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**EPIDEMIOLOGICAL INVESTIGATION OF PINE
FOLIAGE DISEASES BY THE USE OF THE NEEDLE
TRACE METHOD**

**OKKAJÄLJEMEETODI KASUTAMISEST MÄNNI
OKKAHAIGUSTE EPIDEMIOLOOGILISES UURIMISTÖÖS**

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A Thesis
for applying of the degree of Doctor of Philosophy
in Forestry

Väitekirj
filosoofiadoktori kraadi taotlemiseks metsanduse erialal

Tartu 2011

Institute of Forestry and Rural Engineering
Estonian University of Life Sciences

According to verdict No 74 of December 3, 2010 the Doctoral Committee of the Agricultural and Natural Sciences of the Estonian University of Life Sciences has accepted the thesis for the defence of the degree of Doctor of Philosophy in Forestry.

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January 13, 2011, at 10:00.

The English language was edited by Mr. Terry Bush (USA), and the Estonian by
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Publication of this dissertation is supported by the Estonian University of Life Sciences and by the Doctoral School of Earth Sciences and Ecology created under the auspices of European Social Fund.



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ISBN 978-9949-426-93-5

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following papers; in the text the references to these papers are given in Roman numerals. The papers in this thesis are reproduced by the kind permission of the publishers.

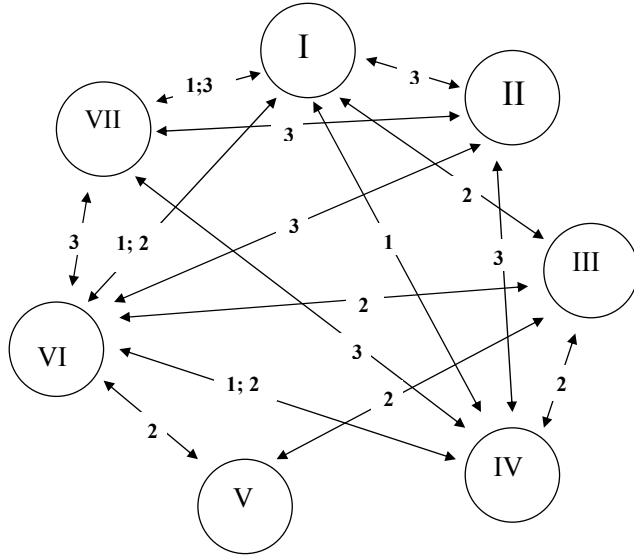
- I **Drenkhan, R.**, Kurkela, T., Hanso, M. 2006. The relationship between the needle age and the growth rate in Scots pine (*Pinus sylvestris*): a retrospective analysis by needle trace method (NTM). European Journal of Forest Research 125: 397–405.
- II Hanso, M., **Drenkhan, R.** 2007. Retrospective analysis of *Lophodermium seditiosum* epidemics in Estonia. Acta Silvatica & Lignaria Hungarica, Special Edition: 31-45.
- III Hanso, M., **Drenkhan, R.** 2008. First observations of *Mycosphaella pini* in Estonia. Plant Pathology 57: 1177.
- IV Kurkela, T., **Drenkhan, R.**, Vuorinen, M., Hanso, M. 2009. Growth response of young Scots pines to needle loss assessed from productive foliage. Forestry Studies/ Metsanduslikud Uurimused 50: 5–22.
- V Hanso, M., **Drenkhan, R.** 2009. *Diplodia pinea* is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia. Plant Pathology 58: 797.
- VI **Drenkhan, R.**, Hanso, M. 2009. Recent invasion of foliage fungi of pines (*Pinus* spp.) to the Northern Baltics. Forestry Studies / Metsanduslikud Uurimused 51: 49–64.
- VII Hanso, M., **Drenkhan, R.** 2010. Epidemiology of *Lophodermium* needle cast of Scots pine (*Pinus sylvestris* L.) in Estonia. Forest Pathology (manuscript, submitted).

The contributions of the authors to the papers were as follows:

Paper	Original idea and study design	Data collection	Data analysis	Preparation of manuscript
I	TK, RD , MH	RD , TK, MH, MV	TK, RD	TK, RD , MH
II	MH, RD	RD , MH	RD , MH	MH, RD
III	RD , MH	RD , MH	RD	MH, RD
IV	TK, RD , MH	RD , TK, MH, MV	TK, RD	TK, RD , MH
V	MH, RD	RD	RD	RD , MH
VI	RD , MH	RD , MH	RD	MH, RD
VII	MH, RD	RD , MH	RD , MH	MH, RD

RD – Rein Drenkhan, MH – Märt Hanso, TK – Timo Kurkela, MV – Martti Vuorinen.

The connections via different subjects between the papers presented in this thesis



1. Investigation of the impact of needle longevity and of needle loss solely from the productive foliage to the growth characteristics of Scots pine.
2. New alien invasive foliage fungi of pines in Estonia: the fungi (*e.g. Mycosphaerella pini et al.*), the process of colonisation in the country, the exotic hosts as vectors of new pathogens to the native Scots pine, specification of the kind of danger of new fungi: chronic needle loss definitely from the productive foliage.
3. Ecology and epidemiology of the *Lophodermium* needle cast.

ABBREVIATIONS AND SOME DEFINITIONS

ANN _n	annual number of needles in the n-th needle set (downward from the top) attached on the stem
ANN ₁	number of current-year needles, age in October four months or 0.3 years
ANN ₂	number of second-year needles, age 1.3 years
ANN ₃	number of third-year needles, age 2.3 years
ANN ₄	number of fourth-year needles, age 3.3 years
AsinNL ₂	arc sine transformation of NL ₂
AsinNL ₍₁₋₂₎	arc sine transformation of NL ₍₁₋₂₎
Chronic disease	a disease which inflicts host through its physiology year by year, or which outbreak lasts several to many years
CNL ₍₁₋₂₎	the percentage classes (with 10% intervals) of NL ₍₁₋₂₎ , combined from NL ₁ and NL ₂
Defoliation	decreased amount of needle sets (or %) from the total main stem in a definite year, which is represented as the number of needle sets lost per year = needle loss = needle shedding
Epidemic	a disease incidence, general and severe in a group of hosts for a time
Epidemic disease	a disease, which severe outbreak lasts not longer than one year
Epidemiology	the study of disease incidence, distribution and control
GIV	<i>Gremmeniella</i> index value (Thomsen 2009), the ratio of monthly precipitation sum (mm) to the monthly mean air temperature (°C) for one or several definite months, mm/°C
GLM	multivariate general linear model
HI	height increment, cm
MEA	malt extract agar
Needle	short shoot with a pair of needles (as used in this disseration)
Needle set	needle age class = needle fascicle = needle cohort
ND	needle density, number of needles/cm
NL	needle loss, = needle shedding, = defoliation, %

NL ₁	needle loss from ANN ₂ , difference between ANN _{1(t-1)} and ANN _{2(t)} , %
NL ₂	needle loss from ANN ₃ , difference between ANN _{1(t-2)} and ANN _{3(t)} , %
NL ₍₁₋₂₎	needle loss percentage combined from ANN ₂ and ANN ₃
PNA	pine needle agar
RI	radial increment, mm
RIM	reconnaissance investigation method
t	the current year
TANL	the total annual needle loss, in needle sets

1. INTRODUCTION

Foliage, the aggregate of leaves (needles), is the assimilating organ of green plants, which assures their life and growth. Most of the coniferous plants form perennial foliage, which complicates evaluations of the essence of the needle sets of different ages. Foliage disease is an important reducing factor for this perennial organ. Epidemiology of foliage diseases deals with the incidence levels and distribution dynamics of old (native) diseases and with early detection of new invasive pathogens. Epidemiology is also an essential tool for determining and applying a control strategy for forest protection.

A disease also may be perennial (chronic) or short-term and severe (epidemic). Studying the influence of diseases of perennial foliage to tree growth is therefore doubly complicated. New methods should be involved in appropriate investigations.

At the end of 1980s the Needle Trace Method (NTM) was created and developed into a new tool in forest pathology and ecology by Kurkela and Jalkanen (1990) in Finland. The method has unique capability of constructing retrospective, long-period chronological series of needle characteristics, enabling juxtaposition of different long-term series for better understanding of the role of foliage and of its losses for tree life. At the beginning NTM helped to understand the normal patterns of foliage development and retention in pines in critical areas, *e.g.*, forest declines (Jalkanen *et al.* 1994). Crown transparency caused by defoliation has been an important measure in forest monitoring assessments (Juknys *et al.* 2003).

Needle shedding (=needle loss, =defoliation) in different needle sets of pines (*Pinus* spp.) in Estonia is quite often caused by fungal pathogens like *Lophodermium seditiosum* Minter, Staley and Millar (Hanso 1963), and others. Most actual invasive foliage pathogens in different countries and continents have recently been *Mycosphaerella pini* Rostr., *M. dearnesii* M.E. Barr, *Diplodia pinea* (Desm.) J. Kickx and *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter (Wakeley 1970; Gibson 1972, 1974; Kowalski 1988; Blodgett *et al.* 2007; Hanso and Drenkhan 2008, 2009). Foliage losses may also be caused by insect pests (Kurkela *et al.* 2005) or abiotic factors, like drought (Bigler *et al.* 2006; Drenkhan and Hanso 2006).

What impacts do foliage diseases represent to the host? Growth is an important characteristic of the vitality of trees (Dobbertin 2005). Although it can be expected that all the attached living needles on pines support growth, and that the number of needles in different needle sets correlates directly and positively with the annual growth rate, this is not sufficiently documented by the research.

It is known that in pine foliage, the youngest needles assimilate most actively (Wang *et al.* 1995; Jach and Ceulemans 2000). In some studies the needle loss from young, less than 3-years-old needle cohorts or the total needle loss has been found to correlate with the growth or growth reduction (Piene *et al.* 2003; Williams *et al.* 2003; respectively). However, this relationship appears uncertain if loss percentages have not been differentiated between loss of growth supporting foliage and loss of senescent foliage. A trial to specify the meaning of and to define the most actual part of the total foliage – the productive foliage – is one of the aims of this work, a subject addressed to better understand the essence of needle losses in pines, accompanied by epidemics of old and new alien foliage diseases.

Lophodermium needle cast has been the most serious foliage pathogen of Scots pine in Estonian forest nurseries and plantations, widely known to the foresters from the beginning of the 20th century (Weiß 1902; Vestergren 1903). This disease is continuously an issue in forest nurseries through today, *e.g.*, in 2004–2007 more than 70% of the Scots pine planting stock was grown in open-land nurseries as bare-root seedlings (Tetlov and Siimon 2008; Jäärats *et al.* 2010). However, for developing a better control strategy, several issues of *Lophodermium* needle cast epidemiology still were analysed insufficiently.

At the beginning of the 21st century the current situation in foliage pathology of pines has deeply changed by the colonisation of this country by several new pathogens. This process has obviously been triggered by the deep changes in the climate. In the wake of this climate change several new invasive foliage fungi, including two quarantined pathogens (*M. pini* and *M. dearnessii*), have been reported by us in Estonia, a sign of change in the long-lasting adaptive relations in Scots pine foliage pathology. Some new invasive disease agents were also just documented on other conifers (*e.g. Phaeocryptopus gaeumannii* (Rohde) Petrak on *Pseudotsuga menziesii* (Mirb.) Franco, *cf.* Drenkhan and Hanso 2010)

and deciduous trees. It means that foliage pathology of forest trees needs more attention and scientific investigations.

Modelling of the *Lophodermium* needle cast epidemics would specify the correct seasonal timing of the control measures. Meteorological characteristics, which support pathogens' dissemination, received therefore particular attention. Documented new diseases need special attention and monitoring already during their acclimatisation period.

2. REVIEW OF LITERATURE

2.1. History and entity of the Needle Trace Method (NTM)

During the final two decades at the end of the last century, many investigations and research papers regarding air pollution and its role in forest health appeared. Many scientists expected that air pollution was the main reason for large-scale forest decline. This was partly based on defoliation, which was suspected to be higher during the second half of the 20th century than it ever had been before. By the use of NTM it was soon demonstrated, that this hypothesis was not correct.

Perhaps these air pollution issues gave rise to this new, unique method, the Needle Trace Method (NTM) (Kurkela and Jalkanen 1990; Aalto and Jalkanen 1996, 1998, 2004; Jalkanen 1998), which can be applied in forest ecology, production biology, pathology, entomology, forest health monitoring, and complement the fields of dendroecology and dendroclimatology (Kurkela and Jalkanen 1990; Jalkanen *et al.* 2000a). NTM offers the opportunity to collect historical needle data series of conifers, useable in the analyses of trees from their young age to the age of 100 years or even much older.

NTM is based on the fact that needle traces remain in wood as long as the wood is not rotten or burnt. The short shoots (further: needles) have connections to the pith by vascular tissue through the annual rings only as long as they are physiologically (even minimally) active and attached to the shoot. After the shedding of a needle, its vascular tissue stops growing and cannot be seen any more in the later (outer) tree rings (Figure 1).

Up to this point, NTM has been used in several countries to generate retrospective needle data series commonly for *Pinus sylvestris* L., less commonly for other *Pinus* spp. (Jalkanen *et al.* 2000b; Konôpka *et al.* 2000; Insinna *et al.* 2007a, 2007b), but also for *Picea abies* L. Karst. (Sander and Eckstein 1994, 1997) and *Picea sitchensis* (Bong.) Carr. (Williams *et al.* 2003; Straw *et al.* 2005). NTM may reveal and has already revealed the history of conifer foliage throughout the full tree life, and can also generate needle data series for very far back in time, when no foliage visual assessments were possible, if only conifer trunks have remained.

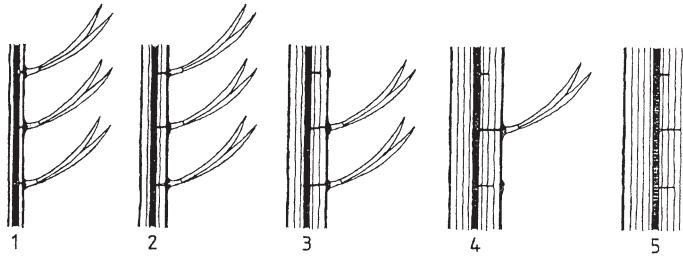


Figure 1. Pine shoots with attached needles and remaining needle traces in wood (Aalto and Jalkanen 1996).

The large-scale visual defoliation assessments (like the program ICP Forests) were subjective and depended much on the estimators. The visual estimates, also, are not sufficiently accurate to find out the mechanisms of foliar recovery, tree growth reaction to the foliage diseases, etc. (Armour *et al.* 2003).

2.2. Extractable by NTM foliage characteristics and growth rate of Scots pine

As said before, pines have perennial foliage, which complicates the attempts to investigate the relations between the foliage and tree growth. Needle longevity in pines has been found to correlate positively with increasing latitude and altitude (Jalkanen *et al.* 1995; Xiao 2003) and the mean needle age increases with tree age (Pensa *et al.* 2001). The needle longevity seems to be a largely phenotypic response (Reich *et al.* 1996; Pensa and Sellin 2002). Although in the pine foliage the youngest needles assimilate most actively (Wang *et al.* 1995; Sullivan *et al.* 1997; Bobkova and Zagirova 1999; Jach and Ceulemans 2000), it is not clear which needle sets are significant to tree growth.

Needle age is not correlated directly with height growth in *P. sylvestris* and *P. ponderosa* Dougl. ex Laws. (Insinna *et al.* 2007a). Different *Pinus* species have even been found to demonstrate a negative correlation between height growth and needle density (Konôpka *et al.* 2000; Jalkanen and Levanic 2001; Kurkela *et al.* 2005; Insinna *et al.* 2007a) and stand-level needle-density variation is mostly due to changes in height growth (Salminen and Jalkanen 2006). A statistically significant correlation

was found between needle production (= current-year needles, ANN_t) and height increment in northern Finland and Germany (Jalkanen and Tuovinen 2001; Insinna *et al.* 2007a, respectively), but the current-year needles had not supported the height growth of trees the same year. The current-year needles mature much later than the shoot to which they are attached (Kurkela 1980, Figure 6). Also the photosynthetic activity of current-year needles in the first half of the summer season is low (James *et al.* 1994). Thus, the current-year needles can affect mainly radial growth during the second part of the season (Leikola 1969).

2.3. Needle loss related to the growth of Scots pine

One foliage characteristic that NTM can explain – the needle loss in Scots pine may originate from one to several needle sets (Ross *et al.* 1986). Both fungal diseases and drought decrease the amount of needles and may be the reason for defoliation. Needle loss is related to growth reduction, but this relationship cannot be correctly quantified unless the actual growth-supporting proportion of the total foliage, *i.e.*, the productive foliage, is not specified and unless the needle loss is not related solely to that proportion. Scots pine in northern Finland sheds approximately one needle set annually (Jalkanen 1998; Muukkonen 2005). It is difficult to determine the extent of growth reduction due to the defoliation if the needle loss is presented as a part of a certain annual needle set (Williams *et al.* 2003), since more than one needle set is supporting the growth.

In some studies the needle loss from young (usually < 3-years-old) needle cohorts or the total needle loss correlated with the growth or growth reduction (Piene *et al.* 2003; Williams *et al.* 2003; respectively). This relationship, however, appears uncertain because loss percentages have not been differentiated between loss of growth-supporting (=productive) foliage and loss of senescent foliage. The needle cohorts of different ages have different values in supporting the growth, because: i) the number of needles in the different needle sets among the total foliage is greatly different (see **Paper I**); ii) physiological activity of different needle cohorts varies (Jach and Ceulemans 2000; Zha *et al.* 2002); iii) there are seasonal functional differences in the different needle sets (Loach and Little 1973; Sullivan *et al.* 1997).

In the literature there may not exist any adequate definitions for the productive or growth-supporting foliage. Many published works have described the effect of natural or artificial defoliation on the growth of trees. The needle loss in these reports has been described as the loss from any particular needle set (Kulman 1971; Ericsson *et al.* 1980; Armour *et al.* 2003) or as the loss from the total green foliage (Alfaro *et al.* 1982; Williams *et al.* 2003). When the rate of defoliation is inaccurately defined, it is clear that the relationship between the needle loss and the growth rate can be based only on correlations and cannot prove cause and effect (Armour *et al.* 2003) or open sink and source (Honkanen *et al.* 1999). This relationship is also essential in foliage pathology.

What is the difference between the loss of foliage caused by insect pests (*i.e.* herbivory) or the artificial experimental removal of needles, compared to the loss of needles by fungal or abiotic (*e.g.*, caused by drought) defoliation? In the artificial defoliation experiments, the effect of the removal of needles varied according to the age of the removed needles and to the season of this human intervention (Linzon 1958; O'Neil 1962; Ericsson *et al.* 1980; Honkanen *et al.* 1999). The growth reduction in conifers, achieved by the artificial removal of green needles or caused by herbivory (Ericsson *et al.* 1980; Honkanen *et al.* 1999; Armour *et al.* 2003; Piene *et al.* 2003; Williams *et al.* 2003; Kurkela *et al.* 2005; Straw *et al.* 2005) cannot be correctly compared with the growth reduction in trees caused by abiotic stress situations (like drought) or by fungal needle diseases (like *Lophodermium* needle cast). The reason is that the first type of needle removal is accompanied by the additional energy-consuming traumatic effects (resin flow etc.), but the second type is not.

2.4. Hitherto use of the NTM in forest pathology

Annual rings are excellent retrospective data sources, which offer to dendrochronology valuable information about past tree life (Fritts 1976). Unfortunately, often annual rings alone cannot give enough explanation for historical events like epidemics of needle pathogenic fungi and calamities of herbivorous insect pests. NTM is an ideal method to study past events in which needles have been influenced by biotic and abiotic factors (Ferretti *et al.* 2002).

Long retrospective needle data is an essential storehouse of historical memory for the comparison of the earlier data with the appropriate current-time information. How long was the mean needle age of Scots pine historically? When, definitely, and why was the needle retention in some years higher and sometimes much lower? Why does the needle age vary greatly, and why does the needle retention change year by year? These questions can be considered if you have an extended time-series needle dataset.

Different studies demonstrate that NTM can be applied successfully to the retrospective quantification of needle losses caused by insect outbreaks, for instance the defoliation of Sitka spruce by *Gilpinia hercyniae* Hartig (Williams *et al.* 2003) or by *Elatobium abietinum* Walker (Straw *et al.* 2005) in Wales, of Scots pines by *Bupalus piniaria* L. in Scotland (Armour *et al.* 2003) and by *Diprion pini* L. in Finland (Kurkela *et al.* 2005).

One of the rather essential needle cast diseases of Scots pine in Finland and northern Scandinavia is caused by *Lophodermella sulcigena* (Rostr.) v. Höhnelt. The first time NTM was successfully used for retrospective identification of the post-epidemic survival and tree growth reactions to a well-documented *L. sulcigena* epidemic in Finland (Jalkanen *et al.* 1994).

Although the first pilot trial of the retrospective analyses of *Lophodermium* needle cast epidemics in Estonia was undertaken by us already seven years ago (Drenkhan and Hanso 2003), the large variety of epidemiological problems still needed more all-round analyses by the use of longer retrospective needle and weather data series, to distinguish timetable and other epidemiological peculiarities of the historical epidemics of this disease. Modelling of the *Lophodermium* needle cast epidemics in Estonia should improve the development of a better control strategy than exists today.

2.5. Retrospective needle and growth data series in forest ecology

Retrospective needle data series are valuable in different aspects, as for estimation of the: 1. tree growth itself (see chapter 2.2), 2. effect of needle loss on tree growth (see chapter 2.3), 3. pointer years of needle losses

for forest pathology (see chapter 2.4). These aspects have been analysed more deeply in this dissertation.

The utility of the NTM lies in the valuable knowledge it yields about the variation in needle retention and natural thinning of tree crowns. The last has been evaluated as an excellent environmental indicator (Jalkanen *et al.* 2000b). NTM also helps to compare the needle retention scores with current visual foliage assessments (Pouttu and Dobbertin 2000).

Before our investigations, different needle retention and long-term needle age values were extracted and compared by this method in northern Estonia and southern Finland (Pensa and Jalkanen 1999, 2005). The minimum needle retention was registered in southern Finland and northern Estonia in the beginning of the 1980s (Jalkanen and Pensa 2000). It demonstrated some parallel tendencies, but also that alkaline deposition in northern Estonia could not be the main influencing factor in the annual variation of needle retention, needle age and radial growth, which had been expected before (Jalkanen and Pensa 2000).

Needle density (the number of needles per shoot length) is species-specific in pines and shows year-to-year variation within each species (Jalkanen *et al.* 1998). The long-term dynamics of needle density in Scots pine increased with the ageing of trees in northern Finland and Estonia (Pensa *et al.* 2006). A similar tendency was registered in Brandenburg, Germany, where the needle density increased with the age for *P. sylvestris* and *P. ponderosa*, but mean needle density was higher in *P. ponderosa* (Insinna *et al.* 2007a). It is known that tree growth decreases with age and it is fact that a negative correlation exists between the needle density and the tree height growth (Drenkhan and Hanso 2000; Jalkanen and Levanic 2001). In other words, the shorter shoot, the more are needles on it.

The predicted climate warming (Logan *et al.* 2003; Walther and Linderholm 2006) would mostly support different southern foliar pathogens in their invasion to the northern areas (see chapter 2.8). On the other hand, in light of climate warming it is essential to find correlations between climate and tree foliage and growth characteristics. For instance, both height increment and needle production were strongly influenced by the temperature regime of the previous summer in Lapland (Pensa *et al.* 2006). In contrast, for the other characteristic of tree growth, the radial

increment, there was a lesser correlation with the mean July temperature of the current-year. Similar correlations in Estonia showed, as expected, that Estonian trees are not so strongly affected by climatic factors as trees in Lapland (Pensa *et al.* 2006). This result was similar to earlier ones that the previous year's July is the best predictor for next year height growth of Scots pines at the northern timberline (Jalkanen and Tuovinen 2001; McCarroll *et al.* 2003; Salminen and Jalkanen 2004). Other investigations also supported it: the best predictors of needle litterfall of Scots pine in northern Finland were the May to mid-July temperature sum with a lag of 4 years, rainfall in May with a lag of 2 years and temperature in September with a long lag of 6 years (Lehtonen *et al.* 2008). Farther south, in Germany for instance, the autumn rainfall of the previous year was equally important for Scots pine and Ponderosa pine, but summer precipitation was more significantly influencing the needle parameters of Scots pine (Insinna *et al.* 2007a), whereas temperature seemed to have a minor impact on needle parameters. However, height growth and needle characteristics should be more sensitive to biotic and abiotic factors than radial growth. The dissertation results connected to the foliage infectious pathology supported these statements.

2.6. *Lophodermium* needle cast and its agent, *Lophodermium seditiosum*

The most common foliage disease in young Scots pine plantations and forest nurseries of northern and central Europe, the *Lophodermium* needle cast, is caused by *Lophodermium seditiosum*. Occurrence of the disease and its agent (historically: *Lophodermium pinastri* Chev.) in the territory of Estonia was first documented at the turn of the 19th to the 20th century, both nearly at the same time, by the Baltic German forester Weiß (1902) and Swedish mycologist Vestergren (1903), respectively. *L. pinastri* was first described in Estonia much earlier (Dietrich 1856).

The first temporary forest nurseries in Estonia (Taebbla and Saulepi) were established in 1847, but wide-scale planting of clear-cut areas by nursery-grown pine seedlings started some decades later, after the century-long practice of pine regeneration by seeding. Already before the year 1870, for instance, the share of afforestations by planting in the northern Tartu region reached 36% (Meikar 1994). Therefore it seems likely that

Lophodermium needle cast as a problem in forest nurseries of Estonia had seriously emerged already by that time.

The research work on *Lophodermium* needle cast started in Estonia at the end of 1950's and has been carried out up today (Hanso 1963, 1965, 1970; Hanso and Hanso 2003; Hanso and Drenkhan 2007b), incl. applied diagnostic service by M. Hanso in forest nurseries and plantations across the entire territory of Estonia during 1972–1985.

During the last quarter of the 20th century, the taxonomic position of the actual pathogen of Scots pine inside the genus *Lophodermium* was specified step by step (Stephan 1973; Minter *et al.* 1978; Minter and Millar 1980; Diwani and Millar 1987). Before that, a trial to characterize the *Lophodermium* needle cast epidemiology was undertaken in France (Lanier and Sylvestre 1971). Lately, using retrospective needle data series a provisional, century-long retrospective time-table of *Lophodermium* needle cast epidemics in Estonia was constructed (Drenkhan and Hanso 2003; Hanso and Drenkhan 2007b).

Regardless of the new invasive fungal diseases of pines, *Lophodermium* needle cast in forest nurseries and plantations is persistently a live issue.

2.7. New alien invasive pathogens

A substantial part of the dissertation deals with the first detection of several new alien invasive foliage fungi of pines, and investigation of the colonisation process of Estonia by these fungi (including two under-quarantine pathogens), together with the hypothetical evaluation of their possible modes of danger. As the new invasive foliage fungi, definitely *Mycosphaerella pini*, *M. dearnessii*, *Diplodia pinea* and *Cyclaneusma minus* were described and investigated.

2.7.1. *Mycosphaerella pini*

A quarantine pathogen *M. pini* (in its anamorphic stage *Dothistroma septosporum* (Dorog.) Morelet) causes *Dothistroma* needle blight. The fungus was first described by Doroguine (1911) in Russia (as *Cytosporina septospora* Dorog.). Thereafter, in Britain, the fungus was found on forest nursery stock in 1954 (Murray and Batko 1962), and later (in 1963) in

British Columbia, Canada (Parker and Collis 1966). Thereafter, *Dothistroma* needle blight has caused serious damages especially in exotic pine plantations in New Zealand, Chile, Australia, central and eastern Africa, *i.e.*, in various countries and continents of the Southern Hemisphere (Gibson 1972, 1974).

After the first outbreaks, the pathogen has caused a few sporadic outbreaks in the Northern Hemisphere (Bradshaw 2004), but during the last two decades, the frequency and severity of *Dothistroma* needle blight has increased in Europe as well in North America (Bradshaw 2004; Barnes *et al.* 2008). Serious epidemics have been reported on Corsican pine (*Pinus nigra* ssp. *laricio* Dougl. ex Loud.) in the UK (Brown *et al.* 2003) and France (Landmann 2000), as well as on *Pinus nigra* Arnold in Hungary (Koltay 2001) and in the Czech Republic (Jankovský *et al.* 2009a). At the end of the 1990s and beginning of the 2000s disease occurrences with severe damages were recorded in both managed and natural stands of Lodgepole pine (*Pinus contorta* var. *latifolia* Dougl. ex Loud) in British Columbia (Woods 2003; Woods *et al.* 2005).

In a sample of damaged needles of *P. nigra*, collected from Järvelja at the autumn of 2006, a quarantine pathogen *M. pini* (in its anamorphic stage *D. septosporum*) was diagnosed for the first time in Estonia (Hanso and Drenkhan 2008a, **Papers III and VI**). In 2008 the fungus was already found on *P. sylvestris* in southern and central Finland (Müller *et al.* 2009).

2.7.2. *Mycosphaerella dearnessii*

M. dearnessii (syn. *Scirrhia acicola*) with its anamorphic stage *Lecanosticta acicola* (Thümen) A. Sydow causes brown spot needle blight. The fungus is an under-quarantine foliage disease on pines in the EPPO region and appears to be of American origin. *M. dearnessii* was first observed and collected in South Carolina, USA, in 1876, and afterwards that fungus has been recognised as a serious disease at least since 1919 (Tainter and Baker 1996). For a long time *M. dearnessii* has been known as a serious disease mainly in the forest nurseries of North America (Wakeley 1970).

The first report of *M. dearnessii* in Europe was made in 1978 (EPPO 2010). The fungus has been reported to date in Europe from Germany (Butin and Richter 1983; Pehl 1995), France (Chandelier *et al.* 1994),

Switzerland, Bulgaria and former Yugoslavia (Holdenrieder and Sieber 1995), Croatia (Novak-Agbaba and Halambek 1997), Italy (La Porta and Capretti 2000), Austria (Kirisits and Cech 2006), Czech Republic (Jankovský *et al.* 2009c), Serbia (Jankovský *et al.* 2009a) and Slovenia (Jurc and Jurc 2009).

In 2007 the fungus was found for first time in Estonia (Drenkhan and Hanso 2009a). Before the findings (**Paper VI**) the earlier, north-eastern-most location of brown spot needle blight was documented in Germany, *i.e.*, at least 1000 km south-west from Estonia. No records of the fungus from Nordic (Scandinavian) and other Baltic countries are still documented.

2.7.3. *Diplodia pinea*

D. pinea (syn. *Sphaeropsis sapinea* (Fr.: Fr.) Dyko & Sutton) causes *Diplodia* tip blight. The fungus has been known in both hemispheres as a harmful pathogen of needles and shoots, but also of cones and even roots (Peterson 1977; Swart *et al.* 1985; Flowers *et al.* 2006; Blodgett *et al.* 2007), and as an important sapstain fungus that spoils logs during export (Thwaites *et al.* 2004). *Pinus* species are most susceptible to *D. pinea*, but the pathogen also infects species of *Cedrus*, *Abies*, *Larix*, *Thuja*, *Juniperus*, *Picea* and *Pseudotsuga* (Flowers *et al.* 2006). In North America the fungus has even killed entire trees in plantings of exotic (commonly *P. nigra*) as well native pine species (Peterson 1977).

In Europe, the epidemics of *D. pinea* have been detected in many countries, as in the Netherlands (De Kam *et al.* 1990), France (Piou *et al.* 1991), Austria (Cech and Tomiczek 1996), Croatia, Slovenia (Diminič and Jurc 1999), Hungary (Tamás 2003), Slovakia, Czech Republic (Jankovský and Palovčikova 2003), the UK (Brown and Macaskill 2005), Germany (Blaschke and Cech 2007) and Italy (Maresi *et al.* 2007). A serious epidemic has been registered in the Czech Republic in *P. nigra* stands since 2001/2002 (Jankovský and Palovčikova 2003) and in Germany on Scots pine stands since 2003 (Blaschke and Cech 2007). Since 2005, there has been an increase in *D. pinea* on both young crops and mature trees of *Pinus* spp. in the UK (Brown and Macaskill 2005).

In southern Europe the occurrence of *D. pinea* in healthy *P. nigra* was positively correlated with a high insolation index (Maresi *et al.* 2007).

Therefore it is not surprising that serious drought in 2003 supported the *D. pinea* epidemic in central Europe (Jankovský and Palovčikova 2003; Steinfath 2006; Blaschke and Cech 2007).

Before the findings of *D. pinea* in Estonia (**Papers V and VI**), the closest documented location of *D. pinea* was in Germany, more than 1000 km south-west from Estonia. Still no information has been published about the findings of the fungus from Fennoscandia, Lithuania or Latvia.

2.7.4. *Cyclaneusma minus*

C. minus (syn. *Naemacyclus minor* Butin) is widely distributed in Europe: in Germany, France, Spain, Italy (Butin 1973), the former Czechoslovakia (Minter 1981), Poland (Kowalski 1988), the Netherlands (Gremmen 1959), the UK (Lehmann and Hudson 1977), Denmark, Switzerland (Millar and Minter 1980), the Crimea region of Ukraine (Minter and Dudka 1996) and Slovenia (Jurč 2007).

In Europe *C. minus* has been reported frequently as an endophyte (Rack and Scheidemann 1987; Kowalski 1982, 1988; Gourbière *et al.* 2001; Jurč 2007), or as a pathogen that damages only individual needles of *P. sylvestris*, *P. nigra* and *P. mugo*. However, sometimes it may cause heavy cast of two year-old needles in young (up to 20 years) Scots pine plantations in Poland (Kowalski 1988). In the Southern Hemisphere, *C. minus* has been classified only as a serious pathogen (Bulman 1993; Bulman and Gadgil 2001).

The first time the fungus was found on the needles of young Scots pines in Estonia was in 1999, but for nearly a decade only in one limited location (Hanso and Hanso 2003). The earlier location of *C. minus* detection most close to Estonia has been in Poland, ca. 600 km south-west of Estonia. No published information exists about the findings of the fungus from Latvia and Lithuania, nor from the Fennoscandian countries, except for Denmark.

2.8. Climatic excesses as the obvious trigger of the recent invasions

Woods *et al.* (2005) demonstrated how even a relatively small change in climate can have serious implications for a tree species, particularly

if the change surpasses an environmental threshold that has previously restricted the development of certain of its pathogens.

The natural environment of Estonia is sensitive to climate change, since the country is located in the transitional zone between regions of different bioclimatic conditions (Kont *et al.* 2007). During the second half of the 20th century mean annual air temperature in Estonia has increased by 1.0–1.7 °C. Statistically significant increases in monthly mean temperatures have occurred mainly from January to May (Jaagus 2006). Zonal atmospheric circulation has brought to Estonia more wet weather than previously, which in winter was warmer and in summer cooler than long-term averages (Keevallik *et al.* 1999). The precipitation trends are less distinct, but an increasing trend can be noticed during the cold half-year and in the summer month of June (Jaagus 2006). These climatic changes obviously might support the arrival and settlement of alien invasive southern fungi in Estonia, but also the epidemics of some earlier pathogens of pine foliage, like *Lophodermium seditiosum*.

Natural habitats in Estonia suffered from two severe droughts, in 2002 and 2006 (Drenkhan and Hanso 2004, 2006; Hanso and Drenkhan 2007a). Similarly, in Germany two severe droughts in 2003 and 2006 (Oldenburgh 2007; Steyrer 2007; Wulf and Schumacher 2007) together with forest diseases (incl. *Diplodia pinea*) and insect pests killed several young pine plantations (Steyrer 2007).

The predicted climate warming (Logan *et al.* 2003; Walther and Linderholm 2006) would mostly favour these southern pathogens, for which the earlier low winter temperatures were a limiting factor. Concerning the spread of *M. pini*, the increase in temperature may be counterbalanced by the decrease in summer rainfall, but *D. pinea* may benefit from the water stress of its host (Desprez-Loustau *et al.* 2007). Concerning the impact of climate change on forest pathology, more attention should be given to different characteristics of weather extremes (*e.g.*, the extent of hardening period in autumns) and not only to the climate warming or winter freezing.

Scots pine is known as a cold- and drought-resistant tree species in Estonia (Laas 2004). In this work, retrospective needle and growth data series of Scots pine were juxtaposed with the series of adverse environmental events during the recent decade. These events began with the hard

epidemic of *Lophodermium* needle cast in 2001, were followed by the very dry summer of 2002 and ended by two stressors: the extremely abrupt autumn of 2002 and abnormally cold first half of the winter of 2002/2003. Most probably it exceeded the tolerance level of a number of pines, leading to the massive death even among Scots pines (Drenkhan and Hanso 2006). Naturally, several other less tolerant exotic and native tree species suffered during these climatic excesses (Hanso and Drenkhan 2007a). Also reported in the scientific literature, for instance, was that in Scots pine seedlings in northern Sweden positive correlations were found between the autumn cold hardiness and tree vitality (Persson *et al.* 2010).

At the same time, neglecting the obvious role of meteorological prerequisites (Drenkhan and Hanso 2009b), *i.e.* underestimating the role of weather extremities in triggering also that epidemic, the reason for massive death and sufferings of common ash (*Fraxinus excelsior* L.) in eastern and central Europe has been continuously tied practically only to the new fungal pathogen *Chalara fraxinea* T. Kowalski.

3. AIMS OF THE STUDY

The hypotheses of the study were:

1. only a part of green (alive) foliage has an essential impact on the growth of pines and the needle loss from this part of the total foliage has to be assessed at the defoliation versus growth investigations;
2. long-known (*Lophodermium seditiosum*) and new (e.g. *Mycosphaerella pini*) foliage diseases of pine demonstrate different offensive epidemiological strategies toward hosts' physiology and ecology.

The main aims of the present doctoral dissertation were to investigate:

1. the relationship between the foliage and the tree growth characteristics of Scots pine (*Pinus sylvestris* L.), including which needle age classes have the strongest impact on radial and height increment of trees; in other words, to define the productive foliage (**Paper I**);
2. the annual foliar dynamics of young Scots pines and the quantitative effect of the annual loss of needles solely from that productive foliage on the radial and height increments of trees (**Paper IV**);
3. the new, invasive, pathogenic fungi on pine foliage in Estonia (and in northern Latvia) during the current wave of weather extremes, including monitoring of the peculiarities of the process of colonisation of Estonia, a fast pilot investigation of their biological and ecological peculiarities (e.g., knowledge about the season of dissemination of spores, essential for the applied forest protection, etc.), and including the assessment of their possible risk to the Estonian forests (**Papers III, V and VI**);
4. the potentiality of the retrospective appraisal of epidemics of the historically most dangerous foliage disease of Scots pine – *Lophodermium* needle cast – from a retrospective, long-term needle dataset, obtained by the use of NTM, together with the juxtaposition and specification of the meteorological prerequisites, which give rise to a next-in-order epidemic (**Papers II, VII**);
5. some epidemiological peculiarities of *Lophodermium* needle cast disease, still poorly analysed in forest pathological research, such as the maximal vulnerable age of Scots pine, comparison of the impacts of needle diseases and herbivorous insect pests on the pine growth, etc. (**Paper VII**).

4. MATERIAL AND METHODS

4.1. Study sites and sample sizes

Paper I:

The radial and height increment of trees relative to foliage status was studied in Scots pines growing in Pyhtää, southern Finland (site 1), and in two stands (site 2 and site 3) in Konguta, south-central Estonia (Figure 2). A total of 25 sample trees were analysed by NTM from 3 stands. The sampled nine pines were 53 years old on site 1. On site 2 eight pines, 109–115 years old, were sampled. The age of eight sample trees on site 3 was between 95 and 105 years.

Paper II:

A total of 103 sample trees from six pine stands (Figure 2) were chosen and analysed by the NTM protocols, including 95 sample trees of *P. sylvestris* and 8 of *P. contorta*.

Only young age periods of tree life (in four studied stands from 5...7 to 22...25 years, in one *P. sylvestris* stand from 3 to 14 years, and in one *P. contorta* stand from 5(7) to 22(25) years were included into the subsequent investigation. In addition to the foliage data, according to the NTM protocols, also the radial growth of sample trees was analysed at breast height, but in the youngest stand the radial growth was analysed at the stump height of trees.

Papers III; V and VI:

In (2006) 2007 symptomatic needle samples were systematically collected from several exotic *Pinus* species and occasionally from native *P. sylvestris*, which revealed infections by the new pathogen *M. pini* (see **Paper III**).

During two years (2007 and 2008), several fallen cones with the visible conidiomata of an unknown fungus on the cone scales were systematically searched and collected from exotic *P. nigra* trees in three places of Järvselja and in the towns of Tartu, Pärnu, Tallinn and in Pädaste, Muhumaa island (see **Paper V**). The systematic collection of fallen cones of *P. nigra* was essential, because *P. nigra* is known as the pine species most susceptible to this universally pathogenic to hosts' different tissues

fungus. *D. pinea*, and its cone scales were known to be the most preferable substrate for the fungus during initial colonisations. Very often they have acted as vectors in the dissemination of this fungus to other tree organs, including needles.

Field samples were collected by the authors according to the reconnaissance investigation method (RIM) in forest pathology (Parmas 1961), from 2006 to 2008 in Estonia and in 2008 in northern Latvia (see **Paper VI**). RIM is also a mycogeographic field research method and is based on qualitative rather than quantitative visual registration by an experienced specialist of the occurrence of definite species, their symptoms and/or fruiting bodies by the route-method, or by sampling during the visits to all of the potentially provocative habitats (*e.g.* exotic trees), or by occasionally selected habitats of native tree species (*e.g.* young stands of *P. sylvestris*). The age of exotic sample trees varied, but the age of native Scots pines was limited to 5–20 years. Using RIM, no quantitative characteristics concerning sampling can be presented. Samples were taken mainly in spring and autumn, as these are the most important seasons for symptom expression in foliage diseases of pines. As a rule, the symptomatic needles were sampled from among short shoots attached to the trees and mostly from the lower parts of canopies, and if needed, also from among the needle litter.

Distribution maps of different fungi (see **Paper VI**) indicate: 1) the sampling areas, and 2) the resulting (laboratory) diagnoses (*i.e.*, whether a sample was symptomatic or asymptomatic).

Samples of all new for Estonia fungal species (except of *Mycosphaerella dearnessii*) were deposited in the Mycological Herbarium of the Estonian University of Life Sciences /TAA(M)/ (see **Papers III, V, VI**).

Paper IV:

The amount of needles in the different annual needle sets was studied retrospectively in a Scots pine provenance experiment in Konguta, south-eastern Estonia. Six provenances were included in this study, two grown from seeds originating from Estonia and four from Finland. In October 2004, eight pines from each provenance were randomly chosen among the dominant or codominant trees, which totalled 48 sample trees, the sample tree age being 14 years, and analysed by NTM.

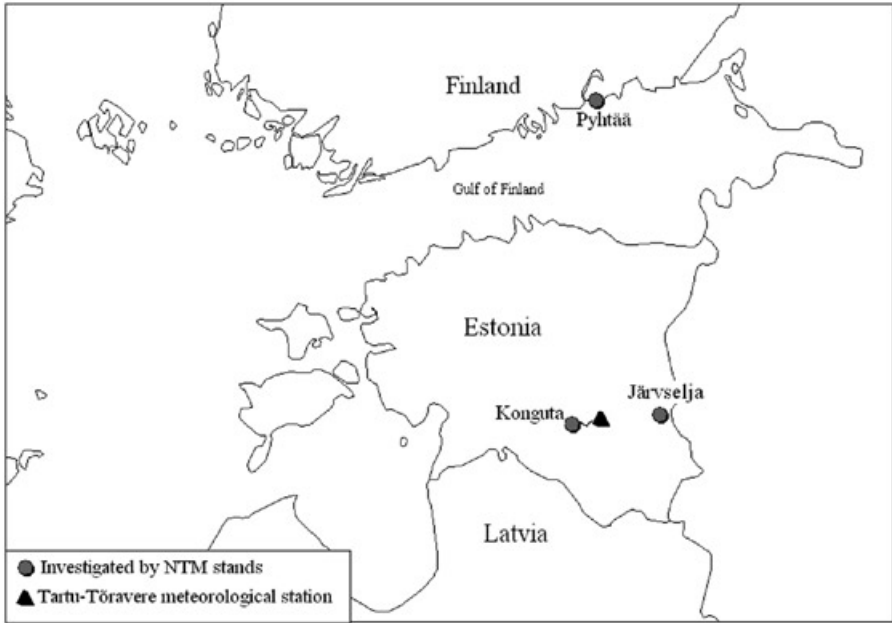


Figure 2. Location of Tartu-Tõravere meteorological station and the sampling stands, investigated by NTM in Konguta (six experimental stands of *P. sylvestris*) and Järvselja (one experimental stand of *P. sylvestris* and one of *P. contorta*), south-central Estonia, and in Pyhtää (one experimental stand of *P. sylvestris*), southern Finland.

Paper VII:

The retrospective NTM data were obtained from six *P. sylvestris* experimental stands, of which five stands were growing in Konguta and one stand in Järvselja (Figure 2). In total 46 sample trees were analysed from these stands. Eight model trees from stand 1 were 109–115 years, eight trees from the stand 2 were 95–105 years old, and the age of four model trees from stand 3 was about 40–56 years. Eight model pine trees from stand 4 were 31–33 years and ten model trees were 13–14 years old from stand 5. Eight model trees from stand 6 were ca. 70 years old. The analysed sample trees represented retrospective growth and needle loss data series for a long-period, lasting from 1887 until 2006. Irrespective of calendar years, the needle loss and growth data were divided by the ages of all 46 sample trees, every group includes this way the data of different trees of the same age, but at the different calendar years. Therefore, the definite age groups of 3–11 (A) and 20–33 years (B) were extracted and investigated.

4.2. FIELD AND LABORATORY MEASUREMENTS

4.2.1. NTM techniques and tree sampling

NTM is destructive, which means that the sample tree has to be felled and its stem cut into the sections (blocks) by every annual shoot, omitting the branch whorls (Figure 3). The prepared sample bodies are transported to the laboratory for NTM analyses. In the laboratory every single sample body, which has at least 10 innermost tree rings, is examined ring by ring until the pith, discovering and registering on definite year-ring surfaces every needle trace. The needle traces are visible and easy to count on the smoothed surface of wood (Figure 4), thereby revealing the numbers of past needle sets per year.



Figure 3. A felled sample tree in the forest with the marked eastern side and a cut sample block (annual shoot) with the contours of the further sample body (original photo).

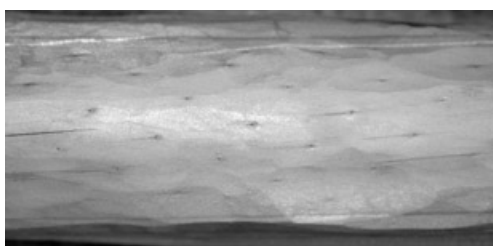


Figure 4. Needle traces on a smoothed surface of pine wood in a sample body (original photo).

In **Papers I, II, IV** and **VII** all sample trees were chosen and analysed (by counting needle traces) according to NTM protocols (Aalto and Jalkanen 1996, 1998). From 123 sample trees in total 2,550 sample bodies and ca. 25,000 annual ring surfaces were analysed by NTM. All the sample bodies were analysed separately by every consecutive tree-ring, where needle traces were counted beginning from the 10th (with the actual arrival of first rings not before the 6th ring) until the first (innermost) tree-ring around the pith (Aalto and Jalkanen 1998).

