to be caused by *C. destructans* associated with the nematode *Xiphinema bakeri* (Sutherland et al. 1972). However, in later studies it was found that inoculations with the nematode alone resulted in typical disease symptoms and no synergistic effects were detected between the nematode and the fungus (Sutherland 1977).

1.3.2 *Fusarium*

Some *Fusarium* species are important pathogens, causing damping-off, root rot and stem rot (Domsch et al. 1980). In fact, *Fusarium* root rot, the symptoms of which are wilting, chlorosis and necrosis, has been considered to be one of the most common disease of conifer seedlings in the world (Bloomberg 1981). In Canada, Bloomberg (1971) showed that some strains of *F. oxysporum* which persisted in seedlings that survived damping-off, later become active and caused root rot. *Fusarium* root rot of bare-rooted seedlings has been most often attributed to the fungus *F. oxysporum* Schlecht. (Bloomberg 1971, Bloomberg 1973, Sutherland 1990). This species is common on containerized seedlings (James et al. 1987, 1989), but other species

such as *F. acuminatum* Ell. & Ev. , *F. avenaceum* (Fr.) Sacc., *F. solani* Mart. Appell &Wollenw., *F. sambucinum* Fuckel and *F. tricinctum* (Corda) Sacc. are also possible (James 1983, James et al. 1989, James et al. 1991).

Hypocotyl rot on Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings was shown to be caused by *F. oxysporum.* The aboveground symptoms are similar to those of *Fusarium* root rot, but the roots remain healthy (Hansen and Hamm 1988). The stem canker on the same conifer species was first attributed to *F. roseum* Link ex Gray (nomen ambioguum) (Morgan 1983), but Hansen and Hamm (1988) later proved that *F. roseum* and *Phoma eupyrena* Sacc. cause the lesions near the root collar. These fungi, acting alone or together, also cause cankers on the upper part of the stem of Douglas fir seedlings (Hansen and Hamm 1988). Expanding cankers may gridle seedlings.

1.3.3 *Godronia*

The most extensively studied fungi causing stem lesions and cankers on birch seedlings in Finnish nurseries is *Godronia multispora* J. Groves (Petäistö 1983, Rikala and Petäistö 1986). This fungus also occurs in young birch stands growing on peat and paludified mineral soils (Kurkela 1974). It has been shown to be pathogenic to yellow (*B. alleghaniensis* Britton) (Martineau and Lavallee 1971), silver and downy birches (Kurkela 1973b, 1974, Romakkaniemi 1986).

The pathogenicity of *Godronia* spp. to birch has been known for a long time. *G. cassandrae* Peck f. sp. *betulicola* Groves causes cankers on many birch species, such as paper birch (*B. papyrifera* Marshall), grey birch (*B. populifolia* Marsh.) and yellow birch (Smerlis 1968, 1969, Desprez-Loustau and Dessureault 1987, 1988). Inoculations with *G. fuliginosa* (Fr.) Seaver, isolated from diseased shoots of paper birch seedlings growing among willows, were successful on paper, gray and yellow birches. Pycnidia with characteristic spores of *G. fuliginosa* developed only on inoculated yellow birch seedling, other birch species had other disease symptoms (Smerlis 1969).

1.3.4 *Phomopsis*

Many *Phomopsis* species (teleomorhp *Diaporthe*) are known to cause seedling blights and stem cankers. *Phomopsis* sp. was found to be associated with dead young shoots and cankers on yellow birch seedlings and saplings in Ontario (Horner 1956). The perfect stage of this *Phomopsis* proved to be *Diaporthe alleghaniensis* R. H. Arnold (*D. eres* f. sp. *betulae* R. H. Arnold) (Arnold 1967). Inoculations into wounds caused canker formation on shoots, stems and branches on yellow birch (Arnold 1970). In

Europe *D. alleghaniensis* was a stress pathogen on *B. maximowicziana* Regel, but no infection occurred on silver birch (Butin 1980).

P. lokoyae is one of several fungi that cause stem canker on Douglas fir seedlings (Hansen 1990).

1.3.5 *Pythium*

Pythium species are common both in soil and in aquatic environments and have a wide host range (Sutherland et al. 1989). These species have been isolated from soil in forest nurseries, e.g. in the USA (Campbell and Hendrix 1967, Hendrix and Campbell 1968), Canada (Vaartaja and Salisbury 1961, Vaartaja 1967, 1968), Australia (Vaartaja and Bumbiers 1964) and New Zealand (Robertson 1973). In particular, the *P. ultimum* Trow.- *P. irregulare* Buisman complex is found world-wide in forest tree nurseries; while other species occur less frequently (Hendrix and Campbell 1973).

The main type of disease caused by *Pythium* spp. is damping-off in forest nurseries (Vaartaja and Cram 1956, Vaartaja et al. 1961, Darvas et al. 1978, Perrin and Sampangi 1986). Another type, *Pythium* root rot, is usually restricted to juvenile or succulent tissues or on older plants to the root tips or lateral roots (Hendrix and Campbell 1973, Endo and Colt 1974, Sutherland et al. 1989). In a pathogenicity tests *P. helicoides* Drechs., *P. irregulare*, *P. rostratum* Butler, *P. spinosum* Sawada, *P. ultimum* and *P. vexans* de Bary reduced the weight of roots of loblolly and shortleaf pines (*Pinus taeda* L. and *P. echinata* Mill.). However, the reduction in root weight did not necessarily result in a corresponding reduction in shoot weight (Hendrix and Campell 1968b). In general, *Pythium* spp. operate as part of a disease complex involving other root pathogens that attack tree species of all ages during nursery culture (Hendrix and Campbell 1973).

1.3.6 *Rhizoctonia*

The genus *Rhizoctonia* is a large, diverse and complex group of fungi. Based on teleomorphs, most *Rhizoctonia* species are associated with one of four Basidiomycotina: *Thanatephorus* and *Waitea*, which have anamorphs, with multinucleate cells in young vegetative hyphae and *Cerotobasidium* and *Tulasnella* with binucleate cells (Sneh et al. 1991). Futher subdivision into anastomosis groups (AG) can be made on the basis of affinities for hyphal fusion (Anderson 1982, Ogoshi 1976, 1987). To date, at least 11 AGs have been identified within the multinucleate *R. solani* complex and over 20 groups (CAG/AG) among the different *Ceratobasidium* spp.; and two groups (WAG) within *Waitea* (Sneh et al. 1991, Vigalys and Cubeta 1994). However, genetic variation is possible

among isolates representing the same AG group (Duncan et al. 1993, Balali et al. 1996). Ogoshi (1987) has introduced intraspesific subgroups (ISG) within an AG. The isolates representing the same ISG anastomose more frequently with each other or have host specificity or possess other distinguishing characteristics. Most ISG are morphologically and genetically different from each other and represent biological species (Vigalys and Gonzalez 1990, Vigalys and Cubeta 1994).

The most studied species within the genus *Rhizoctonia* is *R. solani* Kühn, which is an important pathogen of young tree seedlings throughout the world (Roth and Riker 1943, Vaartaja and Cram 1956, Molin et al. 1961, Saksena and Vaartaja 1961, Hocking 1968, Darvas et al. 1978, Perrin and Sampangi 1986, Huang and Kuhlman 1989, 1990). Saksena and Vaartaja (1960, 1961) isolated *Rhizoctonia* spp. from forest nurseries in Saskatchewan and Manitoba. The pathogenicity of ten different *Rhizoctonia* species was tested, and five were found to cause root rot in pine (*P. sylvesris* and *P. resinosa*) seedlings, such as the binucleate species *R. globularis* Saks. and Vaart., *R. endophytica* var. *endophytica* Saks. and Vaar. and *R. callae* Cast. (Saksena and Vaartaja 1961). Binucleate *Rhizoctonia* isolates representing the anastomosis group CAG-3 (=AG-E, Sneh et al. 1991), were found to cause seedling blight of longleaf (*P. palustris* Mill.) and loblolly pines in the USA (English et al. 1986, Runion and Kelley 1993) and damping-off in Slash pine (*Pinus elliottii* Engelm. var. *elliottii*) in Georgia (Huang and Kuhlman 1990). In Norway, binucleate *Rhizoctonia* spp. have been identified as the cause of needledeath in Norway spruce (Roll-Hansen and Roll-Hansen 1968). A more detailed review about *Rhizoctonia* spp. associated with forest trees has been published by Hietala and Sen (1996).

1.3.7 *Phytophthora*

At least 80 species and varieties have been described in the genus *Phytophthora* (Mitchell and Kannwischer-Mitchell 1992). Most *Phytophthora* species are soil-borne pathogens causing e. g. damping-off, root rots, collar and crown rots, stem rot and foliar blights.

Ph. cinnamomi Rands is one of the most destructive pathogen on various trees and shrubs world-wide. This species may have originated in Papua New Guinea and in South Africa (Brasier and Hansen 1992). In Europe it has been reported to infect, e.g. Douglas fir in the UK (Aldhouse 1972) and in France it is associated with cankers in red oak (*Quercus rubra* L.) (Robin et al. 1992). In the USA, *Ph. cinnamomi* causes littleleaf disese of shortleaf pine and loblolly pine (Lorio 1966) and root and collar rot e.g. to Fraser fir (*Abies fraseri* (Pursh) Poir) (Bruck and Kenerley 1983, Kenerley and Bruck 1987, Kenerley et al. 1989), noble fir (*A. procera* Rhedo), Pacific silver (*A. amabilis* Forb.), Shasta red (*A. magnifica* var. *shastensis*

Lemm.) (Hamm and Hansen 1982), Norway spruce, balsam fir (*Abies balsamea* (L.) Mill.) (Kenerley and Bruck 1981), red pine (*Pinus resinosa* Ait.) (Jackson and Grandall 1935) and Douglas fir (Pratt et al. 1976, Hamm and Hansen 1982). It has also been isolated from Monterey pine (*P. radiata* D. Don) in Australia (Kassaby and Hepworth 1987) and in New Zealand (Basset and Will 1964). In South Africa it is associated with many *Pinus* species (von Broembsen 1981) and eucalypts (Linde et al. 1994).

Ph. cactorum (Leb. and Cohn) Schr., which has a world-wide distribution, is a root, stem and dieback pathogen of many different types of host (Nienhaus 1960). In forest nurseries it has been isolated from root rot seedlings of Douglas fir, Pacific silver fir and noble fir and from the crown of declining seedlings of many other fir species (Hamm and Hansen 1982, Adams and Bielenin 1988). In Sweden it has been reported to cause damping-off of Scots pine (Molin et al. 1961), and in Finland *Ph. cactorum* has been a serious stem pathogen of birch seedlings (Lilja and Hietala 1994a,b). It has been isolated from sweet birch (*B. lenta* L.) suffering from a bleeding canker (Anonymous 1941), and it has been shown to be the cause of seedling blight in beech (*Fagus sylvatica* L.) in the UK (Strouts 1981). It has been isolated with other oomycetous fungi as well from cankers on many tree species, e.g., apple (*Malus domestica* Borkh.) (Harris 1991), *Eucalyptus* spp. (Wardlaw and Palzer 1985), horse-chestnut (*Aesculus hippocastanum* L.) (Werres et al. 1995) and noble fir (Chastagner et al. 1995).

Ph. cryptogea Pethybr. and Laff causes root rot of Douglas fir in the USA (Pratt et al. 1976, Hamm and Hansen 1982) and British Columbia (Hamm et al. 1985). It has also been isolated from stem cankers on noble fir (Chastagner et al. 1995). In artificial inoculations it has been shown to infect many conifer species (Hamm and Hansen 1982, Campbell and Hamm 1989). In Europe, *Ph. cryptogea* is one *Phytophthora* species occurring in woody ornamentals (Smith et al. 1985).

Ph cryptogea, Ph. drechsleri Tucker and *Ph. gonapodyides* (Peterson) Buisman may be conspesific (Brasier et al. 1989, Brasier 1991). However, Hamm and Hansen (1991) prefer separating *Ph. cryptogea* and *Ph. drechsleri* from each other because these two species have certain differences: e.g. *Ph. drechsleri* grows at 35° C and *Ph. cryptogea* does not. *Ph. gonapodyides*, reported as *Ph. drechsleri,* infects a large number of conifer seedlings in the USA (Pratt et al. 1976, Hamm and Hansen 1982, Campbell and Hamm 1989, Chastagner et al. 1995).

Ph. lateralis is almost exclusively responsible for the root rot of Port Orford cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.) in Oregon, Washington and California (Hansen et al. 1989).

Ph. megasperma Drechsler is a complex; isolates representing the species have differences in morphology, physiology, biochemistry and pathogenicity (Hansen 1991). It contains at least nine groups, including

hostspecific biological groups (Hansen et al. 1986, Hansen and Maxwell 1991, Föster and Coffey 1993). Two distinct forms of *Ph. megasperma,* representing DF and BHR groups, are found in conifer nurseries (Hamm and Hansen 1991). Isolates from the BHR group have been less aggressive than isolates from the DF group, but in pathogenicity tests they have had a wider host range (Hamm and Hansen 1981, 1982).

Ph. citricola Saw has been reported to damage Fraser, balsam, white and Douglas firs (Shew and Benson 1981, Adams and Bielenin 1988). In Australia it has also been isolated from nursery soil (Davison and Bumbieris 1973). *Ph. pseudotsugae* has been found in Oregon and Washington in forest nurseries where it attacks Douglas fir (Hamm and Hansen 1983, 1987, 1991) and an *Abies* sp. (Hamm and Hansen 1991).

A new *Phytophthora* species was found to be associated with the current death of the common, grey (*A. incana* (L.) Gaertner) and Italian (*A. cordata* Desf.) alder in Southern England. A crown dieback, lower stem bark lesions and tarry exudations were observed in diseased trees (Gibbs and Lonsdale 1995). In inoculations, lesions developed in the bark above and below all the *Phytophthora* inoculated wounds (Brasier et al. 1995).

2 Aims

The general aims of these papers was to identify and characterize pathogens responsible for root dieback disease on conifer seedlings and stem lesions on silver birch stems in forest nurseries in Finland.

Specific aims of the individual studies:

I To clarify the role of fungi in root dieback. This was carried out by isolating and identifying fungi from the roots of diseased and healthy conifer seedlings and from the growth substrate in forest nurseries. The pathogenicity of all isolated species to Scots pine was also tested.

II To determine the frequency of uni- and binucleate *Rhizoctonia* spp. and Pythiaceae fungi in the roots of diseased conifer seedlings and their effects on the root and shoot development of Norway-spruce and Scots-pine seedlings. This was accomplise through fungal isolations and pathogenicity tests.

III To characterize the Finnish uninucleate *Rhizoctonia* isolates from root dieback seedlings in one nursery together with an isolate from Norway.

IV To test the similarity of uninucleate *Rhizoctonia* isolates from different conifer species and nurseries by means of pathogenicity tests, hyphal anastomosis and Randomly Amplified Polymorphic DNA (RAPD) markers.

V To test the pathogenicity of *Phytophthora cactorum* to silver birch seedlings and to asses its frequency and that of other microbes in stem lesions on nursery seedlings of silver birch, and to determine the effect of the previous year's fertilization on the incidence of *P. cactorum* infection.

VI To compare *P. cactorum* isolates from different hosts in Europe This was carried out by testing the genetic similarity of *P. cactorum* using the Random Amplified Microsatellites (RAMS) technique. In addition, the pathogenicity, morphology and growth rates of isolates from stem lesions on silver birch and strawberry plants suffering from crown rot were compared to confirm the hyphothesis that isolates from birch and strawberry were different from each other.

3 Materials and methods

3.1 Sample collection and fungal isolation

During the period 1986-1995, samples were collected from forest nurseries located throughout Finland (tables 1 and 2, fig. 1). The conifer seedlings showed root dieback symptoms (I, II) and the silver birch seedlings had necrotic lesions on the stems and on the root collar (IV, V). Healthy conifer seedlings were also studied (I, II). The fungal isolations from strawberry plants showing crown rot symptoms were conducted by Päivi Parikka (VI). Details of the samples are presented in Table 1.

Fungal isolations were carried out by plating surface sterilized pieces cut from the marginal zone between dead and healthy tissue on different agar media. After 3-5 days' incubation the hyphal tips were transferred to a new medium (I, II, V). Fungi were also isolated from nursery bed soil and peat from the containers of the rootdieback seedlings using an apple or a cucumber seedling as bait (I, II).

3.2 Pathogenicity tests

In order to complete Koch's postulates the pathogenicity of fungi isolated from root dieback seedlings (I, II, IV) and *P. cactorum* from necrotic lesions on silver birch (V, VI) and crown of strawberry plants suffering from crown rot (VI) was tested and the pathogens reisolated from the diseased seedlings.

The potential pathogenicity of all the fungal species from conifer roots was examined under sterile conditions (I). Most experiments in which the effects of Pythiaceae and *Rhizoctonia* spp. on root and shoot development of Scots pine (I, II, IV) and Norway spruce (II, IV) were investigated, were carried out in normal, unsterile growth conditions in a greenhouse.

Inoculations with *P. cactorum* isolates from silver birch and strawberry were carried out on wounds on host plants and on the intact bark of birch seedlings. Silver birches were also inoculated with strawberry isolates and vice versa. In addition, common alder was inoculated with an isolate from silver birch (VI).

Table 1. Origin of samples presented in fig. 1. Conifer seedlings (■) and birch seedlings (○).

Nursery	Date	Plant species	Type and age of seedlings		Number of seedlings		Number of soil samples	Publication
			containerized	bare-rooted	diseased	healthy		
1 Imari	07.09.87	*Pinus sylvestris*	1				1)	II
	12.11.90	*Pinus sylvestris*	1			20		II
	15.08.91	*Pinus sylvestris*	1		10			III, IV
2 Nuojua	02.11.87	*Picea abies*	1		20			II
	12.05.92	*Pinus sylvestris*	1		10			II
3 Lapinlahti								
- Pekolampi	10.05.86	*Pinus sylvestris*		2A + 1	37			I
	31.07.87	*Pinus sylvestris*	1		15			I
		Pinus sylvestris	2		24			I
	15.09.87	*Pinus sylvestris*		2A + 1	9			I
	23.10.87	*Pinus sylvestris*				10	19	I, IV
		Picea abies				10	19	I
	05.05.89	*Pinus sylvestris*		2A + 1	20	20		I, IV
	11.10.89	*Pinus sylvestris*		2A + 1		20		I, IV
	23.05.91	*Pinus sylvestris*		2A + 1	20	20		II
	14.08.92	*Picea abies*	2		20	20		II, IV
4 Ahvenlampi	10.10.89	*Picea abies*	1		20	20		I
5 Suonenjoki	24.05.91	*Pinus sylvestris*	1		20	20		II
	23.05.94	*Pinus sylvestris*	1		10			IV
6 Tuusjärvi								
- Ruuttula	30.07.87	*Pinus sylvestris*		2A + 1	7			II
7 Onkamo	04.04.95	*Betula pendula*	1		15			IV
8 Mellanå	15.10.90	*Pinus sylvestris*	1		40			I, IV
	15.05.91	*Pinus sylvestris*	1		20	20		II, IV
	10.09.94	*Betula pendula*	1		20			V
	05.06.93	*Betula pendula*	1		10			VI
9 Rantasalmi								
- Puupelto	09.05.86	*Pinus sylvestris*		2A + 1	11			I
10 Syrjälä	23.07.93	*Picea abies*	1					IV
11 Ahlainen								
- Kankaanr.	27.09.87	*Pinus sylvestris*		2A	10			II, IV
	10.10.92	*Picea abies*	2		59			II
12 Metsätyllilä	30.05.87	*Pinus sylvestris*	1		10	10	10	I
- Mäntyharju	05.05.89	*Pinus sylvestris*		2A + 1	20	20		I, IV
- Tyllilä	28.09.93	*Picea abies*	1		10			IV
	10.10.94	*Betula pendula*	1		40			V, VI
	10.10.95	*Abies koreana*						IV
13 Ukonniemi	05.07.91	*Pinus sylvestris*			15	15		II, IV
	05.07.91	*Betula pendula*	1		7			VI

1) Fungi were isolated from the growth substrate of diseased seedlings

Table 1. continues

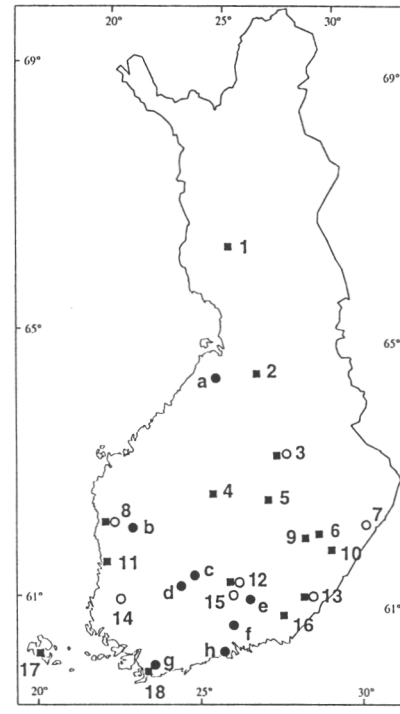
Nursery	Date	Plant species	Type and age of seedlings		Number of seedlings		Number of soil samples	Publi-cation
			contain-erized	bare-rooted	diseased	healthy		
14 Säkylä	11.05.92	*Betula pendula*	1		20			VI
15 Vierumäki	17.09.92	*Betula pendula*	1		50			V, VI
16 Taavetti - Hepoharju	17.05.91	*Picea abies*	2		20	20		II, IV
17 Jomala	29.08.91	*Larix sibirica*	1		5			II, IV
18 Leksvall	13.05.87	*Pinus sylvestris*		2A + 1	32			II
	31.07.87	*Pinus sylvestris*		2A + 1	16			II
	17.09.91	*Pinus sylvestris*		3A	10	10		II

Table 2.
Origin of samples presented in figure 1. Strawberry plants (●). Isolation were conducted by Päivi Parikka.

Strawberry-field	Year	Plant species	Publi-cation
a Rantsila	1991	*Fragaria* x *ananassa*	VI
b Karvia	1992	*Fragaria* x *ananassa*	VI
c Kuhmalahti	1990	*Fragaria* x *ananassa*	VI
d Pälkäne	1990	*Fragaria* x *ananassa*	VI
e Jaala	1990	*Fragaria* x *ananassa*	VI
f Artjärvi	1992	*Fragaria* x *ananassa*	VI
g Pohjankuru	1990, 1991	*Fragaria* x *ananassa*	VI
h Porvoo, Kari	1992	*Fragaria* x *ananassa*	VI

Figure 1. Origin of samples

■ conifer seedling
○ birch seedling
● strawberry plant



3.3 Fungal identification

Traditional taxonomy is largely based on morphological criteria supported by physiological tests. The shape, dimensions and presence of different types of conidia/spores, chlamydospores and conidia-/sporangiaphores and other hyphal chartacteristics were used in all (I, II, III, IV, V, VI) the studies (Booth 1971, Domsch et al. 1980, Sutton 1980, van der Plaats-Niterink 1981, Stamps et al. 1990, Hamm and Hansen 1991, Sneh et al. 1991). In addition to hyphal and cultural morphology, the nuclear condition (I, II, III, IV) and hyphal anastomosis (III, IV) (Sneh et al. 1991) were used in our studies for the identification and characterisation of *Rhizoctonia* spp. A new method, involving axenic liquid culture of the fungus in the presence of Scots-pine seedlings, was introduced to induce uninucleate *Rhizoctonia* sp. to produce teleomorph (III).

More precise classification methods based on genomic structure, the Randomly Amplified Polymorphic DNA (RAPD) technique (Williams et al. 1990, Weising et al. 1995) and the recently developed Random Amplified Microsatellites (RAMS) technique (Zietkiewicz et al. 1994) were also employed. Both methods use a polymerase chain reaction (PCR) in which the target DNA sequence is amplified with the help of primers and a thermostable DNA polymerase. The electrophoretic separation of amplified segments and their staining reveal possible differences in the banding patterns of studied DNA targets. RAPD use short, GC primers of arbitrary sequence, and RAMS primers are based on microsatellite motifs (Williams et al. 1990, Zietkiewicz et al. 1994).

In our work RAPD analysis was used to study the similarity of uninucleate *Rhizoctonia* sp. isolates and their relatedness to binucleate *Rhizoctonia* tester strains and to two Finnish binucleate isolates from root dieback seedlings (IV). RAPD markers have earlier been applied to measure genetic variation within fungi such as *R. solani* (Duncan et al. 1993) and *Phytophthora* spp. (Cooke et al. 1996). RAMS analysis proved to be useful for characterizing genetic variation within a variety of fungi (Hantula et al. 1996). In this study the method was applied to compare *P. cactorum* isolates from different hosts. Additional *Phytophthora* spp. from Group I, *Ph. undulata* (H. E. Petersen) M. W. Dick and *Pythium anandrum* Drechsler were also included in the RAMS analysis for comparision (VI).

4 Results and discussion

4.1 Root dieback

Surveys of fungi present in the roots of diseased seedlings (table 3) and subsequent pathogenicity trials suggest that the root dieback is a complex, both *Rhizoctonia* and *Pythium* spp. being involved (I, II). Seedlings at all developmental stages may be affected (I, II, IV), but the effects are most detrimental at the juvenile stage (I). This is in accordance with the results of studies in Norway (Galaaen and Venn 1979, Venn et al. 1986, Sharma et al. 1993, 1995, Børja et al. 1995).

Common soil fungi representing different genera inhabited the roots of diseased seedlings, but many of them were also present in roots of healthy lookings seedlings (I) (Galaaen and Venn 1979, Venn 1983, Venn 1985 a,b). *Pythium* spp., *Ph. undulata* and *Fusarium oxysporum* f. sp. *pini* were pathogenic to young seedlings (I, II). *Ph. undulata* is infrequently reported and little is known about its pathogenicity. It has been isolated from irrigation water in the UK (Pitts and Calhoun 1984) and also from streams in forested areas of North America (Hamm et al. 1988).

Table 3. The percentage of diseased seedlings infected with *Rhizoctonia* and Pythiaceae fungi

Fungus	*Picea abies*		*Pinus sylvestris*	
	container.	bare-rooted	container.	bare-rooted
Pythium anandrum	2.6		8.9	10.0
P. dissotocum				0.4
P. irregulare			3/10[1)]	
P. ultimum var. *ultimum*		10	6.7	26.1
Pythium spp.	7.1			11.9
Pythium HS-group	2.6		5.2	0.8
Phytophthora undulata	25.3		19.3	6.1
Rhizoctonia				
uninucleate	36.4	30	46.7	18.0
binucleate	23.4		3.0	2.3
multinucleate				1.5
Number of seedlings	154	10	135	261
Number of nurseries	5	1	6	6

[1)] Baited from the growth substrate of diseased seedlings with 10 cucumber seedlings.

The most pathogenic isolate to seedlings at all stages from 2-week-old to 2-year-old was uninucleate *Rhizoctonia* sp (I, II, IV). Hall (1986) has described an uninucleate *Rhizoctonia* sp. which infected wheat roots. However, from his description of the anamorph we can be sure that this fungus is not the same species causing root rot of Scots-pine and Norway-spruce seedlings (III). Isolates of two species, *R. quercus* Cast. and *R. alpina* Cast., were reported to contain uninucleate hyphae (Burpee et al. 1980). However, Sneh et al. (1991) consider them to represent binucleate *Rhizoctonia* spp., having teleomorphs in *Ceratobasidium* spp.

After unsuccessful attempts to obtain the perfect state of uninucleate *Rhizoctonia* sp., the studied fungus fruited in an axenic liquid culture in the presence of Scots-pine seedlings (III). On the basis of basidial characteristics the uninucleate *Rhizoctonia* isolates from Finland and Norway can be placed in the genus *Ceratobasidium* (III). All the known anamorphs of other *Ceratobasidium* spp. have binucleate cells in their vegetative hyphae (Sneh et al. 1991). The percentage of uninucleate tip/subapical cell pairs in the all uninucleate isolates was 96.7 (IV). Futher studies showed that the uninucleate *Rhizoctonia* sp. isolates formed a genetically homogeneous group. They anastomosed readily with each other, but not with tester strains (Sneh et al. 1991) of *Ceratobasidium* (III, IV). In a RAPD analysis, uninucleate isolates had banding patterns different to those of the binucleate isolates and testers used (IV).

Hietala (1995) isolated binucleate and uninucleate *Rhizoctonia* sp. from the same seedling. The tested binucleate *Rhizoctonia* isolates were genetically 10-25 % similar to the uninucleate *Rhizoctonia* sp. The Finnish binucleate *Rhizoctonia* isolates show morphological variation and can be divided into several anastomosis groups (Hietala 1995, IV). The pathogenicity of these isolates varied depending on the isolate. Some isolates were pathogenic (V) and some non-pathogenic (II). The binucleate isolate 268 anastomosed with the tester strain AG-I, but the anastomosis frequency was lower than that observed among uninucleate isolates (IV). This and the low genetic similarity (28 %) between the Finnish isolate 268 and the Japanese tester strain AG-I suggest that we might need new, 'European' testers for a reliable AG grouping. It is known that geographic isolation contribute divergency and dissimilarity even in the same AGs (Duncan et al. 1993, Balali et al. 1996).

It has been known for long that *Rhizoctonia* species cause damping-off and root rot in forest nurseries (Vaartaja and Cram 1956, Saksena and Vaartaja 1961). Nursery inoculations by Venn et al. (1986) with a *Rhizoctonia* sp., which was later shown to be the same uninucleate type as that in Finland (III), reduced the growth of Norway-spruce seedlings and resulted in typical root dieback symptoms such as stunted growth (Venn et al. 1986). In our studies, too, infection with the uninucleate *Rhizoctonia* reduced the main root and lateral root length of both Scots pine and Norway spruce, producing stunted seedlings (II, IV). The proportion of dead

seedlings varied in different inoculation trials from 0 to 100 % (I, II, IV). In Hietala's studies (1995) all the infected Norway-spruce seedlings remained alive, but the total root length was lower compared to the controls.

In Sweden the opportunistic pathogen, *Cylidrocarpon destructans* was, in addition to *Pythium* spp. and some other species, the most common isolate from diseased roots (Beyer-Ericson 1989, Unestam et al. 1989, Beyer-Ericson et al. 1991). A low light intensity and anaerobic root environment were found to predispose Scots pine roots to invasion by *C. destructans* (Unestam et al. 1989). In our studies none of the isolates representing the genus *Cylindrocarpon* were pathogenic. All the isolated Pythiaceae fungi were pathogenic to young, 2-week-old, Scots-pine seedlings (I), but only *P. ultimum* Trow. var. *ultimum* and *Ph. undulata* proved to be pathogenic to older, 12-week-old seedlings (II).

In Norway the most common fungus in the roots of diseased Norway-spruce seedlings was *P. sylvaticum* Campbell and Hendrix (Galaaen and Venn 1979). In laboratory trials *Pythium* sp. (later identified as *P. dimorphum*) was more pathogenic than *P. sylvaticum* (Venn 1985b). In the first nursery inoculation with this *Pythium* sp. some seedlings died suddenly after two weeks, but those that survied grew well (Venn et al. 1986). However, in later nursery inoculations it was pathogenic and reduced the shoot length of Norway-spruce seedlings (Børja 1995).

P. dimorphum readily infected the roots of 10- to 12-day-old Norway-spruce seedlings and caused the upper part, above the root hair zone, to become dark brown and the hypocotyl necrotic, while the root tips remained light coloured (Sharma et al. 1993, Børja et al. 1995). More than 30 different PR proteins accumulated in the infected roots (Sharma et al. 1993), and Børja et al. (1995) showed that the formation of lignin, distribution of flavanols and condensed tannins, were related to the visual disease symptoms and to cellular changes in root tissue.

The infection patterns of *P. dimorphum* and the uninucleate *Rhizoctonia* sp. are different. As described above, *P. dimorphum* did not infect root tips (Børja et al. 1995). In contrast, uninucleate *Rhizoctonia* sp. infection started from the root tips which became pigmented (Hietala 1995). In addition, hyphal aggregates typical of *Rhizoctonia* were observed on the surface of lateral root tips, but there were also hyphae inside the cortical cells in the main root (Hietala 1995).

In general, *Pythium* spp. operate as a part of a disease complex involving other root pathogens that attack tree species of all ages in nurseries (Hendrix and Campbell 1968, 1973). I have presented a hypothesis according to which root dieback is a disease of successive infections: primary infection by uninucleate *Rhizoctonia* sp. results in a high moisture content in the growth media because the decayed roots of infected seedlings cannot utilize the water supply. Wet conditions favour Pythiacea fungi and promote secondary attack by *Pythium* spp. or *Ph. undulata* (II).

Further unpublished results have shown that uninucleate *Rhizoctonia* is capable of infecting both Scots-pine and Norway-spruce seedlings under dry conditions (Lilja and Heiskanen unpubl.).

As described above, uninucleate *Rhizoctonia* sp. and *Pythium* spp. are able to kill small seedlings (I), but older infected seedlings may survive. The surviving seedlings do not fully recover from the disease. (II, IV)

4.2 *Phytophthora cactorum* and stem lesions on silver birch seedlings

Different diseases of many plants have been attributed to *P. cactorum* (Nienhaus 1960). In Europe strawberry crown rot was first reported from Germany in 1952 (Deutschmann 1954), but has since occurred in many other European countries. In Finland *P. cactorum* has regularly caused crop losses in strawberry (*Fragaria* x *ananassa* Duch.) fields since 1990 (Parikka 1990). One year later the first silver birch seedlings with necrotic lesions and *P. cactorum* on stems were sent to the Finnish Forest Research Institute from Metsätyllilä, Ukonniemi and Imari nurseries, locating in different parts of Finland (Fig. 1, Table 1).

Subsequent sampling and isolation revealed that *P. cactorum*, *F. avenaceum* and *Godronia* sp. were present in stem lesions in all nurseries from which diseased birch seedlings have been collected (V). Petäistö (1983) concluded that *G. multispora* infects seedlings only via wounds. *F. avenaceum* has been common in stem lesions of planted birch seedlings wounded by insects (Juutinen et al. 1976) and voles (Henttonen et al. 1994). In our study *P. cactorum* isolates from birch and strawberry effectively infected young silver birch seedlings via wounds, while only isolates from birch caused stem lesions on intact bark (VI). This is in accordance with the results of other pathogenicity test in which the only species observed to cause lesions on the intact bark of 1-year-old silver birch seedlings was *P. cactorum*, isolated from birch. Other inoculated species were *B. cinerea*, *F. avenaceum*, *Godronia* sp., binucleate *Rhizoctonia* sp. and a *P. cactorum* isolate from strawberry (Lilja et al. 1996).

Many of the morphological characteristics used in *Phytophthora* systematics, such as sporangial and oogonial dimensions, may exhibit a high level of variation within a species and may even overlap between species (Waterhouse 1963, Stamps et al. 1990). *P. cactorum* is a homothallic species producing deciduous, papillate sporangia and oogonia with paragynous antheridia. The sporangia should be ovoid according to the original descriptions and figures, but it may also be spherical, ellipsoid or obpyriform (Stamps et al. 1990). The size of oogonia varies between 25-32 µm (Hamm and Hansen 1991), 30.4-35.4 µm (Oudemans and Coffey 1991) or 24-36

µm (Kennedy and Duncan 1995). The mean size of the oogonia of Finnish *P. cactorum* isolates from birch (29.4 µm) and strawberry (28.3 µm) were slightly different (VI), but both values were inside the range presented above by Hamm and Hansen (1991) and Kennedy and Duncan (1995). The sporangia of isolates from strawberry were longer than those from birch ($p<0.01$), the values being 33.6 x 24.6 µm and 27.7 x 23.4 µm respectively (VI). The sporangial dimensions of CACT1 isolates from different parts of the world (USA, Canada, Europe, New Zealand, Australia and South Africa) were 26.1-34.5 x 22.1-29.1 µm (Oudemans and Coffey 1991) and for *P. cactorum* isolates from UK 34-60 x 25-55 µm (Kennedy and Duncan 1995).

