FOLIAGE, SHOOT AND STEM DISEASES OF TREES

Proceedings of the IUFRO WP 7.02.02 Meeting
Quebec City, May 25-31, 1997

G. Laflamme, J.A. Bérubé, R.C. Hamelin (éditeurs/editors)

Centre de foresterie des Laurentides - Laurentian Forestry Centre
Rapport d'information - Information Report
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Ressources naturelles Canada
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Brûlures de pousses sur *Pinus banksiana* causées par *Gremmeniella abietina*, race nord-américaine
(Photo : G. Laflamme)

Cover photo:
Shoot blights on *Pinus banksiana* caused by *Gremmeniella abietina*, North American race
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FOREWORD

This meeting was to be held in St. John's, Newfoundland at the invitation of the Canadian Forest Service. Because of the closure of the Newfoundland Research Centre, it was decided to move the meeting to Quebec City, where it was held from May 25 to May 31, 1997.

Although we live in a society where people can now communicate through electronic mail, this kind of meeting is still necessary as it offers a good opportunity to present data that are not yet ready for publication in refereed journals or information that will never be published elsewhere. It also allows for the exchange of information and presents opportunities for scientists working in the same field of research to discuss work in progress with one another, to visit experimental sites and plan future collaborations. We are therefore grateful to the International Union of Forest Research Organizations (IUFRO) for giving us the chance to meet with colleagues from around the world.

The IUFRO Working Party 7.02.02, Foliage, Shoot and Stem Diseases of Trees was meeting under this name for the first time. Indeed a change was voted at the last meeting in Vallombrosa (June 1994), near Firenze, Italy. Members of the two IUFRO WP, S2.06-04 (Canker and Shoot Blight of Conifers) and S2.06-02 (Foliage Diseases), decided to merge into this broader group of Forest Pathology. The reduction of the number of working groups was in accordance with the organization's restructuring of the divisions. This working group is now classified under division 7: Forest Health. More information on IUFRO can be found on the Internet at the following address: http://iufro.boku.ac.at/.

This meeting was attended by 56 scientists and researchers from 11 countries in North America, Europe and Asia. A total of 35 scientific papers were presented in 7 oral sessions and 16 posters were on display. Two field trips had been planned and proved to be very popular among the participants. As well, they got to stroll through Old Quebec to observe the success the campaign against Dutch elm disease was having in the city.

The following includes most of the contributions that were presented at the meeting with the exception of papers from authors who did not want them published or did not provide their text. In addition, a few manuscripts were included from members of our working parties who were not able to attend the meeting. The papers' contents are the responsibility of the author(s).

Gaston Laflamme, Local Organizer and Deputy IUFRO 7.02.02
Jean Bérubé, Local Organizer
Ursula Heiniger, Coordinator IUFRO WP 7.02.02

ACKNOWLEDGEMENTS

We gratefully thank Mr. Guy Bussières of the Forestry and Geomatics Faculty of Université Laval for his participation within the organizing committee as treasurer. We would also like to mention Mrs. Louise Innes and Mr. Guy Croteau from the Quebec Department of Natural Resources as well as Mr. Robert Blais of Natural Resources Canada for their help in preparing the field trips. Mr. Pierre DesRochers of Natural Resources Canada and Mr. Pierre Côté, representative of Quebec City, must be given credit for preparing and guiding the "elm watch" city tour.

Also, we address our thanks to Ms. Geneviève Roy who helped us during the numerous scientific presentations. As well, we would like to underline the work of Ms. Pamela Cheers, Mr. Claude Heppelle and Ms. Diane Paquet of the publications staff of the Laurentian Forestry Centre. Finally, we would especially like to thank the sponsors who helped us finance this activity: the Laurentian Forestry Centre, Natural Resources Canada, Produits forestiers Bellerive Ka "N" Enda Inc., Les industries James Maclaren Inc. and la Coopérative forestière des Hautes-Laurentides.

J. Bérubé and G. Laflamme

AVANT-PROPOS

Cette réunion devait d'abord avoir lieu à St. John's, Terre-Neuve, à l'invitation du Service canadien des forêts. Toutefois, en raison de la fermeture du Centre de foresterie de Terre-Neuve, il a été décidé de déplacer la réunion à Québec, où elle a effectivement eu lieu du 25 au 31 mai 1997.

Bien que nous soyons dans l'aire des communications instantanées, avec Internet et le courrier électronique, ce type de réunion a encore sa place et offre une excellente occasion de présenter soit des résultats qui ne sont pas encore formulés pour publication dans des revues avec arbitres, soit de l'information qui ne sera jamais publiée ailleurs. Ces rencontres permettent aussi l'échange d'information et offrent la possibilité aux scientifiques oeuvrant dans la même spécialité de discuter entre eux des travaux déjà en marche, de visiter des sites expérimentaux et de planifier des collaborations futures. Nous sommes donc très reconnaissants envers l'Union internationale des organisations de recherches forestières (IUFRO) de nous avoir offert la chance de rencontrer des collègues venant de tous les coins du monde.

Le groupe de travail 7.02.02, « Maladies des pousses, du feuillage et des tiges des arbres », se réunissait en fait pour la première fois sous cette appellation. En effet, un changement fut décidé lors de la dernière rencontre qui a eu lieu à Vallombrosa, près de Florence en Italie, en juin 1994. Les membres des groupes de travail S2.06-04 « Canker and Shoot Blight of Conifers » et S2.06-02 « Foliage Diseases » ont alors décidé de fusionner en un groupe couvrant un plus large spectre de la pathologie forestière. La réduction du nombre de groupes de travail (GT) va dans le même sens que la réorganisation des divisions qui est en cours dans l'organisation. Ce GT fait partie de la Division 7 : Santé des arbres. On peut obtenir plus d'information sur l'IUFRO à l'adresse Internet suivante : http://iufro.boku.ac.at/.

Un total de 56 scientifiques et chercheurs venant de 11 pays d'Amérique du Nord, d'Europe et d'Asie ont participé à la réunion de Québec. Il y a eu en tout 35 présentations réparties en sept sessions et 16 affiches ont été exposées. Les deux excursions sur le terrain ont été très populaires et une marche dans la vieille partie de la ville de Québec nous a permis d'apprécier le succès du programme de lutte contre la maladie hollandaise de l'orme mis en oeuvre par cette municipalité.

Ce compte-rendu comprend les textes de la plupart des communications et affiches présentées lors de la réunion à l'exception de ceux dont les auteurs ne voulaient pas qu'ils soient publiés ou ceux dont les auteurs avaient omis de les remettre. De plus, nous avons inclus quelques textes de membres de notre GT qui n'ont pu assister à la réunion. Enfin, il est à noter que ce sont les auteurs qui sont responsables du contenu de leurs textes/manuscrits.

Gaston Laflamme, organisateur et adjoint IUFRO WP 7.02.02
Jean Bérubé, organisateur
Ursula Heiniger, coordonatrice IUFRO WP 7.02.02

REMERCIEMENTS

Nous tenons à remercier vivement M. Guy Bussières, de la Faculté de foresterie et de géomatique de l'Université Laval, pour sa participation au sein du comité d'organisation, principalement à titre de trésorier du comité.

Nous avons pu compter aussi sur l'assistance de Mme Louise Innes et de M. Guy Croteau, du ministère des Ressources naturelles du Québec, et de M. Robert Blais, du Centre de foresterie des Laurentides de Ressources naturelles Canada, pour leur participation à l'organisation des excursions. Des remerciements doivent aussi aller à M. Pierre DesRochers, du Centre de foresterie des Laurentides de Ressources naturelles Canada, et à M. Pierre Côté, de la Ville de Québec, pour l'organisation et l'animation de la visite reliée aux ormes en milieu urbain.

Nos remerciements s'adressent aussi à Mme Geneviève Roy qui nous a assisté lors des présentations aux différentes sessions scientifiques. Nous avons grandement apprécié l'aide du personnel d'édition du CFL, soit Mme Pamela Cheers, M. Claude Heppelle et Mme Diane Paquet. Enfin, nous voulons remercier tout spécialement nos commanditaires qui ont aidé au financement de cette activité soit le Centre de foresterie des Laurentides de Ressources naturelles Canada, Les produits forestiers Bellerive Ka "N" Enda Inc., Les Industries James Maclaren Inc. et la Coopérative forestière des Hautes-Laurentides.

J. Bérubé et G. Laflamme

FOLIAGE DISEASES

SIGNIFICANCE OF *LECANOSTICTA* spp. TO PINES IN NUEVO LEÓN, MEXICO

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SUMMARY

A survey of the *Lecanosticta* species from Nuevo León revealed that this genus is well represented in the state. *Lecanosticta acicola* (Thüm.) Syd. apud Syd. & Petr. was found on *Pinus ayacahuite* Ehrenb., *P. cembroides* Zucc. and on *P. halepensis* Mill. *Lecanosticta gloeospora* H.C. Evans has been found only on *Pinus pseudostrobus* Lindl. A possible new species of *Lecanosticta* with bigger spores (37-52 x 2.5-3.5 µm) than L. *acicola* was found on *Pinus culminicola* Andresen & Beaman. *Gloeocoryneum cinereum* (Dearn.) Weindlm. [= *Lecanosticta cinerea* (Dearn.) H.C. Evans] was found on *Pinus hartwegii* Lindl. and on *P. arizonica* Engelm.

Keywords: *Pinus* spp., needle blight, Mexico

INTRODUCTION

The genus *Lecanosticta* is a very important fungal forest pathogen, since *Lecanosticta acicola*, anamorph of *Mycosphaerella dearnesii* Barr, causes severe widespread needle blight on several species of pines. Gibson (1978) mentioned this species as the cause of the most important foliage pine diseases in North America. Evans (1984) studied this genus on Central American pines, including some Mexican pines. The same author described a new species of *Lecanosticta*, (*L. gloeospora*) from Nuevo León on *Pinus pseudostrobus* and proposed a new combination: *Lecanosticta cinerea* for *Gloeocoryneum cinereum*. However, this combination was not considered valid, since no basionym was given.

The state of Nuevo León, in northeast Mexico, has a great diversity of pine species. Eleven species of pines are found in the state. Since only a few studies have been made on the fungi associated with these species, this study presents the results obtained from a survey of the genus *Lecanosticta* on pines in Nuevo León and discusses the extent of this disease.

MATERIAL AND METHODS

Collections of pine needles presenting *Lecanosticta*-like fructifications were made. Notes were taken of the host, damage and other ecological features. Specimens were then brought to the laboratory where fungi were placed in a herbarium and examined using usual mycological techniques.

All the examined specimens are deposited at the Herbarium of the Facultad de Ciencias Forestales de la UANL (CFNL). Some specimens of *Lecanosticta* species hosted at the International Mycological Institute (IMI) were also examined for comparison. A complete list of the examined specimens is presented below:

Lecanosticta acicola

IMI 91340 type of *Cryptosporium acicolum* Thümen Flora 1878, p. 78. [*Lecanosticta acicola* (Thüm.) Syd.] USA, South Carolina, Aiken, col. H.W. Ravenel. In *Pinus variabilis* Lamb. 1876. in Herbario Univ. Padova;
IMI 104312 *Lecanosticta acicola*, Durham, North Carolina, USA, 20.04.1940, *Pinus palustris*, F.A. Wolf;
IMI 281599 *Lecanosticta acicola*, Acaxochitlan-Zacatlán, Puebla, Mexico, 9.05.1983, *Pinus patula*, H.C. Evans;
IMI 281582 *Lecanosticta acicola* Montaña de Celaque, Lempira, Honduras, 18.10.1981, *Pinus ayacahuite*, H.C. Evans;
CFNL 318 *Lecanosticta acicola* El Orito, Galeana, Nuevo León, Mexico, 30.07.1987, *Pinus cembroides*, 2300 m.s.l., J.G. Marmolejo;
CFNL 1204 *Lecanosticta acicola* Cerro del Potosí, Galeana, Nuevo León, Mexico, 29.08.1992, *Pinus ayacahuite*, 3000 m.s.l., J.G. Marmolejo;
CFNL 329 *Lecanosticta acicola* Pablillo, Galeana, Nuevo León, Mexico, 25.08.1989, *Pinus cembroides*, 2000 m.s.l., J.G. Marmolejo;
CFNL 1203 *Lecanosticta acicola* Cerro del Potosí, Galeana, Nuevo León, Mexico, 20.06.1992, *Pinus ayacahuite*, 3000 m.s.l., J.G. Marmolejo;
CFNL 319 *Lecanosticta acicola* El Orito, Galeana, Nuevo León, Mexico, 7.06.1990, *Pinus cembroides*, 2400 m.s.l., J.G. Marmolejo;
CFNL *Lecanosticta acicola* Bosque Escuela, Iturbide, Nuevo León, Mexico, 19.01.1995, *Pinus halepensis*, 1600 m.s.l., J.G. Marmolejo;
CFNL 1276 *Lecanosticta acicola* Bosque Escuela, Iturbide, Nuevo León, Mexico, 20.08.1994, *Pinus halepensis*, 1500 m.s.l., J.G. Marmolejo.

Lecanosticta gloeospora

IMI 283812 *Lecanosticta gloeospora* Iturbide-Galeana, Nuevo León, Mexico, 16.05.1983, *Pinus pseudostrobus*, H.C. Evans (Type);
CFNL 505 *Lecanosticta gloeospora*, La Palapa, Bosque Escuela, Iturbide, Nuevo León, Mexico, 27.09.1990, *Pinus pseudostrobus*, 1500 m.s.l., J.G. Marmolejo.

Lecanosticta sp.

CFNL *Lecanosticta* sp. Cerro del Potosí, Galeana, Nuevo León, Mexico, 6.06.1993, *Pinus culminicola*, 3200 m.s.l., J.G. Marmolejo.

Gloeocoryneum cinereum

CFNL 503 *Gloeocoryneum cinereum* Aserradero Ejido 18 de Marzo, Galeana, Nuevo León, Mexico, 15.11.1986, *Pinus arizonica*, 2100 m.s.l., J.G. Marmolejo;
CFNL 507 Gloeocoryneum cinereum Santa Clara, Galeana, Nuevo León, Mexico, 13.03.1991, *Pinus arizonica*, 1800 m.s.l., J.G. Marmolejo;
CFNL 1212 *Lecanosticta acicola* Cerro del Potosí, Galeana, Nuevo León, Mexico, 20.06.1992, *Pinus hartwegii*, 3000 m.s.l., J.G. Marmolejo.

RESULTS

Lecanosticta acicola. This species was found on needles of *Pinus ayacahuite*, *P. cembroides* and on *P. halepensis*. The most severe damage was observed on *Pinus halepensis*, a Mediterranean pine planted at the Faculty's experimental forest for research purposes. The examined specimens showed similar dimensions to those obtained from the revision of the *L. acicola* type.

Lecanosticta gloeospora. It was only found on *Pinus pseudostrobus*. This species, described by Evans from Nuevo León, was found with light needle discoloration. It differs from *Lecanosticta* by its smaller and dark brown ascospores.

Lecanosticta sp. A species of *Lecanosticta* with bigger conidia (37-52 x 2.5-3.5 µm) was collected with needle blight on *Pinus culminicola*, a dwarf pine species with an endemic distribution, that grows at high elevations in Nuevo León and the neighbor state, Coahuila.

Gloeocoryneum cinereum. This species was collected on *Pinus arizonica* and on *P. hartwegii*, with needle blight. Evans (1984) placed this species under *Lecanosticta* based on conidiogenesis and conidial septation. He proposed to name it *Lecanosticta cinerea*; this combination, however, is not valid, because no basionym was given.

This richness of the *Lecanosticta* species could be related with the richness of the pine species (11 species) that are found in Nuevo León. Although no serious problems caused by *Lecanosticta* have yet been reported in the state, foresters should pay attention to this pathogenic genus when pines are used for commercial use (e.g. plantations for wood, pulp or Christmas trees).

We thank the Facultad de Ciencias Forestales, UANL, Linares, Mexico and the Darwin Initiative for the Survival of Species, United Kingdom for supporting this investigation.

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Evans, H.C., 1984. The genus *Mycosphaerella* and its anamorphs *Cercoseptoria*, *Dothistroma* and *Lecanosticta* on Pines. Mycological Papers 153: 1-102

Gibson, I.A.S., 1978. Diseases of Forest Trees Widely Planted as Exotics in the Tropics and Southern Hemisphere. Part II. The Genus *Pinus*. C.M.I., Kew

VARIATION IN MORPHOLOGY OF *LIRULA* ON *PICEA* IN NORTH AMERICA

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SUMMARY

Based on examination of specimens and previous descriptions of fungi in the genus *Lirula*, there appear to be at least four *Lirula* species that occur on *Picea* in North America, only one of which (*L. brevispora*) is currently published as a species. The previously described *Lophodermium filiforme* is likely a taxon distinct from *Lirula macrospora*, with which it has been considered synonymous. Distinguishing traits of the other new taxa are provided. Taxon A occurs in central North America on *P. glauca* (Moench) Voss and *P. pungens* Engelm. Either this taxon or a similar, but distinct, taxon in Europe may be considered equivalent to *Lophodermium filiforme*. Taxon B is a distinctive taxon that occurs in eastern North America on *P. rubens* Sarg. Taxon C is a distinctive taxon that occurs in western North America on *P. sitchensis* (Bong.) Carrière. Seven taxa of *Lirula* on *Picea* are suggested in Europe and North America, as compared with the two currently described.

INTRODUCTION

Darker (1932) provided descriptions of two *Lophodermium* species (*L. macrosporum* (Hartig) Rehm, *L. filiforme* Darker) with elongate hysterothecia on *Picea*. *Lophodermium macrosporum* was basically considered to be a short-spored European species, and *L. filiforme* a long-spored North American species. Terrier (1953) determined that previous measurements of ascospore length of *L. macrosporum* were erroneous, and concluded that the two species represented the same taxon. Darker (1967) subsequently placed *L. filiforme* in synonymy with *L. macrosporum* to form *Lirula macrospora* (Hartig) Darker, but did not provide a description of the new taxon. This left previously described differences unresolved. Cannon and Minter (1984) provided a description of *Lirula macrospora* that was apparently based on descriptions of specimens previously known as *Lophodermium macrosporum*, as, for example, the ascospore length, conidiospore length, and conidiomata appearance fit previous descriptions of that taxon.

When Darker (1967) created the genus *Lirula*, he stated that there is an aggregate of closely related *Lirula* species or varieties on *Picea*, and he noted that further study may necessitate the reestablishment of a taxon distinct from *L. macrospora* based on material he had previously named as *Lophodermium filiforme*. Ziller (1969) subsequently described *Lirula brevispora* Ziller, a notably short-spored (25-35 µm in length) species from British Columbia, Canada, as a species distinct from those previously described. Thus, there are currently two species of *Lirula* described on *Picea*. Stephan and Osorio (1995) described morphological and biological variation among three specimens (two European, one North American) supporting the concept that *L. macrospora* is a complex consisting of at least three different taxa. My observations of specimens and examination of pertinent literature also indicate that *L. macrospora* is a complex of species. The objective of this research is to describe morphological variation in the *L. macrospora* complex in North America.

MATERIALS AND METHODS

Several specimens were examined and published descriptions were used to compare certain morphological and biological characters of fungi considered to be in the *L. macrospora* complex. Specimens and literature examined to provide data for this paper were: for Taxon A: Grand Forks County (Co.), North Dakota (ND), USA, on *P. glauca* (Moench) Voss, collected 25 June 1984 by J. A. Walla; for Taxon B: Yancey Co., North Carolina, USA, on *P. rubens* Sargent, collected 22 June 1995 by G. A. Tuskan; for Taxon C: BPI 651548, Juneau, Alaska, USA, on *P. sitchensis* (Bongard) Carriere, collected 5 July 1953 by J. W. Kimmey; for *Lophodermium filiforme*: BPI 651516 (type specimen), Bear Island, Lake Timagami, Ontario, Canada, on *P. canadensis* (*P. glauca*), collected 1 July 1926 by G. D. Darker, and published description (Darker 1932); for *Lirula macrospora* (*Lophodermium macrosporum*): published description and drawings (Cannon and Minter 1984).

Additional specimens were examined to support the descriptions of these taxa. These included, for Taxon A: Walsh Co., ND, USA, on *P. glauca*, collected 16 May 1984 by J. A. Walla; Walsh Co., ND, USA, on *P. glauca*, collected 4 June 1986 by J. A. Walla; Stark Co., ND, USA, on *P. glauca*, collected 20 July 1987 by J. A. Walla; Pembina Co., ND, USA, on *P. glauca*, collected 15 August 1993 by J. A. Walla; Rolette Co., ND, USA, on *P. pungens* Engelmann, collected 16 August 1984 by J. A. Walla; Pembina Co., ND, USA, on *P. pungens*, collected 8 June 1995 by J. A. Walla; for Taxon B: Yancey Co., North Carolina, USA, on *P. rubens*, collected 19 October 1994 by J. A. Walla; Coos County, New Hampshire, USA, on *P. rubens*, collected 31 May 1996 by W. Merrill; and for Taxon C: BPI 651547, Queen Charlotte Islands, British Columbia, Canada, on *P. sitchensis* collected 28 August 1943 by R. E. Foster; BPI 651549, Youngs River Plantation, Clatsop Co., Oregon, USA, on *P. sitchensis*, collected 23 April 1956 by J. Hunt and E. Wright; BPI 651550, Olney G. S. Highway 202, Oregon, USA, on *P. sitchensis*, collected 26 May 1955 by K. Wright; Juneau, Alaska, USA, on *P. sitchensis*, collected 11 April 1997 by P. E. Hennon; for *L. filiforme*: BPI 651517, Ste. Anne des Monts, Quebec, Canada, on *P. canadensis*, collected 19 September 1928 by J. H. Faull; BPI 651518, Claude, Quebec, Canada, on *P. canadensis*, collected 18 September 1928 by J. H. Faull.

Squash mounts of ascomata were scanned to measure about 10 of the largest individual asci and ascospores. This was intended to somewhat compensate for the expected presence of immature asci and spores in such mounts. Ascomata, conidiomata, and conidia were observed in sections made by hand of fresh material and in sections made by microtome of paraffin-embedded material. Surface characters of ascomata, conidiomata, and discoloration (bands and spots of pigmentation) were made using a stereomicroscope.

RESULTS

Taxa A, B, and C were morphologically distinct (Table 1). The characters that distinguished among these taxa were variations in the ascomata, conidiomata, and width of the basal needle band (a band of pigmentation typical of *L. macrospora* infection). Distinguishing characters of ascomata included position in and on the needle, width of ascomata, relative width of hymenia to width of overall stromata, length of asci, type of fungal tissue to the sides of hymenia, extent of covering by clypei, surface color, and presence of surrounding and underlying pigment. Distinguishing characters of the conidiomata included size of conidiospores, position on the needle, form and size, surface color, and presence of surrounding pigment. In the materials examined, the

three taxa occurred on different hosts and in different regions. Characters not useful in distinguishing the taxa included ascomata length (all variable, extending to length of needle), ascus shape (all a form of cylindric to clavate), ascospore shape (all a form of filiform) and size, and conidiomata position in the needle (all intraepidermal).

Few differences were found between Taxon A and the type of *L. filiforme* (Table 1). The differences consisted of the occasional presence of a black border around ascomata of *L. filiforme* but not Taxon A, somewhat longer asci and ascospores in *L. filiforme*, different shapes of conidiospores (as observed for Taxon A and described for *L. filiforme*), and a somewhat wider basal needle band in Taxon A.

Taxon B appears to vary from all other described taxa primarily by the appearance of ascomata and position of conidiomata (Tables 1, 2). Taxon C appears to vary from all other described taxa primarily by the position of the ascomata in and on the needle (Tables 1, 2). The fungi described as *L. filiforme* and *L. macrosporum* appear to be distinct taxa based on the size of asci and possibly the size of ascospores, dimensions of conidiospores, presence of pigment around and under the ascomata, and presence of pigment around the conidiomata (Tables 1, 2).

DISCUSSION

The variation noted in ascomata and conidiomata among Taxa A, B, and C indicates that they likely represent discrete species of *Lirula*. Caution in assigning species rank is appropriate, however, because the effects of host and environment on these characters need to be considered, particularly in regard to presence and amount of pigment and length of life cycle. Fruiting body position and spore size seem relatively unlikely to be influenced by these external factors. Precedence has been established in using these characters to assign species rank. Darker (1932) wrote that the position of ascomata, that is, the depth of insertion in the host tissues, appears to be a constant feature for each species. He also wrote that length, breadth, and width of ascomata are characteristic in the morphology of individual species. Minter (1981) used position of ascomata in the needles to differentiate species of *Lophodermium* on *Pinus*. Darker (1932) used position and pigmentation of conidiomata to differentiate what are now *Lirula* species on *Abies*. Notably, variation in morphology and pigmentation in different host species and environments is associated with distinct *Lirula* species on *Abies* (Darker 1932). No precedent was found for assigning species rank on the basis of length of the life cycle.

Ascospore length was not distinctive among the specimens observed. However, as Terrier (1953) and Stephan and Osorio (1995) pointed out, measurement of ascospore length is accurate only when using spores that have been released from hysterothecia. This situation essentially means that ascospore length in herbarium specimens should not be used to determine the true value of this character in species in the *L. macrospora* complex.

There appears to be at least seven species of *Lirula* (numbered #1 through #7) that occur on *Picea*. Two are described species, *L. macrospora* (#1) and *L. brevispora* (#2). Another, *L. filiforme* (#3), was described, but later named a synonym of *L. macrospora*; this is likely a distinct species based on characteristics of ascomata, conidiomata, and pigmentation. Type I (#4) as characterized by Stephan and Osorio (1995) would be unique if the ascospores of *L. macrospora* are actually 127-152 µm as described by Terrier (1953); in addition, the dimensions of conidiospores appear distinctive from *L. macrospora* (Table 2). Characters of Type II (Stephan and Osorio 1995) overlapped with characters of Type III except for the appressorium type, but the described

differences in ascospore length may also be diagnostic. Types II and III appear to differ from *L. macrospora* in the lack of a border around the ascomata and conidiomata and in the size of conidiospores. These types could not be differentiated from *L. filiforme* based on the traits provided by Stephan and Osorio (1995). Because Types II and III differ from each other, one must be a distinct taxon (#5), while the other may be considered to be equivalent to *L. filiforme*.

Of the taxa examined for this paper, Taxon A is the same as Type III of Stephan and Osorio (1995). Taxon B appears to be a distinct taxon (#6) based on its unique conidiomata and on a combination of characteristics of ascomata, conidiomata, and pigmentation. Taxon C appears to be a distinct taxon (#7) based on the unique position of the ascomata in and on the host needle and on a combination of characteristics of ascomata, conidiomata, and pigmentation. Type C is likely the only one that can readily be distinguished from all the others in the field, due to the position and color of the ascomata.

Lirula macrospora is a complex of distinct species in North America as well as in Europe. The diversity of *Lirula* species on *Picea* appears similar to that on *Abies*, with seven taxa now suggested.

Table 1. Characters of three taxa in the *Lirula macrospora* complex and of *Lophodermium filiforme*.[1]

Character	Taxon A[2]	Taxon B	Taxon C	*L. filiforme*[3]
Ascomata	- surface glossy black - no black border observed - intraepidermal - between ribs - clypeus extends nearly to edge of stroma - no pigment below stroma	- glossy black in center, light brown beyond - some with narrow black border - intraepidermal - between ribs - edge of stroma extends well beyond clypeus - large pigmented area below stroma	- dull black in center, gray beyond - some with narrow black border - intrahypodermal - centered on abaxial midrib - edge of stroma extends well beyond clypeus - thin line of pigment along base of stroma	- surface glossy black - few with narrow black border - intraepidermal - between ribs - clypeus extends nearly to edge of stroma - no pigment below stroma
Asci	93-125 µm long	155-170 µm long	133-148 µm long	133-140 µm long [135-160]
Ascospores	63-90 µm long	73-88 µm long	63-88 µm long	80-105 µm long [115-160]
Conidiomata	form and size very variable; appear as concolorous pustules between ribs; no pigment observed on border - very difficult to observe	- elongate; centered on adaxial midrib; concolorous with surrounding needle tissue; very narrow black border - difficult to observe	- form and size variable, but tend to be elliptical; no pattern in position; color is light tan on yellowish-brown needles; narrow black border - easy to observe	- form and size very variable; appear as concolorous pustules between ribs; no pigment observed on border - very difficult to observe
Conidia	- cylindrical 5-9 µm long	- cylindrical 4-5 µm long	- not found	- not found [bacillar to allantoid] [5-9.5 µm long]
Host	- *P. glauca* - North Dakota, US	- *P. rubens* - North Carolina, US	- *P. sitchensis* - Alaska, US	- *P. glauca* - Ontario, Canada
Length of life cycle	3 years	2 years	2 years	- unknown
Tissue type on sides of hymenia	- dense pseudoparenchyma	- dense pseudoparenchyma	- loosely packed filamentous mycelium	- dense pseudoparenchyma
Avg. width of hymenia/ stromata	416 µm/471 µm - ratio = 0.88	381 µm/604 µm - ratio = 0.63	379 µm/651 µm - ratio = 0.58	465 µm/504 µm - ratio = 0.92
Basal band width	61-183 µm	168-244 µm	30-76 µm	61-91 µm

[1]Underlined characters are unique to that taxon.
[2]Taxon A is of similar origin to Type III of Stephan and Osorio (1995).
[3]Observations based on examination of type specimen. Information in brackets from Darker (1932).

Table 2. Characters used to differentiate taxa of *Lirula macrospora* for comparison with Table 1.

Character	*Lophodermium macrosporum*[1]	Type I[2]	Type II[2]	Type III[2]
Ascomata	- surface not described - black border present - intraepidermal - between ribs - primarily abaxial - clypeus extends nearly to edge of stroma	- glossy black - mostly with a finely notched dark line - intraepidermal	- glossy black - without a peripheral dark line - intraepidermal	- glossy black - without a peripheral black line - intraepidermal
Asci	110-140 µm long			
Ascospores	56-77 µm long	(52-) 60-88 µm long	136-182 (-210) µm long	102-140 (-156) µm long
Conidiomata	- irregular, often oblong; concolorous with host, with narrow black border, with dark ostiolar area - easy to observe	- form and size very variable; very pronounced differences to the surrounding needle tissue - easy to observe	- form and size very variable - very difficult to observe	- form and size very variable - very difficult to observe
Conidia	- ellipsoidal 1.5-2 µm long	- ovoid (2.4-) 4 µm long	- cylindrical or rod-shaped 4.8-7.2 (-8.0) µm long	- cylindrical or rod-shaped 4.4-9.6 (-10.4) µm long
Host	- *P. abies* - Germany	- *P. abies* - Europe	- *P. abies* - Europe	- *P. glauca* - US
Length of life cycle		2 years	2 to 8 years	3 years
Avg. width of hymenia/stromata	-ratio = near 1.0			

[1]Characters as described by Cannon and Minter (1984).
[2]Types from Stephan and Osorio (1995).

ACKNOWLEDGEMENTS

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LIRULA NERVISEQUIA ON SILVER FIR (*ABIES ALBA*) NEEDLES IN THE MEDITERRANEAN REGION

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SUMMARY

Damage in Italy from *Lirula* (*Hypodermella*) *nervisequia* (DC ex Fr.) Darker has been observed in *Abies alba* stands in the Alps and the Apennines (along the central peninsula). *Lirula nervisequia* causes browning and desiccation on needles two or more years old. In the Alps it affects both young and adult trees, while in the Apennines only young understory trees are colonised. The first symptoms of infection usually appear in the spring. The greatest number of hysterothecia is observed in the autumn. The infected needles generally remain on the shoots and may also be colonised by other fungi. In Italy, the fungus is presumed to be endemic in *A. alba* stands and the epidemic spread of the fungus occurs only after summer drought, when conditions are unfavourable for silver fir growth.

INTRODUCTION

The Mediterranean climate in its annual cycle is characterised by a distinct period of summer drought, a concentration of precipitation in winter, great variability in precipitation from year to year, and hot to very hot summers and intense solar radiation. Most of the trees in this area belong to species that have adapted to these climatic conditions. To give an idea of forest tree distribution during the various ice-ages, it may be mentioned that currently there are ten species of *Abies* in this area, whereas in central Europe there is only one. Many of these tree species grow in mountain areas, isolated from one another, so that they somewhat resemble relict species. Only *Abies alba* Mill. (silver fir) has maintained a more or less continuous range which extends from central Europe to the Mediterranean Basin along the mountain crests of the Italian peninsula (as far as Calabria) and Greece (Seigue, 1985).

Like the tree species of this area, the pathogenic fungi of forest trees are also strongly affected by local climatic conditions, in particular by summer drought, which limits the time of dissemination of pathogens that colonise leaves and needles, so that production and diffusion of spores must be concentrated in the spring and autumn. Many of the pathogens in the area are the same as those found in the temperate regions, but because of their geographic origin, they are subject to water stress in the summer (Biraghi, 1955).

On silver fir, *Lirula nervisequia* (DC ex Fr.) Darker is fairly common. This ascomycete, formerly known as *Hypodermella nervisequia* (DC) Lagerb., usually grows on needles two or more years old, causing dieback, but not abscission. Hysterothecia that are characteristically black and elongated, which often merge to form a single line on the lower leaf surface, form on the needle

venation. The appearance of hysterothecia is preceded by the formation of pycnidial fruiting bodies along the axis of the upper leaf surface. It is usually the needles of the older internodes, understory branches, or branches towards the centre of the crown that become infected (Biraghi, 1963). The fungus is common on *Abies alba* and *A. cephalonica* Loud throughout Europe (Minter and Millar, 1984), but it is only rarely reported as a serious defoliating agent, and then only on particular sites.

MATERIALS AND METHODS

Between 1993-95, serious attacks of *L. nervisequia* were reported on two sites in Italy: at Vallombrosa in the Apennines, and in Val Cauria, an Alpine locality. In both areas, samples of branches with pathological symptoms were collected for inspection.

- At Vallombrosa, periodic inspections were carried out on sample trees from April 1995 to February 1997. At approximately 2-month intervals the number of needles, grouped by age, in each disease category (green; red; with fruiting bodies) was counted and the asci and ascospores were measured.

- In Val Cauria, an assessment of damage was carried out in 1996 with transects of 50 m in the most seriously affected areas.

Precipitation data were recorded on both sites to assess the correlation between precipitation and the development of the disease.

RESULTS

Vallombrosa. Observations in the field and laboratory examination revealed the presence of both known varieties of the fungus: *L. nervisequia* var. *nervisequia* and *L. nervisequia* var. *conspicua* Darker (Darker, 1967). They were found in two separate areas in the Vallombrosa forest.

- *L. nervisequia* with pycnidial fruiting bodies not visible to the naked eye showed elongated hysterothecia that rarely merged into each other. They averaged 2.4 mm long and 0.45 mm wide. There were an average of 4 hysterothecia per needle. The asci were 118-190 x 18-24 µm (avg. 142 x 21 µm) while the ascospores were 46-68 x 2-4 µm (avg. 58 x 2 µm).

- *L. nervisequia* var. *conspicua*, whose pycnidia form a visible dark line along the nervation of the upper needle surface, had the following size: for the asci 140-234 x 20-36 µm (avg. 185 x 28 µm) and the ascospores, 36-102 x 2-4 µm (avg. 70 x 4 µm). The hysterothecia almost always merged into each other, forming a single black fruiting body, of the same length as the needle itself, and of the same width as the other *Lirula* variety (Table 1).

The life cycle of this fungus has not yet been fully established. As with *L. nervisequia* var. *nervisequia*, needle reddening, the first symptom of the disease, is observed in the autumn (Sept.-Oct.) on needles in their second growing season. The hysterothecia appeared the following winter (Nov.-Jan.).

Val Cauria. On this site only the variety *conspicua* was recorded. The percentage of infected needles was extremely variable, and that of needles 2-6 years old with fruiting bodies ranged from 5 to 45%.

On each plot examined the disease affected 20-80% of trees with varying intensity. Trees showing symptoms (comprising more than 70% of all trees) revealed strong infections that were mostly concentrated in the lower third of the crown, where more than 40% of needles were also infected. Many dominant trees suffered damage, though to a lesser degree, in the upper part of the crown, and some even in the apex.

Precipitation data for the growing seasons in May to August of 1992-1994 revealed lower than average precipitation during this period, both at Vallombrosa and in Val Cauria in the Alps. One apparent indication resulting from the examination is that strong outbreaks of the disease tend to occur after a drier than usual growing season. In Vallombrosa, the average number of infected needles during 1992, 1993 and 1994 was respectively 59.6±1.8, 21.4±2.1 and 4.3±0.1. When precipitation levels returned to normal, in 1994, the disease intensity also diminished (Fig. 1).

DISCUSSION AND CONCLUSIONS

The irregular weather pattern of the Mediterranean Basin, with strong periodic swings lasting 10-15 years, favours the spread of pathogens that at any one time are best adapted to a drier or to a more temperate climate. During years with humid and cool springs and summers, pathogens such as *Marsonnina, Dothichiza, Cronartium, Melampsora,* and *Lophodermium* are more likely to thrive. These fungi are normally common in central Europe on poplar and pine, but when condusive conditions occur they will also attack plantations in the Mediterranean area. When the climate moves to a drier part of the cycle, however, these pathogens become sporadic, and are limited to isolated northern mountain slopes, or humid valley bottoms.

L. nervisequia, though very widespread, seems to be a weak pathogen that exploits plants already under stress and that, like a number of other pathogens, becomes more vigorous when the host is subject to water stress during the growing season (Lehtijarvi and Barklund, 1995).

Severe outbreaks of *L. nervisequia* were already reported by Biraghi (1963) in the Vallombrosa forest between 1950-1952, but always on understory trees in humid locations. Biraghi (1963) considered anomalous weather conditions to be the prime reason why the fungus attacked trees growing on the edge of stands with optimal ventilation and sunlight, and then disappeared completely for 10 years.

The fungus outbreak reported here seems to be related to the considerable reduction in precipitation during the 1992-1994 growing seasons. In Val Cauria, where silver fir grows in conditions that are optimal for this fungal species, water stress that occurred in fir stands during this period may be considered the probable inciting cause for the *L. nervisequia* attacks that were reported.

Table 1. Length of ascocarps, asci and ascospores of *Lirula nervisequia* collected from *Abies alba* needles from the Vallombrosa forest.

L. nervisequia	Ascocarps	Asci	Ascospores
var. *nervisequia*	2350 X 450 µm	118-190 X 18-24 µm	46-68 X 2-4 µm
var. *conspicua*	2-3cm X 450 µm	140-234 X 20-36 µm	36-102 X 2-4 µm

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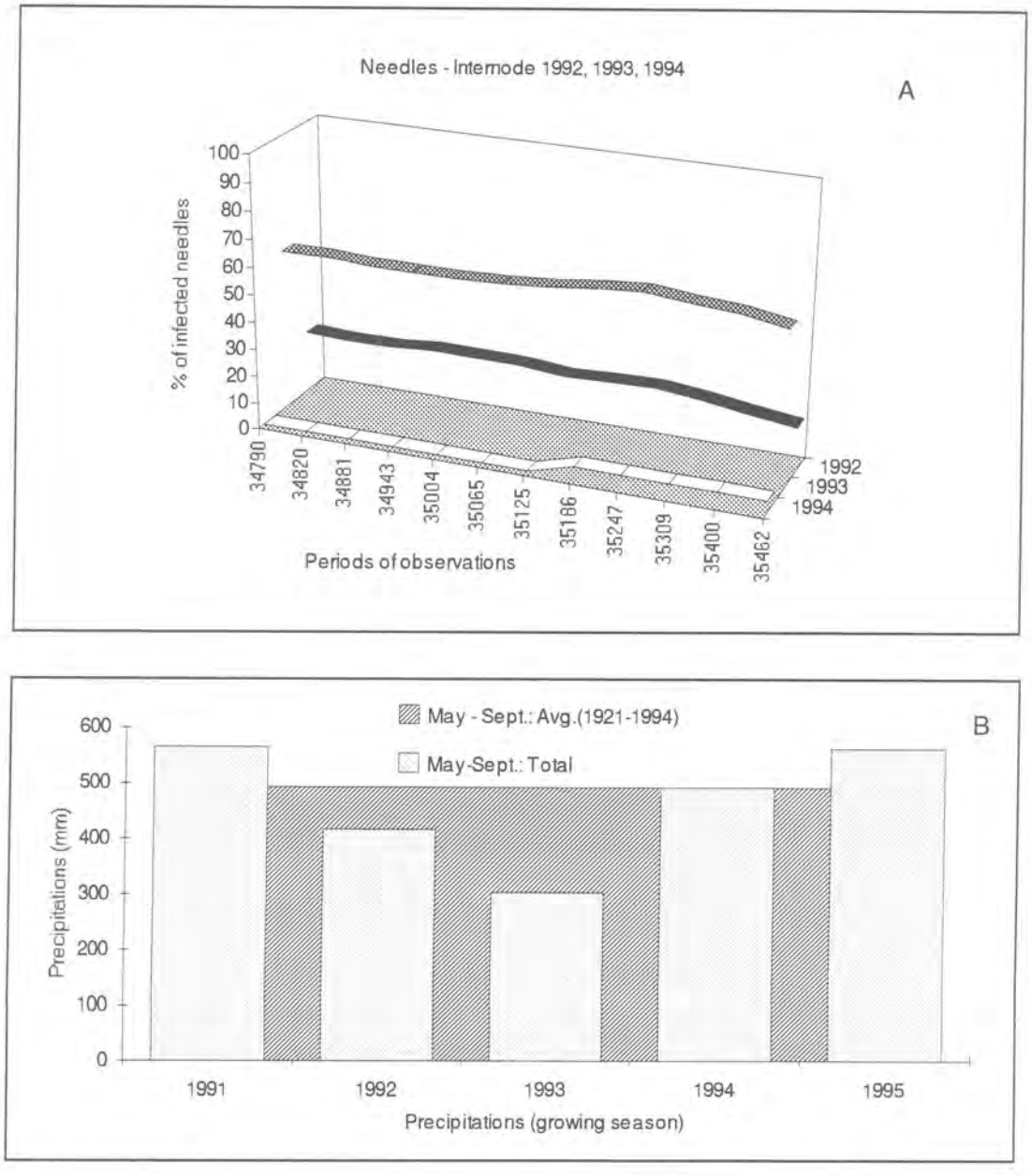

Figure 1. Vallombrosa: A - Percentage of *Abies alba* needles infected by *Lirula nervisequia*.
B - Total precipitation during the growing season (May - September) recorded between 1991 - 1995.

A DESTRUCTIVE SWISS NEEDLECAST EPIDEMIC IN COASTAL OREGON DOUGLAS-FIR PLANTATIONS

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INTRODUCTION

In the 1920's, Swiss needlecast and the fungus that causes the disease, *Phaeocryptopus gaeumannii*, were described as devastating Swiss Douglas-fir Pseudotsugamenziesii plantations (Gaumann 1930). From Switzerland the disease spread to Germany, Austria, and Czechoslovakia, following the prevailing winds (Boyce 1940). In response to this epidemic, a survey was conducted in Oregon & Washington in 1938 (Meinicke 1939). The fungus was found throughout the Douglas-fir range but only on older senescing needles and never causing disease. The disease has since limited the use of Douglas-fir in Europe, New Zealand (Hood & Kershaw 1975), the eastern United States (Boyce 1940), and in Christmas tree plantations (Morton & Patton 1970); wherever Douglas-fir has been grown far from its native range.

Approximately ten years ago, foresters near Tillamook in Oregon's Coast Range began to notice occasional yellow trees. As more and more trees were affected the problem came to be called the Tillamook decline. Just three years ago this was recognized to be an epidemic of Swiss needlecast with the characteristic symptoms of chlorotic needles, low needle retention, and poor height and diameter growth. Last year the Oregon Dept. of Forestry mapped the extent of the disease by aerial survey. They flew the entire Oregon coast and found Swiss needlecast affecting over 50,000 hectares, mostly within a fog belt which extends from the coastline inland about 19 kilometers. This coastal fogbelt has historically been characterized as the sitka spruce-western hemlock zone in which Douglas-fir has always been a relatively minor component of the forest. Today however, these forests have been largely converted to young plantations of Douglas-fir. The worst stands were found in Tillamook County. So what has changed between 1939 and 1997?

The two main hypotheses addressed by the research program underway at Oregon State University are 1) that there is a new, more aggresive strain of the fungus, and 2) that the environment in the spruce-hemlock zone, where the disease is most prevalent, favors the pathogen.

THE NEW STRAIN HYPOTHESIS

Population Structure. We are testing the new strain hypothesis using DNA and pathogenicity markers. So far, we have a culture collection of about 250 single spore isolates, most of which are from the Pacific Northwest. This year we are adding isolates from Europe, New Zealand, Canada, and the eastern United States to our culture collection.

A preliminary study using RAPD (Random-amplified polymorphic DNA) fingerprinting as a means to estimate genetic diversity within *P. gaeumanii* has been completed. We used a small subset of our isolates for the pilot study: three populations in Washington, five in Oregon, three of which were in the Tillamook area, and two isolates from Pennsylvania.

In figure 1, the length of the branches corresponds to relative similarity of the isolate's fingerprints. Although the sample size and number of markers were too small for conclusions, it is evident that there is significant genetic variability in this fungus. There are suggestions of geographical clustering but no discernable correlation with pathogenicity. We are continuing this approach by increasing the sample size and number of markers.

We are also testing pathogenicity by inoculating seedlings in a mist chamber with the macerated mycelium of 5 isolates from stands expressing different levels of disease.

Anamorphic States. It has often been suggested in the literature that *Rhizosphaera* is the anamorph of *Phaeocryptopus*, in part because the two fungi may be found on the same needles. We were skeptical of this claim because not only do the two fungi look very different in culture, but close relatives to *Phaeocryptopus* have hyphomycete anamorphs which produce conidia on unprotected conidiophores. *Rhizosphaera*, a coelomycete, produces conidia within a protective structure. Using cladistic analysis of ribosomal DNA sequences, we have shown that *Phaeocryptopus* and *Rhizosphaera* are not closely related (Fig. 2).

THE CONDUCIVE ENVIRONMENT HYPOTHESIS

Environmental Conditions. The alternate hypothesis is that environmental conditions in the Tillamook area favor spore release, needle infection, and development of the fungus. Nine plots have been installed in three clusters. Each cluster includes one obviously diseased site, one mildly diseased site, and one healthy site, where disease level is estimated by needle color and retention. Each plot has spore traps and a weather station recording temperature, relative humidity, and rainfall.

There were no convincing differences in the weather data. Lower elevation plots with more serious disease were generally warmer through the spring months. These more severely diseased sites were also drier through the spring months. Perhaps too much rain washes spores from the air. Budburst occurred one to two weeks earlier at these sites and *Phaeocryptopus* spores were trapped before budburst at all sites. Earlier work has demonstrated that most infection takes place in newly expanding foliage, so it is easy to imagine that early flushing trees are exposed to more inoculum.

Period of Infection. The next series of experiments was designed to determine period of infection at sites with different disease levels. We are testing the correlation between the length of time needles are exposed to inoculum and subsequent level of infection at test plots with visible symptoms. Last spring, branch tips were bagged before budburst to prevent contact with airborne spores. The bags were removed at two-week intervals through the growing season. This spring we harvested those shoots and counted the pseudothecia emerging from the stomates to determine periods and level of infection. At the severely diseased site, infection occurred until at

least mid-August (Fig. 3). This experiment is being repeated this year to give us a better sample size.

Effect on Host. Although the fungus is constantly associated with diseased trees, it is also present on trees with minimal symptoms. We looked at needle retention to test the relationship between infection and actual damage. Branches were collected from three sites before budburst and pseudothecia were counted on a sample of needles which were infected last spring.

Figure 4 shows the amount of fungus present at different disease levels as determined by needle retention. The severely diseased site, holding only last year's needles, had over 35% of stomata blocked by pseudothecia. The mildly diseased and healthy site, each holding nearly a full needle complement, had far fewer pseudothecia on one-year-old needles.

Height and diameter growth are also reduced by this disease. We measured height and diameter at breast height of ten trees at each of the test plots. The ten trees measured at the severe site averaged much smaller for both height and diameter (Fig. 5).

Wood anatomy is also affected in diseased trees. Healthy Douglas-fir normally has much more spring-wood with large, thin-walled tracheids than it does summer wood, with dense, thick-walled tracheids. Trees infected with *P. gaeumannii* have this ratio reversed, and there is disproportionately more summer wood than spring wood (Fig. 6).

Fungal Biology. We are using microscopy to clarify the conditions and mechanisms of spore infection and subsequent development of the fungus on and within needles. In addition to planned experiments to clarify optimal conditions, we have made several important observations. Using cuticular peels we have found germinating ascospores forming appressoria over stomata. This suggests that the fungus enters the needle through the stomate.

P. gaeumannii colonizes needles by vegetative hyphal growth on the needle surface as well as internally. Using cuticular peels, we have seen a positive correlation between external vegetative colonization and disease intensity. We measured surface hyphae at a high and a low disease site in early summer and again the following spring. At high disease, there is a significant increase in the amount of surface hyphae over the 7-month incubation period (Fig. 7).

We are also interested in seeing how the fungus travels inside needles. Scanning electron microscopy (SEM) shows extensive intercellular travel, with no cell penetration observed. This year will see further SEM work documenting the extent and time-course of travel from initial infection to eventual fruiting of *P. gaeumannii*.

TENTATIVE CONCLUSIONS

1. *P. gaeumannii* is genetically diverse, but there is no evidence yet to suggest a new strain.
2. Rhizosphaera is not the anamorph of *Phaeocryptopus*.
3. Disease is most severe on sites with year round moderate climate and a longer growing season.

4. Infection of current year needles is high on sites with poor needle retention and low on sites with better retention.
5. The fungus increases vegetatively both on the needle surface and internally between cells.
6. Infection is through stomatal penetration.
7. Swiss needlecast severely impacts needle retention, height and diameter growth, and wood anatomy.

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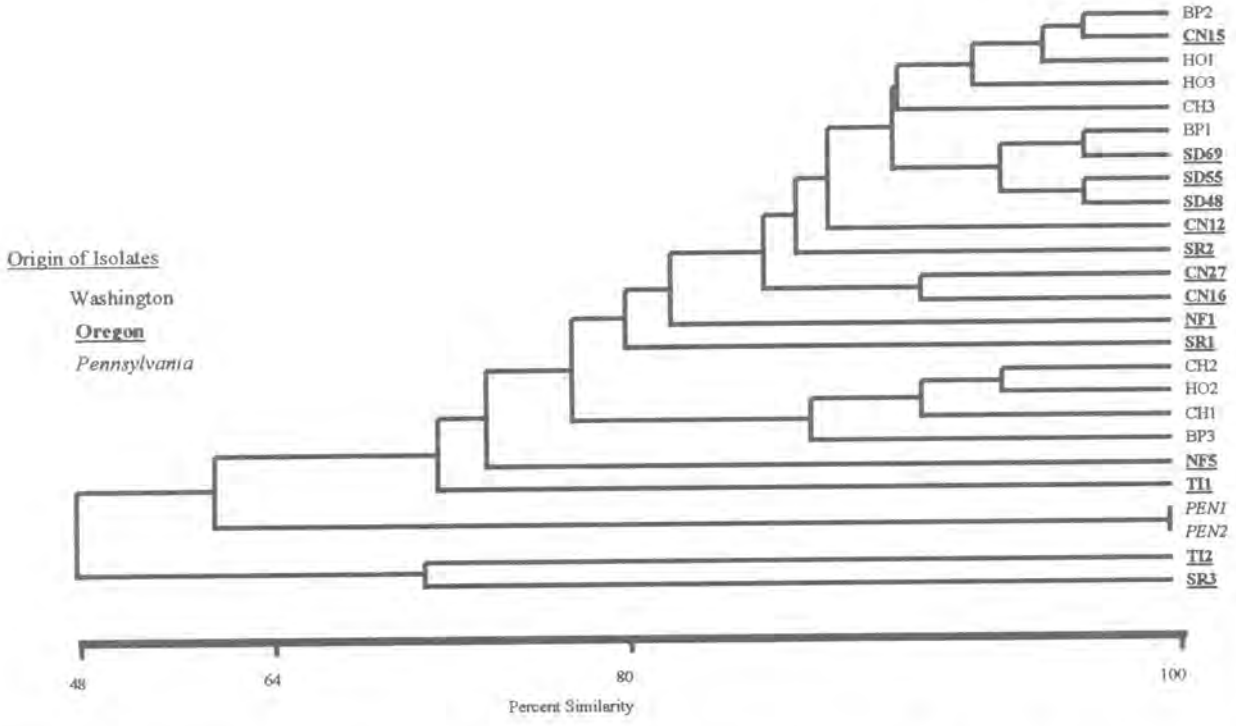

Figure 1. UPGMA phenogram for 17 markers of preliminary RAPD data. Isolates from Oregon are underlined, those from Pennsylvania are italicized, and those from Washington are in normal font. The data were analyzed in NT-SYS using a simple-matching coefficient for the similarity matrix.

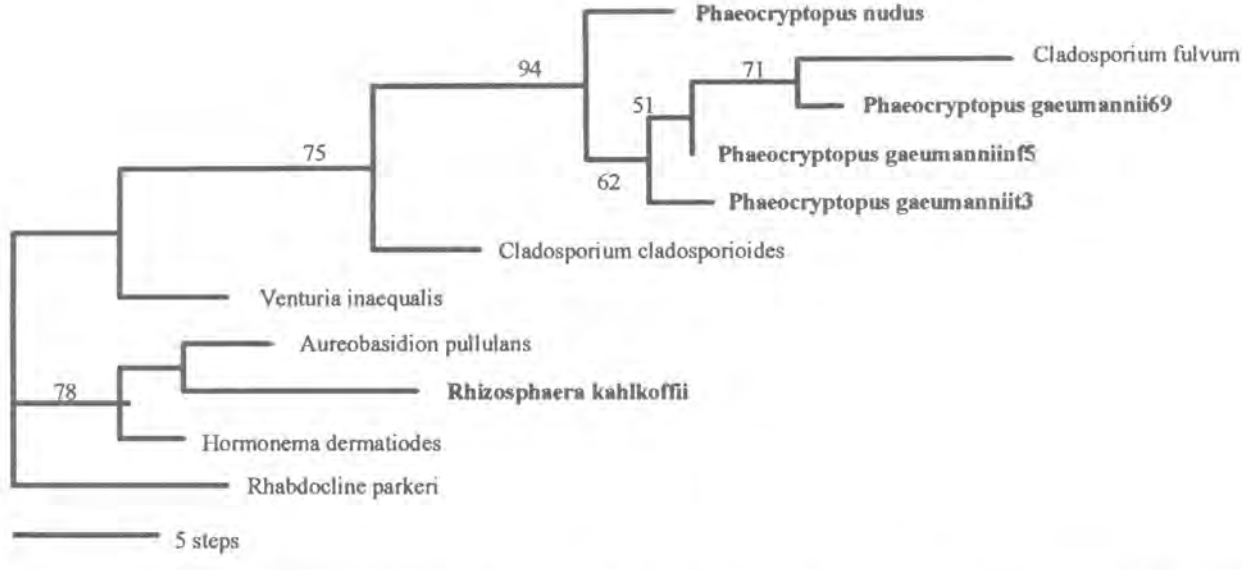

Figure 2. Single most parsimonious tree generated by branch-and-bound weighted parsimony (132 steps) of ribosomal DNA small subunit sequences (PAUP). *R. parkeri* is the designated outgroup. Bootstrap values equal to or greater than 50% are displayed at the nodes. The scale bar represents the number of steps along the branches.

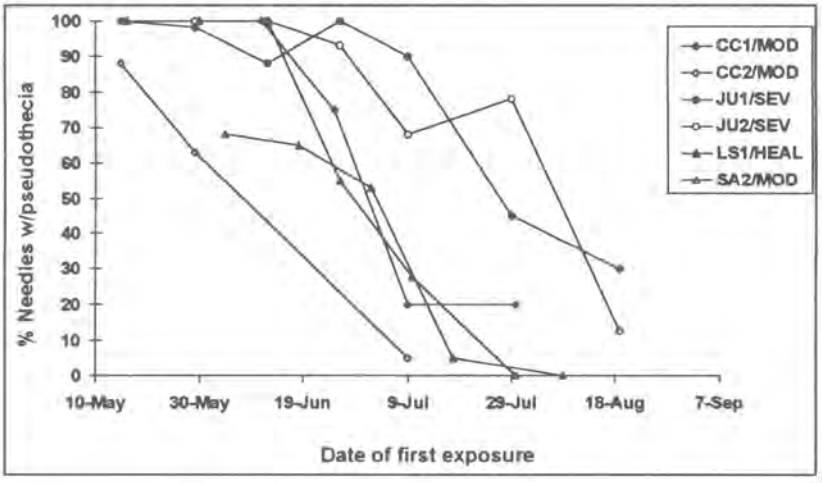

Figure 3. Infection of Douglas-fir needles protected from inoculum for portions of the growing season. Sites differed in disease severity (SEV=severe, MOD=moderate, HEAL=healthy).

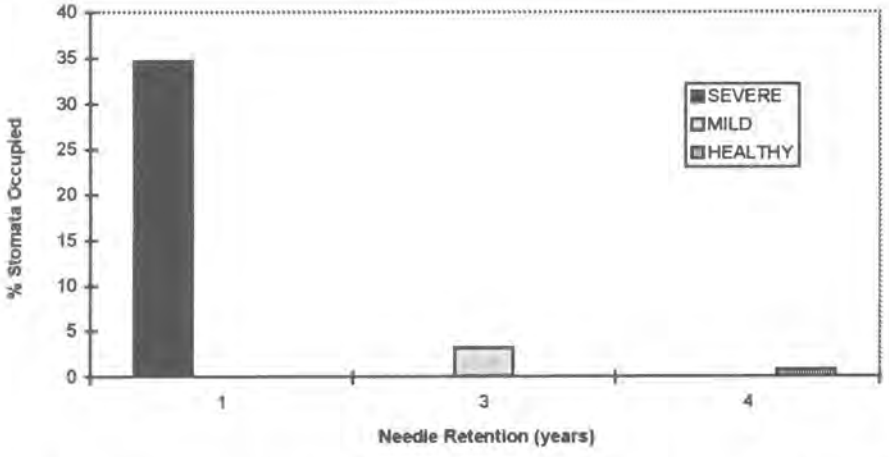

Figure 4. Pseudothecia on stomata of one-year-old-needles at three sites differing in Swiss needle cast severity.

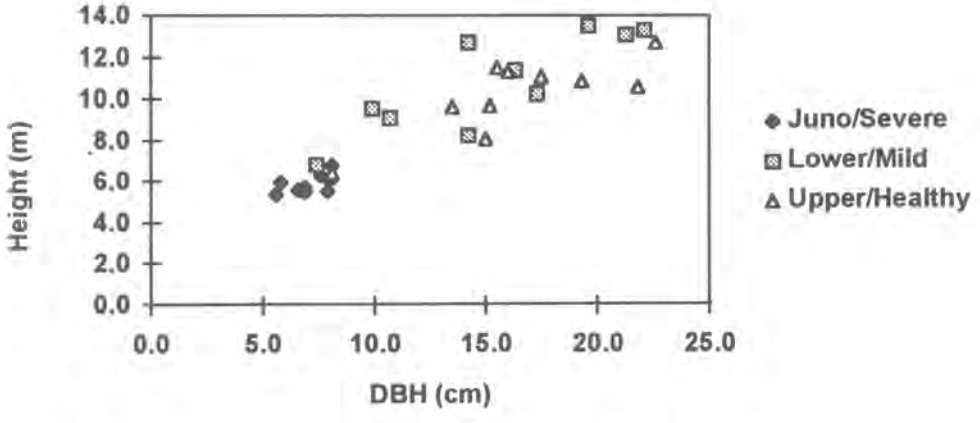

Figure 5. Growth characteristics of trees at three sites with different disease levels.

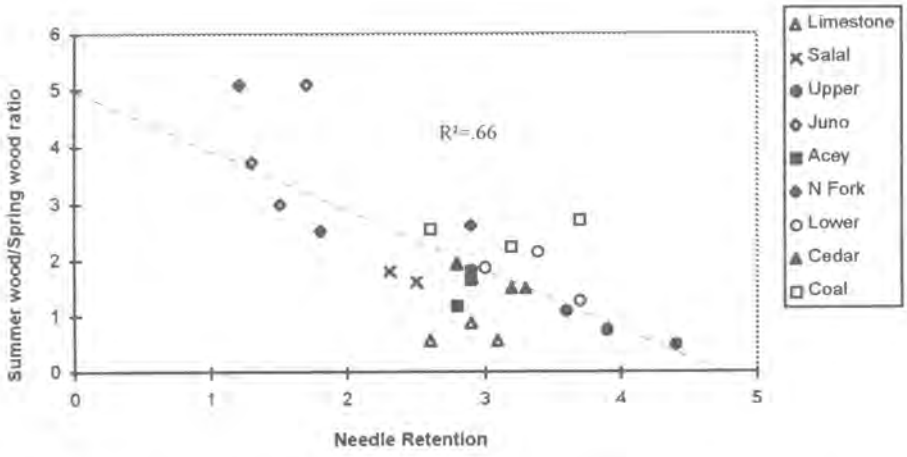

Figure 6. Summer wood to spring wood ratios as a function of the number of needles per year retained by Douglas-fir trees growing at 9 sites of differing disease severity.

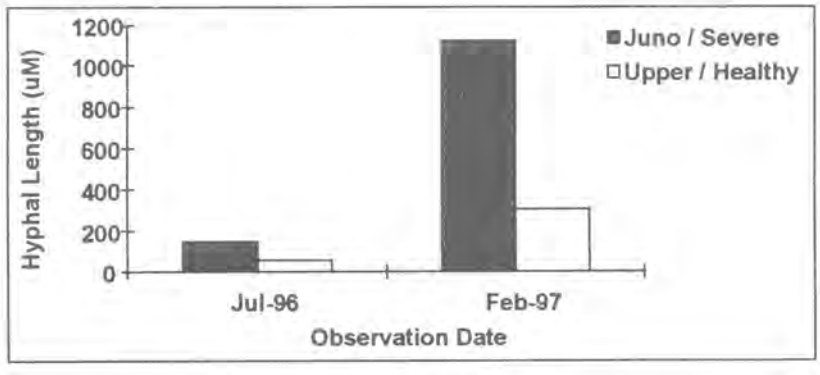

Figure 7. Comparison of average surface hyphae abundance beginning versus end of season at two sites with different disease levels.

NEEDLE FUNGI OF NORWAY SPRUCE - AN INVENTORY IN SWEDEN

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SUMMARY

Litter was periodically collected from four stands of Norway spruce, *Picea abies*, and needle fungi were observed. Needles containing fruiting bodies of *Lophodermium piceae*, *Rhizosphaera kalkhoffii* and *Tiarosporella parca* accounted for up to 47%, 27% and 15% respectively, of the total weight of the needle litter collected over a one-year period. The occurrence of *L. piceae* and *R. kalkhoffii* showed characteristic differences between the stands. The annual fruiting cycle of *L. piceae* was more regular compared with the annual cycle of needle-fall.

Keywords: *Picea abies, Lophodermium piceae, Rhizosphaera kalkhoffii, Tiarosporella parca*

INTRODUCTION

Norway spruce, *Picea abies* (L.) Karst., is indigenous in Sweden where it represents about 45% of the standing volume. The tree species has extended from the north, with the southern boundary of its natural range passing through the very south-western part of Sweden. The planting of indigenous and exotic provenances of Norway spruce, indigenous and exotic provenances has increased markedly during this century, especially in the southern half of the country and also south and west of its natural distribution. Over the last 50 years, faster growth, denser stands and, in some cases, planting on unsuitable sites have all contributed to increasing the susceptibility of Norway spruce forests to drought and to other damages. In addition, the atmospheric deposition of acid and nitrogenous compounds is heavy in the south-west region.

Severe needle loss in Norway spruce occurred in southern Sweden in 1977 and 1983 after very dry summers. There was concern that this indicated the onset of a forest decline in the region. A comprehensive country-wide survey of crown transparency has been under way since 1984. However, to find other criteria for assessing tree vitality an intensive monitoring program was established that covered four mature spruce stands. Among other factors, needle fungi became of interest. Preceding work had shown that fruiting bodies of *Lophodermium piceae* (Fuckel) Höhn. and *Rhizosphaera kalkhoffii* Bubak on dead needles totally dominated the fungal flora of Norway spruce needles in Sweden (Livsey and Barklund 1992). Before it fruits, *L. piceae* lives for several years in the living needles as an endophyte (Barklund 1987), and on the naturally senescent old needles the development of fruit-bodies takes months. *R. kalkhoffii*, however, is regarded as a secondary fungus in *P. abies*, which only invades needles that have sustained abiotic damage (Diamandis 1978). Fruiting takes a couple of weeks on needles that have recently died. *R. kalkhoffii* was never isolated from living needles in Sweden (Livsey 1995).

MATERIAL AND METHODS

Litter was collected in four mature Norway spruce stands. Two stands were situated close to each other at Söderåsen (SÖ, latitude 56°07′) and Tönnersjöheden (TH, lat. 56°46′) south-west of, but close to, the boundary of the natural range of Norway spruce. The third stand is situated at Ydrefors (YD, lat. 57°05′), also in the south but more to the east of the other stands; and the fourth, Svartberget (SV, lat. 64°15′), is located in northern Sweden. At SÖ and TH, both influenced by the Atlantic, the rainfall is about 1100 mm per year, and mean deposition was 20 and 16 kg N/ ha per year respectively for the four-year period investigated. At YD and SV, where there is about 700 mm of rainfall per year, the deposition was 9 and 2 kg N/ ha and per year respectively.

In each stand the litter was collected in eight funnels, each covering 0.25 m^2. The litter was collected every second month from 1988 through 1991 and every third month in 1992. Collections at the SU site could only be made during the growing season because there was too much snow at other times.

The frequency of needles with fruiting bodies of the different fungi was determined by examining 100 needles from each collector under a stereomicroscope. Needles without visible insect damage were chosen. The frequencies were then used to calculate the weights of needles with fruiting bodies of each fungus, which are presented in the figures. There was rarely more than one fungal species per needle, and such data are not included in this report.

RESULTS AND DISCUSSION

On average for the four-year period, the weight of fallen needles per ha and year was 2750 kg at SÖ, 2130 kg at TH, 1610 kg at YD and 930 kg at SV. Amounts collected at each sampling are presented in Figure 1. A large amount of litter is indicative of good tree growth in Norway spruce. Thus the fact that needle-fall was higher at SÖ and TH than at YD suggests that growing conditions were better at the first two sites mentioned. Conditions for good growth in the south-west is the reason why Norway spruce is grown much in this region. The lower litter amounts (i.e. less growth) at SV can be attributed mainly to the much shorter growing season in the north.

In Scandinavia and elsewhere in Europe (Heiniger and Schmid 1986), needle-fall in Norway spruce was reported to show a main peak in the autumn or winter, with a smaller peak sometimes occurring during spring or early summer. In previous work at the sites used in the present investigation (TH and YD) the same pattern was found during 1986-1988 (Livsey and Barklund 1992). However, during 1989-1992 such a pattern in the needle-fall could only be detected at YD and only for one year (1988-89) (Fig. 1). The preceding period (1986-1988) was characterised by unusually cold winters as well as cold and rainy summers. By contrast, during the following years (1989-92), the monitoring period was characterised by very warm winters, with little snow, and dry summers. The unusual weather could be the reason for the different pattern of needle-fall observed. At YD strong peaks in needle-fall in the autumn were observed, but although autumn peaks also occurred at SÖ and at TH, the needle-fall at both sites seemed to be irregular and prolonged, while continuing in the spring (Fig.1). It is also a fact, that at SÖ and TH the winters are influenced by the maritime climate.

At most, about 1300 kg of needles with *L. piceae* was collected per ha during a single year at TH, which represented ca 42% of the needle litter. The corresponding value for *R. kalkhoffii* at SÖ was about 1000 kg, representing ca 27% of the needle litter. Less than 0.1 % (by weight) of the needles collected in the south contained fruiting bodies of *Tiarosporella parca* (Berk. et Br.) Whitney. In the northern SV site, needles with fruiting bodies of *L. piceae* and *R. kalkhoffii* accounted for up to 47% and 15%, respectively, of the needle-fall in one year. The proportion of needles containing fruiting bodies of *T. parca* was, at most, 15%. Solhiem (1989) also reported *T. parca* to be common in northern Norway. Fruiting bodies of other needle fungi were rare at all four sites.

L. piceae fruiting bodies develop in needles falling during autumn and winter (Osorio and Stephan 1991). In most of the collected needles with *L. piceae* the fungus was in the *Leptostroma* stage. Ascomata of *L. piceae* were only found late in the season. Both stages were included when calculating weights of needles with *L. piceae* (Fig. 2). A temporal pattern is evident: Needles with the developing fruiting structures of *L. piceae* started to fall in the autumn, and the main fall of needles with *L. piceae* fruiting bodies was over by February (Fig. 2). At TH, SV and YD, the peak in needle-fall generally coincided with a peak in the proportion of needles with *L. piceae* fruiting bodies but this tendency was somewhat weaker at SÖ. The fruiting of *L. piceae*, which completed its life cycle, seemed to occur more regularly than the needle-fall.

The occurrence of *R. kalkhoffii* (Fig.3) showed a less distinct periodic pattern compared with that of *L. piceae*. The development of *R. kalkhoffii* may be strongly tied to weather events. However, the regular occurrence of *R. kalkhoffii* in the summer indicates that winter can be unfavourable for fruiting of the fungus. Since SÖ and TH were the warmest sites, temperature could explain why the fungus occurred year round at those sites. However, differences in the frequency of *R. kalkhoffii* between SÖ and TH cannot be explained by temperature. The soils at SÖ and TH are of similar type and have about the same water-holding capacity. However, there has been substantial nitrate leaching from the soil at SÖ, whereas no nitrate leaching from the TH soil has been noticed. This difference can be attributed mainly to the higher deposition of nitrogenous compounds at SÖ and suggests that trees are under more stress at SÖ than at TH. One could thus speculate that weather-related damage, and thus needle susceptibility to infection by *R. kalkhoffii*, should be higher at SÖ.

The fungal flora showed characteristic differences between the four sites. However, although the monitoring was continued for more than four years, it is still difficult to generalize the results and identify trends. Needle-fall is greatly influenced by weather, and the results need to be analysed together with accurate weather data.

ACKNOWLEDGEMENTS

Many thanks are due to Dr. D. Tilles for improving the language. This work was funded by The Swedish Forestry Board.

