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The Most Frequent *Lophodermium* spp. on Scots Pine and Austrian Pine and Their Role in the Appearance of Other Fungi on the Needles

Vladimir LAZAREV[a] – Dragan KARADŽIĆ[b] –
Miroslav MARKOVIĆ[a*] – Predrag PAP[a] – Leopold POLJAKOVIĆ-PAJNIK[a]

[a] Institute of Lowland Forestry and Environment, Novi Sad, Serbia
[b]Faculty of Forestry, Belgrade, Serbia

Abstract – Different critical periods for infection, the difference in morphological-anatomical and eco-physiological characteristics and other distinguish support the taxonomic concept on the existence of several *Lophodemium* spp. on pines.

In addition to the study of *Lophodemium* spp. on secondary needles, the study was extended also to primary needles. The results of our long-term research, compared to the results obtained by other researchers in Europe, show that in Serbia there are at least two widely distributed *Lophodemium* species infecting Scots pine and Austrian pine needles. This study is supporting with identification of *L. seditiosum* and *L. pinastri* using species-specific PCR primers from the ribosomal ITS region. In pine plantations were established connection between *Lophodemium* species and other fungi on needles Scots and Austrian pine. On needles of Scots pine, except *L. pinastri* and *L. seditiosum* mostly were established following more important fungi: *Sphaeropsis sapinea*, *Cyclaneusma minus* and *Sclerophoma pythiophylla*. On needles of Austrian pine, except *L. pinastri*, were established following more important fungi: *Dothistroma pini*, *Sphaeropsis sapinea*, *Cyclaneusma niveum* and *Cytospora friesii*.

***Lophodermium* spp. / Scots pine / Austrian pine / needle fungi**

Kivonat – A leggyakoribb *Lophodermium* fajok az erdeifenyőn és a feketefenyőn, szerepük más tűgombák megjelenésében. Különböző kritikus fertőzési időszakok, morfológiai-anatómiai és öko-fiziológiai jellegek és egyéb megkülönböztető bélyegek támasztják alá a több *Lophodermium* faj létezésének taxonómiai elvét a *Pinus* fajokon. A *Lophodermium* fajok másodlagos tűkön végzett vizsgálatát az elsődleges tűkre is kiterjesztettük. Hosszú távú kutatásaink eredményei, összhangban más európai kutatók eredményeivel azt mutatják, hogy Szerbiában legalább két széleskörűen elterjedt *Lophodermium* faj fordul elő az erdeifenyő és a feketefenyő tűin. Ezt a *L. seditiosum* és *L. pinastri* riboszomális ITS szekvenciáin alapuló, fajspecifikus PCR primereket alkalmazó, molekuláris azonosítása is alátámasztja. *Pinus* erdősítésekben összefüggést állapítottunk meg a *Lophodermium* fajok és az egyéb gombák között az erdei- és feketefenyő tűin. Az erdeifenyő tűin, a *L. pinastri* és *L. seditiosum* fajokon kívül a következő jelentősebb gombák telepedtek meg: *Sphaeropsis sapinea, Cyclaneusma minus* és *Sclerophoma pityophila*. A feketefenyő tűin a *L. pinastri*-n kívül a *Dothistroma pini, Sphaeropsis sapinea, Cyclaneusma niveum* és *Cytospora friesii* fajok jelentek meg gyakrabban.

***Lophodermium spp.* / erdeifenyő / feketefenyő / tűgombák**

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[*] Corresponding author: miroslavm@uns.ns.ac.yu; 21000 NOVI SAD, Antona Cehova 13. P.O.Box 117.

1 INTRODUCTION

Lophodermium spp. are identified on all five Continents, on about 30 pine species and varieties, and the most susceptible are *Pinus sylvestris* and *P. resinosa.* More susceptible than other species are *P. brutia, P. densiflora, P. montana, P. mugo, P. nigra, P. tabuliformis, P. contorta, P. halepensis, P. pinea, P. radiata, P. montezumae, P. virginiana.* Pine needles are infested by numerous *Lophodermium* species: *L. pinastri, L. nitens, L. australe, L. pini-excelsae, L. pini-pumilae, L. conigenum, L. seditiosum*, etc. In Europe the most significant species in this genus is *L. seditiosum* Minter, Staley a. Millar (anamorph stage *Leptostroma rostrupii* Minter / = *L. austriacum* Oud./), and the most widespread is *L. pinastri* (Schrad. ex Hook.) Chev. (anamorph stage *Leptostroma pinastri* Desm). Along with these two species, *L. conigenum* (Brunaud) Hilitzer and *L. pini-excelsae* Ahmad are more frequent than other species on pine needles in Europe.