In spite of possible morphological variation, the first genetic analysis based on isozymes (Oudemans and Coffey 1991) and mitochandrial DNA (Föster and Coffey 1991) suggested that *P. cactorum* strains isolated from diverse geographical locations and host plants share a high degree of simillarity. However, later studies using randomly amplified polymorhic DNA demonstrated the presence of two subspecific groups within *P. cactorum* isolates from apple, strawberry and raspberry (Cooke et al. 1996). The Random Amplified Microsatellites (RAMS) technique used by us revealed considerable variation within *P. cactorum* isolates from different host plants. In general, the grouping correlated with the plant species. The UPGMA dendrograms (VI) separated three main groups: isolates from silver birch, beech and all other plant species in this study (red raspberry, strawberry, apple, rhododendron and horse-chestnut).

The genetic variation within isolates originating from single plant species was low, the extreme being the 20 isolates from strawberry which had identical RAMS patterns. This suggests that a single clone causes the crown rot of strawberry within the studied geographical area (Finland, Estonia, Sweden, Germany, England and Scotland). However, small differences between the isolates of this clone were observed in the growth rates at different temperatures and in sporangial dimensions (VI).

The morhology of 11 isolates from silver birch did not differ, but in RAMS analysis three closely related amplitypes were observed. Nine isolates collected from six nurseries in southern and central Finland form one subgroup (nurseries: Metsätyllilä, Säkylä, Mellanå, Onkamo, Lapinlahti and Nuojua). The second amplitype was represented by one isolate from Vierumäki (Ph 11), which did not differ morphologically or physiologically from the other isolates. The isolate from Ukonniemi (Ph 3), forming the third amplitype, was the only one that differed slightly from the other isolates from birch; it grew fast at optimum and high temperatures (VI).

The strawberry plants infected with *P. cactorum* from strawberry died within three weeks. The mortality of silver birch seedlings, wounded and inoculated at the age of 2 months, varied from 10 to 85 % depending on the *P. cactorum* isolate (VI).

Clonal birch plants, wounded and inoculated at the age of 1 year, were still alive after the first summer. However, all the plants broke after second summer, but during the third summer 75 % of the plants produced new shoots from the base of the stem (V).

The nitrogen fertilization and phosphorus-potassium rich fertilizers used in Finnish nurseries were believed to affect the susceptibility of silver birch seedlings to stem lesions, although nitrogen fertization did not increase the incidence of *G. multispora* (Rikala and Petäistö 1986). It is known that ammonium nitrate fertilization increases crown and root rot caused by *P. cactorum* in apple trees (Utkheide and Smith 1995). In our experiment the previous year fertilization, which included two different PK levels and two timing of N application, did not have statistically significant effect on the development of lesions caused by *P. cactorum* during the five-week test period (V). The nutrient ratios (P/K and P/N) of the infected seedlings were higher than recommended by Ingestad (1970), but the possibly imbalanced nutrient status did not explain the results.

Conclusions

The articles presented two fungi: the uninucleate *Rhizoctonia* sp. and *Phytophthora cactorum*, both new findings in Finland. A survey of fungi present in the roots of diseased conifer seedlings and subsequent pathogenicity tests suggest that root dieback is a complex caused by the joint action of uninucleate *Rhizoctonia* and Pythiaceae fungi. All tested uninucleate *Rhizoctonia* isolates reduced the root system development of Scots-pine and Norway-spruce seedlings, resulting in death or stunted growth depending on the age of the seedlings. The low genetic variation observed within uninucleate *Rhizoctonia* proves that it has not occurred in forest nurseries in Finland or Norway for long enough to lead to genetical divergence.

P. cactorum proved to be one fungus responsible for stem lesions on birch seedling. The high genetic variation between *P. cactorum* isolates from birch and strawberry suggest that migration of these pathogens from Europe is probably independent.

References

Adams, G. J. , Jr. & Bielenin, B. 1988. First report of *Phytophthora cactorum* and *P. citricola* causing crown rot of fir species in Michigan. Plant Disease 72: 79-80.

Aldhouse, J. R. 1972. Nursery Practise, Forestry Commission Bulletin, No 43, 1-184.

Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annual Review of Phytopathology 20: 329-347.

Anonymous 1941. Firthy third annual report Rhode Island State College Agricultural Experiment Station. Contributions from Rhode Island State College Agricultural Experiment Station. No 586: 1-71.

Anonymous 1981. Yearbook of Forest Statistics 1980. Folia Forestalia 460: 1-205.

Anonymous. 1995. Statistical Yearbook of Forestry. Aarne, M. (ed.) Agriculture and Forestry 1995:5.

Arnold, R. H. 1967. A canker and foliage disease of yellow birch. 1. Description of the causal fungus, *Diaporthe alleghaniensis* sp. nov. and the symptoms on the host. Canadian Journal of Botany 45: 783-801.

Arnold, R. H. 1970. A canker and foliage disease of yellow birch. II. Artificial infection studies with *Diaporthe alleghaniensis* Canadian Journal of Botany 48: 1525-1540.

Balali, G. R., Whisson, D. I., Scott, E. S. & Neate, S. M. 1996. DNA fingerprinting probe specific to isolates of *Rhizoctonia solani* AG-3. Mycological Research 100: 467-470.

Basset, C. & Will, G. M. 1964. Soil sterilization trials in two forest nurseries. New Zealand Journal of Forestry Science 9: 50-58.

Beyer-Ericson, L. 1989. Rotdöd i skogsplantskolor. Plantnytt 1: 1-4 (In Swedish).

Beyer-Ericson, L., Damm, E. & Unestam, T., 1991. An overview of root dieback and its causes in Swedish forest nurseries. European Journal of Forest Pathology 21: 439-443.

Bloomberg, W. J., 1971. Diseases of Douglas-fir seedlings caused by *Fusarium oxysporum*. Phytopathology 61: 467-470.

Bloomberg, W. J., 1973. *Fusarium* root rot of Douglas-fir seedlings. Phytopathology 63: 337-341.

Bloomberg, W. J. 1981. Diseases caused by *Fusarium* in forest nurseries. In: Nelson, P. E., Toussoun, T. A., Cook, R. J. (eds.). *Fusarium*. diseases, biology, and taxonomy. University Park, PA, Pennsylvania State University Press. pp. 178-187.

Booth, C. 1971. The genus *Fusarium*. Lavenham Press Ltd., Lavenham, Suffolk.

Brasier, C. M., Hamm, P. H. & Hansen, E. M. 1989. *Phytophthora* diseases. Status of *P. gonapodyides*, *P. drechsleri*, and *P. cryptogea*. Report on Forest Research 1989. HSMO, London. pp. 49-50.

Brasier, C. M. 1991. Current questions in *Phytophthora* systematics. In: Lucas, J. A., Shattock, R. C., Shaw, D. S. & Cooke, L. R. 1991. *Phytophthora*. Cambridge University Press. Cambridge. pp. 104-128.

Brasier, C. M., Hansen, E. M., 1992. Evolutionary biology of *Phytophthora*. Annual Review of Phytopathology 30: 173-200.

Brasier, C. M., Rose, J. & Gibbs, J. N. 1995. An unusual *Phytophthora* associated with widespread alder mortality. Plant Pathology 44: 999-1007.

von Broembsen, S. 1981. Control of *Phytophthora* and other soil-borne diseases in forest nurseries. South African Forestry Journal 177: 37-40.

Bruck, R. I. & Kenerley, C. M. 1983. Effects of metalaxyl on *Phytophthora cinnamomi* root rot of *Abies fraseri*. Plant Disease 67: 688-690.

Burpee, L. L., Sanders, P. L. & Cole, H. Jr., 1980. Anastomosis grouping of *Ceratobasidium cornigerum* and related fungi. Mycologia 72: 689-701.

Butin, H. 1980. *Diaporthe alleghaniensis* R. M. Arnold, als Schwacheparasit auf *Betula maximowicziana* Regel. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 32, 1: 5-8.

Børja, I. 1995. The pathogenicity of *Pythium dimorphum* and *Rhizoctonia* sp. to *Picea abies* seedlings and the effect of benomyl on disease development. In: Børja, I. 1995. Root dieback and defence responses in Norway spruce seedlings infected with the fungal pathogen, *Pythium dimorphum*. Ph. D. Thesis, Norsk institutt for skogforskning. Ås, 1995. Paper 1.

Børja, I., Sharma, P., Krekling, T. & Lönneborg, A. 1995. Cytopathological response in roots of *Picea abies* seedlings infected with *Pythium dimorphum*. Phytopathology 85: 495-501.

Campbell, W. A. & Hendrix, F. F. 1967. *Pythium* and *Phytophthora* species in forest soils in the south-eastern United States. Plant Disease Reporter 51: 929-932.

Campbell, S. J. & Hamm, P. B. 1989. Susceptibility of Pacific Northwest conifers to *Phytophthora* root rot. Tree Planters' Notes 40: 15-18.

Chakravarty, P. & Unestam, T. 1987. Mycorrhizal fungi prevent disease in stressed pine seedlings. Journal of Phytopathology 118: 335-340.

Chastagner, G. A., Hamm, P. B. & Riley, K. L. 1995. Symptoms and *Phytophthora* spp. associated with root rot and stem canker of Noble fir Christmas trees in the Pacific Northwest. Plant Disease 79: 290-293.

Cooke, D. E. I., Kennedy, D. M., Guy, D. C., Russell, J., Unkles, S. E. & Duncan, J. M. 1996. Relatedness of Group I species of *Phytophthora* as assessed by randomly amplified polymorphic DNA (RAPDs) and sequences of ribosomal DNA. Mycological Research 100: 297-303.

Dahm, H. & Strzelczyk, E. 1987. Effect of pH, temperature and light on the pathogenicity of *Cylindrocarpon destructans* to pine seedlings in associative cultures with bacteria and actinomycetes. European Journal of Forest Pathology 17: 141-148.

Darvas, J. M., Scott, D. B. & Kotze, J. M. 1978. Fungi associated with damping-off in coniferous seedlings in South African Nurseries. South African Forestry Journal 104: 15-19.

Davison, E. M. & Bumbieris, M. 1973. *Phytophthora* and *Pythium* from pine plantations in South Australia. Australian Journal of Biological Sciences 26: 163-169.

Desprez-Loustau, M.L. & Dessureault, M. 1987. Etiologie du chancre godronien du bouleau jaune. Summary: Aetiology of *Godronia* canker of yellow birch. Canadian Journal of Forest Research 17: 1355-1360.

Desprez-Loustau, M.L. & Dessureault, M. 1988. Influence de stress controles sur la sensibilite du bouleau jaune au chancre godronien cause par *Godronia cassandrae* Peck f.sp. *betulicola* Groves. Summary: Influence of controlled stresses on the sensitivity of yellow birch to canker caused by *Godronia cassandrae* Peck f.sp. *betulicola* Groves. Canadian Journal of Forest Reserach 18: 121-127.

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

Domsch, K., Gams, W. & Anderson, T.-H. 1980. Compendium of soil fungi 1. Acad. Press. London, New York. 859 pp.

Duncan, S., Barton, J. E. & O'Brien, P. A. 1993. Analysis of varation in isolates of *Rhizoctonia solani* by random amplified polymorpic DNA assay. Mycological Research 97: 1075-1082.

Endo, R. M. & Colt, W. M., 1974. Anatomy, cytology, and physiology of infection by *Pythium*. Proceedings of American Phytopathological Society 1: 215-223.

English, J. T., Ploetz, R. C. & Barnard, B. L. 1986. Seedling blight of long leaf pine caused by a binucleate *Rhizoctonia solani* like fungus. Plant Disease 70: 148-150.

Frivold, L., H. & Mielikäinen, K. 1991. The silviculture of birch in Scandinavia. In: Lorrain-Smith, R. & Worrell, R. (eds.). The commercial potential of birch in Scotland. The Forestry Industry Committee of Great Britain. Edinburgh. pp. 55-65.

Föster, H. & Coffey, M. D. 1991. Approaches to the taxonomy of *Phytophthora* using polymorphisms in mitochondrial and nuclear DNA. In: Lucas, J. A., Shattock, R. C., Shaw, D. S. & Cooke, L. R. 1991. *Phytophthora*. Cambridge University Press. Cambridge. pp. 164-183.

Föster, H. & Coffey, M. D. 1993. Molecular taxonomy of *Phytophthora megasperma* based on mitochodrial and nuclear DNA polymorphisms. Mycological Research 97: 1101-1112.

Galaaen, R. & Venn, K., 1979. *Pythium sylvaticum* Campbell, Hendrix and other fungi associated with root dieback of 2-0 seedlings of *Picea abies* (L.) Karst. in Norway. Meddelelser fra Norsk Institutt for Skogforskning 34: 265-280.

Gibbs, J. & Lonsdale, D. 1995. *Phytophthora* disease of alder: The situation in 1995. Forestry Commission Research Information Note 258: 1-4.

Hall, G. 1986. A species of *Rhizoctonia* with uninucleate hyphae isolated from roots of wintwer wheat. Transactions from British Mycological Society 87: 466-471.

Hamm, P. B. & Hansen, E. M 1981. Host specificity of *Phytophthora megasperma* from Douglas-fir, soybean, and alfalfa. Phytopathology 71: 65-68.

Hamm, P. B. & Hansen, E. M. 1982. Pathogenicity of *Phytophthora* spp. to Northwest conifers. European Journal of Forest Pathology 12: 167-174.

Hamm, P. B. & Hansen, E. M. 1983. *Phytophthora pseudotsugae*, a new species causing root rot of Douglas-fir. Canadian Journal of Botany 61: 2626-2631.

Hamm, P. B., Hansen E. M. & Sutherland, J. R. 1985. *Phytophthora cryptogea* and *P. drechsleri* associated with root rotted Douglas fir seedlings in British Columbia. Plant Disease 69, 361.

Hamm, P. B. & Hansen, E. M. 1987. Identification of *Phytophthora* spp. known to attack conifers in the Pacific Northwest. Northwest Science 61:103-109.

Hamm, P. B. & Hansen, E. M. 1991. The isolation and identification of *Phytophthora* species causing damage in bare-root conifer nurseries. In: Sutherland, J. R. and Glover S. G. (eds.). Proceedings of IUFRO Working Party s2.07-09. Forestry Canada, Pacific and Yukon Region Pacific Foresrtry Centre BC-X-311. pp. 169-179.

Hamm, P. B., Hansen, E. M., Hennon, P. E. & Shaw, C. G. 1988. *Pythium* species from forest and muskeg areas of Southeast Alaska. Transactions of the British Mycological Society 91: 385-388.

Hanioja, P. 1969. Taimipoltteen aiheuttajista metsäntutkimuslaitoksen Punkaharjun koeaseman taimitarhalla. Summary: On damping-off fungi in the nurseries of Forest Research Institute at Punkaharju experimental station. Communicationes Instituti Forestalis Fenniae 6: 1-21.

Hansen, E. M. 1990. *Phytophthora* root rot. In: Hamm, P. B., Campbell S. J. & Hansen E. M. (eds.). Growing healthy seedlings. Identification and management of pests in Northwest forest nurseries. Forest Pest Management, U.S. Department of Agriculture, Forest Service, Pacific Northwest Region, Oregon State University. pp. 17-21.

Hansen, E. M. 1991. Variation in the species of the *Phytophthora megasperma* complex. In: Lucas, J. A., Shattock, R. C., Shaw, D. S. & Cooke, L. R. 1991. *Phytophthora*. Cambridge University Press. Cambridge. pp. 148-163.

Hansen, E. M. & Hamm, P. B. 1988. Canker diseases of Douglas-fir seedlings in Oregon and Washington bareroot nurseries. Canadian Journal of Forest Research 18: 1053-1058.

Hansen, E. M. & Maxwell, D. P. 1991. Species of the *Phytophthora megasperma* complex . Mycologia 83: 379-381.

Hansen, E. M., Brasier, C. M. , Shaw, D. S. & Hamm, P. B. 1986. The taxonomic structure of *Phytophthora megasperma*. Evidence for emerging biological species groups. Transactions of the British Mycological Society 87: 557-573.

Hansen, E. M., Hamm, P. B. & Roth, L. F. 1989. Testing Port-Orford-Cedar for resistance to *Phytophthora*. Plant Disease 73: 791-794.

Hantula, J., Dusabenyagasani, M. & Hamelin, R. C. 1996. Random amplified microsatellites (RAMS) - a novel method for characterizing genetic variation within fungi. European Journal of Forest Pathology 26: 159-166.

Harris, D. C. 1991. The *Phytophthora* diseases of apple. Journal of Horticultural Science 66: 513-544.

Heiskanen, J. 1994. Irrigation regime affects water aeration conditions in peat growth medium and the growth of containerized Scots pine seedlings. New Forests 9: 181-195.

Hendrix, F. F. & Campbell, W. A., 1968. Pythiaceous fungi isolated from southern forest nursery soils and their pathogenicity to pine seedlings. Forest Science 14: 292-297.

Hendrix, F. F. & Campbell, W. A., 1973. *Pythium*s as plant pathogens. Annual Review of Phytopathology 11: 77-98.

Henttonen, H., Lilja, A. & Niemimaa, J., 1994: Myyrien ja hyönteisten aiheuttamat sieni-infektiot koivun taimien uhkana. In: Smolander, H. & Rautala, J. (eds.). Taimitarhapäivät Suonenjoen tutkimusasemalla 17.-18.8.1993. Finnish Forest Research Institute Research Paper 496: 125-129. (In Finnish).

Hietala, A. M. 1995. Uni- and binucleate *Rhizoctonia* spp. coexisting on the roots of Norway-spruce seedlings suffering from root dieback. European Journal of Forest Pathology 25: 136-144.

Hietala, A. M. & Sen, R. 1996. *Rhizoctonia* on forest trees. In: Sneh, B., Jabaji-Hare, S.. Neate, S. & Dijst, G. Rhizoctonia species: Taxonomy, Molecular Biology, Ecology, Pathology and Control.

Hocking, D. 1968. Fungi associated with damped-off and healthy pine seedlings and seed in east African pine nurseries. Transactions of the British Mycological Society 51: 221-226.

Horner, R.M. 1956. Diaporthe canker on *Betula lutea*. Proceedings, 22nd Session Canadian Phytopathological Society, Edmonton 1955, 23: 16-17.

Huang, J. W. & Kuhlman, E. G. 1989. Recovery and Pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia*-like fungi in forest nurseries. Plant Disease 73: 968-972.

Huang, J. W. & Kuhlman, E. G. 1990. Fungi associated with damping-off of Slash pine seedlings in Georgia. Plant Disease 74: 27-30.

Hytönen, J. 1995. Peltojen metsitys vaatii taitoa ja tietoa. In: Hytönen, J. & Polet, K. (eds.) Peltojen metsitysmenetelmät. Metsäntutkimuslaitoksen tiedonantoja 581: 5-11. (In Finnish).

Ingestad, T. 1970. A definition of optimum nutrient requirements in birch seedlings. I. Physiologica Plantarum 23: 1127-1138.

Jackson, L. W. R. & Grandall, B. S. 1935. A *Phytophthora* root and collar rot of *Pinus resinosa* seedlings. Phytopathology 25: 22 (Abstr.)

Jalkanen, R. 1985. Uusi tauti taimitarhalla. Metsälehti 11, p. 20 (In Finnish).

Jamalainen, E. A. 1956a. A test on the control of black snow mold (*Herpotrichia nigra* Hartig) in spruce seedlings by the use of pentachloronitrobenzene (PCNB). Valtion Maatalouskoetoiminnan Julkaisuja 148: 68-71.

Jamalainen, E. A. 1956b. Männyn karisteen torjunta kemiallisilla aineilla Leksvallin taimitarhassa. Summary: The control of the needle cast of pine with chemicals at the Leksvall nursery. Silva Fennica 88(2): 1-10.

Jamalainen, E. A. 1961. Havupuiden taimistojen talvituhosienivauriot ja niiden kemiallinen torjunta. Summary: Damage by low-temperature parasitic fungi on coniferous nurseries and its chemical control. Silva Fennica 108(4): 1-15.

James, R. L. 1983. *Fusarium* root disease of containerized seedlings at the Montana State Nursery, Missoula. USDA Forest Service, Cooperative Forestry and Pest Management.

James, R. L., Dumroese, R. K., Wenny, D. L., Myers, J. F. & Gilligan, C. J. 1987. Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. 1. Seed and seedling infection, symptom production and disease progression. USDA, Forest Service Northern Region Missoula, Montana Report 87-13. 1-22.

James, R. L., Dumroese, R. K. & Wenny, D. L. 1991. *Fusarium* diseases of conifer seedlings. In: Sutherland, J. R. and Glover S. G. (eds.). Proceedings of IUFRO Working Party s2.07-09. Forestry Canada, Pacific and Yukon Region Pacific Foresrtry Centre BC-X-311. pp. 181-189.

James, R. L., Dumroese, R. K. & Wenny, D. L. 1994. Observations on the association of *Cylindrocarpon* spp. with diseases of containergrown conifer seedlings in the inland Pacific Northwest of the United States. In: Perrin, R. & Sutherland, J. R. (eds.) Diseases and insects in forest nurseries. Dijon (France), October 3-10, 1993. (Les Colloques, n°68). INRA, Paris. pp. 65-78.

James, R. L., Dumroese, R. K., Gilligan, C. J. & Wenny, D. L. 1989. Pathogenicity of *Fusarium* isolates from Douglas-fir seed and container-crown seedlings. Idaho Forest, Wildlife and Range Experiment Station, Bulletin Number 52: 1-10.

Juutinen, P., Kurkela, T. & Lilja, S. 1976. Ruohokaskas *Cicadella viridis* (L.) lehtipuun taimien vioittajana sekä vioitusten sienisaastunta. Summary: *Cicadella viridis* (L.), as a wounder of hardwood saplings and infection of wounds by pathogenic fungi. Folia Forestalia 284: 1-12. (In Finnish).

Kassaby, F. Y. & Hepworth, G. 1987. *Phytophthora cinnamomi*: Effects of herbicides on radial growth, sporangial production, inoculum potential and root disease in *Pinus radiata*. Soil Biology and Biochemistry 19: 437-441.

Kenerley, C. M. & Bruck, R. I. 1981. *Phytophthora* root rot of Balsam fir and Norway spruce in North Carolina. Plant Disease 65: 614-615.

Kenerley, C. M. & Bruck, R. I. 1987. Distribution and disease progress of *Phytophthora* root rot of Fraser fir seedlings. Phytopathology 77: 520-526.

Kenerley, C. M., Papke, K. & Bruck, R. I. 1989. Effect of flooding on development of *Phytophthora* root rot in Fraser fir seedlings. Phytopathology 74: 401-404.

Kennedy, D. M. & Duncan, J. M. 1995. A papillate *Phytophthora* species with specificity to *Rubus*. Mycological Research 99: 57-68.

Kessler, W. 1988. Wurzelfäule an Jungpflanzen von Eiche und Rotbuche durch *Cylindrocarpon destructans*. Sozialistische-Forstwirtschaft 38, 4: 110-111.

Kurkela, T. 1967. Keväällä havaitusta männyn taimitarhataudista ja *Scleroderris lagerbergis*ta. Metsätaloudellinen Aikakauslehti 84: 391-392. (In Finnish).

Kurkela, T. 1973a. Epiphytology of Melampsora rusts of Scots pine (*Pinus sylvestris* L.) and aspen (*Populus tremula* L.). Communicationes Instituti Forestalis Fenniae 79, 4: 1-68.

Kurkela, T. 1973b. *Godronia multisporan* aiheuttama tauti raudus- ja hieskoivun taimissa eräissä metsänlannoituskokeissa turvemaalla. Summary: A disease caused by *Godronia multispora* Groves on young *Betula verrucosa* Ehr. and *B. pubescens* Ehr. on fertilized peatland. Suo 24, 1: 8-15.

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

Kurkela, T. & Lilja, S. 1983. Taimitarhan sienitauteja. Keskusmetsälautakunta Tapio, 15 p. (In Finnish).

Landis, T. D. 1984. The critical role of environment in nursery pathology. In: Dubreuil S. H. (ed.). 31st Western International Forest Disease Work Conference Proceedings; 1983 August 22-66; Coer d'Alene, ID. Missoula, MT: USDA Forest Service, Cooperative Forest and Pest Management pp. 27-31.

Landis, T. D. 1989. Disease and pest management strategies. In: T. D. Landis, Tinus, R. W. , McDonald, S. E. & Barnett, J. P. The Container Tree Nursery Manual. Volume 5. The Biological Component: Nursery Pests and Mycorrhizae. USDA Forest Service, Agriculture Handbook 674, Public Affairs Office, Washington, DC. pp. 4-99.

Lilja, A. 1979. Koivun siementen sienet ja niiden patogeenisuus. Summary: Fungi on birch seeds and their pathogenicity. Folia Forestalia 408: 1-14. (In Finnish).

Lilja, A. 1996. Versolaikkujen ja värivikojen aiheuttajat koivun taimilla. Folia Forestalia 1996(2): 157-161. (In Finnish).

Lilja, A. & Hietala, A. 1994a. *Phytophthora cactorum* and a novel type *Rhizoctonia* sp. as forest nursery pathogens. In: Perrin, R. & Sutherland, J. R. (eds.) Diseases and insects in forest nurseries. Dijon (France), October 3-10, 1993. (Les Colloques, n°68). INRA, Paris. pp. 59-64.

Lilja, A. & Hietala, A. 1994b. Koivun versolaikku taimitarhalla. In: Smolander H. & Rautala J. Taimitarhapäivät Suonenjoen tutkimusasemalla 17-18.8.1993. The Finnish Forest Research Institute. Research Papers 496. pp. 121-123 (In Finnish).

Lilja, A., Lilja, S. & Poteri, M., 1988. Root dieback in forest nurseries. Karstenia 28: 64 (Abstr.).

Lilja, A., Hallaksela, A. M. & Heinonen, R. 1995. Fungi colonizing Scots-pine cone scales and seeds and their pathogenicity. European Journal of Forest Pathology 25: 38-46.

Lilja, A., Hantula, J. & Nuorteva, H. 1996. Uusimpia tuloksia koivun versolaikkutaudista. In: Smolander, H. & Salonen, T. Taimitarha-päivät Jyväskylässä. Metsäntutkimuslaitoksen tiedonantoja 601: 7-16. (In Finnish).

Lilja, S. 1973. Koivunruoste ja sen torjuminen. Metsänviljelyn koeaseman tiedonantoja 9: 21-26. (In Finnish).

Lilja, S. 1980. Taimitarhan tärkeimmät sienituhot. Metsänviljelyn koeaseman tiedonantoja 35: 11-18. (In Finnish).

Lilja, S. 1986. Diseases and pest problems on *Pinus sylvestris* nurseries in Finland. Bulletin OEPP/EPPO Bulletin 16: 561-564.

Linde, C., Kemp, G. H. J. & Wingfield, M. J. 1994. *Pythium* and *Phytophthora* species associated with eucalypts and pines in South Africa. European Journal of Forest Pathology 24: 345-356.

Lorio, P. L. Jr. 1966. *Phytophthora cinnamomi* and *Pythium* species associated with loblolly pine decline in Louisiana. Plant Disease Reporter 50: 596-597.

Martineau, R. & Lavallee, A. 1971. Quebeck region. Annual report of the forest insect and disease survey, 1970. Canadian Forest Service, Canadian Department of Fishery and Forestry, pp. 32-48.

Mitchell, D. J. & Kannwischer-Mitchell, M. E. 1992. *Phytophthora*. In: Singleton L. L., Mihail J. D. & Rush C. M. (eds.). Methods for Research on Soilborne Phytopathogenic Fungi. APS Press, St. Paul, Minnesota. pp. 31-38.

Molin, N., Persson, M. & Persson, S. 1961. Root parasites of forest tree seedlings. Meddelanden från Statens Skogsforskningsinstitut 49: 1-17.

Morgan, P. 1983. *Fusarium* stem rot of Douglas fir seedlings. Plant Disease 67: 441-442.

Nienhaus, F. 1960. Das Wirtsspektrum von *Phytophthora cactorum* (Leb. & Chon.) Schroet. Phytopathologishe Zeitschrift 38:33-68.

Ogoshi, A., 1976. Studies on the grouping of *Rhizoctonia solani* Kühn with hyphal anastomosis and on the perfect stages of groups. Bulletin of the National Institut of Agricultural Science, Series C. 30: 1-63.

Ogoshi, A., 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annual Review of Phytopathology 25: 125-143.

Oudemans, P. & Coffey, M. D. 1991. Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. Mycological Research 95: 19-30.

Parikka, P. 1990. Nahkamätä vaivaa mansikkaa. Puutarha 9/90: 630-631. (In Finnish).

Perrin, R. & Sampangi, R. 1986. La fonte des semis en pépinière forestière. European Journal of Forest Pathology 16: 309-321.

Petäistö, R.-L. 1983. Rauduskoivun versolaikut taimitarhalla. Summary: Stem spotting of birch (*Betula pendula*) in nurseries. Folia Forestalia 544: 1-9.

Pitts, J. E. & Calhoun, J. 1984. Isolation and identification of Pythiaceous fungi from irrigation water and their pathogenicity to *Antirrhium*, tomato and *Chamaecyparis lawsoniana*. Phytopathologische Zeitschrif 110: 301-318.

Plaats-Niterink, A. J. van der, 1981. Monograph of the genus *Pythium*. Studies in mycology, Centraalbureau voor Schimmelcultures, Baarn 21: 1-242.

Pratt, R. G., Roth, L. F., Hansen, E. M. & Ostrowsky, W. D., 1976. Identity and pathogenicity of *Phytophthora* causing root rot of Douglas-fir in the Pacific Northwest. Phytopathology 66: 710-714.

Rikala, R. 1993. Kastelu ja lannoitus taimitarhalla: Kastelutarpeen määrittäminen ja lannoituksen vaikutus taimien kehittymiseen tarhalla ja istutuksen jälkeen. Licentiate thesis in silviculture. University of Helsinki. Faculty of Agriculture and Forestry. Department of Forest Ecology. 40 p.+ 103 p.

Rikala, R. & Petäistö, R-L. 1986. Lannoituksen vaikutus koulittujen rauduskoivun taimien ravinnepitoisuuteen, kasvuun ja versolaikkuisuuteen. Summary: Effect of fertilization on the nutrient concentration, growth and incidence of stem spotting in bare-rooted birch transplants. Folia Forestalia 642: 1-9.

Robertson, G. I. 1973. Occurrence of *Pythium* spp. in New Zealand soils, sands, pumices and peat, and on roots of container-grown plants. New Zealand Journal of Agricultural Research 16: 357-365.

Robin, C., Deprez-Loustau, M.-L. & Delatour, C. 1992. Factors influencing the enlargement of trunk cankers of *Phytophthora cinnamomi* in red oak. Canadian Journal of Forest Research 22: 367-374.

Roll-Hansen, F. & Roll-Hansen, H. 1968. A species of *Rhizoctonia* DC. ex Fr. damaging spruce plants in nurseries in Southern Norway. Meddelelser fra Norsk Skogforskning Institutt 82: 421-440.

Romakkaniemi, P. 1986. The susceptibility of *Betula pendula* and *B. pubescens* to stem spot disease on different soils. Silva Fennica 20: 23-28.

Roth, L. F. & Riker, A. J. 1943. Seasonal development in the nursery of damping-off of red pine seedlings caused by *Pythium* and *Rhizoctonia*. Journal of Agricultural Research 67: 417-431.

Runion, G. B. & Kelley, W. D. 1993. Characterization of a binucleate Rhizoctonia species causing foliar blight of loblolly pine. Plant Disease 77: 754-755.

Saksena, H. K. & Vaartaja, O. 1960. Description of new species of *Rhizoctonia*. Canadian Journal of Botany 38: 931-943.

Saksena, H. K. & Vaartaja, O. 1961. Taxonomy, morphology and pathogenicity of *Rhizoctonia* species from forest nurseries. Canadian Journal of Botany 39: 627-647.

Salt, G.A. 1979. The increasing interest in monor pathogens. In: Schippers, B. & Gams, W. Soil-borne plant pathogens. Academic Press New York. pp. 289-312.

Sharma, P., Børja, D., Stougaard, P. & Lönneborg, A. 1993. PR-proteins accumulating in spruce roots infected with a pathogenic *Pythium* sp. isolate include chitinases, chitosanases and ß-1,3-glucanases. Physiological and Molecular Plant Pathology 43: 57-67.

Sharma, P., Børja, I. & Lönneborg, A. 1995. A novel function of polyphenols: Pathogenesis related proteins are retained in complex with polyphenols and released after pathogen infection. In: Børja, I. 1995. Root dieback and defence responses in Norway spruce seedlings infected with the fungal pathogen, *Pythium dimorphum*. Ph. D. Thesis, Norsk institutt for skogforskning. Ås, 1995. Paper 4.

Shew, N. D. & Benson, D. M. 1981. Fraser fir, root rot induced by *Phytophthora citricola*. Plant Disease 65, 688-689.

Sinclair, W., A., Lyon, H., H. & Johnson, W., T. 1987. Diseases of trees and shurbs. Cornell University Press, New York. 574 p.

Smerlis, E. 1968. The occurrence and pathogenicity of forms of *Godronia cassandrae* in Quebec. Canadian Journal of Botany 46: 597-599.

Smerlis, E. 1969. Pathogenicity of some species of *Godronia* occurring in Quebec. Plant Disease Reporter 53: 807-810.

Smith, P. M., Ousley, M. A. & Middleton, J. 1985. Epidemiology and control of *Phytophthora* diseases of woody ornamentals. Annual Report Classhouse Crops Research Institute 1984. Littlehampton, Sussex. pp. 102-105.

Sneh, B., Burpee, L. & Ogoshi, A. 1991. Identification of *Rhizoctonia* species. The American Phytopathological Society, St. Paul 133 pp.

Stamps, D. J., Waterhouse, G. M., Newhook, F. J. & Hall, G. S., 1990. Revised tabular key to the species of *Phytophthora*. CAB International Mycological Intitute Mycol Papers 162: 1-28.

Strouts, R. G. 1981. *Phytophthora* diseases of trees and shurbs. Arbocultural Leaflet, Department of the Environment, Uk. No 8.

Sutherland, J. R. 1977. Corky root disease of Douglas fir seedlings: pathogenicity of the nematode *Xiphinema bakeri* alone and in combination with the fungus *Cylindrocarpon destructans*. Canadian Journal of Forest Research 7: 41-46.

Sutherland, J. R. 1990. Fusarium root rot. In: Hamm, P. B., Campbell S. J. & Hansen E. M. (eds.). Growing healthy seedlings. Identification and management of pests in Northwest forest nurseries. Forest Pest Management, U.S. Department of Agriculture, Forest Service, Pacific Northwest Region, Oregon State University. pp. 8-9.

Sutherland, J. R., Sluggett, L J. & Lock, W. 1972. Corky root disease observed on two Spruce species and Western Hemlock. Tree Planters' Notes 23, 4: 18-20.

Sutherland, J., Shrimpton, G. & Sturrock, R., 1989. Diseases and insects in British Columbia Forest seedling nurseries. FRDA Report 065: 27-28.

Sutton, B. C. 1980. The Coelomycetes. CAB, Kew, Surrey.

Unestam, T., Beyer-Ericson, L. & Strand, M., 1989. Involvement of *Cylindrocarpon destructans* in root death of *Pinus sylvestris* seedlings: Pathogenic behaviour and predisposing factors. Scandinavian Journal of Forest Research 4: 521-536.

Utkheide, R. S. & Smith, E. M. 1995. Effect of nitrogen form and application method on incidence and severity of *Phytophthora* crown and root rot of apple trees. European Journal of Plant Pathology 101, 3: 283-289.

Vaartaja, O. 1967. Reinfestation of sterilized nursery seedbeds by fungi. Canadian Journal of Microbiology 13: 771-776.

Vaartaja, O. 1968. *Pythium* and *Mortierella* in soils of Ontario forest nurseries. Can. J. Microbiol. 14: 265-269.

Vaartaja, O. & Cram, W. H., 1956. Damping off of conifers and of Caragana in Saskatchewan. Phytopathology 46: 391-397.

Vaartaja, O. & Salisbury, P. J. 1961. Potential patogenity of *Pythium* isolates from forest nurseries. Phytopathology 51: 505-507.

Vaartaja, O. & Bumbiers, M. 1964. Abundance of *Pythium* species in nursery soils in South Australia. Australian Journal of Biological Sciences 17: 436-445.

Vaartaja, O., Cram, W. H. & Morgan, G. A. 1961. Damping off etiology especially in forest nurseries. Phytopathology 51: 35-42.

