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Evaluation of fungicide application programmes for Septoria tritici blotch control in winter wheat

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Abstract

Zymoseptoria tritici is the causal agent of Septoria tritici blotch (STB) in wheat, and its spread is reported to depend on environmental conditions. Since the spread of disease is usually controlled by using fungicides, selecting timings of their application is crucial. The aim of the present study was to estimate the effect of two fungicide application programmes for STB control in winter wheat and to evaluate the relationship between the severity of disease and quantified DNA of *Z. tritici* in leaves. Two field experiments (in monoculture field and in the field after non-host crops) with winter wheat were designed, and six fungicide application treatments were chosen. For the disease control, fungicide Adexar (a.i. fluxapyroxad 62.5 g l^{-1} + epoxiconazole 62.5 g l^{-1}) in dosages 1.0 and 2.0 l ha^{-1} was used. In 2019, due to unfavourable meteorological conditions, plant infestation with the pathogen was low. Nevertheless, comparing the experiments from both crop rotations, there were marked trends of higher severity of STB in winter wheat monoculture. Meanwhile, in 2020 and 2021, the values of the area under the disease progress curve (AUDPC) were higher in winter wheat grown after non-host crops. Differences between tested application programmes were more precise at higher disease pressure in 2020 and 2021. According to the results of a three-year field experiment, higher effectiveness of controlling STB showed fungicide application programmes based on weather conditions and two applications per season. A more important influence of fungicide application programmes on grain yield increase was found in winter wheat monoculture. Application of fungicides had a higher impact in the 2020 cropping season, when a higher level (AUDPC 358.6) of STB disease infection was observed. In both crop rotations, the highest grain yield increase was obtained by applying fungicide twice: 8–10 days after rain and two weeks after the first application. In both crop rotations, Pearson's correlation test showed a strong relationship between visual assessments of disease severity and DNA quantity of *Z. tritici* in leaves of winter wheat.

Keywords: application programmes, AUDPC, real-time-PCR, Septoria tritici blotch, *Zymoseptoria tritici*.

Introduction

Septoria tritici blotch (*Zymoseptoria tritici*) is a widespread and economically important disease of winter wheat, which has a significant negative impact on grain yield. If left without control, yield losses caused by the fungus *Z. tritici* range from 30% to 50% (AHDB, 2012; Jørgensen et al., 2014). The primary source of inoculum is sexual ascospores, which disperse from previous wheat stubbles and infect young plants in autumn (Suffert et al., 2011). These ascospores infect leaves to produce chlorotic and necrotic lesions and fruiting bodies (pycnidia). In spring, asexual spores (conidia) are formed in pycnidia, which are rain splashed up the plant causing the secondary inoculum (Duvivier et al., 2013).

Meteorological conditions are very important in the development of fungal diseases in winter wheat. The incidence and severity of Septoria tritici blotch (STB) may greatly vary depending on the environmental conditions. Spore transfer and germination are the most important steps in the life cycle of *Z. tritici*, which depends upon the availability of water; therefore, rain in May and June is closely related to disease intensity (Wiik, Ewaldz, 2009; Beyer et al., 2012). At stem extension period, high rainfall promotes rapid spore movement from lower to upper leaves. The time of epidemic onset is very important, because early epidemics cause more severe damage of the disease than those occurring later in the growing period. Meteorological conditions are the main factor in the decision to forecast disease and to determine the time for the effective fungicide application (El Jarroudi et al., 2017).

Another essential trait of *Z. tritici* is the latent phase, which is a period from spore contact with the leaf to visual symptoms. Under field conditions, the latent phase of STB lasts from 14 days in the summer period to 28 days in colder weather conditions (AHDB, 2012). The timing of application is complicated, because it is difficult to predict the progression of the disease. Plant protection against STB is focused on the application of foliar fungicide sprays, for optimum, to protect the upper three leaves, which provide most of the grain-

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filling capacity (Fraaije et al., 1999) and contribute to approximately 75% of the final grain yield (Paveley et al., 2000). A typical programme for STB control includes two to four fungicide sprays from early pre-stem extension to a final application at full flowering stage (AHDB, 2012; Fones, Gurr, 2015; Creissen et al., 2018). Application timing determined by the plant growth stage takes no account of pathogen epidemiology. Therefore, this control strategy should be limited, and a decision should be based on inoculum development, rainfall distribution, cultivar susceptibility, and fungicide activity (Burke, Dunne, 2008). Furthermore, fungicides are hazardous for the environment, as they take up to 30% of all plant protection products used in winter wheat (Holka, Bienkowski, 2020).

In recent years, the development of *Z. tritici* resistance to fungicides (Jørgensen et al., 2018) and legislative constraints regarding the reduction of fungicide product usage (Jess et al., 2014) increased the demand for new plant protection programmes.

