agronomy

MDPI

Article

Sensitivity of Lithuanian *Zymoseptoria tritici* to Quinone Outside Inhibitor and Succinate Dehydrogenase Inhibitor Fungicides

Karolina Lavrukaitė *, Mohammad Almogdad, Jūratė Ramanauskienė and Aurimas Sabeckis

Lithuanian Research Centre for Agriculture and Forestry, Instituto av. 1, LT-58344 Akademija, Lithuania; mohammad.almogdad@lammc.lt (M.A.); jurate.ramanauskiene@lammc.lt (J.R.); aurimas.sabeckis@lammc.lt (A.S.)
* Correspondence: karolina.lavrukaite@lammc.lt

Abstract: Septoria tritici blotch (STB) ais one of the most damaging winter wheat diseases worldwide, presenting a significant threat to its yields. The causal STB agent, *Zymoseptoria tritici*, also presents a challenge to control due to its rapid adaptation to fungicides. This requires researchers to continuously monitor the pathogen and investigate and explore strategies to manage the spread of the disease and the development of resistance in the pathogen. Therefore, this study presents the current situation and describes changes in the sensitivity of *Z. tritici* isolates from Lithuania to quinone outside inhibitors (QoIs) and succinate dehydrogenase inhibitors (SDHIs) for the years 2019–2022. The isolates were tested at five different concentrations of two QoI fungicides (azoxystrobin and pyraclostrobin) and three SDHI fungicides (fluxapyroxad, benzovindiflupyr, and bixafen). During the test period, the EC50 values of the tested QoIs increased, while no clear changes were observed in the SDHIs. The most pronounced shift was observed for the active QoI substance pyraclostrobin. The distribution of the EC50 values of the SDHI fungicides showcased one isolate with an outstandingly high EC50 value of 2.6 mg L^{-1}. The results of this study did not reveal any strong patterns of cross-resistance between the fungicides tested. However, a significant positive, moderate correlation (r = 0.55) was found between fluxapyroxad and benzovindiflupyr. Overall, the results of this study contribute to the understanding of the fungicide-resistance situation of *Z. tritici* in Lithuania and may complement management strategies for the pathogen and its fungicide resistance.

Keywords: Septoria tritici blotch; succinate dehydrogenase inhibitors; quinone outside inhibitors; fungicide resistance; correlation

Citation: Lavrukaitė, K.; Almogdad, M.; Ramanauskienė, J.; Sabeckis, A. Sensitivity of Lithuanian *Zymoseptoria tritici* to Quinone Outside Inhibitor and Succinate Dehydrogenase Inhibitor Fungicides. *Agronomy* **2024**, *14*, 813. https://doi.org/10.3390/agronomy14040813

Academic Editor: Francesco Calzarano

Received: 14 March 2024
Revised: 8 April 2024
Accepted: 11 April 2024
Published: 13 April 2024

1. Introduction

The fungal pathogen *Zymoseptoria tritici* (Desm.) [1], which causes the foliar disease Septoria tritici blotch (STB) in winter wheat, has been threatening farmers' agricultural production for decades. This disease has been recognised as a major limiting factor in wheat cultivation, particularly in regions with temperate climates. It is estimated that yield losses can reach up to 50% if the disease is left uncontrolled under favourable conditions [2]. Despite considerable efforts invested in researching this pathogen, effective control has remained challenging, with chemical control remaining the main approach [3]. Fungicides with different modes of action and target sites are used for STB control. The four main groups of fungicides used against STB in Lithuania are demethylation inhibitors (DMIs—Fungicide Resistance Action Committee (FRAC) group 3), succinate dehydrogenase inhibitors (SDHIs—FRAC group 7), quinone inside inhibitors (QiIs—FRAC group 21), and quinone outside inhibitors (QoIs—FRAC group 11) [4]. Each of the aforementioned groups targets a specific single site; therefore, *Z. tritici* is prone to developing resistance towards each of them [5], especially due to its evolutionary adaptation to new conditions, further complicating control efforts. Additionally, each of these groups is ranked according

to its risk of resistance development. DMIs are ranked as having a medium risk of resistance development, SDHIs are ranked as having a medium to high risk, QoIs are ranked as having a high risk [4], and QiIs are predicted to have a medium to high risk [6]. These risks make it necessary to be more cautious with the use of fungicides, especially those with a medium to high risk [4]. The main aspect mediating resistance against fungicides in pathogens is the occurrence of mutations in the target site [7].

QoIs were registered on the market in the mid-1990s and were widely used for the control of *Z. tritici* and other pathogens until resistance patterns were noticed. As QoI acts on the quinol outer (Qo) binding site by inhibiting the cytochrome b (*cytb*) complex, the major factor contributing to QoI resistance involves a specific nucleotide mutation wherein glycine alters to alanine at position 143 (G143A) within the Cybt gene Qo site [8]. Populations of *Z. tritici* carrying this mutation exhibit high or complete resistance to QoI fungicides [4]. This mutation has been found to have developed independently in different geographical areas of Europe, further increasing fungicide resistance. The presence of these mutations at the Qo site, and hence resistance to QoI fungicides, is widespread, leading to a decline in their use against this disease in Europe and the United States of America [9–11]. However, in Lithuania and other Northeastern European countries, QoI fungicides are still moderately effective and are therefore used to control STB and other diseases [12,13].

SDHI fungicides act by inhibiting the succinate dehydrogenase (SDH) enzyme involved in the mitochondrial respiration chain of the fungus. These fungicides have been used to control other pathogens since the 1960s [14]. The first SDHI fungicide to show efficacy against *Z. tritici*, boscalid, was introduced to agricultural markets in the mid-2000s [15]. SDHI fungicides against STBs (such as fluxapyroxad, benzovindiflupyr, and bixafen) appeared on the market more than a decade ago and are still very effective in many countries [16,17]. Initially, resistance to this group was observed in Ireland and the United Kingdom, but intensive use is leading to the development of resistance in other countries as well [18–20]. The reduced sensitivity of *Z. tritici* to SDHI fungicides is associated with single amino acid substitutions in the target site of the SDH subunits B, C, and D, with subunit SDHC carrying the alterations with the greatest effect on resistance development [14]. At present, these mutations are being detected in most European *Z. tritici* populations together with reduced sensitivity to SDHIs [16,19–21].