Venn, K. 1983. Rotavdøing. Det patogene aspekt. Årsskrift for Nordiske Skogplante Skoler. 1982: 51-53 (In Norwegian).

Venn, K. 1985a. Røtavdøing hos bartreplanter i skogplanteskoler. Summary: Root dieback of coniferous seedlings in forest nurseries. Rapport fra Norsk Insitutt for Skogforskning 3/85: 1-11.

Venn, K. 1985b. Betydningen av soppangrep for rotav døing i planteskoler. Aktuelt fra Statens fagtjeneste for landbruket 3: 23-29 (In Norwegian).

Venn, K., Sandvik, M. & Langerud, B. 1986. Nursery routines, growth media and pathogens affect growth and root dieback in Norway spruce seedlings. Meddelelser fra Norsk Institutt for Skogforskning 39: 314-328.

Vigalys, R. & Gonzalez, D. 1990. Ribosomal DNA restriction fragment length polymorphism in *Rhizoctonia solani*. Phytopathology 80:151-158.

Vigalys, R. & Cubeta, M. A. 1994. Molecular systematics and population biology of *Rhizoctonia*. Annual Review of Phytopathology 32: 135-155.

Viljoen, A. Wingfield, M. J. & Marasas, W. F. O. 1994. First report of *Fusarium subglutinans* f. sp. pini on pine seedlings in South Africa. Plant Disease 78: 309-312.

Wardlaw, T. J., Palzer, C., 1985. Stem diseases in nursery seedlings caused by *Phytophthora cactorum*, *P. citricola* and *Pythium anandrum*. Australasian Plant Pathology 14: 57-59.

Waterhouse, G. M. 1963. Key to species of *Phytophthora* de Bary. Mycological Papers (CMI) 92: 1-22.

Weising, K., Nybom, H., Wolff, K. & Meyer, W. 1995. DNA fingerprinting in plants and fungi. CRC Press, Boca Raton, USA.

Werres, S., Richter, J., Veser, I. 1995. Untersuchungen von kranken und abgestorbenen Rosskastanien (*Aesculus hippocastanum* L.) im öffentlichen Grün. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 47,4: 81-85.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuclear Acids Research 18: 6531-6535.

Zietkiewicz, E., Rafalsski, A. & Labuda, D. 1994. Genome fingerprinting by simple sequent repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20: 176-183.

I

Scand. J. For. Res. 7: 547–556, 1992

Conifer Seedling Root Fungi and Root Dieback in Finnish Nurseries

ARJA LILJA, SAKARI LILJA, MARJA POTERI and LI ZIREN

The Finnish Forest Research Institute, Department of Forest Protection, Section of Forest Pathology, Box 18, SF-01301 Vantaa, Finland

Scandinavian Journal of Forest Research

Lilja, A., Lilja, S., Poteri, M. and Ziren, L. (The Finnish Forest Research Institute, Department of Forest Protection, Section of Forest Pathology, Box 18, SF-01301 Vantaa, Finland). Conifer seedling root fungi and root dieback in Finnish nurseries. Accepted Nov. 30. 1991. Scand. J. For. Res. 7: 547–556, 1992.

Fungi were isolated from the roots and growth substrate of bare-rooted and containerized *Pinus sylvestris* and *Picea abies* nursery seedlings displaying a root dieback. Isolations were also made from visually healthy seedlings. The potential pathogenicity of all isolated species was determined in laboratory trials.

Cylindrocarpon spp., *Fusarium* spp. and *Trichoderma viride* were frequently isolated. The isolation frequency of a uninucleate *Rhizoctonia*-like fungus, *Pythium* spp. and *Phytophthora undulatum* from diseased containerized seedlings and their pathogenicity in tests suggest that these fungi are likely involved in the root dieback disease in containers. The pathogenic *Rhizoctonia*-like fungus in addition to *Pythium* spp. was also isolated from bare-rooted seedlings. In greenhouse tests *Pythium* spp. were more pathogenic to 4-week-old Scots pine seedlings grown before transplantation in unsterile substrate than to those seedlings grown axenically in agar. External factors are considered to have some role in the expression of disease. *Key words: root dieback, Pinus sylvestris, Picea abies, Scots pine, Norway spruce, mycoflora, Rhizoctonia, Pythium, Phytophthora, Fusarium.*

INTRODUCTION

Recently a root dieback of bare-rooted and containerized conifer seedlings was identified in certain Finnish forest nurseries (Jalkanen, 1985; Lilja et al., 1988). The symptoms of the disease on both Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) are discolouration of the needles, a partial or total death of the root system and stunted growth. The symptoms often appear after midsummer, on first-year seedlings, although infection is occasionally not detected until seedlings are approaching their second or third growing season.

In Norway a similar disease has affected containerized Norway spruce seedlings. The most common fungus isolated from diseased seedlings was *Pythium sylvaticum* Campbell & Hendrix which was shown to infect young Norway spruce seedlings *in vitro* (Galaaen & Venn, 1979). However in later nursery pathogenicity tests, inoculations with *Pythium* sp. caused minor damage whereas the inoculations with a *Rhizoctonia* sp. resulted in typical root dieback symptoms (Venn et al., 1986). Root dieback of Scots pine has also been reported in Sweden (Unestam et al., 1989). North American conifers suffering from *Phytophthora* root rot in bare-root nurseries of the Pacific Northwest and North Carolina exhibit symptoms similar to those reported in Scandinavia (Pratt et al., 1976; Kenerley & Bruck, 1981; Hamm & Hansen, 1982, 1986; Cooley et al., 1985).

This study reports on the mycoflora present within diseased and visually healthy conifer seedling roots and the growth substrate in nursery plots. The pathogenicity of all isolated species as determined in laboratory trials is also described.

MATERIAL AND METHODS

Sample collection. Over the period 1986–90, from May to October, 1-year-old, 2-year-old and transplanted 3-year-old bare-rooted Scots pine seedlings (1, 2, 2 + 1) and 1-year-old containerized Scots pine and Norway spruce seedlings showing disease symptoms were collected from Ahvenlampi (62°44′ N, 25°11′ E) Mellanå (62°13′ N, 21°39′ E) Pekolampi (63°21′ N, 27°24′ E), Puupelto (62°04′ N, 28°20′ E) and Tyllilä (61°25′ N, 25°53′ E) nurseries. Fungal isolations were made from diseased and visually healthy seedlings. The nursery bed soil and container peat were also studied (Table 1).

Isolation of fungi. Roots were washed in running tap water for 30 minutes. From each 2-year-old and 3-year-old seedling 4–5 diseased roots were sampled by cutting 4 segments from the zone margin between diseased and healthy tissue. The segments were surface sterilized in 0.5% NaOCl for one minute, rinsed for 15 minutes in each of three changes of sterile water, and plated on agar. From 1-year-old seedlings the whole root system was surface sterilized and plated on agar. The agar medium for fungal isolations was 1% water agar, amended with five drops wheat germ oil per litre.

Fungi present in the growth substrate were baited using apples (Golden delicious) as described by Hansen et al. (1979). After colonization blocks of apple tissue from the margin of the early rotting zone were plated on water agar.

The identification of fungi. The identification of Fungi imperfecti (Booth, 1966, 1971; Domsch et al., 1980) and Phycomycetes (van der Plaats-Niterink, 1981; Dick, 1990) were based on morphological criteria. In order to further characterize the isolated *Rhizoctonia* spp. the nuclear condition of them was determined by phase contrast microscopy.

Preparation of sterile and unsterile seedlings. Seeds of Scots pine were first soaked in water overnight, sterilized using 70% alcohol for 2–3 seconds, 1% $HgCl_2$ for 3 minutes, washed for 15 minutes in each of three changes of sterile water, and plated on water agar in Petri dishes. Germinated seeds were transferred into tubes (22 mm × 150 mm) of flasks (250 ml) containing modified Melin-Norkrans (MMN) medium (Marx, 1969) without malt extract. The unsterile seedlings were produced by sowing seeds in boxes filled with unsterile peat (Finnpeat B6).

Table 1. *Number, species and date of collection of conifer nursery seedlings and soil samples from five nurseries*

Nursery	Date	Bare-rooted seedlings
Puupelto	09.05.86	11 diseased Scots pine (2 + 1)
Pekolampi	10.05.86	37 diseased Scots pine (2 + 1)
	31.07.87	15 diseased Scots pine (1)
		24 diseased Scots pine (2)
	15.09.87	9 diseased Scots pine (2 + 1)
	23.10.87	10 diseased Scots pine (2) and
		19 soil samples
		10 diseased Norway spruce (2) and
		19 soil samples
	05.05.89	20 diseased Scots pine (2 + 1) and
		20 healthy looking Scots pine (2 + 1)
	11.10.89	20 diseased Scots pine (2 + 1)
Tyllilä	05.05.89	20 diseased Scots pine (2 + 1) and
		20 healthy looking Scots pine (2 + 1)

Pathogenicity tests in tubes and flasks. The *in vitro* pathogenicity tests used were modified from the method by Vaartaja & Cram (1956). All isolated species and all isolates from Pythiaceae and *Rhizoctonia* spp. were tested. The roots of two-week-old sterile seedlings, one seedling in each tube and six in each flask, were inoculated with potato dextrose agar (PDA) discs (4 mm diameter) cut from a 1-week-old culture of the test fungus. The control roots were inoculated with sterile PDA discs. The number of seedlings was 15, 20, 20, 60 and 65 in five tube tests and 30, 89 and 90 in three flask tests. Incubation was carried out in a growth chamber (CKS 2000, Kryoservice) with a 12 h day at 20°C and 15°C during the dark period.

Pathogenicity test in Petri dishes. The Petri dish method of Duddridge (1986) was employed with sterile 8-week-old Scots pine seedlings (Unestam et al., 1989). Peat-vermiculite (1 : 3) growth media were moistened with MN-liquid and sterilized by gamma-irradiation (5 Mrad). The roots of seedlings, one seedling per Petri dish, were inoculated with test fungus and incubated as described for the tube and flask tests. Ten seedlings were inoculated with each test fungus.

Pathogenicity test in the greenhouse. Sterile and unsterile 4-week-old seedlings, 30 seedlings for each treatment, were transplanted into pots, which had been inoculated one week earlier. The unsterile peat-vermiculate (8 : 2) growth media in pots had been moistened with MN-liquid and the pH adjusted to 4.5 (Wilcox & Wang, 1987). The inoculum for each pot consisted of a 1-week-old agar culture of the test fungus (for control only water agar), homogenized in 100 ml sterile water with an Ultraturrax homogenizer. For inoculum the Pythiaceae species were grown on water agar and other tested species were grown on PDA. After the transplantation, the pots were watered to saturation over a two week period. The experiment was carried out in the greenhouse in April. The temperature varied between 20°C and 25°C.

Inspection and analysis of the pathogenicity tests. The condition of the seedlings was classified according to their symptoms as follows: 0 = no symptoms; 1 = <25% of the needles were yellowish; 2 = <50% of the needles were yellowish or brown and some stunting may have been observed; 3 = <75% of the needles were brown and seedlings were stunted; 4 = >75% of the needles were brown and seedlings were dying or dead. The infection of diseased seedlings was verified by plating their roots on water agar.

Nursery	Date	Containerized seedlings
Tyllilä	30.05.87	10 diseased Scots pine (1) and 10 peat samples and 10 healthy looking Scots pine (1)
Ahvenlampi	10.10.89	35 diseased Norway spruce (1) and 35 healthy looking Norway spruce (1)
Mellanå	15.10.90	40 diseased Scots pine (1)

The disease index of seedlings in each experiment was calculated after experiment period of four weeks from the frequency of different condition classes:

$$(f_{c1} + 2f_{c2} + 3f_{c3} + 4f_{c4})/N$$

where $f_{c1} \ldots f_{c4}$ are the frequencies of the different condition classes and N is the total number of seedlings. The Kruskall-Wallis one-way analysis of variance and a nonparametric test for comparison of treatment and control in pathogenicity tests were used (Anon., 1988).

RESULTS

The fungal species and their isolation frequencies are listed in Tables 2–5. *Cylindrocarpon* spp., *Fusarium* spp. and *Trichoderma viride* were commonly isolated from the growth substrate and diseased roots and also from the roots of visually healthy seedlings.

Pythiaceae fungi were frequently isolated from the nursery soil and peat. The percentage of diseased seedlings infected with these fungi varied (Tables 2–5). *P. ultimum* var. *ultimum* and *P. anandrum* were isolated from both bare-rooted and containerized seedlings. *Phytophthora undulatum* was only isolated from containerized seedlings.

Rhizoctonia-like fungi were isolated from roots of both bare-rooted and containerized seedlings. Isolates from Ahvenlampi and Mellanå nurseries were uninucleate. Most isolates from Pekolampi were also uninucleate, but binucleate and multinucleate types were also isolated. Isolates from Tyllilä were binucleate.

The potential pathogenicity of all isolated species and all isolates from Pythiaceae and *Rhizoctonia* spp. were tested *in vitro* and the fungi found to be pathogenic to 2-week-old sterile pine seedlings in these tests ($P < 0.05$) were: *Pythium anandrum*, *P. dissotocum*, seven isolates of *P. ultimum* var *ultimum*, two isolates of heterothallic *Pythium* sp. (HS-group), *Phytophthora undulatum*, isolates of uninucleate *Rhizoctonia*-like fungus and *Fusarium oxysporum* f. sp. *pini* (Figs. 1 a, b).

Pathogenic uninucleate *Rhizoctonia*-like fungus and *Phytophthora undulatum* were isolated only from diseased containerized seedlings (Table 5). The isolated fungal flora was not significantly different in visually healthy and diseased bare-rooted seedlings in two samples collected in spring 1989 (Table 4). In other samples it was possible to isolate uninucleate *Rhizoctonia*-like fungus also from bare-rooted seedlings (Table 2 and 3).

Isolates of *P. ultimum* var. *ultimum* displayed variation in pathogenicity. The pathogenicity of the same isolate also varied in the different tests. *P. anandrum* was pathogenic in the *in vitro* tests in tubes and flasks, but not in Petri dishes and pots (Figs. 1 and 2).

In the greenhouse study, *Pythium* spp. were more pathogenic to seedlings that had been grown for four weeks in unsterile peat before transplantation than to those seedlings grown under sterile conditions. The pathogenicity of *Phytophthora undulatum* and the uninucleate *Rhizoctonia*-like fungus was not dependent on the growth conditions of seedlings before transplantation into infected soil (Fig. 2).

DISCUSSION

All the isolated fungi are common soil fungi and the species most frequently isolated were those producing large numbers of spores (Domsch et al., 1980). Many were the same as those observed in the Norwegian nurseries (Galaaen & Venn, 1979).

Among *Cylindrocarpon* spp., *C. cylindroides* and *C. pineum* have not been reported earlier in Finland (Ylimäki, 1986). None of our isolates from the genus showed pathogenic properties in the *in vitro* tests although in Swedish laboratory trials, unfavourable conditions

Table 2. *Root system colonization by various fungi in diseased bare-rooted Scots pine seedlings (%)*
Date of sample collections see Table 1

	Nursery					
	Puupelto	Pekolampi				
	Age of seedlings					
Fungus	2+1	1	2	2+1	2+1	2+1
Broomella spp.	9	–	17	16	11	–
Cephalosporium spp.	18	–	–	11	–	–
Cladosporium cladosporioides (Fres.) de Vries	27	–	–	5	–	–
Cylindrocarpon cylindroides Wollenw. var. tenue Wollenw.	–	–	4	41	11	–
C. destructans (Zins.) Scholten	9	–	54	11	11	10
C. didymum (Hartig) Wollenw.	46	–	8	11	11	–
Cylindrocarpon spp.	–	73	4	5	11	–
Fusarium avenaceum (Corda ex Fr.) Sacc.	–	–	–	11	–	–
F. culmorum (W. G. Smith) Sacc.	–	–	21	32	11	–
F. oxysporum Schl. f. sp. pini (Hartig) Snyder & Hansen	–	–	4	11	–	–
F. sambucinum Fuckel var. coeruleum Wollenw.	46	–	–	35	–	–
Fusarium spp.	18	40	–	–	–	–
Leptosphaeria spp.	–	–	–	62	–	–
Mortierella spp.	–	60	54	–	77	10
Penicillium spp.	–	33	–	11	–	15
Phoma herbarum Westend.	–	–	17	–	44	–
Phoma spp.	46	–	21	8	–	–
Pythium anandrum Drechsler	–	–	–	–	–	10
P. ultimum Trow var. ultimum	–	13	8	16	11	10
Pythium spp.	–	6	13	5	–	–
Rhizoctonia-like fungi uninucleate	–	–	4	–	–	20
Trichoderma hamatum (Bonord.) Bain	9	–	–	5	–	–
T. viride Pers. ex Gray	18	33	–	11	–	95
Trichoderma spp.	18	–	–	–	–	–
Ulocladium spp.	18	–	4	–	–	–
Number of seedlings	11	15	24	37	9	20

proved to predispose Scots pine seedlings to invasion by the normally saprophytic *C. destructans* (Unestam et al., 1989).

The trapping method (Hansen et al., 1979) enabled the isolation of high numbers of *Pythium* spp. from the nursery soil and *Phytophthora undulatum* from the peat substrate (Table 3). Although this method was not used for the investigation of the mycoflora within roots these fungi were also isolated from diseased roots. The roots of seedlings collected from Puupelto were in an advanced state of decay and this was the only sample of diseased seedlings where *Pythium* spp. were not isolated (Tables 2–5).

In greenhouse tests the seedlings grown axenically in agar before transplantation were found to be more resistant than those seedlings grown in unsterile substrate (Figs. 1 and 2). This suggests that the unsterile seedlings had been predisposed to attack by *Pythium* spp. as a result of exposure to the peat microflora and fauna.

Table 3. *Root system and growth substrate colonization by various fungi of diseased conifer seedlings (%)*
The samples were collected from Pekolampi 23.10.87 and from Tyllilä 30.05.87

	Nursery					
	Pekolampi				Tyllilä	
	Bare-rooted seedlings				Containerized s.	
	Scots pine		Norway spruce		Scots pine	
	Age of seedlings					
	2		2		1	
Fungus	root	soil	root	soil	root	peat
Broomella spp.	–	–	10	–	–	–
Cephalosporium spp.	–	–	–	–	10	–
Cylindrocarpon cylindroides Wollenw. var. tenue Wollenw.	20	–	–	–	–	–
C. destructans (Zins.) Scholten	20	5	–	–	–	–
C. didynum (Hartig) Wollenw.	–	5	–	5	–	–
C. magnusianum (Sacc.) Wollenw.	20	–	40	–	–	–
C. obtusisporum (Cooke & Harknes) Wollenw.	30	–	–	–	–	–
C. pineum Booth	–	–	–	–	10	–
Fusarium oxysporum Schl. f. sp. pini (Hartig) Snyder & Hansen	30	–	–	16	–	–
F. sambucinum Fuckel var. coeruleum Wollenw.	10	16	–	70	–	–
Mortierella parvispora Linnem.	–	–	10	–	–	–
Mucor mucedo Mich. ex St. Am.	–	11	–	–	–	–
Penicillium frequentans Westling	–	–	–	11	–	–
Penicillium spp.	–	–	–	–	30	–
Pythium anandrum Drechsler	–	–	–	–	20	–
P. dissotocum Drechsler	10	–	–	–	–	–
P. ultimum Trow. var. ultimum	20	16	10	16	–	–
Pythium spp.	–	84	–	37	–	–
Phytophthora undulatum (H. E. Peterson) M. W. Dick comb. nov.	–	–	–	–	80	100
Rhizoctonia-like fungi uninucleate	70	5	30	–	–	–
multinucleate	20	–	–	–	–	–
Trichoderma viride Pers. ex Gray	40	5	10	5	80	–
Number of seedlings	10		10		10	
soil samples		19		19		10

It is possible that the isolation methods used in this study selected only a portion of the total fungal flora. Thus *Phytophthora* species could have been overrun and masked by fast growing *Pythium* spp. which are common in similar environments (Eckert & Tsao, 1962; Jeffers & Martin, 1986; Hansen et al., 1979).

Phytophthora cactorum (Lebert & Cohn) Schroeter, a fungus responsible for *Phytophthora* root rot (Hamm & Hansen, 1982), has been reported in Finland (Tahvonen, 1976; Parikka,

Table 4. *Root system colonization by various fungi in visually healthy (H) and diseased (D) bare-rooted Scots pine (2 + 1) seedlings (%)*
Date of sample collections was 05.05.89

	Nursery			
	Pekolampi		Tyllilä	
Fungus	H	D	H	D
Cephalosporium spp.	5	–	–	–
Cylindrocarpon cylindroides Wollenw. var. tenue Wollenw.	5	15	15	20
C. destructans (Zins.) Scholten	–	15	15	25
C. didynum (Hartig) Wollenhw.	10	35	5	25
C. magnusianum (Sacc.) Wollenw.	–	5	30	15
C. obtusisporum (Cooke & Harkness) Wollenw.	–	15	10	15
C. pineum Booth	25	55	55	55
Fusarium oxysproum Schl. f. sp. pini (Hartig) Snyder & Hansen	–	–	20	15
F. sambucinum Fuckel var. coeruleum Wollenw.	5	10	25	15
Mortierella parvispora Linnem.	25	–	–	–
Penicillium frequetans Westling	–	5	–	–
Penicillium spp.	25	–	5	5
Pythium ultimum Trow. var. ultimum	–	5	5	–
Pythium spp.	–	5	–	5
Rhizoctonia-like fungi binucleate	5	10	5	15
multinucleate	–	5	–	–
Trichoderma viride Pers. ex Gray	50	40	85	60
Ulocladium spp.	35	25	–	5
Number of seedlings	20	20	20	20

1991). In this study *P. undulatum* was the only *Phytophthora* species isolated. It was recently transferred from the species *Pythium* by Dick (1989).

In containerized stock diseased seedlings were visible in patches. The isolation frequency of a uninucleate *Rhizoctonia*-like fungus, *Pythium* spp. and *Phytophthora undulatum* from these seedlings and preliminary pathogenicity tests, suggest that these fungi are probably able to cause root dieback disease in containers. Although the pathogenic *Rhizoctonia*-like fungus and *Pythium* spp. were also isolated from bare-rooted seedlings, the expression of dieback in these seedlings is more complex. Occasionally the above ground symptoms in nursery could not be detected until after transplantation. The main root of both bare-rooted and containerized diseased seedlings was infected and new healthy lateral root growth was detected in a zone near the soil surface.

The occurrence of Pythiaceae fungi may be an indication of insufficient drainage of the growth substrate. *Pythium* species flourish in wet conditions and may cause root rot of containerized seedlings (Sutherland et al., 1989). Water saturation levels and other factors e.g. the pH, temperature and salt concentration in soil water, may create favourable conditions for Pythiaceae and *Rhizoctonia*-like fungi and at the same time predispose the roots to the attack of these and other soil fungi (Bolton, 1980; Venn, 1985; Moorman, 1986; Unestam et al., 1989).

Table 5. *Root system colonization by various fungi in visually healthy (H) and diseased (D) containerized Scots pine (1) seedlings (%)*
Date of sample collections see Table 1

	Nursery				
	Ahvenlampi		Tyllilä		Mellanå
	Norway spruce		Scots pine		Scots pine
Fungus	H	D	H	D	D
Cephalosporium sp.	–	–	60	10	–
Cylindrocarpon spp.	14	34	20	10	5
Mortierella spp.	–	3	–	–	47
Penicillium spp.	–	–	40	30	–
Pythium anandrum Drechsler	–	–	–	20	–
P. ultimum Trow. var. ultimum	–	–	–	–	13
Phytophthora undulatum (H.E. Petersen) M. W. Dick comb. nov.	–	46	–	80	–
Rhizoctonia-like fungus uninucleate	–	40	–	–	97
Trichoderma viride Pers. ex Gray	100	83	80	80	18
Number of seedlings	35	35	10	10	40

ACKNOWLEDGEMENTS

We wish to thank Kari Korhonen M.Sc., Prof. Lalli Laine and Prof. Timo Kurkela for their comments on the manuscript. Robin Sen B.Sc. also provided suggestions and revised the English text. The study was financially supported by the SNS (Samarbetsnämnden för Nordisk Skogsforskning) and Ministry of Agriculture and Forestry.

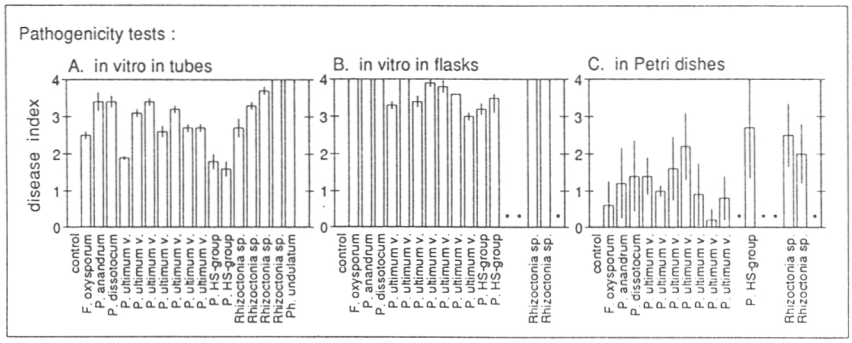


Fig. 1. (*A*, *B*) In vitro disease index of fungi pathogenic to 2-week-old Scots pine seedlings after experiment period of four weeks (*C*) and their pathogenicity to sterile Petri dish grown 8-week-old Scots pine seedlings after experiment period of four weeks ($N = 10$). Bars represent standard deviation. Treatments = control, *Fusarium oxysporum*, *Pythium anandrum*, *P. dissotocum*, 7 isolates of *P. ultimum* var. *ultimum*, 2 isolates of *Pythium* sp. (HS-group), 4 isolates of uninucleate *Rhizoctonia*-like fungus, *Phytophthora undulatum*, * = not in test. Disease index = 0–4, 0 = healthy seedlings, 4 = dead seedlings.

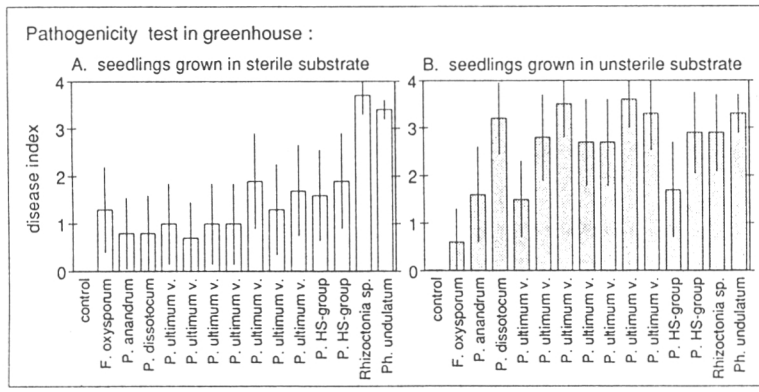


Fig. 2. Disease index of the tested fungi with 4-week-old Scots pine seedlings after experiment period of four weeks in greenhouse tests. (*A*) Seedlings grown axenically in agar before transplantation, (*B*) seedlings grown in unsterile substrate before transplantation. Bars represent standard deviation ($N = 30$). Treatments = control, *Fusarium oxysporum*, *Pythium anandrum*, *P. dissotocum*, 7 isolates of *P. ultimum* var. *ultimum*, 2 isolates *Pythium* sp. (HS-group), uninucleate *Rhizoctonia*-like fungus, *Phytophthora undulatum*. Disease index = 0–4, 0 = healthy seedlings, 4 = dead seedlings.

REFERENCES

Anon. 1988. BMDP Statistical software manual. Eds. Dixon, W. J., Brown, M. B., Engelman, L., Hill, M. A. & Jennrich, R. I. Vol. 1. University of California press, Berkeley, pp 419–428.

Bolton, A. 1980. Effect of temperature and soil pH of soilless media on root rot of poinsettia caused by *Pythium aphanidermatum*. *Canadian Journal of Plant Pathology 2*, 83–85.

Booth, C. 1966. The genus Cylindrocarpon. *Mycological Papers 104*, 56 pp.

Booth, C. 1971. The genus Fusarium. The Lavenham Press Ltd., Lavenham, Suffolk, 237 pp.

Cooley, S. J., Hamm, P. B. & Hansen, E. M. 1985. Management guide to *Phytophthora* root rot in bareroot conifer nurseries of the Pacific Northwest. United States Department of Agriculture, Forest Service. Pacific Northwest Region. December 1985. 14 pp.

Dick, M. W. 1989. *Phytophthora undulata* comb. nov. *Mycotaxon XXXV*, 449–453.

Dick, M. W. 1990. Keys to *Pythium*. The College of Estate Management, Whiteknights, Reading, 64 pp.

Domsch, K., Gams, W. & Anderson, T.-H. 1980. Compendium of soil fungi. Vol. 1, 860 pp., Vol. 2, 405 pp. Academic Press, London, New York.

Duddridge, J. A. 1986. The development and ultrastructure of ectomycorrhizas. III. Compatible and incompatible interactions between Suillus grevillei (Klotzsch) Sing. and 11 species of ectomycorrhizal host *in vitro* in the absence of exogenous carbohydrate. *The New Phytologist 103*, 457–464.

Eckert, J. W. & Tsao, P. H. 1962. A selective antibiotic medium for isolation of *Phytophthora* and *Pythium* from plant roots. *Phytopathology 52*, 771–777.

Galaaen, R. & Venn, K. 1979. *Pythium sylvaticum* Campbell & Hendrix and other fungi associated with root dieback of 2–0 seedlings of *Picea abies* (L.) Karst. in Norway. *Meddelelser fra Norsk institutt for skogforskning 34*, 266–280.

Hamm, P. B. & Hansen, E. M. 1982. Pathogenicity of *Phytophthora* spp. to Northwest conifers. *European Journal of Forest Pathology 12*, 167–174.

Hamm, P. B. & Hansen, E. M. 1986. *Phytophthora* root rot in forest nurseries of the Pacific Northwest. U.S. Department of Agriculture, Forest Service, *General Technical Report RM-137*, 122–124.

Hansen, E. M., Hamm, P. B., Julius, A. J. & Roth, L. F. 1979. Isolation, incidence and management of *Phytophthora* in forest tree nurseries in the Pacific Northwest. *Plant Disease Reporter 63*, 607–611.

Jalkanen, R. 1985. Uusi tauti taimitarhalla. *Metsälehti 11*, p. 20. (In Finnish)

Jeffers, S. N. & Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease 70*, 1033–1041.

Kenerley, C. M. & Bruck, R. I. 1981. *Phytophthora* root rot of Balsam Fir and Norway Spruce in North Carolina. *Plant Disease 65*, 614–615.

Lilja, A., Lilja, S. & Poteri, M. 1988. Root dieback in forest nurseries. *Karstenia 28*, 64.

Marx, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi and soil bacteria. *Phytopathology 59*, 153–163.

Moorman, G. W. 1986. Increased plant mortality caused by *Pythium* root rot of poinsettia associated with high fertilization rates. *Plant Disease 70*, 160–162.

Parikka, P. 1991. Mikä on mansikan nahkamätä? *Kasvinsuojelulehti 24*, 1: 17–20. (In Finnish)

Plaats-Niterink, A. J. van der 1981. Monograph of the genus *Pythium*. CMC Studies in Mycology No. 21, 1–244.

Pratt, R. G., Roth, L. F., Hansen. E. M. & Ostrowsky, W. D. 1976. Identity and pathogenicity of species of *Phytophthora* causing root rot of Douglas-fir in the Pacific Northwest. *Phytopathology 66* (6), 710–714.

Sutherland, J., Shrimpton, G. & Sturrock, R. 1989. Diseases and Insects in British Columbia Forest Seedling Nurseries. FRDA Report 065, 27–28.

Tahvonen, R. 1976. Uusi omenapuun tauti aiheuttanut tuhoja Suomessa. Koetoiminta ja Käytäntö *33* (3), 11. (In Finnish)

Unestam, T., Beyer-Ericson, L. & Strand, M. 1989. Involvement of *Cylindrocarpon destructans* in root death of *Pinus sylvestris* seedlings: pathogenic behaviour and predisposing factors. *Scand. J. For. Res. 4*, 521–536.

Vaartaja, O. & Cram, W. 1956. Damping-off pathogens of conifers and Caragana in Saskatchewan. *Phytopathology 46*, 391–397.

Venn, K. 1985. Rotavdoing hos bartreplanter i skogplanteskoler. Summary: Root dieback of coniferous seedlings in forest nurseries. *Rapport Norsk institutt for skogforskning 3/85*, 1–11.

Venn, K., Sandvik, M. & Langerud, B. 1986. Nursery routines, growth media and pathogens affect growth and root dieback in Norway spruce seedlings. *Meddelelser fra Norsk institutt for skogforskning 39*, 314–328.

Wilcox, H. E. & Wang, C. J. K. 1987. Mycorrhizal and pathological associations of dematiaceous fungi in roots of 7-month-old tree seedlings. *Canadian Journal of Forest Research 17*, 884–899.

Ylimäki, A. 1986. The occurence of Cylindrocarpon species in Finland. *Annales Agriculturae Fenniae 25*, 5–8.

II

Eur. J. For. Path. 24 (1994) 181–192

ISSN 0300–1237

Finnish Forest Research Institute, Department of Forest Ecology, Vantaa, Finland

The occurrence and pathogenicity of uni- and binucleate *Rhizoctonia* and *Pythiaceae* fungi among conifer seedlings in Finnish forest nurseries

By A. LILJA

Abstract

Uni- and binucleate *Rhizoctonia* and *Pythiaceae* fungi were isolated from roots of dieback seedlings. Only binucleate *Rhizoctonia* strains were present in the roots of healthy seedlings. In pathogenicity tests, uninucleate *Rhizoctonia* sp. were pathogenic and binucleate *Rhizoctonia* spp. non-pathogenic. *Pythium ultimum* var. *ultimum* and *Phytophthora undulata* were the only *Pythiaceae* fungi that killed 12-week-old seedlings.

Key words: *Pinus sylvestris* – *Picea abies* – *Larix sibirica* – root pathogens – stunting.

1 Introduction

In recent years, root dieback of conifer seedlings has been a serious problem in certain forest nurseries in Nordic countries. Its symptoms on both Scots pine *(Pinus sylvestris* L.) and Norway spruce *(Picea abies* L. Karst.) seedlings are discolouration of the needles and partial death of the root system. The diseased seedlings occur in patches. Stunted growth has also been reported for containerized seedlings. Bare-rooted seedlings have been affected, but the above-ground symptoms have usually not been detected until after transplantation (LILJA et al. 1992). The disease has been reported in Norway, Sweden and Finland (VENN et al. 1986; LILJA et al. 1988; UNESTAM et al. 1989; BEYER-ERICSON et al. 1991; LILJA et al. 1992). In Finland, some nurseries have had to reject 40 % of their containerized Scots-pine seedlings because of root dieback (LILJA et al. 1993).

Earlier studies have concentrated on the whole mycoflora present on the roots of seedlings showing symptoms of root dieback. *Pythiaceae* fungi have frequently been isolated from diseased seedlings, and these fungi have been shown to be capable of infecting young seedlings (GALAAEN and VENN 1979; LILJA et al. 1992). Moreover, a novel-type uninucleate *Rhizoctonia* sp. (HIETALA et al. 1993) has been isolated, which has proved to be the fungus most pathogenic to 4-week-old Scots-pine seedlings (LILJA et al. 1992). In Norway, nursery inoculations with uncharacterized *Rhizoctonia* sp. have also resulted in typical root-dieback symptoms on containerized Norway-spruce seedlings (VENN et al. 1986).

This study reports on the frequency of uni- and binucleate *Rhizoctonia* strains and *Pythiaceae* fungi in diseased and healthy conifer seedlings collected from 11 nurseries. The pathogenicity of the isolated species to 12-week-old Norway-spruce and Scots-pine seedlings is also described. Furthermore, the second year growth of living but stunted, containerized seedlings collected from one nursery was monitored.

2 Material and methods

2.1 Fungal isolations and identification

Fungal isolations were made from the root tips of healthy and diseased seedlings as described by LILJA et al. (1992), except for one sample collected from the Imari nursery. At Imari, the Scots-pine-seedling roots were in an advanced state of decay and the fungi were baited by sowing cucumber *(Cumcumis sativus* L.) seeds in the growth substrate of the destroyed seedlings and then isolating fungi from the roots of 10 cucumber seedlings (VAN DER PLAATS-NITERINK 1981). Sample collection is presented in detail in Table 1.