According to the NTM protocols a stand as a unit (considering age class or pointer year) have to be covered at least by 5 to 7 sample trees (Jalakanen *et al.* 2000a, b).

4.2.2. Collection of symptomatic needles and fungal species identifications

In **Papers III, V** and **VI** the discoveries of new alien invasive pathogens (*M. pini*, *M. dearnessii*, *D. pinea* and *C. minus*) were recorded. Their course of spreading and colonisation process in Estonia were investigated, using general plant or forest pathological and mycological principles and procedures (Tainter and Baker 1996; Trigiano *et al.* 2004; Agrios 2005; Lundquist and Hamelin 2005), RIM, or special diagnostic methods (Anonymous 2008).

Laboratory diagnoses had to establish whether a collected field sample was symptomatic or asymptomatic. A sample was classified as symptomatic if characteristic symptoms for the appropriate disease were expressed (visually detected during field investigation) and disease agents' fruiting bodies were present (established at the laboratory phase of the investigation). A sample was classified as asymptomatic if no visible symptoms could be found during the field investigation, or if the sample, which appeared likely to be symptomatic in a field investigation, was not carrying fruiting bodies of the pathogens (as it was found by the microscopic examination in laboratory).

The collected samples were investigated under light microscopes (binocular stationary lens CETI and microscope Nikon Eclipse 50i) in the laboratory of forest pathology at the Institute of Forestry and Rural Engineering of the Estonian University of Life Sciences.

The program IMAGE J 1.410 was used for micromorphological measurements of fungi and mostly Student's T-test for statistical comparisons (see **Papers III, V and VI**). The distribution maps (see **Paper VI**) were drawn using the program MapInfo Professional, vers. 7.5.

4.2.3. Fungal isolates

All species of the new alien invasive fungal pathogens discovered and investigated (**Papers III, V and VI**) were repeatedly isolated onto malt extract agar (MEA) (OXOID LP0039, OXOID Agar no.3, LP0013, Oxoid LTD., Basingetoke, Hampshire, England) or pine needle agar media (PNA) (100 g Scots pine fresh green needles boiled in 1 litre tap water for 20 min., 15 g OXOID Agar no.3, LP0013 added, autoclaved at 106 °C for 60 min.). *D. pinea* and *C. minus* were isolated on the MEA and *M. pini* and *M. dearnessii* on the PNA.

For molecular analyses all the isolations were transferred to MEA or PNA plates, which were covered with sterile cellophane membrane. Then, after incubation of ca. 2 weeks at 20°C in the dark, the hyphal mass from culture edges was removed from the membrane and stored in sterilised Eppendorf tubes at –20°C until DNA extractions and research.

4.2.4. Molecular techniques

In **Papers III and V** the DNA of the both fungi was isolated from the hyphal mass grown in pure cultures by using a modification of the method described by Vainio *et al.* (1998). Molecular investigations were performed in the Finnish Forest Research Institute in Vantaa, Finland.

The Internal Transcribed Spacer (ITS) region of ribosomal DNA was amplified from all of the isolates with primers ITS1F and ITS4 (White *et al.* 1990; Gardes and Bruns 1993), according to the temperature cycles given in Gardes and Bruns 1993. The reaction conditions were as suggested by the manufacturer of the Dynazyme II DNA-polymerase (Finnzymes Ltd., Finland), except for the primer concentration (25 µM).

The High Pure PCR purification kit (Roche, Germany) was used to purify the amplification products, which were then sequenced using the Therm EXCEL™ II DNA sequencing kit-LC (for 66 cm gels) (Epicentre®)

with the labelled primer pair ITS1F and ITS4. Resulting sequences were visualised using the LI-COR global edition IR² system (LI-COR Inc., USA) by following the manufacturer's instructions. In GenBank BLAST searches were used to compare Estonian sequences of *M. pini* and *D. pinea* with others from the whole world.

4.3. Data analysis

In **Papers I** and **IV**, using multiplied needle density (ND, the number of needles per cm⁻¹ on a definite annual shoot) and the measured length of annual shoot (HI), the number of needles attached on each annual shoot during the successive years was counted from the pith outwards. Here and elsewhere in this work, the number of needle pairs = short shoots is referred as the number of needles.

With this method the number of needles was classified in different annual needle sets and indicated as ANN₁, ANN₂, ANN₃, etc. (Figure 5). Number of current-year needles (= needle production) on the main stem was marked with ANN₁ (ANN₁ = NDxHI). At the end of each growing season the number of current-year needles was evaluated to be about four months old (0.3 years). The number of the retained needles in the previous year's shoots in the same annual ring was marked by ANN₂ (1.3 years) and ANN₃ with 2.3-years old needles, etc. ANN_n indicates the number of retained needles in the annual shoot grown n-1 years before the current-year shoot but still maintained in the same annual ring.

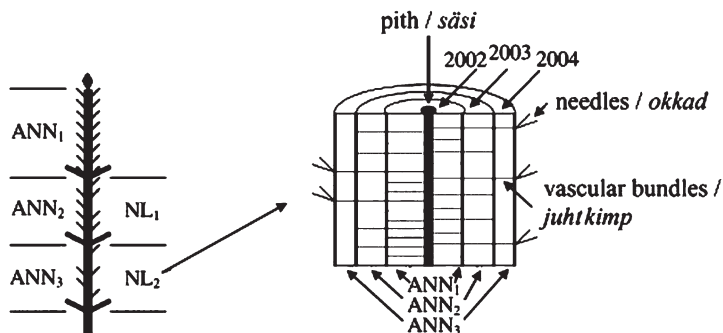


Figure 5. Schematic explanation of the origin of different needle sets (ANN_n) and needle loss values (NL₁ and NL₂) in the pine stem (original drawing).

The effect of different needle sets on the increment was analysed using a covariance analysis method (ANCOVA). Radial (RI) or height (HI) increment was a dependent variable, ND and the number of needles in any of the different needle sets (ANN_1 , ANN_2 , ... ANN_n , respectively) were independent variables. The ANCOVA model includes only one needle set at a time because most needle sets correlated significantly with one or two younger and older needle sets (see **Paper I**).

In the natural conditions of Estonia shoot elongation (HI) of Scots pine stops mostly at the end of June or beginning of July (Pihelgas 1973), and the current-year needles do not mature by that time (Figure 6). On former agricultural land, the bud burst of Scots pine occurred at the end of April 2010, on permanent forest land a week later. Half of shoot elongation was achieved by the beginning of June and almost total shoot elongation (ca. 48 cm) was achieved by the 22nd of June on the former agricultural land. During the next two weeks shoot elongation only by 3–5 (10) cm was registered. Photosynthetic activity at the first half of the season is much lower for the current-year needles than for the older needles (James *et al.* 1994), which means that the current-year needles have only a minimal effect on the current-years' shoot elongation. Therefore, correlation coefficients only between HI and second- and older needle sets were computed (**Papers I and IV**).

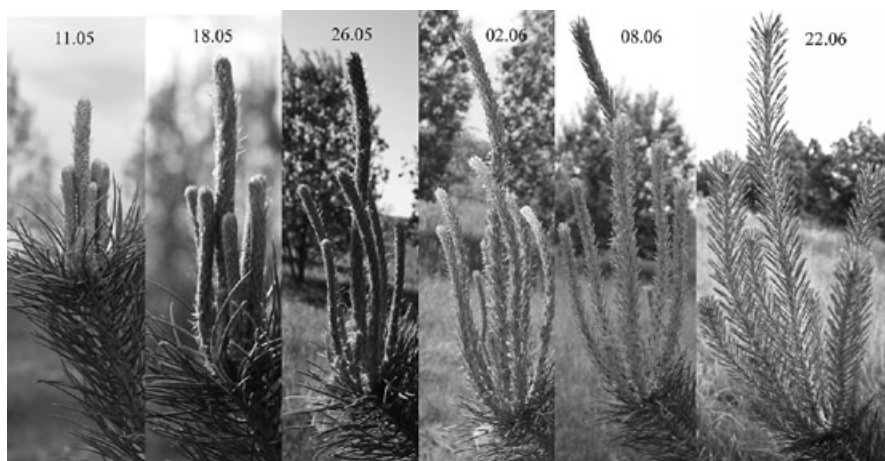


Figure 6. Shoot elongation and needle development of a Scots pine during 7 weeks (from the 11th of May to the 22nd of June 2010, original photos). This series of photos demonstrate that the impact of the current-year needles to the current-year shoot elongation can be minimal.

In **Paper IV**, the annual needle loss (NL) was derived from the needle trace counts as percentage from the number of needles attached in the previous year. The original number of needles, *i.e.* the needles first appearing in the previous year ($ANN_{1(t-1)}$), was used as the base to compute needle losses from the second-year needle sets (NL_1), but NL_2 (*i.e.* the needle losses from ANN_3) was calculated from the ANN_2 of the previous year (Figure 5). Additionally, for determining the total effect of the needle losses on the tree growth, the second and third-year needle losses (*i.e.* from the needle sets ANN_2 and ANN_3) were combined into a new variable $NL_{(1-2)}$.

In **Paper IV**, the annual needle losses (NL) were derived from the needle trace counts as percentages from the number of needles attached in the previous year. The original number of needles, *i.e.* the needles first appearing in the previous year needle sets ($ANN_{1(t-1)}$), was used as the base to compute needle losses from the second-year needle sets (NL_1), but NL_2 (*i.e.* the needle loss from ANN_3) was calculated from the ANN_2 of the previous year (Figure 5). Additionally, for determining the total effect of the needle losses on the tree growth, the second- and third-year needle losses (*i.e.* losses from the needle sets ANN_2 and ANN_3) were combined into a new variable $NL_{(1-2)}$.

Consequently, NL was derived from the needle trace counts as percentage from the number of needles attached in the previous year. The combined needle loss $NL_{(1-2)}$ was computed according to the following equation:

$$NL_{(1-2)} = 100 \times (1 - (ANN_{2(t)} + ANN_{3(t)}) / (ANN_{1(t-1)} + ANN_{2(t-1)})),$$

in which t indicates the current-year and $t-1$ means the year before.

Also, a categorised variable, $CNL_{(1-2)}$ with 10% intervals was used to study the effect of $NL_{(1-2)}$.

Annual differences in RI and HI were analysed statistically in multivariate general linear models (GLM) using the needle loss as an independent covariant or, alternatively, from the needle loss data, transformed to a categorised variable with ten percent intervals (see **Paper IV**).

In **Papers I** and **IV** none of the variables was standardised for final statistical processing operations. Each site was analysed separately. All the statistical operations were performed according to SYSTAT (2000).

In **Papers II** and **VII** the NTM data (the total annual needle loss, TANL) were calculated by the special program NTM_{ENG} ver. 8 (Aalto and Jalakanen 2004). In the **Papers II** and **VII** the TANL means the decrease in the amount of needle cohorts (defoliation) from the total main stem in a definite year, which is represented as the number of needle sets lost per year. The TANLs were used because *L. seditiosum* infects needles in all annual needle sets and in these papers the lost needles were not classified by their status as part of the earlier, different needle sets. The TANL raw data were standardised with a 5-year moving average with one year step (Fritts 1976). The resulted needle loss indexes were calculated separately for every single tree in each experimental stand for the period 1887–2006.

On the base of the support of literature data from the neighbouring countries, which indicated coinciding with the epidemics in Estonia *Lophodermium* needle cast epidemic years in these countries, a sample of larger epidemic years was selected (**Paper VII**). Included in this sample are twelve documented *Lophodermium* needle cast epidemic years (definitely 1923; 1924; 1929; 1955; 1961; 1962; 1975; 1979; 1982; 1999; 2001; 2004) in Estonia inside the more than a century-long period (1887–2006) that were taken into calculations as reference (pointer-) years of epidemics (**Paper VII**). These years, together with the pre-epidemic years, were investigated for detecting the epidemic causality from the meteorological data of these previous years, including retrospective needle loss data from our NTM materials. The original growth data (RI and HI) were used for growth loss analyses for opening the differences in growth characteristics at the epidemic (pointer, 0), one (1) and two post-epidemic (2) years compared with the appropriate long-term (1887–2006) mean data.

Meteorological data were obtained from the Tartu-Tõravere Meteorological Station, which is situated nearly between the investigated stands (Figure 2). In **Paper VII** the GIVs (Thomsen 2009) were also calculated, where GIV means the ratio of monthly precipitation sum (mm) to the monthly mean air temperature (°C) for a definite month.

5. RESULTS

5.1. Correlation between the needle sets and growth characteristics of Scots pine trees

Tree foliage is the most essential element in forest production biology. The state of foliage is also an important characteristic of tree vitality, and the mean needle age and the number of needle sets (Figure 5) represent the definitive estimation values of the vitality of conifers.

Table 1. Correlation between the needle density (Ln(ND) = logarithmic transformation of ND), age of attached needles (CNA), and the mean number of attached needles in different annual needle sets (ANN₁–ANN₁₀) and the sums of the needle sets (ANN_{1–10} = all attached needles; ANN_{5–10} = the sum of the needles older than 4 years, etc.).

Variable	Correlations						Mean number		
	Radial increment (RI)			Height increment (HI)			of needles		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
LNND	-0.460***	-0.310***	-0.202**	-0.726***	-0.450***	-0.363***			
CNA	-0.139	-0.256***	-0.289***	-0.344***	-0.362***	-0.046			
ANN ₁	0.484***	0.411***	0.224***				197.8	232.7	278.4
ANN ₂	0.418***	0.437***	0.232***	0.535***	0.425***	0.120	192.6	220.7	262.6
ANN ₃	0.182*	0.325***	0.247***	0.187*	0.392***	0.286**	150.5	194.6	229.9
ANN ₄	-0.042	0.198*	0.139*	-0.096	0.185	0.079	38.8	147.7	156.5
ANN ₅	0.086	-0.077	0.035	-0.062	-0.113	0.123	2.5	53.3	90.2
ANN ₆		-0.056	-0.025		-0.108	0.160		13.1	43.3
ANN ₇		-0.048	-0.012		-0.090	0.102		1.7	13.7
ANN ₈		0.035	-0.074		-0.060	0.101		0.2	4.5
ANN ₉			-0.049			-0.003			1.3
ANN ₁₀			-0.019			0.090			0.3
ANN _{1–10}	0.312***	0.396***	0.229*				562.3	845.1	1080.3
ANN _{2–10}	0.206*	0.337***	0.186	0.270**	0.347***	0.258**	372.7	624.4	810.3
ANN _{3–10}	0.073	0.235*	0.132	0.056	0.237**	0.244*	186.8	411.8	552.8
ANN _{4–10}	0.017	0.106	0.055	-0.085	0.040	0.168	42.1	222.2	325.9
ANN _{5–10}	0.131	-0.034	-0.008	-0.061	-0.104	0.174	2.5	72.8	163.3
ANN _{6–10}		-0.024	-0.041		-0.103	0.164		16.0	67.6
ANN _{7–10}		-0.032	-0.048		-0.097	0.120		2.0	21.3
ANN _{8–10}		0.062	-0.088		-0.056	0.102		0.2	6.4
ANN _{9–10}			-0.052			0.026			1.6

P-values: * 0.05, ** 0.01, *** 0.001

Our NTM investigation of older Scots pines (see **Paper I**) showed that the correlation of needle sets with RI and HI varied according to the needle age. Younger needle sets (ANN_1 to ANN_3) were significantly positively correlated with the RI in all sites (see chapter 4.1). ANN_4 had a somewhat significant positive correlation with RI only on sites 2 and 3. In most cases, older than the third needle sets were negatively correlated with RI (Table 1). As an annual shoot (=yearly height increment) completes its growth earlier than the needles on it, a study of the correlation between the current-year needles and their shoot elongation is irrelevant (see chapter 4.3). The ANN_2 and ANN_3 on sites 1 and 2, but only ANN_3 on site 3, correlated positively and significantly with HI. For the older than third needle sets, the appropriate correlations were negative in most cases and insignificant (Table 1) in all cases (**Paper I**).

The results showed, that if needle age as an argument was included (see **Paper I**), and moreover, if needle loss was also included, only the second and the third needle sets supported both radial and height growth of young Scots pines (see **Paper IV**).

It can be concluded that a temporary loss of older (> 3 years) foliage sets causes no remarkable growth loss.

5.2. The effect of productive foliage on the tree growth

On sites 1 and 2 (see chapter 4.1) the current, second and third-year needles (ANN_1 , ANN_2 and ANN_3) had a statistically significant effect on RI; on site 3 only the two youngest needle sets (ANN_1 and ANN_2) were significantly correlated (Figure 7). On none of the sites could the pines benefit from needles older than 3 years (ANN_4 and older). Further, some older needle sets (ANN_5 and ANN_6 on site 2 and ANN_6 on site 3) had a negative relationship with the radial growth (Figure 7).

The annual needle sets ANN_2 and ANN_3 on site 1, ANN_2 – ANN_4 on site 2 and only ANN_3 on site 3 had a significant positive effect on HI. The HI had a significant negative relationship with ANN_4 on site 1 and with ANN_5 – ANN_7 on site 2. On site 3, the HI had no significant relationship with older needle sets (Figure 8).

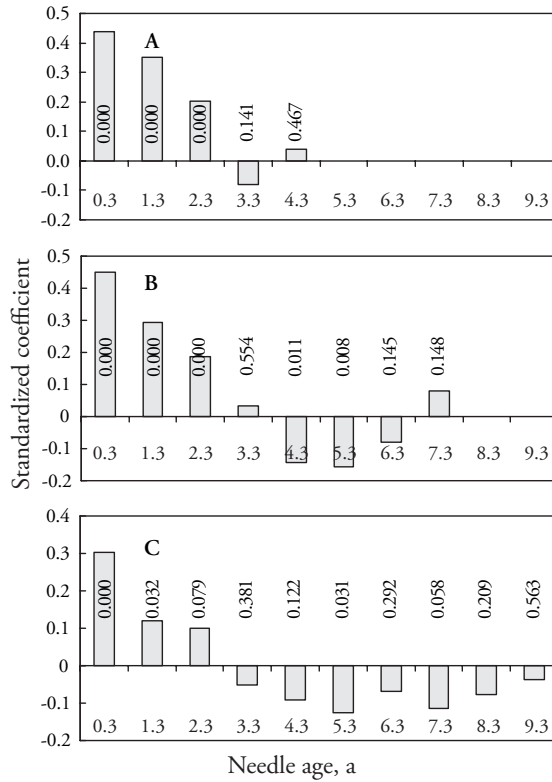


Figure 7. Relative effect of needle sets of different ages on radial increment on the three sites. **a** Site 1, Pyhtää, Finland; **b** Site 2 and **c** Site 3, the both: Konguta, Estonia. Standardised coefficients (columns) indicate the effect on growth. The numbers along the vertical axis are p-values for each needle set.

Since the low correlations of RI and HI with greater than three-year-old needle sets could be due to the low annual numbers of these needle sets, a new set of correlation analyses was computed by using the summed variables (ANN_{1-10} to ANN_{9-10} , see Table 1). Within these variables we obtained the higher numbers of needles than in the single annual needle sets. However, this process yielded fewer cases with significant correlations than the original needle set variables for both RI and HI (Table 1). Thereafter the possible effect of older needles on tree growth was also tested, using these summed variables in ANCOVA, but none of these variables demonstrated a significant effect on any of the sites (see **Paper I**). Similarly, on young Scots pines the needles in the fourth-year and older needle sets had no positive effect on tree growth (**Paper IV**).

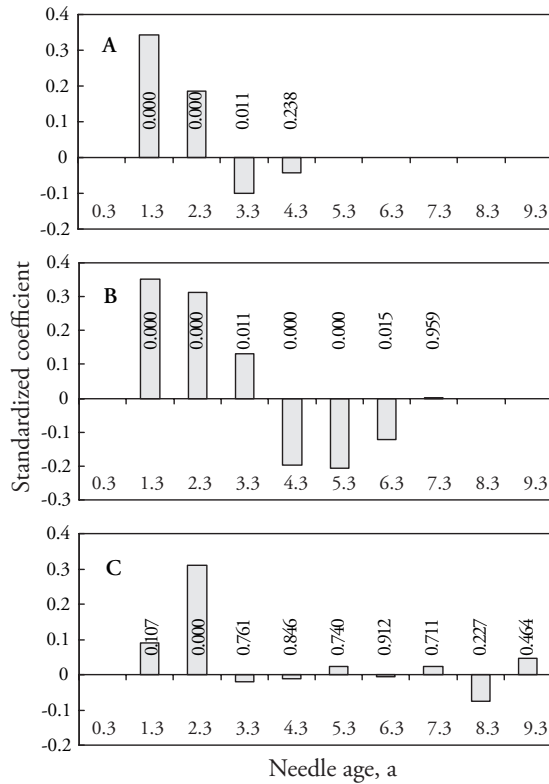


Figure 8. Relative effect of needle sets of different ages on height increment on the three sites. **a** Site 1, Pyhtää, Finland; **b** Site 2 and **c** Site 3, the both: Konguta, Estonia. Standardised coefficients (columns) indicate the effect on growth. The numbers along the vertical axis are p-values for each needle set.

How was the productive foliage detected? Figures 7 and 8 illustrate clearly that the three younger needle sets are productive and affect the growth of Scots pine, positively and in a statistically significant way. It indicates that irrespective of tree age the second (ANN_2) and third (ANN_3) needle sets support HI and the three younger needle sets (ANN_1 , ANN_2 and ANN_3) support RI. However, if the number of remaining needles or, on the contrary, if the needle loss from the older needle sets does not correlate with the growth, it means that these needles did not support the growth and are not productive.

5.3. Needle loss dynamics during the study period

During the study period 1997–2004, the average needle loss NL_1 in ANN_2 from ANN_1 of the previous year was 3.5% and by the third autumn (ANN_3) the loss (NL_2) from the previous years' ANN_2 was 35.7%. NL_2 had the highest average values 67.5% in 2002, and was around 40% in 1999–2001. Before 1999 and after 2002 NL_2 was significantly lower ($p < 0.001$), 21 and 18%, respectively (Figure 9). The average combined needle loss $NL_{(1-2)}$ was 18.5%. Among individual trees the differences in NL_1 , NL_2 and $NL_{(1-2)}$ were highly significant ($p < 0.001$), varying annually from 0 to 100% (Paper IV).

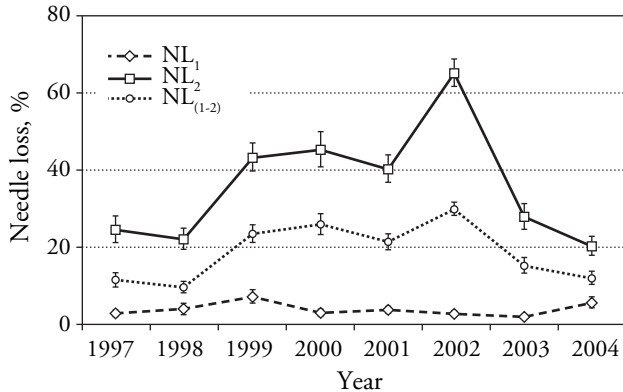


Figure 9. Annual needle loss (%) in the experimental stand in Konguta. NL_1 = needle loss in the second-year needle set (ANN_2), NL_2 = needle loss in the third-year needle set (ANN_3), and $NL_{(1-2)}$ = the combined needle loss from the second and third-year needle sets. Vertical bars show the mean error.

5.4. Impact of needle loss from productive foliage on the tree growth

The relationship between the needle loss and the growth rate was analysed with the model: $Growth = Constant + Provenance + Block + AsinNL + Year$. In the total material the growth-decreasing effect of NL_2 appeared

to be higher for RI ($p < 0.001$) than for HI ($p = 0.012$). The loss (NL_1) in the second needle set had no significant effect on the growth. The effect of $NL_{(1-2)}$, *i.e.* the combined needle loss from ANN_2 and ANN_3 , decreased significantly both RI and HI, with p -values < 0.001 and $= 0.001$, respectively.

The effect of needle loss on the growth rate varied annually. Needle loss was at a relatively high level from 1999 to 2002, but the effect on RI and HI was different. RI was affected significantly by the needle loss (NL_2) in 2000 and 2001 and by $NL_{(1-2)}$ in 2001. Needle loss in the constructed model trees decreased with a slight degree of significance RI and HI also in 1999 ($p = 0.016$ and 0.046 , respectively). In 1997 and in 2001 there was a significant decrease in HI, associated with the both needle loss variables (NL_2 , $NL_{(1-2)}$). Although needle loss was at the highest level ($NL_2 = 65.4\%$) in 2002, GLM-models revealed no significant effect of NL on the growth (both HI and RI). Needle loss had a lagging effect on RI, which was statistically significant in 2001 and 2002 ($p = 0.021$ and 0.005 , respectively). The lagging effect on HI was not significant in either year.

Overall, the combined needle loss value, $NL_{(1-2)}$ gave the highest explanation for the decrease in RI ($F = 13.492$, $p < 0.001$) when the current-year needles (ANN_1) were totally omitted ($C=0$) as a component in the computations of NL-percentages.

The quantitative effect of needle loss on RI was studied in the data of 1999–2001 with the categorised variable $CNL_{(1-2)}$ derived from $NL_{(1-2)}$ with ten percent intervals. In these three years the needle loss had a relatively high effect on RI ($p = 0.002$). Using GLM (Growth = Constant + Provenance + Block + $CNL_{(1-2)}$) we obtained the growth values for different categories of NL. GLM for RI was computed separately for each year (1999–2001). The regression line of the growth data (18 data points obtained), when plotted against $NL_{(1-2)}$ -class means, revealed that at the level of 50% $NL_{(1-2)}$ decreased RI as a mean by 39.2% in these three years (Figure 10). Similarly, the effect of $NL_{(1-2)}$ on HI was computed for the data of 1997, 1999 and 2001, when $NL_{(1-2)}$ had a relatively high effect on HI. Sixteen data-points obtained were plotted against ten-percent classes of $NL_{(1-2)}$, and the regression line for these points revealed a 34.8% decrease in HI with the needle loss of 50% (Figure 11). Whether or not these numbers indicate that the remainder in the degree of growth

reduction (10.8 and 15.2%, respectively) can be attributed to the mitigating effect of the other reserves in the economy of energy of a tree has to be investigated further.

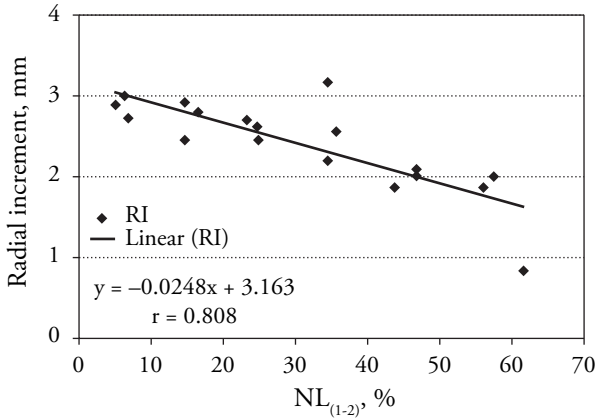


Figure 10. The relationship between the needle loss, represented in ten percent categories (= needle loss classes), and radial growth. The dots represent the means of the appropriate categories (needle loss classes, adjusted for RI) for the three succeeding years, 1999–2001.

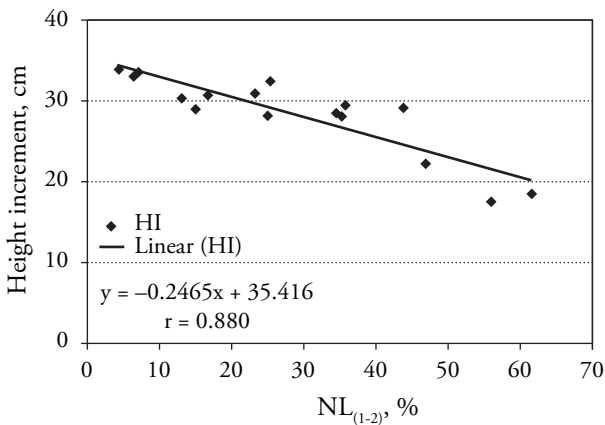


Figure 11. The relationship between the needle loss, represented in ten percent categories, and height growth. The dots represent the means of the appropriate categories (needle loss classes, adjusted for HI) for three selected years, 1997, 1999, and 2001.

5.5. Practical use of retrospective needle and weather datasets to distinguish outbreaks of needle pathogens of Scots pine

Irrespective of the arrival of several new, alien pine foliage pathogens in Estonia, the most common foliage disease in young Scots pine plantations and especially forest nurseries is still *Lophodermium* needle cast.

It is known that: 1) *Lophodermium* needle cast epidemics occur in Estonia almost regularly, but not at precise intervals, and 2) needle cast epidemics are much more frequent in forest nurseries than in plantations. The epidemics are sometimes so frequent in forest nurseries that they can occur during two successive years.

In this investigation the traditional approach in explaining the causes of the epidemics is followed, *i.e.*, considering the meteorological conditions of the preceding year. The timetable of the potential epidemic years of *Lophodermium* needle cast was reconstructed retrospectively in **Paper II** by juxtaposing two time-series: 1) the meteorological data (by selecting out the years with rainy summer + mild winter), and 2) the needle characteristics (high needle loss). A year with an extensive needle loss was considered as a potential epidemic year if it followed a year with an extensive precipitation in summer.

The pathogen of *Lophodermium* needle cast disease needs both high precipitation for successful infection of pine needles in summer, and a mild winter for successful colonisation of infected needle tissues before the new vegetation period. Thus potential *Lophodermium* needle cast epidemic years were considered to be probable if the combination of wet and warm summers followed by mild winters was present before the years of high needle loss. Large epidemics of other infectious needle diseases of pine (for that time) were rare in Estonia, but insect pests were commonly triggered, in contrast, by warm and dry summers. Regarding the meteorological conditions, eight combinations of summers (between May and September) and the following winters (from the previous year December to the pointer year March), were examined in the calculations to find out which combinations were followed by high needle loss in the next year. A year was classified as a “high precipitation” year, and the following winter as a “mild” winter, if they were characterised by higher values of the respective meteorological characteristics than the long-period (1882–2004) means \pm standard errors of the appropriate means.

Two variants of “summers” were included into the investigation. Within all the eight combinations, higher (compared to long-term mean, $p < 0.001$) annual needle loss values followed the years with high precipitation in the period from:

- a) July to September (incl.) + mild winter, and
- b) July to August (incl.) + mild winter.

During the extended period (1884–2004) larger epidemic years of *Lophodermium* needle cast in Estonia and in nearby countries, documented in the literature, were 1923, 1924, 1929, 1961, 1975, 1979, 1982, 1999, 2001 and 2004 (see Table 1 in **Paper II**), and these were taken as the sample of reference years. Comparison of the appropriate meteorological data of documented *Lophodermium* needle cast epidemic years with the long-term (1884–2004) means showed that both combinations of the extent of summer seasons of pre-epidemic years had the mean precipitation values at least close to, but mostly higher than, the long-period means (except for 1975). The low winter temperature and the summer warmth both seemed not to affect the pathogen in triggering a new epidemic.

Paper II lists, starting from the last decades of the 19th century to the present, the potential epidemic years of the *Lophodermium* needle cast disease. The preceding years, supporting the rise of an epidemic through their meteorological peculiarities (two combinations of summer months and following mild winters) are not indicated separately. If the NTM data in a potential epidemic year (*i.e.*, after a year with high precipitation in summer and following mild winter) showed also a “high needle loss” (*i.e.*, higher than the mean needle loss of the years of the entire long-term period covered by our NTM data), then the year was classified as a potential epidemic year for pine plantations. The years following solely the “high precipitation summer and mild winter” years (*i.e.*, without high needle loss in our experimental stands) were classified as potential epidemic years only in forest nurseries.

5.6. New information about different aspects in *Lophodermium* needle cast epidemiology, extracted from the retrospective needle dataset

A retrospective search of *Lophodermium* needle cast epidemic years was undertaken by calculating mean monthly precipitation sums of two or

three summer months of the previous year and of the winter-time mean monthly temperatures (**Paper II**). In a new set of calculations, the mean precipitation sums of every single month (Figure 12), mean monthly temperature and GIV values from pre-previous May to the epidemic year May were analysed separately (**Paper VII**).

The Student's T-test comparisons confirmed that the mean precipitation sums were significantly higher in May, June, July and August of the year before the epidemic, in comparison with the mean characteristics of the same months of the extended period (1887–2006). As a rule, also the pre-epidemic autumns were wet: the mean precipitation sums of October and November were higher than the long-term means (Figure 12), but not significantly.

Generally, the mean temperatures of the summertime months of the years before the sample of epidemic years did not differ from the long-term means, except for August, which was characterised by the statistically significantly ($p < 0.01$) lower mean monthly temperature than the

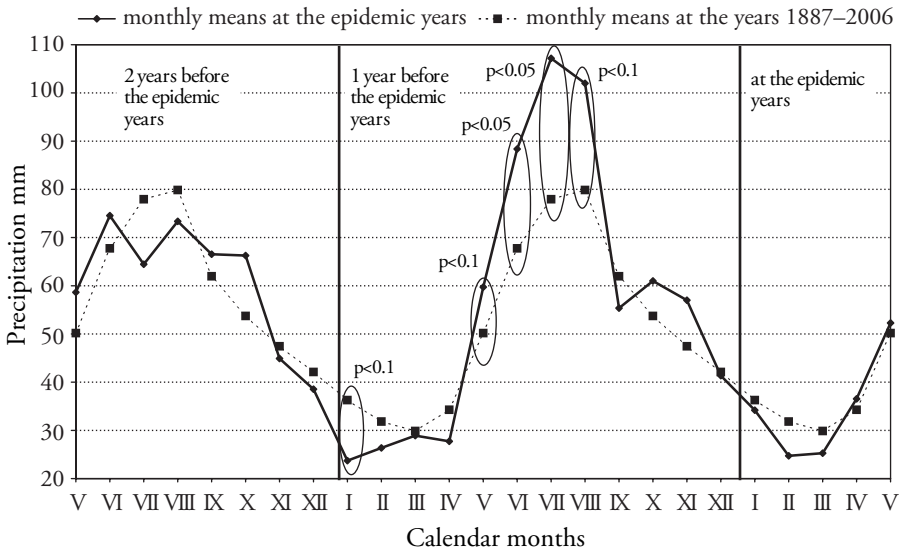


Figure 12. Mean precipitation sums of 20 months previous to the *Lophodermium* epidemic years (1923, 1924, 1929, 1955, 1961, 1962, 1975, 1979, 1982, 1999, 2001 and 2004) in comparison with the long-term means. The months with statistically significant differences in the mean precipitation sums on the two broken lines are united by ovals and supported by the p-values of difference.

appropriate long-term (1887–2006) mean. Obviously, in the Nordic country Estonia the midsummers are already naturally cool enough for the fungus.

Although the autumn and winter seasons of the years preceding the epidemic years were somewhat milder, their mean temperatures did not differ significantly from the appropriate long-term means. However, mean temperatures of some single months within the seasons – October, November and December of the preceding year and January and March of the epidemic years were somewhat warmer than the long-time means.

Which definite cumulative precipitation sums of summer months (from May to August, inclusive) could trigger the *Lophodermium* needle cast epidemics? The mean precipitation sum for these summer months for the long-term period was 276 ± 8 mm, for the sample of twelve epidemic years 361 ± 15 mm, the last varying in different epidemic years from 284 to 487 mm (excluding from this sample the epidemic year 1975, then 301 to 487 mm). Practically, if the precipitation sum reaches or goes over 300 mm, an epidemic will occur in forest nurseries, if 360 mm, an epidemic may also affect pine plantations. No severe epidemic in forest nurseries or plantations will be expected if the precipitation sum does not reach 300 mm.

Thereafter it was possible to count within the extended period (1887–2006) in total 49 years during which precipitation sums from May to August constituted at least 300 mm, which also means 49 potential epidemic years in forest nurseries (Table 2).

GIV analyses ended with quite similar results (concerning pre-epidemic summer months) as compared to the analyses, based solely on the mean precipitation sums: during 1887–2006 in total 52 *Lophodermium* needle cast epidemics should have occurred in Estonia (Table 2).

It has been known for a long time that young pines are suffering from *Lophodermium* needle cast more than older pines, but the host age limit has been rarely specified and was varied greatly. According to the retrospective needle loss and tree growth data for our sample of epidemic years, the vulnerable host age limit in Estonia was 22–24 years (Figure 13).

Table 2. The number per decade of reconstructed epidemics of *Lophodermium* needle cast and the mean needle loss indexes at these epidemics during the period 1887–2010.

Decade (interval)	Reconstructed epidemic years (precipitation >300 mm, GIV > 5.0)	Frequency of epidemic years per decade	Mean needle loss indexes of host trees at their vulnerable age (3–24 years) during the epidemic years
1887–1900	1892, 1893, 1895, 1898 a , 1899	5	1.07
1901–1910	1903, 1904, 1905, 1906, 1907, 1908 b , 1909, 1910 b	8	1.02
1911–1920	1911, 1912, 1916 b , 1917	4	1.02
1921–1930	1923, 1924, 1926, 1928, 1929	5	0.93 d
1931–1940	1931, 1932, 1935, 1936, 1938, 1939	6	1.15
1941–1950	1943, 1944, 1946, 1949, 1950	5	1.08
1951–1960	1953, 1955, 1957, 1958 b	4	0.88
1961–1970	1961, 1962, 1963, 1967	4	1.05
1971–1980	1974 a , 1975 b , 1979	3	1.15
1981–1990	1982, 1986, 1987, 1988	4	1.15
1991–2000	1991, 1994, 1999	3	1.01
2001–2010	2001, 2004, 2005, 2009 c	4	1.17

a – Precipitation sums, but not GIVs between May to August incl., indicated epidemic years.

b – GIV but not precipitation sums for the months May to August indicated epidemic years.

c – No NTM data for the appropriate age of trees at the sample years of epidemics.

d – Included larger hosts' age group (23–33 years).

Without considering the tree age, the mean needle loss index values were significantly ($p < 0.0001$) different at the sample of *Lophodermium* needle cast years (1.072 ± 0.04) from the high needle loss years (but not belonging to our sample of *Lophodermium* needle cast years, 1.295 ± 0.01) and from the low needle loss years (0.704 ± 0.01). When grouping the host trees by ages, the needle losses in the age group of trees of 3–11 (A) years were not significantly different at the *Lophodermium* needle cast years, in comparison with the same tree age group at the high needle loss years (Figure 13).

As expected, during the documented sample of *Lophodermium* needle cast epidemic years the needle loss indexes in pines were larger at the age group A than at the age of 20–33 years (B), 1.23 ± 0.06 and 0.98 ± 0.04 , respectively.

Mean needle loss indexes for the younger A-group differed significantly ($p < 0.01$) from the long-term (1887–2006) mean needle loss indexes (1.00 ± 0.01), but needle loss indexes of the older B-group did not. At the same time, mean needle loss indexes at the A-group differed significantly ($p < 0.01$) from the loss of the B-group. The results of similar analyses, concerning the calamities of herbivorous insect pests (diprinoids), did not differ significantly in any case at the same age groups and in the similar combinations.

Excluding the *Lophodermium* needle cast epidemic years from computations, the mean needle loss indexes for the A-group was 0.899 and for the B-group 1.009. It means that the epidemic years of *Lophodermium* needle cast had clearly increased the needle losses of young pines: the mean needle loss index at the epidemic years was 1.23 ± 0.06 . Although well-known informally for a long time, this fact has very rarely, if ever, been demonstrated experimentally.

Differing from the epidemics of *Lophodermium* needle cast, the calamities of the diprinoid herbivorous insect pests of Scots pine, such as *Bupalus piniarius* and *Neodiprion sertifer*, have been correlated in our material with drier and warmer, rather than cooler and wetter, summer and autumn months during the preceding years (see **Paper VII**). During the last century the epidemic and the calamity years had overlapped only once.

How much has the growth of trees been reduced at and after the sample of epidemic years of *Lophodermium* needle cast and calamities of herbivorous insect pests? For the sample of epidemic years the mean radial growth reduction was decreasing, extending up to the age of ca. 22–24 years. This upper vulnerable age limit of pines for *Lophodermium* needle cast coincided surprisingly well with the age limit obtained by analysing the needle loss index data series (Figure 13), pointing out the same susceptible age limit of pines for *Lophodermium* needle cast.

In the younger, A-group of pines, the radial growth of trees decreased significantly at the *Lophodermium* epidemic and two years after epidemics, in comparison with the long-term mean, by 12 and 18%, respectively (Table 3). An insignificant decrease in radial growth at the first post-epidemic year reached 7%.

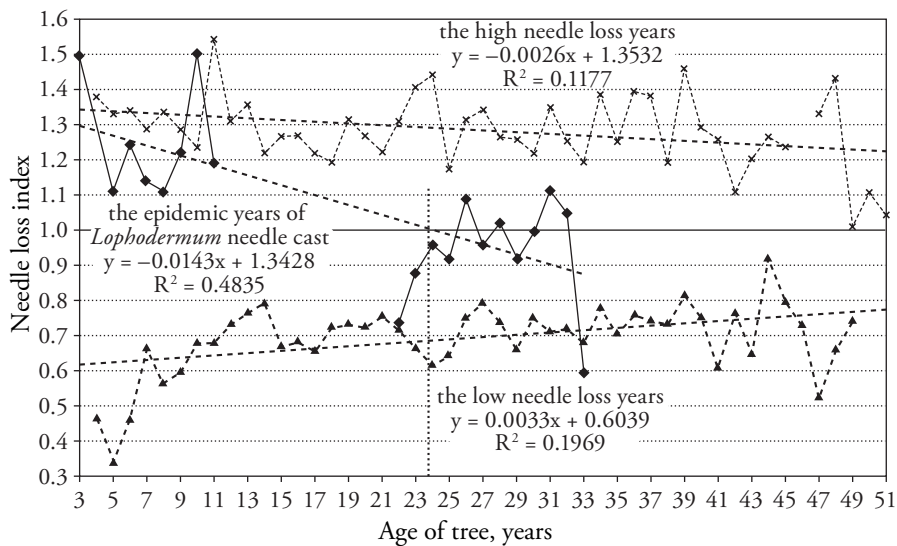


Figure 13. Distribution of the mean needle loss indexes (computed by NTM) in dependence of the tree ages at the twelve *Lophodermium* needle cast epidemic years (1923, 1924, 1929, 1955, 1961, 1962, 1975, 1979, 1982, 1999, 2001 and 2004) (the groups A and B, diamond), and compared to the high needle loss (but not documented as *Lophodermium* needle cast epidemic) years (x) and to the low needle loss years (triangle), respectively, within the long-term period (1887–2006). Mean needle loss index for the long-term period was 1.0 ± 0.01 (bold line).

However, height growth of trees has decreased significantly at the epidemic, and at the first and the second post-epidemic years, 18, 13 and 17%, respectively (Table 3).

In the older, B-group at and during the first years after the *Lophodermium* needle cast epidemics, no statistically significant growth reduction (both in radius and height) could be registered. To the contrary, even some statistically significant cases of growth increase could be registered (Table 3).

Concerning tree growth reductions caused by the calamities of insect defoliators of pines: in the younger, A-group the radial growth rate of pines decreased at the post-calamity and at the second year after the calamity of insect defoliators, 12 and 19%, respectively (Table 4). Height growth of trees, on the other hand, decreased significantly at the calamity years and at the both years after that, by 21, 26 and 34%, respec-

Table 3. Mean growth dynamics (in %) in different age groups of Scots pine at the 12 sampled *Lophodermium* needle cast epidemic years, one and two years after the epidemic years, compared to the same mean growth parameters at the normal (not epidemic and not calamity) years.

Stand age group (years)	Radial growth at the epidemic years	Height growth at the epidemic years	Radial growth one year after the epidemic years	Height growth one year after the epidemic years	Radial growth two years after the epidemic years	Height growth two years after the epidemic years
A(3–11)	-12**	-18***	-7	-13**	-18****	-17****
B(22–33)	4	7	4	21***	6	25***

P-values: * 0.1, ** 0.05, *** 0.01, **** 0.001

Table 4. Mean growth dynamics (in %) in two different age groups of Scots pine during the documented 14 calamity years of herbivorous insect pests of Scots pine, one and two years after the calamity years, compared with the same growth parameters during the normal (not belonging to the sample of *Lophodermium* needle cast epidemics and/or documented calamity) years.

Stand age group (years)	Radial growth at the calamity years	Height growth at the calamity years	Radial growth at the post-calamity years	Height growth at the post-calamity years	Radial growth two years after the calamity years	Height growth two years after the calamity years
A(3–11)	-3	-21***	-12**	-26****	-19****	-34****
B((20)25–33)	-13***	-17***	-6	-5	-1	-4

P-values: ** 0.05, *** 0.01, **** 0.001

tively (Table 4). In the older, B-group both the radial and height growth decreased statistically significantly at the calamity years, 13% and 17%, respectively. Growth reduction during the following the calamity two years was insignificant.

Both defoliators, *Lophodermium* needle cast and herbivorous insect pests of pine, significantly influence the growth characteristics of trees during the year of attack and at least during the two years following the pointer year, especially the height growth and at the younger A-group of trees. At the B-group of pines no significant reduction of *Lophodermium* needle cast epidemics to the tree growth parameters could be registered, but the

influence of insect defoliators continues, especially reducing the height growth of trees (Table 4).

Although some coincidental oscillations were found in the NTM and ICP data-series, *e.g.*, increase in the needle loss/defoliation in 1994, 1998 and 2001, the needle loss dynamics were different in different age groups of Scots pine.

5.7. New alien invasive foliar pathogens and their colonisation process of Estonia

5.7.1. *Mycosphaerella pini*

First time the quarantine pathogen *M. pini* (in its anamorphic *D. septosporum* stage) was diagnosed from the samples of diseased *P. nigra* needles, collected in the autumn 2006 at an arboretum in Agali, Järvelja, south-eastern Estonia. In March 2007 *D. septosporum* was found in Järvelja also on *P. mugo* and on native *P. sylvestris* trees. Thereafter, the fungus was confirmed by partial sequencing of the internal transcribed spacer (ITS) region and data of three Estonian strains were deposited in GenBank. The ITS analyses showed complete (100%) similarity with ca. 50 different sequences of *M. pini* in GenBank (see **Paper III**).

Distribution maps (see **Paper VI**), showing the fast colonisation process of the pathogen, indicate that the spread of *M. pini* began in Estonia from the south and proceeded northward. By the end of 2007 *M. pini* was already documented on *P. sylvestris* in several places in southern Estonia, while several investigated stands in central and northern Estonia stayed asymptomatic. By the spring of 2008, the fungus was widespread in south Estonia and by the end of the same year everywhere in Estonia. Additionally, in autumn 2008 this fungus was for the first time documented in northern Latvia, although obviously this southern fungus colonised Latvia before emerging in Estonia.

5.7.2. *Mycosphaerella dearnessii*

During the isolations of *M. pini* in different regions of Estonia, several colonies were noticed among cultures, which were not producing red pigment, characteristic to *M. pini*. Some of these colonies even had a slightly

greenish colour. In cultures with the same characteristics, similar colonies also produced remarkably similar conidia. Also on the needles *in vivo* these non-typical needle patches were coloured brown rather than red.