The aim of the present study was to estimate the effect of fungicide application programmes for STB control in winter wheat and to evaluate the relationship between severity of disease and DNA quantity of *Z. tritici* in wheat leaves.

Materials and methods

Field experiment and design. The experiment was carried out at the Department of Plant Pathology and Protection of the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, in 2019–2021. Two field experiments with different crop rotations were designed with winter wheat cultivars 'Arkadia' in 2020 and 'Skagen' in 2019 and 2021 with natural infection each year. According to disease assessments performed in the previous years, both cultivars had similar resistance levels to Septoria tritici blotch (STB). One experiment was sited in winter wheat monoculture (since 2005), the other in winter wheat grown after non-host crops: peas, oilseed rape, and peas in 2019, 2020, and 2021, respectively. Winter wheat was sown at a seed rate of 430 viable seeds m^2. The experimental plots were 2.5 m in width and 10 m in length. All plots were arranged in randomised blocks in four replicates. For disease control, the Lithuania-registered (during experimental period) fungicide Adexar (a.i. fluxapyroxad 62.5 g l^{-1} + epoxiconazole 62.5 g l^{-1}) with protective and curative effects was used and spray-applied with a high pressure plot sprayer (Strøby MaskinVærksted, Denmark). During the experiment, common plant growing practices were used.

The experimental design consisted of untreated control and six treatments with two standard fungicide application (A) programmes (Table 1). At treatment No. 2, applications in dosages 1.0 l ha^{-1} were done according to the recommendations of the product manufacturer: applications from plant growth stages (GS) 31 to 69 – at winter wheat stem elongation (the first application (A1) at GS 32) and at heading (the second application (A2) at GS 51–55) stages according to Zadoks growth scale. Treatment No. 3 was applied according to the Agricultural and Horticulture Development Board (AHDB, 2019) at the stages of stem elongation beginning (A1 at GS 32) and flag leaf (A2 at GS 37–39). In treatments Nos. 4–7, applications were done according to the weather conditions: observing weather conditions from plant GS 31 (1^{st} node) stage, 5–7 and 8–10 days after the rain. One application in full dosage (2.0 l ha^{-1}) was done in treatments No. 5 and No. 7, 5–7 and 8–10 days after the rain, respectively.

Table 1. Experimental design and description of fungicide application

No.	Application programme	Fungicide l ha^{-1}	Application timing*					
			2019		2020		2021	
1.	Untreated (control)	–	date	GS	date	GS	date	GS
2.	A1 GS 32; A2 GS 51–55	1.0 1.0	05 23 06 18	32–37 51–55	05 04 06 08	31 55–59	05 18 06 07	32 47–51
3.	A1 GS 32; A2 GS 37–39	1.0 1.0	05 23 06 06	32–37 39–43	05 04 05 19	31 33–37	05 18 05 27	32 37–39
4.	A1 5–7 days after rain; A2 two weeks after A1	1.0 1.0	05 23 06 06	32–37 39–43	05 08 05 25	32 41–43	05 14 05 27	31–32 37–39
5.	A1 5–7 days after rain	2.0	05 23	32–37	05 08	32	05 14	31–32
6.	A1 8–10 days after rain; A2 two weeks after A1	1.0 1.0	05 30 06 18	37–41 51–55	05 11 05 28	33 43–47	05 19 06 04	32 41–43
7.	A1 8–10 days after rain	2.0	05 30	37–41	05 11	33	05 19	32

* – observing from plant growth stage (GS) 31; application: A1 – first, A2 – second

At hard maturity, the plots (2 × 10 m) were harvested with a small plot harvester Haldrup C-85 (Germany). Grain weight and moisture content were measured by weighing and moisture systems of the harvester. Grain yield in t ha^{-1} was adjusted to 14% moisture content. A thousand grain weight (TGW) was calculated with a grain counter Contador (Hoffman Manufacturing, Germany).

Assessments. During the experimental period, three assessments at GS 31, 65, and 75 were done. A total of ten plants randomly selected from each plot were inspected visually, and STB severity was assessed on two or three upper leaves following the plant senescence. The severity of the disease per season was expressed by the area under the disease progress curve (AUDPC) value (Simko, Piepho, 2012):

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

where n is the total number of assessments; y_i – disease severity (%) at the i^{th} assessment; t_i – days at the i^{th} assessment.

Leaf sampling. During 2019, 2020, and 2021 cropping seasons, leaf samples were collected from winter wheat monoculture, when winter wheat were grown after peas (2019), oilseed rape (2020), and peas (2021). Starting from 1 May to 30 June, samples of the three upper leaves (separately 25 leaves for each layer) were randomly collected every week; each sample contained 25 leaves from separate leaf layers. To determine the start of STB spread and its development at different plant growth stages, the leaves were assessed for disease symptoms (Figure 3). Until real-time-PCR (qPCR) analysis, samples were labelled and stored in plastic bags in a refrigerator (at –20°C).

