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THE OCCURRENCE OF PATHOGENIC FUNGI DURING FLAX GROWING SEASON IN CENTRAL LITHUANIA

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Abstract: Experiments designed to study the variation of diversity of pathogenic fungi occurring in fibre flax agrocenosis during the growing season were conducted at the Lithuanian Institute of Agriculture Upytė Experimental Station during 1999–2002. The Upytė Experimental Station is situated in Central Lithuania. Phytopathological analyses of seed, soil and plants were done at the laboratory of the Upytė Experimental Station as well as at the Biodestructor Research Laboratory of the Institute of Botany.

There were identified in the rhizosphere 9 fungal species belonging to 9 genera. On flax seedlings were identified fungi belonging to 13 genera. On flax leaves at green maturity stage was identified only *Oidium lini*. On flax stems at green maturity stage were identified fungi belonging to 12 genera. On mature flax stems were found fungi belonging to 15 genera. On flax seed during the experimental years were identified fungi belonging to 16 genera. The number of fungi identified on seeds varied each year, depending on the year's weather conditions during the seed ripening period.

Having summarised the results, we can assert that pathogenic fungi *Fusarium* spp. and *Colletotrichum lini*, were found both on seed and plant vegetative parts during the whole assessment period. Species composition of fungi present on seed differed depending on the weather conditions. A larger number and more diverse species were found on the seed that ripened during the years with wet growing seasons (2000 and 2001) whereas the seed that ripened during dry growing seasons (1999 and 2002) was less infected with fungi. The spread of fungi on stems and leaves was also determined by the weather conditions. Many species of fungi were identified in 2001, when flax crops were lodged; stems and capsules were damaged by hail, and in 2000, when there was much rainfall.

Key words: flax, fungal diseases, growing season, occurrence, pathogenic fungi

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INTRODUCTION

Fungal species occurring on flax seed and vegetative plant parts have been cited in literature. They can be included into three groups of fungi: a) pathogenic – the ones that can cause fungal diseases; b) dew-retting – the ones that degrade flax stem pectin and separate fibre from wood; c) non-specific to flax occurring sporadically on plants (Komarova 2001).

The most common flax fungal diseases in flax growing countries are wilt, root rots, pasmo disease, mildew, seedling blight, seedling spotting, stem blight, flax rust (Musket and Colhoun 1947; Luchina 1981; Krylova 1994; Peryman *et al*. 2001). Since 1996 in Lithuania were stated flax diseases: fusarium wilt, anthracnose (seedling blight), seedling spotting, stem break and browning (Mikšienė 1995, 1996; Vinickas 1988).

Most of causal agents of flax diseases are soil-borne and seed-borne. The following fungi that can cause flax diseases are identified in Lithuania's soils and plant rhizosphere: *Alternaria alternata*, *A. tenuissima*, *Aureobasidium lini*, *Botrytis cinerea*, *Fusarium avenaceum*, *F. oxysporum*, *Kabatiela lini*, *Phoma exigua*, *Rhizoctonia solani*, *Verticillium albo-atrum*, *V. dahliae*, *Pythium debaryanum*, *Sclerotinia sclerotiorum*, *Thielaviopsis basicola*. The evidence of long-term research carried out at the Lithuanian Institute of Botany suggests that the occurrence of fungi in the soils of Lithuania, species diversity and their abundance are determined by: soil type, its productive moisture, temperature, and the amount of rainfall during the growing season (Lugauskas 1988). *Aureobasidium pullulans* has been identified on plant residues in Lithuania's soils (Lugauskas and Repečkienė 2001). One of this species' strains *A. pullulans* var. *lini,* called *Polyspora Iini* or *Kabatiella lini,* causes stem break and browning disease of flax (Ignatavičiūtė and Treigienė 1998).

Literature sources indicate that flax roots are most often affected by *P. debaryanum*, *T. basicola*, *P. exigua*, *V. albo-atrum*. The main source of infection in the soil are plant residues on which propagules of the most of the mentioned fungi survive. Other fungi – *Polyspora lini*, *Colletotrichum linicola*, *Septoria linicola*, *B. cinerea* are more common on the above-ground parts of flax (Musket and Colhoun 1947; Luchina 1981; Paul and Clark 1989). Brandenburger (1985) in his description of parasitic fungi occurring on plants in Europe supplemented this list by indicating that flax roots are often affected by *Drechslera linicola*, while young plants by *Olipidium brassicae*, *Pythium mamillatum, P. sylvaticum*, *Melampsora liniperda*, *Alternaria linicola*, *Botrytis cinerea*, *Fusarium oxysporum f. lini* and *Sclerotinia sclerotiorum*.

