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ORIGINAL RESEARCH PAPER

Fungal colonization of seeds of three lupine species in different regions of Poland

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Abstract
The health status of seeds of three lupine species (white lupine, narrow-leaved lupine, and yellow lupine) from different regions of Poland was investigated. Seeds were analyzed by microscopic method and PCR. The examined lupine seeds were colonized by saprotrophic fungi of the genera *Alternaria*, *Cladosporium*, and *Penicillium*, and pathogenic fungi of the genera *Fusarium*, *Botrytis*, *Mycosphaerella*, and *Colletotrichum*. The relative frequency (RF) of fungi detected on lupine seeds from the regions of Kujawy, Wielkopolska, Lower Silesia, and Warmia and Mazury was determined. The highest RF values of pathogenic fungi were noted in Lower Silesia in 2012 and 2013, and in Warmia and Mazury in 2011. The RF values of pathogenic and saprotrophic fungi on lupine seeds harvested in different regions of Poland were affected by weather conditions. PCR analyses revealed the presence of *Tri* genes in the seeds of narrow-leaved lupine. The analyzed seeds were relatively free of pathogenic fungi and could be used for sowing and feed production.

Keywords
lupine seeds; fungi; pathogens; saprotrophs; mycotoxins; *Fusarium*; PCR

Introduction

Leguminous plants (Fabaceae) have a beneficial influence on the soil environment, successive crops, and nitrogen fixing, and they constitute a valuable local source of protein in animal feeds [1,2].

Restricted imports of genetically modified soybeans for feed production can be compensated with intensively grown local legumes, including three important lupine species: yellow lupine, white lupine, and narrow-leaved lupine [3].

In Poland, the main avenues of research into lupines include improvements in agricultural technology aimed at increasing crop yield [4] and genetic research aimed at improving the quality of lupine varieties, analyzing the genome of pathogenic fungi that colonize lupine plants, identifying the mechanisms responsible for infections and enhancing plant resistance [5,6]. Bieniaszewski [7] demonstrated that the seed yield of lupines is influenced by genetic factors (variety) and agronomic factors (seeding date, seeding rate, row spacing) as well as by weather conditions (temperature, humidity, precipitation) [8] and pathogenic infections [9–11].

New lupine varieties are characterized by lower alkaloid concentrations, higher protein content, improved protein quality, higher yield, shorter growing season, lower susceptibility to lodging, pests, and diseases [12].

Lupines may be colonized by fungal pathogens (such as lupine anthracnose), which, under supportive conditions, cause diseases, damage plants, or even contribute to the spread of disease epidemics that can lead to the closure of entire seed plantations. Other pathogens are noted sporadically and rarely influence yields, but simultaneous outbreaks of several diseases can also contribute to high crop losses [9].

The aim of this study was to evaluate the health status of seeds of three lupine species grown in different regions of Poland, with special emphasis on seed colonization by toxin-producing fungi of the genus *Fusarium*.

Material and methods

The experimental material consisted of seeds of three lupine species (white lupine 'Butan', narrow-leaved lupine 'Zeus', and yellow lupine 'Mister' and 'Perkoz') grown in different regions of Poland (Lower Silesia, Kujawy, Warmia and Mazury, Wielkopolska). Phytopathological analyses were carried out on batches of 100 seeds from each cultivar based on the morphological characteristics of fungi isolated from the seed material. Seeds were surface disinfected with 1% sodium hypochlorite and 70% ethanol, and they were cultured on potato dextrose agar (PDA). After 10–14 days, fungal cultures were transferred to sterile dishes with PDA and incubated at a temperature of 20–22°C. After 14–20 days, fungal colonies were identified to the genus and species level under an optical microscope based on the available monographs [11,13–15].

The distribution and occurrence of pathogenic and saprotrophic fungi were compared based on their relative frequency (RF) = number of sampling sites containing a given species divided by the sum of the frequencies of all species.

DNA was isolated from ground seeds of three lupine species by means of the column-based method proposed by Kulik et al. [16]. PCR assays were performed with the use of species-specific primers for *Fusarium* pathogens: *Fusarium* spp. [17], *F. avenaceum* [18], *F. culmorum* [19], *F. poae* [20], *F. sporotrichioides* [21], and *F. equiseti* [22] (Tab. 1). The PCR method was also used to identify *Tri5* genes [23] responsible for trichothecene synthesis in lupine seeds (Tab. 1). The reaction mixture of 25 µL was composed of: FailSafe PCR 2× Premix E, 0.2 U Fail Safe Enzyme Mix Only (Epicentre Biotechnologies, USA), 10 pM of each primer, 5.75 to 7.5 µL of deionized water, and 5 µL of DNA template. Samples were subjected to temperature cycling tests in the Eppendorf Mastercycler Gradient thermocycler (Germany). PCR was conducted in three replications for each sample. PCR products were visualized by electrophoresis in 1.5% agarose gel

Tab. 1 Species-specific primers for the detection of *Fusarium* species and *Fusarium Tri* genes in lupine seeds.

