

OCCURRENCE OF *LOPHODERMIIUM* SPP. IN YOUNG SCOTS PINE STANDS IN LATVIA

Kaspars Polmanis^{1,2}, Talis Gaitnieks¹, Viktorija Beļeviča¹, Dainis Rungis¹, Anita Baumanė¹

¹Latvian State Forest Research Institute 'Silava'

²Latvia University of Agriculture

kaspars.polmanis@silava.lv

Abstract

Scots pine (*Pinus sylvestris* L.) is one of the most widespread coniferous species in boreal zones and it has a considerable economic importance in the Baltic countries. The impact of fungi on Scots pine has been known also historically, but it is predicted that it will increase in future due to climate changes that will have a positive effect on incidence and vitality of various species of fungi. The aim of the study was to characterize the occurrence of *Lophodermium* spp. in young stands of Scots pine in Latvia. Needle samples were collected from young pine stands (aged 1 – 14 years) located in all regions of Latvia. DNA extraction from needles was done using modified CTAB protocol, presence of *Lophodermium* in total extracted DNA was detected using a PCR method. Meteorological data – air temperature and precipitation – were obtained from all 34 observation stations of the Latvian Environment, Geology and Meteorology Centre. The presence of *Lophodermium* spp. was detected in all surveyed stands, and occurrence differences between the western and eastern regions were significant ($p = 0.004$). The occurrence of *Lophodermium* spp. in the eastern and western regions was affected by the meteorological conditions in autumn and winter of the previous years, as well as differences in the given years' air temperature and precipitation. In 2016, the eastern region of Latvia had a higher May-August precipitation and, to a lesser extent, temperature, which correlated with a higher occurrence of *Lophodermium* spp.

Key words: needle cast, disease distribution, meteorological conditions.

Introduction

Pine forests have considerable economic importance in the Baltic countries, as demonstrated both by the contribution of the forestry sector to the Gross Domestic Product (GDP) and the proportion of wood and wood products in total export value. Therefore, it is important to understand threats that might negatively affect vitality and growth of Scots pine trees in Latvia. Many threats are associated with rapidly progressing climate changes (Edenhofer *et al.*, 2014). The relationship between meteorological factors and increment (both height and radial) of Scots pine has been well studied and the obtained results mainly demonstrate a slightly positive influence of the predicted changes on tree growth (Jansons *et al.*, 2013a, 2013b, 2015a, 2016a; Rieksts-Riekstins *et al.*, 2014). One of the threats to young Scots pine trees in particular is a needlecast disease caused by fungus *Lophodermium seditiosum*. *Lophodermium* spp. are highly diverse endophytic fungi with woody hosts, representing a range of symbiotic interactions with their host plants, from commensals and mutualists to latent pathogens. They have a one-year development cycle (Ortiz-García *et al.*, 2003). *Lophodermium seditiosum* is one of the most important pathogens of Scots pine (*Pinus sylvestris* L.) needles in nurseries and young stands (Drenkhan, 2011). If the infection of this fungus in the forest nursery is not resolved, it can spread to young pine stands (Kļaviņa *et al.*, 2012). Therefore, research has been done to develop effective fungicides to combat the disease (Millar, 1975; Ormrod, 1976). Weather conditions favorable

for this disease are increasingly observed during recent decades – humid and warm autumns (as well as the end of summer) and mild winters (Martinsson, 1979; Diwani & Millar, 1990; Stenström & Arvidsson, 2001), making this period of time suitable for the studies of occurrence and impact of this disease. Hanso & Drenkhan (2007) indicate that Estonian climatic conditions (cold winters) are not an obstacle to the spread of the disease, which are favorable in late summer and autumn. Host-pathogen co-evolution as well as tree breeding had ensured development of resistance of local Scots pine individuals (genotypes) against the disease (Reich *et al.*, 2003; Booy *et al.*, 2000).

In recent years, other diseases significantly affecting needles have also been identified in Latvia: *Diplodia pinea* (Desmo.) J. Kickx and *Dothistroma septosporum* (Dorogin) M. Morelet (Adamson *et al.*, 2015). The occurrence of new pathogens as well as increased influence of the already present ones is expected in future. Change of dominant tree species is a feasible option only in limited areas (Jansons *et al.*, 2016b) due to specific soil requirements and economic importance of Scots pine. Tree breeding (and use of selected material in forest regeneration) can have an important role in reduction of the *L. seditiosum* and other needlecast diseases, since it is well developed and financially viable activity in Latvia (Jansons *et al.*, 2015b) with proven effects on quantitative traits of Scots pine (Jansons, 2008; Jansons *et al.*, 2006). Therefore, the existing breeding and propagation opportunities such as experimental

trials, infrastructure, as well as seed orchards, can be used to improve the resistance of needlecast simultaneously with other traits. Earlier studies had indicated the potential role of genetics in resistance to the needlecast (Liesebach & Stephan, 1996), but this effect may vary between the populations within a species. The aim of the study was to characterize the occurrence of *Lophodermium* spp. in young Scots pine stands in Latvia, and to investigate the correlation with meteorological factors.

