INnovations in plant Varlety Testing in Europe

Deliverable D1.3

Bioindicators for crop resilience against biotic stress

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Technical References

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[1] PU = Public

PP = Restricted to other programme participants (including the Commission Services)

RE = Restricted to a group specified by the consortium (including the Commission Services)

CO = Confidential, only for members of the consortium (including the Commission Services)

Document history

V	Date	Beneficiary	Author
1	28.04.2023	Teagasc	Ewen Mullins
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Summary

Septoria tritici blotch (STB) is the most important disease for wheat production in Western Europe. STB is caused by the fungal pathogen *Zymoseptoria tritici* and there is currently no variety available with durable resistance to STB. As a result, farmers rely heavily on the use of fungicide inputs to preserve yield and hence the profitability of their farming enterprise. Prior to the appearance of visual STB symptoms in the crop, there is a symptomless phase ('latent period'; for up to 30 days depending on the environmental conditions) during which STB advances unchecked through the host plant. Once the LP concludes, there is a rapid production of spores from the infected leaf, thereby advancing STB spread through the crop and ensuring an epidemic of the disease across the landscape. Previous work has concluded that a longer latent phase is a desirable trait to define quantitative/durable resistance of wheat against STB (Hehir et al. 2018). This deliverable is tasked with investigating the ability of varieties to slow down the LP, which could be used as a bio-indicator to support reducing the potential of STB to spread through a crop and indeed across the landscape. The impact of using molecular diagnostics to explore this phenomenon will be discussed and results from 3 field evaluations across 2 years presented with a list of recommendations on how this approach could be further progressed to assist in VCU testing.

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2 Introduction

Wheat is the cereal grain widely cultivated as a staple food for a majority of the world's population, with Europe producing 33% of total world wheat production (FAOSTAT 2017). Septoria tritici blotch (STB) is a devastating disease for wheat production across Western Europe. Caused by the fungal pathogen *Zymoseptoria tritici*, a typical STB infection initiates in the lower leaves of the crop. Spore transfer from an infected leaf then spreads infection so that STB disease migrates up through the leaf layers of the plant as the plant matures.

Once spores land on a leaf, they infect within ~36hrs and then colonize the inter-cellular spaces of the leaf without producing any visual symptoms. This symptomless phase (latent period, LP) of STB disease is quite long, varying from 9 to 30+ days depending on the environmental conditions. Once the latent period is concluded though, the spore producing bodies will quickly appear on the leaf surface and spores transferred to upper leaves and neighbouring plants via rain splash, thereby spreading the STB epidemic through the crop.

Previous work has indicated strongly that a longer LP is a desirable trait to support quantitative (i.e. durable) resistance in wheat against STB[1]. This is because it delays the appearance of spore producing pycnidia on the infected leaf and hence stalls the further spread of spores to adjacent plants. Although, quantitative resistance is a desired goal it has remained an understudied subject in the study of STB due to the complexity in the measurement of different traits associated with quantitative interactions. One of approach to objectively record quantitative interaction is by measuring *in planta* fungal growth during infection and determining if there is an association or relationship of that data with recorded yield data. Indeed, up to 70% of the yield potential of a wheat crop is supported by the photosynthetic activity of the flag leaf, or uppermost leaf in the canopy. Hence protecting the flag leaf is a key crop management action, as the more STB

[1] Hehir et al. (2017). Temporal and spatial field evaluations highlight the importance of the pre-symptomatic phase in supporting strong partial resistance in Triticum aestivum against Zymoseptoria tritici. Plant Pathology, https://doi.org/10.1111/ppa.12780

development through the flag leaf, the more diseased tissue and hence less yield is returned from the crop (Figure 1).

The importance of STB biomass accumulation during the latent period in *Z. tritici* has been hypothesized as important for successful infection and sporulation[2]. qPCR based quantification of fungal growth has shown its promise in understanding quantitative resistance as reported previously[3]. The objectives of this task was to validate this approach to determine how winter wheat varieties with alternative STB resistance profiles influence the levels of *in planta Z. tritici* growth rate under field conditions. By identifying and supporting an association between components of resistance and *in planta* Z. tritici growth rate it is intended that STB accumulation during the latent period could be an important bio-indicator of increased resistance/tolerance to STB disease in varietal selection protocols.

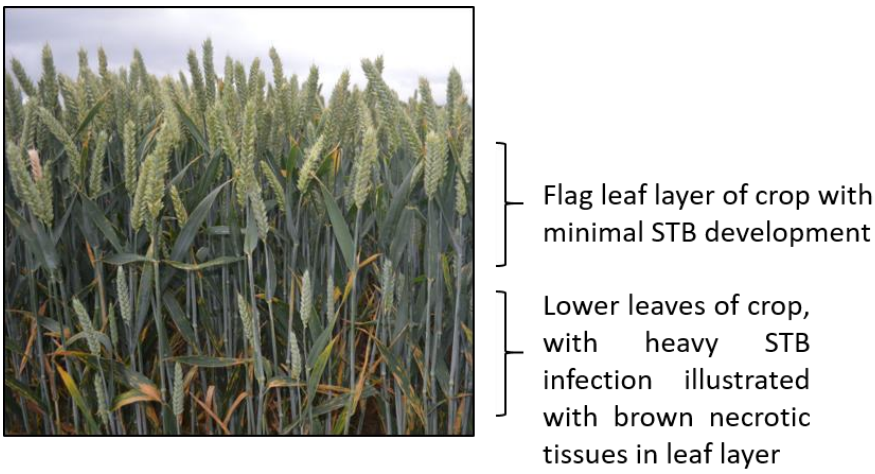

Figure 1: Canopy of winter wheat with STB development in lower leaf layers starting to reach the uppermost flag leaf layer at the top of the crop canopy