Fungal species	Primers	References
Fusarium ssp.	P58SL 5′-AGT ATT CTG GCG GGC ATG CCT GT-3′ P28SL 5′-ACA AAT TAC AAC TCG GGC CCG AGA-3′	Hue et al. [17]
Fusarium avenaceum	FaR 5′-CAA GCA TTG TCG CCA CTC TC-3′ FaF 5′-GTT TGG CTC TAC CGG GAC TG-3′	Doohan et al. [18]
Fusarium culmorum	Fc01F 5′-ATG GTG AAC TCG TCG TGG C-3′ Fc01R 5′-CCC TTC TTA CGC CAA TCT TCT CG-5′	Nicholson et al. [19]
Fusarium poae	Fp28F 5′-CAAGCAAACAGGCTCTTCACC-3′ Fp28R 5′-TGTTCCACCTCAGTGACAGGTT-3	Parry and Nicholson [20]
Fusarium sporotrichioides	FspITS2K 5′-CTT GGT GTT GGG ATC TGT GTG CAA-3′ P28SL 5′-ACA CAA CGG GCT ATA ACA CTC CCC-3′	Kulik et al. [21]
Fusarium equiseti	FEF1 5′-CATACCTATACGTTGCCTCG-3′ FER1 5′-TTACCAGTAACGAGGTGTATG-3′	Mishra et al. [22]
Fusarium spp. (*Tri5* gene)	HATrif 5′-CAG ATG GAG AAC TGG ATG GT-3′ HATrir 5′-GCA CAA GTG CCA CGT GAC-3′	Edwards et al. [23]

Tab. 2 Weather conditions in 2011–2013 (data from meteorological stations in: Mochełek – Kujawy, Bałcyny – Warmia and Mazury, Złotniki – Wielkopolska, Swojec – Lower Silesia).

Month	Mean monthly temperature (°C)				Precipitation total (mm)			
	Kujawy	Wielkopolska	Warmia and Mazury	Lower Silesia	Kujawy	Wielkopolska	Warmia and Mazury	Lower Silesia
Year 2011								
V	13.5	15.3	13.4	14.8	38.4	17.5	41.5	49.4
VI	17.7	18.4	17.5	19.1	100.8	62.4	56.2	95.7
VII	17.5	17.5	18.0	18.2	132.5	214.8	171.9	170.9
Year 2012								
V	14.5	15.1	13.8	15.8	25.4	58.0	42.5	63.7
VI	15.2	15.8	15.2	17.3	133.8	124.4	107.2	94.7
VII	18.8	19.0	19.0	20.0	115.6	149.4	112.2	108.0
Year 2013								
V	14.2	14.4	17.4	14.6	91.7	81.0	46.2	136.0
VI	17.4	17.3	17.9	17.7	49.3	106.0	45.4	171.7
VII	18.8	19.6	18.1	20.5	79.0	46.2	163.8	36.3

with ethidium bromide. Product size was determined based on M 100–1000 molecular weight markers (A&A Biotechnology, Poland).

Weather conditions in 2011–2013 are presented in Tab. 2.

The correlation between the frequency of occurrence of saprotrophic and phytopathogenic fungi and weather conditions in the particular regions of Poland was analyzed by means of Statistica ver. 12.5 (http://www.statsoft.com) using Pearson correlation coefficients – *R*.

Results

The analyzed seeds of white, narrow-leaved, and yellow lupine were colonized by pathogenic and saprotrophic fungi. In all lupine species and cultivars, the most prevalent fungal species were *Alternaria alternata* and *Cladosporium cladosporioides* (Tab. 3–Tab. 5).

The highest number of fungal isolates were obtained from white lupine seeds from both locations in 2011. The prevalence of saprotrophic species was noted, and potentially pathogenic fungi were represented by species of the genus *Fusarium* as well as by *Botrytis cinerea* and *Mycosphaerella lupini* (Tab. 3). A higher number of fungi were isolated from white lupine seeds harvested in the region of Wielkopolska than Kujawy. In Wielkopolska, precipitation was most abundant in July 2011, which contributed to the highest fungal colonization of seeds, including by pathogenic species (Tab. 2, Tab. 3). In 2012, lupine seeds harvested in the region of Kujawy were colonized mostly by pathogenic fungi, which can be attributed to high rainfall in June, during the flowering stage of lupines (Tab. 2, Tab. 3). In 2013, precipitation was low in June, and potentially pathogenic fungi were not observed (Tab. 2, Tab. 3).