As for the last and, for a long time, the most important fungicide group, DMIs, several fungicides in this group are also no longer effective against STB due to the presence of mutations in the CYP51 gene and other mechanisms [22,23]. To this day, DMIs are still widely used for STB control, with prothioconazole acting as the backbone of disease management strategies for several years. However, the decline in efficacy of this fungicide was already noticed in 2015 [22]. Due to the declining efficacy of DMI fungicides such as prothioconazole, control of STB now relies more heavily on products containing active ingredients from the SDHI group, often in combination with DMIs or QoIs. The combination of active ingredients from different groups aims not only to increase efficacy in the field but also to delay the development of resistance [18].

In recent years, the new active DMI ingredient mefentrifluconazole [24] and the QiI active ingredient fenpicoxamide [25] were introduced on the European market. Both have high efficacy against STB and can be incorporated in disease management strategies, thus slightly widening the limited options currently available. With the regulations of the EU becoming increasingly restrictive for fungicide usage [26], it is necessary to monitor the situation of resistance in local field strains of *Z. tritici* towards SDHI-group fungicides. This would allow the desired efficacy of a few products still available on the market to be maintained [22,27].

The objectives of this study were (i) to assess the sensitivity of *Z. tritici* to fungicides of the QoI and SDHI groups and (ii) to test cross-resistance relationships between fungicides within a single chemical group (for QoI, between azoxystrobin and pyraclostrobin; for SDHI, between fluxapyroxad, benzovindiflupyr and bixafen) and between fungicides of different groups.

2. Materials and Methods

2.1. Leaf Sampling and Isolation of Zymoseptoria tritici

Upper winter wheat leaves with a clear septoria leaf blotch infection (distinctive lesions typical of the *Z. tritici* infection) were randomly collected from uniformly treated winter wheat fields throughout Lithuania (primarily from central and northern Lithuania) (22 separate fields in 2019, 25 in 2020, 23 in 2021, and 12 in 2022). The leaves (without prior sterilisation) were cut into 2 cm pieces and placed in Petri dishes on moistened filter paper and incubated in the dark at 18 °C for 18–22 h to stimulate sporulation. Cirrhi from a single pycnidium were picked using a sterile needle and transferred to Petri dishes containing potato dextrose agar (PDA) amended with 100 mg L^{-1} of streptomycin sulphate. After 4–5 days of incubation at 20 °C under a 12 h white light/12 h dark cycle, individual spore colonies were transferred to fresh PDA. A total of 116 *Z. tritici* isolates were collected for the period from 2019 to 2022.

2.2. Fungicide Sensitivity Testing

Suspensions of *Z. tritici* spores were prepared by scraping off the spore colonies and transferring them into sterile water. The final spore concentrations were adjusted to 1×10^5 spores ml^{-L}. The sensitivity to fungicides mentioned above was determined by a microtiter plate assay. The fungicides were dissolved in ≥99.8% acetone (Sigma-Aldrich, St. Louis, MO, USA) and diluted in the PDA media to meet the required concentrations. The wells of the microtiter plates were filled with 200 µL of liquid PDA medium supplemented with fungicides; then, 20 µL of the *Z. tritici* spore suspension was added to each well (after the medium had solidified). Each isolate was prepared in duplicate. Microtiter plates were sealed with parafilm to prevent contamination and desiccation, covered with foil, and incubated in the dark at 20 °C for 5–6 days. To evaluate inhibition, fungal growth assessments were conducted visually at the end of incubation in which the control wells of each isolate were assessed as 100% growth and the remaining concentrations were compared to the control.

2.3. Fungicides

The concentrations for the QoI-group fungicides were set to 100, 10, 1.0, 0.1, and 0.01 mg of active ingredient per litre (mg a.i. L^{-1}) for azoxystrobin and 10, 1.0, 0.1, 0.01, and 0.001 mg a.i. L^{-1} for pyraclostrobin. As the pathogen is more sensitive to SDHI fungicides, the set concentrations were lower for the active ingredients fluxapyroxad, benzovindiflupyr, and bixafen: 3.0, 1.0, 0.33, 0.11, and 0.037 mg a.i. L^{-1}. Each of the sets included a control in which only water was added to the microtiter plate wells. All active ingredients were purchased from Sigma-Aldrich, St. Louis, MO, USA.

2.4. Statistical Analysis

Sensitivity to fungicides was calculated using a non-linear regression (curve fit) as the concentration of fungicide which inhibits fungal growth by half (EC50). To determine cross-resistance between the different fungicides, a correlation analysis was performed using the EC50 values of the fungicides for each isolate. For the correlation analysis, Pearson's r correlation coefficients and probability values were calculated. All calculations were carried out using GraphPad Prism version 10.1.2 (GraphPad Software, La Jolla, CA, USA).

3. Results

The fungicide sensitivity situation and possible changes in the isolated *Z. tritici* field populations during the 2019–2022 period were tested for the QoIs azoxystrobin and pyraclostrobin and for the SDHIs fluxapyroxad, benzovindiflupyr, and bixafen (Table 1). The EC50 values for azoxystrobin ranged from 0.009 to 29.140 mg L^{-1}, and for pyraclostrobin, they ranged from 0.003 to 3.220 mg L^{-1} throughout the years. For both QoI fungicides, there was an increase in the mean EC50 value, though the increase was more pronounced for pyraclostrobin (from 0.282 in 2019 to 1.563 mg L^{-1} in 2022). As expected, the average

EC50 values for QoIs were higher than for SDHIs. Throughout the years, the EC50 values for fluxapyroxad, benzovindiflupyr, and bixafen ranged from 0.072 to 1.568 mg L^{-1}, from 0.019 to 1.597 mg L^{-1}, and from 0.081 to 2.602 mg L^{-1}, respectively. Even though the average EC50 values varied from year to year, no clear increase was observed.

