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Original Research

Effects of Mechanical Weed Control in Barley–Pea Mixture on Colonization of Barley Grain by Fungi, Part 1

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Abstract

The study aimed at being a mycological evaluation of spring barley grain harvested from the barley-pea intercrop, on which different methods of weed control had been used. The field experiment was carried out during 2010-12 and it was conducted using randomized block design in four replicates. Weed control was mechanical (spring-tine harrow treatment of variable intensity) and chemical (herbicide Chwastox Extra 300 SL – 3L per ha). Fungal colonization tests were carried out on disinfected and non-disinfected grains. The research has shown statistically significant differences in the total number of fungi isolated from disinfected and non-disinfected grains in particular years of study, between the objects, and in some cases between the study years. The fungus most frequently isolated from all variants of the experiment was *Alternaria alternata*. *Penicillium chrysogenum* was isolated only from non-disinfected grains. Presented results show that mechanical weed control treatments of appropriately adjusted intensity do not increase fungal colonization of barley grains in mixed culture with peas, as compared to the herbicidal control. The best variant of mechanical weed control for the cereal-legume mixture in terms of mycological quality of barley grain was one passage of spring-tine harrow at full tillering stage of barley. Mechanical weed control may be an alternative to herbicides in weed control in cereal-legume mixtures and may be particularly important for organic and integrated farming.

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

Introduction

Crop mixtures usually offer a more stable yield than pure stands of their component species. Moreover, they are less sensitive to weeds, pathogens, and pest infestations [1]. Cereal-legume mixtures are particularly useful in sustainable and ecological agriculture. Resistance of mixtures to diseases results from reduction, within the canopy, of the amount of tissue of the species sensitive to a particular pathogen, and from the fact that the tissue of the resistant species acts as a physical barrier for the infectious material spreading in the stand [2].

Cereal-legume mixtures are less susceptible to fungal diseases than single stands of their component species. Literature reports that they restrict infection development on aerial parts of the cereal component by airborne pathogens such as *Helminthosporium teres*, *Puccinia recondita*, and *Stagonospora nodorum*. However, such mixtures can be especially vulnerable to infection by fungi of the genus *Fusarium*, in particular by *F. avenaceum* [3].

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Table 1. Experimental design.

Object	Abbreviation of object
Control – without weed control	control
Chwastox Extra 300 SL 3.0 l/ha at full tillering stage of barley	H-3
One pass of spring-tine harrow at the beginning of tillering stage of barley	P-1-0
One pass of spring-tine harrow at full tillering stage of barley	P-0-1
One pass of spring-tine harrow at the beginning of tillering stage of barley and one pass at full tillering stage of barley	P-1-1
Two passes 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 tillering stage of barley and two passes at full tillering stage of barley	P-2-2

After harvest, cereal grains carry a wide range of saprophytic and pathogenic fungi [4]. It has been reported in literature that ca. 100 species of fungi could have been obtained from cereal grains [5], and that the species of *Fusarium* spp. had been most frequently isolated. These fungi can cause *Fusarium* head blight (FHB) and produce mycotoxins [6, 7].

FHB is an important disease that attacks principally wheat and barley but also affects other cereals worldwide. The most common among the *Fusarium* species causing this disease is *F. graminearum*, whereas *F. avenaceum*, *F. culmorum*, and *F. poae* are also responsible for FHB, but they are less often involved [8]. *Fusarium graminearum* predominates in central and southeastern Europe while in the cooler maritime climates there occur mainly *F. culmorum*, *F. avenaceum*, and *F. poae* [9].

In organic and sustainable farming the mechanical method of weed control is a commonly used alternative to chemical method [10-12]. The effectiveness of the mechanical control depends on a number of factors such as the appropriate number of harrow passages, the working depth of the tool, the moisture content of soil and the soil type itself, the species and variety of the crop plant, its phenological phase, weather conditions, and time of harrowing [13, 14]. Currently there are no reports in literature on the effects of harrowing in barley mixtures with legumes or on colonization of barley grain by fungi.

The study aimed at mycological evaluation of spring barley grain harvested from the barley-pea intercrop, in which different methods of weed control were used.

Material and Methods

Field Experiment

The field experiment was conducted in 2010-12 in fields of the Agricultural Experimental Station at Swojec (51º6' N, 17º8' E), part of Wrocław University of Environmental and Life Sciences (WUELS). The 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, size of each plot 36 m^2. Weed control was mechanical and chemical (Table 1).