DNA extraction and qPCR. Samples composed of ten randomly selected winter wheat leaves (from leaves collected as described in the leaf sampling section) for *Zymoseptoria tritici* DNA extraction had been collected in 2019 and 2020. A total of 112 samples were collected, 58 of which were without STB symptoms. The homogenisation of the samples was done using liquid nitrogen. DNA was extracted from 0.1 g of the homogenised sample using a commercial plant DNA kit E.Z.N.A. (Omega Bio-Tek Inc., USA). Assays for *Z. tritici* were carried out in 15 µl of reaction mixture containing 7.5 µl Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific Baltics, Lithuania), 2.5 µl tested DNA, 3.5 µl nuclease-free water, 0.5 µl BSA (bovine serum albumin) and 0.5 µl (10 pM μl^{-1}) of each forward and reverse primer. Two specific primer pairs were used: forward 5'-ATTGGCGAGAGGGATGAAGG-3' and reverse 5'-TTCGTGTCCCAGTGCGTGTA-3' (Duvivier et al., 2013). For the detection of plant DNA and normalisation of the reactions, the EF1α primers Hor1F (5'-TCTCTGGGTTTGAGGGTGAC) and Hor2R (5'-GGCCCTTGTACCAGTCAAGGT) were employed. For individual standard curves, five-fold dilution series with *Z. tritici* DNA isolated from pure culture were used. The amplification reactions were performed in two replications on 7900HT Fast Sequence Detection System (Applied Biosystems, USA) using the following cycling protocol: initial duration at 95°C for 10 min and 40 cycles at 95°C for 15 s each, 60°C for 20 s, and 72°C for 40 s (Duvivier et al., 2013). Values of reactions were calculated as pg of fungal DNA per µg of plant DNA (Nicolaisen et al., 2009).

Weather conditions. Data were collected at the meteorological station located in Akademija, Kėdainiai district. The weather conditions varied over the three experimental seasons (Table 2).

Table 2. Monthly rainfall and average temperatures in autumn from October and November and in spring–summer from March to June for the cropping seasons

Cropping season	October	November	March	April	May	June
Sum of precipitation mm						
2018–2019	32.8 (13)*	12.9 (8)	37.8 (19)	0.0	55.4 (12)	16.1 (5)
2019–2020	34.9 (14)	29.5 (14)	31.7 (12)	9.5 (5)	50.1 (11)	165.9 (14)
2020–2021	49.4 (18)	35.6 (21)	16.3 (13)	26.3 (16)	100.9 (20)	30.1 (9)
Long-time average (1924–2020)	49.6	44.8	36.9	51.4	62.3	76.5
Air temperature, mean °C						
2018–2019	7.9	2.9	3.3	8.9	12.9	20.6
2019–2020	9.3	4.9	3.5	6.8	10.6	18.9
2020–2021	10.2	5.3	2.0	6.4	11.4	19.6
Long-time average (1924–2020)	6.9	2.0	−0.5	6.0	12.4	15.8

* – number of rainy days

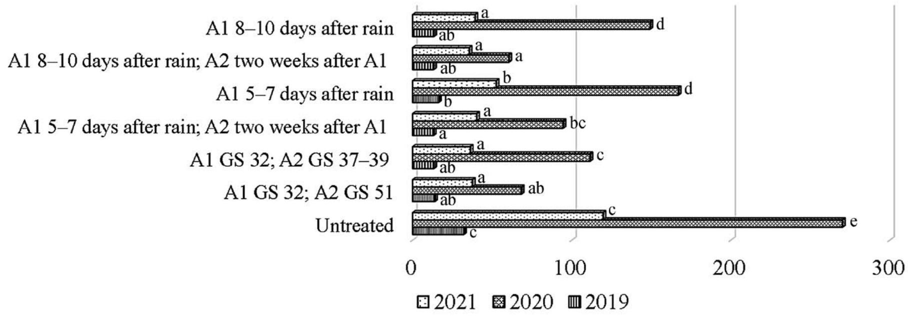
In all three experimental years (2019–2021), the average monthly air temperature was higher compared to the long-time mean. Overall, 2019 was a warm and dry year. The amount of precipitation in October–November and March–June 2019 (155.0 mm) was lower compared to the amount (321.5 mm) of long-time average (1924–2020). In April 2019, it was drastically low (0 mm), whereas in 2020, in the same months, the sum of precipitation was the same as the long-time average (1924–2020), although in April, the amount of precipitation was five times lower than the long-time average. The autumn of 2020 was warm with higher amount of precipitation compared with other years. In summary, 2021 had more rainy days and similar temperatures as the other years.