A. alternata, *A. tenuissima* and other fungi of this genus are widespread in plant rhizosphere. *A. lini* is the most serious for flax. The fungi of *P. exigua* operate in soils on plant residues. The fungi of one of this species forms *P. exigua f. linicola* are considered as causal agents of flax seedling diseases. There have also been identified other fungal species serious for flax crops, they include *S. sclerotiorum*, *Ascochyta lini*, *R. solani* (Lugauskas 1988; Lugauskas and Repečkienė 2001). Young flax plants are often damaged by the fungi of *Pythium* genus. These fungi do more damage to plants when the weather is wet and warm (Musket and Colhoun 1947).

Simay (1994) identified *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. on oil flax seed. According to the data of American phytopathologists, the following fungi have been identified on flax seed: *Pleospora herbarum*, *P. stenospora*, *Comoclathris permunda*, *Mycosphaerella linicola, Curvularia geniculata*. The fungi *Aphanomyces cladogamus*, *Phymatotrichopsis omnivora*, *T. basicola*, *F. acuminatum*, *F. equiseti*, *F. solani*,

Phoma terrestris, *Pythium* spp., *Penicillium* spp., *Helminthosporium* spp. have been found in flax rhizosphere and on seedlings. On seedlings have been found *Colletotrichum lini* (causing seedling blight), *Bipolaris sorokiniana* and *R. solani* (causing shoot rot and root rots), *F. oxysporum* f. sp. *lini* (causing wilt). On leaves, stems and capsules the following fungi have been identified: *Peronospora lini* (causing downy mildew), *Cercospora lini* (causing leaf spots), *Alternaria* spp. *B. cinerea* (causing capsule rots), *Sclerotium rolfsii*, *Pyrenopeziza californica*, *S. sclerotiorum* (causing stem rots), *Melampsora lini* (causing flax rust), *Aureobasidium lini*, *A. pullulans* (causing stem break and browning), *Colletotrichum lini* (causing seedling blight), *Septoria linicola* (causing pasmo disease) *Phoma fusispora* (causing phoma stem blight) and others (Farr *et al*. 1989).

Seedling blight, wilt and seedling spotting are the main seed-borne flax diseases that affect seedlings in Lithuania (Bachelis 1979; Radžiūnaitė 1986; Vinickas 1988). Kudriavtsev (2001) suggests that the causal agent of flax seedling spotting is *Ozonium vinogradovi* found on linseed in Russia. In Lithuania, Vinickas (1988) considered *R. solani* and *Mycelia sterilia* as causal agents of seedling spotting.

F. avenaceum, *F. herbarum*, *F. culmorum* causing fusarium browning are found on flax during the growing season. *F. oxysporum, f. sp. lini* (causal agent of flax wilt) sometimes causes partial browning of plants (Luchina 1981; Loshakova 1991). The fungi of *F. oxysporum* species affect over 150 plant species, therefore they get into the soil and survive well on the residues of various plants. They can cause flax wilt and root rot (Luchina 1981). In Great Britain, pathogenic fungi of *Sclerotinia* spp., *Botrytis* spp., *Phoma* spp., *Oidium* spp., *Alternaria* spp. and other genera have been found in the crops of winter and spring linseed on flax stems, leaves and capsules (Peryman and Fitt 2000).

In Lithuania, *Oidium lini*, causing powdery mildew, was identified on flax leaves (Grigaliūnaitė 1997). In recent years, the occurrence of *S. linicola,* causing pasmo disease has increased in our country (Jovaišienė and Taluntytė 2000; Markevičius and Treigienė 2003). The main source for the spread of the disease is infected seed, but the disease causal agent remains viable in the soil for 9–12 years even where flax is no longer grown (Kornejeva and Loshakova 1976; Neofitova *et al*. 1984; Andruszewska and Korbas 1989). The sexual stage of the disease causal agent *M. linicola* has not been identified in Lithuania, *S. linicola* has been identified on flax stems and leaves since 1999 (Markevičius and Treigienė 2003).

The aim of this work was to identify the fungi found on flax during the growing period and to explore the occurrence of pathogenic fungi on flax seed, stems, leaves and roots.