Seed rate of barley was 99 germinating seeds per 1 m^2, whereas that of pea was 63 germinating seeds per 1 m^2. Agronomical operations performed in the plots in the period of 2010-12 are listed in Table 2.

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 Barley Grains

From each experimental variant 100 grains were surface disinfected in 1.0% NaOCl over 10 min. Another 100 grains were not disinfected. Grain samples were then transferred on PDA medium (potato dextrose agar, Biocorp) in 90 mm 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 [15-17].

Media Used for Isolation and Identification

PDA, 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 grains and for the identification of some species. Czapek-Dox agar medium and MEA were used for identification of the *Penicillii*.

Table 2. Agronomical operations performed in the plots in the period of 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
Harrownig spring-tine harrow I term according to the scheme (Table 1).	08.05	28.04	30.04
Harrownig spring-tine harrow II term according to the scheme (Table 1).	25.05	06.05	08.05
Herbicide treatment - Chwastox Extra 300SL, 3L per ha according to the scheme (Table 1).	26.05	10.05	10.05
Harvest of mixture	12.08	06.08	01.08

* it was performed

Statistical Analysis

The results of the fungal colonization tests were analyzed using ANOVA as available in 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 the respective multiannual values. 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. 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 they were at their lowest in April in all the years of the study (Fig. 1).

The research has shown statistically significant differences between the treatments, and in some cases between years, in the total number of fungi isolated from disinfected and non-disinfected grains. The highest total number of

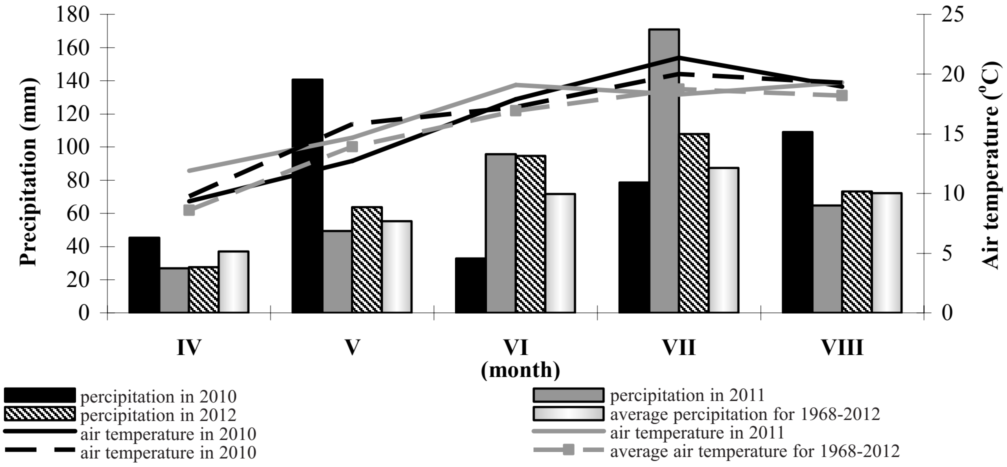

Fig. 1. Temperature and rainfall during the study years (2010-12).

Table 3. The average number of total fungi isolated from disinfected and non-disinfected grains of spring barley in 2010-12 (CFU per 100 grains).

Grains	Year	Abbreviation of object						
		Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Disinfected	2010	84^{b}_{C}*	69^{b}_{E}	92^{a}_{B}	78^{c}_{D}	78^{b}_{D}	97^{ab}_{A}	89^{a}_{B}
	2011	82^{b}_{B}	64^{c}_{D}	76^{b}_{BC}	54^{b}_{E}	70^{c}_{CD}	96^{b}_{A}	88^{a}_{A}
	2012	99^{a}_{AB}	101^{a}_{AB}	98^{a}_{C}	97^{a}_{C}	104^{a}_{A}	103^{a}_{A}	98^{a}_{C}
Non-disinfected	2010	102^{b}_{A}*	101^{b}_{A}	105^{b}_{A}	106^{a}_{A}	103^{c}_{A}	103^{b}_{A}	104^{b}_{A}
	2011	118^{a}_{B}	114^{a}_{C}	122^{a}_{A}	117^{a}_{BC}	114^{b}_{C}	114^{a}_{C}	116^{a}_{BC}
	2012	101^{b}_{D}	114^{a}_{AB}	104^{b}_{CD}	110^{a}_{BC}	122^{a}_{A}	110^{ab}_{BC}	112^{a}_{BC}

* 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 objects on total fungi in a particular research year; they refer to means in rows.

