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Original Research

Effects of Mechanical Weed Control in Barley-Pea Mixture on Colonization of Pea Seeds by Fungi, Part 2

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Abstract

Our study was a mycological evaluation of pea seeds harvested from a barley-pea mixture in which different methods of weed control had been used. The field experiment was carried out during 2010-12 and was conducted using randomized block design in four replicates. Weed control was mechanical and chemical. Fungal colonization tests were carried out on disinfected and non-disinfected seeds. The research showed statistically significant differences in the total number of fungi isolated from disinfected and non-disinfected seeds. The fungus most frequently isolated was *Alternaria alternata*. *Penicillium chrysogenum*, *Sclerotinia sclerotiorum*, and *Trichoderma harzianum* were isolated only from the non-disinfected seeds. Presented results show that suitably chosen mechanical weed control may be an alternative to chemical weed control in the mixtures of cereals and legumes, and may be particularly important for organic and integrated farming. The best variant of mechanical weed control in the cereal-legume mixture in terms of infection pea seeds by fungi is two passes of spring-tine harrow at the beginning of the tillering stage of barley and two passes at the full tillering stage of barley.

Keywords: fungi, barley-pea mixture, weed control, pea seeds

Introduction

Mechanical weed control is particularly important for EC countries because in 2009 the European Parliament and European Council imposed obligations on all member states to follow, beginning in 2014, the principles of integrated pest management [1]. Such principles are currently used often in organic and sustainable farming and are a commonly used alternative to chemical methods [2-4].

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Literature reports on the effectiveness of mechanical control of weeds [5, 6], but there are no reports about the influence of harrowing on colonization of pea seeds by fungi in the barley-pea mixture. Currently there is only one report on this topic, but it analyzed only barley grains. Mechanical weed control of appropriately selected intensity (one passage of spring-tine harrow at full tillering stage of barley) compared to control by herbicides does not increase colonization of barley grains by fungi in the barley and pea mixture [7].

After harvest, legume grains carry a wide range of saprophytic and pathogenic fungi. The most frequent genus

Table 1. Agronomical operations performed in plots in 2010-12.

Agrotechnology	Years		
	2010	2011	2012
Harvest of forecrop seed	winter rye	winter triticale	winter triticale
Harvest of forecrop straw	x*	x	x
Disking	x	x	x
Harrowing heavy harrow – 1 time	x	x	x
Winter plowing (27-29 cm)	x	x	x
Harrowing heavy harrow – 1 time	x	x	x
Superphosphate spreading 40%	40 kg per ha	40 kg per ha	40 kg per ha
Potassium salt spreading 60%	50 kg per ha	50 kg per ha	50 kg per ha
Ammonium nitrate spreading 32%	40 kg per ha	40 kg per ha	40 kg per ha
Aggregate cultivation – 1 time	x	x	x
Pea var. "Milwa" sowing	07.04	01.04	28.03
Spring barley var. "Nagradowicki" sowing	07.04	01.04	28.03
Spring-tine harrow I term	08.05	28.04	30.04
Spring-tine harrow II term	25.05	06.05	08.05
Herbicide treatment – Chwastox Extra 300SL, 3L per ha	26.05	10.05	10.05
Harvest of mixture	12.08	06.08	01.08

* it was performed

of fungi are *Alternaria*, *Cladosporium*, and *Fusarium* [8]. These fungi can cause mycotoxin poisoning, plus allergies in humans and animals, as well as diseases of plants [9-11].

Our study aimed at mycological evaluation of pea seeds harvested from the barley and pea mixture in which different methods of weed control were used.

Material and Methods

Field Experiment

Our field experiment was conducted in 2010-12 in fields of the Agricultural Experimental Station at Swojec (51º6' N, 17º8' E), part of Wroclaw University of Environmental and Life Sciences (further abbreviated as WUELS). A one-factor field experiment was conducted using randomized block design with four replicates. The plots sown with the mixture of spring barley, var. Nagradowicki and pea, var. Milwa, were situated on alluvial loamy sand soil. The number of plots was 28, and size of each plot 36 m^2. Weed control was mechanical and chemical:

