Variety resistance has little effect on ascospore production by wheat pathogens *Zymoseptoria tritici* (STB) and *Pyrenophra tritici-repentis* (YLS)

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Key findings

- *Zymoseptoria tritici* and *Pyrenophra tritici-repentis*, the disease-causing pathogens of septoria tritici blotch (STB) and yellow leaf spot (YLS), can produce ascospores on wheat stubble regardless of their resistance status.
- Any infected wheat stubble has the ability to initiate an epidemic for up to 2 years.
- Reduce harvest cut height to limit *Z. tritici* colonising stubble.
- Excess harvest material must be removed from the paddock to produce a net reduction of *Z. tritici* inoculum.
- Minimise disease impacts by using an integrated approach to management.

Keywords septoria tritici blotch, yellow leaf spot, stubble, resistance, foliar, wheat

Introduction

Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* and yellow leaf spot (YLS) caused by *Pyrenophora tritici-repentis* are major stubble-borne foliar diseases of wheat in the cropping regions of southern New South Wales (sNSW). The diagnostic activities and industry inquiries received in 2021 by NSW DPI pathologists revealed that STB and YLS were the third and fourth most queried diseases across the state. STB does not just infect wheat; durum and triticale are also STB hosts, but there are good levels of genetic resistance in triticale and generally in durum. Therefore, STB is not considered a major disease of these crops in Australia currently. Similarly, YLS also infects triticale and durum, but there are varieties with useful levels of resistance. STB and YLS do not cause significant disease in any other major cereal crop.

Z. tritici and *P. tritici-repentis* have a fungal structure produced on wheat stubble (pseudothecia) that releases airborne spores (ascospores) when environmental conditions are conducive. The ascospores produced can spread by wind to infect host crops. *Z. tritici* ascospores can travel further than *P. tritici-repentis* ascospores. Even after a non-host break crop (e.g. canola) is sown in a paddock, any remaining wheat stubble residues can still be an inoculum source and infect the emerging wheat crop.

STB lesions will appear up to 28 days after infection and produce pycnidia (small black structures inside tan leaf lesions that give a speckled appearance). The pycnidia produce an asexual spore called conidia. The conidia are then splash dispersed by rainfall within the wheat canopy causing new infections and further driving the epidemic. Recent research completed at Wagga Wagga Agricultural Institute (WWAI), found that between 20% and 45% yield loss is possible in a susceptible variety (Baxter, Simpfendorfer and Milgate 2022).

In contrast, YLS lesions are oval, or lens shaped, with a tan centre and chlorotic yellow margin around the lesion. In severe infections or in conducive environmental conditions, lesions can coalesce into each other. The important distinguishing feature between YLS and STB lesions is that YLS do not contain the black pycnidia. *P. tritici-repentis* does, however, produce asexual conidia spores that can be wind dispersed into the upper canopy of the crop and greater distances to infect other susceptible cereal crops. *P. tritici-repentis* has a shorter incubation period than *Z. tritici*, approximately 7 days, resulting in multiple disease cycles per season when environmental conditions are ideal. Fresh conidia spores can be produced every 24 hours on the surface of necrotic YLS lesions. Research conducted at WWAI in 2015 (Milgate and Goldthorpe 2015) found that YLS can cause up to a 26% yield loss in a susceptible variety.

The results of 2 experiments are discussed in this paper, the first demonstrates that variety resistance has no effect on ascospore development for either *Z. tritici* or *P. tritici-repentis*. Further to this, we observed that the in-season disease severity also has no effect on the ability of these necrotrophic pathogens to survive and produce inoculum. The second set of results show how growers can use simple harvest management techniques to mitigate against this survival feature of these important pathogens and reduce the amount of inoculum in paddocks.

Method

Experiment 1

In simple terms, resistance can be thought of as the ability of a plant to limit damage caused by pathogen infection. Current commercial cereal and pulse varieties are screened annually for their resistance to a range of diseases through the National Variety Trials (NVT) system. A sliding resistance rating scale, ranging from resistant (R) to very susceptible (VS) is applied to each entry. A set of 30 commercial wheat varieties were selected from the NVT system and sown in 2 separate field nurseries to assess the foliar disease progression of STB and YLS in varieties sown at different times. There were 4 sowing dates, each approximately 2 weeks apart, starting in early April of each year (Table 1). The nurseries were a randomised complete block design with 3 repetitions. The entries were sown into short rows, 1.5 m long, with 2 entries and a susceptible check per plot. The entries were visually assessed for foliar disease progression, with the trait 'percentage of whole plant coverage' assessed fortnightly during the growing season (data not shown). The experiments were not harvested for yield.

