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Winter wheat leaf blotches development depending on fungicide treatment and nitrogen level in two contrasting years

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Abstract. Tan spot (caused by *Pyrenophora tritici-repentis*) and Septoria tritici blotch (caused by *Zymoseptoria tritici*) are the most widespread winter wheat leaf diseases in Latvia. The aim of the present research was to clarify the development of leaf blotches on winter wheat depending on fungicide treatment schemes under four nitrogen rates. A two-factorial trial was conducted at the Research and Study farm "Pēterlauki" (Latvia) of Latvia University of Life Sciences and Technologies. For this study, data from the 2018/2019 and 2019/2020 growing seasons was used. Four schemes of fungicide application and an untreated variant, as well as four nitrogen rates (N120, N150, N180, and N210 kg ha^{-1}) were used. The total disease impact during the vegetation period was estimated by calculating the area under the disease progress curve (AUDPC). The severity of leaf blotches in winter wheat leaves differed significantly during both vegetation seasons. Tan spot was the dominant disease in 2019 (18.7% in untreated variant). The development of tan spot was reduced by fungicide treatment; however, only in 2019, the influence of fungicide was significant. Septoria tritici blotch was the dominant disease in 2020 (11.4% in untreated variant), and its development was decreased by fungicides. Nitrogen fertilizer rate had no significant effect on the development of Septoria tritici blotches. Yield harvested in 2020 were significantly higher than those in 2019 (on average 5.23 t ha^{-1} in 2019, 8.40 t ha^{-1} in 2020). The using of fungicides provided significant increase of yield but there were no significant differences among fungicide treatment schemes.

Key words: winter wheat, *Pyrenophora tritici-repentis, Zymoseptoria tritici*, values of AUDPC, control.

INTRODUCTION

Wheat is an economically important cereal crop throughout the world, including Latvia. In 2019, the total sown area of winter wheat was 381.5 thousand ha (30.9% from total area of sowings) with the average yield of 5.28 t ha^{-1} (Central Statistical Bureau of Latvia, 2019).

The occurrence of leaf blotches is one of the factors affecting winter wheat grain yields. Septoria tritici blotch (caused by *Zymoseptoria tritici*) and tan spot (caused by *Pyrenophora tritici-repentis*) are the most important winter wheat leaf diseases in Europe (Willocquet et al., 2021), but the severity of both diseases vary significantly among the years (Bankina et al., 2018; Švarta et al., 2020; Willocquet et al., 2021). In

Latvia, tan spot was first identified only in the first half of the 1990s, but in recent years, the disease has become dominant. The severity of Septoria tritici blotch has been significantly lower and has exceeded the severity of tan spot only in a few years (Bankina et al., 2018).

The main source of tan spot infection is wheat straw debris, where pseudothecia with ascospores develop (Cotuna et al., 2015). The spread of disease significantly increased in continuous wheat sowings and in fields under minimum tillage (El Jarroudi et al., 2013; Bankina et al., 2018). The time of the appearance of the first tan spot symptoms and the further development of disease depend on meteorological conditions (Bankina et al., 2018; Schierenbeck et al., 2019; Willocquet et al., 2021) and susceptibility of variety (Kremneva et al., 2021). In years with high humidity and high average air temperatures, the susceptibility response to the disease was observed in more varieties than in years with dry conditions (Kremneva et al., 2021).

In contrast, the development of Septoria tritici bloch mainly depends on meteorological conditions, and agronomic practice is less important (Kuzdralinsky et al., 2015; Bankina et al., 2018). Optimal conditions for the development of Septoria leaf blotch are: minimum temperatures of 8 °C and maximum temperatures between 15 °C and 25 °C, relative humidity higher than 80%, and long periods of leaf wetness.