[2] Brennan et al. (2019). A review of the known unknowns in the early stages of Septoria tritici blotch disease of wheat. Plant Pathol. 68:1427-1438. https://doi.org/10.1111/ppa.13077

[3]Rahman et al. (2020). Quantification of In Planta Zymoseptoria tritici Progression Through Different Infection Phases and Related Association with Components of Aggressiveness, https://doi.org/10.1094/PHYTO-09-19-0339-R

3 Results

2.1 Field Operations

Across the three site-year field trials conducted (Table 1), each site-year had 6 common varieties except for Site-Year 2 (SY2), which contained 8 varieties. SY1 was a 'no fungicide' experiment while SY2 and SY3 included an additional fungicide treatment to assess the yield potential of varieties tested. Septoria severity was measured on the flag (uppermost leaf on plant) and the 2nd leaf (penultimate leaf) across 10 randomly collected plants per replicated plot, on each sampling day. Sampling started once the flag leaf was fully opened. Leaves were collected every week till GS 79-85 (from ~May to end of July) and plots harvested to record yield data. Leaves were collected to measure septoria severity and preserved for fungal biomass analysis via qPCR. Septoria severity was measured visually for SY 1, however an additional assay was developed for SY 2 and SY 3 with leaf samples scanned using a flat-bed scanner and image analysis software APS ASSESS. Total disease was presented as AUDPC (area under disease progress curve) values for (i) necrosis and (ii) lesions bearing pycnidia, which provides a temporal perspective on disease incidence over the time of the experiment.

Table 1 – Experimental sites and years

Site-year	Year	Location	Winter wheat varieties	Fungicide
SY 1	2020-21	Carlow Site 1	Arina, JB Diego, Stigg, Sundance, Torp and Vanilnoir	- fungicide
SY 2	2021-22	Carlow Site 2	Arina, JB Diego, Stigg, Sundance, Torp, Vanilnoir, W9568 and W9997	+ / - fungicide
SY 3	2021-22	Cork	Arina, JB Diego, Stigg, Sundance, Torp and Vanilnoir	+ / - fungicide

2.2 STB Evaluation and Yield Returns

For SY1 (2020-2021) the susceptible varieties 'JB Diego' and 'Vanilnoir' recorded high levels of STB disease as $AUDPC_{necrosis}$ on the penultimate leaf. In contrast and as expected, lower levels of $AUDPC_{necrosis}$ were noted in the more tolerant varieties of 'Stigg', 'Sundance' and 'Torp' (Figure 2). However, due to an extended dry period through late June which was matched with high temperatures, levels of $AUDPC_{necrosis}$ through the uppermost flag leaf layer remained low (Figure 2A). Not surprisingly this low level of disease necrosis translated into a low incidence of lesions bearing pycnidia ($AUDPC_{pycnidia}$) on both the penultimate and flag leaf of the tested varieties, with the relative ranking of varieties the same as for $AUDPC_{pycnidia}$ as was noted for $AUDPC_{necrosis}$ (Figure 2B).

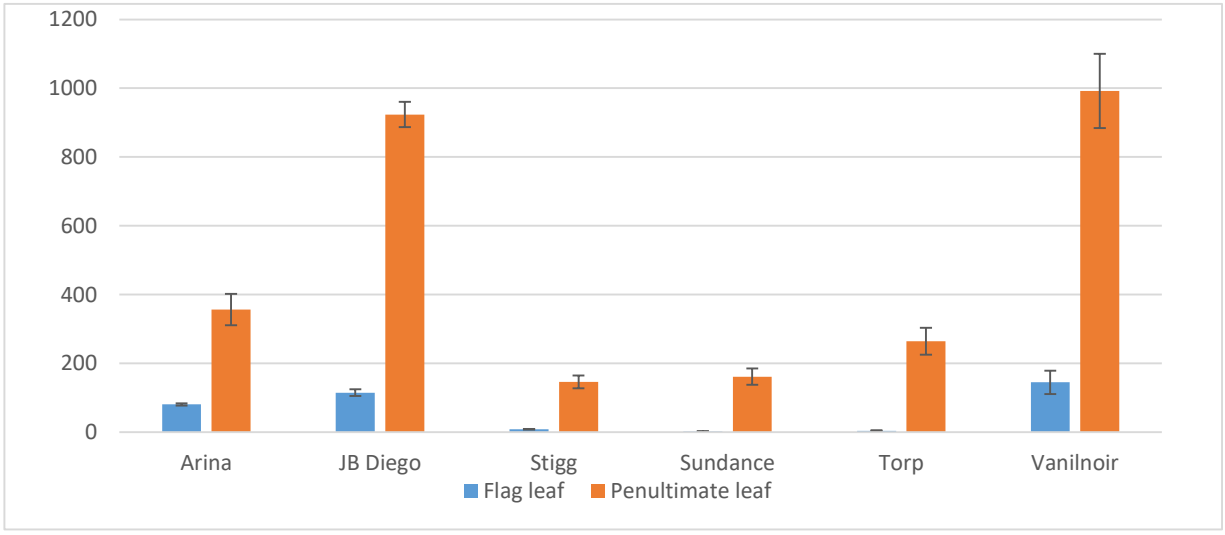

Figure 2A. *SY1 (2020 – 2021), $AUDPC_{necrosis}$ of six varieties. Necrosis was measured as percentage of leaf area covered with septoria necrotic lesion.*