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The weight of needle litter

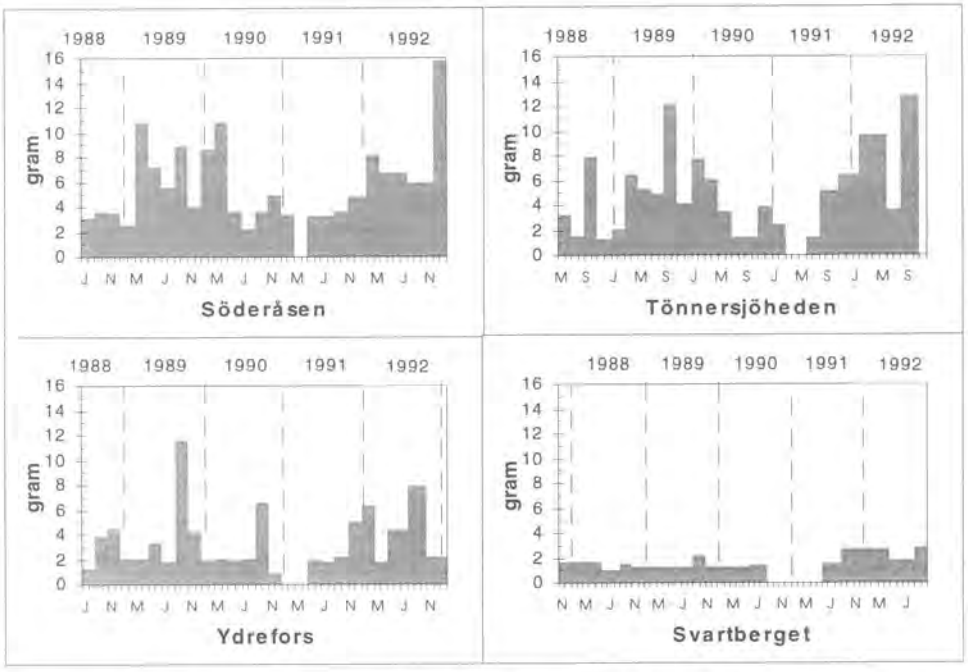

Figure 1. Weight (g) of fallen needles per 0.25 m^2 collecting surface. Each bar represents one sampling period in the mature Norway spruce stands at Söderåsen, Tönnersjöheden and Ydrefors. At Svartberget the litter collected in the spring had fallen during the preceding winter months.

Lophodermium needles

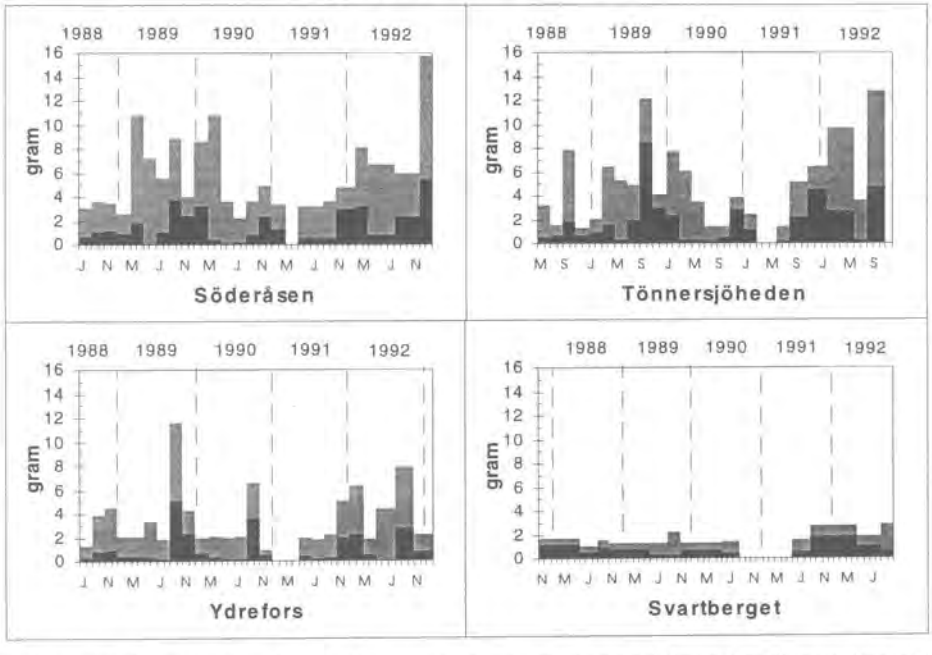

Figure 2. Needles with fruiting structures of *Lophodermium piceae* (black bars) and the weight (g) of all fallen needles (grey bars) per 0.25 m^2 of collecting surface Each bar represents one sampling period in the mature Norway spruce stands at Söderåsen, Tönnersjöheden, Ydrefors and Svartberget.

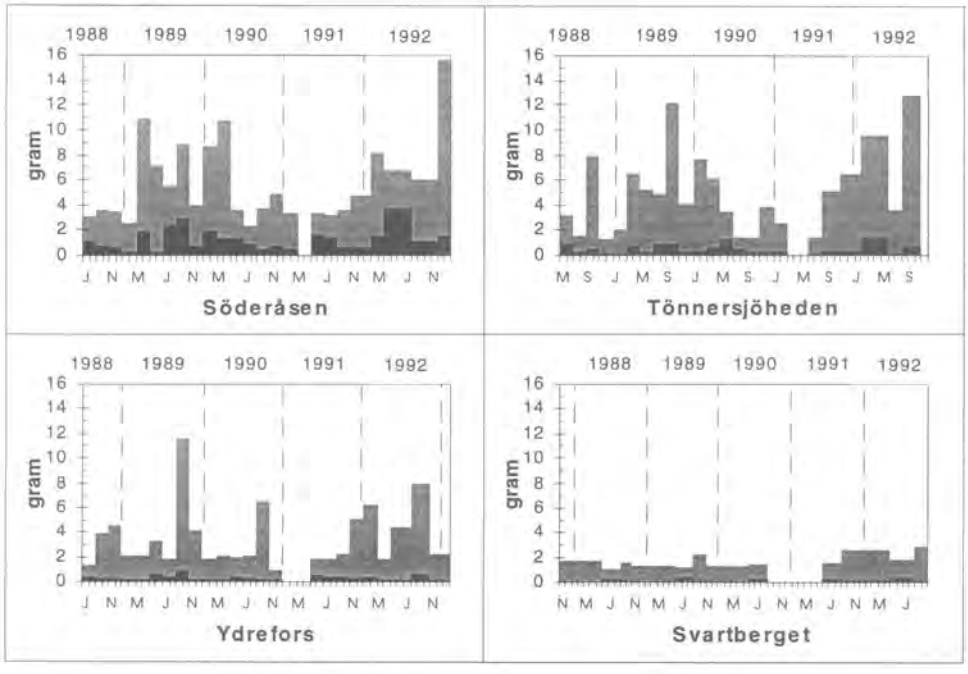

Figure 3. Needles with pycnidia of *Rhizosphaera kalkhoffii* (black bars) and the weight (g) of all fallen needles (grey bars) per 0.25 m^2 of collecting surface. Each bar represents one sampling period in the mature Norway spruce stands at Söderåsen, Tönnersjöheden, Ydrefors and Svartberget.

LOPHODERMIUM SEDITIOSUM IN SWEDISH FOREST NURSERIES DETECTION USING PCR TECHNIQUES AND FUNGICIDE CONTROL

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SUMMARY

The ITS regions of the fungal ribosomal DNA from *Lophodermium seditiosum* and *L. pinastri* were sequenced. After comparing the sequences of these two species, specific primer pairs were designed that made it possible to detect a latent infection of *L. seditiosum* in healthy-looking pine needles.

The fungicide Propiconazol had only a slightly suppressive effect on infection by *L. seditiosum* but the disease was effectively controlled with the fungicides azoxystrobin or fluazinam.

INTRODUCTION

Lophodermium seditiosum Minter, Staley & Millar is a serious needle pathogen on pine. In Sweden *Pinus sylvestris* L. in south and middle Sweden is particularly susceptible. During certain years a heavy infection can result in severe needle loss in young plantations and in nurseries, which can result in reduced growth or even in the death of young seedlings. *Lophodermium* often infects pine seedlings in nurseries. During recent years, serious outbreak of the pathogen have occurred in many nurseries in the southern and middle parts of Sweden, and the recommended fungicides (propiconazol and tolylfluanide) have not been effective in many cases. In other countries various maneb preparations are often used to control this pathogen, but the use of this fungicide is prohibited in Swedish forest nurseries.

Although many different *Lophodermium* species have been found on pine needles (Minter 1981) only *L. seditiosum* is considered to be pathogenic (Diwani and Millar 1987). *L. seditiosum* infects pine during late summer or autumn, and signs of infection (small yellow spots) appear on the infected needles later the same autumn (Butin 1995). In spring after the snow melts red/brown spots appear, and eventually the whole needle turns red. In early summer, small, grey to black pycnidia are formed, resembling a small pencil line. Ascocarps are formed in summer or autumn. Identification of the species is based mainly on the morphology of the ascocarps.

Nursery managers in Sweden have emphasised the need to detect and identify the pathogen during its latent phase and that more effective fungicides are required to control the disease.

MATERIAL AND METHODS

Primer design

DNA was extracted from two isolates each of *L. seditiosum and L. pinastri.* The ITS region of the fungal ribosomal DNA was amplified according to Stenström and Ihrmark (1997a) using PCR (polymerase chain reaction) and the universal primers ITS 1, ITS 2, ITS 3 and ITS 4 (White et al 1990). The whole ITS region from each of two fungi, both containing about 520 base pairs, were sequenced. The sequencing reaction was performed using the Taq Dye Deoxi Terminator cycle in an Applied Biosystem 373A sequencer.

ITS regions for the two fungal species were then compared. At sites with differences in sequence, primers were designed for *L. seditiosum* with as many base-pair differences as possible in the 3' end between the two species. Five primers were placed in the ITS 1 end and four in the ITS 4 end. Each primer consisted of 18 to 24 base pairs.

The PCR amplification procedure described above (Stenström and Ihrmark 1997a) was repeated with an annealing temperature between 58 and 64°C except this time the newly designed primers were used. To determine the specificity of the primers, DNA was extracted from various specimens of fungi in our culture collection that had all been isolated from needles (*Botrytis cinerea* Pers.:Fr., *Cenangium ferruginosum* Fr:Fr, *Cladosporium* sp., *Diplodia* sp., *Lophodermium pinastri* (Schrad:Fr) Chev., *Penicillium* sp.,*Phomopsis* sp., *Phacidium coniferarum* (Hahn) DiCosmo, NagRaj & Kendrick, *Sclerophoma* sp., *Trichoderma* sp.). DNA was also isolated from pine needles (Stenström and Ihrmark 1997) with fruit bodies of *L. seditiosum* and *L. pinastri* and from healthy-looking needles with and without *L. seditiosum.*

Fungicide control

We evaluated the performance of three different fungicides against *L. seditiosum*: propiconazol, which is recommended in Sweden for use against *L. seditiosum* as well as against several other pathogens and which is registered for use in forest nurseries; fluazinam, which is used against fungal pathogens in potatoes in agriculture, but is not registered for use in forest nurseries; and azoxystrobin, a completely new preparation from Zeneca that is being registered for use against fungal pathogens in agriculture (Zeneca 1996).

This investigation was carried out in a nursery, situated outside V„rnamo in the south of Sweden, where there had been serious problems with *L. seditiosum* infection for several years. The effects of the three different fungicides were assessed by comparing treated seedlings with untreated control seedlings. Five plots were treated with each of fungicides once a month during the summer and autumn of 1995, and at the same time seedling were checked for signs (ascocarps) of *L. seditiosum.* The seedlings were treated with fungicides again during the summer of 1996, and a final check for ascocarps was made in August the same year. In each ascocarp check, ten out of 25 randomly chosen needles from every treatment was examined.. The number of infected needles and the number of ascocarps per needle were measured.

RESULTS AND DISCUSSION

Primer design

It was possible to amplify the selected ITS region of the ribosomal fungal DNA from *L. seditiosum* with all the selected primer pairs. In some cases the primer pairs also amplified the ITS region from *L. pinastri* or from the other tested fungi. However, one of the selected primer pairs (Stenström and Ihrmark 1997b) amplified the ITS region from *L. seditiosum* but not those from other tested needle fungi. With this primer pair it was possible to identify *L. seditiosum* in needles with early symptoms of *L. seditiosum*, such as yellow spots, and in needles with pycnidia or ascocarps of *L. seditiosum*. It was also possible to detect an early latent infection in healthy-looking needles from the untreated control seedlings described below under fungicide control. Needles from seedlings testing positive for *L.seditiosum* were incubated and confirmed the presence of *L. seditiosum*.

Fungicide control

Propiconazole did not have any effect on ascocarp formation during the first year (1995), but during 1996, new needles were slightly less infected compared with those on untreated control seedlings. Fluazinam had no effect on ascocarp formation during the first year, but the year after (1996) the new needles did not show any signs of infection. Compared with control seedlings, those treated with azoxystrobin already showed sharply reduced numbers of ascocarps by the first autumn: Almost all of the untreated control seedling had ascocarps on their needles, whereas ascocarps were found on only 10-25% of the needles treated with azoxystrobin. These seedlings were still completely healthy during the following year as well.

CONCLUSIONS

* It is possible to design specific primers against *L. seditiosum* after sequencing the ITS region of the ribosomal DNA. Using these primers in a PCR reaction, a latent infection of *L. seditiosum* in pine needles can be detected before visible symptoms of infections appear.
* An infection of *L. seditiosum* can be effectively controlled with either Azoxystrobin or Fluazinam.

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OBSERVATIONS ON THE MORPHOLOGY AND HISTOLOGY OF *LOPHODERMIUM AUSTRALE* ON ATTACHED NEEDLES OF *PINUS TAEDA*

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SUMMARY

Lophodermium australe on *Pinus taeda* needles was examined morphologically and histopathologically. In general, the morphological features of this fungus were found to be in agreement with previous findings. The histopathology of the affected needle tissue was found to be similar to that of other needle-cast organisms in that the major tissue effect was exhibited as a collapse of the mesophyll cells in the symptomatic tissue with a limited observable presence of *L. australe*.

INTRODUCTION

Lophodermium australe Dearn. is a well documented foliar fungus of *Pinus* spp. with a wide geographical distribution (Hedgcock 1932, Minter 1981). The anamorph of *L. australe* was described by Cooke (1878-9) and the teleomorph later by Dearness (1926). A rather spelling description of this fungus was reported by Minter (1981). This fungus species has been categorized as a saprophyte, pathogen, or both (Bega et al. 1978, Jewell 1993, Minter 1981, Roux and Lundquist 1984, Staley 1975). The present work was initiated by observations over several years in north Louisiana and south Mississippi of *P. taeda* exhibiting symptoms characteristic of "needle cast", but with only *L. australe* present on the dead portion of the attached needles. Only the last flush of needle growth from the previous growing season was observed to be affected by this fungus. When present, the new growth of host pines did not exhibit the presence of *L. australe*.

This report summarizes previous findings and, also, presents observations on the macroscopic features and the pathological anatomy of *L. australe* in needle tissue of *Pinus taeda* L.

MATERIALS AND METHODS

Needle samples (>150) exhibiting needle cast symptoms were collected from April-June on *P. taeda* in Louisiana and Mississippi. The symptoms included: 1) dying back from the needle tip to about one-half or two-thirds with base portion to fascicle green; 2) distinct separation of dead and green tissue, but with a narrow black separation line; 3) twisting and drooping of affected needles; 4) and the uniform and unique presence of ascostroma considered typical for *L. australe* on the dead needle portions (Minter 1981, Dearness 1926). The majority of the samples were prepared for microscopic examination (Jewell 1988), while others were utilized for fresh mounts prepared in lactophenol-aniline blue. In addition, >30 samples from apparently disease free (normal) *P. taeda* needles were collected and examined microscopically.

RESULTS

Normal Needle Tissue

The normal needle samples examined exhibited no visible tissue abnormalities, and were similar to Harlow's (1947) description of *P. taeda* needles. A *Fumago* sp. frequently was observed in stomata of the sample needles, but previous experience indicated this caused no abnormalities in the host tissues (Jewell 1994).

Morphology of the pathogen

The following is a brief summary on the morphology of *L. australe* from work by Cooke (1878-79), Dearness (1926), and Minter (1980, 1981). Also included are more recent observations of this fungus on *P. taeda*.

Stromata were present only on dead needle tissue, which was of a uniform light brown colour. Zone lines were absent. Ascocarps were mostly amphigenous, black, elongate with acute ends and with a common grey border and opened by a longitudinal split (Fig. 1A). Prior to opening, ascocarps often appeared as black lines between two rows of stomata (Fig. 1B). Coalescing ascocarps were rare. Asci were cylindric with an obtuse summit, narrow at the base, and with an obvious terminal pore (Fig. 1C). Ascospores were linear, surrounded by a mucilaginous sheath, discharged through a terminal pore, and frequently coiled in the terminal and/or basal portion of the ascus (Fig. 1D, E). Paraphyses were numerous, linear, and commonly longer than the asci. The paraphyses tip configuration varied considerably; uniformly linear and straight, slightly swollen, or hooked and swollen or non-swollen (Fig. 1F). Some tips appeared to be segmented (Fig. 1F). The sizes of stromata, asci, ascospores, and paraphyses compared favorably with the earlier descriptions (Dearness 1926, Minter 1981).

The anamorph (*Leptostroma durissimum* Cke.) of *L. australe* was frequently observed on the dead portion of the attached needles along with the ascocarps. These stromata were usually exhibited as thin, black lines between rows of stomata, and were usually shorter than the ascocarps (Fig. 1A, B). Conidiomata commonly opened by a longitudinal split at one side of the stroma similar to Minter's Group 3 (1980). Conidiophores appeared to have a swollen base and tapering to a somewhat pointed tip (Fig. 1G). Conidia were hyaline, non-septate, rod-like, linear, much longer than wide, with somewhat obtuse ends, produced terminally on the conidiophores, and expelled in a white mucilaginous mass (Fig. 1H, I).

Pathological anatomy of affected needle tissue

Symptomatic tissue samples from *P. taeda* needles infected with *L. australe* exhibited the characteristic collapse of mesophyll cells considered typical of needle-cast pathogens (Fig. 2A) (Jewell 1990). This cellular collapse was the major cellular abnormality observed in the tissue examined. Hyphae of *L. australe* were scarce in the collapsed mesophyll tissues, but when present were inter- or intracellular, and filamentous (Fig. 2C). For the most part, cellular abnormalities did not extend into or beyond the endodermis (Fig. 2C). However, in some cases, the endodermal cells were slightly crushed, particularly subtending stromatic structures. The hypodermal and epidermal cells appeared unaffected other than in relation to displacement in and around stromata. Hyphae

were often observed in the endodermis, and in the transfusion and vascular tissues. These tissues also appeared unaffected by the pathogen.

A rather distinct separation of non- and symptomatic tissue was exhibited at the juncture of green and dead needle tissue (Fig. 2D). A stationary interface (Jewell 1990) was not observed in the tissue samples examined. Intercellular hyphae were commonly observed in the non-collapsed mesophyll adjacent to the symptomatic tissues (Fig. 2E), however, no obvious cellular abnormality was associated with this hyphal presence.

Stromata of *L. australe*, at maturity, caused the subtending mesophyll to be crushed and pushed onto the vascular system of the host needle (Fig. 2F, G). The stromata were observed to be inserted in the host tissue as subepidermal at the edges, probably subcuticular at the midpoint of the clypeus, and seated on the hypodermis (Fig. 2H, I). There appeared to be displacement of epidermal cells by stromata development (Fig. 2H). Hyphae bordering and subtending stromata were prevalent, conspicuous, and appeared similar to the textura globulosa and textura angularis of Korf (1958). Filamentous hyphae were also observed at the edges in the cavity of the stromata (Fig. 2H, I).

DISCUSSION/CONCLUSION

Examination of *P. taeda* needles infected with *L. australe* indicated this organism caused tissue effects consistent with other pathogenic fungi known to cause needle cast (Jewell 1990). The major abnormality exhibited was the collapse of the mesophyll cells in the symptomatic tissue first described by Hartig (1874) and later assessed to several needle-cast pathogens (Jewell 1990). The anatomical abnormalities associated with *L. australe* in the present work indicate this organism causes pathological reactions in host needle tissue. Subsequently, a reasonable assumption would be that *L. australe* is a pathogen capable of infecting healthy pine foliage. As with other needle-cast pathogens, the considerable collapse of mesophyll cells associated with a scarcity of hyphae of the pathogen, indicates the potential of toxin activity by *L. australe*.

In general, the morphology of *L. australe* was found to agree with the early reports on this species (Cooke 1878-79), Dearness 1926, Minter 1981). The insertion of the stromata in the host tissue was found to be difficult to determine decisively, as indicated by Minter (1981). Variations in particular morphological features may be the result of stages of maturity in the feature or structure observed. Ascospores, prior to release, being longer than the ascus might explain the coiling of such spores in the asci. Indications of segmentation of paraphyses tips may be an expression of age. Young paraphyses may not exhibit such tips. The fine details of the anamorph, *L. durissimum*, require further investigation.

ACKNOWLEDGEMENTS

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Figure Legends

Magnifications of Figures 1 and 2 are approximate. as = ascospore(s); cs = coiled ascospore(s); cm = collapsed mesophyll; ed = endodermis; ep = epidermis or cell(s); hd = hypodermis or cell(s); pf = paraphyses tips; po = pore; a = ascocarp(teleomorph); b = ascus; c = conidiomata(anamorph); d = conidia (um); e = conidiophore(s); h = hypa(e); m = non-collapsed mesophyll cell(s); s = mucilaginous sheath; t = transfusion tissue; v = vascular system.

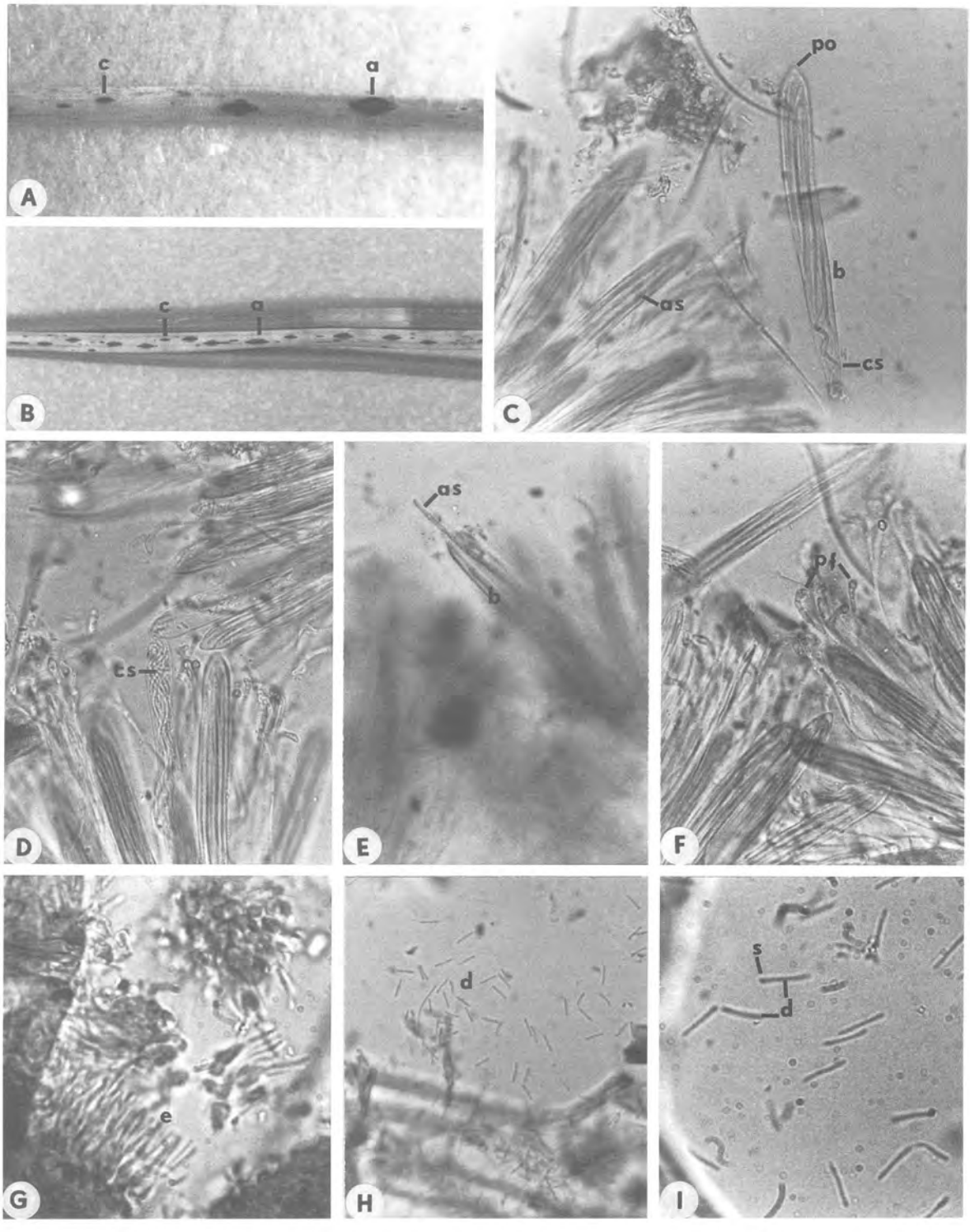

Figure 1. A-B - host needles exhibiting conidiomata and open ascocarps; B - immature conidiomata and ascocarps; C - typical ascus with terminal pore and coiled ascospore in base, 400X; C - coiled ascospores in terminal portion of ascus, 400X; D - ascospore being expelled through terminal pore of ascus, 400X; E - paraphyses tips apparently segmented, 400X; F - conidiophores, 1400X; G-H - conidia, note mucilaginous sheath, 400X, 1400X.

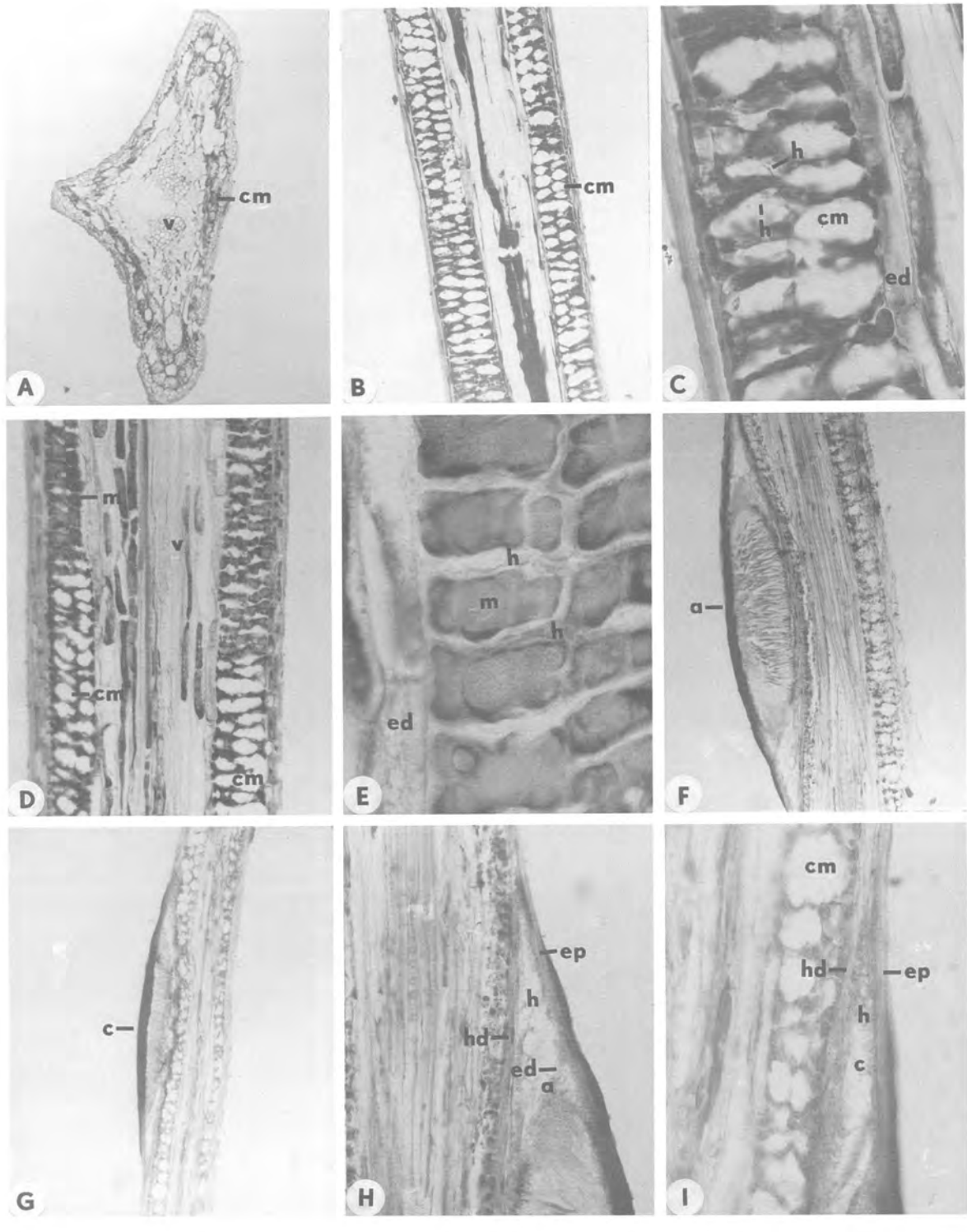

Figure 2. A - transverse view of infected needle, 78X; B - characteristic collapsed mesophyll, cells, 75X; C - collapsed mesophyll exhibiting intercellular filamentous hyphae, 420X; D - juncture of symptomatic and green needle tissue, 131X; E - intercellular hyphae in mesophyll in green tissue, 511X; F - ascocarp - note bending of subtending tissue, 85X; G - conidiomata, 76X; H- sub-epidermal insertion of ascocarp with epidermal cell displacement, 151X; I - subepidermal insertion of conidiomata, 312X.

FOVEOSTROMA MONTENEGRINUM N. SP., A NEW CAUSAL AGENT OF BARK NECROSIS IN SILVER FIR

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SUMMARY

A new species of *Foveostroma* is described. Morphological and cultural characteristics of the fungi are presented. The fungus is associated with occurrence of bark necrosis and dieback branches of silver fir (*Abies alba* Mill.) in the Durmitor National Park, mountain region in Montenegro, southern Europe. It is the first announcement of this genus on conifers in Europe.

Keywords: *Abies alba*, *Foveostroma montenegrinum*, new fungus species, Montenegro/ Yugoslavia.

INTRODUCTION

An investigation of bark necrosis and dieback of silver fir branches (*Abies alba* Mill.) was conducted in the Durmitor National Park (UNESCO World Heritage Site, M&B) in Montenegro, southern Europe (Fig. 1.), from 1991 to 1994. In the late summer, the diseased bark tissue contained fruiting bodies of one undescribed species of *Foveostroma*.

The name *Foveostroma* (*Coelomycetes, Deuteromycotina*) was proposed by DiCosmo (1978) as a new name for previously classified genus *Micropera* Léveillé, and later accepted by the other authors (Funk, 1979, 1981; Sutton 1980; Rossman 1987; Hosagoudar & Balakrishnan 1991). On *Abies* Mill. hosts, the genus represented two species, *F. abietinum* (Peck) DiCosmo was described on *Abies balsamea* and *Tsuga canadensis* (DiCosmo, 1978) and *F. boycei* (Dearn.) Funk on *Abies grandis* and *Pseudotsuga menziesii* (Funk 1967, 1976, 1981). According to Funk (1981), both of the fungi occur with telemorphs in *Dermea* species and are usually considered to be parasites in North America.

In Europe, *Foveostroma* is not a known genus on conifers. Sutton (1980) included one species, *F. drupacearum* (Lév) DiCosmo, the anamorph of *Dermea cerasi* (Pers.:Fr) Fr., which occurs on *Prunus* spp.

MATERIALS AND METHODS

Comprehensive research on healthy and dying coniferous forest ecosystems was carried out from 1991 to 1994 in the Durmitor National Park. Special attention has been paid to five dominant coniferous forest ecosystems where silver fir (*Abies alba* Mill.) is present: I.'Alusko Borje', *Pinetum*

nigrae-illyricum (annual temp. average 7°C or -3°C in January); II.'Stevovica borje', *Pinetum sylvestris* (6°C, -4°C); III.'Razvrsje', *Piceto-Abietum* (5°C, -5°C); IV.'Mlinski Potok', *Piceto-Abieti-Fagetum*, virgin silver fir forest (4°C, -6°C); V.'Crvena greda', *Pinetum mugi montenegrinum* (2°C, -8°C). The elevation gradients of the forest communities were 800-900 m, 950-1100 m, 1200-1300 m, 1400-1700 m, and 1800- 2000 m above sea level respectively, with annual precipitation gradients from 1000 to 2200 mm (Vujanovic 1995).

In each forest community, 24 trees with bark necrosis and dieback branches in the crown (Funk 1981) were recognized. Twelve trees in each of the two high categories, H < 4 and H > 4 m, were selected for sampling. Every year, in early summer, late summer and early autumn, the four samplings of diseased branches from the each of the trees were collected.

Bark from individual branches was examined under a Wild binocular to determine expansion of the bark necrosis or presence of the *Foveostroma* fruiting bodies. Transverse sections of mature conidiomata were examined under a Carl Zeiss light microscope. Pure cultures were obtained by sterilizing bark of 0.4 (long.) x 0.2 cm (diam.) cut portion, rinsed in 70% ethanol for 15 seconds, emerged in 2% sodium hypochlorite for 2 min, then plated out onto 2% potato-dextrose agar (PDA) and 2% malt agar (MA), and incubated for one month at 20°C under diffuse light.

RESULTS

Taxonomy

Foveostroma montenegrinum n.sp. Fig. 2. a; b; c; d; e; f. (Deuteromycotina, Coelomycetes, Phyalostromatinae)

Conidiomata iregularia, eustromatica, pulvinaria, dissita vel gregaria, luteo-olivacea vel olivaceo-brunea, immersa vel erumpentia, originem subperidermalia; irregulatum plurilocularia vel convolutea, raro unilocularia, loculus conidiomatus ad inferiorem vel/et superiorem partem; 700-1600 µm diametro, 500-1000 µm alt.; composita ex cellulis palide-olivaceo-brunneis "textura intricata" ad basim, vel "textura angulatis" ad medium et superiorem partem conidiomatis. Ostiolata absentia, dechiscent in paries apicalem "textura prismatica". Conidiophora circa cavitatem conidiomatis enascentia, simplicia vel ramosa, septata, hyalina, 15-40 x 2.5-3 µm; Cellulae conidiogenae phiallides, hyalinae vel subhyaline, 20-30 x 2.5-3 µm, in muco involutae; Macroconidia blastico-phyalidica, curvata, falcata vel sinuata, hyalina vel pallido-lutea, guttulata vel non guttulata, 0-3-septata (5), 48-87 x 4-7 µm; Microconidia hyalina, aseptata (3), filiforma vel curvata, 15-35 x 2-2.5 µm.

Conidiomata irregular, eustromatic, pulvinate, scattered to gregarious, yellowish-green when young, becoming green-brown at maturity, immersed to erumpent, subperidermal in origin, expanded base immersed in host periderm, conic or flattened, furfuraceous or pruinose; irregularly multilocular or convoluted, rare unilocular, locules situated in the lower part or/and in the upper part of the conidiomata; 700-1600 µm in diameter, 500-1000 µm in height; conidiogenous region subhyaline to pale yellow, when the periphery wall is darker; tissue composed of cells at the base pale olivaceous-brown textura intricata, become textura angularis in the medium and upper region of the conidiomata. Ostiole absent, dehiscence by irregular breakdown and splitting central part of apical wall cells in textura prismatica. Conidiophores completely lining locules, simple or

branched, septate, hyaline, smooth walled, 15-40 x 2.5-3 µm. Conidiogenous cells phialides, hyaline to subhyaline, smooth walled, 20-30 x 2.5-3 µm, invested in mucus. Conidia blastic-phyalidic, hyaline to pale yellow, 0-3 septate (occasionally 4-5 septate in more year-old conidiomata), smooth, thin walled, curved, falcate or occasionally sigmoid, guttulate or not guttulate, 48-87 x 4-7 µm, microconidia hyaline, aseptate (occasionally 1-3 septate in more year-old conidiomata) filiform to curved, 15-35 x 2-2.5 µm.

TYPE: UBFP 5301 (University of Belgrade, Forestry Faculty-Forest Pathology), on branch of *Abies alba* Mill., Zabljak, NP Durmitor, Montenegro/Yugoslavia, 27-VIII-1993. Collected by V. Vujanovic. Determinated by V. Vujanovic.

KNOWN DISTRIBUTION: Montenegro, Yugoslavia
TELEMORPH: Unknown

Cultural characters

Foveostroma montenegrinum appear as slovenly growth on PDA and very slovenly on MA medium (Fig.3). Colonies growing on potato dextrose at 20°C attain a diameter of 5.9 mm and at 4°C of 1.5 mm per month. The cultures produce on PDA, flocosse-moderate aerial mycelium, pale yelow, reverse yellow-brune, margin regular. The fungus growth on the MA at 20°C is 4.6 mm, at 4°C -1.0 mm per month, respectively, and produce appressed, scant aerial mycelium, pale yellow-olivaceous to greyish, reverse olivaceous-brune (Fig. 2.f). Mycelium of this fungus is branching, septate, uniformly narrow, hyalinae, 4-5 µm-wide hyphae.

Disease

The disease was found in 1993 and 1994, only in the virgin silver fir forest *Piceto-Abieti-Fagetum* Blec. in the 'Mlinski potok' basin (1400-1700 m asl.) and mountain pine forest *Pinetum mugi montenegrinum* Blec. of the 'Crvena greda' plateau (1800-2000 m asl.). Most silver fir at these localities, above 1400 m asl., were exposed to severe early frost, -8°C in October 1992. In 1993, the branches (⌀ < 2 cm diam.) and leaders of 11% immature trees (H < 4 m) were covered by fruiting bodies of *Foveostroma montenegrinum*. Two percent of trees over 4 m were attacked. The incidence of fungus infection did not increase in 1994.

The first collection made in late June contained immature pycnidia in reddish necrotic depression areas and on the bark scars of fallen needles followed by the production of well-developed pycnidia that contained mature macroconidia in early August. Callus tissues did not form around bark lesions, but resin was frequently associated with it. Infected branches usually die back to the stem, where the disease can be recognized. Dead needles were often colonized by *Cytospora* sp., *Tiarosporella abietis* (Whitney, Reid & Pirozynski) var. *alba* Karadzic and *Rustroemia elata* (Alb.& Schw. ex Fr.) Rehm. In the other sampling sites, situated under 1400 m asl., *Valsa friesii* (Duby) Fckl. and *Phomopsis abietina* (Hart.) Grove were dominant.

DISCUSSION

Sutton (1980), in the monograph on coelomycetes, gives the generic description of the groups *Corniculariella, Gelatinosporium* and *Foveostroma* in the *Phyalostromatinae,* proposed that the tissue structure of the conidiomata is the primary distinction between types of genera. At the fungus species in this work, conidiomata well developed, eustromatic, unilocular to irregularly multilocular stromata, composed of textura intricata and textura angularis cells; conidiophores completely lining locules, simple or branched, septate; conidiogenous cells phyalides; macroconidia blastic-phyalidic, septate, falcate to sigmoidal; suggest that the fungus should be classified in the coelomycetes genus *Foveostroma.*

Rossman (1987) included, from genus *Foveostroma* on conifers, two species *F. abietinum* (Peck) DiCosmo and *F. boycei* (Dearn.) Funk. *Foveostroma montenegrinum*, in which all morphological characteristics correspond to the typical genus, is readily distinguished from the two other conifer-inhabiting species.

Compared with *F. abietinum* (DAOM 191765) and *F. boycei* (DAOM 56758), including the literature dates (DiCosmo 1978 and Funk 1981), the new species is characterized (Table 1) by larger conidiomata, usually more than 1 mm in diam., immersed or erumpent, multilocular, olivaceous-brown; longer conidiophores and conidiogenous cells, as well as wider macroconidia ($\bar{x}$ diam.> 4 µm ; L/w = 12.3) and microconidia ($\bar{x}$ diam.> 2 µm ; L/w = 11.1).

Table 1. Size of fruiting bodies and spores of *Foveostroma montenegrinum* n.sp. compared with other known species on *Abies* hosts.

Species	Hosts	Conidiomata (µm)	Macroconidia (µm)	Relation L/w	Microconidia (µm)	Authors
Foveostroma abietinum	*Abies balsamea*	500-1000 x 600	40-87 x 3-4	18.1	11-22 x 1.5	DiCosmo (1978)
Foveostroma boycei	*Abies grandis*	300-1000 x 200-500	42-56 x 3-4	14.0	8-14 x 1-2	Funk (1981)
Foveostroma montenegrinum	*Abies alba*	700-1600 x 500-1000	48-87 x 4-7	12.3	15-35 x 2-2.5	Funk (1981) Original

Furthermore, *Foveostroma montenegrinum* is characterized by distinctive culture, compared with *F. abietinum* and *F. boycei* Funk (1967). The fungus growth is slower (on MA is 4.6 at 20°C or 1.0 mm at 4°C per month) than *F. abietinum* and *F. boycei.* Colonies of *F. boycei* (growth on MA at 20°C is 1 cm per month), produce very scant, whitish, yellow, aerial mycelium with 2-3 µm diameter hyphae, while *F. abietinum* produces a floccose culture with abundant white mycelium. *F. montenegrinum* on MA produces appressed, scant aerial mycelium, pale yellow-olivaceous to greyish, reverse olivaceous-brown, with 4-5 µm diameter hyphae.

Moreover, the new species has occurred on *Abies alba* Mill. host, which is different from the two previously mentioned species. Funk (1978) showed that the *Foveostroma* species are specific to host genus, and that the *Dermea* state is apparently very rare in most areas (Funk 1981). Sexual state of *F. montenegrinum* was not discovered.

F. montenegrinum is associated with the bark necrosis of immature trees, especially after early frost, similar to disease in Douglas fir associated with *F. boycei* (Funk 1967, Funk 1981). Other fungi were not present on the diseased bark, and although no pathogenicity tests have been conducted, evidence suggests that *F. montenegrinum* is the causal agent.

CONCLUSION

On the basis of morphological and cultural characteristics, a new species named *Foveostroma montenegrinum* is described on *Abies alba* Mill. This fungus is the first member of the *Foveostroma* to be described on conifers in Europe. Also, this is the first confirmation of the revisionary study of DiCosmo (Can. J. Bot., 1978, 56: 1665-1690) with a newly discovered *Foveostroma* species. The fungus is associated with bark necrosis of silver fir.

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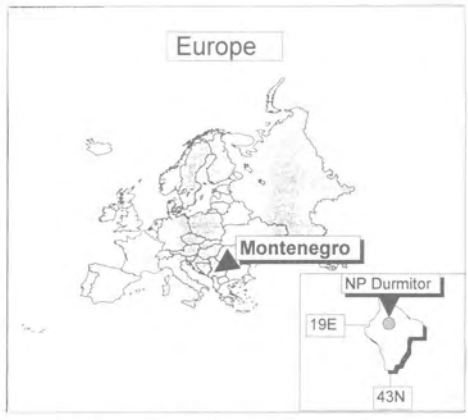

Figure 1. Location of the study area in Montenegro, southern Europe.

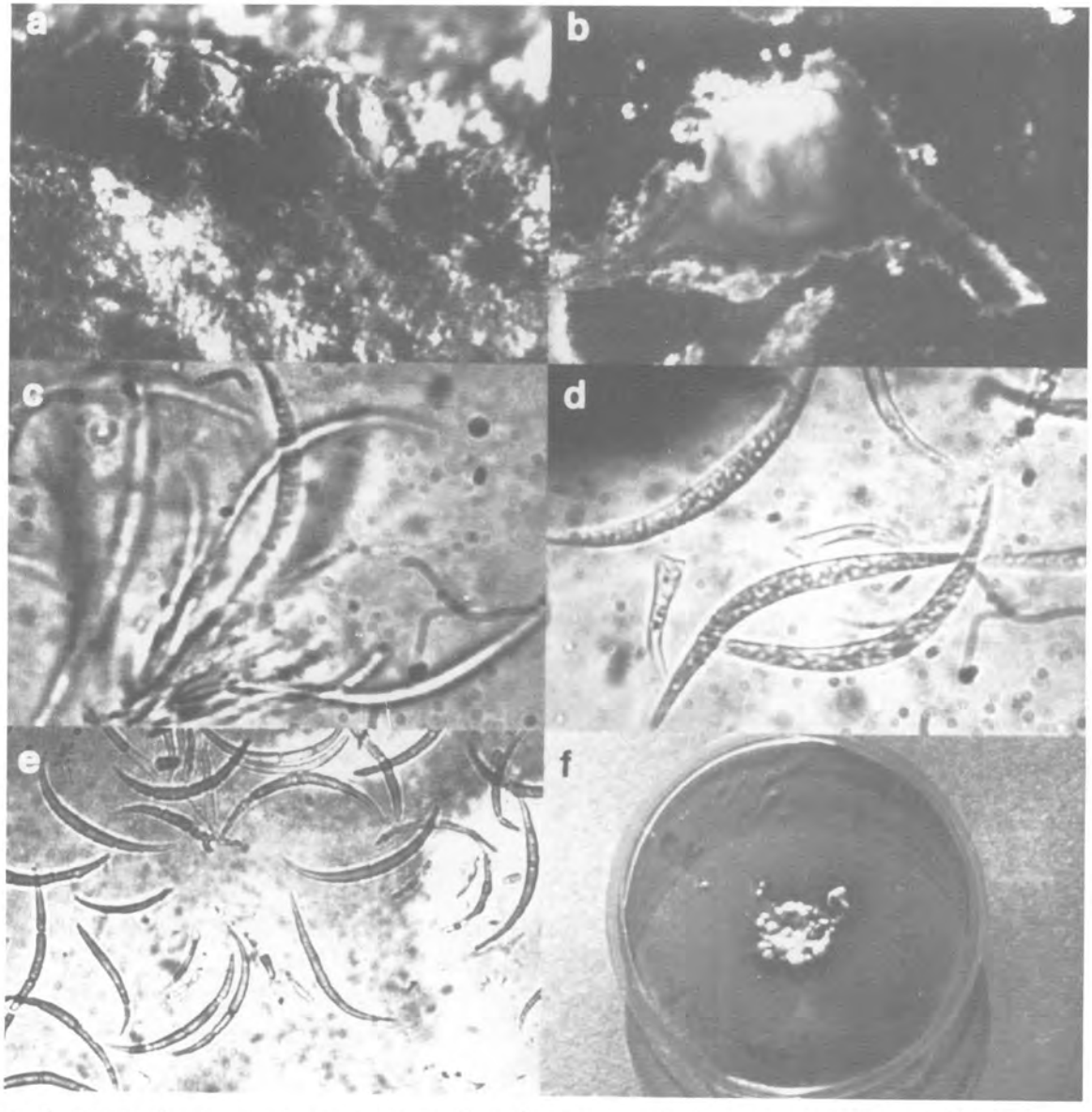

Figure 2. *Foveostroma montenegrinum,* a. Fructifications on bark of the branch from *Abies alba,* with splitting central part of apical wall cells; b. Vertical section of fructification; c. Detail of conidiogenous region; d. Immature conidia; e. Mature conidia; f. 4-month old culture on MA at 20°C.

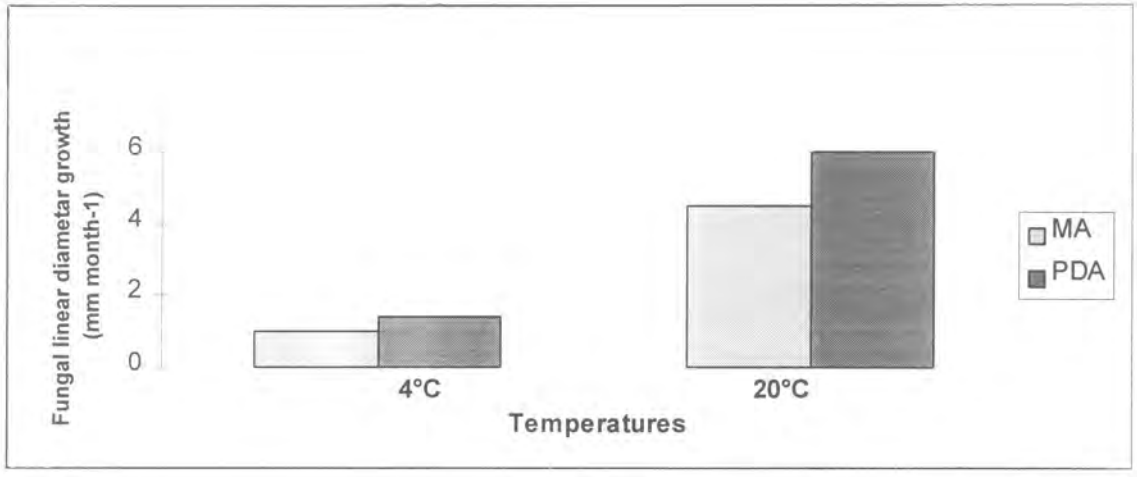

Figure 3. *Foveostroma montenegrinum* n.sp. colony growth on PDA and MA medium.

SEVEN NEW *PINUS* HOSTS FOR *STRASSERIA GENICULATA* (BERK. & BR.) HÖHN

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SUMMARY

Strasseria geniculata is recorded for the first time on *Pinus jeffreyi, P. mugo* 'Hesse', *P. rigida, P. tabulaeformis (Diploxylon)*, and *P. albicaulis, P. cembra, P. strobus* 'Torulosa' *(Haploxylon)*. In the arboretum of the Montreal Botanical Garden, the fungus was isolated from 24% of investigated hosts (n = 29). Based on the morphology and dimension of conidia, as well as basal appendages, the presence of three fungus types - *Chaetosporella geniculata, Strasseria carpophila* and *Plagiorhabdus crataegi* is discussed.

Key words: *Strasseria geniculata*, new pine hosts, needle fungus.

INTRODUCTION

Twenty species and one form have been published in the anamorph-genus *Strasseria* (1982). Sutton (1967) established the modern concept with a description of *Strasseria*, which has proved to produce appendaged phialospores. Also, Sutton (1980) proposed that, regardless of host (coniferous or nonconiferous), only one species of *Strasseria* should be recognized. Recently, comprehensive and revisionary studies by Nag Raj (1982) confirmed Sutton's concept, recognizing only one species, *Strasseria geniculata* (Berk. + Br.) Höhn, from 15 different host genera (trees, shrubs and herbs).

The fungus is known in Australia, Austria, Canada, Germany, Holland, Romania, the U.K., the U.S.A., and the former U.S.S.R. (Sutton 1980, Nag Raj 1982). The first report on this species in Canada was by Parmellee and Cauchon (1979) and it described its presence on *Pinaceae* from Quebec and Newfoundland. Furthermore, Nag Raj (1982) reported the species' presence in Alberta and Ontario, emphasizing the importance of the fungus in Canadian forests where it is associated with branch and twig cankers, and needle necrosis of *Pinus, Abies,* and *Picea*.

More frequently, *S. geniculata* was collected and described from substrates of *Pinus* hosts. Thus, records exist on six pine hosts: *Pinus resinosa* Ait., *P. radiata* D. Don, *P. strobus* L. from North America, and: *P. halepensis* Mill., *P. nigra* Arn. var. *maritima, P. sylvestris* L. from Europe (Parmellee and Cauchon 1979, Sutton 1980, Nag Raj 1982). As a part of a study on the biodiversity inventory of fungi taxa associated with three different pine crown ecological niches in the Montreal Botanical Garden (Vujanovic et al. in preparation), colonization of pine needles by *S.*

geniculata was investigated. This paper redescribes the fungus on seven new pine hosts which were previously never reported.

MATERIALS AND METHODS

An inventory of the coelomycetes found on pines was carried out in the arboretum of the Montreal Botanical Garden (QC) in 1996; 48 pine groups from five sections of the arboretum, representing 21 species, bearing into two subgenuses (*Haploxylon* and *Diploxylon)*, three sections and six subsections. The pines are indigenous on three continents: America, Europe and Asia.

In April 1996, as seen in Table 1, of 29 pine hosts with symptoms of needles necrosis (red-brown needles), approximately four trees for each host were selected. Simultaneously observation, collection and fungus isolation were conducted on 40 needles (current year and second-year needles) from each of the trees. Investigations were carried out at approximately one month intervals, from June to September 1996.

Table 1. Classification* of the investigated *Pinus* species in the Montreal Botanical Garden arboretum and their region of origin

Pine						
Section	**Subsection**	**Subgenus** (No. of needles)	**Species**	**Varieties, form or cultivars**	**Pine region**	**Forest zone**
Strobus	***Cembrae***	*Haploxylon (5)*	***P. albicaulis* Engelm.**	-	Western North America; Mexico	boreal and subalpine
		Haploxylon (5)	***P. cembrae* L.**	-	N.W. and C. Europe	boreal and subalpine; temperate
		Haploxylon (5)	***P. koraiensis* Sieb et Zucc.**	-	Northern Asia	temperate
	Strobi	*Haploxylon (5)*	***P. peuce* Griseb.**	-	Mediterranean	temperate
		Haploxylon (5)	***P. parviflora* Sieb et Zucc.**	-	East and S.E. Asia	temperate
		Haploxylon (5)	***P. strobus* L.**	- 'Pendula' 'Torulosa'	Western North America; Mexico	boreal and subalpine; temperate
		Haploxylon (5)	***P. x shwerinii***	-	-	-
		Haploxylon (5)	***P. flexillis* James**	-	Western North America	boreal and subalpine
		Haploxylon (5)	***P. ayacahuite* Ehr.**	-	Western North America	subtropical
Pinus	***Sylvestris***	*Diploxylon (2)*	***P. resinosa* Ait.**	-	Eastern North America	temperate
		Diploxylon (2-3)	***P. tabulaeformis* Carr.**	-	East and S.E. Asia	boreal and subalpine; temperate
		Diploxylon (2)	***P. densiflora* Sieb et Zucc.**	-	East and S.E. Asia	temperate
		Diploxylon (2)	***P. sylvestris* L.**	- 'Fastigiata' 'Waltereri'	N.W. and C. Europe; Northern Asia	boreal and subalpine; temperate

Table 1 (cont'd)

Pine						
Section	**Subsection**	**Subgenus** (No. of needles)	**Species**	**Varieties, form or cultivars**	**Pine region**	**Forest zone**
		Diploxylon (2)	***P. nigra* Arnold**	- 'Laricio' 'Nigra'	Mediterranean	temperate
		Diploxylon (2)	***P. leucodermis* Ant.**	-	Mediterranean	temperate
		Diploxylon (2)	***P. mugo* Turra**	- 'Gallica' 'Hesse'	Northwestern Europe	boreal and subalpine; temperate
		Diploxylon (2)	***P. uncinata* Mill. Mirb.**	-	Northwestern Europe	temperate
Pinaster	***Australes***	*Diploxylon (3)*	***P. rigida* Mill.**	-	Eastern North America	temperate
	Contortae	*Diploxylon (2)*	***P. contorta* Dougl.**	-	Western North America; Mexico	boreal and subalpine; temperate
	Ponderosae	*Diploxylon (2- 3)*	***P. jeffreyi* Grev. & Balf.**	-	Western North America, Mexico	boreal and subalpine; temperate
		Diploxylon (2-3)	***P. ponderosa* Dougl.**	-	Western North America; Mexico	temperate

*Classification according to A. Farjon (1984)

The needles were examined under a Leitz Orthoplan microscope at 20x, 40x and 100x magnifications, in order to determine morphology and size of fruiting bodies (20 f.b./host) and conidia (100 con./host) of the fungus. Pure cultures were obtained by sterilizing 0.5 cm long needle portions, emerged in a 1% sodium hypochlorite solution (20% Chlorax®, CA, USA) for 2 min, rinsed for 10 s in sterile water, and then plated out onto 2% potato-dextrose agar (PDA, Becton Dickinson) and incubated at 21 °C.

RESULTS

Disease

S. geniculata was isolated from seven or 24% of the investigated hosts (29), as follows: North American - *P. jeffreyi* Grev. + Balf. and *P. rigida* Mill. (*Diploxylon*), *P. albicaulis* Ehrenb. and *P. strobus* 'Torulosa' *(Haploxylon);* European - *P. mugo* Turra 'Hesse' *(Diploxylon), P. cembra* L. (Haploxylon); East Asian - *P. tabulaeformis* Carr. (*Diploxylon*). In these hosts, the symptoms initially emerge as yellow and reddish brown spots, or transverse dispersed bands throughout the surface of the needles. The frequency of fungus pycnidial conidiomata on needles was very low in early summer (0.1%), increased in later summer (1.2%) and was higher in early autumn (5%). Fruiting bodies (pycnidia) of *S. geniculata* developed into reddish-brown spots and bars on both the abaxial and adaxial surfaces of the needles. The dominant symptom was a reddish-browning and casting of 2nd-year foliage in autumn, when more than 50 black, fully mature, pycnidial stroma may be counted on each needle (Fig. 1A). In all cases, only the needles of the lower canopy were infected.

Description of the fungus S. geniculata

From pines, abundant occurrence of fruiting bodies in early autumn on necrotic or fallen needles are picnidial conidiomata, immersed or erumpent, dark brown or black, single or aggregated, lacunose or multilocular, ostiolate, wall of textura angularis, 90-250 (350) µm diam., 60-150 (250) µm deep. Conidiophores arising from the inner cells of the locular walls, around the locule, branched at the base, 1-2 septate, often reduced to conidiogenous cells, smooth, hyaline, in mucus. Conidiogenous cells phialides, hyaline, smooth-walled, 3-10 x 2.3-3.7 µm. Conidia hyaline, blastic-phialidic, allantoid to botuliform, apex obtuse, base truncate, smooth walled, sometimes 2-3 guttulate, 7-15.7 x 2.3-3.7 µm ($\bar{x}$ = 11.3 x 3.0), with an apical mucoid appendage 2-6 µm long, and a basal-filiform appendage 6-27 x 0.5 µm by which the conidium is attached to the conidiogenous cell (Fig. 1. A, B, C, E). The telemorph was not observed for *S. geniculata.*

Mycelium of *S. geniculata* is hyaline, branching, septate, not uniformly narrow, 4-8 µm wide hyphae. The diameter growth of the colonies on PDA was 12 mm per day at 21 °C. Starting as pale brown-grayish, becoming black later (Photo 1D) with a dark brown colour on the inverse side of the Petri dish. There were few differences between the distribution of fruiting body into culture obtained from different hosts. Fruiting bodies abundant, simple or aggregate stroma, uniformly distributed throughout mycelium. Sometimes, the stroma was more aggregate and, as for isolates of *P. mugo* 'Hesse', reduced in the middle of the colonies.

DISCUSSION

A total of 50 synonyms are known for this fungal species (Sutton 1980, Nag Raj 1982). Nag Raj (1982), on the basis of conidial length and width, and basal appendage length, described 11 fungus types of *Strasseria geniculata.* The average size of conidia and appendages, according to the original description from *Pinus* at the Montreal Botanical Garden, corresponded to three of the described fungus types (Table 2).

Table 2. Size of conidia and basal appendages of *Strasseria geniculata* on seven different hosts in the Montreal Botanical Garden arboretum.

Host collections	Conidium (µm)			Fungus specimen types *
	Length	Width	Basal appendage length	
P. albicaulis *P. cembra* *P. rigida* *P. tabulaeformis* *P. jeffreyi*	9-15.7	2.5-3.5	8-17	*Strasseria carpophila*
P. mugo 'Hesse'	7.0-12.5	2.5-4.0	6.0-11.5	*Chaetosporella geniculata*
P. strobus 'Torulosa'	8.0-13.8	2.5-3.5	7-27	*Plagiorhabdus crataegi*
Pinus spp.	7.0-15.7	2.3-3.7	6.0-27	*Strasseria geniculata*

*Our specimens were compared with data from the reference fungus types described by Nag Raj (1982).

The morphology and dimensions of conidia and basal appendages in this work, compared with the Nag Raj (1982) data, suggested that the following fungal types are present in *S. geniculata* on pine species at the Montreal Botanical Garden: *Chaetosporella geniculata*, *Plagiorhabdus crataegi* Shear and *Strasseria carpophila*. Among them, *C. geniculata* basal tubular appendages of conidia were the most reduced. The Nag Raj (1982) list of examined fungus types in *Pinus* hosts includes *C. geniculata* as well as *Pestalozzia rolandii* Fautr., *Sphaeropsis geniculata* Berk. + Br. and *Strasseria nigra* Dearn.

For *Strasseria geniculata*, all seven fungus - host relationships are new reports. It is also the first report of the fungus on an East Asian pine species, *P. tabulaeformis*. With the exception of *Pinus rigida*, all the others (Table 1) originated from boreal and alpine forest zones.

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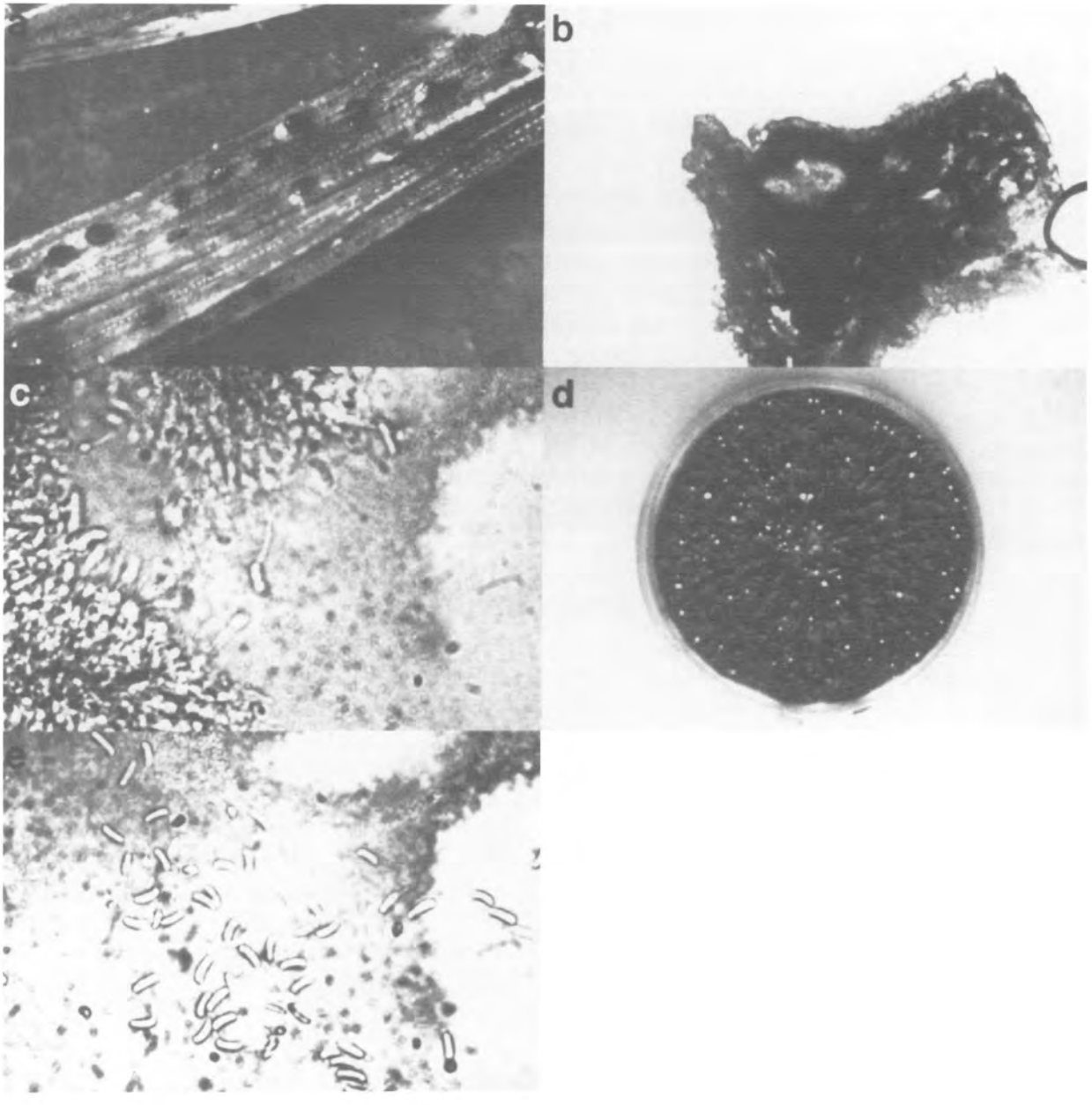

Figure 1. *Strasseria geniculata*: A. Fruiting bodies on needle; B. Vertical section of conidioma; C. Conidiogenous cells; D. 1-month-old colony; E. Mature conidia.

PHAEOCRYPTOPUS GAEUMANNII ON DOUGLAS-FIR PROVENANCES

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SUMMARY

In a 22-year-old field trial with the extensive IUFRO Douglas-fir seed collection of 1966-68, several provenances were selected for investigations about possible differences in the susceptibility against the needle cast fungus *Phaeocryptopus gaeumannii*. From the 18 provenances of the green variety, 8 of the grey variety and 5 of the blue variety were included. The percentage of retained needles and the number of fungal pseudothecia on needles of four consecutive needle age classes (1987 to 1990) generally showed a great genetic variation between provenances, although differences between Douglas-fir varieties and regions were not so clear. The highest numbers of fruit body formation occurred in four provenances from interior eastern British Columbia. Southern interior provenances of the blue variety were generally less attacked. Provenances of the green variety showed an intermediate behaviour. The annual loss of needles did not necessarily correspond to the annual pseudothecia formation of the fungus on the respective provenance.

INTRODUCTION

The fungus *Phaeocryptopus gaeumannii* (Rohde) Petrak causes a serious needle cast disease in Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco). This ascomycete was first found in Switzerland in 1925 (Swiss needle cast disease) and was later described as a new species under the name *Adelopus Gäumanni by* Rohde (1936). In the thirties, the disease spread epidemically from Switzerland through southwestern Germany, and was also found in northern Germany and Denmark. Apparently, it was introduced from the south (Lyr 1958). The disease caused severe losses in Douglas-fir stands. In the last decades, the fungus occurred only locally and sometimes became rare. But during the last ten years a severe increase of infections was observed in various Central European countries (Van Dam 1991, Schultze and Raschka 1992, Küchemann 1993, Bietlot and Malaisse 1994, Schröter et al. 1997). For about 20 to 30 years, Douglas-fir was increasingly used for reforestation, and today the stands are even-aged. The fungus can now be found in many 20 to 30-year-old European Douglas-fir stands.

Since the first occurrence of the Swiss needle cast disease, it has been reported on various individual trees, progenies or provenances of Douglas-fir to *P. gaeumannii* infections (e.g Hood 1982, McDermott and Robinson 1989, Nelson et al. 1989, Bietlot and Malaisse 1994, Hood et al. 1990). In the present study, first results are given about the variation of Douglas-fir provenances regarding the *Phaeocryptopus* needle cast disease under the environmental conditions of northern Germany.

MATERIALS AND METHODS

Douglas-fir provenance trial

The extensive IUFRO Douglas-fir seed collection of 1966-68 offered a good opportunity to study possible variation of provenances regarding different susceptibilities to *P. gaeumannii*. A field trial (Dgl 7) was planted with 3-year-old seedlings in the spring of 1973 in Grosshansdorf (Schleswig-Holstein, northern Germany) and arranged in a randomized complete block design with four replications. Coordinates for the trial were: 10°12'E, 53°39'N at an altitude of 40 m. Annually, the mean temperature was 8.1°C (May to September 14, 3°C) and the mean precipitation was 739 mm (May to September 354 mm).

Beginning in the late eighties one could observe increasing infections of the trees by *P. gaeumannii* in the Douglas-fir plantation. The four replications of the field trial seemed to be homogeneously infected by this needle cast disease. Therefore, the Douglas-fir plantation was investigated to determine whether there were differences in the susceptibility of provenances to this disease. Thirty-one provenances from the natural range in North America were selected with 18 from the coastal regions of British Columbia, Washington, Oregon and California, 8 from the interior of British Columbia, Washington and Oregon, and 5 from the southern interior of Colorado, Arizona and New Mexico (Table 1).

Collection of samples

For further assessments, up to 16 branches were collected in the fifth whorl from the tops of four trees of each provenance in the spring of 1991 before flushing. The internodes of the four consecutive years (1987 to 1990) were investigated in these branches regarding annual shoot length, total number of needles per annual shoot (age class), percentage of retained needles and needle length. Also the number of pseudothecia of *P. gaeumannii* was examined on the undersides of three randomly selected needles per age class by using a dissecting microscope. The average numbers of fruit bodies per 10 mm were calculated for each provenance from the total number of fruit bodies divided by the total length of the three needles.

In the following text, the total number of needles per age class, the percentage of retained needles, and the differences between provenances in the mean number of fungal fruit bodies per 10 mm needle length are shown.

Statistical analysis

Statistical analyses were calculated with the SAS program package (SAS Institute Inc. 1989).

RESULTS

Needle retention

The total number of needles per annual shoot varied between provenances, trees and age classes depending on the respective shoot length. The annual shoots of four investigated trees

per provenance had originally been between 40 and 60 needles. The number of needles was generally highest in provenances of the green variety with longer shoots and low in the blue variety with shorter shoots.

The percentage of retained needles was relatively high in all age classes with some differences between the three Douglas-fir varieties (Fig. 1).

Needle retention was generally highest in the 18 provenances of the green variety. It was about 90% to 100% in age class one (1990) with a decrease to 70% to 80% in age class four (1987). The behaviour and ranking of these provenances were very similar in all age classes (see Fig. 1).

The eight provenances of the grey variety showed a lower percentage of retained needles in all age classes with about 80% to 90% in age class one (1990), decreasing to 70% to 80% in age class four (1987).

Needle retention was lowest in the five provenances of the blue variety. The percentages varied between 50% and 70% for all age classes. Differences between provenances were highest in this Douglas-fir variety.

Needle infection of provenances by *Phaeocryptopus gaeumannii*

The black and spherical pseudothecia of *P. gaeumannii* occurred on the undersides of the needles forming two parallel rows above the stomata and were easy to recognize and to count. The distribution of fruit bodies was inhomogeneous in slightly infected needles. Consequentially, the pseudothecia occurred in small colonies or spots, often near the needle tip. The undersides of heavily infected needles were covered along the stomata rows by a more or less homogeneous black layer. Counting was more difficult.

There was a great variation regarding the number of fruit bodies of *P. gaeumannii* on needles of individual trees, of different provenances and within the four consecutive years. In Figure 1, the mean numbers of pseudothecia counted per 10 mm needle length in the four years (1987 to 1990) are shown for the 31 Douglas-fir provenances.

The 1-year-old needles (1990) of all 31 provenances were covered with fungal fruit bodies. The mean number was relatively low with 36.9 pseudothecia/10 mm needle length. The highest numbers were counted in four provenances from interior eastern British Columbia and in two provenances from Colorado (no. 155) and Arizona (no. 157) with mean values between 70 and 120 fruit bodies/10 mm (Fig. 1). Most of the green variety provenances had fruit body numbers below the average.

