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The variability of the occurrence of *Fusarium culmorum* in winter wheat grain in relation to climatic conditions and cultivation methods

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Abstract

Wheat grains are colonized by a wide range of different saprophytic and pathogenic fungi. Infestation of mycotoxigenic species such as *Fusarium* spp. raises concerns about the safety of the grains for human and animal consumption. The colonization depends upon various environmental and genetic factors. The aim of the study was to analyse the variability of the incidence of the *Fusarium culmorum* fungus in wheat grain in relation to years of experiments – environmental conditions, the height of the preceding crop stubble, tillage system and cultivar. The study was conducted in the years 2009–2011, at the Experimental Farm of the Institute of Soil Science and Plant Cultivation in the Lower Silesia Region. The preceding crop was winter wheat. The study included the following experimental factors: factor I – height of preceding crop stubble: a) low stubble, b) high stubble; factor II – soil tillage systems: a) direct sowing, b) ploughless tillage, c) plough tillage; factor III – winter wheat cultivars: a) ‘Mewa’, b) ‘Rapsodia’, c) ‘Legenda’. Mycological analyses were conducted on the grain from the cultivars harvested at the stage of full maturity. It is noteworthy that in the ploughless tillage system the fungal infestation observed in caryopsis under study was higher than that in the plough tillage system. Within the various tillage systems, the high stubble variant reduced the level of infection of wheat grain with the fungus *F. culmorum*. The wheat cultivars were characterised by varied resistance to grain colonisation by *F. culmorum* depending on the soil tillage system.

Key words: environment, *Fusarium culmorum*, stubble height, tillage system.

Introduction

In many regions in Poland as well as in Western Europe, the relative proportion of cereals in the crop structure has already reached a dangerous level of 70%. The common practice of cereal crop rotations or monoculture in the cultivation of winter wheat and rye contribute to the development of fungal diseases. In recent years, fusarium head blight has become a common disease of wheat all over the world (Klix et al., 2008; Xu et al., 2008; Summerell et al., 2010). The high diversity within the *Fusarium* genus makes it difficult to control fungi on wheat plantations (Infantino et al., 2012). At present, the most frequently recorded species of the fungus in Europe include *F. culmorum* (W.G.Sm) Sacc., *Gibberella zeae* (Schwein.) Petch, *Gibberella avenacea* R.J. Cook, *F. poae* (Peck.) Wollenw. and *Monographella nivalis* (Schaffnit) E. Müll. Fungi belonging to the genus *Fusarium* can produce various mycotoxins, classified as trichothecenes, such as deoxynivalenol (DON), nivalenol (NIV), toxin T-2 and toxin HT-2, as well as other toxins such as zearalenone and fumonisins B1 and B2 (Kammoun et al., 2010; Popowski, Celar, 2013).

Many research reports emphasise the fact that *G. zeae* and *F. culmorum* are among the most dangerous pathogenic fungi in Europe (Maiorano et al., 2008; Blandino et al., 2010). *F. poae*, which is becoming more and more widespread in Poland, is less pathogenic than either *F. culmorum* or *G. zeae*. Research has shown that *G. zeae* requires higher temperatures to develop than *F. culmorum* (Häberle et al., 2007). In northern Europe (Scandinavia, Finland and northwestern Russia) and Asia (Siberia and the Russian Far East) the most common *Fusarium* species responsible for Fusarium head blight (FHB) are the *F. avenaceum* (currently name *G. avenacea*) / *arthrosporioides* / *tricinctum*, *F. graminearum* / *culmorum* / *cerealis* and *F. poae* / *sporotrichioides* / *langsethiae* species groups based on morphology (Yli-Mattila, 2010). Studies conducted during the last decade in Holland and Germany indicate an increasing dominance of *G. zeae* in the areas where so far the dominant species has been *F. culmorum* (Wagacha, Muthomi, 2007; Xu, Nicholson, 2009). The trend may result from the genetic changes in populations of that fungal species (Bateman et al., 2007).

Airborne ascospores of *G. zeae* may infect cereals faster than the conidia of *F. culmorum*, which rely on rain droplets for dispersal.