Identification of the fungi was based on morphological criteria (OGOSHI 1987; VAN DER PLAATS-NITERINK 1981; STAMPS et al. 1990). A safranin stain (BANDONI 1979) was used to determine the nuclear condition of *Rhizoctonia* spp. fungi.

2.2 Pathogenicity test

The fungi used in the pathogenicity test were isolated in this study (Tables 2, 3). A total of 4 strains of uninucleate *Rhizoctonia* sp. were isolated from roots of diseased seedlings: 1. Scots pine in the Ukonniemi nursery (Table 1); 2. Scots pine in the Ruuttula nursery; 3. Scots pine in the Mellanå nursery; 4. Norway spruce in the Kankaanranta nursery. Four strains of binucleate *Rhizoctonia* sp. were isolated from roots of: 1. A healthy Scots-pine seedling in the Pekolampi nursery; 2. A diseased Scots-pine seedling in the Suonenjoki nursery; 3. A healthy Scots-pine seedling in the Imari nursery; 4. A diseased Norway-spruce seedling in the Kankaanranta nursery. The *Pythiaceae* fungi were: 1. *Pythium anandrum* Drechsler, isolated from a diseased Scots-pine seedling in the Suonenjoki nursery; 2. *P. irregulare* Buisman, baited from container peat of diseased Scots-pine seedlings in the Imari nursery; 3. *P. ultimum* Throw var. *ultimum,* from a diseased Scots-pine seedling in the Leksvall nursery; 4. A heterothallic *Pythium* sp. (HS-group), from a diseased Scots-pine seedling in the Ukonniemi nursery; 5. *Pythium* sp., from a diseased Norway-spruce seedling in the Kankaanranta nursery; 6. *Phytophthora undulata* (H. E. Petersen) M. W. Dick, from a diseased Norway-spruce seedling in the Pekolampi nursery.

At the time of inoculation, the Scots-pine and Norway-spruce seedlings were 12 weeks old. They were growing in 0.8 l pots, three seedlings in each pot. The mycelial inocolum consisted of a disc (1-cm diameter) cut from the margin of a 1-week-old, PD-agar culture of the fungus. The disc was buried in the middle of the pot, at a depth of 9 cm. Controls were inoculated with sterile PD-agar. The number of replicates was four. The experiment was carried out in the middle of summer in a greenhouse. The temperature in the greenhouse was maintained at 20°C. The illumination was natural sunlight.

After 2 months' incubation, the condition of the seedlings was examined. The seedlings were divided into three categories: healthy, diseased, and dead. The length of shoots, and the dry weight of shoots and roots, were measured.

2.3 Growth of living seedlings showing root-dieback symptoms

Four styroblock containers with 1-year-old Scots-pine seedlings, 75 seedlings in each container, were collected on 15th May 1991 from an open field at the Mellanå nursery in order to monitor the second-year growth of living seedlings showing symptoms of root dieback. The seedlings in two of the containers displayed stunted growth in comparison to the other two containers where seedlings had grown normally. Fungal isolations were made, as previously described, from 20 healthy-looking and 20 stunted seedlings (Tables 1, 3). The remaining seedlings were transferred to the greenhouse where the temperature was 20°C and illumination was natural sunlight.

Table 1. The seedling material from different forest nurseries: 2A = 2-year old; 2A+1 = 3-year old, transplanted as 2-year old; 3A = 3-year old; 1 = 1-year old; 2 = 2-year old; A = grown in open field; D = diseased; H = healthy

Nursery	Location		Date	Tree species	Type and age of seedlings		Number of seedlings	
	N	E			containerized	bare-rooted	D	H
Hepoharju	60°55′	27°33′	17.05.91	*Picea abies*	1		20	20
Imari 1	66°29′	25°32′	07.09.87	*Pinus sylvestris*	1		[1]	
Imari 2	66°29′	25°32′	12.11.90	*Pinus sylvestris*	1			20
Imari 3	66°29′	25°32′	15.08.91	*Pinus sylvestris*	1		10	
Jomala	60°09′	19°57′	29.08.91	*Larix sibirica*	1		5	
Kankaaranta 1	61°39′	22°44′	27.09.87	*Pinus sylvestris*		2A	10	
Kankaanranta 2	61°39′	22°44′	10.10.92	*Picea abies*	2		59	
Leksvall 1	60°03′	23°16′	13.05.87	*Pinus sylvestris*		2A+1	32	
Leksvall 2	60°03′	23°16′	31.07.87	*Pinus sylvestris*		2A+1	16	
Leksvall 3	60°03′	23°16′	17.09.91	*Pinus sylvestris*		3A	10	10
Mellanå	62°13′	21°39′	15.05.91	*Pinus sylvestris*	1		20	20
Nuojua 1	64°34′	26°39′	02.11.87	*Picea abies*	1		20	
Nuojua 2	64°34′	26°39′	12.05.92	*Pinus sylvestris*	1		10	
Pekolampi 1	63°21′	27°24′	23.05.91	*Pinus sylvestris*		2A+1	20	20
Pekolampi 2	63°21′	27°24′	14.08.92	*Picea abies*	2		20	20
Ruuttula	62°08′	28°48′	30.07.87	*Pinus sylvestris*		2A+1	7	
Suonenjoki	62°39′	27°04′	24.05.91	*Pinus sylvestris*	1		20	20
Ukonniemi	61°12′	28°15′	05.07.91	*Pinus sylvestris*	1		15	15

[1] Fungi were baited from growth substrate of diseased seedlings.

Table 2. **The percentage of diseased bare-rooted Scots-pine seedlings infected with *Rhizoctonia* and *Pythiaceae* fungi. Number of seedlings in parenthesis. See Table 1 for sample collection**

Fungus	Nurseries					
	Kankaan-ranta 1 (10)	Leksvall 1 (32)	Leksvall 2 (16)	Leksvall 3 (10)	Peko-lampi 1 (20)	Ruuttula (7)
Pythiaceae:						
Pythium anandrum		22	25		20	
P. ultimum var. *ultimum*				40		
Pythium sp. HS-group						29
Pythium spp.	20		13			
Phytophthora undulata					80	
Rhizoctonia spp.						
uninucleate	30	13	6	10	20	29
binucleate				10		

On 30th August, fungal isolations were repeated. In addition to isolations, the 1-cm-long tips of the main roots were cut from 10 healthy-looking and 10 stunted seedlings, five seedlings from each container, in order to confirm the fungal infection with longitudinal, razor-blade sections. The length of the shoots and the shoot and root dry weight of 50 healthy-looking control seedlings and 50 stunted seedlings were also measured. All sampling was performed randomly from each container.

2.4 Statistics

The data were analysed using one-way-analysis of variance and the significance of the mean differences determined using Tukey's and Duncan's multiple-range test. Correlations between shoot length and shoot and root dry weight were also calculated (BMDP 1988).

3 Results

3.1 Fungal isolations

Uninucleate *Rhizoctonia* sp. and *Pythiaceae* fungi were isolated only from seedlings suffering from root dieback. Binucleate *Rhizoctonia* strains were also present in the roots of diseased seedlings. Occasionally both bi- and uninucleate *Rhizoctonia* were isolated from the same seedling. The percentage of seedlings infected with uninucleate *Rhizoctonia* sp. varied from 6 % to 80 % (Tables 2, 3). *Pythium anandrum, P. ultimum* var. *ultimum,* heterothallic *Pythium* sp. (HS-group) and *Phytophthora undulata* were isolated from both bare-rooted and containerized Scots-pine seedlings. *Pythium* spp. were isolated from containerized Norway spruce and bare-rooted Scots-pine seedlings. *P. anandrum, Pythium* sp. (HS-group) and *Ph. undulata* were also isolated from containerized Norway-spruce seedlings and *P. ultimum* var. *ultimum* and uninucleate *Rhizoctonia* sp. were present in the roots of containerized larch *(Larix sibirica* Ledeb.) seedlings. *P. irregulare* was baited from the growth substrate of diseased, containerized Scots-pine seedlings. One sample of healthy Scots-pine seedlings collected from Imari in 1990 (Table 1) was 100 % infected with binucleate *Rhizoctonia* spp.

Table 3. **The percentage of diseased containerized seedlings infected with *Rhizoctonia* and *Pythiaceae* fungi. Number of seedlings in brackets. See Table 1 for sample collection**

Fungus	Nurseries										
	Hepo-harju Norway spruce (20)	Imari 1 Scots pine[1]	Imari 3 Scots pine (10)	Jomala Larch (5)	Kankaan-ranta 2 Norway spruce (59)	Mellanå Scots pine (20)	Nuojua 1 Norway spruce (20)	Nuojua 2 Scots pine (10)	Peko-lampi 2 Norway spruce (20)	Suonen-joki Scots pine (20)	Ukon-niemi Scots pine (15)
Pythiaceae:											
Pythium anandrum			20						20	30	
P. ultimum var. *ultimum*				20		20					
P. irregulare		30									
Pythium sp. HS-group	20		10								40
Pythium spp.					15		10				
Phytophthora undulata	15							40	100	30	
Rhizoctonia spp.											
uninucleate	20	20	20	40	58	40	10	20	10		80
binucleate					61					20	

[1] Baited from the growth substrate of diseased seedlings with 10 cucumber seedlings.

3.2 Pathogenicity tests

All the tested uninucleate *Rhizoctonia* sp. strains were pathogenic. The percentage of dead or dying seedlings varied between 17 % and 75 %, depending on the strain and tree species (Fig. 1). Inoculations with uninucleate *Rhizoctonia* sp. caused a decrease in the shoot dry weight of Scots pine ($p < 0.01$) and Norway spruce ($p < 0.05$) seedlings compared with the controls (Fig. 2). The decrease in root dry weight of both species was also significant ($p < 0.01$).

The tested binucleate *Rhizoctonia* strains were not pathogenic. Two strains increased the shoot dry weight of Norway-spruce seedlings ($p < 0.01$) and one strain increased the shoot dry weight of Scots-pine seedlings ($p < 0.01$) (Fig. 3).

P. ultimum var. *ultimum* and *Ph. undulata* were the only *Pythiaceae* fungi which proved to be slightly pathogenic (Fig. 1). Furthermore, they decreased the shoot and root dry weight of Scots-pine seedlings ($p < 0.01$). *P. irregulare* and *Pythium* sp. slightly increased the shoot dry weight of Norway spruce ($p < 0.05$) (Fig. 4).

The shoot length of the seedlings correlated with both the shoot and root dry weights of Scots pine ($r = 0.94$, $r = 0.92$) and Norway spruce ($r = 0.94$, $r = 0.81$).

3.3 Growth of stunted containerized seedlings

The seedlings from Mellanå nursery which had grown poorly during their first growing season and were stunted in the spring also grew poorly during their second growing season, and were much smaller in late August than the healthy seedlings (Fig. 5). In spring, uninucleate *Rhizoctonia* sp. was isolated from 40 % of the stunted seedlings. The frequency of *P. ultimum* var. *ultimum* was 20 % (Table 3). In August, uninucleate *Rhizoctonia* sp. was isolated from 60 % of the stunted seedlings; other isolates were *P. ultimum* var. *ultimum* (40 %) and a heterothallic *Pythium* sp. (10 %). The monilioid hyphae of *Rhizoctonia* were visible inside the main root tip in razor-blade sections. No fungal hyphae were seen in the roots of healthy seedlings. New lateral roots developed near the soil surface appeared to be

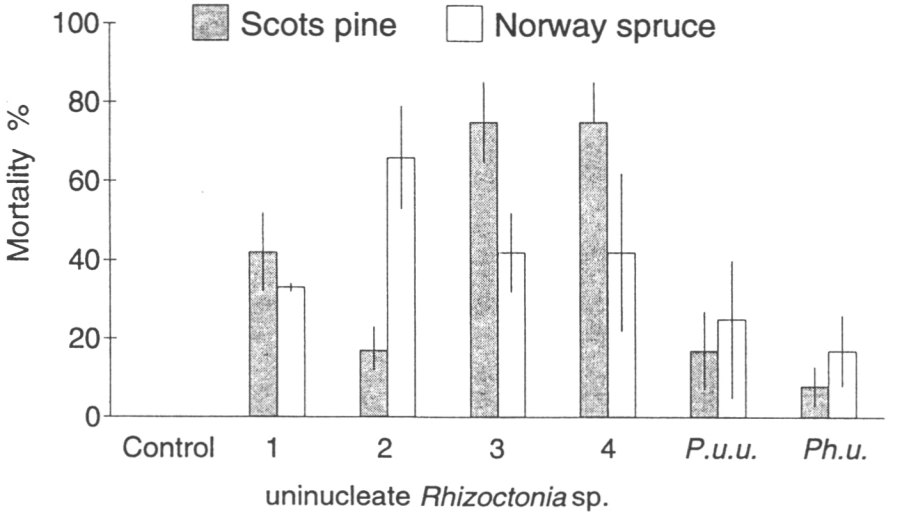


Fig. 1. The isolates showing pathogenicity to 12-week-old Scots-pine and Norway-spruce seedlings 2 months after inoculation. Four isolates of uninucleate *Rhizoctonia* sp., *Pythium ultimum* var. *ultimum* and *Phytophthora undulata*. Number of dying or dead seedlings (%). Bars indicate standard error

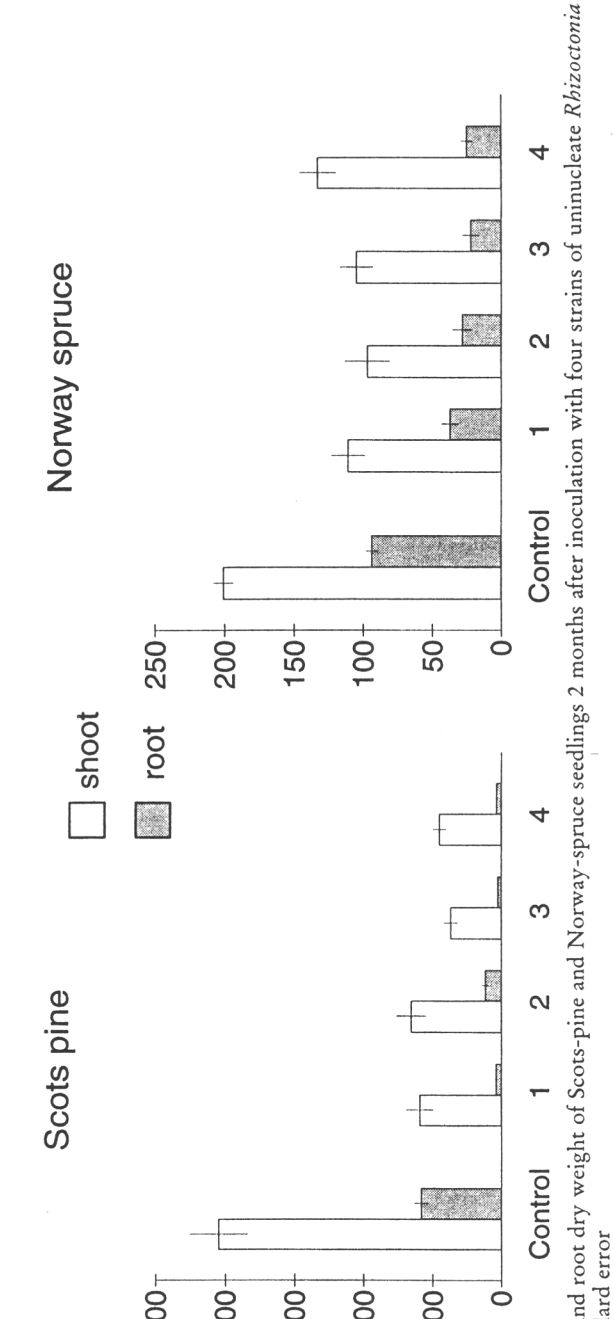


Fig. 2. The shoot and root dry weight of Scots-pine and Norway-spruce seedlings 2 months after inoculation with four strains of uninucleate *Rhizoctonia* sp. Bars indicate standard error

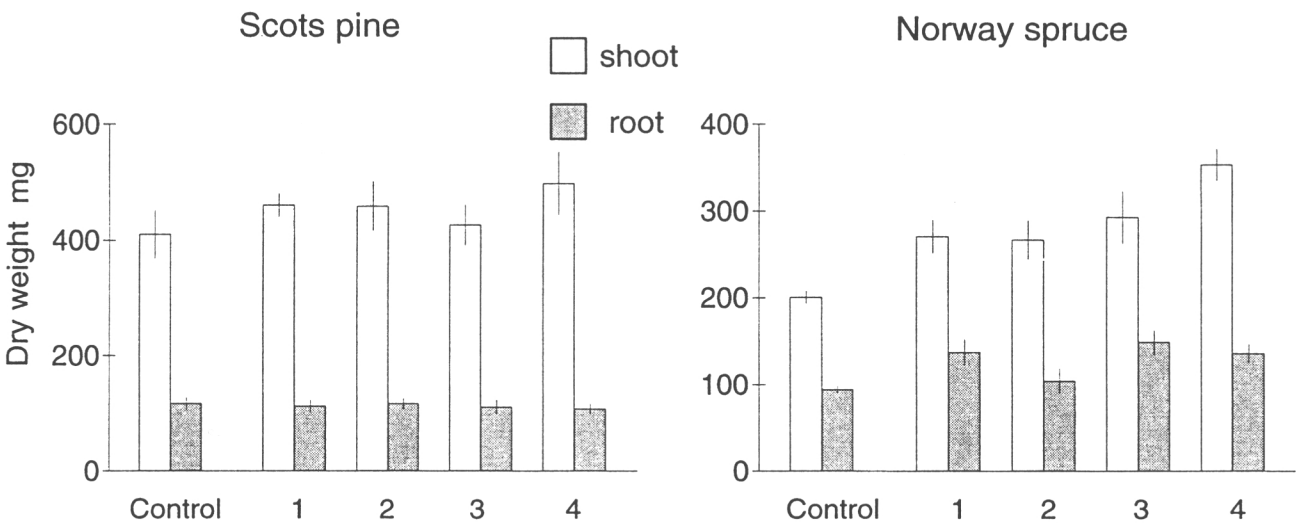


Fig. 3. The shoot and root dry weight of Scots-pine and Norway-spruce seedlings 2 months after inoculation with four strains of binucleate *Rhizoctonia* sp. Bars indicate standard error

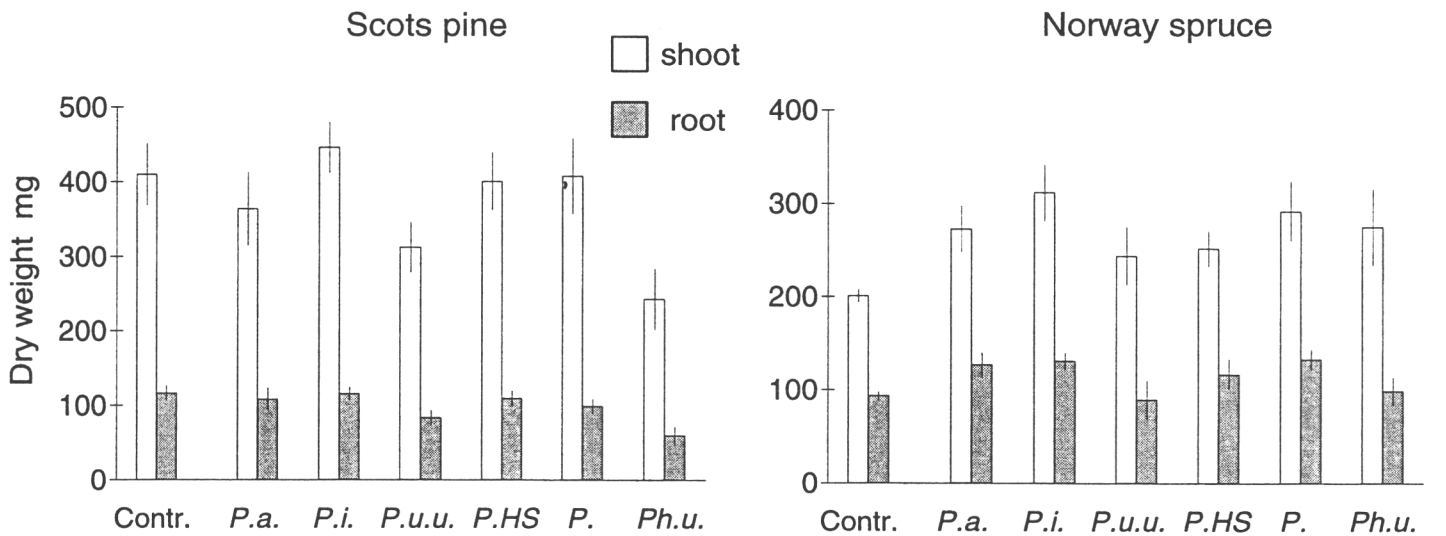


Fig. 4. The shoot and root dry weight of Scots-pine and Norway-spruce seedlings 2 months after inoculation with six *Pythiaceae* fungi: *P. a.* = *Pythium anandrum*; *P. i.* = *P. irregulare*; *P. u. u.* = *P. ultimum* var. *ultimum*; *P.* HS = an isolate of heterothallic *Pythium* sp. (HS-group); *P.* = *Pythium* sp. and *Ph. u.* = *Phytophthora undulata.* Bars indicate standard error

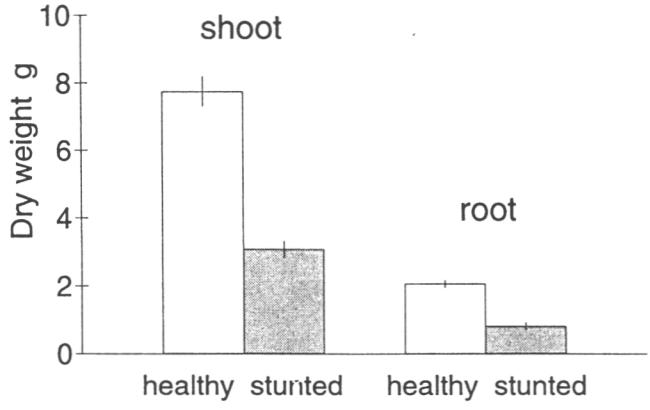


Fig. 5. The shoot and root dry weight of healthy and root-dieback seedlings of containerized Scots pine after their second growing season. Bars indicate standard error

keeping the infected seedlings alive. The difference between the shoot and root dry weight of the healthy and stunted seedlings was significant ($p < 0.01$).

4 Discussion

The isolation frequency of uninucleate *Rhizoctonia* sp. and *Pythiaceae* from the roots of diseased conifer seedlings confirms the earlier hypothesis that these fungi are involved in root dieback (VENN et al. 1986; LILJA et al. 1992). All tested strains of uninucleate *Rhizoctonia* sp. were strongly pathogenic. Suonenjoki was the only nursery from which uninucleate *Rhizoctonia* sp. was not isolated. The roots of the seedlings collected from the Suonenjoki nursery were moderately rotten and *Ph. undulata* was the only isolated fungus to show any pathogenicity in the inoculation trial (Fig. 1).

Binucleate *Rhizoctonia* spp. represents a diverse group of organisms. Some of them are known as pathogens of conifer seedlings (ENGLISH 1986; HUANG 1990). More than 20 species (KATARIA and HOFFMANN 1988) and at least 24 anastomosis groups (BURBEE et al. 1980; OGOSHI et al. 1979, 1983) have been described. The strains tested in this study were not pathogenic. On the contrary, some strains increased the shoot dry weight of the seedlings (Fig. 3), and the Scots-pine seedlings from Imari that were infected 100 % with binucleate *Rhizoctonia* sp. were healthy.

The only *Pythiaceae* species showing any pathogenicity were *P. ultimum* var. *ultimum* and *Ph. undulata* (Figs 1, 4). *Ph. undulata* is a fast growing fungus, recently transferred from the genus *Pythium* to *Phytophthora* on the basis of its DNA sedimentation rate and zoospore differentiation (DICK 1989; BRASIER and HANSEN 1992) and, in general, little is known about its pathogenicity (VAN DER PLAATS-NITERINK 1981). In a Finnish study, it was shown to be the most pathogenic *Pythiaceae* fungus to 4-week-old Scots-pine seedlings (LILJA et al. 1992).

Most *Pythium* species are known to infect juvenile or succulent tissues (HENDRIX and CAMPBELL 1973; ENDO and COLT 1974). They are pathogens of mainly young conifer seedlings that cause damping off and root rot restricted to the root tips or lateral roots (VAARTAJA and CRAM 1956; VAARTAJA et al. 1961; SUTHERLAND et al. 1989; RUSSELL 1990).

The reduction of root mass caused by uninucleate *Rhizoctonia* sp. infection and the presence of *Pythiaceae* fungi suggest that the root dieback may be a disease of successive infections. Primary infection with uninucleate *Rhizoctonia* sp. resulted in a high moisture content in the growth substrate, because the decayed roots of the seedlings could not utilize the water supply. Wet conditions are suitable for the growth of *Pythiaceae* and favourable

for attack by *Pythium* spp. (VAARTAJA et al. 1961; HENDRIX and CAMPBELL 1973; RUSSELL 1990).

The growth of seedlings infected with the root dieback is dependent on their ability to produce new lateral roots near the soil surface. The 1-year-old, stunted, containerized seedlings collected from the Mellanå nursery remained stunted after their second growing season. This shows that the seedlings showing root-dieback symptoms, even living and green ones, do not fully recover from the disease.

Acknowledgements

The author would like to thank ARI HIETALA and Mrs RITVA VANHANEN for their valuable assistance in the laboratory as well as Dr KARI KORHONEN, Prof. TIMO KURKELA, MARJA POTERI and Dr PEKKA TAMMINEN for their critical reading of the manuscript and their helpful suggestions for improvements. JOHN DEROME revised the English text. I also thank SAKARI LILJA and all the nurserymen for their help in sample collection.

Summary

Uninucleate *Rhizoctonia* sp. and several *Pythiaceae* fungi were isolated from the roots of nursery conifer seedlings showing root-dieback symptoms, but these fungi were not isolated from healthy-looking seedlings. In the pathogenicity test the strains of uninucleate *Rhizoctonia* sp. were highly pathogenic to Scots-pine and Norway-spruce seedlings. The only *Pythiaceae* fungi showing some pathogenicity to 12-week-old seedlings were *Pythium ultimum* var. *ultimum* and *Phytophthora undulata*. Binucleate *Rhizoctonia* strains were isolated from both diseased and healthy seedlings. In the pathogenicity test they were nonpathogenic and in fact appeared to increase the seedling shoot growth. Two strains of binucleate *Rhizoctonia* increased the shoot dry weight of Norway-spruce seedlings and one strain increased the shoot dry weight of Scots-pine seedlings.

Résumé

Existence et pouvoir pathogène de Rhizoctonia uni- et binucléé et de Pythiaceae chez des semis de conifères dans les pépinières finlandaises

Des *Rhizoctonia* sp. uninucléés et plusieurs *Pythiaceae* ont été isolés des racines de semis de conifères qui présentaient des symptômes de dépérissement racinaire mais ils n'ont pas été isolés de semis d'apparence saine. Dans les tests de pathogénicité, les souches uninucléés de *Rhizoctonia* sp. étaient très pathogènes pour les semis de *Pinus sylvestris* et de *Picea abies*. Les *Pythiaceae* qui avaient un certain pouvoir pathogène vis-à-vis de semis de 12 semaines étaient *Pythium ultimum* var. *ultimum* et *Phytophthora undulata*. Les isolats binucléés de *Rhizoctonia* ont été isolés de semis malades comme de semis sains. En test de pathogénicité, ils étaient non pathogènes et augmentaient la croissance des pousses. Deux souches de *Rhizoctonia* binucléés augmentaient le poids sec des pousses chez l'Epicéa et une souche augmentait le poids sec des pousses des semis de Pin sylvestre.

Zusammenfassung

Vorkommen und Pathogenität von ein- und zweikernigen Rhizoctonia-Arten und Pilzen aus der Familie der Pythiaceae an Koniferensämlingen aus finnischen Forstbaumschulen

Aus Wurzeln von Koniferensämlingen (Baumschulware) mit Wurzelfäule wurden einkernige *Rhizoctonia*-Arten und verschiedene *Pythiaceae* isoliert. Gesunde Sämlinge waren frei von diesen Pilzen. In Pathogenitätstests waren die einkernigen *Rhizoctonia*-Arten stark pathogen gegen *Pinus sylvestris* und *Picea abies*. Die einzigen Vertreter der *Pythiaceae* mit einer gewissen Pathogenität waren *Pythium ultimum* var. *ultimum* und *Phytophthora undulata*. Zweikernige *Rhizoctonia*-Stämme wurden sowohl von kranken als auch von gesunden Sämlingen isoliert. Sie erwiesen sich als nicht pathogen und

förderten sogar das Sprosswachstum der Sämlinge: 2 Isolate erhöhten das Sprosstrockengewicht bei *Picea abies* und 1 Isolat erhöhte das Sprosstrockengewicht bei *Pinus sylvestris.*

References

BANDONI, R. J., 1979: Safranin O as a rapid nuclear stain for fungi. Mycologia **71,** 873–874.

BRASIER, C. M.; HANSEN, E. M., 1992: Evolutionary biology of *Phytophthora.* Annual Rev. Phytopath. **30,** 173–200.

BEYER-ERICSON, L.; DAMM, E.; UNESTAM, T., 1991: An overview of root dieback and its causes in Swedish forest nurseries. Eur. J. For. Path. **21,** 439–443.

BMDP, Statistical Software Manual, 1988. University of California Press, Berkeley, Vol. 1. 619 pp.

BURBEE, L. L.; SANDERS, P. L.; COLE, H. Jr., 1980: Anastomosis grouping of *Ceratobasidium cornigerum* and related fungi. Mycologia **72,** 689–701.

DICK, M. W., 1989: *Phytophthora undulata* comb. nov. Mycotaxon XXXV, 449–453.

ENDO, R. M.; COLT, W. M., 1974: Anatomy, cytology, and physiology of infection by *Pythium.* Proc. Amer. Phytopath. Soc. **1,** 215–223.

ENGLISH, J. T., 1986: Seedling blight of Longleaf Pine caused by binucleate *Rhizoctonia solani* like fungus. Plant Disease **70,** 148–150.

GALAAEN, R.; VENN, K., 1979: *Pythium sylvaticum* Campbell; Hendrix and other fungi associated with root dieback of 2-0 seedlings of *Picea abies* (L.) Karst. in Norway. Medd. Nor. inst. skogforsk. **34,** 265–280.

HENDRIX, F. F.; CAMPBELL, W. A., 1973: Pythiums as plant pathogens. Annual Rev. Phytopath. **11,** 77–98.

HIETALA, A.; SEN, R.; LILJA, A., 1993: Anamorphic and telemorphic characteristics of a uninucleate *Rhizoctonia* sp. isolated from the roots of nursery grown conifer seedlings. Mycological Research (in press).

HUANG, J. W., 1990: Fungi associated with damping-off of Slash-Pine seedlings in Georgia. Plant Disease **74,** 1, 27–30.

KATARIA, H. R.; HOFFMANN, G. M., 1988: A critical review of plant pathogenic species of *Ceratobasidium* Rogers. J. Plant Dis. Protec. **95,** 81–107.

LILJA, A.; LILJA, S.; POTERI, M., 1988: Root dieback in forest nurseries. Karstenia **28,** 64.

–; –; –; LI, Z. 1992: Conifer seedling root fungi and root diback in Finnish nurseries. Scand. J. For. Res. **7,** 547–556.

–; HIETALA, A.; SEN, R., 1993: Havupuiden lahojuuri- ja koivun versolaikkutauti. In Metsänsuojelututkimuksen tuloksia. Metsäntutkimuspäivä Vantaalla. Metsäntutkimuslaitoksen tiedonantoja No. 460, 1993. Ed. by T. KURKELA and K. LEPPONEN. Vantaa, Finland. pp. 5–12.

OGOSHI, A., 1987: Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annual Rev. Phytopath. **25,** 125–143.

–; ONIKI, M.; SAKAI, R.; UI, T., 1979: Anastomosis grouping among isolates of binucleate *Rhizoctonia.* Trans. Mycol. Soc. Japan **20,** 33–39.

–; –; ARAKI, T.; UI, T., 1983: Studies on the anastomosis groups of binucleate *Rhizoctonia* and their perfect state. J. Fac. Agr. Hokkaido Univ. **61,** 244–260.

PLAATS-NITERINK, A. J. VAN DER, 1981: Monograph of the genus *Pythium.* Studies in mycology, Centraalbureau voor Schimmelcultures, Baarn **21,** 1–242.

RUSSELL, K., 1990: Damping off. In: Growing healthy seedlings. Identification and management of pests in northwest forest nurseries. Ed. by P. B. HAMM, S. J. CAMPBELL and E. M. HANSEN 1990. U.S.D.A. For. Serv., Pac. Northw. Reg., Oregon State Univ. pp. 2–5.

STAMPS, D. J.; WATERHOUSE, G. M.; NEWHOOK, F. J.; HALL, G. S., 1990: Revised tabular key to the species of *Phytophthora.* CAB Int. mycol. inst. Mycol Papers **162,** 1–28.

SUTHERLAND, J.; SHRIMPTON, G.; STURROCK, R., 1989: Diseases and insects in British Columbia Forest seedling nurseries. FRDA Rep. **065,** 27–28.

UNESTAM, T.; BEYER-ERICSON, L.; STRAND, M., 1989. Involvement of *Cylindrocarpon destructans* in root death of *Pinus sylvestris* seedlings: pathogenic behaviour and predisposing factors. Scand. J. For. Res. **4,** 521–536.

VAARTAJA, O.; CRAM, W. H., 1956: Damping off of conifers and of *Caragana* in Saskatchewan. Phytopathology **46,** 391–397.

–; –; MORGAN, G. A., 1961: Damping-off etiology especially in forest nurseries. Phytopathology **51,** 35–42.

VENN, K.; SANDVIK, M.; LANGERUD, B., 1986: Nursery routines, growth media and pathogens affect growth and root dieback in Norway-Spruce seedlings. Medd. Nor. inst. for skogorsk. **39,** 314–328.

Author's address: ATRJA LILJA, Finnish Forest Res. Inst., Dept of Forest Ecology, PO Box 18, SF-01301 Vantaa, Finland

received: 20. 12. 1993, accepted: 10. 3. 1994

III

Anamorphic and teleomorphic characteristics of a uninucleate *Rhizoctonia* sp. isolated from the roots of nursery grown conifer seedlings

ARI M. HIETALA, ROBIN SEN* AND ARJA LILJA

Department of Forest Ecology, Finnish Forest Research Institute, P.O. Box 18, FIN-01301 Vantaa, Finland

Vegetative, anastomosis, fruiting and basidial characteristics were analysed in one Norwegian and five Finnish *Rhizoctonia* isolates from the roots of various nursery grown conifer seedlings. The isolates displayed common hyphal and colony morphology that also confirmed their designation as a *Rhizoctonia* sp. Hyphal cells were predominantly uninucleate except for one isolate which contained a relatively high number (11%) of binucleate tip and subapical cells. All isolates had similar temperature dependent growth rates and formed a single anastomosis group within which killing reactions were detected in opposing fusion cells of all non self pairings.

Fruiting was induced in all but one Finnish isolate using a novel method involving axenic liquid culture of the fungi in the presence of Scots pine (*Pinus sylvestris*) seedlings. Basidial and basidiospore dimensions indicate that the isolates represent a single *Ceratobasidium* sp. although two selected isolates showed no hyphal anastomosis reactions with binucleate testers of the different *Ceratobasidium* anastomosis groups (AG-A to AG-S). The identified teleomorph closely resembles *C. bicorne* but further confirmation was not possible because this fungus is only available as herbarium material.

Rhizoctonia DC. was established in 1815 by de Candolle to accommodate a non-sporulating root rotting fungus, *Rhizoctonia crocorum* DC.: Fr. However, the subsequent description of numerous heterogenic *Rhizoctonia* species prompted a major revision of the taxonomic criteria required for the identification of *R. solani* Kühn that were later expanded to cover the whole genus (Parmeter & Whitney, 1970; Ogoshi, 1975). Accepted vegetative features included the requirements of hyphae with dolipore septa, basally constricted branching near the distal septum of cells, absence of clamp connections, conidia and rhizomorphs and sclerotia with undifferentiated structure.

