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Timing of Scots pine branch damage caused by large-tree type of *Gremmeniella abietina* var. *abietina* and the structure of epidemics in northern Finland

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ROVANIEMI RESEARCH STATION

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Timing of Scots pine branch damage caused by large-tree type of *Gremmeniella abietina* var. *abietina* and the structure of epidemics in northern Finland

Juha Kaitera

Academic dissertation

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public discussion in lecture room Sali 12 of the University's Main Building, Fabianinkatu 33, 3rd floor, on 5 December 1997, at 12 o'clock noon.

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The types of the causal agent of *Gremmeniella abietina* causing Scleroderris canker in northern Finland and the Kola Peninsula in north-western Russia within stands in the first-thinning stage or middle age, were identified using conidia morphology, fatty acid and sterol profiles and random amplified microsatellites of the isolates. Large-tree type (type A) *G. abietina* var. *abietina* was frequently isolated from Scots pine branches formed and injured in the early 1990s above the snow cover. Both small-tree type (type B) and large-tree type were isolated within the same stands from small seedlings below the snow cover. Both types were widely distributed over northern Finland and the Kola Peninsula (north-western Russia).

The patterns of past epidemics caused by *G. abietina* were studied on Scots pine branches in a severely and slightly damaged stand in eastern Lapland. The disease history was determined using a branch analysis method, in which scars and cankers caused by *G. abietina*, leader changes, branch mortality and *Tomicus* spp. attacks were counted systematically from each first-order branch. In the severely damaged stand, *G. abietina* had damaged pines at the stand level annually for decades but a severe epidemic developed in the 1980s, peaking in the mid-1980s. The pattern of the epidemics was confirmed by using the various variables included in the analysis. *Tomicus* spp. attacks increased on pine shoots after the fungal epidemic had subsided in the late 1980s. A similar pattern of epidemics was detected in the slightly damaged stand but the relative frequency of the damage was lower than in the severely damaged stand. In individual, severely damaged trees, the epidemic appeared stronger and lasted for longer period than in moderately or slightly damaged trees.

Fruit body production and sporulation of large-tree type *G. abietina* var. *abietina* was investigated in the slightly damaged stand after a local outbreak of the disease in the early 1990s. The large-tree type mainly produced pycnidia one year after infection and the conidia were disseminated two years after initial infection between late June and early July. Pycnidia were, however, produced on the infected shoots over several years, serving as an annual source of inoculum within the stand. Microconidia were detected inside pycnidia two years after the infection. There was no statistically significant difference between months during the growing period in the frequency of infected shoots, although June-July was the main time of infection.

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Contents

List of publications

This thesis is based on the following articles, which will be referred to in the text by their Roman numerals. All the papers are reprinted with the permission of the publishers.

I Kaitera, J.; Jalkanen, R., 1992: Disease history of *Gremmeniella abietina* in a *Pinus sylvestris* stand. Eur. J. Forest Pathol. 22, 371–378.

II Kaitera, J.; Jalkanen, R., 1994: The history of shoot damage by *Tomicus* spp. (Col., Scolytidae) in a *Pinus sylvestris* L. stand damaged by the shoot-disease fungus *Gremmeniella abietina* (Lagerb.) Morelet. J. Appl. Ent. 117, 307–313.

III Kaitera, J.; Jalkanen, R., 1995: Comparison of *Gremmeniella abietina* historical damage to Scots pines. Canad. J. Forest Res. 25, 1503–1508.

IV Kaitera, J.; Hantula, J.; Jalkanen, R., 1997: Development of fruiting bodies of large tree type of *Gremmeniella abietina* var. *abietina* and timing of infection on Scots pine in northern Finland. Eur. J. Forest Pathol. 27, 115–124.

V Kaitera, J.; Müller, M.; Hantula, J., 1997-8: Occurrence of *Gremmeniella abietina* var. *abietina* large- and small-tree types in separate Scots pine stands in northern Finland and in the Kola Peninsula. Mycol. Res. (in press).

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1 Literature review

1.1 Introduction

Disease monitoring is time-demanding and difficult in remote areas, as they are difficult to reach. Inaccurate damage estimates are, therefore, common and the estimates are often launched out dramatically without studying in detail the causal agents involved with the disease (VÄLIVERRONEN 1996). Disease estimates usually cover only a few-years period, which makes it difficult to study the causal agent involved with the damage symptoms and the factors affecting the damage development. Some efforts to date fungal epidemics in the past have been made (ROBAK 1964), but methods revealing the agents involved with the damage development in the past, are needed. The most destructive pathogen causing successive epidemics on Scots pine (*Pinus sylvestris* L.) is *Gremmeniella abietina* (Lagerb.) Morelet, which was first reported in the 1800s in Finland (KARSTEN 1884).

1.2 Variation in *Gremmeniella abietina*

1.2.1 Variation in the morphological and colonial development of *G. abietina*

Gremmeniella abietina was distinguished into separate types based on conidia septation for the first time by ETTLINGER (1945). He noted about two separate types of *G. abietina* with conidia of either 4 or 4 to 8 cells. Thereafter, DONAUBAUER (1974) and STEPHAN (1979) described the variation in the number of *G. abietina* conidia septa on different hosts in central Europe. Morphological and cultural variation of the European, North-American and Asian races of *G. abietina* var. *abietina* was thereafter investigated by DORWORTH and KRYWIENCZYK (1975).

In Finland, UOTILA (1983) described two distinct types of *G. abietina* var. *abietina*, of which type A produces conidia with 3 septated cells and type B with 3 to 7 septated cells. He also found differences in the colony area development of the corresponding types even among isolates originating from single ascospores within single asci (UOTILA 1990a, 1992, 1993). KAITERA and JALKANEN (1996) also found statistically significant differences in the colony growth of individual isolates both within and among the A and B type of *G. abietina* var. *abietina*, and even among isolates within a single stand in northern Finland, indicating the great variation within *G. abietina* var. *abietina*.

1.2.2 Biochemical and genetical variation in *G. abietina*

Three races of *G. abietina* var. *abietina*, and two types within the European race of the previous variety have been distinguished based on their pectic enzymes (LECOURS et al. 1994), protein patterns (PETRINI et al. 1989, 1990), immunoblotting (PETÄISTÖ et al. 1996), fatty acids and sterols (MÜLLER and UOTILA 1997), RAPDs (HAMELIN et al. 1993, 1996, BERNIER et al. 1994, HELLGREN and HÖGBERG 1995, HANSSON et al. 1996) and randomly amplified microsatellites (HANTULA and MÜLLER 1997). Some of the former Scandinavian studies have (HELLGREN and HÖGBERG 1995, HANSSON et al.1996), however, dealt with isolates from either seedlings or from more southern locations. The number of northern Finnish large-tree type *G. abietina* var. *abietina* isolates has been low in such studies (PETRINI et al. 1990, HANTULA and MÜLLER 1997, MÜLLER and UOTILA 1997), most of the isolates being from the southernmost part of northern Finland representing mainly small-tree type *G. abietina* var. *abietina*.

1.2.3 Variation in symptomatology, infection biology, pathogenicity and fruit body production and maturation of *G. abietina*

Gremmeniella abietina primarily infects the bud of the youngest shoot, where the infection is highly restricted (SKILLING 1972, DONAUBAUER 1974), and penetrates via bract stomata (PATTON et al. 1984). In the infected shoot, the bud becomes resinous (READ 1966, GREMMEN 1972, PATTON et al. 1984) and the fungus colonizes needle and shoot tissues next to the bud during the following dormant period (READ 1966, LANG and SCHÜTT 1974, SIEPMANN 1976). The symptoms appear the following spring or early summer as the new bud is poorly formed (SLETTEN 1971, GREMMEN 1972), needles turn brownish from their bases and lose their retention (BJÖRKMAN 1959, PATTON et al. 1984). Symptoms normally appear in the one-year old shoots of large pines one year after infection (WALDIE 1926) but they have also been reported to appear rarely on seedlings more than one year after the infection (LAFLAMME 1986, MAROSY and PATTON 1988, BARKLUND and UNESTAM 1988).

As an indication of slight and locally restricted infection on the shoot, *G. abietina* also causes cankers of various shape on *Pinus* spp. branches and stems (ROLL-HANSEN 1964, OHMAN 1966, READ 1966, DIETRICHSON 1968, POMERLEAU 1971, DORWORTH 1973, DONAUBAUER 1974, AALTO-KALLONEN and KURKELA 1985, KARLMAN et al. 1994). Cankers occur on stems of pine seedlings near the ground level (OHMAN 1966, SLETTEN 1971, DORWORTH 1973, KURKELA 1981), and some of the cankers may primarily be caused by frost (ROLL-HANSEN 1964, POMERLEAU 1971). The resin content of the cankered wood is higher compared to healthy wood (READ 1966, SLETTEN 1971, DORWORTH 1973, PATTON et al. 1984), and a typical yellow-greenish colour is evidence for the presence of *G. abietina*

mycelia in the canker (OHMAN 1966, READ 1968b, POMERLEAU 1971, SLETTEN 1971). The chemical composition of *G. abietina* metabolites is also typical in the coloured wood (e.g. AYER et al. 1989). These cankers may expand for years (KURKELA and NOROKORPI 1979, AALTO-KALLONEN and KURKELA 1985), and may finally girdle the tree above the canker (ROLL-HANSEN 1964).

In the infected shoots pycnidia appear one year after infection (ROLL-HANSEN 1964, KURKELA 1967, DORWORTH 1972, UOTILA 1985) and apothecia 2 years after infection (KUJALA 1950, ROLL-HANSEN and ROLL-HANSEN 1973a). Conidia are disseminated mainly during the late spring and early summer (SKILLING 1969, NEVALAINEN 1986), and the ascospores are disseminated in the mid and late summer (SKILLING 1969, NEVALAINEN 1986, LAFLAMME and ARCHAMBAULT 1990). According to ROLL-HANSEN (1982) apothecia are more common under continental weather conditions than on coastal ones, as the occurrence is opposite for pycnidia. Both apothecia and pycnidia have been reported on Scots pine in north-eastern Fennoscandia (KRUTOV 1993).

Studies dealing with the time of *G. abietina* infection are few. According to YOKOTA et al. (1974) and PETÄISTÖ and REPO (1988), artificial *G. abietina* inoculation succeeds best when inoculation is performed in the early summer. There is, however, variation in the frequency of successfull infections within the growing period as well as between years (BAZZIGHER et al. 1986).

Studies dealing with the infection biology, occurrence, sporulation or maturation of fruit bodies of different types of *G. abietina* are few or lacking. According to UOTILA (1992), type A *G. abietina* var. *abietina* produces more pycnidia and less apothecia than type B of the same variety *in vivo* but type B produces more conidia than type A *in vitro*. The conidial production, however, varies greatly even among individual ascospore cultures originating from single asci (UOTILA 1990a). According to UOTILA and TERHO (1994), type A *G. abietina* var. *abietina* is more pathogenic than type B of the same variety.

1.3 Historical epidemics caused by *G. abietina* in northern Fennoscandia

The earliest reliable Scleroderris canker epidemic on pine in northern Finland was reported in the 1940s (KUJALA 1950). Prior to this, KANGAS (1937) had noted damage on pine seedlings with symptoms resembling Scleroderris canker in wide areas in northern Finland. Thereafter, BJÖRKMAN (1959) and KOHH (1964) reported a severe epidemic in pine nurseries in 1959 in northern Sweden. During 1960s and 1970s, KRUTOV (1993) described a severe Scleroderris canker epidemic in 1965 and 1967 in northern Russia, and KURKELA (1967), VALTANEN (1970) and NOROKORPI (1971) reported annual damage between 1967 and 1970 in northern Finland in young pine plantations. The next serious epidemic in northern Finland

was reported in pine plantations at high elevation in 1982 (UOTILA and JALKANEN 1982). After this, KARLMAN et al. (1994) reported Scleroderris canker epidemics in 1987, 1988, 1990 and 1992 in young plantations in northern Sweden. The type of *G. abietina* was, however, not identified in any of the previous studies. In northern Finland, KAITERA and JALKANEN (1994a) reported for the first time Scleroderris canker in locally restricted areas near water sheds in naturally regenerated pine stands in the first-thinning stage and middle age. Previous damage had occurred in the 1980s but fresh damage was still present in some areas in the early 1990s indicating that local epidemics were still continuing (KAITERA and JALKANEN 1994a). The last severe epidemic was recorded in 1994, again in artificially regenerated pine plantations over wide areas in northern Finland (KAITERA, unpublished data).

1.4 Agents causing primarily necrosis and potentially associated with *G. abietina* necrosis

1.4.1 Fungi

Only a few fungi cause necrosis on Scots pine branches and stems in northern areas. Cankers caused primarily by these fungi can be distinguished from those caused by *G. abietina* due to lack of yellow-greenish colour and high amount of blue-staining in the wood, and location of the canker in the bark and sapwood. According to KUJALA (1950), *Crumenulopsis sororia* (P. Karst.) Groves and *Lachnellula fuscosanquinea* (Rehm) Dennis are parasitic in northern Finland. High resin flow, blue-staining and zonatic growth patterns in the wood are common for *C. sororia* cankers (LAGERBERG and SYLVEN 1913, KUJALA 1950, VLOTEN and GREMMEN 1953, GREMMEN 1960, 1976). Whereas *C. sororia* causes cankers mainly on pine stems, *L. fuscosanquinea* has been detected only on dead branches of small seedlings (KANGAS 1937, KUJALA 1950). *Lachnellula pini* (Brunch.) Dennis causes resinous cankers near the ground level on young pine stems (HAHN and AYERS 1934, VAARTAJA 1953, KAITERA and JALKANEN 1993), being nearly as pathogenic as *G. abietina* (KURKELA and NOROKORPI 1979). The pathogenicity of *Cenangium ferruginosum* Fr. ex Fr. has been discussed in Scandinavia and in Central Europe, but it is considered saprophytic (LAGERBERG and SYLVEN 1913, KUJALA 1950, VLOTEN and GREMMEN 1953, ROLL-HANSEN 1967, GREMMEN 1976). *C. ferruginosum* may also infect pine branches after *G. abietina* infection (GREMMEN 1960, READ 1968b).

Several saprophytic fungi have been identified on pine branches and stems but their ability to produce necrosis is minimal. Among such species, *Crumenulopsis pinicola* (Rebent) Groves and *Lachnellula subtillissima* (Cooke) Dennis cause no necrosis on pine branches and bark occurring

mainly in southern Finland and more southern climate (KUJALA 1950, GREMMEN 1960, 1976, LEHTIJÄRVI 1992). *Lachnellula chrysophthalma* Pers. ex Karst. is a common saprophyte on bark of fallen branches of pine seedlings (LAGERBERG and SYLVEN 1913, KUJALA 1950, VAARTAJA 1953), and may occur in *L. pini* cankers (ROLL-HANSEN 1967). *Pezicula livida* (Berk & Br.) Rehm is a saprophyte on pine bark and wood of dead lower branches (GREMMEN 1960, 1976), and it may infect secondarily pine after snow damage (KUJALA 1950). Also *Sphaeropsis sapinea* (Fr.) Dyko & Sutton occurs saprophytically on bark of pine shoots (KUJALA 1950). *Sydowia polyspora* (Bref. & Tav.) E. Müller (anam. *Sclerophoma pityophila* (Corda) Höhn.) occurs commonly on pine branches in northern Finland (KUJALA 1950), and may infect secondarily cankers caused by frost (JØRSTAD 1925) or *G. abietina* (BUTIN 1966, READ 1968b). *Tympanis pinastri* Tul. occurs saprophytically on thin bark of dead branches (KUJALA 1950). *Therrya fuckelii* (Rehm) Kujala and *Therrya pini* (Alb. & Schwein.) occur saprophytically on branches all over Finland (KUJALA 1950, LEHTIJÄRVI 1992). *T. fuckelii* is considered as saprophytic on thick branches in Central Europe, and is often associated with *C. pinicola* (GREMMEN 1960, 1976), as *T. pini* occurs only in dead plant material (GREMMEN 1958). *Biatorella difformis* (Fr.) Vain. is associated with cankers in the bark and sapwood that blue-stain strongly, and may be slightly pathogenic (KUJALA 1950). According to KRUTOV (1994), a corresponding canker has sharp edges and dark brown surface occurring on small seedlings. *Biatorella resinae* Fr. Mudd may also be slightly pathogenic on pines, and it is associated with resinous cankers resembling those of *C. sororia* (AYERS 1941, GREMMEN 1960). In addition, *Phacidium coniferarum* (Hahn) Kendrix causes necrosis on pine shoots in pruning wounds, and has occasionally been found in northern Finland (UOTILA 1990b). *Scoleconectria cucurbitula* (Tode: Fr.) Booth infects recently killed pine branches all over Finland (KUJALA 1950), and is considered as saprophyte (GREMMEN 1976). *Valsa pini* (Alb. et Schw.) Fr. occurs also on pine branches in northern Finland (KUJALA 1950), and is considered as saprophyte (KUJALA 1950, GREMMEN 1960).

Some rust fungi may also infect young pine shoots in northern Finland causing in some cases cankers or lesions on the shoots. *Melampsora pinitorqua* (Braun) Rostr. causes necrotic wounds on young seedlings (KUJALA 1950), and in case of a slight infection, these wounds heal and next to the wound, the shoot is bent the following winter (KURKELA 1973). This type of necrosis occurs commonly on Scots pine only in the southern part of northern Finland (KURKELA 1969, JALKANEN and KURKELA 1984). Also *Peridermium pini* (Pers.) Lev. causes necrotic lesions on pine branches and stems all over northern Finland (KAITERA and JALKANEN 1995b). A characteristic feature for the lesion is a resinous, blackish swollen wood on the shoot, with traces of old aecia in the lesion (PAWSEY 1964).

1.4.2 Insects

Several species in the order Hemiptera cause necrotic spots in the cambium of young pine shoots either by sap-sucking or mechanically (GRAHAM 1952). According to SAALAS (1949), *Aradus cinnamomeus* Panz., *Elasmucha grisea* L., *Pineus pini* Macq., *Lachnus pineti* Koch and *L. nudus* De Geer. cause such necrosis on young pine seedlings. *L. pineti and L. nudus* may also suck sap on stems and branches of older pines, the latter one being common also in northern Finland (KANGAS 1937, SAALAS 1949). Also some species in orders Diptera, Lepidoptera and Coleoptera cause damage to the meristem (GRAHAM 1952). *Prosternon tessellatum* (L.) and *Magdalis violacea* (L.) make small holes in thin bark of young seedlings, whereas *Hylobius* spp., *Monochamus sutor* (L.) and *Pissodes* spp. cause such holes on shoots of older pines (SAALAS 1949). *Dioryctria splendidella* H.S. larvae live in the resinous tumors on shoots of both young pine seedlings and older trees, and hollow-out young shoots leaving wood chips inside the tunnel (SAALAS 1949). *Cydia coniferana* (Ratz.) acts similarly on shoots of older trees, and *Ernobius nigrinus* (Sturm.), *Magdalis frontalis* (Gyll.) and *Dioryctria mutatella* Fuchs. larvae cause feeding tunnels only in young pines (SAALAS 1949). Only *D. mutatella* of the previous species occur rarely in northern Finland (KANGAS 1937, SAALAS 1949).

The most important insects causing damage especially in wind-thrown pine trees in northern Finland are *Tomicus* spp. (*T. piniperda* (L.), *T. minor* (Hart.), SAARENMAA 1987). They produce feeding tunnels in the youngest pine shoots, but as a distinguishing feature for larval tunnels, *Tomicus* spp. clean the tunnels of wood chips (TRÄGÅRDH 1921, SAALAS 1949). *T. piniperda* feeding tunnels are also resinous in their margins (SAALAS 1919). Some species of Lepidoptera are common in northern Finland causing damage on pine shoots. *Retinia resinella* (L.) larvae cause resinous tumors especially on leader shoots of young pine seedlings (KANGAS 1931) but also on lower branches of older trees (KANGAS 1931, SAALAS 1949). These tumors often occur only on one side of the shoot, and leader above the tumor is often broken (SAALAS 1949). In the case of a slight tumor, the leader survives and a canker is formed (KANGAS 1931), which may remain open at the central part of the canker (KANGAS 1931). The healed cankers do not, however, reach the pith of the shoot (KANGAS 1931).

According to SAALAS (1949) *Rhyacionia buoliana* (Den. & Schiff.), *Rhyacionia pinicolana* (Doubl.), *Rhyacionia duplana* (Hb.), *Blastesthia turionella* (L.), *Blastesthia posticana* (Zett.), *Rhyacionia pinivorana* (Lienig & Zeller) and *Exoteleia dodecella* (L.) larvae feed in the buds and shoots of young pine seedlings forming a tunnel in the shoot. *R. buoliana*, *R. pinicolana* and *E. dodecella* form tunnels rarely also in young shoots of older trees (SAALAS 1949). Only *R. pinicolana*, *B. turionella*, *B. posticana* and *E. dodecella* of the previous species occur in northern Finland (KANGAS 1937, SAALAS 1949).

1.4.3 Abiotic factors

Cankers caused by abiotic factors are usually annual as the agency causing the disease is operative only for one season. With perennial cankers caused by fungi (e.g. *Lachnellula willkommii* (Hartig) Dennis, see HOPP 1957), the causal agency functions year after year, finally girdling the host above the canker (BOYCE 1948). Frost has been considered as an important predisposing factor for fungal canker formation (LANGNER 1936). According to READ (1967), frost causes necrosis on buds without any resin flow. Later in the case of an open canker, however, resin flow occurs in the brown or dark-brown canker wood (EICHE 1966). On seedlings, frost injures meristem and new xylem cells leaving a clearly detectable frost ring in the wood (DAY and PEACE 1934). In this ring of wood, new xylem cells are wider, more irregular in shape and contain a thinner wall compared to uninjured cells (PEACE 1962). Functionally, frost damage is caused by ice crystalization inside plant cells followed by either mechanical disruption of the protoplasm or absorption of water through cell walls (PEACE 1962).