Materials and Methods

Twelve young stands of Scots pine (further in the text 'objects') (Table 1) were selected – 5 stands in Western (W) Latvia, 5 stands in Eastern (E) Latvia and two stands in the central (C) part of Latvia. A report describing the presence of *Lophodermium* spp. in Scots pine stands in Latvia (Moročko-Bičevska *et al.*, 2010), was utilized to select the sampling locations. In each stand the needle samples from 24 trees (aged 1 – 14 years) were collected. The total surveyed area of young stands was 20.7 ha (Fig. 1). The analyzed stands were located up to 1 km from the young stands that were surveyed in 2009 and had similar tree dimensions and growth conditions (mineral soil, surrounded by other Scots pine stands). Location of young stands was determined by GARMIN eTrex 20 navigator.

In each young stand along the longest diagonal, 6 – 15 needles of the previous year damaged by *Lophodermium* spp. were collected from 24 trees

and inserted in sealable plastic bags. Needle samples were collected in August. Accurate monthly average values of air temperature (°C), precipitation (mm) and relative air humidity (%) for the selected study objects in years – 2008, 2009, 2015 and 2016 were obtained by spline interpolation. With spline interpolation method the image raster cells whose dimensions were 100 x 100 m in nature were created, reflecting accurate weather data information about the above-mentioned areas. Meteorological data – air temperature and precipitation in 2008, 2009, 2015 and 2016 – were obtained from all 34 observation stations of the Latvian Environment, Geology and Meteorology Centre.

In total, 288 samples were collected in 2016 from all regions of Latvia. Both objects from the central part of Latvia were excluded from further analysis to sufficiently differentiate the western and eastern regions. Consequently, results from only 240 needle samples were used for further mathematical analysis. DNA extraction from all samples was done using modified CTAB (Doyle & Doyle, 1987) protocol. Presence of *Lophodermium* in total extracted DNA was detected using a PCR method as described previously (Stenström & Ihrmark, 2005).

Descriptive statistics was used to characterize the data; chi-square criterion was used to analyze differences between the occurrence of *Lophodermium* spp. infection in the western and eastern regions and differences in infection frequency between years in these regions (Arhipova & Balina, 2003).

Table 1

Location and inventory parameters of studied objects

Object No.	Regions	Object location		Stand area, ha	Stand age, years	Stand average H, m	Stand average DBH, cm	Stand density, trees ha ⁻¹
		Latitude	Longitude					
1	Western	56°40'30.13"N	22°16'42.30"E	3.6	6	1.9	3.5*	3100
2	Western	57° 2'15.66"N	23°10'20.08"E	1.6	7	3.2	5.2*	3200
3	Western	57°27'44.09"N	22°40'47.95"E	1.0	10	4.8	5.8*	2800
4	Western	57°20'59.24"N	22° 6'14.71"E	0.7	12	9.2	9.5*	2500
5	Western	57° 3'41.59"N	21°48'44.67"E	0.7	4	1.5	2.7*	3350
6	Eastern	56°36'8.05"N	25°35'5.24"E	0.7	6	2.2	3.7*	2700
7	Eastern	56°48'41.10"N	26° 7'46.21"E	2.7	4	0.9	1.8**	3300
8	Eastern	56°34'43.14"N	26°40'28.90"E	0.7	3	1.1	2.1**	3250
9	Eastern	56°27'11.62"N	27°17'20.52"E	0.5	10	6.5	6.2*	1900
10	Eastern	56° 5'28.54"N	26°21'42.05"E	2.8	5	2.1	2.9*	3400
11	Central	56°43'12.75"N	23°41'9.75"E	1.3	11	8.9	9.3*	2200
12	Central	56°55'55.09"N	24°23'23.74"E	4.4	3	0.9	1.9**	2900

*DBH – mean diameter at breast height; ** mean diameter at root collar; H – mean height.



Figure 1. The location of research objects.

Results and Discussion

Correlations were not found between the young stand height, diameter and density indicators and their impact on *Lophodermium* spp. Therefore, data were further analyzed as a single set of data, evaluating them between years. The study shows that *Lophodermium* spp. were present in needles collected

from all analyzed objects. This correlates with results from a previous report, where *Lophodermium* spp. were present in similar localities (Moročko-Bičevska *et al.*, 2010).

