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Relationship of AUDPC values of tan spot and *Stagonospora glume blotch* with grain infection in winter and spring wheat

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Abstract

Field and laboratory experiments were performed at the Lithuanian Institute of Agriculture over the period 2003–2006. The tests involved three winter wheat cultivars ('Aron', 'Hereward' and 'Taurus') and three spring wheat cultivars ('Hena', 'Munk' and 'Nandu'). Different disease levels (untreated control, leaf spot disease severity 0–1.0%, 1.1–5.0%, 5.1–10.0% and 10.1–25.0%) were initiated by using leaf spot disease – infested wheat straw and fungicide applications. Leaf and ear disease assessments were done during wheat growing season. *Phaeosphaeria nodorum* and *Pyrenophora tritici-repentis* infection levels on grain were estimated in the laboratory under controlled conditions. A correlation analysis was done between tan spot AUDPC (area under the disease progress curve) values and grain infection by *P. tritici-repentis* and between the severity of *Stagonospora glume blotch* and *P. nodorum* infection on grain. Tan spot AUDPC values, in most cases, either did not correlate or correlated poorly (from 0.26 to 0.41) with grain infection by *P. tritici-repentis* in winter and spring wheat. The correlation analysis of *Stagonospora glume blotch* and *P. nodorum* infection on grain showed no relationship between these two parameters.

Key words: tan spot, *Stagonospora glume blotch*, grain infection, *Phaeosphaeria nodorum*, *Pyrenophora tritici-repentis*.

Introduction

Seed-borne diseases have been found to affect the growth and productivity of crop plants (Dawson, Bateman, 2001; Weber et al., 2001). A seedborne pathogen present externally or internally or associated with the seed as contaminant, may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Bateman, Kwasna, 1999; Khanzada et al., 2002).

Literature sources indicate over 100 fungi of various genera occurring on wheat grain, e.g. *Fusarium* spp., *Alternaria* spp., *Pyrenophora* spp., *Septoria* spp., *Epicoccum* spp., *Sporotrichum aurantiacum* and others (Weber et al., 1992; Shah et al., 1995; Fernandez et al., 1998; Mankevičienė et al., 2007).

Tan spot caused by *Pyrenophora tritici-repentis* (anamorph of *Drechslera tritici-repentis*) and

Stagonospora leaf blotch caused by *Phaeosphaeria nodorum* (anamorph of *Stagonospora nodorum*) are a constraint on yields of wheat (Bhathal et al., 2002). Both pathogens are usually called leaf spot diseases (Bhathal et al., 2002). No cultivar is immune, therefore means such as sowing seed that has been certified to contain low percentage of pathogens and that has been further reduced by seed treatment fungicides are effective against the pathogens. Also, application of foliar fungicides, crop rotation and debris management are used to prevent disease epidemic (Shah et al., 1995). On the other hand, seed treatment and foliar fungicide applications cause environmental concerns, therefore plant breeders, geneticists and phytopathologists develop new cultivars, resistant to leaf spot disease. Currently, wheat cultivars partly or moderately resistant to leaf spot diseases account for less than 20.0% of all the cultivars (Ruzgas, Liatukas, 2006; Singh et al., 2006; Singh, Hughes, 2006).

P. nodorum survives on the field debris and is able to infest all above-ground plant organs of wheat. Seed infection is common, and infected seeds can be an important source of primary inoculum for foliar epidemics (Milus, Chalkley, 1997). Other researchers noticed that in some regions winter wheat has a potential seedborne source of inoculum of *P. nodorum* but the level of infection varies widely between years in which the seed is produced and between specific lots (Shah, Bergstrom, 1993). Furthermore, seed-to-seedling transmission efficiencies close to 100% have been recorded in New York field plots sown with seed infected by *P. nodorum* (Shah et al., 1995).

The pathogen *P. tritici-repentis* can survive on diseased seed, infested crop residue, and overwintering grass hosts. Seeds are less important to initiate tan spot disease but it is known that seedlings initiated from infected seeds are poorly developed (Wakuliński et al., 1998).

In the present study, winter and spring wheat grain was analyzed for the incidence of *P. nodorum* and *P. tritici-repentis* fungi that cause *Stagonospora glume blotch* and tan spot, respectively. Attempts were made to find the relationship between AUDPC values of tan spot on wheat leaves and grain infection by *P. tritici-repentis* and severity of *Stagonospora glume blotch* and grain infection by *P. nodorum*.