In the case of frost associated with *G. abietina*, POMERLEAU (1971) showed the presence of frost rings with dead outer cambium, and concluded that frost injury was followed by *G. abietina* infection. Also DIETRICHSON (1968) and SLETTEN (1971) found frost rings in stem cankers of seedlings infected by *G. abietina*. Frost cankers, however, occur mainly in the lower branches or stem of small seedlings near the ground level (EICHE 1966, DIETRICHSON 1968, POMERLEAU 1971), and they occur only rarely in old wood tissues (EICHE 1966). In artificial studies, YOKOTA et al. (1974) also suggested that early frost might have increased the number of successful infections on seedlings. ROLL-HANSEN (1964) described both frost and *G. abietina* cankers, and concluded that they were easily distinguishable, and that only in a few cases *G. abietina* had infected stems after frost damage. Neither SKILLING (1972) nor DORWORTH (1972) consider frost cracks as important infection courts for *G. abietina* infection.

Some other abiotic factors may also cause necrosis on pines. Hail storms can cause cankers mechanically. These cankers are restricted in their occurrence, occurring usually only at one side of the branch or stem with a crack in the middle of the canker (BOYCE 1948). According to COOK (1925), hail damage may occur several mm deep in the wood. Also glaze has been reported to injure stems of woody plants by forming an ice layer on the windy side, followed by a sharp-edged damage in the bark after ice cracking (LUTZ 1936). The damage, however, occurs only near the ground level and is identified by healed protuberance in the bark (LUTZ 1936). Crown snow load may also break, bend or cut off branches mechanically causing necrosis on injured branches (e.g. HEIKINHEIMO 1920).

1.5 Factors affecting epidemics caused by *G. abietina*

Gremmeniella abietina is both favoured (AALTO-KALLONEN and KURKELA 1985, BARKLUND and UNESTAM 1988, PETÄISTÖ and REPO 1988) and unfavoured by low temperatures (DORWORTH 1972). According to UOTILA (1988), also low temperature sum favours *G. abietina* outbreaks. *G. abietina* epidemics are also enhanced by shading (READ 1968a, DONAUBAUER 1972, AALTO-KALLONEN and KURKELA 1985, UOTILA 1988), high relative humidity (DONAUBAUER 1972, AALTO-KALLONEN and KURKELA 1985), free water (SMERLIS 1968, SKILLING 1969), and high total amount of rainfall during the previous summer (UOTILA 1988). Some authors consider frost as an important predisposing factor for *G. abietina* infection (EICHE 1966, DIETRICHSON 1968, SLETTEN 1971, DONAUBAUER 1972, UOTILA 1988), and some authors have an opposite opinion about its role (DORWORTH 1972, SKILLING 1972). Low light intensity (PETÄISTÖ and REPO 1988) and fertilization (DONAUBAUER 1972, PÄTILÄ and UOTILA 1990) may also favour *G. abietina* epidemics. READ (1968a), however, states that pines with poor vigour are more susceptible to *G. abietina* infection than those with good vigour.

Most severe Scleroderris canker occur in deep depressions (READ 1968b, Dorworth 1972, 1973, AALTO-KALLONEN and KURKELA 1985, SAIRANEN 1990, KAITERA and JALKANEN 1995a) that both receive large numbers of spores (DORWORTH 1972) and the microclimate is *G. abietina* favouring (DORWORTH 1972, UOTILA 1988). Neither damage inventory studies (KAITERA et al. 1995a) nor artificial inoculation experiments (LAURENCE et al. 1984, BRAGG and MANION 1984, VUORINEN 1990, VUORINEN and UOTILA 1997) suggest that *G. abietina* is favoured by SO_2 or sulphuric acid. Neither is the frequency of endophytes diminished more strongly than that of *G. abietina* on pine shoots due to heavy metals and SO_2 *in vitro* (RANTA et al. 1994).

2 Study objectives

A preliminary objective for the project this investigation was included was to characterize damage recently observed on Scots pine in northern Finland (e.g. VÄLIVERRONEN 1996), and to determine the most important agents involved with the damage symptoms. After the causal agents were identified (e.g. KAITERA and JALKANEN 1993), it was important to investigate the structure of the epidemics and damage patterns on pine. Pine sapling stands were excluded from all the studies. The specific objectives of the different studies were as follows.

The preliminary objective was to identify the causal pathogen isolated from branches showing typical symptoms on damaged pines using both ecological methods and novel biochemical and genetical techniques (IV, V). In these studies, it was also important to clarify the variation and distribution of the causal type within single pine stands suffering from successive outbreaks of the disease.

The second objective was to investigate the damage pattern of past epidemics of pine in northern Finland. For this purpose, a new determination method was developed based on the presence of damage in annual pine shoots (I). As a case study (I), the damage pattern of epidemics of pine was aimed to clarify in a severely damaged stand in eastern Lapland (Rikkilehto). Subsequently, it was important to confirm the use of the method in a slightly damaged pine stand representing typical damage in northern Finland (KAITERA and JALKANEN 1994a), and also to clarify whether the damage pattern of the epidemics was similar on pines damaged at various levels (III).

The third objective was to clarify the life cycle of *G. abietina* during an epidemic in a damaged stand. This was done to get evidence of damage patterns for past epidemics. Therefore, *G. abietina* fruiting, sporulation and time of infection were investigated in the slightly damaged stand in northern Finland (IV).

Fourthly, insects (*Tomicus* spp.) were supposed to play some role on pines in the damaged stands, and therefore, it was necessary to date *Tomicus* spp. attacks and to study the relationship between the fungal damage pattern of the epidemics and the *Tomicus* spp. damage pattern. Therefore, a new method for detecting *Tomicus* spp. attacks in the past was necessary to develop and further confirm (II, III).

3 Material and methods

3.1 Study areas

The type of *G. abietina* causing Scleroderris canker on Scots pine branches of trees in the first-thinning stage and on suppressed seedlings, was identified in three slightly and one moderately damaged stand in northern Finland and north-western Russia (IV, V, KAITERA et al. 1995b). The past disease history of pine was determined in a severely damaged stand (Rikkilehto) and a slightly damaged stand in northern Finland (I, II, III, KAITERA and JALKANEN 1993). *G. abietina* pycnidia and apothecia production, sporulation and time of infection were investigated during an epidemic in a slightly damaged stand (IV).

3.2 Sample trees

Gremmeniella abietina was isolated and identified from branches above snow cover and seedlings under snow cover of 96 randomly chosen pine trees (one isolate per tree) in 4 stands (IV, V). In the severely damaged pine stand (Rikkilehto), 14 pine trees were selected randomly among a higher number of trees to represent evenly all damage and size classes of the trees. In the slightly damaged stand, 5 pines from 3 damage class of each, were selected systematically for the disease history study (III). In the previous stand, 19 evenly scattered trees were selected randomly for investigating *G. abietina* fruiting and sporulation, and 65 trees were selected similarly for timing the *G. abietina* infection within the growing period.

3.3 Determining *G. abietina* history of epidemics and associated *Tomicus* spp. attacks on branches

3.3.1 Variables determining *G. abietina* damage

Branch damage caused by *G. abietina* was determined systematically from living and dead first-order branches using either healed (scars) or unhealed (cankers; see ROLL-HANSEN 1964, KURKELA 1981) necrosis of the shoots (I, II, III). This necrosis reaches the pith and the first tree ring of the shoot, as the annual infection occurs in the current-year shoot (SKILLING 1972, DONAUBAUER 1974), causing necrosis on the shoot after the first dormant period (LANG and SCHÜTT 1974, SIEPMANN 1976, PATTON et al. 1984). A yellow-greenish colour of the infected wood is distinguishing evidence for the presence of *G. abietina* mycelia in the infected tissues (OHMAN 1966,

READ 1968b, KURKELA 1981). The shape of the canker is also a typical feature for *G. abietina* canker on Scots pine branches (see KURKELA 1981). Cankers caused by frost include frost rings that are rare in mature pine trees compared to seedlings (DAY and PEACE 1934, EICHE 1966, DIETRICHSON 1968), were excluded from the analysis. Also necrosis caused by hail, insects (SAALAS 1949) or other canker pathogens (KUJALA 1950), without symptoms associated with *G. abietina* necrosis, were excluded from the analysis.

The following variables were used as additional variables in the analysis. Leader change (I, III) includes direct symptoms of shoot dieback (READ 1968b) with *G. abietina* fruit bodies, colouring of infected tissues (OHMAN 1966, KURKELA 1981) and death of the bud with high resin flow (DORWORTH 1972), or indirect symptoms via strangulation of the branch in cankers on older shoots (ROLL-HANSEN 1964). Due to difficulties in distinguishing indirect symptoms from symptoms caused by other agents in some cases, all leader changes were counted together. Branch mortality revealed the years when whole branches had died (I, II, III). It gives a rough estimate for the overall damage pattern without, however, providing any specific information about the causes for the damage.

The relative number of each variable was counted per shoot and the annual average figures were compared graphically to each other (I, III). Due to discontinuous nature of these variables, no statistical procedures were used to compare the corresponding relative numbers between individual years.

3.3.2 Variables determining the time of *Tomicus* spp. attacks

Tomicus spp. attacks were determined from each first-order branch (II, III), based on the occurrence of a feeding tunnel in relation to the current-year shoot at the time of attack (II). The age of the attacked shoot was determined using internodes, living side branches and year rings in the leader next to the base of the feeding tunnel. *Tomicus* spp. feeding tunnels were distinguished from tunnels caused by other insects on pine shoots by the lack of wood chips inside the tunnels (TRÄGÅRDH 1921, SAALAS 1949). It was also assumed that the attacked shoot prematurely dies soon after the attack without forming any new growth after the attack. This assumption does not, however, affect the results, as no new growth is formed in the corresponding shoot after the attack (TRÄGÅRDH 1921). Attacks occurring before shoot extension (TRÄGÅRDH 1921) were attributed to the previous year's shoot.

The annual *Tomicus* spp. attack patterns were compared graphically to leader change patterns and patterns of other variables determining *G. abietina* damage and between years (I, II, III).

3.4 *G. abietina* fruit body production and sporulation during an epidemic

A new peak in the pattern of epidemics was observed in the slightly damaged stand in 1992. During the epidemic, 100 shoots formed in 1991 and showing Scleroderris canker (incl. killed bud and leader, coloured needles with poor needle retention; see also BJÖRKMAN 1959, ROLL-HANSEN 1964, KURKELA 1967, 1981) were selected randomly among 19 pines, and the fruit body production and sporulation of *G. abietina* were observed monthly on the shoots for two years (IV). The shoots were observed in the field using a pocket microscope, and the frequency of *G. abietina* fruit bodies, the number of pycnidia with conidia oozing out detected as slimy tendrils (see ROLL-HANSEN 1964), were checked on these shoots once or twice a month between July 1992 and September 1993 (IV). Another 100 shoots formed in 1992 were checked in the field four times between August 1993 and August 1994 with the same variables using the same procedure as for the shoots formed in 1991.

Twenty sample shoots formed in 1991, located next to shoots observed in the field and bearing *G. abietina* pycnidia were selected randomly once or twice a month between October 1992 and September 1993, and were checked under the stereo and light microscopes in the laboratory. The variation in the number of conidia septa, and the frequency of conidia and microconidia were recorded on a microscope slide after smashing one to five pycnidia on the slide (IV).

3.5 Determining *G. abietina* branch infection within growing period during an epidemic

Infection caused by *G. abietina* was dated monthly between June and September 1993 by covering and revealing healthy shoots in 65 trees (IV). First, 100 shoots were covered each month for one month using a pollination bag. As a control, 100 branches were kept uncovered. Second, 250 shoots were covered in late May, followed by exposing 50 shoots monthly for one month. Fifty branches were covered and 50 branches were uncovered during the whole period of investigation. The frequencies of monthly infected branches were compared between months (IV).

3.6 Determining the type of *G. abietina* in the Scots pine stands

3.6.1 Identification based on conidia morphology, host size and disease symptoms

The occurrence of different types of *G. abietina* above the snow cover was described both within separate Scots pine stands, and among diseased branches including shoot dieback, pycnidia and cankers caused by *G. abietina*, and infected seedlings under snow cover (V). The type of 84 *G. abietina* isolates was determined after 54-days' incubation at 15 °C in the light on agar flasks by counting the number of conidia per ml, and determining the variation in the number of septa per conidiospore by using a Burger haemocytometer (V). Those isolates, which did not produce conidia during the 54 day's incubation, were further incubated for a maximum of 124 days. The isolates were then classified either as small-tree type (type B), in case the conidia had more than six septa, or as large-tree type (type A), in case they had less than seven septa (UOTILA 1983). In case the isolate did not produce any conidia during the incubation period, it was classified as either large-tree type or small-tree type based on the host size, and the occurrence and development of pycnidia and apothecia on the shoot. Isolates from branches occurring above the snow cover next to pycnidia were classified as large-tree type, and those occurring under snow cover next to apothecia were classified as small-tree type (V).

3.6.2 Identification based on fatty acid profiles

The isolates were inoculated on modified orange serum agar (MOS) (MÜLLER et al. 1994), incubated at 21 °C in the dark for 36 days, after which 1.5 g of mycelia were harvested for further analysis. The FAST profiles of the isolates were determined using the protocol described in MÜLLER et al. (1994), followed by classification of *G. abietina* into either large-tree type or small-tree type using models based on discrimination analysis earlier described in MÜLLER and UOTILA (1997).

3.6.3 Identification based on randomly amplified microsatellites

Eighty-four (V) and 12 (IV) *G. abietina* isolates were identified using randomly amplified microsatellite technique (HANTULA et al. 1996) with marker specificities described in HANTULA and MÜLLER (1997). The protocol included cell disruption, several extractions and precipitation (HANTULA et al. 1996). After this, PCR-reactions were carried out as in HANTULA and MÜLLER (1997). The amplification products were separated by electrophoresis (HANTULA and MÜLLER 1997), and the types were identified mainly based on the type specificity of markers observed by HANTULA and

MÜLLER (1997). For the identification, CCA1500 marker, two markers with CCA primer with approximate molecular weight of 700-750 bp (see HANTULA and MÜLLER 1997), and CCA150 marker were used for all the isolates. Additional identification markers, ACA700 and CGA500, were used for 15 isolates (see HANTULA and MÜLLER 1997).

3.7 Statistical analysis

Annual relative number of different damage variables were compared graphically between years without any statistical analysis due to the discontinuous nature of the variables used (I, II, III). Number of infected branches was compared between months using χ^2 test by SAS (SAS Inc., Cary, U.S.A.) (IV). Fatty acid and sterol profiles of *G. abietina* were identified using Systat for Windows v. 5.0 (Systat Inc., Evanston, U.S.A.) for discriminant analysis, and fatty acids and sterols of different types were compared using SAS (SAS Inc., Cary, U.S.A.) for variance analysis.

4 Results

4.1 The type of *G. abietina* damaging Scots pine

According to genetical analysis (RAMS), the type of *G. abietina* isolated from pycnidia, cankers or branch wood above the snow cover, was identified as large-tree type of *G. abietina* var. *abietina* in all four stands investigated (IV, V). Based on the previous analysis, both large-tree type and small-tree type were identified from pine seedlings under the snow cover in three out of four stands (V).

The other two methods applied gave almost identical results compared to RAMS analysis. According to *G. abietina* FAST profiles, both types of *G. abietina* var. *abietina* were identified among isolates from both branches and seedlings (V). Twelve isolates could not be classified as either of the types with statistical significance at $p < 0.01$ based on discriminant analyses of their FAST profiles. Two isolates were classified differently at $p < 0.01$ than with both other methods applied. Statistically significant differences between isolate groups were only found between small-tree type and large-tree type isolate groups (V).

According to conidia morphology, 10 isolates were classified as small-tree type, 65 as large-tree type, and 9 isolates did not produce conidia during the incubation period. Eight of these isolates were classified as large-tree type based only on host size and fruit body production *in vivo*, and only one isolate could not be identified. Two isolates from branches were classified as small-tree type, and the rest of the isolates were classified as large-tree type. Eight isolates out of fifteen from seedlings were classified as small-tree type, and 6 isolates were classified as large-tree type.

4.2 The disease pattern of *G. abietina* epidemics on Scots pine

According to branch analysis *G. abietina* had infected pine branches for decades (I, III). The past disease pattern showed several minor peaks in the pattern of epidemics and one long-lasting epidemic at stand level. The first scars and cankers were recorded in the 1940s with peaks in the epidemics in 1948 and 1957 (I, II). After some minor peaks in the late 1960s and early 1970s in the study areas, a severe epidemic broke out in the early 1980s, being at the highest level from 1982 to 1988 and peaking in 1984 (I, III). The epidemic was stronger and lasted for a longer period in trees most severely damaged by *G. abietina* (III). In the late 1980s the epidemic subsided in the severely damaged stand (I), but the epidemic still continued in the slightly damaged stand (III).

The patterns of other variables supported the major *G. abietina* disease pattern (scars and cankers) during 1980s (I, III). The number of leader changes peaked from 1983 to 1991, being at a high level also after the *G. abietina* epidemic (I, III). A similar trend occurred in annual branch mortality, which peaked during the mid and late 1980s (I, III).

4.3 *Tomicus* spp. attacks in relation to pattern of *G. abietina* epidemics

Tomicus spp. attacked pine shoots in the slightly damaged stand in the 1950s (III) and in the severely damaged stand in the 1960s (II). A small peak in the number of attacks occurred in 1983-1984 at the same time as the *G. abietina* epidemic peaked in the severely damaged stand (II) but a higher peak occurred in both areas from 1988 onwards after the *G. abietina* epidemic had subsided (II, III). The highest peak in the number of leader changes occurred at the same time as the number of attacks peaked in both study areas (II, III). *Tomicus* spp., however, played only a minor role in the damage development in the slightly damaged stand (III).

4.4 Production and maturation of *G. abietina* fruit bodies, and timing of infection during an epidemic

Gremmeniella abietina fruit body production and maturation was similar on the 1991- and 1992-formed shoots both in the field and in the laboratory. Pycnidia appeared on the shoots primarily in late summer, one year after infection, although some variation in the frequency and appearing of pycnidia between successive years was observed (IV). Pycnidia started to sporulate in early June, 2 years after infection, and all pycnidia were releasing conidia before the following August (IV). Fresh pycnidia and microconidia inside pycnidia appeared on the previous shoots during the late summer 2 years after infection, between healthy and infected tissues. No *G. abietina* apothecia were formed on the infected and dead shoots during the 3-year period of investigation (IV). Conidia inside pycnidia were 1–6 celled, the majority being 1–4 celled. Based on this, the type of *G. abietina* involved was classified as large-tree type of *G. abietina* var. *abietina* (IV).

The number of 1993-infected shoots was low among the branches studied, varying monthly between 6% and 19%. The infection experiments indicated statistically non-significant differences between months in the number of monthly infected shoots (IV). July, however, appeared to be the main time of infection during the growing period based on the results of both experiments. The low frequency of *G. abietina* fruit bodies and fresh damage on the shoots in 1994 suggested that the epidemic suddenly stopped after an epidemic peak in the area (IV).

5 Discussion and conclusions

5.1 Identification of the type of *G. abietina* within the stands

Large-tree type (type A) *G. abietina* var. *abietina* was associated with pine shoot dieback, cankers and pycnidia on branches occurring both above and below snow cover (IV, V), but also small-tree type (B type) *G. abietina* var. *abietina* was found on seedlings below the snow cover within most of the stands (V). Although both types have been identified earlier in northern Finland based on different identification methods (UOTILA 1983, KAITERA and JALKANEN 1996, PETÄISTÖ et al. 1996, HANTULA and MÜLLER 1997, MÜLLER and UOTILA 1997), this is the first report of both types within the same stand. Earlier B type *G. abietina* var. *abietina* has been reported to be more common in northern Finland than A type of the same variety (UOTILA 1983), but this may also be due to identification of *G. abietina* almost solely concentrated on seedlings in pine regeneration areas. Recently, KAITERA and JALKANEN (1994a) reported Scleroderris canker on trees in the first-thinning stage or middle age in large areas in northern Finland. The former stands were similar to stands in this study, and therefore, there is a reason to believe that the former damage reported in northern Finland was caused by large-tree type *G. abietina* var. *abietina*. Although identification of *G. abietina* was made from less than a decade-old cankers, there is reason to believe that the large-tree type *G. abietina* infected branches decades ago just after the stand canopy was closed, since the small-tree type of the same variety was identified only under snow cover (V). According to ILVESSALO (1937), the average height of a healthy 40-year-old, naturally regenerated Scots pine is about 6 m, and the average dominant height of a corresponding 25-year-old Scots pine is about 3.5 m on sites similar to those in studies I and III. The former heights correspond to tree height during the first *G. abietina* infections and just before the infections became annual in the study areas (I, III).

There was some variation between typing based on different methods, but the results agreed relatively well with one another, even though the results of RAMS seemed to be the most reliable ones. Some contradicting typing based on FAST was probably due to small number of northern Finnish isolates used earlier for discriminant analysis (MÜLLER and UOTILA 1997), used for identification also in this study (V). Identification based only on conidia septation (UOTILA 1983) may not be reliable in some cases for distinguishing isolates of different types, as the septation varies greatly within the types. Elsewhere also ETTLINGER (1945), DONAUBAUER (1974) and STEPHAN (1979) showed the high variation in the number of *G. abietina* conidia septa. UOTILA (1992) even found *in vitro* conidia with both more and less than seven septa from mycelia originating from single ascospores within single asci.

5.2 The history of *G. abietina* epidemics on Scots pine

The disease history on pine branches revealed that *G. abietina* had damaged pines in both investigated stands for decades with the first peaks in the 1940s, but an increasing trend in the damage pattern was detected from 1960s to 1990s (I, III). Earlier BUTIN and HACKELBERG (1978) investigated the pattern of epidemics on pines during several years showing successive annual fresh damage at stand level. KOHH (1964), KURKELA (1967), NOROKORPI (1971), KRUTOV (1993), UOTILA and JALKANEN (1982) and KARLMAN et al. (1990) reported severe *G. abietina* epidemics in sapling stands in northern Fennoscandia from 1950s to 1980s and the same peaks were detected roughly also in the study areas (I, III). This is interesting, as the former reports were dealing with young seedlings without identifying the type of *G. abietina*, and damage on pines in the first-thinning stage or middle age in naturally regenerated pine forests were reported only recently (KAITERA and JALKANEN 1994a).