In age class two (1989), the needles had the highest mean number of all age classes with 45.8 pseudothecia/10 mm needle length (Fig. 1). However, two provenances (no. 14, Eagle Bay, BC; no. 157, Kiabab Plateau, Arizona), which had pseudothecia on the 1-year-old needles, were free of fungal fruit bodies on the 2-year-old needles. The maximal number of fruit bodies ran up to about 180/10 mm needle length (provenance no. 111, Horsefly, BC). The same provenance had already a high number in the age class one needles (1990). Very high figures also showed

provenance nos. 109 (Dunster, BC) and no. 110 (Clemina, BC), all from adjacent seed zones of the same geographical region in eastern British Columbia. The other four grey variety provenances from seed zone 3040 of interior eastern British Columbia were either less attacked by needle cast or uninfected (no. 14). Provenances of the green variety showed great differences from nearly zero to about 97 fruit bodies/10 mm (no. 70, Denny Creek, Washington). Four provenances of the blue variety were only slightly or not infected. One exception was no. 155 (Willow Creek, Colorado) with 113 fruit bodies/10 mm.

In age class three (1988), needles of 28 provenances showed fruit body formation. The needles of three provenances were apparently free of infection (Fig. 1). The average for all provenances was 22.8 pseudothecia/10 mm. The maximal number of fruit bodies was found in provenance no. 110 (Clemina, British Columbia) with a value of 82.2/10 mm needle length. This provenance had also high fruit body numbers in the needle age classes one (1990) and two (1989). Also, some other provenances of the grey and green variety had relatively high numbers of fruit bodies. Four provenances of the blue variety were either not or slightly infected. An exception was provenance no. 169 (New Mexico), which was the only one in this age class to have such a high number of fruit bodies (81.4/10 mm).

The 4-year-old needles (1987) had the lowest number of fungal fruit bodies with an average of 8.7/10 mm (Figure 1). Thirteen provenances showed no fruit body formation whatsoever in this needle age class. Eighteen provenances had needles with fruit bodies. Among them was provenance no. 111 (Horsefly, British Columbia) with the maximal number fruit bodies/10 mm needle length (33). One interesting point was that the five provenances of the blue variety showed no fruit bodies.

DISCUSSION

In old-growth Douglas-fir stands of North America, the ascomycetous fungus *Phaeocryptopus gaeumannii* (Rohde) Petrak is endemic and belongs to the mycoflora of old and young needles, but is not very frequent and does not cause severe damage (Sherwood and Carroll 1974). However, in recently established plantations (see e.g. Winton and Hansen 1997), in Christmas tree plantations (see e.g. Michaels and Chastagner 1982), and especially outside North America, this fungus can cause one of the most important needle cast diseases of Douglas-fir. The results of the present study with Douglas-fir provenances grown under the environmental conditions of northern Germany confirm earlier reports on differences in the susceptibility of Douglas-fir against this fungus (Hood 1982, McDermott and Robinson 1989, Nelson et al. 1989, Hood et al. 1990, Bietlot and Malaisse 1994). In this study, a distinction is made between provenances of the green or coastal form, the grey or northern interior form, and the blue or southern interior form of Douglas-fir.

Concerning the presence of pseudothecia of *P. gaeumannii* on needles, one can state that all investigated Douglas-fir provenances can be attacked, but at different degrees, related to the four consecutive needle age classes. All provenances had infected 1-year-old needles (1990). However, the number of uninfected provenances increased, when 2- to 4-year-old needles were considered. This can be the result of the loss of infected needles over the years or of variation in the resistance of provenances.

Needle loss was relatively low in the four needle age classes. An obvious relationship between the percentage of retained needles and the proportion of needles infected by *P. gaeumannii* could not be found in the present study. That is in contrast to other studies, where the intensity of infection and degree of needle retention were positively correlated (Michaels and Chastagner 1984, McDermott and Robinson 1989, Hood et al. 1990). One explanation could be that the intensity of the infection in the Douglas-fir plantation was still very low compared to other field trials under study. It is known that premature needle loss by Swiss needle cast disease is also related to environmental conditions unfavourable for the host plant, to events of deep frost in combination with drought, and to variation in the sensitivity of a host tree to infection (Lyr 1958, McDermott and Robinson 1989, Schröter et al. 1997). It will, therefore, be necessary to observe the development of the disease incidence in the plantation during the coming years. Until now, the annual loss of needles did not correspond to the annual pseudothecia production on the respective provenance. Although needle loss per year was highest in blue Douglas-firs, it was obviously not only caused by the needle cast fungus *P. gaeumannii*. In some cases, *Rhabdocline pseudotsugae* Sydow, the other important needle cast disease, was also present on 1-year-old needles. The occurrence of both fungi on the same needle was very rare. Provenances of the blue variety from the southern interior are the most susceptible ones to *R. pseudotsugae*, but provenances of the grey variety can also become infectious under the environmental conditions of northern Germany (Stephan 1973, 1981, Liesebach and Stephan 1995). The infection rate of *R. pseudotsugae* was not very high in the investigated provenances, but might have contributed to some extent to the lower proportion of retained needles in blue variety provenances (Fig. 1).

If the average number of pseudothecia formed on the undersides of the needles is an indication of the degree of susceptibility on a Douglas-fir provenance, then there are great differences between the provenances. Nelson et al. (1989) reported that the density of pseudothecia on infected needles was a heritable, additive character in various Douglas-fir progenies. Therefore, one should consider the differences between provenances in the present investigation as genetic variation. For example, the four provenances from interior eastern British Columbia exhibited the highest number of fungal fruit bodies in all needle age classes. That agrees with results of Hood (1982), who studied natural second-growth stands in British Columbia and found high infection rates in the interior. He observed that the mean infection varied regionally and a relation with climatic patterns existed. According to Hood (1982) and McDermott and Robinson (1989), one can assume that provenances from locations with higher rainfall, particularly from May to July, show higher resistance to the Swiss needle cast disease. Therefore, provenances originating from drier locations are presumably more susceptible, especially when grown on new locations with higher rainfall. Besides humidity, water content of the needles, disturbances of the water regime, and density of a stand all favour the development of shadow needles and have on effect on increasing infection rate (Lyr 1958, Strittmatter 1974).

To summarize, one can state that there is pronounced variation between Douglas-fir provenances regarding the intensity of fruit body formation of *P. gaeumannii*, although differences between varieties and regions are not yet very clear.

Table 1. Origin of the Douglas-fir provenances

No.	State/ Province	Location	Latitude [°N]	Longitude [°W]	Altitude [m]
Grey variety					
109	BC	Dunster	5312	119.38	823
110	BC	Clemina	52.85	119.08	884
111	BC	Horsefly	52.30	121.32	823
8	BC	Golden	51.38	117.00	869
14	BC	Eagle Bay	50.93	119.22	442
15	BC	Blind Bay	50.88	119.40	412
18	BC	Salmon Arm	50.73	119.22	472
16	BC	White Lake	50.12	119.25	518
Green variety					
26	BC	Stella Lake	50.28	12547	152
34	BC	Sechelt	49.52	123.88	183
40	BC	Cassidy	49.05	123.95	198
53	Washington	Darrington	48.27	121.63	152
70	Washington	Denny Creek	47.40	121.53	549
73	Washington	Humptulips	47.32	123.90	137
74	Washington	Matlock	47.30	123.43	503
83	Washington	Packwood	46.57	121.67	655
86	Washington	Neselle	46.37	123.73	46
90	Washington	Cougar	46.08	122.30	503
94	Oregon	Vernonia	45.77	123.22	213
117	Oregon	Marion Forks	44.50	122.00	1067
120	Oregon	Oakridge	43.90	122.37	884
103	Oregon	Coquille	43.20	124.17	76
128	California	Gasquet	41.85	123.98	122
131	California	Scott Bar	41.73	123.10	1006
143	California	Wild Wood	40.38	123.00	1188
147	California	Fort Bragg	39.50	123.72	61
Blue variety					
155	Colorado	Willow Creek	37.38	106.35	2743
157	Arizona	Kiabab Plateau	36.30	112.15	2408
158	Arizona	S. Francisco Peak	3520	111.40	2743
162	New Mexico	Otero N. of James Canyon	32.55	105.30	2438
169	New Mexico	--	35.20	106.43	3307

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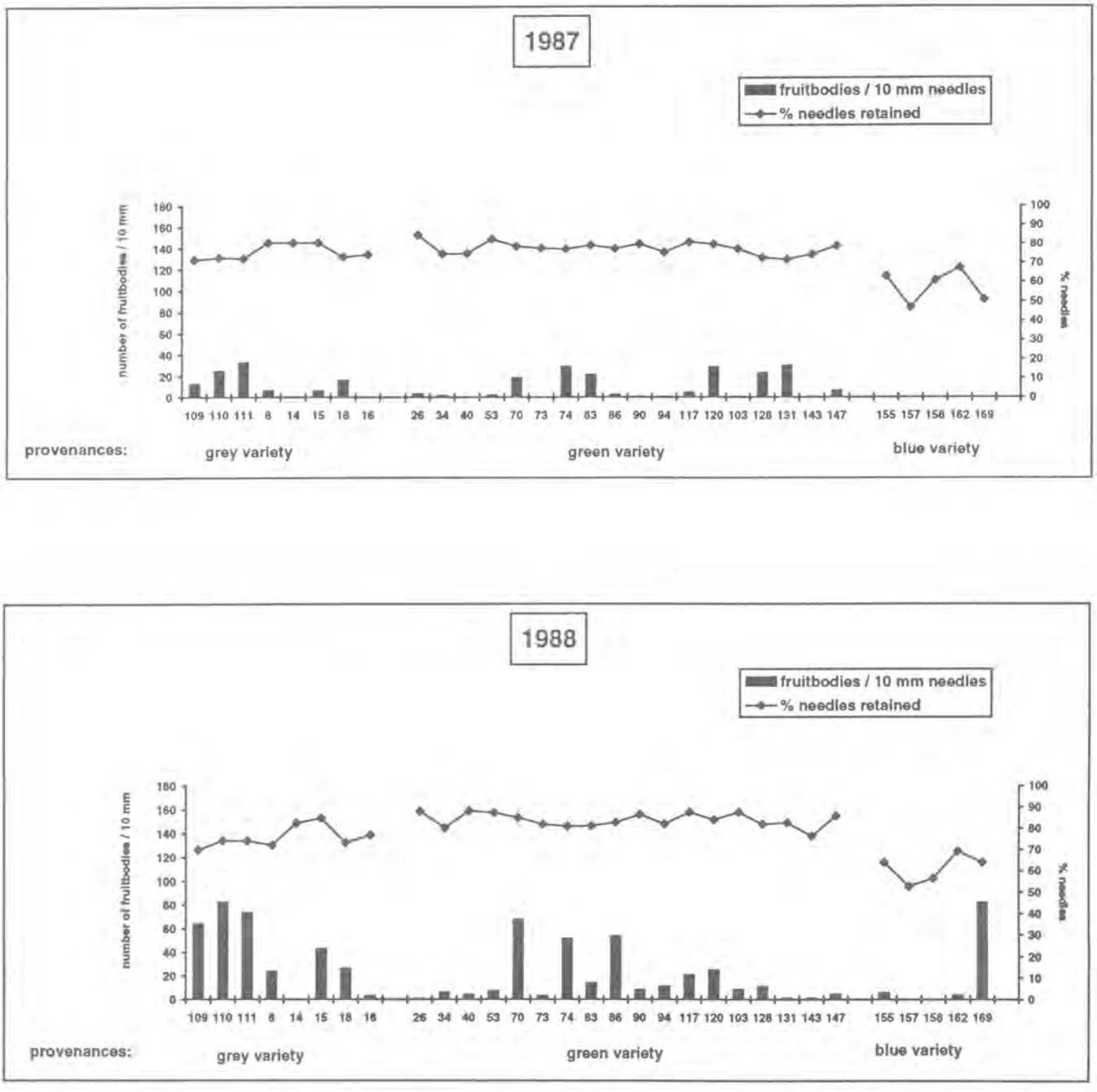
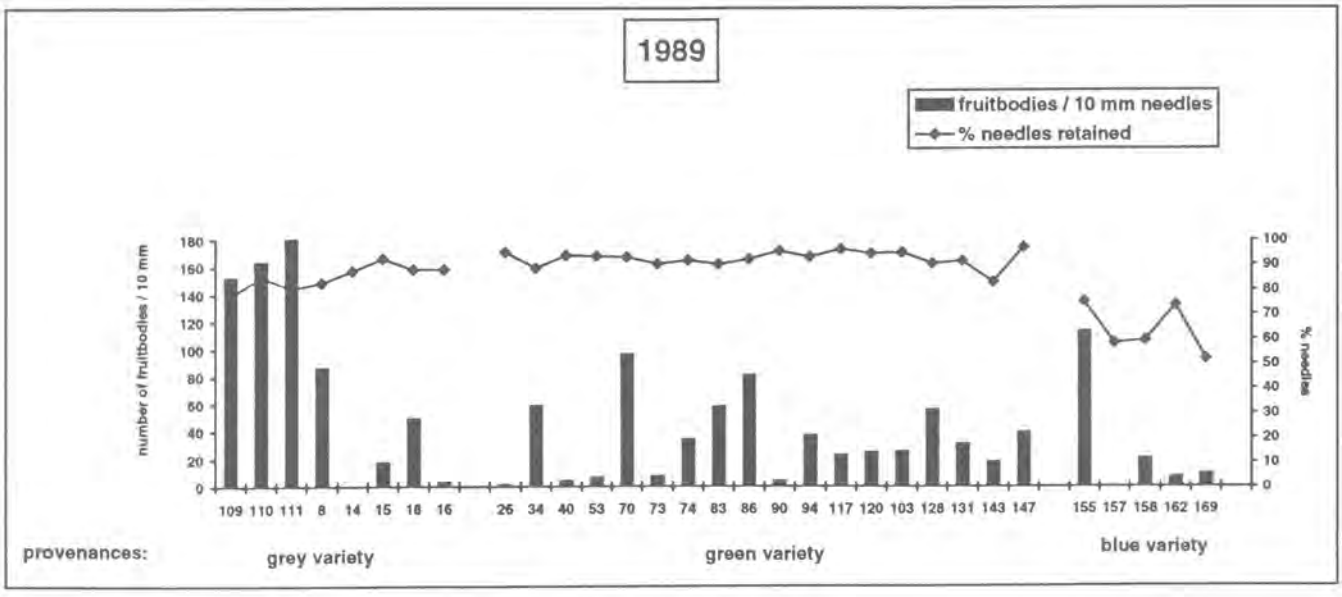


Figure 1. Percentage of retained needles (black dots) and average number of fruitbodies of *Phaeocryptopus gaeumannii* (black columns) on needles of four consecutive needle age classes (1987 to 1990) of 31 Douglas fir provenances.



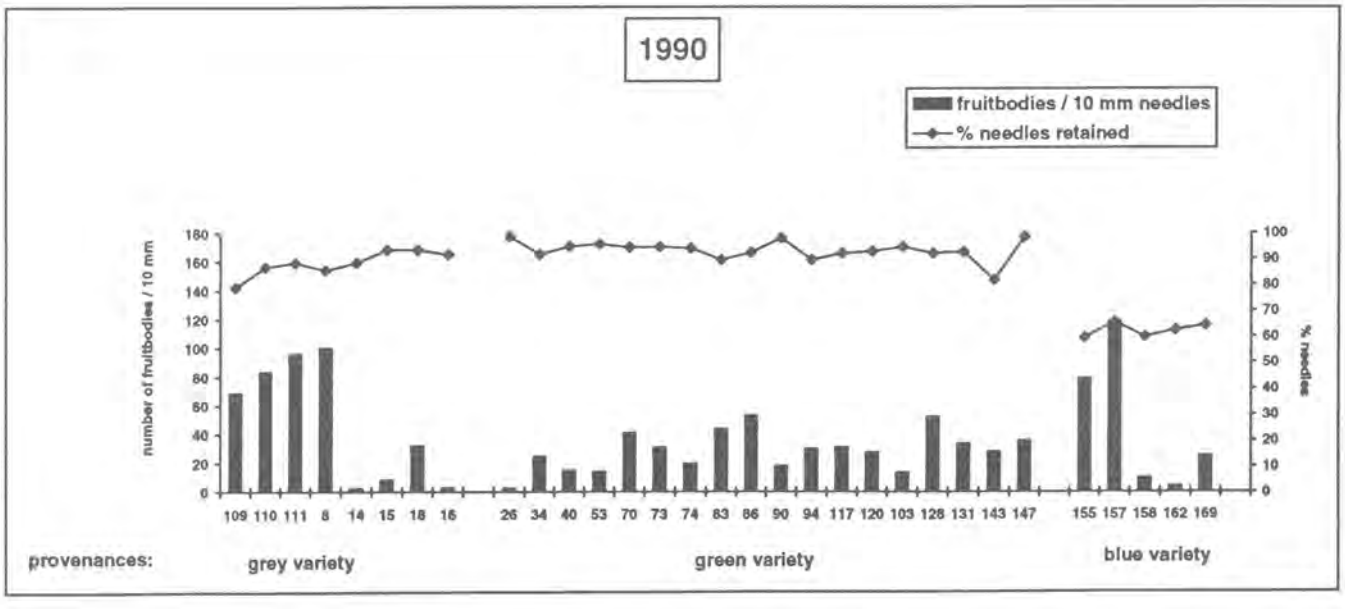


Figure 1 (continued). Percentage of retained needles (black dots) and average number of fruitbodies of *Phaeocryptopus gaeumanii* (black columns) on needles of four consecutive needle age classes (1987 to 1990) of 31 Douglas fir provenances.

SOMATIC INCOMPATIBILITY STUDIES OF *DISCULA UMBRINELLA* STRAINS FROM *QUERCUS ALBA* AND *QUERCUS RUBRA*

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SUMMARY

This study reports on an improved method for assessing mycelial interactions from fungal pairing experiments of *Discula umbrinella* strains derived from *Quercus alba* and *Quercus rubra*. Mycelial interactions were classified as to having compatible or incompatible reactions. Based on compatible reactions, all *D. umbrinella* strains isolated from *Q. alba* and *Q. rubra* were classified in one of six different mycelial compatibility groups. Mycelial compatibility groups distinguished intraspecific genetic variation but not host-species specificity. Microscopic slide cultures were also used to study mycelial interactions of paired cultures. Hyphal fusions were more common in compatible reactions whereas mycelial inhibition and twisting were more common in incompatible reactions. Strains from *Q. alba* and *Q. rubra* produced similar numbers of conidiomata on autoclaved leaf discs on both oak species.

Keywords: mycelial incompatibility, vegetative incompatibility, endophyte

INTRODUCTION

Rayner (1991) described mycelial interactions in ascomycete fungi and defined the concept of fungal individualism. He grouped fungal isolates based on compatible and incompatible mycelial interactions. Anagnostakis (1987) used mycelial incompatibility as an effective means of identifying intraspecific variation within field populations of the forest pathogen, *Cryphonectria parasitica*. Anagnostakis (1987) referred to mycelial incompatible groups as vegetative incompatibility groups (VC). Mycelial incompatibility is but one of many events associated with vegetative incompatibility, the inability of two strains to fuse and form a stable heterokaryon. Kohn et al. (1990) proposed the use of mycelial compatibility groups as an alternative to vegetative incompatibility groups as a means to identifying the genetic variation within field populations of pathogenic ascomycetes. Kohn et al. (1990) defined a compatible reaction as the ability of two paired strains to form a single colony. An incompatible reaction was defined as the ability of two paired strains to form a distinct reaction line within the interaction zone of the two strains.

The purpose of this study was to develop somatic incompatibility techniques for *Discula umbrinella*, an endophyte and plant pathogen of oak species in order to determine the range of genetic variation present in the fungal population in a native plant community. Genetic variability of forest endophytes and pathogens is an important component of forest management. Results from this study will be incorporated into a proposed design for a forest health monitoring system at the Patuxent Wildlife Research Center, Laurel, MD, U.S.A.

MATERIALS AND METHODS

Discula umbrinella (Berk. et Broome) Sutton isolates were collected from leaves of two species of oaks, *Quercus alba* and *Quercus rubra* at Patuxent Wildlife Research Center, Laurel, MD, U.S.A. Cultures were isolated using an improved large scale technique (Cohen, 1997). The fungal isolates were stored in sterile water and transferred to Difco potato dextrose agar when needed. Strains were then transferred to water agar prior to somatic incompatibility testing. Two water agar blocks containing a fungal strain were transferred to a microcentrifuge tube containing 1 ml of water. The agar blocks were smashed with a pestle and suspended in water. For pairing of mycelial strains, a .2 ml suspension of each of the two strains was placed 1 cm apart on oatmeal agar plates. The oatmeal agar was prepared by heating 30 g of oats in 500 ml distilled water. The oat suspension was filtered through cheesecloth. The remaining oat suspension was diluted to 1000 ml with distilled water and 20 g of Bacto agar was added. All possible pairings were included such as *Q. alba* x *Q. alba*, *Q. rubra* x *Q. rubra*, and *Q. alba* x *Q. rubra*. Strains were also self-paired for comparison purposes. Mycelial interactions were judged to be compatible when mycelia of two isolates intertwined easily, no noticeable line formed and hyphal fusions were common. An incompatible reaction occurred when a fine white barrier line formed between two isolates or an empty zone occurred when mycelia did not grow. A partial compatible reaction was defined if a small portion of the contact included either a white barrier line or an empty zone.

Mycelial interactions were also observed microscopically on slide cultures. Three isolates collected from white and red oaks were paired on slide cultures and observed for a range of hyphal interactions such as hyphal fusing, intertwining, coiling and rejection after 3, 7 and 14 days. The slide culture apparatus contained a glass slide elevated on two toothpicks on top of filter paper sitting in a glass petri dish. This apparatus was sterilized and molten 2.0% water agar was dropped onto the slide within the petri dish. Five percent glycerol was added to each petri dish to retard drying out during the experiment. Two isolates were paired per slide by adding a drop of mycelial suspension from each culture equidistant 1 cm from each other to the slide. Mycelial interactions were observed and recorded photographically.

Fungal strains were tested for the ability to produce conidiomata on both white and red oak leaf discs. Leaf discs were taken from one-month old oak seedlings using a 6 mm diameter hole punch. Discs were then autoclaved and placed on 60 mm diameter water agar plates. Each water agar plate was inoculated with an agar disc of the fungal isolate in the center of the plate. Surrounding the inoculum to the left were three white oak leaf discs and on the right, three red oak leaf discs. The plates were incubated for 3 weeks at 24°C under fluorescent lights. The number of conidiomata per leaf disc was counted for each species.

RESULTS

Discula umbrinella strains from oak species did not form strong mycelia interactions when agar blocks were plated directly on potato dextrose agar. Mycelial interactions were strongly enhanced when mycelia were first grown on a weak media such as water agar, followed by inoculation of oatmeal agar plates with mycelial suspensions. Mycelial interactions of paired strains included a single colony formed, a clear inhibitory zone or a dense, white inhibitory zone formed between the two paired strains. Partial compatible/incompatible interactions were also observed but not in high frequency. Examples of compatible and incompatible pairings are found in Table 1.

Table 1. Examples of mycelial pairing interactions of *Discula umbrinella* strains collected from *Quercus alba* and *Quercus rubra* leaf discs

Pairing of Fungal Strains	Mycelial Interactions
Q. alba x *Q. alba*	
WO122 x WO122	Compatible
WO236 x WO236	Compatible
WO546 x WO546	Compatible
WO655 x WO655	Compatible
Q. rubra x *Q. rubra*	
RO122 x RO122	Compatible
RO226 x RO226	Compatible
RO1035 x RO1035	Compatible
Q. alba x *Q. rubra*	
WO122 x RO122	Incompatible
WO236 x RO1035	Compatible
WO546 x RO226	Incompatible
WO655 x RO226	Compatible

Paired compatible reactions were assigned to six different mycelial compatiblity groups. The six mycelial compatibility groups did not distinguish *Q. alba* from *Q. rubra* strains but were able to distinguish intraspecific variation.

Somatic incompatibility interactions were also studied at the microscopic level. Light microscopy revealed many hyphal interactions including coiling, twisting, hyphal fusion and inhibition. Hyphal coiling was common in both compatible and incompatible reactions. Mycelial fusions were common in compatible reactions whereas inhibition and twisting were common in the incompatible reactions.

Fungal strains from *Q. alba* and *Q. rubra* were able to produce conidiomata on autoclaved leaf discs from either oak species (Table 2). The numbers of conidiomata produced by white oak strains did not significantly differ on autoclaved white oak or red oak leaf discs. The same situation occurred with respect to conidiomata numbers produced by red oak strains on white oak or red oak leaf discs.

Table 2. Number of conidiomata produced by *Discula umbrinella* strains on autoclaved *Quercus alba* and *Quercus rubra* leaf discs incubated on water agar

	Average number of conidiomata per leaf disc[1]	
Isolates	***Q. alba***	***Q. rubra***
Q. alba strains		
WO122	22.83 (4.83)	16.67 (7.66)
WO546	24.17 (7.03)	15.17 (7.76)
WO614	14.50 (3.93)	10.00 (6.87)
WO812	21.50 (3.39)	9.50 (5.82)
Q. rubra strains		
RO112	27.50 (8.96)	16.67 (7.84)
RO226	16.16 (6.76)	9.50 (3.78)
RO315	20.17 (11.77)	13.83 (10.98)
RO426	37.67 (11.25)	23.67 (11.72)

[1] (standard deviation)

DISCUSSION

Somatic incompatibility studies with *Discula umbrinella* indicate that this technique is suitable for detecting intraspecific genetic variation of fungal strains but is not sensitive enough to detect host-species specificity. Mycelial compatibility grouping is a simple technique for categorizing individuals within a fungal population. It is an inexpensive, useful technique for surveys of large-scale field experiments to check for genetic diversity prior to employing more sophisticated molecular tools.

It is interesting that mycelial compatibility grouping of *Discula umbrinella* revealed intraspecific genetic variation but not host-species specificity. Further studies should be conducted utilizing molecular tools to verify this finding. Haemmerli et al. (1992) isolated *D. umbrinella* strains from beech and detected intraspecific genetic variation with randomly amplified polymorphic (RAPD) profiles. They were able to determine that four separate individual genotypes were isolated from a single beech leaf. Based on the RAPD work, Haemmerli et al. (1992) were also able to group together oak and beech strains and place the chestnut strains into a separate group.

Mycelial interactions at the light microscopic level were less easy to use for screening pairing reactions. The interactions were not as clear cut or quantifiable. Recording the interactions at the macroscopic level proved to be more reproducible and useful.

Discula umbrinella strains easily produced viable conidia in conidiomata on potato dextrose agar or oak leaf discs under the conditions of 12 h of fluorescent light at 24°C. Strains also produced similar conidiomata numbers on autoclaved white oak and red oak leaf discs. This data may indicate that white oak and red oak strains do not demonstrate fungal-host specificity.

CONCLUSIONS

An improved technique for studying somatic incompatibility of *D. umbrinella* strains has been developed. The technique is based on growing strains under starvation conditions and then pairing macerated cultures on a reduced nutrient medium. This technique enhances the compatible and incompatible hyphal interactions. Results of this study confirm intraspecific genetic variation of fungal strains isolated from *Q. alba* and *Q. rubra*, but do not substantiate fungal-host specificity.

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MYCOSPHAERELLA PINI (= *SCIRRHIA PINI*), THE PERFECT STATE OF *DOTHISTROMA SEPTOSPORA*: FIRST OBSERVATION IN PORTUGAL

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SUMMARY

Mycosphaerella pini, the perfect state of *Dothistroma septospora*, was recorded for the first time in Portugal. The symptoms caused by the fungus, its morphological characters and distribution on pine needles are presented here. The comparison with the related species *Scirrhia acicola* is discussed.

INTRODUCTION

The ascomycete *M. pini* Rostrup in Munk was first found by Funk and Parker (1966) on diseased needles collected on a range of pines on Vancouver Island (Canada). These authors showed the genetic relationship between this fungus and *Dothistroma septospora* Dorog. Morelet (=*Dohistroma pini* Hulbary) by means of single ascospore isolation that gave rise to cultures indistinguishable from those obtained from conidia of *D. septospora*. They described *M. pini* as the perfect state of *D. septospora*.

In 1967, the same ascomycete was found in France, as well as its imperfect state (Morelet, 1967, 1969). The presence of *M. pini* and its imperfect state *D. septospora* was also demonstrated by Gremmen (1968) on needles of *P. ponderosa* from Romania. This ascomycete has also been recorded in California (Wagner,1967, in Peterson, 1981), Oregon (Peterson & Harvey, 1976) and Alaska (Peterson, unpublished, referred to by Peterson, 1981).

In Portugal, the fungus is now identified for the first time on needles of *P. pinaster* Ait. at Alcácer do Sal, on *P. pinea* L.at Montemor-o-Novo, and needles of *P. radiata* D. Don at Azóia (Leiria).

Part of our collection is deposited at the International Mycological Institute-UK Herbarium, under the number 287282, where the identification was confirmed by Dr A. Sivanesan.

MATERIAL AND METHODS

The parasite *Dothistroma septospora* was first found in Portugal on needles of *Pinus pinaster* Ait. collected in S. Miguel Island, Açores (Fonseca, 1980). Collections made during the spring of 1984 and subsequent years on *P. radiata*, *P. pinaster* and *P. pinea*, in several stands in mainland Portugal, revealed the presence of needle blight with some of them dying from the tips downwards. On dead portions, black fructifications which burst through epidermis were observed. Sections were made of these fruitbodies.

RESULTS

Signs and symptoms of infection

Diseased trees showed reddish bands on previous year needles, and on most of them a reddening from the tips downwards is observed. The distal parts of these needles dies and bears linear black fruiting bodies breaking the epidermis (Fig. 1). The reddening on current-year needles is seen by the end of June and July.

The disease causes serious reduction of growth rate through destruction of foliage and in cases of severe attack, it causes the death of the host.

Morphological characters of the fungus

Vertical sections on dead needle parts showed small spherical ascomata 35-80x15-20µ, (Figs. 2-3) developing within the host tissue and a short cylindrical ostiole emerging through the stoma. Asci bitunicate, widest at the base, giving an inverted appearance, 45-50x7-10µ, containing eight ascospores, 12-15x3-4µ, hyaline, fusiform to cuneate, 1-septate, slightly constricted at the septum, the two cells being unequal (Fig. 4). The mycelium is ramified, intracellular and confined to the mesophyll.

These observations led us to identify the ascomycete as *Mycosphaerella pini* Rostrup in Munk (Evans 1984) = *Scirrhia pini* Funk & Parker, the perfect state of *Dothistroma septospora*.

DISCUSSION

Comparison with the Scirrhia acicola

The author of a previous paper (Fonseca, 1980) compared *D. septospora* (=*D. pini*), the imperfect state of the fungus identified here, with *Septoria acicola* (Dearn.) Siggers. The presence of a purplish-red pigment in the tissue of both ascigenous and conidial states of *Mycosphaerella pini* is a valuable diagnostic feature. This pigment is capable of diffusing out in dilute KOH (Funk & Parker,1966). The experiment was done with tissues of the conidial state in 10% KOH and the purplish-red color was observed (Fonseca, 1980). Concerning the ascigenous state, the same experiment was carried out in 5% KOH and a permanent purplish-red color was formed. On the contrary, Funk & Parker found that this purplish-red fades after a short time. No mention is made about the percentage of the KOH solution. This pigment is not present in needles attacked by *S. acicola*.

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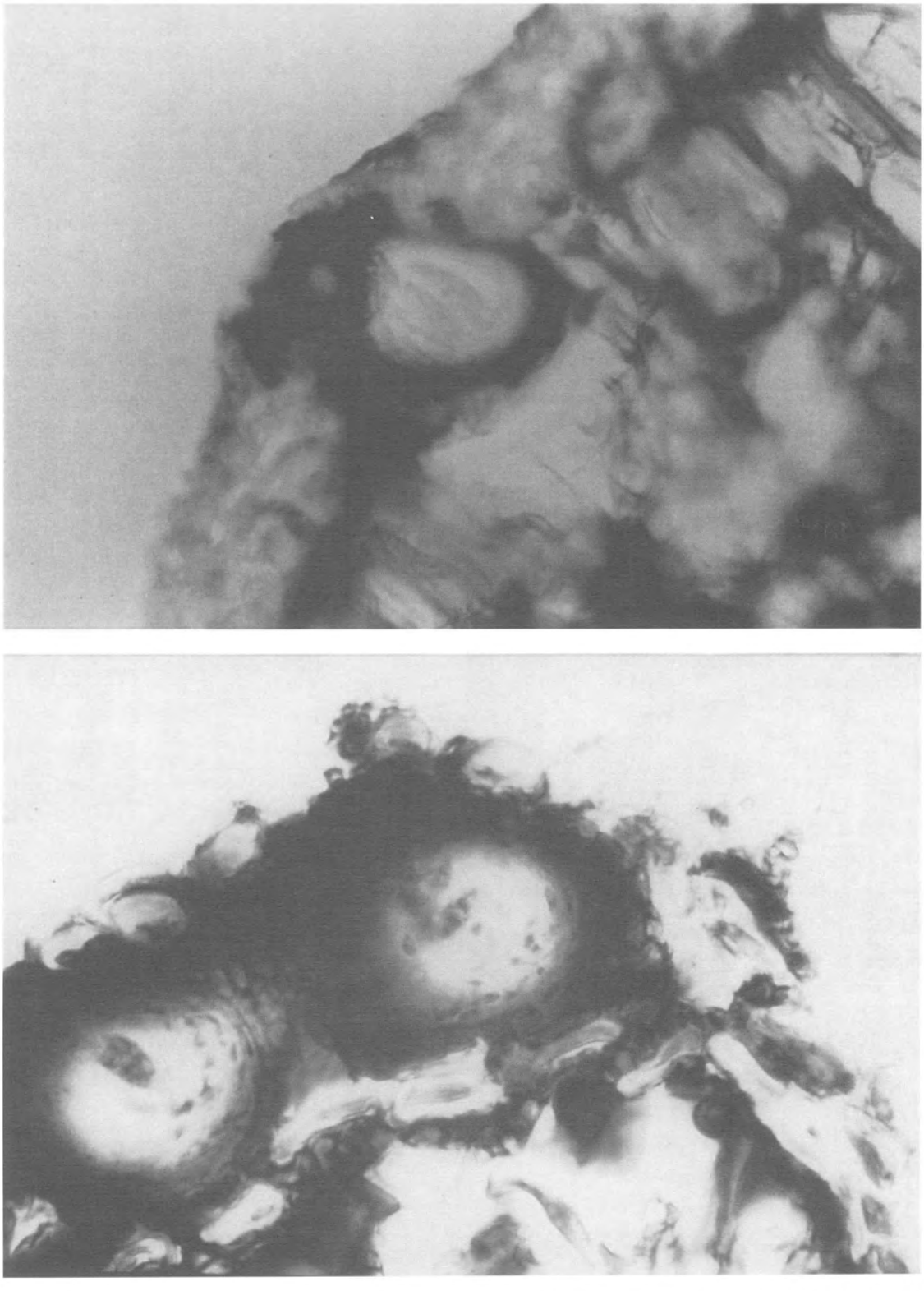
Figure 1. Needles of *Pinus radiata* attacked by *Mycosphaerella pini*.



Figures 2-3. Transversal sections on needles of *P. radiata* showing ascomata and ascospores of *M. pini* (x400).

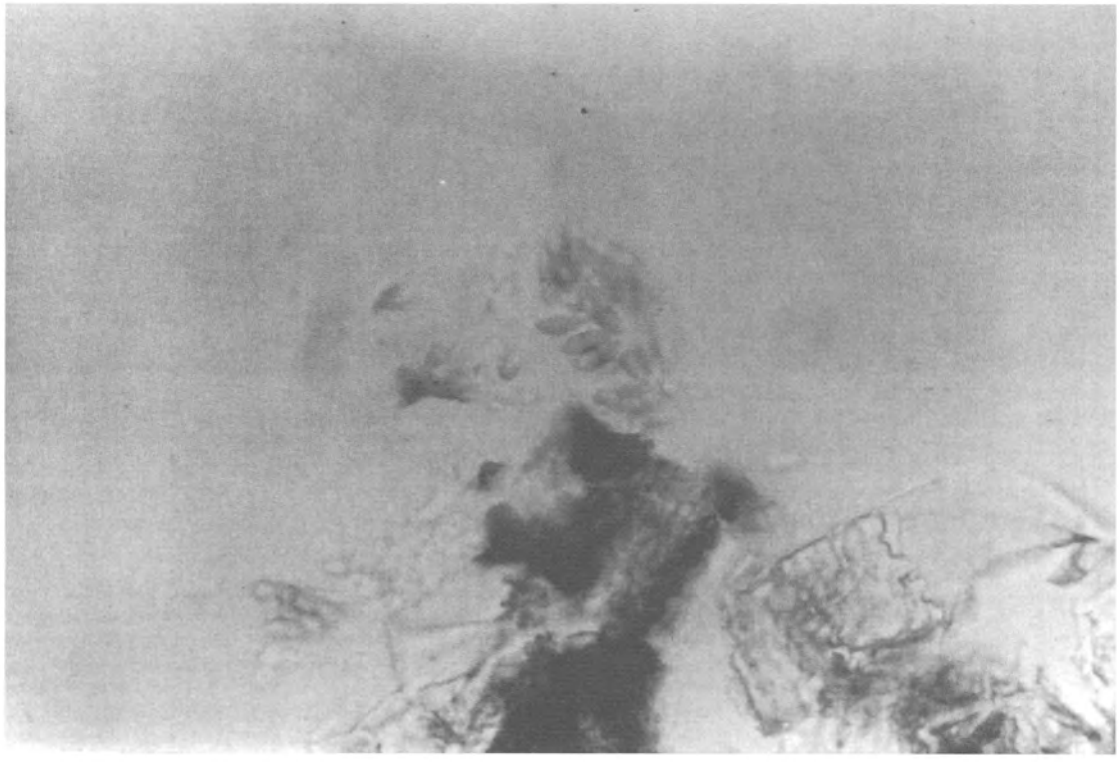


Figure 4. Ascospores of *M. pini* on needles of *P. radiata* (x600).

SHOOT AND TWIG BLIGHTS

A HISTORICAL REVIEW OF THE LARCH SHOOT BLIGHT IN JAPAN

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SUMMARY

A disease called "Larch shoot blight" was extremely serious only within a certain period from 1958 to 1963 after World War II, in Japan. And affected areas became within limited areas in southern Hokkaido and also limited in northern Tohoku, both of which are in northern Japan. Since then, no more serious disease conditions for establishment of larch plantations have been reported from anywhere in Japan. The author presumed that the main reason for the disease development depended upon a trigger, "acid mist", to the disease originated from the combustion of low quality coal (high sulfur content and local production of coal) and refining of various mines for sulfide. These conditions resulted from the reconstruction of Japan after the war. When coal changed from low quality to high quality (low sulfur content and imported ones), reduction or closure of the mines was recorded along with desulfurization. Since then no more serious disease conditions had been recorded for 30 years, even as the other climatic conditions, such as mist, precipitation, wind, etc. have not changed and much more activities on industries have been recognized. Therefore, acid mist originated from the combustion of low quality coal and from refining of sulfide at metal mines was presumed as a main cause, a trigger, for the invasion of the pathogen into the larch and for the development of shoot blight.

Keywords: Larches, Shoot blight, *Botryosphaeria laricis*, Acid mist.

INTRODUCTION

Japanese larch, *Larix kaempferi* Serg. is a larch species native to Japan and is naturally distributed in the central part of Honshu, Japan's main land mass (or island). This is a fast growing tree species especially in young plantations and easily produces seedlings. Because very rapid growth is shown in nurseries and at the young stage, this is quite suitable for establishing plantations. Therefore, after WW II, this was chosen as one of the best planting tree species and was planted in various areas in central and northern Japan. The large-scale plantations of this species were started in 1955-56, after a heavy typhoon. Soon after, a disease, shoot blight, was reported first from the southern part of Hokkaido in 1953 and then from the northern part of Tohoku in 1958. In some cases, disease conditions of saplings became serious, and the establishment of plantations was thought to be "hopeless" and damage at nurseries affected one million seedlings in the early 1960's. Various efforts to control this shoot blight were made, but no effective means of control at plantations were found. Studies at affected plantations were performed and many reports were published on this subject.

As a result, recommendations were as follows: 1) no transplanting at healthy areas with affected seedlings, 2) windbreak forests of larch should be removed, 3) no planting with affected seedlings, 4) seedlings must be treated by dipping into fungicide before being carried into planting

sites, 5) do not plant at windy sites and establish wind protection forests. Cycloheximide for control of this disease at nurseries was recommended.

Studies were mostly performed in and around the affected plantations and no comparisons between diseased and healthy areas were made. Even the serious conditions were reported to be limited only to southern Hokkaido and northern Tohoku, much wider healthy areas with larch were found in Hokkaido, Tohoku and the other areas of Japan.

The author tried to compare various items on the disease development between affected areas and healthy areas and then found that the disease was reported only in a period of combustion with home coal in Hokkaido and mining of sulfide in Tohoku. On the other hand, only the European larch was affected and it has still been affected in the healthy areas for the Japanese larch since 1960's.

Therefore, it is concluded that a trigger for the disease development was "acid mist" and the desulfurization was a decisive action to the reduction of the disease.

PATHOGEN AND SYMPTOMS

The pathogen of the shoot blight is *Boryosphaeria laricis* (Sawada) Shang (= *Physalospora laricina* Sawada; =*Guignardia laricina* Yamamoto et K. Ito). This is an ascomycetous fungus and native to Japan. The pathogen was reported in 1938 and was named by Sawada in 1950. It attacks only current shoots and sometimes kills them, but does not affect twigs of more than two years old (Figs. 1 and 2). When current shoots were affected in the early growing period, the top of shoots turned down and when current shoots were affected in the later stage, they could grow straight, but were killed later. If saplings were affected seriously, no main stem growth was visible and the general feature of the sapling became like a "Witches broom" (bushy shoots), but no saplings were killed.

When environmental conditions became better at the same plantation, a straight main stem appeared among bushy shoots of affected seedlings. Therefore, saplings had bushy shoots on the lower portion of the stem with a main stem growing among the dead bushy parts. This situation indicated that a trigger for the development of the shoot blight had existed for the disease development but this trigger was gone. It was proposed that the existence of some acting agent on larch was promoting the disease.

The author paid attention to the differences between healthy and diseased areas, because being from a healthy area he was able to make comparisons, in an objective manner. He documented the influences on SO_2 from the combustion of coal and refining by mines and clarified these findings by the historical observation of the disease.

CLIMATIC AND TOPOGRAPHIC CONDITIONS

The cold sea water current of the western Pacific Ocean comes down towards the south and the warm current comes up to the north and both are mixed off the coast of southern Hokkaido. There, the thick mist appeared in June and July in general. In this season, the wind

blows southward and mist goes into the inland regions of Hokkaido. The mist is stopped by the surrounding mountain ridges and stays on the ground like a sea.

In Tohoku, the same situation appears. A cold current flows south and a warm current flows north in the Sea of Japan and both are mixed off-shore on the west side of Tohoku. Therefore, a thick mist appears as in Hokkaido and the mist moves inland with the westerly winds. Fairly high mountain ridges run north to south in central Tohoku, stopping the mist at the ridge.

INDUSTRIAL AND MINING ACTIVITIES AND THE ENVIRONMENT

1. Hokkaido is the main industrial area and some coal mines are located off shore in the southern part of Hokkaido. The energy needs of these activities were supported by local coal production (low energy output and high sulfur content) after WW II. This means production of dense acid mist by SO_2. Furthermore the acid mist stagnated in southern Hokkaido. Unfortunately, no observations on the pH in the mist were made at the time, because no one thought about the hazard to planted trees.

2. In Tohoku, there were many mines for copper, iron and sulfur in northern part and they were quite active after WW II. The material were mostly sulfide and SO_2 was exhausted during refining. Hills and mountains around the mines were covered with acid mist, and almost no trees were seen around mines. The acid mist moved beyond the mining areas to plantations and became a trigger of the larch shoot blight. Around the 1970's, almost all mines of every kind were shutting down their activities, because imported raw materials were much cheaper than the local production. With those great changes in various mines, the air became cleaner over plantations and the disease declined remarkably.

3. Coal: Due to large scale bombing in Japan during the war, the systematic industrial activities were completely destroyed. The increased production of coal was the first step for reconstruction of Japan. The reconstruction was planned as follows: increased production of coal→activation of steel production→supply of basic materials→supply of electricity→reinforcement of transportation→reconstruction. Therefore, coal production became extremely active.

In 1950, a war on the Korean Peninsula broke out. This was an opportunity for a step forward in reconstruction and coal, the energy source, was used at factories, power stations, various metal mines and so on. The number of coal mines reached 841 in 1956, and a total production of 55 million t/year was reached, the maximum production of coal in Japan. The coal of local production contained about 2% sulfur on average and released a large quantity of SO_2 into the air.

As shown in Tables 1 and 2, imported coal became cheaper than the local production in 1959, at this high quality imported coal (0.5% sulfur content and high energy output) replaced the local production. Furthermore, conversion of energy source from coal to oil, the fuel revolution, gave a decisive blow to local production. Since 1970, the production of the home coal decreased remarkably to 35 million t/year and also number of coal mines to 74. In 1997, number of coal mines dipped to only 3. On the contrary, imported coal reached 100 million t/year. The local production of coal is in terminal stage.

Beside the coal, various metal mines are also in the same situation of the decline of production by the cheaper raw materials from foreign countries.

HISTORICAL OBSERVATION ON PROGRESS OF THE DISEASE

As shown in Table 1, the whole industrial activities started in 1950 after WW II and the energy needs for every activity were supported by coal of local production. The total amount reached 40 million t/year in 1950 from 20 million t/year in 1945. At the same time, various mines were also accelerated for increasing production. Therefore, many locations were filled with SO_2 gas after combustion of coal from industrial areas and the exhaust SO_2 gas from refining at mines. It is very lucky that most of the industrial areas in Japan faced the Pacific Ocean, in a wide sense, and that the constant wind and the upper air current blow from west to east. This wind carries SO_2 and other pollutants out to the Pacific Ocean.

Therefore, no serious damage to larch plantations has been recorded in Japan. The exceptions to this situation were in southern Hokkaido due to seasonal mist and south wind, and in northern Tohoku again due to seasonal mist and west wind.

In 1955, after the heavy typhoon, large scale plantations were planned for the damaged areas. By this action, young larch saplings were planted widely for the establishment of plantations in northern Japan. The increasing amount of SO_2 (= by combustion of coal and by production at mines) from industries and the disease development existed at the same period and the serious disease conditions continued for several years.

In 1961, the maximum volume of coal production was recorded at 55 million t/year after the war. In the same year, damage to larch plantations by shoot blight was recorded as a maximum of 100 000 ha in total. At this point, the conversion from coal to oil and also from low quality coal of local production to high quality of imported ones progressed rapidly by the cheaper cost and the constant supply. Furthermore, the raw materials for copper, iron and other metals of local production faced difficulties, because of much cheaper imported ones as mentioned above.

As a result, coal mines in Japan declined rapidly. In 1963, closing coal and other mines were recorded in high numbers. 1965 was one of the most remarkable turning points in the history of air pollution in Japan. That is, trees in cities became healthy everywhere in Japan, by decreasing SO_2 from industries. The conditions in Hokkaido and Tohoku were similar and at the same time, the disease conditions suddenly declined.

Therefore, the decline of the disease and decreasing SO_2 appear at the same period. Furthermore, desulfurization of the exhaust gas from combustion of coal and crude oil refining started in 1968 by the law. In 1972, desulfurization became popular in Japan, and the larch shoot blight was repressed completely. No more serious disease conditions have been recorded for 30 years.

CONCLUSION

It is quite clear that the connections between acid mist and shoot blight development can be explained as follows:

When increasing industrial activities with low quality coal combustion, shoot blight developed rapidly and when conversion of coal to high quality or coal of local production to oil, the disease declined rapidly and clearly, in limited plantations in Hokkaido and Tohoku where plantations were located near industrial areas and places of mist stagnation. No serious disease conditions were and have been found in the other large areas in Japan.

Furthermore, stems growing among the affected dead bushy shoots occurred everywhere in affected plantations. These conditions show that the poor conditions for the disease accelerated by the acid mist had disappeared. No changes in the climatic conditions of the mist in June and July were found. Under those circumstances, it was clear that the larch shoot blight that appeared from 1958 to 1963 was triggered by the acid mist.

From another point of view, the European larch has been affected with this disease, from the beginning to nowadays. The author has continued to make observations on the European larch planted at the Tokyo University Forest in Hokkaido where no shoot blight in the Japanese larch was seen, but the disease on the European larch still can be found in a grade of slightly affected ones, but the European larch is surviving with the disease.

A great hope against damage to forests by acid mist is found in the historical progress of the larch shoot blight shown in Japan. By decreasing SO_2 by combustion of high quality coal and the desulfurization of the exhaust gas, the forests may become healthy.

The author wished to express on his own opinion why the larch shoot blight developed and soon after disappeared rapidly. He also collected various data and condensed his opinion with those facts. His main opinion was discussed above and he should say that we have overcome the SO_2 damage to the forests and will be able to establish fine larch plantations.

Table 1. History of the larch shoot blight as connected to industries in Japan.

Year	Industrial activities	Larch and shoot blight
1938		A new disease of larch was reported in Hokkaido. Description of this specimen is still in Univ. of Hokkaido. (Pathogen is thought to be a native species to Japan)
1941	Pacific war, WW II End of war. Because of bombing, industrial activities were mostly stopped. 20 million tons/year of coal production	
1950	War in Korean Peninsula First step for reconstruction of industries after WW II. 40 million tons/year of coal	Shoot blight was described as *Physalospora laricina* Sawada in Tohoku District.
1952	Iron refinery, oil refinery, paper mill, power station, coal and other mining were accelerated, rapidly.	Ascus state was found in southern Hokkaido. Development of the disease in slightly affected grades was reported in wide but limited ranges.
1954	A heavy typhoon hit Hokkaido.	Severe damage to natural forests was recorded. Reforestation was planned with planting larch.
1956	Air pollution became serious by combustion of low quality coal.	Damage by shoot blight in southern Hokkaido and limited areas in Tohoku was reported.
1958 (1959)	Conversion of coal from low to high quality and imported high quality coal were started. Decline of coal production in Japan.	The disease in young plantations was reported from various but limited places in Hokkaido and Tohoku. Serious situation of the disease at young plantation appeared in limited areas of the southern part in Hokkaido.

1961	Large scale oil combustion started. Conversion from coal to oil progressed, rapidly. 55 million tons/year of coal production in Japan. Increased stock.	Severe conditions by the disease were reported from Tohoku. Total damaged areas became 100 000 ha in Japan. The pathogen was changed to *Guignardia laricina* Yamamoto et Ito and the disease was named "Larch shoot blight" (Sakigare-byou in Japanese).
1962	Beginning of reduction in coal mines in Japan.	Rapid decreased condition of the disease was found.
1963	Closing of coal and various other mines.	Continuous decrease in the disease conditions.
1965	Turning point of air pollution problems in Japan.	No more severe situation of the disease had been reported.
1966	Continuation of closing mines.	Decreased severity of the disease appeared sharply.
1968	A law against air pollution by SO_2 was started. Beginning of desulfurization.	Only a few slightly affected areas were found. NO ATTENTION became necessary for the disease.
1972	Generalization of desulfurization and repression of shoot blight	

Table 2. Production of coal in Japan, numbers of mines and in Hokkaido.

Year	Total amount (1000 t/year)	Production in Hokkaido (1000 t/year)	No. of total mines in Japan
1940	56,317		
1945	22,335		
1950	39,330	708	736
1956	48,281	453	841
1961	55,417 (max.)	877	622
1965	50,113	1,087	222
1970	38,329	1,280	74
1975	18,597	852	35
1991	7,880	50	6
1995	6,500		3

ACKNOWLEDGEMENTS

Many thanks for the cooperation on my knowledge of the coal industries by the Japan Coal Industry Society, Tokyo and of the desulfurization by Mitsubishi Heavy Industries Inc., Tokyo are expressed.

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Figure 1. Affected European larch planted in the eastern part of Hokkaido.
Figure 2. Affected European larch planted at the University Forest in Hokkaido, the University of Tokyo, in the central part of Hokkaido.

Both photos taken by Dr. Ikuo Takahashi, the University Forest in Hokkaido, the University of Tokyo, Furano, Hokkaido, Japan.

Both pictures show the general symptom of the shoot blight. The Japanese larch is fairly resistant to the disease without triggers for developing disease. On the contrary, the European larch is extremely susceptible to the disease. These two pictures show symptoms on the European larch planted at healthy areas to the Japanese larch, and indicate that the European larch is susceptible to the disease.

SIROCOCCUS SHOOT BLIGHT (*SIROCOCCUS CONIGENUS*) ON EASTERN LARCH IN NEW BRUNSWICK, NOVA SCOTIA AND PRINCE EDWARD ISLAND, CANADA: 1983 - 1994

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SUMMARY

Sirococcus shoot blight (*Sirococcus conigenus* (DC.) P. Cannon & Minter) caused unprecedented widespread damage on eastern larch (*Larix laricina* (Du Roi) K. Koch) in the Maritime provinces of Canada (New Brunswick, Nova Scotia and Prince Edward Island) in 1983 and again in 1994. Trace infections of Sirococcus shoot blight were found in a few European larch (*Larix decidua* Miller) plantations in Prince Edward Island in 1994.

Keywords: *Larix* spp., *Sirococcus strobilinus*, tamarack.

INTRODUCTION

Prior to its appearance on eastern larch or tamarack in 1983, Sirococcus shoot blight (*Sirococcus conigenus* (DC.) P. Cannon & Minter ≡ *Sirococcus strobilinus* Preuss) was widespread and intensifying in red pine (*Pinus resinosa* Ait.) plantations in Nova Scotia and was present on red pine and spruce (*Picea* spp.) in Nova Scotia, Prince Edward Island and New Brunswick. Records of Sirococcus on larch are rare in the literature. This paper summarizes our observations from 1983 to 1994.

DISCUSSION

An unprecedented shoot blight of eastern larch was widespread in Nova Scotia and Prince Edward Island in 1983 (Magasi 1984) (Fig. 1). A fungus, which was consistently isolated from the affected shoots on malt extract agar, failed to fruit in culture. Shoot browning was observed again in a few areas in central Nova Scotia in 1984 but was much less than in 1983 (Magasi 1985). Shoot browning was also observed at two locations in New Brunswick. In 1985, after storage at about 5°C for about 18 months, the cultures from 1983 produced typical fruiting bodies of *Sirococcus strobilinus*. A culture was confirmed by B.C. Sutton, International Mycological Institute, Kew (now Egham, Surrey, UK) as *Sirococcus conigenus* and he noted that *S. strobilinus* was a synonym of *S. conigenus* (Cannon *et al.* 1983). The culture was retained as IMI 296390. In 1985, *S. conigenus* was found on an eastern larch cone collected at Loch Katrine, Antigonish Co., N.S. In 1986, a trace of shoot blight damage, probably caused by *S. conigenus*, was observed at Lochside, Richmond Co., N.S.

In 1994, Sirococcus shoot blight reappeared on eastern larch and caused shoot dieback from trace to severe intensity on mature and overmature trees in Nova Scotia and New Brunswick

and with trace damage in three eastern larch and two European larch plantations in Prince Edward Island (Hurley *et al.* 1995) (Fig. 2). The disease was also reported on eastern larch from the eastern coastal counties of the State of Maine, U.S.A (Dearborn *et al.* 1995). In the Maritime provinces, most damaged shoots were associated with infected 1993 cones. These dead cones produced *S. conigenus* fruiting bodies when incubated in a moist chamber for a few days. The random pattern of shoot blight on eastern larch became the most reliable method of separating Sirococcus-infected shoots from other damage. By late June, damaged shoots were colonized by rapidly growing secondary fungi which often overwhelmed Sirococcus in culture and made identification difficult.

Sirococcus shoot blight is a well-known shoot pathogen on red pine in the Maritime provinces. Between 1983 and 1993, Sirococcus continued to intensify throughout the Maritime provinces on red pine to the point that: "The deterioration of red pine stands in western Nova Scotia and the spread of the disease to plantations in the eastern half of the province makes Sirococcus shoot blight the major plantation problem in Nova Scotia." (Magasi *et al.* 1994). This inoculum build up is the most likely source of infection for larch. Although it has not been a continuing problem on larch, given favorable conditions over more than a single year, Sirococcus could cause significant growth loss and even tree mortality.

This account, so far as is known, is unique in the known range of eastern larch and Sirococcus shoot blight. It may represent the potential of such diseases to attack other hosts under favorable conditions.

CONCLUSION

In Nova Scotia and to a lesser extent in New Brunswick and Prince Edward Island, Sirococcus shoot blight is a widespread problem on red pine and spruces in plantations and natural stands. The disease is capable of causing significant shoot mortality and has the potential to cause larch mortality if successive infections occur. To date, in spite of a regular source of fungal inoculum from pines and spruces, this has not happened. Sirococcus only occurs intermittently on larch. With its similarity to other insect-caused shoot dieback and its reluctance to fruit in culture, Sirococcus is probably overlooked when it does occur on larch.

ACKNOWLEDGEMENTS

We thank all present and former forest health technicians of the Forest Health Network - Atlantic Region and its predecessor, the Forest Insect and Disease Survey, for their observations and collections.

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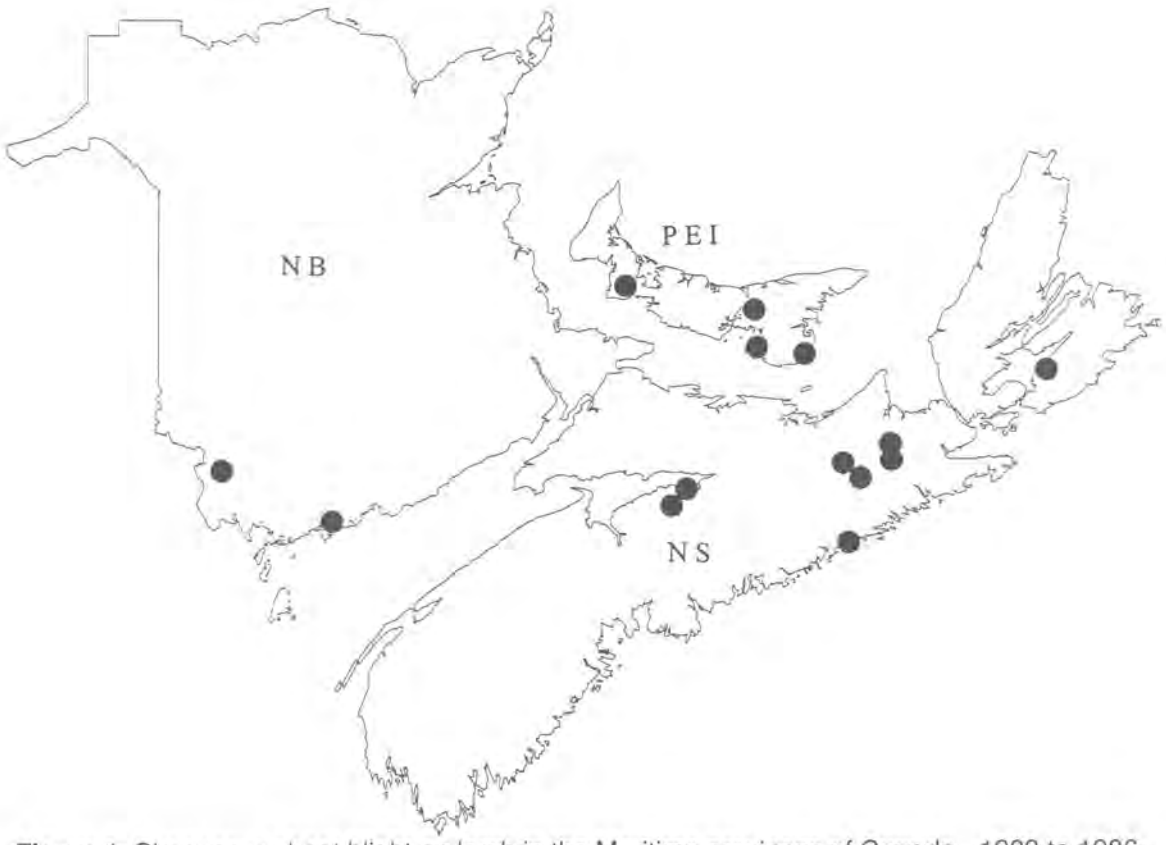

Figure 1. Sirococcus shoot blight on larch in the Maritime provinces of Canada - 1983 to 1986.

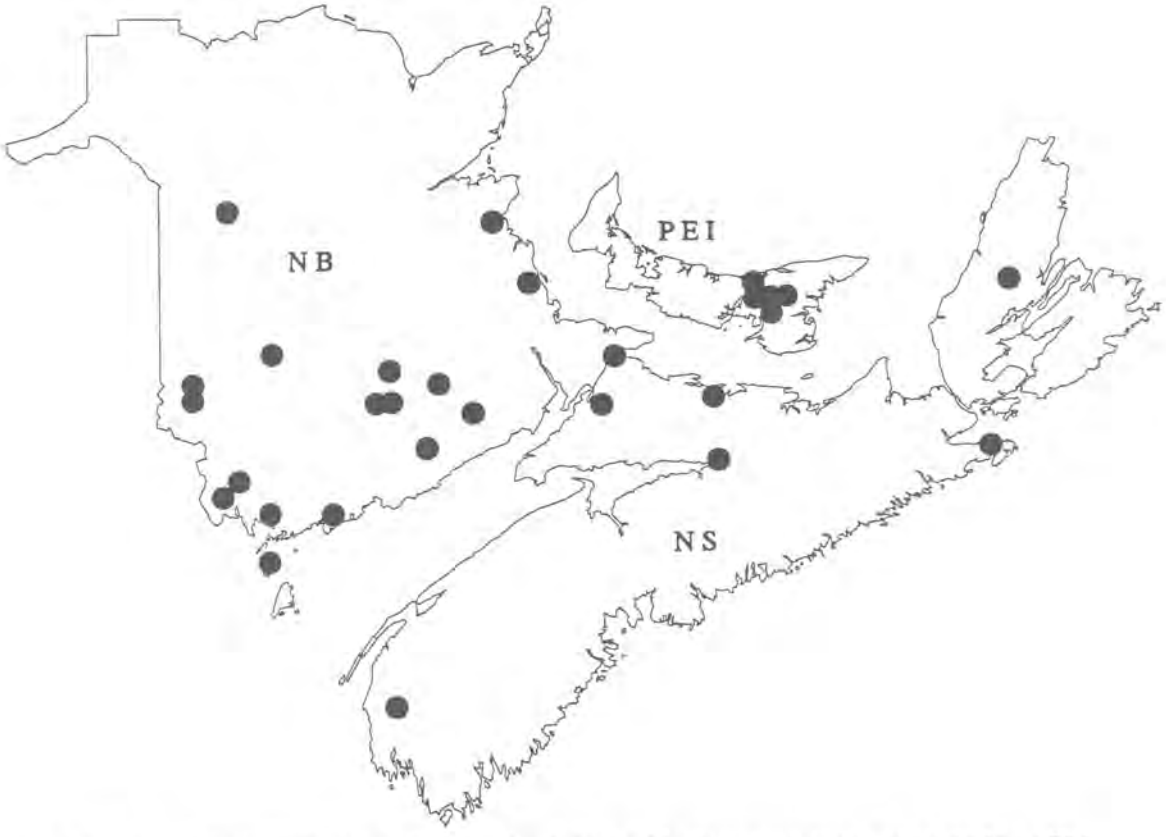

Figure 2. Sirococcus shoot blight on larch in the Maritime provinces of Canada - 1994.

DISEASES CAUSED BY *GREMMENIELLA ABIETINA* (LAGERB.) SCHLAPFER -BERNHARD AND *CENANGIUM FERRUGINOSUM* FR.EX FR. IN SCOTS PINE (*PINUS SYLVESTRIS* L.) STANDS IN POLAND

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SUMMARY

Since the 1980's, the frequency of epiphytosis and the extent of damage to forests caused by *Gremmeniella abietina* and *Cenangium ferruginosum* have increased. Meteorological factors play an important role in the development of these diseases. Weather conditions preceding and accompanying the infection affect its intensity. Frequent insect outbreaks that have taken place in recent years in Poland reduce the resistance of trees and enhance their susceptibility to fungal pathogen attacks. The extent of damage caused by these fungi is still increasing and harmful to forests.

Key word: Scleroderris canker.

INTRODUCTION

Scleroderris occurred for the first time in pine stands of northwestern Poland in 1895. At that time, Schwarz discovered five stations of its occurrence. The disease was found to be caused by a pathogen *Brunchorstia destruens* Eriks., today named *Brunchorstia pinea* being a conidial stage of *Gremmeniella abietina* (Lagerb.) Morelet. A mass infestation of seedlings by this pathogen in this area was recorded by Liese in 1922.

Cenangium dieback caused by a parasitic fungi *Cenangium ferruginosum* Fr. ex Fr. was observed in young pine thickets of central Poland in 1952 and 1959-1963.

During the last twenty years, scleroderris canker occurred in 1979-1983 in pine stands of all age classes in northern and northwestern Poland. In the following years (1983-1985), the weakened stands were mass-attacked by *Cenangium ferruginosum* in both young thickets and 40-year-old stands, particularly in western Poland.

In 1995 and 1996, a mass reddening of crowns in pine thickets and older stands was observed in northwestern Poland, the casual agents of which were gall midges (*Cecidomyidae=Itonididae*) and the fungus *Cenangium ferruginosum*.

Development of the pine shoot disease in Poland

The development rate and range of the disease in trees and stands are not only the result of the presence of the pathogen but also of weather, particularly temperature and moisture conditions. Pathogenic diseases are often preceded by unfavourable conditions of tree growth, such as soil conditions, industrial emissions or intensive insect attacks. These phenomena are usually observed in single-species (pure) stands or stands growing at unsuitable sites. Each stress resulting from the disturbances in the functioning of trees affects resistance processes and activates pathogens (Sierota 1995).

Epiphytoses of pine shoot diseases observed until the middle of the 20th century, and especially the circumstances and causes of their occurrence, have not been thoroughly clarified and described. First, detailed observations were made and studies were conducted during an epidemic infection of stands by *Cenangium ferruginosum* in the periods 1959-1963 and 1983-1985 and by *Gremmeniella abietina* in the period 1979-1983.

According to Lukomski (1968), *Cenangium ferruginosum* infested Scots pine cultures, thickets and older stands in an overall area of 10,228 ha. The severity of the infection was observed at that time almost within the whole range of the disease, i.e. in Poland, Germany, Czechoslovakia and Yugoslavia, which would indicate the influence of macroclimatic factors, however difficult to determine. In Poland, the infection of Scots pine by *Cenangium ferruginosum* was accompanied by *Thecodiplosis brachyntera*. Lukomski stated that the mass occurrence of *Thecodiplosis brachyntera* facilitated the infection of trees though it was not a sufficient condition.

The next occurrence of pine shoot dieback leading to the dieback of whole plantations and stands, even those of age classes II and III, was observed in the period 1979-1985. The main casual agent of the disease was *Gremmeniella abietina* which was accompanied by *Cenangium ferruginosum*. The epiphytosis of scleroderris canker occurred in a total area of 135,354 ha in which trees were removed from an area of 7,000 ha (Duda 1996).

The epidemic of scleroderris canker was noted in those pine stands in which a clean-eating of the nun moth (*Lymantria monacha*) during its outbreak in the 1970's caused severe damage, particularly to older stands of age class III. The spread of the disease was favoured by predisposing weather conditions immediately preceding it (1978) and after it started (1979): the cold and wet fall and the frosty and long winter with a thick snow cover. Heavy snowfall and low spring temperatures at the beginning of 1979 enhanced inoculum concentrations of the pathogen. Moist and cold maritime climate had considerable effect on the scleroderris canker which was expressed in the severity of the infection in the northern regions of Poland.

In addition to weather conditions, some other predisposing factors played an important role in the development of the disease in pure stands on former agricultural lands, at drought-affected sites or cold air drainage areas. The pathogen usually affected non-tended, shaded thickets and stands weakened by insect attacks. The studies on the biotic effects of forest fungal communities on *Gremmeniella abietina* showed that they did not inhibit the pathogen's growth. In effect, the absence of natural enemies of the pathogen resulted in highly damaged stands. At the same time, it was observed that in pine stands infected by *Gremmeniella abietina* pine trees, these exhibited higher susceptibility to *Heterobasidion annosum* attacks.

The attacks of *Cenangium ferruginosum* in pine stands followed a long period of drought in the years 1982-1983 and the draining of meadows in the Oder basin.

In 1996, pine stands of northern and western Poland were under the influence of unfavourable weather conditions. Early winter without snowfall and low temperatures caused soil freezing to a depth of 1.8 m in some regions of Poland. In the spring, a rapid rise in air temperature (at the end of March, daily temperatures were 16 - 23°C) and soil temperature below 0°C resulted in physiological drought in plants as a direct effect of water stress. The opening of the stomata was due to an intensive insolation and constant transpiration of plants. Foliage severely affected by drought caused a rapid needle yellowing and reddening. The outbreak of gall midges, such as *Thecodiplosis brachyntera* and *Cecidomyia baeri*, made the stands more susceptible to fungal diseases. Needle colonization by these insect species was recorded in 1994 in an area of 8,500 ha and in 1995, in an area of 602,000 ha. At the same time, a simultaneous activation of endophytic fungi living on live pine needles and shoots and mass incidence of saprophytic fungi on dying or dead shoots and on logging slash were observed. *Cenangium ferruginosum* and *Scoleconectria cucurbitula* (Tode:Fr) showed the highest sporulating activity.

Surveys of forest health status that were carried out in May and June 1996 revealed that the symptoms of crown dieback occurred in stands of all age classes in a total area of 947,500 ha.

Stands with more than 60% crown damage were observed on 8,000 ha, with 31-60% crown damage on almost 60,000 ha and in an area of 880,000 ha, the symptoms of shoots and needle damage were less than 30% (Fig. 1).

By the end of the summer and at the beginning of the fall, most of the dead needles had fallen and it looked as if the trees had improved their health condition. However, their crown structure was loosened and the mortality of shoots and branches was high. Usually, a few dead trees had an earlier infection by root pathogens. The estimate of the occurrence of *Cecidomyidae* species conducted in November 1996 showed a decrease in the infestation rate.

In his detailed mycological analysis of pine shoot samples collected between June and November 1996, Kowalski (1997) stated that the phenomenon of shoot dieback was of the same nature in the whole northwestern region of Poland, however, it varied locally in the intensity of symptom occurrence. Killed shoots were colonized by fourteen fungal species. Most frequently isolated were *Cenangium ferruginosum*, *Sclerophoma pythiophila* (Corda) Höhn. and *Scoleconectria cucurbitula*. *Gremmeniella abietina* and *Sphaeropsis sapinea* (Fr.:Fr) Dyko & Sutten were found occasionally. Many of the isolated fungi colonized live and healthy needles and shoots which supported their endophytic character.