Table 1 The sowing date windows for STB and YLS nurseries, 2019 and 2020.

Sowing date (SD)	Across seasons (2019–2020) sowing windows for STB and YLS nurseries
SD1	8 April
SD2	21–24 April
SD3	5–6 May
SD4	19–22 May

The second part of Experiment 1 quantified the number of ascospores released from the wheat stubble. This was to determine the risk of the stubble as an inoculum source for the proceeding wheat crop and to quantify any difference in the number of ascospores released from residual stubble of varieties that have a range of resistance ratings and infections during the season.

The standing stubble was left to naturally weather and mature in the paddock after harvest. Due to operational space restraints, the stubble had to be collected before sowing in the following year and placed into breathable calico bags, then secured to a paddock fence line to continue the weathering and maturing process.

Four varieties out of the 30 assessed for foliar disease progression, were selected to quantify ascospore release from the stubble. The selected varieties had a range of resistance ratings to STB and YLS (Table 2). The spore release component of Experiment 1 was conducted under controlled conditions in the laboratory. To stimulate the release of spores from the stubble, 5 tillers per variety were soaked in water for 6 hours. The ascospores were then released from the pseudothecia on the stubble using air suction in a specially designed apparatus, affectionately named the 'spore liberator'. The stubble was in the spore liberator for 18 hours. The ascospores were captured on a microscope slide and counted manually using a microscope. This was repeated 44 times for each variety for each sowing date.

Table 2 Resistance ratings for the 4 varieties that were assessed for ascospore release under laboratory conditions.

Variety	Resistance rating to STB*	Resistance rating to YLS*
Axe(b	S–VS	S
LongReach Kittyhawk(b	MR–MS	MR–MS
LongReach Lancer(b	MS	MS
Sunlamb(b	MR	MR–MS

* as per *NSW DPI Winter crop variety sowing guide*.
Resistance ratings: MR = Moderately resistant; MR–MS = Moderately resistant to moderately susceptible; MS = Moderately susceptible; S = Susceptible; S–VS = Susceptible to very susceptible.

Treatments

Experiment 1

Each year, all entries were inoculated with stubble known to be infected with either *Z. tritici* or *P. tritici-repentis* to promote natural initial infection. Supplementary overhead irrigation was applied daily in short, regular bursts, to further drive the epidemic by maintaining leaf wetness and humidity within the crop canopy. Agronomically, all entries were treated the same within the growing year. There were no fungicides applied.