- Control – without weed control
- H-3 – Chwastox Extra 300 SL 3,0 l/ha at full tillering stage of barley
- P-1-0 – One pass of spring-tine harrow at the beginning of tillering stage of barley
- P-0-1 – One pass of spring-tine harrow at full tillering stage of barley
- P-1-1 – One pass of spring-tine harrow at the beginning of tillering stage of barley and one pass at full tillering stage of barley
- P-2-1 – Two passes of spring-tine harrow at the beginning of the tillering stage of barley and one pass at full tillering stage of barley
- P-2-2 – Two passes of spring-tine harrow at the beginning of the tillering stage of barley and two passes at full tillering stage of barley

The amount of the seed mixture components was as follows: 30% of normal sowing barley in pure (99 germinated grains×m^{-2}) and 70% of normal sowing pea in pure (63 germinating seeds×m^{-2}). Agronomical operations performed in the plots in 2010-12 are listed in Table 1.

Characteristics of Plant Varieties

Spring barley var. Nagradowicki represents very high-yielding potential in the area of Poland, and also on poorer soils. Plants are of medium height and very good lodging resistance. It ripens early, and shows good resistance to soil acidification. The variety presents good health. It is highly resistant to powdery mildew (*Blumeria graminis*), and

Table 2. The average number of total fungi isolated from disinfected and non-disinfected pea seeds in 2010-12 (CFU per 100 seeds).

Seeds	Year	Abbreviation of object						
		Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Disinfected	2010	44^{bC}*	42^{bC}	47^{bC}	84^{aA}	59^{bB}	41^{aCD}	34^{aD}
	2011	50^{bB}	43^{bC}	58^{bA}	59^{bA}	60^{bA}	40^{aC}	31^{aD}
	2012	62^{aABC}	56^{aBC}	70^{aAB}	58^{bBC}	76^{aA}	50^{aCD}	38^{aD}
Non-disinfected	2010	118^{bCD}*	109^{bC}	101^{bD}	147^{bA}	135^{bABC}	140^{bAB}	120^{bBCD}
	2011	197^{aA}	175^{aBC}	138^{aD}	176^{aBC}	190^{aAB}	170^{aC}	184^{aABC}
	2012	104^{bCD}	131^{bB}	100^{bD}	175^{aA}	130^{bB}	161^{abA}	123^{bBC}

*Means followed by the same letter do not differ significantly. Small letters mark the effect of research year on total fungi in a particular object; they refer to means in columns. Capital letters mark the effect of object on total fungi in a particular research year; they refer to means in rows. Fisher's least significant difference (LSD) test, $\alpha \leq 0.05$.

rhynchosporium (*Rhynchosporium secalis*). On the other hand, pea var. Milwa is early variety and very evenly maturing, useful for cultivation in the area of Poland. It is also recommended for sowing in crop mixture. The plants are characterised by very good stiffness of stem. It is highly resistant to diseases, especially to downy mildew and gray mold (*Botrytis cinerea*).

Meteorological Conditions

Meteorological data were obtained from the instruments installed in the Agro-Hydrometeorology Observatory at Swojec (part of WUELS; 51º6' N, 17º8' E; Fig. 1).

Tests of Fungal Colonization of Pea Seeds

From each experimental variant 100 seeds were surface disinfected in 0.5% NaOCl during 1 min. Another 100 seeds were not disinfected. Seed samples were then transferred on PDA medium (potato dextrose agar, Biocorp) in Petri dishes. All variants of the experiment were incubated in four replicates. The incubation of cultures on Petri dishes was carried out at room temperature (22ºC) for 5-10 days in darkness. After incubation, the number of CFUs (colony forming units) per 100 seeds was calculated and the fungi were identified.

Identification of the Fungi

The fungi were identified using diagnostic keys and monographs [12-14].

Media Used for Isolation and Identification

PDA (Biocorp), Czapek-Dox Agar (1.2% agar, Biocorp), and MEA (Malt Extract Agar, Biocorp) were used. PDA medium was used for the isolation of fungi from the seeds and for the identification of some species. Czapek-Dox agar medium and MEA were used for identification of the *Penicillii*.