Thereafter the serious (listed as an A2 quarantine pest by EPPO) pathogen *M. dearnessii* (anamorph *L. acicola*) was also diagnosed in the needle samples of *P. ponderosa* from the Tallinn Botanical Garden by laboratories in Denmark and Austria in 2008. Molecular studies of the Estonian cultures of *M. dearnessii* that we isolated confirmed our diagnoses.

Isolates of both *M. pini* and *M. dearnessii* were difficult to obtain due to slow growth. Both fungi have still not been recorded in Estonia in their sexual stages.

5.7.3. *Diplodia pinea*

D. pinea, one of the most common and widely distributed pathogens of conifers worldwide, known also as a dangerous needle pathogen of pines, was not previously described in Estonia, nor in neighbouring countries. In September 2007, this fungus was observed by us for the first time on scales of fallen cones under a middle-aged *P. nigra* tree in the Järvelja nursery. Cones of other *P. nigra* trees in Järvelja and in several other locations in Estonia were found to be asymptomatic. Thereafter the fungus species was confirmed by its ITS region and deposited in GenBank. A comparison showed 100% similarity with ca. 30 earlier *D. pinea* sequences from around the world, deposited in GenBank (see **Paper V**).

In 2008 the fungus was discovered in all the formerly asymptomatic *P. nigra* trees in Järvelja and in western Estonia. Consequently, during this single year of observations *D. pinea* was found rapidly spreading in Estonia, but still only on cones (see **Papers V** and **VI**).

From shoots of *P. sylvestris* the fungus was detected for the first time in Tallinn, thereafter from shoots of *P. nigra* in Tartu, although in these towns fallen cones of *P. nigra* were still not observed to be infected. It is unknown how fast the fungus can change host organs as substrate in Estonia and spread from cone scales (and shoots) to needles, buds, roots and trunk wood, thereby causing considerable losses. That has been the common process in the southern countries.

5.7.4. *Cyclaneusma minus*

The fourth new alien fungus on Scots pine needles, *C. minus*, has been of particular interest during recent years because of its suddenly higher dissemination rate. In 1999 *C. minus* was found on the needles of young Scots pines in the Tartu forest nursery as a species new to Estonia, but without any evidence of it from other locations in Estonia. Since 2007, suddenly, several findings of *C. minus* were registered, first in southern Estonia (e.g., in Võru county) and by the autumn of 2008 the fungus was found throughout Estonia, still only in small patches. The fungus could not be found in northern Latvia in the autumn 2008, despite special searches, but we found *C. minus* in northern Latvia in 2009.

Isolates of *D. pinea* and *C. minus* grew fast on artificial media and were thus easy to culture.

Fortunately, in Estonia *C. minus* has not acted still as a serious pathogen (see **Paper VI**): our monitoring has not confirmed serious premature needle losses due to the fungus.

6. DISCUSSION

6.1. Needle age, productive foliage and growth of Scots pine

Foliage diseases reduce the assimilating apparatus of trees, thus affecting tree growth rate and storage of nutrients. In perennial trees like pines, investigation of the contribution of different needle sets to the tree growth is complicated.

Assuming that the number of needles attached in the stem correlates with the number of needles or needle sets in the branches, it has to be possible to estimate the contribution of different needle sets to the growth of pines. Needle retention in the main stem and in the branches seems to be approximately equal, with only small variations; *e.g.*, in Finland it was found to be slightly lower in the main stem (Jalkanen *et al.* 1995), whereas in a sample from Estonia the opposite was true (Pensa 2003).

Retrospectively-counted needle traces from different annual shoots have been classified to different needle sets according to needle age. Only young needle sets correlated positively with both radial and height increment (see **Papers I** and **IV**). Some needle sets older than 3 or 4 years seemed to have even a negative relationship with tree growth. Furthermore, the trees that had green older needles grew less than those without such needle sets. Some previous studies have established that slowly growing trees often demonstrate high needle retention, as is the situation with increasing elevation or along a south–north gradient (Schoettle 1990; Jalkanen *et al.* 1995; Xiao 2003).

The effect of older needle sets on growth processes cannot be determined accurately by means of the statistical calculations used in this study, since older needle sets in young pines represent a very small portion of the whole foliage, and their numbers do not vary in the same way as current-year increments (RI and HI). However, all green needles may participate in assimilation and thus support growth, but it is known that the photosynthetic capacity of the needles tends to decrease with their age (Niinemets and Lukjanova 2003).

It is fact that the youngest needles are physiologically most active and therefore most important in dry mass production (Sullivan *et al.* 1997; Bobkova and Zagirova 1999; Wang *et al.* 1995; Oleksyn *et al.* 1997; Zha

et al. 2002). This is logical because all needles in the three youngest needle sets accounted for 97.8% of the total foliage (Xiao and Ceulemans 2004). In light of these numbers, it is not surprising that older needle sets are not at all important for assimilation and increment processes in pine trees. Needles older than 3 years may only serve as storage for reserve nutrients. The needles start to senesce when these nutrients are mobilised and retranslocated for new growth.

It is demonstrated in **Paper I**, that trees grew best on site 3, although some needles there even in the tenth needle set could occasionally be attached. The trees on site 1 were growing slowly, yet the oldest needles were found in the fifth needle set (ANN₅). At the stand level, high availability of nutrients in relation to other growth promoting factors may retard the recycling of the minerals stored in older needle sets and the needles that remain attached for several years. It seems that the difference between the sites in the number of older attached needles might be correlated with the soil fertility rather than with the irradiance gradient in the tree crowns (see Niinemets and Lukjanova 2003).

Apparently, the relationship between high needle retention or delayed retranslocation of mobile minerals and growth within the stand has not been studied. The growth of individual trees may be impeded, *e.g.*, by drought, injuries, needle loss, etc., which always signals a direct or indirect decrease in the assimilative apparatus. On the other hand, the retranslocation of nutrients may be mainly controlled by the growth rate (Nambiar and Fife 1987). The results showed that in certain years the trees that had older needles (ANN₄ to ANN₆) attached grew less than those that had already lost these needle sets.

Only site 1 had a very apparent reason for decreased growth, a heavy needle loss caused by *Diprion pini* (Kurkela *et al.* 2005). On sites 2 and 3 no apparent reason for growth retardation could be identified, but frequent *Lophodermium* needle cast epidemics usually lead to foliage losses in Estonian pine stands of younger ages (see **Papers II** and **VII**).

The results are well in accordance with the earlier knowledge about the effect of needle age on the photosynthetic activity (Wang *et al.* 1995; Jach and Ceulemans 2000). In **Paper I** the needle sets older than 3 years had no positive effect on the growth (RI and HI). They were mostly present in the trees with retarded growth rate, apparently due to the losses in the younger needle sets.

6.2. Loss of needles from the productive foliage and its impact on the tree growth

Scots pine has various numbers of needle sets attached, which seems to be regulated by the local site conditions (Reich *et al.* 1996) and by climatic factors (Jalkanen *et al.* 1995; Xiao 2003; Insinna *et al.* 2007a). In **Paper I** it was shown that only the second and third-year needle sets seem to be most important for the current-year growth, and that (*i.e.* the productive needles) is the most important part of the foliage of Scots pine for further studies of the effect of needle loss on the tree growth.

Normal senescence of the needles of Scots pine in Estonia occurs in September (Sepp 1959; Hanso 1970). The fall of senescent needles in the third autumn (NL_3) was not included in the loss of productive needles. The loss of needles from ANN_4 seems not to be connected with the growth losses.

The *Lophodermium* needle cast affects both height and radial increment (Wühlisch and Stephan 1986) and the fungus has acted as the main pathological problem for young Scots pines in Estonia (see **Papers II and VII**). During the summer *L. seditiosum* infects needles (see **Paper VII**) and the diseased needle fascicles shed during the next spring, often before the beginning of the new growth (Hanso 1970; Diwani and Millar 1987). In every case a great part of the most actively assimilating foliage is lost, and at the time of shedding of the affected needles they drop together with the stored nutrients.

Another frequent stressing factor in Estonia – summer drought – mostly reaches the critical level during the later half of the season (Lagergren and Lindroth 2002). In the current season, therefore, it may affect only the radial growth (Pichler and Oberhuber 2007). Unfortunately, in the NTM material the early season needle loss caused by fungal diseases can not be separated from that caused by summer drought in late season.

Regression between the needle loss and the growth characteristics gave different values depending on the categorisation of the needle loss percentages. Six categories with 10% intervals were chosen to avoid the effect of the few deviations with high needle loss values. It was supposed that needle loss less than 10% does not cause any growth reduction (Jalkanen 1998) and the growth reduction would be steepest when the nee-

dle loss is about 50%. Apparently at least some of the needles lost due to summer drought could have been productive in the beginning of the season, and the use of the stored material from the previous season might have some compensating effect for the lost assimilation capacity. Therefore 50% growth reduction was not reached by the 50% needle loss.

During study period 1997–2004, three years of high needle loss $NL_{(1-2)}$ caused statistically significant annual radial and height growth reductions, but the characteristics of the reactions differed: radial growth was affected more than the height growth. The regression analysis showed highest growth reduction from combined needle loss $NL_{(1-2)}$, which revealed that needle loss at the level of 50% decreased RI as a mean by 39.2% (Figure 10) and HI by 34.8% (Figure 11).

Supposing that the current-year needles mature by the end of June or by beginning of July (Pihelgas 1973) and are productive only in the latter half of the current season (see Clark 1961; Loach and Little 1973), the number of current-year needles was weighted with various coefficients in determining the total needle loss. Inclusion of the current-year needles into the NL-formula as a component decreased the explanation value of needle loss in the seasons of heavy needle loss. It is not surprising, since only about ten percent of the annual radial growth is occurring after the maturation of current-year needles in the middle of July (Leikola 1969; Ericsson *et al.* 1980; Renninger *et al.* 2006; Insinna *et al.* 2007b). In several earlier studies the needle loss from different age classes was weighted equally when analysing the response of the growth of trees to the needle loss (Piene 1989). It might yield results that are incomparable among different studies, because the number of needles in different needle sets is very different.

L. seditiosum was considered to be the main reason for needle cast in 2001 (belonging also to our sample of epidemic years) and the influence of the summer drought was very apparent in the foliage in 2002 (Drenkhan and Hanso 2004, 2006). This shedding from the second needle set (appearing in 1.3-year-old needles) was interpreted as premature, because most of the senescent needles in the sample trees shed at the end of their third growing season (*i.e.*, older than 2.3 years).

The results, presented in **Paper IV**, were not compared with results found in the literature concerning the effects of artificial or herbivory defolia-

tion, since these types of needle removal are accompanied by additional energy-consuming traumatic effects (resin flow etc.).

6.3. Epidemiology of *Lophodermium* needle cast and the retrospective needle dataset

During heavy epidemics, *L. seditiosum* may almost equally infect needles of all age classes (Kurkela 1979). The intensity of infection often increases with the age of the needles (Kowalski 1982). However, the fungus kills needles in all needle sets, and among the lost needles are the most productive needle sets (ANN₂ and ANN₃). The fungus is thus the most significant reason for tree growth loss (see **Papers I and IV**). In addition, *Lophodermium* needle cast should play a certain role in the natural selection of surviving and failing individual trees during the early stages of stand formation (see **Paper II**). Fortunately, there are some or even several intermediate years between the epidemics of *Lophodermium* needle cast.

6.3.1. Recurring of *Lophodermium* needle cast epidemics

From this point of view the question is, are contemporary epidemics occurring more rarely or, on the contrary, more frequently than those in the past century? The results of appropriate computations are represented in Table 2. The mean long-term frequency of epidemics per decade in forest nurseries was 4.6, in forest plantations nearly half of that number.

Lophodermium needle cast epidemics occurred more frequently during the first half of the 20th century, compared to the second half of the 20th century (together with the first decade of the 21st century). The larger and stronger needle losses could be registered in pines at the age of 3–24 years, which is the vulnerable host age for the *Lophodermium* needle cast (see Figure 13).

6.3.2. Seasonal development of *Lophodermium* needle cast

It is known that *L. seditiosum* is pathogenic to several species of pines (Diwani and Millar 1987) and the sporulation period of the fungus falls in the late summer and autumn (Diwani and Millar 1990). As occurs elsewhere, in Estonia the disseminating ascospores of *Lophodermium*

needle casts agent infect green needles by germination and direct penetration of hyphae to the current season's needles. During the late autumn to early spring, visible spots appear on infected needles. By the spring of the epidemic year, the infected needles are entirely brown or even red-brown and premature loss follows from late spring to early summer (Hanso 1963; Minter 1981). Fruitbody formation follows and the subsequent sporulation: the fungus needs considerable moisture during the second half of summer for this. However, it became evident (see **Paper VII**) that high precipitation already in May and June supports *L. seditiosum* epidemics, obviously lengthening the infection period by providing the necessary additional level of humidity for the pathogen. For example, in southern Sweden ascocarps of *L. seditiosum* developed from June to November and the spore dispersal depended on weather conditions during these months (Stenström and Arvidsson 2001).

6.3.3. Forecasting of *Lophodermium* needle cast epidemics

The development of *L. seditiosum* is very irregular and depends on environmental, particularly climatic, factors (Martinsson 1979). As previously known, wet and warm autumns or already ends of summers may provoke new *Lophodermium* needle cast epidemics (Viirik 1931), as do mild winters (Lepik 1930; Stenström and Arvidsson 2001). In Estonia it became evident that the moisture values of the pre-epidemic year months May, June, July and August are important for the *Lophodermium* needle cast epidemics. It shows that neither the mean precipitation sums of autumn months (September, October and November), nor the mean temperatures of summer (except of August) and winter months in Estonia affect the start of *Lophodermium* needle cast epidemics in Estonia.

GIV-analyses similarly revealed the significance of the pre-epidemic-year months June, July and August, but not May, as did the precipitation analyses. The influences of pre-epidemic-year September, October and November were also insignificant. The long-term (1887–2006) mean temperature of November in Estonia is much lower than +5 °C. Consequently, GIVs can be also used for predicting *Lophodermium* needle cast epidemics in Estonia. In Estonia GIVs do not predict so well the epidemics of *Gremmeniella abietina*, as was demonstrated in Denmark (Thomsen 2009).

The GIV analyses pointed out 3 more possible epidemics. The anal-

yses, based on precipitation, pointed especially towards June and July ($p < 0.05$, see Figure 12) and less strongly ($p < 0.1$) May and August, but GIV evaluated with equal strength ($p < 0.05$) the role of meteorological conditions in June, July and August. Both analyses demonstrated that no correlations existed with the appropriate conditions in the summers of the years pre-previous to the epidemic years.

This work was also intended to help practical forestry with the new knowledge. For modelling of the dependence of *Lophodermium* needle cast dissemination and, consequently, the seasonal timing of the control measures, the cumulative precipitation sums and GIV values of the 12 pre-epidemic years, belonging to the sample of *Lophodermium* needle cast epidemics, were calculated and compared to the appropriate long-term means. The question was posed: which definite amount of summer precipitation (or GIV) might provoke the start of a new *Lophodermium* needle cast epidemic?

Summing up the meteorological prerequisites of the epidemic years: if the monthly precipitation sums of the months from May to August (incl.) total 300–360 mm and more, or GIV 5.0–6.4, an epidemic of *Lophodermium* needle cast will follow next year, but only in forest nurseries. If the monthly precipitation sums reach 360 mm, or GIV 6.4, the epidemic may attack pines in plantations as well as in forest nurseries. The seasonally earliest prognoses: if the cumulative precipitation sum during the calendar months May, June and July reaches 250 mm, a serious threat of a new epidemic will be expected the next year.

The preventive (prophylactic) treatment with fungicides can definitely be effective, if timed based on the variation of summer precipitation and GIV values. For instance, based on the results of the recent research, in 2008 (precipitation sum from May to August incl. 394 mm) a strong *Lophodermium* needle cast epidemic was predicted for the next year (Hanso and Drenkhan 2008b) and in the spring of 2009 this prognosis turned out to be correct.

6.3.4. Highest vulnerable age limit of pine toward *Lophodermium* needle cast

It has been known for a long time that *Lophodermium* needle cast infects and damages young pines, but the definite highest affected host age limit

has been less specified. The host age limit in literature has varied largely, with values such as 1–4 years in Germany (Neger 1919), 10–12 years in Estonia (Hanso 1965, 1970), 23 years in Finland (Kujala 1950), 25 years in western Norway (Hagem 1928) and even over 30 years in Poland (Kowalski 1990). According to the results which were obtained by the use of different proxies (see **Paper VII**), including retrospective NTM data of the sample of 12 epidemic years from a long-term (1887–2006) and the data of growth losses, the hosts' age of 22–24 years was found to be the highest vulnerable age limit of Scots pines for *Lophodermium* needle cast in Estonia.

6.3.5. Impact of *Lophodermium* needle cast and of herbivorous insect pests to tree growth

Tree growth is the indicator of tree vitality (Dobbertin 2005). Individual susceptibility of pines to *Lophodermium* needle cast is quite variable (Schütt 1957; Martinsson 1979). Comparison of the changes in the growth rate of affected trees, attributed to the epidemics of *Lophodermium* needle cast, or to the calamities of insect defoliators, demonstrated comparatively similar, significant reduction in both radial and height increments of trees at the younger A-group (3–11 years), as at the epidemic or calamity years, as well as at the next and at the second year after the epidemics (Table 3 and 4). Both characteristics demonstrated stronger decreases in height, rather than in radial growth. As expected, losses in productive needle sets of the epidemic year and of two years after the epidemics at the beginning of seasonal tree growth had to be accompanied by the growth rate decrease (**Papers I and IV**), regardless of the agent, e.g., *Lophodermium* needle cast (Hanso 1963; Minter 1981), or diprinoïd insect defoliator (Larsson and Tenow 1984; Cedervind 2003). Among diprinoïd insects *Neodiprion sertifer* is an early-season defoliator, which leaves intact only the current-year needles of pines (Cedervind 2003), whereas *Bupalus piniaria* is a late-season defoliator, influencing the growth rate of trees with one- or two-year lag (Armour et al. 2003). *N. sertifer* is prevailing in the list of calamities of diprinoïd insect defoliators in Estonia.

Compared to the long-term means, both growth rates (RI and HI) in the older, B-group of pines (20–33 years) had decreased during the known calamity years of herbivorous insect pests, but not during the epidemic years.

6.4. New foliar diseases of pines and their spreading in Estonia

It could be possible that the very first arrival of *D. septosporum* occurred in Estonia even before 2006, but was not registered at the time for lack of a significant outbreak. On the other hand, two different Estonian *D. septosporum* populations showed high genetic diversity (Drenkhan *et al.*, unpublished data). The high diversity suggests an occurrence of sexual proliferation, recent introduction as a massive inoculum or a long presence of *D. septosporum* in these areas (Drenkhan *et al.*, unpublished data). Sexual proliferation of *D. septosporum* could be possible, but no evidence of occurrence of the perfect stage of the fungus was found. Before the first detection in 2006, *D. septosporum* might have existed for some time as an endophyte, but it is known that the needle pathogens *M. pini*, and *M. dearnessii* have not been detected in central Europe as endophytes, although disease symptoms are occasionally observed (Holdenrieder and Sieber 1995; Sieber 2007).

During three years of investigations in Estonia the most abundant formation of conidia of *D. septosporum* was registered in an unusual season for this species – from late October to mid-December and even during the following wintertime. Does it mean that the fungus has recently arrived and still not adapted to the natural conditions of Estonia? Usually the acervuli sporulate and the infection occurs from late spring to late summer (in the Southern Hemisphere, cf. Gilmour 1981), from April to October (in south-central Europe, cf. Kirisits and Cech 2007), or from May to September (in East Anglia, Britain, cf. Archibald and Brown 2007).

In Europe the species *M. pini*, *M. dearnessii* and *D. pinea* have been recorded most often on *P. nigra*, less often on *P. mugo* and *P. sylvestris* (Holdenrieder and Sieber 1995; Jankovský and Palovčíková 2003; Luchi *et al.* 2005; Flowers *et al.* 2006; Hänisch *et al.* 2006; Steinfath 2006; Blodgett *et al.* 2007; Jurč 2007; Thoirain *et al.* 2007; Whitehill *et al.* 2007; Anonymous 2008). *P. nigra* has been a preferable host species as well for some other dangerous pine disease agents, like *Gremmeniella abietina* (Thomsen 2009). Fortunately, *P. nigra* has not been planted much in Estonia and the existing trees are scattered, growing mostly singly or in pairs, except for an old stand in Pädaste, Saaremaa, and some trees in Järvelja, south-east Estonia (Laas 2004). *P. mugo* has been more popular than *P. nigra*, creating verdant areas in towns and greenbelts besides the

roads, but is less affected by these new alien pathogens. Transfer of these pathogens from exotic pine species to *P. sylvestris*, the single native pine and one of the main commercial tree species in Estonia, is worth special attention and care. In contrast to the other three alien species, introduction of *C. minus* seems to have occurred directly from *P. sylvestris* to *P. sylvestris* and, currently, from *P. sylvestris* to *P. mugo* as it was detected in 2009.

In the Southern Hemisphere the spread of *D. septosporum* has been fast, especially on exotic pine species (Bradshaw 2004). In 2002 *D. septosporum* was detected for the first time for the Baltic countries in Lithuania on *P. mugo* (Jovaišienė and Pavilionis 2005), which was the only record of this fungus there for several years afterward, with no records from *P. sylvestris*. *D. septosporum* was found widely spread in Lithuania again only at the end of September 2008, this time already on Scots pine (Markovskaja and Treigienė 2009).

This may explain the peculiarity of dissemination of *M. pini*: it first infects the exotic pine species and after some years (and generations of the pathogen?) native Scots pine will be colonised. The spread has obviously extended from south to north. Since 2008 *M. pini* has been detected all over Estonia (see **Paper VI**). In the same year the fungus was recorded for the first time in south-central Finland (Müller *et al.* 2009), and the next year already in northern Finland (Vuorinen 2009) and in central Norway (Halvor Solheim, personal communication).

Why does the dissemination of pathogenic fungi need attention? *M. dearnessii* has been found for the first time on exotic *P. ponderosa*, and *D. pinea* on exotic *P. nigra*. Additionally, *M. dearnessii* as a serious quarantine pathogen (Anonymous 2008) has been found in some European countries soon after detection of *M. pini* (Pehl 1995; Brandstetter and Cech 2003; Jankovský *et al.* 2009b). The monitoring of alien fungi is essential 1. for early detection of new invasive (incl. quarantine) fungi, which is needful valuable information for institutions of local plant protection, 2. for establishment of hosts' circle, 3. how the fungi affect hosts in our environment, 4. when the fungus will sporulate. The last item is the basis for prophylactic work in forest management.

6.5. Invasive versus native foliar diseases of pines and evaluation of their possible risk in the natural conditions of Estonia

6.5.1. Droughts and invasions

Concerning *D. pinea* as a new species in Estonia: latent infections of *D. pinea* in North America had turned pathogenic with severe symptoms namely after droughts (Stanosz *et al.* 2001). During the last decade in Europe this pathogen has spread northward also mainly during drought periods. Serious drought in 2003 supported a *D. pinea* epidemic in central Europe (Jankovský and Palovčíkova 2003; Hännisch *et al.* 2006; Steinfath 2006; Blaschke and Cech 2007). Spread of the fungus might occur by insect vectors (Whitehill *et al.* 2007) or human activities (Burgess *et al.* 2004). Droughts in 2002 and 2006 (Drenkhan and Hanso 2004, 2006; Hanso and Drenkhan 2007a) obviously supported the invasion and establishment of *D. pinea* after incidental introduction in Estonia, probably by pine seeds or planting material through a forest nursery. In fact *D. pinea* was detected first on cone scales *P. nigra*, growing close to the nursery in Järvelja (see **Paper V**).

Currently, after the discovery of these new diseases, the climate of the Baltic countries and of large areas in Fennoscandia has been evaluated as suitable for *Dothistroma* needle blight (Watt *et al.* 2009). Scots pine, the single native pine species of this region, is known to be susceptible to this disease also in central Europe (Gilmour 1967; Lang and Karadžić 1987).

6.5.2. Epidemic versus chronic diseases

Dothistroma needle blight, although it is not especially emphasised in the literature, can behave as a chronic disease (Woods 2003; Woods *et al.* 2005; Peterson 2006). Its outbreaks have been estimated to proceed for a decade (Lewis and Welsh 2005). According to the first observations, *D. septosporum* may have chronic character in Estonia, or give epidemics from several to many years long. Chronic (extended-period epidemic) *D. septosporum* or other new invasive fungi, although less virulent, can deprive the host, year by year, of all needles older than the current year's production and therefore be even more dangerous to the host trees than the native virulent, but epidemic, disease (see **Papers II, IV and VII**). The main reason is that they permanently deprive the productive needles

of host (see chapters 5.2 and 5.4), and long-term stressed trees finally die out after attack of a secondary pest.

In contrast, the most common needle disease of pines during the 20th century in Estonia, *Lophodermium* needle cast, has clearly been shown to be an epidemic disease (Hanso 1963; Hanso and Hanso 2003; Hanso and Drenkhan 2007b). In 2009, the last epidemic of *L. seditiosum* coincided in south and central Estonia with the serious spread of *D. septosporum* in several young *P. sylvestris* plantations. During this epidemic, *D. septosporum* was found to affect only second-year and older pine needles, while *L. seditiosum* affected also the youngest, current-year needles. This difference could demonstrate higher virulence in *L. seditiosum* rather than in *D. septosporum*. High virulence of *L. seditiosum* is, however, mitigated by few to several intermediate years of rest time between consecutive epidemics, during which the trees can renew their foliage, and produce and relocate resources to the new growth or stores.

It is known (Lyytikäinen-Saarenmaa 1999) that the consequences of defoliation stress depend on defoliation type, intensity and timing, and the source-sink position of damaged needles, which determines whether defoliation predisposes pines to further damage or not. As demonstrated before, since the current-year needles mature after the shoot elongation, they apparently cannot support the current-year height increment, although during the latter half of the growing season they can support only the radial increment (Clark 1961; Ericsson *et al.* 1980). In this work it was also proved by the NTM that the second and third-year needles serve as the main current-year tree growth supporting needle sets (or as productive foliage, see **Papers I** and **IV**). Repeated privation, year by year, of older needles and, therefore, stored nutrients by a new, chronic alien foliage disease may stress the host trees even more than a single, severe loss of needles by an epidemic disease.

Additionally, it is unknown whether *L. acicola* and *D. septosporum*, both quarantine species, behave similarly. Also, *D. pinea* needs special attention, for it is unknown how fast the fungus can change the host organs as substrate. How *C. minus* will behave in the conditions of Estonia is also unknown.

6.6. Future prospects

1. One essential task is to extend the retrospective needle dataset of Scots pine constructed in this work by NTM from both ends as long as possible, but also to complete the existing dataset of trees in the calendar years that are still represented by small numbers of sample trees (annual shoots).
2. Another essential task is to follow by molecular methods the possible changes in virulence of *L. seditiosum* between the different next-in-order epidemics. Can we actually de-emphasise the role of genetics in the triggering of new epidemics, as could be concluded from the investigations in this dissertation? Inclusion of molecular methods for species detection in the early phases of their arrival, *i.e.*, already during at their latent periods, may be needed.
3. It is still unknown the largest territory size that can be occupied by an epidemic of *Lophodermium* needle cast, and which dimensions demonstrate local versus large epidemics. Is it depending solely of meteorological conditions?
4. Continuous monitoring of the epidemiological behaviour of the new alien invasive disease agents (like *Dothistroma pini*, etc.) in the changing natural environment of Estonia will be needed, and perhaps also in some neighbouring countries where appropriate investigations are not undertaken (such as in Latvia).

7. CONCLUSIONS

This dissertation provides information which unites the basic investigations of the biologically most productive part of the Scots pine foliage, the productive foliage, with some comparisons in epidemiology and the offensive strategies of the old native (*Lophodermium seeditiosum*) and new alien (e.g. *Mycosphaerella pini*, *M. dearnessii et al.*) pathogens of pine foliage. This approach became possible thanks only to the creation of the needle trace method (NTM).

An essential part of this thesis includes the analysis of how to define the productive part of pine foliage, and how to determine what part of the foliage affects the growth of Scots pine. Secondly, the impact of needle loss, originating from the productive foliage, on tree growth was analysed. The retrospective needle datasets of the period 1887–2006 were extracted from our experimental stands by the NTM and used in this analysis for solving both of these problems.

The arrival at the beginning of the 21st century of several new, important alien invasive foliage fungi of *Pinus* species, including two quarantine disease agents, has obviously greatly changed, both ecologically and economically, the causal relations in the foliage pathology of pines, for which during the 20th century *Lophodermium* needle cast was the most significant disease. This work also describes the process of colonisation of Estonia by four new, alien invasive pathogens. New epidemiological peculiarities of the most harmful foliage disease agent of Scots pine in the 20th century – *Lophodermium seeditiosum* – were extracted by the use of NTM from the past, by juxtaposing retrospective needle loss, meteorological and other data series.

The most essential results of the present dissertation:

1. NTM analyses, carried out in the experimental stands of Scots pine of different ages, illustrated clearly that three younger needle sets belong to the productive foliage and affect the growth of pine positively and statistically significantly. Irrespective of tree age, the three younger needles sets (ANN_1 , ANN_2 and ANN_3) support radial increment, but second (ANN_2) and third (ANN_3) needle sets support height increment of trees. It is concluded that the two or three youngest needle sets are important and all older green needles mini-

mally contribute to growth, possibly because of their small quantity. The trees that have attached old needles grew less than those that had already totally lost the oldest needle sets: needles older than 3 years had no positive effect on the growth (radial and height increment). The basis for the separation of productive foliage from non-productive was simple: if the number of remaining needles does not correlate with the growth characteristics of the tree, it means that these needles did not support the growth and were not productive. This work makes suspicious the earlier supposition that all attached living needles on pines support the tree growth (**Papers I, IV**).

2. Needle loss or defoliation has been used as an important measure in forest monitoring assessments. Most oscillations in the needle loss dynamics during 1999–2002, caused mainly by fungal needle cast due to *L. seditiosum* and by summer drought, occurred in the third annual needle set. Decreases in both radial and height increments in relation to the needle loss were apparent in the total material for 1997–2004, but needle loss reduced the annual growth significantly only in three years inside that period. The regression analyses revealed that needle loss from productive foliage at the level of 50% from $NL_{(1-2)}$ (the combined needle loss from ANN_2 and ANN_3) decreased RI as a mean by 39.2% and HI by 34.8%. It means that by the point of 50% needle loss from productive part of foliage, growth reduction by 50% was not reached, obviously due to mitigating effect of nutrients reserved in different tissues (**Paper IV**).
3. During the last years four new invasive pathogenic fungi were detected on *Pinus* species and their dissemination process was followed in Estonia. The under-quarantine pathogen *M. pini* (in its anamorphic stage *D. septosporum*) was diagnosed in the samples of diseased *P. nigra* needles in the autumn 2006. The fungus was widespread in south Estonia on the beginning of 2008 and by the end of the same year all over in Estonia. Additionally, *M. pini* was for the first time documented in northern Latvia in 2008. Thereafter, the second serious quarantine pest *M. dearnessii* (in its anamorphic stage *L. acicola*) was diagnosed in the needle samples of *P. ponderosa* from the Tallinn Botanical Garden in 2008. The third dangerous needle pathogen *D. pinea* (syn. *S. sapinea*) was first detected in 2007, first on scales of several fallen cones of *P. nigra*. In 2008 the fungus was found rapidly spreading in Estonia, but still only on cones.

The fourth alien fungus on Scots pine needles *C. minus* suddenly increased dissemination during 2007 and 2008.

During three years of investigations the most abundant formation of conidia of *D. septosporum* was registered from late October to mid-December and during the following wintertime, which is unusual season for this species. It indicates that the fungus is not adopted the condition of Estonia presently and was spread quite recently to here in wave of climate extremities (**Papers III, V, VI**).

M. pini was found to affect only second-year and older pine needles, while *L. seditiosum* at the epidemic year also killed the youngest, current-year needles. This difference demonstrated higher virulence in *L. seditiosum* rather than in *M. pini* only apparently: the new alien invasive quarantine pathogen *M. pini*, for instance, can deprive the host year by year from the productive foliage, but *L. seditiosum*, between the epidemics, gives to the host rest time for restoring the foliage lost during epidemic and for renewal of normal growth and stores.

4. The retrospective appraisal historically indicated specific meteorological conditions which triggered *Lophodermium* needle cast epidemics in Estonia. A particular amount of precipitation or level of GIV (=mm/°C) from May to August (incl.) was found to correlate with the triggering of epidemics of *Lophodermium* needle cast. High precipitation or GIVs during only one preceding summer correlated with epidemics, which means that only one pathogens' generation (life-cycle) is needed for the emergence of an epidemic. The meteorological prerequisites of the epidemic years: if the monthly precipitation sums of the months from May to August (incl.) make up 300 – 360 mm and more, or GIV is 5.0–6.4 and more, an epidemic of *Lophodermium* needle cast will follow next year in forest nurseries. If the monthly precipitation sums reach 360 mm, or GIV 6.4, the epidemic may attack pines in plantations as well as in forest nurseries. Additionally, the retrospective needle and meteorological data indicated that the mean long-term frequency of epidemics per decade in forest nurseries was 4.6, in forest plantations nearly half of that number. These results clarify the situation when will be high risk for epidemic of *Lophodermium* needle cast and it has to be strong basis for the modelling of the disease epidemics in further (**Papers II, VII**).

5. According to the retrospective needle loss and growth data for the sample of *Lophodermium* needle cast epidemic years, the hosts' vulnerable age limit in Estonia was 22–24 years. Statistically significant growth reduction was established only in the younger age pines (3–11 years) at and after the epidemics of *Lophodermium* needle cast. The growth reduction at and during first two years after the calamity years of herbivorous insect pests of pine (diprinoids) was established in the both compared aged groups of pines, as in 3–11, as well in 20–33 years. Generally, if it could be possible that *Lophodermium* needle cast can infect pines irrespective the host age, the disease can inflict the pines only in the younger age, up to the 22–24 years (**Paper VII**).

In summary: after the arrival of several new invasive pathogenic fungi at the beginning of the 21st century in Estonia, the needle pathology of pines has obviously changed substantially. New pathogens have already demonstrated offensive strategies that differ from the most dangerous pine foliage pathogen at the 20th century, *L. seditiosum*. The difference was demonstrated by defining the most essential part of the Scots pine foliage – the productive foliage, which turned to be possible only by the use of long retrospective data-series, extracted by the NTM. By the use of the same method some essential epidemiological peculiarities of *L. seditiosum* were also extracted – for future comparisons with the new alien colonisers, after their adaptation in Estonia.

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SUMMARY IN ESTONIAN

OKKAJÄLJEMEETODI KASUTAMISEST MÄNNI OKKAHAIGUSTE EPIDEMIOLOOGILISES UURIMISTÖÖS

Dendrokronoloogilise meetodi edasiarendus – okkajälje meetod (edaspidi NTM) avastati ja arendati välja Soome metsateadlaste-patoloogide poolt 1980. aastate lõpust alates. NTM unikaalsus seisneb võimaluses konstrueerida retrospektiivseid pikaajalisi igihaljaste puude okastikku iseloomustavaid andmeridu, mis võivad ulatuda kaugele minevikku, veel enne seda, kui üldse alustati okastikuseisundi hindamist. NTM tekkega sai võimalikuks uurida okaspuude, eelkõige männiliikide okaste pikaajalise dünaamika mustreid ning anda adekvaatseid hinnanguid sellest, milline on olnud näiteks okaste eluiga või püsimine läbi aegade, millist okastiku hulka ja seisundit võib hinnata normilähedaseks, jne. NTM on juba andnud vastuseid küsimusele, kui suur on erinevus okaste elueas või püsivuses tänapäeva tööstuse ajastul sajanditaguse ajaga võrreldes. Dateerimist võimaldavad retrospektiivsed okastikudünaamika hinnangud on kahtlemata olulised metsade suremise ja tervise põhjuslike seoste uurimise seisukohalt. Võra läbipaistvus või selle kasvu põhjustav defoliatsioon on tähtsad hinnangulised tunnused metsamonitooringus. NTM omab perspektiivi ka kui abivahend seni vaid visuaalselt teostatava metsamonitooringu märksa täppisteaduslikumal tasemel teostamiseks või kontrollimiseks. NTM on heaks kiidetud ka Euroopa Liidu tasandil ning arvatud üheks oluliseks abivahendiks metsanduslikes ja ökoloogilistes uuringutes ning keskkonnaseisundi iseloomustamisel.

Üldteada on, et okaste eluiga pikeneb geograafiliste laiuskraadide ja mägedes kõrguse tõustes, samuti puu vananedes. Lisaks männiliikide okaste n-ö normaalsele, vanusest tingitud varisemisele võivad erivanuselistes okkaklassides põhjustada varisemist ka mitmed seenpatogeenid, nagu ilmselt meil sajandeid eksisteerinud *Lophodermium seditiosum*, kuid alates 21. sajandist ka *Mycosphaerella pini*, *M. dearnessii*, *Diplodia pinea* ja *Cyclaneusma minus*, mõned putukkahjurid-diprinooidid ja abiootilised tegurid (suvine põud, erinevate sesoonide külmad jt).

Seni on puu kasvuga seostatud kogu rohelist okastikku ja sedagi, et okkad nende vanusele vaatamata seonduvad otse ja positiivselt puu jooksva aasta kasvuga, seega – rohkem okkaid, kiirem kasv. Nimetatud küsimust ei ole seni siiski piisavalt ja selgelt käsitletud, teada oli, et noo-

rem okastik assimileerib intensiivsemalt kui vanem, luues ja suunates orgaanilist ainet sedavõrd rohkem ka puu kasvamisse. Siiski ei leidunud kirjandusest täpsustust, milline osa hariliku männi mitmeaastasest okastikust tegelikult puu kasvu otseselt toetab. Seega polnud ka selge, neist milliste okaste kadu vähendab enam puu kasvu (ning mõjutab ka tema vitaalsust). NTM andmereal, kõrvutades neid teiste (näiteks ilmastiku) andmeridadega, annavad esmakordselt võimaluse retrospektiivselt välja selgitada meie oluliste, 20. sajandil esinenud okkahaiguste epideemiaaastad, hinnata nende sagedust nimetatud sajandi erinevatel kümnenditel, nende mõju puude kasvule ning prognoosida epideemiade esinemist lähiaastatel. Samuti võimaldab NTM määratleda uute, äsja ilmselt drastilisemate kliimamuutuste taustal saabunud haiguste ründe eripärasid meie kodumaisele harilikule männile. Käesoleva teadustöö üks eesmärke ongi suunatud praktilisse metsamajandusse, et võimaldada seal teha teaduslikult põhjendatud metsakaitselisi otsuseid näiteks männi-pudetõve (haigustekitaja *L. seditiosum*) puhangute modelleerimiseks.

Kuna Eesti kliima muutub mitmete prognooside kohaselt niiskemaks, mõjub see kahtlemata provokatiivselt seenhaigustele, suurendades ka metsapatoloogiliste uuringute intensiivistamise vajadust lähiaegadel.

Eelnevast lähtudes seati käesoleva dissertatsiooni koostamisel üles järgmised hüpoteesid.

1. Ainult osa mändide elus- (rohelist) okastikust avaldab olulist mõju mändide kasvule ning okaste kadu vaid sellest okastikust tuleb arvesse võtta defoliatsioon *versus* puu kasv uuringutes.
2. Männi seniste okkahaiguste tekitajate hulgast tuntud ehk 20. sajandil enim esinenuid (*L. seditiosum*) ning vähem tuntuid ehk uusi ehk 21. sajandil meile saabunud invasiivseid (näiteks *Mycosphaerella pini*) patogeene iseloomustavad erinevad peremeestaime füsioloogiat ja ökoloogiat mõjutavad ründestrategiead.

Lähtudes dissertatsiooni hüpoteesidest olid töö eesmärgid järgmised.

1. Selgitada, kas seoste abil hariliku männi (*Pinus sylvestris* L.) okastiku- ja juurdekasvu tunnuste vahel on võimalik määratleda nn produktiivset okastikku, leides selleks, millises vanuses okkad mõjutavad puude radiaal- ja kõrguskasvu ning millised mitte (**Artikkel I**).
2. Uurida noorte mändide okastiku dünaamikat, eriti aga nüüd juba

- üksnes produktiivsest okastikust pärineva okkacao kvantitatiivset efekti puude radiaal- ja kõrguskasvule (**Artikkel IV**).
3. Antud uurimistöö käigus, äsjaste ilmamuutuste taustal avastati esmakordselt Eestis neli uut, invasiivset männiokaste haigust, nende hulgas kaks karantiinset, neist üks ka Põhja-Lätis. Eesmärgiks oli tulnukliikide seire alusel uurida Eesti koloniseerimise käiku nende poolt ning produktiivse okastiku mõiste abil hinnata nende võimaliku riski mändidele meie metsades (**Artikkel III, V ja VI**).
 4. 20. sajandil hariliku männi kõige ohtlikuma okkahaiguse, männi-pudetõve epideemiade retrospektiivne väljaselgitamine (**Artikkel II ja VII**).
 5. Kasutades multiproksiaalset lähenemist (teiste andmeridade hulgas ka NTM andmereal), hinnata mõningaid jätkuvalt puudulikult analüüsitud männi-pudetõvega seonduvaid metsapatoloogilisi isearasusi, nagu männi vastuvõtlik eluiga selle haiguse suhtes, männi-pudetõve ja okkatoiduliste putukate kahjustuse võrdlev mõju männi juurdekasvu-tunnustele, jms (**Artikkel VII**).

Töös analüüsiti NTM protokollide kohaselt mudelpuuliigi hariliku männi 123 isendit. Kokku saeti välja ca 2550 proovipakku ehk aastavõrset, neist omakorda raiuti välja sama arv proovikehi, millistest analüüsiti üksikhaaval ca 25 000 aastarõngast, lugedes igalt neist kõik seal nähtavad okkajäljed.

Aastarõngaste kaupa dokumenteeritud okkajälgede hulgas jagati erinevatesse okka-vanusklassidesse, kus ANN_1 tähistab jooksva aasta, ANN_2 eelmise ja ANN_3 üle-eelmise aasta okaste arvu jne. Erinevate okkaklasside mõju arvutamiseks puude radiaal- ja kõrguskasvule kasutati kovariatsioonanalüüsi ANCOVA meetodil.

Puude radiaalkasvu saavad mõjutada lisaks teatud aastakäikude vanematele okastele ka jooksva aasta okkad, kuid seda vaid kasvusesooni teisel poolel. Kõrguskasvu ei saa jooksva aasta okkad mõjutada, kuna nad küpsevad alles sama aastavõrse kõrguskasvu lõppemise järel.

Okkahaiguste kahjulik toime väljendub arusaadavalt puule olulise assimileeriva pinna kaotuses – okkakaos. Lähtudes erinevate okka-vanusklasside positiivse mõju alusel juurdekasvule määratletud produktiivse okastiku mõistest, analüüsiti ka erinevatest okkaklassidest toimuva okkacao mõju puude juurdekasvule. Selleks analüüsiti erinevate okkaklasside

vahelist okkakadu (NL_1 ja NL_2), seejärel ka kombineeritud okkakadu $NL_{(1-2)}$. Statistiliselt oluliselt reageeris puu oma kasvutunnustega just kombineeritud okkakaole $NL_{(1-2)}$. Vastavalt sellele kategoriseeriti edasisel analüüsil okaste kadu 10%-se intervalliga $CNL_{(1-2)}$. Statistilised analüüsid tehti programmiga SYSTAT (2000).

Männi-pudetõve epideemiade retrospektiivsel analüüsil kasutati kogu puust (s.o erinevatest okka-vanusklassidest) lähtuvat okkakadu, kuna männi-pudetõbi kahjustab igas vanuses okkaid. Analüüsiti okkakao mõju epideemia-aasta (0), sellele järgneva (1) ja ülejäämise (2) aasta juurdekasvule. Okkakao ja juurdekasvu seoseid analüüsiti, võrreldes aastate 1887–2006 (edaspidi: pika perioodi) kohta koostatud NTM andmeridade okkakadusid kalendriaastate kuude sademete summade ja keskmiste temperatuuridega Tartu-Tõravere ilmajaamast. Sama pika perioodi kohta arvutati ka männi-pudetõve suhtes kriitiliste kuude (maist oktoobrini k.a) GIV väärtused. GIV on sisuliselt kuu sademete summa (mm-tes) ja sama kuu keskmise temperatuuri ($^{\circ}\text{C}$ -des) suhe.

Invasiivsete okkahaiguste avastamiseks ja Eesti koloniseerimisprotsessi dünaamika jälgimiseks uute haiguste poolt kontrolliti nende kasvukoh-tadel süstemaatiliselt eelkõige provokatiivseid substraate – eksootmände (*Pinus nigra* ja *P. mugo* jmt), kuid jälgiti mitme aasta jooksul ka uute haiguste arengut juhuslikult valitud hariliku männi puistutes üle Eesti. Igast seirepunktist koguti sümptomaatilisi okkaid, milliseid hiljem laborooriumis kontrolliti mikroskopeerimise teel, küllalt sageli kasutades täppisdiagnoosiks ka puhaskultuuride meetodit.

Levikukaardid Eesti asustamise dünaamika kirjeldamiseks koostati programmiga MapInfo Professional 7.5.

Olulisemad tulemused, vastavalt püstitatud hüpoteesidele ja eesmärki-dele on järgmised.

1. NTM abil analüüsiti olulist produktsiooni-bioloogilist küsimust: okaste eluea mõju hariliku männi juurdekasvule. Selgus, et noorema-test okkaklassidest kaks, eelkõige ANN_2 (s.o eelmisel) ja ANN_3 (üle-eelmisel aastal tekkinud okkad) „toidavad” männi juurdekasvu enim. Noorimasse, ANN_1 (jooksva aasta okkad) okka-vanusklassi kuulu-vad okkad mõjutavad aga vaid puude radiaalkasvu ja sedagi üksnes jooksva kasvuaasta teisel poolel. Olenemata puude elueast vanemad

okkad (okkaklassidest ANN₄ ja vanemad) ei toetanud puude radiaal- ja kõrguskasvu üheski uuritud puistust, isegi korreleerusid puu kasvuga negatiivselt. Kokkuvõtvalt: kaks või kolm noorem okkaklassi on olulised puu kasvule ning kõik vanemad seonduvad minimaalselt kasvuga, üheks põhjuseks nende oluliselt väiksem hulk. Alus produktiivse ja mitte-produktiivse okastiku eristamiseks: kui puul säilivad okkad seonduvad puu kasvuga, on need produktiivsed ja kui ei seonu puu kasvuga, siis mitte-produktiivsed. Töö tulemused seavad kahtluse alla varasemad teadmised, nagu toetaksid kõik puul olevad rohelised okkad puu kasvu (**Artikkel I** ja **IV**).