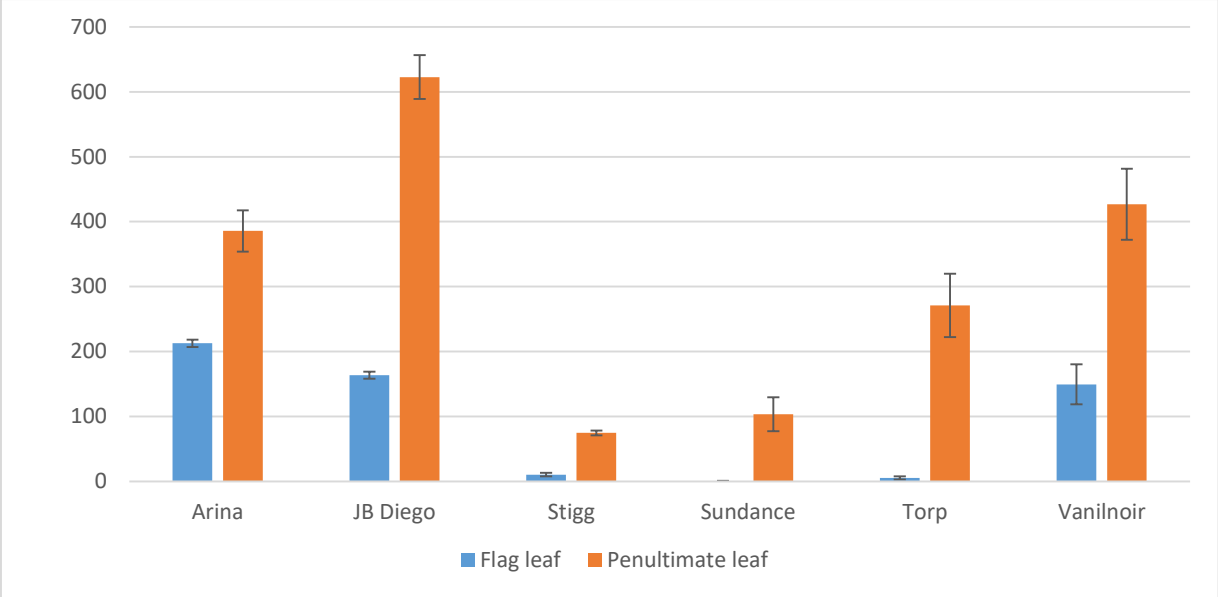

Figure 2B. *SY1 (2020-2021), AUDPC$_{pycnidia}$ of six varieties. Pycnidia coverage was measured as percentage of leaf area covered with septoria lesion bearing pycnidia*

In the case of SY2 (2021-2022) at Carlow, a greater separation between the *AUDPC$_{necrosis}$* for the varieties' penultimate leaf was evident with vars. 'Stigg', 'Sundance' and 'Torp' recording strong partial resistance to STB compared to the susceptible 'JB Diego' and 'Vanilnoir' (Figure 3A). This equated to a similar profile for AUDPC$_{pycnidia}$ of the six varieties (Figure 3B).

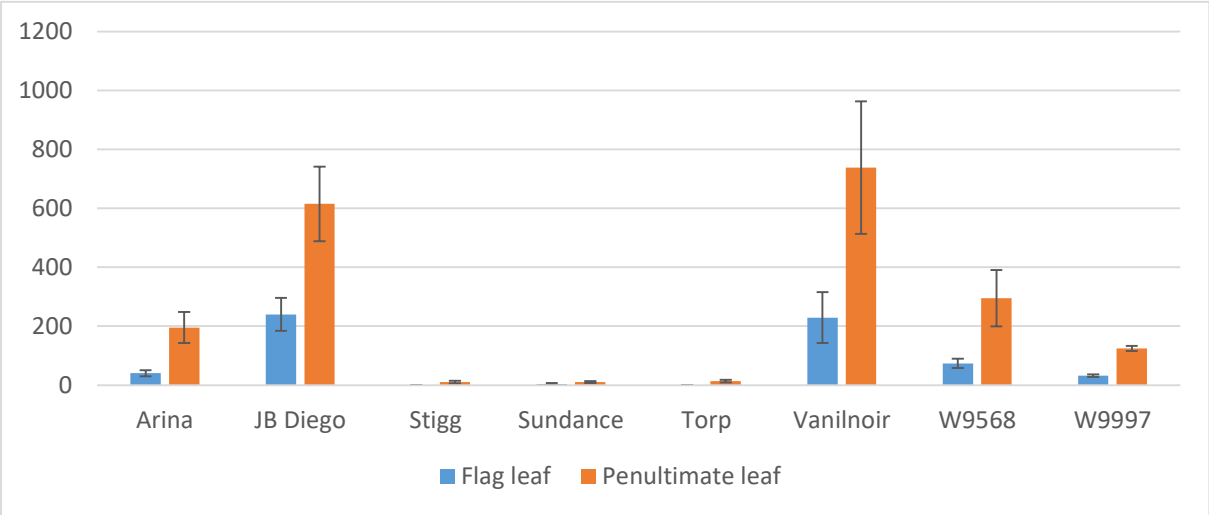

Figure 3A. *SY2 (2021-2022), AUDPC$_{necrosis}$ of 8 varieties. Necrosis was measured as percentage of leaf area covered with septoria necrotic lesion.*