Experiment 2

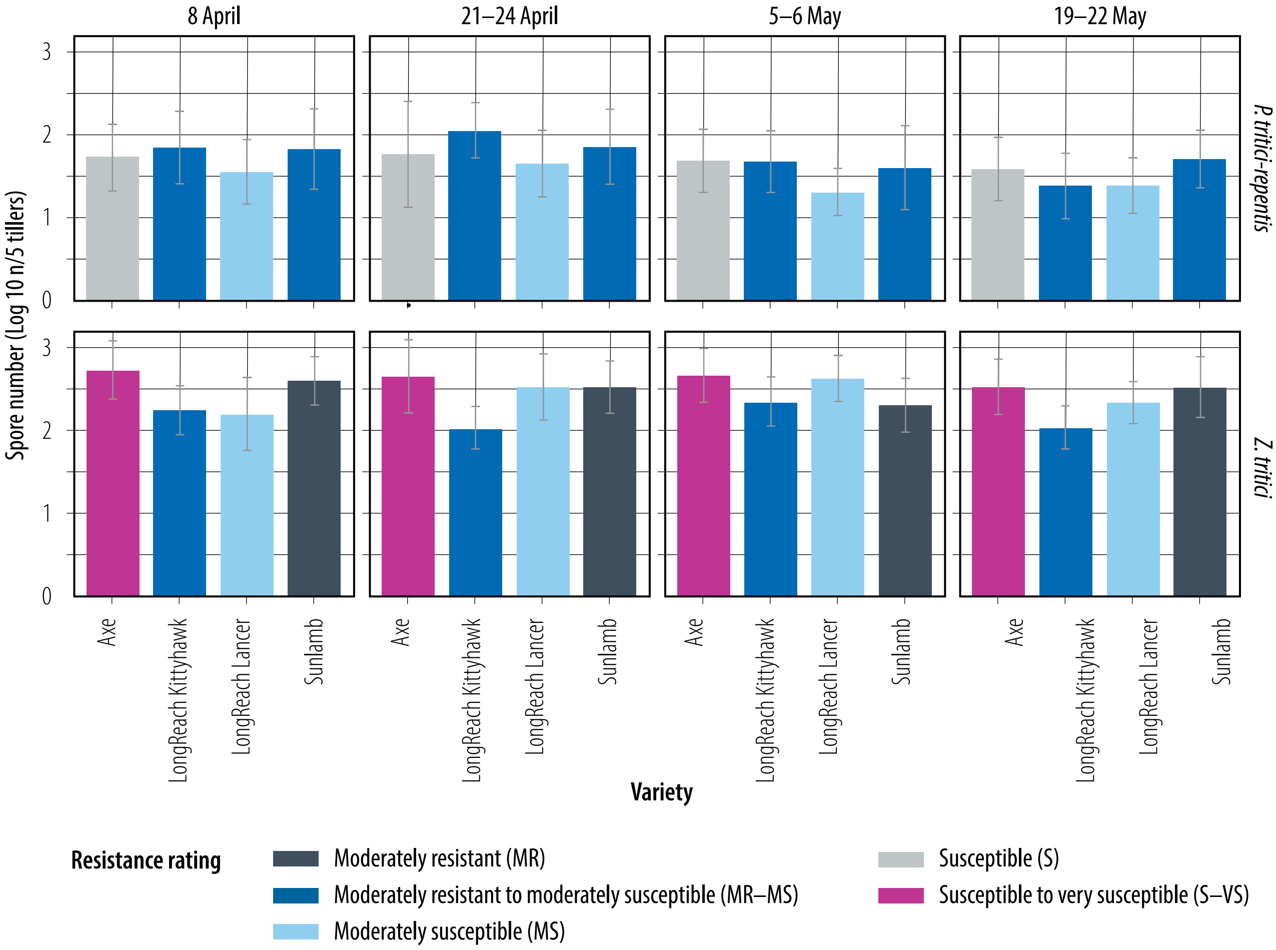
A single variety Beckom(b, S–VS to STB was planted in 10 m^2 plots at WWAI during 2020. The experiment was inoculated with STB and maintained with supplementary irrigation as above. The plots were harvested at 3 heights, with the average cut height of treatments being 32 cm (high treatment), 24 cm (medium treatment) and 14 cm (low treatment) (Figure 2a). Each of the cut heights aimed to lower the straw length by one node on the mainstem. The stubble was weathered naturally in the paddock and collected during May 2021 and ascospore release counted as per Experiment 1. Spore releases from the different heights were repeated 96 times (Figure 2b).

Results

The ascospore release data collected over 2 years (Figure 1) shows that regardless of the variety's resistance rating, there is no significant difference ($P = 0.05$) across the 4 sowing dates, or within a sowing date between varieties, of the number of *Z. tritici* and *P. tritici-repentis* ascospores released from the stubble. In the case of STB, this means that an MR-rated variety is releasing the same amount of ascospores as an MR–MS, MS or S–VS-rated variety.

Generally, there were more *Z. tritici* ascospores released from the stubble than *P. tritici-repentis* ascospores. Across all sowing dates, the minimum number of *Z. tritici* ascospores released was Log10 score of 2.0, or over 100 ascospores per 5 tillers, with many reaching a Log10 score of 2.5, or approximately 300 ascospores per 5 tillers. In contrast, the *P. tritici-repentis* ascospores ranged between Log10 score 1.0 and 2.0: between 10 and 100 ascospores per 5 tillers. Further findings from experiments (data not shown) have shown that stubble infected with the STB-causing pathogen *Z. tritici*, can generate enough ascospores to initiate an epidemic 2 years after the wheat crop was grown. Again, this was irrespective of the variety resistance rating.