The health status of pine stands in 1997 will depend on the resistance of trees and weather conditions affecting both the host plant and the pathogen, the inoculum and fungal parasitic activity.

CONCLUSIONS

Pine shoot disease is, to a great extent, associated with pine's high specific susceptibility. The development of the disease has always been fostered by local climatic effects. The predisposing factors prior to infections, such as reduced tree resistance, mass incidence of insects,

excessive rainfall or drought regime were found to increase the susceptibility of trees to pathogen attacks (as shown in Fig. 2).

During the 1980's, epiphytosis followed the outbreak of *Lymantria monacha* and was enhanced by specific meteorological conditions favoring the development of the disease. The situation, with the present epiphytosis, seems to be similar although the course of its development is different. The infection is caused by a different set of pathogens.

In recent years, we have been faced with frequently reccurring diseases caused by pathogens which used to be called weakness pathogens. The extent of damage caused by these fungi is still increasing and more harmful then ever to forests.

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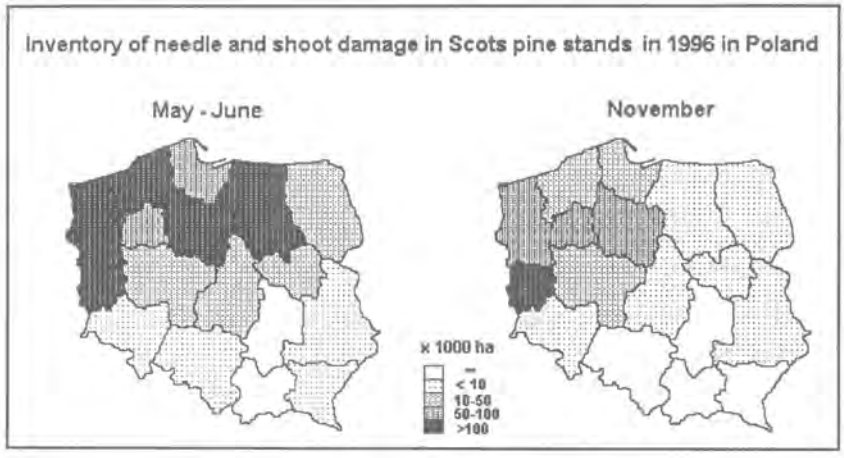

Figure 1. Inventory of needle and shoot damage in Scots pine stands in Poland.

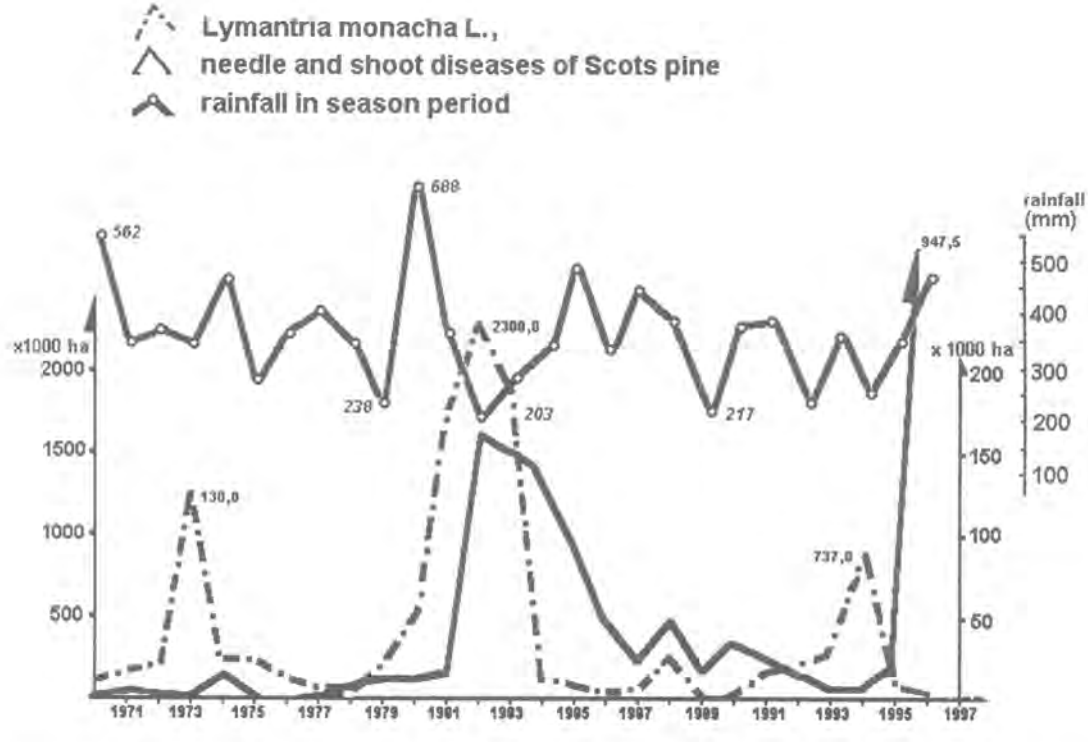

Figure 2. Occurrence of *Lymantria monacha* L., needle and shoot diseases of Scots pine and rainfall in northwestern Poland.

DAMAGE TO JAPANESE PINES CAUSED BY *CENANGIUM FERRUGINOSUM* IN NORTHERN HONSHU, JAPAN

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SUMMARY

Dieback of Japanese pine trees caused by *Cenangium ferruginosum* occurred in Iwate Prefecture, in the northern part of Honshu, in Japan, from 1994 to 1996. The rate of dieback was 97% in the most severely affected plot. In a plot located in a Japanese black pine stand, 13% of the affected trees were killed. Apothecia were observed from May to August on dead twigs and branches, with the greatest abundance occurring in July. Asci and ascospores were produced from late June to early August. A soil survey showed that soil moisture was very high or soil compactness was very hard in the affected plots. Precipitation in the summer seasons from 1993 to 1995 was more than twice the normal precipitation. The results suggest that environmental factors are closely associated with serious occurrences of this disease.

Keywords: *Pinus* spp., *Cenangium ferruginosum*, dieback, precipitation, soil moisture content.

INTRODUCTION

Cenangium twig blight of Japanese pines (*Pinus densiflora* Sieb. et Zucc. and *Pinus thunbergii* Parl.) is one of the most important diseases of pine plantations in Japan (Ito 1974). The dieback of Japanese pine trees caused by *Cenangium ferruginosum* Fr. ex Fr. has been noted in wide areas of Japan (Kobayashi and Mamiya 1963, Ogura et al. 1995, Suto 1970). However, little is known about this disease. In fact, there have been no detailed observations of this disease in mature pine forests in Japan. From 1994 to 1996, Cenangium twig blight of Japanese pine trees occurred in Iwate Prefecture, in the northern part of Honshu, in Japan. Serious outbreaks of this disease were found in some mature pine stands.

We conducted some field surveys to understand the actual conditions and the factors related to this damage in these pine stands.

MATERIALS AND METHODS

1. Distribution of the Disease in Iwate Prefecture and in Japan

From 1994 to 1996, we conducted a survey of the disease incidence of Cenangium twig blight in Iwate Prefecture, in the northern part of Honshu, in Japan. From June to August of 1995 and 1996, twigs or branches that had turned brown were collected and fruit body production was observed.

To make a distribution map of this disease in Japan, records of the incidence of this disease were obtained from the Forest Agency of Japan (all references occurred between 1957 and 1992) and from *Forest Pests* (a monthly journal) (whose references occurred between 1975 and 1982).

2. Actual Condition of the Damage in Survey Stands

The field survey was conducted at two mature pine stands, one of Japanese red pine (*P. densiflora*) and the other of black pine (*P. thunbergii*) in Iwate Prefecture. The Japanese red pine stand was located at the foot of the northern side of Mt. Iwate (elev. 2041 m) at Matsuo village. The Japanese black pine stand was a coastal forest located at the northern part of Iwate Prefecture at Noda village. The age, average DBH and average height were 21 years, 10 cm and 12 m, respectively in the Japanese red pines stand and 60 years, 20 cm and 14 m, respectively in the Japanese black pine stand. Three plots were set up (extent 400 m^2 ~ 1500 m^2) in each pine stand. The severity of damage of each tree was evaluated according to the degree of dieback (Suto 1970) and classified into 5 grades. Detailed surveys were conducted at the Japanese red pine stand in August 1994 and at the Japanese black pine stand in August 1996.

3. Period of Apothecial Development

To confirm the period of development of fruit bodies (apothecia) of the fungus, infected twigs and branches were collected from pine stands and carried to the nursery of Iwate Prefectural Forestry Technology Center in Yahaba village. Fruiting bodies produced on dead twigs and branches were investigated from April to September. The degree of apothecial development of the fungus was visually observed and classified into 4 grades depending on the extent of fruit body formation on the twigs and branches. The degree of development of asci was also investigated by observing cross sections of the apothecia under the microscope. For each period, 10 apothecia were observed and classified into 3 grades. The degree of ascospore development was evaluated by the number of ascospores in asci under the microscope and was classified into 4 grades. To confirm the degree of ascospore development, collected apothecia were placed on the undersides of the lids of petri dishes and kept at 25°C for 72 h. The ascospores were then allowed to drop to the bottom of petri dishes. The number of ascospores that dropped per visual field (X400) of the microscope was counted in 3 petri dishes at each period, and the degree of ascospore development was classified into 4 grades according to the average number of ascospores.

4. Survey of Environmental Conditions

To clarify the relationship between the occurrence of the disease and environmental conditions, soil surveys were conducted. Soil profiles (1 m in width X 1 m in depth) of the layers, textures, structures and moisture conditions were prepared for each plot. Surveys were conducted at the Japanese red pine stand in October 1995 and May 1997 and at the Japanese black pine stand in August 1996 and May 1997.

Climatic data (precipitation and temperature) from 1993 to 1995 were collected from monthly meteorological records (Nihon Kisyoukyoukai Morioka Shibu 1993-1995) at Matsuo village and Kuji city (the closest climate survey station) in Iwate Prefecture.

RESULTS AND DISCUSSION

1. Distribution of the Disease in Iwate Prefecture and in Japan

The distributions of Cenangium twig blight in Japan (based on a literature survey) and in the Iwate Prefecture (based on the present field survey from 1994 to 1996) are shown in Fig. 1. It has been reported that this disease was found in only a limited number of pine stands in the Iwate Prefecture. But the field survey revealed that this fungus was widely distributed in Iwate Prefecture (Li et al. 1995). The literature survey indicates that this fungus is distributed widely in Japan. According to past records, this disease was found on the following pine species: *Pinus densiflora, P. thunbergii*, and the F1 hybrid of *P. thunbergii* X *P. massoniana*. Damage was also found on trees 8 to 50 years old, and especially on trees 10 to 20 years old. The record of maximum extent of the disease was 340 ha in Japan. The range of ages and the range of areas damaged by this disease in Japan are rather small compared with those in Europe (Kessler 1993).

2. Actual Condition of the Damage in Survey Stands

Needles turned brown conspicuously from late spring to early summer and fruit bodies (apothecia) of the fungus were observed on dead twigs and branches (Fig. 2). From field observations, the symptoms of the disease on Japanese black pine appeared approximately two weeks to a month later than those on Japanese red pine.

Results of the field survey are shown in Tables 2 and 3. The incidence of twig blight was 97% in the most severely affected plot. Three percent of affected trees in this plot died. The fruit bodies of the fungus were observed on the upper parts of the stems of dead pine trees. Death of trees by this disease in mature pine stands has never been reported in Japan (Kobayashi and Mamiya 1963, Ogura et al. 1995, Suto 1970). On the other hand, 71% of trees in the most severely affected plot in the Japanese black pine stand were infected. Thirteen percent of the affected trees in this plot were killed by the disease.

3. The Period of Apothecial Development

Fruit bodies were produced on dead twigs and branches once a year. Apothecial development of the fungus is shown in Table 3. Apothecia were observed from late May to mid August on damaged Japanese red pine twigs. Only black stromata were observed until mid May. After mid August, only black and brittle fruit bodies were observed. The field observations showed that the season of apothecium production on twigs occurred later in Japanese black pine than in Japanese red pine. Ascospore development of the fungus is shown in Table 4. No ascospores were recognized until early June in spite of the development of apothecia. Ascospores were observed from late June to early August.

These results suggest that ascospores of *C. ferruginosum* were mainly dispersed between late June and early August in northern Japan.

4. Environmental Conditions of the Pine Stands

The results of the soil survey are shown in Fig. 3. According to the usual classification (Forest Soil Division 1975), soils in both investigated stands were immature. In the Japanese red pine stand, the horizon sequence and soil color indicate that horizons from A_1 to B_2 were artificial banked soil. Because these horizons had poor humus, a massive structure had hard compactness, especially, the B_2 horizon. Thus, healthy roots were hardly observed below this layer. In the Japanese black pine stand, horizons between C_1 and C_2 were ballast layers, because these horizons had poor humus. A reduced horizon about 30 cm in depth was observed. An aquifer was also observed in the soil below 50 cm. It was considered that an aquifer seasonally shift to the reduce horizon. Thus, only rotted roots were observed at depths below 30 cm.

Precipitation for 10-day periods near the two investigated pine stands is shown in Fig. 4. It is well known that Cenangium twig blight is most common after severe winters, especially if the winter is preceded by unusually mild autumn weather (Wayne et al. 1987). But there was no appreciable difference in the temperature between 1993 and 1995 compared with other years. On the other hand, the precipitation during the summers of 1993 and 1995 conspicuously exceeded the normal precipitation. Damage in the Japanese red pine stand first appeared in late spring of 1994. It also appeared in early summer of 1996 in the Japanese black pine stand. Thus, abnormal precipitation was recorded one year before the appearance of the damage in both stands. Kobayashi and Mamiya (1963) reported that the release of ascospores from sporecarps of *C. ferruginosum* is enhanced by rain.

CONCLUSIONS

This is the first report that proves that Japanese pines in mature stands were killed by Cenangium twig blight disease in Japan. It is believed that distinctive soil conditions led to the rotting of roots in the pine stands. Because the pine trees may be stressed continuously by the rotting of roots, it is also believed that the abnormal precipitation caused numerous infections of the fungus. Thus, this suggests that the severe damage in the mature pine stands was the result of two factors, that is, continuous stress on the pine trees by abnormal soil conditions and numerous infections due to unusual amounts of precipitation.

Table 1. Severity of Cenangium twig blight in a mature Japanese red pine stand (21 years old)

Plot	No. of trees surveyed	Severity of damage [1)]					Total trees affected
		Healthy	Slight	Moderate	Severe	Dead	
A-1	194	6(3.1)	60 (30.9)	102 (52.6)	24 (12.4)	2 (1.0)	188 (96.9)
A-2	55	2 (3.6)	34 (61.8)	18 (32.7)	1 (1.8)	0 (0.0)	53 (96.4)
A-3	55	38 (69.1)	17 (30.9)	0 (0.0)	0 (0.0)	0 (0.0)	17 (30.9)

[1)] Healthy-no twigs or branches were infected; Slight-one to three twigs or branches were infected; Moderate-between slight and severe damage; Severe-more than half the number of twigs or branches were infected; Dead-only dead twigs or branches were observed. Numbers in parentheses indicate the percent of number of trees surveyed.

Table 2. Severity of Cenangium twig blight in a mature Japanese black pine stand (60 years old)

Plot	No. of trees surveyed	Severity of damage [1]					Total trees affected
		Healthy	Slight	Moderate	Severe	Dead	
B-1	133	39 (29.3)	39 (29.3)	20 (15.0)	23 (17.3)	12 (9.0)	94 (70.7)
B-2	108	92 (85.2)	13 (12.0)	2 (1.9)	1 (0.9)	0 (0.0)	16 (14.8)
B-3	113	95 (84.1)	18 (15.9)	0 (0.0)	0 (0.0)	0 (0.0)	18 (15.9)

[1] See footnote, Table 1.

Table 3. Period of fruit body development of *C. ferruginosum* on damaged Japanese red pine twigs

Date	Degrees of fruit body development					
	April	May	June	July	August	Sept.
1995			++	+++	+++[1]	-
1996	-	+[2]	++	+++	++	-

Symbols indicate the relative quantity of apothecia observed on the pine twigs or branches : -, None; +, few; ++, many; +++, numerous.

1) Includes black and brittle apothecia because ascospores had already been discharged.

2) Includes not only immature apothecia but also black stromata.

Table 4. Degree of apothecial development of *C. ferruginosum* on damaged Japanese red pine twigs at different times between 21 May and 31 August in 1995 and 1996

Date	Degree of development									
	May 21-31	June 1-10	June 11-20	June 21-30	July 1-10	July 11-20	July 21-31	Aug. 1-10	Aug. 11-20	Aug. 21-31
1995										
apothecia			+++[1]	+++	+++	+++	+++	+++	+++	+++
asci			+[2]	++	++	++	++	+	-	-
ascospores			-[3]	++	++	++	++	+	-	-
1996										
apothecia	+	++	+++	+++	+++	+++	+++			
asci	-	+	++	++	++					
ascospores	-	-	+	++	++					

[1] Relative quantity of apothecia (same as in Table 3): -, None; +, few; ++, many; +++, numerous.

[2] Degree of ascial development (relative number of asci observed on apothecia): -, None; +, some; ++, many.

[3] Degree of ascospore development (relative number of ascospores per visual field (X400) of microscope, average of 3 repetitions): -, 0; +, 1-50 ascospores; ++, 51-100.

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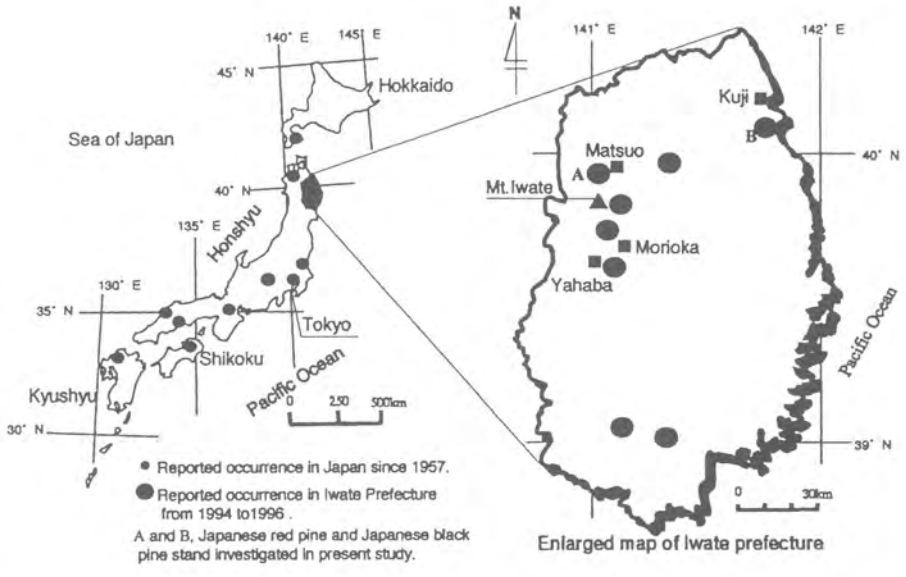

Figure 1. Geographic distribution of Cenangium twig blight in Japan and Iwate Prefecture.

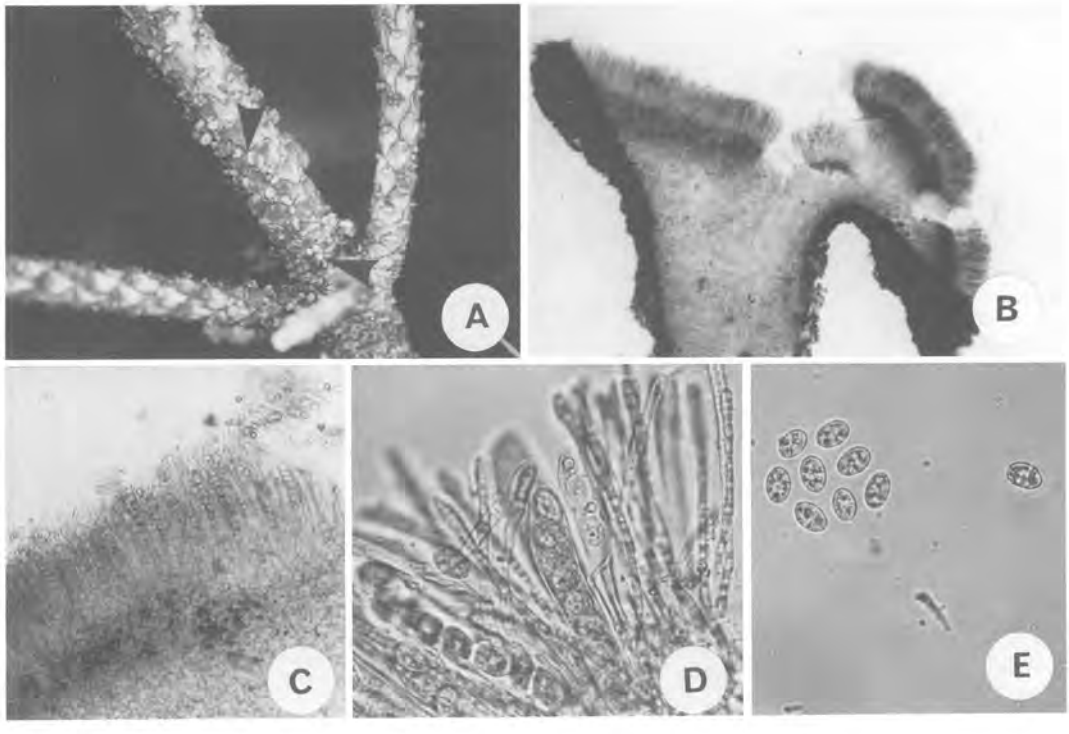

Figure 2. Micrographs showing *Cenangium ferruginosum* on Japanese red and black pines in Iwate Prefecture. A: Twig of damaged black pine tree. Arrows show apothecia of *C. ferruginosum*. B~E, micrographs obtained from Japanese red pines. B: Cross section of apothecium under low magnification (X60). C: Cross section of apothecium, showing clusters of asci (X150). D: Asci with ascospores and paraphyses (X600). E: Ascospores (single-celled) (X600).

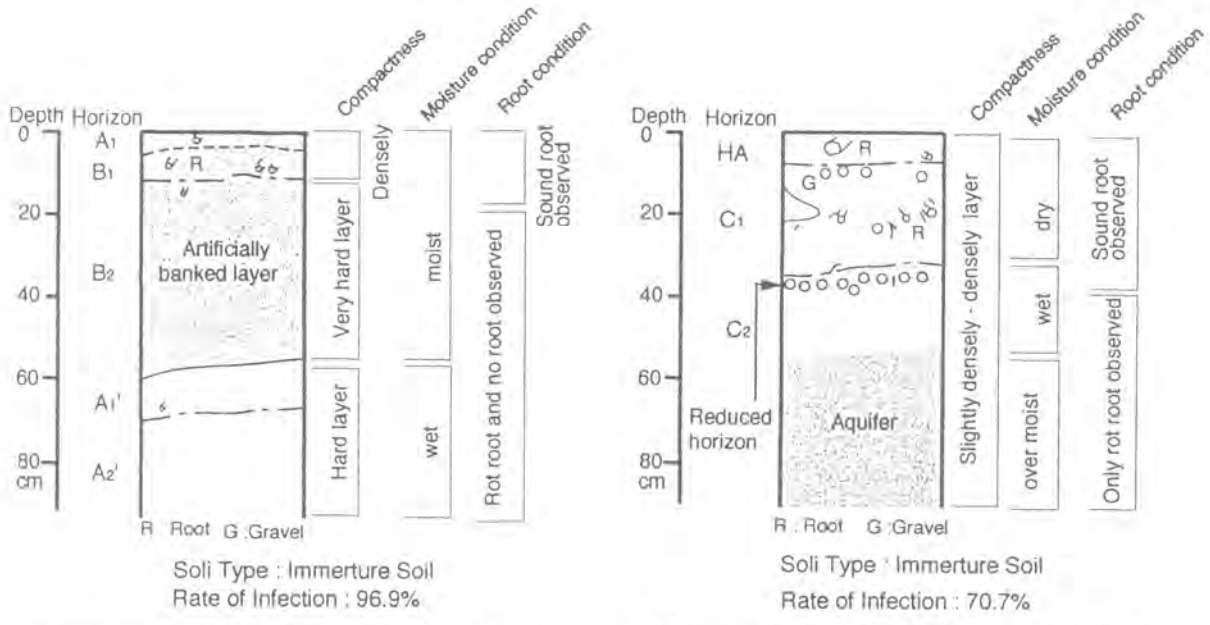

Figure 3. Profiles of soil, soil compactness, soil moisture conditions and root conditions in Japanese red and black pine stands.

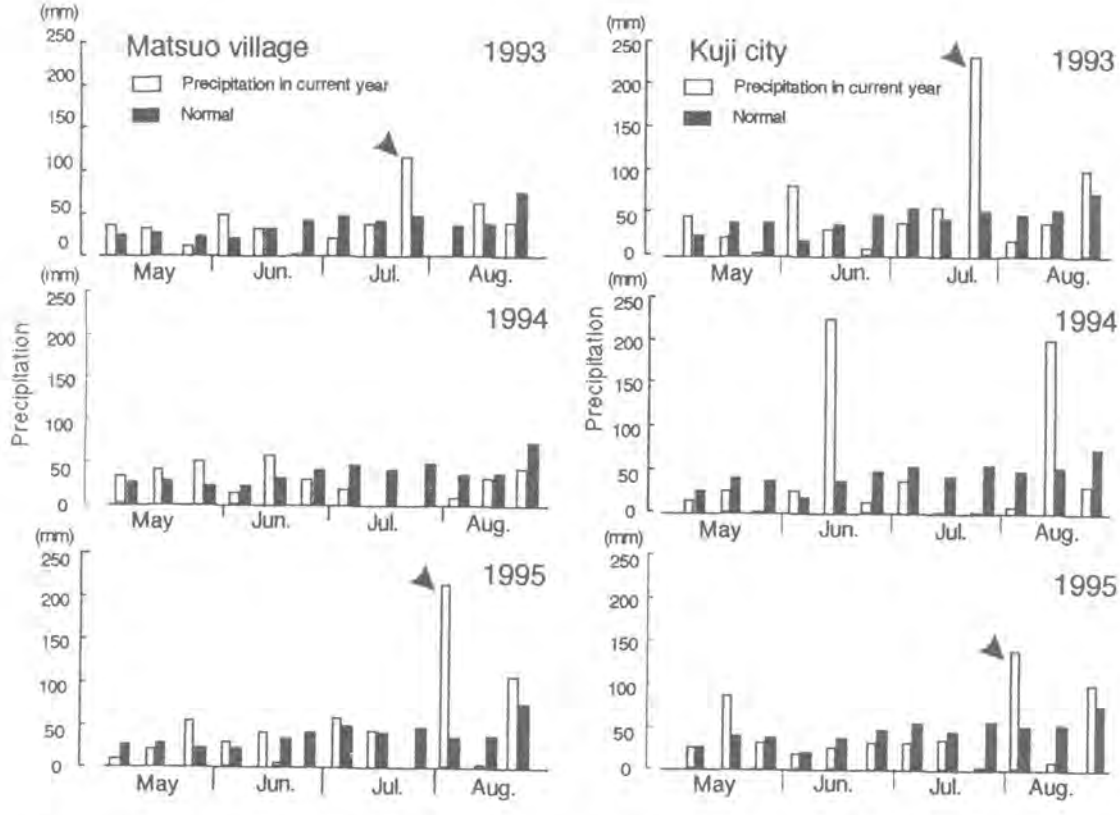

Figure 4. Monthly precipitation for May-August in Iwate Prefecture (1993-1995). Arrows show abnormally high precipitation compared with normal precipitation.

SHOOT BLIGHT OF *PINUS HALEPENSIS* Mill. IN THE ITALIAN PENINSULA

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SUMMARY

Aleppo pine (*Pinus halepensis* Mill.) is a Mediterranean tree which is found in Italy in both natural and cultivated stands. This species is affected by a shoot blight and needle diseases caused by several fungi such as *Brunchorstia pinea, Sphaeropsis sapinea, Lophodermium seditiosum, Thyriopsis halepensis, Cronartium flaccidum, Melampsora pinitorqua, Cyclaneusma minus, Cenangium ferruginosum, Pestalotiopsis funerea* and *Syrococcus conigenus*. In some coastal areas, shoot blight and crown reddening caused by wind-borne sea aerosol have also been observed. All these diseases are briefly described, taking into account the environmental conditions under which they chiefly arise.

INTRODUCTION

Aleppo pine (*Pinus halepensis* Mill.) is a Mediterranean tree species which grows in an area extending from the coasts of North Africa and Spain to Greece and Turkey. Its range is rather discontinuous, especially in the middle section of the Mediterranean Basin. In Italy, the species grows in spontaneous stands in Puglia, Umbria, Liguria, Sardinia, Campania and Sicily. It thus grows in areas that tend to have less severe frosts, to which Aleppo pine is more susceptible than other Mediterranean conifers.

The importance of Aleppo pine derives from its drought resistance characteristics and its ability to grow on poor, thin, rocky soils, including soils containing toxic amounts of minerals. These characteristics render this species suitable for reforestation on degraded or refractory sites (i.e. on bare soils) (Bernetti, 1995).

Recent reforestation with Aleppo pine, often mixed with cypress, is widespread in the Italian coastal areas and adjacent hills. The overall surface area of land planted predominantly with Aleppo pine is estimated to be some 20,000 ha (Seigue, 1985).

The present report provides a brief description of some of the most important fungal diseases hitherto observed in Italy on the needles and twigs of Aleppo pine, with special consideration of the environmental conditions that favour the disease. Some observations on crown damage caused in the coastal areas of Puglia by sea aerosol are also reported.

Microfungi observed on shoots and needles.

1. ***Sphaeropsis sapinea*** (Frifr) Dyko and Suton
(syn. *Diplodia pinea*, *Deuteromycotina*, *Coelomycetes*);
2. ***Brunchorstia pinea*** (Karst.) Höhn.
(*Deuteromycotina*, *Coelomycetes*, teleomorph: *Gremmeniella abietina*, syn. *Ascocalix abietina*, *Scleroderris lagerbergii*);
3. ***Thyriopsis halepensis*** (Cooke) Theiss. and Syd.
(*Ascomycotina*, *Dothideales*);
4. ***Cronartium flaccidum*** (Alb. and Schw.) G. Wint.
(*Basidiomycotina*, *Uredinales*);
5. ***Melampsora pinitorqua*** Rostr.
(*Basidiomycotina*, *Uredinales*);
6. ***Cenangium ferruginosum*** Fr.: Fr.
(*Ascomycotina*, *Helotiales*);
7. ***Lophodermium seditiosum*** Minter, Staley and Millar
(*Ascomycotina*, *Rhytismatales*);
8. ***Pestalotiopsis funerea***, (Desmaz.) Steyaert (*Deuteromycotina*, *Coelomycetes*);
9. ***Sirococcus conigenus***, (DC.) P. Cannon and Minter
(*Deuteromycotina*, *Coelomycetes*);
10. ***Cyclaneusma* (*Naemacyclus*) *minus*** (Butin) DiCosmo, Peredo and Minter
(*Ascomycotina*, *Rhytismatales*).

1. ***Sphaeropsis sapinea*** (Diplodia blight) is a sometimes devastating disease of pines that are already under stress. On Aleppo pine it causes tip blight, resinous cankers on the main stems and branches, blight of seedlings, dieback, and basal cankers accompanied by grey to black stains of the sapwood. It is common in plantations where trees are planted outside their natural range, but it rarely causes damage in natural stands. Generally, trees are predisposed to this infection by conditions such as water shortages, compacted soil, root injury, excess shade, or heat reflected from nearby roofs.

In the Mediterranean basin, the prime cause predisposing to severe *S. sapinea* infection is recurrent summer drought. Severe outbreaks have been recorded in Aleppo pine plantations in Israel (Madar *et al.*, 1996) and Morocco (Stiki, 1994). In Italy damage has been sporadic. The fungus has been found frequently on trees suffering from the aftereffects of the exceptionally cold winter of 1985 and the following drier than usual summer (Capretti *et al.*, 1987). In the central Apennines, *S. sapinea* has also been found on Aleppo pine shoots previously killed by *Brunchorstia* infection (Barbacovi *et al.*, 1979).

2. ***Brunchorstia pinea***. In its typical form, Brunchorstia dieback is characterized by the death of shoots and canker formation in the shoots and branches.

On *P. nigra* Arnold and *P. halepensis* infection can be extensive in the Apennines, where the development of many trees may be put at risk. On Aleppo pine, damage was located especially in the lower portion of the crowns in a 25-year-old plantation (central-southern

Apennines) located at an altitude which is excessive (500-750 m a.s.l.) considering the characteristics of the species (Barbacovi *et al.*, 1979).

3. ***Thyriopsis halepensis***. This fungus causes serious defoliation on various pine species. Two-year-old needles are especially affected, more often those in the lower part of the crown. Generally the apical tufts of the needles are not affected, or affected only in part by the defoliation.

Damage seems to be most serious in pine stands with poor ventilation due to the persistence of humidity, especially after some years characterized by dry springs and summers. This infection has been observed in areas reforested with *P. halepensis, P. pinaster* Ait. and *P. nigra* var. *laricio* (Poir.) Maire in various parts of central and southern Italy (Basilicata, Puglia, Lazio) (Frisullo and Luisi, 1984).

4. ***Cronartium flaccidum***. This fungus causes blister rust on two-needled pines. The pine species most susceptible to blister rust are, in descending order of importance, *Pinus pinea* L., *P. halepensis, P. nigra, P. sylvestris* L. (Raddi and Fagnani, 1978). During its complex life cycle, *C. flaccidum* infects and kills the bark tissue, causing twigs and branches to wither.

Epidemic outbreaks of this rust occurred in Italy on various species of pine from the late 1940´s until the 1970´s. On Aleppo pine such damage was mostly limited to young plantations in Puglia (Moriondo, 1989). These epidemics were probably related to the weather patterns of the time, characterized by exceptionally rainy spring and summer periods, alternating with periods of greatly reduced rainfall, and to the extensive reforestation carried out in the period before and after World War II.

5. ***Melampsora pinitorqua***. This fungus causes pine twist rust. Attacks occur on trees 1 to 10 years old. Seedlings in the first year of growth have no resistance against this rust and easily die from it (Moriondo, 1967).

In Italy, pine twist rust has been reported on various pine species, including Aleppo pine, in Liguria, Tuscany and Campania. The severity of infection depends mainly on a high mean rainfall in spring, when the trees are infected by the basidiospores. Epidemics occurred in the 1960´s, concurrently with the direct sowing of pine trees along the coast and in the hilly areas further inland (Moriondo, 1989).

6. ***Cenangium ferruginosum***. This fungus is mainly saprotrophic on the bark of dead branches of various pine species. It almost always occurs as the teleomorph only.

In Tuscany, Italy, *C. ferruginosum* is found in young plantations on some partly damaged or dead branches of Aleppo and Maritime pines suffering from the particularly cold winter of 1985 and the following long dry summer (Capretti *et al.*, 1987). On these trees other weak pathogens such as *S. sapinea* and *Pestalotiopsis funerea* were present at the same time.

It has also been found on Aleppo pine shoots damaged by attacks of *Brunchorstia* (Barbacovi *et al.*, 1979).

7. ***Lophodermium seditiosum.*** This fungus causes needle reddening of pines. It attacks all pine species at the unlignified seedling stage. At the adult stage only *P. sylvestris* is highly susceptible. Damage produced by *L. seditiosum* consists of the more or less extensive loss of needles from previous growing seasons. Trees infected with the fungus for a number of seasons in succession can die owing to infestation with secondary parasites (Moriondo, 1989).

In Italy, needle reddening causes significant damage only in nurseries where it kills young seedlings of all pine species, and causes defoliation of young *P. sylvestris* trees of various ages. When spring and summer rainfall is much higher than usual, adult trees can also become infected, and this has occurred in *P. sylvestris* stands in Lombardy (Moriondo, 1967).

8. ***Pestalotiopsis funerea.*** This fungus primarily infects gymnosperms on which it acts as a weak pathogen, fruiting on the leaves and stems of weakened or dead trees. In Italy, it may act as a parasite on some *Cupressaceae* (Panconesi, 1994). On pines it is a weak parasite, or a saprophyte taking the place of other pathogens (Capretti *et al.*, 1987).

9. ***Sirococcus conigenus***. This fungus causes shoot blight and seedling mortality on conifers in nurseries, plantations, natural stands and ornamental plantings. Succulent shoots and often one-year-old twigs are killed.

Long wet periods favour both the spread and the severity of infection. Low light intensity beneath the forest canopy, for example, enhances tree susceptibility and hence the severity of Sirococcus blight. Damage on large trees is mainly confined to the lower branches and has but a slight effect on general health.

Cases of sporadic dieback from *Sirococcus* have recently been observed in the Mercadante Forest in Puglia on young Aleppo pines mixed with oak.

10. ***Cyclaneusma minus***. This fungus attacks various species of pine, from the transplant to the thicket stage. Under certain weather conditions it can cause premature cast of needles of all ages.

In Italy, its presence is rare as it has been isolated only in Liguria, on *P. halepensis, P. pinaster* and *P. radiata* D. Don (Butin, 1973).

Damage to the crown from sea aerosol

Crown reddening and dieback due to sea aerosol have been observed extensively on *P. halepensis* in Puglia along both the Adriatic and the Ionian seas. The damage symptoms appear in a typical manner on the shoots that are continually exposed to the sea winds which prevail. Instances of trees with a pillow-like shape or with flag-crowns are common. The aerosol also affects many other tree species, but tree survival is almost never threatened. The question whether damage is due entirely to the salt deposited on the crowns, or also involves marine pollutants (anionic surfactants), as occurred in other cases (Gellini *et al.*, 1983), still remains to be determined.

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A NEW SPECIES OF *ELYTRODERMA* FROM PORTUGAL: BIOLOGICAL CYCLE

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SUMMARY

A new species of the genus *Elytroderma* Darker, causing shoot and needle disease on *Pinus pinea*, is described under the name *Elytroderma lusitanicum* Fonseca-Neves, and is also illustrated. The relationship between the new species and other species already described is discussed. The biology of the fungus was investigated on needles and shoots and it was found to be perennial in shoots, as it was able to infect new buds and needles, thus maintaining an unbroken chain of infection into the developing stems and needles. Twisted branches and witches' brooms as well as brown lesions in the inner bark were seen. Mycelium was observed in woody tissues, buds and current-year green needles.

INTRODUCTION

An ascomycete, in the family Rhytismataceae, was found to cause heavy damage to needles and shoots of *Pinus pinea* L. It is clear that the fungus belongs to the genus *Elytroderma* Darker; it shows morphological similarities to *E. torres-juanii* Diamandis and Minter but differs in its biology and symptoms caused on the host, being similar to *E. deformans* (Weir) Darker. Because this species cannot be identified with *E. deformans*, or *E. torres-juanii*, a new species is suggested to accommodate the Portuguese collections of *Elytroderma*.

Elytroderma lusitanicum sp. nov.

Ascocarpi in bruneis regionibus mortibus acuum sunt qui viridis regiones quoque ostendunt, praecipue abaxiales, plerunque in latitudine maxima acuum incidentes, nigri, nitidi, usque 5mm longis, rima longitudinali mediana aperti sine labiis, subepidermales clypeos habentes lateraliter amplificatos et incorporantes cellulas hypodermaliae. Asci unitunicati, saccati octospori, 240-350 µm, IK2 nihil. Paraphyses filiformes, flexuosae septatae vel non septateae, apices incrassatae. Ascosporae fasciculatae dispositae, baciliformes, primum hyalinae dein palide-brunneae, 1-septatae, cellula apicali parviore, ad septum exiguae constrictae, 110 -160x8-10 µm et muco hyalino 6-8 µm lato circundatae.

In foliis vivis *Pini pinea* L, Portugal, Estremadura, Lagôa de Albufeira Setembre 1981; holotypus LISFA 13422.

Haec descriptio valde afinis est descriptionis *Elytroderma torres-juanii* praccipue differens formatione "witches' brooms".

SIGNS AND SYMPTOMS OF THE DISEASE

The most striking feature of the disease is a spring reddening of attacked needles contrasting with the normal foliage. Infected needles in the spring of their second and third year are reddish from the tip, leaving green bases. Concolorous pycnidial blisters are produced on the red portions. As the season advances, this reddish colour gradually fades to brown and the perfect stage of the fungus begins to form. The first hysterothecia appear as dark brown or black shiny lines on dead portions of the needles and such lines are the most definite sign of the disease. The different stages of the disease development are shown in Fig. 1.

The infected needles are cast from the lower branches at the start of winter and in affected trees (still alive at this time) the lower crown is without needles, while the upper parts have a green and healthy appearance. Affected trees show a reduction in their growth and vigour, upturned branches (Fig. 2) and stunted appearance; witches' brooms were observed (Fig. 3) and the fungus was found to be able to grow through the phloem colonizing shoot tissue (Fig. 4). Many trees have been killed and in some plots all the trees were dead.

An internal symptom is the presence of brown lesions in the inner bark of infected branches. On sectioning the bark tangentially (Fig. 5), transversely or obliquely, these lesions are observed on attacked branches of all ages including the main stem. Brown lesions are also observed in the central axis and scales of cones.

BIOLOGICAL CYCLE

P. pinea needles emerge from their fascicular sheath around March-April and the new developing needles remain symptomless until October. In late October, the ascospores released from one-, two- and sometimes three-year-old needles infect current- year and older needles. Translucent yellow infection spots can be seen on these green needles, at any point along their length. Needles bearing these small spots were labelled in the field in November-December 1981 to follow the fungus development. During the following spring (March 1982), the needles infected in the previous year (labelled in November-December) took on a striking red colour extending from the tip downwards, leaving green needle bases (Fig. 1). *Concolorous pycnidia*, appearing externally as small blisters about 1 mm long, were produced on reddish portions on the needle.

At the end of May and early June, the hysterothecial development begins and the reddish colour of the diseased needles starts to fade from the tip and and the straw colour of the dead needles gradually occurs. The young hysterothecia first appear as black lines, 5-10 mm long. During the summer they grow larger until maturity, sometimes coalescing longitudinally to form longer fruit bodies on both sides of the needle (Fig. 1).

From late August continuing into September, mature ascospores can be found in the hysterothecia. At the beginning of autumn (October), the hysterothecia start to release their ascospores. During October and November, a great number of opened ascocarps (Fig. 6) are found on brown needles, still attached to the branches. At the start of winter (mid- December) we cannot find any spores left in the hysterothecia; most of the needles are entirely dead (brown) and only some remain green at the base.

Needles killed by the disease are usually shed during the winter months. Nevertheless some of them, though dead, remain attached to the branches even a year after having died.

Portuguese *Elytroderma* was found to be a systemic fungus, i.e. it can colonize both needles and shoots from existing infection and grow from one year's shoot into the next. The study of anatomical aspects of pathology of the disease gave us information concerning the limits of systemic invasion and the course of mycelium within buds and twigs of umbrella pine.

Mycelium was observed colonizing not only the axis of the buds, but also the needle primordia contained therein.

Buds appear during the growing season, before ascospore discharge. Thus, if mycelium is found inside these buds, it is unlikely that it is a result of infection of buds by ascospores. Mycelium was found in the phloem of current-year branches, and hyphae could be traced from there into the terminal buds formed at the end of those branches. Therefore, it is clear that hyphae travel distally through the phloem of the twig to the terminal buds, colonizing the needle primordia. Consequently, the apparently healthy green needles emerging in spring contain mycelium as early as a year before reddening.

Mycelium was also observed in branches from 2 up to 11-12 years old. The ondulating, branched, septate, sometimes of unequal diameter hyphae were seen in the parenchymatous tissue which forms the pith, destroying the primary phloem, and its course was traced through the medullary rays, also parenchymatous, up to secondary phloem and cambium. Secondary phloem is damaged, resulting in cavities filled by mycelium and often transformed into resin cysts, seen with the naked eye as the characteristic brown lesions in the inner bark.

The same pattern was observed on sections of branches from witches' brooms, but in these cases, hyphae were seen colonizing the tracheids, passing through the pits to the adjacent cells. There was no evidence of destruction of lignified tissues by the fungus, but the normal growth of the annual rings was affected.

DISCUSSION

Two species of *Elytroderma* have already been recorded; *E. deformans* (Weir) Darker from North America, which is a systemic fungus causing witches' brooms and other twig deformations on infected trees (Childs, 1968; Hunt, 1978; Lightle 1954, 1955; Weir, 1916) and *E. torres-juanii* Diamandis & Minter from Greece and Spain, which seems not to be systemic (Diamandis, 1980; Diamandis & Minter, 1979, 1980). These two species differ from each other in some morphological features. The apothecia of *E. torres-juanii* appear as broad black areas, often as wide as the needle, and sometimes continuing around to the adaxial side (Fig. 7), whereas in *E. deformans* the ascocarps are so narrow as to be contained between two rows of stomata (Fig. 8). The clypeus of *E. deformans* is composed only of blackened fungal cells (Fig. 9), while in *E. torres-juanii*, the clypeus is composed of blackened fungal cells and cells of the needle hypodermis, making the ascocarps partially subhypodermal. The clypeus on *E. deformans* is located below the needle epidermis covering only the central part of the hymenium (Fig. 9), whereas in *E. torres-juanii* the clypeus is located below the needle epidermis, but often extends laterally well beyond the

hymenium on one or both sides. The same feature is observed on Portuguese *Elytoderma* (Fig. 10). *E. deformans* and *E. torres-juanii* differ also in ascocarp, asci and ascocarp sizes.

Glavas (1981) found a species of *Elytroderma* in the former Yugoslavia attacking *Pinus pinea*, *P. halepensis* and *P. pinaster*. This author identified the fungus as belonging to the genus *Elytroderma* and in most characteristics, it is similar to *E. torres-juanii*. However Glavas found different sizes for asci and ascospores for the ex-Yugoslavian *Elytroderma*. As with the Greek and Spanish records, this author found no evidence of witches' brooms. He did notice cases of dying trees, but he believed that it could happen after some years of severe attack, and pointed out that more investigations are needed concerning this fungus.

The species from Portugal clearly shows the characteristics of the genus *Elytroderma* Darker (1932), with 1-septate bacilliform ascospores enveloped in a gelatinous sheath, saccate asci, containing spores fasciculately arranged, subepidermal ascocarps and concave hymenium. This fungus was described in a previous paper (Minter & Fonseca, 1983) and that description, based mainly on morphological characters, corresponded closely to the original description made by Diamandis and Minter (1979) for *E. torres-juanii*. Further research, however, led us to the conclusion that the fungus in Portugal is systemic and capable of growing through the phloem, colonizing woody tissues (Fig. 4) and causing witches' brooms (Fig. 3) and other twig deformations and brown lesions in the inner bark (Fig. 5). In this aspect, it differs from *E. torres-juanii*. The parasite invades buds and needle primordia maintaining an unbroken chain of perennial infection as with *E. deformans*.

The biology in infected needles is similar to that of *E. deformans* in that current-year needles are infected and browning occurs during the following winter months and fruitbodies are formed in the following summer. In *E. torres-juanii*, second- and third-year needles are infected and only third-year needles bear ascocarps (Minter, 1980). Another feature is that in *E. torres-juanii*, there is a synchronous ripening of asci (Minter & Cannon, 1984), while in the Portuguese species the asci do not ripen at the same time. Therefore, the fungus now described cannot be placed in *E. torres-juanii* or in *E. deformans*. A new species must be introduced to accommodate these Portuguese collections of *Elytroderma*, and the name *E. lusitanisum* Fonseca-Neves was proposed. This fungus is readily separated from *E. torres-juanii* by its systemic condition and from *E. deformans* by its morphological characters.

A striking characteristic noted for the first time in this genus is the presence of a purplish-red pigment in the tissues attacked by *E. lusitanicum*. This pigment is able to diffuse out in KOH (5%). This characteristic was observed in tissues of needles attacked by *Dothistroma septospora* (Funk & Parker, 1966; Fonseca, 1980), but no mention is made in the literature about this characteristic in tissues attacked by *E. deformans* and *E. torres-juanii*. When the same experiments were made with needles attacked by these two species, a light purplish coloration was observed, but not as red as with *E. lusitanicum*.

Specimens examined:

Elytroderma deformans: on *Pinus jeffrey*, Sierra National Forest, California, USA, 15 July 1910. leg. E. P. Meinecke; Laguna Mountain, California, USA, 10 January 1983, leg. R. F. Sharpf; on *P. contorta*, Invermere, BC, Canada, 24 July 1961, leg. W.G. Ziller, ex DAVFP Nº 13190; on *P. ponderosa*, Rock Greek, BC, Canada, 21 June 1976, leg. R. D. Erickson, ex DAVFP Nº 76-9-0188-

01. *E. torres-juanii*: on *P. brutia*, Kupsin, N. Arcadia, Central Peloponnese, Greece, leg. S. Diamandis. *E. lusitanicum* on *P. pinea*, Lagôa de Albufeira, Estremadura, Portugal, 16 September 1981, holotype LISFA 13422, leg. Nominanda Fonseca-Neves. Other specimens with the same substrate, locality and collector: LISFA 4 August 1982, 10 September 1982, 13 October 1982, 20 September 1983, 10 October 1983, 19 September 1984, 26 September 1984.

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Help with the Latin translation from Dr Esaú Santos is also acknowledged.

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Figure1. Different stages of the disease development on *Pinus pinea*.

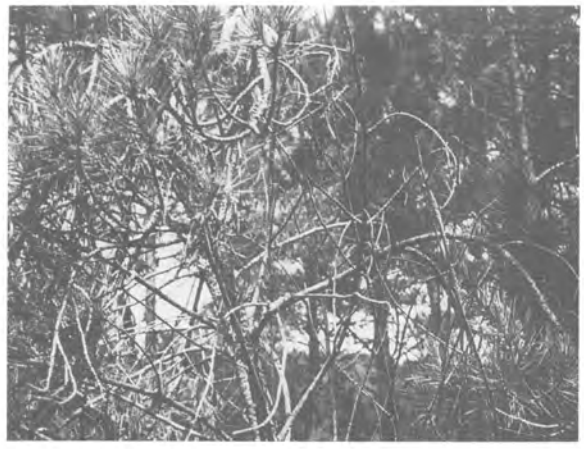

Figure 2. *P. pinea* showing upturned branches.

Figure 3. Witches' broom on *P. pinea*.

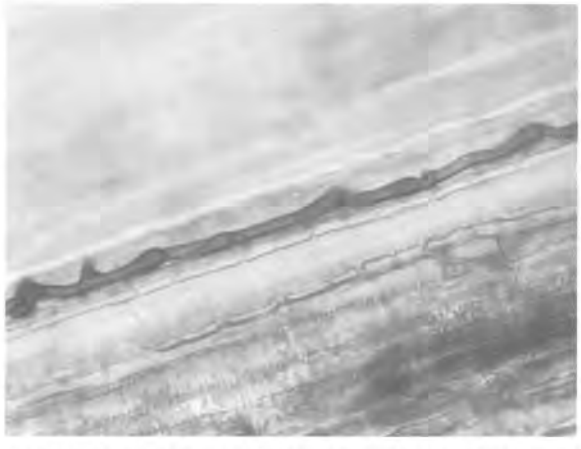

Figure 4. Hypha of *Elytroderma* in the phloem of a *P. pinea* branch, x 250 (longitudinal section).

Figure 5. Longitudinal sections of a branch of *P. pinea* showing brown lesions, x 3.35.

Figure 6. Opened hysterothecia of *Elytro-derma*, x 15.

Figure 7. Hysterothecia of *E. torres-juanii* on needles of *P. brutia*, x 3.3.

Figure 8. Shoot of *P. contorta* with needles showing hysterothecia of *E. deformans*, x 2.3.

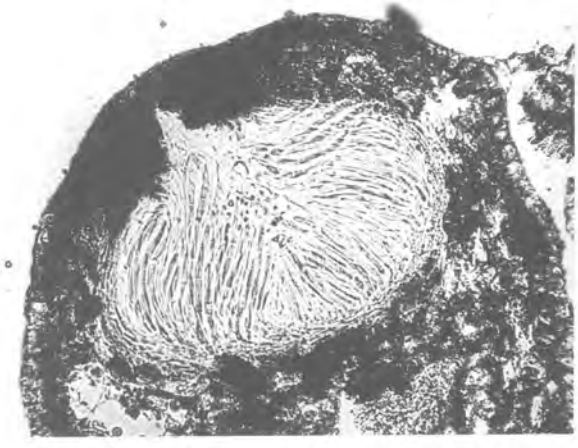

Figure 9. Ascocarp of *E. deformans* on *P. contorta* showing the clypeus covering only the central part, x 200.

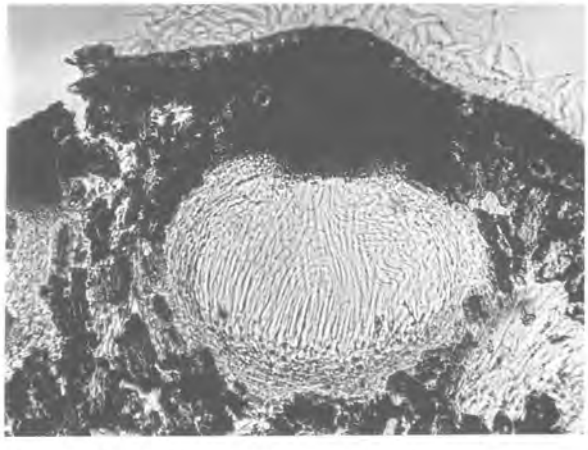

Figure 10. Vertical section on fruitbody of *E. lusitanicum* on *P. pinea* showing hypodermal cells incorporated in the clypeus and clypeus extending laterally, x 200.

MELAMPSORA PINITORQUA ROSTR. IS AN ACTIVE PATHOGEN OF *PINUS SYLVESTRIS* L. IN UKRAINE

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SUMMARY

Melampsora pinitorqua causes death or distortion of the shoots of *Pinus sylvestris*. Spreading of *M. pinitorqua* depends on the ecological situation in a region. Resistance of *P. sylvestris* shoots grown from seeds obtained from different geographic regions is studied.

Keywords: *Melampsora pinitorqua*, *Pinus sylvestris*, *Populus tremulae*.

INTRODUCTION

Pinus sylvestris L. in forest, nursery, garden and youthful cultures is often affected by rust fungus *Melampsora pinitorqua* Rostr., which leads to death or distortion of the shoots. Because of the disease of the phloem and the cambium, rupture of tissues and deformation occurs causing S-form twisting of shoots, and even though a tree survives, timber quality is lost (Shevchenko et al. 1991). Spreading of *M. pinitorqua* essentially depends on the ecological situation in a region (Derjuzshkin et al. 1956). Dry autumn conditions favor formation of teleutospores on *Populus tremula* L. (a foliage alternate host of the pathogen), while long and humid springs promote the germination of basidiospores and pine infection.

RESULTS AND DISCUSSION

Outbreaks of the disease are observed once every 2-3 years in a very dry eastern region of Ukraine (Kreminne and Lisichansk forest station of Lugansk province, Krasnyi Liman and Slavjansk forest station of Donetsk province) that affects from 10 to 25% of all pine shoots. The same level of infection can be found every year in very humid regions of West Ukraine (the Carpathian region). Moreover, massive epiphytotics of the pathogen are detected once every 4-5 years in West Ukraine.

Resistance of *P. sylvestris* shoots grown from seeds obtained from different geographic regions is studied. The seeds' provenance from the northern regions (the Kareliva, Murmansk and Arkhangelsk regions of Russia) produced the shoots that could be affected by *M. pinitorqua* by 15%. Pine shoots from southern regions (the Lugansk and Zaporizhzhya regions of Ukraine) were affected by the pathogen 2.5-3 times more than those from the north.

Disease and deformation of terminal shoots by *M. pinitorqua* took place on trees up to 30 years old. Moreover, deformation occurred more often on trees grown from seeds from the southern region, than on those from seeds from the northern region. The older terminal shoots of

P. sylvestris were diseased too: since they were greater in diameter, shoot distortion did not occur. Taking into account the higher resistance of seeds from the northern regions to M. *pinitorqua*, they could be tested for the expediency of their usage for making seed orchards of P. *sylvestris* in southern regions.

Diseases caused by *M. pinitorqua* appeared on the lateral shoots. They were found in 25-30 year-old plants in single cases because of the self-cleaning of the stem from shoots including distorted ones. The individual plants with a terminal bud perished due to *M. pinitorqua* supplementary buds that were developed below. The latter grew to form a bush, but subsequently, polytop trees of 3 to 5 stems are formed. It should also be noted that the recommended method of felling *P. tremulae* before planting at a distance of up to 500 m from the place of planting does not always give the desired results due to the formation of root sucker with leaves that serve for spore growth. That is why felling must be done with the rooting out and burning of roots following it.

Positive results have been obtained when spraying shoots of pine with 5% solution of bordeaux mixture. Spraying should be done at the beginning of the formation of basidiospores on the deciduous leaves of *P. trumulae*.

Duration of incubation (from the moment of infection to appearance of aecia) is determined within 10-14 days. Aecia of fungus appeared from 20 May to 12 June, depending on weather conditions in the Ukraine regions (optimum temperature is 18-25°C). Urediniospores of fungus develop in June - July on the lower side of leaves of *P. tremulae* and *P. alba* L.; teliospores develop on those leaves too, approximately in September-October. Germination of teliospores and formation of basidiospores usually occurs in May.

The area over which *M. pinitorqua* spreads in Ukraine is quite vast. It covers eastern, central and western forests of Ukraine where most pine plantations grow. Fungus is spread in pine plantations of Russia, Belarussia and a number of countries in Europe and America (Fedorov 1987). In new plantations (10-12 years old in central and eastern Ukraine), the number of aecia on the shoots amount to 8-10.

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HARDWOOD CANKER AND CHESTNUT BLIGHT

BUTTERNUT CANKER IN NORTH AMERICA 1967-1997

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SUMMARY

In 1967 it was first reported that butternut trees, also called white walnut (*Juglans cinerea*), were dying in southwestern Wisconsin from an unknown canker disease. Thirty years later, butternut of all ages are being killed throughout its range in North America by *Sirococcus clavigignenti-juglandacearum*, the fungus demonstrated to be the causal agent of butternut canker and described as a new species in 1979. We now suspect this is an introduced fungus that is seedborne, and may be disseminated long distances by insects. Multiple cankers affect twigs, branches, boles, and buttress roots. Coalescing cankers progressively girdle branches and stems, killing trees. In the United States, recent surveys show that up to 80% of the butternut have been killed in some states, leading to the listing of butternut as rare, sensitive or a species of special concern in those areas. Restrictions have been placed on the harvest of healthy butternut from some state and federal lands. In Canada, widespread butternut mortality has been reported in Ontario. The disease has been detected in Quebec on trees in several natural stands, a plantation, and recently in seedlings in a forest tree nursery. Potentially resistant trees are being selected throughout the United States and southern Ontario, clonally propagated by grafting, and established in several replicated clone banks in order to conserve potentially valuable genotypes. In addition, natural and artificial regeneration systems are being investigated in order to retain butternut in eastern forests for its nuts, wood, and perhaps more importantly, its contribution to biodiversity.

Keywords: *Juglans cinerea*, *Juglans nigra*, *Sirococcus clavigignenti-juglandacearum*

INTRODUCTION

Butternut (*Juglans cinerea*) is valued for its wood for furniture, paneling, specialty products, and is highly sought for carving. Although butternut has not been used for commercial nut production on a large scale, several cultivars with good nut qualities have been identified (Millikan and Stefan 1990). Butternut is also an important source of wildlife mast and significantly contributes to the biodiversity of eastern forests. Butternut is not a common tree anywhere within its range, but grows in several forest types with many other tree species. The range of butternut is similar to black walnut (*J. nigra*) but it extends farther north and not as far south (Rink 1990). Although butternut and black walnut often grow together, they are not known to hybridize naturally (Williams 1990). Butternut does, however, hybridize with English walnut (*J. regia*) and Japanese walnut (*J. ailantifolia*), the latter of which has been widely planted in the United States (Bixby 1919). Recently it was confirmed that butternut is closely related to the Asian heartnuts and should be placed into the taxonomic section *Cardiocaryon*, represented by the Japanese walnut (Fjellstrom and Parfitt 1994).

HISTORY

Butternut decline, common throughout the northeastern United States in the early 1900's, was characterized by branch dieback, colonization by the fungus *Melanconis juglandis* Ellis & Everhart) Graves, and gradual death of trees (Graves 1923). No cankers were associated with affected trees. The decline and death of butternut trees continued to be attributed to the infection by *M. juglandis*, or its imperfect state (*Melanconium oblongum* Berkeley), until the observation in southwestern Wisconsin in 1967 of dieback and stem cankers of butternut (WI Con. Dep. 1967). It was reported that a survey of a 40-acre woodlot revealed that all but two butternut trees had cankers. This evidence, and detailed examinations of canker ages (Nicholls 1979) indicates that butternut canker has been present in the United States at least since the early 1960's, and perhaps much earlier.

In 1979 the cause of butternut canker was reported to be a newly described fungus *Sirococcus clavigignenti-juglandacearum* (Nair et al. 1979). Its origin remains unknown, but several lines of evidence indicate that this is a recently introduced fungus. Factors relating to the fungus and to the disease partially supporting this assertion are: apparent rapid spread of the disease throughout the butternut range since its discovery; the highly aggressive nature of the disease on infected trees; the relative scarcity of resistant trees; the lack of genetic diversity in the fungus (Furnier et al. 1994); and the age of the oldest cankers (40 years) examined in North Carolina (Personal communication, R.L. Anderson, USFS). Thus, this is most likely an example of an exotic pathogen threatening another of our native forest trees (Campbell and Schlarbaum 1994).

DISTRIBUTION AND IMPACT

Surveys of butternut to determine their health are difficult owing to the highly scattered nature of the occurrence of trees in many forest types. However, the disease has been detected throughout the range of butternut in North America. The disease was first reported from Ontario, Canada in 1992 (Davis et al. 1992) where it was said to be common. The disease was first recorded in Quebec in 1990 (Innes and Rainville 1996). The disease has not yet been reported in the Maritime provinces, and efforts are underway to confirm this and to prevent the entry of the fungus, particularly into New Brunswick where a population of healthy butternut still exists (Personal communication, L.A. Cree, Agriculture and Agri-Food Canada).

Butternut canker kills trees of all ages. Branches and young saplings may be quickly killed by a single canker, although older trees are killed over a period of time by multiple, coalescing cankers that either progressively kill the crown or eventually girdle the stem. Sprouts, if they develop, also become infected and are killed usually within the first few years. Butternut is relatively short-lived and shade intolerant, thus older trees of low vigor are declining even in the absence of the canker. Crown dieback, decay, root rot, and damage by wood borers are symptoms commonly associated with these trees.

Based on USDA Forest Service forest inventory data, there has been a dramatic decrease in the number of live butternut trees in the United States in the last 15 years. Live butternut in all size classes combined decreased by 58% in Wisconsin and 84% in Michigan during this period. These data do not distinguish healthy from diseased or declining trees. In 1978 butternut canker had not been reported in Vermont or New Hampshire (Anderson and LaMadeleine 1978), but since

has been observed throughout these states. In 1976 butternut canker was present only in southwestern Wisconsin where it was found on 80% of the trees examined (Prey et al. 1982). A recent Wisconsin Department of Natural Resources survey revealed that 91% of the live butternut in all age classes throughout Wisconsin were diseased (Carlson 1993). Surveys in the southeastern United States revealed that 77% of the butternut have been killed in North Carolina and Virginia, and infected trees continue to be found in new counties in most of the northeast and north central regions (USDA Forest Service 1995a). For these reasons, butternut has been listed as rare, or as a sensitive species of special concern in many states.

DISEASE BIOLOGY

Young cankers are elongated, sunken areas commonly originating at leaf scars and lateral buds, usually in the upper crowns. Later, cankers develop an inky black center with a whitish margin resulting from tissue degradation by the fungus. Removal of the bark reveals the brown to black elliptical area of killed cambium. Small branches are rapidly killed, resulting in dieback and symptoms similar to the Melanconis disease. The fungus *M. juglandis* invades and is often found on branches killed by *S. clavigignenti-juglandacearum*, contributing to the past misidentification of the cause of butternut canker. Most stem cankers become perennial, and are often found in bark fissures or under the bark. Stem cankers commonly occur on the lower stem and on exposed root flares where old cankers become target shaped, caused by the layers of callus that surround each canker.

There is no known sexual state of *S. clavigignenti-juglandacearum*. Conidia develop under infected bark in sticky masses where stromatal pegs break open the bark and the conidia are dispersed by rainsplash and wind during rainfall throughout the growing season. Conidia are only released during rainfall, in small droplets or as aerosols (Tisserat and Kuntz 1983a). Large numbers of conidia are carried in runoff water down tree trunks from branch cankers into wounds and other openings, causing multiple stem cankers. Spores can travel at least 150 feet in rainsplash (Nicholls 1979). Cool, cloudy weather favors spore longevity and conidia can survive for at least 8 hours under these conditions (Tisserat and Kuntz 1983b), indicating the possibility of long distance spread.

Infection of young branches in the crown usually precedes stem infections. The fungus infects trees through buds, and openings such as lenticels and bark cracks. Another common entry is through leaf scars (Kuntz et al. 1979). The fungus continues to sporulate on standing or felled dead trees for at least 20 months (Tisserat and Kuntz 1982, 1984), so unless entire infected trees are removed from the stand, local sanitation by felling trees is not effective.

Since the number of airborne conidia rapidly decreases from an inoculum source (Tisserat and Kuntz 1983b, Nicholls 1979), it has been speculated that birds or insects may be involved in the long-distance spread of the fungus required to infect isolated and widely scattered butternut. Field collections in 1995 in Minnesota and Wisconsin included over 70 insect species and one mite species associated with butternut (Katovich and Ostry, unpublished). On recently dead branches of butternut, a group of small beetles were commonly found under bark in association with the stromatal pegs and pycnidia produced by *Sirococcus*. This group of beetles included families, Laemophloeidae, Monotomidae, Nitidulidae, and Staphylinidae. *Sirococcus* was isolated in pure culture from the following three species; *Laemophlaeus fasciatus, L. testareus* (Laemophloeidae),

and *Bactridium* spp. (Monotomidae). Similar insects have been identified as potential vectors in Vermont (Halik and Bergdahl 1996).

There is evidence that *S. clavigignenti-juglandacearum* can be seedborne. Butternut seedlings were killed soon after emergence from seed collected from infected trees, stratified with intact husks, and sown in a greenhouse (Orchard 1984). The fungus was isolated from lesions at the base of the seedlings where they were attached to the seed. Recently, the fungus was confirmed on nursery seed and seedlings of black walnut and butternut in Quebec (Personal communication, L. Innes; Innes and Rainville 1996).

HOST RANGE

Butternut is the only *Juglans* species significantly damaged by this disease. Black walnut has infrequently been found infected naturally in the field when growing with severely diseased butternut (Kuntz et al. 1979; Ostry et al., unpublished). Recently, the fungus was found on twigs of a heartnut (*J. ailantifolia* var. *cordiformis*) growing in a mixed black walnut and heartnut plantation in Iowa (Ostry, unpublished).

Using artificial inoculations, Orchard et al. (1982) assessed the relative susceptibility of several commercially valuable *Juglans* species and hybrids to *S. clavigignenti-juglandacearum* in 10 to 20-year-old field plantings near Carbondale, IL. Species included black walnut, Persian (English) walnut (*J. regia*), Japanese walnut (*J. ailantifolia*) and heartnut, and various hybrids of these species. All of these species and hybrids are susceptible to some degree. Japanese walnut, heartnut, and hybrids between them and butternut exhibited greater resistance to the fungus, developing smaller cankers than either Persian walnut or black walnut. Caution must be exercised to prevent the accidental introduction of *S. clavigignenti-juglandacearum* into California where it could potentially severely impact the commercial English (*J. regia*) walnut industry.

To determine if hardwoods other than species of *Juglans* could be infected, and perhaps provide a source of inoculum in forest stands, 12 potted 1-year-old seedlings each, of nine hardwood species: white ash (*F. americana*), green ash (*F. pennsylvanica*), pecan (*Carya illinoensis*), shagbark hickory (*Carya ovata*), white oak (*Q. alba*), northern red oak (*Q. rubra*), black oak (*Q. velutina*), bur oak (*Q. macrocarpa*), and black cherry (*Prunus serotina*) were wound-inoculated with mycelium or spores from one of two isolates of *S. clavigignenti-juglandacearum* in the greenhouse and scored for disease development (Ostry, unpublished). Black walnut and butternut seedlings were included for comparison. All of the butternut were nursery seedlings from seed of unknown origins. After 3 months, the greatest canker expansion was on butternut, followed by black walnut and shagbark hickory. A few butternut seedlings were girdled by elongating cankers, and on some seedlings much larger cankers developed than on the others. This may indicate variation in disease susceptibility among seedlings. Non-inoculated control wounds on all species were completely callused-over and no canker symptoms developed.

The majority of inoculated wounds on all of the species other than black walnut, butternut, and shagbark hickory were callused-over. However, the wood directly beneath the inoculated wound and slightly beyond the wound margin on many of the species was stained indicating infection by the fungus. The fungus was re-isolated from these areas on inoculated pecan, shagbark hickory, black cherry, red, black, and white oak. These results suggest that the fungus

might be able to survive on hardwoods other than butternut by causing either inconspicuous cankers or rapid death (a hypersensitive reaction) of small branches, similar to inoculated black walnut (Orchard 1984).

DISEASE RESISTANCE

Currently there are no known cultivars or varieties of butternut in commercial trade with proven resistance to butternut canker since many of them may have been selected for various traits associated with nut production in the absence of the disease. Limited research has been directed at screening butternut selections for resistance. In work determining optimum conditions for screening, succulent butternut seedlings from open-pollinated healthy and diseased parents were inoculated with spores in growth chambers and later examined for infection (Orchard et al. 1981, Orchard 1984). All of the seedlings became infected, although after 12 weeks canker development was significantly greater on seedlings from diseased parents. Although these results are preliminary, they suggest that resistance to butternut canker may be heritable.

Healthy butternut growing among diseased and killed trees have been found. These trees are canker-free in spite of growing directly alongside severely diseased trees. Other trees have few cankers, or they may have cankers that have been completely closed-over by callus, a host response suggesting possible disease resistance or tolerance. These trees may be able to live many years with the disease. Criteria have been developed to assist land managers and woodlot owners interested in identifying butternut that may have disease resistance and may be valuable for tree improvement efforts (Ostry et al. 1994).

MANAGEMENT GUIDELINES AND GENE CONSERVATION

Genetic improvement to develop resistance in butternut is justified if individuals exhibiting disease resistance can be found and propagated. Interspecific breeding with *Juglans* species and varieties such as Japanese walnut and heartnut that have greater canker resistance than butternut may also be a strategy that can be employed to obtain superior trees for reforestation and for nut production in plantations.