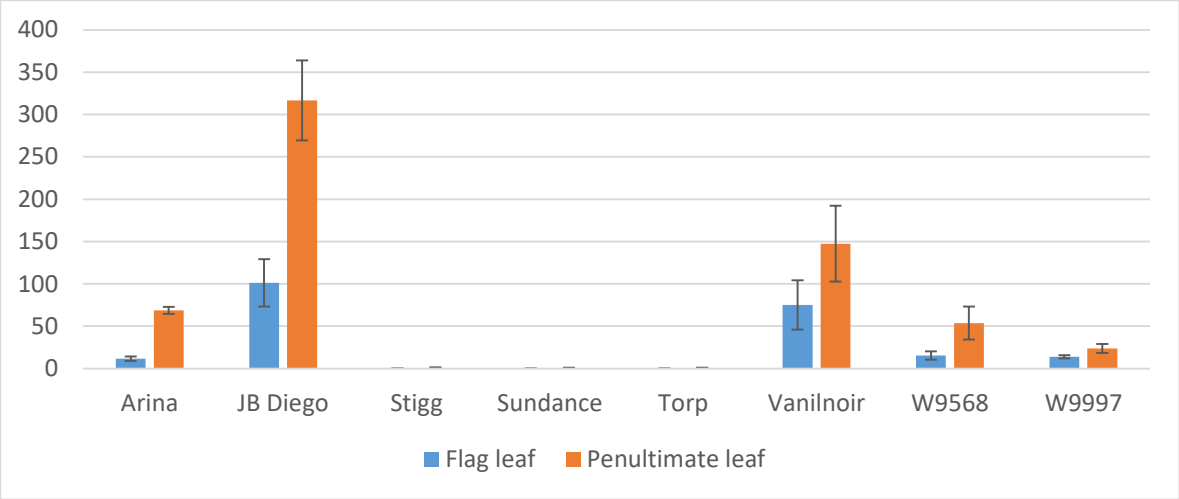

Figure 3B. *SY2 (2021-2022), AUDPC$_{pycnidia}$ of 8 varieties. Pycnidia coverage was measured as percentage of leaf area covered with septoria lesion bearing pycnidia.*

For SY3, a high incidence of STB was recorded at the Cork site, due to its location near the coast and higher relative humidity. As such, this trial saw the highest AUDPC$_{necrosis}$ levels recorded at >2000 for 'JB Diego' and 'Vanilnoir' (Figure 4A). The high disease pressures also challenged the three partially resistant varieties ('Stigg', 'Sundance', 'Torp'), which led to AUDPC$_{necrosis}$ values of ~1000, 1250 and 1500 respectively. However, the AUDPC$_{necrosis}$ values reduced substantially when the flag leaf was scored with values <500 for 'Stigg' and 'Sundance' and <600 for var. 'Torp'. The AUDPC$_{pycnidia}$ at SY3 was comparable to what was seen in SY2 and SY1, with var. 'Stigg', 'Sundance' and 'Torp' reducing the ability of *Z. tritici* to maintain an epidemic due to reduced pycnidia formation on disease leaves (Figure 4B).

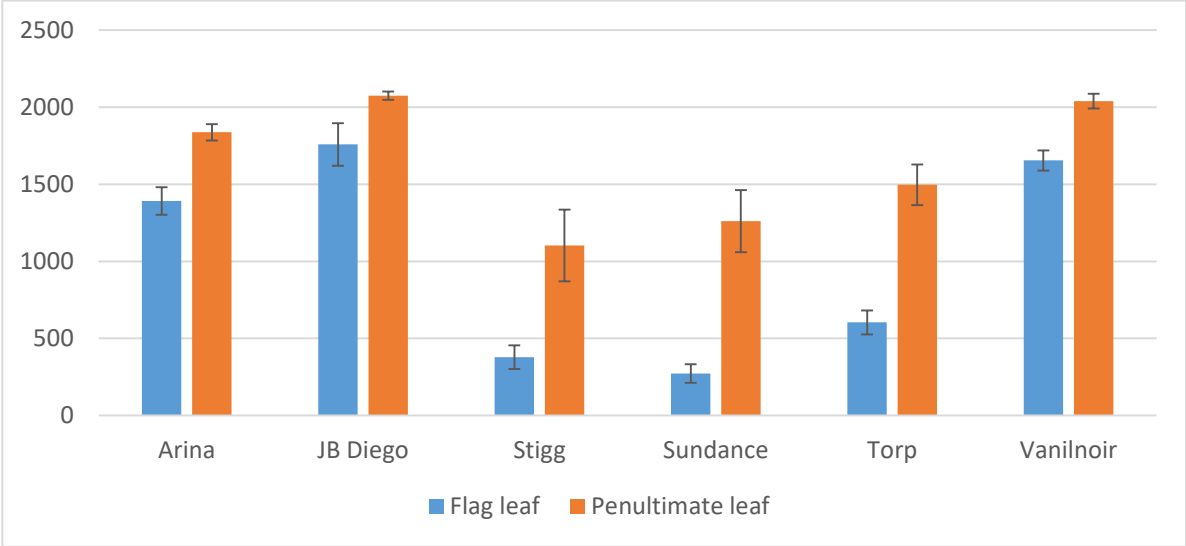

Figure 4A. *SY3 (2021-2022), $AUDPC_{necrosis}$ of six varieties. Necrosis was measured as percentage of leaf area covered with septoria necrotic lesion*