The seeds of narrow-leaved lupine from Kujawy were characterized by the highest levels of fungal colonization, whereas the lowest number of fungi were isolated from the seeds harvested in Wielkopolska. Those differences can be attributed to weather conditions in the experimental years (Tab. 2, Tab. 4). Fungal pathogens identified on the seeds produced in Wielkopolska in 2013 accounted for 50% of all fungal isolates (Tab. 4). The presence of pathogens was not observed in seeds in the remaining years of the study. A high percentage of potentially pathogenic fungi was noted in isolates from seeds harvested in Lower Silesia in 2012. Seeds from Kujawy were not significantly infected in 2011 and 2012, and pathogens were not isolated in 2013 (Tab. 2, Tab. 4).

Tab. 3 Fungal colonization of white lupine seeds harvested in the regions of Kujawy and Wielkopolska in 2011–2013.

No.	Fungal species	Cultivar (region)					
		Butan (B)			Butan (P)		
		2011	2012	2013	2011	2012	2013
1.	*Fusarium avenaceum* (Corda ex Fr.) Sacc.	1			2		
2.	*Fusarium equiseti* (Corda) Sacc.				6		
3.	*Fusarium poae* (Peck) Wollenw.		1		1		
4.	*Fusarium sambucinum* Fuckel	1					
5.	*Fusarium tricinctum* (Corda) Sacc.						1
6.	*Fusarium* spp.	1	1		1		1
7.	*Alternaria alternata* (Fr.) Keissler	22	3	5	61	5	30
8.	*Aspergillus* spp.				3		
9.	*Botrytis cinerea* Pers.	1				1	
10.	*Cladosporium cladosporioides* (Fresen) de Vries	1	2				11
11.	*Epicoccum nigrum* Link	1	1				
12.	*Mucor* spp.						8
13.	*Mycosphaerella lupini* (Kaiser and Crous)		2				
14.	*Penicillium* spp.	1		3			
15.	*Nigrospora sphaerica* (Sacc.) Mason				1		
16.	*Rhizopus nigricans* Ehrenb.		2			6	2
17.	*Trichoderma roseum* Pres.	1			6		
18.	*Trichoderma* spp.		2			1	
Total		30	14	8	81	13	53
% pathogenic fungi		13.30	28.57	0.00	12.35	7.69	3.77
% saprotrophic fungi		86.70	71.43	100.00	87.65	92.31	96.23

B – seeds from Mochełek, Kujawy; P – seeds from Złotniki, Wielkopolska.

In the seeds of narrow-leaved lupine from the region of Warmia and Mazury, the presence of pathogens was noted in 2011 and 2013 due to relatively high precipitation in July during seed ripening (Tab. 2, Tab. 4).

The seeds of two yellow lupine cultivars were analyzed in this study: 'Mister' and 'Perkoz'. The seeds from Wielkopolska were less colonized by pathogens than the seeds from Kujawy in all years of the experiment. The absence of pathogens in lupine seeds 'Mister' can probably be attributed to low precipitation during flowering in 2011 and seed ripening in 2013 (Tab. 2, Tab. 5). The highest percentage of potentially pathogenic fungi was observed in seeds of the 'Mister' cultivar from Kujawy in 2011 and 2012. The seeds of yellow lupine 'Perkoz' harvested in Kujawy were colonized by *Colletotrichum lupini* (three isolates) in 2013 (Tab. 5). In all years of the experiment, high precipitation levels were noted in Kujawy during lupine flowering (June) and seed ripening (July), which definitely influenced the health of yellow lupine seeds (Tab. 2, Tab. 5).

The distribution and occurrence of pathogenic and saprotrophic fungi in all analyzed regions were compared based on their relative frequency (RF) (Tab. 6). The highest RF values of pathogenic fungi detected on lupine seeds were noted in Lower Silesia in 2012 and 2013 and in Warmia and Mazury in 2011 (Tab. 6). Saprotrophic fungi were

Tab. 4 Fungal colonization of narrow-leaved lupine seeds harvested in the regions of Warmia and Mazury, Lower Silesia, Kujawy, and Wielkopolska in 2011–2013.