MATERIALS AND METHODS

Test samples were collected in the fibre flax crops (cvs. Baltučiai and Ariane) grown in the crop rotation at the LIA's Upytė Experimental Station. Analyses of fungal infection of flax rhizosphere, seed and vegetative plant part were done at the Upytė Experimental Station and the Institute of Botany during 1999–2002. About 50 samples of seed and soil were analysed every year. Randomly selected 200 plants per plot were taken for phytopathological analysis, leaves, stems and roots were inspected, per cent of disease-affected plants and disease severity were identified.

Analysis of soil (rhizosphere) micro-organisms was carried out at the Institute of Botany according to D. Zviagincev and K. Killham methodologies (Szegi 1983).

For the determination of internal fungal infection seeds were disinfected with 70% ethyl alcohol for 0.5 minutes, then rinsed 3 times in distilled water, dried on sterile filter paper and sown on malt agar medium. For the evaluation of one sample 400 seeds were analysed. Ten flax seeds were placed per 9 mm Petri dish. After 5–7 days of incubation in a thermostat at 25°C the plates were inspected and checked for seed fungal contamination. Identification was carried out according to Malone and Muskett (1997), Satton *et al*. (2002), Mathur and Kongsdal (2003) descriptors. For the evaluation of seed surface contamination with fungi dilution plating technique was applied. 10 g of seeds were placed in 100 ml of sterile water, shaken for 10 min., dilutions (1 : 100, 1 : 1 000, 1 : 10 000) were made and 1 ml of suspension was transferred into a Petri dish and poured over with malt agar medium with chloramphenicol (50 mg/l). The analysis of each sample was performed in three replications. Fungi were cultivated 7–10 days at a 28°C (Samson *et al*. 1992). Seed washing-off and centrifugation were used to isolate causal agents of pasmo disease from flax seed. 5 g of seeds were placed in a test-tube with 100 ml of sterile water, shaken for 1 min, dilutions poured out into centrifugal test-tubes and centrifuged for 5 minutes. Afterwards samples of sediment from the bottom of centrifugal test-tube were taken and studied using microscope, for typical *Septoria lini* conidia (Rogova 1960).

Phytopathological tests of flax vegetative plant part were conducted following the methodologies developed for phytopathological research (Metodiczeskije ukazanija... 1969; Loshakova *et al*. 2000). Diseases were identified according to visible symptoms, using disease descriptors and following methodological directions (Musket and Colhoun 1947; Metodiczeskije ukazanija... 1969; Pidopliczko 1978; Loshakova *et al*. 2000). The affected plant parts were stored in a wet chamber, and after emergence of mycelium the samples were examined with microscope for conidia shape and colour, according to which causal agents were identified.

Disease sevezity index was calculated using formula:

$$I = \frac{\sum (ab) \times 100}{A \cdot S}$$

Where:
I – disease sevezity index %;
a – number of infected plants;
b – degree of infection;
A – total number of plant samples (healthy and infected);
S – the highest degree if infection.

Weather conditions during flax growing period

During the experimental period flax establishment, growth and development and occurrence of pathogens were determined in relation to the meteorological conditions to calculate Selyaninov hydrothermal coefficient (HTC) (ratio of total precipitation during the period with air temperature above 10°C to the sum of mean daily temperature for the same period) (Dirsė 2001). The optimum humidity is when HTC is 1.0–1.5, excess humidity when HTC is 1.6 and higher, moderate humidity when HTC ranges between 0.7–0.6.

The mean monthly hydrothermal coefficient suggests that in each experimental year conditions were either close to optimal or optimal (Fig. 1). June and July of 1999 and August of 2002 were distinguished by a shortage of rainfall. A very considerable excess of moisture was recorded in the middle of July of 2000, when the hydrothermal coefficient achieved 9.