Wheat diseases management in Europe is mainly based on the use of fungicides and resistant varieties (Willocquet et al., 2021). In general, one to three applications are used, depending on the severity of diseases, susceptibility of variety, and yield potential (Willocquet et al., 2021), but in Ireland - even four applications are used (Creissen et al., 2018). The results of five-year (2013–2017) field experiments in six European countries revealed that disease levels were affected by the level of fungicide use, by cultivar resistance, and by meteorological conditions (Willocquet et al., 2021). In most cases, multiple disease intensities were lower when fungicide use was determined according to local recommendations (for example, one fungicide application in Norway and Sweden, two applications in Belgium), compared to no or limited protection (Willocquet et al., 2021). Gomes et al. (2016) found that susceptible varieties (with high levels of infection coefficient) need two fungicide applications (at GS 34 and GS 47). In moderate resistant varieties (with low levels of infection coefficient), preventive fungicide treatment at GS 34 proves to be beneficial, but resistant varieties do not need a disease control. In turn, Willocquet et al. (2021) confirmed that differences between cultivars reduce as the level of fungicide protection increases.

Nitrogen application significantly increases the grain yields, but nitrogen fertilization may also influence the development of foliar disease due to larger canopies that provide more favourable conditions for pathogens infection and development. Castro et al. (2018) found that severities of Septoria tritici blotch and tan spot significantly decreased with the increase in N rate. In contrast, Jensen & Jørgensen (2016) established that higher N rates was probably a major reason for the increased severity of Septoria tritici blotch in sowings with high densities.

The aim of the present research was to clarify the development of leaf blotches on winter wheat depending on fungicide treatment schemes under four nitrogen rates.

MATERIALS AND METHODS

Two-factor field experiments were carried out at the Study and Research farm "Pēterlauki" of Latvia University of Life Sciences and Technologies (56º 30.658′ N and 23º 41.580′ E). For this study, data from the 2018/2019 and 2019/2020 growing seasons was used. One of the most popular cultivars in Latvia 'Skagen' was used in the trial.

Four schemes of fungicide application and an untreated variant (Table 1), as well as four nitrogen rates (N120 (80+40), N150 (80+70), N180 (80+70+30), and N210 (80+80+50) kg ha^{-1}) were used. In total, 20 variants were arranged using a split plot design in four replications, more detailed information was given in previous publication (Švarta et al., 2020).

The intensity of fungicide treatment was demonstrated by treatment frequency index (TFI) (Nistrup Jørgensen, 2008).

Table 1. Fungicide treatment schemes

Variants	Treatment timing-growth stage (GS) according to BBCH*	Active ingredients of fungicides	Dose, L ha^{-1}	Treatment frequency index
F0 (untreated variant)	Without fungicides	-	-	-
F1 (one treatment)	55–59	Protioconazole 130 g L^{-1}; bixafen 65 g L^{-1}; fluopyram 65 g L^{-1}	0.750	0.5
F2 (one treatment)	55–59	Protioconazole 130 g L^{-1}; bixafen 65 g L^{-1}; fluopyram 65 g L^{-1}	1.500	1.0
F3 (two treatments)	32–33	Protioconazole 160 g L^{-1}; spiroxamin 300 g L^{-1}	0.625	1.0
	55–59	Protioconazole 130 g L^{-1}; bixafen 65 g L^{-1}; fluopyram 65 g L^{-1}	0.750	
F4 (three treatments)	32–33	Protioconazole 160 g L^{-1}; spiroxamin 300 g L^{-1}	0.625	2.0
	55–59	Protioconazole 130 g L^{-1}; bixafen 65 g L^{-1}; fluopyram 65 g L^{-1}	0.750	
	63–65	Metconazole 90 g L^{-1}	1.000	

* BBCH – phenological growth stages of cereals according to the 'BiologisceBundesanstalt, Bundessortenamt und Chemische Industrie' (BBCH) scale.