The primary factor affecting the rate of infection of the after-crop is the post-harvest residue (Maiorano et al., 2008). Therefore, the preceding crop and the soil tillage system play an important role in controlling the infection of ears of cereals by the fungi of *Fusarium* and *Gibberella* genus. In recent years, ploughless tillage has become more and more popular, owing to economic and environmental reasons. The considerable degradation of soil in certain regions of the world, caused by intensive soil tillage, makes it necessary to develop new technologies favouring soil conservation on the one hand, and biodiversity recreating natural biocenoses in areas of intensive agricultural production on the other. Following a previous crop of maize, plough tillage may significantly reduce the occurrence of *F. culmorum*, whereas the application of various forms of ploughless tillage increases the threat of wheat infection by fungi (Blandino et al., 2010). The enhanced biological activity of the upper soil horizons under the conditions of conservation tillage may nonetheless limit the number of pathogenic fungi inoculi (Lenc et al., 2009).

However, the application of various forms of simplified or reduced tillage, and especially of direct sowing, creates additional problems related to an

excessive amount of straw remaining on the soil surface. High stubble has significant advantages in the case of direct sowing. Sowing with drills yields good results as long as the straw is laid along the path of the drill and there is no accumulation of post-harvest residue in the sowing groove. Notwithstanding, high stubble increases the risk of infection by fungi, particularly those belonging to the genus *Fusarium*. This in turn may contribute to a decreased crop yield. Unfortunately, the data concerning the influence of stubble height on the infection of secondary crops by *Fusarium* fungi remains insufficient.

The aim of the study was to analyse the variability of the occurrence of the *F. culmorum* fungus in winter wheat grain in relation to environmental conditions – years of experiments, height of the previous crop stubble, tillage system and plant cultivars.

Material and methods

Environment and meteorological conditions.

The study was conducted in the years 2009–2011, at the Experimental Farm of the Institute of Soil Science and Plant Cultivation in the Lower Silesia Region (Table 1). The experiment was set up in a split-split-plot design in four replicates, on *Stagnic Cutanic Albeluvisol*: loamy sand overlying loam. The following crop rotation was employed: spring wheat → winter oilseed rape → winter wheat (preceding crop) → winter wheat (in experiment).

Table 1. Precipitation and mean air temperature during the vegetative period

Years	March	April	May	June	July	August	March–August
Sum of precipitation mm							
2009	61	25	66	181	145	50	528
2010	44	51	137	49	124	83	488
2011	26	26	41	64	112	77	346
Average air temperature °C							
2009	4	11	14	16	20	19	14
2010	3	9	12	18	21	19	14
2011	4	11	14	19	18	19	14

Plant material. The study included the following experimental factors: factor I – height of preceding crop stubble: a) low stubble (10 cm), b) high stubble (40 cm); factor II – soil tillage systems: a) direct sowing, b) ploughless tillage, c) plough tillage (Table 2); factor III – winter wheat cultivars: a) ‘Mewa’, b) ‘Rapsodia’, c) ‘Legenda’. The examined cultivars featured the same time of maturity and they reached dough stage after 195–197 days starting from the 1st January of analysed year.

In the experiments involving both high and low stubble, the remaining post-harvest residue was shredded into chaff and spread evenly over the field. The surface area of an experimental plot was 15 m². After harvesting the preceding crop, the plots with high and low stubble were treated with Roundup herbicide in the first ten days of August. In the high stubble treatment, winter wheat was cut at a height of 40 cm, while low stubble was 10 cm tall.

Table 2. Tillage systems

Tillage system	Cultivation details
Plough tillage	Post-harvest cultivation – cultivator at 15 cm + string roller Basic land preparation – ploughing to a depth of 25 cm + harrowing Pre-plant tillage – cultivator + string roller
Ploughless tillage	Post-harvest cultivation – cultivator at 15 cm + string roller Pre-plant tillage – cultivator + string roller
Direct sowing	Direct sowing – Great Plains drill with a double disc drilling unit and a cultivating disc

Grain infection. At the milk-dough stage each of the experimental treatments was evaluated according to the frequency of leaves and caryopsis disease. Mycological analyses were performed on the grain of the cultivars harvested at the stage of full maturity. The observations were conducted immediately after the harvest (grain moisture content <12%). In each year of the study, mycological analyses were carried out on 300 wheat grains sampled from each plot of the experiments. The grains were rinsed first with running water and then three more times with distilled water. The material prepared in this way was placed on Petri dishes containing PDA (potato-dextrose agar) medium, previously acidified with citric acid to the pH level of 5.5. The grains were then incubated in a thermostat chamber at 23°C for 7–8 days. The appearing fungal colonies were transplanted to the PDA slants and identified to the species level with the help of monograph keys (Pitt, Hocking, 2009; Watanabe, 2011). The grain samples from particular experiments showed varied numbers of isolated fungal colonies of *Fusarium culmorum*. These numbers were subjected to a statistical analysis.