In Europe, *Lophodermium* spp. have been a serious problem in the nurseries for more than 100 years, while in North America they have been significant since 1966. Infected seedlings are often transported before the visible symptoms, and they die after planting. This method of dissemination results in the occurrence of local epidemics.

The species in the *Lophodermium* genus, as well as other species of widely widespread micro-organisms, are characterised by high variation of bioecological characteristics (morphological characters, host spectre, degree of virulence, point of attack, etc), which points to the presence of numerous species. The determination of the species which differ by the above bioecological characteristics, and especially by pathogenic properties, explains the contradiction between the European and the North American descriptions of this disease, i.e. its parasitic and saprophytic characteristics, as well as the existence of the pathogenic species which is very aggressive (Millar – Watson, 1971; Staas Ebregt – Gremmen, 1975; Staley 1975; Minter 1981; Lazarev 1980, 2004). However, the symptoms of this species have been masked for a long time or covered by the symptoms of widespread species which are frequent on the cast needles.

The variation of *Lophodermium* spp. is proved also by the identification of *L. seditiosum* and *L. pinastri* by the species-specific PCR primers from ribosomal ITS region (Stengström – Ihrmark, 2005).

During the multiannual study of *Lophodermium* species life cycle on pine needles, several species of fungi were found on the same needles. For this reason, this paper presents, along with bioecological characteristics of the most frequent and most significant *Lophodermium* species which infest Scots pine and Austrian pine needles, also a survey of succession and connexion of other fungal species identified on the needles.

2 MATERIAL AND METHODS

Scots pine and Austrian pine needles of different ages, infected by disease agents, are analysed in all development stages (primary and secondary needles) and a year after shedding. The collected samples originate from the nurseries, plantations and stands. During the three-year research, each year and each month, 100 needles of different ages were analysed from each of the 12 plots (4 nurseries, 4 plantations and 4 stands). The development of the disease symptoms was monitored on the still living and on the dead needles, as well as the time of occurrence and ripening of the fruiting bodies in relation to the type, location and needle age. From each needle group (green, chlorotic, yellow, brown, red, with and without fruiting bodies) 10 samples were selected which were superficially sterilised in sodium hypochlorite for 5 minutes, washed in distilled water, cut into 3 mm long segments and placed on artificial media potato dextrose agar (PDA) and malt agar (MA).

The morphological-anatomic characteristics of fruiting bodies and reproductive organs on the needles were analysed by standard mycological and phytopathological techniques.

The succession of fungi on individual needles was observed based on the analysis of the isolates from the infected needles with and without disease symptoms, every 15 days, and based on the fruiting bodies which appeared in different times of the year.

3 RESULTS

The principal diagnostic characteristics of the most significant and most widespread *Lophodermium* species in our conditions (*L. pinastri* and *L. seditiosum*) on Scots pine primary and secondary needles, as the most susceptible species, are presented in *Table 1*.

Table 1. The principal diagnostic characteristics of L. pinastri and L. seditiosum on primary and secondary needles

Characteristics	Primary needle		Secondary needle	
	L. pinastri	*L. seditiosum*	*L. pinastri*	*L. seditiosum*
Location of the pathogen fruiting bodies	coleoptiles	3 months old	in the litter and on needles of dead branches	needles on the plants in the nursery
Position of apothecia in the needles	partly subepidermal	wholly subepidermal	partly subepidermal	wholly subepidermal
Average clypeus thickness (µm)	70	60	95	115
The most frequent colour of lips of apothecia	greygreen	green	red	green
Thickness of apothecium basal layer (µm)	60	50	80	96
Apothecium length (µm)	600-800	500-700	700-1200	800-1500
Average hymenium thickness (µm)	35	30	48	58
Ascus length (µm)	105-135	120-160	110-155	140-170
Ascospore length (um)	60-120	80-120	70-110	90-120
Pycnidia length (µm)	180-250	200-280	300-400	300-500
Conidia length (µm)	3.0-4.5	5.0-6.5	4.5-6.3	6.0-8.0
Cross bands on needles as differential symptoms	not formed or several black bands formed	not formed	many black bands formed	lines not formed or several brown bands formed
Mycelium colour on 2 % malt agar	white with black margin	brown	white with black margin	brown
Period of ascospore dissemination	April-September	August-April	April-September	August-April
Critical period for infection	May-June	mid August-October	May-June	mid August-October

The differences in morphology, anatomic structure and other characteristics between the primary and the secondary needles point to the differences expressed by morphological and anatomical characters of *L. pinastri* and *L. seditiosum* fruiting bodies and reproductive organs on these types of needles. However, the periods of spore dissemination from the fruiting bodies, critical periods of infection and the characteristics of the cultures of both needle types are the same for each species.