Statistical analysis of the experimental data was made using the analysis of variance (ANOVA) by procedure PROC GLM of software SAS, version 9.4 (SAS Institute, USA). To determine the differences between treatments, Duncan's multiple range test was used ($P < 0.05$) (Raudonius, 2017). To conduct Pearson's correlation between disease infection, TGW, and grain yield from the different crop rotations, procedure PROC CORR was used. Standard error (SE) of the mean grain yield and TGW were calculated.

Results and discussion

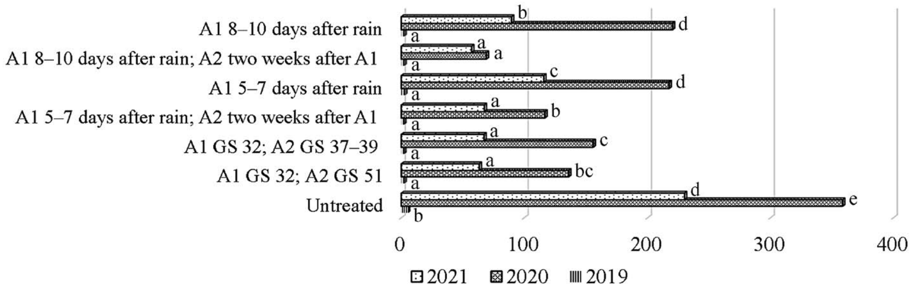
The severity of STB and the effect of fungicide application programmes. The data collected from years 2019–2021 shows that disease severity greatly varied between the experimental years and crop rotations, which frequently occurs in the Lithuanian climate (Ronis et al., 2014). In 2019, dry weather conditions might have been the reason for the low infestation of plants, as the spread of STB infection is reliant on rainfall during the growing period (Pietravalle et al., 2003). In untreated (control) winter wheat crop, AUDPC values were 32.1 and 5.9 in monoculture and winter wheat grown after non-host crops, respectively (Figures 1 and 2). In 2020, the average of AUDPC values was 270.3 in monoculture and 358.6 in winter wheat grown after non-host crop (oilseed rape). In 2021, the average of AUDPC values was 119.7 in monoculture and 229.9 in winter wheat grown after non-host crop (peas). Favourable weather conditions for the development of STB infection are temperate temperatures in winter and high rainfall during the growing period, especially in May and June (Pietravalle et al., 2003; O'Driscoll et al., 2014; El Jarroudi et al., 2017). In 2020, rainy June might have been the reason for higher infestation of plants.

Despite low severity of disease in 2019, both in monoculture and after non-host crops, all application programmes significantly reduced STB severity in comparison with untreated crops. There were no significant differences detected between application programmes in the winter wheat grown after non-host crops. In monoculture, the only significant difference established between fungicide application programmes was where fungicide applications were conducted 5–7 days after rain, once and twice.



Note. Means followed by the same letter do not significantly differ ($P = 0.05$, Duncan's multiple range test).

Figure 1. Effect of fungicide application programmes (A1 and A2) on the area under disease progress curve (AUDPC) values of Septoria tritici blotch in winter wheat monoculture, 2019–2021



Note. Means followed by the same letter do not significantly differ ($P = 0.05$, Duncan's multiple range test).

Figure 2. Effect of fungicide application programmes (A1 and A2) on the area under disease progress curve (AUDPC) values of Septoria tritici blotch in winter wheat grown after non-host crops, 2019–2021

In experimental years 2020 and 2021, all fungicide application programmes significantly reduced disease severity in both experiments. The results of 2020 show that the most effective application timing in both rotations was where fungicide was applied twice: A1 8–10 days after rain and A2 two weeks after A1. Single fungicide applications (5–7 days and 8–10 days after rain) were least effective compared to other programmes, with hardly any difference between the two. According to the results of current experiment, heavy and frequent rains (165.9 mm) in June influenced the longer period of infection of STB, therefore, single fungicide applications were less effective. In 2021, significant differences between fungicide application programmes were detected. Lower efficiency (50–61%) of fungicide was found in winter wheat grown after peas crop in plots, where single application was done, regardless of the number of days after the rain, and in winter wheat monoculture, where fungicide spray was conducted 5–7 days after rain.

The effectiveness of fungicide application programmes was more pronounced in experimental years 2020 and 2021, when disease severity was higher. The results of these two years showed that the programmes, when fungicide was used twice were more effective. Results of current experiment indicate that single application has a lesser effect on disease severity, because higher STB pressure was observed after the emergence of flag leaf (Figure 3).