On the basis of the known teleomorphs the genus is limited to the sub-division Basidiomycotina; class Hymenomycetes (sub-class Holobasidiomycetidae or Phragmobasidiomycetidae) (Sneh, Burpee & Ogoshi, 1991). The four main genera represented are *Thanatephorus* Donk (includes the teleomorph of e.g. *R. solani*), *Waitea* Warcup & P. H. B. Talbot (e.g. *R. zeae* Voorhees), *Tulasnella* J. Schröt. (e.g. *R. repens* Bernard) and *Ceratobasidium* Rogers (e.g. *R. endophytica* var. *endophytica* H. K. Saksena & Vaartaja and *R. cerealis* E. P. Hoeven). A major taxonomic feature of the anamorphs that separates the former and latter two genera is the respective presence of multinucleate and binucleate cells in young vegetative hyphae (Sneh *et al.*, 1991). The multinucleate *R. solani* (*T. cucumeris* (A. B. Frank) Donk and *T. praticola* (Kotila) Flentje and binucleate *Rhizoctonia* spp. (*Ceratobasidium* spp.) have been respectively further sub-divided into 11 and 21 anastomosis groups (AG) based on affinity and fusion of interacting hyphae of paired cultures (Sneh *et al.*, 1991 and references therein).

Isolates of two species, *R. quercus* Cast. and *R. alpina* Cast., were found to contain uninucleate hyphae (Burpee *et al.*, 1980) but this could not be confirmed by Ogoshi *et al.* (1983) who regarded the latter fungus to be binucleate. The anamorph of an authenticated uninucleate *Rhizoctonia* sp. from the roots of winter wheat has been described (Hall, 1986).

Many *Rhizoctonia* species are economically important plant pathogens in agriculture and thus receive considerable attention but less is known about these fungi and their effect on forestry production, e.g. in forest tree nurseries. It has been known for a long time that damping-off in nursery conifer seedlings can be caused by *R. solani* (Vaartaja & Cram, 1956; Saksena & Vaartaja, 1961). More recently in Georgia, U.S.A., Huang & Kuhlman (1990) showed that *R. solani* and a binucleate *Rhizoctonia* sp., representing anastomosis groups AG-4 and CAG-3, respectively, were able to induce damping-off symptoms in nursery Slash pine (*Pinus elliottii* Engelm. var. *elliottii*) seedlings. A binucleate *Rhizoctonia* sp. (CAG-3) was also identified causing seedling blight of longleaf pine (*P. palustris* Mill.) in Florida (English, Ploetz & Barnard, 1986). Ten different *Rhizoctonia* species causing root rot in pine (*P. sylvestris* L. and *P. resinosa* Ait.) seedlings in Canadian nurseries included *R. callae* Cast., *R. globularis* H. K. Saksena & Vaartaja and *R. endophytica* var. *endophytica* (Saksena & Vaartaja, 1961). All have since been confirmed to be binucleate *Rhizoctonia* species and the latter is an anamorph of *Ceratobasidium cornigerum* (Bourdot) Rogers (Sneh *et al.*, 1991).

In the Nordic countries, a binucleate *Rhizoctonia* sp. has been identified killing the needles of Norway spruce (*Picea*

* Present address: Department of General Microbiology, P.O. Box 41 (Mannerheimintie 172), FIN-00014 University of Helsinki, Finland.

Table 1. *Rhizoctonia* isolates from the roots of nursery grown conifer seedlings

Isolate number	Location	Coordinates	Host	Seedling type	Year	Source
250	Lapinlahti, Finland	63° 21′ N, 27° 24′ E	*Picea abies*	Containerized	1992	A. Lilja
256	Jomala, Finland	60° 09′ N, 19° 57′ E	*Larix sibirica*	Containerized	1991	A. Lilja
260	Lapinlahti, Finland	63° 21′ N, 27° 24′ E	*Pinus sylvestris*	Bare-rooted	1989	A. Lilja
263	Lapinlahti, Finland	63° 21′ N, 27° 24′ E	*Pinus sylvestris*	Bare-rooted	1987	A. Lilja
264	Lapinlahti, Finland	63° 21′ N, 27° 24′ E	*Pinus sylvestris*	Bare-rooted	1989	A. Lilja
83-111/1N[a]	Namsos, Norway	64° 29′ N, 11° 29′ E	*Picea abies*	Containerized	1983	K. Venn[b]

[a] Venn *et al.* (1986).
[b] Norwegian Forest Research Institute.

abies (L.) Karst.) seedlings (Roll-Hansen & Roll-Hansen, 1968) and, more recently, an uncharacterized *Rhizoctonia* sp. was also found to cause root dieback in seedlings of the same tree species in Norwegian nurseries (Venn, Sandvik & Langerud, 1986). A nursery survey of fungi present in the roots of both bare-rooted and containerized grown conifer (*P. sylvestris* and *P. abies*) seedlings in Finland included a uninucleate *Rhizoctonia*-like fungus that was found to be an aggressive root pathogen of Scots pine (*P. sylvestris*) in pathogenicity tests (Lilja *et al.*, 1992).

The aim of this work was to further characterize the uninucleate *Rhizoctonia* sp., which has been commonly isolated from roots of Finnish nursery grown conifer seedlings, together with the isolate from *P. abies* in Norway (Venn *et al.*, 1986).

MATERIALS AND METHODS

Fungal isolates

Information on the geographic origin of the isolates studied, host species and seedling type, year and source are given in Table 1. The Finnish isolates were originally isolated on distilled water agar (DWA) (1%) from surface sterilized root pieces as described by Lilja *et al.* (1992).

Anamorphic characteristics

Hyphal characteristics (growth pattern, hyphal diameter, septal structure and the number of nuclei per cell) were examined after growth of the isolates on microscope slides coated with low strength (1/8) potato dextrose agar (PDA) (4·88 g PDA and 13·12 g agar (Difco laboratories, USA) l^{-1} H_2O) that were maintained in a moist atmosphere for 48 h at 24 °C. All the following hyphal and basidial dimensions were measured using a light microscope equipped with a stage and eyepiece micrometer. The diameter of 15 subapical cells of main runner hyphae were randomly measured from four colonies of each isolate at 400 × magnification. The septal condition of hyphae was examined under phase contrast at 1000 × magnification. Nuclei were stained with HCl-Giemsa following the fixing and staining procedures described by Wilson (1992), and numbers in tip and subapical cell pairs (100 cells each) were counted at 400 × magnification.

For characterization of general cultural features, petri-dishes containing PDA were centrally inoculated by transferring a 5 × 5 mm block from the margin of an actively growing colony and incubated in the dark at 24°. Colony colour and the distribution size, shape, colour and structure of sclerotia for each isolate were regularly examined over a period of 21 d. The colours were designated using the mycological colour chart of Rayner (1970).

Growth rates

Five replicate PDA plates, similarly inoculated (as above) with each isolate were incubated in the dark at 7, 14, 18, 21, 24, 28 and 31°. The colony diameter was measured at 24 h intervals along two right angled axes.

Hyphal anastomosis

Hyphal anastomosis was microscopically examined on the surface of distilled water agar (DWA) (15 g agar l^{-1}) in petri-dishes to determine anastomosis groupings (Parmeter, Sherwood & Platt, 1969) and on microscope slides coated with 1/8 PDA for detailed identification of the type of hyphal fusion reaction (Matsumoto, Yamamoto & Hirane, 1932; Yokoyama, Ogoshi & Ui, 1983; Yokoyama & Ogoshi, 1986). Isolates were paired in all combinations at a distance of 2 cm by inoculating the agar surface using a modified Pasteur pipette (Korhonen & Hintikka, 1980) producing small cylindrical inoculum plugs (2 mm × 1 mm diam.). Pairings were incubated at 21° until the margins of opposing colonies began to overlap. Hyphal anastomosis on DWA was directly observed through the petri-dishes at 100 × and confirmed at 400 × magnification. The frequency of perfect and imperfect hyphal fusions on 1/8 PDA was recorded from a total of 75 contact points, where in each case two opposing hypha either fused, crossed or grew in a juxtapositioned manner (Sneh *et al.*, 1991), using phase contrast at 400 × magnification. All pairing combinations were made on two separate occasions.

Two isolates, 263 and 264, were also paired in all combinations on DWA with the binucleate *Rhizoctonia* (*Ceratobasidium* spp.) tester isolates (AG-A to AG-S, deposited in the ATCC) (Sneh *et al.*, 1991), *R. alpina* (CBS 309.35) and *R. quercus* (CBS 313.35) to identify any anastomosis reactions.

Teleomorphic characteristics

A new method utilizing living Scots pine seedlings to induce the perfect state was developed. Sterile Scots pine seedlings

were prepared by first imbibing seeds in distilled water at 5° for 36 h and then washing in a Tween-80 solution (3 drops per 100 ml distilled water) for 5 min. After three separate rinses in distilled water the seeds were treated with hydrogen peroxide (30%, v/v) for 15 min and then washed in three changes of sterile distilled water. The seeds were plated on 1·2% water agar, 15 to 20 seeds per petri-dish, and incubated inverted at 21° in dark for 14 d. Three sterile seedlings were then aseptically transferred to each petri-dish containing 20 ml sterile distilled water. A 5 × 5 mm block from the margin of a 3-d-old colony of an isolate grown on PDA at 21° was transferred to the petri-dish which was then incubated on a laboratory bench with natural indirect lighting. Between 3 and 5 replicate petri-dish culture systems per isolate were prepared in separate experiments over the whole year.

Following the development of hymenia on the water and seedling surface, determined visually and at 40 × magnification, samples were transferred to microscope slides and squash preparates made for measurements of basidial and basidiospore dimensions.

RESULTS

Anamorphic characteristics

All cultures showed the general *Rhizoctonia* hyphal characteristics. Basally constricted branches arose at acute angles behind the apices of the advancing hyphae and at right angles in the older hyphal regions. A dolipore septum was always formed near the point of origin of the branch. The mean width of subapical cells of the main runner hyphae ranged from 6·3 to 7·2 μm (Table 2). All the counted tip and subapical cells of isolates 250, 264 and 83-111/1N were uninucleate (Fig. 1A). The percentages of binucleate (tip:subapical) cells in 256, 263 and 260 were 4:4, 1:3 and 11:11%, respectively. In the latter isolate, hyphae containing many consecutive binucleate cells originating from uninucleate hyphae (Fig. 1B) were mainly restricted to small areas in the central parts of the colony.

The isolates showed almost uniform cultural morphology on PDA. Buff coloured, velvety-looking, young colonies grew in a radial manner and no zonation was observed. Hazel coloured spots first appeared on the central surface of the colony within 5 days and later appeared over the whole surface area to give a spotted appearance. Some of these very characteristic pigmented spots continued to spread covering an area of several mm² after an incubation period of 21 d. Within 14 d the first submerged, usually rounded sclerotia appeared. The individual submerged, but occasionally surface

Table 2. Subapical cell widths of main runner hyphae[a] and dimensions of monilioid cells of sclerotia[b] (μm)

Isolate	Hyphal width ± S.D.	Monilioid cell	
		Length	Width
250	6·6 ± 0·56 (5·4–7·7)	16–29	13–20
256	6·4 ± 0·54 (5·4 ± 7·8)	16–30	11–18
260	7·2 ± 0·51 (5·8–8·4)	18–35	11–19
263	6·7 ± 0·63 (5·2–7·8)	18–32	11–20
264	6·5 ± 0·60 (4·8–7·7)	17–34	11–18
83-111/1N	6·3 ± 0·57 (5·0–7·5)	14–32	10–17

[a] 60 measurements (15 measurements from four separate colonies).
[b] 60 measurements.

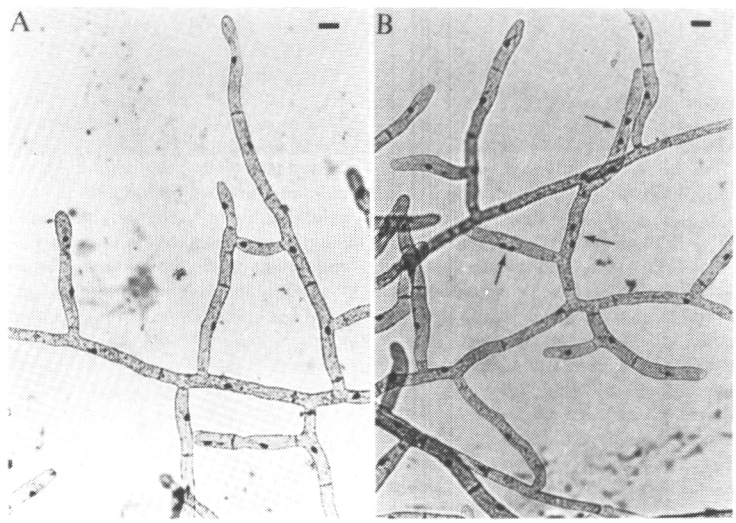


Fig. 1. Nuclei of isolate 260 stained with HCl-Giemsa. Hyphae with (A) only uninucleate cells and (B) a uninucleate hypha giving rise to a sidebranch with binucleate cells (arrowed). Bars = 10 μm.

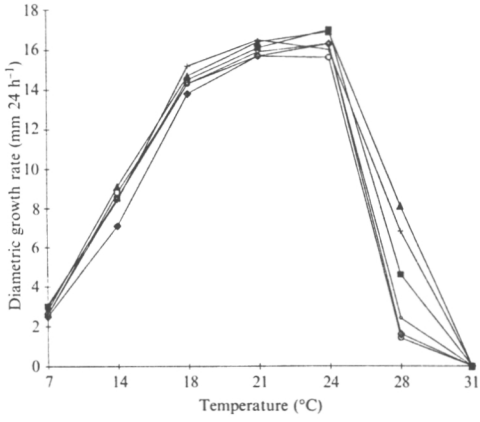


Fig. 2. The effect of temperature on the growth of different isolates on potato dextrose agar. ■—■, 250; ▲—▲, 256; ◎—◎, 260; ◇—◇, 263; o—o, 264; +—+, 83-111/1N.

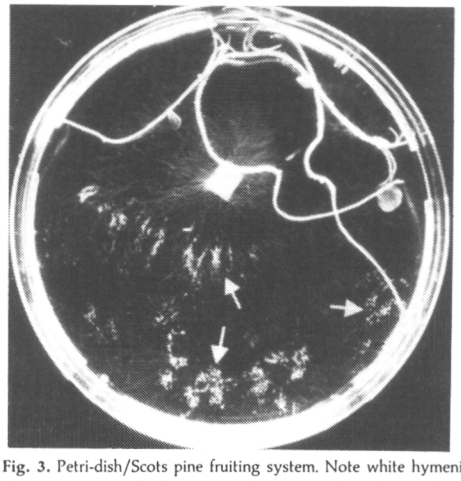


Fig. 3. Petri-dish/Scots pine fruiting system. Note white hymenial clusters (arrowed) of isolate 260 on the water surface after incubation of two weeks.

located, sclerotia were fulvous to umber in colour with diameters up to 900 μm. These submerged sclerotia tended to aggregate to form several mm large cauliflower-like structures after 21 d or longer incubation periods. The sclerotia were constructed of doliform to subglobose monilioid cells that were not organised into a rind and medulla. The dimensions of monilioid cells are presented in Table 2.

Growth rates

All isolates had very similar growth rates up to 24° on PDA, at 28° it was possible to separate some of the isolates (Fig. 2). Maximum growth rates of two isolates, 260 and 83-111/1N, were at 21° and the others at 24° and all were unable to grow at 31°.

Hyphal anastomosis

The hyphae of all the uninucleate isolates anastomosed with each other both on DWA and thin films of 1/8 PDA. In self pairings on the latter agar, opposing hyphae often showed varying degrees of hyphal attraction which was followed by cell wall contact either between tips, a tip and a hyphal side wall or via bridging between adjacent side walls. Following contact a positive anastomosis reaction resulted in a perfect fusion which involved the loss of cell walls at the fusion junction and a maintenance to cytoplasmic continuity which occurred in 95–100% of the fused cells in all pairings. In non self pairings, similar hyphal pre-contact interactions were followed by imperfect fusions identified by a characteristic rapid sequence of events: cell wall dissolution, cytoplasmic granulation, a loss of cell turgor as detected by a narrowing of cell diameter and finally a complete vacuolation of between one and three cells on either side of the fusion junction, a phenomenon termed the killing reaction (Yokojama & Ogoshi, 1986). In most pairings, over 98% of the fusion cells were killed following anastomosis. The overall anastomosis fusion frequencies of self and non self pairings were in the range 49–57 and 45–71%, respectively.

No hyphal fusions were recorded in pairings of 263 and 264 and either the binucleate *Rhizoctonia* isolates representing the known anastomosis groups of *Ceratobasidium* spp. (AG-A to AG-S) or *R. alpina* and *R. quercus*.

Fig. 4. Typical basidia of the uninucleate *Rhizoctonia* sp. under phase contrast; isolates (A) 264 and (B) 83-111/1N. Bars = 10 μm.

Table 3. Basidial[a] and basidispore[b] dimensions (μm) of the uninucleate *Rhizoctonia* isolates

	Metabasidium				Sterigma						Basidiospore	
							Number					
Isolate	Length	Width	Basal width	Width/b.w.	Length	Width	1	2	3	4	Length	Width
250	13·4 (10·7–14·9)	10·8 (9·3–12·1)	5·0 (3·4–6·2)	2·2 (1·6–3·1)	13·9 (10·0–25·8)	2·7 (1·8–3·8)	7	12	1		10·5 (9·6–13·4)	6·8 (5·3–7·6)
260	14·0 (12·0–16·4)	11·1 (10·0–12·3)	5·0 (3·4–6·7)	2·3 (1·8–3·1)	14·6 (9·4–44·0)	2·6 (2·0–3·6)	2	18			11·4 (9·6–13·4)	7·0 (5·7–8·6)
263	13·9 (12·0–16·3)	11·3 (10·0–13·0)	4·9 (3·9–7·7)	2·3 (1·7–2·9)	14·2 (7·0–20·3)	3·0 (1·9–4·8)	6	8	4	2	11·6 (9·6–14·3)	6·9 (5·3–8·4)
264	13·6 (11·1–17·0)	11·1 (8·7–13·4)	5·0 (3·1–6·8)	2·4 (1·6–3·6)	15·9 (8·7–27·2)	2·9 (1·8–4·4)	4	13	3		11·1 (8·6–14·8)	6·9 (5·1–8·6)
83-111/1N	15·7 (13·1–19·9)	12·0 (10·0–14·0)	5·2 (2·8–7·9)	2·5 (1·5–4·6)	16·8 (9·5–28·6)	3·2 (2·2–4·6)	1	14	5		11·6 (9·3–14·3)	6·8 (5·1–7·9)

[a] Measurements from 20 basidia.
[b] Measurements from 40 basidiospores.

Teleomorphic characteristics

All the isolates, except 256, could be repeatedly fruited with the new method between April and August but not at other times of the year. The diurnal day and night room temperature fluctuations during the spring and summer varied between 21 and 30° but remained at a temperature of 20–22° during the autumn and winter. White coloured hymenium usually developed on the water surface near the margins of the petri-dish but occasionally more centrally (Fig. 3) and also on the seedling surface. Basidia appeared within 8 to 15 d arising directly from basal hyphae or short side branches and the shape of metabasidia varied from obovate to subglobose (Fig. 4A and B). The basidial and basidiospore dimensions are given in Table 3. The most frequent number of normally stout sterigmata was two and occasionally an adventitious septum was formed in the central or apical regions of the sterigmata. Branched sterigmata were also infrequently observed and in very rare cases unbranched sterigmata lacking basidiospores grew hundreds of μm long below the water surface, maintaining their characteristic width and forming regularly spaced adventitious septa. The basidiospores were ovoid to ellipsoid and germinated on the water surface by direct germ-tube formation or in a minority of cases following repetition.

DISCUSSION

It is clear, from the anamorphic and teleomorphic data presented, that the isolates described represent a *Rhizoctonia* species. We have further examined the nuclear condition of over 30 isolates of similar cultural morphology, originating from conifer seedling roots taken from nurseries in Finland and Norway and they all contain predominantly uninucleate hyphae as described (unpublished data). It was not possible to compare our isolates with the uninucleate *Rhizoctonia* species described by Hall (1986) as the isolates were not deposited in a culture collection (G. Hall, pers. comm.). However, from his descriptions of the anamorph it is clear that these two species are not the same. No positive anastomosis reactions were detected in pairings of either 263 or 264 with the morphologically dissimilar isolates of *R. alpina* and *R. quercus* which had earlier been identified as being uninculeate by Burpee *et al.* (1980).

In the data presented, only isolate 260 had binucleate cell numbers that were clearly higher than the expected frequencies explainable by random mitotic divisions (Tu, Kimbrough & Aldrich, 1977). The origin of occasional series of binucleate cells amongst the prevailing uninucleate cells in hyphae restricted to the central areas of the colony is not clear. Contamination of cultures can be ruled out as both nuclear types exist in the same hypha as shown in Fig. 1B. The phenomenon does seem to be a general feature of our isolates as it has been observed to occur sporadically on various occasions under the same cultural conditions. Similar unpredictable changes may also explain the differences in nuclear condition reported for *R. alpina* and *R. quercus* (Burpee *et al.*, 1980; Ogoshi *et al.*, 1983). We have also observed consecutive binucleate cells in hyphae of a colony grown out of a single uninucleate tip cell. Whether the two nuclei are identical, being brought together either by self anastomosis or as a result of loss of septal synchronization, or, more interestingly, are dissimilar possibly resulting from a reduction division requires further detailed genetic investigation.

The very similar growth rates of all the isolates studied suggest that they are quite closely related, although at 28° it was possible to separate 260, 263 and 264 from the others. The uniformity of isolates was also further confirmed by the identification of a single anastomosis group. Within this group it is clear that the isolates represent different genotypes as non self anastomoses always resulted in the appearance of a killing reaction in neighbouring cells on either side of the point of hyphal fusion.

Unsuccessful attempts to obtain the perfect state, made using the nutrient step-down (Adams & Butler, 1983*a*, *b*) and antibiotic induction (Kangatharalingham & Carson, 1988) methods, prompted the development of the described method incorporating living seedlings of Scots pine. As no fruiting was observed on the water surface in the absence of seedlings, it is likely that the presence of plant material provides the fungus with a limited nutrient resource that may also actively induce fruiting through production of plant specific metabolites or breakdown products. During the autumn and winter months the centrally heated room temperatures remained very stable and thus the lack of a diurnal temperature gradient and inadequate natural lighting conditions may have contributed to the inhibition of fruiting. The method was reliable enough during spring and summer but the short window for fruiting is rather inconvenient and therefore requires further development. It was not possible to induce basidia of isolate 256, which had originally been isolated from larch (*Larix sibirica* Ledeb.), in the petri-dish system containing *P. sylvestris* although 83-111/1N and 250 from *P. abies* did fruit regularly. It may be the case that fruiting of 256 is host

specific but further work including other larch isolates is needed to confirm this.

On the basis of basidial characteristics, these isolates can be placed in *Ceratobasidium* (Talbot, 1965). The ratio between the widths of metabasidia and their supporting hyphae was generally over two, which is a major feature defining the genus. The development of basidia on basal hyphae or on short side branches and the repetitive germination of basidiospores are also typical of *Ceratobasidium* species. In contrast, there was a lack of affinity between two representative uninucleate isolates (263 and 264) and tester isolates of known *Ceratobasidium* anastomosis groups AG-A to AG-S. The differing cultural morphology of these testers also indicates that they were not related to this uninucleate fungus. There is no description of similar anamorphs that could be related to these Nordic isolates in the anamorph keys of *Ceratobasidium* species summarized by Sneh *et al.* (1991).

The teleomorph of our uninucleate isolates does seem to resemble that of *C. bicorne* J. Erikss. & Ryvarden, described from Danish field material, parasitic on the moss *Polytrichum attenuatum* (Brid.) (*Polytrichastrum formosum* (Hedw.) G. L. Sm.) (Eriksson & Ryvarden, 1973). The basidia of *C. bicorne* are obovate to subglobose (15–20 × 8–10 μm) with two (in one case three) large and stout sterigmata (length, 12–18 μm; basal width, 3 μm). Basidiospores were narrowly ovoid to narrowly ellipsoid or subcylindrical (13–16 × 6–8 μm). Since *C. bicorne* is known only from the type location, the basidial dimensions given above are not necessarily completely representative in terms of the intraspecific variation of this species, but allowing for this variation in the uninucleate isolates they could well be *C. bicorne* (L. Ryvarden, pers. comm.). For confirmation it would be necessary to isolate this fungus and obtain information on the nuclear condition, cultural characteristics and anastomosis reaction with the uninucleate isolates.

The sexuality of the described uninucleate *Rhizoctonia* is at present being further investigated. The presence of predominantly uninucleate cells in all these, and many other isolates, would suggest that they are monokaryons of heterokaryotic bi- or multinucleate fungi. However, no morphological evidence of heterokaryon formation (e.g. aerial tufted mycelium) has yet been detected between paired isolates or within and between their single spore progeny although evidence of vegetative incompatibility, as indicated by strong demarcation zones, between paired progeny and their parents, is common (unpublished data). The fact that these isolates can be frequently isolated from conifer roots only as uninucleate hyphae does not support the monokaryon hypothesis either. Therefore it is possible that the killing reaction between uninucleate *Rhizoctonia* isolates is a sign of somatic incompatibility between the vegetative stage of this fungus, analogous to the one between *R. solani* field isolates (Ogoshi, 1987; Adams, 1988). It has also been possible to induce fruiting of single basidiospore progeny using the described system, as has been achieved in a *Ceratobasidium* sp. (Parmeter, Whitney & Platt, 1967). This may suggest primary homothallism but further analysis of the genetic condition of single basidiospore derived cultures using isozyme or DNA/RFLP markers are needed (see Adams, 1988).

These uninucleate *Rhizoctonia* appear to be important pathogens in the Nordic countries, causing root die-back in nursery seedlings of a range of conifer species, and thus information on the host range, mode of infection, population genetics and sexuality of these fungi are a major priority.

In conclusion, this uninucleate fungus does fit into *Ceratobasidium* but because of the unusual nuclear condition of the field isolates we would prefer to describe it as a uninucleate *Rhizoctonia* sp. having a *Ceratobasidium* fruiting stage.

We would like to thank Professors A. Ogoshi and L. Burpee for providing us with *Ceratobasidium* tester isolates, Professor K. Venn and Dr D. Børja for their Norwegian *Rhizoctonia* sp. and together with Professor L. Ryvarden, Dr K. Korhonen and Dr G. Hall for helpful discussions. A.H. and R.S. also thank The Natural Resources Research Foundation of Finland (Suomen Luonnonvarain Tutkimussäätiö) for funding this work.

REFERENCES

Adams, G. C. (1988). *Thanatephorus cucumeris* (*Rhizoctonia solani*) a species complex of wide host range. In *Advances in Plant Pathology* (ed. D. S. Ingram & P. H. Williams), **Vol. 6**, *Genetics of Plant Pathogenic Fungi* (ed. G. S. Sidu), pp. 535–552. Academic Press: London, UK.

Adams, G. C. Jr & Butler, E. E. (1983*a*). Influence of nutrition on the formation of basidia and basidiospores in *Thanatephorus cucumeris*. *Phytopathology* **73**, 147–151.

Adams, G. C. Jr & Butler, E. E. (1983*b*). Environmental factors influencing the formation of basidia and basidiospores in *Thanatephorus cucumeris*. *Phytopathology* **73**, 152–155.

Burpee, L. L., Sanders, P. L., Cole, H. Jr & Sherwood, R. T. (1980). Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. *Mycologia* **72**, 689–701.

de Candolle, A. P. (1815). Mémoire sur les rhizoctones, nouveau genre de champignons qui attaque les racines des plantes et en particular celle de la luzerne cultivée. *Mémoires du Muséum National d'Histoire Naturelle* **2**, 209–216.

English, T. R., Ploetz, R. C. & Barnard, B. L. (1986). Seedling blight of long leaf pine by a binucleate *Rhizoctonia solani*-like fungus. *Plant Disease* **70**, 148–150.

Eriksson, J. & Ryvarden, L. (1973). The Corticiaceae of North Europe, **Vol. 2**, *Aleurodiscus–Confertobasidium*. Fungiflora: Oslo, Norway.

Hall, G. (1986). A species of *Rhizoctonia* with uninucleate hyphae isolated from roots of winter wheat. *Transactions of the British Mycological Society* **87**, 466–471.

Huang, J. W. & Kuhlman, E. G. (1990). Fungi associated with damping-off of slash pine seedlings in Georgia. *Plant Disease* **74**, 27–30.

Kangatharalingham, N. & Carson, M. L. (1988). Technique to induce sporulation in *Thanatephorus cucumeris*. *Plant Disease* **72**, 146–148.

Korhonen, K. & Hintikka, V. (1980). Simple isolation and inoculation methods for fungal cultures. *Karstenia* **20**, 19–22.

Lilja, A., Lilja, S., Poteri, M. & Ziren, L. (1992). Conifer seedling root fungi and root dieback in Finnish nurseries. *Scandinavian Journal of Forest Research* **7**, 547–556.

Matsumoto, T., Yamamoto, W. & Hirane, S. (1932). Physiology and parasitology of the fungi generally referred to as *Hypochnus* Sasakii Shirai. I. Differentiation of the strains by means of hyphal fusion and culture in differential media. *Journal of the Society of Tropical Agriculture* **4**, 370–388.

Ogoshi, A. (1975). Grouping of *Rhizoctonia solani* Kühn and their perfect stages. *Review of Plant Protection Research* **8**, 93–103.

Ogoshi, A. (1987). Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. *Annual Review of Phytopathology* **25**, 125–143.

Ogoshi, A., Oniki, M., Araki, T. & Ui, T. (1983). Studies on the anastomosis groups of binucleate *Rhizoctonia* and their perfect states. *Journal of the Faculty of Agriculture, Hokkaido University* **61**, 244–260.

Parmeter, J. R. Jr, Sherwood, R. T. & Platt, W. D. (1969). Anastomosis grouping among isolates of *Thanatephorus cucumeris*. *Phytopathology* **59**, 1270–1278.

Parmeter, J. R. Jr & Whitney, H. S. (1970). Taxonomy and nomenclature of the imperfect state. In *Rhizoctonia solani, Biology and Pathology* (ed. J. R. Parmeter Jr), pp. 7–19. University of California Press: Berkeley, U.S.A.

Parmeter, J. R. Jr, Whitney, H. S. & Platt, W. D. (1967). Affinities of some *Rhizoctonia* species that resemble mycelium of *Thanatephorus cucumeris*. *Phytopathology* **57**, 218–223.

Rayner, R. W. (1970). *A Mycological Colour Chart*. Commonwealth Mycological Institute: Kew, U.K.

Roll-Hansen, F. & Roll-Hansen, H. (1968). A species of *Rhizoctonia* DC. ex Fr. damaging spruce plants in nurseries in Southern Norway. *Meddelelser fra Det Norske Skogforsøksvesen* **82**, 421–440.

Saksena, H. K. & Vaartaja, O. (1961). Taxonomy, morphology and pathogenicity of *Rhizoctonia* species from forest nurseries. *Canadian Journal of Botany* **39**, 627–647.

(*Accepted 8 February 1994*)

Sneh, B., Burpee, L. & Ogoshi, A. (1991). *Identification of Rhizoctonia Species*. The American Phytopathological Society: St Paul, U.S.A.

Talbot, P. H. B. (1965). Studies of '*Pellicularia*' and associated genera of Hymenomycetes. *Persoonia* **3**, 371–406.

Tu, C. C., Kimbrough, James W. & Aldrich, H. C. (1977). Cytology and ultrastructure of *Thanatephorus cucumeris* and related taxa of the *Rhizoctonia* complex. *Canadian Journal of Botany* **55**, 2419–2436.

Vaartaja, O. & Cram, W. H. (1956). Damping-off pathogens of conifers and of *Caragana* in Saskatchewan. *Phytopathology* **46**, 391–397.

Venn, K., Sandvik, M. & Langerud, B. R. (1986). Nursery routines, growth media and pathogens affect growth and root dieback in Norway spruce seedlings. *Meddelelser fra Norsk Institutt for Skogforskning* **39**, 313–328.

Wilson, A. D. (1992). A versatile giemsa protocol for permanent nuclear staining of fungi. *Mycologia* **84**, 585–588.

Yokoyama, K. & Ogoshi, A. (1986). Studies on hyphal anastomosis of *Rhizoctonia solani*. IV. Observation of imperfect fusion by light and electron microscopy. *Transactions of the Mycological Society of Japan* **27**, 399–413.

Yokoyama, K., Ogoshi, A. & Ui, T. (1983). Studies on hyphal anastomosis of *Rhizoctonia solani*. I. Observation of perfect fusion with light microscopy. *Transactions of the Mycological Society of Japan* **24**, 329–340.

IV

Plant Pathology (1996) **45**, 000–000 Paper 000

Identification of a uninucleate *Rhizoctonia* sp. by pathogenicity, hyphal anastomosis and RAPD analysis

A. LILJA*, A. M. HIETALA* *and* R. KARJALAINEN†‡
**Finnish Forest Research Institute, Vantaa Research Center, P.O. Box 18, FIN-01301 Vantaa, Finland and † University of Helsinki, Department of Plant Biology, Plant Pathology Section, P.O. Box 28, FIN-00014 Helsinki, Finland*

Finnish and Norwegian uninucleate *Rhizoctonia* sp. isolates, originating from roots of nursery grown conifer seedlings suffering from root dieback and having *Ceratobasidium* perfect state, were tested for pathogenicity and genetic relatedness. All tested isolates of this pathogen considerably reduced the root system development of Scots pine and Norway spruce seedlings resulting in death or stunted growth. The uninucleate isolates anastomosed readily with each other producing a killing reaction. In a RAPD-PCR analysis, the uninucleate isolates had different banding patterns from our reference isolates, two Finnish binucleate isolates (AG-I and R. sp.) and standard tester isolates of genus *Ceratobasidium* representing anastomosis groups AG-A, AG-C, AG-E, AG-G and AG-I. UPGMA analysis clustered the uninucleate isolates together at a greater similarity than 75% while the binucleate isolates formed distinct clusters and were 10–25% similar to the uninucleate *Rhizoctonia* sp. Hyphal anastomosis and DNA data suggest that the uninucleate *Rhizoctonia* sp. is an homogeneous group and distinct from the tested binucleate *Rhizoctonias*.

INTRODUCTION

Root dieback of nursery grown conifer seedlings has caused considerable economic losses in Norway and Finland. Surveys for fungi present in diseased roots and subsequent pathogenicity trials indicate that root dieback is a complex involving both *Rhizoctonia* and *Pythium* spp. (Venn *et al.*, 1986; Lilja *et al.*, 1992). In Finland, the most pathogenic *Rhizoctonia* species is uninucleate (Lilja *et al.*, 1992; Lilja, 1994) and, on the basis of cultural morphology and hyphal anastomosis it represents the same *Rhizoctonia* sp. studied in Norway by Venn *et al.* (1986; Hietala *et al.*, 1994). Uninucleate *Rhizoctonia* sp. can be fruited under laboratory conditions and belongs to the genus *Ceratobasidium* (Hietala *et al.*, 1994). All previously known anamorphs of *Ceratobasidium* have binucleate hyphal cells. In addition, anastomosis tests indicated that this species is not related to the known anastomosis groups (AG-A–AG-S) of *Ceratobasidium* (Hietala *et al.*, 1994).

Besides the uninucleate *Rhizoctonia* sp., binucleate *Rhizoctonia* spp. have also been isolated from roots of conifer seedlings showing root dieback symptoms in Finland. The tested isolates representing binucleate *Rhizoctonia* spp. have not been shown to be pathogenic (Lilja *et al.*, 1992; Lilja, 1994; Hietala, 1995). These binucleate *Rhizoctonia* spp. show considerable morphological variation and can be divided into several anastomosis groups (AG-I, R. spp.) (Hietala, 1995). Anastomosis groups AG-A (Ogoshi *et al.*, 1983) and AG-E (= CAG-3) (English *et al.*, 1986; Huang & Kuhlman, 1990; Runion & Kelly, 1993) have been identified for isolates related to other diseases of conifer seedlings.