Table 1. Mean EC_{50} (mg L^{-1}) values for azoxystrobin, pyraclostrobin, fluxapyroxad, benzovindiflupyr, and bixafen in *Zymoseptoria tritici* isolates from Lithuania.

Fungicides	Years			
	2019	2020	2021	2022
Azoxystrobin	6.292 (0.136–20.250) [1]	4.864 (0.616–22.540)	9.787 (0.009–29.140)	10.790 (0.905–23.925)
Pyraclostrobin	0.282 (0.004–1.198)	1.099 (0.025–2.128)	1.175 0.007–3.197)	1.563 (0.003–3.220)
Fluxapyroxad	0.260 (0.072–0.579)	0.564 (0.232–1.568)	0.331 (0.124–0.864)	0.483 (0.187–0.985)
Benzovindiflupyr	0.194 (0.019–0.444)	0.598 (0.153–1.597)	0.277 (0.120–0.780)	0.549 (0.180–1.144)
Bixafen	0.379 (0.081–1.594)	0.681 (0.171–2.602)	0.283 (0.138–0.590)	0.599 (0.229–1.478)

[1] ranges of values are shown in brackets.

The EC50 distribution patterns of the QoI-group fungicides are presented in Figure 1. The distribution of sensitivity shows that throughout the testing period, only a few isolates had EC50 values below 1 and 0.1 mg L^{-1} for azoxystrobin and pyraclostrobin, respectively. As for the shift towards higher concentrations for azoxystrobin, it happened gradually, whereas for pyraclostrobin, there was a drastic shift from 2019 to 2020. In 2019, for pyraclostrobin, there were only two isolates with EC50 values barely reaching the 1.0 mg L^{-1} mark, but in the following years, a greater proportion of the isolates had values exceeding 1.0 mg L^{-1}. This illustrates a great increase in the average EC50 values for pyraclostrobin.

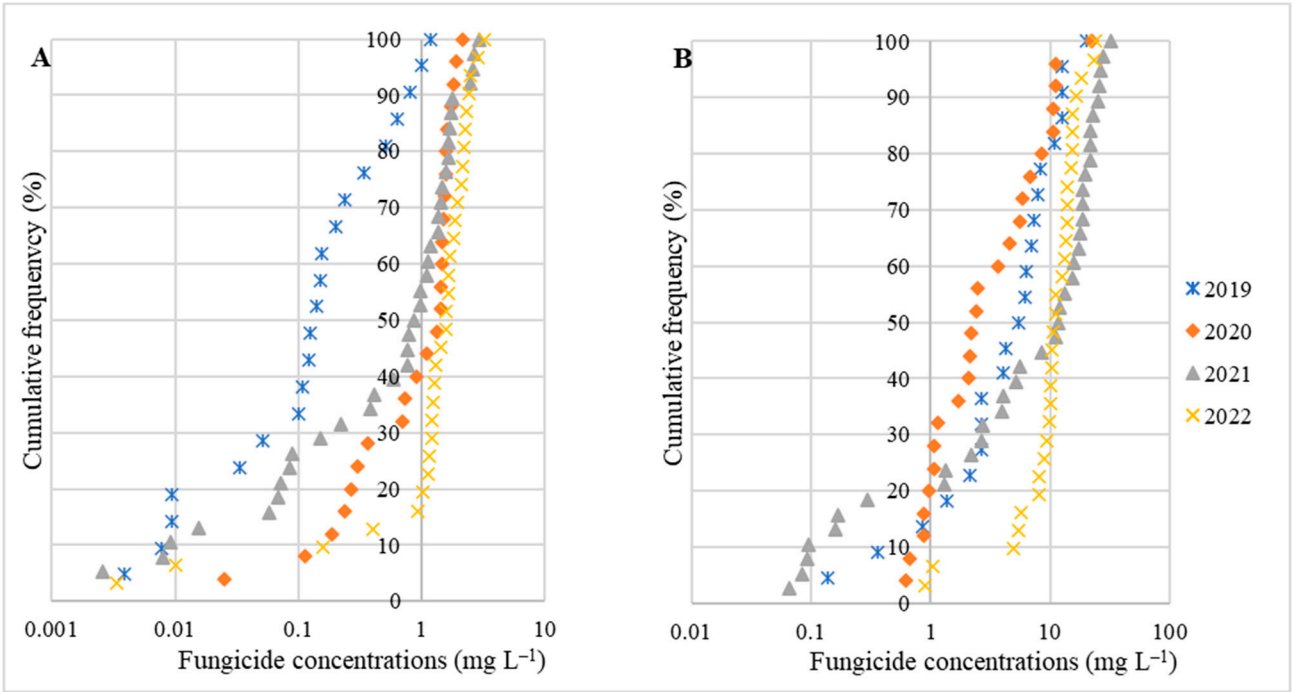

Figure 1. Distribution of sensitivity of *Zymoseptoria tritici* isolates to QoI fungicides: (**A**) pyraclostrobin; (**B**) azoxystrobin. Isolates are ranked according to cumulative EC50 values.