The damaged stands were located in local depressions or water sheds (KAITERA and JALKANEN 1993, III) proved to be highly susceptible to *G. abietina* (DORWORTH 1973, SAIRANEN 1990, KAITERA and JALKANEN 1995a). Despite this, the disease patterns were quite similar in both stands (I, III) suggesting that macroclimate also affects disease patterns. The disease broke out in both study areas as well as in individual pines injured at different degree in the early 1980s probably due to extremely favourable weather conditions for *G. abietina* sporulation at that time (I, III, see KAITERA and JALKANEN 1993). After the extremely wet summer of 1981, a severe *G. abietina* epidemic developed also in pine sapling stands in northern Finland (UOTILA and JALKANEN 1982). Earlier, several authors (e.g. SKILLING 1969, LAFLAMME and ARCHAMBAULT 1990, UOTILA 1988) showed the important role of water and moisture for sporulation and damage development. Also AALTO-KALLONEN and KURKELA (1985) found a correlation between weather conditions and the number of *G. abietina* cankers in the 1970s in southern Finland. In the study areas (I, III), the epidemic temporarily subsided probably due to climate favourable to pine at the end of 1980s. In the late 1980s, the number of scars was high on the branches (I), which was also the case in southern Finland after an epidemic peak (UOTILA 1988). The epidemic, however, seemed to continue longer especially in trees most severely damaged earlier by *G. abietina* (III). This pattern has also been detected in a similar stand in northern Russia (KAITERA et al. 1995b). The number of leader changes has earlier been reported to correlate with the peak of the *G. abietina* epidemic, which was also the case in this study, although this variable was not as specific as scar and canker to detect *G. abietina* damage.

Branch analysis is most effective in determining the patterns of epidemics when used with large number of branches with different degree of injuries. In the case of low number of branches, the pattern of epidemics may not be reliable (KAITERA et al. 1995a). As an assumption in the analysis,

symptomless infections and infection into older shoots were considered rare. Symptomless infections have been reported to occur mainly in a few-year old shoots of seedlings (BARKLUND 1984, RANTA and NEUVONEN 1994), but also in branches of older pines (V). Information about necrosis development in such branches is lacking but as such occasions have not been reported earlier, and symptomless infections rarely develop into disease more than two years after infection on seedlings, cases when symptomless infections one year after infection develop later into Scleroderris canker are probably few. In this study (I, II, III), this uncertainty was considered in the interpretations. It is also highly unprobable that the primary infection would occur in shoots older than the current year. The primary infection has been proved to occur on the youngest shoot via bract stomata (PATTON et al. 1984), after which the colonization of the shoot tissues starts during the first dormant period (LANG and SCHÜTT 1974, SIEPMANN 1976). Although *G. abietina* causes necrosis when artificially inoculated in older pine stems (KURKELA and NOROKORPI 1979, UOTILA 1992), and is probably capable of infecting seedling stems via frost cracks (ROLL-HANSEN 1964, EICHE 1966, DIETRICHSON 1968), these cankers would either not reach the pith of the shoot or there would remain a clear mark of the primary damage in the canker area. The minority of all necrosis is, however, caused by agents other than *G. abietina* in a similar stand as in this study in northern Fennoscandia (KAITERA et al. 1995b).

Annual relative number of infections is reasonable to compare between years, if all the corresponding shoots have the same possibility to get infected. This is probably seldom the case as the number of conidia is higher in depressions (DORWORTH 1972) and the environmental conditions predispose symptom development differently in such depressions and in different parts of the pine canopy.

5.3 *G. abietina* infection, fruit body production and maturation within the growing period

After a few year's period with a little annual fresh damage, a new epidemic developed locally in the slightly damaged stand in 1992, lasting for a few years (IV). KARLMAN et al. (1994) also reported of similar peak in the epidemics in northern Sweden in the early 1990s. The sudden ending of the epidemic was dramatic, as there were plenty of pycnidia on the shoots that even disseminated conidia efficiently during successive years (IV). The large-tree type *G. abietina* produced pycnidia one year after initial infection on the shoots, which has been reported earlier within *G. abietina* var. *abietina* (ROLL-HANSEN 1964, KURKELA 1967, DORWORTH 1972, HELLGREN and BARKLUND 1992), but not within a single type of *G. abietina* var. *abietina*. Conidia were disseminated during the early summer two years after infection (IV). Early summer has been reported to be the main time of *G. abietina* conidia dissemination also in more southern climate (SKILLING 1969, NEVALAINEN 1986, HELLGREN and BARKLUND

1992). The infected shoots also bore a few fresh pycnidia two years after infection (IV). This, added to the capability of *G. abietina* to fruit on green shoots (KAITERA and JALKANEN 1994b), is an indication of the tremendous capacity for the fungus to reproduce. This also explains why *G. abietina* is able to cause annual damage within the stands for a longer period as was detected in I and III. The annual dissemination pattern of large-tree type *G. abietina* var. *abietina* via conidia, which do not spread far from the inoculum source (SKILLING 1969), explains why individual trees will be continuously severely infected as was the case in III. Therefore, the pathogen did not vanish even after several pine-favouring seasons from the stand and was able to reproduce rapidly causing sudden peaks in the epidemic in susceptible sites, as has recently been noticed in eastern Lapland (KAITERA and JALKANEN 1994a). This also suggests that Scleroderris canker will be a permanent problem in susceptible sites as long as the branch volume is high enough for fresh infection.

The large-tree type *G. abietina* var. *abietina* did not produce apothecia on branches during the 3 year's period of investigation (IV). Rarity of apothecia is a common feature for *G. abietina* var. *abietina* on branches of large trees (ROLL-HANSEN 1964, DONAUBAUER 1974), although both types of *G. abietina* var. *abietina* produce pycnidia and apothecia *in vivo* (UOTILA 1992). Microconidia were also observed just before conidia dissemination (IV) but their role in the infection process is unknown. Earlier ROLL-HANSEN and ROLL-HANSEN (1973b) and ZAJCHOWSKI and BERGDAHL (1982) noted *G. abietina* microconidia, and according to UOTILA (1983), both types of *G. abietina* var. *abietina* may produce microconidia.

5.4 *Tomicus* spp. attacks in relation to *G. abietina* epidemics

Tomicus spp. attacks were rare in both study areas until 1980s (II, III). Their relative number increased just after the *G. abietina* epidemic had subsided in the damaged stands (II, III). Such a relationship was earlier noted by REDFERN and GREGORY (1991), and a similar relationship occurs between *G. abietina* and *Pissodes* spp. (LANIER et al. 1984) and *Ips* spp. (JAHN 1960). *Tomicus* spp. attacks occurred preferably in the youngest shoots in the upper canopy (IV), which has been reported earlier (LÖYTTYNIEMI 1978, LÅNGSTRÖM 1980, 1983).

The method for determining the time of *Tomicus* spp. attacks is the most accurate one especially if used just after the damage has occurred and when the number of fallen shoots is still low. *Tomicus* spp. feeding tunnels in the shoot are easily distinguishable from tunnels caused especially by species of Lepidoptera as wood chips are absent in *Tomicus* spp. feeding tunnels (SAALAS 1949).

In conclusion, the large-tree type of *G. abietina* var. *abietina* was associated with Scleroderris canker on Scots pine branches reported in the 1980s and 1990s in northern Finland. *G. abietina* had damaged pines for

decades in the corresponding stands, and several peaks in the epidemics were detected over the past decades. A serious epidemic developed in the 1980s, subsiding in the late 1980s. Epidemics, however, continued locally in the 1990s. The fungus was found to sporulate annually via conidia, which offers a tremendous capacity for the fungus to cause long-lasting epidemics in the diseased stands. This also makes Scleroderris canker a permanent problem in susceptible pine stands. After the fungal epidemic, insects (*Tomicus* spp.) were found to cause additional damage in the surviving branches.

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Disease history of Gremmeniella abietina in a Pinus sylvestris stand

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Abstract

A new method has been developed for studying the history of *Gremmeniella abietina* infection in Scots pine (*Pinus sylvestris*) stands. The branch analysis method is based mainly on the occurrence of necrotic cankers and scars caused by *G. abietina* in annual shoots of branches. The supporting factors are the number of leader changes of branches and branch death model.

Key words: *Gremmeniella abietina* – *Pinus sylvestris* – Methods – Disease history – Cankers.

1 Introduction

Gremmeniella abietina (Lagerb.) Morelet affected Scots pine (*P. sylvestris* L.) seedlings in northern Finland in the 1930's and 1940's (KANGAS 1937; KUJALA 1950). The first northern Finnish epidemic was noticed, when *G. abietina* had killed many Scots pine seedlings in nurseries in the late 1960's (KURKELA 1967) and saplings in plantations in the late 1960's and early 1970's (NOROKORPI 1972). A new epidemic occurred in 1982 in high-elevation areas in central and eastern Lapland and in Kuusamo (JALKANEN 1987). In 1988–1989, stands damaged by *G. abietina* were found in the remote easternmost Finnish Lapland near the Soviet border. The history of the disease in the area was, however, not known.

G. abietina causes necrosis in the secondary phloem of stems and branches. Some of the necrotic areas heal while others expand year after year and may eventually lead to the death of the tip of the infected branch (KURKELA 1981). In expanding canker necrotic areas, *G. abietina* infection appears as a resinous spot, yellow-greenish in colour, in the wood underneath the canker necrotic area and as yellow-brownish in colour in the inner part of the bark (KURKELA 1981).

The time of infection of a tree can be dated by estimating the time of formation of a canker on the stem (AALTO-KALLONEN and KURKELA 1985). The number of infections can be estimated by counting the cankers, scars and changes in annual leaders of the main stem (AALTO-KALLONEN and KURKELA 1985; UOTILA 1988). However, nobody has tried to estimate when infection occurred and the number of infections in annual shoots by using branches, which contain an abundance of material for a historical check.

The aim of this study was to create a method that would be of help in identifying the past infection times of a single tree. Such a method was seen as useful in clarifying the history of *G. abietina* in the eastern Lappish forests.

2 Material and methods

Fourteen Scots pines varying in height from 5 to 11.5 m and with breast-height diameter varying between 3.5–13.7 cm were felled in the winter of 1990–1991 in a 70-year-old stand

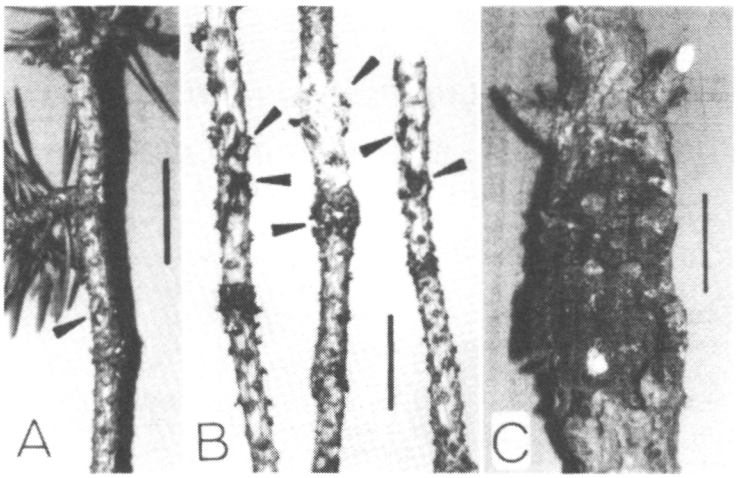

Fig. 1. Symptoms caused by *Gremmeniella abietina* in a branch of Scots pine. A. a scar; B. small, young cankers; C. a large, old canker. Bar = 2 cm

heavily damaged by *G. abietina* in Rikkilehto, Salla, eastern Lapland (67°12′N, 29°26′E). According to UOTILA's (1985b) classification, the range of damage classes of the sample trees was 40–95 %. All the living and dead branches were cut from their bases and thoroughly investigated in the laboratory.

The following variables were used in the branch analysis: 1. scars, 2. cankers, 3. leader changes, 4. death year of whole branch, and 5. holes made by *Tomicus* spp. In the case of each first-order branch, the number of scars and cankers caused by *G. abietina* were counted in each shoot. A scar was defined as being a necrotic area caused by *G. abietina* in the phloem of branches and subsequently occluded by the tree so that the fungus had not been able to spread further. Canker is an unhealed necrotic area many years of age (Fig. 1 a–c).

The time of formation of a scar or a canker was checked carefully by splitting the shoot in the middle of the canker or by digging the resin out from the canker, with a knife, to see whether the canker reached the pith of the shoot. This was done under the stereoscope. It was presumed that a *G. abietina* infection occurs mainly in autumn and that the formation of the scar or canker begins in the infected shoot during the dormant period following infection when the pathogen penetrates the shoot. The penetration area is characterised by the presence of a totally or partly untouched tree ring between the pith of the shoot and the infected tree ring. As the canker spreads year after year, resinous wood remains in the cankered area of the infected shoot.

G. abietina cankers were distinguished from cankers of different origin by checking whether the canker reached the pith of the shoot. The yellow-greenish colour of the wood underneath the wound was used as an aid in the identification of the infection caused by *G. abietina* (see KURKELA 1981). The shape of the canker is also something of a distinguishing feature of *G. abietina* infections. In the canker, the shoot has burst and expanded at the sides, and there is often bark above the deep bottom of the canker centre. Pine twisting rust [*Melampsora pinitorqua* (Braun.) Rostr.], a common canker forming pathogen in more southerly areas, does not occur in pines in the northern Salla region. Hail shower injuries have not been recorded.

In the branch analysis, the cankers and the scars were considered to have occurred during the infection year. The analysis was based on the fact that latent infections are rare, and thus infections were most likely to have occurred only in the youngest shoots.

All the leader changes in the first-order branches were counted, too, in order to elucidate whether *G. abietina* had killed a great number of shoots without leaving any scars or cankers on shoots. The number of holes caused by *Tomicus* spp. in shoots was also counted. A description of this method will be published shortly. The years when branches had died were also determined by counting the tree rings from the cross section of the base of the branches with the help of a stereoscope. The total number of investigated shoots was 11 564 (Fig. 2).

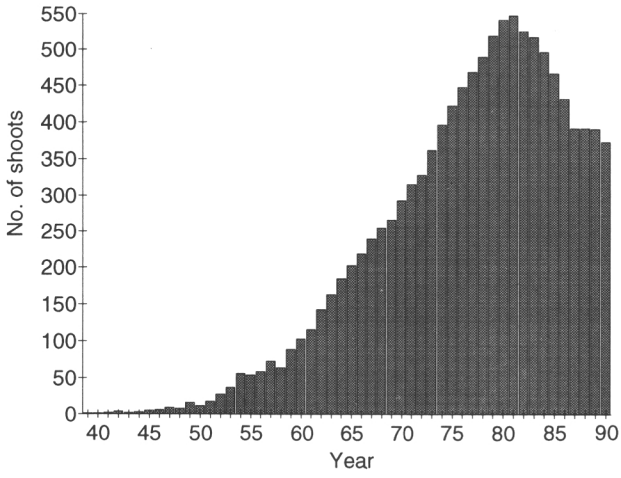

Fig. 2. The number of shoots examined for each year from 1938. Year 90 means that the shoots were formed in the summer of 1990

3 Results

First-order branches bore abundant scars (total 520) and cankers (total 608) caused by *G. abietina*. Thus, the average number of necrotic wounds was 80.6 per examined tree and 0.1 per examined shoot. The oldest scars found, a few only, dated back to the years 1948, 1956 and 1957, and cankers to the years 1947, 1948 and 1954. Since 1963 scars (and since 1961 cankers) were found annually up to 1989. No scars or cankers were found in the 1990 shoots.

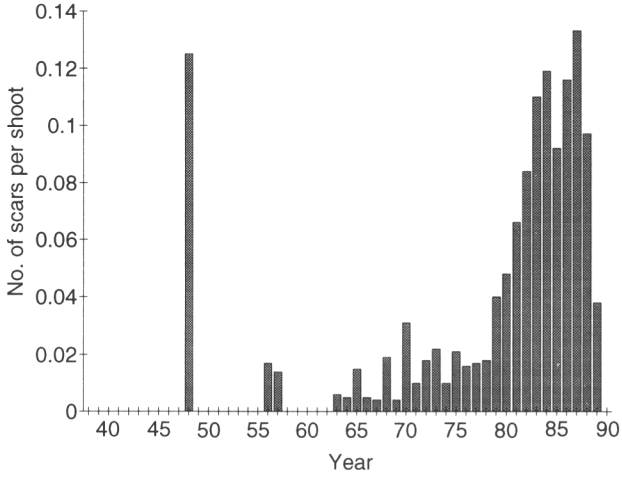

Fig. 3. Annual variation of the average number of scars per shoot from 1938–1990 in Rikkilehto, eastern Lapland

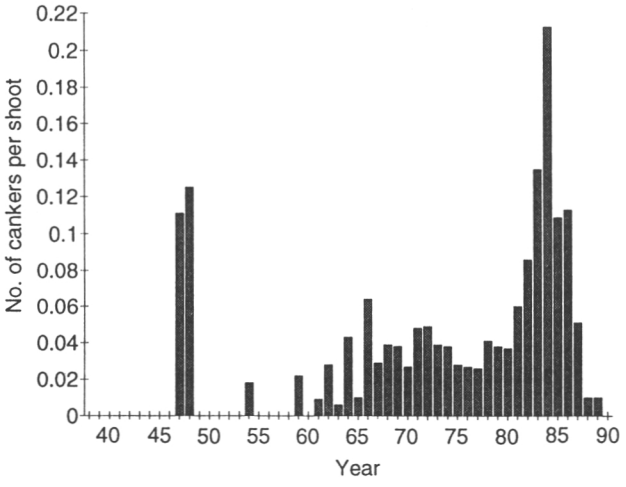

Fig. 4. Annual variation of the average number of canker per shoot from 1938 – 1990 in Rikkilehto, eastern Lapland

A slight increase in the number of scars per shoot occurred in 1970 and a larger generally sustained increase from 1979–1988 with most occurring in the 1983, 1984, 1986 and 1987 shoots (Fig. 3). In 1963–1978, the average number of scars per shoot was 0.005–0.03, while the corresponding figure for 1981–1988 was about 0.07–0.14. In 1989, the number of scars fell to 0.04.

A small number of shoot cankers occurred between 1961 and 1980 with peaks in 1966, 1971 and 1972. Before 1981, and excluding the peaks, an average number of cankers per shoot was 0.03 to 0.04. The greatest number of cankers per shoot (0.09–0.14) occurred in 1982–1986 with a clear peak (0.21) in 1984 (Fig. 4). In 1987, the cankers decreased to 0.05 while in 1988–1989 the figure was about 0.01.

A total of 634 leader changes were observed in first-order branches. The first leader changes had occurred in 1954. A slight increase in leader changes per shoot occurred in 1976, 1981 and 1982; on the average, about 3.5 % of shoots had died. The number of leader changes per shoot was at its highest level in 1983–1989 when about 10–16 % of shoots were lost (Fig. 5). In 1990, the number of leader changes decreased rapidly.

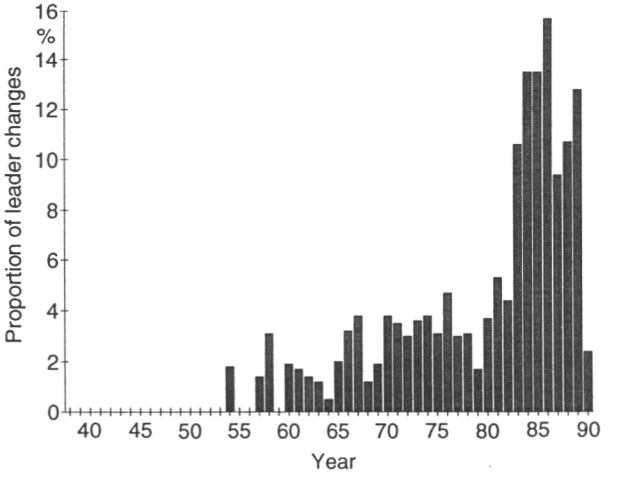

Fig. 5. Annual variation of the relative number of leader changes in first-order branches from 1938–1990 in Rikkilehto, eastern Lapland

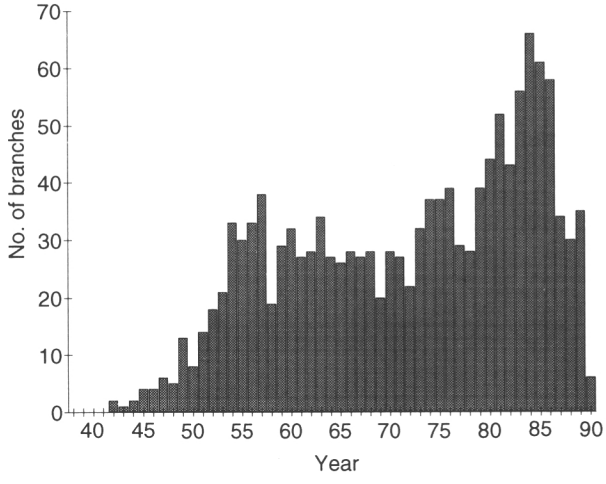

Fig. 6. Variation in annual mortality of first-order branches from 1938–1990 in Rikkilehto, eastern Lapland

The oldest branch deaths found had occurred in 1942 when the trees were about 20 years old. Since 1942, branches have died annually, the number of branch deaths first increasing geometrically to the mid-1950's, then levelling to 1.5–2.0 branches/tree/year in the late 1950's – early 1970's after which they again increased geometrically until 1986 (Fig. 6). In 1981 and 1983–1986, a large number of branches (about 50 to 70) died annually, whereas the number was only about half of that in 1987–1989. Seventy dead branches means, on the average, 5 branches/tree/year. In 1990, the number of dead branches was very small. In part, this may be due to the fact that some shoots could still have died in the dormant season 1990–1991 after the branches had been collected for research purposes.

4 Discussion

According to the scar and canker observations, *G. abietina* infection has taken place regularly since 1961. Some cankers were found to date as far back as 1947, 1948 and 1954. This means that the fungus had infected pines already when the stand canopy was closing. A small increase in the number of scars per shoot occurred in 1970 and in the number of cankers in 1966, 1971 and 1972. These peaks agree with earlier findings (KURKELA 1967; NOROKORPI 1972). Thus, there are signs that an epidemic was going on both in nurseries and plantations, which indicates that the pathogen had infected branches in older stands such as those in the study area, too.