In order to perform mycological analysis, grain samples were collected from each experimental plot, although *Fusarium* spp. infection is not usually regular on the whole field. Not infected plots (with zero values) always appear in this kind of experiment. Nevertheless, these values cannot be considered as missing data. Therefore, results of this study were subjected to log-linear analysis that enabled analysis of the results, despite the occurrence of occasional zero values in some replications.

Statistical analysis. In biological sciences, the method most frequently used to test the statistical significance of the effect of various factors on the variability of the qualitative trait under study is the log-linear analysis. Under the procedure, all significant deviations of the observed values from the expected ones indicate the existence of a relationship (interaction) between the analysed variables. After a logarithmic transformation of the expected values, the model assumes a linear form that, at its simplest, can be represented by the formula:

$$\ln(E_{ij}) = M. + \lambda_i^X + \lambda_j^Y + \lambda_{ij}^{XY},$$

where E_{ij} are the expected values, $M.$ is the general mean based on equal values in each cell, λ_i^X – the effect of the i^{th} value of variable X ; λ_j^Y – the effect of the j^{th} value of variable Y , λ_{ij}^{XY} – the effect of the interaction of the i^{th} value of variable X and the j^{th} value of variable Y .

The log-linear model enables the validation of null hypothesis, which assumes a lack of interaction between two or more of the analysed variables. It also allows, after rejecting all insignificant interactions, an assessment of the effect of particular factors on the variability of the population under study. The interactions between the widespread occurrence of *F. culmorum* on the wheat grain and the wheat cultivars, soil tillage systems, stubble height as well as the duration of the research were demonstrated by means of the log-linear analysis. The analysis of correspondence was applied in order to analyse the variability of the isolated numbers of *F. culmorum* in relation to the wheat cultivars, soil tillage systems and stubble height. The canonical analysis of correspondence allows an assessment of the structure of relations between the cultivars and the remaining experimental factors under study.

Results

Regardless of the soil tillage system and the height of the stubble, the analysed wheat grain was infected by the following fungi species: *Alternaria alternata* (Fr.) Geissler, *Botrytis cinerea* (Preuss) Woudenberg & Crous, *Epicoccum nigrum* Link, *Fusarium culmorum* (W.G. Smith) Sacc., *Penicillium notatum* Westling and *Sclerotinia sclerotiorum* (Lib.) de Bary. Lower infestation was observed among *Gibberella avenacea*, *G. intricans* Wollenw., *Trichoderma harzianum* Rafii, *T. viridae* Pers. ex S.F. Gray and *Trichothecium roseum* Link ex Fr.

The statistics provided for the model with the second and third order interactions showed significant values (Table 3). Therefore, the hypothesis regarding the lack of dependence of the *F. culmorum* populations on the duration of the research, soil tillage systems, stubble heights and wheat cultivars should be rejected

Table 3. Tests of partial and marginal associations and interactions between the investigated factors

Effect	Degrees of freedom	Test χ^2 partial association	Significance level (p)	Test χ^2 marginal association	Significance level (p)
Years (1)	2	1360.70	<0.001	1360.70	<0.001
Stubble height (2)	1	91.75	<0.001	91.75	<0.001
Tillage systems (3)	2	220.20	<0.001	220.20	<0.001
Cultivars (4)	2	61.64	<0.001	61.64	<0.001
1 × 2	2	326.26	<0.001	328.57	<0.001
1 × 3	4	333.31	<0.001	330.76	<0.001
1 × 4	4	32.89	<0.001	33.69	<0.001
2 × 3	2	45.94	<0.001	35.28	<0.001
2 × 4	2	37.59	<0.001	30.29	<0.001
3 × 4	4	149.91	<0.001	137.75	<0.001
1 × 2 × 3	4	138.15	<0.001	83.44	<0.001
1 × 2 × 4	4	82.06	<0.001	30.64	<0.001
1 × 3 × 4	8	124.26	<0.001	176.62	<0.001
2 × 3 × 4	4	107.82	<0.001	72.11	<0.001

at the level of $p < 0.01$. The inclusion of second and third order interactions in the model improved its level of fit, as indicated by the significant values of the Chi² test. The analysis showed a considerable variability of *F. culmorum* occurrence in the grain of wheat cultivars during the research. This indicates the importance of the effects of atmospheric conditions on the variability of the occurrence of the fungus under study. It was also found that the occurrence of *F. culmorum* strongly correlated with the height of the preceding crop stubble remaining in the field. The results of the log-linear analysis show a strong relationship between tillage systems and stubble

height. The varied height of the preceding crop stubble enhanced the impact of the soil tillage systems under study on the occurrence of *F. culmorum* on wheat grain obtained from the cultivars which were the subject of the research.