Soil at the site was loam, sod-calcareos soil. Soil is characterized by the following indicators depending on the year: pH_{KCl} = 6.4–7.0, organic matter content 29–40 g kg^{-1}, P_2O_5 content 118–167 mg kg^{-1} and K_2O content 262–244 mg kg^{-1} of the soil.

The rate of nitrogen fertilizer was divided into two applications for variants N120 and N150, and into three applications - for variants N180 and N210. Before sowing, fertilizer was applied N - 11–25 kg ha^{-1}, P_2O_5 - 33–65 kg ha^{-1} and K_2O - 65 kg ha^{-1}.In spring, after resumption of vegetative growth, ammonium nitrate (NH_4NO_3; N 34.4%) was used for all variants. The second top-dressing consisting of ammonium sulphate ($(NH_4)_2SO_4$; N21, S 24%) at the rate of 100 kg ha^{-1} was done in winter wheat at

GS 29–31, and the remaining amount of needed nitrogen was added using ammonium nitrate. The final application was done at GS 47–51 with ammonium nitrate.

Weeds were controlled using foliar commercial herbicides: in 2019, tritosulfuron (714 g kg^{-1}) + florasulam (54 g kg^{-1}) 70 g kg^{-1} and adjuvant Dash 0.5 L ha^{-1}, in 2020, florasulam 9100 g kg^{-1}) + halauxifen-methyl (104.2 g kg^{-1}) 0.04 kg ha^{-1} and MCPA (750 g L^{-1}) 1.5 L ha^{-1} were used.

The severity of leaf blotches was assessed: for the whole plant - at GS 31–32, for three upper leaves - at GS 37 and GS 63–65, and for two upper leaves - at GS 73 and GS 75–79. Growth stages were noted according to BBCH scale. In the first assessment, 25 plants were evaluated. In further assessments, 50 leaves were evaluated from every plot, proportionally taking flag leaves, first leaves and second leaves from each plot. The severity was expressed in percentages.

The total disease impact during vegetation period was estimated by calculating the area under the disease progress curve (AUDPC) using the formula (1) (Simko & Piepho, 2012):

$$A_k = \sum_{i=1}^{N_{i-1}} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i) \qquad (1)$$

where n – data-set extent; a_i – variable at the i index of a data-set, N.

The yield was harvested at GS 89–90, and yield data was adjusted to 14% moisture. Mathematical data processing was done by using R-studio, multi-way Anova analysis, and correlation analysis. Bonferroni test was used for the comparison of means; the differences were considered statistically significant when $p < 0.05$.

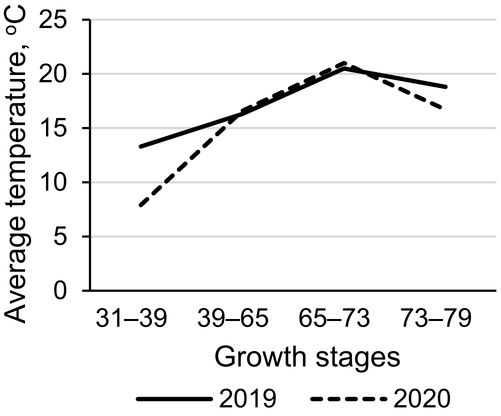

Figure 1. Average temperatures in 2019–2020 (data from the Study and Research farm “Pēterlauki” meteorological station).

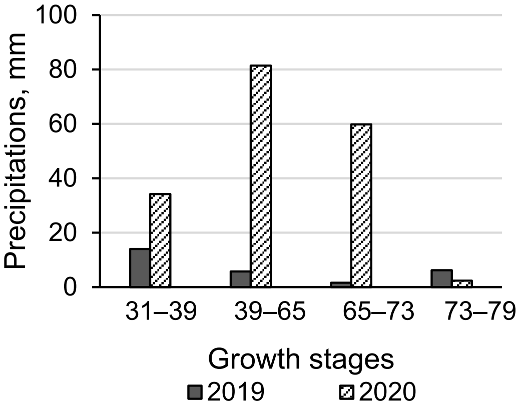

Figure 2. Amounts of precipitation in 2019–2020 (data from the Study and Research farm “Pēterlauki” meteorological station).