No.	Fungal species	Cultivar (region)											
		Zeus (O)			Zeus (W)			Zeus (B)			Zeus (P)		
		2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
1.	*Fusarium avenaceum* (Corda ex Fr.) Sacc.				2	4	1	1	1				
2.	*Fusarium equiseti* (Corda) Sacc.						1						
3.	*Fusarium poae* (Peck) Wollenw.					1							3
4.	*Fusarium sporotrichioides* Sherb.						1						1
5.	*Fusarium tricinctum* (Corda) Sacc.					1	7						
6.	*Fusarium* spp.			2		4	1	1					
7.	*Alternaria alternata* (Fr.) Keissler	28	14	30	27	13	43	65	2	48	56	5	4
8.	*Aspergillus* spp.				1								
9.	*Botrytis cinerea* Pers.	11		3	2	1		1					
10.	*Cladosporium cladosporioides* (Fresen) de Vries	3	2	1		3	8	1	3	34			
11.	*Epicoccum nigrum* Link			3	1	2	1	14					
12.	*Mucor* spp.								1	1	1		
13.	*Penicillium* spp.	29		1			1	4	8		2	1	
14.	*Rhizopus nigricans* Ehrenb.					4	1				3	2	
15.	*Trichoderma roseum* Pers.						29						
16.	*Trichoderma* spp.								2				
Total		71	16	40	33	33	95	87	19	83	62	8	8
% pathogenic fungi		15.15	0.00	12.5	12.12	33.33	11.58	3.45	5.26	0.00	0.00	0.00	50.00
% saprotrophic fungi		84.85	100.00	87.5	87.88	66.67	88.42	96.55	94.74	100.00	100.00	100.00	50.00

B – seeds from Mochełek, Kujawy; O – seeds from Bałcyny, Warmia and Mazury; P – seeds from Złotniki, Wielkopolska; W – seeds from Swojec, Lower Silesia.

Tab. 5 Fungal colonization of yellow lupine seeds harvested in the regions of Kujawy and Wielkopolska in 2011–2013.

No.	Fungal species	Cultivar (region)											
		Mister (B)			Mister (P)			Perkoz (B)			Perkoz (P)		
		2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
1.	*Fusarium avenaceum* (Corda ex Fr.) Sacc.	3											
2.	*Fusarium culmorum* (W. G. Smith) Sacc.		1						2				
3.	*Fusarium poae* (Peck) Wollenw.		8		1	1				4			1
4.	*Fusarium sambucinum* Fuckel	1											
5.	*Fusarium* spp.	1	1					1				1	
6.	*Alternaria alternata* (Fr.) Keissler	36	4	47	84	5		6	15	40	44	15	20
7.	*Botrytis cinerea* Pers.	13	13			1						1	
8.	*Cladosporium cladosporioides* (Fresen) de Vries			2			2	1	2	2			7
9.	*Colletotrichum lupini* (Bondar) Damm, P. F. Cannon and Crous									3			
10.	*Epicoccum nigrum* Link	4		1	1	1	1		1		2	2	
11.	*Mucor* spp.						1		1		4		
12.	*Nigrospora sphaerica* (Sacc.) Mason											1	
13.	*Penicillium* spp.				1						1		2
14.	*Rhizopus nigricans* Ehrenb.	2				3	2		3	2	2		1
15.	*Trichoderma* spp.								5				
16.	Non sporulating fungi										2		
Total		60	27	50	88	11	6	8	29	51	55	20	31
% pathogenic fungi		30.00	85.18	1.14	18.18	6.90	0.00	12.50	6.90	13.73	0.00	10.00	3.22
% saprotrophic fungi		70.00	14.82	98.86	81.82	93.10	100.00	87.50	93.10	86.27	100.00	90.00	96.78

B – seeds from Mochełek, Kujawy; P – seeds from Złotniki, Wielkopolska.

Tab. 6 Frequency of occurrence of pathogenic and saprotrophic fungi on lupine seeds in different regions of Poland (%).

Region	Group of fungi	2011	2012	2013
Kujawy	Pathogenic	6.50	7.50	1.75
	Saprotrophic	39.75	14.25	46.25
Wielkopolska	Pathogenic	2.75	1.25	1.75
	Saprotrophic	68.00	11.75	22.75
Warmia and Mazury	Pathogenic	11.00	0.00	5.00
	Saprotrophic	60.00	16.00	35.00
Lower Silesia	Pathogenic	4.00	11.00	11.00
	Saprotrophic	29.00	22.00	83.00

characterized by the highest RF in Lower Silesia in 2013 and in Wielkopolska and Warmia and Mazury in 2011. The above results suggest that the RF values of pathogens and saprotrophs were affected by weather conditions in each year of the study. In order to verify this hypothesis, an analysis of the linear correlation between the RF of fungi and climatic conditions (mean monthly temperatures and precipitation totals) was performed. The statistical analysis confirmed that total rainfall in May ($R = 0.93$) and June ($R = 0.59$), and average temperature in July ($R = 0.84$) had a significant effect on the RF of pathogenic fungal species on lupine seeds (Fig. 1c–e). The RF of saprotrophic species was affected by average temperature in June ($R = 0.57$) and, to a lesser extent, precipitation (Fig. 2a–f).