The proportion of individuals where *Lophodermium* spp. were detected using a PCR method ranged from 50.0% (Stand 4) up to 100% (Stands 7, 8,

Table 2

Detection of *Lophodermium* spp. and meteorological data at each object

Object No.	Region	<i>Lophodermium</i> spp. detection, % in 2016	Average autumn precipitation, mm		Average winter temperatures, °C		Average temperature from May to August, °C		Average precipitation from May to August, mm	
			2008	2015	2008	2015	2009	2016	2009	2016
1	Western	83.3	206.0	235.0	-2.0	-0.7	14.6	15.8	282.2	317.7
2	Western	87.5	166.8	199.3	-1.2	-0.2	15.0	16.3	307.2	357.3
3	Western	87.5	207.2	190.4	-1.1	-0.1	14.2	15.4	386.0	360.0
4	Western	50.0	260.0	228.6	-1.4	-0.3	14.3	15.2	328.7	315.9
5	Western	95.8	300.8	253.5	-1.5	-0.3	14.2	15.9	282.2	279.1
Average western		80.8	228.1	221.4	-1.4	-0.3	14.5	15.7	317.3	326.0
6	Eastern	79.2	206.9	233.8	-2.9	-2	14.7	16.4	315.7	425.4
7	Eastern	100	199.0	174.9	-3.3	-2.5	14.4	16.1	308.4	341.5
8	Eastern	100	176.3	241.4	-3.1	-2.4	14.8	16.4	294.2	320.3
9	Eastern	87.5	175.7	285.0	-3.5	-2.4	14.4	16.2	334.0	291.0
10	Eastern	100	141.7	180.4	-2.8	-2.1	15.0	16.5	315.8	366.9
Average eastern		93.3	179.9	223.1	-3.1	-2.3	14.7	16.3	313.6	349.0
Total average		87.1	204.0	222.2	-2.3	-1.3	14.6	16.0	315.5	337.5

10). The overall proportion of Scots pine individuals where *Lophodermium* spp. were detected was 87.1% (Table 2).

Objects were divided into two groups (eastern and western region) to find the regional differences between detection of *Lophodermium* spp. *Lophodermium* spp. were more frequent in the eastern region ($p = 0.004$) (Fig. 4). Differences in occurrence of *Lophodermium* spp. in young stands of Scots pine in eastern and western regions could be explained by differences in meteorological (e.g. temperature and precipitation) conditions in the previous year (autumn and winter temperature) and during the survey year during *Lophodermium* spp. development from May to August.

A previous study (Moročko-Bičevska *et al.*, 2010) had surveyed the occurrence of *Lophodermium* spp. in Latvian pine stands using a fungal culturing method. Samples for this study were collected in 2009, and the current samples were collected in similar locations, in order to enable the comparison of meteorological data. In 2016, using PCR detection, *Lophodermium* spp. were found in 87.1% of analyzed needle samples. The occurrence data were obtained using different approaches (cultivation on media in 2009 and PCR detection in 2016), however, the occurrence of *Lophodermium* spp. was lower in 2009. A significant difference in occurrence between the eastern and western regions was found in 2016. Based on the fact that autumn and winter meteorological weather conditions of the previous year affect the development of *Lophodermium* spp. in the next year (Drenkhan, 2011), it was concluded that in 2015 winter was warmer (-1.3 ± 0.8 °C) than in 2008 (-2.3 ± 0.3 °C), and the amount of precipitation in autumn of 2015 was higher (222.2 ± 2.7 mm) compared to the amount of precipitation in year 2008 (204.0 ± 5.9 mm). The autumn precipitation in 2015 was similar between

the western and eastern regions (221.4 mm and 223.1 mm), and the average winter temperatures were lower in the eastern region (Table 2). However, the average precipitation in May-August 2016 was higher in the eastern region, and the average temperature in May-August 2016 was also slightly higher in the eastern region. These differences in May-August precipitation and temperature were not as pronounced in 2009 (when no significant differences in occurrence of *Lophodermium* spp. were found between the eastern and western regions).