2. Teine oluline metsa produktiooni-bioloogiline küsimus on okaste varisemine (okkakadu, defoliatsioon). Seda tunnust kasutatakse laialdaselt kui üht olulist metsade tervise hindamise kriteeriumi. Senistes arvukates defoliatsiooni-uuringutes pole reeglina tehtud aga vahet puult varisevate okaste tähenduse üle puu juurdekasvu „toitmisel”, s.t kas varisevad okkad olid produktiivsed või mitte. Antud uurimistöö originaalsed tulemused, kus esmalt määratleti produktiivne okastik ning seejärel just sealt pärineva okkakao seosed puude juurdekasvuga kinnitasid, et männi radiaal- ja kõrguskasvu reageerimine produktiivsete okaste kaole sõltub ka okaste varisemise aastaajast (**Artikkel I**). Esmakordselt õnnestus retrospektiivselt hinnata üksnes produktiivsest okastikust pärineva okkakao mõju noorte mändide kasvule mitme üksteisele järgneva aasta jooksul. Regressioonanalüüsi tulemused näitasid, et 50%-line okkakadu produktiivsest okastikust vähendas puude radiaalkasvu 39.2% ja kõrguskasvu 34.8% võrra, mis näitab, et 50% okkakadu ei tähenda sama suurt juurdekasvu vähenemist (**Artikkel IV**).
3. Käesoleva sajandi esimesel kümnendil oleme Eestis kasvavatel mändidel avastanud neli uut invasiivset seenpatogeeni: *Mycosphaerella pini*, *M. dearnessii*, *Diplodia pinea* ja *Cyclaneusma minus*. Neist kaks esimest, karantiinset haigustekitajat, vaid anamorfsetes arengujärkudes (vastavalt *Dothistroma septosporum* ja *Lecanosticta acicola*). Kõik need liigid on mitmetes maades ja mandritel tuntud kui ohtlike okkahauguste tekitajad. Sealhulgas: esmakordselt nii Eesti kui ka Balti- ja Põhjamaade jaoks tervikuna avastati 2007. aastal harilikult männilt ohtlik punavöötaud (haigustekitaja *M. pini*), mustalt männilt (*P. nigra*) registreerisime nimetatud seent juba 2006. aastal (**Artikkel III**). Esmakordselt Eestis ning Balti- ja Põhjamaades ter-

vikuna avastati ohtlik universaalne männiliste seenhaiguse tekitaja *D. pinea* (sünonüüm *Sphaeropsis sapinea*), esmalt küll vaid musta männi käbidelt 2007. aastal. *D. pinea* ohtlikkus seisneb tema universaalsuses: nimetatud seen kahjustab nii mändide okkaid, võrseid, oksi, käbisid, juuri ning tekitab isegi puidusinetust (**Artikkel V**). Rohkem on see haigus maailmas tuntud siiski kui okkahaigus. Sooritatud molekulaarsete uuringute tulemusena deponeeriti ülemaailmses geenipangas mõlemate haigustekitajate, *M. pini* ja *D. pinea* Eesti päritoluga tüvede ITS nukleotiidide järjestused ning võrreldi neid mujalt maailmast pärinevatega.

Perioodil 2007–2008 registreeritud pooleaastaste vahe-etappide järel oli punavöötaud mitmel männiliigil 2008. aastaks levinud juba üle kogu Eesti, valgevöötaud aga juhuslikult ning enamasti vaid harilikul männil. *D. pinea* esines selle ajani ainult musta männi käbidel. On oodata aga, nagu see mujal maailmas juhtunud, uute männikudede (*incl.* okkakudede) hõivamist tema poolt ning esimesi arvestatavaid kahjusid. Avastatud karantiinne pruunvöötaud Põhja-Eestis on seni aga ainus nimetatud seene dokumenteeritud esinemise koht terves Põhja-Euroopas (**Artikkel VI**).

Punavöötaudi uuringutel avastati, et see liik on Eestis võimeline sporuleerima ja levima tema jaoks väga ebatavalisel sesoonil – pehmetel talvedel, mis võib tähendada, et see patogeen ei ole veel kohastunud meie kliimaga. Lisaks leiti, et punavöötaud Eestis surmab peamiselt teise ja kolmanda aasta okkaid, mitte aga jooksva aasta okkaid. Produktsiooni-bioloogiliste uuringute (**Artikkel I ja IV**) tulemustel põhinedes püstitati esmakordselt maailmas hüpotees, mis seletab meile invasiivsete tulnukatena saabunud patogeenide suuremat ohtlikkust mändidele: erinevalt epideemilisest männi-pudetõvest (**Artikkel VII**) on nimetatud haigused kroonilise iseloomuga. Kui pudetõve epideemiade vahelistel aastatel suudab mänd taas luua normaalse juurdekasvu ning „toita” ka varuainete hulka, siis uutel kroonilistel haigustel selliseid kahjustuse vaheaastaid ei esine. Aasta-aastalt hävitades juurdekasvu põhilise toiteallika – produktiivse okastiku, s.o 2. ja 3. aastakäigu okkad, võivad uued haigused viia männi kiratsemise ning surmani kindlamini kui seda suudab teha näiliselt virulentsem (ka viimase aasta, s.o noorimaid okkaid surmav) männi-pudetõbi. Krooniliselt nõrgestatud puid nakatavad seejärel ja viivad surmani aga hoopis teised patogeenid või kahjurid.

4. Dokumenteeritud männi-pudetõve tekitaja (*L. seditiosum*) epideemiade ja ilmastikuandmete kõrvutamisel leiti esmalt epideemiaid vallandanud ilmastikutingimuste kriteeriumid; seejärel, okkajälje meetodil saadud retrospektiivsete okkakadude ja möödaniku ilmastikuandmete baasil saadud kriteeriume aluseks võttes, rekonstrueeriti tõenäolised männi-pudetõve epideemia-aastad enam kui terve 20. sajandi kohta.

Taanis kasutusele võetud, sademeid ja temperatuure ühendava GIV (mm/°C) väärtusi kasutati seal okaspuu võrsevahi (*Gremmeniella abietina*) epideemiade retrospektiivsel analüüsil. Taanist erinevalt sobis GIV Eestis pigem männi-pudetõve epideemiade vallandumise määratlemiseks. Leiti, et nii GIV abil kui ka üksnes sademete hulga põhjal on võimalik prognoosida männi-pudetõve puhanguid. Männi-pudetõve dokumenteeritud epideemia-aastate hulgast analüüsiks valitud 12 aasta ilmastikuandmeid analüüsides selgus, et kriitiliseks teguriks epideemia tekkel osutusid sademed perioodil eelmise aasta maist kuni augustini (kaasa arvatud): kui sademete summa augusti lõpuks oli saavutanud 300 mm või enam või GIV ulatunud väärtuseni 5.0 või enam, siis järgmisel aastal järgnes sellele männi-pudetõve epideemia metsataimlates. Kui suvekuude sademete summa oli saavutanud augusti lõpuks vähemalt 360 mm või GIV väärtus 6.4 või enam, oli epideemiat oodata nii metsataimlates kui ka metsakultuurides. Püüdes anda võimalikult varajast signaali männi-pudetõve epideemia tekkeks, tuleb arvestada, et kui maist juulini (kaasa arvatud) on sademeid 250 mm ja enam, on põhjust karta ja tuleb valmistuda uue epideemia algamiseks. Retrospektiivse analüüsi tulemusena selgus, et perioodil 1887–2006 oli dekaadi kohta esinenud metsataimlates keskmiselt 4.6 männi-pudetõve puhangut, metsakultuurides poole vähem. Tulemused täpsustavad seniseid teadmisi männi-pudetõve epideemia initsieerimisest ja on aluseks kõnealuse ohtliku okkahai-guse prognoosimudeli väljatöötamisel (**Artiklid II ja VII**).

5. 20. sajandi ohtlikuma männi okkahaiguse Eestis – männi-pudetõve tekitaja (*L. seditiosum*) mõningate epidemioloogiliselt oluliste küsimuste lahendamiseks kasutati pilootprojektina multiproksiaalset lähenemist (**Artikkel VII**). See tähendab, et üritati kõrvutada mitmeid erinevaid asjasse vähegi puutuvaid andmeridu retrospektiivselt NTM abil saadud andmeridadega ja ilmastikuandmetega, üle puude

juurdekasvude, kuni defoliatsiooni andmeridadeni rahvusvahelise metsade monitooringu (ICP Forests) vaatlusaladel. Kinnitust leidis ammu teadaolev, kuid esmakordselt arvväärtusteni täpsustatud, epideemia puhkemist vallandava sademete kumuleeruva summa väärtus teatud suvekuudel. Uudsenä ka sügiskuude niiskustingimuste väheolulisus ning hoopis suve alguskuude (mai ja juuni) normi ületava niiskuse olulisus. Eesti oludes enamiku suviste kalendrikuude õhutemperatuurid ei omanud männi-pudetõve epideemiate tekkes erilist tähtsust, erandiks oli augustikuu keskmisest jahedam õhutemperatuur. Ilmseks põhjuseks see, et Eesti geograafilise asukoha tõttu on suvised temperatuurid siin enamasti niigi piisavalt jahe-
dad. Võrreldes männi-pudetõvest ja männi okkatooidulistest putukatest-diprioniididest põhjustatud okkakadusid ning nendejärgseid puude juurdekasvu muutusi, selgus, et seenhaigus mõjutab oluliselt enam puude kasvu nende noorimas eas, putukad aga kahjustavad mände nii nooremas kui vanemas eas. Männi-pudetõve epideemia ulatus on aga võrreldamatult suurem männi-okkatooiduliste putukate kalamiteedi ulatusest ja summaarsed okkakaod seepärast ka võrreldamatult suuremad. Mitme proksiaalse andmerea abil leiti, et männi-pudetõbi võib kahjustada vaid noori mände kuni vanuseni 24 eluaastat.

Mitmete uute invasiivsete seenpatogeenide saabumine Eestisse 21. sajandil on oluliselt muutnud varasemaid suhteid ja seoseid männiokaste patoloogias. Uued patogeenid demonstreerivad teistsugust ofensiiv-strateegiat võrreldes senistega. Ajal, mil uued patogeenid on kohastumas Eesti muutumises olevate keskkonnatingimustega, tuleb seepärast intensiivistada vastavaid metsapatoloogilisi uuringuid.

ACKNOWLEDGEMENTS

Dokoritöö ma pühendan oma kallile Siirile ja lastele, kes on pidanud ootama issit pikki päevi ja öhtuid usus, et kunagi saab töö ehk valmis. Ma olen siiralt tänulik teile kannatlikkuse eest!

I would like to thank my supervisor and mentor, Märt Hanso. There have been times when I was feeling hopeless about my work, like a mountaineer who can see the top of a mountain that is still very far away. Then Märt always found new and positive energy which he shared with me, encouraging me to continue the work. I am very grateful to him for supervising so consistently. Märt always has explained and stressed that forest pathology does not mean only disease agents, but disease is a complex relationship between pathogen, host and environment.

I am deeply thankful to Prof. Timo Kurkela, who helped me to detect new and wonderful possibilities with the needle trace method. Timo was one author of the method and I have had unique chance to learn from and share valuable ideas and thoughts with him.

Timo and Märt both are strong researchers-pathologists and very good specialists in Finland and Estonia, respectively. I have had an exceptional chance to work together with both of them and gain valuable knowledge in forest pathology. Thank you very much!

I would like to thank Dr. Risto Jalkanen, who taught me new skills and principles of the needle trace method. It was the first time that I was so far north in Finland, in Rovaniemi.

I am also grateful to Tarmo Aalto, Martti Vuorinen, Kalev Adamson, Kairi Kell, Katrin Drenkhan and Triinu Punder, who helped me to take samples from trees. Kairi, Kalev, Triinu and Kati also have analysed many of the sample bodies and counted numerous needle traces using the needle trace method.

I thank also the persons who helped me to collect needle samples at different sites in Estonia, and special thanks to my sister Kati.

I would like to thank Prof. Jarkko Hantula, who taught me principles of

ITS analyses, and Marja-Leena Santanen for her help in the laboratory as well as the other very kind and helpful people at the Finnish Forest Research Institute.

I thank Allar Padari and Regino Kask for help with technical questions. Additionally I am thankful to Merje Toome for her good remarks and recommendations regarding my thesis. I also offer many thanks to the entire staff at the Department of Silviculture in our institute.

Erilised tänud isale ja emale, kes on olnud alati toeks ja andnud mulle võimaluse liikuda nii kaugele nagu ma just parajasti olen. Tänud õdedele ja vendadele toetava suhtumise eest minu tegemistesse. Olulised tänud toetuse eest metsamehest onule, Agu Treial'ile, kes on olnud heas mõttes süüdi selles, et ma üleüldse metsandust õppima asusin.

My studies were supported by the Environmental Investment Centre through a number of important projects. The studies were partly supported by SNS project PATHCAR and the project SF0170021s08.

I

PUBLICATIONS

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The relationship between the needle age and the growth rate in Scots pine (*Pinus sylvestris*): a retrospective analysis by needle trace method (NTM)

Received: 10 January 2006 / Accepted: 10 April 2006 / Published online: 10 June 2006
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Abstract Variation in annual radial and height increments (RI and HI, respectively) as well as the number of needles belonging to different needle sets of successive annual shoots, determined by the needle trace method (NTM), was studied retrospectively in Scots pine stands, one in southern Finland and two in Estonia. The connection between different needle sets and tree growth was studied by correlation and covariance analyses. The numbers of needles of the three youngest needle sets were positively correlated with growth, whereas the number of needles older than 3 years had no significant correlation. The covariance analysis emphasized the negative relationship of older needle sets with tree growth. It is generally concluded that the two or three youngest needle sets are important and all older needles minimally contribute to growth, possibly because of their small quantity. Older needles may remain on the tree if assimilation is impeded for some reason, e.g. due to premature loss in the two youngest needle sets. The trees having attached old needles grew less than those that had already totally lost these needle sets.

Keywords Crown thinning · Annual needle set · Needle age · Radial increment · Height increment · Needle trace method

Communicated by Hans Pretzsch

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Introduction

Premature and accelerated shedding of older needle sets is called crown thinning (Kronenverlichtung), particularly in studying forest health condition as affected by air pollution. Crown thinning has been expressed as loss from the total foliage in ten (Jukola-Sulonen et al. 1987; Schmid-Haas 1990) or sometimes five (Dobbertin and Brang 2001) percentage classes determined by visual observations regardless to the age of the fallen needles. Relative needle weight has also been determined as the number of needle sets in succeeding annual shoots (Skuhravý 1990) or as the percentage of an assumed normal number of needle sets (Dong 1986). However, the foliage weight, or the number of needle sets that pine should normally have, cannot be determined unambiguously because it is not possible to decide whether pine needles abscise timely or untimely (maturely or prematurely; see Drenkhan and Hanso 2000) solely on the basis of the duration of needle retention. Needle longevity in pines (*Pinus sylvestris* L. and *P. tabulaeformis* Carr.) has been found to correlate positively with increasing latitude (Jalkanen et al. 1995b; Xiao 2003, respectively). The availability of mineral nutrients (Reinikainen 1967; Oleksyn et al. 1997; Niinemets and Lukjanova 2003), accumulation of certain mineral elements as air pollutants (Lamppu and Huttunen 2003), or extensive root injuries impeding the uptake of water and nutrients (Jalkanen et al. 1995a) may also decrease the lifetime of needles. Tree ageing itself appears to increase needle longevity (Pensa et al. 2001); therefore longevity seems to be a largely phenotypic response (Reich et al. 1996; Pensa and Sellin 2002). Needle loss in the youngest annual shoots is quite often caused by fungal pathogens like *Lophodermella sulcigena* Rostr. (v. Höhn.) in Scots pine (Jalkanen 1986) or *Chrysomyxa* needle rusts in spruces (Hartmann et al. 1988; Bauer et al. 2000).

Although it can be expected that all the attached living needles support growth and that the number of

needles in different needle sets correlates directly and positively with the annual growth rate, the youngest pine needles are apparently the most important factor in dry mass production in a tree. In pine foliage, the youngest needles assimilate most actively (Wang et al. 1995; Sullivan et al. 1997; Bobkova and Zagirova 1999; Jach and Culemans 2000).

In this paper, we studied the relationship of radial and height increments with the number of attached needles in succeeding annual shoots based on retrospective data from three pine stands. The data were obtained using the needle trace method (NTM).

Materials and methods

The radial and height increment relative to foliage status were studied in Scots pines (*P. sylvestris*) growing on a dry sandy soil in Pyhtää, Southern Finland, 60°26' N, 26°39' E (site 1) and in two stands at Konguta, Elva Forest District, South Central Estonia, 58°13' N, 26°10' E (site 2) and 58°12' N, 26°08' E (site 3).

On site 1, the stand had naturally regenerated. The pines were 53 years old, with an average dominant height of 11–13 m in 2001. The nine trees selected had been used previously to study the effect of defoliation on the growth and development of general foliage status after the attack of the common pine sawfly (*Diprion pini* L.) in the 1980s (Kurkela et al. 2005).

On site 2, a fine-structured sandy soil, eight pines 109–115 years old were sampled. Site 3 had, more than a century ago, been in agricultural use. The age of eight sample trees on site 3 was between 95 and 105 years. The stands on sites 2 and 3 were established by planting. All sample trees were growing in the main storey; each had a straight healthy stem. The trees were cut and their eastern sides were marked with a chain saw.

The height of the trees was measured, as was the length of the internodes (= annual height increment, HI). From each stem, disks were sawn at the height of 1.3 m, from which annual radial increments (RI) were recorded with a tree-ring measuring system in the main compass directions (site 1) or only eastern and western directions (sites 2 and 3) with an accuracy of 0.01 mm. The averages of these measurements were used in this study.

In all sample trees, a bolt about 15 cm long was taken from each internode above breast height for counting needle traces according to the procedure presented by Aalto and Jalkanen (1998). To calculate the effect of the needles in each needle set, the number of needles in the bolts of succeeding years was derived from the needle trace counts (ANN_1 = the number of current-year needles, ANN_2 = the number of retained needles in the previous year's shoot but in the same annual ring, ANN_3 , etc. to ANN_n = the number of retained needles in the annual shoot grown $n-1$ years before the current year shoot but still in the current year annual ring). We classified the annually attached needles by their age. The

age of current-year needles was 4 months (≈ 0.3 year, from needle birth to the time of yellowing of senescent needles). On sites 1, 2 and 3 the age of the oldest attached needles were 4.3, 7.3 and 9.3 years, respectively. ND = needle density (the number of needles cm^{-1} on an annual leader shoot) and CAN = the age of currently attached needles, derived from the original needle trace counting, were also used to explain increment variation. The growing year of each annual shoot and the code number of each tree were registered as category variables. The NTM variables were used as independent regression variables and radial increment (RI) and height increment (HI) as dependent variables.

The effect of different needle sets on increment was analysed using a covariance analysis method (ANCOVA). RI or HI was a dependent variable, ND and the number of needles in any of the different needle sets (ANN_1 , ANN_2 , ..., ANN_n , respectively) were independent variables. The ANN_1 was not used in HI analysis, since $ANN_1 = \text{annual needle production} = \text{ND} \times \text{HI}$. The ANCOVA model could include only one needle set at a time because most needle sets correlated significantly with one or two younger and older needle sets. Due to the low annual numbers of 5-year-old or older needle sets, we computed a new set of variables for the sums of different annual needle sets, e.g. ANN_{1-10} = total number of needles attached (ANN) and ANN_{5-10} = the sum of needle sets from ANN_5 to ANN_{10} . In this way we obtained a higher number of needles for these summed variables than there were in any corresponding original annual needle set. These summed variables were used the same way to compute correlations and ANCOVA models as the original ANN_n variables. The effect of the mean age of all the attached needles on RI and the effect of the age of the needles older than 1 year on HI were also tested. The tree code was used as a category variable since each tree in a stand has its own growth rate that it tends to retain. None of the variables was standardized for final statistical processing operations. Each site was analysed separately. All the statistical operations were performed according to SYSTAT® (2000).

Results

The data from site 1 for studying the increments and NTM variables consisted of 35–40 annual internodes per individual tree from 1955 to 2001. On site 2, the data comprised 34–46 annual internodes per tree from 1884 to 1931, and on site three 33–42 annual internodes per tree from 1899 to 1945. Although the stands had been growing in different times, the data for each one represented the same stage in stand development, i.e. tree ages from about 5 to 45 years. The mean increments (RI and HI, cf. Table 1) were different on these three sites, indicating great differences in site fertility.

Needle density (ND) is the NTM variable logically demonstrating a high dependence on the internal and

Table 1 Mean of radial increment at the height of 1.3 m (RI_{1.3}), and height increment (HI), needle longevity, age of currently attached needles (CNA), and needle density (ND) in the sample trees of each site

Site	<i>N</i>	RI _{1.3} (mm)	HI (cm)	Needle longevity (a)	CNA (a)	ND (Ncm ⁻¹)
1	347	1.47	26.8	2.25	1.35	7.58
2	334	1.99	34.5	2.94	1.88	6.96
3	308	2.16	47.5	2.95	2.02	5.98

N number of measurements; *a* year; Ncm⁻¹ number of needles per cm of shoot

external factors conducive to growth in pines. The ND or its transformed logarithmic values, Ln(ND) correlated negatively with RI and HI on each site. The age of the attached needles (CNA) correlated negatively, in most cases to a significant degree, with the increment variables (Table 2). The ND was lowest on site 3 and highest on site 1, the CNA was lowest on site 1 and highest on site 3 (Table 1). The differences in ND and CNA were statistically significant between the sites with *P* 0.001 for each pairwise comparison, except for the difference in the CNA between the sites 2 and 3 where the *P* value was 0.015.

The correlations of the needle sets with RI and HI varied according to needle age. Younger needle sets (ANN₁ to ANN₃) were significantly positively correlated with radial increment (RI) in all sites. ANN₄ had a somewhat significant positive correlation with RI on sites 2 and 3. In most cases, older needle sets were negatively correlated with RI (Table 2). As an annual shoot completes its growth earlier than the needles on it, a

study of the correlation between the current-year needles and shoot elongation is irrelevant. Therefore, correlation coefficients were computed only for second-year and older needle sets and HI. The ANN₂ and ANN₃ on sites 1 and 2, but only ANN₃ on site 3, correlated positively and significantly with HI. As for the older needle sets, the correlations were negative in many cases and insignificant (Table 2) in all cases.

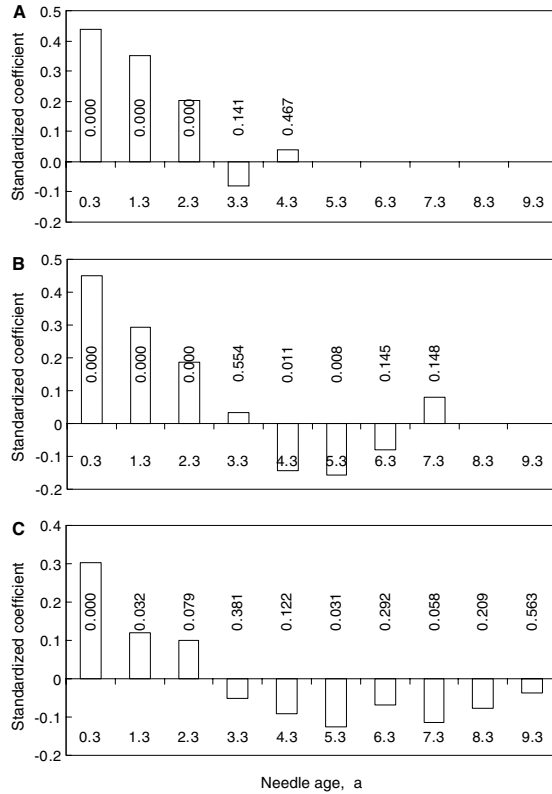
In addition, the relationships of different needle sets with RI and HI were examined with ANCOVA, having ND and either of ANN_{*n*} as explanatory variables and the tree code as a category variable on each site. The effect of different needle sets on tree growth was about the same as indicated by the correlation coefficients. On sites 1 and 2 the current, second and third-year needles (ANN₁, ANN₂ and ANN₃) had a statistically significant effect on RI; on site 3; only the two youngest needle sets (ANN₁ and ANN₂) were significantly correlated (Fig. 1). On none of the sites the pines could benefit from needles older than 3 years (ANN₄ and older).

Table 2 Correlation between needle density (Ln(ND) = logarithmic transformation of ND), age of attached needles (CNA), and the mean number of attached needles in different annual needle sets (ANN₁-ANN₁₀) and the sums of the needle sets (ANN₁₋₁₀ = all attached needles; ANN₅₋₁₀ = the sum of the needles older than 4 years, etc.)

Variable	Correlations						Mean number of needles		
	Radial increment (RI)			Height increment (HI)			Site 1	Site 2	Site 3
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3			
Ln(ND)	-0.460***	-0.310***	-0.202**	-0.726***	-0.450***	-0.363***			
CNA	-0.139	-0.256***	-0.289***	-0.344***	-0.362***	-0.046			
ANN ₁	0.484***	0.411***	0.224***				197.8	232.7	278.4
ANN ₂	0.418***	0.437***	0.232***	0.535***	0.425***	0.120	192.6	220.7	262.6
ANN ₃	0.182*	0.325***	0.247***	0.187*	0.392***	0.286**	150.5	194.6	229.9
ANN ₄	-0.042	0.198*	0.139*	-0.096	0.185	0.079	38.8	147.7	156.5
ANN ₅	0.086	-0.077	0.035	-0.062	-0.113	0.123	2.5	53.3	90.2
ANN ₆		-0.056	-0.025		-0.108	0.160		13.1	43.3
ANN ₇		-0.048	-0.012		-0.090	0.102		1.7	13.7
ANN ₈		0.035	-0.074		-0.060	0.101		0.2	4.5
ANN ₉			-0.049			-0.003			1.3
ANN ₁₀			-0.019			0.090			0.3
ANN ₁₋₁₀	0.312***	0.396***	0.229*				562.3	845.1	1080.3
ANN ₂₋₁₀	0.206*	0.337***	0.186	0.270**	0.347***	0.258**	372.7	624.4	810.3
ANN ₃₋₁₀	0.073	0.235*	0.132	0.056	0.237**	0.244*	186.8	411.8	552.8
ANN ₄₋₁₀	0.017	0.106	0.055	0.085	0.040	0.168	42.1	222.2	325.9
ANN ₅₋₁₀	0.131	-0.034	-0.008	-0.061	-0.104	0.174	2.5	72.8	163.3
ANN ₆₋₁₀		-0.024	-0.041		-0.103	0.164		16.0	67.6
ANN ₇₋₁₀		-0.032	-0.048		-0.097	0.120		2.0	21.3
ANN ₈₋₁₀		0.062	-0.088		-0.056	0.102		0.2	6.4
ANN ₉₋₁₀			-0.052			0.026			1.6

P values: *** 0.001, ** 0.01 and * 0.05

Fig. 1 Relative effect of needle sets of different ages on radial increment on the three sites. **a** Site 1, Pyhtää, Finland; **b** Site 2, Konguta, Estonia (forestland); and **c** Site 3, Konguta, Estonia (former agricultural land). Standardized coefficients (columns) indicate the effect on growth obtained using ANCOVA. The numbers along the vertical axis are *P* values for each needle set



Further, some older needle sets (ANN_5 and ANN_6 on site 2 and ANN_6 on site 3) had a negative relationship with (effect on) radial growth (Fig. 1). The annual needle sets ANN_2 and ANN_3 on site 1, ANN_2 – ANN_4 on site 2 and only ANN_3 on site 3 had a significant positive effect on HI. The HI had a significant negative relationship with ANN_4 on site 1 and with ANN_5 – ANN_7 on site 2. On site 3, the HI had no significant relationship with older needle sets (Fig. 2).

Since the low correlations of RI and HI with older needle sets could be due to the low annual numbers of these needle sets, we computed new sets of correlation analyses using the summed variables (ANN_{1-10} to ANN_{9-10} , see Table 2). Within these variables we got the higher number of needles than in the single annual needle sets. However, this process yielded fewer cases with significant correlations than the original needle set

variables for both RI and HI (Table 2). Then we also tested the possible effect of older needles on tree growth using these summed variables in ANCOVA, but none of these variables demonstrated a significant effect on any of the sites. Finally, we examined whether there were any growth differences between the observed 0-values and the values > 0 in each needle set on the sites. The tests included as covariate variables $\ln(\text{ND})$ and the number of all currently attached needles, and as category variables the tree code and any of the annual needle sets with two classes ($ANN_n = 0$ or > 0). Significant differences, if any, could be expected in needle sets from ANN_4 to ANN_7 , since some of these needle sets, depending on the site, were covered by a number of observations adequate for comparisons in both classes (Table 3). On site 1, both the RI and HI were lower when some needles were attached in ANN_4 than when the values for this needle

Fig. 2 Relative effect of needle sets of different ages on height increment on the three sites. **a** Site 1, Pyhtää, Finland; **b** Site 2, Konguta, Estonia (forestland); and **c** Site 3, Konguta, Estonia (former agricultural land). Standardized coefficients (*columns*) indicate the effect on growth

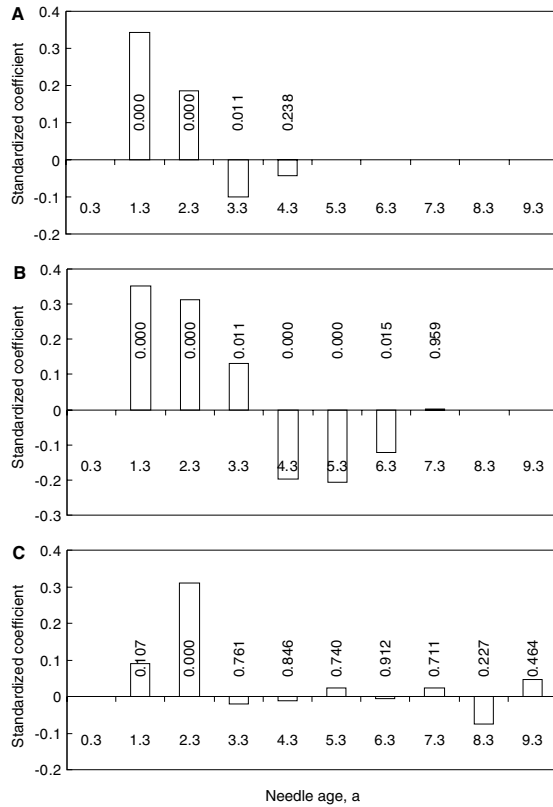


Table 3 Number of observations in each needle set with one or more needles attached (> 0) or without any needles (0) by the sites

Needle set	Site 1		Site 2		Site 3	
	> 0	0	> 0	0	> 0	0
ANN ₁	347	0	328	0	308	0
ANN ₂	338	0	322	0	300	0
ANN ₃	317	12	314	0	292	0
ANN ₄	146	174	303	3	263	21
ANN ₅	22	289	198	100	197	79
ANN ₆	0	302	74	216	122	146
ANN ₇			14	268	46	214
ANN ₈			3	271	15	237
ANN ₉			0	275	5	239
ANN ₁₀					2	234

Bold fonts show the needle sets in which attached needles were correlated with a significantly ($P < 0.05$) lower growth rate (of RI, HI or both) and with a lower number of needles in at least one of the youngest needle sets (ANN₁–ANN₃) compared to the cases where the needle sets indicated had no needles

set were zero, with $P = 0.002$ and 0.001 , respectively. On site 2, the RI had lower values when some needles were attached in ANN₅ than when the needle values were zero, with $P = 0.005$, and HI had significantly higher values when the values in three needle sets, ANN₅–ANN₇, were zero than when some needles were attached, the P values being 0.006, 0.008 and 0.041, respectively. On site 3, RI was higher in ANN₅ and ANN₆ when the observed needle values were zero than when some needles were attached, with P 0.001 and $P = 0.040$, respectively, and HI had higher values in ANN₅ when the needle values were zero than when some needles were observed to be attached ($P = 0.006$). Older needle sets (ANN₄ and older) had no significant growth-promoting effect in any of the sites.

Since the trees with older needles apparently were growing less than those without such needles, we examined possible differences in the number of needles of the most active needle sets (ANN₁–ANN₃) where older needle sets had a value of either 0 or > 0 . The results of this test corresponded well to the differences in RI and HI between these two groups determined in previous tests. On site 1, there were significantly more needles (P 0.001) in the two youngest needle sets (ANN₁ and ANN₂) at ANN₄ = 0 rather than at ANN₄ > 0 . The same type of differences were established on site 2 at ANN₆ = 0 or > 0 , with P values of 0.042 and 0.003 for ANN₁ and ANN₂, respectively, and on site 3 at ANN₅ = 0 or > 0 , with P values of 0.011 and 0.015 for ANN₁ and ANN₂, respectively.

Discussion

Between the sites of this study, the maximum number of needle sets was different, equalling five, eight and ten on sites 1, 2 and 3, respectively. Therefore, the statistical tests for examining the effect of the annual needle sets on RI and HI were processed separately for each site. Other factors restricting the statistical processing were a high correlation between succeeding needle sets as well as the autocorrelation in radial and height increments. In fact, all variables measured as “independent” explanatory or as dependent variables in pines were endogenous in the system, which rendered the interpretation of causality difficult.

To elucidate the relative importance of the separate needle sets to the growth, we made a hypothetical model for the distribution of needles of different ages in young Scots pines that have the oldest needles in the fifth needle set. We assumed that the leader shoot of the tree has constant annual growth rate (a), and that the leader growth in each first-order branch decreases annually according to an exponential function (ap^t), in which p is 100 minus the percentage of the decrease and t is the age of the branch whorl. We also assumed that the lateral shoots of any internodes in the successive branching are growing according to the function ap^{t+n} , where n shows the branching order from the main branch. For example,

the length of the apical shoot in the 6-year-old branch is ap^6 and that of the second-order branch in the same 6-year-old branch is ap^8 . When $p = 0.7$ the growth of apical shoots obtained using these formulas follows very closely the annual branch elongation measured by Ilonen et al. (1979) in young Scots pines. Assuming that the branches are longest in the sixth whorl when counted from the top of the tree (Kellomäki and Oker-Blom 1983), and below that level the relative needle mass and its effect on the total photosynthesis are negligible (Kellomäki and Hari 1980), and also assuming that the needle density is constant throughout the crown system, we computed the relative number of needles for the annual needle sets for the six uppermost whorls. Combining these numbers gave the original numbers of the needles in each needle set for the part of the crown most important for photosynthesis (Kellomäki and Oker-Blom 1983). Applying the percentages of the remaining needles in each needle set in site 1, we found that the ratios between five annual needle sets (from ANN₁ to ANN₅) were 770:430:200:30:1, respectively.

These numbers for each annual needle set seem to be quite realistic, although in nature apical shoots in the branches may produce more than two lateral shoots depending much on light irradiance (Kellomäki and Oker-Blom 1983; Niinemets and Lukjanova 2003), and decreasing shoot growth tends to increase needle density (Ross et al. 1986; Kurkela et al. 2005). However, the growth decreases in both leader and lateral shoots in the ageing branches, when the increasing needle density may not change the ratio much between the different needle sets.

All needles in the three youngest needle sets accounted for 97.8% of the total foliage (see also Xiao and Ceulemans 2004). In light of these numbers, it is not surprising that older needle sets are not at all important for assimilation and increment processes in pine trees. Needles older than 3 years may only serve as storage for reserve nutrients. The needles start to senesce when these nutrients are mobilized and retranslocated for new growth.

The assessment of needle loss has usually been made by means of rather approximate visual inspection, irrespective of the cause. Needle disease epidemics in conifers may often continue for several years (e.g. Shaw and Toes 1977; Burleigh et al. 1982; Ostry and Nicholls 1989). In such cases it is difficult to distinguish the effect of annual infections from that of the total needle loss in the succeeding years on the tree growth. The quantity of currently attached needles can be determined accurately by counting or, even more precisely, by weighting the needle fascicles of each annual shoot in the sample material. To obtain the relative number of retained needles one should also count the traces of the fallen needles (Kurkela 1981). Using the NTM, this data, especially in pines, can be extended to studying retrospectively the history of foliage conditions right from the start of stand development (Kurkela and Jalakanen 1990; Aalto and Jalakanen 1998). Needle traces could be

counted from succeeding annual shoots and tree rings in the stem of pine. In this manner it is possible to obtain retention data for each needle set in each succeeding year of tree growth. Assuming that the number of needles (needle fascicles) attached in the stem correlates with the number of needles or needle sets in the branches, it is possible to estimate the contribution of different needle sets to the growth in pines. Needle retention in the main stem and in the branches seems to be approximately equal, with only small variations; in Finland it was found to be slightly lower in the main stem (Jalkanen et al. 1995b) whereas in a sample from Estonia the opposite was true (Pensa 2003).

The youngest needles are physiologically most active and therefore most important in dry mass production (Sullivan et al. 1997; Bobkova and Zagirova 1999; Wang et al. 1995; Oleksyn et al. 1997; Zha et al. 2002). However, current-year needles mature much later than the shoot to which they are attached (e.g. Kurkela 1980). Thus, current-year needles may have no contribution to shoot elongation, and they can affect radial growth only during the latter two-thirds of the season (Leikola 1969).

The effect of older needle sets on growth processes cannot be determined accurately by means of the statistical calculations used in this study since older needle sets in young pines represent a very small portion of the whole foliage, and their numbers do not vary in the same way as current-year increments (RI and HI). However, all green needles may participate in assimilation and thus support growth, but it is known that the photosynthetic capacity of the needles tends to decrease with their age (Niinemets and Lukjanova 2003).

Only young needle sets correlated positively with both radial and height increment. Some needle sets older than 3 or 4 years seemed to have a negative relationship with tree growth. Further, the trees that had green older needles grew less than those without such needle sets. Some previous studies have established that slowly growing trees often demonstrate high needle retention, as is the situation with increasing elevation or along a south-north gradient (Schoettle 1990; Jalkanen et al. 1995b; Xiao 2003). The main reason for slow growth in the north is the low rate of photosynthesis resulting from a harsh climate. On the other hand, pathogens infecting young needle sets may reduce the actively assimilating foliage and, consequently the growth rate and then the fall of older needles can be retarded (see Jalkanen et al. 1994).

In the material of this study, a high growth rate at stand level was associated with high needle retention, needle age and a large number of needles in older needle sets. Within the stands, however, a large number of attached old needles was correlated with a low growth rate. During needle senescence some mobile mineral nutrients (e.g. N, P and K) are redistributed to younger needles but increased mineral nutrition, for example by fertilization, may reduce this retranslocation (Helmisaari 1992). If the availability of nutrients is balanced and their level exceeds the growth requirement in certain

climatic situations, nutrient recycling may be delayed. In this study, trees grew best on site 3, although some needles in the tenth needle set could occasionally be attached. The trees on site 1 were growing slowly, yet the oldest needles were found in the fifth needle set (ANN₅). At the stand level, high availability of nutrients in relation to other growth promoting factors may retard the recycling of the minerals stored in older needle sets and the needles remain attached for several years. It seems that the difference between the sites in the number of older attached needles was correlated with soil fertility rather than the irradiance gradient in the tree crowns (see Niinemets and Lukjanova 2003).

Apparently, the relationship between high needle retention or delayed retranslocation of mobile minerals and growth within the stand has not been studied. The growth of individual trees may be impeded, e.g. by drought, injuries, needle loss, etc., which always signals a direct or indirect decrease in the assimilative apparatus. On the other hand, the retranslocation of nutrients may be mainly controlled by the growth rate (Nambiar and Fife 1987). We found that in certain years the trees that had older needles (ANN₄ to ANN₆) attached grew less than those that had already lost these needle sets. Only site 1 had a very apparent reason for decreased growth, a heavy needle loss caused by the common pine sawfly, *D. pini* (Kurkela et al. 2005). On sites 2 and 3 no apparent reason for growth retardation could be identified; however, frequent needle cast (*Lophodermium sedditiosum* Minter, Staley and Millar) epidemics usually lead to foliage losses in Estonian pine stands of younger ages (Hanso 1970; Drenkhan and Hanso 2003).

The two youngest, physiologically most active needle sets represent about 80–90% of the total needle mass in Scots pines. Unfortunately, we have no well-examined cases available with tree-wise records of losses in the youngest needle sets and of the growth in Scots pine. In 3-year-old Norway spruce seedlings, a 70% loss in the youngest needles (presumably accounting for about a 35% loss in total needle mass) lead to an approximately 30% loss in dry mass production (Plattner et al. 1999; Bauer et al. 2000). According to van Sickle (1974), 40% of artificial infection of current year needles by *Pucciniastrum goeppertianum* (Kuehn.) Kleb. in balsam fir (*Abies balsamea* Mill.) in two successive periods of growth led to an approximately 30% loss in the radial growth of the trees. Infections targeted at second-year or older needles may not lead to significant growth losses, as appeared from foliar infection by *Lirula abietis-concoloris* (Mayr ex Dearn.) Dark on white fir [*Abies concolor* (Cord. and Glend.) Lindl.] in California (Scharpf 1986), if not repeated in several years in succession. It seems that the extents of losses in the two youngest needle sets and the reductions in growth are of about the same magnitude (where both are expressed in percentages).

Our study shows the relative importance of the youngest needle sets for the growth in Scots pine. The results are well in accordance with the earlier knowledge

about the effect of needle age on the photosynthesis activity (for example, Wang et al. 1995; Jach and Ceulemans 2000). Needle sets older than 3 years had no positive effect on the growth (RI and HI). They were mostly present in the trees with retarded growth rate, apparently due to the losses in the younger needle sets. It can be concluded that a temporary fall of older (> 3 years) foliage sets causes no remarkable growth loss. Instead, any loss in the youngest (ANN₁ and ANN₂) needle sets may be directly proportional to subsequent growth reduction.

Acknowledgements We are grateful to Docent Dr. Risto Jalkanen and Forest Engineer Mr. Tarmo Aalto (Finnish Forest Research Institute, Rovaniemi) for their participation in the fieldwork and invaluable discussions during the preparing of this paper. We thank Prof. Jarkko Hantula (Finnish Forest Research Institute, Vantaa) and Prof. Hardi Tullus (Estonian Agricultural University, Tartu) for reading the manuscript and for their valuable comments and suggestions. Mr. Sulev Kivastik (Tartu) and Mr. Terry Bush (MA, USA) corrected the English.

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Retrospective Analysis of *Lophodermium seditiosum* Epidemics in Estonia

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Abstract – The needle trace method (NTM), created and developed by the Finnish forest pathologists prof. T. Kurkela, dr. R. Jalkanen and T. Aalto during the last decade of the XX century, has been already used by several researchers of different countries for retrospective analysis of needle diseases (*Hypodermella sulcigena*, by R. Jalkanen et al. in Finland) or herbivorous insect pests of Scots pine (*Diprion pini*, by T. Kurkela et al. in Finland; *Bupalus piniaria*, by H. Armour et al. in Scotland), but as well of pests of Sitka spruce (*Gilpinia hercyniae*, by D.T. Williams et al. in England). Scots pine in forest nurseries and young plantations of Estonia is often but irregularly suffering from the epidemics of the needle cast fungus *Lophodermium seditiosum*. Current environmental regulations exclude from the regulatory (control) measures all the others except of well-argued prophylactic systems, built up on reliable prognoses. The last is inconceivable without the availability of a reliable, as well, and long-lasting retrospective time-series of *L. seditiosum* epidemics, which, as it is known from the last half of the XX century, are occupying large forest areas, usually not least than a half of (the small) Estonia. An appropriate time-series would be useful, as well, for the more basic understanding of the accelerated mortality processes during the stand formation in early pole-age Scots pine plantations. Methodological principles of the use of NTM in an appropriate investigation together with the preliminary results of our research work, looking back for more than a century, are introduced and discussed in this investigation.

needle trace method (NTM) / needle diseases / annual needle loss / *Lophodermium seditiosum* / *Pinus sylvestris* / *P. contorta*

Kivonat – A *Lophodermium seditiosum* járványok visszatekintő elemzése Észtországban. A tűnyomok módszerét (NTM) finnországi kutatók (T. Kurkela, R. Jalkanen és T. Aalto) fejlesztették ki a XX. század utolsó évtizedében. Különböző országokban több kutató alkalmazta a tűbetegségek (*Hypodermella sulcigena*, R. Jalkanen et al. Finnországban), az erdeifenyő rovarkárosítások (*Diprion pini*, T. Kurkela et al. Finnországban; *Bupalus piniaria*, H. Armour et al. Skóciában), a szitkalc károsítások (*Gilpinia hercyniae*, by D.T. Williams et al. Angliában) utólagos elemzésére. A tükarc gomba (*Lophodermium seditiosum*) gyakran, de nem rendszeresen támadja az erdeifenyőt az észtországi csemetekertekben és fiatal erdősítésekben. A jelenlegi környezetvédelmi szabályok kizárják a védekezési módszereket, a megbízható előrejelzésen alapuló, jól megindokolt megelőző rendszerek kivételével. Ez utóbbiakhoz elengedhetetlen, hogy rendelkezünk a *L. seditiosum* járványok megbízható, és hosszú távra visszatekintő idősorával. E járványok a múlt század második felében nagy, rendszerint fél Észtországnyi területeket sújtottak. Egy megfelelő idősor hasznos lehet a felgyorsult pusztulási folyamatok alaposabb megértéséhez is az erdeifenyő erdősítések állományá

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alakulása során. Bemutatjuk és megvitatjuk az NTM alkalmazásának metodológiai elveit, kutatómunkánk több mint egy évszázadra visszatekintő előzetes eredményeivel együtt.

tünyomok módszere (NTC) / tübetegségek / éves tüvesztés / *Lophodermium seditiosum* / *Pinus sylvestris* / *P. contorta*

1 INTRODUCTION

Scots pine (*Pinus sylvestris* L.) is one of the main commercial tree species in Estonia, growing here in congenial to this species environment – climate and soils. The most common foliage disease in young Scots pine (*Pinus sylvestris* L.) plantations and forest nurseries of Estonia is caused by *Lophodermium seditiosum* Minter, Staley and Millar (*Rhytismatales*, *Ascomycota*). Occurrence of the needle cast disease and the disease agent (historically: *Lophodermium pinastri*) on the territory of Estonia was first documented at the turn of the XIX-XX century, both nearly at the same time, by the Baltic German foresters (Weiß 1902) and mycologists (Vestergren 1903), respectively. In Germany the problem of *Lophodermium* needle cast ("KiefernSchütte") had risen for the first time ca 50 years before, accompanying the beginning of the large-scale afforestations of the cutted areas by the use of nursery-grown pine seedlings (Stein, 1852. Thar. Forstl. Jahrb., Bd. 8; Holzner, 1877. Die Beobachtungen über die Schütte der Kiefer. Freiburg).

By today we know, that: 1) *Lophodermium* needle cast epidemics occur in Estonia almost regularly, but not precisely after definite intervals, and 2) needle cast epidemics are much more frequent in forest nurseries than in plantations, sometimes so frequent that the successive epidemics can not be distinguished from each other.

Still ignoring the possibility of genetic improvement of the pathogens virulence before the next-in-order epidemic, the explanation for the rise of a new epidemic has been still behold solely in the peculiar meteorological preconditions - rainy summers and following mild winters - of the years before the epidemics.

2 MATERIAL AND METHODS

2.1 Time-series and additional data sources

In this investigation we followed the traditional approach in the explaining of causes of the epidemics, i.e. considering the meteorological conditions of the preceding years. We tried to specify retrospectively the potential epidemic years of *Lophodermium* needle cast by juxtaposing two time-series: 1) the meteorological data (by selecting out the years with rainy summer + mild winter), and 2) the needle characteristic (extensive needle loss of the year, behold as a potential epidemic year).