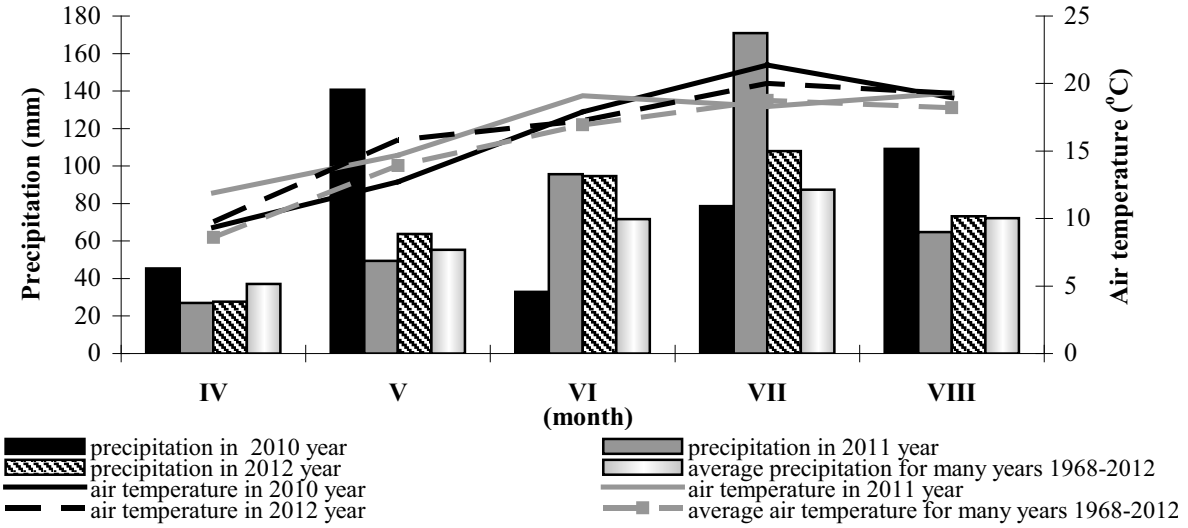

Fig. 1. Temperature and rainfall in the growth seasons during the study years (2010-12).

Table 3. The average number of fungi isolated from disinfected and non-disinfected pea seeds in 2010 (CFU per 100 seeds).

Fungi species		Abbreviation of object						
		Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Disinfected seeds	*Alternaria alternata*	24^{aB}*	15^{aC}	25^{aB}	30^{aA}	11^{abD}	15^{aC}	10^{aD}
	Aspergillus niger	0^{fB}	0^{dB}	0^{dB}	1^{dA}	0^{dB}	0^{eB}	0^{dB}
	Botrytis cinerea	0^{fB}	3^{cB}	1^{dB}	10^{bA}	3^{cdB}	3^{cB}	1^{dB}
	Cladosporium cladosporioides	1^{efB}	1^{dB}	0^{dB}	2^{dAB}	4^{cA}	0^{eB}	0^{dB}
	Cladosporium herbarum	$2^{de B}$	7^{bA}	0^{dC}	7^{bcA}	8^{bA}	2^{cdB}	2^{dB}
	Epicoccum nigrum	0^{fC}	0^{dC}	0^{dC}	3^{dA}	1^{cdB}	0^{eC}	3^{cdA}
	Fusarium avenaceum	0^{fB}	1^{dB}	1^{dB}	3^{dA}	1^{cdB}	1^{deB}	0^{dB}
	Fusarium culmorum	3^{cdD}	6^{bC}	9^{cB}	10^{bAB}	12^{aA}	9^{bB}	6^{bcC}
	Fusarium equiseti	1^{efCD}	1^{dCD}	0^{dD}	4^{cdB}	3^{cdBC}	10^{bA}	3^{cdBC}
	Fusarium graminearum	8^{bA}	3^{cBC}	0^{dD}	4^{cdB}	2^{cdBCD}	1^{deCD}	2^{dBCD}
	Fusarium oxysporum	0^{fC}	1^{dB}	0^{dC}	3^{dA}	0^{dC}	0^{eC}	0^{dC}
	Rhizopus stolonifer	5^{cCD}	4^{cCD}	11^{bAB}	7^{bcBC}	14^{aA}	0^{eD}	7^{abBC}
Non-disinfected seeds	*Alternaria alternata*	53^{aABC}*	46^{aC}	48^{aBC}	70^{aA}	69^{aAB}	60^{aABC}	48^{aBC}
	Aspergillus niger	0^{dB}	1^{bA}	0^{dB}	0^{dB}	0^{cB}	0^{eB}	0^{cB}
	Botrytis cinerea	3^{cdA}	0^{bC}	1^{dB}	0^{dC}	0^{cC}	0^{eC}	1^{cB}
	Cladosporium cladosporioides	7^{cA}	2^{bAB}	3^{cdB}	0^{dC}	0^{cC}	1^{deBC}	0^{cC}
	Cladosporium herbarum	38^{bB}	49^{aA}	26^{bC}	55^{bA}	40^{bB}	53^{bA}	49^{aA}
	Epicoccum nigrum	4^{cdB}	5^{bAB}	8^{cAB}	10^{cA}	6^{cAB}	6^{cdAB}	5^{bcAB}
	Fusarium avenaceum	0^{dC}	0^{bC}	0^{dC}	1^{dB}	5^{cA}	0^{eC}	1^{cB}
	Fusarium culmorum	1^{dC}	0^{bC}	5^{cdB}	5^{cdB}	6^{cAB}	0^{eC}	9^{bA}
	Fusarium equiseti	7^{cA}	2^{bC}	3^{cdBC}	3^{dBC}	5^{cAB}	2^{deC}	1^{cC}
	Fusarium graminearum	3^{cdA}	0^{bB}	0^{dB}	1^{dAB}	0^{cB}	3^{deA}	0^{cB}
	Fusarium oxysporum	1^{dAB}	2^{bA}	1^{dAB}	0^{dB}	1^{cAB}	0^{eB}	0^{cB}
	Penicillium chrysogenum	0^{dB}	0^{bB}	3^{cA}	2^{dA}	0^{cB}	0^{eB}	2^{cA}
	Rhizopus stolonifer	1^{dBD}	2^{bBCD}	3^{cdBC}	0^{dD}	3^{cBC}	11^{cA}	4^{bcB}
	Sclerotinia sclerotiorum	0^{dB}	0^{bB}	0^{dB}	0^{dB}	0^{cB}	4^{deA}	0^{cB}