The meteorological data (average temperature (°C) and the amount of precipitation (mm)) were summarised according to growth stages of winter wheat when the evaluation of leaf blotches was done (Figs 1, 2). The meteorological conditions were significantly different in both research years. Overall, the air temperatures in the vegetation period were higher than long-term observations in both trial years - in some decades the increase of average air temperature reached even +5.0 °C (in 2019: - 3rd decade of June; in 2020 - 3rd decade of April, 2nd decade of May, 3rd decade of June.

There was a significant difference between the amounts of precipitation during the research years. In 2019, the amount of precipitation was low (for most decades only 20–30% from long-term observations). In contrast, second research year was characterised with rainfall (for example, 1st decade of June - 359%, 3rd decade of June - 219% from long -term observations).

RESULTS AND DISCUSSION

The development of leaf blotches. The severity of leaf blotches in winter wheat leaves differed significantly during both years of investigations (Fig. 3). In 2019, the total amount of precipitation was low and the lack of moisture was observed in all winter wheat growth stages; however, the severity of tan spot reached a significant level (18.7% in untreated variant). Although the first symptoms of tan spot were observed already at GS 31–32 (severity 0.03%), rapid development began only at GS 73. In contrast, the level of Septoria tritici blotch was relatively low. The first symptoms were observed later - only at the stage of early milk (severity 0.6%). Further development of the disease was slow, and at the end of vegetation period, the severity of Septoria tritici blotch in untreated plots was only 7.4%.

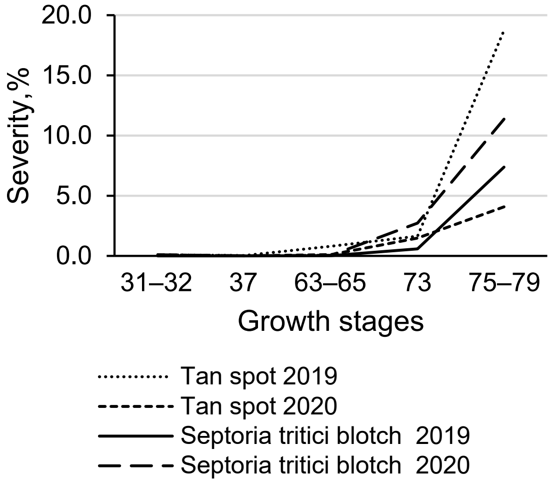

Figure 3. Severity of winter wheat leaf blotches in untreated plots in 2019–2020.

In 2020, the amount of precipitation at GS 39–65 was 81.4 mm, but at GS 65–73 - 59.8 mm (at this time in 2019 - respectively only 5.8 and 1.6 mm). These conditions promoted the development of Septoria tritici blotch. It is known that the causal agent of Septoria tritici blotch requires a moist leaf surface for infection and weather was favourable for disease development. This corresponds to the results from other studies (El Jarroudi et al., 2013; Bankina et al., 2018; Castro et al., 2018). The first symptoms were observed at an early stage of wheat development (GS 31–32) but rapid development of Septoria tritici blotch started at GS 73 (2.7% in untreated variant) and coincided with rainfall (on 29.06.2020. - 56 mm). At the end of vegetation period, the severity of Septoria tritici blotches in untreated plots reached already 11.4%. For the first time, tan spot was observed also at GS 31–32 (0.1%), but its development was slow, and at the end of vegetation, it reached only 4.1% in untreated plots. This is an unexpected result, because usually higher amounts of precipitation increase the level of tan spot (Fleitas et al., 2018; Schierenbeck et al., 2019).

Total impact of both diseases expressed as values of AUDPC (Fig. 4) differed significantly depending on year ($P < 0.001$).