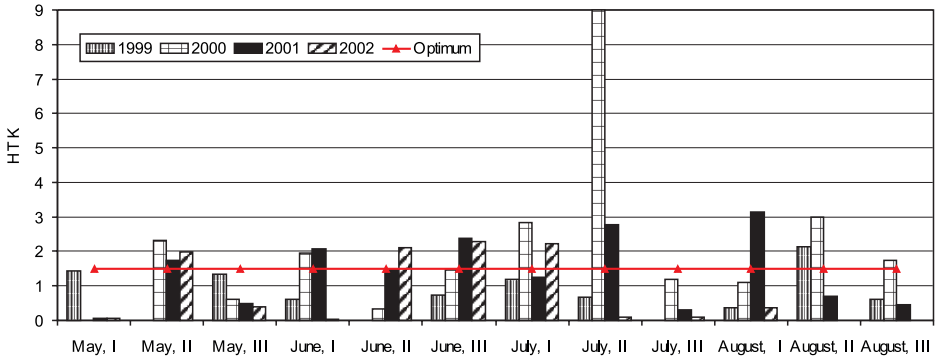

Fig. 1. Hydrothermal coefficient of the flax growing seasons, Upytė, 1999–2002

RESULTS

Nine fungal species belonging to 9 genera in the flax rhizosphere were identified, and 7 species belonging to 7 genera on the seed in 2001 (Fig. 2). On seed and in the rhizosphere the prevailing fungus was *F. oxysporum* (finding frequency 75%). The species *A. alternata,* (frequency 75%) *P. expansum* (frequency 75%), and *Drechslera sorokiniana* (frequency 62.5%) considerably prevailed on seed. *Trichoderma viride*, *P. sylvaticum*, and *V. albo-atrum* were found frequently in flax rhizosphere.

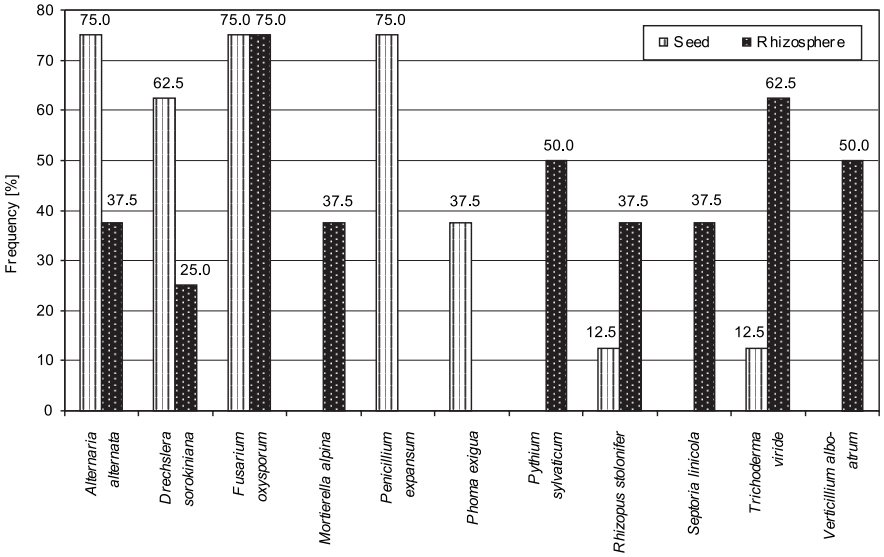

Fig. 2. Frequency of fungal species found on flax seed and in the rhizosphere, Upytė, 2001

Table 1. Fungi identified on flax seedlings, Upytė, 1999–2003

Genus, species	Infected plants [%]			
	1999	2000	2001	2002
Colletotrichum lini	79.5	33.0	68.5	5.69
Fusarium oxysporum	5.0	1.0	7.4	2.67
Thielaviopsis basicola		5.0	7.0	2.1
Verticillium albo-atrum			8.5	7.9
Fusarium semitectum		5.1	2.0	
Fusarium graminearum	1.9		1.5	2.0
Epicoccum nigrum		0.5	2.9	
Glomerularia corni			2.0	
Glocotinia granigena			5.0	
Ascochyta hordei var. hordei			2.5	
Mucor spp.		8.4	5.9	2.3
Aspergillus spp.			1.0	
Acremonium spp.			1.5	
Mortierella spp.			2.1	
Penicillium spp.			4.0	
Trichoderma viride			6.0	
Total genera	2	5	13	5

Table 2. Fungi found on plant vegetative parts, Upytė, 1999–2003

Genus, species	Infected plants [%]			
	1999	2000	2001	2002
On leaves at green maturity				
Oidium lini	75.0	53.1	29.3	83.9
On stems				
Alternaria spp.	35.0	25.9	60.0	25.1
Cladosporium spp.*	50.1	56.8	85.0	29.7
Geomyces pannorum			2.3	
Diplodina spp.			2.0	
Drechslera sorokiniana		7.9	15.2	3.7
Mucor spp.*	7.9	12.0	25.0	10.0
Penicillium spp.*	8.0	5.3	7.9	5.0
Trichothecium roseum			15.0	
Verticillium albo-atrum		3.0	3.6	
Colletotrichum lini	20.0	16.0	75.0	31.0
Fusarium spp.	39.0	23.0	22.0	15.67
Septoria linicola		82.0	96.0	12.1
Kabatiella lini		5.0	3.0	
Botrytis cinerea		12.0	25.5	
Sclerotinia sclerotiorum		28.8	12.9	
Total genera	7	13	16	9

* – found only on mature stems

On flax seedlings were identified fungi belonging from 2 to 13 genera (Table 1). *F. oxysporum* and *C. lini* were identified on flax seedlings each year of investigation.