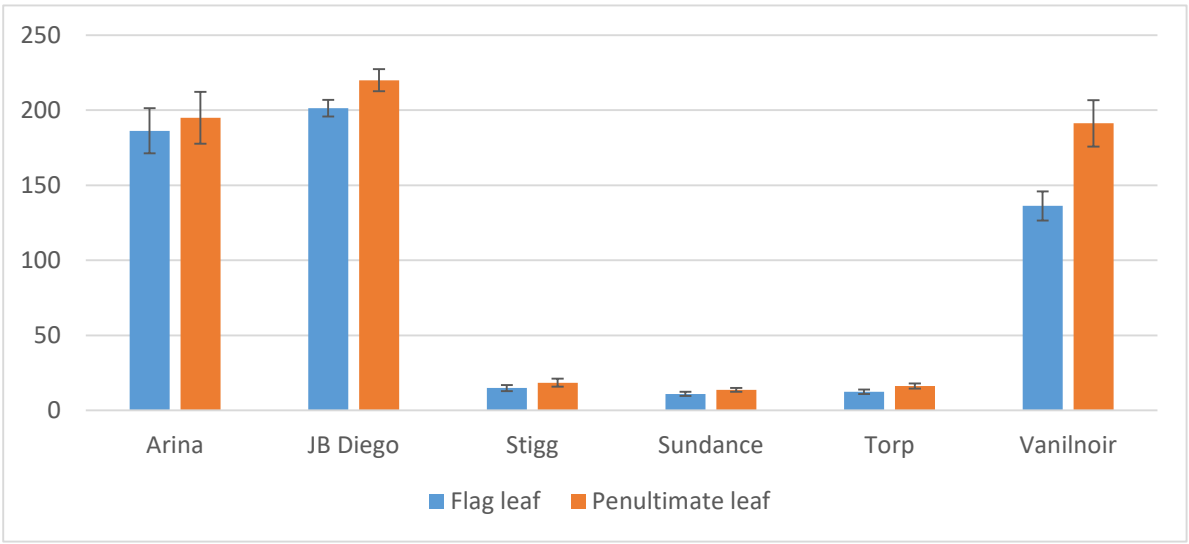

Figure 4B. *SY3 (2021-2022), $AUDPC_{pycnidia}$ of six varieties. Pycnidia coverage was measured as percentage of leaf area covered with septoria lesion bearing pycnidia*

The consistent response across the site seasons for these three varieties ('Stigg', 'Sundance' and 'Torp') indicates the potential of this varietal material to reduce STB epidemic potential at a landscape level; highlighting the importance of breeding for durable STB resistance in winter wheat collections. This is highlighted further by the yield returns (Figure A1 – A4), where the average yield

for the combined 'resistant' varieties ('Stigg', 'Sundance', 'Torp') in the absence of the standard 3-spray commercial fungicide programme was 10.8 t/ha, contrasting with an average value of 7.6t/ha for the combined susceptible varieties of 'Ariana', 'JB Diego' and 'Vanilnoir'. The national average yield of winter wheat in 2022 was 11.1t/ha, returned from a commercially sprayed programme.

2.3 Quantifying STB fungal biomass

The developed quantitative PCR (qPCR) assay returned high sensitivity for each of the respective wheat and *Z. tritici* standard curves, detecting as low as 250 pg wheat and as low as 0.1 pg *Z. tritici* genomic DNA per assay. Importantly, the spiking of *Z. tritici* samples with wheat had no impact on the sensitivity of the qPCR assay to detect the fungal pathogen.

A previous study reported that the delay in STB disease progression is related to the lengthened latent period in varieties with partial STB resistant phenotypes[4]. From the qPCR analyses completed in this work, a consistent and incremental increase in fungal biomass was observed in varieties across the site seasons (Figure A4) through week 1 and 2 for all varieties sampled, which continued increasing for the susceptible varieties (e.g 'Ariana', 'JB Diego' and 'Vanilnoir'). This increase was not noted for the more tolerant 'Stigg', 'Sundance' and 'Torp'.

2.4 Association between STB biomass accumulation and durable STB resistance

The potential association between STB biomass accumulation (recorded with qPCR assay) and STB disease progression (recorded with AUDPC), was determined through a pooled association that computed correlation values within each week and then also compared across the weeks of each field season. The data presented (Figure 5A) indicates that for the two most susceptible varieties ('JB Diego', 'Arina'), a strong association was returned (combining both flag leaf and penultimate leaf datasets) between the recording of STB biomass (via qPCR) at week 1 (indicated with blue

[4] Rahman et al. (2020). Quantification of In Planta Zymoseptoria tritici Progression Through Different Infection Phases and Related Association with Components of Aggressiveness, https://doi.org/10.1094/PHYTO-09-19-0339-R

arrow) versus the subsequent AUDPC values that developed through week 2 (r = 0.99), week 3 (r = 0.93), week 4 (r = 1.00) and week 5 (r = 0.97). If the qPCR detection was completed in week 2 (indicated with green arrow), similarly high associations with the visually recorded AUDPC were recorded at week 3 (r = 0.96), week 4 (r = 0.99) and week 5 (r = 0.93). When the qPCR detection was completed at week 3, the r values for the associated AUDPC values of week 4 and week 5 were 0.93 and 0.82 respectively (indicated with red arrow).