The classification of *Rhizoctonia* spp. is based on hyphal and cultural morphology, nuclear condition, hyphal anastomosis and morphology of teleomorphs (Sneh *et al.*, 1991). The perfect stage of *Rhizoctonia* is often difficult to obtain and the presence of bridging isolates has led to the search for biochemical tools for better taxonomic resolution (Mordue *et al.*, 1989; Vilgalys & Cubeta, 1994). *Rhizoctonia* strains have been identified by electrophoresis of soluble proteins (Reynolds *et al.*, 1983) and by isozymes (Sweetingham *et al.*, 1986; Cruickshank, 1990; Damaj *et al.*, 1993; Masuhara & Neate, 1994). DNA/DNA hybridization analysis has been

‡ To whom correspondence should be addressed.

Table 1 Identities and sources of *Rhizoctonia* spp. isolates

Country	Nursery	Location	Date	Tree species	Code of isolate
Finland	Taavetti	60°55′N, 27°33′E	17.05.91	*Picea abies*	298
Finland	Mellanå	62°13′N, 21°39′E	15.10.90	*Pinus sylvestris*	290
Finland	Mellanå	62°13′N, 21°39′E	15.05.91	*Pinus sylvestris*	297
Finland	Imari	66°29′N, 25°32′E	15.08.91	*Pinus sylvestris*	257
Finland	Jomala	60°09′N, 19°57′E	29.08.91	*Larix sibirica*	256
Finland	Ahlainen	61°39′N, 22°44′E	27.09.87	*Pinus sylvestris*	261
Finland	Lapinlahti	63°21′N, 27°24′E	23.10.87	*Pinus sylvestris*	263
Finland	Lapinlahti	63°21′N, 27°24′E	05.05.89	*Pinus sylvestris*	264
Finland	Lapinlahti	63°21′N, 27°24′E	11.10.89	*Pinus sylvestris*	260
Finland	Lapinlahti	63°21′N, 27°24′E	14.08.92	*Picea abies*	250
Finland	Metsätyllilä	61°25′N, 25°53′E	05.05.89	*Pinus sylvestris*	268
Finland	Metsätyllilä	61°25′N, 25°53′E	28.09.93	*Picea abies*	248
Finland	Metsätyllilä	61°25′N, 25°53′E	10.10.95	*Abies koreana*	245
Finland	Suonenjoki	62°39′N, 27°04′E	23.05.94	*Pinus sylvestris*	246
Finland	Syrjälä	61°53′N, 29°10′E	23.07.93	*Picea abies*	249
Finland	Ukonniemi	61°12′N, 28°15′E	05.07.91	*Pinus sylvestris*	255
Norway	Gvarv	59°23′N, 09°11′E	27.03.87	*Picea abies*	87-691/3
Norway	Kvatningen	64°29′N, 11°29′E	12.09.83	*Picea abies*	83-111/1N
Norway	Prestebakke	58°55′N, 11°40′E	25.03.85	*Picea abies*	85-387/Na

successfully used to show that multinucleate and binucleate *Rhizoctonia* spp. are unrelated (Vilgalys, 1988; Vilgalys & Cubeta, 1994). Restriction analysis of amplified PCR products (Cubeta *et al.*, 1991) has been used to study the genetic relationships among groups of binucleate *Rhizoctonia*, and their results supported anastomosis grouping.

Randomly Amplified Polymorphic DNA (RAPD) markers have been used for genetic mapping (Welsh & McClelland, 1990; Williams *et al.*, 1990), population variation studies (Hadrys *et al.*, 1992) and taxonomic problems (Demeke *et al.*, 1992; Heun *et al.*, 1994). RAPD markers have been widely applied to measure genetic variation with *Fusarium* spp. (Manulis *et al.*, 1994; Yli-Mattila *et al.*, 1996) and *R. solani* (Duncan *et al.*, 1993). The reliability and reproducibility of RAPD fingerprinting in standard reaction conditions was recently demonstrated (Tommerup *et al.*, 1995). In this study we investigated the similarity of isolates representing the recently characterized uninucleate *Rhizoctonia* sp. by pathogenicity tests, nuclear condition, hyphal anastomosis and RAPD markers.

MATERIALS AND METHODS

Rhizoctonia spp. isolates

Seventeen Finnish and Norwegian uninucleate *Rhizoctonia* isolates isolated from roots of nursery-grown conifer seedlings showing root dieback symptoms were used in this study (Table 1). Using basidial dimensions and hyphal anastomosis, six isolates (five Finnish: 250, 256, 260, 263, 264; one Norwegian: 83-111/1N) were characterized previously as an anamorph of the genus *Ceratobasidium*, not related to the known anastomosis groups AG-A–AG-S (Hietala *et al.*, 1994). In addition, nine uninucleate Finnish and two Norwegian *Rhizoctonia* sp. isolates wee examined in further studies. Two Finnish binucleate *Rhizoctonia* spp. isolates, 268 and 245, were included as reference isolates. Standard tester strains (see Sneh *et al.*, 1991) of selected anastomosis groups of genus *Ceratobasidium* (AG-A, AG-C. AG-E. AG-G and AG-I), supplied by Akira Ogoshi (Hokkaido University), were also included as reference isolates in RAPD analysis.

Pathogenicity tests

Ten-week-old seedlings

In the first experiment, 10-week-old Scots pine and Norway spruce seedlings growing in fertilized (N 15%, P 5%, K15% plus micronutrients, 1 kg/m^3), low-humified Sphagnum peat (Finn peat M6) were inoculated with each of

the 17 uninucleate isolates and with the two Finnish binucleate isolates. The experimental conditions and design were as described by Lilja (1994), with the exception that the number of replicates per treatment was six. The controls were inoculated with pure agar blocks. The seedlings were harvested after an incubation period of 8 weeks. Root systems were carefully washed under tap water and the main root length and the lengths of the three longest lateral roots and shoot and root dry weights were measured. Before the determination of the dry weight of the roots, 1-mm segments were cut from the main roots of one randomly chosen living seedling representing each treatment and from all dead seedlings. Fungal isolation was done from these segments as described before (Lilja *et al.*, 1992) to assess the presence of the inoculated fungus.

One- and 2-year-old seedlings

In the second experiment, 1- and 2-year-old Scots pine seedlings were transplanted into 0·8 L pots containing the same kind of peat as described before. Uninucleate *Rhizoctonia* isolate 264 was grown on cellophane on water agar (12 g/L, Bacto agar, Difco) for 1 week, and two 3·5 cm^2 pieces of the mycelium on cellophane were buried 9 cm deep on both sides of the seedling 2 cm from the edge of the pot. There was one 1-year-old and one 2-year-old seedling in each pot. Twenty inoculated and 20 uninoculated pots were arranged randomly in a greenhouse, where the temperature was 20°C and day length 16 h. The seedlings were watered so that the peat in the pots dried between waterings, and they were fertilized (0·1% Suprex 5: N 11%, P 4%, K 25% plus micronutrients) every second week. The dry weight of the root system and the length of the main root were measured after the test period of 7 months. The data from both experiments were analysed using ANOVA and the significance of the mean differences in the first pathogenicity test was determined with Duncan's multiple range test using BMDP statistical software (Anonymous, 1990).

Nuclear condition and hyphal anastomosis

The nuclear condition of all Finnish and Norwegian isolates was examined after growing the isolates on microscope slides coated with low strength (1/8) potato dextrose agar (4·88 g/L PDA, Difco and 13·12 g/L Bacto agar) that were maintained in a most atmosphere at 24°C for 48 h (Hietala *et al.*, 1994). The nuclei were stained with HCl–Giemsa following the fixing and staining procedures described by Wilson (1992), and nuclear numbers in tip and subapical cell pairs (100 cells each) were counted at 400× magnification.

Hyphal anastomosis was examined in Petri dishes containing water agar (15 g/L Bacto agar) amended with malt extract (1 g/L). Uninucleate *Rhizoctonia* isolates were paired against each other in all combinations at a distance of 2 cm by inoculating the agar surface using a modified Pasteur pipette (Korhonen & Hintikka, 1980). The two Finnish binucleate isolates were similarly paired against each other and the included tester isolates of *Ceratobasidium*. Pairings were incubated at 21°C until the margins of opposed colonies overlapped. Hyphal anastomosis was scanned at 110× and confirmed at 400× magnification using light microscopy.

RAPD analysis

DNA isolation

Isolates were cultured for 5–7 days at 21°C on PDA (39 g/L) with a sterile cellophane membrane on the surface. The mycelium was scraped off and ground to fine powder under liquid N_2 with a mortar and pestle. DNA was purified essentially as described by Lee & Taylor (1990), but including additional phenol extraction and RNAse treatments (Karjalainen & Kammiovirta, 1994). The genomic DNA pellet was dissolved in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8·0), and stored at –20°C until used.

DNA amplification

Amplification conditions were modified from those of Williams *et al.* (1990) in the following way: 50 μL reaction mixtures were used, including 25 ng of genomic DNA, 200 μM of each dNTP, 200 nM of primer, and 5 μL 10 × polymerase buffer (0·1 M Tris–HCl, pH 8·3, 1 mg/ml BSA, 0·5 M KCl, 10–50 mM $MgCl_2$) to give a final Mg concentration in the PCR mixture ranging from 1·0 to 5·0 mM according to suggestions by D. Howland, G. Arnan and R. Oliver from the University of East Anglia, UK, and 1 U of Dyna-Zyme polymerase (Finnzymes Co, Finland). Premixtures (without DNA) of each reaction were made to minimize the risk of contamination. The reaction was overlaid with sterile paraffin oil (two drops).

Table 2 The effects of inoculation with different *Rhizoctonia* isolates on the root growth of Scots pine seedlings after 2 months' incubation

Code of isolate	Nuclear condition	The percentage of dead seedlings	Main root length (cm) mean±SE	Lateral root length (cm) mean±SE	Dry weight of roots (mg) mean±SE	Dry weight of shoots (mg) mean±SE
Control		0	60·8 ± 5·6	32·0 ± 2·3	278·0 ± 32·7	559·3 ± 61·9
245	Binucleate	5·6 ± 5·6	49·7 ± 4·2	21·5 ± 1·3[a]	234·5 ± 25·9	590·0 ± 54·5
268	Binucleate	16·7 ± 11·4	43·8 ± 4·8[a]	18·5 ± 3·1[a]	205·2 ± 55·3	583·5 ± 122·1
Mean±SE		11·1 ± 6·3	46·7 ± 3·2	20·0 ± 1·7	219·8 ± 29·4	586·7 ± 63·7
246	Uninucleate	11·0 ± 7·0	15·7 ± 2·3	5·5 ± 0·4[a]	44·0 ± 8·5[a]	250·0 ± 41·6[a]
248	Uninucleate	11·1 ± 11·1	29·5 ± 4·2[a]	13·7 ± 1·9[a]	118·8 ± 25·9[a]	463·8 ± 50·4
249	Uninucleate	5·6 ± 5·6	35·2 ± 1·8[a]	14·0 ± 1·9[a]	123·5 ± 18·9[a]	400·8 ± 56·4
250	Uninucleate	0	38·2 ± 4·2[a]	18·0 ± 2·4[a]	170·8 ± 22·0[a]	572·3 ± 54·2
255	Uninucleate	11·1 ± 7·0	32·5 ± 4·0[a]	13·0 ± 1·8[a]	116·5 ± 23·1[a]	433·2 ± 37·2
256	Uninucleate	5.5 ± 5·6	31·7 ± 3·4[a]	12·2 ± 1·3[a]	125·0 ± 17·8[a]	434·7 ± 52·6
257	Uninucleate	0	24·2 ± 2·5[a]	10·3 ± 0·9[a]	90·7 ± 9.6[a]	412·5 ± 49·8
260	Uninucleate	5·6 ± 5·6	26·2 ± 4·3[a]	10·5 ± 2·2[a]	84·8 ± 16·6[a]	366·0 ± 56·9
261	Uninucleate	0	28·5 ± 8·3[a]	9·7 ± 2·2[a]	102·5 ± 31·6[a]	384·0 ± 68·7
263	Uninucleate	0	32·2 ± 2·5[a]	11·2 ± 1·0[a]	105·2 ± 11·7[a]	435·0 ± 30·0
264	Uninucleate	5·6 ± 5·6	30·7 ± 4·5[a]	12·8 ± 1·9[a]	125·2 ± 36·5[a]	372·2 ± 73·5
290	Uninucleate	11·1 ± 7·0	22·7 ± 3·3[a]	10·0 ± 1·1[a]	97·5 ± 16·9[a]	487·2 ± 53·7
297	Uninucleate	16·6 ± 7.0	22·5 ± 1·9[a]	10·8 ± 1·1[a]	98·7 ± 17·7[a]	344·0 ± 18·3[a]
298	Uninucleate	11·1 ± 7.0	27·8 ± 3·5[a]	10·5 ± 1·5[a]	98·8 ± 27·7[a]	430·0 ± 103·5
83-111/1N	Uninucleate	0	37·2 ± 5·6[a]	11·7 ± 1·6[a]	136·3 ± 19·3[a]	464·8 ± 43·1
85-387/Na	Uninucleate	16·6 ± 7·4	34·8 ± 3·5[a]	12·8 ± 1·3[a]	126·3 ± 10·8[a]	502·7 ± 20·4
87-691/3	Uninucleate	0	48·0 ± 4·4[a]	19·0 ± 1·8[a]	227·5 ± 22·2	611·5 ± 32·6
Mean±SE		6·5 ± 1·4	30·4 ± 1·1	12·1 ± 0·5	117·2 ± 5·9	433·2 ± 14·4
F			6·40	10·64	5·50	2·52
P			0·001	0·001	0·001	0·002

[a] Means differ significantly ($P = 0·05$) from control using Duncan's multiple range test.

Amplification was performed in an MJ Research Programmable Thermal Controller programmed for 40 cycles of 1 min at 94°C, min at 35°C and 2 min at 72°C. Amplification products were electrophoresed in 1·4% agarose gels with 1 × TBE (0·089 M Tris–borate, 0·002 M EDTA) and stained with ethidium bromide. Amplifications were repeated twice for each isolate and only reproducible bands were scored.

The nucleotide sequences of primers were generated randomly using the Applied Biosystems DNA synthesizer (Model 381A) at the Biotechnology Institute of the University of Helsinki. Seven primers were tested, and the three best ones (91298: GGA CGA TTC G; 91299: CGA TTC GGC G; 91300: CGA GGT TCG C) were selected for further study. Primers 91299 and 91300 have been used in previous studies for identifying strains of *Heterobasidion annosum* (Fr.) Bref. (Fabritius & Karjalainen, 1993; Karjalainen & Kammiovirta, 1994) and *Fusarium avenaceum* (Fr.) Sacc (Yli-Mattila *et al.*, 1996). The statistical analysis of RAPD data, similarity coefficients (DICE) and clustering analysis was based on the NTSYS program (Rohlf, 1989).

RESULTS

Pathogenicity

In the first pathogenicity experiment 0–16·6% and 5·6–16·7% of Scots pine seedlings and 0–22·2% and 0–5·6% of Norway spruce seedlings were killed, 2 months after inoculation with uninucleate and binucleate *Rhizoctonia*, respectively (Tables 2 and 3). All control seedlings were alive (Tables 2 and 3). In general, both uni- and binucleate *Rhizoctonias* decreased the growth of Scots pine and Norway spruce seedlings, but the

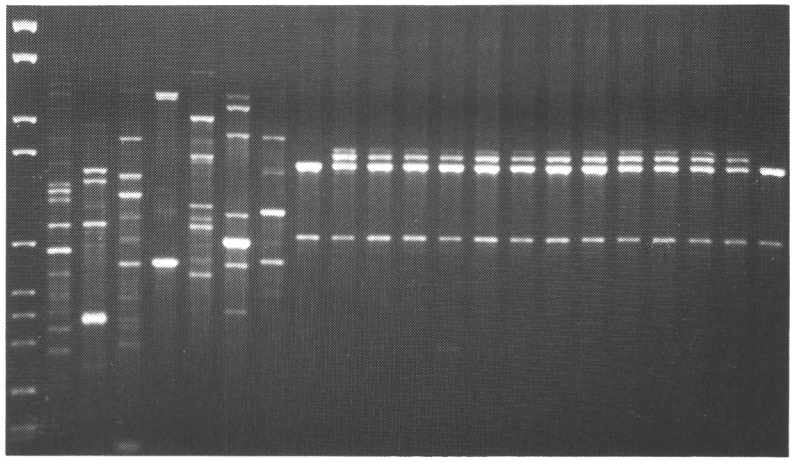
Table 3 The effects of inoculation with different *Rhizoctonia* isolates on the root growth of Norway spruce seedlings after 2 months' incubation

Code of isolate	Nuclear condition	The percentage of dead seedlings	Main root length (cm) mean±SE	Lateral root length (cm) mean±SE	Dry weight of roots (mg) mean±SE	Dry weight of shoots (mg) mean±SE
Control		0	21·2 ± 1·4	12·5 ± 0·9	34·7 ± 3·4	78·0 ± 6·2
245	Binucleate	5·6 ± 5·6	14.3 ± 1·1[a]	8·2 ± 0·7[a]	22·0 ± 1·3[a]	54·7 ± 4·3[a]
268	Binucleate	0	17·8 ± 1·2	9·3 ± 1·2[a]	20·5 ± 2·9[a]	53·3 ± 5·9[a]
Mean±SE		2·8 ± 2·8	16·1 ± 0·9	8·7 ± 0·7	21·2 ± 1·5	54·0 ± 3·5
246	Uninucleate	0	12·8 ± 1·0[a]	4·0 ± 0·4[a]	19·5 ± 2·1[a]	48·0 ± 4·9[a]
248	Uninucleate	0	14·6 ± 1·7[a]	5·2 ± 0·4[a]	20·2 ± 4·4[a]	45·3 ± 8·3[a]
249	Uninucleate	0	14·8 ± 1·1[a]	6·0 ± 0·5[a]	22·3 ± 3·3[a]	59·3 ± 7·3
250	Uninucleate	11·1 ± 7·0	15·8 ± 1·5[a]	6·7 ± 0·6[a]	21·7 ± 3·2[a]	48·7 ± 7·1[a]
255	Uninucleate	5·6 ± 5·6	13·1 ± 1·8[a]	6·0 ± 0·8[a]	16·5 ± 3·4[a]	44·3 ± 6·9[a]
256	Uninucleate	22·2 ± 7·0	12·8 ± 1·8[a]	3·8 ± 0·5[a]	14·0 ± 3·5[a]	41·7 ± 10·6[a]
257	Uninucleate	0	13·7 ± 1·4[a]	4·1 ± 0·6[a]	15·5 ± 3·0[a]	40·3 ± 6·4
260	Uninucleate	11·1 ± 7·0	13·0 ± 0·7[a]	4·5 ± 0·6[a]	15·8 ± 3·9[a]	41·3 ± 10·9[a]
261	Uninucleate	5·6 ± 5·6	13·5 ± 1·5[a]	5·3 ± 0·8[a]	22·5 ± 3·1[a]	55·5 ± 4·9
263	Uninucleate	11·1 ± 7·0	18·3 ± 2·7	5·5 ± 0·9[a]	19·0 ± 3·1[a]	41·8 ± 5·3[a]
264	Uninucleate	5·6 ± 5·6	15·8 ± 2·2[a]	7·2 ± 1·3[a]	25·0 ± 2·9[a]	43·5 ± 7·3[a]
290	Uninucleate	5·6 ± 5·6	13·0 ± 1·7[a]	4·0 ± 0·9[a]	17·3 ± 3·8[a]	50·7 ± 7·8[a]
297	Uninucleate	0	11·8 ± 1·0[a]	3·7 ± 0·3[a]	13·7 ± 1·5[a]	38·3 ± 1·9[a]
298	Uninucleate	5·6 ± 5·6	15·3 ± 2·1[a]	5·2 ± 0·8[a]	17·0 ± 2·0[a]	37·6 ± 5·5[a]
83-111/1N	Uninucleate	11·1 ± 7·0	17·0 ± 1·4	5·2 ± 1·0[a]	17·1 ± 2·9[a]	45·7 ± 5·3[a]
85-387/Na	Uninucleate	5·6 ± 5·6	15·5 ± 0·9[a]	5·1 ± 0·4[a]	18·0 ± 1·5[a]	49·8 ± 3·7[a]
87-691/3	Uninucleate	5·6 ± 5·6	16·6 ± 1·0	7·0 ± 1·1[a]	23·1 ± 3·6[a]	54·1 ± 10·3[a]
Mean±SE		5·2 ± 1·4	14·6 ± 0·4	5·2 ± 0·2	18·7 ± 0·8	46·2 ± 1·7
F			2·18	7·34	2·37	1·79
P			0·006	0·001	0·003	0·03

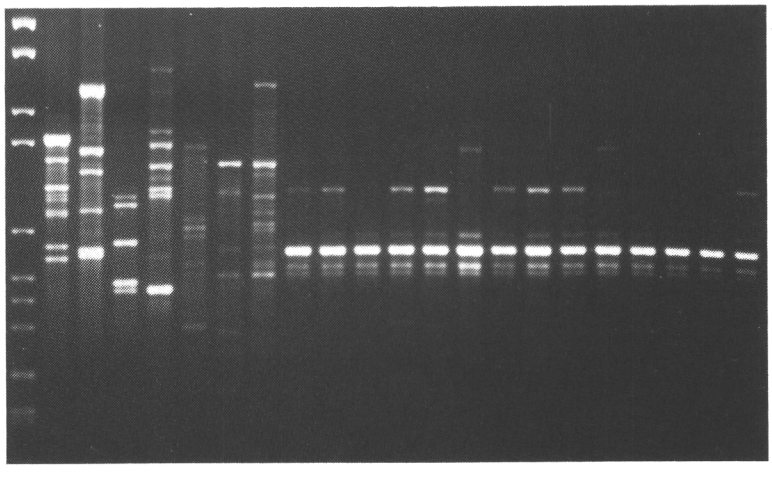
[a] Means differ significantly ($P = 0·05$) from control using Duncan's multiple range test.

uninucleate isolates were more virulent based on all measured seedling parameters. The effect of *Rhizoctonia* inoculation was most clearly shown in the root parameters, particularly in the lateral root length. All *Rhizoctonia* isolates, despite the nuclear number or isolate, significantly reduced ($P < 0·05$) the lateral root length of both tree species (Table 2 and 3). The main root length and the dry weight of whole root system of both tree species were also decreased, but the difference was not statistically significant in all cases (Table 2 and 3). The shoot growth of inoculated Norway spruce seedlings was reduced compared to controls; shoot dry weights were significantly lower ($P < 0·05$) in all treatments excluding uninucleate isolate 249 (Table 3), while on Scots pine only two uninucleate *Rhizoctonia* isolates, 246 and 297, significantly decreased the shoot dry weights ($P < 0.05$). Re-isolation of *Rhizoctonia* from inoculated seedlings was successful in most cases.

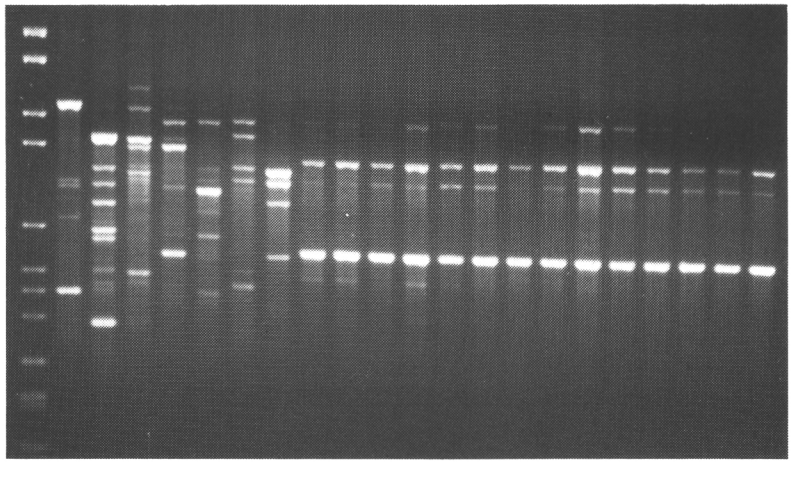
In the second pathogenicity test, all 1-year-old and 2-year-old seedlings inoculated with uninucleate isolate 264 were alive after the 7-month test period. Inoculation significantly decreased the main root length and root dry weight of both 1-year-old and 2-year-old seedlings. The main root length of 1-year-old seedlings was 16·41 ± 0·75 cm, while the control value was 44·10 ± 7·09 cm ($F = 18·13$, $P = 0·0004$). The root dry weight of the same seedlings was 186 ± 24 mg for treatment and 289 ± 55 mg for control ($F = 3·20$, $P = 0·08$). The root parameters for inoculated 2-year-old seedlings were 19·90 ± 2·32 cm and 326 ± 44 mg while the control values were 56·77 ± 8·29 cm and 478 ± 39 mg. The decreases both in the main root length ($F = 20·10$, $P = 0·0003$) and root dry



A



B



C

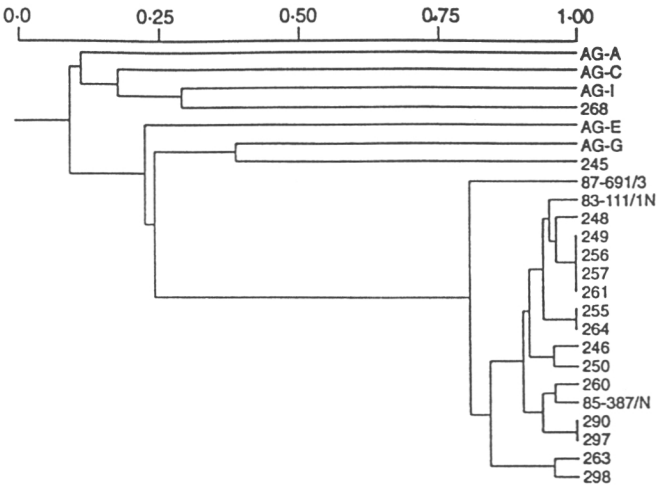


Fig. 2 UPGMA dendrogram of relationships among *Rhizoctonia* isolates based on similarity (DICE) coeffecients using SAHN computer program (NTSYS-pc, Rohlf, 1989). Uninucleate isolates form a separate group from the other tester strain.

weight ($F = 6{\cdot}32$, $P = 0{\cdot}02$) were statistically significant.

Nuclear condition and hyphal anastomosis

Isolates identified previously as uninucleate *Rhizoctonia* sp. possessed predominantly uninucleate hyphal tips. All the counted tip/subapical cell pairs of isolates 246 and 250 were uninucleate. Fourteen isolates had 94–99% of uninucleate tip/subapical cell pairs. Isolate 87-691/3 had the lowest number of uninucleate tip/subapical cell pairs, 87%. The total number of observed cell pairs for these 17 isolates was 1700; of this amount, 56 cell pairs were uninucleate. The types and frequencies of the aberrant nuclear condition in tip/subapical cell pairs were uninucleate/binucleate (8), binucleate/uninucleate (11) and binucleate/binucleate (37). The reference isolate 268 had 98% binucleate/binucleate and 2% trinucleate/binucleate tip/subapical cell pairs. The other reference, isolate 245, had 96% binucleate/binucleate, 2% trinucleate/binucleate, 1% trinucleate/trinucleate and 1% tetranucleate/binucleate tip/subapical cell pairs.

All uninucleate *Rhizoctonia* isolates anastomosed readily with each other, several anastomosis points were commonly detected in a single microscopic field. In self pairings, anastomosed cells fused together forming living bridges between the opposed colonies. In non-self pairings, hyphal anastomosis resulted in a killing reaction: after the cell wall fusion, between one and three cells died on either anastomosing hypha. In non-self pairings, living bridges between the opposed colonies were observed only when isolates 260, 263 and 264 were paired against each other, but even in these three pairing combinations the killing reaction was observed frequently. The binucleate isolate 268 anastomosed with the tester isolate of AG-I producing a killing reaction, but the anastomosis frequency

Fig 1 Analysis of uninucleate *Rhizoctonia* isolates by RAPD-PCR method. A, B and C: lane 1 = molecular weight markers (MW, Boehringer IV) are 2176, 1766, 1230, 1033, 653, 517, 473, 394, 298, 234, 220 and 154 bp. (A) Primer 91298, lanes 2–8, binucleate reference isolates = AG-A, AG-C, AG-E, AG-G, AG-I, 245, 268; lanes 9–22, uninucleate isolates 87-691/3, 83-111/1N, 246, 248, 249, 250, 255, 256, 257, 260, 261, 263, 264 and 85-387/Na. (B and C) Primers 91299 and 91300; lanes 2–8, binucleate reference isolates = AG-A, AG-C, AG-I, 268, AG-E, AG-G, 245; lanes 9–22, uninucleate isolates = 87-691/3, 83-111/1N, 246, 248, 249, 250, 255, 256, 257, 260, 261, 263, 264 and 85-387/Na.

was lower than that observed among uninucleate isolates. The other binucleate isolate, 245, did not anastomose with isolate 268 nor with the tester isolates of *Ceratobasidium*.

RAPD analysis

RAPD-PCR analysis using three primers indicated that all uninucleate isolates had different RAPD-DNA profiles compared with the Finnish binucleate isolates and tester isolates (Fig. 1A–C). Amplification of DNAs by the primers 298 and 300 revealed three common bands for almost all uninucleate isolates (size of the fragments, 298: 650–1100 bax pairs (bp), 300: 500–950 bp). One dominant band (*c.* 650 bp) was typical of all uninucleate isolates produced by the primer 299. Generally, uninucleate isolates showed relatively homogenous DNA-profiles compared with the high variability found in Finnish binucleate and *Ceratobasidium* anastomosis tester isolates (Fig. 1A–C).

The uninucleate *Rhizoctonia* sp. showed high similarity within the group, over 75–80% similarity coefficients in a dendrogram analysis (Fig. 2). UPGMA clustering (Fig. 2) indicates that uninucleate *Rhizoctonia* isolates are different at the DNA level from Finnish binucleate isolates and *Ceratobasidium* anastomosis testers which were only about 10–25% similar to the uninucleate isolates. The similarity of the Japanese tester strain of AG-I and the Finnish isolate 268 anastomosing with this group was about 28%.

DISCUSSION

The data suggest that uninucleate *Rhizoctonia* sp., causing root stunting of Scots pine and Norway spruce seedlings, forms a genetically homogenous group that is distinct from the binucleate Finnish *Rhizoctonia* isolates, and *Ceratobasidium* anastomosis tester isolates employed in this study.

All tested *Rhizoctonia* isolates decreased the lateral root length of both Scots pine and Norway spruce. The pathogenicity of uninucleate *Rhizoctonia* sp. has been shown in previous studies, but all Finnish binucleate isolates tested before have been non-pathogenic (Lilja *et al.*, 1992; Lilja, 1994; Hietala, 1995). However, the two binucleate isolates tested here decreased the length of laterals. The most comprehensive studies related to *Rhizoctonia* associated to root diseases of conifer seedlings were carried out by Saksena & Vaartaja (1960, 1961). Later studies have shown that the species studied by them were multi- or binucleate *Rhizoctonia* spp. (see e.g. Sneh *et al.*, 1991); one binucleate species causing stunted root growth of pine seedlings, *R. endophytica* var. *endophytica* Saks. & Vaar., was confirmed to belong to the AG-A of genus *Ceratobasidium* (Ogoshi *et al.*, 1983). The phenomenon that older seedlings infected with uninucleate *Rhizoctonia* sp. can survive but become stunted because of decreased root mass (Lilja, 1994; Hietala, 1995) was obvious also in this study especially with Norway spruce seedlings (Table 3). The root systems of Scots pine seedlings infected at the age of 1 or 2 years were also decreased after the 7-month incubation period.

Anastomosis tests indicated that there is a single anastomosis group within the uninucleate *Rhizoctonia* sp. This is in agreement with earlier observations that culturally the species is extremely homogeneous (Hietala *et al.*, 1994; Hietala, 1995). In *R. solani*, the killing reaction is regarded as a somatic incompatibility reaction (see e.g. Sneh *et al.*, 1991); based on this interpretation, all the uninucleate isolates represent different genotypes. In addition to the killing reaction, a low frequency of perfect fusions was observed in the pairing combinations between isolates 260, 263 and 264. At present, the genetic background of the killing reaction in this species is not known but possibly these three isolates, originating from the same nursery, are more closely related than the other isolates.

Nuclear staining confirmed that the fungus is predominantly uninucleate. As previously shown (Hietala *et al.*, 1994), binucleate cells can co-exist with prevailing uninucleate cells in the same mycelium. The high frequency of binucleate/binucleate cell pairs in the isolate 87-691/3, as observed by Hietala *et al.* (1994) for the isolate 260 (11/11%), gives further evidence that these binucleate cells do not represent random mitotic events prior to cell division. However, the explanation behind this phenomenon remains unknown.

Results from the anastomosis test and RAPD analysis are in agreement: uninucleate isolates had rather uniform RAPD-PCR profiles in all tested primers. In contrast, the banding patterns of binucleate isolates were very heterogeneous with all primers. The low degree of genetic similarity between uni- and binucleate isolates was at a similar level that has often been found between different species of several other fungi

(Smith & Andersen, 1989; Taylor & Natwig, 1989; Maclean *et al.*, 1993).

Geographic isolation may contribute to divergency and subsequent dissimilarity. Low similarities were observed in a RAPD analysis by Duncan *et al.*, (1993) for some *R. solani* isolates obtained from various geographic locations and representing the same AGs; correspondingly, there was a relatively low similarity between the Finnish AG-I isolate and the Japanese tester strain.

The high homogeneity of the uninucleate *Rhizoctonia* sp. may reflect that the isolates have not been geographically separated for a time long enough to lead to significant divergence. Alternatively high genetic homogeneity within the uninucleate *Rhizoctonia* sp. may be an implication of a narrow source population. A small, homogenic source population may be a consequence of seedling exchange between nurseries. Spruce seedlings have also been imported from Norway, where pathogenic uninucleate *Rhizoctonia* sp. was first reported (Venn *et al.*, 1986). The influence of pathogen migration on reducing genetic variability has been previously found in a *Phytophthora infestans* (Mont.) de Bary population (Goodwin *et al.*, 1994) where contaminated seed tubers were an important source of pathogen spread.

REFERENCES

Anonymous, 1990. *BMDP Statistical Software*. Berkeley: University of California Press.

Cruickshank RH, 1990. Pectic zymograms as criteria in taxonomy of *Rhizoctonia*. *Mycological Research* **94**, 938–46.

Cubeta MA, Echandi E, Abernethy T, Vilgalys R, 1991. Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology* **81**, 1395–400.

Damaj M, Jabaji-Hare SH, Charest P-M, 1993. Isozyme variation and genetic relatedness in binucleate *Rhizoctonia* species. *Phytopathology* **83**, 864–71.

Demeke T, Adams RP, Chibbar R, 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in *Brassica*. *Theoretical and Applied Genetics* **84**, 990–4.

Duncan S, Barton JE, O'Brien PA, 1993. Analysis of variation in isolates of *Rhizoctonia solani* by random amplified polymorphic DNA assay. *Mycological Research* **97**, 1075–82.

English JT, Ploetz RC, Barnard EL, 1986. Seedling blight of longleaf pine caused by binucleate *Rhizoctonia solani*-like fungus. *Plant Disease* **70**, 148–50.

Fabritius, AM, Karjalainen R, 1993. Variation in *Heterobasidion annosum* detected by Random Amplified Polymorhic DNAs. *European Journal of Forest Pathology* **23**, 193–200.

Goodwin SB, Cohen BA, Fry WE, 1994. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 11591–5.

Hadrys H, Balick M, Schierwater B, 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology* **1**, 55–63.

Heun M, Murphy JP, Phillips TD, 1994. A comparison of RAPD and isozyme analyses for determining the genetic relationships among *Avena sterilis* L. accessions. *Theoretical and Applied Genetics* **87**, 689–96.

