

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

Rein Drenkhan* and Märt Hanso

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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

Authors' address: Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Kreutzwaldi, 5, 51014 Tartu, Estonia, *e-mail: rein.drenkhan@emu.ee

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-Ayanz *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-

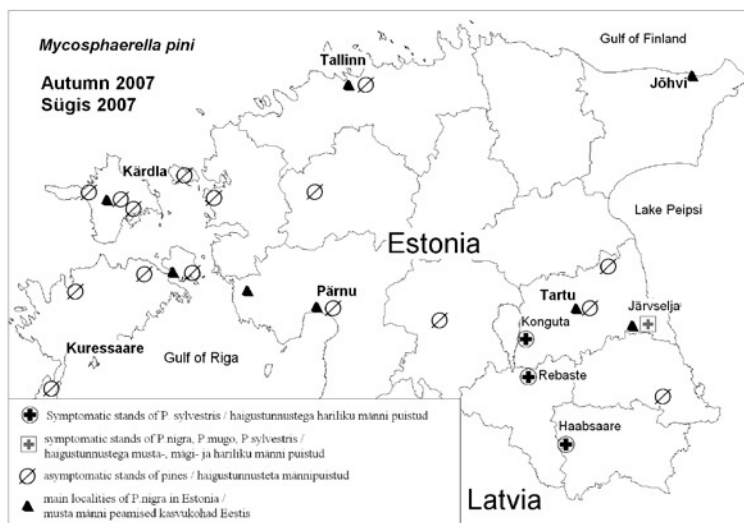


Figure 1. Distribution map of *Mycosphaerella pini* in autumn 2007.

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

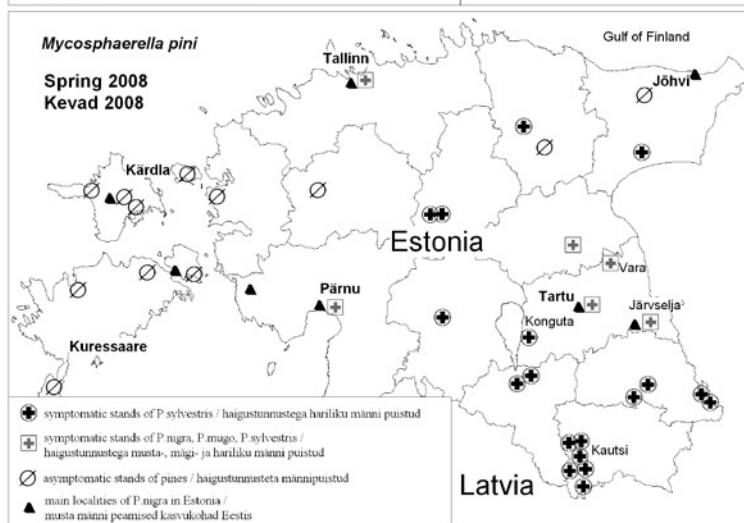


Figure 2. Distribution map of *Mycosphaerella pini* in spring 2008.

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

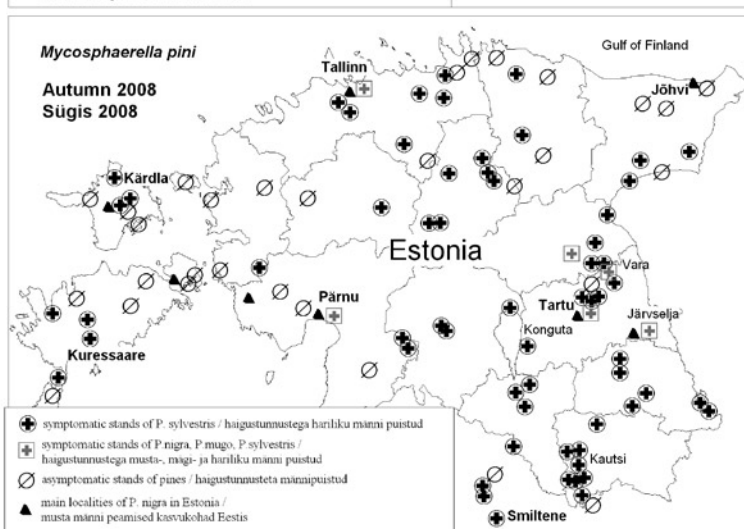


Figure 3. Distribution map of *Mycosphaerella pini* in autumn 2008.

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)