Fungal species of the genus *Fusarium* were identified in seed samples by PCR (Tab. 7). *Fusarium* fungi were not noted in the seeds of white lupine cv. 'Butan' and yellow lupine cv. 'Perkoz' from Wielkopolska. Species-specific primers supported the identification of *F. avenaceum*, *F. poae*, and *F. sporotrichioides* only in individual samples, which could be related to the presence of other *Fusarium* species, such as *F. sambucinum* and *F. tricinctum*, which were detected microscopically (Tab. 3–Tab. 5).

The presence of *Fusarium* fungi capable of producing trichothecene mycotoxins was noted in the seeds of narrow-leaved lupine cv. 'Zeus' from Warmia and Mazury and from Kujawy in 2011 and in the seeds from Lower Silesia in 2013 based on the presence of *Tri* genes responsible for trichothecene synthesis (Tab. 7). Trichothecene-producing fungi were not isolated from the seeds of the remaining lupine species and cultivars.

Discussion

Lupine seeds produced in Poland are used mainly as animal feed (63%), food (29%), and plant propagating material [1].

The average losses caused by pathogenic infections in legume production are estimated at 15%, but they can be as high as 70–80%. Leguminous plants are exposed to infectious factors throughout the entire growing season, from germination until pod formation and seed filling. Legumes are particularly susceptible to infections caused by pathogenic fungi during flowering [9].

In the present study, lupine seeds were colonized by pathogenic and saprotrophic fungi. In all lupine species and cultivars, the most prevalent saprotrophic fungi were *Alternaria alternata*, *Cladosporium cladosporioides*, *Rhizopus nigricans*, *Epicoccum nigrum*, and the genus *Penicillium*, whereas potentially pathogenic fungi were represented mainly by *Fusarium* species, *Botrytis cinerea*, and single isolates of *Mycosphaerella lupini* and *Colletotrichum lupini*.

Numerous studies have demonstrated the presence of fungi of the genera *Alternaria*, *Penicillium*, *Cladosporium*, *Aspergillus*, *Rhizopus*, *Ulocladium*, *Chaetomium*, *Arthrinium*, *Stemphylium*, *Fusarium*, *Botrytis*, *Colletotrichum*, and *Bipolaris* on the seeds of various