fungi species among all the years of the study were isolated from disinfected grains of barley in the treatment 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) in 2012. In contrast, their lowest number was isolated in 2011 from the P-0-1 (one pass of spring-tine harrow at full tillering stage of barley) treatment. As for the non-disinfected grains, the highest total number of fungi were isolated also from P-1-0 (one pass of spring-tine harrow at the beginning of tillering stage of barley) in 2011 and from 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) in 2012, whereas the lowest number was recorded in 2010 from H-3 (Chwastox Extra 300 SL 3,0 l/ha at full tillering stage of barley), and in 2012 from reference plots. Most fungi isolated from the disinfected grains were isolated in 2012 and the least in 2011, while from the non-disinfected grains most of the fungi were isolated in 2011 and the least in 2010. The numbers of fungi isolated from the non-disinfected grains in 2010 did not significantly differ between objects. The variants of weed control most effective in terms of mycological seed quality in the cereals and legumes was P-0-1 (one passage of spring-tine harrow at full tillering stage of barley). The worst variant, that had most badly affected seed quality of barley, was P-2-1 (two passages of spring-tine harrow at the beginning of tillering stage of barley and one passage at full tillering) (Table 3).

From the non-disinfected grains there were more fungi species isolated than from the disinfected ones, with exception for 2012. Generally, from all the variants of the experiment 16 fungi species were isolated. The highest number of species were isolated from both the disinfected and non-disinfected grains in 2010, and the lowest number in 2012. In all years of the experiment there were significant differences in the number of fungi species isolated from disinfected and non-disinfected grains. The fungus most frequently isolated from all the treatments and in all years of the study was *Alternaria alternata*, with two exceptions: one for the 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) variant (*Epicoccum nigrum*) and the other for 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) variant (*Drechslera avenae*) in 2012, both being isolated from the non-disinfected grains. The fungi species least isolated in 2010 from disinfected grains were *Botrytis cinerea* and *Mucor mucedo*, whereas from the non-disinfected grains was *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Sordaria fimicola*. On the other hand, the species of *Cladosporium herbarum* and *Rhizopus stolonifer* were isolated in 2011 from the disinfected grains, and from the non-disinfected *Botrytis cinerea*, *Cladosporium herbarum*, *Fusarium oxysporum,* and *Mucor mucedo*. In 2012 *Cladosporium herbarum* was the least frequently isolated fungi species from disinfected grains, and *Fusarium oxysporum* from the non-disinfected ones (Tables 4-9).

Penicillium chrysogenum was isolated only from the non-disinfected grains. The fungi such as *Cladosporium cladosporioides* and *Mucor mucedo* were isolated only in 2010 and 2011, whereas *Drechslera avenae* and *Sclerotinia sclerotiorum* in 2010 and 2012. Interestingly, *Sordaria fimicola* was not isolated from the non-disinfected grains only in 2012 (Tables 4-9).

Discussion

Environmental factors show considerable influence on the occurrence of fungal species and disease severity. Temperature and rainfall affect the production and dispersal of fungal inoculum, the two key factors responsible for the occurrence and severity of disease. The infection of barley grains before and after maturity is greatest at high relative air humidity [18] and it may reduce the grain yield of a crop, seed vigor, and germination [19]. However, during the growth seasons of the presented study, the average rainfall and temperatures seem to have no significant effect on the colonization of barley grains by fungi.

Literature reports about colonization of single and multi-species mixtures by fungi [20, 21], but there are no reports concerning the influence of mechanical treatments in the canopy of cereal-legume mixtures on colonization of

Table 4. The average number of fungi isolated from disinfected grains of spring barley in 2010 (CFU per 100 grains).