On flax leaves at green maturity stage only *O. lini* was identified in each year (Table 2).

On flax stems at green maturity stage fungi belonging to 3–12 genera were identified. On green flax stems the prevalent fungi were *C. lini, F. avenaceum, F. oxysporum, Alternaria* spp. *and S. linicola*. On mature flax stems there were found fungi belonging to 15 genera (Table 2). Fungi belonging to *Cladosporium, Mucor, Penicillium* genera were found only on mature flax stems, whereas *B. cinerea* and *S. sclerotiorum* were found only on lodged flax stems.

On flax seed during the experimental years were identified fungi belonging from 6 to 16 genera (Table 3).

Table 3. Fungi identified on flax seed, Upytė, 1999–2002

Genus, species	Infected seed [%]			
	1999	2000	2001	2002
Alternaria spp.	12.0	25.0	35.0	12.5
Aspergillus spp.	0.5	2.0	0.5	0.2
Aureobasidium pullulans		0.7		
Colletotrichum lini	30. 0	25.9	23.4	12.2
Curvularia lunata		0.5		
Drechslera sorokiniana		5.0	4.5	
Fusarium spp.	2.5	12.1	4.0	10.0
Trichoderma spp.		3.5	2.1	2.1
Mycelia sterilia	1.5	1.9	12.8	0.5
Phoma exigua		0.7		
Penicillium spp.	0.5	0.2	1.5	1.8
Pythium spp.		0.1	0.5	
Rhizoctonia solani		12.5	10.5	5.8
Mucor spp.		0.5	2.8	0.5
Thielaviopsis basicola		0.8	0.5	
Verticillium albo-atrum		0.5	0.5	
Total genera	6	16	13	9

Studies on the incidence and severity of fungal diseases suggest that seedlings were more heavily affected by anthracnose in 1999 and 2001 (79.5 and 68.5% seedlings, respectively), and in 2002 the incidence of this disease was as low as 5.69% (Table 4). The severity of anthracnose differed between the experimental years: the highest disease severity (8.92%) was recorded in 1999, while the lowest severity (1.99%) in 2002.

Fusarium wilt affected from 1.0 to 7.4% of seedlings and the disease severity was weak.

In 1999, 2000 and 2002, at green maturity stage flax was heavily affected by powdery mildew (affected plants accounted for 75.0; 53.1 and 83.9%). In 2001 powdery mildew was less severe and affected 29.3% of plants.

Table 4. Incidence and severity of flax fungal diseases, Upytė, 1999–2003

Disease (pathogenic fungus)	Percentage of disease							
	1999		2000		2001		2002	
	incidence	index	incidence	index	incidence	index	incidence	index
On seedlings								
Anthracnose (*Colletotrichum lini*)	79.5	8.92	33.0	2.94	68.5	6.11	5.69	1.99
Wilt (*Fusarium oxysporum*)	5.0	1.23	1.0	0.01	7.4	2.36	2.67	0.02
On leaves at green maturity								
Powdery mildew (*Oidium lini*)	75.0	10.93	53.1	10.32	29.3	12.8	83.9	15.6
On stems								
Anthracnose (*Colletotrichum lini*)	20.0	2.00	16.0	5.67	75.0	10.33	31.0	3.31
Wilt and stem browning (*Fusarium* spp.)	39.0	2.33	23.0	9.67	22.0	7.33	15.67	4.54
Pasmo (*Septoria linicola*)			82.0	9.77	96.0	55.42	12.1	3.65
Stem break and browning (*Kabatiella lini*)			5.0	1.67	3.0	1.33		

Flax stems were annually affected by the following fungal diseases: anthracnose and fusarium blight. Steam break and browning lesions were found on stems only in 2000 and 2001, the disease incidence was not high, affected plants accounted only for 3–5% of the tested plants. Wet and cool weather is conducive to the occurrence of steam break and browning.

DISCUSSION

The number of fungi on flax plants varied each year as influenced by the weather conditions during the flax growing season. A considerably small number of genera were found in 1999 and 2002 (6 and 8 respectively) when the weather was dry and hot.