The number of scars per shoot has increased since 1979 and that of cankers since 1982 very rapidly in the study area. An extremely serious epidemic was noticed in eastern Lapland in 1982 specially in high-elevation plantations. This was due to the very cold and rainy summer of 1981 (JALKANEN 1984). The number of scars and cankers per shoot shows that the last epidemic started in the study area at the same time or a little earlier than in plantations in the same area. In their study conducted in southern Finland, AALTO-KALLONEN and KURKELA (1985) found that the cold growing season and the number of days of frost correlated well with the number of stem cankers.

The epidemic was at its worst during the years 1982–1986 and began to weaken in 1987, according to the number of cankers. However, the number of scars was still very high in 1987 and 1988. This would appear to indicate that, although the epidemic had begun to recede (probably due to environmental conditions unfavourable for the fungus), the fungus still made efforts to infect host trees. In southern Finland, UOTILA (1988) found a large number of scars the year after the peak of the infection; these scars were due to libera-

tion of conidia by the fungus. Since 1989 the number of scars per shoot has decreased very noticeably in the study area. The rapid general decrease of the epidemic from 1988 to 1990 in eastern Lapland is documented (SALEMAA et al. 1991). In this study, no scars or cankers were observed in the youngest shoots. This supports the hypothesis that the infection is detectable at earliest in the spring following infection. However, no visible signs of the 1990 infection were observed in the summer 1991.

The number of leader changes was at its highest in 1983–1989. This occurred partly at the same time as the peak of the epidemic in 1980's. UOTILA (1988) found in southern Finland that the number of leader changes caused by *G. abietina* correlated well with the peak of the epidemic. Also, the number of dead branches was at its highest in 1981 and 1983–1986, at the same time as the peak of the epidemic. The high number of leader changes in 1988–1989 may be explained by the secondary shoot damage caused by *Tomicus* spp.

While the branch analysis method should be used systematically only on living branches, individual cankers on old dead branches can tell if *G. abietina* has occurred in a stand at all. A scar is not as useful as a canker for detecting infection especially in older branches. A scar is useful in the case of the youngest shoots, because very often there is only a small necrotic area in the phloem of the branch, which can then develop into a canker or remain at the scar stage.

If the number of branches studied is very small or if some of them have disappeared (as in the case of the oldest whorls) and when most of the branches have died after a serious epidemic, the average number of scars or cankers may indicate too strong an infection by *G. abietina*. Also, if the majority of the branches have died after a serious epidemic, the availability of information about epidemics after a serious epidemic is more limited. The method is therefore most suitable for stands where the trees have recovered after an epidemic.

Latent infection and infection into older shoots cannot be taken into consideration when using this method. They are probably of only minor importance in the infection process. The major channel of infection is, according to PATTON et al. (1984), the stomata, and penetration is believed to happen during the next dormant period following infection. AALTO-KALLONEN and KURKELA (1985) have noticed that it is very difficult to tell afterwards, whether a canker was initially caused by a microbe or frost and whether it was formed during the preceding autumn or during the following spring. In this method the infection year is defined as the period from the early summer to the next spring. It has also been noticed with Norway spruce [*Picea abies* (L.) H. Karst.] that *G. abietina* can spread from the youngest shoots into older ones (BARKLUND and ROWE 1981).

The method is also based to the fact that all of the youngest shoots have the same chance of becoming infected; this makes it possible to compare years to each other within the scope of the data for whole trees. However, the conidia spread only after rain (SKILLING 1969) and significant increases in the disease are limited to a distance of no more than 4–6 meters from the infection point (UOTILA 1985a). This may mean that conidial infection does not have the same chance of infecting every youngest shoot. Also, the length of the shoot, the susceptibility of the shoot and the relative humidity of the air can vary in different parts of the canopy.

The branch analysis method can also be used in different parts of the tree. This would give a different weight to each variable in different parts of the canopy. It would also make it possible to estimate more accurately the importance of the infection within a tree. The method is useful in clarifying the disease history of *G. abietina* in areas, which are remote and which are reached only long after the epidemic.

Summary

The history of *Gremmeniella abietina* in a 70-year-old heavily infected Scots pine (*Pinus sylvestris*) stand was studied. Both dead and living branches were cut from 14 living trees. In the branch analysis, the number of cankers, scars, and leader changes caused by *G. abietina* were counted in each annual shoot of each first-order branch. The years when whole branches had died, were determined. A total of 1727 branches including 11 564 branch leader shoots were examined. Both the total and the average number of cankers and scars showed that *G. abietina* had been present in the stand frequently since 1961 and infrequently as early as the 1940's. A strong epidemic began in the 1980's. The peak of the epidemic, according to cankers formed, occurred during the years 1982–1986 and according to scars in 1981–1988. The branch analysis method can be used accurately in the examination of the history of *G. abietina* for at least 20 to 25 years retrospectively.

Résumé

Histoire de la maladie à Gremmeniella abietina dans un peuplement de Pinus sylvestris

L'étude historique de *G. abietina* a été faite dans un peuplement de *Pinus sylvestris* de 70 ans très infecté. Les branches mortes et vivantes ont été coupées sur 14 arbres vivants. Dans l'analyse des branches, le nombre de chancres, de cicatrices et de changements de flèches provoqués par *G. abietina* ont été comptés sur chaque pousse annuelle des branches de premier ordre. Les années au cours desquelles des branches entières sont mortes ont été déterminées. Au total, 1727 branches, comprenant 11 564 branches latérales, ont été examinées. Le total comme le nombre moyen de chancres et de cicatrices, montrent que *G. abietina* était fréquent depuis 1961 et peu fréquent dès les années 1940. Une forte épidémie avait commencé dans les années 1980. Le maximum de l'épidémie, d'après les chancres formés, a eu lieu en 1982–1986, et d'après les cicatrices, en 1981–1988. La méthode d'analyse des branches peut être utilisée efficacement dans l'étude rétrospective de *G. abietina*, sur au moins 20–25 ans.

Zusammenfassung

Krankheitsgeschichte von Gremmeniella abietina in einem Pinus sylvestris-Bestand

In einem 70jährigen, stark von *G. abietina* befallenen Kiefernbestand (*P. sylvestris*) wurde der Krankheitsverlauf untersucht. Von 14 lebenden Bäumen wurden tote und lebende Äste erster Ordnung entnommen und für jeden Jahrestrieb-Abschnitt folgende Parameter erhoben: Anzahl der Nekrosen, Anzahl der Narben und Häufigkeit ersetzter Leittriebe. Bei abgestorbenen Ästen wurde der Zeitpunkt des Absterbens bestimmt. Insgesamt wurden 1727 Zweige mit 11 564 Leittrieben untersucht. Die gesamte und die durchschnittliche Anzahl der Nekrosen zeigte, daß *G. abietina* seit 1961 in hoher Frequenz im Bestand vorhanden war und in geringer Häufigkeit bereits seit ca. 1940. In den 80er Jahren begann eine starke Epidemie. Der maximale Befall, bezogen auf die neugebildeten Nekrosen, trat zwischen 1982 und 1986 auf, bezogen auf die Narben lag der Peak zwischen 1981 und 1988. Die Methode der Astanalyse ermöglicht eine zuverlässige Beurteilung des Befallverlaufes durch *G. abietina* für zumindest die letzten 20–25 Jahre.

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The history of shoot damage by *Tomicus* spp. (Col., Scolytidae) in a *Pinus sylvestris* L. stand damaged by the shoot-disease fungus *Gremmeniella abietina* (Lagerb.) Morelet

By J. KAITERA and R. JALKANEN

Abstract

A method has been developed for studying the history of attack by *Tomicus piniperda* L. in Scots pine (*Pinus sylvestris* L.) stands. Attacks in a stand were dated by means of external evidence on living shoots and young branches, and from anatomical evidence in older shoots. The method revealed that *Tomicus* outbreak was associated with epidemics caused by *G. abietina*. The role of *Tomicus* in the decline of a *G. abietina* infected stand is discussed.

1 Introduction

Pine shoot beetles (*Tomicus piniperda* L., *T. minor* Hart., Scolytidae) breed readily in freshly felled logs or windthrown trees but living trees are only attacked after weakening by some other agent. Beetle damage has been noticed recently in trees weakened by pruning (RÄISÄNEN et al. 1986) and by artificial snag production (VITIKKA et al. 1991). Beetles cause growth losses through maturation feeding on the youngest shoots of Scots pines, which fall down in the next autumn and winter (SAALAS 1949; LÖYTTYNIEMI 1978; LÅNGSTRÖM 1980). The shoot damage occurs both in weakened and healthy trees.

Beetle populations have been higher than normal during 1980's in large areas in Lapland. In trees which were felled by autumn storms in 1982 and 1985 in northern Finland, the only attacked insect, which existed in masses, was *Tomicus piniperda* L. (SAARENMAA 1987). In windthrown trees, successful colonization can lead in maximum production of 18 000 new beetles per tree (LÅNGSTRÖM 1984).

The shoot-disease fungus, *Gremmeniella abietina* (Lagerb.) Morelet causes the most serious disease of Scots pine (*Pinus sylvestris* L.) in Lapland. Epidemics have occurred in nurseries (KURKELA 1967), in man-made plantations (VALTANEN 1970; NOROKORPI 1972; UOTILA and JALKANEN 1982) as well as in natural forests (JALKANEN and KAITERA 1992). Trees weakened by *G. abietina* may supply suitable breeding material for shoot beetles. In Britain, the role of *T. piniperda* in dieback of Scots pine is considered to be secondary, merely causing growth losses in the late stage of the dieback (REDFERN and GREGORY 1991). A similar relationship has been noticed between *G. abietina* and *Pissodes* (LANIER et al. 1984).

The aim of this study was to determine the history of attack by *Tomicus* in a stand damaged by *G. abietina*, and to study the possible role of shoot beetles in the damage process.

2 Material and methods

The study area was situated in Rikkilehto (67° 12′ N, 29° 26′ E) in eastern Lapland. The experimental stand of 70-year-old Scots pine at first thinning age was heavily damaged by *G. abietina* and about

60 % of the trees had been killed. None of the dead trees had been removed and no felling had taken place within 2–3 km of the study area. For a more detailed description of the stand, see KAITERA and JALKANEN (1992, 1993). The stand was inventoried in autumn 1990 and all the living and dead branches from fourteen Scots pines were cut and brought to laboratory for further examination.

Signs of *Tomicus* injury were recorded from every shoot of all the first-order branches. The year of

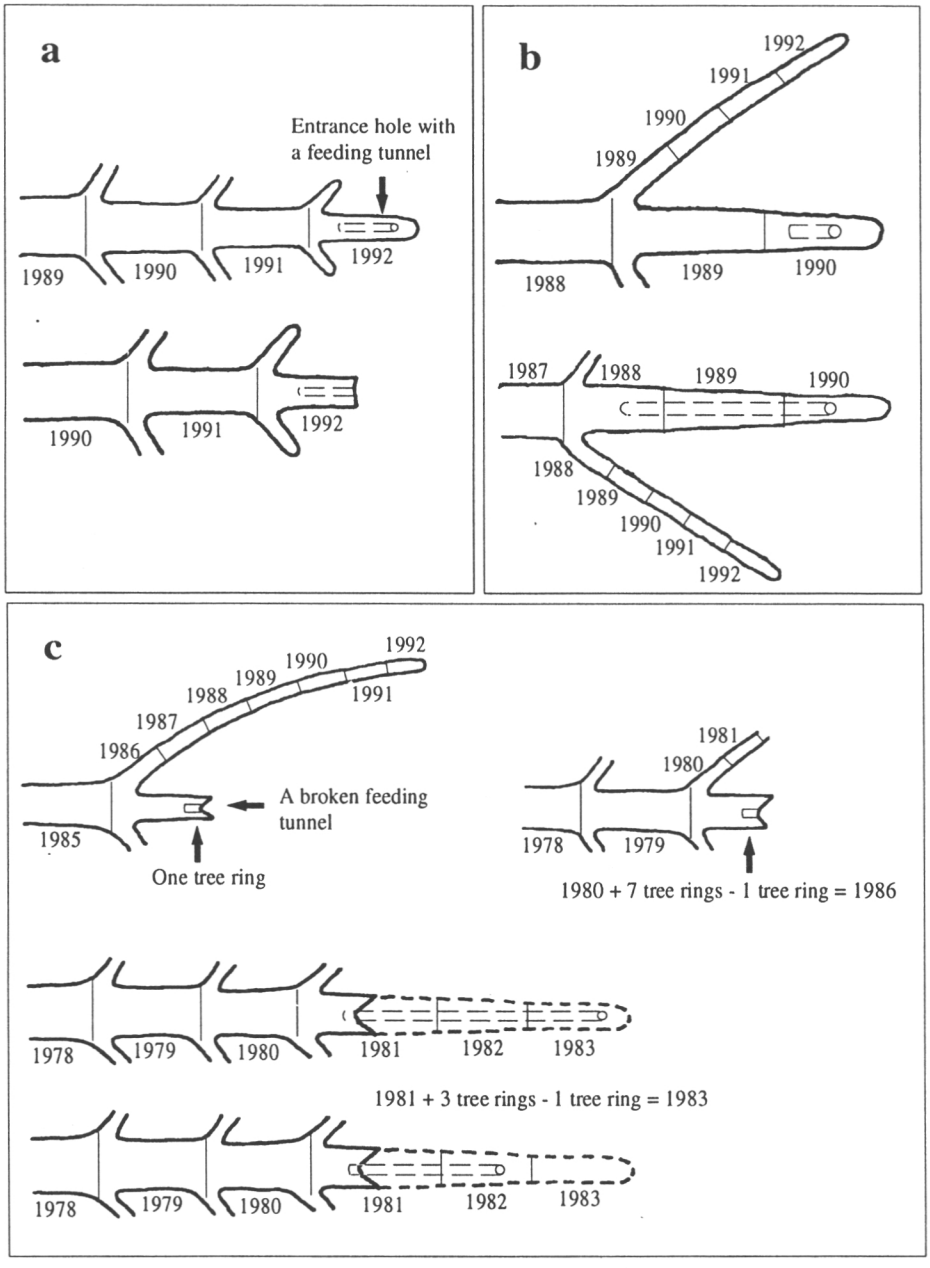

Fig. 1. The occurrence of a *Tomicus* spp. attack in a Scots pine shoot. a: a current-year shoots; b: an older shoot while the attacked shoot is still intact in the branch; c: as b but the attacked shoot has broken off

the attack (YRAT) was determined as follows. If a *Tomicus* entrance hole was in a current-year shoot, the YRAT was the same as the year in which the shoot was formed (fig. 1a). If an entrance hole or a feeding tunnel was in an older, still intact shoot, the YRAT was the same as the birth year of the youngest shoot in the attacked leader, where the entrance hole and the feeding tunnel were situated. The age of the youngest shoot was counted from internodes of the leader and the living side branches (fig. 1b). It was assumed that the attacked shoot and its terminal bud die prematurely after the attack without forming a new growth in the following years. If an entrance hole or a feeding tunnel was in a dead, broken shoot, the birth year of the attacked shoot was determined by counting its age with the help of living side branches and then its death year by counting the tree rings in the base of the shoot (fig. 1c). The following formula was then used.

$$\text{YRAT} = \text{birth year of the shoot} + (\text{number of tree rings} - 1).$$

Attacks occurring before shoot extension in any year were attributed to the previous year. In shoots subject to repeated attacks, the number of attacks could be determined only in shoots, which remained on the tree. This was found on one occasion only.

The relationship between infections caused by *G. abietina* and the leader changes has been shown to be quite clear. The history and intensity of *G. abietina* infection in the experimental area and in the same sample trees is described by KAITERA and JALKANEN (1992).

3 Results and discussion

The total number of beetle holes found was 171. The first holes were in 1963 shoots (fig. 2). Between 1973 and 1978 there were on average 0.01 attacks per youngest shoot. After 1982 the attacks increased dramatically with the peaks of 0.025–0.035 in 1983–1985, and 0.065–0.085 in 1988–1989. The number of the attacks was still high in 1990. Significant damage by shoot beetles therefore only occurred in the last decade (1980–1990).

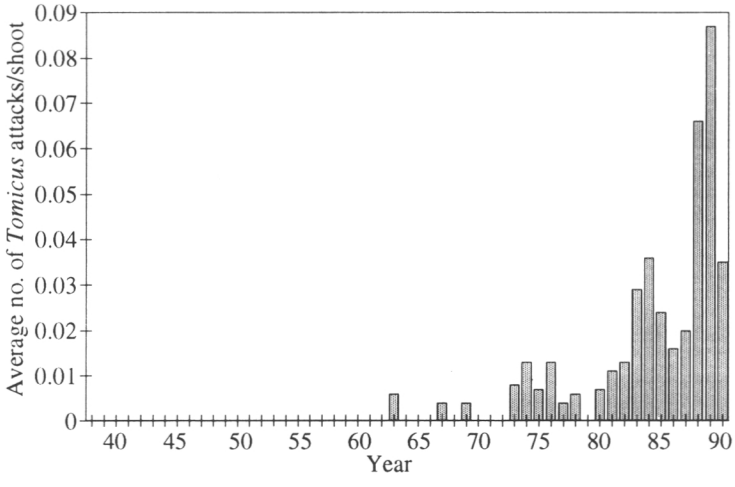

Fig. 2. Annual variation in the average number of *Tomicus* holes per shoot in 1938–1990 in Rikkilehto, eastern Lapland

A broad relationship between shoot boring by *Tomicus* species and infection by *G. abietina* is indicated by the incidence of scars and cankers (fig. 3a) and leader changes on first-order branches (fig. 3b). The outbreak of *G. abietina* was associated with an initial moderate increase in *Tomicus* activity during period 1983–1985 but there is some evidence that the second, much greater peak of activity occurred as the outbreak declined. This strongly suggests that the *G. abietina* outbreak was the primary cause of damage in the stand and that it provided breeding material for the subsequent development of a large *Tomicus* population.

Pine shoot beetles preferentially attack the outermost shoots of the crown (LÖYTTYNIEMI 1978; LÅNGSTRÖM 1980, 1983). Thus, in a dense stand attacks are concentrated the shoots of the topmost whorls. In LÅNGSTRÖM's (1980) study over 50 % of the youngest shoots over 5 cm in length in the upper canopy, about 40 % in the central part and 10–25 % in the lower part were attacked. These results are confirmed by observations made in this study. For instance in 1984, the lower canopy had not been attacked, and the average number of attacked shoots was 0.02 in the middle canopy and about 0.09 in the four uppermost whorls. In 1985, the average number of holes per shoots in the youngest whorl was 0.085 whereas it was 0.02–0.03 in the lower canopy. In 1986–1987 the attacks were very few in the whole canopy (0.01–0.035). However, in 1988–1989 the attacks increased especially in the uppermost 8 whorls being 0.135–0.165, whereas the attack level in the lower canopy was 0.04–0.05. The high attack level of the upper canopy continued in 1990, too.

The size of the bark beetle population around the experimental stand was not known. Road-side timber storage was, however, lacking and only occasional windthrown trees have maintained a small beetle population in the area. Therefore, *Tomicus* beetles seemed to have succeeded in bilding higher population in the experimental stand than in the

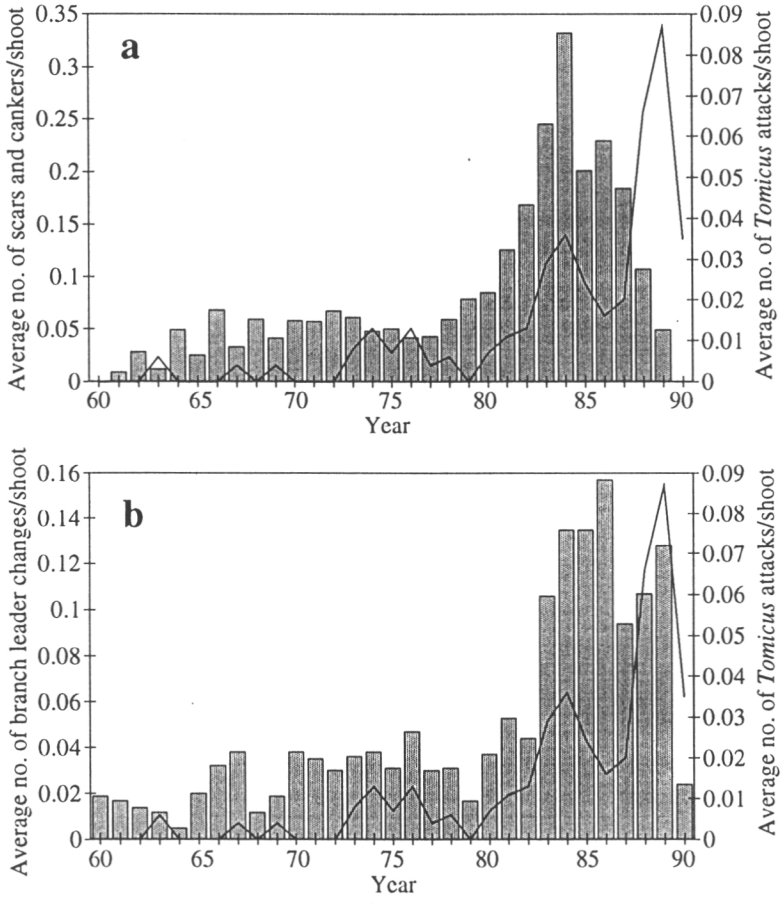

Fig. 3. Relationships between *Tomicus* attacks (solid line) and infection by *G. abietina* (columns) at Rikkilehto, eastern Lapland in 1960–1990 as shown by a, the number of scars and cankers per shoot, and b, the number of branch leader changes per shoot

surroundings in general. This is surely due to the primary injuries caused by *G. abietina* to Scots pines, because *G. abietina* had weakened and killed trees in the stand more than 30 years (KAITERA and JALKANEN 1992). The number of trees killed by *G. abietina* has probably been at the highest level in the mid- and late-1980's and thus served beetles for breeding material at that time. The increase in shoot beetle attacks in *G. abietina* weakened Scots pines has been noticed earlier, too (REDFERN and GREGORY 1991).