Fungi species	Abbreviation of object						
	Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Alternaria alternata	60^{a}_{C}*	38^{a}_{F}	66^{a}_{A}	46^{a}_{E}	50^{a}_{D}	64^{a}_{B}	65^{a}_{AB}
Botrytis cinerea	0^{e}_{B}	0^{g}_{B}	1^{de}_{A}	0^{f}_{B}	0^{f}_{B}	0^{e}_{B}	0^{e}_{B}
Cladosporium cladosporioides	0^{e}_{B}	0^{g}_{B}	1^{de}_{AB}	2^{de}_{A}	2^{de}_{A}	0^{e}_{B}	1^{de}_{AB}
Cladosporium herbarum	2^{d}_{B}	5^{c}_{A}	0^{e}_{C}	4^{bc}_{A}	0^{f}_{C}	1^{e}_{BC}	1^{de}_{BC}
Drechslera avenae	0^{e}_{B}	4^{cd}_{A}	0^{e}_{B}	5^{b}_{A}	1^{ef}_{B}	5^{c}_{A}	0^{e}_{B}
Epicoccum nigrum	1^{de}_{CD}	3^{de}_{AB}	1^{de}_{CD}	4^{bc}_{A}	2^{de}_{BC}	0^{e}_{D}	0^{e}_{D}
Fusarium avenaceum	1^{de}_{D}	7^{b}_{A}	5^{c}_{B}	3^{cd}_{C}	7^{c}_{A}	5^{c}_{B}	1^{de}_{D}
Fusarium culmorum	10^{b}_{A}	2^{ef}_{C}	5^{c}_{B}	5^{b}_{B}	1^{ef}_{C}	9^{b}_{A}	2^{cd}_{C}
Fusarium equiseti	0^{e}_{D}	2^{ef}_{BC}	0^{e}_{D}	4^{bc}_{A}	3^{d}_{AB}	1^{e}_{CD}	3^{c}_{AB}
Fusarium graminearum	8^{c}_{B}	5^{c}_{C}	9^{b}_{B}	3^{cd}_{D}	9^{b}_{B}	8^{b}_{B}	11^{b}_{A}
Fusarium oxysporum	1^{de}_{B}	0^{g}_{B}	1^{de}_{B}	1^{ef}_{B}	0^{f}_{B}	3^{d}_{A}	1^{de}_{B}
Mucor mucedo	0^{e}_{B}	0^{g}_{B}	0^{e}_{B}	0^{f}_{B}	0^{f}_{B}	1^{e}_{A}	0^{e}_{B}
Rhizopus stolonifer	0^{e}_{C}	0^{g}_{C}	2^{d}_{AB}	1^{ef}_{BC}	3^{d}_{A}	0^{e}_{C}	2^{cd}_{AB}
Sclerotinia sclerotiorum	0^{e}_{B}	1^{fg}_{A}	0^{e}_{B}	0^{f}_{B}	0^{f}_{B}	0^{e}_{B}	1^{de}_{A}
Sordaria fimicola	1^{de}_{AB}	2^{ef}_{A}	1^{de}_{AB}	0^{f}_{B}	0^{f}_{B}	0^{e}_{B}	1^{de}_{AB}

*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.

Table 5. The average number of fungi isolated from non-disinfected grains of spring barley in 2010 (CFU per 100 grains).

Fungi species	Abbreviation of object						
	Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Alternaria alternata	70^{a}_{A}*	49^{a}_{C}	54^{a}_{B}	47^{a}_{D}	55^{a}_{B}	49^{a}_{C}	50^{a}_{C}
Botrytis cinerea	0^{f}_{B}	0^{h}_{B}	1^{fg}_{A}	0^{f}_{B}	0^{f}_{B}	0^{f}_{B}	0^{f}_{B}
Cladosporium cladosporioides	3^{d}_{AB}	0^{h}_{C}	0^{g}_{C}	0^{f}_{C}	2^{e}_{B}	0^{f}_{C}	4^{e}_{A}
Cladosporium herbarum	3^{d}_{BC}	2^{fg}_{C}	4^{e}_{AB}	3^{e}_{BC}	5^{d}_{A}	4^{d}_{AB}	3^{e}_{BC}
Drechslera avenae	0^{f}_{D}	9^{d}_{AB}	0^{g}_{D}	4^{e}_{C}	4^{d}_{C}	10^{b}_{A}	8^{c}_{B}
Epicoccum nigrum	5^{c}_{F}	16^{b}_{B}	10^{c}_{D}	12^{c}_{C}	22^{b}_{A}	8^{c}_{E}	6^{d}_{F}
Fusarium avenaceum	2^{de}_{C}	3^{f}_{BC}	2^{f}_{C}	8^{d}_{A}	4^{d}_{B}	2^{e}_{C}	0^{f}_{D}
Fusarium culmorum	0^{f}_{F}	13^{c}_{A}	2^{f}_{E}	7^{d}_{C}	0^{f}_{F}	10^{b}_{B}	4^{e}_{D}
Fusarium equiseti	1^{ef}_{B}	0^{h}_{C}	0^{g}_{C}	0^{f}_{C}	0^{f}_{C}	7^{c}_{A}	0^{f}_{C}
Fusarium graminearum	12^{b}_{C}	6^{e}_{F}	22^{b}_{B}	25^{b}_{A}	10^{c}_{D}	8^{c}_{E}	22^{b}_{B}
Fusarium oxysporum	1^{ef}_{A}	0^{h}_{B}	1^{fg}_{A}	0^{f}_{B}	0^{f}_{B}	0^{f}_{B}	0^{f}_{B}
Mucor mucedo	1^{ef}_{A}	0^{h}_{B}	1^{fg}_{A}	0^{f}_{B}	0^{f}_{B}	0^{f}_{B}	0^{f}_{B}
Penicillium chrysogenum	0^{f}_{D}	2^{fg}_{C}	6^{d}_{A}	0^{f}_{D}	0^{f}_{D}	0^{f}_{D}	4^{e}_{B}
Rhizopus stolonifer	3^{d}_{B}	1^{gh}_{CD}	2^{f}_{BC}	0^{f}_{D}	0^{f}_{D}	5^{d}_{A}	3^{e}_{B}
Sclerotinia sclerotiorum	1^{ef}_{A}	0^{h}_{B}	0^{g}_{B}	0^{f}_{B}	0^{f}_{B}	0^{f}_{B}	0^{f}_{B}
Sordaria fimicola	0^{f}_{B}	0^{h}_{B}	0^{g}_{B}	0^{f}_{B}	1^{ef}_{A}	0^{f}_{B}	0^{f}_{B}