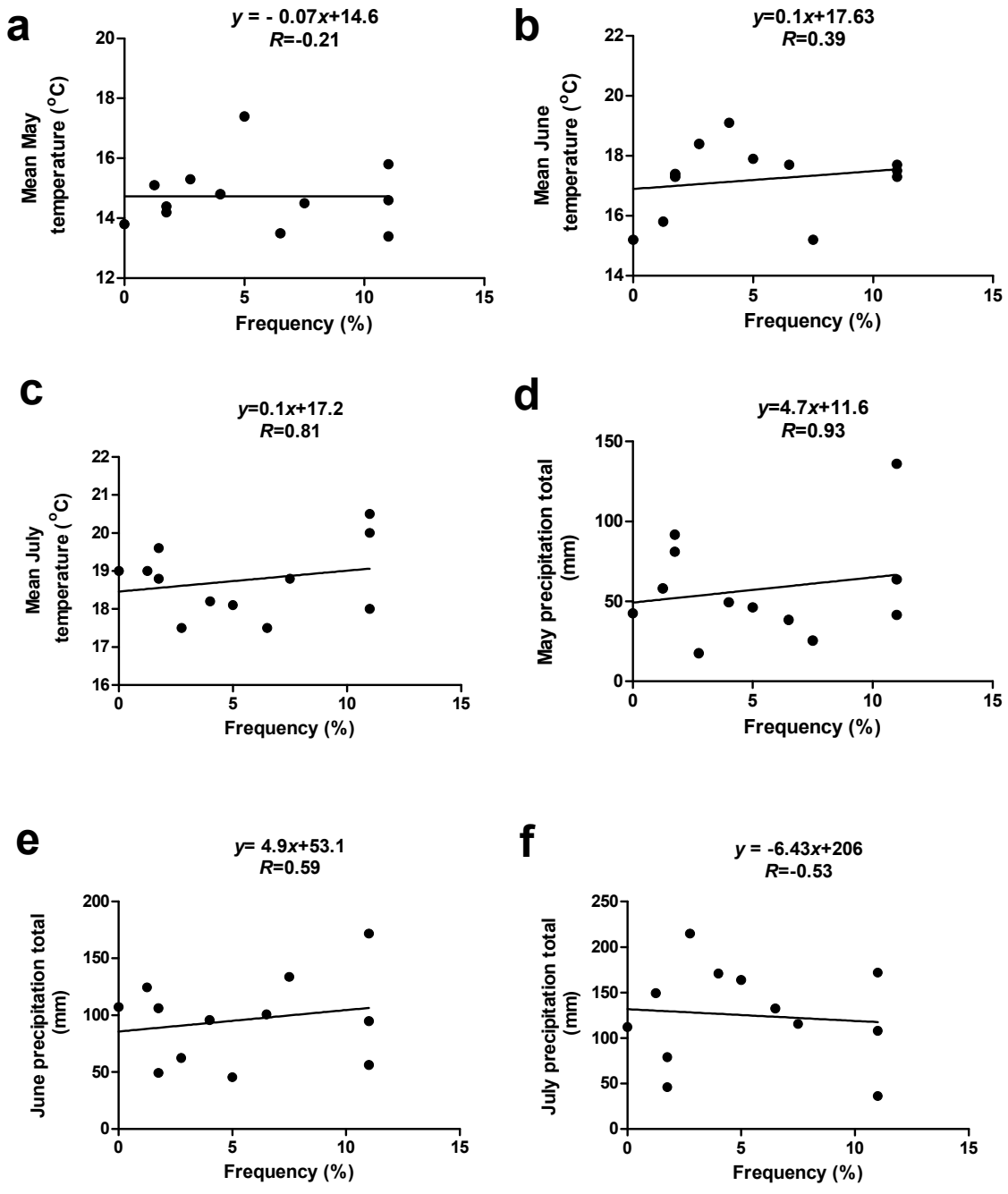

Fig. 1 Correlation between the frequency of occurrence of pathogenic species, mean temperatures, and monthly precipitation totals in particular months.