Hietala AM, 1995. Uni- and binucleate *Rhizoctonia* spp. co-existing on the roots of Norway-spruce seedlings suffering from root dieback. *European Journal of Forest Pathology* **25**, 136–44.

Hietala AM, Sen R, Lilja A, 1994. Anamorhic and teleomorphic characteristics of a uninucleate *Rhizoctonia* sp. isolated from the roots of nursery grown conifer seedlings. *Mycological Research* **98**, 1044–50.

Huang JW, Kuhlman EG, 1990. Fungi associated with damping-off slash pine seedlings in Georgia. *Plant Disease* **74**, 27–30.

Karjalainen R, Kammiovirta K, 1994. Identification of *Heterobasidion annosum* (Fr.) Bref., a root and butt rot disease of forest trees by PCR-fingerprinting. In: Schots A, Dewey FM, Oliver R, eds. *Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification*. Cambridge: University Press, 111–6.

Korhonen K, Hintikka V, 1980. Simple isolation and inoculation methods for fungal cultures. *Karstenia* **20**, 19–22.

Lee SB, Taylor JW, 1990. Isolation of DNA from fungal mycelia and single spores. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR Protocols: A Guide to Methods and Applications*. San Diego: Academic Press, 282–322.

Lilja A, 1994. The occurrence and pathogenicity of uni- and binucleate *Rhizoctonia* and *Pythiaceae* fungi among conifer seedlings in Finnish forest nurseries. *European Journal of Forest Pathology* **24**, 181–92.

Lilja A, Lilja S, Poteri M, Ziren L, 1992. Conifer seedling root fungi and root dieback in Finnish nurseries. *Scandinavian Journal of Forest Research* **7**, 547–56.

Maclean DJ, Braithwaite KS, Manners JM, Irwin JAG, 1993. How do we identify and classify fungal plant pathogens in the era of DNA analysis? In: Andrews JH, Tommerup IC, eds. *Advances in Plant Pathology*, Vol. 10. London: Academic Press, 207–44.

Manulis S, Kogan N, Reuven M, Ben-Yephet Y, 1994. Use of the RAPD technique for identification of *Fusarium oxysporum* f. sp. *dianthi* from carnation. *Phytopathology* **84**, 98–101.

Masuhara G, Neate SM, 1994. Characteristics of some *Rhizoctonia* spp. from South Australian plant nurseries. *Mycological Research* **98**, 83–7.

Mordue JEM, Currah RS, Bridge PD, 1989. An integrated approach to *Rhizoctonia* taxonomy: cultural, biochemical and numerical techniques. *Mycological Research* **92**, 78–90.

Ogoshi A, Oniki M, Araki T, Ui T, 1983. Studies on the anastomosis groups of binucleate *Rhizoctonia* and their perfect states. *Journal of the Faculty of Agriculture Hokkaido University* **61** 2, 244–60.

Reynolds M, Weinhold AR, Morris TJ, 1983. Comparison of anastomosis groups of *Rhizoctonia solani* by polyacrylamide gel electrophoresis of soluable proteins. *Phytopahtology* **73**, 903–6.

Rohlf FJ, 1989. *NTSYS-pc. Numerical taxonomy and Multivariate Analysis System*. Setauket, New York: Exeter Publishing, Ltd.

Runion GB, Kelly WD, 1993. Characterization of a binucleate *Rhizoctonia* species causing seedling blight of loblolly pine. *Plant Disease* **77**, 754–5.

Saksena HK, Vaartaja O, 1960. Description of new species of *Rhizoctonia*. *Canadian Journal of Botany* **38**, 931–43.

Saksena HK, Vaartaja O, 1961. Taxonomy, morphology and pathogenicity of *Rhizoctonia* species from forest nurseries. *Canadian Journal of Botany* **39**, 627–47.

Smith ML, Anderson JB, 1989. Restriction fragment length polymorphism in mitochondrial DNAs of *Armillaria*: identification of North American biological species. *Mycological Research* **93**, 247–56.

Sneh B, Burpee L, Ogoshi A, 1991. *Identification of* Rhizoctonia *species*. St. Paul: APS Press.

Sweetingham MW, Cruickshank RH, Wong DH, 1986. Pectic zymograms and the taxonomy and pathogenicity of the *Ceratobasidiaceae*. *Transactions of the British Mycological Society* **86**, 305–11.

Taylor JW, Natwig DO, 1989. Mitochondrial DNA and evolution of heterothallic and pseudohomothallic *Neurospora* species. *Mycological Research* **93**, 257–72.

Tommerup IC, Barton JE, O'Brien PA, 1995. Reliability of RAPD fingerprinting of three basidiomycete fungi, *Laccaria*, *Hydnangium* and *Rhizoctonia*. *Mycological Research* **99**, 179–86.

Venn K, Sandvik M, Langerud B, 1986. Nursery routines, growth media and pathogens affect growth and root dieback in Norway spruce seedlings. *Meddelerser fra Norsk Institutt for Skogforskning* **39**, 314–28.

Vilgalys R, 1988. Genetic relatedness among anastomosis groups in *Rhizoctonia* as measured by DNA/DNA hybridization. *Phytopathology* **78**, 698–702.

Vilgalys R, Cubeta MA, 1994. Molecular systematics and population biology of *Rhizoctonia*. *Annual Review of Phytopathology* **32**, 135–55.

Welsh J, McClelland M, 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nuclear Acids Research* **18**, 7213–8.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nuclear Acids Research* **18**, 6531–5.

Wilson AD, 1992. A versatile giemsa protocol for permanent nuclear staining of fungi. *Mycologia* **84**, 585–8.

Yli-Mattila T, Paavanen S, Hannukkala A, Parikka P, Tahvonen R, Karjalainen R, 1996. Isozyme and RAPD-PCR analyses of *Fusarium avenaceum* strains from Finland. *Plant Pathology* **45**, 126–34.

V

Eur. J. For. Path. 26 (1996) 89–96

ISSN 0300–1237

[1]*Finnish Forest Research Institute, Vantaa Research Center, Vantaa; and* [2]*Finnish Forest Research Institute, Suonenjoki Research Station, Suonenjoki, Finland*

Stem lesions on *Betula pendula* seedlings in Finnish forest nurseries and the pathogenicity of *Phytophthora cactorum*

By A. LILJA[1], R. RIKALA[2], A. HIETALA[1] and R. HEINONEN[1]

Summary

Phytophthora cactorum was isolated from necrotic stem lesions on 20–80% of diseased *Betula pendula* seedlings sampled from three nurseries in Finland. *Fusarium avenaceum* and *Godronia* sp. were also found in all nurseries. Inoculation of *P. cactorum* on *B. pendula* seedlings caused necrotic lesions and the spread of these lesions around the stem was detrimental. Neither the timing of N application nor two different PK-levels applied to the seedlings the previous year significantly affected the size of lesions caused by *P. cactorum.*

1 Introduction

In Finland, the annual production of birch seedlings increased from 3 million to 28 million seedlings in 1980–93 and currently makes up 15% of the total seedling production (ANONYMOUS 1981, 1994). During this increased production, various disease problems appeared, of which stem lesions and cankers were among the most serious. Several fungi are known to cause these lesions. The most extensively studied of these is *Godronia multispora* J. W. Groves (PETÄISTÖ 1983; RIKALA and PETÄISTÖ 1986). Other fungi isolated from birch-stem lesions include *Fusarium avenaceum* (Fr.) Sacc., *Alternaria alternata* (Fr.) Keissl. and *Botrytis cinerea* Pers. ex Nocca & Balb. (PETÄISTÖ 1983; LILJA and HIETALA 1994).

In 1991, *Phytophthora cactorum* (Leb. and Cohn) Schr. was isolated for the first time from necrotic stem lesions of silver-birch (*Betula pendula* Roth) seedlings in Finland (LILJA and HIETALA 1994). Earlier, *P. cactorum* had been isolated from sweet birch (*B. lenta* L.) suffering from a bleeding cancer (ANONYMOUS 1941), and it has been shown to be the cause of seedling blight in beech (*Fagus sylvatica* L.) in the United Kingdom (STROUTS 1981), as well as cankers on the trunk of many tree species, e.g. apple (HARRIS 1991), *Eucalyptus* spp. (WARDLAW and PALZER 1985) and horse chestnut (*Aesculus hippocastanum* L.) (WERRES et al. 1995).

The nutrition of plants affects, in addition to growth and hardening, the susceptibility of plants to diseases (HUBER 1980). Deficiency of nutrient elements needed to synthesise chemical and physical barriers can result in decreased resistance (SCHMITTHENNER and CANADAY 1983; DESPREZ-LOUSTAU and DESSUREAULT 1988). The different nitrogen fertilization and phosphorus-potassium rich fertilizers used in Finnish nurseries has been suggested to affect the susceptibility of birch seedlings to stem lesions (RIKALA and PETÄISTÖ 1986), frost (JOZEFEK 1989) and damages caused by voles (HENTTONEN 1993).

The objectives of this study were to test the pathogenicity of *P. cactorum* to silver-birch seedlings and to assess its frequency and that of other microbes in stem lesions on samples of seedlings. The effect of the previous year's fertilization on incidence of *P. cactorum* infection was also studied.

2 Materials and methods

2.1 Sampling and fungal isolations

The material for this study was collected from three nurseries where diseased *B. pendula* seedlings had been found during routine inventory of seedling stock. In Vierumäki nursery (61°12′ N, 28°15′ E), 9.7% of a total of 800 000 seedlings had to be rejected because of stem lesions in the spring of 1993. Mellanå nursery (62°13′ N, 21°39′ E) produced 250 000 birch seedlings in 1994; in the autumn, 1% of these seedlings showed stem lesions. Mäntyharju nursery (61°25′ N, 25°53′ E) produced 230 000 birch seedlings in 1994, and in the autumn 26% were rejected owing to stem lesions.

In Mäntyharju, all silver-birch seedlings in 40 randomly sampled container trays (PS 1008, 35 seedlings in each tray) were assessed and divided into two categories: healthy (no lesions) and diseased (lesions). The height and root-collar diameter of one healthy and one diseased seedling, which were randomly sampled within each container tray, were measured.

For fungal isolations, 50 diseased seedlings (origin, seed orchard 363) were collected in Vierumäki nursery in the autumn of 1992, 20 seedlings (origin, Lapua) were collected in Mellanå in the autumn of 1994, and 40 seedlings (origin, seed orchard 378) were collected in Mäntyharju in the autumn of 1994. Necrotic lesions on the stems were surface sterilized with 70% alcohol, and the outer layer of bark was peeled off before small pieces were cut from the marginal zone between diseased and healthy tissue. Six pieces taken from one lesion on each seedling were plated on malt-extract agar (12 g/l Difco malt extract, 12 g/l Difco agar) and on malt-extract agar (5 g/l Difco malt extract, 12 g/l Difco agar) amended with benomyl (0.02 g/l Benlate) and incubated at 20°C. After 3–5 days incubation, hyphal tips were transferred to malt agar or potato-dextrose agar (39 g/l Difco). Identification of the fungi was based on morphological criteria (DOMSCH et al. 1980; SUTTON 1980; HAMM and HANSEN 1991).

2.2 Pathogenicity tests

The pathogenicity of *P. cactorum* was tested in two inoculation experiments. The first experiment was performed outdoors with silver-birch plants from a single clone. Cloned plants were used to decrease the genetic variation of the plant material. The second experiment was carried out in a greenhouse with nursery seedlings that had been fertilized the previous year with different combinations of N, P and K.

In both experiments the young trees were artificially inoculated by removing a 16-mm^2 piece of periderm with a scalpel at a height of 7 cm above the soil. The inoculum, a 16-mm^2 agar block from a 1-week-old PDA-culture of *P. cactorum*, was placed on the wound and secured with parafilm. Pure agar blocks were used as controls. After 2 weeks incubation, the parafilm and agar block were removed.

2.2.1 Experiment with plants from a single clone

In the spring of 1992, 30 1-year-old silver-birch plants (clone 36 Ristiina), which had been tissue cultured at Punkaharju Research Station (RYYNÄNEN and RYYNÄNEN 1986), were transplanted into pots (5 l) containing fertilized, low-humified Sphagnum peat (Finn peat M6, Kekkilä Corp., Finland). The amount of fertilizer (N 15%, P 5%, K 15% plus micronutrients) in peat was 1 kg/m^3. On May 15, 1 week after transplantation, when the leaves had not yet burst, the plants were inoculated with two strains of *P. cactorum*. One strain (Ph2) had been isolated from stem lesions of silver-birch seedlings grown at Mäntyharju nursery, and the other (Ph3) was from Ukonniemi nursery (61°12′ N, 28°15′ E). The plants

were arranged outdoors in a completely randomized design. The plants were not further fertilized but they were watered every day.

The condition of the plants was monitored on 1 October 1992, 30 April 1993, and 22 June 1993. Vertical and horizontal spread and size of the necrotic lesions was measured in October and April, and the root-collar diameter were measured in April. On 22 June 1993, attempts were made to isolate *P. cactorum* from the necrotic lesions using malt-extract agar (0.5% ME) amended with benomyl.

2.2.2 *Experiment with differently fertilized seedlings*

The silver birch seedlings (origin, seed orchard 379) were produced according to standard nursery practice at Suonenjoki nursery (62°39′ N, 27°04′ E) in 1992. The seedlings were grown in plastic container trays (Plantek-25, 25 cell per tray, cell volume 380 cm^3, Lännen Tehtaat, Finland) filled with the same type of peat as in the clone experiment. Six combinations of NPK fertilization were used: two potassium-phosphorus levels (PK1, PK2) combined with three application periods of nitrogen (N1, N2, N3). N as ammonium nitrate (Kemira Corp.) and PK as Superex-7 (Kekkilä Corp.) was given as water solution once a week. The nitrogen application periods lasted 6 weeks, starting on June 9 (N1), June 30 (N2), and July 21 (N3). PK-fertilization was started on July 7 and finished on September 8. Total amounts of nutrients per seedling (including premixed fertilizers) were: N 164 mg (all treatments), P 74 mg and K 126 mg (PK1 treatment), P 129 mg and K 195 mg (PK2 treatment). Fertilizer treatments were randomized within two replicates, two trays per treatment in each replicate.

At the end of the growing season 1992, the height and root-collar diameter of 60 seedlings per treatment, randomly sampled among blocks and trays, were measured. The seedlings were dried (48 h, 65 °C) and their N concentrations were determined with a LECO CHN-600 analyser (Leco Co., USA). The P and K concentrations were determined from dry-digested 2 M HCL samples (HALONEN et al. 1983) using plasma-emission spectrophotometric analysis (ICP, ARL 3800).

On April 1 1993, 72 seedlings were taken from the cold storage (−4 °C) where they had been kept over winter, and on 8 April 1993 they were transplanted into pots (5 l) containing the same type of peat as before. Three weeks after transplantation, when growth had started, the stems were inoculated with two strains of *P. cactorum*, as described previously. One strain (Ph10) had been isolated from stem lesions of silver birch in Lapinlahti nursery (63°21′ N, 27°24′ E) and the other strain (Ph11) from Vierumäki nursery. Twelve seedlings from two nursery replicates represented each fertilization treatment, so that six seedlings were inoculated with each strain of *Phytophthora*. The seedlings were arranged in a randomized block design with three blocks in a greenhouse with a temperature range of 19–22 °C. The seedlings were not fertilized but were watered every second day. After 5 weeks incubation, the stems were cut off and both the area of stem lesions and the height of the seedlings were measured. Attempts were made to isolate the pathogen from necrotic lesions, as described above.

2.3 Statistics

All data except the results in the second inoculation experiment were analysed using ANOVA, and the significance of the mean differences was determined with Tukey's multiple-range test (ANONYMOUS 1990).

The data in the second inoculation experiment in greenhouse were analysed using the mixed-model ANOVA. The replication in the fertilization treatments was used as a first random effect and the block in inoculation experiment as a second random effect. The fixed

effects were fertilizations: N-application times and PK-levels, and *P. cactorum* strain. The first error term was all interactions between fertilizations and replication. The second error term was the error of variance (ANONYMOUS 1983).

3 Results

3.1 Inventory in Mäntyharju nursery

According to the inventory in Mäntyharju nursery, the percentage of seedlings with necrotic lesions (24, SD = 13) was very similar to that recorded by the nursery (26). In 15% of the sampled container trays all the seedlings were healthy. The average height of the diseased seedlings in Mäntyharju was 117 cm (SD = 14) and the average diameter of root collar was 9 mm (SD = 1.0). The corresponding values for the healthy seedlings were 127 cm (SD = 10) and 10 mm (SD = 1), respectively. The decrease in height was statistically significant ($p < 0.01$).

3.2 Isolated micro-organisms

Using malt agar as an isolation medium, *P. cactorum, F. avenaceum, Godronia* sp. and bacteria were isolated from stem lesions in all nurseries. The frequencies of these microbes and of *A. alternata, B. cinerea, Chaetomium* sp., *Cladosporium herbarum* (Pers.) Link ex Gray, *Mucor* sp., *Phoma* sp., *Phomopsis* sp., binucleate *Rhizoctonia* sp., *Trichothecium roseum* Link and two unidentified fungi are presented in Table 1. Benomyl in malt extract agar (0.5% ME) restricted the growth of Ascomycetes and Fungi imperfecti. In these plates, *P. cactorum* often grew as a pure culture.

Table 1. **Fungi and bacteria isolated from stem lesions in three nurseries. The numbers indicate the percentage of lesions from which each micro-organism was isolated**

	Forest nursery		
Micro-organisms	Vierumäki	Mellanå	Mäntyharju
Alternaria alternata (Fr.) Keissl.	20		15
Botrytis cinerea Pers. ex Nocca & Balb	4		10
Chaetomium sp.	20		7
Cladosporium herbarum (Pers.) Link ex Gray	4	10	
Fusarium avenaceum (Fr. Sacc.	8	20	7
Godronia sp.	2	15	5
Mucor sp.			7
Phoma sp.	10		
Phomopsis sp.		10	
Phytophthora cactorum (Leb. and Cohn) Schr.	60	20	80
Rhizoctonia sp. binucleate	2		
Trichothecium roseum Link	4		
Unidentified, gray sterile fungus			5
Unidentified, yeast-like fungus		15	
Bacteria	40	25	30
Number of seedlings	50	20	40

3.3 Inoculation experiments

3.3.1 Experiment and plants from a single clone

Within 2 weeks of inoculation, necrotic lesions were observed that were identical to those on birch seedlings grown in nurseries. No difference was found between the two fungus strains. In October, 20 weeks after inoculation, the average size of stem lesion was 78 mm^2 (SD = 18) on plants infected with *P. cactorum* strain Ph2 and the diameter of the lesion was 18 mm (SD = 4) vertically and 8 mm (SD = 1) horizontally. The corresponding values on plants infected with strain Ph3 were 74 mm^2 (SD = 16), 18 mm (SD = 4) and 7 mm (SD = 2), respectively. The wounds on the control plants healed well without any signs of disease. There were no differences between the mean root-collar diameters (7 mm) of controls and infected birches.

The size of the lesions did not increase during the winter, but by June 22 in the following summer, the necrotic lesions had spread around the stem and the shoots were dying. *P. cactorum* was isolated from the lesions. One year later, in the spring of 1994, 75% of the plants produced new shoots from the base of the stem.

3.3.2 Experiment with differently fertilized seedlings

The results of fertilization treatments in 1992 were not in all respects those expected. Different N application times or PK-levels did not affect ($p > 0.05$) the average height and root-collar diameter of seedlings. In contrast to expectations, the fertilization treatments did not affect ($p > 0.05$) foliar nitrogen and potassium contents of seedlings. The only significant difference ($p = 0.008$) was in foliar P-concentrations in two PK-treatments (PK_1: 8.3 ± 0.9 mg/g dry matter, PK_2: 10.1 ± 0.5 mg/g dry matter); (Table 2). The data from nutrient analysis were not available at the time of seedling inoculation.

In 1993, the fertilization treatments during the previous year did not affect ($p > 0.05$) growth of the second-year shoots (31 cm, SD = 5). N application time ($p = 0.38$), PK-level ($p = 0.83$) and their interaction ($p = 0.42$) had no effect on the size of lesions. The strains of *P. cactorum* did not differ from each other ($p = 0.72$). After 5 weeks incubation in all treatments infected with the strain Ph 10, the average size of the stem lesions on the seedlings was 159 mm^2 (SD = 89); the vertical diameter of the lesion was 28 mm (SD = 11) and the horizontal diameter was 11 mm (SD = 4). The corresponding values for seedlings infected

Table 2. **Average height, root-collar diameter and foliar nitrogen, phosphorus and potassium concentrations of silver-birch seedlings in six treatment combinations of fertilization at the end of the growing season. Treatment combinations: application periods (6 weeks) for nitrogen started (N1) June 9, (N2) June 30 and (N3) July 21, and phosphorous and potassium levels were P 74 mg and K 126 mg/seedling for the PK_1-level, and P 129 mg and K 195 mg/seedling for the PK_2-level. Standard deviation is indicated in parentheses**

Treatment	Height cm	Diameter mm	N	P (mg/g dry matter)	K
$N1PK_1$	89 (17)	6.9 (1.1)	27.9 (4.8)	7.8 (1.5)	24.9 (2.2)
$N1PK_2$	84 (16)	6.6 (0.9)	29.9 (1.3)	9.9 (0.2)	28.2 (2.6)
$N2PK_1$	84 (17)	6.6 (0.8)	28.4 (0.9)	8.4 (0.2)	24.6 (0.5)
$N2PK_2$	84 (16)	6.6 (0.7)	30.3 (1.1)	10.3 (0.8)	29.1 (1.5)
$N3PK_1$	82 (13)	6.4 (0.5)	32.9 (1.9)	8.8 (0.7)	25.9 (3.4)
$N3PK_2$	79 (16)	6.3 (0.6)	31.5 (2.0)	10.1 (0.4)	26 (0.1)

with Ph11 were 161 mm^2 (SD = 71), 30 mm (SD = 11) and 11 mm (SD = 2). Re-isolation of *P. cactorum* from necrotic lesions was, in most cases, successful.

4 Discussion

A number of the fungi isolated from the stem lesions are thought to be pathogenic to birch. *F. avenaceum* and *A. alternata*, for example, have been isolated from necrotic tissue around the wounds made by *Cicadella viridis* (L.) for oviposition (JUUTINEN et al. 1976) and from cankers on birch seedlings debarked by voles (HENTTONEN et al. 1994). *G. multispora* has commonly been isolated from stem lesions on silver birch in some nurseries (PETÄISTÖ 1983; RIKALA and PETÄISTÖ 1986), and also from cankers in young birch stands growing on peat and paludified mineral soils (KURKELA 1974). Because the *Godronia* sp. isolates in this experiment did not form the perfect state, it was not possible to identify them to particular species.

The high isolation frequency of *P. cactorum* from Mäntyharju and Vierumäki nurseries, and pathogenicity tests, suggests that this species is probably involved in birch-stem lesions. It was also present in Mellanå nursery, although the frequency was lower. Direct evidence for its pathogenicity was obtained from the inoculation experiments as, within 2 weeks, necrotic lesions identical to those found in the nursery were formed. After a cessation of development during the winter, further host invasion during the following summer resulted in breakage of plants. In this respect, *P. cactorum* differs markedly from *G. multispora* where most development occurs in the dormant season (KURKELA 1974).

Most investigations concerning the role of nutrition in development of *Phytophthora* diseases have dealt species other than *P. cactorum*, and have given contradictory results (SCHMITTHENNER and CANADAY 1983). The nutrient ratios (P/K and K/N) of infected seedlings were clearly higher than recommended by INGESTAD (1970). In this study, the possible unbalanced nutrient status did not explain the results. None of the fertilizer treatments applied during the year before inoculation significantly affected the development of *P. cactorum* over a 5-week period. Further work on the influence of environmental factors on the disease is merited, together with a detailed study on the infection process.

Acknowledgements

The authors thank Ritva VANHANEN and Sonja SARSILA for assistance with the experiments, as well as Dr Kari KORHONEN and Prof. Timo KURKELA for critical reading of the manuscript. The authors are also grateful to the nursery managers Tarmo NOREN, Antti SULONEN and Marja LINDQVIST for their help, to Dr Matti ROUSI, who provided the clonal silver-birch seedlings, and to Dr Joann VON WEISSENBERG, who revised the English.

Résumé

Lésions de tige chez des semis de Betula pendula en pépinières forestières finlandaises et pathogénicité de Phytophthora cactorum

Phytophthora cactorum a été isolé de lésions nécrotiques de la tige de 20 à 80% des semis malades de *Betula pendula* prélevés dans trois pépinières de Finlande. *Fusarium avenaceum* et *Godronia* sp. ont aussi été trouvés dans toutes les pépinières. L'inoculation de *P. cactorum* à des semis de *B. pendula* a provoqué des lésions dont l'extension autour de la tige était nuisible. Ni le rythme d'application d'azote ni deux niveaux différents de P et K appliqués aux semis l'année précédente, n'ont affecté significativement la taille des lésions à *P. cactorum.*

Zusammenfassung

Stammläsionen an Sämlingen von Betula pendula in finnischen Baumschulen und die Pathogenität von Phytophthora cactorum

In finnischen Baumschulen konnte *Phytophthora cactorum* aus Stammläsionen von 20–80% der geschädigten Sämlinge von *Betula pendula* isoliert werden. *Fusarium avenaceum* und *Godronia* sp. wurden ebenfalls in allen Baumschulen nachgewiesen. Inokulation von *B. pendula* Sämlingen mit *P. cactorum* führte zu nekrotischen Läsionen. Stammumfassende Läsionen waren letal. Weder der Zeitpunkt von N-Düngung noch von PK-Gaben (zwei unterschiedliche Konzentrationen) im Vorjahr beeinflussten die Größe der durch *P. cactorum* verursachten Läsionen signifikant.

References

ANONYMOUS, 1941: Fifty-third annual report Rhode Island State College Agricultural Experiment Station. Contr. Rhode Isl. St. Coll. agric. Exp. Sta. No. 586, 1–71.

–, 1981: Yearbook of Forest Statistics 1980. Folia For. **460**, 1–205.

–, 1983: SAS' User's Guide: Statistics. Version 5 Edition. SAS Institute Inc. Cary.

–, 1990: BMDP Statistical Software Manual, Vol 1. Berkeley: Univ. Calif. Press.

–, 1994: Yearbook of Forest Statistics 1993–94. Ed. by M. AARNE. The Finnish Forest Research Institute, SVT Agriculture and Forestry **7**, 1–348.

DESPREZ-LOUSTAU, M. L.; DESSUREAULT, M., 1988: Influence de stress contrôlés sur la sensibilité du bouleau jaune au chancre godronien causé par *Godronia cassandrae* Peck f. sp. *betulicola* Groves. (In French, English summary). Can. J. For. Res. **1**, 121–127.

DOMSCH, K.; GAMS, W.; ANDERSON, T.-H., 1980: Compendium of Soil Fungi, 1. London, New York: Acad. Press.

HALONEN, O.; TULKKI, H.; DEROME, J., 1983: Nutrient analysis methods. Bull. Finn. For. Res. Inst. **121**, 1–28.

HAMM, P. B.; HANSEN, E. M., 1991: The isolation of *Phytophthora* species causing damage in bare-root conifer nurseries. In: Proc. IUFRO Work. Part. s2.07–09. Ed. by Sutherland, J. R.; Glover, S. G. For. Can. Pac. Yukon Reg. Pac. For. Cent. BC-X-311. pp. 169–179.

HARRIS, D. C., 1991: The *Phytophthora* diseases of apple. J. Hortic. Sci. **66**, 513–544.

HENTTONEN, H. 1993: Myyrätorjunnan nykynäkymiä. (in Finnish). In: Metsänsuojelututkimuksen tuloksia. Metsäntutkimuspäivä Vantaalla. Ed. by KURKELA, T.; LIPPONEN, K. Metsäntutk. tied. **460**, 13–18.

–; LILJA, A.; NIEMIMAA, J., 1994: Myyrien ja hyönteisten aiheuttamat sieni-infektiot koivun taimien uhkana. (in Finnish). Finnish For. Res. Inst. Res. Pap. **496**, 125–129.

HUBER, D. M., 1980: The role of mineral nutrition in defence. In: Plant Disease. An Advanced Treatise, Vol V. Ed. by HORSFALL, J. G.; COWLING E. B., New York: Academic Press. pp. 381–406.

INGESTAD, T., 1970: A definition of optimum nutrient requirements in birch seedlings. I. Physiol. Plant. **23**, 1127–1138.

JUUTINEN, P.; KURKELA, T.; LILJA, S., 1976: *Cicadella viridis* (L.), as a wounder of hardwood saplings and infection of wounds by pathogenic fungi. (in Finnish, English summary). Folia For. **284**, 1–12.

JOZEFEK, H. 1989: The effect of varying levels of potassium on the frost resistance of birch seedlings. Silva Fennica **23**, 21–31.

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

LILJA, A.; HIETALA, A., 1994: *Phytophthora cactorum* and a novel type *Rhizoctonia* sp. as forest nursery pathogens. In: Diseases and Insects in Forest Nurseries, Dijon, France, October 3–10, 1993. (Les Colloques, no 68). Ed. by PERRIN, R.; SUTHERLAND, J. R., Paris: INRA. pp. 59–64.

PETÄISTÖ, R.-L., 1983: Stem spotting of birch (*Betula pendula*) in nurseries (in Finnish, English summary). Folia For. **544**, 1–9.

RIKALA, R.; PETÄISTÖ, R.-L. 1986. Effects of fertilization on the nutrient concentration, growth and incidence of stem spotting in bare-rooted birch transplants. (in Finnish, English summary). Folia For. **642**, 1–9.

RYYNÄNEN, L.; RYYNÄNEN, M., 1986: Propagation of adult curly-birch succeeds with tissue culture. Silva Fennica **20**, 139–147.

SCHMITTHENNER, A. F.; CANADAY, C. H., 1983: Role of chemical factors in development of *Phytophthora* diseases. In: *Phytophthora*. Its Biology, Taxonomy, Ecology and Pathology. Ed. by ERWIN, D. C.; BARTNICKI-GARCIA, S.; TSAO, P. H., St. Paul. Minnesota: APS Press. pp. 189–196.

STROUTS, R. G., 1981: *Phytophthora* diseases of trees and shrubs. Arbocultural Leaflet, Department of the Environment, UK. No 8.

SUTTON, B. C., 1980: The Coelomycetes. Surrey: CAB Kew.

WARDLAW, T. J.; PALZER, C., 1985: Stem diseases in nursery seedlings caused by *Phytophthora cactorum, P. citricola* and *Pythium anandrum*. Australasian Plant Pathology **14**, 57–59.

WERRES, S.; RICHTER, J.; VESER, I. 1995: Untersuchungen von kranken und abgestorbenen Rosskastanien (*Aesculus hippocastanum* L.) im öffentlichen Grün (in German). Nachrichtenbl. Deut. Pfanzenschutzd. **47**, 81–85.

Authors' addresses: Arja LILJA, Ari HIETALA and Riitta HEINONEN, Finnish Forest Research Institute, Vantaa Research Center, PO Box 18, FIN-01301 Vantaa, Finland; Risto RIKALA, Finnish Forest Research Institute, Suonenjoki Research Station, FIN-77600 Suonenjoki, Finland

Received: 20.7.1995; accepted: 23.11.95

VI

Mycol. Res. 000 (0): 000–000 (1996) *Printed in Great Britain* 96-76

Genetic variation and host specificity of *Phytophthora cactorum* isolated in Europe

JARKKO HANTULA*[1], ARJA LILJA[1] AND PÄIVI PARIKKA[2]

[1] *Finnish Forest Research Institute, Vantaa Research Centre, P.O. Box 18, FIN-01301 Vantaa, Finland*
[2] *Agricultural Research Centre, FIN-31600 Jokioinen, Finland*

Analysis of *Phytophthora cactorum* using Random Amplified Microsatellites (RAMS) revealed considerable variation among isolates, most of which correlated with the original host plants. The lack of variation among isolates originating from strawberry suggests that crown rot of strawberry is caused by a single clone within the geographical area studied. It was also shown that *P. cactorum* isolates form a unique group different from other *Phytophthora* spp. from Group I.

P. cactorum isolates from necrotic stem lesions on *Betula pendula* seedlings or *Fragaria* × *ananassa* plants suffering from crown rot were highly pathogenic to their original host plants. *P. cactorum* isolates from strawberry inoculated via wounds also caused necrotic lesions on *B. pendula*. On the other hand isolates from *B. pendula* did not cause disease symptoms on strawberry plants. On *Alnus glutinosa* the percentage of successful inoculations with a birch isolate was 40.

Phytophthora cactorum (Lebert and Cohn) J. Schröt. is a worldwide omnivorous plant pathogen with nearly 160 host species (Nienhaus, 1960). Different diseases have been attributed to this oomycetous fungus on diverse genera including carrot (Vaartaja & Salisbury, 1961), raspberry (Duncan, Kennedy & Seemüller, 1987), strawberry (Deutschmann, 1954), apple (Strouts, 1981; Harris, 1991), Noble fir (Hamm & Hansen, 1982; Chastagner, Hamm & Riley, 1995), horse chestnut (Caroselli, 1953; Werres, Richter & Veser, 1995), *Acer* spp. (Strouts, 1981), Scots pine (Molin, Persson & Persson, 1960; Vaartaja & Salisbury, 1961), *Betula* spp. (Caroselli, 1953) and *Eucalyptus* spp. (Wardlaw & Palzer, 1985).

In Finland, *P. cactorum* was isolated for the first time in 1990 from strawberry (*Fragaria* × *ananassa* Duch.) plants showing crown rot symptoms (Parikka, 1990). A year later, it was found in necrotic stem lesions on silver birch (*Betula pendula* Roth) seedlings growing in forest nurseries (Lilja *et al.*, 1996*b*). Recently, in summer 1995, it was also isolated from stem lesions on nursery seedlings of common alder (*Alnus glutinosa* (L.) Gaertner) (Lilja *et al.*, 1996*a*).

Many of the morphological characteristics used in *Phytophthora* systematics, such as sporangial and oogonial dimensions, may exhibit high levels of variation within a species and they may even overlap between species (Waterhouse, 1963; Stamps *et al.*, 1990). More than sixty years ago Leonian (1934) concluded that *P. cactorum* is one of the 'good' species, e.g. it can be identified by morphological key characteristics. Isozyme analysis and mtDNA studies have shown that *P. cactorum* strains isolated from diverse geographical locations and host plants share a high degree of similarity (Förster & Coffey, 1991; Oudemans & Coffey, 1991).

The Random Amplified Microsatellite (RAMS) technique originally described by Zietkiewicz, Rafalski & Labuda (1994) has been shown to be applicable for fungi (Hantula, Dusabenyagasani & Hamelin, 1996; Hantula & Müller, 1997). The technique combines most of the benefits of Random Amplified Polymorphic DNA (RAPD) and microsatellite analyses, and is therefore promising for studies of genetic variation. In RAMS analysis the DNA between the distal ends of two closely located microsatellites is amplified and the resulting PCR products are separated electrophoretically (Hantula *et al.*, 1996).

In this study we tested the homogeneity of *P. cactorum* species by studying isolates from different host plants.

MATERIALS AND METHODS

The Finnish *P. cactorum* isolates originated from strawberry plants suffering from crown rot or from necrotic stem lesions on silver birch seedlings growing in forest nurseries. The isolation from diseased strawberry plants was carried out by plating surface sterilized (96% alcohol and flaming) pieces cut from root collar on potato-dextrose agar (PDA, 39 g l^{-1}, Difco). After 3–5 d incubation at 21–23 °C, hyphal tips were transferred to fresh plates of PDA. Isolations from birch-stem lesions were conducted as described by Lilja *et al.* (1996*b*). *P. cactorum* isolates from other hosts, and *Phytophthora* species belonging to Waterhouse's (1963) main Group I: *P. iranica* Ershad, *P. pseudotsugae* Hamm & E. M. Hansen, *P. clandestina* P. A. Taylor, Pascoe & Greenh. (Stamps *et al.*, 1990) and *P. idaei* D. M. Kenn. (Kennedy & Duncan, 1995) and other

* Corresponding author.