Materials and methods

Field experiments. Field experiments were done in the experimental field of the Lithuanian Institute of Agriculture's Plant Pathology and Protection Department during 2003–2006. Experiments were set up in the crops of different cultivars of winter and spring wheat (*Triticum aestivum* L.). The tests involved winter wheat cultivars 'Aron', 'Taurus' and 'Hereward', and spring wheat cultivars 'Nandu', 'Hena', and 'Munk'. The cultivar 'Hereward' is characterized as susceptible to tan spot and *Stagonospora blotch*, cultivars 'Aron' and 'Taurus' are moderately susceptible to the diseases. Spring wheat cultivar 'Munk' is moderately susceptible to tan spot and *Stagonospora blotch*. Both cultivars 'Hena' and 'Nandu' are characterized as moderately resistant to the diseases.

Conventional soil tillage and drilling technology was applied. Treated seed of winter wheat was sown at a rate of 4.5 million, and spring wheat at 5.5 million viable seed ha⁻¹ at a sowing depth of 3–4 cm. Winter rape preceded winter wheat, except for the year 2004 when the pre-crop was pea. The pre-crop for spring wheat in 2003 and 2006 was spring rape and in 2004 and 2005 peas. Herbicides and in-

secticides were used to protect winter and spring wheat crops against weeds and pests.

Artificial inoculation was generated by covering post-emergence wheat plots with the straw infected by necrotrophic pathogens (approx. 400 g m²). Leaf spot diseases (tan spot, *Stagonospora* leaf blotch) and *Stagonospora glume blotch* level during the growing season was controlled by fungicide treatments. Plots (size 3 m²) were arranged using a randomized complete-block design with six replications.

The experimental design: 1) natural field infection, 2) maintained leaf spot diseases severity 0–1.0%, 3) maintained leaf spot diseases severity 1.1–5.0%, 4) maintained leaf spot diseases severity 5.1–10.0%, 5) maintained leaf spot diseases severity 10.1–25.0%.

Triazole fungicide treatments were made to maintain the desirable disease severity during the whole growing season. Fungicide Tilt, a.i. propiconazole 250 g l⁻¹, at a dose of 0.5 l ha⁻¹ was used. Experimental plots were monitored for the powdery mildew routinely, and morpholine fungicide Corbel (a.i. fenpropimorph 750 g l⁻¹), at a dose of 0.75 l ha⁻¹ was used according to the need to eliminate influence of the disease. Fungicides were applied at 400 l spray volume per ha using a knapsack-sprayer Hardi with a constant boom pressure of 2.5 bars.

Top three, two or one fully expanded and green leaves were scored for percent of leaf area affected by tan spot. The percentage of diseased area was visually assessed on 10 randomly selected main tillers from each plot (Anonymous..., 2004). The area under disease progress curves (AUDPC) was calculated using the percent estimates ratings in each cultivar (Introduction..., 1990).

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i),$$

where: y_i – disease severity at the i^{th} observation (in percentage),

t_i – time (days) of the i^{th} observation; n – total number of observations.

At wheat early dough maturity (GS 83–85), assessments of *Stagonospora glume blotch* were made on wheat ears (Anonymous..., 2004). Evaluation scales for both leaf and ear diseases are shown in Figure 1.

Laboratory analyses. For the laboratory test, 300 grains of each sample were used to determine infection level by both pathogens *Phaeosphaeria nodorum* and *Pyrenophora tritici-repentis*. The grains were not surface-disinfected prior to plating on moist filter paper in plastic Petri dishes 9 cm in diameter, 25 grain per dish.