The results of the multiannual study of succession and connexion of the more significant fungi on Scots pine and Austrian pine needles are presented in *Table 2.*

Table 2. Succession and connexion of the more significant fungi on Scots pine and Austrian pine needles

Pine species	Needle type and age	Succession and connexion of the pathogens
Pinus sylvestris	primary; younger than one year	*Lophodermium seditiosum (++);* isolated from the needles about 3 months old;
	primary; one year old	*Lophodermium pinastri (++);*
	secondary; younger than one year	*Lophodermium seditiosum (++);* *L. pinastri (+);* /both fungi isolated from green needles/;
	secondary; one year old	*Lophodermium seditiosum (++)*/pycnidia/*; (+);* *Lophodermella sulcigena (+);* *L. pinastri (+)*/isolate/;
	secondary; two years old	*Lophodermium pinastri (++);* *Cyclaneusma minor (++);*
	secondary; more than two years old, in the litter	*Lophodermium pinastri (++);* *Cyclaneusma minor (++);* *Cytospora friesii (++);*
Pinus nigra	primary; younger than one year	*Lophodermium seditiosum (+);* isolated from the needles about 3 months old;
	primary; one year old	*Lophodermium pinastri (+);*
	secondary; younger than one year	*Lophodermium seditiosum (+);* *L. pinastri (+);* /both fungi isolated from green needles/
	secondary; one year old	*Dothistroma pini (++);* *Sphaeropsis sapinea (++);* *Lophodermium seditiosum (+)*/pycnidia/; *L. pinastri* /isolate/;
	secondary; two years old	*Dothistroma pini (++);* *ophodermium pinastri (++);* *Cyclaneusma niveus (++);*
	secondary; more than two years old, in the litter	*Dothistroma pini (++);* *Cyclaneusma niveus (++);* *Sphaeropsis sapinea (++),* *Lophodermium pinastri (+);* *Cytospora friesii (++);*

The data in *Table 2* indicate that the older primary needles of Scots pine and Austrian pine are infested by *Lophodermium pinastri,* and the younger ones by *L. seditiosum.* Based on the symptoms and the obtained isolates, it can be concluded that Scots pine seedlings (primary needles) are more susceptible to the attack of *Lophodermium* species than Austrian pine. The same conclusion also refers to secondary needles.

Primary pathogens on Scots pine secondary needles are: *Lophodermium seditiosum, Lophodermella sulcigena* and *Lophodermium pinastri* (on older needles), and on Austrian pine *Dothistroma pini* and *Sphaeropsis sapinea.*

The secondary pathogens, which occur massively on the needles diseased by primary pathogens, are the species in the genera *Cyclaneusma* and *Cytospora friesii.*

4 DISCUSSION AND CONCLUSIONS

Different periods of mass infection during a year, then different types of ascospore germination, penetration of germ tubes directly through the cuticle or through the stomata, significant anatomic-morphological differences of fruiting bodies (in particular apothecia) on primary and secondary needles, as well as numerous other ecological, biological, physiological and pathological characteristics point to the existence of a number of *Lophodermium* species which differ significantly (Millar – Watson 1971; Staley 1975; Minter 1981; Lazarev 1980, 2004).

The species in the *Lophodermium* genus can cause damage in different climate conditions. The intensity of attack depends, *inter alia,* on geographic position and macroclimate, first of all on the climate humidity. It can be taken that pines are more at risk in the more humid areas and in the higher mountain regions. The intensity of attack of the disease agents is maximal in the areas with precipitation 700-800 mm, while in the areas with precipitation between 600 and 700 mm, it is significantly lower (Lazarev 1981). The precipitation below 300 mm during the vegetation period is not sufficient for the infection (Pagony, 1975). Consequently, these pathogens are a far more difficult problem in the countries of North and central Europe (Germany, Holland, Sweden, Bosnia and Herzegovina) than in the countries of South Europe. The disease can reach the epidemic proportions in the nurseries during one year, because of the high density of seedlings and the favourable moisture, due to irrigation. In the plantations, epidemics last for 2-3 years, and they become severe in the effects of favourable microclimate and during the rainy period.