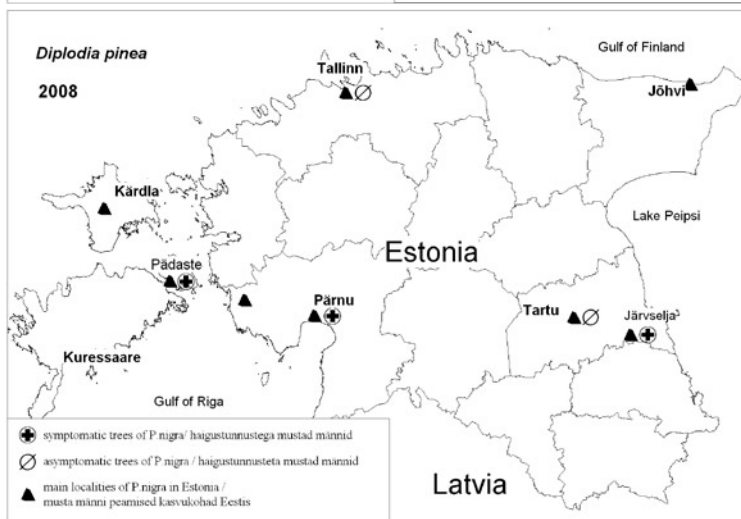
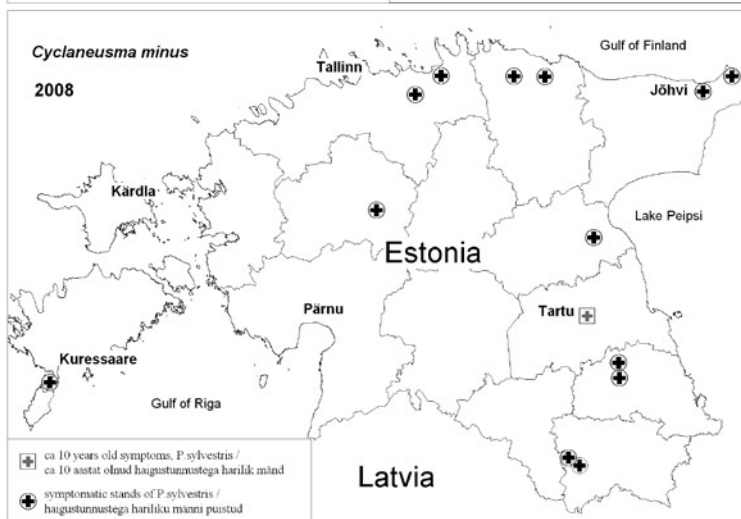


Figure 6. Distribution map of *Cyclaneusma minus* in 2008.

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 viljakehades 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>Substraat</i>		<i>Koniidide pikkus (μm)</i>		<i>Koniidide laius (μm)</i>			<i>Kirjandusallikas</i>	
				<i>Keskmine</i>		<i>Vahemik</i>	<i>Keskmine</i>	<i>Vahemik</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	30	24.8	4.4	18.6-36.1	2.5	0.4	1.8-3.7	"
		Culture	58	22.6	4.4	13.2-31.4	2.4	0.3	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	40	27.8	4.4	18.7-38.2	2.4	0.3	1.9-3.3	"
		Culture	60	27.3	3.9	15.9-36.4	2.4	0.2	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	93	21.4	3.2	14.2-31.5	3.0	0.3	2.1-4.0	Barnes <i>et al.</i> 2008
		Culture	45	28.1	7.1	12.9-43.3	3.0	0.4	2.2-4.3	
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 Proovi päritolu	Vara (N=30)		Järvselja (N=30)		Konguta (N=40)		Kautsi (N=30)		Latvia (N=40)	
	length pikkus (µm)	width laius (µm)	length pikkus (µm)	width laius (µm)	length pikkus (µm)	width laius (µm)	length pikkus (µm)	width laius (µm)	length pikkus (µm)	width laius (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 EPPO, 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
<i>Proovi päritolu</i>	<i>Peremees-taim</i>	<i>Substraat</i>	<i>Koniidi tunnus</i>							<i>Kirjan-dusallikas</i>	
Estonia, Järvselja	<i>Pinus nigra</i>	Cone scales	0-septate	36	37.3	3.5	30.6-47.1	14.0*	1.0	11.8-16.5	Original material
Estonia, Pädaste	<i>Pinus nigra</i>	Cone scales	0-septate	30	33.8	3.3	28.9-40.9	12.8*	1.3	9.7-15.4	"
Estonia, Pädaste	<i>Pinus nigra</i>	Cone 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>
USA, central	<i>P. mugo</i>	Cultures		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 proviides 0- ja 1-vaheseinaga 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 easkottide mõõtmed

Sample origin	Host	Substrate	Microstructure	Length (μm)				Width (μm)			Reference
				Pikkus (μm)				Laius (μm)			
				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>	
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	"
		Needle	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. Ascum dimensions of *Cyclaneusma* spp.Tabel 5. *Cyclaneusma* spp. viljakehade mõõtmed.

Sample origin	Host	Substrate	Microstructure	Length (mm)				Reference
				Pikkus (μm)				
				N	Mean	SD	Range	
<i>Proovi päritolu</i>	<i>Peremees-taim</i>	<i>Substraat</i>	<i>Mikrostruktuur</i>		<i>Keskmine</i>		<i>Vahemik</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
	<i>Cyclaneusma minus</i>							
Ukraine, Crimea	<i>Pinus</i> spp.	Needle	Ascomata				most <0.5	Minter and Dudka 1996
	<i>Cyclaneusma niveum</i>							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 Järvselja, 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.

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

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 mitmete 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 uurimistöö 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 rekognostseerimeetodil, 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 herbaariumis 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 *Dothistroma 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 üldsuunaga 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 eksootilike, 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 puistuid. 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 sinetusseenaena 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 (haigusetekiataja *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 tagamiseks kui ka varuainetesse.

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