Previous studies suggest that disease control on the upper three leaf layers of the crop canopy is very important, because those are critical to grain yield formation (Paveley et al., 2012; van den Berg et al., 2013). Researchers from the United Kingdom (Paveley et al., 2000; 2012) as the main fungicide application programme recommend two sprays (A1 and A2): A1 is done at the full emergence of the third leaf (GS 32),

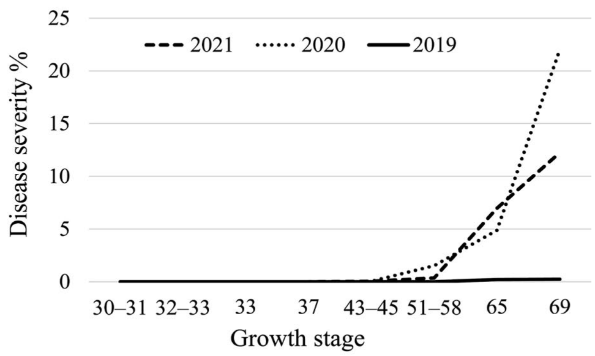


Figure 3. Developement of Septoria tritici blotch at different plant growth stages on the upper three leaves of winter wheat, 2019–2021

and A2 is done at the full emergence of the flag leaf (GS 39). In the present study, in application programmes with two fungicide sprays, the second application (A2) was done at full emergence of the flag leaf, which maximised fungicide life. These fungicide application programmes resulted in lower severity of STB.

Effect of crop rotations on the severity of STB. In the comparison of two crop rotations, AUDPC values differed in 2019–2021. In 2019, higher disease severity was recorded in monoculture, meanwhile in 2020 and 2021, AUDPC values were higher in winter wheat grown after non-host crops. Results of the studies show that winter wheat as pre-crop might increase disease intensity (Wenda-Piesik et al., 2016), whereas the data from 2020 and 2021 of current experiment coincides with the conclusions made by Latvian researchers. The STB infection level is influenced more by meteorological conditions than crop rotation (Bankina et al., 2018). O'Driscoll et al. (2014) noted that STB flourishes, when temperatures in winter are temperate and rainfall during the growing period is high. Thus, weather conditions in the cropping seasons of 2020 and 2021 were perfect for intensive development of STB infection. Other researchers suggest that aerial canopy architecture and plant architectural features such as the number of tillers, leaf dimensions, and vertical distance between leaves influence splash-dispersed pathogens and are important factors in disease management (Tivoli et al., 2012). Canopy density has an effect on disease spread by reducing the distance of spore dispersal (Schoeny et al., 2008).

In the present study, winter wheat as monoculture was grown for more than ten successive years. After 15 years, in winter wheat monoculture, a reduction in disease severity was even more pronounced compared to non-host crops. Also, in spring of 2020 and 2021, very dry weather conditions with a lower average temperature influenced plant development (Table 2). Winter wheat grown as monoculture had sparser canopy compared to wheat grown after oilseed rape (2020) and peas (2021). These factors had an impact on spore dispersal and disease reduction and explain higher STB severity in 2020 and 2021 in winter wheat grown after non-host crops.

Quantification of Zymoseptoria tritici in winter wheat leaves. To increase the efficiency of a minimum number of sprays, carrying out applications during the early stage of infection is most important. Early application can be ineffective, as the pathogen is not yet present, whilst late application can be inefficient, because the infection has already occurred (Beyer et al., 2012). To assess disease spread and quantify *Z. tritici* in winter wheat leaves, leaf samples were collected in 2019 and 2020 (Figure 3). Higher concentrations of the pathogen were identified in leaves from winter wheat monoculture. Both in winter wheat monoculture and winter wheat grown after non-host crops statistical analysis of data highlighted a strong correlation ($r = 0.870$ and $r = 0.813$, respectively) between visual assessment of disease severity and DNA quantity of *Z. tritici* (Figure 4). Hence, the higher quantity of *Z. tritici*, the higher STB severity. *Z. tritici* DNA was detected in 96.5% of the tested leaf samples (n = 58) without visible symptoms of the disease.