*Explanation as in Table 4.

Table 6. The average number of fungi isolated from disinfected grains of spring barley in 2011 (CFU per 100 grains).

Fungi species	Abbreviation of object						
	Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Alternaria alternata	62 $^{a}_{A}$*	34 $^{a}_{F}$	58 $^{a}_{C}$	36 $^{a}_{E}$	28 $^{a}_{G}$	60 $^{a}_{B}$	54 $^{a}_{D}$
Botrytis cinerea	0 $^{f}_{B}$	0 $^{f}_{B}$	0 $^{d}_{B}$	0 $^{e}_{B}$	0 $^{g}_{B}$	0 $^{f}_{B}$	4 $^{d}_{A}$
Cladosporium cladosporioides	0 $^{f}_{C}$	0 $^{f}_{C}$	0 $^{d}_{C}$	4 $^{c}_{A}$	0 $^{g}_{C}$	2 $^{e}_{B}$	2 $^{e}_{B}$
Cladosporium herbarum	0 $^{f}_{B}$	0 $^{f}_{B}$	0 $^{d}_{B}$	2 $^{d}_{A}$	0 $^{g}_{B}$	0 $^{f}_{B}$	0 $^{f}_{B}$
Epicoccum nigrum	0 $^{f}_{C}$	2 $^{e}_{B}$	0 $^{d}_{C}$	0 $^{e}_{C}$	0 $^{g}_{C}$	0 $^{f}_{C}$	18 $^{b}_{A}$
Fusarium avenaceum	2 $^{e}_{D}$	6 $^{c}_{B}$	2 $^{c}_{D}$	0 $^{e}_{E}$	10 $^{c}_{A}$	4 $^{d}_{C}$	0 $^{f}_{E}$
Fusarium culmorum	8 $^{b}_{A}$	2 $^{e}_{C}$	2 $^{c}_{C}$	0 $^{e}_{D}$	0 $^{g}_{D}$	8 $^{c}_{A}$	6 $^{c}_{B}$
Fusarium equiseti	0 $^{f}_{D}$	6 $^{c}_{B}$	0 $^{d}_{D}$	4 $^{c}_{C}$	8 $^{d}_{A}$	4 $^{d}_{C}$	4 $^{d}_{C}$
Fusarium graminearum	6 $^{c}_{C}$	4 $^{d}_{D}$	12 $^{b}_{A}$	8 $^{b}_{B}$	6 $^{e}_{C}$	12 $^{b}_{A}$	0 $^{f}_{E}$
Fusarium oxysporum	0 $^{f}_{C}$	2 $^{e}_{B}$	2 $^{c}_{B}$	0 $^{e}_{C}$	0 $^{g}_{C}$	4 $^{d}_{A}$	0 $^{f}_{C}$
Mucor mucedo	0 $^{f}_{C}$	0 $^{f}_{C}$	0 $^{d}_{C}$	0 $^{e}_{C}$	16 $^{b}_{A}$	2 $^{e}_{B}$	0 $^{f}_{C}$
Rhizopus stolonifer	0 $^{f}_{B}$	0 $^{f}_{B}$	0 $^{d}_{B}$	0 $^{e}_{B}$	2 $^{f}_{A}$	0 $^{f}_{B}$	0 $^{f}_{B}$
Sordaria fimicola	4 $^{d}_{B}$	8 $^{b}_{A}$	0 $^{d}_{C}$	0 $^{e}_{C}$	0 $^{g}_{C}$	0 $^{f}_{C}$	0 $^{f}_{C}$

*Explanation as in Table 4.