Efficacy of leaf blotches control. In both years, application of fungicides significantly decreased the level of leaf blotches ($p < 0.001$ for both years). The results of the current study coincide with the results of numerous researches, i.e., fungicide treatments reduced the values of AUDPC of leaf blotches in comparison with the untreated variant (Willyerd et al., 2015; Castro et al., 2018; Fleitas et al., 2018; Schierenbeck et al., 2019).

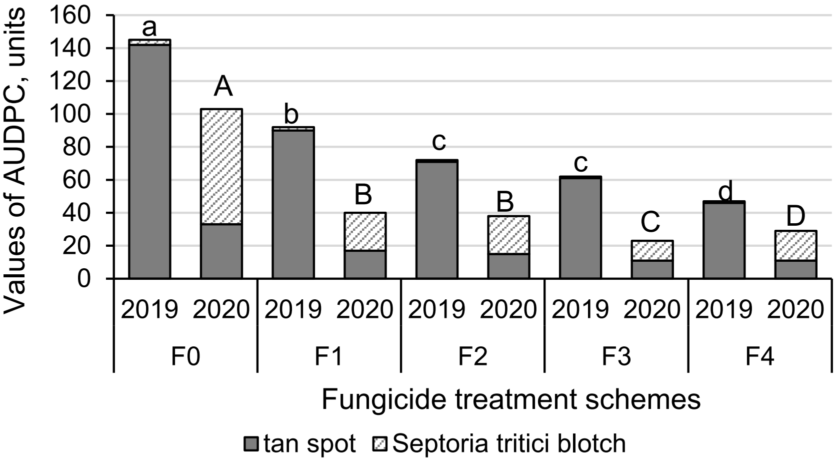

Figure 4. Values of areas under the disease progress curve (AUDPC) of leaf blotches depending on fungicide treatment schemes in 2019–2020.

Significantly different means are labelled with different letters in superscript: $^{a, b, c, d, e}$ – significant difference for average values of AUDPC for 2019; $^{A, B}$ – significant difference for average values of AUDPC for 2020.

More intensive application of fungicides was effective under conductions of higher pressure of leaf diseases. In 2019, when the differences in AUDPC values for tan spot (Fig. 4) among variants ranged between 35 and 147 (without fungicide treatment) units, the efficacy of fungicides in variant F1 (treatment frequency index (TFI) = 0.5) was lower than in variants F2 and F3. Although TFI in F2 and F3 was equal, the efficacy differed. A better efficacy was obtained in F3, where dose of fungicide was divided and the treatment was done in two applications. Variant F4, where TFI = 2, showed an even better efficacy. In 2020, when the AUDPC values ranged only between 8 and 49 units, the differences between fungicide treatment strategies on the severity of tan spot were not significant.

In both years, all fungicide treatments ensured the protection of wheat cultivars against Septoria tritici blotch ($P < 0.001$ for both years). In turn, the differences in the efficacy of fungicide treatment schemes were significant only in 2020. Although TFI in F2 and F3 varied (respectively - 0.5 and 1.0), the efficacy of those fungicide treatment schemes for limiting Septoria tritici blotch did not differ. Similar efficacy was ensured also in F4, where TFI = 2.0. A better effect was obtained in F3, where fungicide application was done two times.

Many studies revealed that used fungicide treatment schemes varied and their effectivity differed. Researches showed that one application of fungicides at the time of flag leaf formation decreased tan spot severity by 83.7% and the values of AUDPC by 86.6% (Bhatta et al., 2018). Other researchers included also early treatment until stem elongation to prevent primary infection (Schierenbeck et al., 2019; Fleitas et al., 2018). Kutcher et al. (2018) found that split fungicide application at both stem elongation (GS 30) and flag leaf (GS 39) tended to decrease the severity of leaf blotches on flag leaves and increase the yield relative to single application of fungicide applied at GS 39. Wegulo et al. (2009) obtained that the fungicides used at flowering can prevent significant yield losses. The present study confirms the results of researches in Luxembourg when in years with major tan spot outbreaks, El Jarroudi et al. (2013) found that during early grain development (at GS 75), the disease severity was well controlled by a single (at GS 55) and double (at GS 31 + GS 59) fungicide treatments, but later (at GS 85) the severity of disease increased. Those researchers used fungicide treatment schemes similar to those in our experiments but with different active ingredients.