These results show that visual diagnosis leads to partial success in fungicide use as the latent period of *Z. tritici* is long enough, from 14 to 28 days. Therefore,

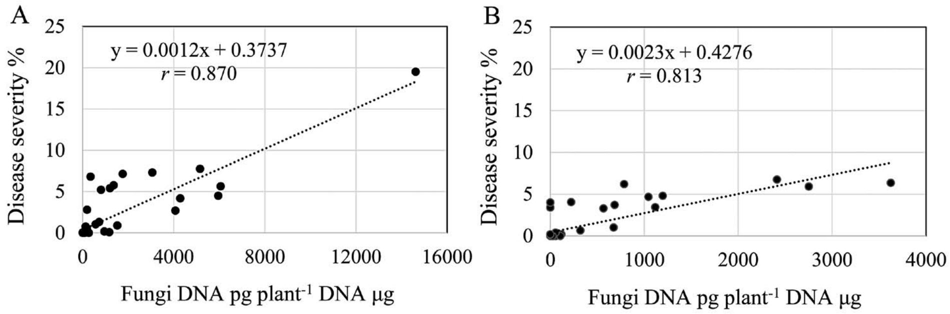


Figure 4. Relationship between severity of Septoria tritici blotch and DNA quantity of *Zymoseptoria tritici* in winter wheat monoculture (A) (n = 58) and in winter wheat grown after non-host crops (B) (n = 54) in 2019 and 2020

in disease control, an essential role may play early diagnosis of disease by qPCR (Fones, Gurr, 2015; Tonti et al., 2019). The best effect of fungicides against STB is approximately the seventh day of the latent period, when the symptoms of the disease are not visible yet. Good plant protection practice requires the use of fungicides prophylactically, before the disease emerges, to protect the newly formed leaves (Fones, Gurr, 2015).

Effect of fungicide application programmes on winter wheat grain yield. The findings of the three-year experiment indicated that grain yield differed between years and rotations. The highest grain yield of wheat monoculture was recorded in 2020, meanwhile of winter wheat grown after non-host crops in 2019. It is known that wheat grown after wheat as monoculture result in grain yield losses (Darguza, Gaile, 2019). Diversification of crop rotation has a considerable impact on winter wheat grain yield and its quality. In all experimental years, the grain yield of winter wheat grown after non-host crops was higher than that of monoculture: 68.5% in 2019, 32.6% in 2020, and 52.6% in 2022 (Table 3).

However, more important influence of fungicide application programmes on grain yield increase was found in winter wheat monoculture. Grain yield increase due to fungicide applying is highly dependable on weather conditions, especially rainfall (Byamukama et al., 2019). In the experiment, it was possible to observe such tendency in winter wheat monoculture grain yield: in 2019, increases per hectare varied from 0.04 to 0.36 t ha^{-1}, in 2020 from 0.07 to 0.70 t ha^{-1}, and in 2021 from 0.08 to 0.35 t ha^{-1}. However, these tendencies did not establish themselves in winter wheat grown after non-host crops. Grain yield increase due to different fungicide application programmes in 2019 from 0.34 to 0.90 t ha^{-1}, in 2020 from 0.37 to 0.96 t ha^{-1}, and in 2021 from 0.02 to 0.28 t ha^{-1} was observed.

As shown by the results of the present study, the best benefits of using fungicides were in the 2020 cropping season, when a higher level of STB disease infection occurred. The averaged grain yield results showed the highest yield increase from the application programme, in which fungicide was applied twice (A1 8–10 days after rain and A2 two weeks after A1) in both crop rotations.

Effect of fungicide application programmes on TGW. All application programmes in 2019 affected TGW positively; however, in winter wheat grown after non-host crops, the increase in TGW was insignificant (Table 4).

In winter wheat monoculture, all fungicide application programmes significantly increased TGW compared with the untreated (control) crop. It should be noted that there were no significant differences between application programmes. Hence, double fungicide applying could be not profitable due to low disease severity, as reported by El Jarroudi et al. (2015). In 2020, all application programmes increased TGW: the highest increase was in the crops, where fungicide using was chosen according to weather conditions, and double fungicide sprayings were made. Comparing all experimental years, TGW increase was the lowest in 2021.

Relationships between STB severity, grain yield, and TGW. Pearson's correlation test showed that disease severity had a higher impact on TGW than on grain yield (Table 5).

Table 3. Effect of fungicide application on winter wheat grain yield (± SE), 2019–2021