Table 7. The average number of fungi isolated from non-disinfected grains of spring barley in 2011 (CFU per 100 grains).

Fungi species	Abbreviation of object						
	Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Alternaria alternata	82 $^{a}_{A}$*	72 $^{a}_{B}$	70 $^{a}_{C}$	58 $^{a}_{F}$	70 $^{a}_{C}$	64 $^{a}_{D}$	62 $^{a}_{E}$
Botrytis cinerea	0 $^{g}_{B}$	0 $^{f}_{B}$	0 $^{e}_{B}$	0 $^{e}_{B}$	0 $^{f}_{B}$	0 $^{f}_{B}$	2 $^{d}_{A}$
Cladosporium cladosporioides	4 $^{e}_{A}$	0 $^{f}_{B}$	0 $^{e}_{B}$	0 $^{e}_{B}$	0 $^{f}_{B}$	0 $^{f}_{B}$	0 $^{e}_{B}$
Cladosporium herbarum	2 $^{f}_{A}$	0 $^{f}_{B}$	0 $^{e}_{B}$	0 $^{e}_{B}$	0 $^{f}_{B}$	0 $^{f}_{B}$	0 $^{e}_{B}$
Epicoccum nigrum	0 $^{g}_{F}$	8 $^{c}_{B}$	6 $^{c}_{C}$	5 $^{d}_{CD}$	4 $^{d}_{D}$	2 $^{e}_{E}$	40 $^{b}_{A}$
Fusarium avenaceum	8 $^{c}_{B}$	8 $^{c}_{B}$	6 $^{c}_{C}$	6 $^{d}_{C}$	14 $^{c}_{A}$	0 $^{f}_{D}$	8 $^{c}_{B}$
Fusarium culmorum	0 $^{g}_{E}$	18 $^{b}_{B}$	0 $^{e}_{E}$	8 $^{c}_{C}$	4 $^{d}_{D}$	20 $^{c}_{A}$	0 $^{e}_{E}$
Fusarium equiseti	0 $^{g}_{C}$	0 $^{f}_{C}$	0 $^{e}_{C}$	0 $^{e}_{C}$	0 $^{f}_{C}$	4 $^{d}_{A}$	2 $^{d}_{B}$
Fusarium graminearum	16 $^{b}_{D}$	2 $^{e}_{E}$	38 $^{b}_{A}$	32 $^{b}_{B}$	16 $^{b}_{D}$	22 $^{b}_{C}$	2 $^{d}_{E}$
Fusarium oxysporum	0 $^{g}_{B}$	0 $^{f}_{B}$	0 $^{e}_{B}$	0 $^{e}_{B}$	2 $^{e}_{A}$	0 $^{f}_{B}$	0 $^{e}_{B}$
Mucor mucedo	0 $^{g}_{B}$	0 $^{f}_{B}$	0 $^{e}_{B}$	0 $^{e}_{B}$	2 $^{e}_{A}$	0 $^{f}_{B}$	0 $^{e}_{B}$
Penicillium chrysogenum	0 $^{g}_{B}$	2 $^{e}_{A}$	2 $^{d}_{A}$	0 $^{e}_{B}$	0 $^{f}_{B}$	0 $^{f}_{B}$	0 $^{e}_{B}$
Rhizopus stolonifer	6 $^{d}_{B}$	0 $^{f}_{D}$	0 $^{e}_{D}$	8 $^{c}_{A}$	2 $^{e}_{C}$	2 $^{e}_{C}$	0 $^{e}_{D}$
Sordaria fimicola	0 $^{g}_{B}$	4 $^{d}_{A}$	0 $^{e}_{B}$	0 $^{e}_{B}$	0 $^{f}_{B}$	0 $^{f}_{B}$	0 $^{e}_{B}$

*Explanation as in Table 4.

cereal grains by fungi. The fungus most frequently isolated in our research was *Alternaria alternata*. This finding conforms to the the reports by Roháčik and Hudec [18], who studied fungal infection of barley grains, although they did so in a pure culture of barley. Other authors also report that *Alternaria* spp. are the fungi most often isolated from barley grains [21, 22].