The radial increment of the sample trees began to decline in 1977 (fig. 4) although the average radial increment in eastern Lapland did not decline at the same period (NÖJD 1992). The decline may partly be caused by normal competition. However, the low tree vigour, mainly caused by *G. abietina* and very little by beetles, is seen the major reason to reduction in radial increment of the trees. The decline coincided with *G. abietina* epidemics. No recovery after the most recent epidemic and the attacks was measured (fig. 4). In ANDERSSON's (1973) study the volume growth was still very low 8 years after

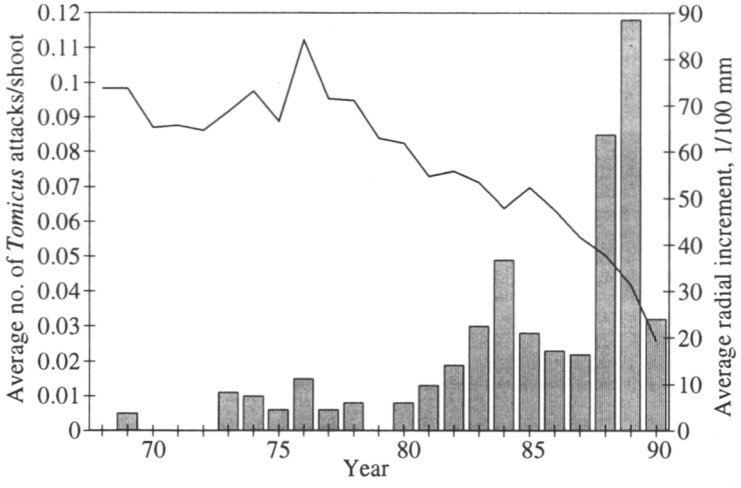

Fig. 4. Annual variation of the average number of *Tomicus* holes per shoot (columns) and the average annual radial increment (solid line) in 10 trees

beetle attacks. In LÅNGSTRÖM's and HELLQVIST's (1991) study the basal area growth decreased for 2–3 years after beetle attacks and recovered during the subsequent 3 years, the total period of loss thus being 5–6 years. Therefore the recovery of the trees in the study area should not yet be seen in radial increment in 1990. The low tree vigour can also be due to the very low number of living branches killed by both *G. abietina* and *Tomicus* spp. One beetle can destroy 1–5 shoots during one summer and autumn (LÅNGSTRÖM 1974). In ELFVING's and LÅNGSTRÖM's (1984) study the number of dropped shoots was 50–150 per tree, though growth losses seemed to occur with at least 200 shoots lost (LÅNGSTRÖM and HELLQVIST 1991). In this study the number of lost shoots in the whole tree is, however, not known, since the intensity of attack was estimated only from first-order branches.

The method gives information about the attacks occurred in the past in that part of the canopy, where the branches are not broken. This method for dating the outbreaks caused by pine shoot beetles can for example be used in windthrown forests. The method is, however, the most accurate when used just after the damage in windthrown forests or in young stands, where the number of fallen shoots is still low. Normally the attacked shoots remain on the trees for only a short period after the attack. In LÖYTTYNIEMI's (1978) study, the number of shoots remaining on the trees the following spring was only 9 %.

The timing of the attack to a certain year by counting the tree rings is accurate, if the attack has occurred in a growing shoot. If the attack occurs before the growing period starts in spring, the attack is counted to the previous years's attack. This is possible because also adult beetles can attack the shoots in the canopy (LÅNGSTRÖM 1980). The first adults fly into the canopy only a few days after the swarming period has started in the spring. The proportion of attacks by adult beetles as compared to the attacks by young beetles is, however, probably very low.

In this method the estimation of the number of attacks in a single shoot can be made only in shoots, which have remained as a whole or partly broken in the tree. In SALONEN's (1973) study the great majority of the attacked shoots had only one feeding tunnel in the shoot. Also in this study the great majority of the studied shoots had only one feeding tunnel. SALONEN (1973) found, that nearly all of the holes were situated in the youngest shoots. According to LÅNGSTRÖM (1980), the majority of the feeding of the new beetle generation was directed to the current-year shoots. However, LÅNGSTRÖM (1983) found, that 40 % of the attacks occurred in current-year shoots, 50 % in 1-year-old shoots and 10 % in older ones. In case the youngest shoot has suddenly died for example due to infection by *G. abietina* and the attack has occurred later in the same shoot, the estimation of the year of attack cannot be done with our method. However, this is propably a very rare occasion, because dead shoots dry very soon and do not serve beetles as feeding material.

Summary

By investigating systematically all the annual shoots of all the first-order branches of fourteen Scots pines (*Pinus sylvestris* L.) infected by *Gremmeniella abietina* (Lagerb.) Morelet, the history of *Tomicus* spp. outbreaks in the stand, too, was clarified from the 1960 to 1990. Oldest traces of *Tomicus* shoot damage dated back to early 1960's. Excluding the year 1979, *Tomicus* spp. had attacked shoots since 1973 to present (1990). Most damage had happened in the 1980's and especially during the last three years 1988–1990. As compared to *Gremmeniella abietina* history in the stand, the pathogen was responsible on the main decline of the stand which was then secondarily fulfilled by *Tomicus* spp.

Zusammenfassung

Über Beziehungen zwischen dem Triebbefall bei Pinus sylvestris durch Tomicus spp. (Col., Scolytidae) und der Kieferntriebkrankheit, verursacht durch den Pilz Gremmeniella abietina (Lagerb.) Morelet

Es wurde eine Methode zum Studium des Triebbefalls bei Kiefer durch den Waldgärtner *Tomicus piniperda* L. entwickelt. Der Befall wurde mit Hilfe der Datierung der äußerlich sichtbaren Einbohrlöcher in lebenden Jungtrieben und des anatomischen Nachweises in älteren Trieben erfaßt. Dabei ergab sich, daß die *Tomicus*-Massenvermehrung zu der Infektion der Triebe durch den Pilz *G. abietina* in Beziehung stand. Die Rolle des Waldgärtners in einem mit *G. abietina* infizierten Kiefernbestand wird erörtert.

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III

Comparison of *Gremmeniella abietina* historical damage to Scots pines

Juha A. Kaitera and Risto E. Jalkanen

Abstract: Fifteen Scots pine (*Pinus sylvestris* L.) trees that were slightly, moderately, or severely damaged by *Gremmeniella abietina* (Lagerb.) Morelet were felled in northern Finland to determine the disease history of the stand. The annual level of damage was determined by counting the scars and cankers on all the first-order branches. Annual branch leader changes (dead shoots), branch mortality, and attacks caused by shoot beetles, *Tomicus* spp., were also determined. Most of the *G. abietina* damage occurred in the middle and late 1980s. However, the damage occurred at low levels in the stand as early as in the 1940s, demonstrating that the history of the disease followed the established pattern noticed earlier in eastern Lapland. For slightly damaged trees, most damage occurred in the mid-1980s, while for their severely damaged counterparts most damage occurred in the late 1980s.

Résumé : Quinze pins sylvestres (*Pinus sylvestris* L.), légèrement, moyennement ou sévèrement endommagés par *Gremmeniella abietina* (Lagerb.) Morelet, furent abattus dans le nord de la Finlande pour reconstituer l'historique de la maladie dans le peuplement. La sévérité de la maladie a été déterminée en comptant les cicatrices et les chancres sur toutes les branches de premier ordre. Les modifications annuelles causées par la mort de la pousse apicale, la mortalité parmi les branches et les attaques par l'hylésine du pin, *Tomicus* spp., furent également notées. La majorité des dégâts causés par *G. abietina* sont survenus au milieu et à la fin des années 1980. Par contre, il y avait eu des dégâts légers dans le peuplement dès les années 1940, indiquant que l'historique de la maladie était semblable à ce qui a été observé précédemment dans l'est du Lapland. Chez les arbres légèrement endommagés, la majorité des dommages sont survenus au milieu des années 1980 alors qu'ils se sont produits à la fin des années 1980 chez les arbres sévèrement endommagés.

[Traduit par la Rédaction]

Introduction

Gremmeniella abietina (Lagerb.) Morelet severely damaged Scots pine (*Pinus sylvestris* L.) during the 1980s in Lapland, northern Finland (Uotila and Jalkanen 1982; Kaitera and Jalkanen 1994*b*). However, the exact years in which damage occurred has been based more on subjective, visual observations than on data. Estimating damage in the field is both laborious and time consuming, especially in remote forest areas and gives only one-time estimates of damage. Recently, a branch analysis method was developed for determining the history of *G. abietina* epidemics (Kaitera and Jalkanen 1992) in which the occurrence of scars and cankers caused by *G. abietina* is related to shoot and branch mortality. In developing the method, Kaitera and Jalkanen (1992) worked in a severely damaged stand where the number of living branches per tree was relatively low.

The high variation in damage estimates within the stand raised the question of whether or not the same infection history could be detected for pine trees with different amounts of damage. In addition, since most affected Scots pine stands in Lapland are only slightly damaged (Kaitera and Jalkanen 1994*b*) there was the question of whether or not the pattern of past disease development is similar in both a slightly damaged stand and a severely damaged stand with the same macroclimatic and environmental conditions, but different microclimatic conditions, in eastern Lapland. The purpose of this study was to determine the disease history of *G. abietina* in a typical, slightly damaged Scots pine stand.

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Materials and methods

Study area and disease assessment

The study was made in a naturally regenerated Scots pine stand which had been slightly damaged by *G. abietina*. The stand, which was at the first-thinning stage (70 years old), was located in Kemihaara in eastern Lapland, Finland (67°57′N, 28°57′E; Fig. 1). Damage caused by *Gremmeniella abietina* was determined by examining all the pine trees on 100-m^2 sample plots at 50-m intervals within the stand. Using the UOT classification of Uotila (1985) *G. abietina* damage was assessed on a scale of 0–100%, where 0% represented a healthy tree and 100% a dead one. Trees receiving ratings of 0–20% were slightly damaged, those

Fig. 1. Location of the study area in which the Scots pine trees were damaged by *Gremmeniella abietina* and the surrounding healthy area in relation to contour lines and the Kemijoki river. Solid circles with two letters represent sample plots with the amount of damage on the plot according to the classification of Hopkins et al. (1979). Symbols for damage are SD, severe damage; HD, high damage; MD, medium damage; LD, light damage; and ND, no damage. Location of the sample trees in the stand; *x*, severely damaged; ⊗, moderately damaged; *o*, slightly damaged.

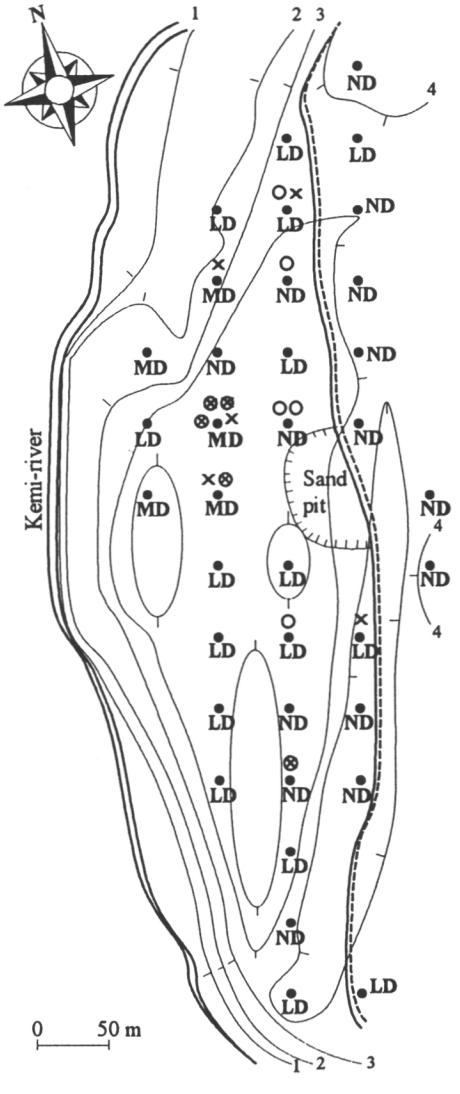


Fig. 2. Scots pine trees damaged by *Gremmeniella abietina* using Uotila's UOT classification (1985). (*a*) Slightly damaged tree (UOT = 0–20%); (*b*) moderately damaged tree (UOT = 30–60%); and (*c*) severely damaged tree (UOT = 70–90%).

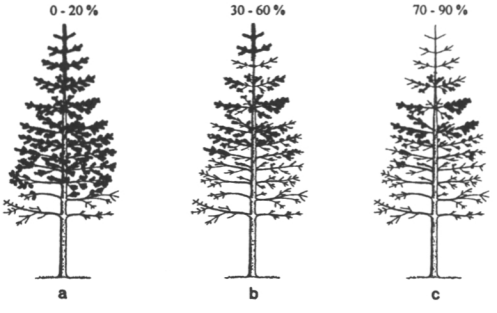
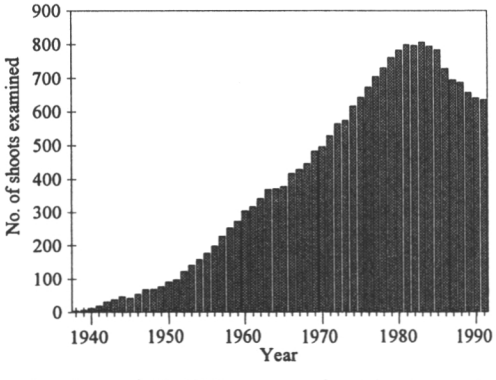


Fig. 3. The number of studied Scots pine shoots examined by year of growth from 1938 to 1991 in Kemihaara, eastern Lapland.



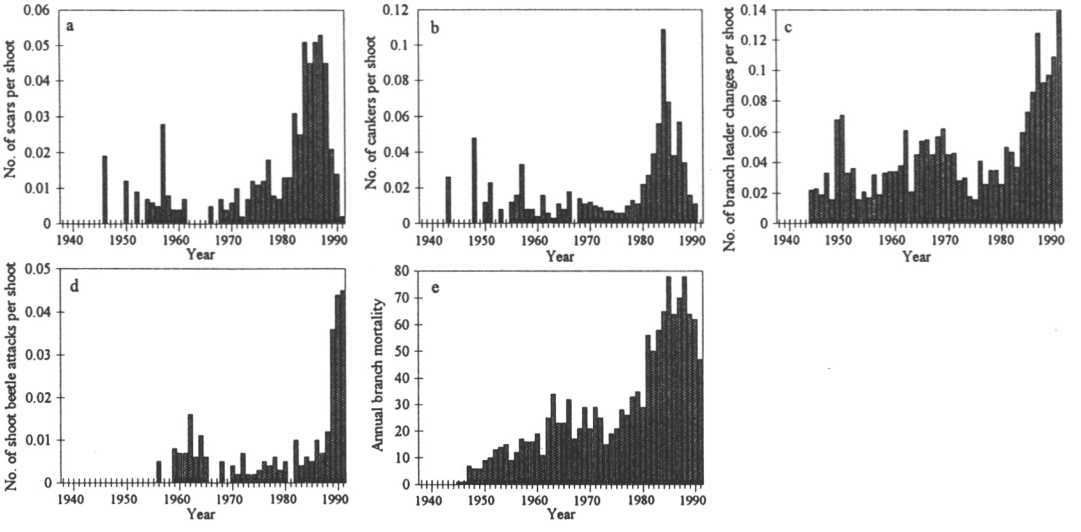
with values of 30–60% were moderately damaged, and those with values of 70–90% were severely damaged (Fig. 2).

Branch analysis

One live tree from each of the five most frequent diameters at breast height (DBH) classes in each of the three damage classes was selected for study. Thus, 15 Scots pine trees with average DBH of 9.6±2.9 cm (mean ± SD) and average age of 70 years were examined. Between October 1991 and June 1992, all the live and dead first-order branches were removed from these trees for branch analyses (Kaitera and Jalkanen 1992). For the branch analyses, scars were defined as being healed-over necrotic areas in each internode of the first-order branch, while cankers were defined as open, swollen, and restricted necrotic areas in the corresponding internodes (Kaitera and Jalkanen 1992). Kurkela (1981) has illustrated typical cankers caused by *G. abietina* and frost.

Scars and cankers typified slight damage while branch leader changes represented severe damage. However, since it was not always possible to ascertain that branch leader

Fig. 4. Annual variation of branch analysis variables from 1938 to 1991 in the Kemihaara Scots pine stand in eastern Lapland. Scars (*a*) and cankers (*b*) caused by *Gremmeniella abietina*, (*c*) branch leader changes (= dead shoots), (*d*) attacks by *Tomicus* shoot beetles, and (*e*) branch mortality. Fifteen trees were examined.



changes (= dead shoots; the following year another shoot acted as a leader) had been caused by *G. abietina*, especially in old branches, all such damage was tallied into a single class. Annual mortality of the dead, first-order branches was determined by tallying the number of tree rings at the base of each first-order branch and then comparing the result with the age of its branch whorl in the stem. The bark and phloem of each swollen internode was removed with a knife to check for necrosis. The year of attack by shoot beetles (*Tomicus* spp.) was also determined by counting the number of their feeding tunnels in every shoot of all the first-order branches and determining the age of the corresponding shoot at the time of attacking by the age of side branches or counting tree rings near the tunnel (Kaitera and Jalkanen 1994*a*).

The history of *G. abietina* infection was determined for each of the three damage classes and compared with one another. Because observations within and among shoots of different ages were not independent, the different years among and within the classes were not tested statistically.

Results

Infection history at the stand level

The total of 21 156 pine shoots, which had formed since 1938, were studied. The number of the examined shoots increased linearly up to the early 1980s, remained constant between 1981 and 1985, and then decreased towards the 1990s (Fig. 3). The maximum number exceeded 800 shoots per annum.

At the stand level, the oldest scars caused by *G. abietina* occurred in shoots formed in 1946. A peak in the number of scars was observed in shoots formed in 1957 (Fig. 4*a*). Since 1968, scars occurred annually, with the annual number of scars being greatest (0.05 scars per shoot) between 1984 and 1988, after which the numbers decreased to the 1970s level (Fig. 4*a*).

The oldest cankers occurred in shoots formed in 1943, and cankers continued to occur at an annual level of 0.01–0.02 per shoot, excluding the peaks in 1948 and 1957 (Fig. 4*b*). Between 1982 and 1988, the annual number of cankers increased to the relatively high level of 0.04–0.07, peaking at 0.11 cankers per shoot in 1984. After 1988, the number of cankers decreased (Fig. 4*b*).

The annual number of branch leader changes (= dead shoots) per shoot was 0.02–0.06 between 1944 and 1984, with small peaks in 1949, 1950, and 1962. Between 1985 and 1991, leader changes increased from 0.08 to 0.14, with peaks in 1987 and 1991 (Fig. 4*c*). Branch leader mortality occurred each year.

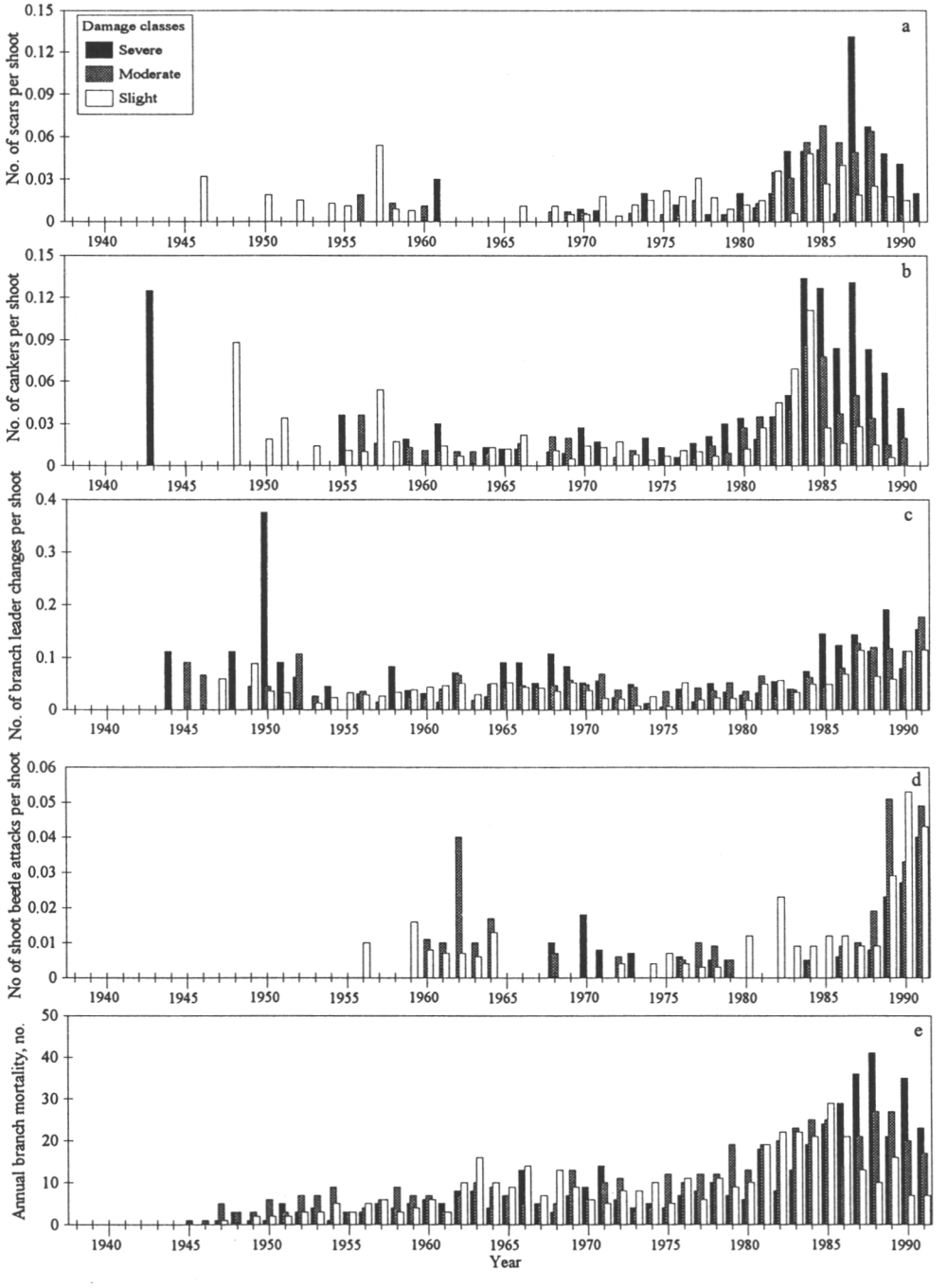
The earliest attacks by *Tomicus* spp. occurred in 1956, and the annual number of attacks was low with less than 0.01 attacks per shoot until 1988, when the number of attacks increased to about 0.04 between 1989 and 1991 (Fig. 4*d*). No evidence of *Tomicus* was found during the 1940s or early 1950s.