The number of fungi identified on seed varied each year, depending on the weather conditions at ripening. Fungi of the 4 genera *Alternaria, Fusarium, Aspergillus, Penicillium* and 2 kinds of *Mycelia sterilia* and *C. lini* were found on seed each year. Fewer fungal genera (6) were identified in the years 1999 and in 2002 (9) because during flax seed ripening the weather was dry and hot. The number of fungi considerably increased in 2000 (16), and 2001 because the weather was wet and hot during flax seed ripening. Flax crop lodged and there were more of common saprophytic fungal species on seed that year.

Having summarised the results, we can assert that pathogenic fungi *Fusarium* spp. and *C. lini*, causing flax fungal diseases were found both on seed and plant vegetative parts during the whole assessment period. These fungi are common flax pathogens and this is in agreement with the data of flax researchers from Byelorussian and Rus-

sian flax Institutes (Neofitova *et al*. 1984; Loshakova 1991; Kudriavtseva 1994; Zasczita l'na-dolgunca... 2003).

Experimental evidence suggests that the spread of harmful fungi in flax agrocenoses is determined by a range of agroecological factors, however, no major factors have been singled out. One of main factors are weather conditions during flax growing season (Rozhmina 2004). Species composition of fungi present on seed depended on the weather conditions. A larger number and more diverse species were found on the seed that ripened during the years with wet growing seasons (2000 and 2001) whereas the seed that ripened during drier growing seasons (1999 and 2002) was in a small degree infected with fungi. The spread of fungi on stems and leaves was also determined by the weather conditions. Many species of fungi were identified in 2001, when flax crops were lodged; stems and capsules were damaged by hail, and in 2000 when there was a lot of rainfall. The weather during the flax growing season in Lithuania has been hot and wet recently. Pasmo is considered to be one of the most devastating flax diseases in Lithuania. It affected flax stems in 2000–2002; its incidence and severity were the highest in 2000 and 2001, when the weather was warm and wet. In the drier year 2002 the disease was less severe.

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POLISH SUMMARY

WYSTĘPOWANIE GRZYBÓW CHOROBOTWÓRCZYCH W OKRESIE WEGETACJI LNU W WARUNKACH LITWY CENTRALNEJ

W Stacji Doświadczalnej Litewskiego Instytutu Uprawy Roli (LIUR) w Upyte w latach 1999–2002 badano zmiany różnorodności grzybów chorobotwórczych w okresie wegetacji w agrocenozie lnu. Stacja Doświadczalna w Upyte znajduje się w centralnej części Litwy. Badania fitopatologiczne przeprowadzono w Stacji Doświadczalnej LIUR w Upyte i w Laboratorium Biodestruktorów Instytutu Botaniki w Wilnie.

W rizosferze lnu zidentyfikowano 9 gatunków grzybów chorobotwórczych, należących do 9 rodzajów. Na kiełkach lnu zidentyfikowano 2–13 rodzajów grzybów. Na liściach lnu w okresie zielonej dojrzałości pojawił się tylko *Oidium lini*. Na łodygach lnu w okresie zielonej dojrzałości zidentyfikowano 12 rodzajów, a w okresie pełnej dojrzałości – 15 rodzajów chorobotwórczych grzybów.

Na nasionach lnu zidentyfikowano 6–16 rodzajów grzybów. Liczba gatunków grzybów zależała od warunków meteorologicznych w okresie dojrzewania nasion.

Grzyby *Fusarium* spp., i *Colletotrichum lini* na nasionach lnu i organach wegetatywnych były identyfikowane w ciągu całej wegetacji. Zestaw gatunkowy patogenów w nasionach różnił się w różnych latach badań zależnie od dominujących warunków atmosferycznych – większa liczba i bardziej zróżnicowane gatunki rozprzestrzeniły się w warunkach większej ilości opadów i ciepłej pogody (2000 i 2001 r.), a mniejsza zidentyfikowana liczba gatunków grzybów, gdy w okresie dojrzewania nasion lnu wystąpił brak wilgotności (1999 i 2002 r.). Zestaw gatunkowy grzybów na łodygach lnu także zależał od warunków meteorologicznych w okresie wegetacji roślin. Wielka różnorodność gatunkowa grzybów była obserwowana w roku 2001, kiedy zasiewy lnu wyległy i były mechanicznie uszkodzone przez grad, a także w roku 2000, gdy obserwowano dużą ilość opadów.