Similarly to tan spot, the strategies used for the limiting of Septoria tritici blotch varied. In Ireland, where winter wheat fungicide programs for Septoria tritici blotch comprised even four applications, first application was done until GS 31 with the aim to slow the progression of Septoria tritici blotch in the upper canopy. Other researchers - Sylvester & Kleczewski (2018) - established that the inclusion of early treatment in fungicide programs (GS 30 + GS 37 or GS 30 + GS 60) did not result in significantly lower disease severity compared to single application at GS 37 or GS 60. Researchers found that fungicide treatment schemes with fungicide application at GS 60 resulted in the lowest leaf blotch severity on the flag leaf. Brinkman et al. (2014) confirmed that early application did not control the disease on flag leaves during grain fill and the most effective fungicide application timing was at GS 35–40 or at GS the 60–65. Triple application of fungicides resulted in the lowest leaf disease during grain fill, but efficacy of triple application did not usually differ from one application done at flowering.

Nitrogen rates. Although the development of tan spot was not influenced by nitrogen fertilizer rate ($P = 0.07$ for 2019; $P = 0.95$ for 2020), the influence of fungicide atment scheme on the development of tan spot (Table 2) depended on nitrogen fertilizer rates. In the year with higher values of AUDPC, the efficacy of treatment schemes where treatment was done in two or three applications (TFI = 1.0 and 2.0) in variants F3 and F4 was higher at all nitrogen rates. In the next year, with low pressure of tan spot, only at N210 the influence of fungicide treatment scheme was significant.

The results of present study confirm that the effectivities of fungicides on the development of tan spot increased with the increase in nitrogen dose (Castro et al., 2018).

Table 2. Values of AUDPC of tan spot depending on fungicide treatment schemes and nitrogen rate in 2019–2020

	Values of AUDPC			
Fungicide treatment schemes	N120	N150	N180	N210
	2019			
F0	146^{a}	142^{a}	147^{a}	133^{a}
F1	97^{b}	93^{b}	88^{b}	84^{b}
F2	85^{b}	$68^{b,c,d}$	$63^{b,c,d}$	70^{b}
F3	$67^{b,c}$	$65^{b,c,d}$	$56^{c,d}$	$56^{b,c}$
F4	48^{c}	46^{d}	54^{d}	35^{c}
	2020			
F0	31^{A}	40^{A}	34^{A}	26^{A}
F1	19^{B}	15^{B}	13^{B}	19^{A}
F2	18^{B}	14^{B}	15^{B}	12^{B}
F3	10^{B}	9^{B}	10^{B}	15^{A}
F4	11^{B}	12^{B}	11^{B}	10^{B}

Significantly different means are marked with different letters in superscript: a, b, c, d, – significant difference for average values of AUDPC for 2019; A, B – significant difference for average values of AUDPC for 2020.

The development of Septoria tritici blotch was not influenced by nitrogen fertilizer rate ($P = 0.67$ for 2019; $P = 0.94$ for 2020). In contrast, Jensen & Jørgensen (2016) found that severity of disease increased significantly with the increase in crop biomass in the untreated plots. The research of

Brinkman et al. (2013) revealed that severity of Septoria tritici blotch on the flag and penultimate leaves weakly associated with N rate. However, in certain years, the severity of disease increased in the untreated plots at higher N rates (32% of the leaf area was infected at a rate of 170 kg ha^{-1}, compared to 26.9% with N rate of 100 kg ha^{-1}). Castro et al. (2018) also found that weather conditions explained the differences in the effect of N fertilization on the AUDPC of Septoria tritici blotch. In the wettest year with more disease pressure, nitrogen fertilization significantly decreased disease severity. Conversely, in the driest year, no differences were detected.