Branch mortality increased steadily until 1962, whereupon it levelled out at 20–30 branches per year (Fig. 4*e*) until 1980. After 1981 annual branch mortality increased to 50–80, with peaks in 1985 and 1988 (Fig. 4*e*).

Infection history in different damage classes

All the scars caused by *G. abietina* in the 1940s occurred on slightly damaged trees. Not until in the late 1950s were scars found on moderately damaged trees and not until 1961 on severely damaged trees (Fig. 5*a*). This resulted in part from the lower number of branches examined between the 1940s and 1960s in the moderately and slightly damaged tree classes as compared with the one in the severely damaged classes. During the late 1960s and 1970s,

Fig. 5. Annual variation of branch analysis variables by damage class between 1938 and 1991 in the Kemihaara Scots pine stand in eastern Lapland. Scars (*a*) and cankers (*b*) caused by *Gremmeniella abietina*, (*c*) branch leader changes (= dead shoots), (*d*) *Tomicus* shoot beetles, and (*e*) branch mortality. Five trees per each of the three damage classes were examined.



scars were most abundant in the slightly damaged trees and less prevalent in the moderately and severely damaged trees. However, scars were common in all damage classes. As the number of scars increased in the early 1980s, there was a corresponding increase in the number of scars on trees of all damage classes, but less so for slightly damaged trees. The peak in the number of scars occurred in 1984 and 1986 in the slightly damaged trees and from 1984 to 1986 on moderately damaged trees. For severely damaged trees, the longest period with the most scars was from 1983 to 1990 with a peak in 1987. While there were fewer of scars in the slightly and moderately damaged tree classes from 1989 to 1991, the number of scars remained high on severely damaged trees. Collections made in June 1992, showed that a few scars also occurred in 1991 on shoots of severely damaged trees (Fig. 5*a*).

Slightly damaged trees were the most commonly cankered, but random trees in all damage classes had *G. abietina* cankers during the 1950s and 1960s. The annual number of cankers was less than 0.04, except in 1943 (in the severely damaged tree class) and in 1948 and 1957 (in the slightly damaged tree class) when 0.13, 0.09, and 0.05 cankers per shoot, respectively, were detected. The number of cankers increased in the late 1970s, especially the severely damaged tree class (Fig. 5*b*), and continued to do so from 1982 onwards for trees in all damage classes. The peak in the number of cankers occurred in trees in the slightly damaged class in 1983 and 1984; in the moderately damaged class in 1984, 1985, and 1987; and in the severely damaged class from 1984 to 1989. The most cankers occurred in all damage classes in 1984, but whereas the number of cankers suddenly decreased in the slightly damaged class, many cankers were recorded for 1985 in the moderately damaged class and later from 1986 to 1989 in the severely damaged class (Fig. 5*b*).

Branch leader changes occurred at random from 1944 to 1959 in all damage classes, with less than 0.09, 0.10, and 0.10 leader changes per shoot in the slightly, moderately, and severely damaged trees, respectively, excluding a peak of 0.38 in 1950 in the severely damaged class. Between 1960 and 1984, when leader changes occurred annually, their relative number were 0.01–0.06 in the slightly damaged class, 0.02–0.07 in the moderately damaged class, and 0.01–0.11 in the severely damaged class. Between 1986 and 1991, the number of annual leader changes varied from 0.06 to 0.12 from 1986 to 1991 in the slightly damaged class, with peaks in 1987 and 1990–1991. The same pattern occurred in the moderately damaged class with an annual level of 0.08–0.18 leader changes per shoot, with peaks from 1987 to 1991. In the severely damaged class, the annual number of leader changes was high level (0.11–0.20) from 1985 to 1989, followed by a peak in 1991 (Fig. 5*c*).

From 1956 to 1988, the number of annual *Tomicus* attacks varied in all damage classes, with less than 0.02 attacks per shoot, except in 1962 when there were 0.04 attacks on trees in the moderately damaged class. The number of attacks increased to 0.03–0.05 between 1989 and 1991 in all damage classes (Fig. 5*d*), but the attacks were more concentrated in the slightly and moderately damaged classes than in the severely damaged class.

Annual branch mortality was less than 16 in all the damage classes from 1945 to 1978. After 1978 it increased to a peak of 19–29 killed branches in 1981–1985 in the slightly damaged tree class and 19–27 branches in 1979, 1981–1985, and 1987–1990 in the moderately damaged tree class (Fig. 5*e*). In the severely damaged tree class, 19–41 branches were killed from 1984 to 1991 with devastating peaks occurring in 1987, 1988, and 1990 (Fig. 5*e*).

Discussion

Earlier Roll-Hansen (1964), Roll-Hansen and Roll-Hansen (1973), and Aalto-Kallonen and Kurkela (1985) related the time to subsequent development of *G. abietina* cankers in Scandinavia. Uotila (1988) also studied leader changes caused by the pathogen in Finland. However, all of these studies were made using data from the main stem of diseased trees. The present method, the first to use branches to determine infection history, showed that *G. abietina* had affected Scots pine in the study area for decades. Two peaks in canker formation were observed in the 1940s and one in leader changes in the 1950s, but the low number of branches examined does not allow other conclusions to be made for this time frame. According to the number of cankers and scars, the last epidemic started in the early 1980s, increased at the end of the 1980s, then declined. The same pattern was detected in all damage classes, but the epidemic declined earlier in the slightly damaged tree class and continued longer in the severely damaged tree class. Perhaps the damage peak from 1986 to 1987 resulted from infections during the mid-1980s, and this in turn resulted in the devastation of the trees in the severely damaged tree class. Also the high occurrence of branch leader changes and branch mortality in the middle and late 1980s suggests that damage was greater and lasted longer in the severely damaged tree class than in the moderately or slightly damaged classes. At the end of the 1980s, the number of shoot beetle attacks increased, but the low number of attacks (see Kaitera and Jalkanen 1994*a*) especially in severely damaged tree class suggests that shoot beetles played a minor role in the damage development.

The development of the *G. abietina* epidemic in our study area was similar to that in a badly damaged Scots pine stand with about the same macroclimatic, but different microclimatic, about 90 km away in Rikkilehto (Kaitera and Jalkanen 1992, 1993, 1994*a*). For severely damaged trees, the scar, canker, and branch leader change patterns were almost the same as at Rikkilehto at the same time during the peak of the epidemic. The number of infected shoots in all tress studied was, however, much lower in the study area than at Rikkilehto (Kaitera and Jalkanen 1992, 1994*a*).

The lack of meteorological data in the study area does not allow us to make correlations between annual damage and climate. The summer of 1981 was, however, extremely rainy near Rikkilehto (June–August rainfall 100% greater than the average for the 1975–1990 period), after which the epidemic seemed to increase dramatically, but there was no clear relationship between monthly rainfall and peaks in the epidemic (Kaitera and Jalkanen 1993). Other factors such as cold weather may also be important in disease

development. For example, Aalto-Kallonen and Kurkela (1985) found a correlation between the number of stem cankers and cold growing seasons and the number of frost days in the 1970s in southern Finland. According to Uotila (1988) the low temperature sum in the growing season and low total irradiation may favor epidemics. Severe *G. abietina* damage was noted in pine plantations throughout Lapland, especially at high elevations, in 1982 (Uotila and Jalkanen 1982), further supporting the idea that the prevailing macroclimatic has a major effect on epidemics. A decline in new damage in the late 1980s was reported by Salemaa et al. (1991). Our results support their findings. The reasons for this decline in the study area may be due to warmer and dryer growing periods in the late 1980s, which may have predisposed pine growth and probably reduced the dissemination of conidia, as occurred in the badly damaged stand at Rikkilehto (Kaitera and Jalkanen 1993).

The macroscopic symptoms of *G. abietina* damage and other biotic or abiotic factors such as frost may be mixed with each other when observed in the field (Kurkela 1981). However, using a microscope, the typical yellow-greenish color of *G. abietina* infected wood is very clear compared with wood damaged by other agents including frost, after which the wood may be blue stained very easily (Jalkanen 1985).

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IV

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Development of fruiting bodies of large tree type of *Gremmeniella abietina* var. *abietina* and timing of infection on Scots pine in northern Finland

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Summary

The formation and maturing of the large tree type *Gremmeniella abietina* var. *abietina* fruiting bodies and their sporulation were investigated for 3 years on Scots pine (*Pinus sylvestris*) in northern Finland. This was done by monthly assessment of shoots in the field and in the laboratory. Infection caused by *G. abietina* var. *abietina* was dated on Scots pine by monthly covering with pollination bags and exposing branches during the growing season. Pycnidia appeared between August and September, 1 year after infection, and they started to release conidia between late June and early July, 2 years after infection. Fresh pycnidia and microconidia were formed during the following August and September in the infected shoots. The causal large tree type of *G. abietina* var. *abietina* did not produce apothecia on branches within 3 years of infection. Monthly covering and exposing branches showed that infection took place mainly between June and July.

1 Introduction

Gremmeniella abietina (Lagerb.) Morelet has severely damaged older Scots pine (*Pinus sylvestris* L.) in recent decades in northern Finland (KAITERA and JALKANEN 1994a; KAITERA et al. 1995). Such damage has been found only in topographical depressions along riversides (KAITERA and JALKANEN 1993, 1995a). The disease symptoms and conidia and apothecia production in the field and in the laboratory (see UOTILA 1983; KAITERA and JALKANEN 1996) indicate that the cause of the damage is the large tree type (type A) *G. abietina* var. *abietina* (later *G. abietina*).

Although *G. abietina* mainly produces pycnidia 1 year (ROLL-HANSEN 1964; KURKELA 1967) and apothecia 2 years after infection (ROLL-HANSEN and ROLL-HANSEN 1973a; HELLGREN and BARKLUND 1992), large tree type *G. abietina* fruiting body production in Fennoscandia has not been described. In addition, there appear to be no studies dealing with timing of infection during the growing season.

The aim of this study was to investigate the timing of fruiting of *G. abietina*, maturing and sporulation of *G. abietina* fruitbodies during an outbreak of the disease, and to determine the infection caused by *G. abietina* on Scots pine during the growing season after an outbreak in northern Finland. This is the first time the type of *G. abietina* has been accurately identified, both genetically and using traditional characterization methods, during an infection or sporulation study. Identification is essential as both types of *G. abietina* occur in northern Fennoscandia.

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2 Materials and methods

2.1 Study area

The study was carried out in a stand of Scots pine slightly damaged by *G. abietina* while at the first-thinning stage (age 70 years), representing typical damage observed in the 1980s in northern Finland. The stand is located in Kemihaara in northern Finland (67°57′N, 28°57′E). The distribution and severity of the *G. abietina* damage, the past disease development pattern of Scots pine in the stand (KAITERA and JALKANEN 1995b), and also the *in vitro* growth (KAITERA and JALKANEN 1996) of the large tree type *G. abietina* isolated from the stand has been published.

2.2 Identification of the pathogen

G. abietina was isolated (one isolate per tree) within a 1-ha area from branches, occurring above the annual snow cover, which showed typical symptoms of Scleroderris canker and bore *G. abietina* pycnidia (eight isolates), from the corresponding symptomless shoots (one isolate), and from small seedlings growing under the annual snow cover (three isolates). The isolates were identified using amplitype-specific random amplified microsatellites (RAMS) markers. For DNA isolation procedures, primer sequences and reaction conditions see HANTULA et al. (1996), and for marker specificities see HANTULA and MÜLLER (1997).

2.3 Timing of fruiting, maturing and sporulation of pycnidia

2.3.1 Experiment 1

In July 1992, 100 shoots formed in 1991 and bearing symptoms caused by *G. abietina*, were marked at random on 19 Scots pine trees. The assessment of the damage symptoms in the field was based on the occurrence of dead buds and dead parts of the shoot, weakly intact needles brownish at the base, or the presence of *G. abietina* pycnidia in the shoot (KURKELA 1967, 1981).

Between July 1992 and late September 1993, the occurrence of *G. abietina* pycnidia and apothecia in the shoots were recorded monthly; between June and August 1993 this was done twice monthly using a × 25 pocket microscope. The occurrence of pycnidia at the base, midway along and at the tip of the shoot, and in the needles, was detected towards the end of 1992 (21 July, 2 October and 31 October). The frequency of pycnidia per shoot was recorded in mid-July 1993 using the following frequency classes: none, a few (1–9) and abundant (10 or more).

Maturation of the pycnidia was determined monthly by counting the number of shoots bearing pycnidia that were releasing conidia. Sporulation was identified by the presence of creamy-pinkish spore tendrils on the pycnidium; the pycnidia were ruptured shortly after sporulation. The spore release period of the pycnidia was determined as the time between the first and the last pycnidia to sporulate. In addition, the number of infected shoots bearing fresh pycnidia (pycnidia appearing 2 years after the infection in shoots formed and infected in 1991 and already bearing pycnidia 1 year after infection) was also counted. The occurrence of apothecia was assessed monthly between July 1992 and September 1993, and then in August 1994 when the experiment was terminated.

2.3.2 Experiment 2

Another 100 shoots that were formed and infected in 1992 were selected in mid-August at random and marked in Scots pine trees adjacent to those used in experiment 1. The number

of shoots bearing *G. abietina* pycnidia, the number of such shoots releasing conidia, and the number of pycnidia per shoot (for classes used, see experiment 1 above) were recorded four times in mid and late August and late September 1993, and in early August 1994.

2.3.3 Experiment 3

Twenty shoots, formed and infected in 1991 and scattered among the same and adjacent pine trees as those used in experiment 1, were sampled monthly between early October 1992 and late September 1993 (twice a month between June and August 1993). The shoots bearing *G. abietina* pycnidia and those bearing sporulating pycnidia (see experiment 1) were counted under a stereomicroscope. The number of pycnidia per shoot (for classes used, see experiment 1) was estimated monthly from early March 1993.

One to five pycnidia per shoot were crushed on a microscope slide in lactic acid and observed under a light microscope; the number of conidia per glass was estimated using the classes 'no spores', 'a few spores' (< 10) and 'abundant spores' (10 or more). The highest number (one figure for the whole sample after visual estimation) and variation in number of septa in the conidia were estimated, and the occurrence of microconidia (ROLL-HANSEN and ROLL-HANSEN 1973b) was recorded.

A sample of 20 shoots, formed and infected in 1992 and adjacent to the shoots investigated in experiment 2, was cut in late August and September 1993. The number of pycnidia per shoot and the number of sporulating pycnidia were determined. In a crushed sample of pycnidia as described above, the number of conidia (for classes used, see above), the occurrence of microconidia, and the highest number and variation in number of the conidia septa were determined.

2.4 Timing of *G. abietina* infection during the growing season

2.4.1 Experiment 4

One to six clusters per Scots pine tree (five to six healthy branches within three metres from each other made up a cluster) were marked in 65 trees by early June 1993. An asymptotic, healthy-looking ('healthy') branch was allowed to have small *G. abietina* cankers in the shoots formed in 1992 or earlier, but branches bearing *G. abietina* fruitbodies were rejected. The infection by *G. abietina* was cross-dated by two experiments. First, 100 branches (total = 500, excluding controls) were covered each month from June to September 1993 in 52 Scots pine trees by a pollination bag for 1 month to prevent *G. abietina* spore infection in the current year's shoots. As a control, 100 branches were kept uncovered in the same trees. Second, a total of 300 branches were covered at the end of May in 36 pine trees among the former 65 trees. Fifty of these branches were exposed monthly for 1 month between June and September 1993 to allow *G. abietina* spore infection in the current year's shoots. Fifty branches were kept uncovered and 50 branches were unexposed for the total period of the investigation.

Branches were cut in early August 1994, and all *G. abietina* symptoms in the shoots formed in 1993 were checked under a stereomicroscope in the laboratory. The symptoms of direct infection in terms of cankers caused by *G. abietina*, killed shoots, *G. abietina* pycnidia, browning of needles from needle base and weakly intact needles (KURKELA 1967, 1981; KAITERA and JALKANEN 1992), and those of indirect infection via strangulation of the shoot by a canker occurring in older shoots (see ROLL-HANSEN 1964) were recorded.

2.5 Statistical analysis

The number of healthy and infected branches between the months was tested by the χ^2 test using SAS statistical software (ANONYMOUS 1989).

3 Results

3.1 Identification of the pathogen

The primary identification was carried out by a RAMS analysis using the CCA-primer, which results in amplitype specific banding patterns at the molecular weight area ≈ 1500 bp (HANTULA and MÜLLER 1997). In this study we observed only one banding pattern type, which has previously been observed only in the large tree type *G. abietina* (and not in the small tree type) suggesting that all the isolates were of large tree type. This single-marker based identification was strengthened using other markers obtained with CCA-primer. They showed markers previously shown to be common in the large tree type, but rare in the small tree type (HANTULA and MÜLLER 1997). In three cases the identification was further confirmed by analysing the ACA700 (dominant marker in the small tree type) and CGA500 markers (dominant marker in the large tree type; HANTULA and MÜLLER 1997). These analyses identified all the isolates as the large tree type *G. abietina*.

3.2 Timing of fruiting, maturing and sporulating of pycnidia

3.2.1 Experiment 1

Less than 20% of the shoots investigated bore *G. abietina* pycnidia in July 1992; the pycnidia were located either at the base (35.3% of the shoots), midway along (35.3%) or at the tip (29.4%) of the shoot. In September 1992, most of the shoots bore pycnidia, and in the early October 1992 over 80% did so (Fig. 1). The pycnidia occurred in both the shoot and its needles in 41% of the shoots bearing pycnidia in late October 1992. In mid-July 1993, 76% of all shoots and 86% of the shoots bearing pycnidia bore abundant pycnidia per shoot.

The pycnidia started to release conidia during the first 2 weeks of June 1993, 2 years after the initial infection. At the beginning of July, over 60% of the shoots bearing pycnidia were releasing conidia, and by mid-July 1993, all such shoots were sporulating. Sporulation continued during July, and all pycnidia on a shoot were releasing conidia in almost 90% of the shoots bearing pycnidia by early August 1993 (Fig. 1).

Fresh pycnidia appeared in 6% of all shoots at the margins between the infected and healthy tissues in early August 1993, and the relative number of such shoots increased to

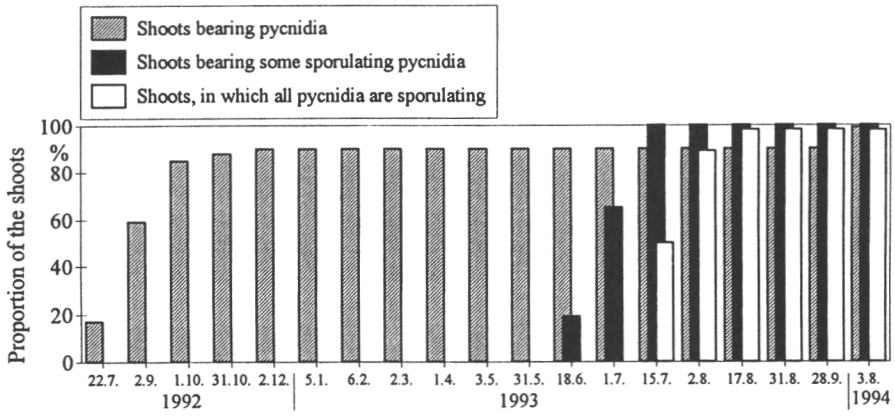

Fig. 1. Temporal variation (%) among shoots formed in 1991 and bearing *Gremmeniella abietina* var. *abietina* pycnidia (grey columns), bearing some sporulating *G. abietina* var. *abietina* pycnidia (black columns) and bearing *G. abietina* var. *abietina* pycnidia, of which all were sporulating (white columns)

43% by the following September (Fig. 2). No fresh pycnidia were detected after that up to the completion of observations in August 1994. No apothecia were produced in any of the shoots investigated during the period of the study.

3.2.2 Experiment 2

In 1993, *G. abietina* pycnidia appeared later in shoots formed in 1992 than in 1992 in shoots formed in 1991, 1 year after infection. In mid and late August, late September 1993, and early August 1994, 6%, 43%, 65% and 92%, respectively, of the shoots investigated bore pycnidia. None of the shoots bearing pycnidia bore abundant pycnidia per shoot in August 1993. The portion was 9% at the end of the following September and 63% at the beginning of August 1994. No pycnidia sporulated during 1993; by August 1994, 92% of the shoots bearing pycnidia released conidia and 65% of such shoots bore only sporulating pycnidia.

3.2.3 Experiment 3

In late October 1992, *G. abietina* pycnidia were found in shoots formed in 1991; the number of pycnidia per shoot varied greatly among the months thereafter. The pycnidia were abundant in 55% of the shoots in March 1993 and in 90–100% from the following July. In shoots formed in 1992, the proportions of shoots bearing pycnidia were 83% and 100% in late August and September 1993, respectively. At that time, 47% and 75% of the sample shoots bearing pycnidia bore abundant numbers of pycnidia.

The pycnidia of shoots formed in 1991 contained conidia sporadically in late 1992, but the number of pycnidia with abundant spores increased from February 1993 (Fig. 3). Eighty per cent and 95% of the shoots formed in 1992, and bearing pycnidia, contained abundant conidia in August and September 1993.

Microconidia were found in 5% of the shoots formed in 1991 and bearing pycnidia in the early June 1993. After that date, the proportion of shoots with such pycnidia increased, peaking between August and September 1993 at 45% to 50% (Fig. 4). Among the shoots formed in 1992, microconidia occurred on 0% and 10% of the shoots in August and September 1993, respectively.