Pathogen biology is characterised by four phases of development: infection phase (in which pathogenic action starts), latent phase (latency), phase of transition to saprophyte feeding, and the phase of reproduction. Infection phase is restricted to the period of ascospore release and their dissemination. According to Raspopov (1966), the ascospore dissemination dynamics depends on the number of apothecia with mature ascospores, air and needle humidity, and air temperature. At mean daily air temperatures of 15-20^0 C, ascospore dissemination increases and it decreases at the temperatures of 10-12^0 C. Ascospore dissemination increases significantly after daily precipitation of 8-10 mm. Rendl (1967) reports the positive correlation between humidity and intensity of attack. In his study of dissemination rhythm, Rack (1975) reports that apothecia contain up to 6,000 spores which are, at the optimal conditions of humidity and temperature, released in about 120 hours. The germination of ascospores on the surface of the needles starts in about 16-36 hours after the release from the apothecia, but no ascospores germinated after 48 hours. Although the above authors did not observe the infection breakthrough, they presumed the possibility of direct penetration through the cuticle. However, Lanier (1968, 1969) believes that penetration develops mainly through the natural openings (stomata). Staley (1975) shows that the germ tube is formed first after the ascospore release, and then it elongates into a colourless appressorium with a relatively thin wall. Germ tube after germination grows through the cuticle and epidermis. In these ascospores there is no penetration through the stomatal aperture, even when the appressoria are formed in the direct vicinity of the stomata. Therefore, the species in the genus *Lophodermium* can have a different mechanism of infection. After fungus penetration in the needle, an amorphous mass appears in individual cells of the epidermis and between the cuticle and the epidermis. After 6-8 days, the needles first become yellow variegated, and then yellow-brown spots are formed, when usually the infection process terminates. It is taken that the disease requires minimum 10 infections per one needle (Rack, 1963).

In the latency phase, there are no visible changes on the infected needles. The decisive factors for the latency period are temperature conditions, but it usually lasts for 20-30 days. This phase, however, can also be absent.

The phase of transition to the saprophyte feeding habit includes at the beginning the pathogenic activity. The germ tubes, diameter 0.5-2 μm, develop from the amorphous mass and penetrate the epidermal cells creating a vigorous mycelium. The mycelium develops best periclinally along the axis of the needles, but it also develops radially filling the resin channels. In this phase, the chlorotic spots gradually become brownish-red and the pycnidia are formed in them. Primary necroses then unite, and at the end of this phase, the whole needle is necrotised.

The phase of generative reproduction is characterised by the formation of fruiting bodies - apothecia in which asci with ascospores are formed. Depending on the pathogen species and climate conditions, apothecia form on the needles during the year. When they are mature they open along the longitudinal aperture and asci with ascospores are released, and they then cause infection. In this phase, depending on the species, cross bands are formed on some needles, which are the differential symptoms of the disease, along with pycnidia and apothecia.

Ševčenko (1960) reports that the life cycle of the fungi in this genus depends for the most part on site conditions. In lowland regions, the development cycle lasts for one year, and in mountain regions, for two or more years (Lebkova 1967).

Lophodermium species, as the primary pathogens, have a significant role in the succession and inter-relations with other pathogens on the needles. This is especially the case in Scots pine, which is much more susceptible to the attack of *Lophodermium* species than Austrian pine. By all means, the succession and connexion of other fungi is affected by the factors mentioned in this paper.

Based on our study, the following can be concluded:

- that the older primary needles of Scots pine and Austrian pine are infested by *Lophodermium pinastri*, and the younger ones by *L. seditiosum*;
- Based on the symptoms and the obtained isolates, it can be concluded that Scots pine seedlings (primary needles) are more susceptible to the attack of *Lophodermium* species than Austrian pine. The same conclusion also refers to secondary needles;
- Primary pathogens on Scots pine secondary needles are: *Lophodermium seditiosum, Lophodermella sulcigena* and *Lophodermium pinastri* (on older needles), and on Austrian pine *Dothistroma pini, Sphaeropsis sapinea* and *Lophodermium pinastri*;
- The secondary pathogens, which occur massively on the needles diseased by primary pathogens, are the species in the genera *Cyclaneusma (C. minor, C. niveus)* and *Cytospora friesii.*

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