Air temperature and precipitation during the time of *Lophodermium* spp. development (from May to August) in 2009 was 14.6 ± 0.2 °C and the amount of precipitation was less than 315.5 ± 6.2 mm, but temperature in 2016 was 16.0 ± 0.3 °C and precipitation was 337.5 ± 6.5 mm. The previously mentioned differences between the analyzed years were statistically significant ($p < 0.001$). When analyzing air temperature and precipitation differences between eastern and western regions in 2009 and 2016, the differences during the period from May to August are small, but could affect the development of *Lophodermium* spp. Air temperature in eastern region in 2009 was 14.7 ± 0.5 °C, the amount of precipitation was 313.6 ± 9.0 mm, but in western region air temperature was 14.5 ± 0.6 °C, the amount of precipitation was 317.3 ± 10.5 mm. In 2016, the air temperature in eastern region was 16.3 ± 0.3 °C and amount of precipitation was 349.0 ± 13.8 mm, but in western region it was 15.7 ± 0.4 °C and 326.0 ± 4.5 mm, respectively. In several other studies – temperature and precipitation were mentioned as some of the main factors for the occurrence, development and spread of needlecast (Drenkhan, 2011; Rajkovic, Markovic, & Rakonjac, 2013). Researchers in their studies have found that the fungus is capable of sporulation and its spores germinate in any time during the year

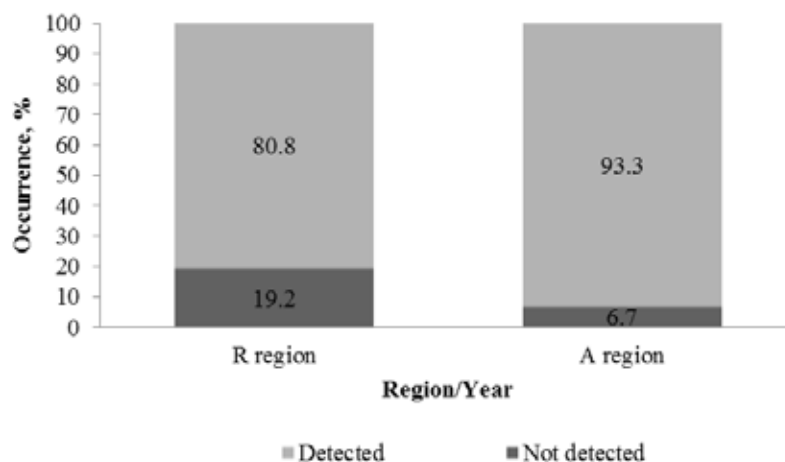


Figure 4. Occurrence of *Lophodermium* spp. in eastern and western regions.

when air temperature is above +5 °C and there is enough moisture (Thyr & Shaw, 1964). The optimum temperature for the development of ascospores (including *Lophodermium* spp.) is from +14 °C to +22 °C, minimum temperature is from -2 to +1 °C, with a maximum from +25 °C to +35 °C (Karadžić & Milijašević, 2008; Peterson, 1967; Gadgil, 1974). In the study, *Lophodermium* spp. in majority of cases have been observed directly on the previous year's needles, and in some cases also on the current year's needles. This shows that the degree of *Lophodermium* spp. infection significantly affects the current year's height increase of a tree and tree growth potential, and it is also noted by other scientists (Staley & Nicholls, 1989; Kanaskie, 1990). Needlecast primarily damages the current year (autumn infected) needles, which have a greater role in ensuring the growth of tree overground parts than older needles (Drenkhan, Kurkela, & Hanso, 2006). Martinsson (1979) found a significant negative correlation ($r = -0.80$, $\alpha = 0.01$) between the current year's relative increase of tree length and needle loss determined by needlecast infection degree. A similar correlation was found by other researchers (Baumanis, 1975; Squillace, La Bastide, & Van Vredenburch, 1975).

The two surveys of the occurrence of *Lophodermium* spp. in young Scots pine stands (Moročko-Bičevska *et al.*, 2010, and this report) in Latvia utilized different methods for fungal detection (culturing vs PCR). In both cases, although only needles showing needlecast symptoms were collected for analysis, *Lophodermium* spp. were not detected in all samples. It is not clear, if this is due to technical

limitations of the utilized detection techniques leading to false negatives, or additional, undetected pathogens could be inducing similar symptoms. In addition, the differences in occurrence frequency between the two reports may be due to differences between the detection techniques utilized. Therefore, additional studies are needed to obtain more accurate data about correlations between *Lophodermium* spp. occurrence and meteorological weather conditions in eastern and western regions, as well as throughout the entire territory of Latvia.

Conclusions

1. The presence of *Lophodermium* spp. was detected in all surveyed stands, and occurrence differences between the western and eastern regions were significant ($p = 0.004$).
2. The occurrence of *Lophodermium* spp. in the eastern and western region was affected by the previous year's autumn and winter meteorological weather conditions and differences in the given years' air temperature and precipitation.
3. In 2016, the eastern region of Latvia had a higher May-August precipitation and, to a lesser extent, temperature, which correlated with a higher occurrence of *Lophodermium* spp.

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