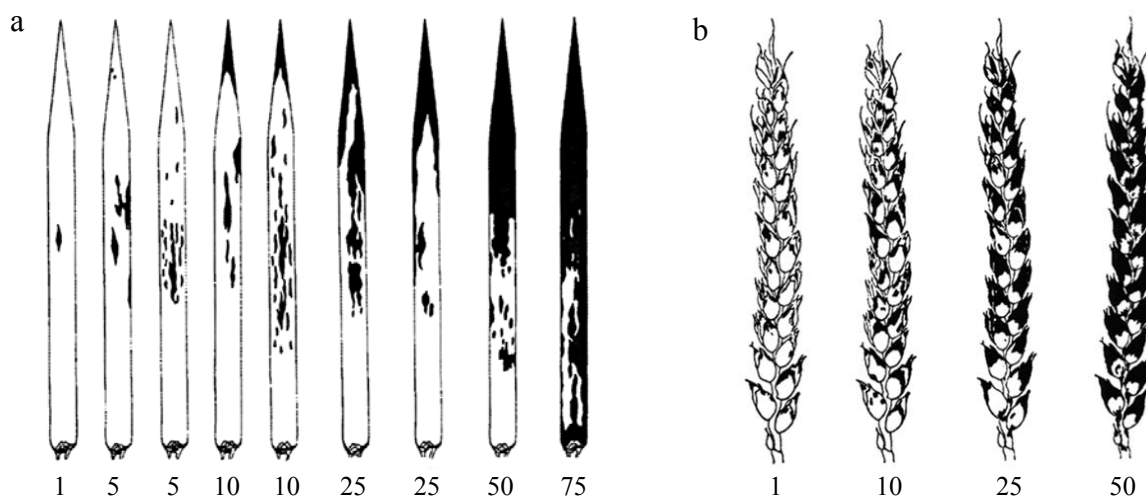


Figure 1. Percentage scales for tan spot evaluation on wheat leaves (a) and for Septoria glume blotch evaluation on wheat ears (b)

For the evaluation of *P. nodorum* infection on wheat grains, the dishes were incubated at 18–20°C for 72 hours, followed by 7–8 hours at –20–21°C to cause grain or seedling killing, and finally for 4–7 days at 20–23°C. After incubation, the grains were examined for the occurrence of *P. nodorum* by using NUV light (“Cole Parmer”, US). Under UV light, mycelium of fungus is detectable from typical green colour (Limonard, 1966; Kietreiber, 1984).

Evaluation of grain infection by *P. tritici-repentis* was done by osmotic method. Filter paper was moistened in sucrose solution (175–200 g of sucrose / 1 l distilled water) prior to placing in dishes. Petri dishes were incubated under mix light using fluorescent day-light and NUV tubes. Incubation period was 6–7 days under 16 h day and 8 h night regime at 24 ± 1°C. Under these conditions, sucrose solution inhibits the germination of wheat grain, light and temperature suppress mycelium growth and spore formation but this favours pigment formation. Pigments of *P. tritici-repentis* fungi under NUV light shine in brick-red colour (Joelsson, 1983).

Statistical analysis. The correlation analysis method was employed to estimate the interaction between the tan spot AUDPC values and *P. tritici-repentis* infected grain, severity of Stagonospora glume blotch and *P. nodorum* infected grain. The significance of data was estimated at 95 and 99% probability level. The statistical analyses were made using *Stat* software from the statistical data processing package *Selekcija* (Tarakanovas, Raudonius, 2003).

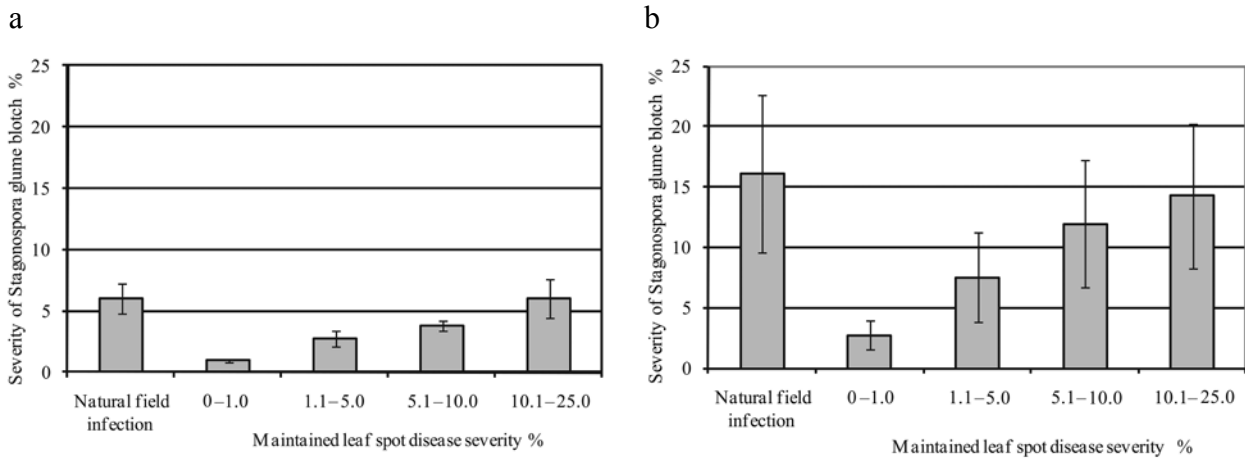
Results and discussion

Our research showed that winter wheat was less affected by Stagonospora glume blotch com-

pared to spring wheat (Fig. 2). Severity of Stagonospora glume blotch in winter wheat was by on average 2.7 times lower compared with that in spring wheat. This trend was recorded in all 4 years. Literature sources also indicate that Stagonospora glume blotch is more common in spring wheat than winter wheat (Zhuk, 2007).