Butternut is an excellent candidate for using the techniques of gene conservation (Ledig 1988; McVey and Nielsen 1996) to retain butternut in our forest ecosystems. However, there is an immediate need to collect and preserve butternut selections for future breeding and restoration efforts. Butternut trees with potential canker resistance are rapidly being eliminated from forest stands due to causes ranging from root disease, old age, harvest, weather damage, and changing land use. Reproduction is often scarce, caused by periodic low seed production, consumption of seed by animals, lack of stump sprouts, and the absence of necessary site conditions for seedling establishment and survival. Management guidelines for land managers and woodlot owners who are interested in maintaining butternut as a component in their stands have been suggested (Ostry et al. 1994, 1996).

In the U.S., several conservation groups, and state and federal agencies have formed a coalition to conserve butternut (USDA Forest Service 1995b). Several states have done surveys to assess the health of the butternut resource and to detect potentially resistant trees for grafting. Results of these surveys have led to the listing of butternut as rare, sensitive or a species of special

concern in many states. The USDA Forest Service and several states have established harvest guidelines for butternut including restrictions on cutting healthy butternut in order to conserve the species. In addition, research is examining various silvicultural strategies to regenerate butternut.

The USDA Forest Service and several states are propagating butternut that may have resistance to the canker. Butternut trees that may have resistance are being selected throughout the United States, clonally propagated by grafting, and established in replicated clonal archives. These trees will be challenged with the fungus using various inoculation techniques. Superior tree selections will then be available for possible future breeding and as a seed source for reforestation or for nut production in plantations. A similar conservation effort is underway in Canada where the Ontario Ministry of Natural Resources has developed a strategy for southern Ontario (Fleguel 1996).

Although we have been aware of its existence for only 30 years, butternut canker is threatening the existence of viable populations of butternut throughout most of North America. The fungus has recently been found affecting black walnut and heartnut in the field and has been shown to have the potential to infect other *Juglans* species, notably the commercially important English walnut, underscoring the threat of this pathogen.

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SIROCOCCUS CLAVIGIGNENTI-JUGLANDACEARUM ON BUTTERNUT AND BLACK WALNUT FRUIT

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SUMMARY

In Quebec, butternut canker, caused by *Sirococcus clavigignenti-juglandacearum*, has induced damage to butternut, *Juglans cinerea*, and to black walnut, *Juglans nigra*. The disease is present in natural stands, in forest nurseries and in plantations. In forest nurseries, we noticed that fruit sown in the soil were associated with mortality of butternut and black walnut seedlings. The objective of this study was to verify whether or not the disease is seedborne. Fruit collected in different natural stands were examined in the laboratory in order to detect the presence of diseased tissues on the surface and in the husk. The infected tissues were plated on an agar medium. *S. clavigignenti-juglandacearum* and other pathogens (various *Fusarium* spp., *Marssonina* spp. and *Cylindrocarpon* spp.) were isolated from the surface and from the inside of butternut and black walnut fruit, collected in different natural stands.

Keywords: Butternut, Black walnut, *Sirococcus clavigignenti-juglandacearum*, *Juglans* spp.

INTRODUCTION

In Quebec, the butternut canker, caused by *Sirococcus clavigignenti-juglandacearum* Nair, Kostichka & Kuntz, was first detected in 1990 on butternut, *Juglans cinerea* L., in a natural stand located in the Outaouais region. Since then, the disease has been reported in other natural stands, in two forest nurseries and in plantations (Innes & Rainville 1996; Innes, Croteau & Rainville 1997). In natural stands, *S. clavigignenti-juglandacearum* has only been found on butternut and is responsible for multiple cankers, with cracked and shredding bark on the trunk, and small cankers on the branches and twigs (Kuntz et al. 1978). In plantations, the disease is present on *J. cinerea* and on black walnut, *J. nigra* L., but seems to be less pathogenic on the latter where only small cankers are present on branches and twigs (Kuntz et al. 1978). The diseased butternut in plantations has similar cankers to those seen on big trees in natural stands. We also found sapling mortality and girdling cankers on the main stem in a one-year-old butternut plantation. In forest nurseries, the disease is also present on both *Juglans* but as in plantations, black walnut is less vulnerable. The symptoms of the disease are similar on both species: elongated cankers that can girdle the upper part of the stem, small cankers on the lower portion of the stem and lesions just below the collar that can eventually kill the seedling. These last lesions seem to be associated with the scar left by the nut once it is detached from the seedling. This last observation led us to think that the fruit could transmit the disease to the seedlings. Transmission by seed to young seedlings has already been mentioned by Sinclair et al. 1987. The objective of this study is to verify if the disease is seedborne in our Quebec nurseries.

MATERIALS AND METHODS

Plant material

In the fall of 1996, 15 batches of about 50 fruit of each species were obtained from different regions of Quebec and sent to the *Direction de la conservation des forêts* (DCF) (Forest Conservation Administration) forest pathology laboratory for inspection. The six batches of butternut fruit were collected in the following locations: Westbury township in the Estrie region, Granby township in the Montérégie region, Mansfield township in the Outaouais region, Seigneurie-de-Berthier in the Lanaudière region, Arthabaska township and Seigneurie Cap-de-la-Madeleine in the Mauricie-Bois-Francs region. The black walnut fruit came from seven locations: a seed orchard and a plantation in Saint-Roch-de-Richelieu in the Montérégie region and plantations from Saint-Nicolas in the Quebec region, Buckingham in the Outaouais region, Pointe-Platon in the Quebec region, Granby township in the Montérégie township, Compton township in the Estrie region and Aylmer township in the region.

Methods

All the fruit obtained were individually examined and the presence and size of the lesions in or on the husk were noted. We also looked for the presence of fruiting bodies on the surface of the fruit. If none were observed, infected tissues from the surface and the interior of the lesions were placed on potato dextrose agar (PDA) with streptomycin in plastic Petri plates. The plates were kept at room temperature and in the light for about 10 days. They were later examined and the most frequent pathogens were identified.

RESULTS

Pathogens associated with the lesions

After examining the butternut and the black walnut fruit, we noticed that an important number of fruit had lesions on and in the husk. However, pathogenic fungi were not always isolated from all the lesions. When pathogens are responsible for the lesions, we found that the fungi fruited directly on the infected tissues or, were isolated in culture if they did not. The percentage of fruit with lesions caused by pathogenic fungi varied from 22 to 85% on butternut, to 50 to 75% on black walnut (Table 1). The most frequent pathogenic fungi were *S. clavigignenti-juglandacearum*, *Fusarium* spp. and *Cylindrocarpon* spp. *Marssonina* spp. was isolated from fruits restricted to two distinct areas.

Lesions caused by *S. clavigignenti-juglandacearum*

The fungus *S. clavigignenti-juglandacearum* was present in all of the six batches of butternut fruit examined but not on all of the fruit lesions. The number of fruit per batch, with lesions caused by *S. clavigignenti-juglandacearum,* varied from 8 to 63%. On black walnut, *S. clavigignenti-juglandacearum* was isolated from fruit obtained in 6 of the 7 locations and from 2 to 67% of the fruit. The fungus was not detected on black walnut fruit obtained from Pointe-Platon.

Description of the lesions on *Juglans* fruits caused by *S. clavigignenti-juglandaceaum*

The lesions that were found on the surface of the fruit from both species of *Juglans* were brown to dark brown. They were usually more or less round and had a diameter of a few centimeters. However, it is not uncommon on older fruit to notice bigger lesions several centimeters across that can cover nearly the entire fruit. Black pycnidia were occasionally seen fruiting directly on the infected tissues. In the husk, the diseased tissues were also brown and the lesions varied from shallow to quite deep. In some instances, the lesions could reach the outer surface of the nut.

DISCUSSION

In this study, we found that butternut and black walnut fruit could be infected by a number of pathogenic fungi. The most frequent were *S. clavigignenti-juglandacearum*, *Fusarium* spp., *Cylindrocarpon* spp. and *Marssonina* spp. The butternut canker fungus was detected on a number of fruit in all of the six butternut batches and in eight of the nine black walnut batches. The fungus causes the same kind of lesions on and in the fruit of both species. Pycnidia could be found fruiting directly on the surface of these lesions. Since the fruit are in close contact with the young seedlings in the nursery, the disease could possibly be transmitted from the fruit lesions to these seedlings. The fungus probably penetrates the young seedling via the scar left by the detached nut.

The next step in our studies will be to try to grow healthy *Juglans* spp. seedlings in our nurseries and since the disease was not detected on black walnut fruit where the husk had been removed, we will try to decontaminate the fruit or take the husk off before sowing.

Table 1. Pathogenic fungi on butternut and black walnut fruits.

Species	Region	Location	Type	% of fruits with lesions caused by pathogens	% of fruits with *S. clavigignenti-juglandacearum*	% of fruits with *Fusarium* spp.	% of fruits with *Cylindrocarpon* spp.	% of fruits with *Marssonina* spp.
butternut	4	Seig. Cap-de-la-Madeleine	forest	22	8	14	0	0
butternut	4	Arthabaska township	forest	80	6	76	0	0
butternut	5	Westbury township	forest	26	26	**	**	**
butternut	7	Mansfield township	forest	84	63	32	0	0
butternut	14	Seig. Berthier	forest	64	52	20	2	2
butternut	16	Granby township	forest	72	28	40	0	12
black walnut	3	Aylmer township	plantation	58	33	17	19	0
black walnut	3	St-Nicolas	plantation	75	19	66	3	0

Table 1 (cont'd)

Species	Region	Location	Type	% of fruits with lesions caused by pathogens	% of fruits with *S. clavigignenti-juglandacearum*	% of fruits with *Fusarium* spp.	% of fruits with *Cylindrocarpon* spp.	% of fruits with *Marssonina* spp.
black walnut	3	Pointe Platon	plantation young trees	72	0	62	40	0
black walnut	3	Pointe Platon	plantation old trees	62	0	34	58	0
black walnut	5	Compton township	plantation	50	4	14	28	0
black walnut	7	Buckingham	plantation	62	2	58	18	0
black walnut	16	Granby township	plantation	0	0	0	0	0
black walnut	16	Granby township	plantation	72	67	6	0	0
black walnut	16	St-Roch de Richelieu	seed orchard	**	0	**	**	**
black walnut	16	St-Roch de Richelieu	plantation old trees	62	54	10	0	2

* fruit without the husk

** information unavailable

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THE BUTTERNUT CANKER FUNGUS RECOVERED FROM INSECTS COLLECTED ON *JUGLANS CINEREA*

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SUMMARY

During the summers of 1994-1996, several insect species were collected from stems, logs and branches of butternut (*Juglans cinerea*) trees in northwestern Vermont and individually placed in sterile vials. Sterile distilled water was added to each vial and swirled before the insect was removed and sacrificed on an agar medium. The rinse water from each vial was then streaked on another agar plate. All plates were incubated for 14 days at 22°C and then examined for *Sirococcus clavigignenti-juglandacearum* (SCJ), the butternut canker fungus. In 1994, SCJ was isolated from *Cossonus platalea* (3 of 67) and in 1995, from *Eubulus parochus* (=*Cryptorhynchus parochus*) (1 of 37). In 1996, SCJ was isolated from bodies of *Astylopsis macula* (=*Amniscus maculus*) (2 of 44), *C. platalea* (1/240) and *E. parochus* (4 of 181) and from vial rinse water of *A. macula* (11 of 45) and *E. parochus* (11 of 32). The remaining water in each vial was stained with trypan blue in lactic acid and examined microscopically. SCJ conidiospores were found in stained rinse water from *A. macula* (22 of 58), *C. platalea* (7 of 106) and *E. parochus* (34 of 63) but conidiospore viability was not determined. Most *A. macula* and *E. parochus* found carrying conidiospores of SCJ were collected from freshly cut logs or branches on which the beetles were mating and/or ovipositing. Also, conidiospores of *Melanconium oblongum* (Melanconis dieback fungus) were observed frequently on *A. macula and E. parochus*. Further studies are planned to determine the life cycles and the SCJ spore-vectoring potential of these beetles and other insects.

Keywords: *Sirococcus clavigignenti-juglandacearum*, *Juglans cinerea*, butternut canker, insect vectors

INTRODUCTION

During the past 30 years, the butternut canker fungus (*Sirococcus clavigignenti-juglandacearum* Nair, Kostichka, and Kuntz) has decimated butternut (*Juglans cinerea*) throughout its range in eastern North America. The Minnesota Department of Natural Resources placed a moratorium on cutting healthy butternut trees growing on state lands in 1992 and the USDA Forest Service introduced butternut harvesting guidelines on national forest lands in 1993 (Ostry et al. 1994). This tree may soon be placed on the federal government's threatened or endangered species list because of butternut canker (Ostry et al. 1994).

Recent forest inventory data indicate that *S. clavigignenti-juglandacearum* has had a major impact on butternut. For example, in North Carolina and Virginia, inventory data showed a 77% decrease in butternut from 1966 to 1986 (Ostry et al. 1994) and a recent Wisconsin survey revealed 91% of the live butternut trees were diseased and 27% of the total population surveyed were dead (Cummings Carlson and Guthmiller 1993). Butternut canker also has been found throughout Vermont. In 1993-94, a total of 1317 living butternut trees were evaluated on 18 field

sites in northwestern Vermont and 94% of the trees examined were found to be cankered, but incidence ranged from 68 to 100% among sites (Bergdahl et al. 1996).

Butternut canker was first reported from Wisconsin in 1967 (Renlund 1971) and *S. clavigignenti-juglandacearum* was described as a new species by Nair et al. (1979). No sexual stage of the fungus has been described. The origin of the causal fungus remains unknown (Ostry and Skilling 1995) but it is believed to be introduced (Ostry et al. 1993).

On cankered and other dead areas of the tree, *S. clavigignenti-juglandacearum* produces stromatal columns (hyphal pegs) that lift and split the bark, exposing pycnidia. Pycnidia grow amidst the stromatal columns and produce abundant conidiospores in a sticky matrix. The fungus can sporulate throughout the growing season for up to two years after the tree dies (Nichols 1979, Tisserat and Kuntz 1981, 1984). Stromatal pegs and pycnidia are more likely to be formed on dead branches than on cankered areas of the main stem (Tisserat and Kuntz 1983a). The fungus is known to infect butternut through wounds or other openings in the bark and kills its host by causing multiple cankers that coalesce and girdle the tree (Tisserat and Kuntz 1981, 1984). Rainsplash dissemination of conidiospores is reported to be the primary means of dissemination within trees and among neighbouring trees up to about 40 metres away (Tisserat and Kuntz, 1983b).

Because of the rapid spread of butternut canker throughout the range of its host, insects or other animal vectors are suspected of being involved in the epidemiology of this disease but there is no proof (Nichols 1979, Tisserat and Kuntz 1983a, Ostry et al. 1994). However, in a recent study of incidence and spatial relationships of butternut canker, it was found that cankered trees had significantly more cankered trees surrounding them than canker-free trees, but this was not so at or beyond a 40-metre radius. Therefore, the authors concluded that rainsplash dissemination explains only some of the spatial distribution of butternut canker and it is likely that other factors play a role in the dissemination of *S. clavigignenti-juglandacearum* (Landis and Bergdahl 1996). The objective of this paper is to report the association of *S. clavigignenti-juglandacearum* with the following three species of insects: *Astylopsis macula* (=*Amniscus maculus*) (Coleoptera: Cerambycidae) (Figure 1A), *Cossonus platalea* (Coleoptera: Curculionidae) (Figure 1B) and *Eubulus parochus* (=*Cryptorhynchus parochus*) (Coleoptera: Curculionidae) (Figure 1C).

MATERIALS AND METHODS

During the months of May through September 1994-1996, we collected insects from stems and branches of standing and recently cut butternut trees at three sites in northwestern Vermont. Using sterile forceps, we placed each insect into a sterile, capped vial for transport to the laboratory. Sterile distilled water was added to each vial, which was then gently swirled to dislodge spores before the insect was aseptically removed from the vial and placed on a plate containing 1.5% malt extract agar or 2% water agar. After plating, each insect was allowed to walk around before the insect was macerated and the resulting parts distributed on the agar surface. The rinse water in each vial was then streaked on another agar plate using a sterile loop. All plates were incubated at 22°C for 14 days and examined for growth of *S. clavigignenti-juglandacearum*. For 1996 collections only, the remaining water in each vial was stained with a drop of 0.1% trypan blue in 50% lactic acid and mixed by gentle agitation. Two drops of water were then removed from each vial, placed on a glass slide and then examined for conidiospores of *S. clavigignenti-juglandacearum* using a compound microscope set at 100X and 400X magnification. Presence of

conidiospores of *Melanconium oblongum* (perfect: *Melanconis juglandis*) (Melanconis dieback fungus) was also recorded.

RESULTS

In preliminary studies, *S. clavigignenti-juglandacearum* was isolated from 3 of 67 *C. platalea* collected from beneath dead bark of cankered butternut trees in 1994. In 1995, the fungus was isolated from 1 of 37 *E. parochus*, all of which were collected from the outer bark of dead, dying and freshly cut butternut trees.

In 1996, we collected a total of 596 insects including: 58 *A. macula*, 252 *C. platalea and* 207 *E. parochus*. However, not all insects were used in every isolation method described above. *Astylopsis macula* was found mating and ovipositing on freshly cut logs and branches of butternut. *Sirococcus clavigignenti-juglandacearum* was isolated from the bodies of 2 of 44 *A. macula*, 1of 240 *C. platalea* and 4 of 181 *E. parochus* and from vial rinse water of 11of 45 *A. macula* and 11 of 32 *E. parochus* (Table 1). Conidiospores of *S. clavigignenti-juglandacearum* were viewed in stained rinse water from 22 of 58 *A. macula*, 7 of 106 *C. platalea* and 34 of 63 *E. parochus*. We found the greatest percentage of each of the beetle species carrying spores when we stained the rinse water and looked for spores microscopically. Thirty-eight percent of *A. macula*, 7% of *C. platalea* and 54% of *E. parochus* were found to be carrying conidiospores of *S. clavigignenti-juglandacearum* using this method. Conidiospores of *M. oblongum* were also frequently recovered from the guts, exoskeleton and inner wings of *E. parochus* (Figure 2A).

Table 1. *Sirococcus clavigignenti-juglandacearum* isolated from or observed on beetles collected on butternut trees in 1996

	Number and percentage of beetles infested					
Beetle Species	**Body Isolation**		**Rinse Water Isolation**		**Rinse Water Observation**	
	#	%	#	%	#	%
Cossonus platalea	1/240	<1	0/92	0	7/106	7
Eubulus parochus	4/181	2	11/32	34	34/63	54
Astylopsis macula	2/44	4	11/45	24	22/58	38

*Numerator equals number of beetles with spores and denominator equals total beetles treated with method.

After the oviposition period of *E. parochus* (June-July), we found the larvae, pupae and adults of the weevil living beneath the bark in areas on butternut logs where *S. clavigignenti-juglandacearum* was producing stromatal columns, pycnidia, and conidiopores (Figure 2B). We also isolated *S. clavigignenti-juglandacearum* from unidentified insect wounds on a young butternut shoot (Figure 2C).

DISCUSSION

Rainsplash is well-documented as the primary means of dissemination of *S. clavigignenti-juglandacearum* (Tisserat and Kuntz 1983b). However, there has been much speculation about

the possibility of insects acting as vectors (Nichols 1979, Tisserat and Kuntz 1983a, Ostry et al. 1994). The work presented in this paper documents that three different species of coleopterous insects are carriers of conidiospores of *S. clavigignenti-juglandacearum* should therefore be considered potential vectors.

Now that we have confirmed the existence of potential insect vectors for *S. clavigignenti-juglandacearum*, we need to determine 1) if any of these three beetles truly serve as vectors by transmitting spores to healthy host tissues and 2) the long-term viability or longevity of these conidiospores once attached to the insect.

Most insects we collected, especially *A. macula* and *E. parochus*, were from recently cut logs or tops of butternut trees, many of which had *S. clavigignenti-juglandacearum* fruiting on them throughout the summer. The collected beetles were often found amongst or near stromatal pegs where the insect could very easily contact the sticky conidiospores during life cycle activities that include: emergence, feeding, mating and oviposition at different times during the growing season. However, we need to determine if these infested insects actually visit healthy trees or uninfected portions of trees at any time during the insect's life cycle. It is possible that these potential vectors feed on new healthy host tissue produced each spring or older bark tissues, or they may simply visit wounds made by other agents and passively transmit the fungus. Also, since we found conidiospores of *M. oblongum* on both *A. macula* and *E. parochus*, it is likely that this fungus has an insect relationship as well.

Tisserat and Kuntz (1983c) reported that conidiospores of *S. clavigignenti-juglandacearum* survive for appreciable lengths of time in an airborne state but that survival is very dependent on meteorological conditions. For example, in a forest setting, spore longevity was favored by cool temperatures and overcast skies and dehydration extended the life of conidiospores while they were airborne. We think the viability of conidiospores on an insect's body will be influenced by these same meteorological factors. The difficulty we have isolating *S. clavigignenti-juglandacearum* directly from the insect may be to some extent related to conidiospore viability. There is a need to determine the long-term viability of conidiospores found on or in an insect if we are to have an understanding of an insect's true vectoring potential.

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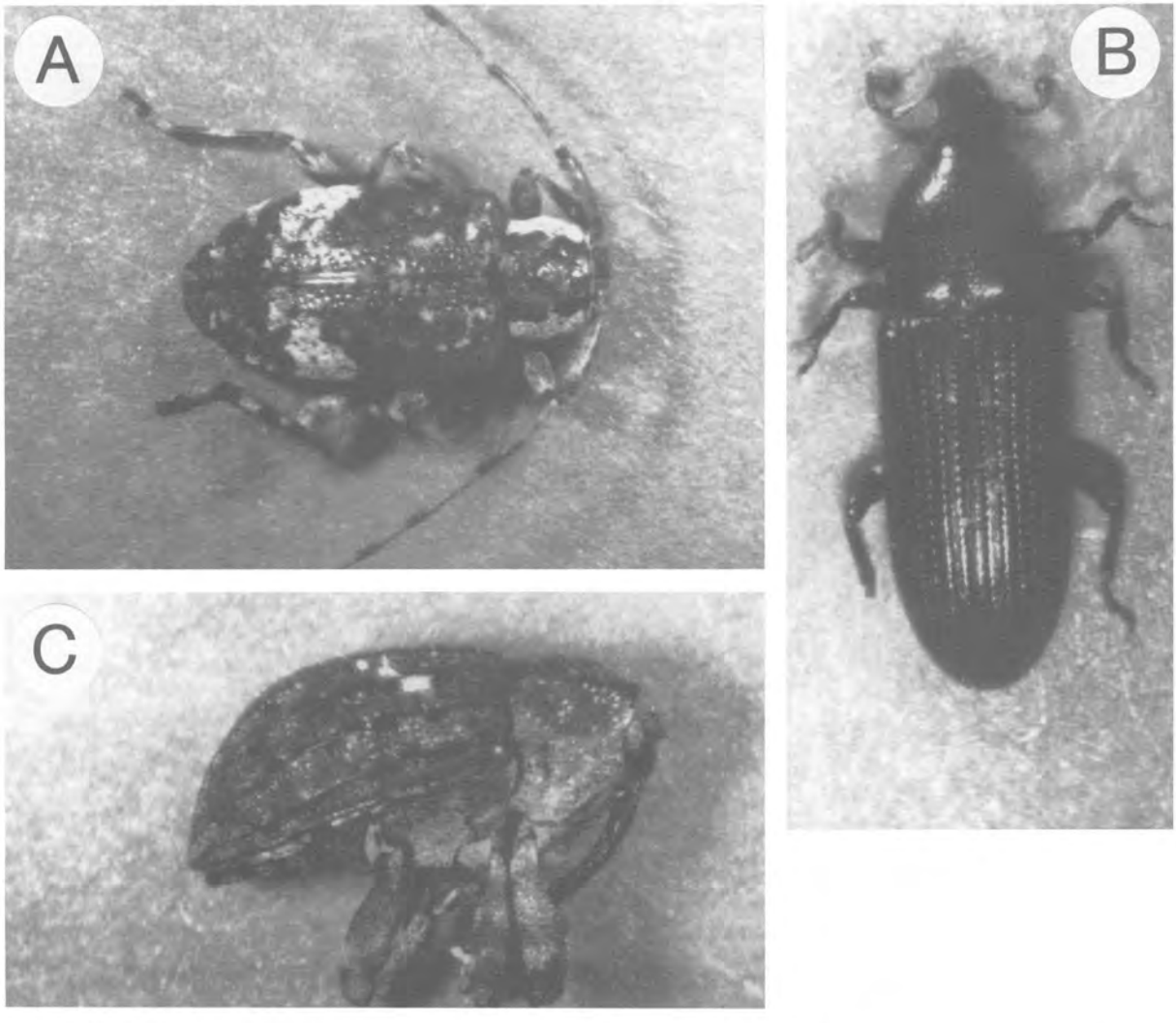

Figure 1A. *Astylopsis macula* (=*Amniscus maculus*) (Coleoptera: Cerambycidae) found on dead and dying butternut trees. This insect has been found carrying conidiospores of both *Sirococcus clavigignenti-juglandacearum* and *Melanconium oblongum.*

Figure 1B. *Cossonus platalea* (Coleoptera: Curculionidae) found under the bark of dead and dying butternut trees, especially in cankered areas. This insect has been found carrying conidiospores of *Sirococcus clavigignenti-juglandacearum.*

Figure 1C. *Eubulus parochus* (=*Cryptorhynchus parochus*) (Coleoptera: Curculionidae) found on dead and dying butternut trees. This insect has been found carrying conidiospores of both *Sirococcus clavigignenti-juglandacearum* and *Melanconium oblongum.*

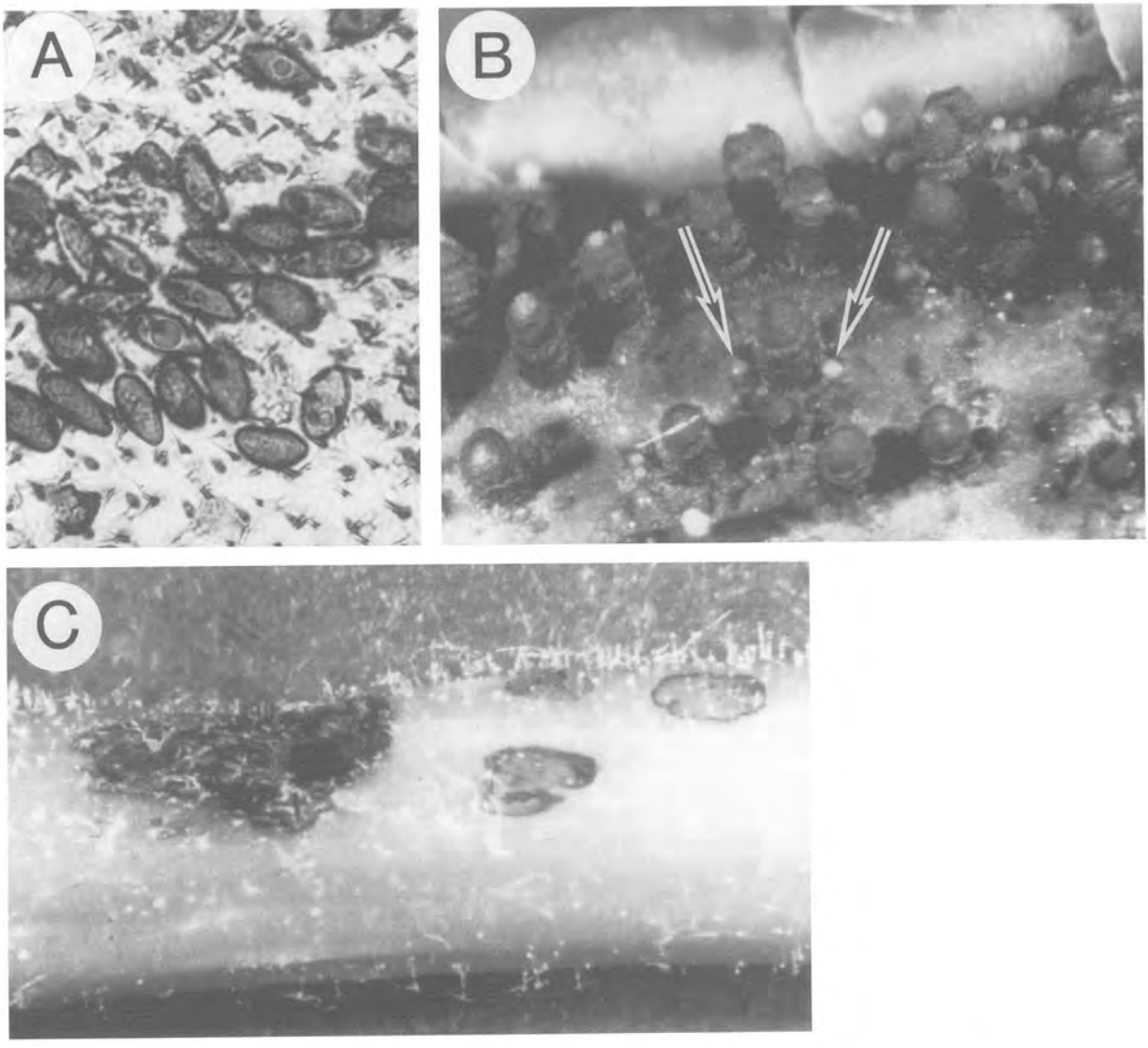

Figure 2A. Conidiospores of *Melanconium oblongum* on the inner wing of Eubulus parochus.

Figure 2B. Stromatal pegs of *Sirococcus clavigignenti-juglandacearum* lifting the thin, outer bark on a branch of *Juglans cinerea*. Note pycnidia with masses of sticky conidiospores near the base of a stromatal peg (see arrows).

Figure 2C. Insect wounds on a young (< 1-month-old) shoot of *Juglans cinerea*. *Sirococcus clavigignenti-juglandacearum* was isolated from the larger wound area on the left. The insect responsible for this wounding is unknown.

PATHOGENIC VARIABILITY OF *NECTRIA GALLIGENA*

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SUMMARY

The fungus *Nectria galligena* causes a perennial canker on many species of northern hardwoods, as well as on fruit trees belonging to the genera *Malus* and *Pyrus*. The pathogenic variability within a population of *N. galligena* from Quebec was studied under greenhouse conditions. Inoculation of saplings of seven deciduous forest tree species with isolates from different host species and locations within Quebec showed the occurrence of a large spectrum of pathogenicity, irrespective of host- or geographic provenance. Among the host species tested, white ash was totally resistant, bur oak was moderately resistant, whereas yellow birch, red oak, silver maple, balsam poplar and American elm were susceptible. Inoculation of yellow birch saplings from three different geographic provenances indicated the occurrence of slight but significant variation for susceptibility within this species. Cross-inoculation of apple and yellow birch saplings with isolates from either host further confirmed the lack of specialization in *N. galligena*. Inoculation of single-ascospore progeny from naturally occurring perithecia and from controlled crosses established the polygenic control of pathogenicity and led to the identification of a nuclear gene with a major effect on this trait.

Keywords: Perennial Nectria canker, *Nectria galligena*, northern hardwoods, pathogenicity.

INTRODUCTION

Hardwoods are an important component of the North American forest ecosystems. In the northeastern part of the continent, species such as yellow birch, sugar maple and red oak also possess a high commercial value since good quality logs can be sold and used for veneer and lumber.

During the 1980's, the Ministère des Ressources naturelles du Québec launched a program for the genetic improvement of northern hardwoods. While the primary objectives of this program were to breed trees with enhanced growth rate and shape, it was deemed desirable to investigate, and possibly increase, the resistance of trees to disease. Perennial Nectria canker, caused by the ascomycete fungus *Nectria galligena* Bres., was identified as a major concern, given its high economic impact (cankered stems are unsuitable for veneer or lumber production) and its broad host range which includes many species of northern hardwoods, as well as *Malus* and *Pyrus* fruit trees (Sinclair et al. 1987). Before resistance to perennial nectria canker could be improved through genetic selection and breeding, however, several questions concerning the pathogen and its interaction with its hosts needed to be addressed. The work described hereafter was thus initiated with the following objectives in mind: 1) to develop accurate, rapid and quantifiable pathogenicity

tests; 2) to evaluate the pathogenic range of a population of *N. galligena* isolated from different hosts and regions within Quebec; and 3) to investigate the variability of pathogenicity towards one host species, yellow birch, and to examine its genetic basis.

MATERIALS AND METHODS

Fungal cultures

Over 100 pieces of bark bearing red, pear-shaped perithecia were collected from European cankers on apple trees (*Malus* sp.), from American beech (*Fagus grandifolia* Ehrh.) with beech bark disease, and from perennial nectria cankers on 12 additional species of northern hardwoods from the following eight genera: *Acer*, *Aesculus*, *Betula*, *Juglans*, *Populus*, *Quercus*, *Tilia* and *Ulmus*. Samples originated from six administrative regions within southern and eastern Quebec, an area representing over 60,000 km^2. Single-ascospore cultures were isolated from individual, surface-sterilized perithecia following the method of Bernier and Hubbes (1990). Isolates of *N. galligena* were identified by their cultural characteristics on PDA and MEA, and by their electrophoretic DNA banding patterns following PCR amplification with both specific (rDNA) and random (RAPD) primers (Bernier and Hamelin 1993; Plante and Bernier 1997).

Pathogenicity tests

Pathogenicity tests were conducted on saplings and cuttings, on calli, and on Golden Delicious apples. Saplings of silver maple (*Acer saccharum* L.), bur oak (*Quercus macrocarpa* Michx.), red oak (*Q. rubra* L.), yellow birch (*Betula allegnaniensis* Britt.), American elm (*Ulmus americana* L.) and white ash (*Fraxinus americana* L.) were grown from seed sources collected in southern Quebec. Saplings of yellow birch included three distinct geographic provenances (Harvey, Blake and Fossambault). Woody material used in pathogenicity tests also included cuttings of balsam poplar (*Populus balsamifera* L.) and Macintosh apple cultivars grafted onto Spartan rootstock.

Saplings and cuttings were inoculated during dormancy, by placing a disk of mycelium into an artificial wound exposing the peridermic layer of the wood (Plante and Bernier 1997). The wound was then covered with a strip of Parafilm and the inoculated material was placed in a greenhouse at 10°C, under natural light, for a period ranging from six weeks to five months before the size of internal and external necrotic lesions was measured.

An *in vitro* assay was developed by obtaining callus tissue from leaf buds excised from dormant 3-year old yellow birch saplings (Yang and Bernier 1996). Initial establishment of calli free of contaminants and recovery of cells with an appropriate texture were made possible by using a double sterilization procedure, and by growing the material on modified MS growth medium (Murashige and Skoog 1962) supplemented with auxins and cytokinins.

The third bioassay consisted in inoculating ripe Golden Delicious apples (Fulbright 1984). The fruits were thoroughly washed under running tap water, allowed to dry at air temperature, and then inoculated by placing a disk of mycelium into a circular wound made with a cork borer. The wound was sealed with a piece of adhesive tape and apples were stored (at 10°C or at room temperature) for up to four weeks.

Genetic analyses

Full-sib single-ascospore progeny from naturally occurring perithecia were recovered as described above. In addition, controlled crosses between selected isolates were carried out in liquid synthetic medium (Dehorter and Lacoste 1980). Since *N. galligena* is homothallic (El-Gholl et al. 1986) and can thus self, single-ascospore progeny recovered from laboratory crosses between genetically distinct parents were subjected to RAPD analysis to ascertain their biparental origin (Plante 1995).

RESULTS

Pathogenicity tests

With the exception of white ash, which was totally resistant to all isolates tested, saplings of northern hardwoods proved to be suitable material for the evaluation and comparison of aggressiveness among isolates of *N. galligena* from Quebec (Plante and Bernier 1997). Results confirmed that wounds were required for successful infection and showed that incubation of inoculated saplings kept dormant and at 10°C resulted in the expression of a broad range in necrotic zone length. Necroses typically appeared as black, oblong lesions with a characteristic sunken appearance.

Yellow birch calli produced from dormant buds supported the growth of *N. galligena*. The four isolates tested varied in their aggressiveness towards calli. The related species *N. coccinea* var. *faginata* Lohman, Watson and Ayers, which does not infect yellow birch in nature nor in greehouse inoculations (Plante and Bernier 1997), was able to colonize the calli but to a very limited extent.

Golden Delicious apples provided a rapid and reproducible means to assess the aggressiveness of *N. galligena*. Final results were obtained within four weeks, compared to three to six months for saplings. Results from inoculated apples were usually correlated with those from saplings, although there were exceptions. One drawdack of this assay, however, was the frequent contamination by *Penicillium* and *Trichoderma* spp.

Pathogenic range and aggressiveness of N. galligena

As mentioned before, all host species tested except white ash were susceptible to *N. galligena*. Isolates of *N. galligena* showed a wide variation in their aggressiveness towards the susceptible species (Plante and Bernier 1997). However, there was no evidence for the occurrence of host specificity, since highly aggressive isolates towards one species tended to retain their phenotype when inoculated to other species. Similarly, some isolates were found to be poorly aggressive to all host species tested (Fig. 1). Results from inoculations also suggested that bur oak, while susceptible, was slightly more resistant than most other species. Lack of specialization was also evident when strains of *N. galligena* isolated from a forest- and from a fruit tree species were cross-inoculated (Fig. 2). On yellow birch and on bur oak, the two strains induced necroses of similar sizes, whereas on apple saplings, the yellow birch strain was more aggressive than the strain originating from a Macintosh apple orchard (Camy 1997). Once again, bur oak displayed some resistance to *N. galligena*.

The three geographic provenances of yellow birch tested exhibited a slight but significant variation in their susceptibility towards *N. galligena* (Fig. 3). The provenance from Fossambault was more susceptible than the provenance from Harvey township, whereas the provenance from Blake township was intermediate (Camy 1997).

Large-scale inoculation of yellow birch from Fossambault with 43 isolates of *N. galligena* from various host species and geographic regions within Quebec allowed us to further quantify the range of variation in aggressiveness. The length of necroses measured after five months of incubation at 10°C in a greenhouse ranged from 0.5 mm for the least aggressive isolate to over 40 mm for the most aggressive ones (Plante and Bernier 1997). There was no evidence, however, that differences in aggressiveness were associated with either host- or geographic origin of the isolates.

Genetic basis of pathogenic variability

Inoculation of small families of full-sibs of *N. galligena* showed the occurrence of wide variations in aggressiveness within some of the families investigated (Fig. 4; Plante and Bernier 1997). This observation provided the basis for a more thorough investigation of the genetic control of pathogenicity in *N. galligena*. Analysis of larger sets of progeny obtained from naturally occurring perithecia confirmed that pathogenic variability involved several nuclear loci. Laboratory crosses between selected isolates with known levels of aggressiveness allowed us to identify one set of progeny which segregated 1:1 for high:low aggressiveness, indicating the effect of two alleles at a single nuclear locus. Both the chromosomal location and the product of this gene with a major effect on aggressiveness remain unknown.

DISCUSSION

Earlier studies on perennial nectria canker of northern hardwoods had already shown the polyphagous nature of *N. galligena* (Welch 1934a, b; Spaulding et al. 1936; Lohman and Watson 1943), as well as the opportunistic behavior of the fungus which needs a wound in order to successfully infect forest tree species (Lortie 1969). However, detailed analysis of pathogenicity of *N. galligena* towards northern hardwoods has usually been hampered by the small number of isolates tested, the inoculation of mixed cultures, the nature of the experimental design, or the lack of control over environmental conditions.

Results from the inoculation of dormant saplings, under greenhouse conditions, showed that juvenile (2-3 year old) material was suitable for pathogenicity tests. The comparison of different geographic provenances of yellow birch revealed the occurrence of a slight but significant amount of intraspecific variability for susceptibility to *N. galligena*. This observation suggests that it may be possible to breed and propagate yellow birch with higher resistance, although a large-scale screening would be required for identifying the most promising material.

The use of calli allowed a very rapid (3 to 7 days) assessment of the aggressiveness of isolates of *N. galligena* towards yellow birch (Yang and Bernier 1996). Mycelial growth of the isolates on calli was well correlated with their aggressiveness towards saplings. Furthermore, we observed that inoculation of *N. galligena* resulted in physical damage to the callus cells following intracellular growth of the pathogen, whereas growth of the beech bark pathogen, *N. coccinea* var. *faginata*, was confined to the intercellular spaces, at least for the 7-day incubation period. Calli appear to

be promising material for the study of host-pathogen interactions associated with perennial Nectria canker. They may also provide a rapid and sensitive bioassay for screening both host and fungal material, especially in laboratories that are equipped and have the necessary expertise for plant tissue culture.

The use of Golden Delicious apples provided another rapid but simpler alternative to saplings for pathogenicity tests. Apples offer several advantages: they are available at any time of the year, their handling requires no specific skills or equipment, they allow rapid observation of symptoms, and they can be stored conveniently in very little space. In some populations of *N. galligena*, however, results observed on apples and on saplings were not correlated. Therefore, it would be desirable that before a large population of isolates is screened on apples, a subset of isolates be inoculated to both apples and saplings to verify whether results on both hosts are correlated. Since apples also seem prone to bacterial and fungal contamination, inoculated material must be checked regularly for the detection and removal of contaminated apples before contamination spreads to other apples.

European investigators reported the occurrence of host specialization in *N. galligena*, and thus described two physiological races, *N. galligena* f.sp. *mali* and *N. galligena* f. sp. *fraxina* occurring on apple and on ash, respectively (Flack and Swinburne 1977). Results from our cross-inoculation experiment with isolates from yellow birch and from apple suggest that there may be no apple-specific race within the Quebec population of *N. galligena*. Analysis of a larger set of representative isolates is, however, needed to further validate this hypothesis and to test whether it holds true for the entire North American population of *N. galligena*.

On the other hand, the observation that white ash was totally resistant to strains of *N. galligena* isolated from other species suggests that, either white ash is resistant to all strains of *N. galligena*, or that there is an ash-specific race of *N. galligena* in North America. Older litterature reports the occurrence of perennial Nectria cankers on ash species in North American forests (Welch 1934a, b; Lohman and Watson 1943), which suggests the second hypothesis is true. However, we have not been able to find perennial Nectria canker on ash and consequently could not recover isolates from this species and conduct cross-inoculations.

When hardwood species other than white ash were considered, the polyphagous and highly variable nature of *N. galligena* were readily apparent. Our results thus confirmed the apparent lack of specificity reported for *N. galligena* by other investigators (Lortie 1969; Barnard et al. 1988). The influence of geographic origin within Quebec was examined by inoculating isolates from several locations in the southern and eastern parts of the province and, once again, no specificity was apparent. Our data thus suggest that *N. galligena* consists of a single, heterogenous population, at least in Quebec. Work is under way in our laboratory to further assess genetic variability and gene flow in a larger population of *N. galligena* composed of individuals from several Canadian provinces and American states.

While the pathogenic variability of *N. galligena* could not be ascribed to environmental factors such as host- or geographic origin, the genetic component of this variability was demonstrated for the first time. Although *N. galligena* is homothallic, outcrossing seems to be frequent since perithecia harboring genetically variable progeny were commonly found. Analysis of small sets of full-sib ascospore progeny clearly showed that the full range of aggressiveness could be observed

in isolates from a single perithecium (Plante and Bernier 1997). This variation in aggressiveness results, in turn, from the presence of different alleles at several pathogenicity loci, as shown by the analysis of large sets of progeny from both naturally-occurring- and laboratory-produced perithecia (Plante 1995). The number and nuclear location of genes involved in pathogenicity, however, remain unknown.

A surprising outcome of our work was the frequent recovery of strains of *N. galligena* with no or little aggressiveness towards northern hardwoods, including the species from which they were isolated (Plante and Bernier 1997). Since low and high aggressiveness were found to segregate in Mendelian fashion, the action of debilitating cytoplasmic mycoviruses conferring a hypovirulent phenotype is ruled out. Alternatively, isolates which failed to infect the seven hardwood species which were tested could be aggressive towards another, undetermined host on which populations could build up. This hypothesis is not appealing either, since one would expect to detect a genetically identifiable host-specific race, which is not the case so far. We believe that it is more likely that poorly aggressive isolates maintain themselves saprophytically in tissues recently killed by more aggressive isolates, where the two can sexually mate. The recovery of perithecia harboring highly variable progeny supports this hypothesis, although results from mixed inoculations would provide further proof.

Research on Dutch elm disease has shown that, over the years, a highly aggressive biotype rapidly displaced the less aggressive biotype which was responsible for the first pandemic in Europe and in North America (Brasier 1987; Houston 1991). Non-aggressive isolates of *N. galligena* seem unlikely to be replaced, at least in the near future, since our results suggest that they hybridize freely with their more aggressive counterparts, giving rise to genetically diverse progeny. This situation is thus quite different from the strong reproductive isolation between the highly- and less aggressive biotypes of the Dutch elm disease pathogen which were eventually recognized as two distinct species, *Ophiostoma novo-ulmi* and *O. ulmi*, respectively (Brasier 1991). Long-term studies would be needed to assess the fate of non-aggressive isolates of *N. galligena* and determine whether they provide the species with any ecological advantage.

CONCLUSIONS

We believe that the studies undertaken by our group are the first ones to quantify and explain pathogenic variability in North American populations of *N. galligena*. This was achieved by inoculating different types of plant material (saplings, calli, apple fruits) with single-ascospore cultures of known host and geographic origin, under reproducible experimental conditions.

These studies have revealed the occurrence of a high variability for pathogenicity in the population studied. This variability was quantitative rather than qualitative: there was no evidence for host specificity (with the possible exception of white ash), whereas aggressiveness varied among isolates. This variability had some genetic origin since families of single-ascospore siblings also exhibited a range of aggressiveness. Not surprisingly, the pathogenicity of *N. galligena* seems under polygenic control. There is no evidence yet that either host- or geographic origin accounted for the differences in aggressiveness observed in the population studied. Work is currently being conducted on a larger collection of isolates from several parts of North America to further address this question.

Like many other fungal pathogens of trees, *N. galligena* remains poorly studied. Our work shows that this pathogen, which is easily grown in the laboratory, is highly amenable to physiological, genetic and molecular investigations. A clearer understanding of the biology and epidemiology of *N. galligena* would undoubtly be useful to tree breeders interested in selecting and propagating northern hardwoods with improved characteristics, including higher resistance to perennial Nectria canker.

ACKNOWLEDGEMENTS

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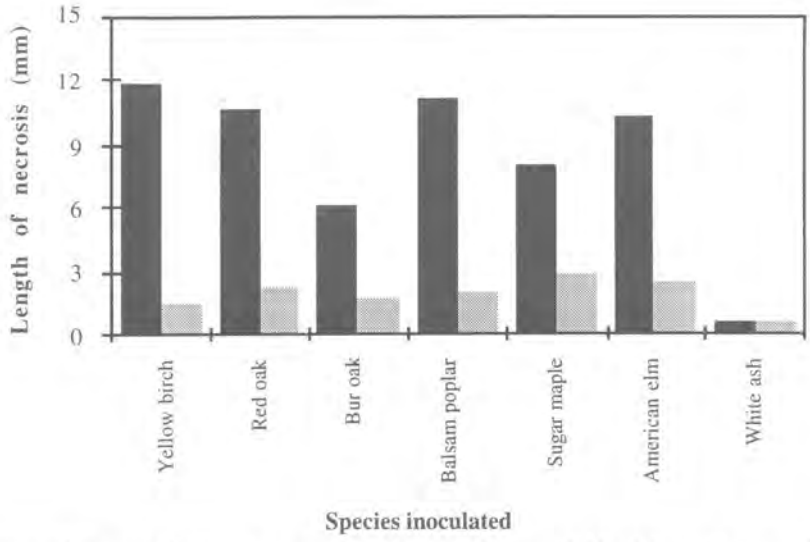

Figure 1. Response of seven deciduous species to inoculation of *Nectria galligena*. Results shown here were obtained by pooling data for 6 highly aggressive (■) and 3 poorly aggressive (□) isolates.

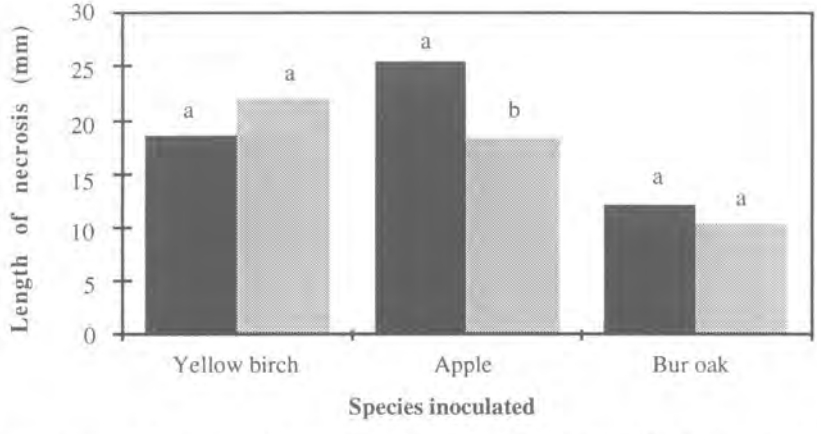

Figure 2. Cross-inoculation of strains of *Nectria galligena* from yellow birch (■) and Macintosh apple (□). Within a pair, different letters indicate a significant ($p < 0.05$) difference (t-Test).

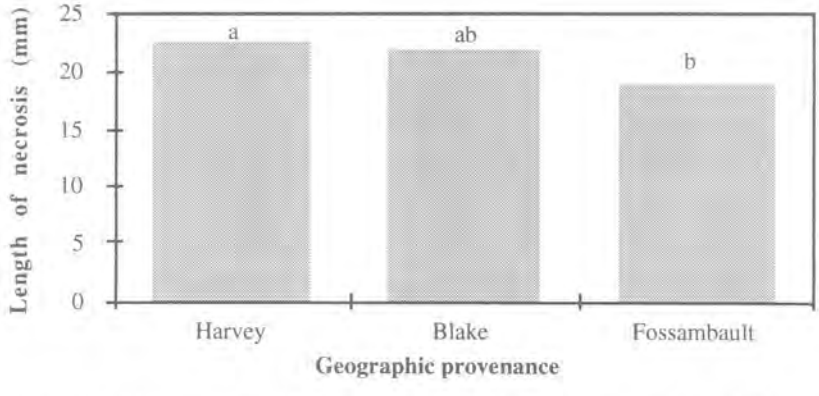

Figure 3. Response of three geographic provenances of yellow birch to inoculation of *Nectria galligena*. Different letters indicate a significant ($p < 0.05$) difference (Tukey's Studentized Range Test).

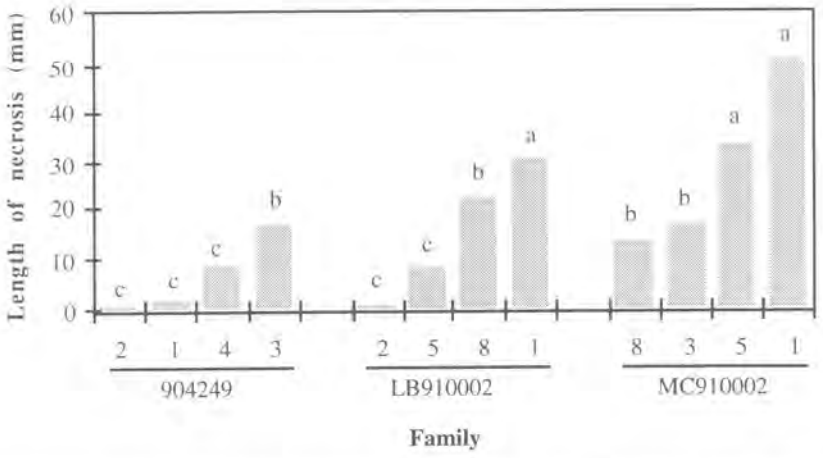

Figure 4. Aggressiveness of full-sibs of *Nectria galligena* towards yellow birch. Families 904249, LB910002 and MC91002 originated from single perithecia collected on American beech, yellow birch and America basswood, respectively. Different letters indicate a significant ($p < 0.05$) difference following Scott-Knott's cluster analysis.

CHESTNUT BLIGHT DEVELOPMENT IN A COPPICE STAND IN SOUTHERN SWITZERLAND

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SUMMARY

The development of chestnut blight (*Cryphonectria parasitica*) was observed in a coppice stand of *Castanea sativa* in southern Switzerland, where hypovirulence has been present for more than 20 years. The stand was clearcut in 1984. Thirty-six sprout clusters with 440 sprouts were observed between 1990 and 1996. All the cankers were localized and measured. During the observation period, 163 sprouts (37%) were lost to mortality. As the dying sprouts were significantly thinner at breast height than the surviving sprouts their mortality is mainly attributed to competition. At the same time, however, chestnut blight incidence was high. Only 79 (28%) of the surviving sprouts remained blight free. The growth rate of the cankers was highest in their first year of appearance and then declined gradually. Hypovirulence had a strong effect. A total of 54% of the cankers were considered as healing on the basis that no reddish canker margin was visible. These healing cankers had a reduced growth rate compared to active cankers. Seventy-two percent of the healing cankers did not grow at all. No effect of the cankers on the increase in stem diameter could be observed.

INTRODUCTION

The chestnut blight fungus (*Cryphonectria parasitica* (Murrill) Barr; *Endothia parasitica* (Murrill) P.J. Anderson & H.W. Anderson) was first identified in Europe in 1938 (Biraghi 1946). A serious epidemic developed, similar to the chestnut blight epidemic in the USA at the beginning of this century (Anagnostakis 1987). However, the heavily infected chestnut (*Castanea sativa* Mill.) stands began to recover due to the natural occurrence of hypovirulent *C. parasitica* strains (for a review see Heiniger and Rigling 1994). Although the blight incidence is still high, the chestnut stands in Italy and southern Switzerland are no longer threatened. In addition to the effect of hypovirulence, some authors assumed that *C. sativa* has a lower blight susceptibility in comparison to *C. dentata* Borkh. (Bazzigher 1981). Young *C. sativa* plants used for infection tests are very susceptible. Bazzigher (1981) attributed the observed survival of thicker and older plants to an ontogenetic change in predisposition.

The objectives of this study were (i) to describe the canker development in a *C. sativa* coppice stand in southern Switzerland where virulent and hypovirulent strains of *C. parasitica* are widespread, (ii) to assess the impact of *C. parasitica* on sprout growth, and (iii) to discuss the possibility of an ontogenetically determined resistance of *C. sativa* against chestnut blight.

MATERIALS AND METHODS

Study site. The Gnosca site is situated in southern Switzerland at 250 to 280 m above sea level on a slope with an inclination of 20 to 60%. The *C. sativa* coppice stand was clearcut in 1984. An approximately 40 x 40 m plot was established with 36 resprouting chestnut stumps. No other hardwood species are competing with the chestnuts. The plot is surrounded by old grown chestnut coppice stands.

Field observations. The measurements were performed as described by Bissegger et al. (1997). In 1990 all dead sprouts were removed. All living sprouts were numbered and the centre of each sprout cluster was mapped. Between 1990 and 1996 the plot was examined annually in August or September. Growth of the sprouts was followed by measuring the diameter at breast height (dbh). All the sprouts were checked for survival and the presence of chestnut blight cankers. On living sprouts all cankers up to 2 m above ground level were recorded. The length of all cankers was measured. Cankers with reddish sunken bark areas were classified as active; cankers with black or undiscolored bark as healing. This classification based on bark color was used independent of whether the cankers were superficial or a depth reaching the cambium. The data were analyzed with Excel 5.0.

RESULTS AND DISCUSSION

Canker incidence. Although chestnut blight is no longer threatening the chestnut forests in southern Switzerland, the question remains of how the disease is affecting the development of the European chestnut stands. In Gnosca, the chestnut blight incidence is high and increasing with time (Table 1). Figure 1 shows the situation in 1990 and in 1996. In 1990, four of the 36 sprout clusters had more than 75% of their sprouts cankered; by 1996, sixteen sprout clusters showed a canker incidence of more than 75%. As many as 120 sprouts carried more than one canker. On one sprout 6 cankers were counted.

A number of new cankers developed each year. Between 1990 and 1991, 106 new cankers developed. In the following years, the number of new cankers varied between 32 and 48. At the same time the number of living sprouts in the plot decreased considerably. Thus the portion of cankered sprouts rose steadily, resulting in 72% cankered living sprouts in 1996 (Table 1).

Canker growth. The growth rate of cankers on living sprouts was calculated (Table 2). Cankers on sprouts that died during the observation period were excluded from the calculation. In the first year of observation the growth rate of the cankers was always considerably higher than in the following years, independent of the year of their appearance. This growth rate is probably overestimated, as newly developing, small cankers ($\leq$ 2 cm) may have been overlooked the year before. During the course of the investigation the growth rate dropped steadily with the exception of 1996.

Bazzigher (1981) found an inverse correlation between mortality and age (based on stem diameter) of the infected trees. He postulated an 'ontogenetic change in predisposition' of *C. sativa* to chestnut blight. The results of this study do not support this hypothesis. All sprouts in the plot were the same age as they developed after the clearcutting in 1984. The data obtained by canker measurements suggest that canker growth is independent of the sprout age (Table 2).

Hypovirulence is present and effective in southern Switzerland. To judge the blight development, the canker incidence itself may not be important, but rather the proportion of cankers that do not grow. Among the 280 cankers that were found on living sprouts in 1995 and 1996, 54% were visually assessed as healing cankers (Table 3). These cankers had an average growth of 1.2 cm in this period. As much as 72% of these cankers grew less than 2 cm. In contrast, the 129 cankers that were judged as active, grew 5.5 cm on average. Only 43% of these grew less than 2 cm. The visual appearance of a canker is therefore a useful indication for classifying cankers as growing or non-growing. Non-growing cankers harbor a high portion of *C. parasitica* strains with a white culture type carrying hypoviruses (Bissegger et al. 1997). Therefore it is concluded that hypovirulence is widespread at Gnosca.

Sprout development. Between 1990 and 1996 a total of 88 sprouts died due to a *C. parasitica* infection and 74 additional sprouts died due to competition. As shown in Figure 2, the dbh of the sprouts that died in the subsequent year was much smaller than the dbh of the surviving sprouts. This suggests that competition and natural thinning are the major factors for mortality. The loss of 37% of the sprouts within the 6 years of observation is low compared to an investigation in France, where an annual loss of 10% of the sprouts was found (Pagès and Cabanettes 1993). Thus, *C. parasitica* does not have a major impact on the development of the coppice stand. In addition, no effect of *C. parasitica* on the increment growth of the sprouts was observed. This was not surprising as *C. parasitica* does not affect the foliage as long as the cambium is alive. In contrast, cankered sprouts were significantly thicker than sprouts without cankers (Table 4). We have no explanation for this observation. There is no evidence that fast growing sprouts are more prone to infection since even at the age of 10 years they still have smooth and crackless bark.

CONCLUSION

Cryphonectria parasitica cankers were very frequent in a 10 year-old coppice stand of C. *sativa* in southern Switzerland. Seventy-two percent of all living sprouts had cankers. These cankers did not affect the increment growth of the sprouts. As the dying sprouts were significantly thinner at breast height, their mortality is mainly attributed to competition. In the last year of observation more than half of the cankers did not grow, indicating a high presence of hypovirulence.

Table 1. Chestnut blight canker development between 1990 and 1996.
[a] From Bissegger et al. (1997).
[b] All the cankers found in the observation period, including cankers on dead sprouts.

	Living sprouts	Living sprouts with cankers	Number of cankers on living sprouts	Total cankers[b]
1990[a]	440 (100%)	162 (37%)	212	212
1994[a]	291 (66%)	219 (75%)	291	463
1996	287 (65%)	208 (72%)	329	544

Table 2. Mean vertical growth rate of all new *Cryphonectria parasitica* cankers that developed between 1990 and 1996. (Only cankers developing on sprouts that remained living until 1996 were included in the calculation). Confidence interval p = 95%.

Cankers (new)	N	1990-1991	1991-1992	1992-1993	1993-1994	1994-1995	1995-1996
1991	46	11.6±2.3	3.9±1.8	2.4±1.0	2.4±1.3	1.1±0.7	1.6±1.2
1992	19		11.4±2.4	6.4±2.7	3.7±2.2	2.0±1.2	2.7±1.7
1993	25			10.9±2.3	4.7±2.1	2.9±2.6	6.3±2.9
1994	28				17.5±4.6	3.9±2.6	5.4±3.0
1995	45					5.9±2.5	4.1±1.6
1996	33					12.6±2.3	10.6±2.2

Table 3. Mean vertical growth rate of active and healing *C. parasitica* cankers between 1995 and 1996. Cankers with red margins were identified as active, cankers with no reddish discoloration were identified as healing. a-Confidence interval p = 95%, b-canker growth < 2 cm, c-canker growth > 10 cm.

	N	Mean canker growth (cm)[a]	Non-growing cankers[b]	Fast growing cankers[c]
Active cankers	129 (46%)	5.5±1.7	43%	21%
Healing cankers	151 (54%)	1.2±0.5	72%	3%

Table 4. Diameter at breast height (dbh) (± standard deviation) of living chestnut sprouts in 1996 with no or 1 to 6 *C. parasitica* cankers. Confidence interval $p_{0.05}$=*, $p_{0.01}$=**.

	N	Dbh (cm)	Significance level
Sprouts without canker	82	6.7±2.98	
Sprouts with 1 canker	86	7.8±3.09	*
Sprouts with 2 cankers	63	8.4±3.12	**
Sprouts with 3 cankers	32	8.4±2.28	**
Sprouts with 4 cankers	17	8.4±3.67	*
Sprouts with 5 cankers	6	9.1±1.79	ns
Sprouts with 6 cankers	1	13.1	-
Sprouts with cankers	205	8.2±2.9	**

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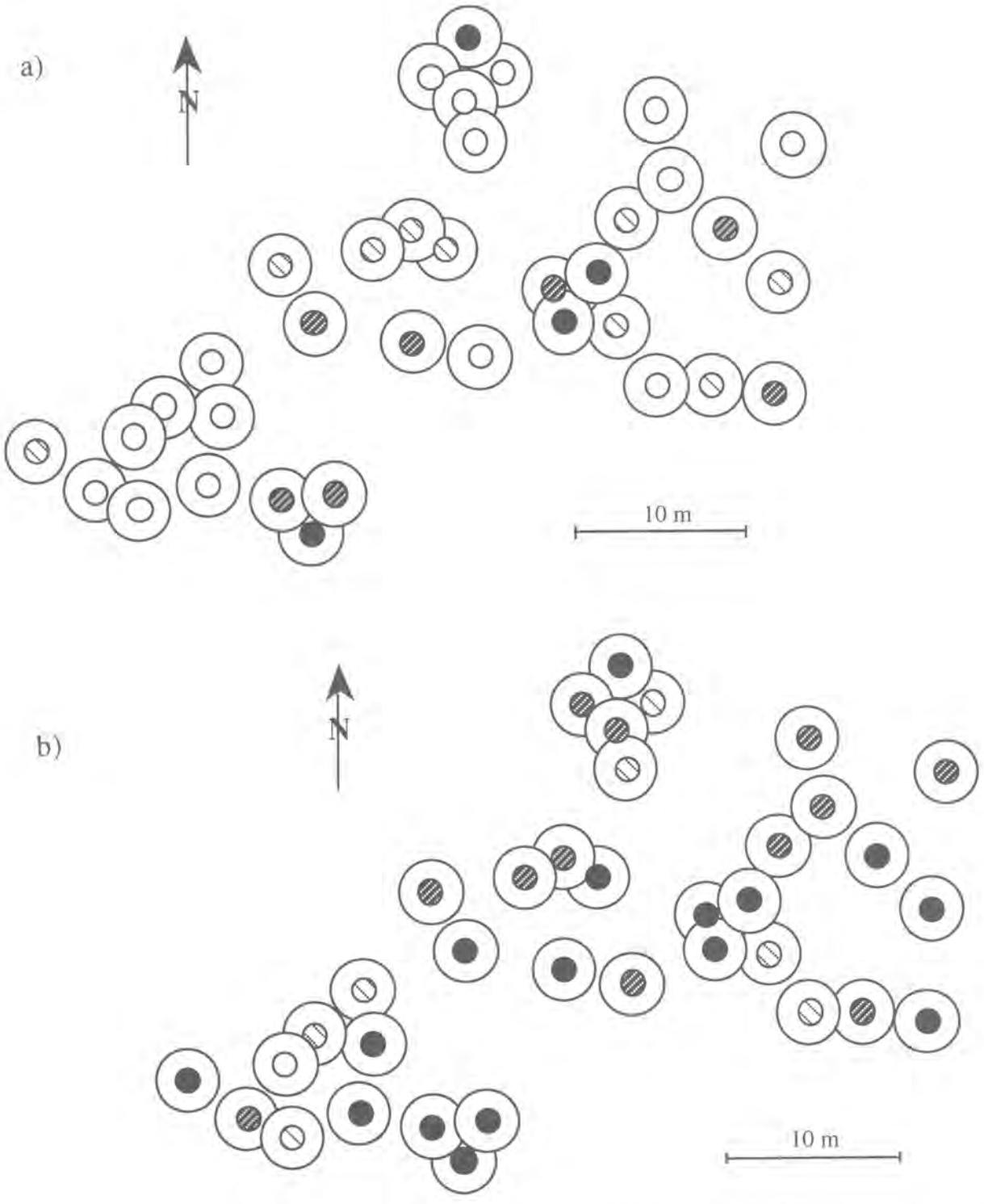

Figure 1. Chestnut blight incidence in Gnosca in 1990 (a) and 1996 (b). Open circles: stumps with ≤25% infected sprouts; slightly hatched circles: stumps with >25 to ≤50% infected sprouts; dark hatched circles: stumps with >50 to ≤75% infected sprouts; solid circles: stumps with >75% infected sprouts.

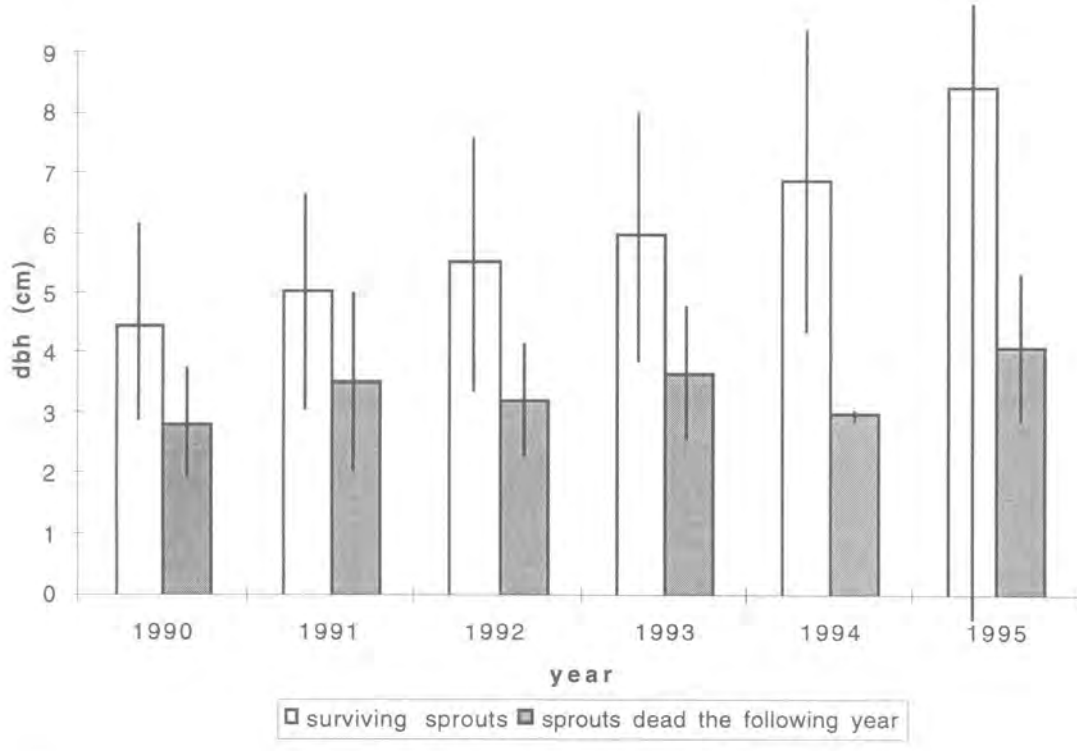

Figure 2. Diameter of sprouts that survived the following year and of sprouts that died the following year.

SCLERODERRIS CANKER

THE HOST RANGE AND GEOGRAPHIC DISTRIBUTION OF THE NORTH AMERICAN AND EUROPEAN RACES OF *GREMMENIELLA ABIETINA* IN ONTARIO

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ABSTRACT

In Ontario, two races of the fungus *Gremmeniella abietina* (Lagerb.) var. *abietina* Morelet occur. Information from surveys conducted between 1964 and 1995 was assessed to determine the geographic distribution and host range of the North American (NA) and European (Eur) races in Ontario. The NA race, first identified in Ontario in 1960, was found throughout the range of pine species north of about 44°N. The Eur race, not identified in Ontario until 1985, was confined to a portion of southern Ontario between 44° 30'N and 45° 45'N. The number of plantations infected with the Eur race increased from three in 1985 to 40 in 1995. This increase was due to the cessation of control activities in infested plantations. In Ontario, the NA race was collected primarily from *Pinus banksiana* and *P. resinosa*, with less frequent isolations from *P. sylvestris, P. strobus, P. nigra,* and *P. contorta* var. *latifolia*. The Eur race was isolated primarily from *P. resinosa*, with several collections from *P. sylvestris, P. strobus* and *P. nigra*, but none have been made from *P. banksiana* or *P. contorta*. Isolations have also been made from *Picea glauca* and were presumed to be *G. abietina* var. *balsamea*. *Pinus resinosa,* the principal host of the Eur race, occurs primarily in the eastern portion of Ontario, south of 47°N and with extensive areas of spruce fir and *P. banksiana* forest to the north. Despite an increase in activity in recent years, the Eur race showed a reduced host and geographic range, as compared to the NA race of *G. abietina*. This might suggest the Eur race might not spread northward and maintain its present distribution.

INTRODUCTION

Scleroderris canker, caused by the fungus *Gremmeniella abietina* (Lagerb.) Morelet var. *abietina*, has been regarded as a major pest of pine in Ontario for approximately 30 years. The fungus was first collected in Ontario from white pine (*Pinus strobus* L.), May 20 1960, in Haughton township, Lot 11, concession I, near Thessalon, Ontario. The fungus was identified in that year as *Scleroderris lagerbergii* Gremmen, (MFB 6724) by J. Reid of the Canadian Forestry Service (Punter, 1967) and confirmed as *Crumenula abietina* (Groves, 1965), which later became synonymous with *Gremmeniella abietina* (Petrini et al. 1989). Later the disease was found to be associated with a red pine disease (*Pinus resinosa* Ait.) (Punter, 1967) which surveys showed to be widespread across northern Ontario (Sippell et al. 1966).