Alternaria spp. are cosmopolitan, ubiquitous fungi and they are saprobic, endophytic, or pathogenic species. They can be found in soils, on plants, in food, animal feed, and in animals and in the atmosphere. Species of *Alternaria* are known as serious plant pathogens, causing major losses on a wide range of crops and bringing about post-harvest decay of many plant products [23]. Studies of the atmos-

Table 8. The average number of fungi isolated from disinfected grains of spring barley in 2012 (CFU per 100 grains).

Fungi species	Abbreviation of object						
	Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Alternaria alternata	61 $^{a}_{BC}$*	38 $^{a}_{F}$	62 $^{a}_{B}$	52 $^{a}_{E}$	60 $^{a}_{C}$	55 $^{a}_{D}$	68 $^{a}_{A}$
Cladosporium herbarum	0 $^{f}_{B}$	0 $^{f}_{B}$	0 $^{e}_{B}$	1 $^{f}_{A}$	0 g$_{B}$	0 g$_{B}$	0 $^{f}_{B}$
Drechslera avenae	10 $^{d}_{D}$	36 $^{b}_{A}$	8 $^{e}_{E}$	20 $^{b}_{B}$	8 $^{d}_{E}$	18 $^{b}_{C}$	6 $^{d}_{F}$
Epicoccum nigrum	2 $^{e}_{C}$	4 $^{d}_{B}$	2 $^{d}_{C}$	6 $^{d}_{A}$	4 $^{e}_{B}$	2 $^{f}_{C}$	2 $^{e}_{C}$
Fusarium avenaceum	0 $^{f}_{D}$	11 $^{c}_{A}$	2 $^{d}_{C}$	4 $^{e}_{B}$	0 g$_{D}$	0 g$_{D}$	0 $^{f}_{D}$
Fusarium culmorum	12 $^{c}_{A}$	0 $^{f}_{D}$	12 $^{b}_{A}$	6 $^{d}_{C}$	12 $^{c}_{A}$	12 $^{c}_{A}$	8 $^{c}_{B}$
Fusarium equiseti	0 $^{f}_{C}$	0 $^{f}_{C}$	0 $^{e}_{C}$	0 $^{f}_{C}$	2 $^{f}_{B}$	4 $^{e}_{A}$	0 $^{f}_{C}$
Fusarium graminearum	14 $^{b}_{B}$	10 $^{c}_{C}$	8 $^{c}_{D}$	8 $^{c}_{D}$	16 $^{b}_{A}$	8 $^{d}_{D}$	10 $^{b}_{C}$
Fusarium oxysporum	0 $^{f}_{B}$	0 $^{f}_{B}$	2 $^{d}_{A}$	0 $^{f}_{B}$	0 $^{g}_{B}$	0 $^{g}_{B}$	2 $^{e}_{A}$
Rhizopus stolonifer	0 $^{f}_{C}$	0 $^{f}_{C}$	2 $^{d}_{B}$	0 $^{f}_{C}$	0 $^{g}_{C}$	4 $^{e}_{A}$	2 $^{e}_{B}$
Sclerotinia sclerotiorum	0 $^{f}_{B}$	0 $^{f}_{B}$	0 $^{e}_{B}$	0 $^{f}_{B}$	2 $^{f}_{A}$	0 $^{g}_{B}$	0 $^{f}_{B}$
Sordaria fimicola	0 $^{f}_{B}$	2 $^{e}_{A}$	0 $^{e}_{B}$	0 $^{f}_{B}$	0 $^{g}_{B}$	0 $^{g}_{B}$	0 $^{f}_{B}$

*Explanation as in Table 4.

Table 9. The average number of fungi isolated from non-disinfected grains of spring barley in 2012 (CFU per 100 grains).