This experimental approach could become possible only after the discovering and developing of the needle trace method (NTM, short description will follow) by the Finnish forest pathologists prof. T. Kurkela, dr. R. Jalkanen and T. Aalto (Kurkela and Jalkanen 1990, Aalto and Jalkanen 1996, 1998).

All available published data on the occurrence of *Lophodermium* needle cast disease during the last century in Estonia were used as additional sources for comparison with our experimental results with the purpose to make up the retrospective list of the disease epidemics.

During more than 40 years the elder author (M. Hanso) had carried out several investigations (Hanso, 1963, 1968, 1970, 2001, 2003) about the biology, ecology and regulation (control) of *Lophodermium* needle cast fungus and the disease, respectively, on

pine. Additionally, his laboratory served in the years from 1972 to 1985 as the diagnostic centre for the whole forest nursery management system in Estonia. All the appropriate materials were used in this investigation, as the additional data.

2.2 Experimental study areas

The retrospective NTM data were obtained from six pine stands, of which four *Pinus sylvestris* stands were growing in Konguta, Elva Forest District, south-eastern Estonia, but two stands (*P. sylvestris* and *P. contorta*, respectively) in Järvelja Training and Experimental Forestry District (Figure 1). Eight model pine trees from stand 1 (situated at 58°13' N, 26°10' E) were 109–115 years, eight trees from the stand 2 (58°12' N, 26°08' E) - 95-105 years old. The area of stand 2 had been more than a century ago in agricultural use. Eight model trees from stand 3 (58°16' N, 27°19' E) were ca 70 years old. The age of three model trees from stand 4 (neighbouring to stand 1) was about 45-50 years. Stand 5 (neighbouring to stands 1 and 4 from the other side) is a Scots pine provenance experiment and the age of altogether 68 model trees was 14 years. The age of eight model trees (*P. contorta*) from stand 6 (neighbouring to stand 3) was about 60-70 years. All stands were established by planting. All sample trees were growing in the main storey, each had a straight healthy stem.



Figure 1. Location of the investigated by the needle trace method (NTM) stands of *Pinus sylvestris* (in Konguta and Järvelja) and *P. contorta* (in Järvelja), and location of the meteorological station (Tõravere).

NTM data from the *P. contorta* stand were included, as this pine species was considered to be resistant to *Lophodermium* needle cast. Comparison of the needle cast dynamics in *P. contorta* and *P. sylvestris* stands could hopefully serve therefore as an additional argument in making decisions, e.g., was a hard needle loss in *P. sylvestris* stand caused by *Lophodermium* needle cast or by some other, more universal agents.

In this work an epidemic has been defined as any increase of disease in a population (Agrios 2005). The term “needle” actually means the term “fascicle” (or “short shoot”), less used in forest pathology than in plant anatomy.

2.3 Meteorological data

Meteorological data were obtained from the Tartu-Tõravere Meteorological Station, which is situated nearly between the investigated stands (*Figure 1*). For comparisons with the appropriate meteorological characteristics of the interesting us years (“pointer years”) the long-period (1884-2004) mean monthly temperatures and precipitation sums were calculated first.

The pathogen needs both, the high precipitation for successful infection of pine needles in summer and the mild winter for successful colonisation of infected needles before the new vegetation of pine.

This way the potential *Lophodermium* needle cast epidemic years were considered to be probable, if the combination of wet (and warm?) summers and following mild winters will be met before the years of high needle loss. Epidemics of other infectious needle diseases of pine are rare in Estonia, but insect pests are triggered, on the contrary, by warm and dry summers. Regarding the meteorological conditions, eight combinations of summers (between May and September) and following winters (from the previous year December to the pointer year March), were taken into the calculations to find out, which combinations were followed by the high needle loss in the next year. A year was classified as a “high precipitation” year and the following winter as a “mild” winter, if they were characterized by higher values of the respective meteorological characteristics, than the long-period (1882-2004) means +/- standard errors of the appropriate means.

Inside all the 8 combinations, higher (compared to long period mean, $p < 0,001$) annual needle loss values followed the years with high precipitation in the period from:

- a) July to September (VII-IX) + mild winter, and
- b) July to August (VII-VIII) + mild winter.

Summer warmth, at the same time and concerning the same combinations of months inside “summer” (i.e.: a and b) had no effect on the amount of needle loss in the next (pointer) year, with no statistical differences between the warmer years and long period mean.

2.4 NTM analysis

One hundred and three sample trees from six pine stands were chosen and analysed according to NTM protocols (Aalto and Jalkanen 1998), including 95 sample trees of *Pinus sylvestris* and 8 of *P. contorta*. According to NTM protocols, a stand is already sufficiently represented by 5 to 10 model trees per age class (Jalkanen et al. 2000). Only 3 sample trees were analysed from the stand 4, which means that in our list the years 1958-1980 are represented insufficiently. The periods 1926-1937 and 1981-1994 are still not covered by NTM analysis at all.

After selecting out in a stand and felling of a sample tree the total height and annual height increments (the distances between the neighbouring branch whorls) were measured. From every annual shoot a 10-25 cm long section (bolt) was cut off. The western, northern and southern sides of each bolt were removed by axe already in forest, and only eastern side was transported to and analysed in laboratory according to NTM. Although all the bolts were marked (numbered), the full eastern side served for the control of the correct bolt age, i.e. the definite calendar year, as well. In laboratory, before the analyzes, only ten innermost rings of each bolt were left intact, because needles never keep attached to pine stem longer than 10 years (in Estonia they stay normally only 2.0 – 3.0 years). One disk per every sample tree was sawed from the main stem for measuring the annual radial increments.

Needle trace method (NTM) was created for retrospective uncovering of different needle characteristics, from which only the characteristic “needle loss” was used in this investigation. NTM is based on the fact that the short shoots of living conifer needles remain attached to the pith of the main stem through the vascular tissue (Kurkela – Jalkanen 1990; Jalkanen – Kurkela 1992; Williams et al. 2003). The vascular connection extends through the each tree-ring, leaving there a trace for so long time as the needle is alive and functioning (Figure 2). When the needle dies the needle trace stops growing (into the next year ring). A needle trace is observed as a brown dot on the surface of the wood and can be seen with naked eye.

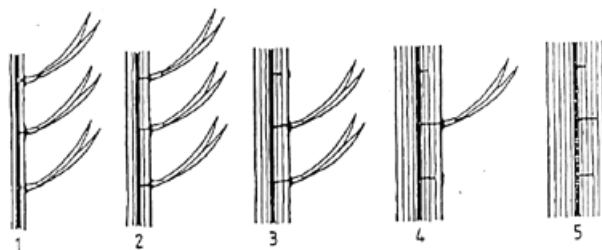


Figure 2. Pine shoots with attached needles and with the remains (needle traces) of fallen needles in five years (Aalto and Jalkanen 1996).

Every annual section of tree have to be analysed, tree ring by tree ring, towards the pith to get the retrospective dataset of needle characteristics. An angle (usually from 45 to 90 degrees) has to be drawn on the both ends of the bolt, extending it from the pith outwards. Lines to connect the two angles have to be then drawn on the longitudinal surface of the eastern side. All needle traces within viewing plane have to be counted and the data have to be entered to the computing program NTMENG.

NTM has been already successfully used in retrospective studies of the growth losses of host trees, subsequent to the defoliation of pines by *Lophodermella* needle-cast disease and by insect *Diprion pini*, both in Finland (Jalkanen et al. 1994 and Kurkela et al. 2005, respectively), but, as well, to the defoliation of spruces by *Gilpinia hercyniae* in Wales (Williams et al. 2003) and of Scots pines by *Bupalus piniaria* in Scotland (Armour et al. 2003). The first trial of retrospective analyses of *Lophodermium* needle cast in Estonia was undertaken some years ago (Drenkhan – Hanso 2003).

2.5 Data analysis

Different years from 1887 to 2003 were covered by different numbers of the model trees, analysed by NTM (Table 1, column 5). Only young age periods of tree life (from 5...7 to 22...25 years in stands 1. – 4., from ca 3 to 13 years in stand 5.) in *P. sylvestris* stands (Figure 3) and from 5...7 to 22...25 years in *P. contorta* stand were included into the computations. According to the NTM protocol the radial growth of sample trees was analysed on the breast height but in the youngest (5.) stand the radial growth was analysed on the stump height.

Although the older sample trees were analysed by NTM until the age of 35-45 years, the needle data, used in this investigation, were restricted by the younger period of tree life, not more than 25 years, because *Lophodermium* needle cast is not dangerous to older Scots pines. In this work recording of the needle trace data were used for calculating annual needle retention and, after that, the annual needle loss.

Annual needle retention (ANR), defined as the sum of the percentages of needles remaining on the leader sections of a tree in a particular year (Aalto and Jalkanen 1998; Pensa and Jalkanen 1999; Williams et al. 2003), was calculated using the equation:

$$ANR_t = \sum [x_t, (x-1)_t, \dots, (x-n)_t] / 100,$$

where ANR_t is the annual needle retention in year t (needle sets), x_t is the percentage of needles on leader section x present in year t , $(x-1)_t$ is the percentage of needles in year on the leader section formed on year earlier, etc.

Annual needle loss was calculated as:

$$ANL_t = (ANR_t - ANR_{t+1}) + 1,$$

where ANL_t is the total loss of needles (needle sets) in year t , and the term 1 represents the new flush of needles in year $(t+1)$ (Aalto – Jalkanen 1998; Pensa – Jalkanen 1999).

The annual needle loss means the decrease in the amount of needle cohorts (defoliation) from the total main stem in a definite year, which is represented as the number of needle sets lost per year. The size of a needle set was calculated by evaluating a complete cohort of needles (i.e. a set of needles produced in a single year) with the value of 1 (100%), a needle cohort, where 75% needles had remained with the value of 0,75, and a needle cohort, where 50% of the needles had remained with the value of 0,5, etc. (Jalkanen 1998; Armour et. al. 2003).

NTM data were calculated by the special program NTMENG version 8 (Aalto – Jalkanen 2004). Statistical analyses were carried out by MS Excel and statistical program SAS.

3 RESULTS AND DISCUSSION

The development of *Lophodermium seditiosum* is very irregular and depends on environmental, particularly climatic factors (Martinsson 1979). The main source of *L. seditiosum* inoculum for infections in plantations is young infected fallen needles on which ascocarps of the fungus form in late summer and autumn (Diwani and Millar 1990). Wet and warm autumns provoke the epidemics in Estonia (Viirik 1931) as do the mild winters (Lepik 1930). Increased problems with *Lophodermium* needle cast in Sweden during the 1990s might be due in part to the occurrence of several consecutive mild winters (Stenström – Arvidsson 2001). Epidemiology of *Lophodermium* needle cast was first analysed by L. Lanier – G. Sylvestre (1971). A. van Maanen – F. Gourbière (2000) conducted an investigation into whether *Lophodermium pinastri* dynamics is determined by the balance between colonization and fructification. Both of these processes were found to be controlled by climatic factors, particularly rainfall. The conclusion was made, that spore production of the pathogen correlates with fructification, and colonization correlates with spore dispersal. It is the best short explanation, why the climatic factors determine the success of an epidemic.

In the earlier (first half of the XX century) literature *Lophodermium* needle cast has been mentioned in Estonia several times, sometimes presumably after the noticeable epidemics of the disease (Weiß 1902, Anonymous 1906 and 1907, S.-K. 1907, Krüger 1910, Aun 1922, Reim 1924 and 1925, Sepp 1928, Viirik 1931, Lepik 1930, Kohh 1933, Stegman 1936). During the Soviet occupation time (i.e. until the 1990s) *Lophodermium* needle cast has been investigated in Estonia in several works (Hanso 1963, 1968, 1970 and 1995, Hanso – Hanso 2001, 2003), but official registration of the data concerning the epidemics and economic losses in forest nurseries of the former (i.e. Soviet) Estonia has been always concealed and/or neglected. Therefore there is still a blank in our knowledge, although theoretical approaches

by that time were solved sufficiently, if not even well, and the disease and its control peculiarities introduced to the practical foresters.

Table 1 represents the potential epidemic years of the disease, therefore the preceding years, supporting the rise of the epidemic through its meteorological peculiarities (two combinations of the summer months and following mild winters, cf. columns 1 and 3, respectively) are not indicated separately. If the NTM data in a potential epidemic year (i.e. after a year with high precipitation in summer and following mild winter) showed, as well, a “high needle loss” (i.e. higher than the mean needle loss of the years of all the period, covered by our NTM data), then the year was classified as an epidemic year for pine plantations (bold numbers in column 7 of the Table 1). Following solely the “high precipitation and mild winter” years (i.e. without high needle loss) were classified as potential epidemic years only for forest nurseries (indicated in the Table 1 with “?” after the year number). Letters after the year numbers in column 7 indicate other important threshold years, concerning *Lophodermium* needle cast in Estonia (e.g. first records, published in German and Estonian, respectively, cited in literature epidemic years, etc., the piths of the letters are specified under the Table 1).

Table 1. The result table

High precipitation in VII-IX + mild winter	High precipitation in VII-IX + mild winter + high annual needle loss	High precipitation in VII-VIII + mild winter	High precipitation in VII-VIII + mild winter + high annual needle loss	The number of sample trees, which NTM data cover the appropriate year	High annual needle loss in <i>Pinus contorta</i>	Important threshold and epidemic years in history attributed to <i>Lophodermium seditiosum</i>
1	2	3	4	5	6	7
1884	1884	1884	1884		1884	1884 ?
1885	1885	1885	1885		1885	1885
1886	1886	1886	1886		1886	1886
1887	1887	1887	1887	1	1887	1887 ?
1888	1888	1888	1888	4	1888	1888
1889	1889	1889	1889	4	1889	1889
1890	1890	1890	1890	6	1890	1890
1891	1891	1891	1891	6	1891	1891
1892	1892	1892	1892	7	1892	1892
1893	1893	1893	1893	8	1893	1893
1894	1894	1894	1894	8	1894	1894
1895	1895	1895	1895	8	1895	1895
1896	1896	1896	1896	8	1896	1896
1897	1897	1897	1897	8	1897	1897
1898	1898	1898	1898	8	1898	1898 ?
1899	1899	1899	1899	8	1899	1899 ?
1900	1900	1900	1900	8	1900	1900
1901	1901	1901	1901	8	1901	1901
1902	1902	1902	1902	8	1902	1902 ^a
1903	1903	1903	1903	8	1903	1903 ^b ?
1904	1904	1904	1904	8	1904	1904
1905	1905	1905	1905	4	1905	1905 ?
1906	1906	1906	1906	4	1906	1906 ?
1907	1907	1907	1907	7	1907	1907 ^c

Table 1. cont. The result table

High precipitation in VII-IX + mild winter	High precipitation in VII-IX + mild winter + high annual needle loss	High precipitation in VII-VIII + mild winter	High precipitation in VII-VIII + mild winter + high annual needle loss	The number of sample trees, which NTM data cover the appropriate year	High annual needle loss in <i>Pinus contorta</i>	Important threshold and epidemic years in history attributed to <i>Lophodermium seditiosum</i>
1	2	3	4	5	6	7
1908	1908	1908	1908	8	1908	1908
1909	1909	1909	1909	8	1909	1909
1910	1910	1910	1910	8	1910	1910 ?
1911	1911	1911	1911	8	1911	1911
1912	1912	1912	1912	8	1912	1912
1913	1913	1913	1913	8	1913	1913
1914	1914	1914	1914	8	1914	1914
1915	1915	1915	1915	8	1915	1915
1916	1916	1916	1916	8	1916	1916
1917	1917	1917	1917	8	1917	1917
1918	1918	1918	1918	8	1918	1918 ?
1919	1919	1919	1919	7	1919	1919
1920	1920	1920	1920	7	1920	1920
1921	1921	1921	1921	7	1921	1921
1922	1922	1922	1922	5	1922	1922 ^d
1923	1923	1923	1923	4	1923	1923 ^e
1924	1924	1924	1924	2	1924	1924 ^f
1925	1925	1925	1925	1	1925	1925 ?
1926	1926	1926	1926		1926	1926
1927	1927	1927	1927		1927	1927
1928	1928	1928	1928		1928	1928
1929	1929	1929	1929		1929	1929 ^g
1930	1930	1930	1930		1930	1930 ?
1931	1931	1931	1931		1931	1931
1932	1932	1932	1932		1932	1932
1933	1933	1933	1933		1933	1933
1934	1934	1934	1934		1934	1934 ?
1935	1935	1935	1935		1935	1935 ?
1936	1936	1936	1936		1936	1936
1937	1937	1937	1937		1937	1937 ?
1938	1938	1938	1938	5	1938	1938
1939	1939	1939	1939	7	1939	1939
1940	1940	1940	1940	8	1940	1940
1941	1941	1941	1941	8	1941	1941
1942	1942	1942	1942	8	1942	1942
1943	1943	1943	1943	8	1943	1943
1944	1944	1944	1944	8	1944	1944
1945	1945	1945	1945	8	1945	1945
1946	1946	1946	1946	8	1946	1946
1947	1947	1947	1947	8	1947	1947
1948	1948	1948	1948	8	1948	1948

Table 1. cont. The result table

High precipitation in VII-IX + mild winter	High precipitation in VII-IX + mild winter + high annual needle loss	High precipitation in VII-VIII + mild winter	High precipitation in VII-VIII + mild winter + high annual needle loss	The number of sample trees, which NTM data cover the appropriate year	High annual needle loss in <i>Pinus contorta</i>	Important threshold and epidemic years in history attributed to <i>Lophodermium seditiosum</i>
1	2	3	4	5	6	7
1949	1949	1949	1949	8	1949	1949
1950	1950	1950	1950	8	1950	1950
1951	1951	1951	1951	8	1951	1951
1952	1952	1952	1952	8	1952	1952
1953	1953	1953	1953	8	1953	1953
1954	1954	1954	1954	8	1954	1954
1955	1955	1955	1955	8	1955	1955
1956	1956	1956	1956	6	1956	1956
1957	1957	1957	1957	1	1957	1957
1958	1958	1958	1958	2	1958	1958
1959	1959	1959	1959	2	1959	1959
1960	1960	1960	1960	2	1960	1960
1961	1961	1961	1961	2	1961	1961^h
1962	1962	1962	1962	2	1962	1962 ?
1963	1963	1963	1963	2	1963	1963
1964	1964	1964	1964	3	1964	1964
1965	1965	1965	1965	3	1965	1965
1966	1966	1966	1966	3	1966	1966
1967	1967	1967	1967	3	1967	1967
1968	1968	1968	1968	3	1968	1968
1969	1969	1969	1969	3	1969	1969
1970	1970	1970	1970	3	1970	1970
1971	1971	1971	1971	3	1971	1971
1972	1972	1972	1972	3	1972	1972
1973	1973	1973	1973	3	1973	1973
1974	1974	1974	1974	3	1974	1974
1975	1975	1975	1975	3	1975	1975 ⁱ
1976	1976	1976	1976	3	1976	1976
1977	1977	1977	1977	1	1977	1977
1978	1978	1978	1978	1	1978	1978
1979	1979	1979	1979	1	1979	1979 ^j
1980	1980	1980	1980	1	1980	1980
1981	1981	1981	1981		1981	1981
1982	1982	1982	1982		1982	1982 ^k
1983	1983	1983	1983		1983	1983
1984	1984	1984	1984		1984	1984
1985	1985	1985	1985		1985	1985
1986	1986	1986	1986		1986	1986
1987	1987	1987	1987		1987	1987
1988	1988	1988	1988		1988	1988 ?
1989	1989	1989	1989		1989	1989 ?

Table 1. cont. The result table

High precipitation in VII-IX + mild winter	High precipitation in VII-IX + mild winter + high annual needle loss	High precipitation in VII-VIII + mild winter	High precipitation in VII-VIII + mild winter + high annual needle loss	The number of sample trees, which NTM data cover the appropriate year	High annual needle loss in <i>Pinus contorta</i>	Important threshold and epidemic years in history attributed to <i>Lophodermium seditiosum</i>
1	2	3	4	5	6	7
1990	1990	1990	1990		1990	1990
1991	1991	1991	1991		1991	1991?
1992	1992	1992	1992		1992	1992
1993	1993	1993	1993		1993	1993
1994	1994	1994	1994		1994	1994 ?
1995	1995	1995	1995	1	1995	1995 ?
1996	1996	1996	1996	3	1996	1996
1997	1997	1997	1997	9	1997	1997
1998	1998	1998	1998	37	1998	1998
1999	1999	1999	1999	62	1999	1999^l
2000	2000	2000	2000	68	2000	2000
2001	2001	2001	2001	67	2001	2001^m
2002	2002	2002	2002	67	2002	2002
2003	2003	2003	2003	66	2003	2003
2004	2004	2004	2004		2004	2004 ⁿ

Legend: The gray field covers the years, for which the NTM data are still missing.
The bold numbers in column 7 indicate the epidemic years in forest plantations.
The bold numbers and normal black numbers in column 7 indicate the possible epidemic years in forest nurseries.
The bold numbers in column 6 indicate the years with high needle loss in the *Pinus contorta* stand.

- a – the first published information about Lophodermium needle cast in Estonia (in German),
- b – the first published record of the agent fungus in Estonia,
- c – the first published in Estonia instructions for the control of the disease,
- d – the first published information about Lophodermium needle cast in Estonia (in Estonian),
- e – k - documented epidemics of Lophodermium needle cast in Estonia.

Our experimental material (NTM data concerning the dynamics of needle loss in pines) is presented on *Figure 3*. According to our computations, including and considering, as well, the literature and other data, the definite Lophodermium needle cast epidemic years in Estonia were:

1) in young pine plantations: 1894, 1896 (the both before the first records of the disease and its agent in literature!), 1904, 1911, 1939, 1943, 1949, 1955, 1957, 1961, 1974, 1999 and 2001; and 2) in forest nurseries: 1884, 1887, 1894, 1896, 1898, 1899, 1903, 1904, 1905, 1906, 1910, 1911, 1918, 1925, 1930, 1934, 1935, 1937, 1939, 1943, 1949, 1955, 1957, 1961, 1962, 1974, 1988, 1989, 1991, 1994, 1995, 1999, 2001 and 2004.

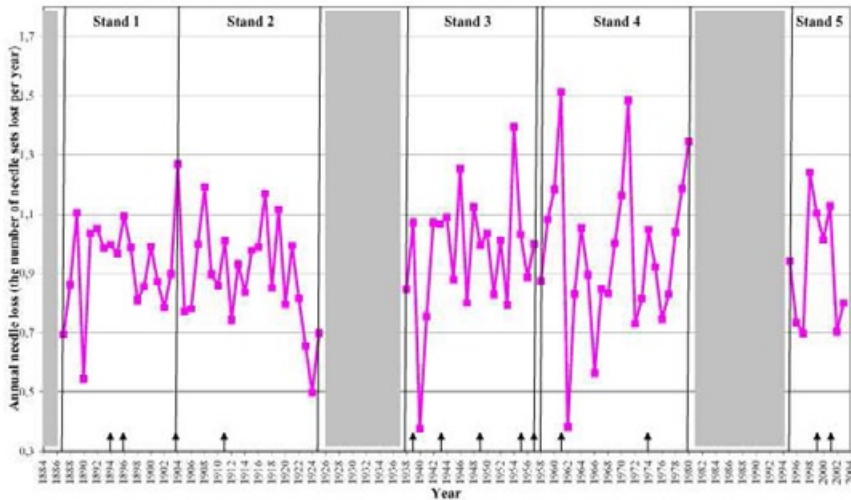


Figure 3. Annual needle loss dynamics during the young age (<25 years) of the analysed by NTM five Scots pine stands in South-East Estonia. Arrows below indicate the possible *Lophodermium* needle cast epidemic years (on the base of meteorological + NTM data). Grey fields cover the years, for which the NTM data are still missing.

Inside the long period (1884–2004) the documented in the literature (or by the services in forest pathology) epidemic years of *Lophodermium* needle cast in Estonia were 1923, 1924, 1929, 1961, 1975, 1979, 1982, 1999, 2001 and 2004 (Table 1). The years of high annual needle loss coincided with the documented epidemic years in 1961, 1979, 1999 and 2001 (Figure 3), which mean values (1,233 +/- 0,1) were higher than the long period (1887–2003) mean annual needle loss (0,966 +/- 0,013). Regarding the meteorological characteristics, the years 1961, 1999, 2001 and 2004 were selected out namely as the years, characterized by the combinations of high precipitation summer (VII–IX or VII–VIII) of the preceding year and following mild winter. At the same time the years 1923, 1924, 1929, 1975, 1979 and 1982, although documented in the literature as epidemic years, were not characterized by the supporting the pathogen preceding conditions (wet summers + mild winters). The years 1923, 1924 and 1975 (but not 1979) had low annual needle loss values compared to the long period mean. The reasons of these low values would be: a) trees in the appropriate age were not any more seriously affected by the *Lophodermium* needle cast, b) the amount of sample trees, covering the appropriate years were not sufficiently represented in the NTM material (at least 5 trees per year have to be analyzed, but e.g. the year 1979 was represented by only a single sample tree, although with high needle loss) and c) the weather data combinations were not perfectly selected for opening the potential epidemic years, in other words: we still do not know the actual needs of the pathogen. For the years 1929, 1982 and 2004 the NTM data are missing (Table 1).

Comparison of the appropriate meteorological data of documented *Lophodermium* needle cast epidemic years with the long period (1884–2004) means showed us, that: 1) the both combinations of summers of epidemic years had the mean precipitation values close to the long period mean (except of 1975), and 2) the mean winter temperatures had even lower values than long period mean in the epidemic years 1923, 1924, 1929, 1979 and 1982 (Table 2).

The low winter temperature, therefore, seems not to be a serious problem for the pathogen in raising a new epidemic, and if the level of summer precipitation is close to the long period mean, the epidemic can get start.

Table 2. Mean meteorological characteristics (summer precipitation and winter temperature) of the years preceding to the documented *Lophodermium* needle cast epidemic years

Year	Precipitation sum during VII-IX (mm)	Precipitation sum during VII-VIII (mm)	Monthly XII-III temperature (°C)
1923	216,0	189,0	-5,4
1924	270,0	185,0	-6,6
1929	329,0	217,0	-7,9
1961	186,0	157,0	-0,1
1975	190,0	145,0	-0,7
1979	381,0	272,0	-8,4
1982	247,0	181,0	-5,2
1999	292,0	268,0	-3,6
2001	237,0	225,0	-2,2
2004	201,5	187,2	-3,1
Long period (1884-2004) mean	220,1+/-6,6	157,9+/-5,5	-4,9+/-0,2

Our NTM material concerning *Pinus contorta* (stand 6) betrayed high annual needle loss values of this pine species in the years 1936, 1938, 1944 and 1961 (Table 1, column 6). These losses were caused, seemingly, by the other agents (perhaps by the starting epidemic of *Gremmeniella abietina*, which was first documented in Estonia in 1964, cf. Hanso, 1969, 1972). *P. contorta* is considered to be resistant to *Lophodermium* needle cast. However, our hopes to find clear differences in the dynamics of needle losses in these pine species (*P. sylvestris* and *P. contorta*) failed, as during some of the presumably epidemic years of *Lophodermium* needle cast (e.g. 1961) both the pine species had high needle loss. During the years of diagnostic service in the forest nursery management system of Estonia (1972-1985) we had diagnosed (by personal communication of the elder author) serious *Lophodermium* needle cast attack on *Pinus contorta* seedlings twice, in 1978 and 1979.

We can set aside the possibility, that any insect defoliator could cause the extensive needle loss of pines during the study years, as it is generally known, that insect pests accompany namely hot and dry, and not wet summers as do fungal diseases. However, we decided to look for the appropriate (i.e. hot and dry) summers and see, which kind of needle losses were following these, presumably congenial to insects, years. It was found, that all the years with hot summers were followed by the years with annual needle loss, less than the long period mean. Therefore we can believe, that none of our five Scots pine stands had lost considerable amount of needles in their youth through the insect attack.

Large scale epidemics of *Coleosporium* needle rust – another possible interfering our conclusions fungal disease of pine needles - are seldom in Estonia. Although the fungus (fungi) can be found year by year, they are few in numbers (personal communication by the elder author). Epidemics of *Hypodermella sulcigena* or *H. conjuncta* are rear, as well, in Estonia.

4 CONCLUSIONS

As the bases of the modern disease regulation (control) system in growing pine seedlings in forest nurseries can be, only and strictly, prophylactic, the prognoses must lie in the foundation of an appropriate system. A retrospective list of epidemics from the history will hopefully improve the raising of a more acceptable prognostic system than it exists today.

According to our computations by the use of NTM (needle trace method), including and considering, as well, the literature and other data, the definite *Lophodermium* needle cast epidemic years in Estonia were:

1) in young pine plantations: 1894, 1896 (the both before the first records of the disease and its agent in literature!), 1904, 1911, 1939, 1943, 1949, 1955, 1957, 1961, 1974, 1999 and 2001; and 2) in forest nurseries: 1884, 1887, 1894, 1896, 1898, 1899, 1903, 1904, 1905, 1906, 1910, 1911, 1918, 1925, 1930, 1934, 1935, 1937, 1939, 1943, 1949, 1955, 1957, 1961, 1962, 1974, 1988, 1989, 1991, 1994, 1995, 1999, 2001 and 2004.

The appropriate analyses, represented in this investigation, is not ended as there are some large periods which are not covered at all or are covered by insufficient amount of the NTM material.

Hopefully, the NTM will serve us in the future as well in the investigations, which have to elucidate the role of *Lophodermium* needle cast epidemics in the selection of surviving and failing trees during the rich-in-victims formation of a pine stand. As well the question: how large territory is occupied by a *Lophodermium* needle cast epidemic, is still not answered.

Acknowledgements: The authors acknowledge highly the encouragement from and personal participation in the field work during the felling of several model trees and separating the analyze bolts from tree stems for the NTM analyses by prof. T. Kurkela, dr. R. Jalkanen and T. Aalto. Prof. T. Kurkala and M. Vuorinen participated already in the establishment of the analyzed youngest (5.) pine plantation. K. Kell has helped the younger author (R. Drenkhan) in the laborious NTM processing work of the trees from the stand 5. K. Karoles participated in the work of diagnostic laboratory in 80-es of the last century. We thank all of them.

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First observations of *Mycosphaerella pini* in Estonia

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An aggressive disease of pines – red belt blight, caused by the fungus *Mycosphaerella pini* (anamorph *Dothistroma septospora*) was recorded for the first time in Estonia, in Järvelja Forest District, South Eastern Estonia.

Estonia is located in the centre of a geographic area subject to climatic change (Aasa *et al.*, 2002). Forests have just experienced two series' of extreme weather events, accompanied by the simultaneous deterioration of the health of different tree species (Hanso & Drenkhan, 2007). Not only has there been declining health of the tree species growing in Estonia, close to the northern limit of their natural range or introduced from the south, but even the native, comparatively cold- and drought-resistant Scots pine (*Pinus sylvestris*) has suffered (Drenkhan & Hanso, 2006). It is therefore not surprising that new invasive diseases may occur on stressed pines, including red belt blight (Woods *et al.*, 2005).

Examination of needle samples, collected in the autumn 2006 from a diseased *Pinus nigra* stand in Järvelja, resulted in the first observation of red belt blight in Estonia. Typical symptoms of *M. pini* infection (Anonymous, 2005) were observed, consisting of bright red belts, stripes and patches on pine needles, together with the conidia of the anamorph, *D. septospora* (21.6–35.8 × 1.8–2.8 µm, average 27.8 × 2.6 µm).

Before the autumn of 2007 several new records of the disease, always accompanied by the spores of *D. septospora* were made from native Scots pine in several young plantations in South Estonia. During the autumn of 2007, several ornamental *P. mugo* and *P. sibirica* trees were found that were badly affected by the disease, and one record was made from *P. ponderosa*. The teleomorph stage was not found during the first year of observations in Estonia.

Herbarium samples were deposited in the Mycological Herbarium of the Estonian University of Life Sciences, TAA(M) 178675. The first Estonian isolation of *M. pini* was made from *P. sylvestris* on the pine needle agar from the

conidia of *D. septospora* on this host. The identity of the isolation was confirmed by partial sequencing of the internal transcribed spacer (ITS) region at the Finnish Forest Research Institute and deposited in GenBank (Acc. Nos. EU330226; EU330227; EU330228). The analyses showed complete (100%) similarity with *ca.* 50 different sequences of *M. pini* in GenBank, originating from different countries (e.g. AY808291, isolated from *P. nigra* in Cracow, Poland; AY808294, isolated from *P. sylvestris* in Slovakia; and AY808295, isolated from *P. mugo* in Bavarian Alps, Germany).

Acknowledgements

We thank Prof. Jarkko Hantula (Finnish Forest Research Institute) for help with the ITS analyses and for suggestions for the manuscript.

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Growth response of young Scots pines to needle loss assessed from productive foliage

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Kurkela, T., Drenkhan, R., Vuorinen, M., Hanso, M. 2009. Growth response of young Scots pines to needle loss assessed from productive foliage. – Forestry Studies | Metsanduslikud Uurimused 50, 5–22. ISSN 1406-9954.

Abstract. The amount of productive foliage was assessed with the help of the relative significance of each annual needle set in their contribution to the growth of young Scots pines (*Pinus sylvestris*). The number of needles in the subsequent needle sets was studied retrospectively in twelve-year-old stand, and the worst needle loss years were detected. The growth rate of trees was compared with the number of needles in the annual needle sets and with the loss of needles from the assessed productive foliage. Needles in the 4th and older needle sets remaining in the trees were interpreted as useless or less important if their amount did not correlate with the growth. The second and third needle sets supported both radial and height growth. Most variation in the needle loss during 1999–2002, caused mainly by summer drought and by fungal needle cast due to *Lophodermium seditiosum*, occurred in the third annual needle set. Decrease in both radial and height increments in relation to the needle loss were apparent in the total material for 1997–2004, but needle loss reduced the annual growth significantly only in two-three years inside that period.

Key words: Scots pine, needle loss, productive needles, senescent needles, *Lophodermium seditiosum*, drought, radial growth, height increment.

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Introduction

Needle loss, shedding of needles or defoliation in Scots pine (*Pinus sylvestris* L.) may originate from one to several needle sets or needle cohorts (Ross *et al.*, 1986). Needle loss is related to growth reduction, but this relationship can not be correctly quantified unless the actual growth supporting proportion of the total foliage, i.e. productive foliage, is not specified and unless the needle loss is not related solely to that proportion. Scots pine sheds needles in northern Finland approximately one needle set (needle cohort) annually (Jalkanen, 1998; Muukkonen, 2005). It is difficult to determine the extent of growth reduction due to the defoliation if the needle loss is presented as a part of a certain annual needle set (Williams *et al.*, 2003), since more than one needle set is supporting the growth. Moreover, in natural conditions it has also been difficult or has seemed impossible to determine the amount of the shedding needles belonging to the normal annual shed of the useless to the tree senes-

cent needles, or to the productive needles shedding prematurely (e.g. Zierl, 2004). Appropriate scientific information is, therefore, very sparse.

In the artificial defoliation experiments the effect of the removal of needles had varied according to the age of the removed needles and to the season of this violent action (Linzon, 1958; O'Neil, 1962; Ericsson *et al.*, 1980; Honkanen *et al.*, 1999). The growth reduction in conifers, achieved by the artificial removal of green needles or caused by herbivory (Ericsson *et al.*, 1980; Honkanen *et al.*, 1999; Armour *et al.*, 2003; Piene *et al.*, 2003; Williams *et al.*, 2003; Kurkela *et al.*, 2005; Straw *et al.*, 2005) can not be correctly compared with the growth reduction in trees caused by abiotic stress situations (like drought) or by fungal needle pathogens (like *Lophodermium* needle cast). The reason is that the first type of needle removal is accompanied by the additional energy-consuming traumatic effects (resin flow etc.), but the second type is not.

The needle loss from young (usually < 3 years old) needle cohorts or the total needle loss correlates with the growth or growth reduction (Piene *et al.*, 2003; Williams *et al.*, 2003; respectively). This relationship, however, appears uncertain because loss percentages have not been differentiated between loss of growth supporting foliage and loss of senescent foliage. The needle cohorts of different age have different values in supporting the growth, because: i) the number of needles in the different needle sets among the total foliage is greatly different (Drenkhan *et al.*, 2006); ii) physiological activity (productivity) of different needle cohorts varies (Jach & Ceulemans, 2000, Zha *et al.*, 2002); iii) there exist seasonal functional differences in the different needle sets, e.g. current year needles do not become productive before the middle of the season and the older needles lose their activity when senescence approaches (Clark, 1961; Loach & Little, 1973; Sullivan *et al.*, 1997). Analyzing the foliage structure retrospectively by the needle trace method (NTM), we can assess the relative importance of each needle set even when some loss of needles from a definite needle set has occurred. If the number of remaining needles or, on the contrary, if the needle loss from the older needle sets does not correlate with the growth, it means that these needles did not support the growth, but were merely remaining on the tree because of slow growth (Drenkhan *et al.*, 2006).

Using the NTM, i.e. counting the number of needle traces in year rings in annual shoots toward the pith of a tree, it is possible to obtain the data concerning annual production and shed of the needles in each annual shoot. Most conveniently, the needle trace counting in pines is restricted to the main stem (Aalto & Jalkanen, 1998).

In the literature there may not exist any adequate definitions for the productive or growth supporting foliage. Many published works have described the effect of natural or artificial defoliation on the growth of trees. The needle loss there has always been described as the loss from any particular needle set (Kulman, 1971; Ericsson *et al.*, 1980; Armour *et al.*, 2003) or as the loss from the total green foliage (Alfaro *et al.*, 1982; Williams *et al.*, 2003). When the rate of defoliation is inaccurately defined, it is clear that the relationship between the needle loss and growth rate can be based only on correlations and cannot prove cause and effect (Armour *et al.*, 2003) or open sink and source (Honkanen *et al.*, 1999). Drenkhan *et al.* (2006) suggested that only three youngest needle sets in Scots pine (*P. sylvestris*) are supporting the growth in southern Finland and in Estonia.

Based on our definition of the productive, growth supporting foliage (Drenkhan *et al.*, 2006) and using the NTM, we studied foliar dynamics and quantitative effect of the annual loss in the productive foliage on the radial and height increment of young pines in a provenance experiment.

Material and methods

Needle loss and its consequences to the growth of trees, including the number of needles in the annual needle sets, were studied retrospectively in a Scots pine provenance experiment established in 1993. Six provenances were included in this study, two from Estonia and four from Finland (Table 1). The experiment was planned according to a randomized complete block design. Two-year-old nursery grown seedlings were planted in four parallel lines with 100 plants in each and with the replications in four blocks in a clear cut area in Konguta, Tartu Forest District, south eastern Estonia (58°13'N, 26°09'E). Planting density was 0.5×1 m, *Vaccinium* forest site type (Lõhmus, 2004).

In October 2004 twelve pines per block, two from each provenance, were chosen by randomized sampling among the dominant or codominant trees, which totalled 48 sample trees. After cutting of the trees the annual height increments from 1997 to 2004 were measured. At the height of 5–10 cm a disk was sawn from each stem. From the discs, annual radial increments (RI) were recorded with a tree-ring measuring system in two random directions with an accuracy of 0.01 mm. The averages of these measurements were further used as characters of radial increments of the trees. Bolts (10–25 cm) were cut from each annual shoot of the stems. The number of currently attached needles was directly counted from the youngest annual shoots. The numbers of needles which had been attached on each annual shoot in the succeeding annual rings were counted from the sample bolts using the NTM (cf. Kurkela & Jalkanen, 1990). To find needle traces in the tree rings of the previous years, the bolts of each annual shoot were handled according to the protocol presented by Aalto and Jalkanen (1998). As the single difference from the protocol the lowest bolts analyzed by NTM represented the internodes of the stems grown up one to three years after the planting.

NTM is based on the fact that needles (actually short shoots) of conifers are connected to the pith by vascular bundles through the annual rings as long as they are attached to the shoot (Figure 1). When the needles shed from the shoot the vascular bundles stop growing and can not be seen in the later (outer) tree rings. The transverse sections of these vascular bundles on the planed surface of annual rings are called needle traces. By planning ring by ring the latewood surfaces and counting the appearing needle traces, we can find the number of the needles, which have been attached during every definite year. Usually only a certain section, restricted e.g. by an angle (α) of 45 or 90 degrees from the pith is planed. The counted number is now divided by the length of the bolt and multiplied by eight or four, respectively to the angle, which gives the needle density (ND) for the bolt in the specified annual ring

Table 1. Seed origin of the tested Scots pine provenance plantations.

Tabel 1. *Katsealuste hariliku männi geograafiliste puistute seemne päritolu.*

Provenance Päritolu	Latitude, N Laiuskraad, N	Longitude, E Pikkuskraad, E
1 Saaremaa Estonia	58° 22'	23° 30'
2 Rakvere Estonia	59° 18'	26° 30'
3 Tenhola Finland	60° 03'	23° 20'
4 Ruokolahti Finland	61° 26'	28° 45'
5 Korpilahti Finland	62° 00'	25° 30'
6 Kinnula Finland	63° 32'	24° 55'

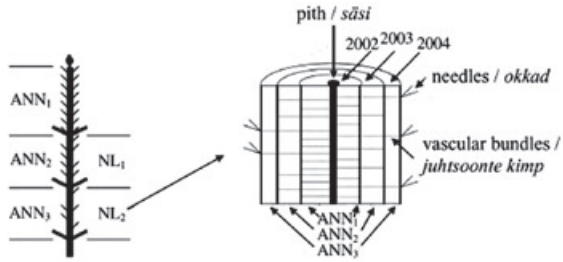


Figure 1. Schematic explanation of the origin of different needle sets (ANN_n) and needle loss values (NL_1 and NL_2) in the pine stem.

Joonis 1. Asendiskeem erinevate okka vanusklasside (ANN_n) ja neist arvatavate okkade väärtuste (NL_1 ja NL_2) kohta männi tüvelõigul.

of each annual shoot. By multiplying ND by the measured length of annual shoot we obtain the number of needles attached on each annual shoot during the successive years counted from the pith outwards (for the method, see also Armour *et al.*, 2003). As noted earlier, the number of needles in the later text actually means the number of needle pairs = short shoots.

An abbreviation, ANN_n , was used for the variable showing the number of needles in an annual needle set. Current-year needles on the main stem were marked with ANN_1 (Figure 1). At the end of each growing season the current year needles were about four months old (0.3 years-), the situation which our needle trace countings are describing. The number of the retained needles in the previous year's shoots in the same annual ring was marked by ANN_2 (1.3 years-) and ANN_3 with 2.3-years-old needles, etc. ANN_n indicates the number of retained needles in the annual shoot grown $n-1$ years before the current year shoot but still maintained in the same annual ring (see the list of symbols and abbreviations in the Appendix 1).

As reference value for the needle loss we used the number of the needles attached in the previous season on the same shoot. The original number of needles, i.e. the needles born the previous year ($ANN_{1(t-1)}$), was used as the base to compute needle loss from the second year needle set (NL_1), but NL_2 (i.e. the needle loss from ANN_3) was calculated from the ANN_2 of the previous year. To present the total effect of the needle loss we combined the second and third year needle losses into a new variable $NL_{(1-2)}$.

Consequently, annual needle losses (NL) were derived from the needle trace counts as percentages from the number of needles attached in the previous year. The combined needle loss $NL_{(1-2)}$ (from the needle sets ANN_2 and ANN_3) was computed according to the following equation:

$$NL_{(1-2)} = 100 \times (1 - (ANN_{2(t)} + ANN_{3(t)}) / (ANN_{1(t-1)} + ANN_{2(t-1)})),$$

in which t indicates the current year and $t-1$ means the year before. To test the effect of the current year needles on RI, we included ANN_1 into the $NL_{(1-2)}$ equation:

$$NL_{(1-2)} = 100 \times (1 - (C \times ANN_{1(t)} + ANN_{2(t)} + ANN_{3(t)}) / (C \times ANN_{1(t)} + ANN_{1(t-1)} + ANN_{2(t-1)})),$$

in which C is a weighting coefficient for current year needles. Alternative values

from 0 to 1.0 were given for C (1.0, 0.5, 0.3, 0.2, 0.1, and 0). The needle fall from ANN₄ was not included as a component in NL, since the most important reason for the needle fall after the third season was natural senescence. NL percentages for statistical analysis were then transformed according to the equation:

$$y = 2 \arcsin \sqrt{x}$$

Also, a categorized variable, CNL₍₁₋₂₎ with 10% intervals was used to study the effect of NL₍₁₋₂₎ (Table 2).

Assuming that six whorls from the top could include the most productive foliage (Kellomäki & Oker-Blom, 1983), we constructed a model for the six uppermost branch whorls (Drenkhan *et al.*, 2006). The model was based on the assumption that the annual shoots in each branch decrease exponentially in the succeeding years and according to the branching order. The first order branches were *p* times the leader shoot (for example *p* = 70%). The next order shoots in the same branch were again 70% of the previous shoot. Further we assumed that the top end of each branch produced five side branches being again 70% of the tip shoot.

The cause of the needle loss is not possible to determine from the NTM data. We considered the loss from the third needle set (the difference between ANN_{3(t-1)} and ANN₄) as the shedding of the senescent needles since the fourth needle sets (ANN₄) had only a few needles attached throughout the study period. In the period from 1999 to 2002, there were two main causes for the premature shedding of pine needles, drought and fungal needle cast (*L. seditiosum*). In 2001 the needle loss was caused mainly by *L. seditiosum* (Hanso & Hanso, 2001) and in 2002 by the late summer drought (Drenkhan & Hanso, 2006).

Annual differences in radial (RI) and height increments (HI) were analyzed statistically in multivariate general linear models (GLM) using the needle loss as independent covariant or, alternatively, from the needle loss data transformed to a categorized variable with ten percent intervals. Since the NTM-variables were derived from the same needle trace counts, only one NTM-variable could be included in the same model describing the growth. No standardization was used for the increment and needle data. Statistical operations were performed according to SYSTAT® (2000) software. Symbols and abbreviations are listed in the Appendix 1.

Table 2. Needle loss categories used in studying the effect of NL₍₁₋₂₎ from the growth supporting foliage (ANN₂ and ANN₃) on tree growth. N = the number of observations, mean % = mean NL₍₁₋₂₎ percentage for each needle loss class.

Tabel 2. Okkakao kategooriad, milliseid kasutati puu kasvu tagavast okastikust (ANN₂ ja ANN₃) pärineva okkavarise NL₍₁₋₂₎ mõju uurimiseks puu kasvule. N = vaatluste arv, mean % = keskmine NL₍₁₋₂₎ protsent igas okkakao klassis.

Needle loss percentage class of CNL ₁₋₂ Okkakao protsendi klass, CNL ₁₋₂	Class code Klassi kood	N	Mean, % Keskmine, %
0–10	1	148	4.68
10.1–20	2	77	15.39
20.1–30	3	63	24.48
30.1–40	4	45	34.73
40.1–50	5	30	45.26
>50	6	10	57.14
Total / Kokku		373	18.53

Results

State of needle sets and needle loss

The mean number of the current year needles (= short shoots) per shoot (ANN_1) for the total material was 239. In most years our sample trees had their oldest needles in the fourth needle set (ANN_4) which, however, represented as a mean only 2.6% of the total number of needles attached to the main stem (Table 3). One single tree in one year had some needles in the fifth needle set (ANN_5). As expected, the number

Table 3. Mean number of the needles in the annual needle sets (ANN_n) attached in the main stem in the autumn by the provenances.