Plough tillage contributed to the reduction in the number of *F. culmorum*, while direct sowing seemed conducive to its development (Table 4). The cultivars displayed varied resistance to grain infection with *F. culmorum*. In high stubble conditions, the cv. 'Legenda' showed increased resistance to the *F. culmorum* fungus. The cv. 'Rapsodia' proved susceptible to the fungus.

Table 4. The number of *Fusarium culmorum* isolates in wheat grain in relation to the cultivar and cultivation methods (sum for 2009–2011)

Tillage systems	Number of <i>F. culmorum</i> isolates			Mean
	'Legenda'	'Rapsodia'	'Mewa'	
High stubble (40 cm) of preceding crop – examined cultivars				
Direct sowing	207	229	263	233.0
Simplified tillage	123	99	107	109.7
Plough tillage	68	208	119	131.7
LSD	54.8	107.2	110.4	87.4
Means	132.7	178.7	163.0	158.1
LSD for cultivars	42.4			
Low stubble (10 cm) of preceding crop – examined cultivars				
Direct sowing	248	299	305	284.0
Simplified tillage	225	221	197	214.3
Plough tillage	181	173	134	162.7
LSD	34.7	82.7	101.5	114.2
Means	218.0	231.0	212.0	220.3
LSD for cultivars	ns			

LSD – lowest significant difference, ns – no significant difference

Smaller differences in the number of *F. culmorum* isolates occurred in low stubble conditions. The data shown in Tables 3 and 5 indicate that in the years 2009 and 2010, during which the vegetative period was characterized by high levels of precipitation, the incidence of the fungal infection was much higher than in 2011, when the precipitation levels were much lower. Low rainfall appeared to significantly reduce the population of *F. culmorum*. In the years 2011 and 2010, low preceding crop stubble caused an increase in the number of *F. culmorum*. In 2009, however, the reverse was observed.

Table 5. The number of *Fusarium culmorum* isolates in wheat grain in relation to stubble height and year of study

Stubble height	Number of isolates			Total
	2009	2010	2011	
High	293	968	162	1423
Low	997	885	101	1983
Total	1290	1853	263	–

The aim of the second part of the study was to analyse the dependence of the *F. culmorum* fungus on the following experimental factors: duration, soil tillage systems, stubble height and cultivars. In order to accomplish this, the analysis of correspondence was applied. The graphs representing the cultivars' response to the analysed experimental factors are presented in a two-dimensional space in order to retain the complete information on the varied effects of the experimental factors on the population of *F. culmorum* (Fig.). By analysing the distribution of particular wheat cultivars in a two-dimensional space we can observe that each of the cultivars forms a separate single-element homogeneous group differing considerably with respect to the occurrence of *F. culmorum*, which, in turn, correlates with the duration of the research, tillage systems and height of stubble. This is indicated by the significant distances between the cultivars in the analysed biplot diagram. Drawing on the length of the vector, it may be concluded that the cultivars exhibited the greatest variability of infection by *F. culmorum* under the following conditions: high preceding crop stubble and plough tillage. The angles between the vectors indicate that in the case of

