Maintaining fungicide effectiveness: Monitoring local *Zymoseptoria tritici* populations for sensitivity shifts and mutations in Estonian fields

Regina Pütsepp, Riinu Kiiker, Lee Põllumaa, Liis Andresen, Kersti Lilleväli, Andres Mäe
Centre of Estonian Rural Research and Knowledge
E-mail regina.pytsepp@metk.agri.ee

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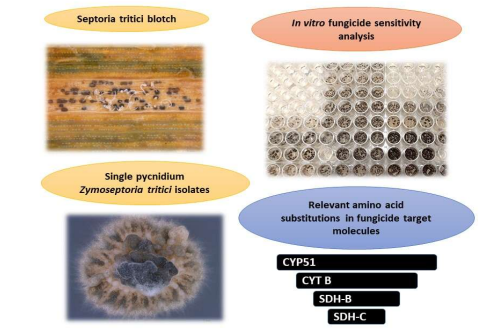


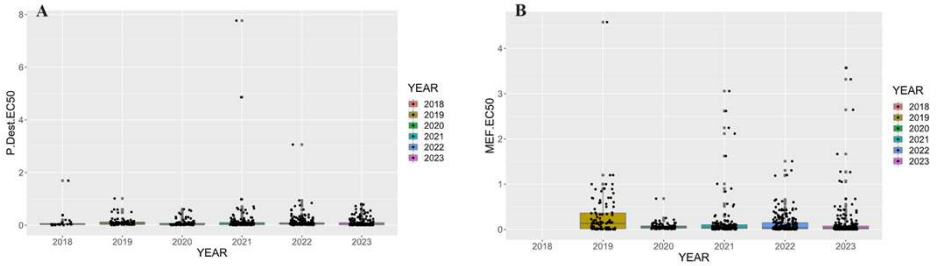
Introduction

A decrease in effectiveness to control Septoria tritici blotch has been observed in Western Europe, while in North-East Europe including Estonia, sensitivity to fungicides tends to be relatively high [1]. It is crucial to monitor the local *Z. tritici* population for mutations related to fungicide adaptation and shifting sensitivity levels to detect changes before field performance declines. We assessed over 800 *Z. tritici* isolates collected over a **six year period (2018-2023)** from Estonian commercial winter wheat fields.

Methods

Microtiter plate assay was used for sensitivity assessment. We tested several active substances of **DMIs, SDHIs, QoIs**, and a new **QiI** (fenpicoxamid).
Relevant amino acid substitutions were identified with PCR/Sanger sequencing in SDH-subunits and with KASP (Kompetitive Allele Specific PCR) genotyping [2] was applied for CYP51 and CytB targets.
EC_{50} values were calculated with GraphPad Prism and statistical analysis and graphs were done in BlueSky Statistics and Microsoft Excel. Significant differences between groups were calculated with Kruskal-Wallis test with Wilcoxon rank sum test.

Figure 1. Frequency (%) of CYP51 mutations in *Z. tritici* population in 2018 – 2023.



Results - DMI

Median EC_{50} values to DMIs ranged from 0.03 to 0.07 ppm for prothioconazole-desthio, and from 0.03 to 0.13 ppm for mefentrifluconazole. For both, the highest median EC_{50} were in 2019. Difenoconazole and metconazole are mainly use for seed treatment, and they had comparable median values to previous ones, 0.05 and 0.07 – 0.17 ppm respectively.
The **CYP51** gene is frequently mutated, with mutations D134G, V136A/C and A379G occurring with a frequency of 2% to 50% depending on the year. Almost every isolate has a mutation I381V, and a noticable increase of mutation S524T frequency (from 10% to 35%) have occurred in the population.

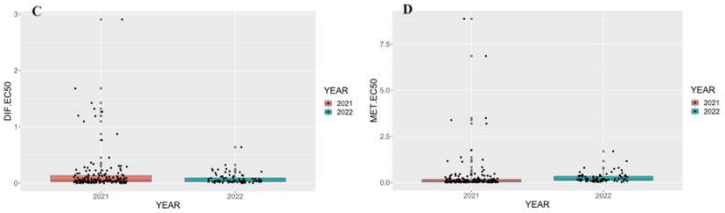


Figure 2. DMI sensitivity (EC_{50} in ppm) of *Z. tritici* population in 2018-2023. Prothioconazole-desthio (A, P.dest), mefentrifluconazole (B, MEF), difenoconazole (C, DIF) and metconazole (D, MET) were used in microtiter plate assay.