The EC50 distribution patterns of the SDHI-group fungicides are presented in Figure 2. In contrast to the QoI fungicides, a slightly different situation was observed for the three tested SDHIs. For all the fungicides, the majority of EC50 values lay in concentrations lower than 1.0 mg L^{-1}. For benzovindiflupyr, there are two distinct groups with similar distributions of EC50 values in 2019 and 2021 and in 2020 and 2022. In the first group, the EC50 values are distributed among the lower concentrations not exceeding 1.0 mg L^{-1}, while in the second group, few isolates surpass this concentration, with half of the isolates having EC50 values greater than 0.5 mg L^{-1}. Of all the SDHIs studied, the distribution of EC50 values for bixafen was the most scattered. The distribution of EC50 values for bixafen, although showcasing similar patterns to benzovindiflupyr, has a few more the isolates with EC50 values exceeding 1.0 mg L^{-1}, with one isolate reaching a concentration of 2.6 mg L^{-1}. In contrast, the EC50 values for fluxapyroxad were the least dispersed; the majority of the isolates tested throughout the years had EC50 values lower than 0.5 mg L^{-1}, with only one isolate from 2020 exceeding a concentration of 1.0 mg L^{-1}.

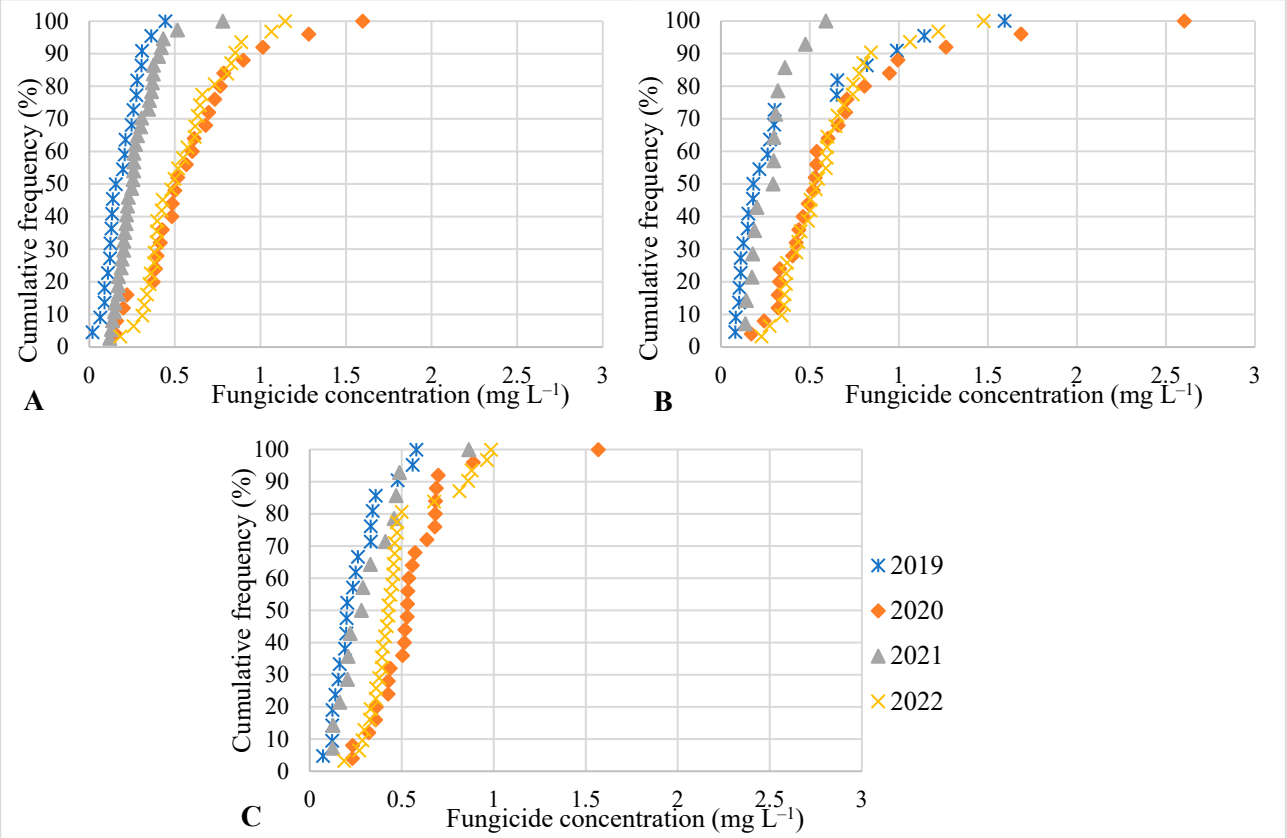

Figure 2. Distribution of sensitivity of *Zymoseptoria tritici* isolates to SDHI fungicides: (**A**) benzovindiflupyr; (**B**) bixafen; (**C**) fluxapyroxad. Isolates are ranked according to cumulative EC50 values.

The EC_{50} values for all tested fungicides were further analysed utilising Pearson's correlation (Table 2). Looking into correlations between fungicide EC50 values, moderate, positive cross-resistance appeared only between the SDHIs fluxapyroxad and benzovindiflupyr ($r = 0.55$, $p \leq 0.001$), and the correlation was significant. Additionally, a significant, albeit weaker, positive cross-resistance was observed between benzovindiflupyr and bixafen. No significant correlation was found between fluxapyroxad and bixafen. The QoIs azoxystrobin and pyraclostrobin exhibited a weak to moderate positive correlation ($r = 0.42$, $p \leq 0.001$) between their EC50 values. Pyraclostrobin also exhibited comparatively weak positive cross-resistance ($r = 0.20$ to 0.36) with all tested SDHIs, and this correlation was statistically significant. No correlation was found between azoxystrobin and SDHIs; the r values for these comparisons ranged from −0.095 to −0.05, indicating no meaningful relationship.

Table 2. Cross-resistance between QoI and SDHI fungicides based on sensitivity data (EC50) in 2019–2022.

	Azoxystrobin	Pyraclostrobin	Fluxapyroxad	Benzovindiflupyr	Bixafen
Azoxystrobin	1				
Pyraclostrobin	0.418 **	1			
Fluxapyroxad	0.080	0.262 *	1		
Benzovindiflupyr	−0.095	0.203 *	0.550 **	1	
Bixafen	−0.050	0.359 **	0.418	0.374 **	1

* denotes significance at the level of $p \leq 0.05$; ** denotes significance at the level of $p \leq 0.01$.