*Means followed by the same letter do not differ significantly. Small letters mark the effect of a particular object on isolates fungi; they refer to means in columns. Capital letters mark the effect of object on a particular fungi species; they refer to means in rows. Fisher's least significant difference (LSD) test, $\alpha \leq 0.05$.

Statistical Analysis

The results of the fungal colonization tests were analyzed using ANOVA as available in the Statistica 9.0 package. Means were compared using Fisher's least significant difference (LSD) test at $\alpha \leq 0.05$.

Results

The average sums of rainfall and mean temperatures in the growth seasons during the experiment were higher than average value for many years. The minimum average rainfall in 2010 was recorded in June, and in 2011 and 2012 in April. The average temperatures during 2010 and 2012 were the highest in July and at their lowest in April in all the years of the study. The highest average sum of rainfall in 2010 was observed in May, but in 2011 as well as in 2012 it was the highest in July (Fig. 1).

The research generally has shown statistically significant differences between the treatments, and years, in the total number of fungi isolated from disinfected and non-disinfected seeds. More fungi were isolated from non-disinfected seeds than from disinfected. The highest total

Table 4. The average number of fungi isolated from disinfected and non-disinfected pea seeds in 2011 (CFU per 100 seeds).

Fungi species		Abbreviation of object						
		Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Disinfected seeds	*Alternaria alternata*	22^{aA}*	18^{aB}	24^{bA}	22^{aA}	22^{bA}	22^{aA}	8^{bC}
	Aspergillus niger	0^{eB}	0^{dB}	2^{cdA}	0^{eB}	0^{dB}	0^{dB}	0^{dB}
	Botrytis cinerea	2^{deB}	0^{dC}	0^{dC}	16^{cA}	0^{dC}	0^{dC}	0^{dC}
	Cladosporium cladosporioides	0^{eB}	3^{dA}	0^{dB}	0^{eB}	0^{dB}	0^{dB}	0^{dB}
	Cladosporium herbarum	6^{bcAB}	8^{cA}	0^{dC}	2^{dBC}	2^{cdBC}	8^{bA}	2^{cdBC}
	Epicoccum nigrum	2^{deA}	0^{dC}	0^{dC}	1^{deB}	0^{dC}	0^{dC}	0^{dC}
	Fusarium avenaceum	$4^{c}d^{A}$	0^{dC}	4^{cA}	0^{eC}	0^{dC}	2^{cdB}	0^{dC}
	Fusarium culmorum	0^{eB}	0^{dB}	0^{dB}	0^{eB}	4^{cA}	0^{dB}	4^{cA}
	Fusarium equiseti	2^{deAB}	0^{dB}	0^{dB}	0^{eB}	0^{dB}	2^{cdAB}	3^{cA}
	Fusarium graminearum	4^{cdA}	0^{dB}	0^{dB}	0^{eB}	0^{dB}	0^{dB}	2^{cdAB}
	Rhizopus stolonifer	8^{bDE}	14^{bBC}	28^{aA}	18^{bB}	32^{aA}	6^{bcE}	12^{aCD}
Non-disinfected seeds	*Alternaria alternata*	100^{aA}*	67^{bD}	56^{aE}	94^{aB}	82^{bC}	84^{aC}	68^{bD}
	Aspergillus niger	0^{eB}	2^{fA}	0^{eB}	0^{eB}	0^{dB}	0^{dB}	0^{eB}
	Botrytis cinerea	0^{eB}	0^{gB}	0^{eB}	2^{deA}	0^{dB}	0^{dB}	0^{eB}
	Cladosporium cladosporioides	10^{cA}	0^{gD}	2^{deC}	0^{eD}	0^{dD}	0^{dD}	4^{cB}