Scleroderris canker is most common to pines, although a new variety (var. *balsamea*) has also been found in association with *Picea mariana* [Mill.] BSP (black spruce) (Laflamme 1988), *Abies balsamea* L. (balsam fir) and *Picea glauca* [Moench] Voss (white spruce) (Petrini et al. 1989). Based on serological comparisons, two distinct races of the fungus are recognized as existing on pines in North America. These are referred to as the North American (NA) and European (Eur)

races of *G. abietina* var. *abietina*. Numerous planting failures in Ontario have been attributed to the NA race of the fungus since the 1950's (Dorworth, 1970) in both *P. resinosa* and *P. banksiana* Lamb.(jack pine) stands (Dorworth 1972). While the origin of the NA race is uncertain, the Eur race is considered as an introduced pest to North America since it shares serological similarities to isolates found in Europe (Dorworth et al. 1977). The Eur race of *G. abietina* is of particular concern due to the extensive damage it caused to *Pinus sylvestris* L. (Scots pine) and *P. resinosa* in New York state (Skilling, 1977) and to *P. contorta* Dougl. ex Loud. var. *latifolia* Englem. (Lodgepole pine) in Europe (Karlman et al. 1994). In addition, based on artificial inoculation studies (Skilling et al. 1986), the Eur race possesses the ability to infect most conifers native to North America. This, at least in theory, provides it with a broader host range than the NA race, which is found only on pines (Petrini et al. 1989).

In Canada, the Eur race was first isolated from red pine plantations in Quebec in 1978 (Lachance and Benoit, 1978) and in Ontario in 1985 (Myren and Davis, 1986). The Eur race has been the subject of concern in Ontario since its discovery. The level of damage caused by the pathogen was previously summarized by Hopkin and McKenney (1995). The present document describes the host range and the geographic distribution of the two races in Ontario, based on analysis of thirty years of survey data.

MATERIALS AND METHODS

Since 1964, annual collections were made by field technicians of the Forest Insect and Disease Survey (FIDS) in Ontario. At each plantation visited, a minimum of 500 trees were inspected along a series of 1 m wide transects distributed throughout each plantation. Information recorded at each plantation included percentage of trees infected, host species, height, stocking, plantation size (ha), and location coordinates. A record was also made of each plantation where the disease was not observed. Samples from suspected diseased trees were submitted to the disease identification unit at the Canadian Forest Service laboratory in Sault Ste. Marie for confirmation. Records of all observations were entered into FIDS INFOBASE (Power, 1986), a national database for forest pest records designed using an INGRES relational database. Records were retrieved from this, and from site locations that were plotted using a PC ARC/INFO geographical information system (ESRI Inc., Redlands, CA, USA.).

In Ontario, serological methods have been employed for routine race identification since 1985. Tissue samples received in the laboratory were visually inspected for signs of the disease and isolations from suspected diseased samples were transferred onto 20% (v/v) V–8 agar medium. Failure to identify the fungus from the submitted sample constituted a negative sample. Serological testing was carried out on positive samples. Water-soluble protein extracts from cultures were challenged against serotype standards to determine race (Dorworth and Krywienczyk, 1975).

RESULTS AND DISCUSSION

Analysis of FIDS INFOBASE data from 1964 to 1995 suggested that the two races of *G. abietina* var. *abietina* show differences in host range in Ontario (Table 1). Both races were found infecting *P. resinosa, P. sylvestris, P. strobus* and *P. nigra* Arnold (Austrian pine). However, only the NA race was isolated from *P. contorta*, and *P. banksiana* (Table 1). Likewise in Quebec, only

the NA race was isolated from *P. banksiana* (Laflamme and Bussières, 1990). In Ontario, the NA race was collected infrequently from *P. strobus* (Table 1) and caused little damage; the Eur race was not collected from this host in Ontario until 1995. In Quebec, of 1397 isolates collected between 1979 and 1989 only one isolate of the Eur race and four of the NA race were collected from *P. strobus* (Laflamme and Bussières, 1990), further suggesting the insignificance of the disease on this host. Three collections were made from *Picea glauca* between 1972 and 1973 (Table 1), but were likely *G. abietina* var. *balsamea* (Petrini et al. 1989).

The extent of the geographic distribution of the two races in Ontario was also different (Fig. 1). Annual surveys determined that both races have a southern-most range of about 44°N (Fig. 2). This is consistent with other reports as neither race is found elsewhere south of 44°N (Marosy et al. 1989). The NA race was found throughout Ontario north of 44°N (Fig. 1) on both red and jack pine (Fig. 3, Table 1). Unlike the NA race, the Eur race was restricted to a portion of southern Ontario between 44° 30'N and 45° 45' N (Figs. 1 and 2). In this region both races are usually found in combination, often in the same plantation, as earlier reported in Quebec (Laflamme and Bussières, 1990). The number of plantations infested by the Eur race increased from three in 1985 to 40 in 1995 (Table 2). This represents a significant increase in incidence, particularly after 1991. Between 1985 and 1995 the Eur race was located in a total of 17 townships (each township covering an area of less than 200 km^2), with 29 of the infested plantations located in four townships where the disease has occurred since 1987 (Table 2). However, it is worth noting that the spread of the disease in the period between 1992 and 1995 was much greater than would have been expected. The Eur race is believed to have a limited ability for long range dispersal, as unlike the NA race, ascospore production is rare or absent. Conidia produced by *G. abietina* are splash-dispersed and generally travel only a few metres from their source, although some have been trapped over 600 m from the point of origin (Skilling et al. 1986). This, however, is insufficient to explain the spread of the pathogen in Ontario. Punter (1967) suggested that infected nursery stock was a major cause of spread of the NA race in northern Ontario. Since the identification of the Eur race in Ontario, the Ontario Ministry of Natural Resources has maintained a strict fungicide program in its nurseries to eliminate scleroderris on pine nursery stock, making this an unlikely source of infection. However, the control program for infested plantations in Ontario which involved removal and sanitation of diseased trees ended around 1992, coincident with the increased incidence of the disease.

In Ontario, the Eur race is likely limited at the southern extent by a warmer climate (Marosy et al. 1989) and in the north by the distribution of red pine. The general range of red pine occurs over much of Ontario, but this species does not normally grow continuously over large areas unless planted and is usually found in small pockets often mixed with white pine and jack pine In Ontario. Red pine occurs in significant numbers on only about 190,000 ha, about 80% of which occurs south of 47°N (OMNR, 1996). The NA race which can readily infect jack pine was recovered from red pine in the mixed northern forest (Fig. 3). However, the Eur race appeared restricted to the areas where red pine was planted over continuous areas. In Ontario, jack pine occurs on 5,880,000 ha of land that contains jack pine as a major component (OMNR, 1996). The greatest distribution of this tree species is in more northern areas but the southern limits extend into the area where the Eur race exists. This tree species is often present in infected red pine plantations as a volunteer, however the Eur race has not to date been isolated from it in Ontario (Table 1). Laflamme (1991, 1993) reports that jack pine can be infected by the Eur race, but the disease seldom develops on this host, and causes only minor damage in the form of tip blight. In Quebec, this tree species is recommended for planting in areas where the Eur race is chronic (Laflamme, 1991). The more

restricted distribution of red pine might provide limited opportunities for the spread of the Eur race north of its present distribution into a region where jack pine dominates. However, it should be noted that the presumed tolerance of jack pine to the Eur race is based only on field observations in Ontario and Quebec (G. Laflamme, pers comm.) and has not been confirmed experimentally.

The results of this study suggests a limited host range and geographic distribution for the Eur race of *G. abietina* in Ontario. Control of the disease involves relatively simple silvicultural practices (Laflamme 1991, Hopkin and Laflammme 1995) which are most successful if conducted when infection levels are low (Laflamme and Blais 1993). The broad host range for the Eur race suggested by the work of Skilling et al. (1986) was not apparent under field conditions in Ontario. This and the present geographic distribution of the Eur race in Ontario would suggest the pathogen is primarily a hazard to red pine but can be controlled with existing silvicultural methods, and might not pose a threat to Ontario plantation forestry given that sufficient efforts are made to contain its spread.

Table 1. Confirmed collections of the European and North American races of *Gremmeniella abietina* var. *abietina,* by host, collected in Ontario, 1964-1995.

Host species	Eur. race	Years collected	NA race	Years
Pinus resinosa	163[a]	1985-1995	396	1964-1995
Pinus banksiana	0		473	1964-1995
Pinus sylvestris	6	1987-1995	13	1967-1995
Pinus strobus	1	1995	14	1960-1995
Pinus nigra	1	1986	3	1972-1994
Pinus contorta	0		4	1972-1976
Picea glauca	0		3[b]	1972-1973

[a] Number of collections

[b] *Gremmeniella abietina* var. *balsamea* (Petrini et al. 1989)

Table 2. Distribution of the European race of *Gremmeniella abietina* var. *abietina* in Ontario, 1985-1994.

Township	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995
Mayo	1[a]	2	2	1	1	1	3	1	1	2	2
McMurrich	1	1	3	2	1	1	5	6	13	18	20
Macauley	1	[b]	2	-	-	-	-	-	1	1	2
Ryerson			2	1	2	1	2	4	6	6	5
Strong					1	-	-	-	1	1	1
Stephenson						1	1	1	1	-	1
Perry								1	1	1	1
Joly								1	-	-	-
Galway									1	-	-
Somerville									1	-	1
Ryde									2	2	2
Stisted									2	2	2
Armour									1	-	-
Chaffey									1	1	2
Watt									1	-	-
Minden										1	-
Monck											1

[a] Number of plantations infected by the Eur race.

[b] Surveys failed to recover the Eur race.

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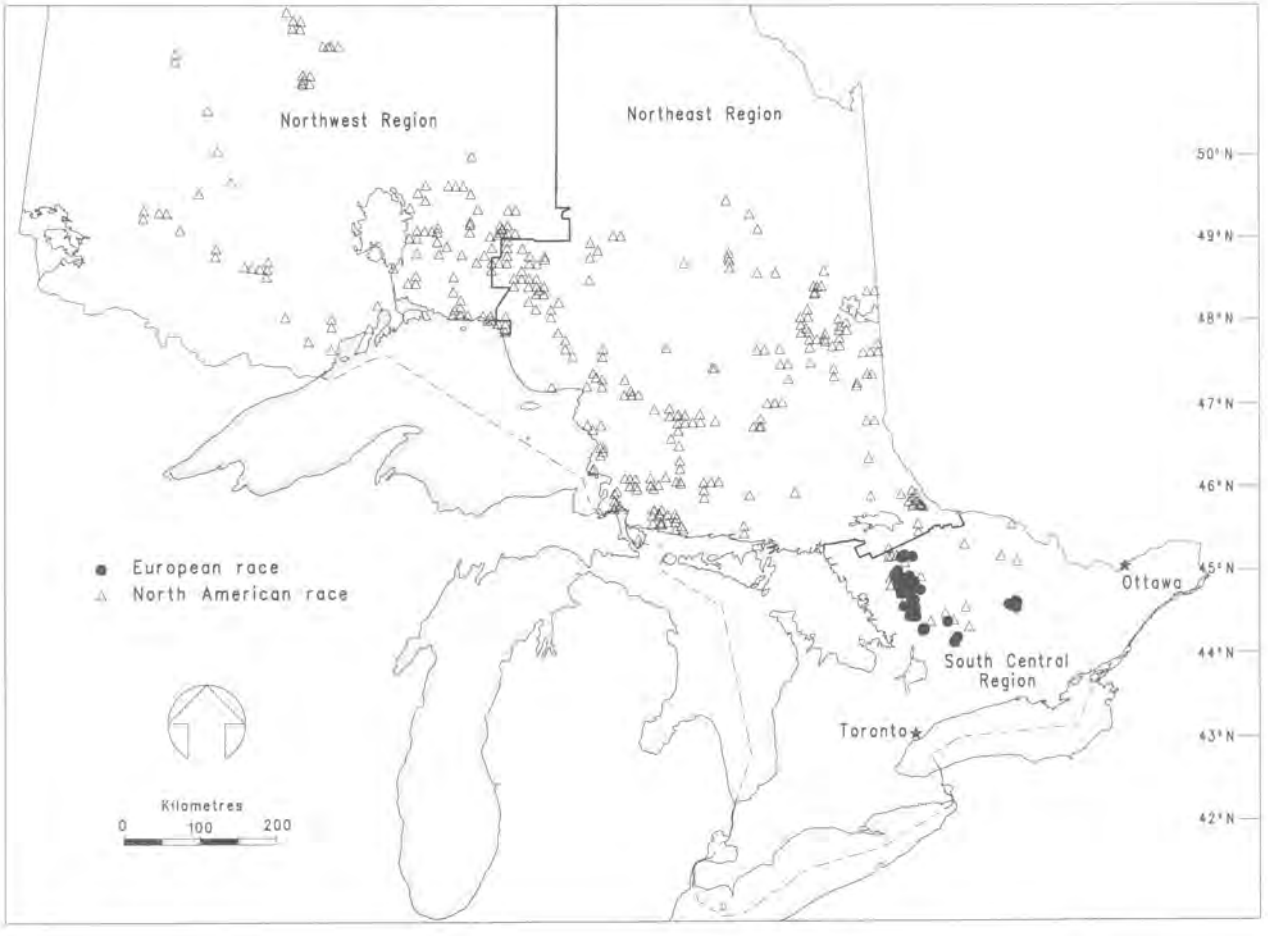

Figure 1. The historical distribution of the North American race (1964-1995) and the European race (1985-1994) of *Gremmeniella abietina* var. *abietina* in Ontario. Region names refer to Ontario Ministry of Natural Resources administrative boundaries.

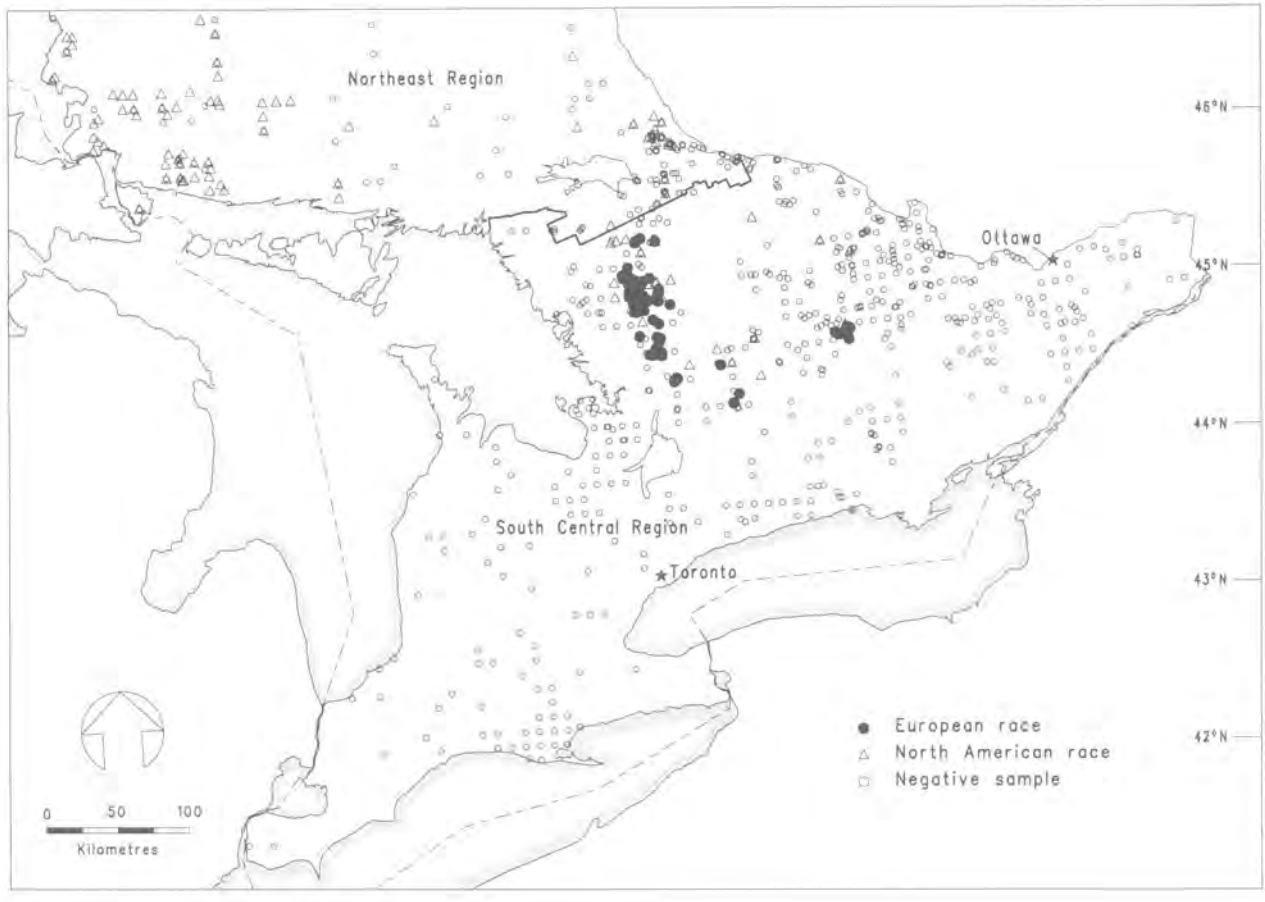

Figure 2. The locations of the North American and European races of *Gremmeniella abietina* var. *abietina* and surveyed plantations where the fungus has not been recovered in Ontario. Region names refer to Ontario Ministry of Natural Resources administrative boundaries.

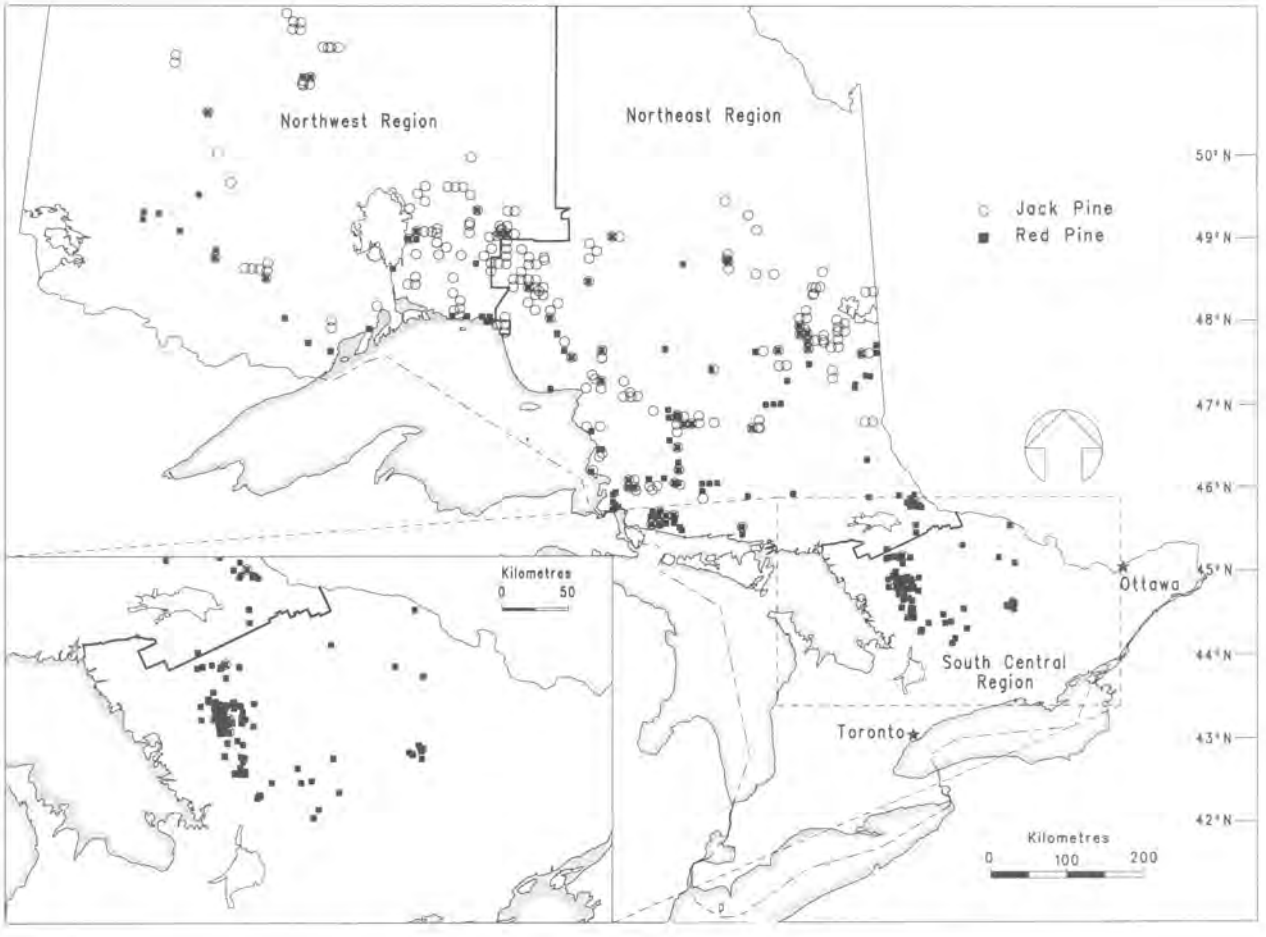

Figure 3. The historical distribution of *Gremmeniella abietina* var. *abietina* in Ontario on red and jack pine, 1965-1995. Region names refer to Ontario Ministry of Natural Resources administrative boundaries.

A TWENTY-FIVE YEAR HISTORY OF SCLERODERRIS CANKER IN VERMONT, USA (1971 - 1996)

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SUMMARY

Scleroderris canker caused by *Gremmeniella abietina* (=*Ascocalyx abietina*) was first reported on red pine (*Pinus resinosa*) and Scots pine (*P. sylvestris*) in Vermont (VT) in 1971. The European race of *G. abietina* was isolated from Scots pine growing in Greensboro, VT in 1976. In 1977, the state of Vermont initiated an annual statewide survey for *G. abietina* and then established a quarantine designed to prevent the movement of susceptible conifers outside infested areas. Since 1971, a total of 126 pine plantations (107 red pine and 19 Scots pine) have been found infested with *G. abietina*. All plantations are located in north central Vermont (in 64 of 251 towns) and total 997 acres. However, during the past 10 years (1986-1996), only one red pine and one Scots pine plantation have been added to the quarantine list. Today, most plantations exhibit only a trace amount of infection, and mortality caused by *G. abietina* is rare. We postulate that an infested nursery unknowingly played a role in the dissemination of *G. abietina* because the county in which the nursery is located has the highest incidence of scleroderris canker in the state.

Keywords: *Gremmeniella abietina*, *Pinus* spp., survey, nurseries

INTRODUCTION

Symptoms of scleroderris canker in the United States were first noticed in the Upper Peninsula of Michigan in 1951 (Skilling and Cordell 1966). The causal fungus was identified as *Scleroderris lagerbergii* Gremmen (Ohman 1966), but it is now known as *Gremmeniella abietina (lagerb.) Morelet* (=*Ascocalyx abietina*) (Petrini *et al.* 1989). However, the asexual form of the fungus (*Brunchoristia pinea* (Krast.) Hohn) is most commonly associated with the disease in the northeastern United States (Bergdahl 1983), as it is in Europe (Gibbs 1983). This asexual form produces abundant conidia in pycnidia or cryptopycnidia on symptomatic host tissues. Conidia are passively liberated during periods of wet weather and are believed to be primarily disseminated by rainsplash (Bergdahl 1983, Dorworth 1972). *Gremmeniella abietina* also has a documented history of being moved to new areas on diseased nursery stock (Skilling and O'Brien 1969, Dorworth 1970a). Once the fungus is established in a plantation it will sporulate, and dissemination will be by natural means (Bergdahl 1983).

Scleroderris canker has been a major problem on young red and jack pines in the Lake States and in Ontario, Canada for the past 30 years. In these areas, the fungus causes a severe canker disease, especially on trees growing on poor quality sites or in cool, moist environments, such as frost pockets or other topographical depressions. The fungus incites disease by causing cankering on the main stem and/or a dieback of the tips of branches to a maximum height of about

2 metres above the ground. In the past, this disease was most often associated with plantation failure (Dorworth 1972, Skilling 1972). However, in the early 1970s, *G. abietina* was found attacking and even killing large red and Scots pines in northern New York (Setliff et al. 1975). This form of disease expression was not typical of scleroderris canker in North America but was typical in Europe, which lead Skilling (1977) to speculate that this more virulent form of the fungus may have a European origin. The presence of the European strain of *G. abietina* was confirmed in New York using serological methods (Dorworth et al. 1977).

Today, there are three distinct serotypes (Asian, European and North American) of *G. abietina* that are recognized within the geographic range of the fungus (Dorworth and Krywienczyk 1975). These three serotypes also form the basis for the different pathogenic races or strains currently designated for the fungus. The European strain appears to be the most virulent because it can attack mature trees, may also be able to cause infection under a wider range of environmental conditions and has a much wider host range than either the Asian or North American strains (Skilling 1977, 1981; Yokota 1983). In addition, a serotype that is intermediate between the European and North American types has been reported but the biological significance of this variant remains unknown (Wendler et al. 1980).

Gremmeniella abietina was first found in Vermont in 1971 (Tattar et al. 1973). However, this find was not considered significant until the European strain was confirmed in 1976 (H.B. Teillon, unpublished). Because of the presence of the European strain, New York and Vermont established internal quarantines in 1977 that were designed to restrict the movement of coniferous trees, including any parts of trees, out of areas known to be infested with *G. abietina*. The federal government also enacted an emergency regulation (1977) for the same purpose but only included restrictions against the European strain. However, this federal regulation was rescinded in 1982 (Laidlaw 1983), but the two state quarantines remain in effect. Upon enactment of the quarantines, New York and Vermont established annual surveys designed to determine the host range and distribution of *G. abietina* within their state boundaries. In 1978, the United States Department of Agriculture (Animal Plant Health Inspection Service) and the Forest Service (State and Private Forestry) also conducted surveys in an effort to determine the extent of infestation in the other northeastern states. Vermont has maintained its survey for the past 20 years (1977-1996). The purpose of this paper is to report the historical findings of these surveys and the current status of *G. abietina* in Vermont.

MATERIALS AND METHODS

In 1977, the Vermont Department of Forests, Parks and Recreation, the Vermont Department of Agriculture and the Forestry Department of the University of Vermont jointly established an intensive statewide survey for *G. abietina*. The Vermont survey was designed to inspect every pine plantation and entailed an examination of every tree for symptoms of scleroderris canker. In addition, any pines located within 1000 feet (about 300 metres) of the surveyed plantation were also examined. All inspections were done by either department personnel or trained seasonal employees. Information recorded for each plantation included: tree species, age, plantation size and location. Also, any symptomatic tissues found during the survey were collected, placed in bags and transported to the forest pathology laboratory at the University of Vermont for isolation and verification. If *G. abietina* was confirmed, the entire town in which the collection was made was then placed on the quarantine list and no restricted materials could be shipped outside of the quarantine area without a certificate stating the materials had been

inspected and found free of scleroderris canker. Also, selected isolates from the 1977-78 survey were shipped to Dr. Skilling's laboratory in St. Paul, MN for strain evaluation using methods of Dorworth and Krywienczyk (1975).

RESULTS

Since 1971, *G. abietina* has been found on a number of different pine species but most commonly on red and Scots pines growing in relatively small plantations in north central Vermont. The other pine species included Austrian pine *(P. nigra*), eastern white pine *(P. strobus)* and jack pine *(P. banksiana)*. Symptoms were usually confined to the tip portion of the lower branches, and cankers were rarely observed on the main stem of infected trees. Most plantations exhibited only trace amounts of infection, and tree mortality was rare. When mortality did occur, it involved either red or Scots pines (<20-years-old) and was usually confined to low areas (depressions) within a plantation. In these areas, *G. abietina* was commonly found in the tops of trees including the terminal stem.

Following the 1977 survey, 25 red and 3 Scots pine plantations were identified as being infested with *G. abietina* (Table 1). This represents approximately 11% of the 253 plantations surveyed. These plantations were located in 22 towns in north central Vermont (Figure 1) and totaled 350 acres (142 hectares). Following the statewide survey in 1978, 3123 additional plantations were surveyed and 27 (<1%) plantations were found infested with *G. abietina*. Of these 27 plantations, 22 (81%) and 5 (19%) were red and Scots pine, respectively, and all were located in north central Vermont (Figure 2). The European strain was confirmed in 13 plantations through 1978. Between 1978 and 1984, the number of plantations with *G. abietina* totaled 108, which is nearly double the number found infested through 1978. These 108 plantations totaled 887 acres (359 hectares).

In 1985, the four southern counties were resurveyed but *G. abietina* was not found. However, the northern survey found an additional 14 plantations that were infested (13 red pine and 1 Scots pine) and as a result, 9 towns were added to the quarantine list (Table 1). Since 1986, only 2 plantations (1 red pine and 1 Scots pine) have been added to the list but no additional towns have been added. During the past 25 years, a total of 126 plantations (107 red pine and 19 Scots pine) in 64 towns have been found infested with *G. abietina* (Table 1). These 126 plantations represent about 997 acres (403 hectares), therefore, the average size of an infested plantation is about 8 acres (3.2 hectares). To date, all infested plantations have been found in northern Vermont and there has been no significant change in the quarantine area since 1986 (Table 1, Figure 3). These data were summarized from the "Forest Insect and Disease Conditions in Vermont" annual reports (1971-1996) published by the Department of Forests, Parks and Recreation, Agency of Natural Resources, State of Vermont.

DISCUSSION

When scleroderris canker was first found in Vermont in 1971, there was little concern except that plantation owners had yet another tree disease problem for which to manage. However, after the Setliff (1975) report that described *G. abietina* killing large trees in New York, there was increased concern about the potential of scleroderris canker, especially after finding a second plantation in 1976 and learning that it was infested with the European strain.

This heightened concern prompted the design and implementation of the internal "state-administered" quarantine that is still in effect (H. B. Teillon, unpublished). This quarantine requires annual field surveys for scleroderris canker and regulates towns known to have plantations infested with *G. abietina*. All other towns are considered disease-free and plantation owners are issued proof-of-origin certificates.

During the past 25 years, scleroderris canker has only been found in northern Vermont. This pattern of distribution has not changed, suggesting *G. abietina* is not actively moving either within or between plantations which differs markedly from the Ontario report (Hopkin and McKenney 1995). Our field observations suggest the fungus is able to persist in young plantations at very low levels without causing significant impact. However, on occasion, the fungus has been responsible for destroying plantings of red or Scots pines but this only occurred in several small plantations prior to 1986.

Gremmeniella abietina has a documented history of being moved around on diseased nursery stock (Dorworth 1970a, 1970b; Skilling and O'Brien 1969). Our survey data shows the highest incidence of scleroderris canker occurs in and around Lamoille County. This pattern of distribution suggests that a nursery could have been involved in the dissemination of *G. abietina* in northern Vermont. In 1978, it was determined that a small private nursery produced red pine, Scots pine and other conifer seedlings for the local trade prior to 1977. This nursery was located adjacent to a 35-year-old stand of red pine, which was found infested with *G. abietina* during our survey in 1978. In addition, a few volunteer red pine seedlings growing in the abandoned nursery area also were found infected. On this basis, we concluded the nursery unknowingly played a significant role in the dissemination of diseased nursery stock to plantations in north central Vermont prior to 1977. Unfortunately, nursery records were not available so there was no way to trace the primary source of infection back to the nursery. However, since the nursery stopped selling trees 20 years ago, it is now interesting to note there have been only two plantations added to the quarantine list during the last 10 years. This change in disease incidence was also observed in reference to a Michigan nursery infested with *G. abietina* and noted as follows: "Once the production and shipment of diseased planting stock ended, the disease was essentially brought under control" (O'Brien 1983). We believe the pattern of disease development associated with scleroderris canker in Vermont is consistent with what one should expect if the primary means of plantation infestation involves introduction of diseased nursery stock.

Table 1. Occurrence of *Gremmeniella abietina* in Vermont, USA (1971-1996)

Year	Number of towns, plantations and acreage infested[1]					Total Plantations Surveyed	Total Acreage Surveyed
	Towns with *G. abietina*	*P. resinosa*	*P. sylvestris*	Total	Acreage		
	#	#	#	#	Ac	#	Ac
1971[2]	1	1	—	1	8	—	—
1976	2	1	1	2	13	—	—
1977[3]	22	25	3	28	350	253	1 156
1978	25	47	8	55	500	3 123	15 120
1979	35	64	10	74	600	136	653*
1980	41	74	11	85	700	132	634*
1981	41	82	11	93	763	140	672*
1982	41	83	11	94	767	103	494*

Table 1 (cont'd)

Year	Number of towns, plantations and acreage infested[1]					Total Plantations Surveyed	Total Acreage Surveyed
	Towns with *G. abietina*	*P. resinosa*	*P. sylvestris*	Total	Acreage		
1983	41	84	14	98	777	125	600*
1984	51	91	17	108	887	123	590*
1985[4]	62	104	18	122	956	258	1 238*
1986-91	64**	106	18	124	992	884	4 243*
1992-96	64**	107	19	126	997	694	3 331*

*Estimates based on an average of 4.8 ac/plt. for the 1977-78 surveys

**No new towns w/*G. abietina*

[1]Cumulative

[2]First Discovery of *G. abietina* in New England

[3]Statewide intensive survey began (251 towns and 14 counties)

[4]Resurvey of 4 southern counties — negative for *G. abietina*

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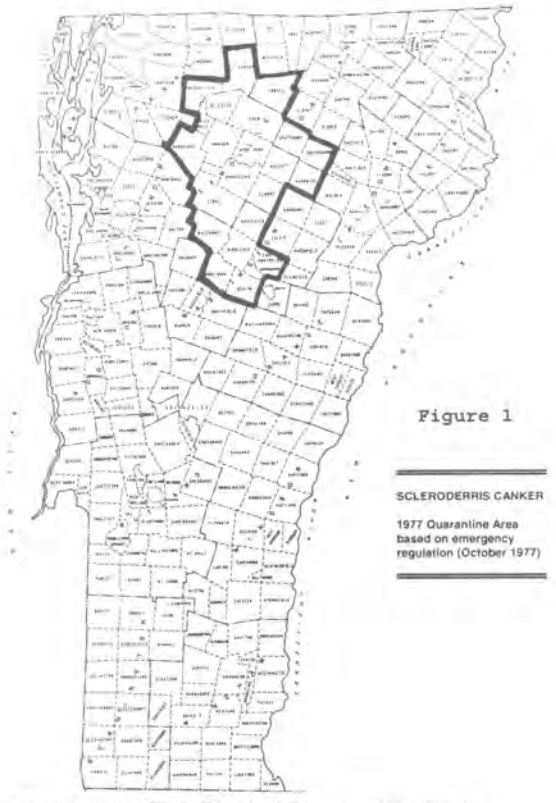

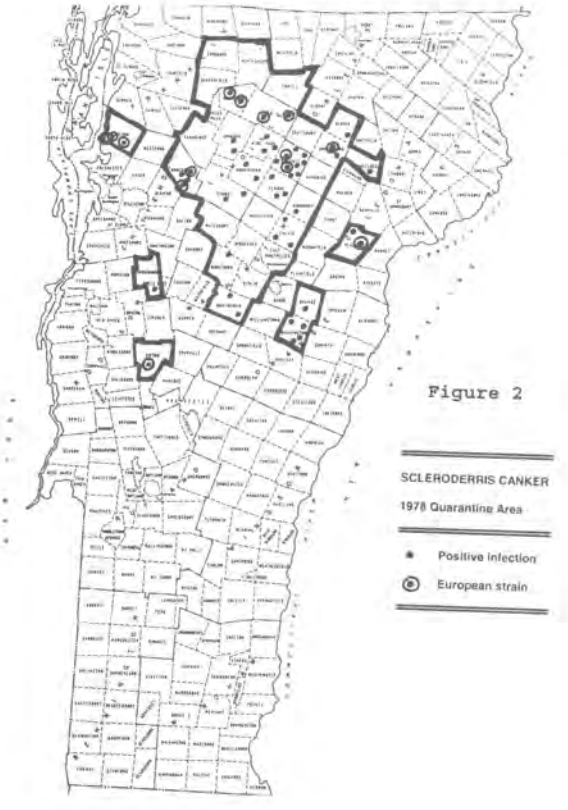

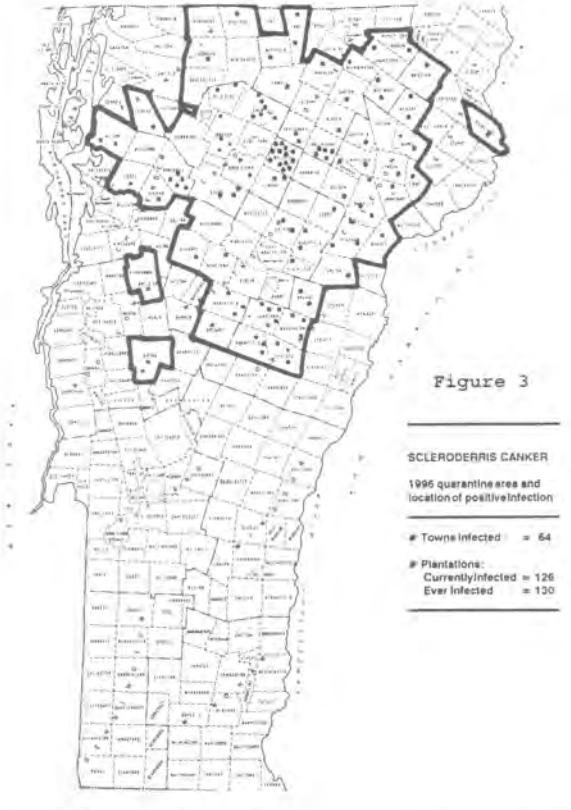

Figures 1, 2, 3. Scleroderris canker quarantine maps for Vermont (1977, 1978 and 1996). Note location of plantations confirmed to have European strain of *Gremmeniella abietina* (1978 map).

PINUS BANKSIANA NOT DAMAGED BY THE EUROPEAN RACE OF SCLERODERRIS CANKER

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SUMMARY

In 1982, red pine (*Pinus resinosa*) plantations were severely damaged by *Gremmeniella abietina*, European race; up to 90% mortality was recorded in the Mont-Laurier region, 200 km north-west of Montreal. Surrounding jack pines (*Pinus banksiana*) did not show any symptoms of the disease. Based on this observation, foresters began to reforest these severely damaged red pine plantations with jack pines in 1985. Plots containing 100 jack pines were set up in each of the three plantations. Red pine seedlings were planted under similar conditions and were used as control. Observations were carried out annually from 1989 to 1992. Mortality for red pine reached 70% in 1992 while all jack pines on the three sites were healthy. European race symptoms on jack pine were identified and can be described as a tip blight. The incidence of trees with these symptoms was high (52, 70 and 96%). The North American race symptoms were present on two jack pine sites at a very low incidence. Ten years after plantation, no jack pines have yet to be damaged by the European race of *G. abietina* because they show resistance to that race. However, all the red pines eventually died.

INTRODUCTION

The fungus *Gremmeniella abietina* (Lagerg.) Morelet causes a disease called scleroderris canker. In North America, this disease is found in the north-east and causes important damage to tree nurseries and conifer plantations, especially pine species (Laflamme and Lachance 1987; Skilling et al. 1986). The taxonomy of the genus *Gremmeniella* has been revised recently by Petrini et al. (1989) and a new variety of *G. abietina var. abietina* has been created. This variety includes the three serological races as defined by Dorworth and Krywienczyk (1975). Two of these races are present in North America: the North American race (NA) and the European race (EU). The NA race only infects pine shoots that are in the snow (Marosy et al. 1989), thus meaning the lower branches of a tree. As for the EU race, it can infect shoots in the upper crown of large trees, and could thus damage the trees more extensively than the NA race. The result of the EU race ravages on large red pine (*Pinus resinosa* Ait.) in the State of New York (Setliff et al. 1975) and on many younger plantations in Quebec (Laflamme and Lachance 1987) has created great concern for all pine species. The possibility of seeing both pine species, *Pinus banksiana* Lamb.(jack pine) and *Pinus contorta* Dougl. var. *latifolia* (lodgepole pine) being invaded by the EU race over a very large geographical was taken very seriously (Dorworth and Muir 1993).

In 1982, we began to study the epidemiology of the EU race in red pine plantations north of Montreal and Ottawa (Laflamme and Lachance 1987). We observed that even if red pine were severely damaged or killed by *G. abietina*, EU race, the surrounding or mixed jack pine seedlings or trees that had been planted or that grew from natural regeneration, were not affected. This did not convince foresters of this region to plant jack pine under or near red pines infected with the EU race.

The objective of this study was to measure on a yearly basis the impact of the EU race on jack pine over many years in three jack pine and one red pine plantations located near or under red pine infected by the EU race of *G. abietina*.

MATERIAL AND METHODS

We selected four differents sites situated from 120 km to 200 km north-west of Montreal (Canada). In each of the sites, a red pine plantation was infested with *G. abietina*, EU race, at the epidemic level (100% of the trees were infected). One permanent plot of 100 jack pines was situated in 1988 on sites 1, 2 and 3 and field observations started in 1989. Site 4 was the control where red pine seedlings were planted in 1989 and field observations started the following year. Here is a brief description of each site:

Site 1: Jack pines were planted in 1986 under residual red pines of about 4 m high with infected shoots up to 3 m.

Site 2: Jack pines were planted in 1985 in a clearcut of diseased red pines, near a few residual red pines of about 4 m high with infected shoots up to 3 m.

Site 3: Jack pines were planted in 1985 in a clearcut of diseased red pine, near a plantation of red pines of about 7 to 8 m high with infected shoots up to 5 m.

Site 4: In 1989, 436 red pines were planted in an opening of about 3,000 m^2 located in a red pine plantation of 5 to 6 m high with infected shoots up to 4 m.

Race identification was conducted as descripted by Petrini et al. (1989).

RESULTS

No mortality of jack pine seedlings was recorded on sites 1, 2 and 3 while mortality of red pine seedlings on site 4 had reached 70% four years after their plantation.

On jack pine, the EU race of *G. abietina* caused tip blight. The incidence of jack pine seedlings with this symptom on all three sites was as high as the EU race incidence on red pine at the control site (Table 1). The difference between the infection on these two pine species is that *G. abietina*, EU race, killed the red pine shoots the year of the infection and progressed towards the stem while only the tip of the jack pine shoot had been infected and died out without any further progress on the shoot.

Table 1. Percentage (%) of disease incidence in trees showing symptoms of *G. abietina* on Jack pine (I, II, III) and red pine (IV) seedlings planted under or near red pine trees infected with the European race of *G. abietina.*

Plots	1989	1990	1991	1992
I	75	98	92	96
II	10	38	39	52
III	20	35	43	70
IV (C)	---	44	55	76

DISCUSSION

These observations demonstrate quite clearly that jack pine shows resistance to the EU race of *G. abietina* while red pine is very sensitive to the disease. On site 1, we can see that jack pine was literally showered with conidia as 96% of the trees showed some infection; this is quite obvious as jack pine seedlings were planted under the infected red pine. In spite of that, they survived, and in 1997, they overgrew the dying red pines. On site 2, one jack pine was found dead in 1990, as the NA race was identified on that tree; natural jack pine stands occured in the area where the native NA race of *G. abietina* was found.

These observations also show that the probability of seeing the EU race progressing towards western Canada through natural jack pine stands is not likely to occur. But to be sure that native lodgepole pine stands are not affected by the EU race, inoculation tests should be undertaken. In the mean time quarantine should prevent any transportation of green material from pines originating from infected area with the EU race.

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IMPORTANCE OF SITE AND TREE SPECIES ON DISEASE INCIDENCE OF *GREMMENIELLA ABIETINA* IN NORTHERN SWEDEN

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SUMMARY

To relate the importance of site and tree species on disease incidence of *Gremmeniella abietina*, 32 regeneration areas of *Pinus contorta* and *Pinus sylvestris* situated on sites supporting pure stands of *P. sylvestris* and *Picea abies* respectively, were studied in northern Sweden. Each pine tree was examined for the occurrence of *G. abietina*. Trees planted on spruce sites were found to be more severely diseased than trees planted on pine sites. *P. contorta* was found to be more severely attacked than *P. sylvestris* on both site types.

Keywords: site type, field vegetation.

INTRODUCTION

Sweden has only two native conifer species of economic importance - the Scots pine, *Pinus sylvestris* L., and the Norway spruce, *Picea abies* (L.) Karst. In order to increase the forest yield, and to compensate for a shortage of raw material for the pulp industry, the lodgepole pine, *Pinus contorta* Dougl. ex Loudon var. *latifolia* Engelm., was introduced into Scandinavia from western North America in the early 20th century. Large-scale plantings began in northern Sweden during the 1970s and the 1980s (Karlman 1981). Up to 1987, the introduction seemed successful and the fast growth of lodgepole pine gave hope for shorter rotation and increased production. The lodgepole pine was considered especially hardy for areas with a harsh climate, and a large proportion of the plantations were carried out in such areas during the 1980s (von Segebaden 1992).

Up to 1986, the lodgepole pine plantings were damaged mostly by voles (Karlman 1984, 1987) but after a series of years with extreme weather conditions (1984-1987), the lodgepole pine was seriously attacked by the pathogen *Gremmeniella abietina* (Lagerb.) Morelet, which occurs throughout Sweden (Karlman et al. 1994). *Gremmeniella abietina* causes damage to both Scots pine and lodgepole pine by killing young shoots and buds, and also by forming cankers on stems and branches (Lagerberg 1912; Roll-Hansen 1964; Kurkela 1984; Ahlqvist et al. 1996). We have studied the development of the disease in 110 conventional plantations of lodgepole pine in northern Sweden from 1987 to 1991 (Karlman et al 1994). Results from the study (Table 1) show that several site factors play important roles on the disease incidence of *G. abietina*. Best-producing tree species is a variable that summarizes many factors and gives a description of the site. The results show that the infection by *G. abietina* was heavier on sites where Norway spruce is the best producing tree species.

Table 1. Effect of variables, in order of importance, on the frequency of *Gremmeniella abietina*.

	F-value	P-value
Temperature sum	84.55	0.0001
Topography	9.24	0.003
Best-producing tree species	5.13	0.0076
Frequency of birch	2.78	0.011

A comparison between the locations of the most severely damaged plantations in our earlier study and the distribution of conifers show that these plantations were primarily located in areas that formerly supported Norway spruce.

In northern Sweden, Scots pine is a primary tree species. Under natural conditions, forest fires occurred repeatedly in these areas, and were necessary for the regeneration of pure stands of Scots pine. Pure pine forests are often situated on dry moorlands along the river valleys. The soil is coarse and mostly poor. The humus layer is thin. The vegetation of the bottom layer contains lichens such as *Cladonia* (Malmström 1949; Sjörs 1956).

Norway spruce, on the other hand, is a secondary tree species. Pure spruce sites are mostly concentrated close to the mountain region. The soil has a fine texture, the ground is moist and nutritious and often covered with a thick raw humus layer. The vegetation of the bottom layer contains mostly mosses such as *Hylocomium*, *Polytrichum* and *Sphagnum* (Malmström 1949; Sjörs 1956).

The basic objective of this study was to find a simply applicated way to describe and predict areas with high risks of attack by *G. abietina*.

MATERIAL AND METHODS

To test if a site or former tree species was a useful description of hazard areas, eight blocks of four conventional regeneration areas of lodgepole pine and Scots pine were laid out on pine and spruce sites respectively in northern Sweden. Within each block, both tree species and sites were represented.

Pine and spruce sites were defined as areas having more than 80% of the respective species. The sites were chosen according to data from the Swedish National Forest Inventory 1923-26 (Hesselman 1935). We are well aware of the fact that the proportion of Scots pine was significantly larger in some areas south of latitude 64°N, where the pine forests were exploited when the timber frontier (Björklund 1984) expanded at the end of the 19th century. However, further north, large-scale clearcuts were done and monocultures were established before the 1923-6 Forest Inventory was collected. The description is therefore accurate.

When creating the blocks the present tree species and age of surrounding stands were also considered.

The occurrence of *G. abietina*, and the infected percentage of foliage were measured for each pine tree within ten circular 100 m^2 plots per plantation. The occurrence of *Phacidium infestans* P. Karst. was also measured.

RESULTS AND DISCUSSION

When comparing the two site types, without regarding tree species, *G. abietina* was found more frequently among trees planted on spruce sites than among trees planted on pine sites (Table 2). When comparing the two tree species without regarding site, *G. abietina* was found more frequently in the lodgepole pine regenerations than in the Scots pine regenerations (Table 3). Only in one block was Scots pine found to be more severely diseased than lodgepole pine.

Table 2. Percent of trees damaged by *Gremmeniella abietina* with respect to site (values with different letters are significantly separated ($p < 0.05$)).

spruce site	pine site
45.7 a	11.0 b

Table 3. Percent of trees damaged by *Gremmeniella abietina* with respect to tree species (values with different letters are significantly separated ($p < 0.05$)).

P. contorta	*P. sylvestris*
41.3 a	15.3 b

When comparing the four combinations of site and tree species, lodgepole pine planted on spruce sites had the highest infection rate, Scots pine on spruce sites the second and lodgepole pine and Scots pine on pine sites the lowest (Table 4).

Table 4. Percent of trees damaged by *Gremmeniella abietina* with respect to site and tree species (values with different letters are significantly separated ($p < 0.05$)).

spruce site		pine site	
P. contorta	*P. sylvestris*	*P. contorta*	*P. sylvestris*
63.2 a	28.2 b	19.5 c	2.4 c

Even though the frequency of damaged trees was higher in the lodgepole pine plantations, the frequency of dead trees was higher in Scots pine. The mean of dead trees for lodgepole pine on spruce sites was 9%, and for Scots pine on spruce sites, 12%. The lower frequency of dead lodgepole pine trees might be a result of the young age of the studied regenerations, and the severely damaged lodgepole pines might die later. From our earlier studies (Karlman et al. 1994) we know however that the lodgepole pine survives severe damage better than Scots pine, even though the quality of the surviving lodgepole pine trees is very bad with cankers, dead tops, double stems, etc. It will be interesting to follow the disease development in these regenerations.

Our results diverge from the Finnish results by Sairanen (1990), who found no typical vegetational pattern or forest site type of Scots pine stands severely affected by *Gremmeniella abietina*. However, her study was concentrated to poorer mineral soils and she suggests that differences in disease development might have been explained by forest site type had richer sites been included in the study.

To summarize, this study was an attempt to find a simple way to predict hazard areas, and our results suggest that the original distribution of tree species is a good way to predict which areas might be at high-risk of attack by *G. abietina*.

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SILVICULTURAL ASPECTS OF THE DISEASE CAUSED BY *GREMMENIELLA ABIETINA* IN NORTHERN SWEDEN

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SUMMARY

Since the 1970s, the North American *Pinus contorta* has been planted on more than 550,000 ha in northern Sweden. A monitoring of 110 plantations of lodgepole pine showed that this introduction was not problem free. In harsh areas, an extensive epidemic of *Gremmeniella abietina* caused severe damage and mortality in young plantations of lodgepole pine during the late 1980s. The damage was worst in topographic depressions and on sites where Norway spruce was estimated to have a higher wood yield than Scots pine. Results from experiments indicate that the "genuine" susceptibilities of Scots pine and lodgepole pine to scleroderris canker are probably about the same, although the exotic lodgepole pine has a higher "induced" susceptibility owing to its exposure to recurrent stress situations. The findings also indicate that provenances of lodgepole pine recommended for broad-scale use are not adapted to cope with the oceanic influences of weather that regularly affect northern Sweden. For the native species of Scots pine and Norway spruce, the susceptibility to scleroderris canker decreases with increasing latitude of origin. Mortality caused by scleroderris canker appears to be lower after mounding, and leaning trees run a greater risk of being killed by scleroderris canker compared with those standing upright. Cleaning out broad-leaved species in young stands of lodgepole pine does not necessarily enhance the development of scleroderris canker or other damaging agents. Trees on both cleaned and control plots were exposed to the range of temperatures conducive to scleroderris canker development for about the same amount of time. G. abietina is not host-specific with regard to Scots pine and lodgepole pine in northern Sweden. In view of the fact that the exotic lodgepole pine has been severely infected during the last ten years, the risk of spread to adjacent plantations of indigenous Scots pine should be considered high.

Keywords: Scleroderris canker, silviculture, control, risks, treatments, *Pinus contorta*, *Pinus sylvestris*

INTRODUCTION

The boreal forest ecosystem in northern Sweden, naturally dominated by Scots pine, *Pinus sylvestris* L. and Norway spruce, *Picea abies*, (L.) Karst. has recently changed through the rapid introduction of a third conifer - the North American lodgepole pine, *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm. (Karlman 1984, Hansson 1996). The first experiences of this exotic pine was overwhelming concerning establishment and early performance (Hagner 1983). In 1979, the National Board of Forestry even recommended planting of lodgepole pine in harsh areas where reforestation efforts with the indigenous Scots pine had failed repeatedly. Some hundred thousand hectares were planted up to 1985.

At the end of the 1980s, severe epidemics of scleroderris canker caused by *Gremmeniella abietina* (Lagerb.) Morelet, developed in plantations of this exotic after a period of unusually cold summers (Karlman 1987, 1990).

To control a pathogen, such as G. abietina, in a forest area requires detailed knowledge about the distribution and incidence of the disease. Such knowledge is especially crucial when introducing an exotic species into a new environment (Karlman 1981). Furthermore, it is important to determine how host resistance, weather conditions, topography and other site factors affect the incidence and severity of disease (Donaubauer *et al.* 1992). Over the years, many silvicultural control measures have been suggested, including choice of regeneration system, conifer species, provenance, site, scarification method, cleaning, thinning and pruning (Kurkela 1984, Skilling and Riemenschneider 1984, Aalto-Kallonen and Kurkela 1985, Skilling 1989, Roll-Hansen *et al.* 1992, Laflamme 1993).

The objectives of our studies were to test some silvicultural strategies and measures in terms of their usefulness in reducing the adverse effects of disease caused by scleroderris canker in young stands of lodgepole and Scots pine in northern Sweden. The focus has been on choice of tree species, choice of provenance, planting period, scarification type, and cleaning of broad-leaved species. Furthermore, the host specificity of *G. abietina* in northern Sweden was investigated.

MATERIAL AND METHODS

In 110 1.5-3 m high conventional plantations of lodgepole pine, and in 28 adjacent, comparably aged plantations or naturally regenerated stands of Scots pine, the occurrence of *Scleroderris* canker was recorded (Karlman *et al.* 1994). To find the main variance components for the frequency of *Scleroderris* canker in lodgepole pine plantations, a regression analysis with backward elimination was carried out on the 588 circular plots within the 110 plantations. The same analysis was then carried out with the remaining eleven variables, but this time mean values at the plantation level were used.

To test the susceptibility under good growing conditions of the two indigenous conifers and the introduced lodgepole pine, an inoculation experiment was arranged (Hansson 1997a). The NBF recommended that 1 800 seedlings of four provenances per species be planted even though they originated from widely separated latitudinal areas. The seedlings were inoculated with a shower of 0, 20,000 or 1,000,000 viable conidia of *G. abietina* and covered with a thin fibre cloth for five days after inoculation. The occurrence of dead tissue on the annual shoot was recorded 13 and 26 months after inoculation. Logit analysis was performed on data from each treatment. The effects of species and provenance within species were calculated for each section. To test for differences between species and between provenances within species, a number of contrasts were calculated.

Another comparison of the susceptibility of the two pine species was verifiable through a scarification experiment located at a harsh site near latitude 67° (Hansson and Karlman 1997). Here 2,880 two-year-old seedlings of very hardy provenances were planted after no soil scarification, patch scarification and mounding. This area is characterized by a cold, temperate, humid climate. The proportions of damaged and killed trees were recorded 18 years after planting,

when the trees were 20 years old. Only trees with apothecia or pycnidia of *G. abietina* were considered as being infected by this pathogen. Analysis of variance was performed on all experimental variables. A Chi-square test of the distribution of scleroderris canker damage degrees was performed on all leaning trees and an equal number of trees still standing upright at the same age, selected at random from each plot.

To study the genetics of *G. abietina*, DNA was extracted from 81 different isolates collected from lodgepole pine and adjacent Scots pine stands in 11 areas in northern Sweden (Hansson *et al.* 1996). In each stand, four well separated pairs of affected trees of each species were selected. The distance between individuals in the pairs was 1 to 5 m. From each tree, two to four twigs with apothecia, or in some cases, pycnidia of *G. abietina*, were collected. PCR amplifications were performed in a DNA thermal cycler PTC 100 and are programmed as described by Hamelin *et al.* (1996). Amplification products were separated by electrophoresis and the amplification patterns were examined. Fragment sizes were determined by measuring images on the photographs and comparing the values with those of the 1 kb ladder. Thirteen arbitrary primers were screened on ten representative isolates (from all three locations and two species) to find primers showing extensive RAPD variation between isolates and reproducible amplification products.

In order to test the result in the large-scale monitoring, because the incidence of disease of scleroderris canker was low where the abundance of broad-leaved species was high (Karlman *et al.* 1994), nine pair-wise comparisons between plots of 1-2 m high lodgepole pines with or without dense broad-leaved trees were laid out (Hansson 1997b). The comparisons were laid out in slightly infected plantations. The winter temperature was measured in one such comparison. The relative frequency of scleroderris canker was used in the t-test.

RESULTS AND DISCUSSION

The results from the monitoring showed that more than 70% of the studied plantations of lodgepole pine were severely infected by scleroderris canker, and where it was possible to make comparisons, 65% of the lodgepole pine plantations were severely infected while none of the Scots pine regenerations had been infected. The main results in this study were that the disease incidence increased with increasing harshness of the site, in topographic depressions and on sites where Norway spruce gave the highest wood yield.

Only the highest spore dose in the inoculation experiment resulted in significant infection. Both pine species were infected to a degree of 53%, 13 months after inoculation, which is significantly higher than the infection degree for Norway spruce at 39%. Unfortunately the Scots pine seedlings were negatively affected by the insect shelter used. Consequently, after twenty-six months, 42% of the affected lodgepole pine seedlings had recovered compared to only 13% of the Scots pine seedlings, and the mortality was 4% for lodgepole pine, which is significantly lower than 33% for Scots pine. Lodgepole pine had a higher ability than Scots pine to recover from a scleroderris canker attack and a lower proportion of dead tissues on the annual shoots. Aitken (1993), who took the individual degree of damage into consideration when calculating the susceptibility, reported similar results.

As a result of heavy wet snow that fell in late December 1993, the 2-m-high lodgepole pines in the scarification experiment were bent down to the ground and were covered by snow until late

April 1994. A significantly higher number of lodgepole pines were leaning (compared to Scots pine) and had poor stem quality at the revision in the autumn of 1995, when the trees were 20 years old. As much as 93% of the lodgepole pines were infected and 22% were killed by scleroderris canker compared to 21 and 5% for Scots pine. Both differences were significant. Due to the use of very hardy provenances of both species, we regard this as an objective species comparison. Our monitoring of lodgepole pine plantations in northern Sweden, where 28 comparisons with the indigenous Scots pine was done, suggests that this is generally valid across large areas in northern Sweden (Karlman *et al.* 1994). The contradictory results in the inoculation experiment probably depends on differences in growing conditions and age of the trees. The young lodgepole pine seedlings in this experiment grew under stress-free conditions, and were vital. The 1.5 to 3 m high trees investigated in the practical plantations in northern Sweden have been exposed to a harsh environment, including recurrent damage from heavy snow loads, often resulting in instability problems (Karlman *et al.* 1994; Hansson and Karlman 1997). Leaning lodgepole pines were more severely damaged by scleroderris canker than those that were not leaning.

Mounding significantly reduced the mortality caused by scleroderris canker in lodgepole pine and by snow blight, *Phacidium infestans* P. Karst. in Scots pine, which is in accordance with results presented by Roll-Hansen *et al.* (1992).

In the inoculation experiment, the susceptibility to scleroderris canker for the native species decreased with increasing latitude of origin. A more complex pattern was revealed for lodgepole pine. Here the most south-eastern provenance was less susceptible than the most north-western one, which together with results recently presented by Wu *et al.* (1996) indicate that the closer a lodgepole pine provenance is to the distribution edge of jack pine, *Pinus banksiana* Lamb., the higher its resistance to pests and pathogens.

Cleaning out broad-leaved species did not enhance the development of scleroderris canker or other damaging agents. Trees on both cleaned and control plots were exposed to the range of temperatures conducive to *scleroderris* canker development for about the same amount of time.

Gremmeniella abietina showed no host-specificity with regard to Scots and lodgepole pine. In view of the fact that the exotic lodgepole pine has been severely infected during the last ten years, the risk of spread to adjacent plantations of indigenous Scots pine should be considered high.

Taking the results from the five studies into consideration, a couple of silvicultural recommendations that could help to reduce damage caused by scleroderris canker on conifers in northern Sweden can be stated: 1) Avoid the use of lodgepole pine in harsh areas where there is a risk of even occasional heavy snow loads; 2) Question the use of lodgepole pine at sites where Norway spruce is the best producing native conifer species. 3) Question the use of lodgepole pine in topographic depressions within clear-felled areas; 4) Use only very hardy provenances of Scots pine nearby areas infected with scleroderris canker; 5) Apply all silvicultural measures that increase the vitality of lodgepole and Scots pine in harsh areas, including early planting and mounding; 6) Use all silvicultural measures that increase the stability of lodgepole pine, especially avoid root deformation and 7) Cleaning out broad-leaved species in lodgepole pine plantations will not increase pathogen-related problems in the stands. The release cutting of double stems during

autumn could, however, result in an increased frequency of scleroderris canker, and cannot therefore be recommended.

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INFECTION OF PINE BY *GREMMENIELLA ABIETINA*: ULTRASTRUCTURAL AND CYTOCHEMICAL ASPECTS

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The infection of *Pinus resinosa* and *P. sylvestris* seedlings by *Gremmeniella abietina* was studied by applying colloidal gold techniques. The pathogen cells, surrounded by the fibrillar material of extracellular sheath, colonized sparsely both intercellular and intracellular spaces of host bracts and short shoot tissues. Microhyphae-like cells were observed as having penetrated host cell walls. Based on our results from gold-labelings using exoglucanase for cellulose and antibodies against pectin or fungal laccase, we suggest that *G. abietina* is able to degrade cellulose and pectin in host cell walls and that phenoloxidases secreted by the pathogen could be involved in the degradation. The results indicate that the extracellular sheath of *G. abietina* is implicated in host-pathogen interactions such as attachment of hyphae to the host surface and cell wall degradation during colonization of host tissues.

MOLECULAR EVIDENCE OF DISTINCT INTRODUCTIONS OF THE EUROPEAN RACE OF *GREMMENIELLA ABIETINA* INTO NORTH AMERICA

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ABSTRACT

The presence of the European race (EU) of *Gremmeniella abietina* var. *abietina*, causing scleroderris canker of conifers, was first reported in North America in 1975 in the northeastern U.S.A., and subsequently in southern Quebec and Newfoundland in the late 1970s, where it quickly became established. We have analyzed DNA profiles in samples from a historic collection of *G. abietina* var. *abietina* that included some of the first isolates of the EU race reported in the U.S.A., to test hypotheses concerning the epidemic of the disease in North America. The hypothesis of a single introduction of that pathogen in the northeastern U.S.A. followed by secondary spread to northeastern Canada was rejected. Genetic diversity calculated by an analysis of molecular variance using haplotypic frequencies and distances was high between populations in continental North America and Newfoundland (Φ_{ct}=0.665, p<0.001) indicating that they did not belong to the same epidemiological unit. By contrast, small levels of genetic differentiation were observed between continental North American populations (Φ_{ct}=0.047, p=0.079), suggesting gene flow is taking place between these populations. A single haplotype of *G. abietina* var. *abietina* dominated the continental populations (80% of the isolates) but was absent from Newfoundland or from Europe, whereas five haplotypes made up the Newfoundland population, all of which were either absent or very rare on the continent. A genetic distance analysis yielded a dendrogram resulting in a close cluster of populations from continental North America and a second cluster comprising European and Newfoundland populations. A phylogenetic analysis of the haplotypes indicated that some of the rare haplotypes may have derived from somatic mutations, while others probably occurred as the result of new introductions. The results are consistent with a scenario of distinct primary introductions of this pathogen into Newfoundland and continental eastern North America followed by secondary asexual propagation.

INTRODUCTION

Gremmeniella abietina (Lagerb.) Morelet var. *abietina* (Petrini et al. 1989), the causal agent of Scleroderris canker, is a very damaging pathogen of conifers in North America, Europe, and Asia (Setliff, Sullivan et al. 1975; Yokota 1975; Laflamme and Lachance 1987). In North America, a North American (NA) race that is believed to be indigenous, and a European (EU) race which was introduced earlier this century presumably from Europe, are present (Dorworth, Krywienczyk et al. 1977). These two races have overlapping morphological characteristics (Petrini, Petrini et al. 1989), but they were shown to be fairly divergent based on their ecological, etiological, and molecular characteristics (Dorworth and Krywienczyk 1975; Hamelin, Bernier et al. 1993; Bernier, Hamelin et al. 1994; Lecours, Toti et al. 1994; Hantula, Dusabenyagasani et al. 1996; Mueller and

Uotila 1996; Hamelin and Rail 1997). Subsequent analyses within the EU race also revealed genetic differentiation within the EU race (Hellgren 1994; Hamelin, Lecours et al. 1996) and genetic profiles in North America were often different from those from Europe (Hamelin, Lecours et al. 1996).

In North America, scleroderris canker was first reported in Michigan and Ontario pine plantations in the 1950s (Martin 1964; Ohman 1966). Subsequently, it was established that the EU race was causing mortality of large trees while the NA race was restricted to infection on the portion of the crowns that remains under snow cover (Marosy 1989). The EU race was first reported in New York State, then in Vermont, Maine, and New Hampshire (Skilling, Schneider et al. 1977; Skilling, Schneider et al. 1986). The first report in Canada was in the late 1970s from a plantation located near the U.S.-Quebec border (Lachance 1979). The pathogen rapidly spread to Quebec and is now prevalent throughout the distribution of red pine in that province (Laflamme 1993). In Newfoundland and in New Brunswick, the disease was also found in 1979, only a year after it was reported in Quebec (Singh, Dorworth et al. 1980; Magasi 1982). The chronology of these new reports was consistent with the scenario of an introduction from Europe to the eastern U.S.A. followed by secondary spread to northeastern pine stands, but the precise routes and means of dissemination were impossible to establish based on the data available.

If the EU race of *G. abietina* var. *abietina* was introduced into northeastern North America followed by secondary asexual spread, the genetic structure of the population would be expected to be uniform among regions with low overall levels of genetic diversity. If several introductions took place but were followed by extensive gene flow, larger levels of genetic diversity combined with low inter-regional genetic diversities would be expected. By contrast, if several introductions occurred followed by local secondary spread but no inter-regional gene flow, a discontinuous population structure would be expected with large genetic diversity among regions. The objective of this study was to analyze the genetic structure of this exotic pathogen using molecular approaches to test the null hypothesis of a single introduction of the EU race into North America, followed by secondary asexual spread.

MATERIALS AND METHODS

Isolates

Two hundred and twenty isolates of GAA-EU were sampled from 7 regions in Quebec over a period of 20 years and conserved in liquid nitrogen (Fig. 1). Isolates were obtained either from a single-spore or from a single pycnidium. The sampling was not particularly structured as the objective of the initial survey was not to study the genetic composition of the pathogen but to record disease incidence in Canada. Nevertheless, the sampling represents a cross section of the scleroderris canker epidemics in North America. Confounding effects of time of collection and geographic origin are treated in the analysis.

In addition, fifteen samples were obtained from several European countries. Only isolates belonging to the European amplitype (Hamelin, Lecours et al. 1996) were used in the present study since the Scandinavian and Alpine amplitypes have not been reported in North America.

Some of the first isolates originally identified as EU race representing the beginning of the *G. abietina* var. *abietina* epidemics were also obtained. However, only 10 of the 25 isolates

previously identified as EU race or intermediate using serology (Dorworth, Krywienczyk et al. 1977) were found to belong to the European race when rDNA-ITS and RAPD markers were used (Hamelin, Bernier et al. 1993; Bernier, Hamelin et al. 1994) and were retained for this study.

All isolates were grown for 1 month on dialysis membranes that were placed in petri dishes containing an agar medium made with 25 ml of Campbell's V-8 juice, 15 g bactoagar, 7.5 g malt extract and 475 ml of water dispensed at 20 ml per petri dish. Alternatively, the strains were inoculated in a V-8 liquid medium and grown in the dark for 4 weeks at 18°C. The mycelia were harvested by filtration, lyophilized and stored at -20°C until needed.

DNA extraction

Approximately 10 mg of lyophilized mycelium were mixed with an equal amount of diatomaceous earth (Sigma, St. Louis, MO) and ground with a mortar and pestle. Six hundred microliters of extraction buffer (1.4 M NaCL, 100 mM Tris-HCL (pH 9.5), 20 mM EDTA, 0.25% 2-mercaptoethanol, 2% CTAB, 1% PEG 6000) were added to the macerated mycelium and incubated at 65°C for 30 min. The mixture was emulsified by adding an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), vortexing and centrifuging for 5 min at 12000x g. The resulting pellet was washed with 70% cold ethanol, dried and resuspended in 50 μl of 10 mM Tris-HCL, 1 mM EDTA (pH 8). All DNA was diluted 1:50 after it was determined that such a dilution yielded the most reproducible RAPD patterns.

DNA amplification

Race identification was performed for each sample according to one of the methods previously described (Hamelin, Bernier et al. 1993; Bernier, Hamelin et al. 1994). Random amplified polymorphic DNA (RAPD) analyses were performed as described previously (Hamelin, Bernier et al. 1993; Hamelin, Lecours et al. 1996) except that 2.5 mM $MgCl_2$ were used for primers OPA12 and C08 and 3.5 mM $MgCl_2$ were used for primer OPD05. Additional primers were screened for polymorphism within the EU race. Primer kits (Operon kits C, D, F) were tested with 7 isolates originating from diverse geographic sources. Most primers were monomorphic even when including isolates from North America and Europe. Only two additional primers, OPC08 and OPD5, were polymorphic and were included in this study. Random amplified microsatellite (RAMS) reactions were performed as described previously with primer DBH$(CGA)_5$ (Hantula, Dusabenyagasani et al. 1996) except that TAQ EXPAND (Boehringer Mannheim, Laval, Quebec) polymerase mixture was used.

Amplifications were carried out in a MJ-Research thermal cycler (MJ-Research, Watertown, MA) programmed for a denaturation step at 94°C for 2 min, followed by 1 cycle at 40°C for 4 min, 72°C for 2 min, then 45 cycles of 94°C for 1 min, 40°C for 1 min and 72°C for 2 min. The reactions ended with a 7 min extension at 72°C.

All isolates were assayed with the 4 selected primers and DNA amplicons were separated by electrophoresis on 1.5% agarose gels in TAE buffer at 3V/cm for 2 h or on 1.5% agarose gels and 1.5% synergel (Diversified Biotech Inc., Boston, MA) in TPE buffer at 3V/cm for 4 h. The gels were stained in ethidium bromide and digitized under U.V. light using the Canberra IS1000 system. Fragment lengths were calculated using the available software. Amplifications of DNA templates and primer combinations were repeated regularly to ensure reproducibility of the amplification profiles. All major diagnostic DNA fragments used in our analysis were reproducible between runs.