Tabel 3. Keskmise sügisel registreeritud okaste arv geograafiliste päritolude kaupa puude peatüvedele kinnitunud okaste vanusklassides (ANN_n).

Provenance / Päritolu	ANN_1	ANN_2	ANN_3	ANN_4	ANN_5
1	261.9	245.2	156.1	13.1	0.0
2	229.7	216.6	133.0	11.7	0.0
3	225.2	208.5	118.1	15.0	0.0
4	213.5	200.1	138.6	17.5	0.3
5	221.0	207.7	136.1	14.8	0.0
6	191.7	180.6	116.9	20.9	0.0
Mean / Keskmine	239.1	217.0	133.6	15.5	0.1

of needles in different needle sets on the main stem varied during the learned period (1997 to 2004, cf. Figure 2). ANN_1 remained below ANN_2 in 1999 and 2000. ANN_3 had the lowest value in 2002. In about six years after the planting the proportion of needles in each needle sets (ANN_1 to ANN_4) had set to their own level in the pines constructed according to the model including the six uppermost branch whorls. In the model trees ANN_1 and ANN_2 varied around 40%, ANN_3 slightly below 20%, and the rest were for ANN_4 .

During the entire study period, no needles from the current year needle sets were lost in the autumns. The average needle loss NL_1 in ANN_2 from ANN_1 of the previous year was 3.5% and by the third autumn (ANN_3) the loss (NL_2) from the previous years' ANN_2 was 35.7%. NL_2 had the highest average values 67.5% in 2002, and was around 40% in 1999-2001. Before 1999 and after 2002 NL_2 was significantly lower ($p < 0.001$), 21 and 18%, respectively (Figure 3). The average combined needle loss $NL_{(1-2)}$ was 18.5%. Among individual trees the differences in NL_1 , NL_2 and $NL_{(1-2)}$ were highly significant ($p < 0.001$), varying annually from 0 to 100%.

Radial increment

The average annual radial increment (RI) of the sample trees was 2.4 mm per year. Among the provenances, there were some differences but without a clear north-south gradient. The highest average growth was registered in 1998, 3.0 mm, and the lowest in 2003, 1.8 mm. Radial increments had a decreasing trend from 1997 to 2004, especially after canopy closure in 1999-2000. ANN_2 and ANN_3 were in a significant positive relationship with RI, when analysed separately with year and two categorical variables $RI = \text{constant} + \text{Provenance} + \text{Block} + \text{Year} + ANN_n$. The effect of ANN_4 was insignificant ($p = 0.179$). ANN_1 correlated significantly with RI, but possibly without mutual relationship.

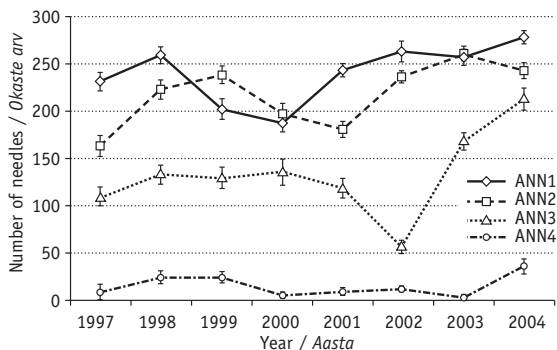


Figure 2. Average number of the needles counted on the main stem and belonging to the different needle sets (ANN_n). Vertical bars show the standard error of the mean.

Joonis 2. Keskmine erinevatesse okka-vanusklassidesse (ANN_n) kuulunud okaste arv puutüvel. Vertikaalsed jooned näitavad aritmeetilise keskmise standardvigu.

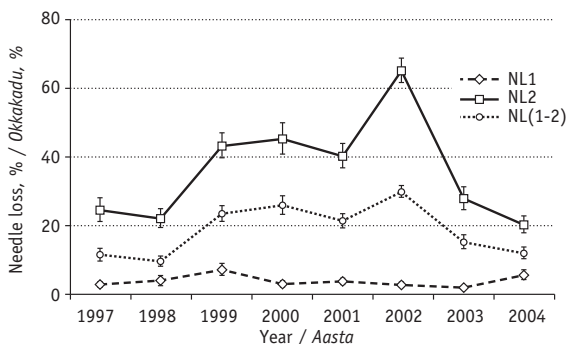


Figure 3. Annual needle loss (%) in the experimental stand in Konguta. NL_1 = needle loss in the second year needle set (ANN_2), NL_2 = needle loss in the third year needle set (ANN_3), and $NL_{(1-2)}$ = the combined needle loss from the second and third year needle sets. Vertical bars show the standard error of the mean.

Joonis 3. Aastane okkakadu (%) Konguta katsepuistus. NL_1 = aastane okkakadu teise vanusklassi okastikust (ANN_2 -st), NL_2 = aastane okkakadu kolmanda vanusklassi okastikust (ANN_3 -st) ja $NL_{(1-2)}$ = kombineeritud okkakadu teisest ja kolmandast vanusklassist. Vertikaalsed jooned näitavad aritmeetilise keskmise standardvigu.

Height increment

The provenances had similar rates of height growth (HI) with the mean annual growth varying between 39.5 and 29.2 cm, the lowest characterising the northernmost provenance, Kinnula. After canopy closure in 1999-2000, in contrast to the RI, the HI had a clear accelerating trend. ANN_2 and ANN_3 affected significantly HI ($p < 0.001$), but ANN_4 did not.

Effect of needle loss

The relationship between the needle loss and growth rate was analysed with the model: Growth = constant + Provenance + Block + AsinNL + Year. In the total material the growth decreasing effect of NL_2 appeared to be higher for RI ($p < 0.001$) than for HI ($p = 0.012$). The loss (NL_1) in the second needle set had no significant effect on the growth. The effect of $NL_{(1-2)}$, the combined needle loss from ANN_2 and ANN_3 decreased significantly both RI and HI, with p-values < 0.001 and $= 0.001$, respectively.

The effect of needle loss on the growth rate varied annually. Needle loss was at a relatively high level from 1999 to 2002, but the effect on RI and HI was different. RI was affected significantly by the needle loss (NL_2) in 2000 and 2001 and by $NL_{(1-2)}$ in 2001 (Figure 4). Needle loss in the constructed model trees decreased slightly significantly RI and HI also in 1999 ($p = 0.016$ and 0.046 , respectively). In 1997 and in 2001 there was a significant decrease in HI, associated with the both needle loss variables (NL_2 , $NL_{(1-2)}$) (Figure 5). Although the needle loss was at the highest level ($NL_2 = 65.4\%$) in 2002, GLM-models revealed no significant effect of NL on the growth (both HI and RI). Needle loss had a lagging effect on RI, which was statistically significant in 2001 and 2002 ($p = 0.021$ and 0.005 , respectively). The lagging effect on HI was not significant in either year.

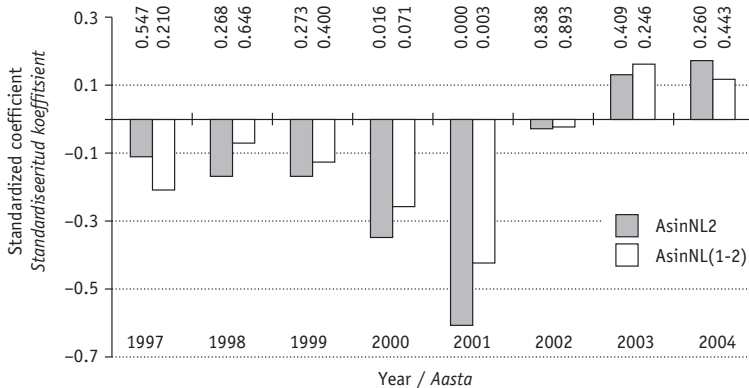


Figure 4. Effect of needle loss on the radial growth (RI) during the study period. The standardized coefficient of regression (ordinate) shows the relative effect of needle loss obtained with the equation: Growth = constant + Provenance + Block + AsinNL. $AsinNL_2$ represents needle loss from ANN_3 , and $AsinNL_{(1-2)}$ represents the combined needle loss of NL_1 and NL_2 . Standardized coefficients are obtained in computing a stepwise regression and are called beta weights by some social scientists (SYSTAT 2000). The numbers in vertical alignment show p-values of the effect of needle loss.

Joonis 4. Okkasko mõju radiaalkasvule (RI) antud uuringuperioodi jooksul. Standardiseeritud regressioonikoefitsient (ordinaat-teljel) näitab okkasko suhtelist mõju, mis on arvatatud valemiga: Kasv = konstant + päritolu + plokk + AsinNL, kusjuures $AsinNL_2$ = tähistab okkaskadu ANN_3 -st ja $AsinNL_{(1-2)}$ tähistab kombineeritud okkaskadu NL_1 -st ja NL_2 -st. Standardiseeritud koefitsientid saadakse sammuviisilisel regressioonil ning neid nimetatakse sotsiaalteadustes ka beeta-kaaluks (SYSTAT 2000). Numbrid vertikaaljoonel näitavad okkasko mõju p-väärtusi.

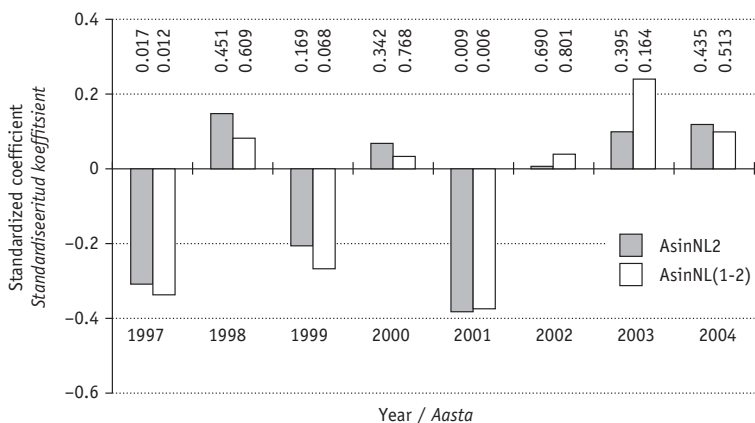


Figure 5. Effect of needle loss on the height increment (HI) during the study period. The standardized coefficient of regression (ordinate) shows the relative effect of needle loss obtained with the equation: Growth = constant + Provenance + Block + AsinNL. AsinNL₂ = represents needle loss from ANN₃, and AsinNL₍₁₋₂₎ represents the combined NL of NL₁ and NL₂. The numbers in vertical alignment show p-values for the effect of needle loss.

Joonis 5. Okkakao mõju kõrguskasvule (HI) antud uuringuperioodi jooksul. Regressiooni standardiseeritud koefitsient näitab suhtelist okkakao mõju, mis on arvutatud valemiga: Kasv = konstant + päritolu + plokk + AsinNL, kusjuures AsinNL₂ = kujutab okkadu ANN₃-st ja AsinNL₍₁₋₂₎ tähistab kombineeritud okkadu NL₁-st ja NL₂-st. Numbrid vertikaaljoonel näitavad okkakao mõju p- väärtusi.

We also tested the dependence of RI from the needle loss when ANN₁ was included partially in the formula for computing the needle loss, giving different weight to the current year needles. Supposing that the current year needles do not affect RI during the whole season, we weighted the number of current year needles by C having different values from one to zero when computing the value for needle loss. Overall, the combined needle loss value, NL₍₁₋₂₎ gave the highest explanation for the decrease in RI ($F = 13.492$, $p < 0.001$) when the current year needles (ANN₁) were totally omitted ($C=0$) as a component in the computations of NL-percentages (Material and Methods).

The quantitative effect of needle loss on RI was studied in the data of 1999-2001 with the categorized variable CNL₍₁₋₂₎ derived from NL₍₁₋₂₎ with ten percent intervals. In these three years the needle loss had a relatively high effect on RI ($p = 0.002$). Using GLM (Growth = constant + Provenance + Block + CNL₍₁₋₂₎) we obtained the growth values for different categories of NL. GLM for RI was computed separately for each year (1999-2001) and adjusted to the same level with the help of the annual mean growth (Snedecor & Cochran, 1968). The regression line of the growth data (18 data points obtained), when plotted against NL₍₁₋₂₎-class means, revealed that at the level of 50% NL₍₁₋₂₎ decreased RI as a mean by 39.2% in these three years (Figure 6).

Similarly, the effect of NL₍₁₋₂₎ on HI was computed for the data of 1997, 1999 and 2001, when NL₍₁₋₂₎ had relatively high effect on HI. Sixteen data-points obtained were plotted against ten-percent classes of NL₍₁₋₂₎, and the regression line for these points revealed 34.8% decrease in HI with the needle loss of 50% (Figure 7).

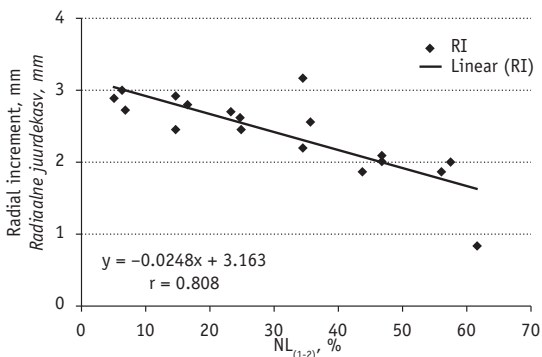


Figure 6. The relationship between the needle loss, represented in ten percent categories (= needle loss classes), and radial growth. The dots represent the means of the appropriate categories (needle loss classes, adjusted for RI) for the three succeeding years, 1999–2001.

Joonis 6. Seos okkakao (jagatuna 10% kaupa kategooriateks või okkakao klassideks) ja puude radiaalkasvu vahel. Punktid näitavad kolme järjestikuse aasta (1999 – 2001) kohta arvutatud okkakao klasside keskmiste väärtuste mõju puude radiaalkasvule.

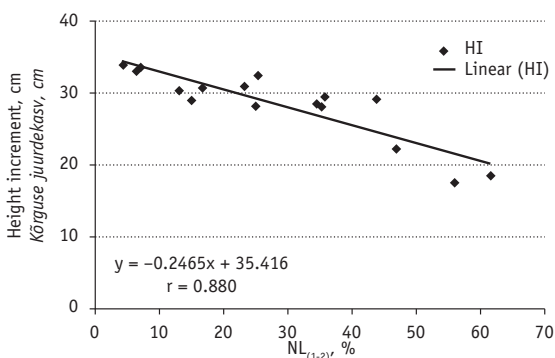


Figure 7. The relationship between the needle loss, represented in ten percent categories, and height growth. The dots represent the means of the appropriate categories (needle loss classes, adjusted for HI) for three selected years, 1997, 1999, and 2001.

Joonis 7. Seos okkakao (jagatuna 10% kaupa kategooriateks või okkakao klassideks) ja puude kõrguskasvu vahel. Punktid näitavad kolme valitud aasta 1997, 1999 ja 2001 kohta arvutatud okkakao klasside keskmiste väärtuste mõju puude radiaalkasvule.

Discussion

Scots pine has various numbers of needle sets attached, which seems to be regulated by the local site conditions (e.g. Reich *et al.*, 1996) and by climatic factors (Jalkanen *et al.*, 1995; Xiao, 2003; Insinna *et al.*, 2007a). In the previous paper Drenkhan *et al.* (2006) showed that only the three youngest needle sets correlated positively with the growth, in conditions prevailing in the studied sites in Estonia and southern

Finland, and the effect of the older needle sets appeared to be negligible. The needles in the fourth or fifth needle sets may still assimilate if remaining on the tree, but the younger needles appear to be physiologically most active (Oleksyn *et al.*, 1997; Sullivan *et al.*, 1997; Zha *et al.*, 2002). In pines, it is uncertain whether the changes in the physiological capacity of ageing needles are the result of general degradation or whole-plant reallocation of resources (Muukkonen, 2005). Possibly, the older needles serve as storage for surplus nutrients if assimilation is impeded for some reason. For example, Scots pine definitely stored nitrogen during the winter in the needles grown the previous summer (Millard *et al.*, 2001). Normal senescence of the oldest needles of Scots pine in Estonia occurs in September (Sepp, 1959, Hanso, 1970). The fall of senescent needles in the third autumn (NL₃) was not included in the loss of productive needles. The loss from ANN₄ seems not to be connected with the growth losses. This needle set may merely influence the dry matter production for the new tissues in the next season, if the accelerated reallocation of nutrients stored in older needles is required (Nambiar & Fife, 1987; Pensa & Sellin, 2003).

Since the current year needles mature after the shoot elongation, they apparently can not support the current year height increment. Similarly, they can support the radial increment only during the latter half of the growing season (Clark, 1961; Ericsson *et al.*, 1980). By this reasoning, only the second and third year needle sets seem to be most important for the current year growth. It seems that the studies on the relationship between the current foliage and growth, or on the effect of needle loss on the growth, should be focused on that most important part of the foliage of Scots pine.

The needle cast fungus *Lophodermium seditiosum* Minter, Staley and Millar affects both height and radial increment (Wühlisch & Stephan, 1986). The fungus infects needles in the second half of summer and autumn, and the diseased needle fascicles shed during the next spring, often before the beginning of the new growth (Diwani & Millar, 1987). In Estonia, after a hard *L. seditiosum* epidemic in 1961 the massive shedding of damaged needles occurred in the first half of June (Hanso, 1970). In every case a great part of the most actively assimilating foliage is lost, together with the nutrients stored in the shedding needles.

Another frequent stressing factor in Estonia – summer drought – mostly reaches the critical level during the later half of the season (Lagergren & Lindroth, 2002). In the current season, therefore, it may affect only the radial growth (Pichler & Oberhuber, 2007).

Ericsson *et al.* (1980) found that even quite drastic needle removal did not cause an immediate decrease in growth, but it occurred merely with a two-year lag. Growth loss after the defoliation seems to be directly proportional to the loss of productive needles (Van Sickle, 1974; Plattner *et al.*, 1999; Långström *et al.*, 2001). As shown before, in our material the two youngest needle sets in the spring belonged to the productive mass of the foliage. That is also supported by the facts that: i) the elongation period of the current year shoots in the northern conifers may be completed approximately in the middle of the season (Clark, 1961; Kurkela, 1980), and ii) two- and three-year-old needles are not much different in their physiological activity (Oleksyn *et al.*, 1997; Zha *et al.*, 2002; Straw *et al.*, 2005).

The role of needles in the lateral branches can not be neglected as well, because in the branches the proportion of different needle sets may vary from that in the main stem. It could be more correct to count all needles in the uppermost whorls in each needle set in order to reveal the total effect of NL on the growth. Assuming that six

whorls from the top could include the most productive foliage (Kellomäki & Oker-Blom, 1983), we constructed a foliage model for the tops of young pines. Needle loss values obtained using this hypothetical number of needles gave similar results concerning the growth losses as our original needle loss variables which did not include errors generated from the model. For that reason we concentrated to the original NL-variables in our analyses.

Regression between the needle loss and the growth characteristics gave different values depending on the categorization of the needle loss percentages. Six categories with the 10% intervals (Table 2) were chosen to avoid the effect of the few deviations with high needle loss values. Supposing that needle loss less than 10% does not cause any growth reduction (Jalkanen, 1998), the estimation of the growth reduction should be compared to that variant (the growth with needle loss from 0 to 10%). This yields a regression line with 2 to 3% less growth reduction than if the line would start from the zero point. Moreover, as the growth reduction could be expected to follow a sigmoid curve, the low level needle loss causes a negligible growth reduction. The growth reduction would be steepest when the needle loss is about 50%. Apparently at least some of the needles lost to summer drought could have been productive in the beginning of the season, and the use of the stored material from the previous season might have some compensating effect for the lost assimilation capacity. Therefore 50% growth reduction was not reached by the 50% needle loss.

Early season needle loss from ANN₂ and ANN₃ apparently causes the highest reduction in the current season growth. This kind of needle loss is typically caused by the needle cast fungi, e.g. *L. seditiosum*. *Lophodermium* needle cast caused a heavy needle loss in Estonia in 2001 (Hanso & Hanso, 2001). Late season needle loss, like that caused by summer drought, may decrease the growth in the next season, apparently detected in RI in our total material as a lagging effect. Although in 2002 the late season drought caused a high needle loss (Drenkhan & Hanso, 2006), its effect was not revealed in our examinations of either the current or the next year's growth, apparently because there were not much differences in NL between the trees. However, NL in 2000 and 2001, lagging by one year, affected the radial growth almost as strongly as it did to the current year growth, suggesting that the lag effect was real and not connected only with the autocorrelation of the variables. Low growth during the recovery period as a lagging effect of defoliation has been found to last several years in some cases (Kurkela, 1981; Wühlisch & Stephan, 1986; Jalkanen *et al.*, 1994; Lyytikäinen-Saarenmaa *et al.*, 2003).

The lagging effect of NL could also be expected in the height increment but it was not revealed there. The explanation may be that, starting with crown closure, the trees are allocating more resources to the height than to the radial growth. Early senescence in ANN₃, because of the late summer drought, might have no observable effect, direct or lagging, on the growth since buds for the next season's growth are formed during the shoot elongation period. Unfortunately, we could not separate for each year the early season needle loss caused by fungal disease from that caused by summer drought in late season.

Supposing that the current year needles mature by the beginning of July and are productive only in the latter half of the current season (see Clark, 1961, Loach & Little, 1973), we weighted the number of current year needles with various coefficients in determining the total needle loss. After some statistical tests we had to totally exclude the current year needles from the foliage supporting both height and radial growth of trees. Inclusion of the current year needles into the NL-formula as a component

decreased the explanation value of needle loss in the seasons of heavy needle loss. It is not surprising since only about ten percent of the annual radial growth is coming up after the maturation of current year needles in the middle of July (Leikola, 1969; Ericsson *et al.*, 1980; Renninger *et al.*, 2006; Insinna *et al.*, 2007b). In several earlier studies the needle loss from different age classes weighted equally when analyzing the response of the growth of trees to the needle loss (e.g. Piene, 1989). It may give results that are incomparable among different studies. The number of needles in different needle sets (age classes) is very different. Therefore, the growth-inhibiting defoliation should be determined solely as the lost proportion from the effective foliage, and the only reference level should be the number of needles during the previous season for each particular needle set. Applying an appropriate coefficient for the efficiency of each needle set may still improve the models for studying the growth response to the defoliation (Jach & Ceulemans, 2000).

High leaf longevity in pines occurs generally in connection with the slow growth rate (Schoettle, 1990; Jalkanen *et al.*, 1995; Pensa & Jalkanen, 2005; Drenkhan *et al.*, 2006). In the model describing height increment, the effect of the ageing of needles could not be used in the same model with NL since NL had strong correlation with the age of the needles.

During heavy epidemics, *L. seditiosum* may almost equally infect needles in all age classes (e.g. Kurkela, 1979). However, the intensity of infection often increases with the age of the needles (Kowalski, 1982). *L. seditiosum* was considered to be the main reason for needle cast in 2001 and summer drought was very apparent in the foliage in 2002. We interpreted this shedding from the second needle set (appearing in 1.3-year-old needles) as premature, because most of the senescent needles in the sample trees shed at the end of their third growing season (i.e. older than 2.3 years).

Conclusions

The results of this study: i) indicate that needle loss at different times of the season may affect differently the height and radial growth, and ii) represent the first report where long-run needle loss solely from the productive foliage was retrospectively assessed and used to explain the effect of the needle loss on the annual growth rate of young Scots pines. In the earlier studies concerning the impact of artificial defoliation or herbivory on the growth rate of conifers, the authors did not present the appropriate definition for the productive foliage nor use it to compute the proportion of total lost foliage. The present results also confirm earlier observations that only the three youngest needle sets correlate with both radial and height increment in Scots pine, but the current year needles have very limited effect even on the radial growth. It seems that only needle loss in the early season, expressed as percentages from the physiologically active (productive) foliage, can explain the relationship between the needle loss and the current year growth. Needle loss in the late season has a minimal effect in the current season, but it may reduce more the radial growth in the next season. To distinguish the early seasons' and late seasons' needle loss from each other when using the NTM, one should count the needle traces separately at the outer surface of the late wood and, additionally, at the surface between the early and late wood. That was not done in our material since many of our sample trees had quite thin annual rings. Generally, more studies and more modified methods are needed to confirm our findings.

Acknowledgements. The authors gratefully acknowledge Mr. Terry Bush (Madison, Wisconsin, USA) for review of the English in this article. The study was supported partly by the target financing project SF0170021s08 of the Estonian Ministry of Education and Research and project of the Estonian Environmental Investments Centre.

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Appendix 1. Symbols and abbreviations.

Lisa 1. Sümboolid ja lühendid.

Symbol / Abbreviation Sümbol / Lühend	Definition / Definiitsioon
ANN _n	annual number of needles in the n th needle set (downward from the top) attached on the stem
ANN ₁	number of current year needles, age in October four months or 0.3 years
ANN ₂	number of second year needles, age 1.3 years
ANN ₃	number of third year needles, age 2.3 years
ANN ₄	number of fourth year needles, age 3.3 years
AsinNL ₂	arc sine transformation of NL ₂
AsinNL ₍₁₋₂₎	arc sine transformation of NL ₍₁₋₂₎
CNL ₍₁₋₂₎	the percentage classes (with 10% intervals) of NL ₍₁₋₂₎ , combined from NL ₁ and NL ₂ (Table 2)
GLM	multivariate general linear model
HI	height increment (height growth of tree), cm
RI	radial increment (radial growth of tree), mm
ND	needle density, number of needles/cm
NL	needle loss, %
NL ₁	needle loss from ANN ₂ , difference between ANN _{1(t-1)} and ANN _{2(t)} , %
NL ₂	needle loss from ANN ₃ , difference between ANN _{1(t-2)} and ANN _{3(t)} , %
NL ₍₁₋₂₎	needle loss percentage combined from ANN ₂ and ANN ₃
t	the current year

Noorte harilike mändide kasvu sõltuvus produktiivse okastiku kaost

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Kokkuvõte

Okkakadu ehk defoliatsioon on rahvusvaheliselt kasutatav oluline tunnus puude ja puistute seisundi hindamiseks. Samal ajal kui okkakao laiemat ökoloogilist tähtsust metsa elus on uuritud suhteliselt palju, on teadmised okkakao produktiooni-bioloogilisest aspektist veel ebapiisavad. Heitlehiste puudega võrreldes on mitme-aastase okastikuga okaspuude lehestiku ja selle kaotuse (ebasoodsate ilmatikuolude, haiguste ja kahjurite läbi) mõju uurimine puude juurdekasvule tunduvalt keerukam. Käesolevas uurimistöös püüti aga just seda teha.

Okkakao saadus – okkavaris sisaldab alati erivanuselisi (s.o. erinevatest okka-aastakäikudest pärinevaid) okkaid, nii füsioloogiliselt vanu, puule tarbetuid kui ka alles aktiivseid (produktiivseid) okkaid. Seepärast tuleb okkakao mõju täpsemaks kindlakstegemiseks puude juurdekasvule arvesse võtta üksnes kadusid produktiivsest okastikust. Mändide juurdekasvu uuringuid okaste vägivaldse eemaldamise (noppi-mise ja lõikamise), samuti okkatoiduliste kahjurputukate hulgipaljuse järel on maailmas tehtud mitmeid. Neid ei saa aga võrrelda okkahaiguste ja põuakahjustuste järgsete juurdekasvumuutuste uuringutega, sest esimesed mõjurid põhjustavad puudele selliseid energiakadusid (vaiguerituse, jms. näol), mida teised ei põhjusta.

Käesolevas artiklis, kasutades selleks nn. okkajälje meetodil (NTM = Needle Trace Method) saadud uurimistulemusi Tartu metskonda Kongutale (58°13'N, 26°09'E) rajatud katsealadelt, analüüsitakse noorte harilike mändide (*Pinus sylvestris* L.) okkakao mõju puude juurdekasvule ajavahemikus 1997 kuni 2004, sealhulgas ka kahe olulisema noorte mändide okaste kadu mõjutava keskkonnateguri – põua ning männi-pudetõve (haigustekitaja *Lophodermium seditiosum* Minter, Staley and Millar) toimetel.

Produktiivne okastik määratleti selle alusel, kas okaste arv erivanuselistes okka-vanusklassides (ANN_n) seondus puude radiaal- ja kõrguskasvuga või mitte. 12 aasta vanustes hariliku männi geograafilistes kultuurides määrati okkajälje meetodil (NTM – Needle Trace Method) retrospektiivselt nii okaste arv erinevates okka-vanusklas-sides kui ka karmimad, põhiliselt männi-pudetõvest (haigustekitaja *Lophodermium seditiosum*) ning põuast tingitud okkakao aastad. Töös uuritud mudelpuud raiuti 1993. a. rajatud geograafilistest männikultuuridest 2004. aasta oktoobris. Seeme vii-maste rajamiseks pärines transsektilt, mis algas Eestist ja ulatus Põhja-Soomeni. Ana-lüüsi kokku 48 mudelpuud (kahest Eesti ja neljast lõunapoolsemast Soome männi geograafilisest algupärast, antud uuringus täpsemat päritolu eristamata).

Uurimistöö tulemused kinnitavad, et männi radiaal- ja kõrguskasvu reageeri-mine produktiivsete okaste kaole sõltub okaste varisemise aasta-ajast. Esmakord-selt õnnestus retrospektiivselt hinnata üksnes produktiivsest okastikust pärinevate okaste kao mõju noorte mändide kasvule mitme üksteisele järgneva aasta jooksul. Nimetatud kaheksa-aastaselt perioodil, mil suurim okkakao varieeruvus registreeriti aastatel 1999-2002, vähenes produktiivsest okastikust tingitud radiaal- ja kõrguskasv mändidel oluliselt ainult kahel-kolmel aastal. Regressioonanalüüsi tulemused näita-sid, et 50%-line okkakadu produktiivsest okastikust vähendas puude radiaalkasvu 39,2% ja kõrguskasvu 34,8% võrra.

Käesolev uuring kinnitab, seejuures okkajälje (NTM) meetodikat kasutades, et vaid kolme noorima okka-vanusklassi okkad seonduvad Eestis ja Lõuna-Soomes mändide radiaal- ja kõrguskasvuga. Noorimate, s.o. jooksva aasta okaste mõju puude juurdekasvule on piiratud, avaldudes vaid puude radiaalkasvus. NTM-uuringuid oli maailmas seni tehtud vaid vanematel puudel.

Received April 30, 2009, revised October 6, 2009, accepted October 22, 2009



***Diplodia pinea* is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia**

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Diplodia pinea (syn. *Sphaeropsis sapinea*), one of the most common and widely distributed pathogens of conifers worldwide (Whitehill *et al.*, 2007), was found in Estonia for the first time in the autumn of 2007. The last decade is characterised by the fast movement of this pathogen from southern Europe to the north. Generally, a southern fungus moves north during drought periods: it is known that *D. pinea* can be released from its quiescent stage in the host by host water stress (Stanosz *et al.*, 2001). Hard drought in 2003 encouraged *D. pinea* to become epidemic in Central Europe (e.g. Blaschke & Cech, 2007). Similarly hard droughts were registered in Estonia in 2002 and 2006, following the general trend of climatic change.

In September 2007 *D. pinea* was found on the fallen cones of a middle-aged Austrian pine (*Pinus nigra*) tree in Järvelja (southeastern Estonia). Cones of an old Austrian pine 3 km to the west and of another middle-aged tree 3 km to the east were uninfected. During the same autumn, fallen Austrian pine cones in Tartu, Tallinn and Pärnu were not observed to be infected. By the middle of May 2008 the fungus was already documented from all the three Austrian pines in Järvelja, but not from the towns. However, by the middle of September 2008 *D. pinea* was recorded in Pärnu and in Mõnumaa island (Päidaste), where there is a single large stand of old Austrian pine. How the fungus entered Estonia is not known – this may have been through insect vectors (Whitehill *et al.*, 2007) or, more probably, by human activities (Burgess *et al.*, 2004).

Conidial dimensions in the sample from Järvelja were 30.6–44.7 (47.1) × (11.8) 14.1–16.8 µm. This sample was deposited in the Mycological Herbarium of the Estonian University of Life Sciences, TAA(M) 178

688. The identity of the *D. pinea* isolate was confirmed by partial sequencing of the internal transcribed spacer (ITS) region at the Finnish Forest Research Institute and deposited in GenBank (Accession No. EU330229). Comparison showed 100% similarity with thirty *D. pinea* sequences in GenBank (e.g. DQ458895, isolated from *P. nigra*, the Netherlands; DQ458898, *P. patula*, Indonesia; and DQ458897, *P. radiata*, South Africa).

Acknowledgements

We thank Prof. Jarkko Hantula (Finnish Forest Research Institute) for help with the ITS analyses.

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Recent invasion of foliage fungi of pines (*Pinus* spp.) to the Northern Baltics

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Drenkhan, R., Hanso, M. 2009. Recent invasion of foliage fungi of pines (*Pinus* spp.) to the Northern Baltics. – Forestry Studies | Metsanduslikud Uurimused 51, 49–64. ISSN 1406-9954.

Abstract. In the wake of severe climatic extremes during recent years, several new invasive foliage fungi of pines have been reported in Estonia. In this paper the peculiarities of the fast dissemination process, particularly from south to north of two quarantined species, *Mycosphaerella pini* and *M. dearnessii*, but also of *Diplodia pinea* and *Cyclaneusma minus* on different host species are described. These species were known as destructive pathogens in several countries and continents. By today, the range of *M. pini* has already reached northern Finland. In its dissemination process we could follow, with the half-year steps, colonization of Estonia from south to north. Some micro-morphological features of these alien species were investigated. Hypothetical risk for pine forests was shortly introduced.

Key words: invasive foliage fungi, *Mycosphaerella pini*, *Mycosphaerella dearnessii*, *Diplodia pinea*, *Sphaeropsis sapinea*, *Cyclaneusma minus*, *Pinus sylvestris*, *Pinus nigra*, *Pinus mugo*, climate change

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Introduction

In the field of invasive ecology, fungi have received scant consideration until recently (Desprez-Loustau *et al.*, 2007). In the wake of worldwide climatic extremes during the last few decades (*e.g.* San Miguel-Ayaz *et al.*, 2000; Logan *et al.*, 2003), including the severe drought of 2003 in Central and Western Europe (Zaitchik *et al.*, 2006; Blaschke & Cech, 2007; Pichler & Oberhuber, 2007), attention to alien pathogens has grown enormously. In addition to several local monitoring programmes (*e.g.* Immler *et al.*, 2007), an all-European network FORTHREATS* was created to identify the potential threats to forests, including alien pathogens, their origins and paths of movement.

In recent years, on the background of climate change (Jaagus, 2006; Walther & Linderholm, 2006; Hanso & Drenkhan, 2007a) several new invasive foliage fungi including two quarantined pathogens (*Mycosphaerella pini* and *M. dearnessii*), have been reported on pines (*Pinus* spp.) in Estonia (Hanso & Drenkhan, 2007b, 2008a, 2008b, 2009). *Diplodia pinea* (syn. *Sphaeropsis sapinea*), which is one of the most common and widely distributed pathogens of conifers worldwide (Whitehill *et al.*, 2007) and *Cyclaneusma minus* were also found for the first time (Hanso & Drenkhan, 2009;

* European Network on emerging diseases and threats through invasive alien species in forest ecosystems under the Sixth Framework Programme for European Research & Technological Development of the EU, Priority 8.1, SSP.

Hanso & Hanso, 2003, respectively). All of these species have been described as serious pathogens in several countries and continents, confirming a fast spread of their ranges across the world, including Europe. The aims of this investigation were: 1) to follow the rate and the moving pathway during the colonization process of different hosts in Estonia (and northern Latvia) by these alien species, 2) to present some micro-morphological characteristics of these southern fungi in the northern Baltics, 3) to pose a hypothetical risk of these pathogens on the basis of the production-biological investigations carried out by the use of needle trace method (NTM, cf. Kurkela & Jalkanen, 1990) and compared with the kind of damage of the hitherto most dangerous needle disease – the pine needle cast (*Lophodermium seditiosum*).

Material and methods

The pathogens (*Mycosphaerella pini*, *Mycosphaerella dearnessii*, *Diplodia pinea* and *Cyclaneusma minus*) were recorded and their spread investigated using general plant or forest pathological and mycological principles and procedures (e.g. Trigiano *et al.*, 2004; Agrios, 2005, Lundquist & Hamelin, 2005) or special diagnostic methods (e.g. Anonymous, 2008). The field samples were collected by the authors according to the reconnaissance investigation method (RIM; see Parmas, 1961) from 2006 to 2008 in Estonia (Figures 1, 2, 4-6) and in 2008 in north Latvia (Figure 3). RIM is widely used in mycogeographic field research and is based on qualitative rather than quantitative visual registration by an experienced specialist of the occurrence of definite species, their symptoms and/or fruiting bodies by the rout-method, by sampling during the visits to all of the potentially provocative habitats (e.g. exotic tree individuals like *Pinus nigra* in our investigation) and by occasionally selected habitats (e.g. young stands of *P. sylvestris*). Using RIM, no quantitative characteristics concerning sampling can be presented. Mainly spring and autumn sampling were used, as these are the most important seasons for symptoms expression in foliage diseases of pines. Maps (Figures 1-6) indicate: 1) the sampling areas, and 2) the resulting (laboratory) diagnoses (*i.e.*, whether a sample was symptomatic or asymptomatic). A sample was classified as symptomatic if characteristic symptoms for the appropriate disease were expressed (visually detected during field investigation) and disease agents' fruiting bodies were present (established at the laboratory phase of the investigation). A sample was asymptomatic if no visible symptoms could be found during the field investigation, or if the sample, which appeared likely to be symptomatic in a field investigation, was not carrying pathogens' fruiting bodies (as it was found by the microscopic examination in laboratory).

The collected samples were investigated under light microscopes (binocular stationary lens CETI and microscope Nikon Eclipse 50i) in the laboratory of forest pathology at the Institute of Forestry and Rural Engineering of the Estonian University of Life Sciences. The program IMAGE J 1.410 was used for morphological measurements and Student's T-test for statistical comparisons. The maps (Figures 1-6) were drawn using the program MapInfo Professional, Version 7.5. All species of the investigated pathogens were repeatedly isolated onto malt extract (OXOID LP0039, OXOID Agar no.3, LP0013) or pine needle agar media (100 g Scots pine fresh green needles boiled in 1 litre tap water for 20 min., 15 g OXOID Agar no.3, LP0013 added, autoclaved at 106°C for 60 min.). Isolates of *D. pinea* and *C. minus* grew fast on artificial media and were thus easy to culture, while *M. pini* and *M. dearnessii* were difficult to isolate due to slow growth. Samples of the fungal species were deposited in the Mycological Herbarium

of the Estonian University of Life Sciences /TAA(M)/, two Estonian strains of *M. pini* and one of *D. pinea* were confirmed by sequencing of their ITS regions and deposited in GenBank (Hanso & Drenkhan, 2008a and 2009, respectively).

Results and discussion

Mycosphaerella pini

A few years ago (in 2006/2007) *Mycosphaerella pini* E. Rostrup apud Munk. in its anamorphic stage *Dothistroma septosporum* (Dorog.) Morelet has been observed for the first time in Estonia (Hanso & Drenkhan, 2008). This fungus, known to cause red-band needle blight, has been reported as a pathogen in North America since the first half of the last century (Barnes *et al.*, 2008). At the end of the 20th century, accompanying the climate change, this fungus has caused serious losses in North America (Woods *et al.*, 2005), and almost simultaneously it became one of the most damaging pathogens of pines in the Southern hemisphere (Anonymous, 2008). In these regions its spread has been fast especially on the exotic pine species (Bradshaw, 2004). Just before the escalation of damages on exotic pines in the Southern hemisphere, it was demonstrated that among plantation forests, the healthiest are those of exotic species (Gadgil & Bain, 1999). This demonstrates how quickly the fungus became established and spread.

In 2002 *D. septosporum* was detected for the first time for the Baltic countries in Lithuania on *P. mugo* (Jovaišienė & Pavilionis, 2005), which was the only record of this fungus for several years afterward. *D. septosporum* was found widely spread in Lithuania again at the end of September 2008, this time on Scots pine (Markovskaja & Treigienė, 2009).

Samples of diseased *P. nigra* needles, collected in autumn 2006 at an arboretum in Agali, Järvselja, southeast Estonia, having strikingly unusual appearance - bright red patches and transversal bands on needles - were found to be infected by *M. pini* in its anamorphic stage *D. septosporum*. In March 2007 *D. septosporum* was found in Järvselja also on *P. mugo* and already on native Scots pine (*P. sylvestris*) (Hanso & Drenkhan, 2008a).

Distribution maps (Figures 1–3), showing the moving pathways of the pathogen, indicate that the spread of *M. pini* began in Estonia from the south and proceeded northward. By the end of 2007 *M. pini* was already documented on *P. sylvestris* in southern Estonia in several other localities, e.g. in Konguta, Haabsaare and Rebaste, while several investigated stands in central and northern Estonia stayed asymptomatic (Figure 1). By the spring 2008 the fungus was widespread in south Estonia, and several new occurrences from central and even northern Estonia were noted. The first records of the fungus in the parks of Pärnu and Tallinn were noted on *P. nigra*, the trees which a half-year earlier had been asymptomatic (Figure 2). In 2008 *M. pini* was recorded first in south and south-central Finland (Müller *et al.*, 2009), next year even in northern Finland (Vuorinen, 2009). In autumn 2008 this fungus was for the first time documented by us in northern Latvia (Figure 3), whereby the fungus had obviously colonized Latvia before emerging in Estonia. It is possible that the very first arrival of *M. pini* happened in Estonia even before 2006, but was not registered for its decent occurrence.

In vivo symptoms of *D. septosporum* are bright red belts, stripes and patches on needles and the figure of acervuli of the anamorphic stage on these red areas. The acervuli emerge from subepidermal needle tissues as small dark nodules and typically have an irregular shape. In some cases the red colour, indicating the produc-



Figure 1. Distribution map of *Mycosphaerella pini* in autumn 2007.

Joonis 1. Punavöötaudi tekitaja *Mycosphaerella pini* levik sügisel 2007.

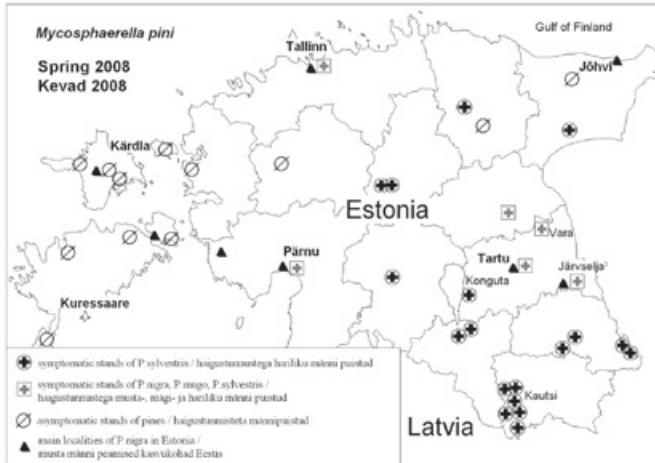


Figure 2. Distribution map of *Mycosphaerella pini* in spring 2008.

Joonis 2. Punavöötaudi tekitaja *Mycosphaerella pini* levik kevadel 2008.

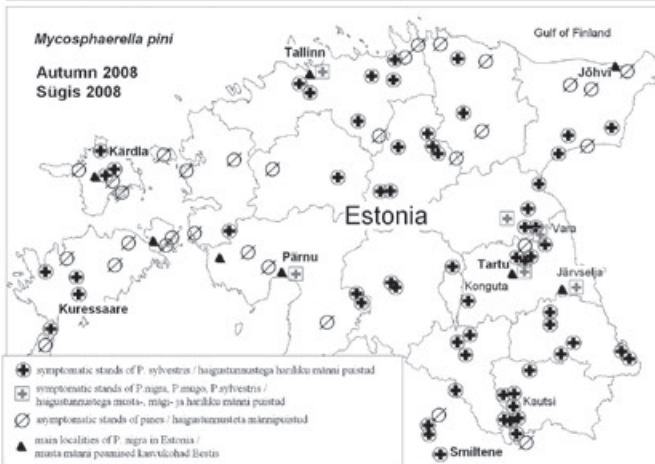


Figure 3. Distribution map of *Mycosphaerella pini* in autumn 2008.

Joonis 3. Punavöötaudi tekitaja *Mycosphaerella pini* levik sügisel 2008.

Figure 4. Distribution map of *Diplodia pinea* on *Pinus nigra* cone scales in 2007.

Joonis 4. *Diplodia pinea* levik 2007. aastal (ainult musta männi käbisoomustel).



Figure 5. Distribution map of *Diplodia pinea* on *Pinus nigra* cone scales in 2008.

Joonis 5. *Diplodia pinea* levik 2008. aastal (ainult musta männi käbisoomustel)



Figure 6. Distribution map of *Cyclaneusma minus* in 2008.

Joonis 6. Valgevöötaudi tekitaja *Cyclaneusma minus* levik 2008. aastal.



tion by the fungus of the highly poisonous polyketide toxin dothistromin (Bradshaw, 2004), is not so obvious.

During three years of investigations in Estonia the most abundant formation of conidia was registered in an unusual for this species season - from late October to mid-December and even during following wintertime. Usually the acervuli sporulate and the infection occurs from late spring to late summer (in the southern hemisphere, cf. Gilmour, 1981), from April to October (in south-central Europe, cf. Kirisits & Cech, 2007), or from May to September (in East Anglia, Britain, cf. Archibald & Brown, 2007). No sexual stage of the fungus was observed in Estonia.

Conidia of *D. septosporum* were measured in samples originating from four different young pine stands in Estonia and one in northern Latvia. Conidial dimensions (Table 1) resembled the dimensions from other European countries, conidial lengths varying statistically more than widths (Table 2). Conidial dimensions from Vara (East Estonia) were significantly ($p < 0.01$) different from the dimensions from Kautsi (South Estonia) and Smiltene (North Latvia). Conidia grown up in pure cultures from the two last localities were also statistically different ($p < 0.001$). Long and thick conidia were characteristic for samples from Järvelja (on *Pinus nigra*) and Smiltene (on *P. sylvestris*) (Table 1). Molecular investigations should demonstrate if there is any taxonomical sense for the use of these variations in betraying the pathways of the arrival of this and other three alien species to Estonia.