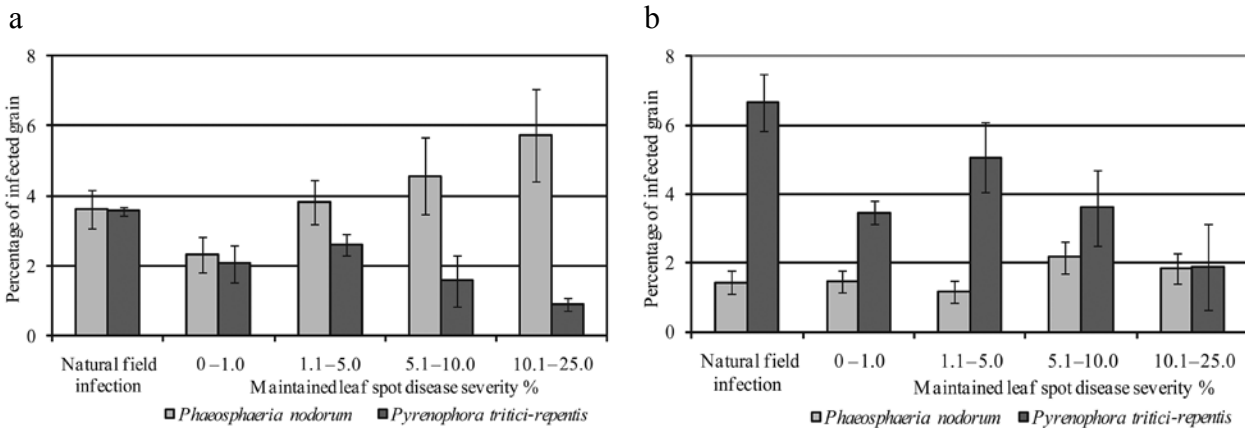
The lowest content of *P. nodorum* – infected grain in winter wheat was in the treatments where plants had been relatively healthy (maintained leaf spot diseases severity 0–1.0%). On average 2.3% of grain were infected in this treatment (Fig. 3 a). The percent of infected grain (3.8%) from the plots where the severity level of leaf spot diseases ranged from 1.0 to 5.0% was at the similar level (3.6% infected grain) to that from the plots with natural field infection of leaf spot diseases. Some authors suggest that fungi on grains or seeds compete with each other (Weber et al., 1992); therefore it is likely that low level of *P. nodorum* infection of unprotected from diseases winter wheat resulted from *P. nodorum* competition with other fungi. At the higher maintained leaf spot diseases severity (> 5.1%) the percent of infected grain varied between 4.6 and 5.7%. It was determined by Shah et al. (1995) that up to 10% seed infection was sufficient to cause severe epidemics in wheat crop.

Leaf spot diseases severely affected winter wheat cultivar ‘Hereward’, while cultivars ‘Taurus’ and ‘Aron’ were less affected by the diseases (Table 1). Comparison of spring wheat cultivars showed that ‘Nandu’ was more affected by leaf spot diseases, while ‘Hena’ and ‘Munk’ were found to be less affected.



Note. Each column represents the mean of 12–18 plots over 2003–2006 experimental years. Standard error bars are shown.

Figure 2. Severity of *Stagonospora glume blotch* in winter (a) and spring (b) wheat under different leaf spot diseases' infection level



Note. Each column represents the mean of 12–18 plots over 2003–2006 experimental years. Standard error bars are shown.