Application programme	2019			
	monoculture		after non-host	
1. Untreated (control)	4.35 a	± 0.27	7.03 a	± 0.17
2. A1 GS 32; A2 GS 51–55	4.38 abc	± 0.08	7.37 abc	± 0.10
3. A1 GS 32; A2 GS 37–39	4.46 abc	± 0.17	7.50 abc	± 0.08
4. A1 5–7 days after rain; A2 two weeks after A1	4.68 bc	± 0.19	7.91 bc	± 0.31
5. A1 5–7 days after rain	4.71 c	± 0.29	7.93 c	± 0.38
6. A1 8–10 days after rain; A2 two weeks after A1	4.40 abc	± 0.13	7.81 bc	± 0.34
7. A1 8–10 days after rain	4.45 abc	± 0.11	7.39 abc	± 0.14
	2020			
	monoculture		after non-host	
1. Untreated (control)	4.91 a	± 0.21	6.41 a	± 0.21
2. A1 GS 32; A2 GS 51–55	4.99 a	± 0.41	7.05 bcd	± 0.19
3. A1 GS 32; A2 GS 37–39	5.27 bcd	± 0.27	7.14 bcd	± 0.29
4. A1 5–7 days after rain; A2 two weeks after A1	5.37 abc	± 0.37	7.02 bcd	± 0.18
5. A1 5–7 days after rain	4.98 a	± 0.26	6.95 b	± 0.10
6. A1 8–10 days after rain; A2 two weeks after A1	5.62 bc	± 0.26	7.37 d	± 0.17
7. A1 8–10 days after rain	5.62 c	± 0.23	6.78 ab	± 0.21
	2021			
	monoculture		after non-host	
1. Untreated (control)	4.50 a	± 0.17	7.08 ab	± 0.13
2. A1 GS 32; A2 GS 51–55	4.85 cde	± 0.17	7.21 ab	± 0.09
3. A1 GS 32; A2 GS 37–39	4.58 ab	± 0.18	7.35 b	± 0.11
4. A1 5–7 days after rain; A2 two weeks after A1	5.09 e	± 0.14	7.31 ab	± 0.13
5. A1 5–7 days after rain	4.64 abc	± 0.15	7.23 ab	± 0.14
6. A1 8–10 days after rain; A2 two weeks after A1	4.85 bcde	± 0.16	7.28 ab	± 0.15
7. A1 8–10 days after rain	4.63 abc	± 0.17	7.09 ab	± 0.13

Note. The difference between the values with the same letter was not significant between different application programmes according to Duncan's multiple range test.

Table 4. Effect of fungicide application on winter wheat thousand grain weight (± SE), 2019–2021

Application programme	2019			
	monoculture		after non-host	
1. Untreated (control)	34.93 a	± 0.40	45.85 ab	± 0.69
2. A1 GS 32; A2 GS 51–55	37.49 c	± 0.61	47.10 ab	± 0.76
3. A1 GS 32; A2 GS 37–39	37.02 bc	± 0.87	46.19 ab	± 0.66
4. A1 5–7 days after rain; A2 two weeks after A1	36.87 bc	± 0.67	47.17 ab	± 0.63
5. A1 5–7 days after rain	36.00 bc	± 0.69	47.29 b	± 0.60
6. A1 8–10 days after rain; A2 two weeks after A1	37.03 bc	± 0.79	46.81 ab	± 0.84
7. A1 8–10 days after rain	36.91 bc	± 0.88	46.45 ab	± 0.60
	2020			
	monoculture		after non-host	
1. Untreated (control)	46.85 a	± 0.32	45.41 a	± 0.14
2. A1 GS 32; A2 GS 51–55	50.11 bcd	± 0.41	49.37 cde	± 0.30
3. A1 GS 32; A2 GS 37–39	49.92 bcd	± 0.37	48.67 c	± 0.27
4. A1 5–7 days after rain; A2 two weeks after A1	50.47 d	± 0.30	49.78 de	± 0.32
5. A1 5–7 days after rain	49.52 b	± 0.26	47.33 b	± 0.25
6. A1 8–10 days after rain; A2 two weeks after A1	50.19 bcd	± 0.31	49.98 e	± 0.16
7. A1 8–10 days after rain	48.31 e	± 0.07	47.34 b	± 0.03
	2021			
	monoculture		after non-host	
1. Untreated (control)	40.62 a	± 0.06	38.97 a	± 0.39
2. A1 GS 32; A2 GS 51–55	41.02 abc	± 0.20	39.90 bcd	± 0.06
3. A1 GS 32; A2 GS 37–39	40.63 a	± 0.07	40.18 bcd	± 0.25
4. A1 5–7 days after rain; A2 two weeks after A1	41.16 abc	± 0.26	40.25 cd	± 0.27
5. A1 5–7 days after rain	40.63 a	± 0.09	39.47 ab	± 0.28
6. A1 8–10 days after rain; A2 two weeks after A1	41.23 c	± 0.34	40.52 d	± 0.20
7. A1 8–10 days after rain	40.67 abc	± 0.39	40.21 cd	± 0.30

Note. The difference between the values with the same letter was not significant between different application programmes according to Duncan's multiple range test.