	Cladosporium herbarum	76^{bD}	80^{aC}	46^{bG}	62^{bF}	88^{aB}	70^{bE}	96^{aA}
	Epicoccum nigrum	3^{dE}	14^{cA}	12^{cAB}	10^{cBC}	6^{cD}	8^{cCD}	2^{dE}
	Fusarium avenaceum	4^{dAB}	0^{gC}	2^{deBC}	0^{eC}	6^{cA}	0^{dC}	0^{eC}
	Fusarium equiseti	4^{dA}	0^{gB}	4^{dA}	4^{dA}	0^{dB}	0^{dB}	0^{eB}
	Fusarium graminearum	0^{eB}	4^{eA}	0^{eB}	0^{eB}	0^{dB}	0^{dB}	0^{eB}
	Penicillium chrysogenum	0^{eB}	0^{gB}	2^{deA}	2^{deA}	0^{dB}	0^{dB}	2^{dA}
	Rhizopus stolonifer	0^{eC}	8^{dB}	14^{cA}	2^{deC}	8^{cB}	8^{cB}	12^{cA}

* Means followed by the same letter do not differ significantly. Small letters mark the effect of a particular object on isolates fungi; they refer to means in columns. Capital letters mark the effect of object on a particular fungi species; they refer to means in rows. Fisher's least significant difference (LSD) test, $\alpha \leq 0.05$.

number of fungi in the year-long study from disinfected seeds of pea were isolated generally in 2012 and from non-disinfected in 2011 (the differences were statistically significant). The significantly lowest number of fungi was isolated from disinfected seeds for all years in the object P-2-2, whereas from non-disinfected seeds in P-1-0. The significantly highest total number of fungi from disinfected seeds of pea were isolated in 2010 from object P-0-1; in 2011 from P-1-0, P-0-1, and P-1-1; and in 2012 from P-1-1. As for the non-disinfected seeds, the significantly highest total number of fungi were isolated in 2010 from P-0-1, in 2011 from control, and in 2012 from P-0-1 and P-2-1 (Table 2).

Generally, from all the variants of the experiment 15 fungi species were isolated (12 from disinfected seeds and 15 from non-disinfected). In all years of the experiment, there were significant differences in the number of fungi species isolated from disinfected and non-disinfected seeds. The fungus most frequently isolated from pea seeds was *Alternaria alternata*, with the following exceptions: in 2010 from disinfected *Fusarium culmorum* (P-1-1), in 2011 from disinfected *Rhizopus stolonifer* (P-1-0, P-1-1, P-2-2) and non-disinfected *Cladosporium herbarum* (H-3, P-1-1, P-2-2), and in 2012 from disinfected *Fusarium culmorum* (H-3, P-1-1, P-2-1, P-2-2) and *Cladosporium herbarum* in non-disinfected (P-2-1). The fungi species least isolated in 2010 from disinfected and non-disinfected seeds was *Aspergillus niger*, while in 2011 *Cladosporium cladosporioides* from disinfected and *Aspergillus niger* and *Botrytis cinerea* from non-disinfected. In 2012, the species of *Epicoccum nigrum* was isolated last from disinfected seeds and *Aspergillus niger* from non-disinfected (Tables 3-5).