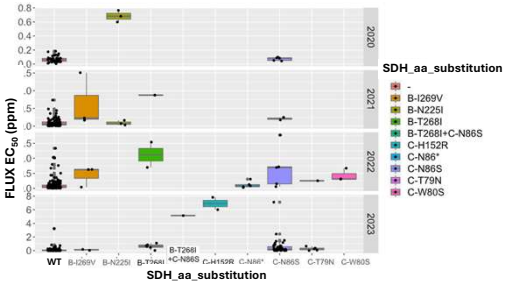


Figure 3. Fluxapyroxad (FLUX) sensitivity (EC_{50} in ppm) of *Z. tritici* isolates from 2020 – 2023 with relevant amino acid substitutions in SDH B and C subunits. Median EC_{50} values are marked with thick horizontal line. "– " in substitutions represents isolates with no substitutions, e.g. wild type.

Results - SDHI

Although mutations in **SDH subunits** are rare, isolates with mutations B-N225I, B-T268I, B-I269V, C-T79N, C-W80S, C-N86S, or C-H152R are usually present, accounting for up to 5% of the population. In 2023, there was a rapid increase of isolates with C-N86S mutations (25% of the population). **C-N86S** substitution had significant impact on fluxapyroxad sensitivity ($p < 0.0001$) and **B-T268I** was also considered significant ($p = 0.0016$). There was also an unusual isolate in 2023, with double-mutations B-T268I+C-N86S, which had reduced sensitivity to fluxapyroxad (EC_{50} = 5.14 ppm).
Median EC_{50} to fluxapyroxad ranged from 0.05 to 0.1 in study years. Other tested fungicides´ EC_{50} ranged from 0.3 to 0.6 ppm for boscalid (2 years data), from 0.07 to 0.1 ppm for bixafen (3 years), and from 0.06 to 0.1 ppm for fluopyram (2 years).

Results – QoI, QiI

The **CytB** gene has relevant G143A mutation, which exceeded the frequency of 50% in Estonian population in 2021. Pyraclostrobin was tested for 2 years (2022, 2023) and median EC_{50} was evenly 0.3 ppm. In 2023, azoxystrobin was also tested (EC_{50} = 2.4 ppm).
Fortunately, *Z. tritici* was mainly sensitive to the new fungicide fenpicoxamid (QiI), median EC_{50} ranged from 0.06 to 0.08.

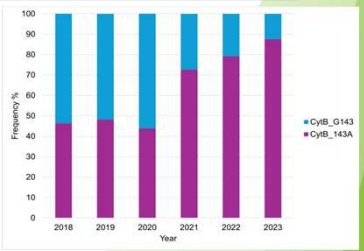


Figure 5. Frequency (%) of *Z. tritici* isolates from 2018 – 2023 with glycine (G) representing wild-type isolates and substituted to alanine (A) in amino acid position 143 in CytB.

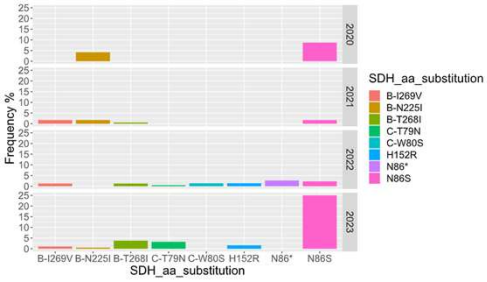


Figure 4. Frequency (%) of *Z. tritici* isolates from 2020 – 2023 with relevant amino acid substitutions in SDH B and C subunits. Asterisk in N86* substitution being either change to K (lysine) or T (threonine).

Conclusion

- Sensitivity to **most of the tested active substances were high**, except for QoIs associated with a high G143A mutation frequency (around 80%) in CytB.
- At the same time, some of the amino acid substitutions in target molecules are becoming more prevalent and more diverse.
- Overall, these findings suggest that CYP51 and SDH genes are important targets for mutations risk surveillance.

References

1. Hellin, P., Duvivier, M., Heick, T.M., et al. (2021). DOI:10.1002/ps.6601
2. Kildea S., Mehenni-Ciz J., Spink J., O'Sullivan E. Changes in the frequency of Irish *Mycosphaerella graminicola* CYP51 variants 2006–2011; Proceedings of the Modern Fungicides and Antifungal Compounds; Friedrichroda, Germany. 21–25 April 2014; pp. 143–144.

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