Table 5. Pearson's correlation coefficient (*r*) for relationships between Septoria tritici blotch AUDPC values, winter wheat thousand grain weight (TGW), and grain yield

Year	TGW		Grain yield	
	r	*p*-value	*r*	*p*-value
Monoculture				
2019	−0.952***	*0.0011*	ns	*0.4480*
2020	−0.914**	*0.0040*	ns	*0.2593*
2021	ns	*0.3200*	ns	*0.2215*
After non-host				
2019	ns	*0.1631*	ns	*0.1187*
2020	−0.9843****	*<0.0001*	−0.9533***	*0.0009*
2021	−0.9015**	*0.0055*	ns	*0.0933*

* – significant at **** <0.0001, *** <0.001 and ** <0.01; ns – not significant

In winter wheat monoculture, a strong significant correlation between AUDPC values and TGW was established in 2019 and 2021; meanwhile, in winter wheat grown after non-host crops it established itself in 2020 and 2021. In most cases, the correlation between AUDPC and grain yield was not significant; statistical analysis demonstrated a strong significant correlation only in winter wheat grown after non-host crops in 2020.

Conclusions

1. In the experiment, the severity of Septoria tritici blotch (STB) varied between experimental years and crop rotations. Different weather conditions during the experimental period were responsible for the differences of STB severity in winter wheat. The highest disease severity (AUDPC values) was recorded in 2020, when the weather was warm and humid. Between the crop rotations, higher disease severity in winter wheat growing as monoculture was in 2019. Meanwhile, in 2020 and 2021, AUDPC values were higher in winter wheat grown after non-host crops.

2. The effectiveness of fungicide application programmes was more pronounced in experimental years 2020 and 2021 with higher disease severity. The average data showed that more effective control of STB was, when fungicide applying was chosen based on weather conditions and fungicide was used twice.

3. A more important influence of fungicide application programmes on grain yield increase was found in winter wheat monoculture. In all three experimental years, the grain yield of winter wheat grown after non-host crops was higher than that of monoculture: 68.5% in 2019, 32.6% in 2020, and 52.6% in 2022.

4. Pearson's correlation test showed a strong relationship between visual assessment of disease severity and DNA quantity of *Zymoseptoria tritici* in wheat leaves in both crop rotations. *Z. tritici* DNA was detected in 96.5% of the tested leaf samples without visible symptoms of the disease.

These results show that early diagnosis of disease may play an important role in disease control.

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Fungicidų purškimo programų įtaka lapų septoriozės intensyvumui žieminiuose kviečiuose

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Santrauka

Zymoseptoria tritici sukelia kviečių lapų septoriozę, kuriai plisti vienas svarbiausių veiksnių yra aplinkos sąlygos. Ligos plitimas kontroliuojamas naudojant fungicidus, todėl labai svarbu tinkamai pasirinkti jų panaudojimo laiką. Tyrimo tikslas – įvertinti dviejų fungicidų panaudojimo programų efektyvumą septoriozės kontrolei žieminiuose kviečiuose ir nustatyti ryšį tarp ligos intensyvumo bei *Z. tritici* DNR kiekio kviečių lapuose. Vykdyti du lauko eksperimentai su žieminiais kviečiais, augintais kaip monokultūra ir lauke po augalo ne šeimininko, ir taikyti šeši purškimo fungicidais variantai. Ligos kontrolei naudotas fungicidas Adexar (v. m. fluksapiroksadas 62,5 g l^{-1} + epoksikonazolas 62,5 g l^{-1}) 1,0 ir 2,0 l ha^{-1}. Dėl nepalankių meteorologinių sąlygų 2019 m. augalų užkrėstumas septorioze buvo mažas. Vis dėlto, lyginant abi sėjomainas, septoriozės didesnio intensyvumo tendencijos nustatytos žieminių kviečių monokultūroje. 2020 ir 2021 m. sezono metu ligos progreso AUDPC reikšmės buvo didesnės žieminiuose kviečiuose, augusiuose po augalo ne šeimininko. Skirtumai tarp purškimo fungicidais programų buvo ryškesni esant didesniam ligos intensyvumui 2020 ir 2021 m. Remiantis trejų metų lauko eksperimento rezultatais, septoriozės kontrolei efektyviausias buvo fungicidų purškimas du kartus atsižvelgiant į oro sąlygas. Didesnė fungicidų purškimo programų įtaka grūdų derliaus padidėjimui nustatyta žieminių kviečių monokultūroje. Fungicidų panaudojimas didesnį poveikį turėjo 2020 m., kai septoriozės intensyvumas buvo didesnis (AUDPC 358.6). Abiejose sėjomainose didžiausias grūdų derliaus padidėjimas gautas fungicidą panaudojus du kartus: 8–10 dienų po lietaus ir dvi savaitės po pirmojo panaudojimo. Pirsono koreliacijos testas parodė stiprų ryšį tarp ligos intensyvumo ir *Z. tritici* DNR kiekio žieminių kviečių lapuose.

Reikšminiai žodžiai: purškimų programos, AUDPC, realaus laiko PGR, septoriozė, *Zymoseptoria tritici*.