Penicillium chrysogenum, *Sclerotinia sclerotiorum*, and *Trichoderma harzianum* were isolated only from the non-disinfected seeds. *Penicillium chrysogenum* was isolated only in 2010 and 2011, *Sclerotinia sclerotiorum* in 2010

Table 5. The average number of fungi isolated from disinfected and non-disinfected pea seeds in 2012 (CFU per 100 seeds).

Fungi species		Abbreviation of object						
		Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Disinfected seeds	*Alternaria alternata*	20^{aB}*	14^{bC}	26^{aA}	28^{aA}	6^{bcD}	14^{bC}	12^{bC}
	Botrytis cinerea	0^{eB}	4^{deAB}	2^{dB}	2^{dB}	10^{bA}	0^{cB}	0^{dB}
	Cladosporium cladosporioides	0^{eB}	0^{fB}	0^{eB}	0^{eB}	4^{bcA}	0^{cB}	0^{dB}
	Cladosporium herbarum	0^{eC}	4^{deB}	0^{eC}	4^{cB}	10^{bA}	0^{cC}	2^{dBC}
	Epicoccum nigrum	0^{eB}	0^{fB}	0^{eB}	0^{eB}	2^{cA}	0^{cB}	0^{dB}
	Fusarium avenaceum	0^{eC}	0^{fC}	0^{eC}	2^{dB}	4^{bcA}	0^{cC}	0^{dC}
	Fusarium culmorum	12^{cD}	18^{aB}	26^{aA}	14^{bCD}	26^{aA}	24^{aA}	16^{aBC}
	Fusarium equiseti	4^{dC}	6^{cdBC}	4^{cC}	2^{dC}	10^{bAB}	12^{bA}	6^{cBC}
	Fusarium graminearum	16^{bA}	8^{cB}	0^{eD}	2^{dC}	2^{cC}	0^{cD}	0^{dD}
	Fusarium oxysporum	0^{eB}	0^{fB}	2^{dA}	0^{eB}	0^{cB}	0^{cB}	2^{dA}
	Rhizopus stolonifer	10^{cA}	2^{efC}	10^{bA}	4^{cB}	2^{cC}	0^{cD}	0^{dD}
Non-disinfected seeds	*Alternaria alternata*	39^{aE}*	49^{aD}	62^{aBC}	70^{aB}	81^{aA}	62^{bBC}	54^{aCD}
	Aspergillus niger	0^{eB}	1^{cdA}	0^{eB}	0^{dB}	0^{eB}	0^{fB}	0^{eB}
	Botrytis cinerea	7^{dA}	0^{dB}	0^{eB}	0^{dB}	4^{dAB}	0^{fB}	0^{eB}
	Cladosporium cladosporioides	0^{eB}	10^{bA}	0^{eB}	0^{dB}	0^{eB}	0^{fB}	0^{eB}
	Cladosporium herbarum	26^{bCD}	48^{aB}	22^{bD}	72^{aA}	18^{bD}	66^{aA}	34^{bC}
	Epicoccum nigrum	3^{deB}	3^{cdB}	2^{deB}	7^{cA}	3^{deB}	3^{defB}	5^{dAB}
	Fusarium avenaceum	0^{eB}	2^{cdAB}	0^{cB}	4^{cA}	0^{eB}	0^{fB}	0^{eB}
	Fusarium culmorum	15^{cBC}	6^{bcE}	8^{cD}	14^{bC}	16^{bB}	2^{efF}	20^{cA}
	Fusarium equiseti	8^{dA}	6^{bcA}	6^{cdA}	4^{cA}	8^{cA}	6^{dA}	4^{dA}
	Fusarium graminearum	6^{dA}	2^{cdAB}	0^{eB}	0^{dB}	0^{eB}	2^{efAB}	2^{deB}
	Fusarium oxysporum	0^{eB}	0^{dB}	0^{eB}	0^{dB}	2^{deA}	0^{fB}	0^{eB}
	Rhizopus stolonifer	0^{eC}	4^{cdB}	0^{eC}	0^{dC}	0^{eC}	16^{cA}	4^{dB}
	Sclerotinia sclerotiorum	0^{eB}	0^{dB}	0^{eB}	0^{dB}	0^{eB}	4^{deA}	0^{eB}
	Trichoderma harzianum	0^{eB}	0^{dB}	0^{eB}	4^{cA}	0^{eB}	0^{fB}	0^{eB}