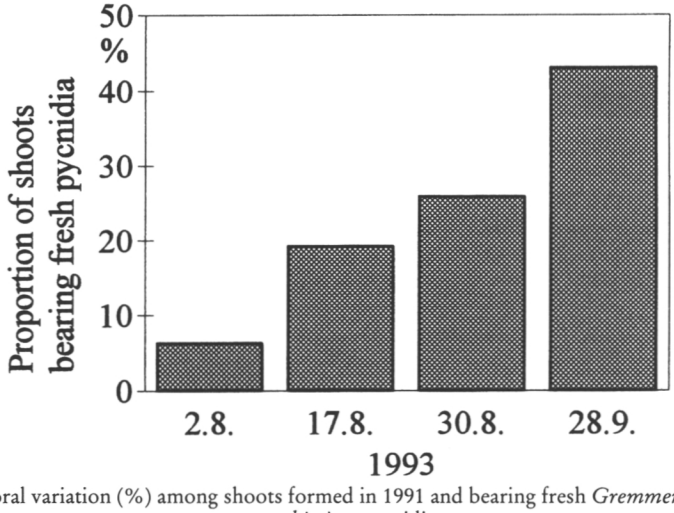

Fig. 2. Temporal variation (%) among shoots formed in 1991 and bearing fresh *Gremmeniella abietina* var. *abietina* pycnidia

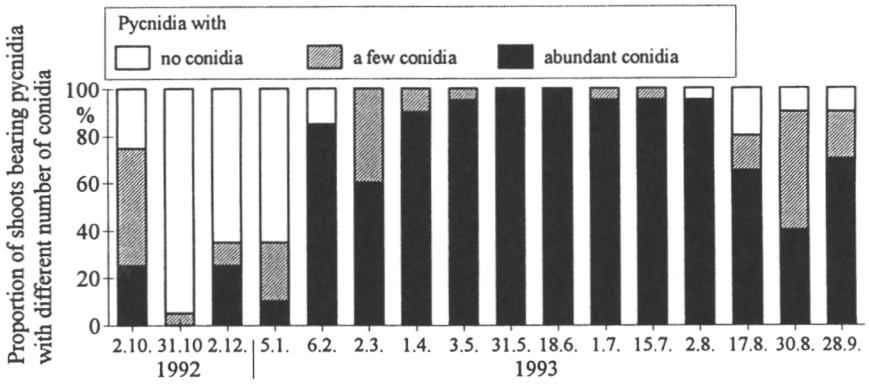

Fig. 3. Temporal variation (%) among shoots formed in 1991 and bearing *Gremmeniella abietina* var. *abietina* pycnidia with no conidia (white column), a few conidia (< 10, grey column) and abundant conidia (10 or more, black column)

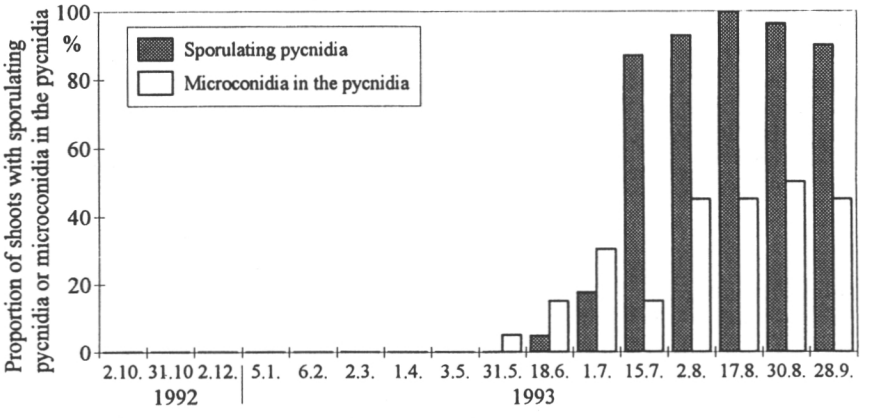

Fig. 4. Temporal variation (%) among shoots formed in 1991 and bearing *Gremmeniella abietina* var. *abietina* pycnidia containing microconidia (white column) or bearing sporulating *Gremmeniella abietina* var. *abietina* pycnidia (grey column)

A total of 5% of the shoots formed in 1991, and bearing pycnidia, released conidia in mid-June 1993; the majority of the shoots started to sporulate between early (18%) and mid-July (87%), and nearly all pycnidia were sporulating by mid-August 1993 (Fig. 4). Not a single shoot formed in 1992 bore sporulating pycnidia in 1993.

The pycnidia on shoots formed in 1991 contained one- to six-celled conidia, the majority being one- to four-celled — these were judged to represent large tree type *G. abietina* (see also KAITERA and JALKANEN 1996). The frequency of four-celled conidia increased from mid-June 1993, with conidia with less than four cells dominating prior to this. The pycnidia on shoots formed in 1992 contained one- to four-celled conidia in 1993.

3.3 Timing of *G. abietina* infection during the growing season

3.3.1 Experiment 4

Of the 500 branches, 19 were lost through breakage during the investigation period. Infection took place during the entire period of investigation. The number of infected shoots was low among the branches studied, varying monthly between 6% and 19% (Fig. 5a). The number of shoots with symptoms of direct *G. abietina* infection was lowest in July, indicating that July was the main time of infection in 1993 (Fig. 5a). Covering of branches in September resulted in the highest number of shoots strangled by *G. abietina* cankers occurring in shoots formed either in 1991 or 1992. The number of healthy and infected branches did not, however, significantly differ between the months ($df = 4$, $\chi^2 = 7.76$, $p = 0.101$). Less than 1% of the investigated shoots bore *G. abietina* pycnidia in August 1994. In the second experiment, the number of monthly exposed branches varied between 47 and 52. The infection took place mainly in the early summer; the number of infected shoots was lowest in August, while the highest numbers were detected in June and July (Fig. 5b). There was, however, no clear difference between the months in the frequency of healthy and infected branches, the number of branches with *G. abietina* pycnidia or the shoots strangled by old *G. abietina* cankers. The difference was not tested statistically because of the low number of observations in most of the test classes. A large number of shoots exposed in September and a few shoots covered during the entire period of investigation, were also infected. Pycnidia occurred in less than 1% of the shoots in August 1994.

4 Discussion

G. abietina pycnidia were found in almost all the shoots investigated, indicating that the criteria for identifying the disease symptoms of infected shoots were valid. According to KAITERA and JALKANEN (1996), *G. abietina* isolated from Scots pine branches, in the same stand as in this study, demonstrated typical characters of large tree type (type A) as described by UOTILA (1983) in Finland both in the field and in the laboratory. In this study, the corresponding *G. abietina* type was identified using a novel DNA-based RAMS analysis (HANTULA and MÜLLER 1997). In this study, the large tree type *G. abietina* began to produce pycnidia in the late summer of the year following infection. This is supported by earlier

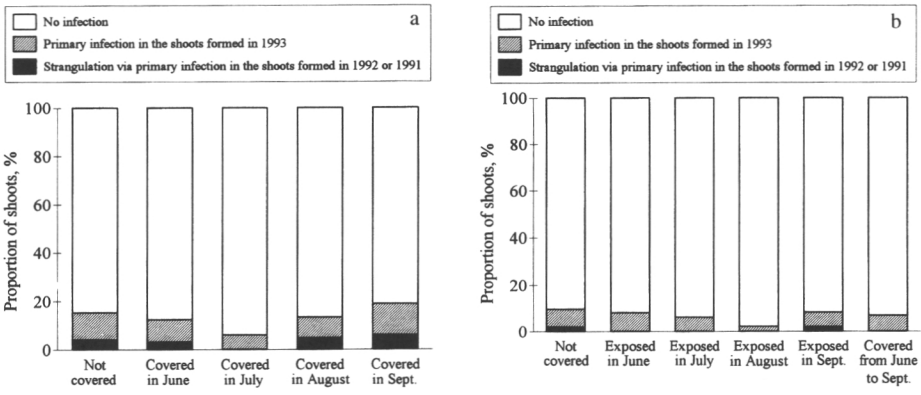

Fig. 5. Temporal variation among healthy, *Gremmeniella abietina* var. *abietina*-infected and *Gremmeniella abietina* var. *abietina*-strangled shoots formed in 1993, and which were not covered, covered (a) and exposed (b) in June, July, August and September, and covered from June to September (b) 1993

general observations made in Fennoscandia (KURKELA 1967; ROLL-HANSEN and ROLL-HANSEN 1973a; HELLGREN and BARKLUND 1992). The fruiting pattern of an individual type of *G. abietina* in Fennoscandia, however, has previously not been presented.

The maturing of the pycnidia in terms of the number and septation of the conidia inside the pycnidia occurred mainly during the late winter and early spring nearly 2 years after infection. However, no similar studies have been conducted elsewhere in Fennoscandia to establish the generality of these results. The conidia started to ooze out of the pycnidia in early June; sporulation peaked during the first 2 weeks of July, and continued until August, 2 years after infection. There was, however, slight variation between the years in the timing of both pycnidial formation and sporulation. This may have been caused by suboptimal moisture (SMERLIS 1968; SKILLING 1969; DORWORTH 1972; LAFLAMME and ARCHAMBAULT 1990) or light intensity and temperature (HUDLER et al. 1984) for conidial release. NEVALAINEN (1986) has reported briefly on a similar dissemination pattern of conidia in northern Finland using spore trapping after a severe *G. abietina* outbreak in 1982. However, he did not identify the causal type of the pathogen. In more southern climates late spring and early summer have been reported to be the main times for conidial release (SKILLING et al. 1986; HELLGREN and BARKLUND 1992).

The peak release of ascospores occurs in midsummer (July–August) in northern climates (NEVALAINEN 1986; LAFLAMME and ARCHAMBAULT 1990). In this study, large tree type *G. abietina* did not produce apothecia in the branches 2–3 years after initial infection. This has been reported to be the time without characterizing the causal type of *G. abietina* (KUJALA 1950; HELLGREN and BARKLUND 1992). According to UOTILA (1992), large tree type *G. abietina* (type A) produces apothecia less frequently than small tree type *G. abietina* (type B) following artificial inoculation, which is similar to the results of this study.

In this study, large tree type *G. abietina* produced microconidia principally 2 years after the infection. ROLL-HANSEN and ROLL-HANSEN (1973b) were the first to remark on microconidia, but their role in the life cycle of *G. abietina* is still unknown. According to SKILLING et al. (1986) and ZAJCHOWSKI and BERGDAHL (1982), both the European and the North American races of *G. abietina* produce microconidia. However, not all isolates of *G. abietina* produce microconidia (ZAJCHOWSKI and BERGDAHL 1982; UOTILA 1983).

Fresh pycnidia developed in late summer, 2 years after infection in the margins between infected and uninfected tissues. This phenomenon indicates a tremendous capacity for the pathogen to survive and reproduce in living branches for several years. Recently, large tree type *G. abietina* was also found to produce pycnidia in cankers on green shoots (KAITERA and JALKANEN 1994b). This is probably one reason for the continuous annual infections at stand level in susceptible sites, the survival of *G. abietina* during unfavourable years, and the successive epidemics that have occurred in northern Finland in the 1980s (KAITERA and JALKANEN 1992, 1995b).

Although a great number of pycnidia disseminated conidia during 1993, there was a low frequency of fresh disease in 1994. This may partly be due to the low number of branches investigated and to favourable climatic conditions for Scots pine during 1993 and 1994. In this study, the main period of infection during the growing season was July, when covering of the branches caused the greatest reduction in the number of infected shoots. The result is reasonable considering the dissemination pattern of the conidia in the stand. The covering of branches in late summer (September) probably delayed the over-wintering of the shoots and enhanced the growth of *G. abietina* in the cankers developed in recent years, which resulted in a large number of strangled shoots. This phenomenon has been noted to occur on Scots pine (ROLL-HANSEN 1964). Some shoots were probably infected before early June, or after the investigation period in late September or early October. The results of some earlier inoculation experiments have suggested that late spring and early summer are the best times for successful infection, i.e. during the growing period (PETÄISTÖ and REPO 1988). KURKELA and NOROKORPI (1979), however, stated that infection between August and September results in larger cankers than infection between May and July. The previous

statement is supported by UOTILA (1990), who noted that large tree type *G. abietina* produced more cankers when inoculated in late summer (August–October) than in early summer.

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Résumé

Développement des fructifications de Gremmeniella abietina var. abietina, 'type grands arbres', et déroulement de l'infection chez le pin sylvestre dans le nord de la Finlande

La formation et la maturation des fructifications ainsi que leur sporulation ont été étudiées pendant 3 ans chez des pins sylvestres dans le nord de la Finlande. Cela a été fait par l'examen mensuel de rameaux, en forêt et au laboratoire. L'infection par *G. abietina* var. *abietina* a été datée sur pin sylvestre en protégeant et en exposant mensuellement des branches pendant la saison de végétation. Les pycnides apparaissaient entre août et septembre, un an après l'infection et elles commençaient à émettre des conidies entre fin juin et début juillet, deux ans après l'infection. Des pycnides et des microconidies fraîches étaient formées au cours des mois d'août et septembre suivants chez les rameaux infectés. L'agent causal, le *Gremmeniella abietina* var. *abietina*, 'type grands arbres', n'a pas produit d'apothécies sur les branches au cours des 3 ans qui ont suivi l'infection. La protection et l'exposition mensuelle des branches ont montré que l'infection avait lieu surtout entre juin et juillet.

Zusammenfassung

Entwicklung von Fruchtkörpern von Gremmeniella abietina var. abietina Typ A (large tree type) und Zeitpunkt der Infektion von Kiefern in Nord-Finnland

Die Bildung und der Reifungsprozeß von Fruchtkörpern sowie die Sporulation von *Gremmeniella abietina* var. *abietina* Typ A (large tree type) auf befallenen Kiefern (*Pinus sylvestris*) in Nord-Finnland wurden während 3 Jahren untersucht. Dazu wurden Triebe im Feld und im Labor monatlich beurteilt. Um den Zeitpunkt der Infektion durch *G. abietina* var. *abietina* festzustellen, wurden Äste während der Vegetationsperiode jeweils für nur einen Monat exponiert, indem man sie während der restlichen Zeit in Betäubungssäcke eingepackt hielt. Pyknidien wurden ein Jahr nach der Infektion zwischen August und September sichtbar, und die Sporulation mit Konidien erfolgte 2 Jahre nach der Infektion Ende Juni–Anfang Juli. Neue Pyknidien und Mikrokonidien wurden im darauffolgenden August und September auf den befallenen Trieben gebildet. *Gremmeniella abietina* var. *abietina* Typ A bildete innerhalb von 3 Jahren nach der Infektion keine Apothezien auf den Ästen. Das Experiment mit den in Betäubungssäcken eingepackten Ästen zeigte, daß die Infektion hauptsächlich in den Monaten Juni und Juli stattfand.

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V

Occurrence of *Gremmeniella abietina* var. *abietina* large- and small-tree types in separate Scots pine stands in northern Finland and in the Kola Peninsula, Russia

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Variation in *Gremmeniella abietina* var. *abietina* was studied in three stands of Scots pine in northern Finland and in the Kola Peninsula. Eighty-four isolates of large- and small-tree types of *G. abietina* var. *abietina* (LTT and STT, respectively) were identified on the basis of tentative characteristics (spore morphology, disease type and host size), fatty acid and sterol profiles (FAST), and random amplified microsatellite technique (RAMS). Both LTT and STT occurred in all three stands. In general, the classifications obtained using the three methods agreed with one another, although a few contradicting results were observed. Variation in fatty acids and sterols in *G. abietina* var. *abietina* was rather low, although the amounts of some individual extractives showed statistically significant differences between the stands. All pathogenic and asymptotic *G. abietina* var. *abietina* isolates originating from branches located at heights above the annual snow cover were identified as LTT based on RAMS, but some were grouped to STT according to their FAST profiles. Both STT and LTT were detected among the isolates obtained from seedlings according to both FAST and RAMS. In addition, in two cases RAMS markers thought to be STT- or LTT-specific were found in the same isolate. The results presented here suggest that LTT of *G. abietina* var. *abietina* caused the devastating epidemics on pines in the first-thinning stage or middle age similar to pines in this study reported in northern Finland and in the Kola Peninsula during the 1980s.

Gremmeniella abietina (Lagerb.) M. Morelet has been divided into races in terms of its morphological, serological and genetical variation (Dorworth & Krywienczyk, 1975; Hamelin, Ouellette & Bernier, 1993; Bernier, Hamelin & Ouellette, 1994; Lecours *et al.*, 1994; Hamelin *et al.*, 1996). In Finland, two different types of *G. abietina* var. *abietina* (A and B) have been distinguished according to their production of conidia and apothecia on Scota pine (*Pinus sylvestris* L.) in the field and in the laboratory (Uotila, 1983). Also, these types differ from one another in terms of their fatty acid and sterol profiles (Müller & Uotila, 1997), pectic enzymes (Lecours *et al.*, 1994) and random amplified microsatellites (RAMS) (Hantula & Müller, 1997). The large-tree type (LTT) and small-tree type (STT) of *G. abietina* var. *abietina* described by Hellgren & Högberg (1995) are identical to types A and B based on immunoblotting (Petäistö *et al.*, 1996). Thus we shall from now on refer to these types as LTT and STT, respectively. Variation between LTT or STT types within a single pine stand using either of the recent protocols has not, however, been investigated.

The aim of this study was to clarify whether different types of *G. abietina* var. *abietina* could be found within the same stand, which *G. abietina* var. *abietina* type caused the devastating epidemics reported in the 1980s in northern Finland (Kaitera & Jalkanen, 1994), and whether *G. abietina* var. *abietina* varies over northern Finland and the Kola Peninsula.

MATERIALS AND METHODS

G. abietina var. abietina isolates

Eighty-four isolates were collected from three naturally regenerated and moderately or severely damaged Scots pine stands in the first-thinning stage or of middle age (average age 70–200 yr) in northern Finland (stands B and C) and in the Kola Peninsula, Russia (stand A, Fig. 1). Disease assessment and the long-term disease history of Scots pine in these stands has been published earlier (Kaitera *et al.*, 1995 *a*; Kaitera, Isaeva & Jalkanen, 1995 *b*). Within each stand, *G. abietina* var. *abietina* was isolated from the wood of recently killed shoots or pycnidia on branches of dominant trees (branches located at heights above the annual snow cover, bearing *G. abietina* var. *abietina* pycnidia and showing symptoms of scleroderris canker), and they were named as pathogenic isolates (A1–A14, B1–B16 and C1–C11, Table 1). *G. abietina* var. *abietina* was also isolated from one-year-old or two-year-old, healthy looking shoots adjacent to infected shoots of the same age, and bearing no visible symptoms of *G. abietina* over the length of the corresponding shoot; these were named asymptotic isolates (A21–A27, B21–B30 and C21–C31, Table 1). In addition, the pathogen was isolated from yellow-greenish stem or branch wood of killed small seedlings (less than 1 m tall, and thus occurring below the annual snow cover) next to *G. abietina* var. *abietina* apothecia (A41–A48, B41–B44 and C41–C43, Table 1). The samples (wood pieces

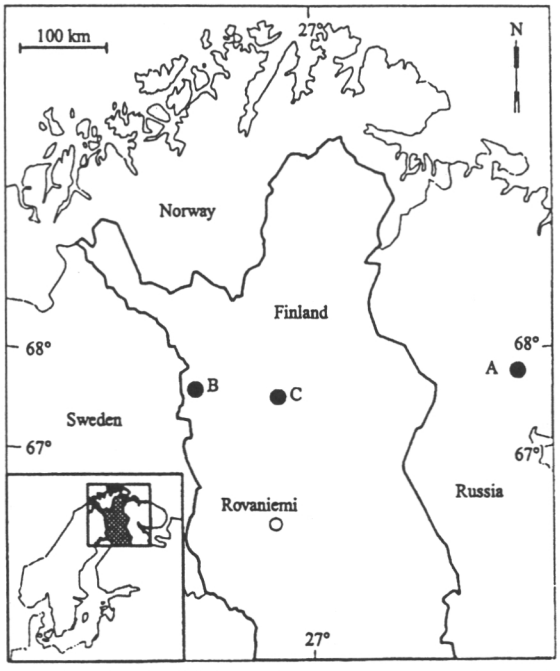

Fig. 1. The location of the investigated stands in the Kola Peninsula, Russia (A = Tsuna), and in northern Finland (B = Muonio, and C = Nuttio).

and fruit bodies) were surface-sterilized by dipping them into 10% sodium-hypochlorite for 1 min, and then placed on malt agar amended with pine needle extract. Each isolate was randomly chosen from among a greater number of isolates.

Tentative estimation of the G. abietina *var.* abietina *type*

The isolates were classiried as LTT or STT type of *G. abietina* var. *abietina* based on several characteristics. These included host size, the occurrence and abundance of fruit bodies observed on the host tree, and the abundance and septation of conidia produced *in vitro* in barley corn agar flasks (Uotila, 1983). The most important criterion employed was the number of conidia septa. When isolates did not produce any conidia *in vitro*, other criteria were used for characterization. The corresponding flasks were inoculated with fluffy and young *G. abietina* var. *abietina* mycelia, incubated in light at 15 °C for 54 d after which 25 ml of 0·4% (w/v) Tween solution was added into the flasks, shaken for 1 min, rested for 10 min, and again shaken for 30 s. After this, 1 µl of the conidial suspension was dropped on a Bürger haemocytometer, and the number of conidia and the variation in their septa were counted, followed by grouping the isolates into either LTT (all conidia having less than seven septa) or STT (some conidia having seven or more septa) types of *G. abietina* var. *abietina*. If an isolate did not produce conidia during the 54 d incubation period, it was reinoculated and reincubated from 68 to 124 d.