Data analysis

A matrix of isolates x markers was recorded and haplotypic frequency was computed. Because the data was collected from various geographic regions over a number of years, frequency data was calculated by year as well as by region to detect chronological trends. Since no important chronological trends were noted in this preliminary analysis, data were then pooled by region. Because of the perennial nature of this canker pathogen, large year-to-year variation such as that observed for agricultural crop pathogens would not be expected.

RESULTS

Twenty-seven haplotypes were found among the 245 strains of *G. abietina* var. *abietina* from North America and Europe based on 22 DNA markers (Table 1). Haplotypes were sorted according to their binary code and arbitrarily given an identification number. A single haplotype (#5) dominated the *G. abietina* var. *abietina* populations in Quebec and the U.S.A., independently of the year or region of sampling (Tables 2 and 3). Haplotype #5 comprised between 53-86% of the isolates sampled in the 7 geographic regions in Quebec and the eastern U.S.A. When strains were pooled by year, between 47-89% of the samples belonged to haplotype #5 (Table 2). In 1985, a survey was conducted over a large portion of the range of distribution of red pine in Quebec and 62% of the samples belonged to haplotype #5 (Table 2).

However, haplotype #5 was completely absent from Newfoundland and Europe (Table 3). The *G. abietina* var. *abietina* population from Newfoundland contained only 5 haplotypes that were either rare or absent from Quebec and the eastern U.S.A. For example, haplotypes #14 and #19 comprised 15% and 19% of the isolates from Newfoundland, respectively, but were absent from Quebec and the U.S.A. (Table 3).

Additionally, 25 rare haplotypes were found in Quebec. None of these haplotypes were present in Newfoundland or in Europe (Table 3). This observation should be viewed with caution, however, since a smaller sample size was obtained from Newfoundland and Europe than from Quebec and the probability of obtaining rare haplotypes is proportional to sample size. Nevertheless, rare haplotypes were also found in Europe and Newfoundland which were absent from Quebec and the eastern U.S. (e.g. #24, #26, #27).

A single haplotype (#15) was common to continental North America, Europe, and Newfoundland (Table 3). However, the frequency of that haplotype differed between regions. It represented 58% of the samples in Newfoundland, 27% of the European samples, and only 8% of the continental North American samples (Table 3). But two additional haplotypes were common to Newfoundland and Europe but absent from Quebec and the eastern U.S.A., one of which (#19) was equally frequent in both sampling regions (Table 3).

Some of the rare haplotypes in continental North America were present in several geographic regions (#6, #7, #22), while others were restricted to a single region (e.g. #1, #2, #3). Direct comparisons between populations in different areas have to be made carefully because of the differences in sample size between regions and because of the potential chronological gradient in sampling. Nevertheless, rare haplotypes were more frequent in the central Mauricie region (9 haplotypes; n=34) than further east in the Gaspé Peninsula (4 haplotypes; n=20).

DISCUSSION

The discovery of the European race of scleroderris canker in New York State in 1975 was followed by intensive surveys to identify infection foci. The disease was almost simultaneously reported in three other states as well as in three Canadian provinces (Lachance 1979; Singh, Dorworth et al. 1980; Magasi 1982). The first report of the disease in Canada was in southern Quebec near the U.S.-Quebec border (Lachance 1979). In the following years the disease was reported further north, west and east and now occurs from Ontario and the Lake States to Newfoundland.

The chronology of these events suggested that the pathogen was introduced from the northeastern U.S.A. followed by spread to other states and provinces. However, the results presented in this study are not consistent with that hypothesis. The uneven distribution of *G. abietina* var. *abietina* haplotypes in continental North America and Newfoundland and apparent discontinuity in the epidemic strongly suggests that there were at least two distinct introductions of this fungus into North America, one on the continent and a second one in Newfoundland with little or no gene flow between these two founding populations. On the other hand, populations in continental North America are clearly part of a single epidemiological unit, as indicated by the low level of gene diversity and low genetic distance between regions on the continent.

In Scandinavia, population structure of *G. abietina* var. *abietina* belonging to the biotype found in North America resembled the population structure observed in continental North America. The same banding patterns occurred in several geographically isolated populations and genetic differentiation among populations was moderate (F_{st}=0.097) and comparable to that observed among continental North American populations in our study (Hellgren 1994). Nevertheless, genetic diversity within *G. abietina* var. *abietina* may be higher in Scandinavia as 37 M13 banding patterns were found among 71 isolates compared with 20 among 204 isolates in continental North America. Although that comparison may not be valid because different markers were used, we also found 6 profiles among 15 isolates from Europe, a proportion similar to that reported for M13 (Hellgren 1994). The presence of a sexual cycle in Scandinavia and its absence from North America could explain these differences (Uotila 1992).

Two hypotheses can be proposed to explain the dominance of a single haplotype of *G. abietina* var. *abietina* on continental North America. First, it is possible that this haplotype is better adapted or more aggressive than the other rare haplotypes. If adaptation were an important factor in the population structure of this pathogen, however, the same haplotype would not be expected to dominate all of the eco-climatic zones represented in this survey. Also, it would be expected that the Newfoundland and Gaspé populations would share the same adapted haplotype since maritime climates prevail in these regions.

A second hypothesis is that a founder effect followed by loss of mating type and genetic drift resulted in an unbalanced population structure with dominance of a few haplotypes (Brasier 1995). Founder effects have been proposed to explain low diversity in some populations of *C. parasitica* (Yir-Chung, Cortesi et al. 1996). The founding population could have been composed mostly of haplotypes #5 and #15 and the rare haplotypes were either introduced later or were simply at a much lower frequency. This hypothesis is supported by the dominance of haplotype #5 (82%) in

the isolates representing the earliest records of the EU race of *G. abietina* var. *abietina* in North America.

The hypothesis of a founder effect in this pathogen is particularly plausible if the frequent haplotypes were present in a few large nurseries and were disseminated on nursery stock. Dissemination of this pathogen has been suspected to occur via infected symptomless seedlings that escaped inspections prior to outplanting (Magasi and Manley 1974; Skilling, Schneider et al. 1986). The endophytic nature of this pathogen predisposes its dissemination on nursery material (Yokota 1975; Laflamme 1986; Petrini, Toti et al. 1990).

The present study highlights the importance of understanding the global epidemics of exotic pathogens. Several countries are regulating this pathogen through quarantines and seedling inspection programs. The present study indicates that Newfoundland and continental North America can be considered as distinct epidemiological units. Also, European populations of *G. abietina* var. *abietina* are genetically very distinct from continental North American populations of *G. abietina* var. *abietina* EU race. A larger sampling of Europe and Asia would be necessary to fully elucidate the center of origin of this pathogen.

Table 1. Binary coding of the DNA profiles of 27 haplotypes of the European race of *Gremmeniella abietina* in North America

Haplotype designation	DNA profile[a]
1	00101000101100000000000
2	00111000011100000000000
3	00111000100000100001100
4	00111000100100000000000
5	00111000101100000000000
6	00111000101100000100100
7	00111010101100000100010
8	00111010101100000100100
9	00111100101100000000000
10	00111100101100010000000
11	00111110101100011000100
12	01001001000100000001000
13	01010000100010100010100
14	01011001000100000001000
15	01111000010100000001000
16	01111000010100000101100
17	01111000100100000000000
18	01111000100100000001000
19	01111001000100000001000
20	01111010010100000101100

Table 1 (cont'd)

Haplotype designation	DNA profile[a]
21	10111100100010001100100
22	10111100101100010000000
23	10111100101100011001000
24	01111000100100000000001
25	11111100010100010010000
26	00111000010000000001000
27	01111100010100000001000

[a] Sequential order of DNA markers are (primer-size in bp of scored fragment): A12-500, A12-800, A12-1100, A12-1350, CGA-150, CGA-300, CGA-500, CGA-550, CGA-580, CGA-620, D5-350, D5-800, D5-750, D5-500, C8-250, C8-350, C8-700, C8-1200, C8-1300, C8-1400, C8-1500, C8-1250.

Table 2. Frequency of haplotypes of the European race of *Gremmeniella abietina* sampled in Quebec pooled by year of survey

		Haplotypes[b]				
Year[a]	N	1, 2, 3, 4	5	6-15	15	16-27
1985	61	<0.06	0.62	<0.08	0.10	<0.05
1986	19	<0.06	0.47	<0.08	0.16	<0.05
1990	28	<0.06	0.89	<0.08	0.07	<0.05
1991	18	<0.06	0.89	<0.08	0.11	<0.05
1995	18	<0.06	0.83	<0.08	0.00	<0.05

[a] all regions were pooled within a year; years 1984, 1988, 1992 and 1996 are not presented since sample size was less than 10.

[b] haplotypes are based on 22 RAPD and RAMS markers and are defined in Table 1.

Table 3. Frequency of *Gremmeniella abietina* haplotypes by region

Region[a]	Haplotypes[b]																	
	N	1, 2, 3	4	5	6, 9	7	8	9, 10, 11, 12, 13	14	15	16, 21, 22, 23, 25	17	18	19	20	24	26	27
Gaspe	22	<0.05	0.00	0.86	<0.05	0.00	0.00	<0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beauce	10	0.00	0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mauricie	34	0.00	0.00	0.53	<0.06	0.00	0.06	0.00	0.00	0.15	<0.03	0.00	0.00	0.00	0.09	0.00	0.00	0.00
East. Townships	14	0.00	0.00	0.75	0.00	0.08	0.08	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Abitibi/Lac St-Jean	12	0.00	0.17	0.67	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Montreal	20	0.00	0.00	0.70	<0.05	0.00	0.10	0.00	0.00	0.10	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Outaouais	80	<0.02	0.00	0.78	<0.04	0.01	0.04	<0.01	0.00	0.05	<0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
U.S.	12	0.00	0.09	0.82	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Newfoundland	26	0.00	0.00	0.00	0.00	0.00	0.00	<0.04	0.15	0.58	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.04
Europe	15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.00	0.07	0.40	0.13	0.00	0.07	0.07	0.00

[a] haplotypic frequencies a were computed for each region but pooled over years sampled between 1984 and 1996.

[b] haplotypes are based on 22 RAPD and RAMS markers and are defined in Table 1.

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NEW MOLECULAR APPROACHES TO STUDY MOLECULAR EPIDEMIOLOGY OF SCLERODERRIS CANKER

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Scleroderris canker caused by *Gremmeniella abietina* (Lagerb.) Morelet cause extensive damage to conifers throughout the northern hemisphere. This fungus comprises several varieties, races and differentiated subspecific groups that have been characterized by several biochemical assays (Dorworth and Krywienczyk 1975; Hamelin et al. 1993; Lecours et al. 1994). The *abietina* variety is the most economically important pathogen within the *G. abietina* complex. It comprises two races, a North American (NA) race, which is believed to be indigenous to North America, and a European one (EU), which was introduced into North America earlier this century. This disease has been spreading in North America since its introduction on nursery material and by rain-splashed spores. A better understanding of the molecular epidemiology of this pathogen is necessary in order to prevent new introductions and further spread.

Within the EU race, three differentiated populations were described and correlated to ecotypic origin in Europe (Morelet 1980; Uotila 1993). These populations had characteristic DNA amplification profiles and were termed amplitypes (Hamelin et al. 1996). The Scandinavian profile was only present in northern Europe and the Alpine profile was found in the Alps at altitudes above 2000 m on *P. cembra* L., *P. mugo* Terra, *P. sylvestris* L. and Dutherity *Larix lyalli* Parl., and the European profile was present throughout Europe from the Scandinavian countries to Italy. In North America, however, the Alpine and Scandinavian amplitypes are absent (Hamelin et al. 1996).

The low genetic variability observed within the European race hampers epidemiological studies of scleroderris canker, especially in North America, where this race is considered to be introduced. However, recent studies using random amplified microsatellites (RAMS) pointed out appreciable intraracial variation in *G. abietina* (Hantula et al. 1996; Hantula and Muller 1997).

The aim of this study was to develop a set of PCR primers that could amplify DNA markers that could be used in the diagnostic and epidemiological studies of the European race of *G. abietina*.

MATERIALS AND METHODS

Fungal isolates

Twenty-three isolates of the EU race of *G. abietina* from Europe and North America were used in this study (Table 1). The DNA was extracted from mycelium as previously described (Hamelin et al. 1996).

Primer design

We used both random amplified microsatellites (RAMS, Hantula et al. 1996) and DNA sequencing with arbitrary primer pairs (SWAPP, Burt et al. 1994) to design PCR primers that flanked regions of the genome that were polymorphic. Six samples of the European race of *G. abietina* P86, US15, J7, M1024, A139p7 and Fi0003, including two individuals randomly chosen from each of the three RAPD amplitypes previously identified within the European race of *G. abietina* were used. RAMS were realized with ACA, GT, and CGA primers with amplification conditions previously described (Hantula et al. 1996).

DNA fragments from RAMS and SWAPP were reamplified and sequenced with an ABI automatic sequencer system. When some mutations were detected in DNA fragments, new PCR primers that flanked the polymorphic region were designed. Potential primers were analyzed for the likelihood of primer-dimers, primer-loops and hair-pin formation using the Generunner software. Five sets of primers were designed.

Amplification and DNA sequencing of markers

In order to confirm the mutations previously observed in the amplified fragments, all sets of primers were used to amplify and sequence amplicons from the selected samples. The primers were also used to amplify genomic DNA from the remaining samples in order to verify if there was also variation within the three populations. PCR products obtained were either separated by electrophoresis in agarose-synergel (Diversified Biotech, Newton Centre, MA), analyzed by low ionic strength single stranded conformation polymorphism (LIS-SSCP, Maruya et al. 1996) or by RFLP.

RESULTS

Since mutations were identified in five DNA fragments obtained from preliminary PCR tests, one from SWAPP and four from RAMS, five PCR primer pairs that flank those variable regions were designed (Table 2).

Three of the five markers had an identical sequence in the Alpine and Scandinavian amplitypes but differed in the European amplitype. A 6 bp insertion and two point mutations were present for the CGA3 marker in the Alpine and Scandinavian samples but absent from the European samples. This insertion was detectable on 1.5% agarose and 1.5% synergel (results not shown). It was located in a microsatellite whose motif unit, AATGAG, was repeated three times in the European samples but four times in the Alpine and Scandinavian samples. However, when PCR products from all other samples were analyzed by LIS-SSCP, no other differences were found within these populations.

Single base pair substitutions that were present in markers GT6 and MLITS differentiated the European amplitype from the Alpine and Scandinavian ones. These markers in these two loci were successfully separated by LIS-SSCP (Fig. 1). Alternatively the mutation in the MLITS locus in the European amplitype resulted in the loss of an *Nla*III restriction enzyme site. The European samples were separated from the Alpine and Scandinavian ones by restriction enzyme digestion using that enzyme (Fig. 2)

GAcga6-61f / GAcga6-511rc and Gaaca9-452f / Gaaca9-800rc primers amplified highly polymorphic regions in all populations (Fig. 3). The CGA6 locus comprised seven different alleles in the European population, four alleles in the Alpine population and three alleles in the Scandinavian population (Table 2). Both indels located in microsatellite stretches of DNA and point mutations were observed in DNA sequences of these regions of the genome. These indels often resulted in length polymorphisms in agarose gels. The CGA6 locus was rich in polymorphic microsatellites such as (G)n, $(GGCA)_n$, $(GACA)_n$, $(CAGA)_n$ and $(ATCT)_n$, while the ACA9 locus contained polymorphic microsatellites such as (G)n, (CCAA)n, (A)n, (TAGA)n,and (TCCAC)n.

DISCUSSION

Three of the five DNA markers developed in this study made it possible to separate the European amplitype from the Alpine and the Scandinavian ones. This could be useful in diagnostics. These markers were apparently in conserved regions since they showed no intragroup polymorphism. However, searching for the sequences that were available in Genbank did not reveal significant homology with these three markers. One of these markers has already been used to detect hybridization between the European and Scandinavian amplitypes (Uotila et al. See this document). LIS-SSCP (Maruya et al. 1996) and RFLP were used to separate samples that differed by a single base pair substitution.

The primer pairs generated from CGA and ACA RAMS primers contained several microsatellites with variable numbers of repeat units that produced length polymorphisms on agarose gels. Although these microsatellites were different in their composition from the motif unit used in the RAMS primers, it appears that we have sampled hypervariable regions. Some of the motif repeats (e.g. AGGC) were also found in hypervariable regions of human and mouse genomes.

Nineteen different alleles were identified in the ACA9 and CGA6 loci. Some of these alleles could be clearly separated on agarose gels, but others could not and needed the use of LIS-SSCP technique because in some samples, insertions and deletions in different repeat motifs could compensate each other and produce whole DNA fragments with few base pairs difference.

A microsatellite locus was observed in *Epichloë typhina*, a fungal endophyte, comprising five alleles that were different in size in a population of 91 field isolates (Groppe et al. 1995). This marker is currently used to study the population genetics of that fungus.

DNA markers sampled by this study showed that despite the geographic separation, Alpine and Scandinavian populations are more closely related than their European counterparts which, however, has an overlapping distribution. Our results confirm observations from spore septation (Morelet 1980; Uotila 1993) and soluble protein electrophoresis (Petrini et al. 1990) indicating a more recent common ancestor to the Alpine and Scandinavian populations and a more distinct one for the European amplitype.

Table 1. Characteristics of *G. abietina* samples used

Isolates	Origin	RAPD amplitype*
P80	Germany, Rendsburg	European
P85	Germany, Nordhorn	European
P82	Germany, Schmalenbeck	European
P83	Germany, Haard	European
P86	Germany, Rendsburg	European
P89	Germany, Schmalenbeck	European
NF87-0492	Canada, Newfoundland	European
NF87-0494	Canada, Newfoundland	European
NF87-0500	Canada, Newfoundland	European
US15	United States, Vermont	European
CF87-0032	Canada, Québec, L'Ascension	European
CF87-0036	Canada, Québec, Lac Saguenay	European
CF88-0007	Canada, Québec, Chute St-Philippe	European
J5	Italy, Adriatic coast	European
J7	Italy, Ortesi, Alps	Alpine
M1019	Switzerland, Chichenberg, Alps	Alpine
M1023	Switzerland, Chilchenberg, Alps	Alpine
M1024	Switzerland, Ahorni, Alps	Alpine
M1042	Switzerland, Ahorni, Alps	Alpine
Fi-0003	Finland, Ylikiminki	Scandinavian
A139p7	Sweden, Adak, Västerbotten	Scandinavian
A319p1	Sweden, Adak, Västerbotten	Scandinavian
SL149s	Sweden, Springliden, Västerbotten	Scandinavian
SL229p3	Sweden, Adak, Västerbotten	Scandinavian
SL548p1	Sweden, Springliden, Västerbotten	Scandinavian

* Hamelin et al. (1996)

Table 2. Characteristics of primers designed in this study

Primer	Putative locus	Number of alleles	Size of PCF product (bp)
GAcga3-350D GAcga3-int	CGA3	2	214-220
GAcga6-61f GAcga6-511rc	CGA6	14	380-420
GAgt6-134f GAgt6-568rc	GT6	2	456
GAaca9-452f GAaca9-800rc	ACA9	5	386-400
GAm16its3-11f GAml6its3-280rc	MLITS	2	290

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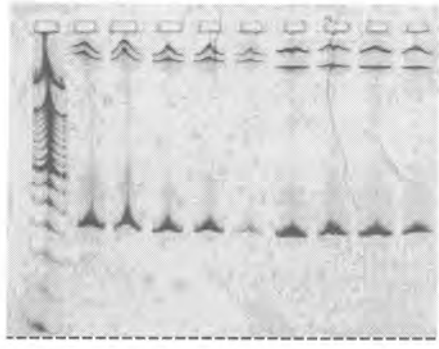

Figure 1. LIS-SSCP analysis of *G. abietina* PCR products amplified by GAgt6-134f and GAgt6-568rc primers. Lane 1: 100 bp molecular weight ladder; lanes 2-6: samples with the European RAPD amplitype P85, P86, NF87-0494, NF87-0500 and US15; lanes 7-8: samples with the Alpine RAPD amplitype J7 and M1024; lanes 9-10: samples with the Scandinavian RAPD amplitype Fi0003 and A139p7.

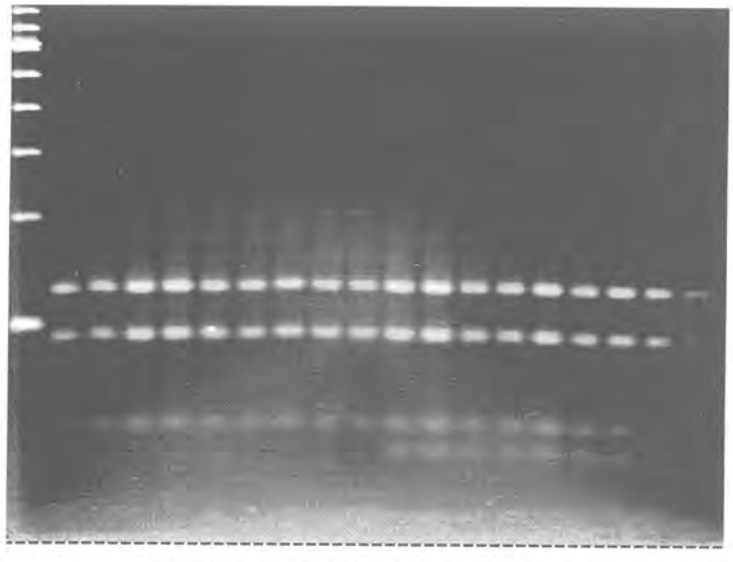

Figure 2. Agarose-Synergel electrophoresis of *G. abietina* *Nla*III digests from PCR products amplified by GAml6its3-11f and GAml6its3-280rc primers. Lane 1: 100 bp molecular weight ladder; lanes 2-10: samples with the European RAPD amplitype P80, P85, P86, P89, NF87-0492, NF87-0494, NF87-0500, US15 and CF87-0032; lanes 11-15: samples with the Alpine RAPD amplitype J7, M1019 M1023, M1024 and M1042; lanes 16-19: samples with the Scandinavian RAPD amplitype Fi0003, A139p7, A319p1, and SL548p1.

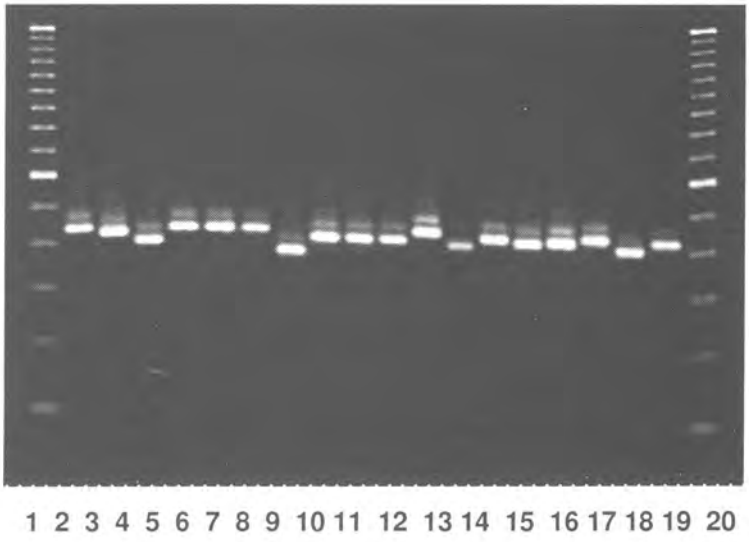

Figure 3. Agarose-Synergel electrophoresis of *G. abietina* PCR products amplified by GAcga6-61f and GAcga-511rc primers. Lanes 1 and 20: 100 bp molecular weight ladder; lanes 2-12: samples with the European RAPD amplitype P80, P85, P86, P89, NF87-0492, NF87-0494, NF87-0500, US15, CF87-0032, CF88-0007; and J5; lanes 13-16: samples with the Alpine RAPD amplitype J7, M1019, M1024 and M1042; lanes 17-19: samples with the Scandinavian RAPD amplitype Fi0003, A139p7, and A319p1.

DO THE TYPE A AND TYPE B OF *GREMMENIELLA* CROSS WITH EACH OTHER?

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Gremmeniella abietina (Lagerb.) var. *abietina* comprises two types, type A and type B with different ecotypic adaptations. These types have different morphological characteristics and are also genetically differentiated. The objective of this study was to determine whether or not they can hybridize. Apothecia was produced by pairing isolates of opposite mating types within (A X A or B X B) as well as between (A X B) types. However, the germination rate was extremely low (0-1%) in all but of the hybrid matings. DNA analyses confirmed the presence of DNA markers of both parents in some of the progeny. The low germination rate in the hybride progeny indicates that such hybrids may be rare or unfit in nature.

POLYMERASE CHAIN REACTION (PCR) DETECTION AND RACE IDENTIFICATION IN PINE SEEDLINGS INFECTED WITH SCLERODERRIS CANKER

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ABSTRACT

Scleroderris canker is a damaging disease of conifers caused by species in the genus *Gremmeniella*. The European race (EU) of *G. abietina* was introduced into North America early in this century, most likely on symptomless but infected seedlings. We have evidence that dissemination on infected nursery material is still taking place. In order to detect such latent infections, we developed oligonucleotide primers that specifically amplify portions of the internal transcribed spacers of the ribosomal DNA of *G. abietina*, including a *MspI* restriction site that is absent in the European race but present in the North American (NA) race. Extracted DNA directly from symptomless infected needles, or from single fruiting bodies, followed by PCR amplification and *MspI* digestion allowed the detection and race identification of both races of *G. abietina* in Red Pine, *Pinus resinosa* Ait. and in Jack Pine, *Pinus banksiana* Lamb. seedlings. It was possible to detect a single- infected seedling in a bulk sample containing 1000 healthy seedlings. DNA hybridization protocols using the oligonucleotide as a probe were also developed. In validation tests with 99 samples derived from 28 seedlings and 31 branches of red pine, 100% of the seedlings from which *G. abietina* was isolated yielded a positive PCR reaction. However, in several cases, seedlings from which *G. abietina* was not isolated by culture yielded a positive PCR assay. In most of those cases, aggressive contaminants had apparently overgrown *G. abietina*.

INTRODUCTION

Scleroderris canker is a damaging disease of conifers caused by species in the genus *Gremmeniella*. In North America, two races are present which are genetically and biologically distinct. Molecular approaches have been used to characterize the different taxonomic groups within *Gremmeniella*. These approaches all rely upon the use of extracted genomic DNA since universal DNA primers were used (Hamelin, Bernier et al. 1993; Bernier, Hamelin et al. 1994; Hamelin, Lecours et al. 1996; Hamelin and Rail 1997). Recently, specific primers have been used to detect root rot pathogens directly from infected nursery seedlings (Hamelin 1996), thereby alleviating the need for lengthy extractions.

The European race of *G. abietina* was introduced to North America early this century, most likely on symptomless infected seedlings and there is evidence that several introduction events took place (Hamelin, Lecours et al. 1998). Such evidence shows that dissemination is still taking place on infected nursery material. A diagnostic PCR approach was developed to detect latent infections, as well as to differentiate between the two races directly from infected tissues or fruiting bodies.

MATERIALS AND METHODS

The variable portion (ITS) of the ribosomal DNA from different races, varieties and species of *Gremmeniella* was amplified using universal primers ITS-1F and ITS-4, and then sequenced (Fig. 1). These PCR products were sequenced and the results were compared with those from other fungi in Genbank and with one another to identify portions that were conserved within groups but variable between taxa. Specific regions were identified for *Gremmeniella*, which allowed the development of primers (GA-UNI and GA-NA) that exclusively amplified members of this genus (Fig. 1). The DNA from needles of infected seedlings with and without symptoms was extracted and used for PCR assay. Amplification with GA-UNI and ITS-4 was followed by *MspI* digestion, a restriction enzyme that only cuts the ribosomal DNA of the EU race of *G. abietina* twice, but cuts the rDNA of the NA race 3 times, in order to identify and differentiate these two races of *G. abietina*. A 10 min boiling extraction method not necessitating the lengthy grinding process normally required, was developed to isolate DNA from fruiting bodies and mycelium without the need to culture the fungus. To validate the PCR assay, fascicles were removed from seedlings with symptoms. For each fascicle, one of the two needles was plated on culture medium for isolation, while the other was used for extraction, followed by PCR with GA-UNI and ITS-4.

RESULTS AND DISCUSSION

The PCR assay combined with *MspI* digestion was an accurate and practical way to detect and identify the causal agent of scleroderris canker. Detection was achieved using DNA isolated from needles of symptomless-infected seedlings, making it possible to get an early diagnostic of the disease. High sensitivity of the PCR method was demonstrated with the detection of a single, infected seedling in a bulk sample containing 1000 healthy seedlings (Fig. 2). A simple and easy-to-perform boiling extraction method allows DNA extraction from a single fruiting body within minutes, enabling fast detection and identification without the need to culture. Validation tests revealed the PCR assay as being more sensitive and reliable than standard isolation on culture medium (Table 1).

Table 1.

		PCR detection	
		+	—
Isolation	+	17	0
	—	26*	56

* 16 samples were heavily contaminated, the remainder showed no growth.

CONCLUSION

The PCR detection proved to be an accurate, sensitive, reliable and rapid method when compared with conventional isolation, which may lack sensitivity due to aggressive contaminants and can require up to 30 days for diagnostic and race identification. Potential applications include quarantine inspections and seedling certification prior to outplanting.

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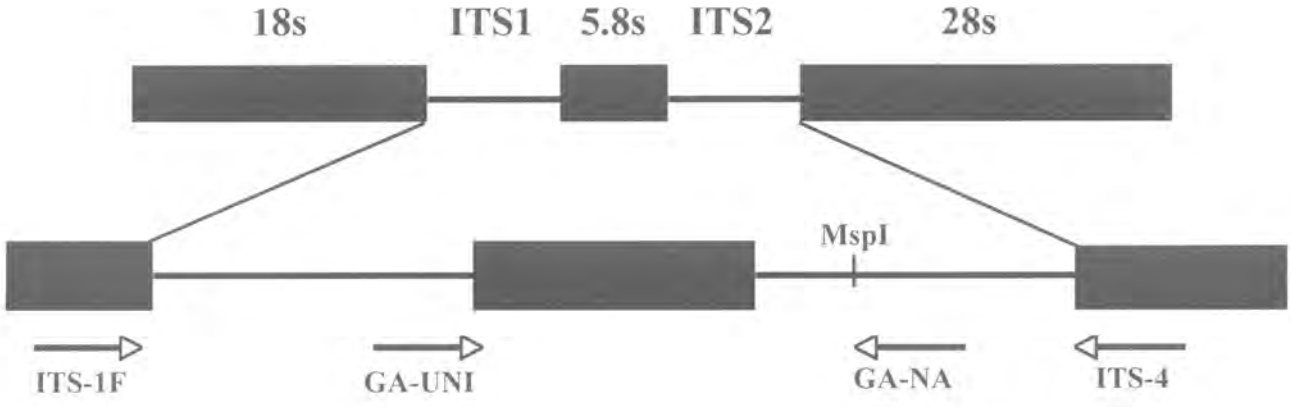

Figure 1. Diagram showing the ribosomal DNA gene, comprising conserved regions (coding for ribosomal subunits 18s, 5.8s and 28s) and the variable internal transcribed spacers (ITS) from which specific primers GA-UNI and GA-NA were developed using universal primers ITS-1F and ITS-4. The position of the restriction site that is used to differentiate among the 2 races of *G. abietina* is indicated.

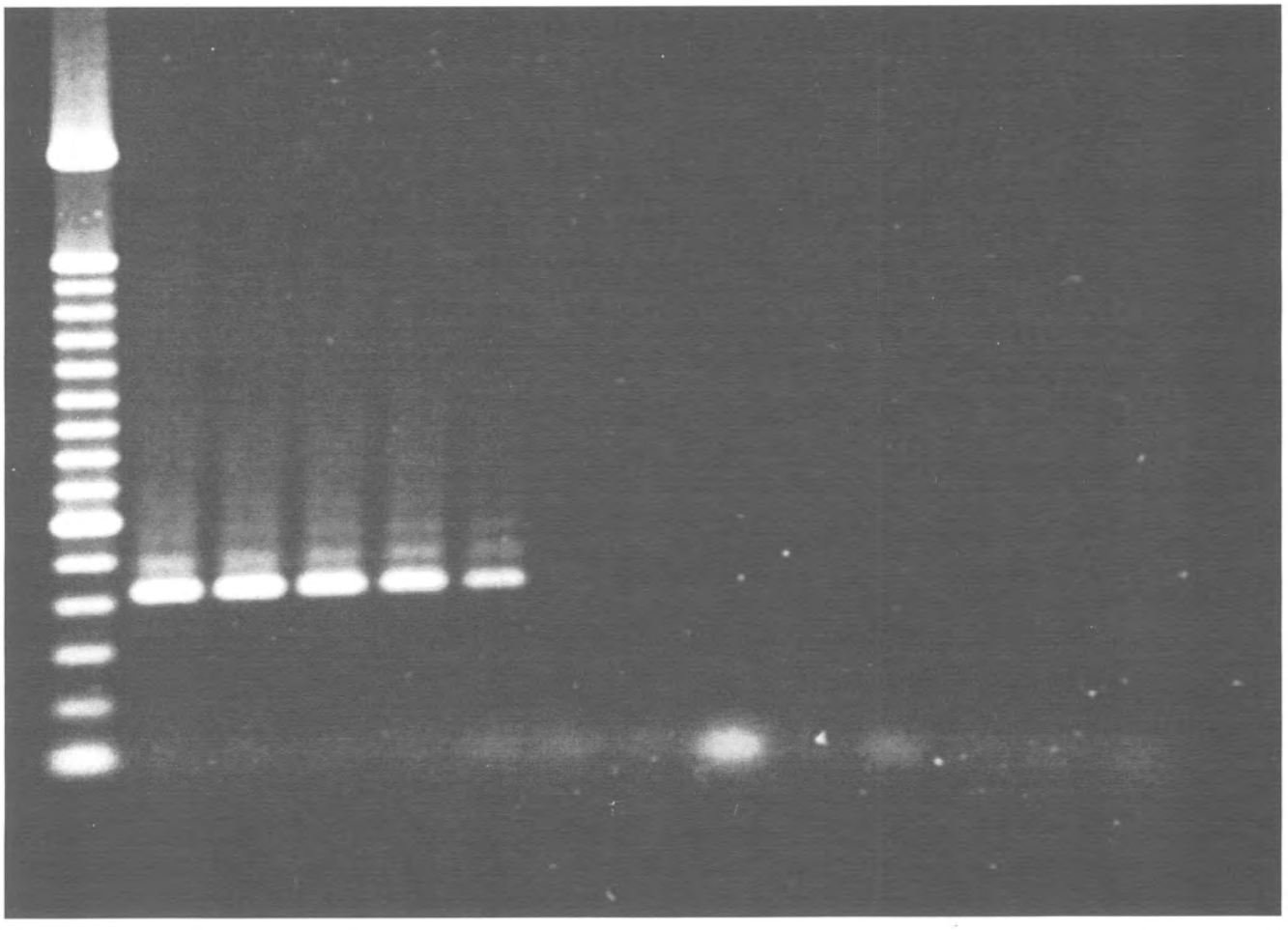

Figure 2. Sensitivity test. DNA extract from one infected seedling was mixed with increasing amounts of DNA extracts from healthy seedlings. Amplification with GA-UNI and ITS-4 was carried out from these dilutions. Lane 1, 100-bp ladder; lane 2, positive control; lanes 3 to 12 respectively, dilutions 1:2, $1:10^{-1}$, $1:10^{-2}$, $1:10^{-3}$, $1:10^{-4}$, $1:10^{-5}$, $1:10^{-6}$, $1:10^{-7}$, $1:10^{-8}$ and $1:10^{-9}$; lane 13, DNA extracts from healthy seedlings; lane 14, negative control.

NEW OUTBREAK OF SCLERODERRIS CANKER IN NEWFOUNDLAND

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ABSTRACT

Scleroderris canker in Newfoundland is caused only by the European race of *Gremmeniella abietina* var. *abietina*. It was first recorded in 1979 on Austrian pine (*Pinus nigra*) on the Avalon Peninsula. A severe outbreak on red pine (*P. resinosa*) at Bauline Line in 1981 followed with regular new infections on Austrian pine (*P. Nigra*) and Scots pine (*P. silvestris*) in and around St. John's. Another oubreak was found at the old Salmonier Line National Tree Nursery in 1987. This may have been the source of the disease. In 1996, two severe outbreaks on red pine were surveyed and results indicated incidence of 91% at Tilton Barren and 100% at Upper Island Cove with very high rate of mortality. Numerous cankers as old as 17 years were found in those infected plantations, indicating an old infection event that probably originated from the production of those trees in 1950 at the Salmonier Line National Tree Nursery.

INTRODUCTION

Scleroderris canker, caused by the pathogen *Gremmeniella abietina* var. *abietina* (Laberg) Morelet, has been a problem on the Avalon Peninsula since it was first recorded on Austrian pine (*Pinus nigra* Arnold) near St. John's, Newfoundland in 1979 (Singh et al., 1980). Over the years, most detected infections centered around urban red (*P. resinosa* Ait.), Austrian, Scots (*P. sylvestris* L.) and jack pine (*P. banksiana*) trees in St. John's. The disease would occasionally break out with extreme severity in plantations or in private gardens outside St. John's. Sanitation methods usually meant cutting and burning.

In 1996, the occurence of scleroderris canker symptoms was wide spread and damages were the worst since the infection of red pine in a 2-hectare plantation on the Bauline Line in 1981.

A new and severe outbreak of this disease occurred in two red, Scots and jack pine plantations, one at Tilton Barren and the other near Upper Island Cove in Conception Bay.

MATERIALS AND METHODS

At the Tilton Barren site, two transects of 50 red pine trees were tallied for incidence of scleroderris cankers symptoms. At the Upper Island Cove, two transects of 100 trees were tallied. For each sampled tree, the infection was classified as slight if only a few shoots on lower branches were affected. It was classified as moderate category when lower branches were all affected. It

was classified as severe category when mid- to upper branches were affected. At each location a few cankers were dissected for age counting.

RESULTS

Results (Table 1) showed that only 9% of the red pine appeared to be healthy at the Tilton Barren site while 27% were slightly infected, 22% were moderately infected, 36% were severely infected and 6% were dead.

No apparently healthy trees were recorded at the Upper island Cove site. In the three categories of infection, 4.5% of the trees showed slight infection, 6% showed moderate infection, 41% showed severe infection and the other 48.5% of red pine were dead.

None of the Scots pine and jack pine at Tilton Barren or Upper Island Cove showed any signs or symptoms of scleroderris canker.

DISCUSSION

This disease, first recorded in 1979 near St. John's, was observed almost yearly on various Austrian and Scots pines. In 1981, an outbreak occured in a red pine plantation on Bauline Line (Sterner and Davidson, 1983), and almost all of the trees died suddently. Another outbreak on Scots pine occured in 1987 near Colliers (Moody, 1989).

In 1996, the red pine plantations at Tilton Barren and Upper Island Cove were affected and cankers as old as 17 years were observed, leading to an infection event before 1978.

In all cases, the material for the Bauline Line, Colliers, Tilton Barrens and Upper Island Cove had been produced at the Salmonier Line National Tree Nursery in the early 1950's (Baker and Pitt, 1996). In 1987, the disease was recorded on Scots pine at the Salmonier Line National Tree Nursery established with European material in 1935 and in operation until 1952. Canker age from Salmonier Line indicated that the infections had actually occurred 16 to 17 years before (circa 1971). The trees did not die however until 1987, apparently a year that favoured abundant spore development and infection. Since trees in the Salmonier Line nursery displayed the earliest infection with scleroderris canker and were European in origin, it is our hypothesis that material brought from Europe during the establisment of the nursery may be responsible for infecting seedlings in that nursery in the late 1940's and early 1950's. The nursery stopped its operation in 1952 and no more plantations established later were found to be infected with scleroderris canker.

Many individual black Austrian pine infected in and around St. John's are old trees dating from the Salmonier Line nursery, but new infections on ornamental saplings may originate from nurseries in Ontario where they were produced in the 1990's. Hence the possibility of two distinct introductions of scleroderris canker in Newfoundland, but that has yet to be demonstrated.

The two outbreaks of 1981 and 1996 are most probably related to conducive climatic conditions for *G. abietina*. On these two occasions, February and March months were much warmer than usual; this may have favoured the infection process of the disease.

CONCLUSION

In order to protect the natural red pine areas of central and western Newfoundland, scleroderris canker has to be carefully monitored in the natural forest, urban areas and in the commercial and provincial tree nurseries. The quarantine has to be strictly enforced. Continued education of the public is needed.

Table 1. Incidence of scleroderris canker survey results on red pine at Tilton Barren and Upper Island Cove in Newfoundland.

Plantation	Total trees surveyed	Incidence of disease (%)				
		Low	Moderate	Severe	Dead	Total
Tilton Barren	100	27	22	36	6	91
Upper Island	200	4.5	5	41	48.5	100

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OTHER DISEASES

GENETIC VARIATION AND THE INFLUENCE OF FERTILIZATION ON THE DEVELOPMENT OF CURRENT SEASON NEEDLE NECROSIS ON NOBLE FIR CHRISTMAS TREES

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SUMMARY

Current season needle necrosis (CSNN) is a common needle disorder of noble (*Abies procera*) and grand fir (*A. grandis*) Christmas trees grown in Oregon, Washington, northern Idaho, and British Columbia, Canada. Initial symptoms of CSNN appear on current season needles during early June. Symptoms consist of tan discolored bands which expand and turn reddish-brown in color by mid summer. Although the exact etiology of CSNN is unknown, foliar applications of calcium chloride and shading trees during shoot elongation are known to be effective in reducing the incidence of CSNN. The incidence of CSNN in growers' plantations varies from year to year and site to site and very little is known about genetic variability and the influence cultural practices like fertilization have on the development of this disorder. Data collected in 1996 from a noble fir seed orchard located near Sheridan, OR indicate that there were significant differences in the susceptibility of different Riley/Fanno (RF) noble fir mother trees to CSNN. RF1 and RF10 were much more susceptible than RF5, RF6, and RF8. RF15 was intermediate in susceptibility. Location of trees within the seed orchard also had a significant effect on the development of CSNN. Data collected on six-year-old progeny from these mother trees indicated that there was a significant correlation between the percentage of unmarketable progeny trees due to CSNN and the CSNN ratings of the mother trees. Annual applications of N and S or S, K_2O, and MgO had no influence on CSNN development on noble fir Christmas trees at seven sites during a seven yearlong fertilizer study. The total amount of nitrogen applied ranged from 0 to 1715 kg/ha. Levels of CSNN were strongly related to site.

Keywords: *Abies* spp., needle disorder, genetics, fertilization, Christmas trees

INTRODUCTION

One-third of the 36 million Christmas trees harvested in the United States in 1996 were grown in western Washington and Oregon. Noble fir (*Abies procera* Rehd.) is becoming an increasingly important species for use as a Christmas tree in this area (Douglas 1983, Proebsting 1983, U.S. Forest Service 1960). In 1969, noble fir only accounted for about 5% of the total production. By 1982, its market share increased to about 18%, and in 1996 it accounted for 40% of the harvested trees. Demand for noble fir has been steadily increasing because of its high quality foliage and superior postharvest keeping qualities (Chastagner 1990 and Nanny 1986).

There are currently two diseases which limit the production of high quality noble fir Christmas trees in the Pacific Northwest. In poorly drained sites, growers can experience high losses from Phytophthora root rot and stem canker (Chastagner et al. 1995). The other disease which can limit the production of high quality trees is current season needle necrosis (CSNN) (Chastagner et al. 1990). CSNN is a common needle disorder of noble and grand fir [*Abies grandis* (Dougl.) Lindl.] Christmas trees grown throughout production areas in western Oregon, Washington, and British Columbia (Chastagner and Staley 1985). CSNN also occurs on grand fir Christmas trees grown in northeastern Washington and northern Idaho. The same or very similar disorder also occurs on noble fir grown in Ireland and Denmark. In Denmark, the condition is referred to as "Røde nåle på nobilis" (red needles on noble fir).

In the Pacific Northwest, initial symptoms of CSNN appear on current season needles during early June. Symptoms consist of tan discolored bands. In some cases, the area of discoloration expands and involves the distal portion of needles or the entire needle. Damaged portion of needles turn reddish-brown in color by midsummer. The incidence of symptomatic needles increases rapidly during June and July. CSNN symptoms can be confused with needle rust and damage caused by high temperatures, herbicides, and moisture stress.

Grand fir generally have a higher incidence of symptomatic needles than noble fir. Unlike grand fir, where symptomatic needles are distributed throughout the tree, branches in the upper portion of noble fir trees have been found to have twice as many symptomatic needles compared with branches in the middle and bottom portion of trees. Growers' observations indicate that CSNN is less of a problem at higher elevation sites and in natural stands. Growers have also associated initial symptom development with high temperatures and observed that certain trees tend to show symptoms year after year.

Although the etiology is unknown, Chastagner et al. (1990) have shown that shading trees or applications of calcium chloride during shoot elongation significantly reduced the incidence of symptomatic needles on noble fir. Although foliar applications of calcium chloride are effective in reducing the incidence of this disorder on both noble and grand fir, this treatment cannot be recommended as a management tool because of phytotoxicity associated with the rates and frequent number of applications required to reduce disease levels.

Very little information is currently available regarding the effect of cultural practices, such as fertilization and genetic variation, with respect to this disorder. This paper reports on observations in the Pacific Northwest during the past several years relating to the effect of fertilization on the development of CSNN and genetic variability associated with this disorder.

MATERIALS AND METHODS

Fertilization Study

Data on the effect of fertilization on the incidence and severity of CSNN on noble fir Christmas trees was collected during a dose-rate fertilizer experiment that was conducted between 1988 and 1994 in western Oregon and Washington. A series of 7 plots were established in the spring of 1988 in commercial Christmas tree plantations located near Rochester, Onalaska, and La Center, WA, and Hillsboro, Canby, Silverton, and Alsea, OR.

Plots were located on either well drained valley soils (Rochester, Canby, Alsea) or on well-drained hill soils (Onalaska, La Center, Hillsboro, Silverton). Applications of nitrogen and sulphur, or sulphur, potassium, and magnesium were applied during the crop rotation to trees which had been planted on either 1.5 x 1.5 m or 1.7 x 1.7 m spacings in 1987. At the time of initial treatment, seedlings measured at least 30 to 38 cm in height.

The plot design was a randomized split block, with each of the seven study sites serving as a block. The fertilizers used in this study included a blend of urea and ammonium sulfate called Urea-Sul, which had a 33-0-0-12 analysis and Sul-Po-Mag, which is also known as K-Mag, and is a combination of sulphur, potassium, and magnesium. The analysis of this material is 22-22-11 (S-K_2O-MgO). Urea-Sul treatments were applied annually beginning in 1988, while the annual Sul-Po-Mag treatments were started in 1990.

Three of the Urea-Sul treatments consisted of constant rates being applied throughout the study, while the rates of Urea-Sul applied in three additional treatments increased as tree height increased during the rotation. The initial rate increase occurred when the trees reached 1 m in height, with the final increases occurring at a height of 1.5 m (Table 1).

Table 1. Annual N, S, K_2O, and MgO applied for each treatment (kg/ha).

Fertilizers	1988	1989	1990	1991	1992	1993	1994
None	0	0	0	0	0	0	0
Sul-Po-Mag	0	0	103/103/52	103/103/52	103/103/52	103/103/52	103/103/52
Urea-Sul	17/6	17/6	17/6	17/6	17/6	17/6	17/6
Urea-Sul	17/6	17/6	17/6	28/10	28/10	28/10	56/20
Urea-Sul	50/18	50/18	50/18	50/18	50/18	50/18	50/18
Urea-Sul	50/18	50/18	50/18	84/30	84/30	84/30	168/61
Urea-Sul	151/55	151/55	151/55	151/55	151/55	151/55	151/55
Urea-Sul	151/55	151/55	151/55	252/92	252/92	252/92	504/184

Each treatment was applied to an area containing approximately 60 trees at each site. A subset of 15 trees within the centre of each treatment area was used for measurement and analysis purposes. Tissue nutrient analyses were conducted each year in September from 1988 through 1994. Sampling was done by removing a pinch of 3 to 6 needles from current season foliage at approximately 10 to 20 locations around the tree crown. Sample locations were limited to the upper one-half to two-thirds of the crown. A number of growth and quality variables were also measured during this study, including CSNN. Annually, starting in 1989, CSNN was rated each September. CSNN severity was rated on a scale of 0 to 3, where 0 = none, 1 = <10%, 2 = 10-50%, and 3 = >50% of the current season needles affected by necrosis.

Genetic Variation

Several noble fir provenance tests have been conducted during the past 30 years in the Pacific Northwest in an effort to identify superior sources of noble fir seed for the production of high quality Christmas trees (Anonymous 1983, Brown and Landgren 1995, Douglas 1984 and 1991).

In the most recent provenance test, trees from the Riley/Fanno area in the Oregon coast range produced the highest quality Christmas trees. To obtain additional information about trees from this area, a series of noble fir progeny tests were established from specific, open, pollinated mother trees in the Riley/Fanno area in 1990. In addition, the top six Riley/Fanno mother trees were grafted into a noble fir seed orchard that was established in 1991/92 (Brown 1990). Although a number of different sources of noble fir were included in the seed orchard, ramets of the six Riley/Fanno trees account for about 50% of the 247 trees in the seed orchard.

The noble fir seed orchard was established by grafting scions of selected noble fir material onto seven-year-old noble fir under stock. The site where the seed orchard is located has a Nekia silty clay loam soil and is located near Sheridan, OR. The seed orchard covers an area of about 0.8 ha. There are approximately 19 evenly spaced trees in each of 18 rows that are 91 metres long and run south to north. The site is at an elevation of about 300 m, fully exposed and relatively flat. From the southeast corner of the seed orchard, there is a drop of approximately 4.3 m to the northern edge. Going west, there is a drop of about 2.4 m along the southern edge of the orchard to the western edge, which is about 85 m away.

In 1996, CSNN developed on trees in the seed orchard and at one large commercial plantation located 10 km west of Salem, OR containing several hundred trees from each of the Riley/Fanno mother trees represented in the seed orchard. Initial observations in the progeny planting and the seed orchard indicated that there was considerable variation in the susceptibility of trees to CSNN. In an effort to demonstrate this, evaluations were made on 48 randomly selected trees from each of the families in the progeny planting and all of the trees in the seed orchard. Trees were also examined in two additional 1990 plantings containing the Riley/Fanno progeny to determine if similar patterns of CSNN susceptibility were evident in these plantings. One of these plantings was located approximately 37 km due east of Salem, OR at about 365 m elevation, while the other was located about 5 km east of Forest Grove, OR at about 243 m elevation. The severity of CSNN was rated on a scale of 0 to 10, where 0 = none, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, . . ., and 10 = 91-100% of the current season needles affected by necrosis between late August and October, 1996. Data were also collected on tree heights at the seed orchard.

RESULTS

Fertilization Study

Over the seven years that data were collected, only 12% of the trees showed any evidence of CSNN. Most of these trees (68%) never had a CSNN rating >1. There were 110 trees which had a CSNN rating ≥2 at some time during the study. Of these, 50 had such a rating only one year, while 60 received such a rating during two or more years. Levels of CSNN were strongly related to site (Table 2). Although fertilization increased tree color, foliage nitrogen levels (percent and total content) and needle weight (data not presented), it did not affect the level of CSNN on the noble fir trees in this study nor was there a correlation between %N in the foliage and CSNN (Figure 1).

Table 2. Overall summary of CSNN ratings for noble fir Christmas trees between 1988 and 1994.

	No. of trees by plot location						
CSNN rating[1]	Rochester	Onalaska	La Center	Hillsboro	Canby	Silverton	Alsea
0	800	797	825	738	564	823	784
1	30	38	15	80	165	14	50
≥2	10	5	0	22	121	3	6

[1]There were a total of 120 trees rated at each plot annually, thus the total number of potential observations over the 7-year period was 840/plot.

Genetic Variation

Only the CSNN data for the six Riley/Fanno mother trees within the orchard will be presented in this paper. Each of these sources had 20 to 21 ramets that were randomly dispersed throughout the orchard. Analysis of the CSNN ratings for these six trees indicated that RF1 and RF10 had significantly higher ratings than RF8, RF5, and RF6 (Table 3). RF15 was intermediate in its susceptibility to CSNN. To determine if the location of the trees within the seed orchard and/or tree height had an effect on the CSNN ratings, multiple regression analyses were done for ramets of the three Riley/Fanno trees with the highest CSNN ratings (RF1, RF10, and RF15). Neither tree height ($P = 0.239$), which increased as one moved from east to west across the orchard, nor tree position ($P = 0.232$) within the row correlated with CSNN levels. There was less disease as one moved from east to west across the seed orchard (Figure 2) and CSNN ratings were highly correlated to the row trees that were located in within the seed orchard ($r = 0.629$, $P < 0.001$).

Table 3. Severity of CSNN on six Riley/Fanno noble fir mother trees in the seed orchard.

	Trees					
	RF1	RF10	RF15	RF8	RF5	RF6
CSNN rating[1] (30 August 1996)	2.40 a	2.05 ab	1.29 bc	0.48 cd	0.10 d	0.05 d

[1] Numbers followed by the same letter are not significantly different, $P = 0.05$, Fisher's test.

In the progeny planting west of Salem, OR, data were collected from 48 randomly selected progeny from each of the six Riley/Fanno mother trees that were evaluated at the seed orchard. The percentage of trees with CSNN ranged from 29% for RF6 progeny to 48% for RF1 and RF15 progeny (Table 4). Trees with CSNN ratings ≥2 were considered unmarketable by the grower and there was a significant correlation between the percentage of unmarketable progeny trees and the CSNN ratings of the mother trees in the seed orchard (Figure 3). Virtually no CSNN was present on the trees in the two other progeny plantings (data not presented).

Table 4. Prevalence of CSNN on the progeny from six Riley/Fanno mother trees[1].

Progeny source	% of trees w/CSNN	Average CSNN rating	% Unmarketable trees (rating ≥2)
RF1	47.9	0.8	22.9
RF15	47.9	0.9	20.8
RF10	37.5	0.6	14.6
RF5	39.6	0.5	8.3
RF8	35.4	0.5	6.2
RF6	29.2	0.5	6.2

[1] Data collected August 30, 1996 from 48 randomly selected trees for each source.

DISCUSSION

Data collected during the seven-year-long noble fir fertilization study indicates that fertilization with Urea-Sul or Sul-Po-Mag did not affect the level of CSNN which develops on noble fir Christmas trees in the Pacific Northwest. Levels of CSNN were strongly related to site, which is consistent with grower observations. Although sites in the fertilization study varied from valley to hillsides, it was not possible to determine if valley sites were more prone to CSNN because of the low number of sites in the test and generally low levels of CSNN. The variation in CSNN levels on trees in the three progeny plantings and on ramets of the Riley/Fanno trees in the seed orchard also indicates the importance of site variation in the development of this disorder. Ramets of RF10, RF1, and RF15, which had CSNN ratings as high as 6 within rows along the eastern edge of the seed orchard, had ratings of 0 to 1 in the rows near the western edge. Although it is unclear why this disease gradient occurred, there was a slight increase in tree height as one moved from east to west, suggesting that trees were growing faster on the western edge of the planting. This may be due to deeper soils and/or better soil moisture levels during the growing season, which may in some way influence the development of CSNN.

Data collected from the seed orchard and the progeny plantings suggest that the development of CSNN is also under strong genetic control. In Denmark, Nielsen (personal communication) found that there was considerable genetic variability in the susceptibility of different noble fir provenances to CSNN. In a comparison of Oregon and Washington provenances to Danish provenances, he found that the Oregon and Washington provenances were much more prone to CSNN than the Danish ones. There has been some speculation that the limited susceptibility of the Danish provenances is somehow related to their dark blue coloration. This appears to be unlikely since the highly susceptible Riley/Fanno parents in the seed orchard also exhibited mature dark blue foliage characteristics similar to that which is associated with the highly selected Danish provenances.

Efforts are currently underway to utilize seedlings grafted with scions from susceptible trees to develop procedures to induce CSNN under controlled conditions. If successful, it should be possible to utilize seed obtained from controlled crosses between CSNN-susceptible and resistant parents in the seed orchard to obtain additional information concerning the genetic control of this important disorder of noble fir Christmas trees.

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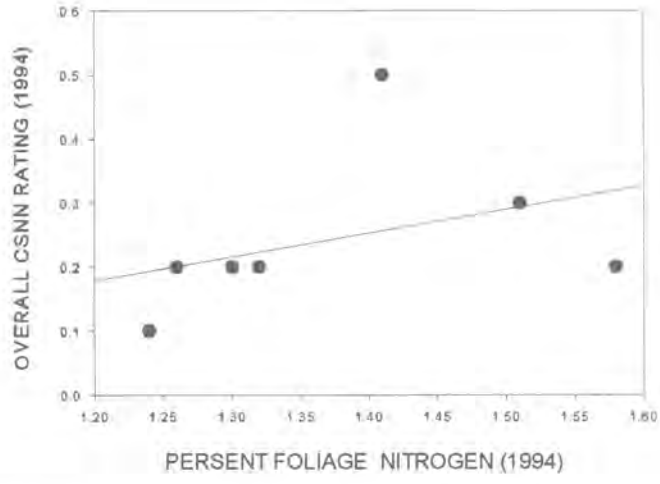

Figure 1. Correlation between percent foliage nitrogen and CSNN ratings (r = 0.380, P = 0.401).

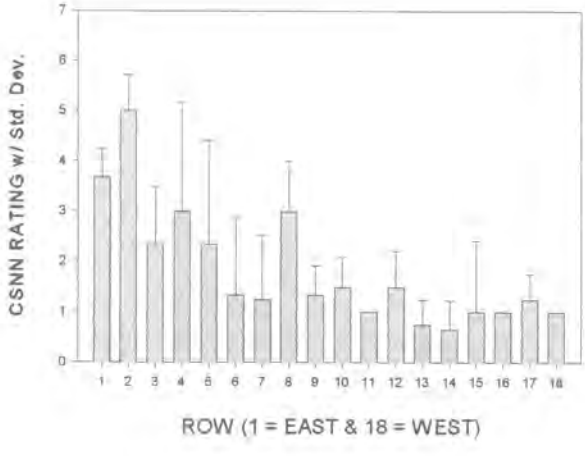

Figure 2. CSNN ratings for RF-1, RF-10 and RF-15 mother trees by row within seed orchard.

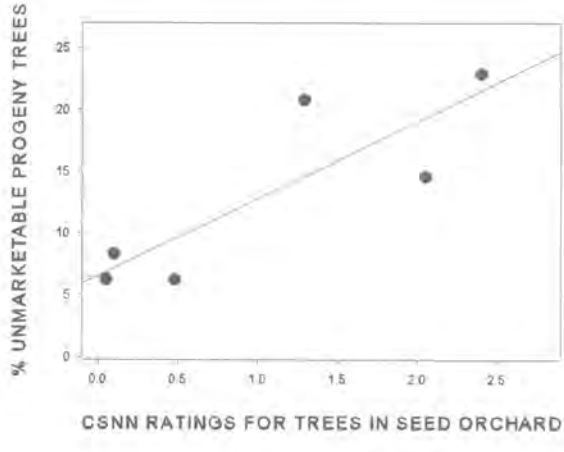

Figure 3. Correlation between CSNN ratings for Riley/Fanno mother trees and levels of damage to their progeny (r = 0.852, P = 0.0315).

CHANGING CONCEPTS OF THE PITCH CANKER DISEASE OF PINES

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SUMMARY

Pitch canker, caused by *Fusarium subglutinans* f. sp. *pini*, is a serious disease of species of *Pinus*. Since pitch canker was first described in 1946, the parameters of the disease have been constantly changing. The potential impact of this disease on pine forests was fully recognized by phytopathologists in 1974 when the disease became epidemic on planted slash and loblolly pines in the southeastern United States. Subsequent studies conclude that most, if not all, southern pines are hosts of the pitch canker fungus. In the last two decades, pitch canker has evolved from a regional disease, to one of national and international importance. Since 1986, pitch canker has seriously damaged Monterey pine in coastal central California and has all the earmarks of an introduced disease. Pitch canker has been reported on pines in Haiti, Japan, and Mexico. In South Africa, the pathogen induces a root rot of *Pinus patula* seedlings. In the southern United States, the pitch canker fungus is frequently an ecological component of fusiform rust galls. Although the major damage from this disease typically results from the infection of vegetative structures, the pitch canker fungus also infects reproductive structures causing mortality of female flowers and mature cones and deteriorates seeds of several pine species.

Keywords: *Pinus* spp., *Fusarium subglutinans*, *Cronartium quercuum*

INTRODUCTION

Pitch canker, caused by *Fusarium subglutinans* f. sp. *pini*, was first reported in North Carolina in 1946. We now know that *F. s.* f. sp. *pini* infects a variety of vegetative and reproductive pine structures at different stages of maturity and produces a diversity of symptoms. When the pathogen causes bleeding, resinous cankers on the main stem, branches, shoots, and exposed roots, the resultant disease is referred to as pitch canker. The pitch canker fungus also causes the mortality of female flowers and mature cones and deteriorates seeds of several pine species. Further confounding the situation is the involvement of insects, the interaction with other pine diseases, and the marked influence of biotic and abiotic factors on the incidence and severity of infections by *F. s.* f. sp. *pini*.

THE CAUSAL FUNGUS

The causal fungus has gone through a number of name changes. In 1946, when pitch canker was first described, it was referred to as an undescribed species of *Fusarium* belonging in the Section Liseola (Hepting and Roth 1946). Three years later, it was designated *F. lateritium* f. sp. *pini* (Snyder et al. 1949). In the 1970's, the most common isolates of *Fusarium* from pitch canker tissue had abundant microconidia in heads and no chlamydospores and were assigned to

Fusarium moniliforme var. *subglutinans* in the section Liseola (Kuhlman et al. 1978). In 1983, the variety was raised to species level as *F. subglutinans* (Nelson et al. 1983). In 1991, Correll et al. justified assigning strains of *F. subglutinans* pathogenic to pines to a specific forma specialis. They proposed that the pitch canker pathogen be designated *F. s.* f. sp. *pini* (Correll et al. 1991).

PITCH CANKER

Description

The disease pitch canker derives its name from the copious pitch flow associated with most cankers (Hepting and Roth 1946). The classic symptom is a bleeding, resinous canker of the trunk, terminals, large branches, and exposed roots. The bark is retained, while the wood beneath the canker is deeply resin-soaked (Dwinell et al. 1985). Dieback in the upper crown results from cankers forming on the branches or shoots. As the branches or shoots are girdled by the fungus, the needles turn yellow to reddish brown; they later turn greyish brown to dark gray (Dwinell and Phelps 1977). The pitch-soaked wood is a diagnostic character useful in separating pitch cankers from most other maladies of pines (Dwinell et al. 1985; Hepting and Roth 1946).

The symptoms of pitch canker frequently vary by pine host and management practices (Dwinell et al. 1985). Trunk cankers are the most common on Monterey (*P. radiata*), Virginia (*P. virginiana*), longleaf (*P. palustris*), and eastern white (*P. strobus*) pines. Dieback is common on slash (*P. elliottii var. elliottii*), loblolly (*P. taeda*), shortleaf (*P. echinata*), sand (*P. clausa*), and Monterey pines (Correll et al. 1991; Dwinell et al. 1985). Trunk cankers on slash pine are common in seed orchards and are usually associated with the use of tree shakers for cone removal (Dwinell and Phelps 1977). Cankers on exposed roots can be found on slash pine in seed orchards and landscape plants, and on Monterey pine in landscape settings. In Mexico, trunk cankers occur on *P. estevezi* and branch and shoot dieback are common on *P. douglasiana*, *P. leiophylla* and *P. arizonica* var. *stormiae* (Dwinell, unpublished; Santos and Tovar 1991). Dieback and truck cankers were found on luchu pine (*P. luchuensis*) on 2 islands of Japan--Amamiooshima and Okinawa (Muramoto and Dwinell 1990; Muramoto et al. 1988).

Incidence

From 1945 to 1973, limited outbreaks of pitch canker were noted in the southeastern United States, but the disease was not economically important. In 1974, a shoot dieback identified as pitch canker reached epidemic proportions on slash pine in Florida plantations and seed orchards and on loblolly pine in North Carolina and Mississippi seed orchards (Dwinell and Phelps 1977; Dwinell et al. 1985). These outbreaks spawned considerable research on pitch canker. Over the last two decades, pitch canker outbreaks in the South have occurred sporadically in time and place (Dwinell et al. 1985). Most, if not all, southern pines are considered susceptible to pitch canker pathogen.

Until the late 1980's, pitch canker was considered a problem for pines in the southeastern United States. In 1986, pitch canker became a major component of dieback and mortality of Monterey pine in California (McCain et al. 1987). Pitch canker was unknown in California until disease outbreaks occurred almost simultaneously at 3 widely-separated geographical locations (Santa Cruz, Hayward, and Santa Barbara). Subsequent surveys found the disease extending from Mendocino County to San Diego; however, the disease is most severe on the central coast of

California (Adams 1989). The pathogen has also been isolated from several pine species native to California and Douglas-fir (*Pseudotsuga menziesii*) (Adams 1989; Correll et al. 1991; Storer et al. 1994). The extensive dieback of Monterey pine suggests that the pathogen was introduced (Gordon et al. 1997). Research on the genetic diversity of vegetative compatibility groups in California populations of *F. s.* f. sp. *pini* (Correll et al. 1992) supports this concept.

In the southeastern United States, the pitch canker disease is a problem on planted pines (i.e., plantations) (Dwinell et al. 1985); in California, the disease has become established in natural Monterey pine stands at the Point Año Nuevo on the Monterey Peninsula, and at Cambria (Gordon et al. 1997; Storer et al. 1994). Pitch canker is a severe threat to the Monterey pine resource in California Gordon et al. 1997; Templeton et al. 1997). The accidental introduction of pitch canker into countries such as New Zealand, Australia and Chile, that have extensive Monterey pine plantations could be disastrous if insects are available to drive the disease.

Until the late 1980's, the only report of pitch canker outside the United States came from Hepting and Roth (1953) who noted that the disease "was found to be abundant in Haiti on *P. occidentalis* in February 1953." In the late 1980's, pitch canker had caused trunk cankers and dieback of luchu pine on Amamiooshima and Okinawa islands of Japan (Muramoto and Dwinell 1990; Muramoto et al. 1988). At about the same time, pitch canker was identified in Mexico. The disease is prevalent on planted *P. halepensis* and in natural stands of *P. douglasiana* and *P. leiophylla* (R. A. Blanchette, personal communication; Santos and Tovar 1991). In 1995, I observed pitch canker on *P. estevezi* in a plantation and *P. arizonica* var. *stormiae* in a natural stand in the State of Nuevo León. In South Africa, *F. s.* f. sp. *pini* is reportedly responsible for root rot of container-grown *P. patula* seedlings (Viljoen et al. 1994).

Infection

The pitch canker fungus is frequently an ecological component of fusiform rust galls caused by *Cronartium quercuum* f. sp. *fusiforme*. Hepting (1971) suggested that some of the greatest damage in plantations was associated with secondary invasion of fusiform rust galls by *Dioryctria* spp. and the pitch canker fungus. Dwinell and Barrows-Broaddus (1985) found that *F. s.* f. sp. *pini* rapidly colonized rust-infected tissue and hastened mortality of slash and loblolly pine seedlings. Infection of rust galls by the pitch canker fungus further weakens stems of mature trees and increases chances of breakage and tree mortality. In the late 1980's, I was unsuccessful in attempts to isolate the pitch canker fungus from galls caused by *Endocronatium harknessii* on Monterey pine in California (unpublished data).

Any fresh wound, regardless of cause or location, provides an infection court for the pathogen. Insects can create wounds which can be infected by airborne spores of the pathogen or serve as vectors. In the southeastern United States, the deodar weevil (*Pissodes nemorensis*) (Blakeslee et al. 1978) and the pine-tip moth (*Rhyacionia* spp.) (Matthews 1962) create wounds that may become infected by airborne spores of the pathogen. In California, Fox et al. (1991) reported that *Ips mexicanus* and *I. paraconfusus* can transmit the pitch canker fungus to Monterey pines. A plethora of other insects, such as species of *Pityophthorus* (twig beetles), *Conophthorus* (cone beetles), and *Ernobius* may be involved in the disease complex in California (Adams 1997; Gordon et al. 1997). In slash pine seed orchards, main stem cankers often develop after injury caused by mechanical cone harvesters. Furthermore, weather-related injuries caused by wind and

hail may serve as entry points. Hurricanes and tornadoes, in particular, have contributed to the intensification of the disease in some seed orchards (Dwinell et al. 1985).

Inoculum seems to be available in all seasons. In Florida, Blakeslee et al. (1978) reported that sporodochia containing macroconidia occurred routinely on infected branches within the upper crown of infected trees during the entire year. Sporodochia can also be found on infected shoots of Monterey pine (Dwinell, unpublished). In a loblolly pine seed orchard, Kuhlman et al. (1982) found spores of the pitch canker fungus throughout the growing season on dead branches in the crown, in rainwater falling through infected trees, and in the air. The pathogen has been isolated from the surface of shortleaf pine cones (Dwinell and Fraedrich, in press). Fraedrich and Dwinell (in press) reported that *F. s.* f. sp. *pini* was primarily detected in spore traps beneath asymptomatic longleaf pine trees during nights when it rained. In California, airborne inoculum of *F. s.* f. sp. *pini* was detected through the year in an area with a high incidence of pitch canker disease in Monterey pine but not in areas where the disease was absent (Correll et al. 1991). A bark wash survey conducted on the central coast of California revealed that where the disease was present, both symptomatic and asymptomatic trees could test positive for the presence of the pitch canker fungus (Adams 1989).

DAMAGE

To Trees

Pitch canker is a dynamic disease. Each outbreak in each specific location has its own unique case history (or sequence of events) (Dwinell et al. 1985). Southern pines recover from shoot dieback, but stem and branch cankers are usually perennial. Factors that drive the disease in loblolly pine seed orchards are different from those that impact slash pine plantations. Pitch canker on Monterey pine in California acts like an introduced disease; whereas, pitch canker in the Southeast is considered endemic.

Damage to pines by *F. s.* f. sp. *pini* includes growth suppression, stem deformation, and tree mortality. The annual mortality in the southeastern United States is low. Southern pines, particularly loblolly, pond (*P. serotina*), and shortleaf pines, generally recover from outbreaks of shoot dieback (Barrows-Broaddus and Dwinell 1985, Dwinell et al. 1985). In California, the mortality of Monterey pines resulting from attack by the pathogen, bark or twig beetles or both has not been quantified. Engraver beetles (*Ips* spp.) and chain saws tend to eliminate trees before the disease kills them. Templeton et al. (1997) has summarized the economic damages of pitch canker in California.