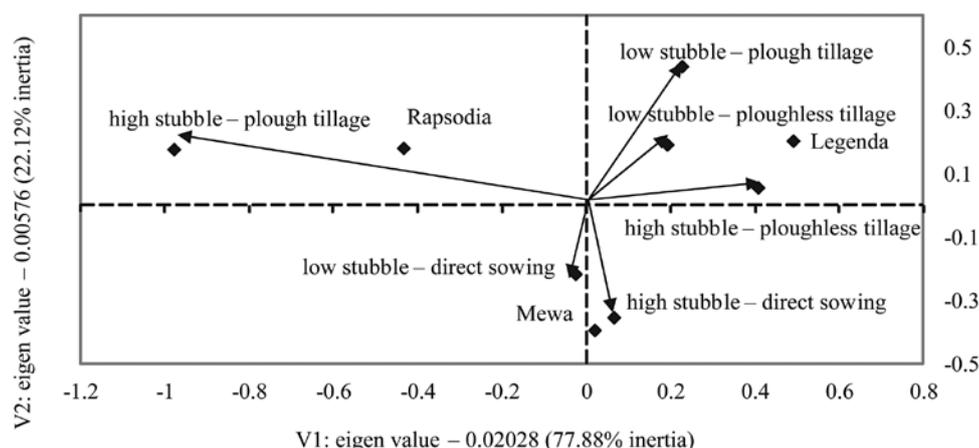


Figure. Biplot – the variability (V1, V2) of the susceptibility of wheat cultivars to infection by *Fusarium culmorum* in relation to the height of the preceding crop stubble, tillage systems and vegetative season

ploughless tillage or zero tillage the height of the stubble did not cause any considerable changes in the occurrence of *F. culmorum*. Conversely, the orthogonal projections of points representing the cultivars onto the vectors of variables show increased infection of cv. 'Rapsodia' with *F. culmorum* in plough tillage and high stubble systems. Direct sowing, regardless of stubble height, was conducive to the development of the fungus on the grain of cv. 'Mewa', while in ploughless tillage system the greatest number of *F. culmorum* was observed on the grain of cv. 'Legenda'.

Discussion

The results of the study indicate that in the years during which the vegetative season was characterised by high levels of rainfall and higher air temperatures in July, the occurrence of *F. culmorum* was higher than in 2011. Bateman et al. (2007) emphasise the significant impact of rainfall on the degree of wheat infection with the *F. culmorum* fungus. Rain droplets carrying the macroconidia of *F. culmorum* contribute to the infection of the plant's stem base and, subsequently, the head. The most important factors determining the degree of wheat ear infection are: temperature, air humidity, cultivar resistance and availability of water (Brennan et al., 2005; Popovski, Celar, 2013). Temperatures exceeding 25°C and high air humidity lasting 24 hours create the most favourable conditions for the infection of plants with *Fusarium* spp. The log-linear analysis confirmed a considerable variation of the susceptibility of the wheat cultivars to infection with the *F. culmorum* fungus. Fungicides used against fusarium head blight showed limited effectiveness (Płaskowska, Chrzanowska-Drożdż, 2009; Blandino et al., 2010; Gilbert, Haber, 2013). Therefore, the selection of cultivars characterised by an increased resistance to the most frequently occurring pathogenic fungi is the primary yield-forming factor. Studies conducted in Canada demonstrated a varied response of the fungi to soil tillage systems (Fernandez et al., 2008).

Winter oilseed rape preceding crop and direct sowing were responsible for a greater rate of infection with *Gibberella avenacea* observed on wheat ears compared to plough tillage, whereas the reverse was demonstrated in the case of *F. graminearum* (Fernandez et al., 2008). Varied responses of *G. avenacea*, *G. zeae* and *F. culmorum* to ploughless tillage systems were observed in Germany and Poland (Guo et al., 2010; Weber, Kita, 2010). These studies indicate that a one-year break in the cultivation of cereals increases the occurrence of *F. culmorum* in plough tillage systems, to the point that it becomes greater than when direct sowing is applied. Ploughed over to the depth of 20–25 cm, the thick-walled chlamydospores are not destroyed, as opposed to the ascospores of *G. zeae* and *G. avenacea* (conservation effect of plough tillage). The experiment showed that annual tillage following a preceding winter wheat crop did not cause an increase in the number of *F. culmorum* isolates. The small number of isolates of other species of fungi may be due to low rainfall in Lower Silesia. *F. culmorum* can develop and sporulate even when the water potential of soil is low. Studies conducted in Luxembourg have shown that in each year when there was a decrease in precipitation during the growing season, 90% of sick ears were infected by *F. culmorum*, and only 10% by *F. graminearum* (Giraud et al., 2010).

The subsequent ploughing brings the spores of the fungus back to the surface of the soil, where cereal cultivation creates optimum conditions for their growth and development. On the other hand, surface (ploughless) tillage, characterised by greater biological activity of the soil, may largely inhibit the growth of that fungus species. Temperature changes during winter also contribute to the destruction of the spore forms of *F. culmorum*. The study shows that the increased occurrence of *F. culmorum* in the case of direct sowing, as opposed to plough tillage, results from the fact that preceding winter wheat crop was applied before the planting of winter wheat cultivars.