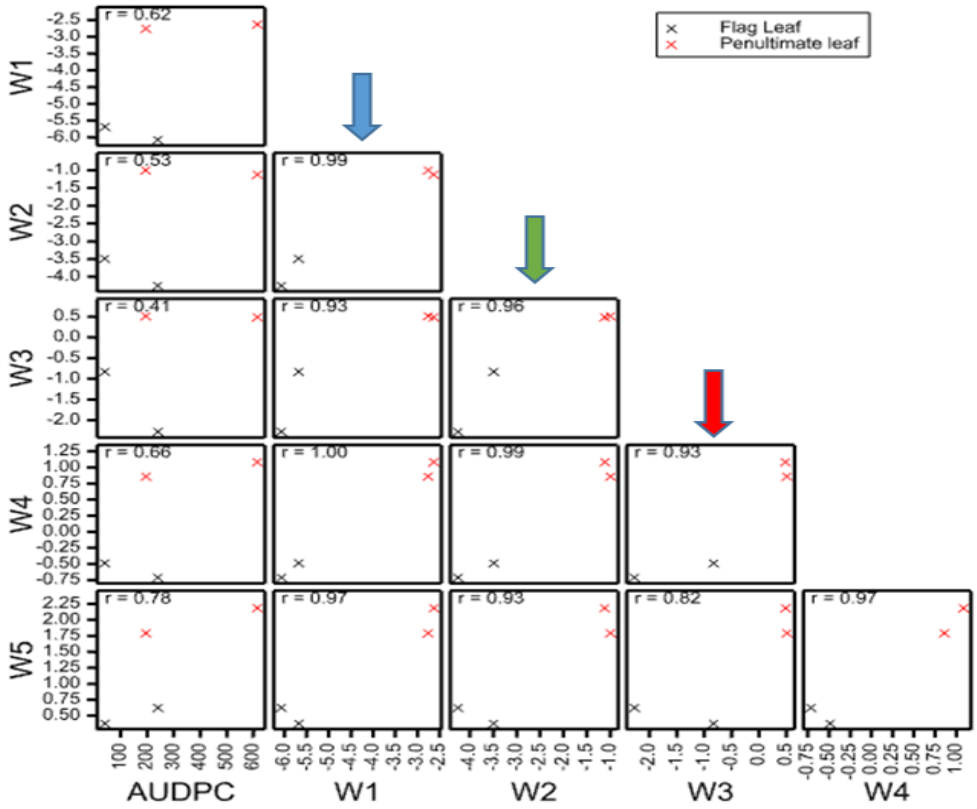

Figure 5A. *Pooled association analysis comparing visually recorded AUDPC values of STB progression versus levels of STB biomass accumulation (via qPCR) across both flag leaf and the penultimate leaf for the susceptible wheat varieties 'JB Diego' and 'Arina'.*

Examining the association between AUDPC and STB biomass accumulation in the more resistant varieties ('Stigg', 'Sundance' and 'Torp'), the calculated r values (Figure 5B) for a qPCR analysis in week 1 associated strongly (r = 0.97) for AUDPC at week 2 and week 4 (r = 0.95) but less at week 3 (r = 0.86) and week 5 (r = 0.64); indicated plots with blue arrow. If the qPCR detection was completed in week 2 (indicated with green arrow), associations with the visually recorded AUDPC were r = 0.82 (week 3), r = 0.91 (week 4) and r = 0.17 at week 5. When the qPCR detection was completed at week 3, the r values for the associated AUDPC values of week 4 and week 5 were 0.94 and 0.61 respectively (indicated with red arrow).

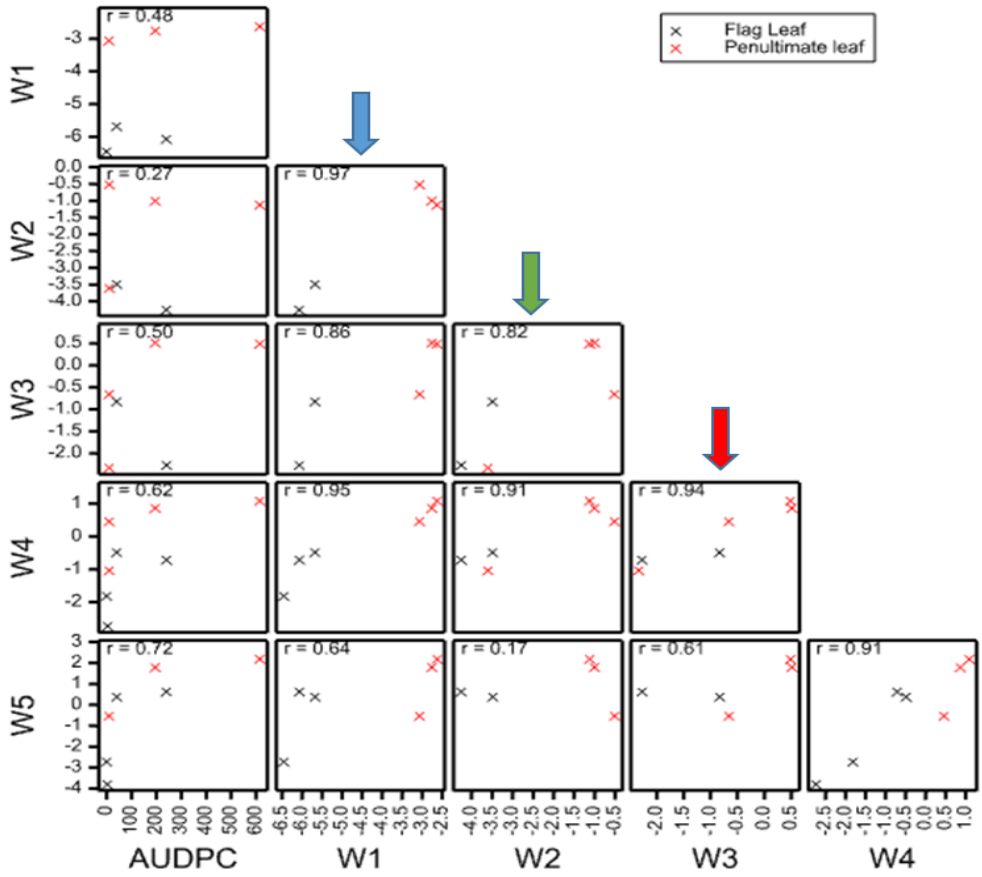

Figure 5B. *Pooled association analysis comparing visually recorded AUDPC values of STB progression versus levels of STB biomass accumulation (via qPCR) across both flag leaf and the penultimate leaf for the resistant wheat varieties 'Stigg', 'Sundance' and 'Torp'.*