Fungi species	Abbreviation of object						
	Control	H-3	P-1-0	P-0-1	P-1-1	P-2-1	P-2-2
Alternaria alternata	54 $^{a}_{A}$*	34 $^{a}_{D}$	38 $^{a}_{C}$	32 $^{a}_{E}$	30 $^{b}_{F}$	30 $^{b}_{F}$	46 $^{a}_{B}$
Cladosporium herbarum	0 $^{g}_{C}$	0 $^{f}_{C}$	12 $^{c}_{B}$	0 $^{g}_{C}$	14 $^{d}_{A}$	0 $^{g}_{C}$	0 $^{f}_{C}$
Drechslera avenae	8 $^{d}_{F}$	34 $^{a}_{B}$	10 $^{d}_{E}$	30 $^{b}_{C}$	26 $^{c}_{D}$	40 $^{a}_{A}$	30 $^{b}_{C}$
Epicoccum nigrum	12 $^{c}_{E}$	24 $^{b}_{B}$	20 $^{b}_{C}$	16 $^{c}_{D}$	36 $^{a}_{A}$	20 $^{c}_{C}$	12$^{c}_{E}$
Fusarium avenaceum	0 $^{g}_{C}$	0 $^{f}_{C}$	0 $^{h}_{C}$	12 $^{e}_{A}$	0 $^{g}_{C}$	0 $^{g}_{C}$	6 $^{d}_{B}$
Fusarium culmorum	2 $^{f}_{D}$	8 $^{c}_{A}$	6 $^{f}_{B}$	6 $^{f}_{B}$	0 $^{g}_{E}$	6 $^{d}_{B}$	4 $^{e}_{C}$
Fusarium equiseti	4 $^{e}_{A}$	0 $^{f}_{B}$	0 $^{h}_{B}$	0 $^{g}_{B}$	4 $^{f}_{A}$	4 $^{e}_{A}$	0 $^{f}_{B}$
Fusarium graminearum	18 $^{b}_{A}$	4 $^{d}_{E}$	2 $^{g}_{F}$	14 $^{d}_{B}$	12 $^{e}_{C}$	6 $^{d}_{D}$	4 $^{e}_{E}$
Fusarium oxysporum	0 $^{g}_{B}$	2 $^{e}_{A}$	0 $^{h}_{B}$	0 $^{g}_{B}$	0 $^{g}_{B}$	2 $^{f}_{A}$	0 $^{f}_{B}$
Penicillium chrysogenum	0 $^{g}_{C}$	4 $^{d}_{B}$	8 $^{e}_{A}$	0 $^{g}_{C}$	0 $^{g}_{C}$	0 $^{g}_{C}$	4 $^{e}_{C}$
Rhizopus stolonifer	3 $^{ef}_{CD}$	4 $^{d}_{C}$	8 $^{e}_{A}$	0 $^{g}_{E}$	0 $^{g}_{E}$	2 $^{f}_{D}$	6 $^{d}_{B}$

*Explanation as in Table 4.

pheric air of various regions of Europe show that the spores of *Alternaria* spp. and *Cladosporium* spp. dominate in the atmosphere and their peak season is in the summer [24]. *Alternaria* ssp., along with other fungi including *Cladosporium* ssp., can cause Black Point (BP). This disease can affect all cereal species, although wheat and barley are most commonly affected. These same fungi can cause discolouration of oats. Symptoms are only visible after harvest. Affected grain shows darkening of the outer coat, particularly at the embryo end of the grain. The disease reduces the commercial grade of barley, causing economic losses to producers. BP incidence exceeding 10% results in downgrading of the grain and can have serious implications for the quality of milling wheat, barley, and oats for processing. The discoloration of the grain can lead to poor flour quality and brown color, and may result in yield rejection on the basis of discolored grains [25].

Penicilium chrysogenum was isolated in all research years, but only from non-disinfected grains. Pląskowska [20] also reports that *P. chrysogenum* (syn. *P. notatum*) is only isolated from non-disinfected grains, because this fungi is generally a saprophyte.

Presented results show that mixtures of cereals and legumes are less exposed to grain infestation by fungi in terms of their quantity and species composition, as compared to pure stands. This conforms to the results reported by other authors [20, 26, 27].

A number of authors note that mechanical treatments may be seen as an alternative to chemical weed control [10-12]. In particular, 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 and thereby yield reduction is prevented [28]. In our research, the variant of weed control that proved the best in terms of mycological quality of the cereals and legumes was P-0-1 (one passage of spring-tine harrow at full tillering stage of barley). According to literature, this procedure also has a beneficial effect on reducing weed infestation, while at the same time inducing the least possible infection level of barley grains by the fungi.

Mechanical weed control is particularly important for the EC countries, as on the 24th November 2009 the European Parliament and European Council had imposed an obligation upon all member states to follow, since 2014, the principles of integrated pest management [29].

Conclusions

1. In mixed cultures of barley and peas, the mechanical weed control of appropriately selected intensity does not increase colonization of barley grains by pathogenic fungi, as compared to the control by herbicides.
2. The best variant of mechanical weed control for the cereal-legume mixture in terms of mycological quality of barley grain was one passage of spring-tine harrow at full tillering stage of barley.
3. 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.

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