* Means followed by the same letter do not differ significantly. Small letters mark the effect of a particular object on isolates fungi; they refer to means in columns. Capital letters mark the effect of object on a particular fungi species; they refer to means in rows. Fisher's least significant difference (LSD) test, $\alpha \leq 0.05$.

and 2012, and *Trichoderma harzianum* in 2012. Interestingly, *Aspergillus niger* was not isolated from the disinfected seeds only in 2012, *Fusarium culmorum* in 2011, and *Fusarium oxysporum* in 2011 from both variants of experience (Tables 3-5).

Discussion

Roháčik and Hudec [15] reported that environmental factors such as temperature and rainfall are the main factors influencing the infectioned plants by fungi. They are responsible for the occurrence and severity of disease. High temperature and relative humidity of air during the growth seasons may reduce the seed yield of a crop, seed vigor, and germination [16]. However, the average rainfall and temperatures in the presented study seem to have no significant effect on the colonization of pea seeds by fungi.

Today there are many scientific reports about colonization of single- and multi-species mixtures by fungi [17, 18], but only one about the influence of mechanical weed control on the colonization of seeds. According to Lejman et al. [7], mechanical weed control of appropriately selected intensity does not increase colonization of barley grains by pathogenic fungi, as compared to the control by herbicides in mixed cultures of barley and peas. The results of our

study on pea seeds confirms this reports and may be particularly important for organic and integrated farming. Presented results also confirm reports by other authors that mixtures of cereals and legumes are less exposed to seed infestation by fungi in terms of their quantity and species composition, as compared to pure stands (only pea) [8, 19, 20].

Mechanical treatments may be seen as an alternative to chemical weed control [3, 4]. Velykis et al. [21] report that the best results in mechanical weed control can be achieved at the early stages of plant development, because mechanical treatment during this period does not damage cereal plants, thereby preventing yield reduction. In our research, the variant of weed control that proved the best in terms of mycological quality of pea seeds in the barley-pea mixture was two passes of spring-tine harrow at the beginning of the tillering stage of barley and two passes at full tillering stage of barley (P-2-2). The least number of fungi was recorded for this procedure most likely by reducing the humidity conditions within a crop and probably reducing weed infestation.

Gleń et al. [8] report that the most frequent fungus from legume seeds are *Alternaria alternata*. Our research conforms this report. However, depending on the method of weed control also dominated species such as *Cladosporium herbarum*, *Fusarium culmorum*, and *Rhizopus stolonifer.* The fungi species very rarely isolated were *Aspergillus niger*, *Botrytis cinerea*, and *Epicoccum nigrum*. Fungi from the genus *Alternaria* and *Cladosporium* are cosmopolitan and occur in soils and in the atmosphere [22]. Studies of the atmospheric air in Poland show that the spores of these fungi dominate in the atmosphere and their peak season is in the summer [23, 24]. High availability of inoculums of these fungi may by be one of the reasons for infection of some plants.

Fungi such as *Penicillium chrysogenum*, *Sclerotinia sclerotiorum*, and *Trichoderma harzianum* were isolated in all research years only from the non-disinfected seeds. Our research about *P. chrysogenum* confirms reports by Pląskowska [17] and Lejman [7], because this species is generally a saprophyte. Gleń et al. [8] reported that *S. sclerotiorum* are not dominant fungi on legume seeds, but in comparison to *P. chrysogenum* is a legume parasite.

Conclusions

1. Suitably chosen mechanical weed control may be an alternative to chemical weed control in barley-pea mixtures and may be particularly important for organic and integrated farming.
2. The best variant of mechanical weed control in the barley-pea mixture in terms of infected pea seeds by fungi is two passes of spring-tine harrow at the beginning of the tillering stage of barley and two passes at the full tillering stage of barley.
3. Pea seeds were colonized mostly by the species *Alternaria alternata*.

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