On the other hand, in the crop rotation pattern winter wheat – preceding winter rape crop – winter wheat, a higher occurrence of *F. culmorum* is attributable to its preserving effect (Weber, Kita, 2010). However, the study in Lithuania showed no significant influence of tillage system on wheat infection by *Fusarium* fungi (Supronienė et al., 2012). The discrepancy in results of the investigated issue can be due to variable species composition of fungi population.

The development of *Fusarium* spp. can be successfully suppressed by antagonistic rhizospheric microorganisms, species of fungi belonging to the *Trichoderma* genus as well as *Pseudomonas* and gram-positive *Arthrobacter* bacteria (Schermer et al., 2013). Under laboratory conditions, the addition of two microbiological strains *P. fluorescens* MKB 100 and *Pseudomonas* spp. MKB 158 and 249, helped in containing head blight in seedlings caused by *F. culmorum* (Khan et al., 2006). In the cases of wheat – wheat rotation, phytosanitary mechanism is triggered off by all kinds of catch crops, especially fodder plants such as oilseed rape, white mustard and oilseed radish. Catch crops consisting of winter vetch, white clover or hybrid alfalfa may significantly reduce the incidence of *Fusarium* spp. in crops (Moszczyńska, Płaskowska, 2005). However, sowing wheat after a preceding crop of corn may induce considerable infection of grain by mycotoxins (DON, NIV and ZEA) of *Fusarium* fungi (Vogelgsang et al., 2011).

Conclusions

1. Various environmental conditions, especially rainfall, in the highest degree contributed to the diversity of the number of *Fusarium culmorum* in winter wheat grain in particular years of the experiment.

2. The number of *F. culmorum* isolates was greater under no-tillage conditions as compared to conventional tillage system.

3. Smaller infection of winter wheat grain by *F. culmorum* was observed at high stubble for each tillage system.

4. Under high stubble conditions, winter wheat cultivars featured diversified resistance to *F. culmorum* infection depending on tillage system. In contrast, low stubble did not differentiate wheat resistance.

5. The results of this research proved interactions between cultivar, stubble height and tillage system. The significance of these interactions points out diversified response of the cultivars with respect to their resistance to *Fusarium* fungi depending on stubble height and tillage system.

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***Fusarium culmorum* paplitimo ant žieminių kviečių grūdų kitimas priklausomai nuo klimato sąlygų ir auginimo būdų**

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Santrauka

Kviečių grūdus kolonizuoja įvairūs saprofitiniai ir patogeniniai grybai. Mikotoksigeniškų rūšių, pavyzdžiui, *Fusarium* spp., paplitimas kelia grėsmę grūdų, skirtų žmonėms ir gyvuliams, saugumui. Grūdų užterštumas grybais priklauso nuo įvairių aplinkos ir genetinių veiksnių. Tyrimo tikslas – išanalizuoti *Fusarium culmorum* grybų paplitimo ant kviečių grūdų kitimą, priklausomai nuo bandymo metų aplinkos sąlygų, priešsėlio ražienų aukščio, dirvožemio dirbimo būdo ir veislės. Tyrimas atliktas 2009–2011 m. Dirvožemio ir augalininkystės instituto, esančio Žemutinės Silezijos regione, bandymų ūkyje. Priešsėlis buvo žieminiai kviečiai. Bandymą sudarė šie veiksniai: I – priešsėlio ražienų aukštis: a) žema ražiena, b) aukšta ražiena; II – žemės dirbimo būdas: a) tiesioginė sėja, b) neariminis dirbimas, c) ariminis dirbimas; III – žieminių kviečių veislės: a) ‘Mewa’, b) ‘Rapsodia’, c) ‘Legenda’. Grūdų mikologinės analizės buvo atliktos juos nuėmus pilnos brandos tarpsniu. Nustatyta, kad taikant neariminį dirbimą grybų paplitimas tirtuose grūduose buvo didesnis nei dirvožemį ariant. Grūdų užsikrėtimo *F. culmorum* grybu lygis buvo mažesnis aukštų ražienų variante taikant visus žemės dirbimo būdus. Kviečių veislių atsparumas grūdų užkrėstumui *F. culmorum* grybais įvairavo priklausomai nuo žemės dirbimo būdo.

Reikšminiai žodžiai: aplinka, *Fusarium culmorum*, ražienų aukštis, žemės dirbimo sistema.

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