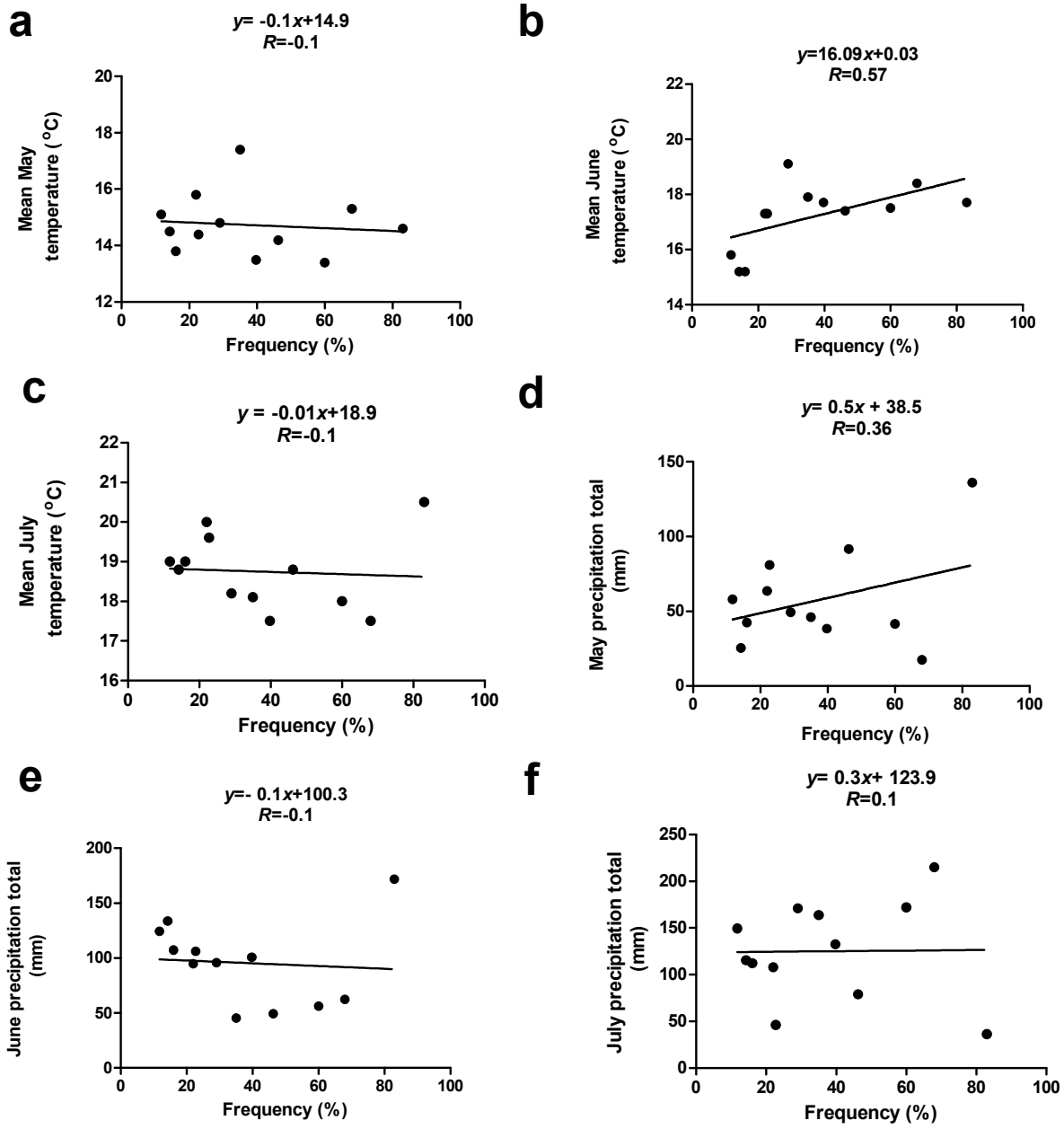

Fig. 2 Correlation between the frequency of occurrence of saprotrophic species, mean temperatures, and monthly precipitation totals.

Tab. 7 Diagnostic analysis of PCR assays of lupine seeds harvested in 2011–2013.

	Fusarium spp.			*Fusarium sporotrichioides*			*Fusarium avenaceum*			*Fusarium poae*			Gen *Tri5*		
	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
1. White lupine 'Butan' (B)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2. White lupine 'Butan' (P)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3. Narrow-leaved lupine 'Zeus' (O)	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-
4. Narrow-leaved lupine 'Zeus' (W)	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
5. Narrow-leaved lupine 'Zeus' (B)	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-
6. Narrow-leaved lupine 'Zeus' (P	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
7. Yellow lupine 'Mister' (B)	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
8. Yellow lupine 'Mister' (P)	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9. Yellow lupine 'Perkoz' (B)	+	+	+		-	+	-	-	-	-	-	-	-	-	-
10. Yellow lupine 'Perkoz' (P)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

"+" – positive result of PCR assays (the presence of *Fusarium* DNA); "-" – negative result of PCR assays PCR (absence of *Fusarium* DNA). B – seeds from Mochełek, Kujawy; O – seeds from Bałcyny, Warmia and Mazury; P – seeds from Złotniki, Wielkopolska; W – seeds from Swojec, Lower Silesia.

lupine species [24–29], which is consistent with our findings.

In this study, the most prevalent species of potentially pathogenic fungi colonizing all three lupine species belonged to the genus *Fusarium* and were represented by *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. poae*, *F. sambucinum*, *F. sporotrichioides*, and *F. tricinctum*. According to Holtz et al. [30], selected *Fusarium* species (*F. oxysporum* f. sp. *lupini*, *F. solani*, *F. avenaceum*, and *F. culmorum*) may cause seedling blight in lupine.

Toxin-producing fungi of the genus *Fusarium* directly affect the quantity and quality of crops and pose a serious threat to human and animal health. *Fusarium* pathogens cause disease and produce mycotoxins that contaminate plant material. Zearalenone and trichothecenes are the most ubiquitous mycotoxins in plants [31–34].

Studies investigating *Fusarium* fungi based on their morphological characteristics and genetic sequences which are identified by PCR with the use of species-specific primers often deliver different or contradictory results. Such discrepancies could be attributed to errors in species classification during microscopic analyses or the absence of specific primers for selected species. Kulik et al. [35] and Schilling et al. [36] also observed that certain *Fusarium* fungi may be difficult to classify to the species level based on their morphological traits, which could lead to errors in taxonomic identification. According to Kulik et al. [35], PCR is a reliable method which supports the identification of a broad range of fungal species. It is highly useful in diagnosing diseases that are transmitted by seeds and pose a serious threat in early stages of plant development, where an accurate diagnosis is required to effectively control the spread of infections.

PCR analyses revealed the presence of *F. avenaceum*, *F. poae*, and *F. sporotrichioides* in the seeds of the analyzed lupine species. PCR is a sensitive, potentially specific, and rapid method for detecting and identifying pathogens directly in plant tissues [33].