4 Conclusion

Previous work[5, 6] has already highlighted the importance of the LP in supporting strong partial resistance to STB disease in commercially grown wheat, with an extended LP correlated with lower rates of STB disease progression both on individual plants but also across the landscape. Logistically, recording the LP in a crop is challenging due to the asynchronous development of plants within a field; due to environmental factors and soil parameters. As the timing of leaf emergence is variable it hence makes it very difficult to record accurately when STB disease first occurs on a newly opened leaf, as it could require twice daily assessments. Consequently, recording LP as a bio-indicator for resistance is not practical for the purposes of varietal selection. In response, the goal of Task 1.3 was to investigate the association between the rate of STB disease progression (AUDPC) through the season and the levels of STB biomass accumulation, in leaf tissues sampled at the earlier stages of the season. Hence, could the molecular-based quantification of STB biomass in the pre-symptomatic latent period be used as a novel bio-indicator for resilience against the biotic stress of STB disease? Based on the work completed across 3 site seasons at two locations in Ireland, the association analyses concluded that the qPCR approach is a valid method to identify highly susceptible material in a pre-commercial varietal collection. For more resistant material, the association values returned were not fully supportive of this conclusion. This was most likely due to the lower levels of STB present in the more resistant material and indicates a possible threshold at which the use of qPCR as an indicator for STB resilience loses its validity. The fact that the approach employed was strongly associated with AUDPC values, does highlight a potential route to facilitate the identification (and ultimately removal) of STB susceptible material from varietal evaluations. As the EU has identified ambitious goals under Farm to Fork, reducing chemical applications through the development of disease resistant varietal material is essential. From these results the output of this deliverable provides an innovative tool and method to assess an important sustainability and

[5] Hehir et al. (2017). Temporal and spatial field evaluations highlight the importance of the pre-symptomatic phase in supporting strong partial resistance in Triticum aestivum against Zymoseptoria tritici. Plant Pathology, https://doi.org/10.1111/ppa.12780

[6] Rahman et al. (2020). Quantification of In Planta Zymoseptoria tritici Progression Through Different Infection Phases and Related Association with Components of Aggressiveness, https://doi.org/10.1094/PHYTO-09-19-0339-R

resilience criteria and ultimately improve the speed and efficiency of DUS and performance testing to discard material that is too reliant on the use of chemical crop protectants to preserve yield.

5 Appendix

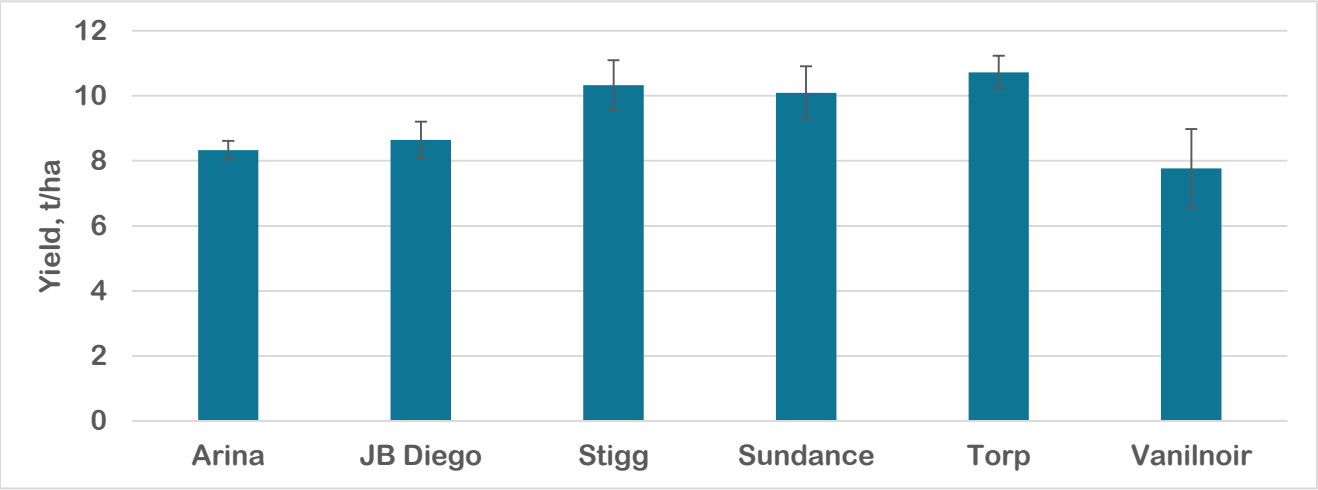

Figure A1. Untreated yield from 2020-21 trial at Oak Park, Carlow site. Yield as t/ha was averaged for three replications and adjusted to 15% moisture.

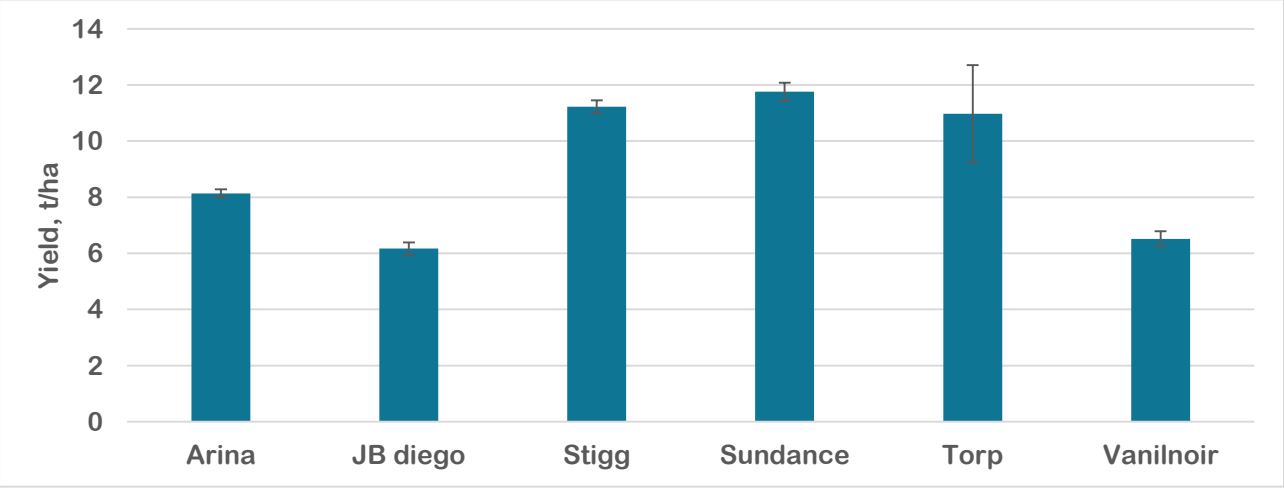

Figure A2. Untreated yield from 2021-22 trial at Oak Park, Carlow site. Yield as t/ha was averaged for three replications and adjusted to 15% moisture.