4. Discussion

Every year, hundreds of isolates of *Z. tritici* are screened worldwide for a possible loss of sensitivity to various fungicides [3,20,28,29]. These screenings provide researchers and manufacturers with valuable information for future decisions. Fungicide sensitivity testing of the Lithuanian *Z. tritici* population was conducted in several attempts over the last decade, with a focus on DMI fungicides [12,13,22,30]. All these previous studies have already showcased slight changes in the sensitivity of *Z. tritici* to various fungicides. The findings of this article are focused on QoI and SDHI fungicide resistance monitoring.

As the QoIs demonstrate high efficacy and additional beneficial effects on plant development (a greening effect), these fungicides are widely used for STB control [31]. Due to their popularity, the resistance of *Z. tritici* to QoIs was first detected in the early 2000s [8], not long after their introduction to the market. In the years that followed, the G143A mutation conferring resistance to QoI fungicides was detected in several countries in Europe [9,10], and later, it was observed in North America [32], Africa [33], and Oceania [34]. The findings of this study confirm that the sensitivity of the *Z. tritici* population to azoxystrobin and pyraclostrobin has been observed to decrease and that the use of these fungicides against the pathogen should be reconsidered, if not completely discontinued. Previous studies of the Lithuanian *Z. tritici* population for the G143A mutation showed that over the past decade, the frequency of isolates carrying this mutation has almost quadrupled, and in 2021, more than 75% of the isolates tested had this mutation [13,22]. This increase in the frequency of this alteration could also explain the shift in EC50 values towards higher concentrations over the 4-year period observed in the current study. Moreover, the efficacy of these fungicides in field experiments in a study by Lavrukaite et al. [13] was also on the lower side, achieving only up to 55 percent disease control, thus explaining and depicting the higher EC50 values obtained in this study. And although a relatively new compound for *Z. tritici* control, fenpicoxamide, also acts on the respiration complex III, it is fortunately unaffected by the alteration of G143A in *cytb* [25]. Additionally, metyltetraprole, a recently developed fungicidal compound, also binds at the Qo site and is unaffected by the G143A mutation; it is therefore categorised into FRAC Group 11A for its unique efficacy against QoI-resistant fungi. It is expected to be a novel addition to the list of products against STB [35].

The first reports of Z. tritici's decreasing sensitivity towards SDHIs were confirmed in laboratory mutants [36,37]. Later, this decreased sensitivity was detected in field strains as well [16,18]. The main mutations associated with a decrease in sensitivity towards these fungicides are C-T79N, C-N86S, and C-H152R. The latter confers the highest levels of resistance; however, it is rarely detected in field conditions, which is usually associated with fitness penalties, whereas the other two mutations are found in most Western European countries [16,19]. Fortunately, to the authors' knowledge, none of these substitutions have been detected in Lithuania or Latvia so far. However, in Estonia, according to a study by Kiiker et al. in 2020 [38], 9% of the isolates tested had the C-N86S mutation. Despite the presence of this mutation, it did not affect the sensitivity of the isolates to SDHI-group fungicides. The data presented in the study by Lavrukaite et al. [13] showed that no mutations associated with resistance to SDHIs were detected in the Lithuanian *Z. tritici* population from 2021. Therefore, relatively low EC50 values for the SDHIs observed in

the current study may be explained by previous data from Lithuania. However, there is a question regarding the few isolates in this study with high EC50 values and whether this is due to other mutations that have not been studied or if it is related to the in planta degradation of SDHI induced by a plant or fungus, as suggested by Mäe et al. [12].

As mentioned in the Introduction, fungicide resistance is mainly driven by specific target site mutations, but there is also a non-specific mechanism associated with the loss of fungicide sensitivity, namely increased efflux, referred to as multidrug resistance (MDR) [39]. It is known that MDR leads to moderate resistance to all three of the main fungicide groups mentioned previously (DMI, SDHI, and QoI) [39,40]. MDR is caused by insertions in the promoter region of the major facilitator gene (MFS1). At present, 13 insertions have been identified [12,13,40,41], although not all of these confer resistance. In a previous study by Lavrukaite et al. [13], an insert with size of 1940 bp (type V) was found among Lithuanian isolates; however, it did not result in MDR. Meanwhile, in a study by Glaab et al. [42], a small proportion of isolates carrying insertions with sizes ranging from approximately 250–400 to 400–600 base pairs were found. The insertion of 519 bp has been one that correlates with the highest resistance towards all fungicide groups [40] Thus, the insertion between 400 and 600 bp found by Glaab et al. [42] might indicate the beginning of the spread of these insertions in Lithuania.

All the investigated SDHIs belong to the same chemical group within SDHIs called pyrazole carboxamides and have similar chemical structures [3,10]. Therefore, it is expected that these fungicides will show cross-resistance [43]. Despite the possibility of cross-resistance patterns between SDHIs, specific mutations with different influences on different active substances have been detected [21,37]. Even though in the current study, there was a moderate positive correlation between fluxapyroxad and benzovindiflupyr, the correlation was not as strong as that reported by other researchers. Steihauer et al. [44] found a very strong, positive correlation between these two fungicides, as did Jørgensen et al. [43], who found a strong correlation between all three of our tested fungicides. Yamashita and Fraaije [21] as well as other researchers found a very strong positive correlation between fluxapyroxad and bixafen. In contrast to other studies, Hagerty et al. [45] found no correlation between fluxapyroxad and benzovindiflupyr. Both SDHIs and QoIs act on the pathogen's respiration mechanisms; however, they act at the different steps of the process. SDHIs act in respiration chain complex II, and QoIs act in complex III [4]. Lithuanian farmers use a combination of fluxapyroxad and pyraclostrobin to control diseases as this combination is registered on the Lithuanian market, and fortunately, the correlation between the two was one of the weakest of all the combinations tested in this study. In terms of cross-resistance between QoIs, it is known that isolates harbouring the G143A mutation tend to exhibit high levels of cross-resistance to different fungicides from QoIs [4,12]. However, the fungicides investigated in this study showcased only a weak correlation. Overall, the presented study showcased only moderate or close-to-moderate cross-resistance relationships between fungicides within same group and low or almost non-existent relationships between fungicides of different groups.