Table 1. Conidial dimensions of *Dothistroma septosporum* in the acervuli on pine needles and in pure cultures in Estonia and elsewhere in Europe

Tabel 1. Punavöötaudi tekitaja anamorfi *Dothistroma septosporum* koniidide mõõtmed okastelt päri-nevates viljakahades ja puhaskultuurides Eestis ja mujal Euroopas

Sample origin	Host	Substrate	Conidial length (μm)				Conidial width (μm)			Reference
			N	Mean	SD	Range	Mean	SD	Range	
<i>Proovi päritolu</i>	<i>Peremees-taim</i>	<i>Sub-straat</i>		<i>Koniidide pikkus (μm) Keskmine</i>		<i>Vahemik</i>	<i>Koniidide laius (μm) Keskmine</i>	<i>Vahemik</i>	<i>Kirjandusallikas</i>	
Estonia, Konguta	<i>Pinus sylvestris</i>	Needle	30	23.1	3.3	16.5-30.6	2.6	0.7	1.8-3.7	Original material
Estonia, Kautsi	<i>P. sylvestris</i>	Needle Culture	30 58	24.8 22.6	4.4 4.4	18.6-36.1 13.2-31.4	2.5 2.4	0.4 0.3	1.8-3.7 1.9-3.6	" "
Estonia, Järvelja	<i>Pinus nigra</i>	Needle	30	27.8	3.8	21.6-35.7	2.6	0.4	1.8-2.7	"
Estonia, Vara	<i>Pinus mugo</i>	Needle	30	22.5	4.0	11.1-28.6	2.7	0.5	1.8-3.7	"
Latvia, Smiltene	<i>P. sylvestris</i>	Needle Culture	40 60	27.8 27.3	4.4 3.9	18.7-38.2 15.9-36.4	2.4 2.4	0.3 0.2	1.9-3.3 1.9-2.9	" "
EPPPO region	<i>Pinus</i> spp.	Needle		28.0		12.0-48.0			2.0-3.0	Anonymous 2008
Finland	<i>P. sylvestris</i>	Culture		29.4		15.0-37.0	2.4		2.0-2.7	Müller <i>et al.</i> 2009
Lithuania	<i>P. mugo</i>	Needle				21.0-45.0			2.0-2.5	Jovaišienė and Pavilions 2005
	<i>P. sylvestris</i>	Needle				15.5-39.0			(1.5) 2.0-3.0	Markovskaja and Treigienė 2009
Austria, Vienna	<i>Pinus peuce</i>	Needle Culture	93 45	21.4 28.1	3.2 7.1	14.2-31.5 12.9-43.3	3.0 3.0	0.3 0.4	2.1-4.0 2.2-4.3	Barnes <i>et al.</i> 2008
Hungary, Sopron	<i>P. mugo</i>	Needle	51	29.6	4.1	18.8-36.3	2.9	0.3	2.3-3.8	"

Table 2. Comparison of conidial dimensions of *D. septosporum* in the acervuli on needles between different origins in northern Baltics, expressed in P values.Tabel 2. Punavöötaudi tekitaja anamorfi *Dothistroma septosporum* okastelt pärinevate koniidide mõõtmete võrdlus erinevate päritolude vahel Põhja-Baltikumis, väljendatuna P väärtuses.

Sample origin <i>Proovi päritolu</i>	Vara (N=30)		Järvselja (N=30)		Konguta (N=40)		Kautsi (N=30)		Latvia (N=40)	
	length <i>pikkus</i> (µm)	width <i>laius</i> (µm)	length <i>pikkus</i> (µm)	width <i>laius</i> (µm)	length <i>pikkus</i> (µm)	width <i>laius</i> (µm)	length <i>pikkus</i> (µm)	width <i>laius</i> (µm)	length <i>pikkus</i> (µm)	width <i>laius</i> (µm)
Vara	1.0	1.0	<0.001	>0.05	>0.05	>0.05	<0.05	<0.05	<0.001	<0.01
Järvselja	<0.001	>0.05	1.0	1.0	<0.001	>0.05	<0.01	>0.05	>0.05	>0.05
Konguta	>0.05	>0.05	<0.001	>0.05	1.0	1.0	>0.05	>0.05	<0.001	>0.05
Kautsi	<0.05	<0.05	<0.01	>0.05	>0.05	>0.05	1.0	1.0	<0.01	>0.05
Latvia	<0.001	<0.01	>0.05	>0.05	<0.001	>0.05	<0.01	>0.05	1.0	1.0

Mycosphaerella dearnessii

A serious (listed as an A2 quarantine pest by EPP0, cf. Anonymous, 2008) pathogen *Mycosphaerella dearnessii* M.E. Barr with its anamorphic stage *Lecanosticta acicola* (Thümen) A. Sydow has been found in some European countries soon after detection of *M. pini* (e.g. Pehl, 1995; Brandstetter & Cech, 2003; Jankovský *et al.*, 2009). *M. dearnessii* was known as a serious disease of pine needles in forest nurseries of North America (Wakeley, 1970).

During isolations of *M. pini* in Estonia several colonies were noticed among cultures which were not producing red pigment, characteristic to *M. pini*. Some of these colonies even had a slightly greenish colour. Both of the different types of colonies (typical and non-typical to *M. pini*) produced conidia, visually resembling each other. On needles *in vivo* these non-typical needle patches were coloured brown rather than red. This new fungus turned out to be *Lecanosticta acicola*, the anamorphic stage of *M. dearnessii*. Still no sexual stage of *M. dearnessii* was recorded in Estonia.

Later, at the request of Plant Health Department of the Plant Protection Inspectorate of Estonia, *M. dearnessii* was diagnosed in the samples from the Tallinn Botanical garden by the laboratories in Denmark (Plantedirektoratet, analyse protocol no. PD 3651 from 14.01.2008) and Austria (Bundesforschungs- und Ausbildungszentrum für Wald, Naturgefahren und Landschaft, Institut für Waldschutz, analyse protocol no. 1912/IV/08/C from 05.08.2008).

Diplodia pinea

In September 2007 *Diplodia pinea* (Desm.) J. Kickx (syn. *Sphaeropsis sapinea* (Fr.: Fr.) Dyko & Sutton) has been observed for the first time in Estonia (Hanso & Drenkhan, 2009). *D. pinea* was known as a harmful pathogen of shoots, needles, cones and roots (Flowers *et al.*, 2006; Blodgett *et al.*, 2007), but also as an important sapstain fungus that spoils logs during export (Thwaites *et al.*, 2004). In North America, *Diplodia pinea* has killed current-year shoots, branches, and entire trees in plantings of exotic (commonly *P. nigra*) and native pine species (Peterson, 1977). Latent infections of *D. pinea* had turned pathogenic with severe tip blight symptoms after droughts (Stanosz *et al.*, 2001). In Southern Europe the occurrence of *D. pinea* in healthy Austrian pines was positively correlated with a high insolation index (Maresi *et al.*, 2007). During the last decade in Europe the pathogen has spread northward during drought periods. Serious drought in 2003 supported a *D. pinea* epidemic in Central Europe (e.g.

Jankovský & Palovčikova, 2003; Hänisch *et al.*, 2006; Steinfath, 2006; Blaschke & Cech, 2007). Spread of the fungus might occur by insect vectors (Whitehill *et al.*, 2007) or human activities (Burgess *et al.*, 2004). Droughts in Estonia in 2002 and 2006 obviously supported to the establishment of *D. pinea* after incidental introduction, probably by pine seeds or planting material through a forest nursery.

In autumn 2007, for the first time in Estonia, *D. pinea* was found on the scales of several fallen cones from a single middle-age *P. nigra* tree growing on the edge of a small forest nursery in Järvselja. Cones of other *P. nigra* trees in Järvselja and in several other locations in Estonia, investigated soon thereafter were asymptomatic (Figure 4). In May 2008 the fungus was found from all the formerly asymptomatic *P. nigra* trees in Järvselja, but not yet from other locations. As demonstrated on Figure 5, in September 2008, some samples of *P. nigra* cones from western Estonia were already symptomatic (Hanso & Drenkhan, 2009). During this one year of observations *D. pinea* was rapidly spreading in Estonia. Still it is unknown how fast the pathogen can change host organs as substrate and spread from cone scales to needles, buds, twigs, roots and trunk wood, causing by that first considerable losses.

Large, brown, 0- to 1-septate conidia of the fungus from Järvselja (southeast Estonia) and Pädaste (western Estonia) were measured and statistically compared (Table 3). The widths and lengths of conidia of the two Estonian origins were found to differ significantly ($p < 0,001$): In Järvselja longer and thicker conidia were found than in Pädaste. Comparing the dimensions of 0- and 1-septate conidia in both of the two Estonian origins a significant ($p < 0,05$) difference was found between the widths, but not between the lengths.

Table 3. Conidial (0- and 1-septate) dimensions of *Diplodia pinea*.

Tabel 3. 0- ja 1-ristvaheseinaga *Diplodia pinea* koniidide mõõtmed.

Sample origin	Host	Substrate	Conidia	Conidial length (µm)				Conidial width (µm)			Reference
				N	Mean	SD	Range	Mean	SD	Range	
Proovi päritolu	Peremees-taim	Substraat	Koniidi tunnus		Keskmine		Vahemik	Keskmine		Vahemik	Kirjandusallikas
Estonia,	<i>Pinus</i>	Cone	0-septate	36	37.3	3.5	30.6-47.1	14.0*	1.0	11.8-16.5	Original
Järvselja	<i>nigra</i>	scales	1-septate	20	36.5	2.7	30.6-42.4	14.6*	1.0	14.1-16.5	material
Estonia,	<i>Pinus</i>	Cone	0-septate	30	33.8	3.3	28.9-40.9	12.8*	1.3	9.7-15.4	"
Pädaste	<i>nigra</i>	scales	1-septate	20	35.3	2.3	28.5-38.4	13.4*	0.7	12.2-14.8	"
Czech Republic	<i>Pinus nigra</i>	Cones, twigs, needles	2-septate				25.0-40.0			10.0-15.0	Jankovský <i>et al.</i> 2003
South-	<i>P. taeda</i>	Cultures		100	42.0			18.8			Swart <i>et al.</i>
Africa	<i>P. radiata</i>	on		100	39.7			17.3			1991
	<i>P. patula</i>	sterile		100	35.0			16.7			"
	<i>P. elliottii</i>	needles		100	45.2			19.0			"
	<i>P. virginiana</i>			100	44.8			18.7			"
USA, north-	<i>P. resinosa</i>		0-1-septate	100	36.0			12.8			Palmer <i>et al.</i>
central	<i>P. mugo</i>	Cultures	septate	100	39.4			12.6			1987
	<i>P. nigra</i>			100	37.6			12.6			"

* 0-septate and 1-septate conidial widths in Estonian samples were significantly different, $p < 0,05$

* Eesti proovides 0- ja 1-ristvaheseinaga koniidide laiused olid omavahel statistiliselt oluliselt erinevad, $p < 0,05$, mitte aga pikkused

Cyclaneusma minus

In Europe *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter (syn. *Naemacyclus minor* Butin) has been reported frequently as an endophyte (Rack & Scheidemann, 1987; Kowalski, 1988; Jurč, 2007), which damages only individual needles of *P. sylvestris*, *P. nigra* and *P. mugo*. However, sometimes it may cause heavy cast of two-year-old needles in young (up to 20 years) Scots pine plantations (Kowalski, 1988). In the Southern hemisphere, *C. minus* has been classified as a serious pathogen (e.g. Bulman, 1993; Bulman & Gadgil, 2001).

In 1999 *C. minus* was found on the needles of young Scots pines in Tartu forest nursery as a new to Estonia species (Hanso & Hanso, 2003). After that this fungus could be found every year in a single, extremely dense pine seed bed of that nursery, but not elsewhere in Estonia. In 2007, however, several findings of *C. minus* were registered, first in south Estonia (e.g. in Võru county) and by the autumn 2008 the fungus was found throughout Estonia (Figure 6), but still only in small patches. The fungus could not be found in northern Latvia in the autumn 2008, despite of special searching trials. Fortunately, *C. minus* has not acted still in Estonia as a real pathogen.

Table 4. Dimensions of ascospores and asci of *Cyclaneusma* spp. (former *Naemacyclus* spp.).Tabel 4. *Cyclaneusma* spp. (varem *Naemacyclus* spp.) kotteoste ja eoskottide mõõtmed

Sample origin	Host	Substrate	Microstructure	Length (μm)				Width (μm)			Reference
				N	Mean	SD	Range	Mean	SD	Range	
<i>Proovi päritolu</i>	<i>Peremees-taim</i>	<i>Substraat</i>	<i>Mikrostruktuur</i>		<i>Keskmine</i>		<i>Vahemik</i>	<i>Keskmine</i>		<i>Vahemik</i>	<i>Kirjandusallikas</i>
Estonia, Saaremaa	<i>Pinus sylvestris</i>	Needle	Ascospores	20	54.0	18.0	23.8-82.0	2.9	0.3	2.3-3.4	Original material
			Asci	20	93.1	6.4	83.9-110.3				"
Estonia, Tartu	<i>P. sylvestris</i>	Needle	Ascospores	30	75.9	14.0	51.2-104.4	3.3	0.3	2.4-4.1	"
			Asci	13	86.6	8.0	75.7-97.0				"
Not defined	<i>P. radiata</i> , <i>P. sylvestris</i>	Needle	Ascospores of <i>Naemacyclus minor</i>				75.0-85.0			2.5-3.0	Butin 1973
			Asci of <i>N. minor</i>				up to 120				"

Table 5. Ascumatal dimensions of *Cyclaneusma* spp.Tabel 5. *Cyclaneusma* spp. viljakehade mõõtmed.

Sample origin	Host	Substrate	Microstructure	Length (mm)				Reference
				N	Mean	SD	Range	
<i>Proovi päritolu</i>	<i>Peremees-taim</i>	<i>Substraat</i>	<i>Mikrostruktuur</i>		<i>Keskmine</i>		<i>Vahemik</i>	<i>Kirjandusallikas</i>
Estonia, Saaremaa	<i>Pinus sylvestris</i>	Needle	Ascomata	57	0.48	0.1	0.28-0.84	Original material
Estonia, Tartu				53	0.53	0.1	0.28-0.88	"
Not defined	<i>Pinus</i> spp.	Needle	Ascomata of <i>Naemacyclus minor</i>				up to 0.5	Butin 1973
Ukraine, Crimea	<i>Pinus</i> spp.	Needle	Ascomata				most <0.5	Minter and Dudka 1996
Ukraine, Crimea	<i>Pinus</i> spp.	Needle	Ascomata				most >0.5	"

The morphological dimensions of *C. minus* in Estonia were similar to dimensions in other countries (Table 4). Ascospores of the fungus in samples from Tartu nursery were significantly ($p < 0.001$) longer and wider than in samples from Saaremaa, but the ascomata were significantly ($p < 0.05$) wider. Taxonomically the fungus from Tartu seems to be closer to *C. niveum* than *C. minus* (Table 5). In the infected needles from Tartu the ascomata of that fungus had emerged on white or nearly colourless needle areas, whereas in the samples from several other localities in Estonia the pale areas were lacking and the ascomata were formed on brownish needle areas.

Host tree species

With origins in southern countries having warmer climates and different host assortments, some new natural prerequisites in the Northern Baltics had to form for the alien invasive fungi.

In Europe the species *M. pini*, *M. dearnessii* and *D. pinea* have been recorded most often on *P. nigra*, less often on *P. mugo* and *P. sylvestris* (Holdenrieder & Sieber, 1995; Jankovský & Palovčíková, 2003; Luchi *et al.*, 2005; Flowers *et al.*, 2006; Hänisch *et al.*, 2006; Steinfath, 2006; Blodgett *et al.*, 2007; Jurč, 2007; Thoirain *et al.*, 2007; Whitehill *et al.*, 2007; Anonymous, 2008). *P. nigra* has been a preferable host species as well for some other dangerous pine disease agents, like *Gremmeniella abietina* in Denmark (Thomsen, 2009). Fortunately, *P. nigra* has not been planted much in Estonia and trees are scattered, growing mostly singly or in pairs, except of an old stand in Pädaste, Saaremaa and a middle-aged stand in Järvelja, South-East Estonia (Sander, 1998; Laas, 2004). *P. mugo* has been more popular than *P. nigra*, creating verdant areas in towns and green belts besides the roads, but is less affected by these new alien pathogens. Transfer of these pathogens from exotic pine species to *P. sylvestris*, as to the single native pine and one of the main commercial tree species in Estonia, is worth special attention and care. In contrast to other three alien species, introduction of *C. minus* seems to have occurred directly from *P. sylvestris* to *P. sylvestris*.

Climate change

Woods *et al.* (2005) demonstrated how even a relatively small change in climate can have serious implications for a tree species, particularly if the change surpasses an environmental threshold that has previously restricted the development of certain pathogens.

The natural environment of Estonia is sensitive to climate change, as it is located in the transitional zone between regions of different bioclimatic conditions (Kont *et al.*, 2007). During the second half of the 20th century mean annual air temperature in Estonia has increased by 1.0-1.7 °C. Statistically significant increases in monthly mean temperatures have occurred mainly from January to May (Jaagus, 2006). Zonal atmospheric circulation has brought to Estonia more wet weather than previously, which in winter was warmer and in summer cooler than long-term averages (Keevallik *et al.*, 1999). The precipitation trends are less distinct, but an increasing trend can be noticed during the cold half-year and in the summer month of June (Jaagus, 2006). These climatic changes obviously might support the arrival and settlement of alien invasive southern fungi in Estonia.

Natural habitats in Estonia suffered from two severe droughts - in 2002 and 2006 (Hanso & Drenkhan, 2007a). Similarly, in Germany two severe droughts in 2003 and 2006 (Oldenburgh, 2007; Steyrer, 2007; Wulf & Schumacher, 2007) together with forest diseases (incl. *Diplodia pinea*) and insect pests killed several young pine planta-

tions (Steyrer, 2007).

The predicted climate warming (e.g. Logan *et al.*, 2003; Walther & Linderholm, 2006) would mostly favour these southern pathogens, for which the earlier low winter temperatures were a limiting factor. For the spread of *M. pini* the increase in temperature may be counterbalanced by the decrease in summer rainfall, but *D. pinea* may benefit from the water stress of its host (Desprez-Loustau *et al.*, 2007).

Are the new diseases in the Northern Baltics chronic or epidemic?

Dothistroma needle blight, although it is not especially emphasized in the literature, can behave as a chronic disease (Woods, 2003; Woods *et al.*, 2005; Peterson, 2006). Its outbreaks have been estimated to proceed for a decade (Lewis & Welsh, 2005). According to the first observations, *D. septosporum* may obtain chronic character also in Estonia.

The most common needle disease of pines during the 20th century in Estonia, *Lophodermium* needle cast, caused by *Lophodermium seditiosum*, has been shown to be clearly an epidemic disease (Hanso, 1963; Hanso & Hanso, 2003; Hanso & Drenkhan, 2007c). In 2008, the last epidemic of *L. seditiosum* coincided in south and central Estonia with the serious spread of *D. septosporum* in several young *P. sylvestris* plantations. During this epidemic, *D. septosporum* infected only second year and older pine needles, while *L. seditiosum* infected also the youngest, current year needles. This difference demonstrated higher virulence in *L. seditiosum* rather than in *D. septosporum*. High virulence of *L. seditiosum* is, however, mitigated by few to several intermediate years of rest time between consecutive epidemics, during which the trees can renew their foliage, produce and relocate resources to the new growth or stores. Chronic *D. septosporum* or other new invasive fungi, although less virulent, can deprive the host, year by year, of all needles older than the current year's production.

The hypothetical danger of the new invasive foliage fungi

It is known (e.g. Lyytikäinen-Saarenmaa, 1999) that the consequences of defoliation stress depend on defoliation type, intensity and timing, and the source-sink position of damaged needles, which determines whether defoliation predisposes pines to further damage or not. Since the current year needles mature after the shoot elongation, they apparently cannot support the current year height increment, although during the latter half of the growing season they can support the radial increment (Clark, 1961; Ericsson *et al.*, 1980). It was experimentally proved by the NTM (needle trace method) that in Estonia the second and third year needles serve as the main current year tree growth supporting needle sets (Drenkhan *et al.*, 2006b; Kurkela *et al.*, 2009). Repeated privation, year by year, of older needles and, therefore, stored nutrients by a chronic foliage disease may stress the host trees even more than a single severe loss of needles by an epidemic disease. Certainly, more studies are needed to confirm our hypothesis.

Acknowledgements. The study was supported by the Estonian Environmental Investments Centre and partly financed by the project SF0170021s08. The authors acknowledge Ülle Metsman and Epp Kõiva from the Plant Health Department of the Plant Protection Inspectorate of Estonia for delivering information concerning diagnosis of *Mycosphaerella dearnessii* in samples from Tallinn by the central European laboratories, the unknown revisers and Mr. Terry Bush from Madison, Wisconsin, USA for the language revision.

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Mändide (*Pinus* spp.) okkaid asustavate seente invasioon Põhja-Baltikumini

Rein Drenkhan ja Märt Hanso

Kokkuvõte

Invasiivökoloogias on seentele kuni viimase ajani pööratud suhteliselt vähe tähelepanu. Ülemaailmselt registreeritud ilmastikuäärmuste ja kliimamuutuste taustal on paari viimase aastakümne kestel, sealhulgas ka 2003. aasta sügava põua järel Kesk- ja Lääne-Euroopas, hakatud tähelepanu pöörama tulnuk-patogeenidele. Lisaks mitmetele piirkondlikele monitooringuprogrammidele on loodud üle-Euroopaline nn. FORTHREATS (European Network on emerging diseases and threats through invasive alien species in forest ecosystems) – võrgustik, mille tegevuse I etapp äsja lõppes. Nimetatud võrgustiku eesmärgiks on metsade suhtes potentsiaalsete ohtude, sealhulgas ka invasiivsete patogeenide identifitseerimine ning nende päritolu ja levimisteede kindlakstegemine.

Käesoleva sajandi esimesel kümnendil on töö autorite poolt Eestis kasvavatel mändidel avastanud neli uut seenpatogeeni (*Mycosphaerella pini*, *M. dearnessii*, *Diplodia pinea* ja *Cyclaneusma minus*). Kõik neli liiki on aga mitmetes maades ja mandritel tuntud kui ohtlike okkahaiguste tekitajad. Kaks liiki (*M. pini* ja *M. dearnessii*) kuuluvad kogu Euroopas, sealhulgas ka Eestis karantiinsete liikide nimekirja.

Käesoleva uurimistö eesmärgiks oli 1) jälgida ja kirjeldada nimetatud haigustele eriti vastuvõtlike männiliikide ning meie kodumaise hariliku männi asustamise käiku kõnealuste patogeenide poolt ning Eesti (ja Põhja-Läti) koloniseerimise üldsuunda ja iseärasusi, 2) esitada kõnealuste patogeenide mõningaid mikromorfoloogilisi tunnuseid Põhja-Baltikumis, s.o. kuni tuhande kilomeetri kaugusel põhja pool nende senistest põhjapoolsematest leiukohtadest Euroopas, ning 3) püstitada hüpoteetiline riskihinnang nimetatud patogeenide toimele meie männikutes, sidudes selle võrdlusega meie eelmise sajandi olulisima männi okkahaiguse – männi-pudetõve (haigusetekitaja *Lophodermium seditiosum*) toime ning okkajälje meetodil (NTM) saadud uurimistulemustega.

Väliuuringud sooritati klassikalisel rekognostseermeetodil, mille käigus koguti proove haigustele vastuvõtlikumatelt eksootliikidelt (eelkõige must mänd) ja harilikult männilt, viimase proovivõtu kohad valiti juhuslikult. Laboratoorsetel uuringutel kasutati statsionaarset binokulaarluupi CETI ja mikroskoopi Nikon Eclipse 50i. Uusliikide herbaareksemplarid on deponeeritud Eesti Maaülikooli Mükoloogilises herbariumis TAA(M).

Kahel *M. pini* ning ühel *D. pinea* Eesti päritoluga tüvel eraldati DNA, täpseks liigi kinnituseks sekveneeriti DNA nukleotiidide järjestus ning vastavad andmed deponeeriti ka maailma geenivaramusse (GenBank).

Nii karantiinsetel punavöötaudil (*M. pini*), pruunvöötaudil (*M. dearnessii*), mõlemad seni registreeritud Eestis vaid suguta arengujärkudena (vastavalt *Dothiostroma septosporum* ja *Lecanosticta acicola*) kui ka seni eestikeelse nimeta *D. pinea* 1 (sünonüüm *Sphaeropsis sapinea*), milliste saabumine registreeriti sajandi I aastakümne II poolel, õnnestus jälgida Eesti kiire asustamise käiku üldsuumaga lõunast põhja poole (vt. joonised 1-5). Neist esimene (*M. pini*) on tänaseks välja jõudnud isegi juba Põhja-Soome. Neljas liik, valgevöötaudi tekitaja *C. minus* saabus meile ilmselt mõnevõrra varem, kuid rea aastate vältel võis teda leida vaid ühest metsataimlast. Alates 2007. aastast

on aga nimetatud liigi levila tormiliselt laienenud, seejuures pole aga võimalik olnud eristada tema levila kindlamat laienemise suunda.

Nii puna- (*M. pini*) kui pruunvöötaud (*M. dearnessii*), niisamuti ka *D. pinea* asustasid esmalt männi eksootliike, eriti musta mändi (*Pinus nigra*). Neist vaid punavöötaud (*M. pini*) on jõuliselt ja laialdaselt asustanud juba ka meie kodumaise hariliku männi (*P. sylvestris*) noorendikke ning keskealisi puustuid. Maailmas paljudel männiliikidel esinevat, isegi mändide universaalseimaks patogeeniks peetavat *D. pinea*'t oleme aga seni leidnud vaid musta männi (*P. nigra*) käbidelt. Nagu see on juhtunud mujal maailmas, võib aga varem või hiljem oodata meilgi viimati nimetatud patogeeni majanduslikult olulisemaid kahjustusi eriti männiokastel, võrsetel, juurtel ja isegi sinetusseenena puitmaterjalis.

Puna- ja pruunvöötaud võivad kujuneda meilgi kroonilisteks okkahaigusteks. Samas pole nad võimelised kahjustama mändide noorimaid, s.o. jooksva aasta okkaid. Puhanguaastatel on suutnud seda teha meie möödunud sajandi kõige ohtlikum okkahaigus – männi-pudetõbi (haigusetekitaja *Lophodermium seditiosum*). Okkajälje meetodil (NTM) oleme aga hiljuti kindlaks teinud, et männi aastavõrse juurdekasvu „toidavad” eriti just vanemad - teise ja kolmanda aasta okkad. Kui nüüd kroonilised vöötaudid peaksid mändid aasta-aastalt neist juurdekasvu toitvatest vanematest okastest ilma jätma, võib neist uutest invasiivsetest haigustest kokkuvõttes oodata mändidele isegi suuremat kahju kui hooajalisest-epideemilisest männi-pudetõvest, mis real epideemiatevahelistel aastatel laseb mändidel okkakaost toibuda ning suunata uusi ressursse nii juurdekasvu tagamisse kui ka varuainetesse.

Received March 25, 2010, revised March 25, 2010, accepted March 30, 2010



Epidemiology of *Lophodermium* needle cast of Scots pine (*Pinus sylvestris* L.) in Estonia

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Summary

We studied the epidemiology of needle cast caused by *Lophodermium seditiosum* on Scots pine using a retrospective approach based on needle loss, meteorological data, and annual radial and height increment of sample trees from 1887–2006, but also ICP forest monitoring data and regional needle cast disease and insect pest records. If the sum of precipitation from May to August reaches 300 mm (or GIV reaches 5.0), an epidemic of *Lophodermium* needle cast will follow next year in forest nurseries, if it exceeds 360 mm (or GIV 6.4), an epidemic will follow as in forest plantations as well as in nurseries. Increased needle losses and growth reduction of trees for *Lophodermium* needle cast epidemics were detected until the age of pines 22–24 years, but for diprinoïd sawfly insect pests also in the older pines. The relevance of these findings for the prediction of *Lophodermium* needle cast in a changing climate is discussed.

Keywords: *Pinus sylvestris*, *Lophodermium seditiosum*, epidemiology, defoliation, needle trace method (NTM), insect damages, radial growth, height growth.

1 Introduction

Lophodermium needle cast caused by *Lophodermium seditiosum* Minter, Staley and Millar, (historically as *L. pinastri* Schrad.) has been known in Estonian forest nurseries and young plantations as a serious foliage disease of *P. sylvestris* from the beginning of the 20th century (Weiß 1902; Vestergren 1903). The first temporary forest nurseries in Estonia (Taebla and Saulepi) were established in 1847, but wide-scale planting of clear-cut areas by the nursery-grown pine seedlings started some decades later, after the century-long practice of pine regeneration by sowing. Already before the year 1870, for instance, the role of afforestations by planting in the northern Tartu region reached 36% (Meikar 1994). It seems likely, that *Lophodermium* needle cast became a serious disease in forest nurseries of Estonia, like it happened in Germany (Stein 1852; Holzner 1877) at the time of the beginning of forest nursery management.

The role of bare-root pine seedlings in forest regeneration in Estonia is still high (Jäärats et al. 2010), e.g., in 2004–2007 more than 70% of the pine planting stock was grown in open-land nurseries (Tetlov and Siimon 2008). Consequently, *Lophodermium* needle cast in forest nurseries is persistently a live issue. In addition to its role in nurseries, *Lophodermium* needle cast plays a certain role in the natural selection of surviving and failing tree-individuals during the early stages of stand development (Hanso and Drenkhan 2007).

During the last quarter of the 20th century the taxonomic position of the actual pathogen of Scots pine inside the genus *Lophodermium* was specified (Stephan 1973; Minter et al. 1978; Minter and Millar 1980; Diwani and Millar 1987). Before that a trial to characterise the *Lophodermium* needle cast epidemiology was undertaken in France (Lanier and Sylvestre 1971). Lately, Hanso and Drenkhan (2007) built a provisional century-long retrospective time-table of *Lophodermium* needle cast epidemics in Estonia.

In this paper several approaches were used to study the connection of meteorological conditions and the emergence of *Lophodermium* needle cast epidemics in Estonia. In the work we used: 1) literature data about the epidemic occurrences of *Lophodermium* needle cast, 2) data of M. Hanso's diagnostic work from 1972–1985, 3) retrospective data of needle losses in

pinus from the NTM computations, 4) foliage monitoring data from the international ICP Forests, which were compared to meteorological data, including the GIV-values (cf. Thomsen 2009), 5) the time-table of calamity years of herbivorous insect pests of pine (from the literature), and 6) the annual growth rate of trees at and after the epidemic and calamity years, respectively. The purpose of the investigation was to improve the forecasting of the *Lophodermium* epidemics in Estonia for a more efficient control.

2 Materials and methods

2.1 Experimental study areas and retrospective needle data series

The NTM data originate from six *P. sylvestris* stands, five stands in Konguta and one in Järvelja, south-eastern Estonia (Fig. 1, Table 1), established by planting. Sample trees were growing in the main storey, each had a straight healthy stem.



Fig. 1. Location of the stands of *P. sylvestris* investigated by NTM (in Konguta and Järvelja), sample points of ICP forest monitoring and the Tartu-Tõravere meteorological station.

Table 1. Investigated by NTM stands of Scots pine.

Stand no.	The number of sample trees	The age of sample trees, years	Coordinates	Forest site type* (according to Lõhmus, 2004)
1	8	109–115	58°13' N, 26°10' E	<i>Vaccinium</i>
2	8	95–105	58°12' N, 26°08' E	<i>Oxalis</i>
3	4	40–56	58°14' N, 26°09' E	<i>Vaccinium-Myrtillus</i>
4	8	31–33	58°11' N, 26°08' E	<i>Vaccinium</i>
5	10	13–14	58°14' N, 26°09' E	<i>Vaccinium</i>
6	8	ca. 70	58°16' N, 27°19' E	<i>Vaccinium-Myrtillus</i>

* - all stands were growing on sandy soils.

In total 46 sample trees from 6 *P. sylvestris* stands (Fig. 1) were felled and processed according to the NTM manual (Aalto and Jalkanen 1998). From the trees 1,600 sample blocks from annual shoots and ca 15,000 annual ring surfaces were analysed. Annual shoot means the height growth of the trees' main stem at a definite year, which thereafter, year by year will be covered in living tree by younger year-rings. In Scots pine the annual shoots are easily distinguished for being divided by branch whorls. NTM is based on the fact that the short shoots of living conifer needles remain connected to the pith through the vascular tissue (Kurkela and Jalkanen 1990; Williams et al. 2003; Insinna et al. 2007a, b). Needle loss means the decrease in the amount of needle cohorts from the total main stem in a definite year (Aalto and Jalkanen 2004). The yearly needle loss data from the period 1887–2006 were used (Fig. 2). The needle loss raw data were standardised with a 5-year moving average with one-year step (Fritts 1976). The resulted needle loss indexes were calculated separately for every single tree in each experimental stand inside the period of 1887–2006.

This way extracted yearly needle loss indexes characterise the full-life needle loss dynamics of every of these 46 sample trees. At first, irrespective of definite calendar years, the needle loss data were grouped by the ages of annual shoots of sample trees, every group including this way needle loss data of different trees at the same ontogenetic age, but at the different calendar years (Fig. 6). Secondly, all this material was ordered and analysed considering twelve sampled *Lophodermium* needle cast epidemic years (see chapter 2.2). This way different numbers of trees were represented, which annual shoots of the same age were found to cover interesting us definite pre-epidemic, epidemic and following calendar years of sampled 12 epidemics. With the aim to analyse the vulnerable age limit of pines at the sampled *Lophodermium* needle cast epidemics and other epidemic peculiarities of the disease, tree (= annual shoot) ages at the pointer years were divided into two groups. Therefore, depending of the amount and extent of the material, which we could extract from our model trees for the epidemic and calamity calendar years, the definite age groups of 3–11 years and of 20–33 years were formed and investigated (Fig. 6). Unfortunately, in our NTM material no annual shoots were found to be of the age of 12–19 years during these definite epidemics and calamities, which were sampled for analyses (Table 2).

According to the NTM protocols (Jalkanen et al. 2000a, b) a unit (an age class or a pointer year) has to be covered at least by 5 to 7 sample trees (=annual shoots).

After felling of trees, but before cutting of sample bodies from them, the lengths of the annual shoots or internodes (= annual height increments, HI) were measured (in cm), and a disk for measuring yearly radial increments (RI) was sawn at the base of the stem (in the stands 4 and 5) or at the height of 1.3m (in the stands 1, 2, 3 and 6). RI of sample trees was measured with an accuracy of 0.01 mm using a LINTAB tree-ring measuring table with the computer program TSAP-Win version 0.59 (Rinn 2003).

2.2 Selection of the years of *Lophodermium* needle cast epidemics

From the diagnosed and suspected *Lophodermium* needle cast epidemic years in Estonia from 1887 to 2006 (see Hanso and Drenkhan 2007), the next sample of epidemic years was chosen for the analyses: 1923, 1924, 1929, 1955, 1961, 1962, 1975, 1979, 1982, 1999, 2001 and 2004. This selection was based on the literature: if an outbreak registered in Estonia had coincided with the outbreak in some other nearby countries, the appropriate year was included into the sample. It means that only larger than local epidemics were included (Table 2).

In addition to Estonia, remarkable occurrences of *Lophodermium* needle cast were documented in 1923, 1924, 1961 and 1962 in Norway, 1923 and 1924 in the Netherlands, in 1955 and 1962 in Germany, in 1975 and 1982 in south Finland and in north-west Russia, in 1979 again in north-west Russia, in 1982 in Scotland and Lithuania, in 2001 in Lithuania and

Britain, and in 2004 again in Lithuania (Hesslink 1927; Hagem 1928; Jung 1960; Veldmann and Kontzog 1963; Roll-Hansen 1967; Greig et al. 1982; Kurkela and Löyttyniemi 1982; Aminev and Minkevich 1986; Magnesen 1992; Anonymous 1982, 2001, 2004; Hendry et al. 2002).

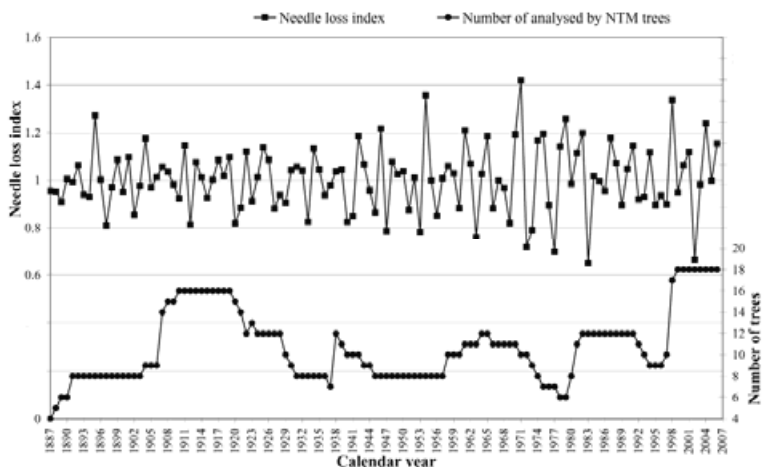


Fig. 2. Mean standardized needle loss indexes of sample trees from the pine stands investigated by NTM (the upper broken line) and the size of sample, covering years from 1887 to 2006 (the lower broken line).

Table 2. Numbers of sample trees, representing by the needle loss indexes of their appropriate annual shoots the documented and sampled 1) *Lophodermium* needle cast epidemic years (see Fig. 6), and 2) calamity years of herbivorous insect pests (*Bupalus piniaria* L. and *Neodiprion sertifer* Geoffr.) of pines in southern Estonia. Incomplete numbers of trees (for representing a stand or pointer year according to the NTM protocols) in different tree age classes are indicated by an asterisk (*).

Age of tree (yrs.) at the year of outbreaks	<i>Lophodermium</i> needle cast		Herbivorous pest	
	Number of trees (=annual shoots of analysed trees)	Represented epidemic years	Number of trees (=annual shoots of analysed trees)	Represented calamity years
3	1*	1979		
4			3*	1980, 1981
5	7	1982, 1999	5	1980, 1981
6	9	1982, 1999	2*	1981
7	6	1982, 2001	5	1958, 1938
8	6	2001	9	1938, 1939, 1959
9	1*	1961	4*	1939, 1960
10	7	1961, 1962, 2004	2*	1962, 1965
11	8	1962, 2004	3*	1962, 1966
20			2*	1950
22	3*	1999		
23	6	1923, 1955, 1975, 1999		
24	14	1924, 1955, 1975, 1979, 1999, 2001		
25	8	1923, 1955, 2001	1*	1980
26	6	1923, 1924, 2001	2*	1958, 1980
27	8	1923, 1924, 1979,	6	1958, 1959

28	7	1982, 2004	9	1958, 1959, 1960, 1980
29	4*	1924, 1979, 2004	10	1959, 1960, 1980, 1981
30	8	1929, 1961, 2004	6	1930, 1960, 1962, 1981
31	13	1923, 1961, 1962	6	1931, 1962
		1924, 1929, 1961,		
		1962, 1982		
32	3*	1929, 1962	6	1930, 1932, 1962
33	2*	1929	5	1930, 1931, 1965

Several indirect data can be found in the Estonian forestry literature, referring to the great planting stock deficiency, coinciding with some definite years of our sample of *Lophodermium* needle cast epidemics, e.g. in 1955 in Saaremaa (Raid 1963) and in 1961 in south-central and in north-eastern Estonia (Nõmmsalu 1963; Ojamaa 1963).

2.3 Analysis of meteorological data

An epidemic year in this paper indicates the year of symptoms expression. Meteorological data from the Tartu-Tõravere meteorological station were used. This station is situated between our two experimental NTM areas in south-east Estonia (Fig. 1).

As the decisive epidemic-triggering meteorological prerequisites are wet and cool summers and mild winters (e.g. Neger 1919; Lanier and Sylvestre 1971; Stenström and Arvidsson 2001), the mean monthly precipitation sums in summer and the mean monthly temperatures in winter one year prior to the epidemic, but also the data of two years (24 months) prior to May of each epidemic year, were compared to the appropriate long-term (1887–2006) means (Fig. 2).

2.4 Computations of GIVs

Index value GIV is computed by dividing the monthly mean temperature into the precipitation sum (mm/°C). When this index value for May, August and September, but also for some combinations of these months in Denmark was higher than 8, an epidemic of *Gremmeniella abietina* followed (Thomsen 2009).

For *Lophodermium* needle cast we computed mm/°C for 24 months prior to May of each epidemic year (see chapter 2.3). Mean mm/°C values were calculated only for the months, which mean monthly temperatures of the long-term period (1887–2006) reached +5.0 °C or were higher, i.e. for May, June, July, August, September and October.

2.5 Needle losses in pines

From our NTM material the mean needle losses were computed for the selected years of *Lophodermium* needle cast epidemics, separately for the host tree ages of 3–11 and 20–33 years (Table 2). For the comparison, needle loss indexes of the high needle loss years (which did not belong to the sample of epidemic years) and of the low needle loss years, were computed and presented for pines of different ages, regardless of calendar years (Fig. 6). The years of high or low needle loss were sorted concerning the difference of their mean values from the total mean needle loss indexes (the total mean was 1.0 ± 0.01): a year of high needle loss if the index was higher, and a year of low needle loss if it was lower than the total mean.

2.6 Juxtaposition of forest monitoring (ICP Forests) and NTM data

Defoliation data of pines from the ICP Forests monitoring plots, nearest to our NTM experimental stands (Fig. 1), were obtained from the Estonian Environment Information Centre (EEIC). The defoliation assessments were performed by the EEIC from 1988 to 2007 according to the ICP manual instructions (e.g., Eichhorn et al. 2010).

Needle loss data from five ICP Forests monitoring stands, no. 104, 121, 135, 147 and 159 (Fig. 1), were compared with our NTM needle loss data. All the plantations and stands were growing within a circle of maximum radius of ca. 50 km. Needle loss indexes of two younger age groups of pines, A and B (youngest, 13–14 and young, 31–33 years, respectively), were obtained by NTM investigations in our experimental stands 5 and 4, respectively (Table 1). The trees in the monitoring stands were divided into two age groups, C and D (adult, 52–79 and old, 90–164 years; 57 and 42 trees, respectively). Thus the exact NTM needle loss data were compared with the visually obtained ICP data.

2.7 Calamities of herbivorous insect pests of Scots pine in Estonia

The most harmful herbivorous insect pests of pine in northern Europe are diprionid sawflies. Of diprionids, the calamities of *Bupalus piniaria* were documented in southern Estonia in 1930–1932, 1980–1981 and 1990–1992 (Mihkelson 1986; Voolma 2000; Voolma and Luik 2001), of *Neodiprion sertifer* in 1938–1939, 1948–1950, 1958–1960, 1962, 1965–1966, and 2007 (Karu 1940; Voolma et al. 1995; Voolma 2000, 2001, 2008) and of *Diprion pini* in 1981–1982 (Voolma et al. 1995). The numbers of analysed annual shoots of sample trees, which cover the 14 diprionid sawfly calamity years in Estonia, are represented in Table 2.

2.8 Influence of *Lophodermium* needle cast and diprionid sawflies on the growth of pines

In different age groups the mean annual radial and height growth rates of trees at the different years of epidemics and at the calamity years, respectively, as well as one or two post-epidemic and post-calamity years were compared with the mean growth rates of similar age trees at the non-epidemic and non-calamity years inside the period 1887–2006 (Tables 5 and 6, respectively).

The annual increments of the trees (RI and HI) were analysed for the:

1. epidemic or calamity (pointer) year;
2. next two post-epidemic or -calamity years.

As the reference, the appropriate mean data of both age groups of trees from the extended period (1887–2006) were used, omitting the pointer years. As said, our NTM material (Fig. 6) enabled to study separately only two age groups of 3–11 and 20–33 years, respectively.

2.9 Data analyses

Statistical operations were performed according to the software of R version 6.6.2 and Microsoft Excel (2007). The differences in tree growth and needle characteristics were expressed in percentages and compared by Student's t-test. Pearson correlation coefficients were used.

3 Results

3.1 Meteorological preconditions of the epidemics

The mean precipitation sums particularly for the month of July of the year preceding the epidemic year were higher than that of the long-term, 107.2 and 78.0 mm, respectively (Fig. 3). This characteristic was significantly higher during the majority of single years inside the sample of *Lophodermium* epidemics, except for 1923, 1924 and 2004. During these three years the pathogens' moisture demands were compensated by the higher precipitation in the adjoining months of June or August, $p < 0.05$ and $p < 0.1$, respectively. Pre-epidemic autumns were also wet: the mean precipitation sums of October and November were higher than long-term means (Fig. 3), although not significantly.

Summer-time temperatures of preceding an epidemic year were generally cooler compared with the long-term (1887–2006) mean temperatures, but not significantly (except of August). The autumn and winter seasons of the years preceding the epidemic were somewhat milder, but their mean temperatures did not differ significantly from the appropriate long-term means.

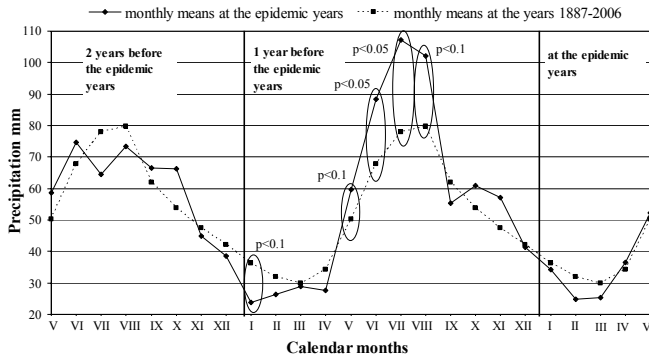


Fig. 3. Mean precipitation sums of 20 months previous to the *Lophodermium* epidemic years (1923, 1924, 1929, 1955, 1961, 1962, 1975, 1979, 1982, 1999, 2001 and 2004) in comparison with the long-term means. The months with statistically significant differences in the mean precipitation sums on the two broken lines are united by ovals and supported by the p-values of difference.

The single common prerequisite for the occurrence of epidemics and calamities was a mild winter, except for February. Differences in the triggering agents of epidemics and calamities were very visible in the amount of summer-time precipitation: in contrary to the epidemics, a summer of the year preceding a calamity year was rather dry (Table 3).

Table 3. Mean monthly temperatures and precipitation sums of the preceding to the starting years of calamities of insect defoliators of pine in southern Estonia

Period	Temperature (C°)					Precipitation (mm)						
	V	VI	IX	X	XI	V-IX	VIII-X	IX-XI	XII-III	V-IX	VIII-X	IX-XI
At the years, preceding to the calamity years	11.7**	16.0*	11.3	6.4**	1.2**	14.4	11.3*	6.4**	-3.9	298.3	173.2	144.0
Mean for 1887-2006	10.7	14.9	10.8	5.4	0.1	13.8	10.6	5.4	-4.9	337.5	196.3	164.0

P-values: * 0.1, ** 0.05

3.2 Which amount of prior precipitation will initiate an epidemic?

The mean long-term precipitation sum of summer months (from May to August) was 276 ± 8 mm, but in preceding to the twelve epidemic years 361 ± 15 mm, varying in different epidemic years from 284 to 487 mm and if to exclude 1975 from the sample, then from 301 to 487 mm. Consequently, if the precipitation sum from May to August (incl.) reaches ca. 300 mm, the next year an epidemic of *Lophodermium* needle cast in forest nurseries can be expected. If the precipitation sum reaches 360 mm, an epidemic may also follow in pine plantations. No epidemic will be expected if the appropriate precipitation sum up to the end of August does not reach 300 mm. If the precipitation sum during May, June and July reaches 250 mm, a new epidemic will be expected for the next year.

During the extended period (1887–2006) there were in total 49 years when precipitation sums from May to August (incl.) reached 300 mm, consequently pointing out 49 potential epidemic years in forest nurseries. The mean needle loss index of these 49 years was significantly ($p < 0.1$) higher than the appropriate mean of the extended period. If to consider as critical the interval of precipitation over 360 mm, then in total 20 epidemics could also occur in pine plantations.