Figure 3. Percentage of grain infected by *P. nodorum* and *P. tritici-repentis* pathogens in winter (a) and spring (b) wheat under different leaf spot disease infection level

Table 1. AUDPC values of tan spot in winter and spring wheat
Average data of 2003–2006 experimental years

Cultivar	Natural field infection	AUDPC value			
		Maintained severity of leaf spot diseases			
		0–1.0%	1.1–5.0%	5.1–10.0%	10.1–25.0%
Winter wheat					
‘Aron’	377.6	155.7	232.5	462.0	502.9
‘Hereward’	566.5	257.5	296.8	406.0	644.9
‘Taurus’	360.4	160.1	245.3	343.7	530.2
Spring wheat					
‘Hena’	288.2	111.0	150.0	202.9	389.8
‘Nandu’	465.7	152.4	233.3	311.3	390.7
‘Munk’	401.3	136.2	197.7	328.9	414.3

Although results on *Stagonospora glume* blotch and grain infection by *P. nodorum* show some relationship, the correlation analysis revealed that these two parameters were mostly unrelated (Table 2). In winter wheat, correlation coefficients

varied from -0.27 to 0.37^* . Only in cultivar ‘Taurus’ the severity of *Stagonospora glume* blotch poorly but significantly correlated with the percent of grain infected by *P. nodorum* ($r = 0.37^{**}$).

Table 2. Correlation coefficients among AUDPC values of tan spot and grain infection by *P. tritici-repentis* (GPT) and between the severity of *Stagonospora glume* blotch (SGB) and grain infection by *P. nodorum* (GPN) in winter wheat cultivars

Dotnuva, 2003–2006

Trait	Correlation coefficient			
	2003	2004	2005	2006
	‘Aron’			
AUDPC x GPT	0.16	0.09	0.60**	0.20
SGB x GPN	0.19	-0.08	0.24	-0.36
	‘Hereward’			
AUDPC x GPT	0.20	0.31	0.06	0.29
SGB x GPN	0.11	0.14	-0.27	-0.09
	‘Taurus’			
AUDPC x GPT	0.29	0.33	0.11	0.26
SGB x GPN	0.37**	0.07	0.17	-0.09

** – significant at 99% probability level

The level of spring wheat grain infected by *P. nodorum* was nearly the same in all treatments (Fig. 3 b). Also, the correlation analysis revealed that percent of spring wheat grain infected by *P. nodorum* and severity of *Stagonospora glume* blotch were unrelated (Table 3). Only in one case with cultivar ‘Munk’ there was obtained poor but significant correlation ($r = 0.34^*$). The results were similar to those in winter wheat. Gaurilėikiene (2005) found that after the use of triazole fungicides epoxiconazole and propiconazole for *Stagonospora glume* blotch control, the incidence and severity of the diseases were significantly reduced two times out of

four but no significant effects were achieved in reducing grain number infected by *P. nodorum*. These results demonstrate that control of *Stagonospora* blotch in the field does not always result in significant reduction of grain infection. Fernandez et al. (1994) established that different resistance mechanisms exist in different host tissues. Also, Van Ginkel and Rajaram (1999) indicate the same reasons in the case with leaf and glume *Septoria*. Probably the findings of the mentioned authors can explain why there were either no relationships between *Stagonospora glume* blotch and *P. nodorum* infected grain or they were poor.

Table 3. Correlation coefficients among AUDPC values of tan spot and grain infection by *P. tritici-repentis* (GPT) and between the severity of *Stagonospora glume* blotch (SGB) and grain infection by *P. nodorum* (GPN) in spring wheat cultivars

Dotnuva, 2003–2006

Trait	Correlation coefficient			
	2003	2004	2005	2006
	‘Hena’			
AUDPC x GPT	–	-0.26	0.40	0.30
SGB x GPN	–	0.26	0.17	0.24
	‘Nandu’			
AUDPC x GPT	0.06	0.31	0.41*	0.03
SGB x GPN	0.24	-0.17	-0.02	-0.01
	‘Munk’			
AUDPC x GPT	-0.24	-0.08	0.11	0.08
SGB x GPN	0.34*	-0.18	-0.06	0.06

* – significant at 95% probability level

Some authors have concluded that *P. nodorum* frequently reproduces sexually and that ascospores are the dominant source of primary inoculum initiating foliar epidemics on wheat (Keller et al., 1997). However, according to the other findings of essentially the same population structure in seedborne populations mean that – from genetic perspective – seeds are an equally plausible source of primary inoculum as ascospores (Bennett et al., 2005). The seedborne inoculum hypothesis cannot be rejected because of strong epidemiological evidence (Luke et al., 1985; Shah et al., 1995).

In our trial, infection level of *P. nodorum* on winter wheat grain varied from 2.3 to 5.7%, on spring wheat from 1.2 to 2.2%. Shah et al. (1995) concluded that infection of seed by *P. nodorum* ensures not only the survival but also the distribution of the pathogen wherever the seed is sown. The control of seedborne *P. nodorum* to the levels of 0.05% or less infected seed will require integration of cultural and chemical control.