To avoid further the development of pathogen resistance to fungicides, it is essential and even mandatory to implement all possible measures recommended by other researchers [17,46] and FRAC working groups for anti-resistance strategies [4]. These measures include implementing host resistance, adopting agronomic plant protection practices, minimising the number of fungicide applications, ensuring appropriate application timings, and the use of mixtures or alterations of fungicides with different modes of action, and, finally, conducting systematic sensitivity monitoring to detect changes in products performance [4].

5. Conclusions

The conclusions drawn from the study on *Zymoseptoria tritici's* sensitivity to various fungicides provide significant insights into the current landscape of fungicide resistance in Lithuania. The findings indicate a notable decrease in the sensitivity of *Z. tritici* populations

to azoxystrobin and pyraclostrobin, suggesting a growing resistance to these fungicides. This decrease in sensitivity aligns with the detection of the G143A mutation and highlights a concerning scenario already observed in most European countries. Regarding sensitivity towards SDHI fungicides, although this study shows that the sensitivity of *Z. tritici* to SDHIs has changed only slightly, further monitoring of the situation is necessary, especially regarding the possibility of mutation emergence. The findings of this study also emphasise the importance of ongoing research for effective disease management strategies to mitigate the risk of fungicide resistance development in *Z. tritici* populations.

Author Contributions: Conceptualization, K.L.; methodology, K.L.; formal analysis, K.L.; investigation, K.L. and A.S.; writing—original draft preparation, K.L.; writing—review and editing, M.A., J.R. and A.S.; supervision, J.R. All authors have read and agreed to the published version of the manuscript.

Funding: Part of this research was supported by the long-term research program "Harmful organisms in agro and forest ecosystems" implemented by the Lithuanian Research Centre for Agriculture and Forestry.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Quaedvlieg, W.; Kema, G.H.J.; Groenewald, J.Z.; Verkley, G.J.M.; Seifbarghi, S.; Razavi, M.; Mirzadi Gohari, A.; Mehrabi, R.; Crous, P.W. *Zymoseptoria* gen. nov.: A new genus to accommodate Septoria-like species occurring on graminicolous hosts. *Persoonia Mol. Phylogeny Evol. Fungi* **2011**, *26*, 57–69. [CrossRef] [PubMed]
2. Jørgensen, L.N.; Hovmøller, M.S.; Hansen, J.G.; Lassen, P.; Clark, B.; Bayles, R.; Rodemann, B.; Flath, K.; Jahn, M.; Goral, T.; et al. IPM Strategies and their dilemmas including an introduction to www.eurowheat.org. *J. Integr. Agric.* **2014**, *13*, 265–281. [CrossRef]
3. Jørgensen, L.N.; Matzen, N.; Hansen, J.G.; Semaskiene, R.; Korbas, M.; Danielewicz, J.; Glazek, M.; Maumene, C.; Rodemann, B.; Weigand, S.; et al. Four azoles' profile in the control of septoria, yellow rust and brown rust in wheat across Europe. *Crop Prot.* **2018**, *105*, 16–27. [CrossRef]
4. FRAC (Fungicide Resistance Action Committe). Available online: https://www.frac.info/ (accessed on 1 March 2024).
5. Lucas, J.A.; Hawkins, N.J.; Fraaije, B.A. The evolution of fungicide resistance. *Adv. Appl. Microbiol.* **2015**, *90*, 29–92. [PubMed]
6. Fouché, G.; Michel, T.; Lalève, A.; Wang, N.X.; Young, D.H.; Meunier, B.; Debieu, D.; Fillinger, S.; Walker, A.S. Directed evolution predicts cytochrome *b* G37V target site modification as probable adaptive mechanism towards the QiI fungicide fenpicoxamid in *Zymoseptoria tritici*. *bioRxiv* **2021**. [CrossRef] [PubMed]
7. Deising, H.B.; Reimann, S.; Pascholati, S.F. Mechanisms and significance of fungicide resistance. *Braz. J. Microbiol.* **2008**, *39*, 286–295. [CrossRef] [PubMed]
8. Fraaije, B.A.; Lucas, J.A.; Clark, W.S.; Burnett, F.J. QoI resistance development in populations of cereal pathogens in the UK. In Proceedings of the BCPC International Congress—Crop Science and Technology, Glasgow, UK, 10–12 November 2003; pp. 689–694.
9. Torriani, S.F.F.; Brunner, P.C.; McDonald, B.A.; Sierotzki, H. QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. *Pest Manag. Sci.* **2009**, *65*, 155–162. [CrossRef] [PubMed]
10. Drabešová, J.; Ryšánek, P.; Brunner, P.; McDonald, B.A.; Croll, D. Population genetic structure of *Mycosphaerella gramicola* and Quinone Outside Inhibitor (QoI) resistance in the Czech Republic. *Eur. J. Plant Pathol.* **2013**, *135*, 211–224. [CrossRef]
11. Hagerty, C.H.; Anderson, N.P.; Mundt, C.C. Temporal dynamics and spatial variation of *Zymoseptoria tritici* azoxystrobin and propiconazole fungicide resistance: A hierarchical survey of commercial winter wheat fields in the Willamette Valley of Oregon. *Phytopathology* **2017**, *107*, 345–352. [CrossRef]
12. Mäe, A.; Fillinger, S.; Sooväli, P.; Heick, T.M. Fungicide sensitivity shifting of *Zymoseptoria tritici* in the Finnish-Baltic region and a novel insertion in the MFS1 promoter. *Front. Plant Sci.* **2020**, *11*, 519898. [CrossRef]
13. Lavrukaitė, K.; Heick, T.M.; Ramanauskienė, J.; Armonienė, R.; Ronis, A. Fungicide sensitivity levels in the Lithuanian *Zymoseptoria tritici* population in 2021. *Front. Plant Sci.* **2023**, *13*, 1075038. [CrossRef] [PubMed]
14. Sierotzki, H.; Scalliet, G. A review of current knowledge of resistance aspects for the next-generation succinate dehydrogenase inhibitor fungicides. *Phytopathology* **2013**, *103*, 880–887. [CrossRef] [PubMed]
15. Glättli, A.; Grote, T.; Stammler, G. SDH-inhibitors: History, biological performance and molecular mode of action. In *Modern Fungicides and Antifungal Compounds*; Dehne, H.W., Deising, H.B., Gisi, U., Kuck, K.H., Russell, P.E., Lyr, H., Eds.; DPG: Braunschweig, Germany, 2011; Volume VI, pp. 159–170.
16. Rehfus, A.; Strobel, D.; Bryson, R.; Stammler, G. Mutations in sdh genes in field isolates of *Zymoseptoria tritici* and impact on the sensitivity to various succinate dehydrogenase inhibitors. *Plant Pathol.* **2018**, *67*, 175–180. [CrossRef]