Table 1. Identities and sources of isolates of *Phytophthora* spp. and *Pythium anandrum*

	Collection code	Host	Geographical origin	Source
P. cactorum	S3, S5, S6, S7, S9, S10, S13, S14, S15, S19	Strawberry	Finland	P. Parikka
P. cactorum	145(H)	Strawberry	England	D. Harris
P. cactorum	TAM(1)	Strawberry	Scotland	D. Kennedy
P. cactorum	S17	Strawberry	Finland	J. Tegel
P. cactorum	CH09, CH12, CH15, CH19	Strawberry	Sweden	C. Olsson
P. cactorum	CH17	Strawberry	Estonia	C. Olsson
P. cactorum	W1	Strawberry	Germany	S. Werres
P. cactorum	2/94 III	Horse-chestnut	Germany	S. Werres
P. cactorum	A1	Strawberry	Germany	S. Werres
P. cactorum	Ph2, Ph3, Ph4, Ph5, Ph8, Ph10, Ph11, Ph14, Ph15, Ph18, Ph20	Silver birch	Finland	A. Lilja
P. cactorum	EM294	Apple	England	D. Harris
P. cactorum	9/88/92, 1557	Rhododendron	Germany	S. Werres
P. cactorum	R12	Red raspberry	Scotland	D. Kennedy
P. cactorum	5/94	Beech	Germany	S. Werres
P. idaei	R66	Red raspberry	England	D. Kennedy
P. clanestina	CBS 347.86			CBS
P. iranica	CBS 374.72			CBS
P. pseudotsugae	CBS 446.84			CBS
P. citrophthora	Ph7	Poinsettia	Finland	P. Parikka
P. undulata	Ph12	Scots pine	Finland	A. Lilja
Pythium anandrum	P.a.	Scots pine	Finland	A. Lilja

Table 3. Primers used in this study

Primer	Sequence*
CCA-primer	5′DDB(CCA)$_8$
CGA-primer	5′DHB(CGA)$_8$
GT-primer	5′YHY(GT)$_7$G

* The following designations are used for degenerate sites: B (G, T, or C), D (G, A, or T), H (A, T, or C), and Y (A, C or G).

oomycetous fungi: *P. citrophthora* (R. E. Sm. & E. M. Sm.) Leonian, *P. undulata* M. W. Dick and *Pythium anandrum* Drechsler, were included as reference isolates in DNA-analysis. Details of the isolates are given in Table 1. It should be noted that none of the isolates was from the fruits of strawberry. The Finnish isolates were from eight forest nurseries and eight strawberry fields located in different parts of Finland.

DNA techniques

DNA extractions. DNA was isolated as described by Hantula *et al.* (1996). The protocol included cell disruption, two phenol:chloroform (1:1) extractions, a chloroform:isoamyl alcohol (24:1) extraction, and precipitation with alcohol followed by drying under vacuum. The DNA was resuspended in 10 mM Tris-HCl buffer (pH 8·0) containing 1 mM EDTA.

PCR-reactions. The PCR-reactions were carried out in reaction conditions suggested by the manufacturer of the Dynazyme II DNA-polymerase (Finnzymes Ltd, Finland), except the concentration of the primer was 2 μM (Table 2). The samples were denatured by 10 min incubation at 95° after which 35 (CGA- and GT-primers) or 37 (CCA-primer) cycles of amplification were carried out (30 s denaturation at 95°, 45 s annealing at a temperature depending on the primer, 2 min primer extension at 72°). The annealing temperatures for the primers were as follows: CCA-primer 64°, CGA-primer 61°, and GT-primer 58°. After the cycles, the reaction was ended with a 7 min extension at 72°.

Electrophoresis. Amplification products were separated by electrophoresis in gels containing 0·8% SynerGel (Diversified Biotech) and 0·8% agarose (FMC BioProducts). The electrophoresis was run in TAE-buffer (40 mM Tris-Acetate pH 8·0, 1 mM EDTA), and the amplification products were visualized by ethidium brimide in uv-light. The lengths of the amplification products were estimated by comparing them to a 100 bp DNA ladder (Gibco BRL). In the case of closely migrating markers (possibly length polymorphisms) several electrophoresis runs were carried out to allow the identification of different markers only from lanes next to each other.

Dendrogram construction

The differences between *Phytophthora cactorum* isolates were calculated by scoring the number of differences in 19 RAMS markers. From these data we constructed a similarity matrix according to formula

$$S = 2 \times n_{ab}/(n_a + n_b),$$

where n_a and n_b represent the total number of bands present in lanes *a* and *b*, respectively, and n_{ab} is the number of bands

which are shared by both lanes. The matrix was used to create UPGMA dendrogram using the MEGA software (Kumar, Tamura & Nei, 1993).

Morphology

Colony morphology, sporangial and oogonial dimensions and growth rates of Finnish *P. cactorum* isolates were described. Oogonia and oospores were measured from 2-wk-old cultures on corn meal agar (17 g CMA, Difco l^{-1}) grown at $20 \pm 2°$. Sporangia were measured from the same kind of cultures having an extra incubation for 24 h under a shallow layer of water. The number of sporangia, oogonia and oospores measured was 50, 24 and 24 respectively for each isolate.

Radial growth of the same *P. cactorum* isolates was compared on CMA after 7 d in the dark at 4°, 10°, 21°, 30° and 35°. The number of replicates was 24 per isolate.

Pathogenicity test

Pathogenicity tests were carried out in a greenhouse with silver birch, strawberry and common alder. Four *P. cactorum* isolates from silver birch (Ph10, Ph11, Ph18 and Ph20) and two from strawberry (S3 and S13) were used. Common alder seedlings were also inoculated with a *P. cactorum* isolate from silver birch.

Experiment with 2-month-old silver birch seedlings. The silver birch seeds (origin, seed orchard SV 378) were sown on 12 Apr. 1995. On 15 May 1995 200 seedlings were transplanted into pots (0·5 l) containing fertilized, low-humidified Sphagnum peat (Finn peat M6). Two seedlings were growing in each pot and one of them was wounded just before inoculation. Twenty wounded and twenty intact seedlings were inoculated on 20 June 1995 with each *P. cactorum* isolate (Ph18, Ph20, S6 and S16) or with pure agar blocks as a control. Thus the number of treatments was 10. The wound was done by removing a 3×3 mm piece of periderm with a scalpel 5 cm above the soil. A 9 mm^2 agar block from a 1-wk-old *P. cactorum* culture on PDA or a pure PDA-block was placed on the would or on the sound bark at the same height and secured with parafilm (Parafilm M, Amer. Nat. Can.). Before inoculation the *P. cactorum* cultures had been in a refrigerator for 2 d.

Each treatment had two replications. Twenty pots, one wounded and one intact seedling in each pot, representing the same treatment were placed on two trays. The trays were arranged in a randomized design in a greenhouse with a temperature range of 19–22°. The illumination was sunlight and the seedlings were watered 3–4 times during a week without further fertilization. The condition of seedlings was monitored on every second week and the last measurements were done on 29 Aug. 95. The seedlings were divided to five categories according to the spread of fungal infection: (i) no lesion or lesion < 9 mm^2, (ii) lesion > 9 mm^2, but less than half of the stem diameter, (iii) lesion had spread more than half of the stem diameter, (iv) lesion had spread around the stem and the seedling was broken, (v) lesion had spread around the stem and the seedling was dead. The disease index of seedlings was calculated from the frequency (f) of different condition categories (c1–c5):

$$DI = (f_{c1} + 2 \times f_{c2} + 3 \times f_{c3} + 4 \times f_{c4} + 5 \times f_{c5})/n.$$

The same index was used to indicate the health condition of plants in all pathogenicity tests.

Experiment with 3-month-old silver birch and common alder seedlings. Twenty 3-month-old silver birch seedlings representing the same batch as above and 20 alder seedlings (origin, seed collection stand 502), were inoculated with *P. cactorum* isolate Ph20 from silver birch or with pure agar blocks as a control. The inoculation technique, growth substrate and greenhouse conditions were as above, but the inoculum was placed on intact bark or on a leaf scar. The leaf was removed from the stem just before inoculation (10 July 1995). One seedling was growing in each pot (0·4 l) and the pots were arranged in a randomized design in a greenhouse. The condition of seedlings was monitored every second week and the last measurements were done on 18 Sept. 95. The seedlings were divided into five categories according to the spread of fungal infection as before.

Experiment with strawberries. The strawberry variety Jonsok was used in pathogenicity tests, because in previous experiments it had been very sensitive to *P. cactorum* infection (Parikka, 1991). Strawberry runner plants were cut on 4 May 1995 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 fertilizer l^{-1} peat). After 40 d 60 plants were removed from the rooting substrate and the roots were washed with tapwater. Twelve plants were inoculated with each *P. cactorum* isolate (Ph18, Ph20, S3 and S13) or with pure PDA blocks as described by Seemüller (1977) with modifications. The crown of the plants was wounded near soil level with a sharp stick. The 2 mm deep and 2 mm wide wound was covered with fungal mycelium from 3-wk-old PDA cultures kept for 2 d 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 before. Three plants represented a replicate and the replicates of all five treatments were arranged in a randomized design in a greenhouse at a temperature of 24°.

The condition of the plants was monitored before and 3 wks after inoculation. The number of fully expanded leaves/plant was counted and the condition of the plant was marked with (i) good, (ii) moderate, (iii) weak, (iv) nearly dead and (v) dead.

Statistics

The parametric data (the sporangial and oogonial dimensions, the growth rates of cultures at different temperatures, and the number of leaves of strawberry plants in the inoculation experiment) were analysed using ANOVA, and the significance of the mean differences was determined with Tukey's multiple-range test. Non-parametric data in pathogenicity tests (disease indexes) were analysed with Kruskal–Wallis

one-way analysis of variance and a non-parametric test was used for comparison of treatments (Anonymous, 1990).

RESULTS

Interspecific variation

The genetic variation between different *Phytophthora* species belonging in main group I, *P. cactorum*, *P. iranica*, *P. pseudotsugae*, *P. clandestina* and *P. idaei*, was considerable compared to variation between different *P. cactorum* isolates. An example is shown in Fig. 1 where the patterns obtained using the CCA-primer are shown. Similar results were obtained with other primers.

Variation in RAMS patterns among P. cactorum *isolates*

The patterns of amplification products obtained with all three primers showed variation within *P. cactorum*. To simplify the description and discussion of the results we designate the different sized markers after the primer used for the amplification and the approximate molecular weight of the band. For example an approximately 750 bp amplification product obtained with CGA-primer is designated as CGA750 marker.

Variation observed using the CGA-primer. Nine easily detectable bands were detected in patterns obtained with the CGA-primer. Three of these were polymorphic. The area of about 750 bp consisted of three banding types: two sharp bands with different mobilities (Fig. 2, lanes e–g and lane h) and a fuzzy band, probably composed of two closely migrating bands (Fig. 2, lanes a–d). These markers are designed as CGA750, CGA740 and CGA730, respectively. Two polymorphic markers were observed in the area of about 300–350 bp (Fig. 2, lanes a–d and e–h). These markers are designated as CGA330 and CGA320, respectively. The CGA250 marker contained a same size marker for all isolates, but there was a reproducible difference in the intensity of the bands. Therefore the fainter (Fig. 2, lanes a–c) and darker forms (lanes d–h) of the bands are considered as separate markers CGA250 and CGA240.

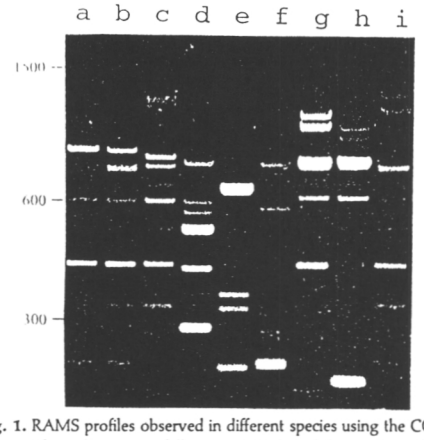


Fig. 1. RAMS profiles observed in different species using the CGA-primer. The isolates are as follows. (a) S3 (*Phytophthora cactorum* from strawberry), (b) Ph4 (*P. cactorum* from silver birch), (c) *P. idaei* R66, (d) *P. citrophthora* Ph7, (e) *P. undulata* Ph12, (f) *Pythium anandrum* P.a., (g) *Phytophthora clandestina* CBS 347.86, (h) *P. iranica* CBS 446.84, and (i) *P. pseudotsugae* CBS 446.84. Molecular weight markers are indicated on the left.

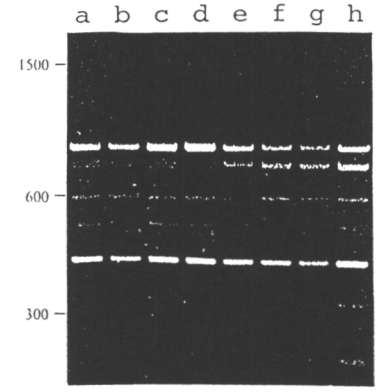


Fig. 2. RAMS-profiles observed within the isolates of *P. cactorum* using the CGA-primer. One representative isolate is selected from each branch of the UPGMA dendrogram in Fig. 5, and the order of patterns is the same as that of the branches in the dendrogram. The isolates are as follows. (a) 9/88/92, (b) R12, (c) 1557, (d) S3, (e) 5/94, (f) Ph11, (g) Ph3, and (h) Ph4. Molecular weight markers are indicated on the left and the markers used in the analysis are marked with short lines on the right.

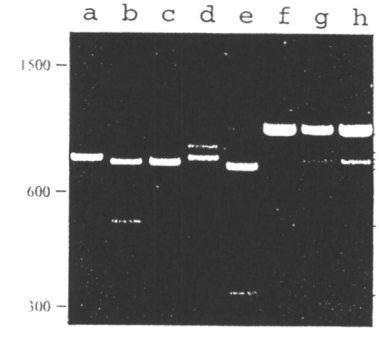


Fig. 3. RAMS-profiles observed within the isolates of *P. cactorum* using the GT-primer. One representative isolate is selected from each branch of the UPGMA dendrogram in Fig. 5, and the order of patterns is the same as that of the branches in the dendrogram. The isolates are as follows. (a) 9/88/92, (b) R12, (c) 1557, (d) S3, (e) 5/94, (f) Ph11, (g) Ph3, and (h) Ph4. Molecular weight markers are indicated on the left and the markers used in the analysis are marked with short lines on the right.

Variation observed using the GT-primer. The amplification cycles using the GT-primer produced several very strong bands (Fig. 3). These markers are designated as GT900 (Fig. 3,

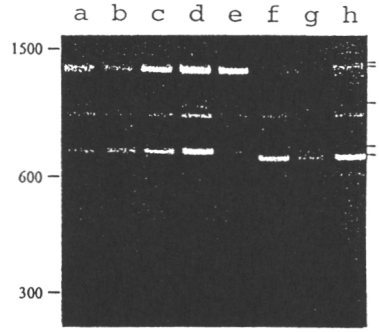


Fig. 4. RAMS-profiles observed within the isolates of *P. cactorum* using the CCA-primer. One representative isolate is selected from each branch of the UPGMA dendrogram in Fig. 5, and the order of patterns is the same as that of the branches in the dendrogram. The isolates are as follows. (a) 9/88/92, (b) R12, (c) 1557, (d) S3, (e) 5/94, (f) Ph11, (g) Ph3, and (h) Ph4. Molecular weight markers are indicated on the left and the markers used in the analysis are marked with short lines on the right.

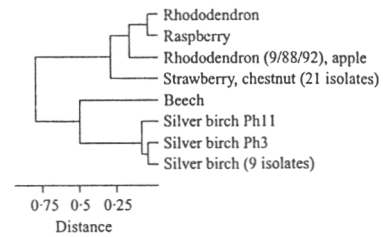


Fig. 5. An UPGMA dendrogram of *P. cactorum* isolates. Host origins are indicated at the ends of the branches. The isolate names are given after the host plant only when informative. The number of identical isolates is given in parentheses where two or more isolates cluster together. The genetic distance is given the band sharing index in the RAMS-loci studied.

lanes e–h), GT800 (Fig. 3, lanes a–d), GT750 (Fig. 3, lanes a and d), GT740 (Fig. 3, lanes b–c, g–h), GT730 (Fig. 3, lane e), GT550 (Fig. 3, lanes a–c) and GT350 (Fig. 3, lane e).

Variation observed using the CCA-primer. Five clear and completely reproducible amplification products were obtained with the CCA-primer. In the area with a size of approximately 1350 bp two different banding types were observed: a single band (Fig. 4, lanes a–e) and a more diffuse set of bands (lanes f–h). Here these two forms are scored as two different markers: CCA1350 and CCA1300. In the area of 1000 bp (CCA1000 marker) variation was observed only in one isolate, as isolate 1557 lacked this marker (Fig. 4, lane c). The marker with approximate size of 950 bp did not vary within the isolates, but in the area of about 700 bp two markers were observed: CCA720 and CCA700 (Fig. 4, lanes a–e, g and f–h, respectively).

In addition to the four clear loci described above, several less clear bands were observed in the amplification pattern obtained using the CCA-primer. Although reproducible, they were either not informative (e.g. found only in one isolate) or difficult to score, and therefore not used in this study.

Distribution of markers. The distribution of polymorphic markers within the isolates is shown in Table 3. Altogether eight different banding patterns were observed. The largest two banding pattern types were composed of 21 isolates originating from strawberry and horse-chestnut, and nine isolates originating from silver birch. The rest of the five banding pattern types were composed of only one or two isolates.

Dendrogram based on RAMS-variation. The data described above were used to construct UPGMA dendrogram (Fig. 5). The grouping of isolates into the dendrograms correlated with the host plants, the only exception being the two isolates originating from *Rhododendron* spp. Furthermore, the dendrograms had three deep branches separating isolates originating from (i) silver birch, (ii) beech and (iii) all other plant species studied (red raspberry, strawberry, apple, rhododendron and horse-chestnut).

Morphology

Colonies of all *P. cactorum* isolates were uniform with a slight rosette pattern, and abundant oogonia and sporangia were

Table 2. Distribution of markers among isolates. Isolates with identical banding patterns are grouped together

Isolate	No of isolates*	Locus CGA 750	CGA 740	CGA 730	CGA 330	CGA 320	CGA 250	CGA 240	GT 900	GT 800	GT 750	GT 740	GT 730	GT 550	GT 350	CCA 1350	CCA 1300	CCA 1000	CCA 720	700
S3	21	1†	0	0	1	0	1	0	0	1	1	0	0	0	0	1	0	1	1	0
Ph2	9	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	1	1	0	1
EM294	2	1	0	0	1	0	0	1	0	1	1	0	0	1	0	1	0	1	1	0
1557	1	1	0	0	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	0
R12	1	1	0	0	1	0	0	1	0	1	0	1	0	1	0	1	0	1	1	0
Ph11	1	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	1	1	0	1
Ph3	1	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	1	1	1	1
5/94	1	0	0	1	0	1	1	0	1	0	0	0	1	0	1	1	0	1	1	0

* Number of identical isolates.

† 1 and 0 indicate the occurrence and lack of the marker, respectively.

Table 4. Mean size of oogonia, oospores and sporangia (μm) of *Phytophthora cactorum* isolates from strawberry suffering from crown rot and from necrotic stem lesions on silver birch seedlings

	Oogonium	Oospore	Sporangia
All isolates from strawberry			
Mean	28·3	27·7	33·6 × 24·6
S.D.	2·3	3·3	4·3 and 3·1
All isolates from silver birch			
Mean	29·4	25·8	27·7 × 23·4
S.D.	3·3	2·5	4·3 and 3·3

formed within 2 wk on CMA. The mean size of oogonia and oospores of *P. cactorum* isolates from strawberry and silver birch differed slightly from each other. The sporangia of strawberry isolates were longer than those of birch isolates ($P < 0{\cdot}01$; Table 4). The sporangia of all isolates were papillate and caducous.

None of the *P. cactorum* isolates grew at 35° and only the isolates from silver birch grew at 4°. High temperature (30°) favoured the growth of strawberry isolates; especially isolates S14 and S19 grew well at this temperature. All silver birch isolates but Ph3 grew slower than strawberry isolates at 30° ($P < 0{\cdot}05$). The Ph3 isolates grew also at 10° and 21°, faster than other silver birch isolates ($P < 0{\cdot}05$). Details of the growth of all *P. cactorum* isolates is presented in Table 5.

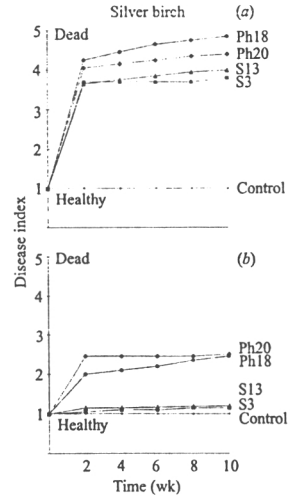


Fig. 6. (*a*) The condition of silver birch seedlings 10 wk after inoculation on wounded bark of 2-month-old seedlings with *Phytophthora cactorum* isolates from stem lesions on silver birch (Ph18, Ph20) and strawberry plants suffering from crown rot (S3, S13). (*b*) The condition of silver birch seedlings 10 wk after inoculation on intact bark of 2-month-old seedlings with *Phytophthora cactorum* isolates from stem lesions on silver birch (Ph18, Ph20) and strawberry plants suffering from crown rot (S3, S13).

Pathogenicity

Two-month-old silver birch seedlings. Within 2 wk after inoculation on wounded bark, necrotic lesions were observed on stems. All tested *P. cactorum* isolates were detrimental to young silver birch seedlings (Fig. 6*a*). The differences between controls and all inoculations on wounds were significant ($P < 0{\cdot}05$). However, the percentage of dead seedlings varied depending on the isolate and ranged from 85 in inoculations with Ph18 to 45, 15 and 10 in inoculations with Ph20, S16 and

Table 5. The growth of Finnish *Phytophthora cactorum* isolates after 1 wk on corn meal agar at different temperatures. Standard deviations are indicated in parentheses

Collection code	Host	4 °C (mm)	10 °C (mm)	21 °C (mm)	30 °C (mm)	35 °C (mm)
S3	Strawberry	No growth	11·8 (0·9)	28·9 (2·2)	33·8 (1·8)	No growth
S5	Strawberry	No growth	11·4 (0·2)	29·7 (1·2)	33·3 (1·8)	No growth
S6	Strawberry	No growth	13·2 (0·7)	34·7 (1·1)	32·4 (1·8)	No growth
S7	Strawberry	No growth	12·9 (0·7)	31·1 (1·4)	25·7 (2·3)	No growth
S9	Strawberry	No growth	12·6 (1·1)	33·6 (1·7)	27·5 (2·3)	No growth
S10	Strawberry	No growth	10·5 (0·6)	25·5 (0·6)	33·2 (3·0)	No growth
S13	Strawberry	No growth	12·9 (1·0)	32·0 (0·9)	36·7 (1·4)	No growth
S14	Strawberry	No growth	12·4 (1·0)	30·2 (0·9)	59·7 (1·4)	No growth
S15	Strawberry	No growth	10·6 (0·7)	30·5 (0·7)	65·1 (3·4)	No growth
S19	Strawberry	No growth	10·0 (0·7)	27·1 (0·8)	32·7 (1·9)	No growth
Ph2	Silver birch	1·3 (0·6)	11·3 (1·1)	28·5 (1·7)	13·7 (2·4)	No growth
Ph4	Silver birch	1·3 (0·8)	11·4 (1·9)	31·2 (1·8)	21·3 (1·8)	No growth
Ph5	Silver birch	0·5 (0·5)	8·9 (0·6)	28·0 (1·2)	20·6 (1·0)	No growth
Ph8	Silver birch	0·3 (0·4)	8·9 (1·3)	26·0 (0·7)	19·6 (2·8)	No growth
Ph10	Silver birch	0·5 (0·5)	11·0 (1·2)	30·6 (0·6)	23·5 (1·3)	No growth
Ph14	Silver birch	0·5 (0·5)	11·0 (1·0)	30·7 (1·3)	23·7 (1·1)	No growth
Ph15	Silver birch	0·5 (0·5)	11·2 (0·9)	29·9 (1·2)	23·9 (0·9)	No growth
Ph18	Silver birch	0·5 (0·5)	11·4 (1·9)	30·6 (0·6)	21·3 (1·8)	No growth
Ph20	Silver birch	0·9 (0·5)	11·0 (1·2)	29·7 (1·2)	13·7 (2·4)	No growth
Ph11	Silver birch	0·9 (0·3)	9·7 (0·9)	28·5 (1·4)	19·0 (2·1)	No growth
Ph3	Silver birch	1·2 (0·6)	12·9 (1·1)	33·1 (1·4)	26·8 (1·1)	No growth

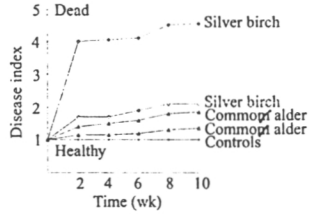


Fig. 7. The condition of silver birch and common alder seedlings 8 wk after inoculation with a *Phytophthora cactorum* isolate from stem lesions on silver birch (Ph20). Inoculations were done on wounded (black markers) or intact bark (grey markers) of 3-month-old seedlings.

Fig. 8. The condition of strawberry plants 3 wk after inoculation with *Phytophthora cactorum* isolates from strawberry plants suffering from crown rot (S3, S13) and stem lesions on silver birch (Ph18, Ph20).

S6, respectively. Inoculations on intact bark with birch isolates also resulted in lesions. The difference in disease index was significant between these and controls for birches infected with strawberry isolates ($P < 0{\cdot}05$) (Fig. 6*b*). The percentage of broken seedlings was 20, while only a few seedlings showed symptoms in inoculations with strawberry isolates on intact bark (Fig. 6*b*).

Three-month-old silver birch and common alder seedlings. The condition of birch seedlings inoculated on leaf scars was weaker than that of control or inoculated seedlings of common alder ($P < 0{\cdot}05$) (Fig. 7). This was also evident from the observation that inoculation killed 40% of 3-month-old silver birch seedlings. Only 40% of the common alder seedlings had small lesions (Fig. 7). Inoculation on intact bark was not very harmful either to birch or to common alder. The percentage of silver birch and common alder seedlings showing small lesions was 30 (Fig. 7).

Strawberry. The isolates from strawberry were highly pathogenic to strawberry plants (Fig. 8). The difference in disease index was significant between these and control plants ($P < 0{\cdot}05$) or plants inoculated with isolates from birch ($P < 0{\cdot}05$) (Fig. 8). The *P. cactorum* isolates from birch were not pathogenic to strawberry plants. The increase in the number of fully expanded leaves was at the same level in these treatments (Ph18 3·5 and Ph 20 3·7) as in the control (3·0) ($P < 0{\cdot}01$).

DISCUSSION

The variation observed in RAMS-markers reflected the plant origin of *P. cactorum* isolates. This indicates that a separate *P. cactorum* type has adapted for each host. In general the genetic variation among isolates originating from single plant species was low, the extreme being the 20 isolates from strawberry, which were identical in their RAMS-patterns. This suggests that a single clone causes crown rot of strawberry within the geographical area studied (Finland, Estonia, Sweden, Germany, England and Scotland). This result is also supported by Cooke *et al.* (1996), who found no variation in RAPD-markers among their isolates from strawberry. It should, however, be noted that small differences between Finnish isolates were observed in growth rates and in sporangial dimensions. In this context it should also be noted that crown rot has been shown to be caused by another pathotype than the leather rot of strawberry fruit (Seemüller & Schmidle, 1979), and that these two pathotypes may differ genetically from each other.

The possible clonal nature of *P. cactorum* (causing strawberry crown rot) is in accordance with the observation that the Irish potato famine may have been caused by a single clonal genotype of *P. infestans* (Goodwin, Cohen & Fry, 1994). Thus, it seems that epidemics on single host plants may be caused by single clones of *Phytophthora*.

The other large group, the 11 isolates from silver birch, varied in RAMS analysis more than isolates from strawberry, and three closely related amplitypes were observed. The morphology of isolates in these amplitypes did not differ from each other. However, their morphology and physiology differed slightly from the Finnish strawberry isolates: dimensions of sporangia were slightly different and only the isolates from birch grew at 4°. In addition, the isolates from strawberry infected birch only *via* wounds and the isolate from birch did not infect strawberry seedlings at all suggesting that there are differences in host specificities between the isolates from birch and strawberry. This is in accordance with the observation of Seemüller & Schmidle (1979) who showed that *P. cactorum* isolates from fruits or crown rot of strawberry and from apple bark differ in their capability to cause symptoms on strawberry and apple. These observations lead to a suggestion that *P. cactorum* isolates from different host plants have unique host specificities, and thus would generally form different pathotypes in a given geographic area.

In RAMS analysis the birch groups are separated only by single differences, which could be explained by mutations within individuals of single clones. This is not unexpected, as microsatellites are known to have an extremely high mutation rate (Charlesworth *et al.*, 1994). However, the clonality of *P. cactorum* from birch should be studied more thoroughly before it can be considered completely resolved.

In RAMS analysis the only isolate from horse-chestnut is identical to strawberry isolates, which is in accordance with the results of the mtDNA analysis (Förster & Coffey, 1991), and the isolate from beech is distantly related to those from silver birch. Isolates from apple, red raspberry and

Rhododendron spp. are closely related to those from strawberry. Of these the two isolates from rhododendron are interesting, as they are separated in dendrogram (Fig. 5). This separation is due to three differences between the RAMS markers suggesting that the population infecting *Rhododendron* spp. in Germany is more variable than that infecting strawberry. However, more information is needed to solve the genetic variation of *P. cactorum* infecting *Rhododendron* species.

In addition to variation within *P. cactorum* the RAMS patterns of this species were compared to *Phytophthora* spp. from Group I, *P. undulata* and *Pythium anandrum*. This analysis showed *Phytophthora cactorum* to be distinct from the other species studied. However, the patterns were so dissimilar that a phylogenetic analysis would not have been meaningful, and therefore was not done.

As a conclusion, the results of this investigation suggest that *P. cactorum* infecting birch and strawberry are genetically and pathogenically separate. Thus their origin is probably different and they have been dispersed independently by man within Europe.

We are very grateful to all those colleagues listed in Table 1 for supplying isolates of *P. cactorum*. Ms Maarit Niemi and Ms Ritva Vanhanen are acknowledged for their skilful technical assistance. We thank Dr Kari Korhonen, Mr Ari Hietala and Mr Sakari Lilja for critical reading of the manuscript.

REFERENCES

Anonymous (1990). *BMDP Statistical Software Manual*, Vol 1. University of California Press: Berkeley.

Caroselli, N. E. (1953). Bleeding canker diseases of hardwoods. Barlett Tree Research Laboratory. *Science of the Tree Topics* **2**(1), 1–6.

Charlesworth, B., Sniegowski, P. & Stephan, W. (1994). The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* **371**, 215–220.

Chastagner, G. A., Hamm, P. B. & Riley, K. L. (1995). Symptoms and *Phytophthora* spp. associated with root rot and stem canker of Noble fir christmas trees in the Pacific Northwest. *Plant Disease* **79**, 290–293.

Cooke, D. E. L., Kennedy, D. M., Guy, D. C., Russell, J., Unkles, S. E. & Duncan, J. M. (1996). Relatedness of Group I species of *Phytophthora* as assessed by randomly amplified polymorphic DNA (RAPDs) and sequences of ribosomal DNA. *Mycological Research* **100**, 297–303.

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

Duncan, J. M., Kennedy, D. M. & Seemüller, E. (1987). Identities and pathogenicities of *Phytophthora* causing root rot of red raspberry. *Plant Pathology* **36**, 276–289.

Förster, H. & Coffey, M. D. (1991). Approaches to the taxonomy of *Phytophthora* using polymorphisms in mitochondrial and nuclear DNA. In *Phytophthora* (ed. J. A. Lucas, R. C. Shattock, D. S. Shaw & L. R. Cooke), pp. 164–183. Cambridge University Press: Cambridge, U.K.

Goodwin, S. B., Cohen, B. A. & Fry, W. E. (1994). Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences, U.S.A.* **91**, 11591–11595.

(*Accepted 6 September 1996*)

Hamm, P. B. & Hansen, E. M. (1982). Pathogenicity of *Phytophthora* species to Pacific Northwest conifers. *European Journal of Forest Pathology* **12**, 167–174.

Hantula, J., Dusabenyagasani, M. & Hamelin, R. C. (1996). Random amplified microsatellites (RAMS) – a novel method for characterizing genetic variation within fungi. *European Journal of Forest Pathology* **26**, 159–166.

Hantula, J. & Müller, M. (1997). Variation within *Gremmeniella abietina* in Finland and other countries as determined by Random Amplified Microsatellites (RAMS). *Mycological Research* **101**, in press.

Harris, D. C. (1991). The *Phytophthora* diseases of apple. *Journal of Horticultural Science* **66**, 513–544.

Kennedy, D. M. & Duncan, J. M. (1995). A papillate *Phytophthora* species with specificity to *Rubus*. *Mycological Research* **99**, 57–68.

Kumar, S., Tamura, K. & Nei, M. (1993). *MEGA: Molecular Evolutionary Genetics Analysis*. Version 1.01. The Pennsylvania State University: University Park, PA 16802.

Leonian, L. H. (1934). Identification of *Phytophthora* species. *West Virginia Agricultural Experiment Station Bulletin* **262**, 1–36.

Lilja, A., Hantula, J. & Nuorteva, H. (1996*a*). Uusimpia tuloksia koivun versolaikusta. *The Finnish Forest Research Institute. Research Papers* **601**, 7–16. (In Finnish).

Lilja, A., Rikala, R., Hietala, A. & Heinonen, R. (1996*b*). Stem lesions on *Betula pendula* seedlings in Finnish forest nurseries and the pathogenicity of *Phytophthora cactorum*. *European Journal of Forest Pathology* **26**, 89–96.

Molin, N., Persson, M. & Persson, S. (1960). Root parasites on forest tree seedlings. *Meddelanden från Statens Skogsforskningsinstitut*, Band **49** (1), 1–17.

Nienhaus, F. (1960). Das Wirtsspektrum von *Phytophthora cactorum* (Leb. et Cohn) Schroet. *Phytopathologische Zeitschrift* **38**, 33–68.

Oudemans, P. & Coffey, M. D. (1991). Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. *Mycological Research* **95**, 19–30.

Parikka, P. (1990). Nahkamätä vaivaa mansikkaa. *Puutarha* 9/90, 630–631. (In Finnish).

Parikka, P. (1991). Nahkamädän alttius testattu. *Puutarha* 11/91, 634–635. (In Finnish).

Seemüller, E. (1977). Resistenzverhalten von Erdbeersorten gegen den Erreger der Rhizomfäule, *Phytophthora cactorum*. *Nachrichtenblatt des Deutschen Pflanzenshutzdienstes* **29**, 124–126. (In German).

Seemüller, E. & Schmidle, A. (1979). Einfluß der Herkunft von *Phytophthora cactorum*-Isolaten auf ihre Virulenz an Apelrinde, Erdbeerrhizomen und Erbeerfrüchten. *Phytopathologische Zeitschrift* **94**, 218–225. (In German, English summary).

Stamps, D. J., Waterhouse, G. M., Newhook, F. J. & Hall, G. S. (1990). Revised key to the species of *Phytophthora*. *Mycological Papers* (CMI) **162**, 1–28.

Strouts, R. G. (1981). *Phytophthora* diseases of trees and shrubs. *Arbocultural Leaflet, Department of the Environment*, U.K. No. 8, 16 pp.

Vaartaja, O. & Salisbury, P. J. (1961). Potential pathogenicity of *Pythium* isolates from forest nurseries. *Phytopathology* **51**, 35–42.

Wardlaw, T. J. & Palzer, C. (1985). Stem diseases in nursery seedlings caused by *Phytophthora cactorum*, *P. citricola* and *Pythium anandrum*. *Australasian Plant Pathology* **14**, 57–59.

Waterhouse, G. M. (1963). Key to species of *Phytophthora* de Bary. *Mycological Papers* (CMI) **92**, 1–22.

Werres, S., Richter, J. & Veser, I. (1995). Untersuchungen von kranken und abgestorbenen Roßkastanien (*Aesculus hippocastanum* L.) im öffentlichen Grün. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **47** (4), 81–85. (In German).

Zietkiewicz, E., Rafalski, A. & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* **20**, 176–183.

ISBN 951-40-1538-X
ISSN 0358-4283