In Nurseries

Pitch canker occurs in bareroot and container nurseries. Diseased pine seedlings show chlorotic or reddish brown needles and wilting. Pitch-soaked lesions usually occur at or near the soil line, but occasionally are found in the region of the cotyledonary node (Barnard and Blakeslee 1980). In a North Carolina nursery, mortality of shortleaf pine seedlings in 1994 was attributed to *F. s.* f. sp. *pini* (M. Cram, personal communication). The pitch canker fungus has also been associated with late-season mortality in longleaf pine nurseries (Cary and Kelly 1994). Fraedrich and Dwinell (in press) concluded that *F. s.* f. sp. *pini* is a wound pathogen of longleaf pine

seedlings. Finally, as previously noted, the pitch canker fungus causes root rot in container-grown *P. patula* in South Africa (Viljoen et al. 1993).

To Cones and Seeds

The pitch canker fungus causes mortality of female flowers and mature cones and deterioration of seeds of several pine species (Miller and Bramlett 1979; Dwinell and Fraedrich in press). Infected loblolly pine cones tend to be misshapen and smaller. Some infected loblolly pine cones have a necrotic tip characterized by internal resin pockets (Barrow-Broaddus 1987). We (Dwinell and Fraedrich, in press) isolated *F. s.* f. sp. *pini* from the surface and interior of immature shortleaf pine cones from a North Carolina seed orchard. We concluded that interior contamination by *F. s.* f. sp. *pini* was not correlated with necrotic regions, caused primarily by insects, on the cone surface. How the pitch canker fungus enters cones is unknown.

The pitch canker fungus is frequently associated with the seeds of southern pines. Entire slash pine seed lots or entire longleaf pine seed crops have been lost because infection by *F. s.* f. sp. *pini* caused low viability and germination (Dwinell et al. 1985). Current research investigates whether the pathogen is primarily on the seed surface or infects the embryo. Longleaf and shortleaf pines contamination is mostly on the seed surface (Dwinell and Fraedrich, in press; Fraedrich and Dwinell, in press). In shortleaf pine 61% of the seeds were contaminated, but only 1.6% of the contamination appeared to be internal (Dwinell and Fraedrich, in press). In the late 1980's, I isolated the pitch canker fungus from seeds of Monterey pine in areas where the disease was prevalent, but not from seeds where the disease was absent (Dwinell and Adams 1993).

MANAGEMENT

Because each outbreak has its own unique history, no specific management strategy has been developed to reduce or eliminate the threat of pitch canker disease. An integrated management approach, including chemical control, biocontrol, genetic selection for resistance, and altered cultural practices, should be considered for specific hosts and growing conditions (Dwinell et al. 1985).

Because wounds can become as infection courts for *F. s.* f. sp. *pini*, understanding the cause(s) of the wounding is tantamount to managing pitch canker (Dwinell et al. 1985). In cases where the wounding agent is an insect, chemical control may reduce disease intensification. Federal and State regulations on the use of chemical pesticides has, however, severely limited this option. Biocontrol organisms have been ineffective (Barrows-Broaddus et. al. 1985; Barrows-Broaddus and Dwinell 1987). Eradication is not a viable option. Variation in the incidence of pitch canker is common among clones within seed orchards, suggesting that genetic selection for resistance is possible (Barrows-Broaddus and Dwinell 1985; Dwinell et al. 1985). Monterey pines resistant to pitch canker has been confirmed by artificial field inoculations (Dwinell and Adams 1993). The long-term management of Monterey pine in California may depend on the development of resistant varieties (Storer et al. 1994).

In seed lots, appropriate seed treatments could eradicate external seed contamination. Soaking longleaf and shortleaf pine seeds in 30% solution of hydrogen peroxide prior to planting has shown promise (Dwinell and Fraedrich, in press). Although the relationship between the

surface contamination of pine seeds by *F. s.* f. sp. *pini* and pre- and post-emergence damping-off and disease in older seedlings is not fully understood, seed treatments currently represent the best insurance against seedling losses.

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BIOLOGICAL CONTROL OF WHITE PINE BLISTER RUST ON RED CURRANT LEAVES USING *CONIOTHYRIUM* SPP.

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ABSTRACT

The fungus *Coniothyrium* spp. capable to achieve biocontrol of apple scab was tested for its potential to control white pine blister rust. *Ribes glandulosum* leaf disks were preinoculated with *Cronartium ribicola* aeciospores followed by *Coniothyrium* spp. mycelial suspension application. Two strains of *Coniothyrium* spp. were tested and both were shown to infect and greatly inhibit *C. ribicola*. Strain P-130 infected on average 253.8 uredinia per leaf disk (96.4%) leading to an average of only 3 healthy uredinia per leaf disk. Strain P-176 was similarly effective with 148.1 infected uredinia per leaf disk (89.2%) and only 7.7 healthy uredinia per leaf disk. The control had on average 440 healthy uredinia per leaf denoting the high incidence of infection.

INTRODUCTION

White pine (*Pinus strobus* L.) is one of the most valuable timber species in eastern Canada. However it is highly susceptible to white pine blister rust caused by *Cronartium ribicola* J.C. Fisher. Since the introduction of this disease around 1910, white pine blister rust has become the major limiting factor in the natural and artificial regeneration of white pine and is responsible for the annual growth loss in excess of 200 million cubic feet (Benedict, 1967). Seedlings and young saplings are most susceptible to infection, especially under cool, wet environmental conditions such as those found in the Northeastern American continent.

A biocontrol agent effective against *C. ribicola* would be a useful tool for the management of young white pine plantations and naturally regenerating stands. Effective control of blister rust would permit reintroduction of white pine to its pre-blister rust range which covered most of deciduous and acadian forest in eastern Canada, and the boreal forest of Newfoundland.

White pine blister rust has a complex life cycle and a long disease development span. The disease spends the winter as a canker on white pine, then infects its alternate host, currants (*Ribes* spp.) in late spring. *Cronartium ribicola* produces urediniospores on *Ribes* and in mid-late summer develops teliospores which are the infectious propagules for white pine. Lethal infections typically occur in the first 6 years of a pine plantation, thus it must be treated throughout this period. Once a pine is infected, it will take 2 years for obvious symptoms (a canker) to appear and up to twenty years for mortality to occur. Other than mechanical pruning or carving of cankers there is no known way to eradicate an infection on pine once it has happened. Since teliospores of *Cronartium ribicola*

are the infectious propagules leading to blister rust on white pine, inhibition of teliospore production or its precursor uredinia could be an effective way to control that disease.

Various candidate biocontrol agents such as *Scytalidium uredinicola* (Hiratsuka et al. 1979), *Darluca filum* (Kendrick 1985), *Tuberculina maxima* (Bergdahl et al. 1978), *S. album* (Pickard et al. 1983), *Cladosporium gallicola* (Tsuneda and Hiratsuka, 1979) and *Monocillium nordii* (Tsuneda and Hiratsuka, 1980) have been studied or proposed to control stem rusts such as *C. ribicola*, but none have shown great efficacy during *in vivo* trials. We report here on *in vivo* test trials of four potential biocontrol agents, two of them belonging to *Coniothyrium* spp. Corda, against *C. ribicola* on *Ribes glandulosum* leaves.

MATERIAL AND METHODS

Strain P-176 (*Coniothyrium* spp), P-130 (*Coniothyrium* spp), P-11 and P-164 which have shown some potential to control apple scab were isolated by co-author Odile Carisse of Agriculture and Agri-Food Canada St-Jean-sur-Richelieu research station. The strains were grown on PDA (Difco) in the dark at 20C until the petri was covered with mycelium. A mycelial suspension was made by gently crushing the collected surface mycelium in sterile distilled water with Tween 20 added.

Mature leaves of red currant (*Ribes glandulosum*) were surface sterilized by dipping them for 2 minutes in Javex 20%, then 1 minute in 70% ETOH and then rinsed in distilled water. All these solutions have Tween 20 added.

Leaf disks 18 mm diameter in preliminary trials or 37 mm in final trials were punched from the leaves and transferred onto petri dishes containing 2% water agar with holes to receive the leaf disks. The disks were then inoculated with previously frozen (-80°C) *Cronartium ribicola* aeciospores which were submitted to a heat shock treatment of 40°C for 5 minutes. Spores were inoculated with an air gun directly on *Ribes* leaf disks and on water agar for spore count and viability assessment. Inoculation of biocontrol agents followed *C. ribicola* inoculation.

Leaf disks were transferred to a growth chamber at 18°C, receiving 8 hours of light for 21 days. At this time, uredinia with urediniospores are present on the surface of leaf disks. They are then transferred to another growth chamber at 13°C, receiving 8 hours of light for 14 days. Telia bearing the teliospores usually appear then.

Tests for potential biocontrol agents: In preliminary trials, one petri dish with four 18-mm leaf disks was inoculated with biocontrol agents P-176, P-130, P-11 and P-164, zero, one, two and three weeks following inoculation with *Cronartium ribicola*. Two petri plates were used for controls with only *C. ribicola*, four with the biocontrol agents to test their effect on healthy leaves alone. A total of 16 petri plates were used for treatment, four to be sprayed with control agents alone and two used as controls. Screening for telia production was done after the 14 days of 13°C temperature and proceeded for as long as the leaf disk survived. Number of telia, health and number of uredinia was assessed.

In final trials, 5 petri plates with two 37-mm leaf disks was inoculated with biocontrol agents P-176 and P-130, zero, one and two weeks following inoculation with *Cronartium ribicola*. Three

petri plates of two leaf disks were used as controls. Measurement of aeciospore density and germination rate was done on the water agar between leaf disks. PDA petri plates were also inoculated along with trials to measure biocontrol agent inoculum density.

RESULTS

In the preliminary tests, the controls indicated that the *Ribes* leaves were well-infected with blister rust . On average, there were 28 uredinia and 60 telia per 18-mm leaf disk (Table 1). The uredinial and telial values were much higher than what is usually observed in the field.

Of the four biocontrol agents tested in the preliminary trials only P-176 and P-130, both *Coniothyrium* spp. strains showed potential for further tests (Table 1). Inoculation of biocontrol agents 7 and 14 days after *C. ribicola* gave the best results. P-176 and P-130 inoculated after *C. ribicola* were able in most cases to completely infect uredinia, and P-176 diminished telial count by over 90% compared to control or some petries of P-11 and P-164. Thus, P-176 and P-130 had a dramatic effect on blister rust, causing the numbers of sickly-looking and dead uridia to increase and inducing large necrotic zones on the *Ribes* leaves where *C. ribicola* was present. Results for P-11 were more mitigated. It caused a significant proportion of uredinia to be sickly looking but there was also some large healthy uredinia and the telia count averaged 59 per leaf (Table 1). P-164 did not inhibit blister rust in this assay and telial count on leaf disks inoculated with that strain was not very different from controls.

Final trials testing of *Coniothyrium* spp. strain P-130 and P-176 were performed on 38-mm leaf disks. The controls exhibited on average 440.3 (±80.5) uredinia, a high value and uncommon in nature. It resulted from an inoculation of 16.7 *C. ribicola* aeciospores per mm^2 of *Ribes* leaf surface with a germination rate of 43,4%. The inoculum density of P-130 was 8.1 colony-forming-unit per cm^2 and 9.2 colony-forming-unit for P-176. The high density of uredinia needed to diminish variation among repeats was responsible for the massive infection of the *Ribes* leaf disks. Under these conditions the leaf disk was necrotic before it could produce telia, so it just remained at the uredinial stage. For this reason we only counted the uredinia.

Strain P-130 infected on average 253.8 uredinia per leaf disk (96.4%) leading to an average of only 3 healthy uredinia per leaf disk (Table 2). Strain P-176 was similarly effective with 148.1 infected uredinia per leaf (89.2%) and only 7.7 healthy uredinia per leaf disk. There was no statistical differences between the two *Coniothyrium* strains or between the different timing of repeats.

DISCUSSION

Since the telia is the structure responsible for producing basidiospores which are the propagules for infection on white pine, we wanted to see if the *Coniothyrium* spp. biocontrol agents were able to inhibit their production. Telia originate from uredinia so inhibition of uridia was also sought. Biocontrol agents *Coniothyrium* spp. strain P-179 and P-130 inoculated after blister rust infection were the most efficient to inhibit uredinia and telia formation. This is the first time a biocontrol agent has given such a result on *C. ribicola*. The inhibition of uredinia and teliospore production may be an effective way to control blister rust. In practical field treatment this means the biocontrol agents would have to be applied in late June or early July for maximum efficacy.

Since basidiospores can travel a few kilometers, the area around the plantation must also be treated.

The blister rust artificial inoculation dose was much higher than natural inoculation indicated by the density of uredinia on the control. Even at such high inoculum load, P-176 and P-130 were effective. It may be suggested that the efficacy will be similar or higher under the lower natural inoculum load.

Coniothyrium allows the biocontrol of *C. ribicola* on *Ribes*, an important control option for commercial currant growers in an area where there is quarantine restriction on such culture.

To see white pine plantations and orchards, along with commercially grown currants, is a common sight in rural Quebec where farmers often diversify income sources. Since *Coniothyrium* was initially developed to control apple scab, a multi-purpose use against blister rust on naturally occurring wild *Ribes*, on commercially grown currants and on apple orchards can be made available for farmers, solving many problems at once and generating a large scale market for the product.

Table 1. Preliminary biocontrol tests of four fungi against *Cronartium ribicola* on *Ribes glandulosum* leaf disks. *C. ribicola* uredinial and telial count per 18-mm leaf disks inoculated with biocontrol agents P-176, P-130, P-11 and P-164, zero, one, two and three weeks after inoculation with *C. ribicola*.

	Uredinial count				Telial count			
	P-176	**P-130**	**P-11**	**P-164**	**P-176**	**P-130**	**P-11**	**P-164**
21 days	2	0	4	11	6	43	171	-
14 days	0	0	0.8	11.1	2.5	14.9	51	94.2
7 days	0	0	4.2	2	0	4	11.9	10.2
0 days	5	6.7	18	42.2	5.8	19.2	4	36
Control		28.2				60		

Table 2. Incidence of symptoms of two strains of *Coniothyrium* spp. on *C. ribicola* uredinia grown on 37-mm *Ribes* leaf disks inoculated with biocontrol agents P-176 and P-130, zero, one and two weeks after inoculation with *Cronartium.*

	14 days	7 days	0 days
P-130	0.6% healthy (1.6±1.6*) 0.7% infected (1.7±1.3) 98.7% dead (246.1±48.9)	0% healthy 5.8% infected (11.8±11.8) 94.2% dead (191.1±53)	2.2% healthy (7.4±6.6) 1.4% infected (4.6±3.4) 96.4% dead (324.5±33.9)
P-176	5% healthy (8.7±4.5) 4.7% infected (8±5.4) 90.3% dead (155.2±30.2)	10.5% healthy (14.9±13.7) 6% infected (8.5±3.8) 83.5% dead (118.4±17)	0% healthy 6.2% infected (11.2±7.3) 93.8% dead (170.8±24)
Controls	94.3% healthy 5.7% infected** 0% dead		

*Average number of healthy, infected and dead uridia per leaf with standard error.

**Uridia infected with another fungus, probably of endophytic nature on *R. glandulosum* leaves.

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ON PROBLEMS AFFECTING PLANTED *TILIA* TREES IN QUEBEC

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ABSTRACT

A pronounced decline associated with various anomalies has led to the annual cutting (since 1992) of about 1% of the planted linden trees in Quebec City (QC), Canada. Such trees were mostly of the *Tilia cordata* parentage. Of the anomalies observed on declining trees with reduced foliage, the occurrence of numerous shoot-bearing galls on the trunks and/or swellings at the base, and of pronounced splitting and peeling of the bark, ranked as the most prominent. The soil and foliage analyses made did not provide a clear indication as to whether mineral imbalance or impoverishment could have contributed to the decline. A search for other causes was undertaken to verify the possible occurrence of pathogens. From microscopical observations made, no evidence was obtained that microorganisms were initially involved as decline agents. Dissections of some 30 trees cut in 1996 showed, however, that opportunistic fungi, bacteria, and insects, as well as detrimental physical factors, could have been active as secondary agents in the decline process. The origin of gall formation could be traced back to the very centre of the stem, with traces of shoot inclusions increasing gradually over the years. Results of surveys conducted in other regions in Quebec and in neighbouring provinces indicated that gall formation and buttress-type outgrowths were general, not only on the large trees, but also on a high percentage of the more recently planted trees. As a result, it seems evident that preventive control measures have to be developed and applied to trees at a young age to prevent a persistent development of such outgrowths and swellings in the future.

INTRODUCTION

Since 1993, the City of Quebec *Service de l'environnement* has had to cope with an exceptional mortality rate of planted linden trees, mostly of the *Tilia cordata* parentage. Indeed, nearly 1% of these trees from a population of over 3,500, had to be cut down annually. Although the number of *Tilia* trees cut in 1997 was lower than in the preceding years, of the 4,343 trees examined (City of Quebec, 1997 internal report), about 1% of the larger diameter trees (over 15 cm dbh) are classified as being in an advanced (class 3) state of decline (similar to 1996), and more than 5% of them have been grouped in an intermediate condition of deterioration (class 2). The main disorders in the class 3 category are characterized by sparse, small, and yellowing foliage, by bark splitting and peeling off, and by the presence of large galls on the trunk. Trees in class 2 show similar but less pronounced anomalies. The decline symptoms prompted Quebec

City's *Service de l'environnement* to sponsor analyses of foliage and soil in order to determine whether any mineral impoverishment or imbalance was associated with the decline syndrome. Although the Ca/N ratios were found to be high at places, no evident differences in element contents between healthy and affected trees and between sites were observed that could explain the causes of the decline (De Chantal 1995).

Other investigations to further study the problem were undertaken in 1996. As most of the trees belonging to class 3 had several galls bearing large numbers of buds and shoots (a condition analogous to crown gall), or outgrowths at the tree base, one objective of the investigation was to conduct ultrastructural studies to verify if microorganisms (bacteria, phytoplasmas and viruses in particular) could have been the causal agents of the anomalies observed. *Tilia cordata*, however, has been reported to be immune to crown gall (Peace 1962). It seemed appropriate, meanwhile, to dissect as many as possible of the cut trees to characterize the anatomical changes occurring in the evolution of these disorders. As a follow-up to the recommendation made in the initial study, a comparative survey was also carried out on a representative number of trees in regions located in and outside the Quebec City area to map the confines of this decline.

Concerning the first objective, samples from tissues producing pronounced bud and shoot proliferation were sampled and processed for ultrastructural observations according to standard procedures of double fixation (glutaraldehyde and osmium tetroxide) and embedding in Epon. With regard to the second objective, disks from some 30 trees (from parts shown to bear the most pronounced disorders) were examined and compared. For the third objective, over 1,000 trees of all ages were examined in Quebec City and surrounding municipalities as well as in the Montreal region, in New Brunswick, and in other regions bordering the province of Quebec. Some trees in Ontario from the Ottawa region (thanks to Dr. L. Carlson, Canadian Forest Service) and Thunder Bay were also examined.

RESULTS

Anatomical observations

These observations did not reveal the presence of microorganisms in the highly proliferating tissues as being part of or surrounding the developing galls. Such galls appeared to be increasing in size by means of large masses or long bands of meristematic-like tissues from which leaf buds were forming. The stimulated galls were apparently beginning to split, having contracted other disorders of the inner and outside bark (Fig. 1). At the margin of these developing galls, or surrounding the growing buds and shoots, some patches of necrotic inner bark were noticeable at times, reaching the cambium, and seemed to result from the presence of various microorganisms (bacteria, fungi, cyanophycae, etc.) but without clear evidence that these organisms were true pathogens. Nevertheless, as a tissue reaction to such patches of necrosis, the bark was thicker in these regions and formed fissures that were larger and deeper (Fig. 1) than in normal bark. Traces of a blue stain fungus representing some localized bark mortality (Fig.1) were noticeable, in some of the tree disks examined; in some instances, the entrance of such fungi likely followed a scolyte-like feeding attack. On some other trees, bark splitting was associated with patches of a whitish compound (Fig. 2) containing rhomboid crystals; similar crystals were present in the layers of cork-like cells bordering the longitudinal, sometimes long bark fissures, as well as in cells located deeper in the bark. The wood surface in such regions displayed numerous spicules; in

drying, the wood cylinder from such affected trees showed ray-like bands of wood, that extended to the heart of the stem, whereas such a feature was not observed in normal-looking wood.

Tree dissections

Most of the disks examined showed, at the level sampled, the presence of galls or similar disorders, sometimes amounting to as many as five that completely encircled the stem (Figs. 1, 2). Abnormal, numerous bark fissures and splitting were common features on such trees. A few gall-free disks included trees of as many as four stem-overgrown branches, which had led to bark necrosis and similar splitting, whereas others had pronounced cracks. Such cracks, at first sight, seemed to correspond to frost cracks (Butin and Shigo 1981), but in fact appeared to have resulted from an overactive formation of callus tissue at the margins of a wound or other types of local damage; upon meeting, such lips of cicatricial tissue acted as a lever causing at times an almost complete diagonal splitting of the trunk. A similar type of reaction was observed in and near the galls. In addition, some trees showed very large swellings at the base (Fig. 3) that bore often a few too many shoots. Dissected swellings disclosed included shoots and pronounced deep bark and wood cracking, with some trees having signs of pronounced frost damage at this level, and bands of necrotic inner bark extending vertically to the tree crown. In other cases, shoot proliferation and inclusion in the bark at the tree base was also related to localized bark mortality that led to severe fissures and the entry of bark-destroying fungi.

Whatever the anomalies were, their point of origin could generally be traced back to the centre of the stem, with the severity of such anomalies having started to become pronounced some 12 to 15 years ago (Fig. 1). Concerning gall formation, the re-activation of suppressed buds (Carter 1955; Boyce 1961; Peace 1962; Kramer and Kozlowski 1966, 1979; Kozlowski 1971; Zimmerman and Brown 1971; Pirone 1972; Tattar 1989; Blanchard and Tattar 1981) near some types of wounds, and a concomitant proliferation of adventitious buds were obviously part of the process. Following these observations, younger trees were examined for disclosing the first signs of gall initiation on the stem, and of swellings at the base of the trees. It could be established that at the median or lateral margins of pruning wounds some tissue proliferation was already prominent (Fig. 4), also associated in some cases with the formation of hundreds of new buds (in 1996, as many as 30 such buds per cm^2 were counted). The emergence of suppressed buds also, but not always, occurred near the wounds. Generally, shoots produced by these buds died off when emerging away from a wound.

Survey data

All visible anomalies were recorded on approximately 160 large trees (over 20 cm in diameter) and about 900 smaller ones (mostly between 15 and 20 cm dbh, thus more recently planted). As swellings and shoot proliferation at the crown level and gall formation on the stems were the main disorders observed that seemed to favour the coming and action of secondary, important detrimental agents, including the lasting interference effect with sap movement, only the number of trees showing such anomalies is given here. Thus, in the regions surveyed, 40 (25%) of the larger trees had tumorous galls, and 32 (20%) had large swellings (which at times were also gall-like) at the base of the tree. The latter trees rarely also had galls on the trunk. Of the smaller trees, 98 (over 10%) already had relatively big galls on the stems, 376 (42%) displayed bud and shoot proliferation near pruning wounds (Fig. 4), 65 (7%) had signs of suppressed bud protrusions,

over 100 (9%) had swellings near the crown level, and 235 (26%) showed some outgrowths at the base. Although only a limited number of trees were examined in Ontario and New Brunswick, the proportions were similar for the listed disorders. It should be mentioned that on the few trees that were re-examined in 1997, bud proliferation was less pronounced than in 1996 at the end of September; in some locations (outside Quebec City) such patches of buds on trees had been pruned twice during the summer, and a new crop of buds was just appearing.

CONCLUSION

The various causes, given in the literature consulted, to explain the anomalies affecting trees (Klein 1908; Carter 1955; Kramer and Kozlowski 1960, 1979; Boyce 1961; Pirone 1972; Kozlowski 1971; Harlow et al. 1979; Blanchard and Tattar 1981; Butin and Shigo 1981; Manion 1981; Phillips and Burdekin 1982; Sinclair et al. 1987; Shigo 1991; De Chantal 1995; IQDHO 1997) did not seem adequate to explain the disorders discussed here. Trees with similar disorders were also found to succumb to the action of secondary agents (Sinclair et al. 1987). If the developing galls present on the young trees were to progress to proportions observed on large trees, a high rate of tree decline related to this condition could be expected to eventually occur on such trees. Preventive measures to circumvent such an eventuality must be developed as quickly as possible. As an imbalance in cytokinins or auxins (Zimmerman and Brown 1971) might be involved in bud proliferation, it could seem plausible to formulate treatments to counteract the action of such compounds, together with the testing of the appropriate horticultural practices that would prevent the outburst of such disorders. As swellings at the tree base may be related to a graft effect, the development of such swellings on existing planted trees may not be completely halted; adverse factors may also be involved. At any rate, in future tree production, arboriculturists may succeed in developing new varieties or new selective methods for the varieties available that may prove effective in obviating the problems under discussion. In all instances, it seems that a concerted effort should be envisaged to group all the parties interested (municipalities, universities and government institutions, arboriculturists, and so on) to hastily develop means to safeguard the planting of this otherwise very interesting ornamental tree. A recent inquiry on the work being carried out worldwide on similar disorders of *Tilia*, conducted by *l'Institut québecois du développement de l'horticulture ornementale* (IQDHO 1997), has revealed that a number of investigations are being conducted on similar problems elsewhere in Canada, the United States and Europe. Organizing a working group, possibly together with the IUFRO 7.02.02 working party, might help to promote a comprehensive research study on the problem. A deplorable stand would be to stop planting linden trees because of such problems (as was the case for elm, white pine, and red pine), at least without having conducted a minimum number of experiments to try and solve them; an equally undesirable attitude would be to continue planting profusely the same linden varieties in the regions under study as if the problem did not exist.

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Figure 1. A cross section through a gall (asterisk) whose origin can be traced back to the stem centre (small arrowhead). Unrestricted expansion of the gall has occurred about 15 years ago (large arrow) where numerous shoots and bark patches started to become included in the stem. During the last years of the outgrowths, patches of outside bark and cambium have died leading to or resulting from bark fissures; fissures (large, short arrow) are visible in the bark surrounding the galls (right portion). It was also evident that the bark detached itself because it likely resulted from the pressure exerted by the expanding gall, and apparently from the action of secondary fungi, some of which was identifiable as blue stain and expanded from the margin of the gall (curved arrow). Opportunistic insects were also present in the bark at the base of the gall.

Figure 2. The base of a tree circumscribed by a necklace of galls. Dead patches of bark were present between the galls, hosting diverse insects. The numerous shoots occurring in these galls have been pruned out. Abundant bark fissures, some associated with the presence of a white substance (arrow) are present on the trunk.

Figure 3. An expanding buttress-like swelling (arrow) at the base of a medium-sized tree. A root that had developed at this level is encircling this swelling.

Figure 4. Beginning of bud and shoot proliferation and outgrowth (arrows) on a recently planted tree. Photographs taken by Mr. Claude Moffet, formerly of LFC.

LOGISTIC ANALYSIS OF RELATIONSHIPS BETWEEN HEALTH AND ENVIRONMENTAL VARIABLES OF SUGAR MAPLES IN THE ARNEWS NETWORK: A FIRST STEP IN MODELLING SUGAR MAPLE HEALTH

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The ARNEWS (Acid rain national early warning system) network contains 99 plots across Canada that were established in 1984-1985. Eighteen plots were in maple stands. In total, those plots contained 276 sugar maples. A preliminary analysis of the relationship between certain variables measured in the network as well as the deposition of sulfate and nitrate and weather conditions with the health status of trees is presented here.

Logistic regression, using a stepwise variable selection procedure, was used to assess the proportion of healthy or affected trees, as predicted by current defoliation, acid rain symptoms, bole damage index, dominance and DBH classes, every year from 1988 to 1992. The predicted proportion of trees in each health class was modeled from the logistic regression equation. Among the health variables, current defoliation and bole damage index were the most frequently selected predictor variables. Current defoliation had the largest impact in reducing the proportion of healthy trees in stands. Acid rain symptoms had some impact when they were healthy.

Furthermore, the same statistical method was used to model the proportion of healthy or affected sugar maple as predicted by 1) sulfate and nitrate deposition in exceedance of critical loads; 2) the number of days of deep frost (-10 C or less) from December to January or in March; 3) the number of days with thick snow cover (10 cm or more) on the ground in March; 4) the amount of rain in July and August and 5) the amount of sunshine from May to August. All variables were related to the health status of the trees. However, weather variables were more adequate in predicting changes in the proportion of healthy trees than deposition variables. Frost from December to February and snow cover in March had more impact in reducing the proportion of healthy maple than any other environmental variable.

QUEBEC CITY INTEGRATED DUTCH ELM DISEASE MANAGEMENT

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Quebec City has been fighting Dutch Elm Disease (DED) since it first hit in the early 1960's. Now, the city has a complete integrated management program. In 1995, all of the 7,600 elms (*Ulmus americana* L.) on the municipal land were inspected at least once during the summer. Of this number, 125 trees were removed because of infection, 11 had some branches removed and four elms were injected with thiabendazole. Furthermore, 2,681 American elms had their stems sprayed with chlorpyrifos from the stump to three metres above the around in order to reduce the native bark beetle (*Hylurgopinus rufipes* (Eichh.)) population. Finally, 125 elms were fertilized in 1995 and 12 trees were planted. The total cost of the management program was $44,900 CAN (1995), averaging $5.90 CAN per tree. Mortality decreased 1% annually.

COLLETOTRICHUM DEMATIUM, A CAUSAL PATHOGEN OF DAMPING-OFF DISEASE IN JAPANESE BEECH SEEDLINGS AND ITS ROLE IN RAPID SEEDLING DEATH

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SUMMARY

The pathogenicity of *Colletotrichum dematium* was evaluated as the agent causing post-emergence damping off of current-year beech seedlings and the role of this fungus in rapid seedling death of current beech seedlings occurring in the fields is discussed. Field observations revealed that damping off caused by fungal pathogen(s) was one of the most important causes of rapid seedling death. *Colletotrichum dematium* was frequently isolated from the hypocotyls of the seedlings that exhibited damping off in five different sites and in every year of the study. Current-year beech seedlings inoculated with *C. dematium* died within 20 days after both wound- and nonwound-inoculation, indicating that this fungus was pathogenic to current-year seedlings. *C. dematium* was also detected in all litter samples examined, although the incidence of detection differed depending on the site and/or detection method.

These results strongly indicate that *C. dematium* is the pathogen that caused damping off and plays an important role in rapid seedling death during the early stages of beech regeneration.

Keywords: *Colletotrichum dematium*, beech seedlings, *Fagus crenata*, pathogenicity, post-emergence damping off

INTRODUCTION

The Japanese beech (*Fagus crenata* Blume) forest is one of the most important forests not only for timber production but also because of its role in conserving the landscape, and for its genetic support in northeastern Japan. In spring, many beech (*Fagus* spp.) seedlings newly emerge on the forest floor during a short period. However, almost all beech seedlings disappear within a few years of emergence (Hashizume and Noguchi 1977; Maeda 1988; Ohkubo et al. 1989). Many studies have identified fungal infection or damping off disease as the principal cause of this rapid disappearance (Kobayashi et al. 1984; Kessler 1988; Nakashizuka 1988; Maeda 1988; Mosandl and Aas 1986). However, little is known about the identity and mode of action of the causal pathogens.

The objectives of this study were (I) to identify the causal pathogen and (II) to evaluate the role of the pathogen in the rapid death of these seedlings.

MATERIALS AND METHODS

Observation of Dead Seedlings and the Causes of Death

To observe the temporal sequence of the dead beech seedlings, we set up a total of nine quadrats in two experimental forests (Tazawako and Hakkohda) in 1990. Experimental Forest I (Tazawako, 39°48'N, 140°47'E, 700 m above sea level) in the Oou Mountains in Akita Prefecture consists of four forest stands (T1-T4) with different stand densities. In each of the four forest stands, two quadrats termed *a* and *b* (2 X 2 m) were set up. In the other experimental forest, Forest II, (Hakkohda, 40°40'N, 140°50'E, 700 m above sea level), in the Hakkohda Mountains in Aomori Prefecture, one quadrat (1 ha, 1 X 1 m) was set up. Current-year beech seedlings with well-expanded cotyledons were marked by inserting a wire with a colored numbered tape near individual seedlings in each quadrat. The number of dead seedlings was examined for three growing seasons until late October when almost all of the leaves of the seedlings had fallen. Damaged seedlings, including those damaged by wilt, were collected to examine the cause of damage. The incidence of mortality was expressed as the percentage of total marked seedlings at the time of each examination.

Isolation of C. dematium from Naturally Infected Seedlings

To investigate whether *Colletotrichum dematium* causes damping off in the field, current-year seedlings exhibiting typical symptoms of post-emergence damping off were collected from June to July in 1990, 1993 and 1994 in five locations in Tazawako, Hakkohda, Appi kohgen, Hachimantai and Kanumazawa. Damaged hypocotyls were cut into small pieces, the surface sterilized with 80% ethanol for a few seconds, and with a 0.1% solution of $HgCl_2$, it was washed three times for 30 sec. with sterile distilled water, plated on PDA, and incubated at 15°C. Twelve to 14 days after incubation, the number of *C. dematium* colonies that emerged from the tissues was counted. The incidence of isolation was expressed as a percentage of the total seedlings tested.

Plant, Fungus and Inoculation

Beech seeds collected in October 1992 in the Hakkohda Mountains were submerged in tap water overnight and germinated on a filter paper bed for 3 to 5 days. Germinated seeds with a uniform size were planted in a pot and grown in a growth chamber controlled at 20°C under 10 h of fluorescent light followed by 14 h darkness for 18 to 25 days until the cotyledons were fully expanded and the first pair of true leaves was developing. *C. dematium* (C-4b106) was used in this study. The fungus was isolated from diseased seedlings exhibiting damping off in July 1990 in Tazawako and maintained on potato dextrose agar (PDA) at 15°C.

Several 6-mm-diameter mycelial disks of *C. dematium* were taken from the edge of a 14-day-old culture and were used as an inoculum. Hypocotyls of the seedlings were wounded with a sterilized blade and were inoculated with either mycelial or non-mycelial (control) disks (Fig. 2). Nonwound inoculation was carried out by placing mycelial disks on hypocotyls and then covering the hypocotyls with aluminum foil. Seedlings subjected to wounds only served as additional controls. In order to provide a high relative humidity, inoculated seedlings were covered with polyethylene bags, which were removed 6 days after inoculation when the first visible symptoms

appeared. These seedlings were incubated in the growth chamber described above. Symptom development and the number of dead plants were recorded periodically over 20 days.

Survey of C. dematium *in the Litter Layer*

To determine whether or not *C. dematium* inhabits the litter layer on the forest floor, hypocotyls of the current-year seedlings were used as bait to isolate the fungus because preliminary results indicated that it was difficult to isolate the fungus directly from the litter falls. Hypocotyls, which were collected in June 1994, were surface-sterilized as described above, cut into 2-cm pieces and were buried in litter collected from various beech forests using tall petri dishes (9 X 10 cm) and maintained in a growth chamber at 20°C. After incubation for 18 to 20 days, when hypocotyls were soaked in water, they were recovered from the litter, surface-sterilized, and incubated on PDA at 15°C. The incidence of isolation of the fungus was determined 2 weeks after incubation. Production of acervuli of *C. dematium* on hypocotyls recovered from litter was also observed.

RESULTS

Temporal Occurrence and Types of Dead Seedlings

Death of the current-year seedlings occurred mainly from the end of May to the end of July. After the middle of August there was no prominent change in the number of surviving seedlings except for a small decrease in October (Fig. 1). The mortality rate was the greatest in the T1 stand where light illuminance was less than in the others. In this forest stand, more than 90% of the seedlings died before August. In the T2 stand, the mortality rate of the seedlings was less than in the T1 stand. Forty-five to 50% of the seedlings died by the middle of July. T3 and T4, which had similar patterns of mortalities, had the smallest percentages of mortalities, although illuminances on these sites were similar to that of the T2 stand. At the end of the first year, about 50% of the seedlings remained. At Hakkohda (Ha), the profile of mortality was similar to that in the T2 stand. Mortalities of one- and two-year-old seedlings were less than those of the current-year ones. The number of seedlings that survived through the previous year decreased gradually during the growing season, and no specific pattern of mortality was detected.

The causes of death of current-year seedlings observed in 1990 were categorized into four main types: A, biting off of hypocotyls under cotyledons by insects or small mammals; B, damping off of seedlings characterized by lesions soaked in water and collapsed lesions in the hypocotyls at the soil level; C, wilt accompanied by curling of leaves with injuries or bites of the tap roots by herbivores; D, wilt in which the main roots grew only in the litter layers. Among the above four types, types A and B were the principle causes.

Isolation of C. dematium *from Naturally Infected Seedlings*

C. dematium was frequently isolated from seedlings with damping off in five field locations and every year of the study. In all the collections except for the sample in July 1990 in Tazawako, the fungus was isolated with a high incidence ranging from 73.0% in July 1994 in Kanumazawa to 98.6% in July 1993 in Hakkohda (Table 1). The incidence of isolation in Tazawako in July 1990 was 55.9%. The incidence of fungi other than *C. dematium* in 1990 was relatively low, and there

was no substantial difference in the relative abundance of fungal species among fields studied (data not shown).

Development of Disease

Figure 2 shows the temporal sequence of symptom development by current-year beech seedlings inoculated with *C. dematium*. When the hypocotyl was wound-inoculated with a mycelial disk, the first visible symptom, a water-soaked lesion, developed 5 to 7 days after inoculation (Figs. 2). This lesion then expanded with time and 90% of the seedlings inoculated died within 14 days of inoculation (Table 2, Fig. 2).

In the case of nonwound-inoculation, the appearance of the first visible symptom occurred 2 or 3 days after its appearance in wound-inoculated seedlings (Fig. 2). However, over 80% of the seedlings died within 20 days of inoculation (Table 2, Fig. 2). In two batches of controls, neither death nor visible symptoms were observed in seedlings within 20 days of inoculation, although browning at inoculation sites occurred (Table 2, Fig. 2). *C. dematium* was reisolated from all dead seedlings tested.

Detection of C. dematium *in the Litter Layer*

C. dematium was detected in all samples by the production of acervuli or isolation of the fungus in hypocotyls recovered from the litter (Table 3). However, there were some cases in which *C. dematium* was not isolated from the hypocotyls but acervuli were produced.

DISCUSSION

The present studies showed that the death of current-year seedlings occurred for two months after emergence, which is in accord with other studies made by Konishi et al. (1990), Hashizume and Noguchi (1977) in *F. crenata* and by Ohkubo et al. (1989) in *F. japonica* that gradual death occurred in one-year-old seedlings.

The greatest mortality rate was observed in Stand T1 with the least illuminance, suggesting that light conditions might be one of the important factors in the survival of seedlings, as pointed out by previous workers (Hashizume and Noguchi 1977; Hashizume and Yamamoto 1975; Kudo 1985; Maeda 1988). The results of the present studies also revealed that there were four major types of death of current-year seedlings. It was shown that among them, herbivory (Type A) and damping off by fungal pathogen(s) (Type B) played important roles in the mortality in the first growing season. Type A occurred mainly during late May to early July. Type B mortality followed later that month. Similar phenomena were reported by Maeda (1988) who studied the temporal sequence of dead seedlings. Type B increased with decreasing illuminance. Mosandl and Aas (1986) showed that opening the canopy reduced the amount of fungal damage. Therefore, light conditions may affect Type B mortality.

Many studies have pointed out the importance of fungal damage and damping-off disease in the rapid disappearance of beech seedlings (Nakashizuka 1988; Maeda 1988; Mosandl and Aas 1986; Sahashi et al. 1994). However, there is little knowledge of the causal pathogens and their roles in this death. *Cylindorocarpon destructans* was isolated from roots of naturally damped-off

beech seedlings, and has been considered a candidate of causal pathogens, but this has not been confirmed (Kobayashi et al. 1984).

The present study showed that current-year beech seedlings inoculated with *C. dematium* died within 20 days after both wound- and nonwound-inoculation (Table 2), suggesting that this fungus was pathogenic to current-year seedlings. These results were consistent with field observations in this study and with observations made by Maeda (1988) that damping off of current-year seedlings occurred mainly from early June to mid-July. However, these results contrast with Sasaki's report (1977) that *C. dematium* was weakly pathogenic to 1-year-old seedlings. This was supported by the finding that there was little death of 1-year-old seedlings from damping-off disease. It is, therefore, unlikely that the death of older seedlings could be caused by the same fungus. The age of the seedlings, which affects biotic and/or abiotic stresses, may explain the difference.

Wound-inoculation accelerated symptom development and seedling death (Table 2, Fig. 2). On the forest floor, some seedlings had small injuries on hypocotyls caused by insects or small mammals (Sahashi, personal observation). These injuries may result in acceleration of seedling death in the field.

C. dematium was isolated from hypocotyls exhibiting damping-off collected from different sites. The incidence of isolation was high and was not very different among sites or among years (Table 1). This suggests that the fungus may generally play an important role in seedling death during the early regeneration stage of beech forests. *C. dematium* was also detected in all litter samples examined (Table 3), suggesting that this fungus inhabits the litter layer. This fungus, therefore, may infect current-year seedlings through contact with the litter during emergence.

Further studies are needed to clarify the infection process of this fungus and the effect of light intensity on disease development, and to quantify the densities of the fungus in different litter layers in order to reach a better understanding of the mechanisms of rapid beech seedling death during the early regeneration stage of beech forests.

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Table 1. Isolation of *Colletotrichum dematium* from current-year seedlings with damping off disease collected from different fields

Field	Year	Month of collection	No. of seedlings tested	Incidences of isolation (%)
Hakkohda	1990	July	23	95.7 (22) [a]
Aomori Pref.	1993	June	90	4.4 (85)
		July	71	98.6 (70)
Tazawako	1990	July	68	55.9 (38)
Akita Pref.	1993	July	31	83.9 (26)
	1994	June	50	80.0 (40)
		July	62	91.9 (57)
Appi Kohgen	1994	June	58	91.4 (53)
Iwate Pref.		July	58	87.9 (51)
Hachimantai	1994	July	84	97.6 (82)
Iwate Pref.				
Kanumazawa	1994	July	37	73.0 (27)
Iwate Pref.				

a) Number of seedlings from which *C. dematium* was isolated.

Table 2. Inoculation test of Japanese beech seedlings with *Colletotrichum dematium*

Innoculation	Days after inoculation	Number of seedlings inoculated	Number of dead plants
Inoculation with wound	26	23 (88.5)	25 (96.2)
Inoculation without wound	18	8 (44.5)	16 (88.9)
Control with wound	17	0 (0.0)	0 (0.0)
Control with wound + PDA disk	17	0 (0.0)	0 (0.0)

a) Total number of seedlings in three replications.
b) Percent dead plants.

Table 3. Detection of *Colletotrichum dematium* in litter layer

Litter layer examined	No. of hypocotyls recovered	Incidence (%) of acervulus	Isolation
Hakkohda	25	76.0 (19)	72.0 (8)
Tazawako	27	29.6 (8)	29.6 (8)
Appi Kohgen	26	73.1 (19)	50.0 (13)
Control	30		0.0 (0)

a) Hypocotyls were incubated on PDA just after surface sterilization without burying.
b, c) Number of hypocotyls on which acervuli were produced and number of hypocotyls from which *C. dematium* was isolated, respectively.

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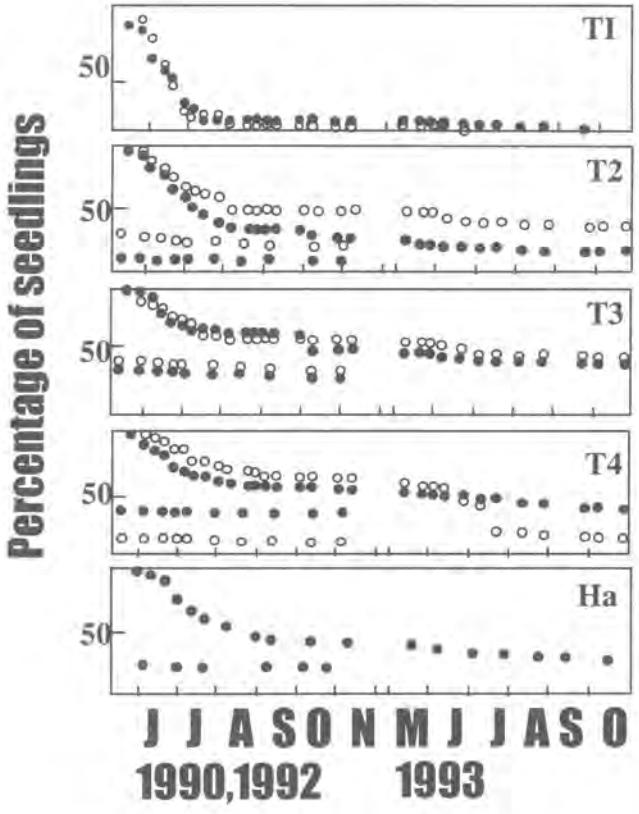

Figure 1. Survival rates of beech seedlings expressed as percentages of total marked seedlings. Black and white symbols show the data obtained in quadrats a and b, respectively, in the same forest stand. T1-T4, Forest stands in Tazawako. One ha, Forest stand in Hakkohda.

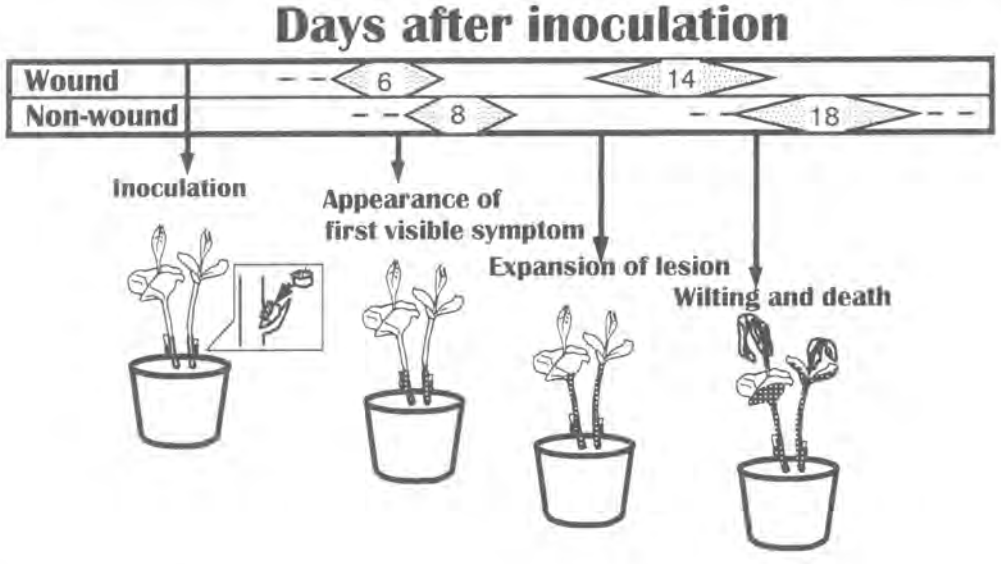

Figure 2. Diagrammatic representation of symptom development of current-year beech seedlings after wound and non-wound inoculation with *C. dematium*.

ANATOMICAL ANALYSIS OF THE SEASONAL DEVELOPMENT OF RESINOUS STEM CANKER OF HINOKI CYPRESS (*CHAMAECYPARIS OBTUSA*)

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SUMMARY

Resinous stem canker of hinoki cypress is one of the most important diseases in Japanese forestry. Conspicuous resinosis from stem and perennial canker formation are symptoms of the disease. A fungus species, *Cistella japonica*, is supposed to be a biotic agent of the disease, but relationships between the fungal invasion and the disease development are not fully clear. Environmental factors predisposing the disease development are also not understood. To support the determination of abiotic factor(s) and the role of biotic agent, season of traumatic resin canal formation and necrotic lesion development in the phloem was determined anatomically in hinoki cypress trees affected by resinous stem canker. Newly formed resin canals were observed in August. The stimulus that causes resin canal formation appears to occur between May and July. This result and the beginning of resin exudation in June indicate that resin exuded before August originated from resin canals formed in previous year(s). Development of a phloem necrotic lesion begins in June and continues until October, and is conspicuous in August samples. These results suggest that causal agent activity increases in summer.

Keywords: hinoki cypress (*Chamaecyparis obtusa*), resinous stem canker, *Cistella japonica*, traumatic resin canal, lesion development

INTRODUCTION

Resinous stem canker ("Rooshi" pitch canker) of hinoki cypress (*Chamaecyparis obtusa* Endlicher) is one of the most important diseases in Japanese forestry. Conspicuous resinosis from stem and perennial canker formation are symptoms of the disease. A fungus species, *Cistella japonica* Suto *et* Kobayashi, is supposed to be a biotic agent of the disease (Suto 1991, 1992, 1997), but relationships between the fungal invasion and the disease development are not fully clear. Environmental factors, such as low temperature, predisposing the disease development are also not understood. New resinosis from the trunk of a diseased tree is observed from June to October, and resinosis is remarkable in June and July (Yada 1989). Both normal and traumatic

resin canals are not observed in the xylem of hinoki cypress. Only traumatic resin canals are formed in the phloem. Thus, traumatic resin canal formation in the phloem is necessary for resinosis. Season of resin canal formation and necrotic lesion expansion in the diseased tree, however, has not been determined.

A resinous stem canker-affected tree often has resin canals in many phloem annual rings (Kuroda and Suzuki 1985, Suto 1995). In hinoki cypress, phloem resin canal, induced by wounding, are formed within two to three annual rings from the cambium, and not formed in outer annual rings (Kuroda 1995). Therefore, many layers of resin canals indicate the presence of repetitive stimuli for several years.

Necrotic lesions are formed in the phloem of diseased trees, and lesions expand year after year. In the early stage, cambium is not killed for several years. Then, lesions reach the cambium and expand even more. The trunks of a heavily affected trees are deformed by such large cambial death.

To obtain basic information about the clarification of abiotic factors of the disease, we tried to determine when resin canals formed and when necrotic lesions developed in naturally affected trees.

MATERIALS AND METHODS

Two sampling sites were established in resinous stem canker-affected hinoki cypress stands in both the Fukushima Prefecture and the Saitama Prefecture (Table 1). Early in December 1995, and February, April, May, June, August and October 1996, two to three diseased trees and one healthy tree were selected, and bark blocks were taken from the lesions and around them.

Sample blocks were fixed in formalin-acetic acid-alcohol, and dehydrated in alcohol series. Then, blocks were embedded in celloidin, and sectioned with a sliding microtome. Sections of 20 μm in thickness were stained with safranin-fastgreen and observed under a light microscope.

A year of resin canal formation was analyzed from the phloem annual ring where resin canals formed, from the shape of resin canals and the state of surrounding parenchyma cells. Lesion development was analyzed from the distribution of necrotic area and the stage of wound periderm formation.

RESULTS

Traumatic resin canal formation

No newly formed resin canals were observed in healthy trees. In diseased trees from every sampling site, newly formed resin canals were observed in August (Figs. 1, 2, 3). Various progress stages of resin canal formation, from the beginning to its near completion were observed in August. Before June, most resin canals in annual rings formed one to two years before they were predicted as forming in previous years, though only a small number of them were hard to determine if they had formed in the current year (Figs. 1, 2, 4).

Resin canal formation was induced mainly in the the phloem annual ring formed in the previous year. Resin canals were sometimes formed in the current annual ring where they were affected by cambial death or intense resin canal formation (Figs. 1, 2).

Artificial wounding on diseased tree trunks in April slightly induced a sequence of resin canal formation in May (Fig. 5). Wounding in December induced resin canals in advanced stages until May.

Relationships between resin canal formation and resin exudation

In every sampling site, new resinosis was observed in June, though no resin canal was formed during that month. Resin canals formed in the sampling year were not observed around trunk lesions with only new resinosis in sampling year, as they were formed in previous year(s).

Necrotic lesion development

Only necrosis with wound periderm and very small necrosis without wound periderm was observed all-year-round in the phloem of diseased trees (Figs. 6, 7). Development of necrotic lesions began in June and continued until October, and was conspicuous in August samples (Figs. 6, 8).

DISCUSSION

Observations in this study show that new resin canal formation begins in June or July. The beginning of resin canal formation within one month after wounding in April suggests that stimuli causing resin canal formation act from mid-May to July. Kuroda and Suzuki (1985) estimated that spring was the season of resin canal formation in resinous stem canker-affected trees. No resin canal formation, however, was observed in June samples in the present study. Climatic conditions may affect the season of resin canal formation.

Resin exudation before new resin canal formation in the same year showed that the resin originated from resin canals formed in previous year(s). This suggests that resinosis in the first year of the disease development occurs after August or does not occur that year. This inference is consistent with the observation of Kameyama *et al.* (1992) that resin does not exude even though resin canals are formed in the early stages of disease development.

Necrotic lesions developed with wound periderm formation following local denaturation or necrosis of phloem tissue. Thus, the mode of lesion expansion is different from outer bark formation in which periderm formation precedes phloem tissue necrosis.

Resin exudes from resin canals or resin pockets through cracks or resin-soaked tissue in the phloem (Kameyama *et al.* 1991). It is likely that lesion development induces resin exudation by stimulating resin production in previously formed resin canals, and by resin canal itself being the ways of resin exudation. If this is true, certain stimuli would cause lesion development, resin exudation and then new resin canal formation.

Suzuki *et al.* (1988) described the possibility of small necrotic lesion induction by low temperature. Small necrotic lesions are often observed in the outer layer of the phloem tissue of the diseased trees. It is necessary to examine the relationships between the small lesion formation and the disease development.

CONCLUSIONS

In the phloem of resinous stem canker-affected hinoki cypress trees, traumatic resin canal formation occurs between June or July and August, and necrotic lesion development occurs from June to October. Although resin exudation was observed in June, resin exuded before August originated from resin canals formed in the previous year(s). It is suggested that expansion of necrotic lesions induces resin exudation from resin canals already formed, and induces new resin canal formation. Stimuli causing necrotic lesion development are also assumed to occur between May and July.

Table 1. Sampling sites

Site	Location	Tree age (Year)	Elevation (m)	Soil (type)	Mean slope (degree)	Exposition
F1	Kawauchi, Fukushima Pref.	34	470	Bd	35	NE
F2	Koriyama, Fukushima Pref.	32	390	Bd	35	NE
S1	Kamiizumi, Saitama Pref.	24	810	Bd	15	E
S2	Minano, Saitama Pref.	28	900	Bd	20	SE

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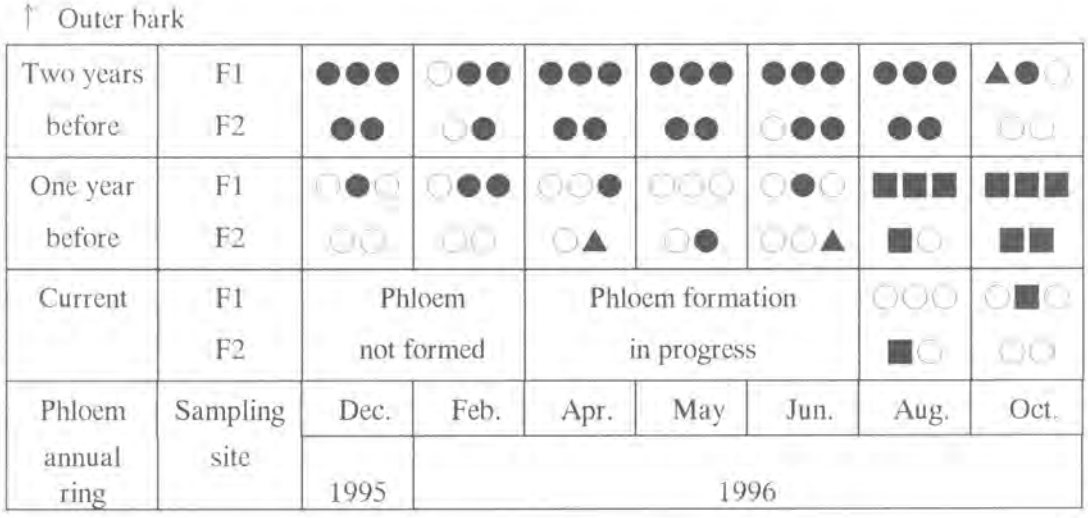
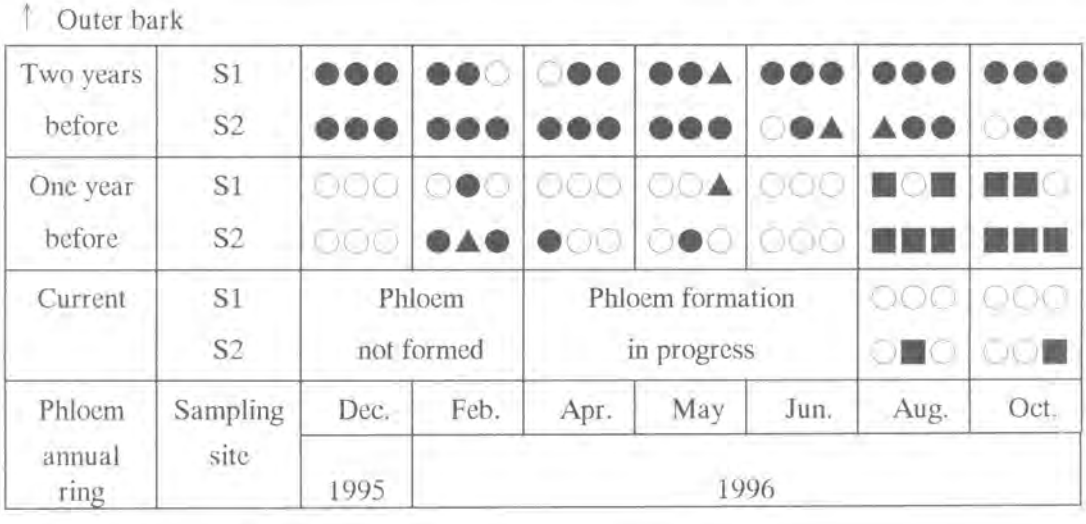


○, No resin canal; ●, Resin canals formed before 1995;
▲, Resin canals with the year of formation unclear; ■, Resin canals formed in 1996.

Figure 1. Traumatic resin canal formation in each phloem annual ring of resinous stem canker-affected hinoki cypress trees from sampling sites in Fukushima Prefecture. Each symbol in the cell corresponds to each trunk lesion.



○, No resin canal; ●, Resin canals formed before 1995;
▲, Resin canals with the year of formation unclear; ■, Resin canals formed in 1996.

Figure 2. Traumatic resin canal formation in each phloem annual ring of resinous stem canker-affected hinoki cypress trees from sampling sites in Saitama Prefecture. Each symbol in the cell corresponds to each trunk lesion.

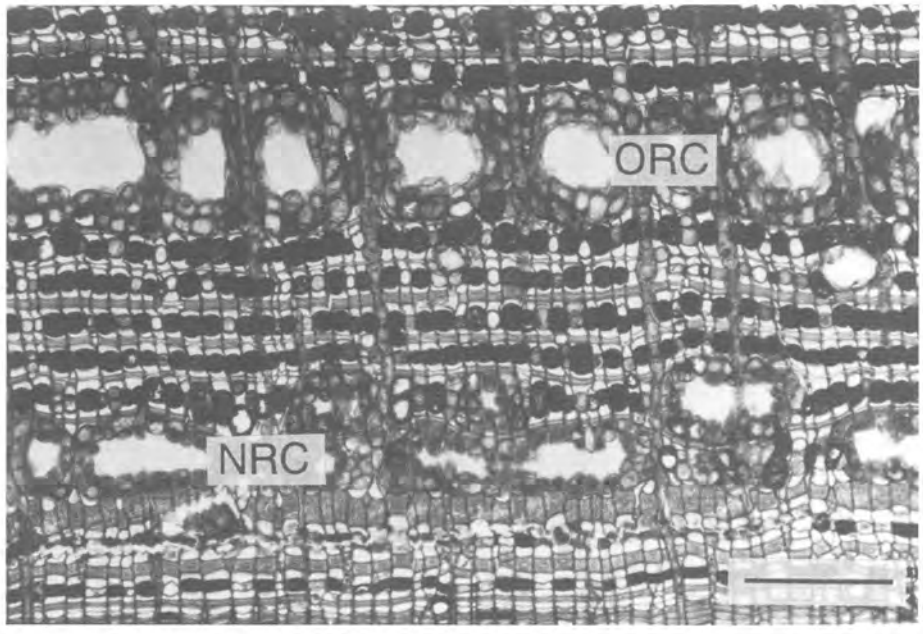


Figure 3. Newly formed resin canal (NRC) and resin canal formed in the previous year (ORC), August sample. Bar = 200 µm.

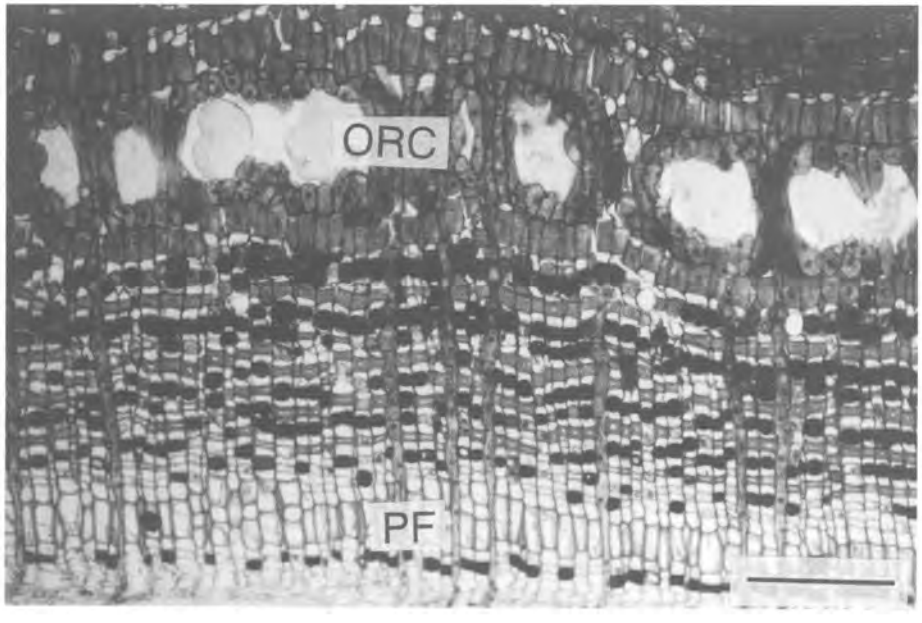


Figure 4. Differentiation of new phloem fiber (PF) and resin canal formed in the previous year (ORC), June sample. Bar = 200 µm.

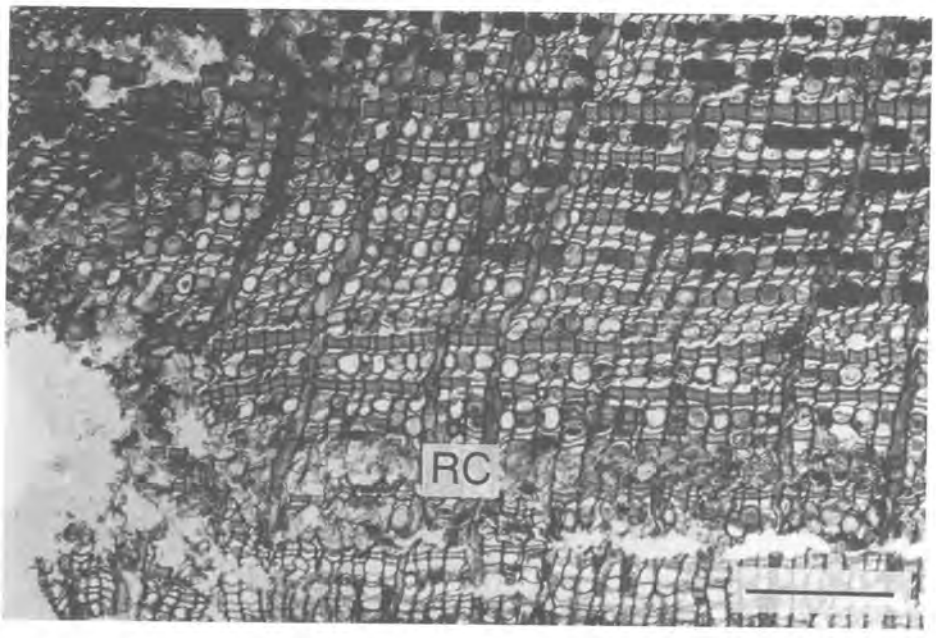


Figure 5. Early stage of resin canal (RC) formation in May sample induced by an artificial wound in April. Bar = 200 μm.

F1	○○○	−◎−	◎−○	○◎−	−◎●	○●●	●○○
F2	○○	−	○◎	−○	●●◎	◎●	○◎
S1	○−◎	− −	○○−	○ ○	○−◎	●◎●	○◎◎
S2	○◎○	−−◎	◎○○	○ ○	◎−○	●●●	○●●
Sampling site	Dec.	Feb.	Apr.	May	Jun.	Aug.	Oct.
	1995	1996					

Degree of necrotic lesion development: −, no.; ○, slight; ◎, a little; ●, conspicuous.

Figure 6. Necrotic lesion development in the phloem of resinous stem canker-affected hinoki cypress trees. Each symbol in the cell corresponds to each trunk lesion. Degree of necrotic lesion development was analyzed in relation to wound periderm formation.

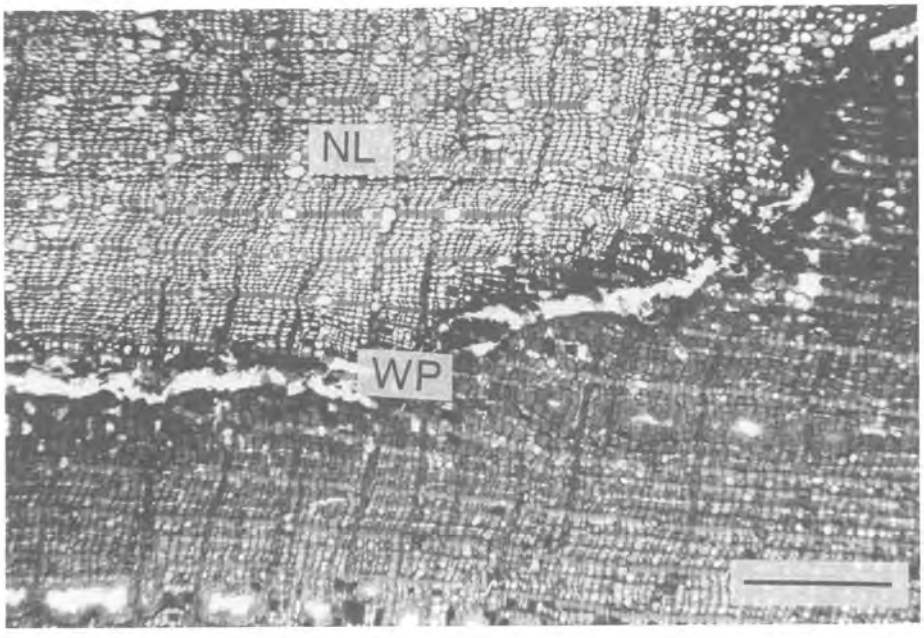


Figure 7. Necrotic lesion (NL) walled off by wound periderm (WP), May sample. Bar = 400 µm.

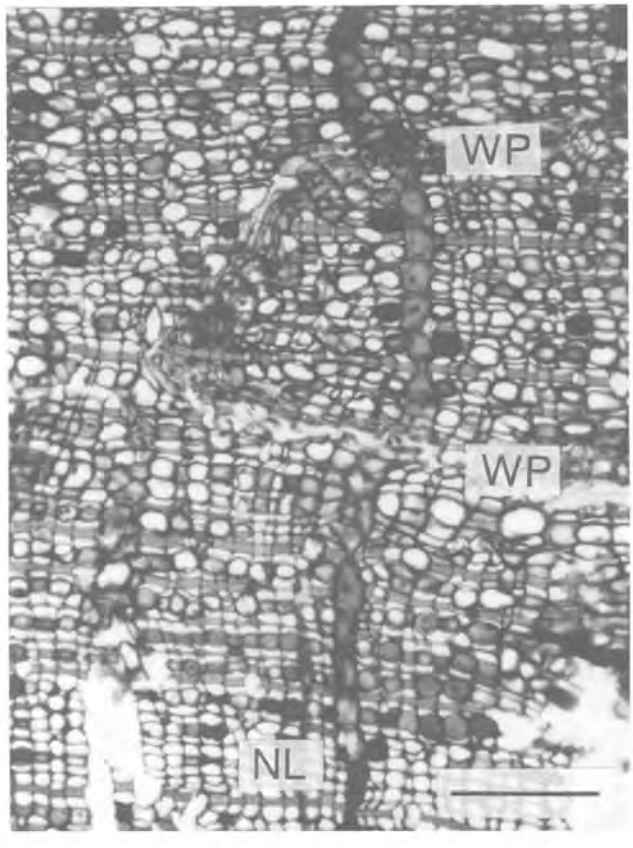


Figure 8. Expansion of phloem necrotic lesion in August sample. Wound periderm (WP) is being formed to wall off necrotic lesion (NL) including living cells. Bar = 200 µm.

APPENDIX I

Topics related to each station visited during the field trips

A) Pre-meeting field trip

1. **Forest Health**: relation between the health variables of sugar maple and the environment (P. DesRochers).
2. **From maple sap to maple syrup**: visit to a sugar camp.
3. **White pine blister rust**: untreated plantation (G. Laflamme).
4. **White pine blister rust**: plantation treated by pruning at a young age (G. Laflamme).
5. **Christmas tree plantation**: balsam fir.

B) Stroll through Old Quebec City

Dutch Elm Disease Control Program by Quebec City (P. DesRochers and P. Côté).

C) Post-meeting field trip

1. **Salt spray symptoms on white pine and ice damage.**
2. **Tree nursery diseases:** *Sphaeropsis sapinea*, Cylindrocladium root rot, grey mold (*Botrytis cinerea*), Scleroderris canker and butternut canker (L. Innes et al.).
3. **Scleroderris canker, European race**: epidemiology. Control trial by pruning and thinning (G. Laflamme).
4. **Scleroderris canker, European race**: validation of control measures. Stratification of symptoms (G. Laflamme).
5. **Techniques of log-house construction.**
6. **White pine blister rust**: examples of severe damage; examples of a variety of symptoms and signs.
7. **Scleroderris canker, European race**: resistance trial of *Pinus banksiana* (G. Laflamme). **Other diseases**: hypoxylon canker on aspen and cherry tree black knot.
8. **Porcupine damage to red pine.**
9. **Butternut trees**: northern limit of the species and search for butternut canker.
10. **Scleroderris canker, European race**: epidemiology (snow pockets).

APPENDIX II

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