17. Vestergård, N.F.; Jørgensen, L.N.; Hellin, P.; Heick, T.M. Fungicide spraying intensity in the field drives the selection of amino acid alteration conferring resistance in *Zymoseptoria tritici*. *Eur. J. Plant Pathol.* **2023**, *166*, 385–401. [CrossRef]
18. Dooley, H.; Shaw, M.W.; Mehenni-Ciz, J.; Spink, J.; Kildea, S. Detection of *Zymoseptoria tritici* SDHI-insensitive field isolates carrying the SdhC-H152R and SdhD-R47W substitutions. *Pest Manag. Sci.* **2016**, *72*, 2203–2207. [CrossRef] [PubMed]
19. Hellin, P.; Duvivier, M.; Heick, T.M.; Fraaije, B.A.; Bataille, C.; Clinckemaillie, A.; Legrève, A.; Jørgensen, L.N.; Andersson, B.; Samils, B.; et al. Spatio-temporal distribution of DMI and SDHI fungicide resistance of *Zymoseptoria tritici* throughout Europe based on frequencies of key target-site alterations. *Pest Manag. Sci.* **2021**, *77*, 5576–5588. [CrossRef] [PubMed]
20. Birr, T.; Hasler, M.; Verreet, J.-A.; Klink, H. Temporal Changes in Sensitivity of *Zymoseptoria tritici* Field Populations to Different Fungicidal Modes of Action. *Agriculture* **2021**, *11*, 269. [CrossRef]
21. Yamashita, M.; Fraaije, B.A. Non-target site SDHI resistance is present as standing genetic variation in field populations of *Zymoseptoria tritici*. *Pest Manag. Sci.* **2018**, *74*, 672–681. [CrossRef]
22. Heick, T.M.; Justesen, A.F.; Jørgensen, L.N. Resistance of wheat pathogen *Zymoseptoria tritici* to DMI and QoI fungicides in the Nordic-Baltic region—A status. *Eur. J. Plant Pathol.* **2017**, *149*, 669–682. [CrossRef]
23. Blake, J.J.; Gosling, P.; Fraaije, B.A.; Burnett, F.J.; Knight, S.M.; Kildea, S.; Paveley, N.D. Changes in field dose-response curves for demethylation inhibitor (DMI) and quinone outside inhibitor (QoI) fungicides against *Zymoseptoria tritici*, related to laboratory sensitivity phenotyping and genotyping assays. *Pest Manag. Sci.* **2018**, *74*, 302–313. [CrossRef] [PubMed]
24. Bryson, R.J.; Stammler, G.; Hu, A.; Strobel, D.; Meyer, L.; Moronval, M.H. Mefentrifluconazole—The first Isopropanol-azole fungicide for the control of Zymoseptoria tritici including field isolates with known complex CYP51 haplotypes. In Proceedings of the 12e Conference International Sur les Mal. des Plantes, Vegephyl, Tours, France, 11–12 December 2018; pp. 222–231.
25. Owen, W.J.; Yao, C.; Myung, K.; Kemmitt, G.; Leader, A.; Meyer, K.G.; Bowling, A.J.; Slanec, T.; Kramer, V.J. Biological characterization of fenpicoxamid, a new fungicide with utility in cereals and other crops. *Pest Manag. Sci.* **2017**, *73*, 2005–2016. [CrossRef] [PubMed]
26. European Commission. *Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions: A Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System COM (2020) 381 Final*; European Commission: Maastricht, The Netherlands, 2020.
27. Barrès, B.; Corio-Costet, M.-F.; Debieu, D.; Délye, C.; Fillinger, S.; Grosman, J.; Micoud, A.; Siedwart, M.; Walker, A.-S. Trends and Challenges in Pesticide Resistance Detection. *Trends Plant Sci.* **2016**, *21*, 834–853. [CrossRef]
28. Sykes, E.M.; Sackett, K.E.; Severns, P.M.; Mundt, C.C. Sensitivity variation and cross-resistance of *Zymoseptoria tritici* to azole fungicides in North America. *Eur. J. Plant Pathol.* **2018**, *151*, 269–274. [CrossRef]
29. Heick, T.M.; Matzen, N.; Jørgensen, N.L. Reduced field efficacy and sensitivity of demethylation inhibitors in the Danish and Swedish *Zymoseptoria tritici* populations. *Eur. J. Plant Pathol.* **2020**, *157*, 625–636. [CrossRef]
30. Ronis, A.; Jørgensen, L.N.; Semaškienė, R.; Gaurilčikienė, I.; Ramanauskienė, J. Sensitivity of *Mycosphaerella graminicola* isolates to demethylation-inhibiting (DMI) fungicides. *Zemdirbyste-Agriculture* **2014**, *101*, 177–184. [CrossRef]
31. Bartlett, D.W.; Clough, J.M.; Godwin, J.R.; Hall, A.A.; Hamer, M.; Parr-Dobrzanski, B. The strobilurin fungicides. *Pest Manag. Sci.* **2002**, *58*, 649–662. [CrossRef] [PubMed]
32. Estep, L.K.; Zala, M.; Anderson, N.P.; Sackett, K.E.; Flowers, M.D.; McDonald, B.A.; Mundt, C. First report of resistance to QoI fungicides in North American populations of *Zymoseptoria tritici*, causal agent of Septoria tritici blotch of wheat. *Plant Dis.* **2013**, *97*, 1511. [CrossRef] [PubMed]
33. Taher, K.; Graf, S.; Fakhfakh, M.M.; Salah, H.B.; Yahyaoui, A.; Rezgui, S.; Nasraoui, B.; Stammler, G. Sensitivity of *Zymoseptoria tritici* isolates from Tunisia to pyraclostrobin, fluxapyroxad, epoxiconazole, metconazole, prochloraz and tebuconazole. *J. Phytopathol.* **2014**, *162*, 442–448. [CrossRef]
34. Stewart, T.M.; Perry, A.J.; Evans, M.J. Resistance of *Zymoseptoria tritici* to azoxystrobin and epoxiconazole in the lower North Island of New Zeland. *N. Z. Plant Protect.* **2014**, *67*, 304–313. [CrossRef]
35. Matsuzaki, Y.; Kiguchi, S.; Suemoto, H.; Iwahashi, F. Antifungal activity of metyltetraprole against the existing QoI-resistant isolates of various plant pathogenic fungi: Metyltetraprole against QoI-R isolates. *Pest Manag. Sci.* **2020**, *76*, 1743–1750. [CrossRef] [PubMed]
36. Fraaije, B.A.; Bayon, C.; Atkins, S.; Cools, H.J.; Lucas, J.A.; Fraaije, M.W. Risk assessment studies on succinate dehydrogenase inhibitors, the new weapons in the battle to control septoria leaf blotch in wheat. *Mol. Plant Pathol.* **2012**, *13*, 263–275. [CrossRef] [PubMed]
37. Scalliet, G.; Bowler, J.; Luksch, T.; Kirchhofer-Allan, L.; Steinhauer, D.; Ward, K.; Niklaus, M.; Verras, A.; Csukai, M.; Daina, A.; et al. Mutagenesis and functional studies with succinate dehydrogenase inhibitors in the wheat pathogen *Mycosphaerella graminicola*. *PLoS ONE* **2012**, *7*, e35429. [CrossRef] [PubMed]
38. Kiiker, R.; Juurik, M.; Heick, T.M.; Mäe, A. Changes in DMI, SDHI, and QoI fungicide sensitivity in the Estonian *Zymoseptoria tritici* population between 2019 and 2020. *Microorganisms* **2021**, *9*, 814. [CrossRef] [PubMed]
39. Leroux, P.; Walker, A.S. Multiple mechanisms account for resistance to sterol 14alpha-demethylation inhibitors in field isolates of *Mycosphaerella graminicola*. *Pest Manag. Sci.* **2011**, *67*, 44–59. [CrossRef] [PubMed]
40. Omrane, S.; Audéon, C.; Ignace, A.; Duplaix, C.; Aouini, L.; Kema, G.; Walker, A.S.; Fillinger, S. Plasticity of the MFS1 promoter leads to multidrug resistance in the wheat pathogen *Zymoseptoria tritici*. *mSphere* **2017**, *2*, e00393-17. [CrossRef]