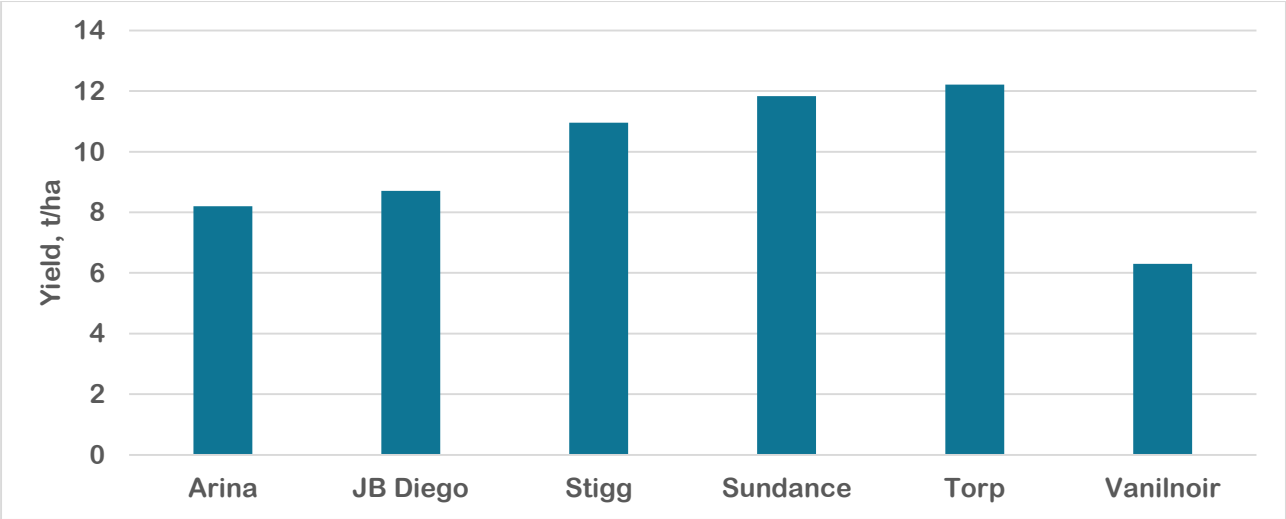

Figure A3. Untreated yield from 2021-22 trial at Mallow, Cork site. Yield as t/ha was averaged for three replications and adjusted to 15% moisture.

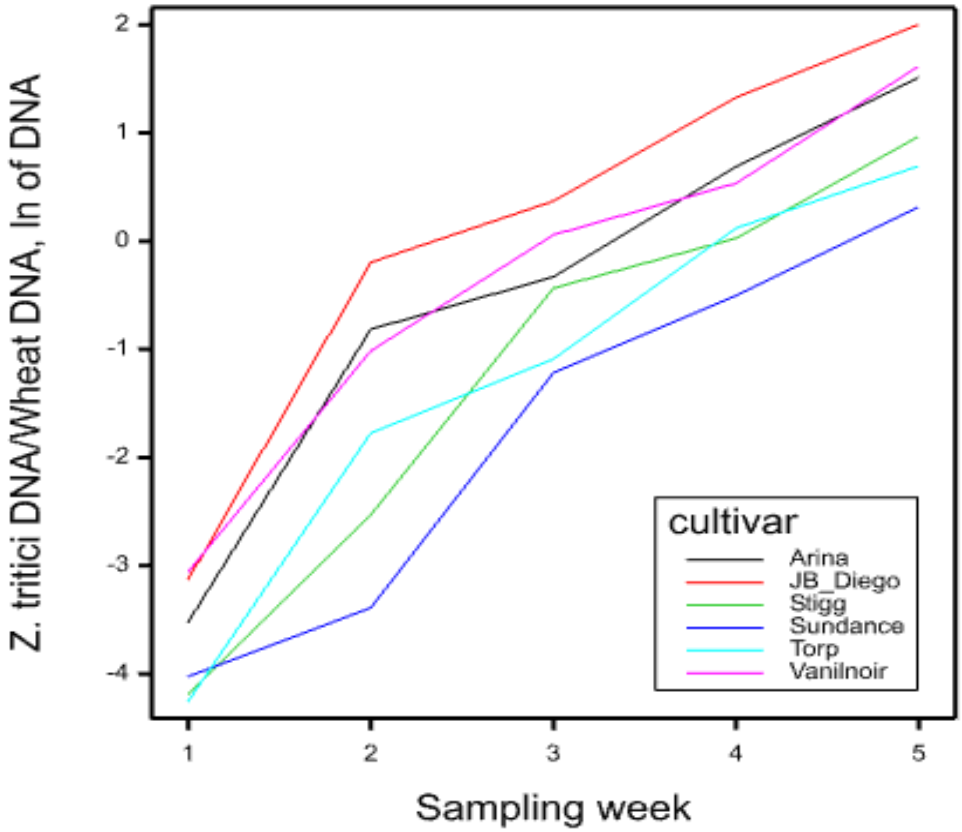

Figure A4. Rate of increase in STB fungal biomass, based on a pooled analyses across site seasons for varieties listed.