Our investigation showed that tan spot AUDPC values either did not correlate with grain infestation by *P. tritici-repentis* in winter wheat or the correlation coefficients were poor (varied from 0.26 to 0.33). Strong and significant correlation coefficient (0.60**) was detected only for cultivar 'Aron' during the year 2005.

Similar results were also obtained in spring wheat. Four correlation coefficients were poor (varied from 0.30 to 0.41) for the cultivars 'Hena' and 'Nandu'. In other cases tan spot AUDPC values did not correlate with grain infestation by *P. tritici-repentis*.

Poor relationship could be explained by available inoculum source from adjacent plots or other fields. Conidia of fungi *P. tritici-repentis* could be distributed by hundreds of meters (Shah et al., 2001). According to other authors, viable conidia could be disseminated over a distance of more than a few kilometres (Singh, Hughes, 2006).

In 2003, in cultivar 'Munk' and following year in cultivar 'Hena' the correlation coefficients were negative (–0.25 and –0.26, accordingly). Analysis of the causes of these results revealed that in this particular year seeds from untreated control were less infected by *P. tritici-repentis* compared with other treatments, where severity of leaf spot diseases was lower. The grains from plots with the higher tan spot infection were found to be less infected by *P. tritici-repentis* compared with the grains from plots where tan spot infection was low. It is likely that competing species of fungi on grain influenced the variable results in different years. It is known

that cereal grains during ripening are colonized by mixed mycobiota, which are influenced by different abiotic factors. The fungi interact with each other as they compete to utilize the available nutrients. Changes in the environment or other stress factors such as fungicide applications may also lead to one species having an advantage over competitors (Magan, Lacey, 2004; Magan, Aldred, 2007).

Conclusions

1. The severity of *Stagonospora glume blotch* in spring wheat was slightly higher than that in winter wheat; however the latter had higher content of *P. nodorum* – affected grain. With increasing severity of leaf spot diseases the content of *P. nodorum* – infected grain tended to increase.

2. *P. tritici-repentis* was more common on spring wheat than on winter wheat grain. However, the AUDPC values of tan spot in most cases did not have any significant effect on wheat grain infection by *P. tritici-repentis*.

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Kviečių dryžligės AUDPC reikšmių ir varpų septoriozės ryšiai su vasarinių bei žieminių kviečių grūdų užsikrėtimu patogeniniais grybais

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Santrauka

Lauko ir laboratoriniai tyrimai atlikti 2003–2006 m. Lietuvos žemdirbystės institute. Buvo pasirinkta po tris žieminių ('Aron', 'Hereward', 'Taurus') ir vasarinių kviečių ('Hena', 'Munk', 'Nandu') veisles. Naudojant lapų dėmėtligių pažeistus smulkintus šiaudus ir purškimą fungicidais, buvo dirbtinai palaikomas bandymo schemoje numatytas ligos intensyvumo lygis (natūrali lauko infekcija, lapų dėmėtligių intensyvumas 0–1,0, 1,1–5,0, 5,1–10,0 bei 10,1–25,0 %). Lapų ir varpų ligų apskaita atlikta kviečių vegetacijos metu. Nuėmus derlių grūdų ėminiai analizuoti laboratorijoje kontroliuojamose sąlygose. Koreliacinė analizė atlikta tarp kviečių dryžligės AUDPC reikšmių bei *Pyrenophora tritici-repentis* užkrėstų grūdų ir tarp varpų septoriozės intensyvumo bei *Phaeosphaeria nodorum* užkrėstų grūdų. Nustatyta, kad kviečių dryžligės AUDPC reikšmės su grūdų užsikrėtimu grybu *P. tritici-repentis* nekoreliavo arba koreliavo labai silpnai (koreliacijos koeficientas varijavo nuo 0,26 iki 0,41). Šios tendencijos buvo panašios ir žieminiuose, ir vasarinių kviečiuose. Nepavyko nustatyti esminio koreliacinio ryšio tarp varpų septoriozės ir grūdų užsikrėtimo grybu *P. nodorum* žieminiuose bei vasariniuose kviečiuose.

Reikšminiai žodžiai: kviečių dryžligė, varpų septoriozė, grūdų infekcija, *Phaeosphaeria nodorum*, *Pyrenophora tritici-repentis*.