Fatty acid and sterol analysis (FAST)

The isolates were inoculated on three separate occasions on modified orange serum agar (MOS) (Müller, Kantola & Kitunen, 1994), incubated at 21° in the dark for 36 d, after which 1·5 g of mycelia (fresh weight) sectors were harvested and preserved in glass vials at −20°. The samples were then homogenized, vacuum-dried for 24 h, and then stored at −18° until the fatty acids and sterols were extracted and analysed by means of gas chromatography (Müller *et al.*, 1994). The extracts were identified by GLC-MS using a mass spectrum database, and confirmed by comparing retention times to those of standard compounds. Those extracts including the unidentified extracts, which, in three successive cultivations, amounted to at least 1% of the total area in samples of one or more isolates, were used for statistical analysis. For a more detailed description of the used protocols, see Müller *et al.* (1994).

Statistical evaluations were done using two programs; SAS (SAS Inc., Cary, U.S.A.) was used for variance analysis and Systat for Windows *v.* 5·0 (Systat Inc., Evanston, U.S.A.) for discriminant analysis.

Random Amplified Microsatellites (RAMS)

DNA was isolated as described by Hantula, Dusabenyagasani & Hamelin (1996); the protocol included cell disruption, two phenol-chloroform (1:1) extractions, a chlorophorm:isoamyl alcohol (24:1) extraction, and precipitation with alcohol followed by drying. The obtained DNA was resuspended into 10 mM Tris-HCl buffer (pH 8·0) containing 1 mM EDTA. The PCR reactions were carried out as described in Hantula & Müller (1997). In short, the samples were denatured after which 37 cycles of amplification were carried out at the annealing temperatures of 64° for CCA, 61° for CGA primer and 49° for ACA primer.

The amplification products were separated by electrophoresis, run in a TAE buffer, and visualized by ethidium bromide in uv light. The lengths of the amplification products were estimated by comparing them to a 100 bp DNA ladder (Life Technologies, Gaithersburg MD, U.S.A.) and, as length polymorphisms occurred, several electrophoresis runs were carried out to allow identification of the different markers only from lanes adjacent to each other. For a more detailed description of the protocol used, see Hantula & Müller (1997).

The identification of isolates to *G. abietina* var. *abietina* types by RAMS analysis was mostly based on the type specificity of markers observed by Hantula & Müller (1997). All the isolates were analysed for markers obtained with CCA primer. Of these, the CCA1500 marker (a marker with an approximate size of 1500 bp) has been reported to be *G. abietina* var. *abietina* type-specific (Hantula & Müller, 1997). In addition, advantage was taken from their observation that at most two markers with approximate molecular weight of 700–750 bp occur in LTT, whereas in STT there may be up to four such markers (Hantula & Müller, 1997). Therefore, the number of markers in this size class can be used to differentiate the *G. abietina* var. *abietina* types in many cases. In addition to these markers, a band with a molecular weight of approximately 150 bp (CCA150), not scored in Hantula & Müller (1997), was analysed during the course of this study. For 15 isolates (see the results), additional information was gathered by analysing *G. abietina* var. *abietina* type-specific markers of ACA700 (marker occurs in STT) and CGA500 (marker occurs in LTT) (Hantula & Müller, 1997).

RESULTS

Tentative classification of the isolates

Based on conidial morphology, both types of *G. abietina* var. *abietina* were detected in two stands (A and B). The number of conidia of the LTT isolates ranged between one and 691 ml^{-1} after 54 d incubation, and between 1 and 4×10^3 after 124 d incubation, and that of the STT isolates between 27 and $1{\cdot}8 \times 10^5$, and $1{\cdot}7 \times 10^3$ and $3{\cdot}5 \times 10^4$ ml^{-1}.

Ten isolates were tentatively classified as STT, 65 as LTT, and nine remained unclear in this respect as they did not produce conidia during the 124 d incubation period (Table 1), although eight of them could be classified as being of the LTT type based on other criteria, and only C41 could not be identified. Among the STT isolates, the proportion of conidia with seven or more septa ranged between 1% and 4%. Isolates A44 and B42 were exceptions as 11% and 6% of their conidia had seven or more septa. In addition, A11, A41, A43, A46, A47, B4 and B43 produced conidia typical for STT after one cultivation, but ones typical for LTT only after a repeated cultivation. All the LTT isolates produced conidia with less than five septa, except A1, A2, A6, A21, A24, A45, B7, B9, B16, B23, B26, C3 and C42, which produced 1–7%, respectively, of five-septate conidia. Isolates A3, B1 and C7 produced 1% of conidia with six septa.

Only two pathogenic isolates from dominant trees produced conidia typical for STT (A11 and B4). Both *G. abietina* var. *abietina* types were detected among isolates from seedlings, as eight out of 15 isolates (A41, A43, A44, A46, A47, B42, B43 and B44, Table 1) produced conidia typical for STT, and six isolates (A42, A45, A48, B41, C42 and C43) produced conidia typical for LTT.

Variation in G. abietina *var.* abietina *based on FAST*

Representatives of both STT and LTT groups were found in all three stands, as well as among pathogenic and asymptotic isolates and isolates from seedlings according to FAST profiles. Twelve out of the 84 isolates investigated could not be classified as belonging to either LTT or STT groups with statistical significance at $P < 0{\cdot}01$ based on discriminant analysis of their FAST profiles (Table 1). This may be expained by the fact that the discriminant model applied here was calculated earlier with isolates originating predominantly from central and southern Finland. Two isolates (C4 and C6) were grouped differently at $P < 0{\cdot}01$ (to STT) than with both other methods applied (Table 1).

Table 2 presents average FAST profile data for the various isolate groups. Group STT contains ten isolates classified as belonging to the STT group with statistical significance at $P < 0{\cdot}01$ (see Table 1), while the other isolates are included in the LTT groups. This grouping agrees well with the results obtained from RAMS. The variation between the FAST data for the *G. abietina* var. *abietina* groups listed in Table 2 was low. Statistically significant differences were found mainly between the STT and the LTT groups. The FAST profile variation between LTT isolates from the three stands A, B and C were small; significant differences were observed only between Cis-6,9-octadecadienoic acid (6,9-18:2) and Ergost-22-en-3-on (Ergostenon). No significant variation was found between FAST profiles of pathogenic and asymptotic isolates.

The average mismatch values of the isolates show a small within-stand variation (Table 2). The average mismatch value of all LTT isolates is in the same range as that within the group in Table 2.

Identification of G. abietina *var.* abietina *types by RAMS*

The amplification products obtained using the CCA primer identified 73 isolates as belonging to the LTT group and six to the STT group (Table 1). There were two exceptions; C42 had a LTT-specific CCA1500 marker, whereas the other studied markers were STT-specific, and B43, which had other markers STT-specific, but revealed one marker with an apparent size of about 150 bp (Table 1). This marker was observed only among the LTT isolates in the rest of our data. These two observations led us to study these and 13 other isolates using CGA and ACA primers. These analyses confirmed the identification of the 13 control isolates (based solely on markers amplified using the CCA primer; Table 1). In addition, the markers obtained with these primers were STT-specific for B43 and C42 (Table 1). Thus, both of these isolates had one marker typical for LTT and four for STT, suggesting that the markers in CCA1500 and CCA150 locus were either not completely *G. abietina* var. *abietina* type-specific, or that these two isolates were hybrids between LTT

Table 1. Classification of *Gremmeniella abietina* var. *abietina* isolates in three stands (A = Tsuna, Russia, B = Muonio, and C = Nuttio, northern Finland), by initial identification (based mainly on conidia morphology *in vitro*), FAST profiles, and type-specific RAMS markers. Isolates A1–A14, B1–B16 and C1–C11 represent pathogenic *Gremmeniella abietina* var. *abietina*, and A21–A27, B21–B30 and C21–C31 asymptotic *Gremmeniella abietina* var. *abietina*. Isolates A41–A48, B41–B44 and C41–C43 were from seedlings

		FAST profiles			RAMS markers				
		Probability of belonging to*			CCA			ACA	CGA
	Inital identification	LTT	STT	Result	1500	700–750	150	700	500
A1	LTT	1	0	LTT	LTT	?[1]	LTT	—[3]	—
A2	LTT	0·056	0·944	STT	LTT	?	LTT	—	—
A3	LTT	0·986	0·014	LTT	LTT	?	LTT	—	—
A4	LTT	1	0	LTT	LTT	?	LTT	—	—
A5	LTT	1	0	LTT	LTT	?	LTT	—	—
A6	LTT	1	0	LTT	LTT	?	LTT	—	—
A7	LTT	0·993	0·007	LTT	LTT	?	LTT	—	—
A8	LTT	0·507	0·493	LTT	LTT	?	LTT	—	—
A9	LTT	0·635	0·365	LTT	LTT	?	LTT	—	—
A10	LTT	1	0	LTT	LTT	?	LTT	—	—
A11	STT	0·960	0·040	LTT	LTT	?	LTT	—	—
A12	LTT	1	0	LTT	—	—	—	—	—
A13	LTT	1	0	LTT	LTT	?	LTT	—	—
A14	LTT	0·155	0·845	STT	LTT	?	LTT	—	—
A21	LTT	1	0	LTT	LTT	?	LTT	—	—
A22	LTT	1	0	LTT	LTT	?	LTT	—	—
A23	LTT	0·003	0·997	STT	—	—	—	—	—
A24	LTT	1	0	LTT	—	—	LTT	LTT	LTT
A25	LTT	1	0	LTT	LTT	?	LTT	—	—
A26	LTT	1	0	LTT	LTT	?	LTT	—	—
A27	LTT	1	0	LTT	LTT	?	LTT	—	—
A41	STT	0	1	STT	STT	STT	STT	STT	STT
A42	LTT	0·998	0·002	LTT	LTT	?	LTT	LTT	LTT
A43	STT	0	1	STT	STT	STT	STT	STT	STT
A44	STT	0	1	STT	STT	STT	STT	STT	STT
A45	LTT	1	0	LTT	LTT	?	LTT	LTT	LTT
A46	STT	1	0	LTT	LTT	?	LTT	LTT	LTT
A47	STT	0·927	0·073	LTT	STT	STT	STT	STT	STT
A48	LTT	1	0	LTT	LTT	?	LTT	—	—
B1	LTT	1	0	LTT	LTT	?	LTT	—	—
B2	LTT	1	0	LTT	LTT	?	LTT	—	—
B3	LTT	1	0	LTT	LTT	?	LTT	—	—
B4	STT	1	0	LTT	LTT	?	LTT	—	—
B5	LTT	0·995	0·005	LTT	LTT	?	LTT	—	—
B6	LTT	1	0	LTT	LTT	?	LTT	—	—
B7	LTT	1	0	LTT	LTT	?	LTT	—	—
B8	LTT	1	0	LTT	LTT	?	LTT	—	—
B9	LTT	1	0	LTT	LTT	?	LTT	—	—
B10	LTT	1	0	LTT	LTT	?	LTT	—	—
B11	LTT	1	0	LTT	LTT	?	LTT	—	—
B12	LTT	1	0	LTT	LTT	?	LTT	—	—
B13	LTT	1	0	LTT	LTT	?	LTT	—	—
B14	LTT	1	0	LTT	LTT	?	LTT	—	—
B15	LTT	1	0	LTT	LTT	?	LTT	—	—
B16	LTT	1	0	LTT	LTT	?	LTT	—	—
B21	LTT	1	0	LTT	LTT	?	LTT	—	—
B22	LTT	1	0	LTT	LTT	?	LTT	—	—
B23	LTT	1	0	LTT	LTT	?	LTT	—	—
B24	LTT	1	0	LTT	LTT	?	LTT	—	—
B25	LTT	1	0	LTT	LTT	?	LTT	—	—
B26	LTT	1	0	LTT	LTT	?	LTT	—	—
B27	LTT	1	0	LTT	LTT	?	LTT	—	—
B28	LTT	1	0	LTT	LTT	?	LTT	—	—
B29	LTT	1	0	LTT	LTT	?	LTT	—	—
B30	LTT	0·629	0·371	LTT	LTT	?	LTT	—	—
B41	LTT	0·937	0·063	LTT	LTT	?	LTT	LTT	LTT
B42	STT	0	1	STT	STT	STT	STT	STT	STT
B43	STT	0	1	STT	STT	STT	LTT	STT	STT

Table 1. *Continued.*

		FAST profiles			RAMS markers				
		Probability of belonging to*			CCA			ACA	CGA
	Inital identification	LTT	STT	Result	1500	700–750	150	700	500
B44	STT	0	1	STT	STT	STT	STT	STT	STT
C1	LTT	1	0	LTT	LTT	?	LTT	—	—
C2	LTT	1	0	LTT	LTT	?	LTT	—	—
C3	LTT	1	0	LTT	LTT	?	LTT	—	—
C4	LTT	0	1	STT	LTT	?	LTT	—	—
C5	LTT	1	0	LTT	LTT	?	LTT	—	—
C6	LTT	0	1	STT	LTT	?	LTT	—	—
C7	LTT	1	0	LTT	LTT	?	LTT	—	—
C8	LTT	1	0	LTT	LTT	?	LTT	—	—
C9	LTT	0·107	0·983	STT	LTT	?	LTT	—	—
C10	LTT	1	0	LTT	LTT	?	LTT	—	—
C11	LTT	1	0	LTT	LTT	?	LTT	—	—
C21	LTT	1	0	LTT	LTT	?	LTT	—	—
C22	LTT	1	0	LTT	LTT	?	LTT	—	—
C23	LTT	1	0	LTT	LTT	?	LTT	—	—
C24	LTT	1	0	LTT	LTT	?	LTT	—	—
C25	LTT	1	0	LTT	LTT	?	LTT	—	—
C26	LTT	0·981	0·019	LTT	LTT	?	LTT	—	—
C27	LTT	1	0	LTT	LTT	?	LTT	—	—
C28	LTT	0·999	0·001	LTT	LTT	?	LTT	—	—
C29	LTT	1	0	LTT	LTT	?	LTT	—	—
C30	LTT	1	0	LTT	LTT	?	LTT	—	—
C31	LTT	0·397	0·603	STT	LTT	?	LTT	—	—
C41	?	1	0	LTT	LTT	?	LTT	LTT	LTT
C42	LTT	0	1	STT	LTT	?	STT	STT	STT
C43	LTT	1	0	LTT	LTT	?	LTT	STT	LTT

* According to discriminant analysis by Systat version 5.0.
[1] Not conclusively identifiable.
[2] Not determined.

and STT, or perhaps back-crosses between a hybrid and a strain of STT.

All the isolates from dominant trees regardless of the stand or pathogenicity belonged to LTT, but six out of 15 isolates from seedlings belonged to STT (A41, A43, A44, A47, B42 and B44), and seven to LTT (A42, A45, A46, A48, B41, C41 and C43). In addition, both isolates B43 and C42 were isolated from seedlings.

DISCUSSION

This study confirms the earlier suggestion that the damage occurring in Scots pine branches located above the annual snow cover in the 1980s in northern Finland and the Kola Peninsula (see Kaitera & Jalkanen, 1994, 1995; Kaitera *et al.*, 1995*a*, *b*), was caused by LTT of *G. abietina* var. *abietina*. Earlier epidemics caused by *G. abietina* in northern Fenno-scandia have only been reported on pine seedlings and showed symptoms resembling those caused by STT (Kohh, 1964; Norokorpi, 1971; Karlman, Hansson & Witzell, 1994). The results of both FAST and RAMS showed that both types of *G. abietina* var. *abietina* were detected within each stand, indicating a wide distribution of both types over northern Fennoscandia. The results also demonstrated that LTT dominated on pine branches located above the annual snow cover, while STT (based on RAMS or FAST and tentative determinations) was lacking or almost lacking in such branches. Hence, STT injured almost solely small seedlings with heights not exceeding the thickness of the snow cover, but LTT also occurred in such seedlings, which is in good agreement with Uotila's (1983) description for A and B types of *G. abietina* var. *abietina* in Finland.

In this study, the three most abundant extractives of the isolates of LTT and STT groups, both among the stands and in the isolate groups, were equal to those listed earlier by Müller & Uotila (1997). Previously Ranta & Neuvonen (1994) isolated *G. abietina* frequently from symptomless one-year-old shoots after inoculation. Petrini *et al.* (1990), did not find any differences in the protein patterns between endophytic and pathogenic *G. abietina* isolates, and this supports the results obtained in the present study. Conidia morphology has proved to be a good criterion for distinguishing the two types of *G. abietina* var. *abietina* from one another (Uotila, 1983, 1990, 1992; Kaitera & Jalkanen, 1996). Based on conidia morphology, STT of *G. abietina* var. *abietina* resembles *G. abietina* var. *cembrae* (Ettlinger, 1945), but the latter variety has not been found in Finland (Hantula & Müller, 1997; Müller & Uotila, 1997). According to Uotila (1990), STT (type B)

Table 2. Average FAST-profiles of eighty-four isolates of three stands (A–C) and pathogenic and asymptotic isolates with average within group-mismatch values

	LTT						
	Stand A	Stand B	Stand C	All LTT	All STT	Pathogenic	Asymptotic
Number of isolates	25	27	22	74	10	46	28
Total extractive (% w/w)	2·7	2·5	2·7	2·6	4·2	2·5	2·9
Fatty acids[1]							
15:0	0·7[a]	0·7[a]	0·7[a]	0·7[a]	0·6[b]	0·8[a]	0·7[a]
9-16:1	1·0	1·1	1·0	1·0	0·7	1·0	1·0
16:0	12·2	12·3	12·6	12·3	11·3	12·5	12·0
17:1	0·8	0·9	0·9	0·8	0·9	0·9	0·8
OH-16:0	1·3	1·1	1·3	1·2	0·8	1·3	1·1
9,12-18:2	43·1	45·3[a]	45·0	44·5	41·6[b]	45·0[a]	43·6
C 18:1	24·8[a]	23·4[a]	23·2[a]	23·8[a]	29·5[b]	23·0[a]	25·2[a]
T 18:1	1·6	1·8	1·7	1·7	1·7	1·7	1·7
18:0	1·7	0·8	0·8	0·8	0·8	0·8	0·8
6,9-18:2	0·9[ac]	0·7[b]	0·8[bd]	0·8[bd]	0·9[cd]	0·8	0·8[bd]
x,x,x-18:3	0·2	0·3	0·3	0·3	0·4	0·3	0·3
OH-22:0	0·5	0·5	0·6	0·5	0·4	0·5	0·5
25:0	0·2	0·6	0·5	0·5	0·3	0·4	0·5
OH-24:0	1·4	1·2	1·2	1·3	0·9	1·3	1·3
Sterols[1]							
Ergostatrienol	0·4	0·4	0·2	0·3	0·4	0·4	0·3
STA	0·2	0·2	0·3	0·2	0·1	0·2	0·3
Ergostenon	2·9[a]	2·7[a]	2·9[a]	2·8[a]	1·5[b]	2·8[a]	2·8[a]
STE	1·1	1·1	0·9	1·0	0·7	1·1	1·0
Ergosterol	4·9	4·3	3·9	4·4	5·9	4·3	4·5
Ergostadienol	0·2[a]	0·0[b]	0·2	0·2	0·1	0·1	0·2
STB	0·7	0·7	0·9	0·8	0·2	0·8	0·7
Average mismatch	9	7	8	8	11	7	9

[1] STA, STE and STB are unknown sterols; for explanations of other abbreviations see Müller & Uotila (1997).

[ab] Values in each column with a different letter differ statistically significantly at $P < 0{\cdot}01$ according to Tukey's test.

produces conidia more abundantly on barley corn agar as well as apothecia *in vivo* (Uotila, 1992) than LTT does, and the conidia production varies greatly even among mycelia originating from ascospores of a single ascus (Uotila, 1990). In this study, however, some isolates did not produce conidia during the incubation period, or did so very poorly. Differences in colony appearance and in symptoms demonstrated by small seedlings were not clear enough to allow accurate typing of the isolates. Two isolates produced conidia typical for STT, although they belonged to LTT according to FAST and RAMS. This suggests that LTT may also produce conidia with more than six septa.

The FAST and RAMS results agree quite well, especially if the FAST classification succeeds at the 1·00 probability level. Nevertheless, two out of 84 isolates were grouped differently based on FAST and RAMS. One of these (C4) belonged also to LTT according to immunoblotting (Petäistö *et al.*, 1996) and RAMS, but to STT according to FAST. This failure may be due to the small number of isolates from northern Finland collected from naturally regenerated stands used for creating the model for the discriminant analysis used in this study (see Müller & Uotila, 1997). However, RAMS or RAPD markers should be more reliable than morphological or chemotaxonomical characteristics in determining variation in *G. abietina* as they are at least in most cases based directly on selectively neutral genetic information. Hellgren & Högberg (1995) demonstrated with DNA markers two distinct ecotypes in Scandinavia, and Hamelin *et al.* (1996) found three amplitypes of *G. abietina* in Europe. Hantula & Müller (1997) using RAMS markers showed earlier four banding pattern types within *G. abietina*, and markers within LTT from southern Europe and North America that do not occur within LTT in Finland.

The grouping of isolates becomes more difficult if hybridization occurs. This appears to have occurred in the case of two isolates in this study, and in two cases reported earlier by Hamelin *et al.* (1996) and Hantula & Müller (1997). The situation is, however, more complicated as the type-specificity of markers is based on the distribution of these markers within a moderate number of tested isolates. This is probably the reason why Hansson *et al.* (1996) observed variation in the occurrence of two dominant RAPD markers previously thought to be type-specific (Hamelin *et al.*, 1996). Therefore, the occurrence of single LTT-specific markers in two of our isolates with all other markers being STT-specific could be due to these markers not being completely type-specific. Thus, they are not necessarily indications of hybridization. To completely solve this question of hybridization between LTT and STT, offspring from (potential) crosses with known parental isolates must be studied.

Müller & Uotila (1997) also suggested that the European STT of *G. abietina* should not be included to the European race, since its FAST profile resembles more that of the North American STT than that of the European LTT.

A study clarifying whether both types of *G. abietina* var. *abietina* cause the classical symptoms of STT in artificially regenerated pine sapling stands reported in northern Fenno-

scandia during the past four decades (Kurkela, 1967; Norokorpi, 1971) is still needed.

Ms Leena Seitamäki prepared the used agar media and helped in preparing the isolates. Ms Rauni Valjakka performed most of the FAST and RAMS-analysis for the isolates. We also wish to thank Dr Ludmila Isaeva for her help in getting some of the preliminary branch material from Russia. This study was financed by the Finnish Ministry of Agriculture and Forestry and the Finnish Forest Research Institute.

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