41. Patry-Leclaire, S.; Neau, E.; Pitarch, A.; Walker, A.S.; Fillinger, S. Plasticity of the MFS1 promotor is not the only driver of Multidrug resistance in *Zymoseptoria tritici*. *bioRxiv* **2023**. [CrossRef]
42. Glaab, A.; Weilacher, X.; Hoffmeister, M.; Strobel, D.; Stammler, G. Occurrence and distribution of CYP51 haplotypes of *Zymoseptoria tritici* in recent years in Europe. *J. Plant Dis. Prot.* **2024**, *2024*, 1–8. [CrossRef]
43. Jørgensen, L.N.; Matzen, N.; Heick, T.M.; O'Driscoll, A.; Clark, B.; Waite, K.; Blake, J.; Glazek, M.; Maumene, C.; Couleaud, G.; et al. Shifting sensitivity of septoria tritici blotch compromises field performance and yield of main fungicides in Europe. *Front. Plant Sci.* **2022**, *13*, 1060428. [CrossRef] [PubMed]
44. Steinhauer, D.; Salat, M.; Frey, R.; Mosbach, A.; Luksch, T.; Balmer, D.; Hansesn, R.; Widdison, S.; Logan, G.; Dietrich, R.A.; et al. A dispensable paralog of succinate dehydrogenase subunit C mediates standing resistance towards a subclass of SDHI fungicides in *Zymoseptoria tritici*. *PLoS Pathog.* **2019**, *15*, e1007780. [CrossRef] [PubMed]
45. Hagerty, C.H.; Klein, A.M.; Reardon, C.L.; Kroese, D.R.; Melle, C.J.; Graber, K.R.; Mundt, C.C. Baseline and temporal changes in sensitivity of *Zymoseptoria tritici* isolates to benzovindiflupyr in Oregon, U.S.A., and cross-sensitivity to other SDHI fungicides. *Plant Dis.* **2021**, *105*, 169–174. [CrossRef] [PubMed]
46. Corkley, I.; Fraaije, B.; Hawkins, N. Fungicide resistance management: Maximizing the effective life of plant protection products. *Plant Pathol.* **2022**, *71*, 150–169. [CrossRef]