Species of the genus *Fusarium* synthesize mycotoxins that are dangerous to plants. In this study, HATrif/HATrir primers developed by Edwards et al. [23] were used to identify *Tri5* gene responsible for the production of trichothecenes. The presence of *Tri5* gene was noted only in the seeds of narrow-leaved lupine in 2011 and 2013.

Anthracnose, the most devastating disease in lupines, is caused by the fungal species *Colletotrichum lupine* (Bondar) Nirenberg, Feiler & Hagedorn, which is part of the *C. acutatum*

complex [11,13,37]. The disease is widespread around the world, and its incidence is particularly high in regions characterized by high precipitation, high humidity and temperature, which contribute to the development of secondary infections throughout the growing season [38,39]. *Colletotrichum* spp. is spread by seeds, which are considered to be the primary source of infection [38]. In our study, *Colletotrichum lupini* was detected only in the seeds of yellow lupine cv. 'Perkoz' from Kujawy in 2013, which can be attributed to low precipitation during flowering and seed ripening. The degree of colonization by *Colletotrichum* spp. can vary subject to weather conditions during the growing season [29]. Podleśny et al. [40] also reported a correlation between the development of plant diseases and weather conditions. Anthracnose was more prevalent in Polish regions characterized by high precipitation and high temperatures during lupine flowering. According to Snarska and Szczygielski [41], even a single abundant rainfall event can promote the spread of anthracnose in lupine if it is accompanied by high ambient temperature. Thomas et al. [42] reported that even very low levels of colonization by *Colletotrichum* spp. (0.001% seeds) can decrease lupine yields by even 30%. Podleśny et al. [40] observed a 30% drop in the seed yield of narrow-leaved lupines in regions affected by anthracnose.

Fungal pathogens not only decrease seed yield, but also lower the quality of lupine seeds [10,40]. Many plant diseases are spread by seeds, which is why seed health and seed dressing should be prioritized in lupine production [27].

Conclusions

The seeds of white, narrow-leaved and yellow lupine constitute valuable raw material to produce animal feed. Lupine seeds have to be closely monitored for the presence of mycotoxins – toxic metabolites produced by fungi. The seeds analyzed in this study conformed to high quality standards and were generally free of fungal pathogens. A comparison of the RF values of pathogenic and saprotrophic fungi in view of weather conditions revealed that the colonization of lupine seeds by pathogens was considerably influenced by total precipitation in May and average temperature in July, whereas average temperature in June had a significant effect on the occurrence of saprotrophic species.

Despite the above, the health and microbiological purity of lupine seeds have to be regularly controlled to ensure that adequate prevention methods are undertaken in the event of massive colonization by fungal pathogens to minimize potential losses in yield. The study was conducted on seeds from different regions of Poland. The risk of anthracnose was minimal, but due to the high virulence of *C. lupini*, lupine populations should be regularly monitored with the involvement of molecular methods. The use of healthy certified seeds can significantly reduce the prevalence of fungal diseases, improve plant health and increase the quantity and quality of seed yields.

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Grzyby zasiedlające nasiona trzech gatunków łubinów uprawianych w różnych rejonach Polski

Streszczenie

Badania dotyczyły oceny zdrowotności nasion trzech gatunków łubinów (biały, wąskolistny, żółty) pochodzących z różnych rejonów Polski. Analizy wykonano metodą mikroskopową oraz PCR. Wykazano, że nasiona badanych łubinów były zasiedlone przez grzyby saprotroficzne: *Alternaria* spp. *Cladosporium* spp. *Penicillium* spp. oraz patogeniczne z rodzajów: *Fusarium*, *Botrytis*, *Mycosphaerella* i *Colletotrichum*. Obliczono wskaźnik częstotliwości występowania grzybów (RF) w nasionach łubinów pochodzących z Kujaw, Wielkopolski, Dolnego Śląska oraz Warmii i Mazur. Wykazano, że najwyższe wartości wskaźnika częstotliwości występowania gatunków patogenicznych dotyczyły nasion łubinów pochodzących z rejonu Dolnego Śląska w roku 2012 i 2013 oraz z Warmii i Mazur w roku 2011. Stwierdzono, że na częstotliwość występowania grzybów patogenicznych i spartotroficznych na nasionach pozyskanych z różnych regionów Polski wpływ miały warunki pogodowe. Wykazano ponadto obecność genów *Tri* w nasionach łubinu wąskolistnego metodą PCR. Stwierdzono, że nasiona badanych łubinów były w niewielkim stopniu zasiedlone przez grzyby patogeniczne i mogą stanowić dobry surowiec do produkcji pasz oraz jako materiał nasienny.