3.3 Does GIV reveal the *Lophodermium* needle cast epidemics in Estonia?

The GIVs of May, June, July and August (and several combinations of these months), previous to the *Lophodermium* epidemic years were calculated and compared to the appropriate long-term (1887–2006) means. The combination of all summer months from May to August (Fig. 4) turned to indicate best the *Lophodermium* epidemics. The mean-term GIV for that period in Estonia was 4.8 ± 0.1 , but for the epidemic years it was 6.4 ± 0.3 , with the minimum 5.0.

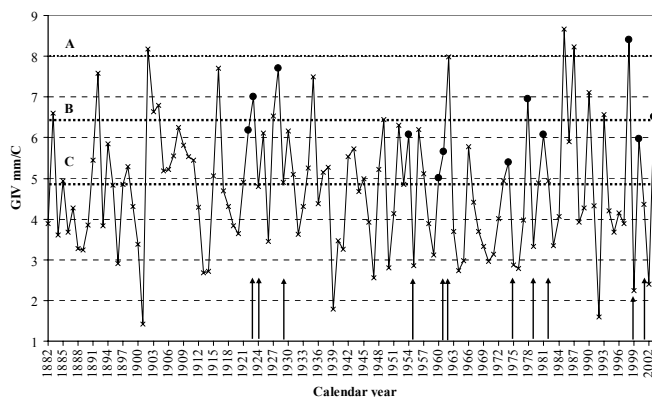


Fig. 4. Mean GIVs of the summer months in Tartu-Tõravere (south-east Estonia) for the period 1887–2006. Vertical arrows indicate the epidemic years of *Lophodermium* needle cast, black dots indicate the GIVs at the pre-epidemic years. The three horizontal dotted lines indicate the threshold values of GIVs for the triggering of *Lophodermium* epidemics in Estonia: 4.8 – in nurseries, 6.4 – in plantations, and 8.0 – of *Gremmeniella* dieback in Denmark.

According to the GIVs, during 1887–2006 in total of 52 *Lophodermium* epidemics should have occurred in Estonia. So the GIV analyses predicted 3 epidemics more than the monthly precipitation sums alone did. The analyses based on precipitation strongly indicated June and July ($p < 0.05$, see Fig. 3) and less strongly ($p < 0.1$) May and August as predictor months. GIV indicated with equal strength ($p < 0.05$) June, July and August (Fig. 5). Both analyses demonstrated that no correlations appeared with the meteorological conditions, which prevailed during the 24th to 17th months prior to the epidemics (Fig. 5).

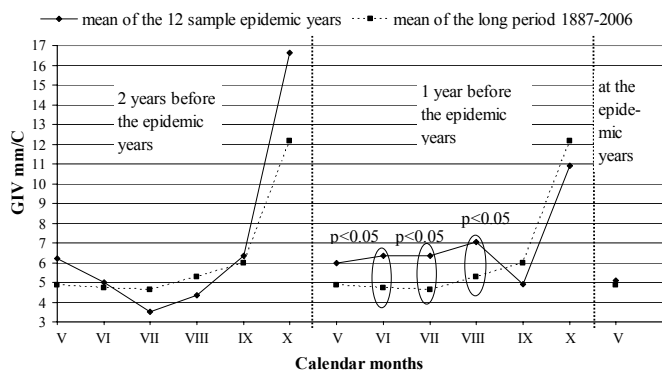


Fig. 5. Mean GIVs of the calendar months preceding twelve *Lophodermium* epidemic years (1923, 1924, 1929, 1955, 1961, 1962, 1975, 1979, 1982, 1999, 2001 and 2004) in comparison with the means of the period from 1887 to 2006. The appropriate months with the statistically significant differences in the mean GIVs on the two broken lines are displayed with ovals and supported by the p-values of difference.

3.4 Analyses of needle loss data

At the epidemic years the needle losses in pines were significantly higher at the age of 3–11 years than at the age of 20–33 years, 1.23 ± 0.06 and 0.98 ± 0.04 , respectively (Table 4). Mean needle loss at the younger group differed significantly ($p < 0.01$) from the total mean needle loss (1.00 ± 0.01) of the period 1887–2006, but needle loss of the older group did not.

The range of 3–11 years at the *Lophodermium* needle cast epidemics was not significantly different from the same tree age range of the high needle loss years, which were obviously containing some of local epidemic years not included to our sample of larger epidemics. Also, abundant high GIVs (Fig. 4) point out several other possible *Lophodermium* needle cast years among the reference group.

Without considering the host tree age, the total mean needle loss indexes were significantly ($p < 0.0001$) different for the sample of *Lophodermium* needle cast years versus the high needle loss years, as well from the low needle loss years. Rising tree age was accompanied by diminishing of the difference between the mean needle losses at the high and low needle loss years (Fig. 6).

Comparison of damages attributed here to the disease or to the insects revealed (Table 4) that at the age of 3–11 years needle losses caused by *Lophodermium* needle cast were higher (1.23 ± 0.06) than losses caused by the insects (1.09 ± 0.08). On the contrary, for the age of 20–33 years needle losses caused by insects were higher. Needle losses in both age groups of pines were significantly ($p < 0.01$) different for disease, but not for insect outbreaks.

As several local epidemics obviously incurred into the reference-group, the actual differences between the needle losses at the epidemic and reference years should be larger.

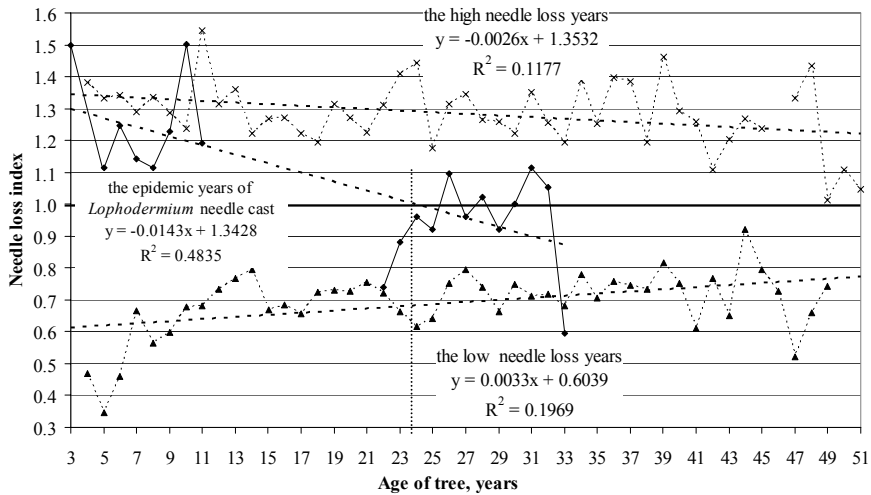


Fig. 6. Distribution of the mean needle loss indexes (computed by NTM) in dependence of tree ages at twelve *Lophodermium* epidemic years (1923, 1924, 1929, 1955, 1961, 1962, 1975, 1979, 1982, 1999, 2001 and 2004; diamond), compared to the high needle loss (but not included into the sample of *Lophodermium* epidemics) years (x) and to the low needle loss years (triangle), respectively, during the period of 1887–2006. The mean needle loss index for this long-term period was 1.0 ± 0.01 (bold line).

Table 4. Mean needle loss indexes at the 1) sample of *Lophodermium* needle cast epidemic years, and, 2) documented calamity years of diprinoid herbivorous insect pests of pine in southern Estonia

Year	The age group of trees, years	The mean needle loss index
At <i>Lophodermium</i> needle cast epidemic year	3–11	1.23 ± 0.06
	22–33	0.98 ± 0.04
At <i>B. piniaria</i> and <i>N. sertifer</i> calamity year	3–11	1.09 ± 0.08
	(20) 25–33	1.02 ± 0.06

3.5 Comparison of NTM needle loss data with the ICP forest monitoring defoliation data

Although some coincidental oscillations could be found in the NTM and ICP data-series, e.g., an increase in the needle loss/defoliation in 1994, 1998 and 2001 (Fig. 7), the needle loss dynamics were different in the four (A, B, C and D, see chapter 2.6) age groups of pine. During these three years with only 2001 belonging to the sample of epidemic years, also some other agents might cause high needle losses in different age groups. In contrast, for the epidemic year 1999, three older age groups of pines (B, C and D) demonstrated high needle losses, and only the youngest age group (A), which should have to be most vulnerable to *Lophodermium* needle cast, demonstrated a sharp decrease in needle loss. The explanation for that may lie in the high needle loss in the youngest age group for the previous year 1998 (Fig. 7). Obviously insufficient resources (needles) remained on surviving trees for the larger needle loss at the next, sample epidemic year 1999. The weakest trees obviously died for two successive outbreaks, fell out and could not be represented in the NTM material.

Our NTM data series of foliage losses in two younger age groups (A and B, respectively) correlated poorly ($r=0.43$), with regard to the different causal relations of needle losses in these age groups. On contrary, the ICP visual monitoring data of foliage loss dynamics in two older age groups of pines (C and D, respectively) demonstrated much higher correlation ($r=0.63$). Obviously needle losses, attributed to *Lophodermium* needle cast in the youngest age group could not be reflected in the needle loss (defoliation) dynamics in two oldest age groups. The needle loss dynamics of the D group correlated, although poorly, even somewhat more with A group than with the B group, coefficients 0.38 and 0.28, respectively. Only poor correlations were found in the needle loss dynamics of the age group C with younger age groups of pines.

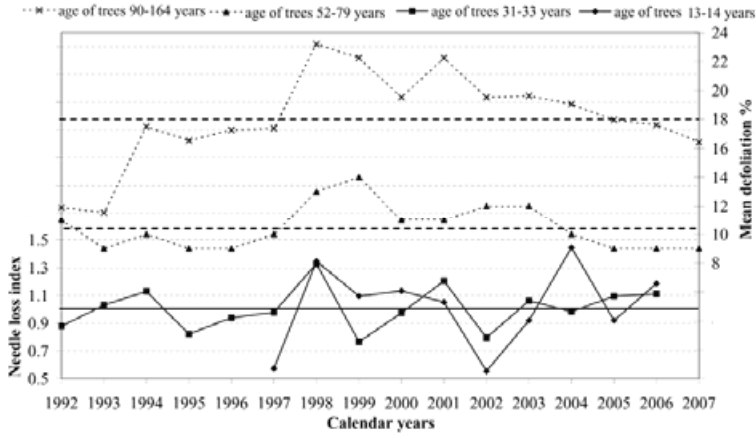


Fig. 7. Juxtaposition of needle loss indexes obtained by NTM (solid lines) with the defoliation rates visually evaluated in the ICP Forests monitoring program (dashed lines) of pine plantations and stands of different ages during the last decades. Horizontal lines indicate the long-term mean values.

3.6 The impact of *Lophodermium* needle cast on the growth of pines

During the epidemics the radial growth reduction was visible only in young pines (Fig. 8), extending at a decreasing rate up to the age of ca. 22–24 years. This upper vulnerable age of pine coincides with our other result, based on another proxy, the needle loss index data series (Fig. 6).

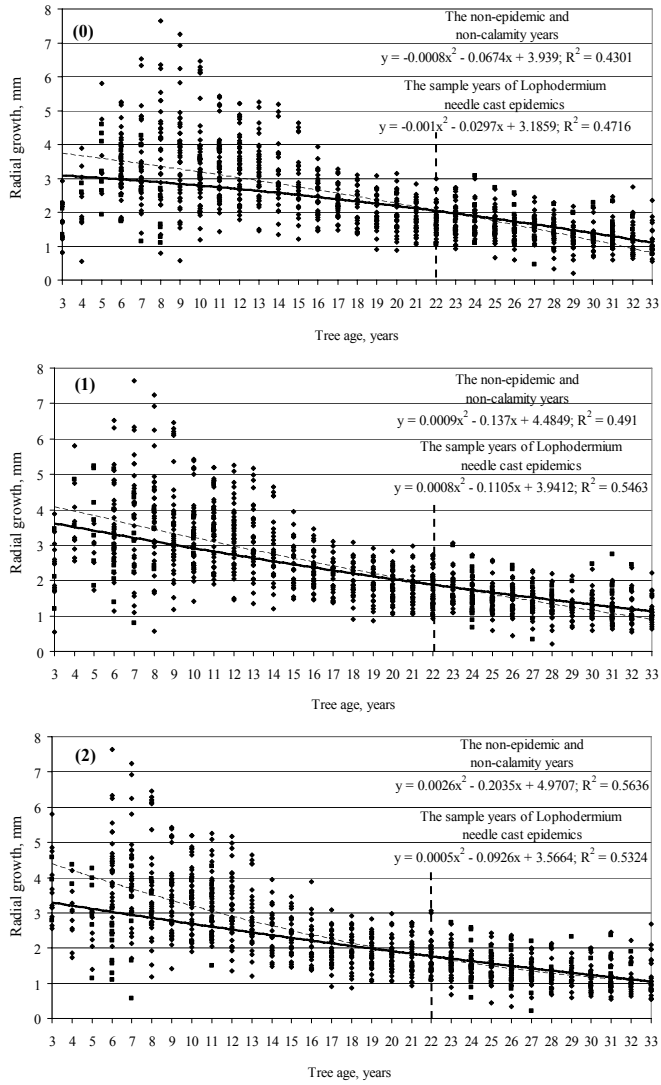


Fig. 8. Mean radial growth of trees of various ages at the sample of *Lophodermium* epidemic years (0), first post-epidemic years (1) and at the second post-epidemic years (2) (squares, bold solid line), compared to the long-term mean radial growth of the same-age trees at the non-epidemic and non-calamity years (diamonds, thin dashed line).

Table 5. Mean growth dynamics (in %) in different age groups of pine at the 12 *Lophodermium* epidemic years, one and two years after the epidemics, compared to the same mean growth parameters at the normal (not epidemic and not calamity) years

Age group (years)	Radial growth at the epidemic years	Height growth at the epidemic years	Radial growth one year after the epidemics	Height growth one year after the epidemics	Radial growth two years after the epidemics	Height growth two years after the epidemics
3–11	-12**	-18***	-7	-13**	-18****	-17****
22–33	4	7	4	21***	6	25***

P-values: ** 0.05, *** 0.01, **** 0.001

In the younger group (3–11 years) of pines, the radial growth of trees decreased significantly at the year of *Lophodermium* epidemic and two years after the epidemics, in comparison with the long-term mean, by 12 and 18%, respectively (Table 5), an insignificant decrease in radial growth at the first post-epidemic year reached 7%.

Height growth of trees has decreased significantly at the epidemic, and at two first post-epidemic years, 18, 13 and 17%, respectively (Table 5).

In the older group (22–33 years) of pines at and during the first years after the *Lophodermium* needle cast epidemics, no statistically significant growth reduction (both in radius and height) could be registered, to the contrary – some significant growth increase cases could be registered (Table 5).

3.7. The impact of herbivorous insect pests to the growth of pines

In the younger group (3–11 years), the radial growth rate of pines decreased at the two post-calamity years of insect defoliators, 12 and 19%, respectively (Table 6). Height growth of trees, on the other hand, decreased significantly already at the calamity years and after that, by 21, 26 and 34%, respectively (Table 6).

In the older group, (20)25–33 years, the radial and height growth decreased significantly at the calamity years, 13% and 17%, respectively, but growth reduction during the following the calamity two years was insignificant.

Table 6. Mean growth dynamics (in %) in two different age groups of pine during the documented 14 calamity years of herbivorous insect pests of pine, one and two years after the calamity years, compared with the same growth parameters during the normal (not belonging to the sample of *Lophodermium* needle cast epidemics and/or documented calamity) years

Age group (years)	Radial growth at the calamity years	Height growth at the calamity years	Radial growth at the post-calamity years	Height growth at the post-calamity years	Radial growth two years after the calamity years	Height growth two years after the calamity years
3–11	-3	-21***	-12**	-26****	-19****	-34****
(20)25–33	-13***	-17***	-6	-5	-1	-4

P-values: ** 0.05, *** 0.01, **** 0.001

Both defoliators such as *Lophodermium* needle cast as well herbivorous insect pests of pine significantly influence the growth characteristics of trees at and at least during two years after the pointer year, especially the height growth and for the younger group of trees (3–11 years). At the older group of pines, no significant reduction by the *Lophodermium* needle cast epidemics to the tree growth parameters could be registered, but the influence of insect defoliators continues, especially influencing the height growth of trees (Table 6).

3.8 The frequency of *Lophodermium* needle cast epidemics

The mean long-term frequency of epidemics per decade in forest nurseries was 4.6, in plantations about half of that. *Lophodermium* needle cast epidemics occurred more frequently during the first half of the 20th century, rather than the second half and the first decade of the 21st century (Table 7).

Table 7. The number per decade of reconstructed epidemics of *Lophodermium* needle cast and the mean needle losses at these epidemics during the period 1887–2010

Decade (interval)	Frequency of epidemic years per decade	Mean needle loss indexes of host trees at their vulnerable age (3–24 years) at the epidemic years
1887–1900	5	1.07
1901–1910	8	1.02
1911–1920	4	1.02
1921–1930	5	0.93a
1931–1940	6	1.15
1941–1950	5	1.08
1951–1960	4	0.88
1961–1970	4	1.05
1971–1980	3	1.15
1981–1990	4	1.15
1991–2000	3	1.01
2001–2010	4	1.17

a – Included larger host age group (23–33 years, i.e. partly older than the vulnerable age limit).

Discussion

Needle pathology of Scots pine at the 21st century in Estonia has obviously essentially changed after the arrival of several invasive pathogenic fungi like *Mycosphaerella pini*, *M. dearnessii*, *Diplodia pinea* and *Cyclaneusma minus* (Hanso and Drenkhan 2008a,b; 2009; Drenkhan and Hanso 2009). However, *Lophodermium* needle cast is continuously a serious negative factor in Scots pine nurseries and plantations of northern Baltics and Scandinavia (Hanso and Hanso 2001, 2003; Stenström and Arvidsson 2001; Stenström and Ihrmark 2005).

Meteorological predisposition to epidemics: precipitation and GIVs

The development of *L. seditiosum* is irregular and depends on environmental, particularly on climatic, factors (Martinsson 1979). Already Tubeuf (1901), Haack (1911) and Hagem (1928) noticed that high humidity in summer supports infection. However, some deviations can be also found in the literature. Wet and warm autumns may provoke epidemics (Viirik 1931), as do mild winters (Lepik 1930; Stenström and Arvidsson 2001).

Zonal atmospheric circulation has currently brought to Estonia more wet weather than previously (Keevallik et al. 1999), which obviously could support the development of the pathogen.

Ascospores of *Lophodermium seditiosum* mature, disseminate, and infect current season's needles. During the period from late autumn to early spring, visible spots appear on infected needles. By the spring of an epidemic year, the infected needles are entirely red-brown and premature loss follows from late spring to June (Hanso 1963; Minter 1981). In our work it became evident that high precipitation already in May and June may stimulate ascocarp formation in *L. seditiosum* and thereby provide the necessary level of humidity, which is

normally needed during the second half of summer. In southern Sweden, ascocarps of *L. seditiosum* developed from June to November, with the spore dispersal depending on weather conditions during these months (Stenström and Arvidsson 2001).

For modelling of the *Lophodermium* needle cast dissemination and, consequently, the seasonal timing of the control measures, the cumulative precipitation sums of the year preceding to the sample of 12 *Lophodermium* needle cast larger epidemics were calculated and compared to the appropriate long-term means.

Precipitation (moisture value) of the pre-epidemic year months May, June, July and August were important for the start of *Lophodermium* needle cast epidemics in Estonia. Neither mean rainfall sums of autumn months (September, October and November), nor the mean temperatures of summer (except of August) and winter months affect the *Lophodermium* needle cast. Obviously, the summers in Estonia are already naturally cool enough for the fungus.

GIV analyses indicated June, July and August, but not May of the pre-epidemic year, as did the precipitation analyses. The mean temperature of November in Estonia is already much lower than +5°C. The much lower, than long-time mean, temperature of the pre-epidemic year August was reflected also in the GIVs. It can be expected that if the GIV of June, July and August is 5.0, an epidemic of *Lophodermium* needle cast will follow next year in forest nurseries, but if it goes over 6.4, the epidemic may occur also in forest plantations.

Consequently, if the monthly precipitation sums from May to August (incl.) will make up 300–360 mm, or GIV 5.0–6.4, next year an epidemic of *Lophodermium* needle cast will follow in forest nurseries. If these monthly precipitation sums reach and pass 360 mm, or GIV 6.4, an epidemic may hit pines as in forest nurseries, as well in plantations.

Vulnerable age limit of hosts

Young pines in nurseries and plantations suffer from *Lophodermium* needle cast more than older pines. The highest vulnerable host age has been rarely specified in the literature, and varied largely even in these rare cases, from 1–4 years (e.g., Neger 1919), through the medium term of 10–12 years (Hanso 1965), until the highest documented length of 23 (Kujala 1950) and 25 years (Hagem 1928), sporadically reaching even 30 and more years (Kowalski 1990). According to the results of this work, the hosts' age of ca. 22–24 years was found to be the highest vulnerable age of Scots pine.

Conceivably prolonged linear mean values of the needle loss indexes of high and low needle loss years (Fig. 6) intersected at the age of pines of approximately 120 years. It may mark that from this age it is not possible to divide different years into the low and the high needle loss years. It may indicate that an upper age limit should be established for Scots pine in the ICP Forests defoliation monitoring programs. As it is generally expected, Scots pines reach their full maturity at the age of 100–150 years, when also their height growth ceases (e.g., Sarvas 1964).

Juxtaposition of NTM needle loss and ICP Forests defoliation data

Kowalski and Lang (1983) evaluated the occurrence of *L. seditiosum* in 70–100-year old Scots pine stands in Poland as negligible. However, in several published ICP Forests monitoring data *Lophodermium* needle cast has been denoted as one of the causes of needle losses even in adult Scots pine stands (e.g., Hendry et al. 2001, 2002; Asi et al. 2005). Since one of the aims of this study was a trial to specify the highest vulnerable age limit of pines to *Lophodermium* needle cast, we also juxtaposed NTM data series with the ICP Forests monitoring data.

Pouutu and Dobbertin (2000) revealed the annual needle retention and needle density patterns in *P. sylvestris* over the past 100 years in Rhone Valley, Switzerland. By comparing

the visually assessed Swiss Forest Inventory data between 1985 and 1996 with their original NTM data, a significant correlation between the mean values was revealed – in two thirds of all years the direction of the annual changes was identical. The decrease in foliage was connected there mainly with severe droughts, but the reaction of pines to drought should be less dependent on tree age than to *Lophodermium* needle cast.

In Estonia a weak positive correlation ($r=0.38$) was found between the needle loss (defoliation) dynamics in the oldest, D-group (90–164 years, ICP Forests) and the youngest, A-group (NTM) of pines. There were no correlations between the needle losses in the C-group (52–79 years, ICP Forests) with younger age groups, A and B (both NTM). Needle loss dynamics in both middle-age groups of pines, B and C, in comparison with the youngest and oldest age groups, were also characterized by smaller variations (Fig. 7). Needle loss in all age groups of pines increased in 1994 and 1998, but also the year 2001 was characterised by the visibly higher needle losses. In 1994 a local *Lophodermium* needle cast epidemic was registered (Hanso and Drenkhan 2007), but the wide-scale epidemic year 2001 (Hanso and Hanso 2001), represented one of the sample epidemic years (see chapter 2.2). The common high needle loss in pines in 1998 might be caused by hard summer drought a year before, or by a small-scale attack of insect defoliators. When the high needle losses in young pines could be attributed to the *Lophodermium* needle cast epidemics, then the hard droughts registered in southern Estonia in 1992, 1997, 1999, 2002 and 2006, could inflict needle retention and cause loss also in older pines.

Herbivorous insect pests

Meteorological prerequisites that trigger calamities of herbivorous insect pests are obviously different from those that trigger fungal diseases. In comparing the meteorological conditions, it became evident, that for an insect calamity the warmer month-pairs of May-June and October-November occurred as decisive prerequisites (Table 3). At preceding to the calamity years, the mean temperatures of the entire summer- (from May to September) and of the winter-time (from December to March) were also slightly warmer than those, preceding to the *Lophodermium* epidemics. Concerning calamities, these results are in full agreement with the earlier investigations (Kulman 1971; Larsson and Tenow 1984; Mihkelson 1986; Olofsson 1987; Voolma and Luik 2001).

The impact of needle loss on tree growth at and after the epidemics and calamities

Tree growth is the indicator of tree vitality (Dobbertin 2005). Individual susceptibility of pine to *Lophodermium* needle cast varies greatly (Schütt 1957; Martinsson 1979). In a 12 year old Scots pine stand in Estonia, needle loss from the productive foliage at the level of 50%, caused by *Lophodermium* needle cast and summer drought, decreased radial and height increment of trees by 39.2% and 34.8%, respectively (Kurkela et al. 2009).

In this study, comparison of the changes in the growth rate of affected trees, attributed to the epidemics of *Lophodermium* needle cast or to the calamities of insect defoliators, demonstrated comparatively similar, significant reduction in both radial and height increments of trees at the younger age (3–11 years), as at the epidemic or calamity years, as well as at the first and second year after the pointer years (Table 5 and 6; Fig. 8). Both characteristics demonstrated stronger decreases in height, rather than in radial growth. The height growth of trees at this age of stand formation is decisive for their fate. As expected, losses from the productive needle sets at the beginning of seasonal tree growth of the next and of the second year after the epidemic had to be accompanied by the growth rate decrease (Drenkhan et al. 2006; Kurkela et al. 2009), regardless of the agent, e.g., *Lophodermium* needle cast (Hanso 1963; Minter 1981) or diprinoid insect defoliator (Larsson and Tenow 1984; Cedervind 2003). *Lophodermium* needle cast causes early summer defoliation. Among diprinoid insects

Neodiprion sertifer is also an early-season defoliator, which leaves intact only the current year needles of pines (Cedervind 2003), whereas *Bupalus piniaria* is a late-season defoliator, influencing the growth rate of trees with one- or two-years long lag (Armour et al. 2003). In the list of calamities of diprionid insect defoliators in Estonia *N. sertifer* is prevailing.

As the spatial extension of a calamity of diprionid sawflies is generally smaller than that of an epidemic of *Lophodermium* needle cast, the resulting needle losses of both types of defoliators would be only hardly comparable, whereby no documental proof exists, that our NTM stands and sample trees were definitely affected or non-affected by the insect pests during the documented in southern Estonia calamity years. Infection of pines at the epidemic years of *Lophodermium* needle cast is more general, if not total.

During the calamity years of herbivorous insect pests, but not during the epidemic years both growth rate characteristics (RI and HI) decreased in the older age group of pines (20–33 years), if to compare with the long-term means.

For the evaluation of the influences of insect defoliators on the growth rate of trees, one also has to consider the negative influence of drought, since insect calamities were dependent on warm (or even droughty) summers (Table 3). On the contrary, moist and rather cool summers that trigger *Lophodermium* needle cast epidemics are generally characterised as pointer years with the large coherent positive tree response (Neuwirth et al. 2007). However, extreme weather conditions alone may increase needle losses and decrease growth rate in pines (Löhmus 1992; Pouttu and Dobbertin 2000; Drenkhan and Hanso 2004; Hordo et al. 2009). It should not be forgotten that one calamity year registered in Estonia preceded the century's coldest winter, that of 1939/40 (Löhmus 1992; Drenkhan and Hanso 2006).

During three years (at the pointer-, the next and second year after the epidemic), the radial growth of trees in the youngest age group of pines (3–11 years) decreased in the case of *Lophodermium* needle cast by 37% and in the case of insect defoliators by 34%, but height growth by 48% and by 81%, respectively. In difference from the needle diseases, insect defoliations of pines are accompanied by the additional energy losses (resin exudation, etc., cf. Kurkela et al. 2009).

In Finland, in trees, which suffered from the late defoliator *Diprion pini*, the greatest yearly radial increment loss reached about 95%, whereas the effect of defoliation on the height increment was not so abrupt and drastic (Kurkela et al. 2005). Långström et al. (2001) reported that in Sweden a single year of complete defoliation of Scots pine by *Diprion pini* incurred a total loss in radial growth about 35%, which lasted during 4 years. In the east of Scotland the radial and volume increments of 20–25 years old Scots pine trees were substantially reduced, by up to 50%, for 2–3 years after the calamities of *B. piniaria* (Armour et al. 2003). In Sweden, growth losses increased with the degree of defoliation by *B. piniaria*, and trees with 90 and 100% defoliation lost 56% and 59% of their volume growth, respectively, even during the six years following the defoliation (Cedervind 2003).

Consequently, the impact of needle losses through the *Lophodermium* needle cast and diprionid defoliators of pines could be investigated for even longer time than the two to three years examined in this work, if the high frequency of *Lophodermium* needle cast epidemics would not hinder to do that.

Acknowledgements

The authors thankfully acknowledge prof. emer. Timo Kurkela from Helsinki, Finland and prof. Ottmar Holdenrieder from Zürich, Switzerland for valuable comments and suggestions concerning the manuscript, Mr. Allar Padari from Tartu, Estonia, for technical assistance, Mr. Kestutys Grigaliunas from Kaunas, Lithuania, for the data of disease outbreaks in Lithuania, and Mr. Terry Bush from Madison, Wisconsin, USA, for the language revision. The study was supported by the Estonian Environmental Investments Centre and partly financed by the project SF0170021s08.

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1995–1999 Bakalaureuseõpe metsamajanduse erialal, Metsandus-
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1983–1995 Antsla Keskkool

Võõrkeelte oskus: inglise keel , soome keel, vene keel

Teaduskraad:
2003 Teadusmagister metsakasvatuse erialal, väitekirj: Hari-
liku männi (*Pinus sylvestris* L.) okastust iseloomustavate
tunnuste ning puude juurdekasvu võrdlev uurimine
okkajälje meetodil põlisele metsamaale ning endisele
põllumaale rajatud puistus. Eesti Põllumajandusülikool

Teenistuskäik:
Alates 2005 Eesti Maaülikool, Metsandus- ja maehitusinstituut,
lektor
2004–2007 Metsahoiu Sihtasutus, juhataja
2003–2004 Eesti Põllumajandusülikool, Metsanduslik Uurimisins-
tituut, laborant
2002–2004 Eesti Metsakeskus OÜ, spetsialist
1998–2001 Karula Rahvuspark, teadusspetsialist

Uurimistöe põhisuunad:
Metsapatoloogia; okaste-, lehtede- ja võrsete haigused;
puudehaigused kliimamuutuste kontekstis

Teaduspreemiad ja stipendiumid:

2007	Kristjan Jaagu riiklik välislähetus-stipendium
2003	Eesti Teaduste Akadeemia preemia magistritööle
1999	Eesti Teaduste Akadeemia preemia bakalaureusetööle
1998	Eestlaste Kesknõukogu Kanadas, stipendium

Projektid:

2010–2011	„Uute invasiivsete (s.h. karantiinsete) metsahaiguste areng ja mõju meie metsaloodusele kliimamuutuste taustal“, SA Keskkonnainvesteeringute Keskus, vastutav täitja
2008–2013	Sihtfinantseeritav teema SF0170021s08: „Biomassi produktsioon metsaökosüsteemides, selle metsanduslikud ja ökofüsioloogilised alused“, osaline täitja
2005–2010	Põhjala-Balti metsapatoloogia koostöövõrgustik (PATHCAR), Põhjamaade ministrite nõukogu, osaleja ja täitja
2008–2009	„Uute invasiivsete metsahaiguste ohtlikkuse ja leviku uuring Eestis“, SA Keskkonnainvesteeringute Keskus, vastutav täitja
2007–2008	„21. sajandi alguse süvenevate metsakahjustuste põhjuste analüüs“, SA Keskkonnainvesteeringute Keskus, vastutav täitja
2003–2005	LIFE projekt „Prioriteetsete metsaelupaikade kaitse Eestis“, Euroopa Komisjon, täitja.

Erialane täiendõpe:

2008	Kursus „Taimede DNA eraldamine ja PCR“, Eesti Maaülikool, Tartu
2006	Doktorikursus „Puu võra – struktuur ja funktsioneerimine – alt ja ülevalt“, Tartu Ülikool, Eesti
2006	NOVA „Välikursus metsapatoloogias“, Helsinki Ülikool, Soome
2004	Okkajälje meetodika alane täiendamine, METLA, Rovaniemi katsejaam, Soome
2001–2002	Magistriõppe programm „Säästlik metsandus Balti mere regioonis“, Rootsi Põllumajandusülikool (SLU), Alnarp, Rootsi

Teadusorganisatsiooniline tegevus:

1999–	Eesti Loodusuurijate Seltsi liige
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CURRICULUM VITAE

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Education:
2003–2010 PhD studies in Forest Management, Institute of Forestry and Rural Engineering, Estonian University of Life Sciences
1999–2003 Master studies in Forest Management, Faculty of Forestry, Estonian Agricultural University
1995–1999 Bachelor studies in Forest Management, Faculty of Forestry, Estonian Agricultural University
1983–1995 Antsla Secondary School

Foreign languages:
English, Finnish, Russian

Academic degree:
2003 Master's Degree, Thesis: Comparative investigation of the foliage describing characteristics by needle trace method and growth rate of Scotch pine (*Pinus sylvestris* L.) in the stands on permanent forest soil and on former arable soil. Estonian Agricultural University

Professional employment:
Since 2005 Estonian University of Life Sciences, Institute of Forestry and Rural Engineering, lector
2004–2007 Metsahoiu Sihtasutus, manager
2003–2004 Estonian Agricultural University, Forest Research Institute, laboratory assistant
2002–2004 Eesti Metsakeskus OÜ, specialist
1998–2001 Karula National Park, specialist of science

Research interests:
Forest pathology; especially foliage and shoot diseases; diseases in the context of climate change

Awards and grants:

- 2007 Kristjan Jaak Mobility Scholarship
2003 Estonian Academy of Sciences, prize for Master's thesis
1999 Estonian Academy of Sciences, prize for Bachelor's thesis
1998 Estonian Central Council in Canada, Scholarship

Projects and cooperation:

- 2010–2011 “The development and impact of new invasive (incl. quarantine) forest diseases to our forests on the wave of climate change.” Environmental Investment Centre. Chief performer.
2008–2013 TF project SF0170021s08: “Production of biomass in forest ecosystems, its silvicultural and ecophysiological bases.” Partial performer.
2005–2010 Centre of Advanced Research in Forest Pathology (PATHCAR), Nordic Forest Research Co-operation Committee. Participant and performer.
2008–2009 “Investigation of riskiness and extension of new invasive forest diseases in Estonia.” Environmental Investment Centre. Chief performer.
2007–2008 “Analysis of extraordinary pathological processes in forests at the beginning of the 21st century.” Environmental Investment Centre. Chief performer
2003–2005 LIFE-Natura project “Protection of priority forest habitat types in Estonia.” European Commission. Performer.

Professional trainings:

- 2008 Graduate course, “Plant DNA extraction and polymerase chain reaction (PCR).” Estonian University of Life Sciences, Tartu, Estonia.
2006 Ph.D. course, “Tree canopy – structure and functioning – from below and above.” University of Tartu, Estonia.
2006 NOVA course, “Field course in Forest Pathology,” University of Helsinki, Finland.
2004 Complement course of Needle Trace Method. Finnish Forest Research Institute, Rovaniemi, Finland.
2001–2002 M.Sc. programme “Sustainable forestry around the Baltic Sea,” Swedish University of Agricultural Sciences, Alnarp, Sweden

Membership:

- 1999– Member of the Estonian Naturalists Society

LIST OF PUBLICATIONS

Publications indexed in the ISI WEB of Science database:

- Drenkhan, R.,** Hanso, M. 2004. Recent natural disturbances in Scots pine (*Pinus sylvestris* L.) plantations and stands of South-East Estonia: Causes and consequences. Transactions of the Faculty of Forestry, Estonian Agricultural University 37: 17–22.
- Drenkhan, R.,** Kurkela, T., Hanso, M. 2006. The relationship between the needle age and the growth rate in Scots pine (*Pinus sylvestris* L.): a retrospective analysis by needle trace method. European Journal of Forest Research 125: 397–405.
- Hanso, M., **Drenkhan, R.** 2008. First observations of *Mycosphaerella pini* in Estonia. Plant Pathology 57: 1177.
- Hanso, M., **Drenkhan, R.** 2009. *Diplodia pinea* is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia. Plant Pathology 58: 797.
- Rytkönen, A., Lilja, A., **Drenkhan, R.,** Gaitnieks, T., Hantula, J. 2010. First record of *Chalara fraxinea* in Finland and genetic variation among samples from Åland, mainland Finland, Estonia and Latvia. Forest Pathology DOI: 10.1111/j.1439-0329.2010.00647.x (accepted 05.03.2010).
- Drenkhan, R.,** Hanso, M. 2010. New host species for *Chalara fraxinea*. New Disease Reports 22: 16.
- Hanso, M., **Drenkhan, R.** 2010. Epidemiology of *Lophodermium* needle cast of Scots pine (*Pinus sylvestris* L.) in Estonia. *Forest Pathology* (manuscript, submitted).

Papers published in other peer-reviewed international journals with a registered code (or international editorial board):

- Drenkhan, R.,** Hanso, M. 2000. Needle retention, needle density and growth rate of Scots pine (*Pinus sylvestris* L.). Forstry Studies / Metsanduslikud Uurimused 34: 85–91.
- Drenkhan, R.,** Hanso, M. 2006. Alterations of Scots pine needle characteristics after severe weather conditions in south-eastern Estonia. *Aktuelt fra skogforskningen* (Oslo) 1: 69–74.

- Hanso, M., **Drenkhan, R.** 2007. Retrospective analysis of *Lophodermium seditiosum* epidemics in Estonia. Acta Silvatica & Lignaria Hungarica, Special Edition: 31–45.
- Kurkela, T., **Drenkhan, R.**, Vuorinen, M., Hanso, M. 2009. Growth response of young Scots pines to needle loss assessed from productive foliage. Forestry Studies/ Metsanduslikud Uurimused 50: 5–22.
- Drenkhan, R.**, Hanso, M. 2009. Recent invasion of foliage fungi of pines (*Pinus* spp.) to the Northern Baltics. Forestry Studies / Metsanduslikud Uurimused 51: 49–64.
- Hanso, M., **Drenkhan, R.** 2010. Two new Ascomycetes on twigs of Silver birches (*Betula pendula*) in Estonia. Folia Cryptogamica Estonica 47: 21–26.

Papers published in other peer-reviewed research journals with a local editorial board:

- Drenkhan, R.** 2000. Männikokaste säilimise retrospektiivne uurimismeetod ja selle kasutamise esimesi tulemusi Eestis [Needle trace method for the retrospective analysis of Scots pine (*Pinus sylvestris* L.) needle retention and first results of its use in Estonia]. Transactions of the Faculty of Forestry, Estonian Agricultural University 33: 13–20. [In Estonian, summary in English].
- Drenkhan, R.**, Hanso, M. 2003. Kas okkajälje meetod võib abiks olla männi-pudetõve (*Lophodermium seditiosum*) epideemiae retrospektiivsel analüüsil? [Could the needle trace method be of help in the retrospective analysis of *Lophodermium seditiosum* epidemics?]. Transactions of the Faculty of Forestry, Estonian Agricultural University 36: 11–20. [In Estonian, abstract in English].
- Drenkhan, R.**, Hanso, M. 2005. Hariliku männi (*Pinus sylvestris* L.) okastustunnuste hälvetest karmide talvede järel Kagu-Eestis [Deviations in needle characteristics of Scots pine (*Pinus sylvestris* L.) caused by the severe winters in South-East Estonia]. Transactions of the Faculty of Forestry, Estonian Agricultural University 38: 9–20. [In Estonian, abstract in English].

Applied research publications:

- Drenkhan, R.** 1999. Männikute uuenemisest Kabala metskonnas [Regeneration of pine stands in the Kabala Forest District]. Eesti Mets [Estonian Forest] 7: 20–21.
- Hanso, M., **Drenkhan, R.** 1999. Kas männiokkad varisevad enneaegu? [Do pine needles shed prematurely?] Eesti Loodus [Estonian Nature] 6: 235–237.
- Drenkhan, R.** 2000. Männiokaste eluea määramine okkajälje meetodiga [Determination of the age of pine needles by the Needle Trace Method]. Eesti Mets [Estonian Forest] 5: 16–18.
- Hanso, M., **Drenkhan, R.** 2007. Metsa- ja linnapuud ilmastiku äärmuste vaevas [Forest and town trees are suffering from the extreme weather conditions]. Eesti Loodus [Estonian Nature] 58: 6–13.
- Hanso, M., **Drenkhan, R.** 2007. Punavöötaud on jõudnud Eestisse! [Red belt blight has reached Estonia!]. Eesti Loodus [Estonian Nature] 38: 52.
- Hanso, M., **Drenkhan, R.** 2008. Puna-, pruun- ja valgevöötaud Eestis [Red-, brown- and white-belt blights in Estonia]. Eesti Mets [Estonian Forest] 23: 16–22.
- Drenkhan, R.**, Hanso, M. 2009. Hariliku saare allakäik Eestis ja mujal Euroopas [Common ash dieback in Estonia and elsewhere in Europe]. Eesti Loodus [Estonian Nature] 60: 14–19.
- Drenkhan, R.**, Hanso, M. 2009. Sauresurma tekitaja suguline arengujärk on leitud [Sexual state of the ash dieback agent has been discovered]. Eesti Loodus [Estonian Nature] 60: 21.
- Drenkhan, R.**, Hanso, M. 2010. Uus pudetõbi ebatsuugal [New needle cast disease in Douglas-fir]. Eesti Mets [Estonian Forest] 3: 21–23.

PRESENTATIONS

Presentations at international conferences and meetings

1. **Drenkhan, R.**, Hanso, M. “Recent natural disturbances in Scots pine (*Pinus sylvestris* L.) plantations and stands of South-East Estonia: Causes and consequences.” International Workshop on Natural disturbances and ecosystem-based forest management. 27–29 May **2004**, Otepää, Estonia.
2. **Drenkhan, R.**, and Hanso, M. “Alterations of Scots pine needle characteristics after severe weather conditions in south-eastern Estonia.” SNS Nordic-Baltic Meeting on Forest Pathology. 28–31 August **2005**, Biri, Norway.
3. **Drenkhan, R.**, and Hanso, M. “First use of NTM in the diagnosis of complicated cases of natural disturbances in Scots pine.” International Workshop on the scale of natural disturbances from tree to stand. 28–30 September **2005**, Palanga, Lithuania.
4. Hanso, M., **Drenkhan, R.** “Retrospective analyses of *Lophodermium seditiosum* epidemics in Estonia.” IUFRO International Conference on foliage, shoot and stem diseases of forest trees. 21–26 May **2007**, Sopron, Hungary.
5. Hanso, M., **Drenkhan, R.** “Retrospective survey of the *Lophodermium* needle cast epidemics in Estonia.” SNS PATHCAR Nordic-Baltic Meeting on Forest Pathology. 26–30 August **2007**, Hyytiälä, Finland.
6. **Drenkhan, R.**, Hanso, M. “Alterations of Scots pine growth and needle characteristics after abrupt winter onsets: A retrospective analyses using the needle trace method (NTM).” SNS PATHCAR Nordic-Baltic Meeting on Forest Pathology. 26–30 August **2007**, Hyytiälä, Finland.
7. Hanso, M., **Drenkhan, R.** “First observations of *Mycosphaerella pini* in Estonia.” SNS PATHCAR Nordic-Baltic Meeting on Forest Pathology. 26–30 August **2007**, Hyytiälä, Finland.
8. Hanso, M., **Drenkhan, R.** “Alien fungal colonizers of pine needles in Estonia: A signal of climate warming?” International SNS Workshop on the Network of Climate Change Risks on Forests. 28–30 August **2008**, Umeå, Sweden.

9. Hanso, M., **Drenkhan, R.** “Alien fungal colonizers of pine needles in Estonia: A signal of climate warming?” XVII Symposium of Baltic Mycologists and Lichenologists. 17–21 September **2008**, Mändjala, Saaremaa Island, Estonia.
10. Hanso, M., **Drenkhan, R.** “Multiproxiol approach to the epidemiology of *Lophodermium* needle cast of Scots pine (*Pinus sylvestris* L.) in Estonia.” SNS PATHCAR Nordic-Baltic Meeting on Forest Pathology. 28 September–2 October **2009**, Palanga, Lithuania.
11. Hanso, M., **Drenkhan, R.** “Forest health decline after the climatic extremes in Estonia.” International SNS Workshop on the Network of Climate Change Risks on Forests. 26–28 October **2009**, Tvärminne, Finland.
12. **Drenkhan, R.**, Hanso, M. “*Chalara fraxinea* on exotic ash trees in Estonia.” International EPPO Workshop on *Chalara fraxinea*. 30 June–2 July **2010**, Oslo, Norway.

Presentations in Seminars

1. **Drenkhan, R.**, Hanso, M. “Alterations of Scots pine needle characteristics after severe weather conditions in south-eastern Estonia.” Seminar of Forest Pathology, 4 April **2005**, METLA, Helsinki, Finland.
2. **Drenkhan, R.**, Hanso, M. “Recent and past damages on Scots pine in Estonia as revealed by the needle trace method (NTM).” Seminar of Forest Pathology, 3 April **2006**, METLA, Helsinki, Finland.
3. Kurkela, T., **Drenkhan, R.** “Needle loss and growth rate in young Scots pines.” Seminar of Forest Pathology, 20 November **2006**, METLA, Helsinki, Finland.
4. Hanso, M., **Drenkhan, R.** “Alien fungal colonizers of pine needles in Estonia: A signal of climate warming?” Seminar of Forest Pathology, 16 April **2008**, METLA, Helsinki, Finland.
5. Hanso, M., **Drenkhan, R.** “Uued metsahaigused Eestis seonduvalt vapustustega ilmastikus“ [New forest diseases connected with the weather extremes in Estonia]. XVIII Taimekaitsepäeval [XVIII Day of Plant Protection], 18 November **2008**, Tartu.
6. Hanso, M., **Drenkhan, R.** “Ilmastik ja uued metsahaigused” [Weather and the new diseases in forests]. ELUS'i Mükoloog-iaühingu aastakoosolek [Yearly Meeting of the Estonian Mycological Society]. 12 December **2009**, Tartu.

7. **Drenkhan, R.**, Hanso, M. “Invasiivsetest seenhaigustest lehtpuudel” [New invasive diseases on broadleaf trees]. Metsakaitse seminaril “Uued invasiivsed seenhaigused ja putukkahjurid Eestis kliimamuutuste foonil” [Seminar on “New invasive fungal diseases and insect pests on the wave of climate change in Estonia”]. 6 April **2010**, Tartu.
8. Hanso, M., **Drenkhan, R.** “Ilmastikuäärmustest ja nende võimalikust toimest metsa- ja pargipuude üldseisundile” [Weather extremities and their potential impact on the health situation of the forest and ornamental trees]. Metsakaitse seminaril [Seminar on Forest Protection], 6 April **2010**, Tartu.
9. Hanso, M., **Drenkhan, R.** “Invasiivseid, sh. karantiinseid okaspuude seenhaigusi” [New invasive, incl. quarantine fungal diseases on conifers]. Metsakaitse seminaril [Seminar on Forest Protection], 6 April **2010**, Tartu.
10. **Drenkhan, R.** “What is the origin of the Estonian and Finnish *Dothistroma septosporum* populations?” Seminar of Forest Pathology, 16 April **2010**, METLA, Helsinki.