Wheat yield

The average winter wheat grain yield in both trial years differed significantly ($P < 0.001$). Nitrogen fertilization significantly increased the average grain yield per both years. In 2019, a significant yield increase was observed at the nitrogen rate N180 ($P = 0.015$) (Švarta et al., 2020), but in 2020 - at the rate N150 ($P < 0.001$). Further increase of nitrogen rate did not provided a significant yield increase.

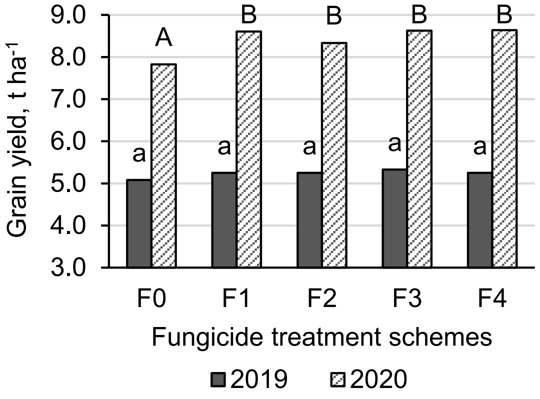

Figure 5. Winter wheat yield (t ha^{-1}) depending on fungicide treatment schemes in 2019–2020 at the average nitrogen background.

Significantly different means are marked with different letters in superscript: ^{a} – significant difference for 2019; $^{A, B}$ – significant difference for 2020.

As leaf blotch severity was influenced by fungicide treatment schemes but no by nitrogen rate, this article further analyses the grain yield depending on fungicide treatment schemes at the average nitrogen background.

In our experiment, a negative moderate correlation between total AUDPC of leaf blotches and grain yield was established ($r = -0.58$). In 2019, winter wheat grain yields was 5.08–5.25 t ha^{-1} (Fig. 5) and fungicide treatment schemes did not influenced yield ($P = 0.80$). In 2020, when the average winter wheat grain yield was higher (7.82–8.64 t ha^{-1}), the using of fungicides provided significant increase of yield ($P < 0.001$) compared to untreated variant but there were no significant differences among fungicide treatment schemes. It coincides with results of research in the Baltic–Nordic region what indicate a positive yield response even after one fungicide treatment of wheat (Jalli et al., 2020). Researchers obtained that yield responses from fungicide treatment range between 0.5 and 2 t ha^{-1} and depends on region, meteorological conditions, cultivar and soil type.

CONCLUSIONS

1. The development of tan spot and Septoria tritici blotch differed significantly in both years and depended on meteorological conditions. In 2019, tan spot dominated and the severity of disease reached a significant level (18.7% in untreated variant). In 2020, weather conditions promoted the development of Septoria tritici blotch (11.4% in untreated variant).

2. All used schemes of fungicide treatment significantly decreased the level of tan spot and Septoria tritici blotches. More intensive fungicide application contributed better leaf blotches control, but it did not give additional yield under Latvian conditions in 2019–2020.

3. Nitrogen fertilizer rate had no significant effect on the development of leaf blotches; however, more intensive fungicide treatment was more effective in variants with higher rates of nitrogen.

4. Nitrogen fertilizer rate significantly increased the average grain yield per both years. A significant yield increase was observed: 2019 - at the nitrogen rate N180, in 2020 - at the rate N150. Further increase of nitrogen rate did not provided a significant yield increase.

5. A negative moderate correlation between total AUDPC of leaf blotches and grain yield was established. The using of fungicides increased grain yield only in year when obtained significantly higher yields (grain yields reached 7.00 t ha^{-1}) although the severity of leaf blotches was lower.

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