The foliar disease data (not shown) revealed an interaction with the ascospore release data. It showed that earlier sowing dates (SD1 and SD2) had a much higher level of foliar disease than the later sowing dates (SD3 and SD4), with SD1 having the highest disease levels and SD4 the lowest. For example, Axe(b in SD1 scored 85% STB disease severity while in SD4 the variety only scored 34%. However, this did not correlate to higher ascospore numbers released from the stubble from the earlier sowing dates (Figure 1) compared with the later sowing dates. The number of ascospores released within a single sowing date, remained relatively stable.



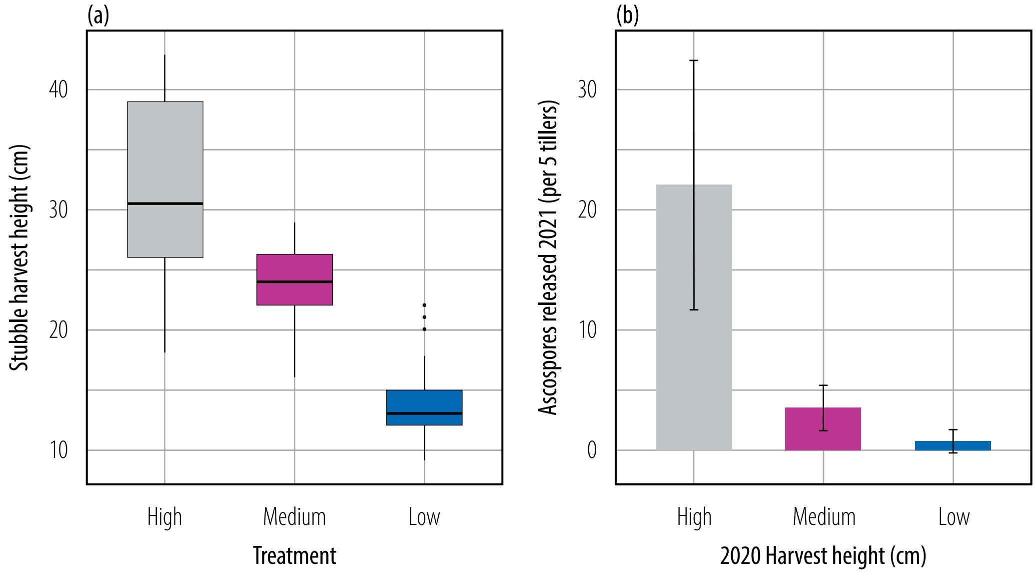
Note: The Log10 average combines all data from 2 years of spore release repetitions conducted in the laboratory.
There is no significant difference in the number of ascospores released between resistance ratings for both STB and YLS.
Log1 = 10 spores, Log 2 = 100 spores and Log 3 = 1000 spores.
Vertical bars represent 95% confidence intervals (*P* = 0.05).

Figure 1 The average Log10 number of *Zymoseptoria tritici* and *Pyrenophora tritici-repentis* ascospores released from wheat stubble, from 4 varieties with different resistance ratings to the diseases septoria tritici blotch (STB) and yellow leaf spot (YLS).

Figure 2a and 2b show results from Experiment 2, the STB stubble management experiment. Both figures are specific to STB and the results from these cannot be extrapolated to YLS.

Figure 2a shows the harvest cut heights imposed across the plots.

Figure 2b shows the reduced number of *Z. tritici* ascospores by lowering the harvest cut height. Using the 32 cm cut height as a base level, lowering the cut height to 24 cm reduced the number of ascospores produced from the stubble by 87%. When comparing the 32 cm cut height with the 14 cm cut height, the number of ascospores released from the stubble was reduced by 97%.



Vertical bars represent 95% confidence intervals ($P = 0.05$).

Note: average harvest cut heights for treatments: High – 32 cm, Medium – 24 cm and Low – 14 cm.

Figure 2 (a) Box plot showing the harvest cut height in centimetres (cm) of the high, medium and low treatments for the 2020 STB stubble management experiment. (b) The number of *Zymoseptoria tritici* ascospores released from 5 tillers from the 3 stubble cut height treatments. This figure displays the reduction in ascospore numbers as the harvest cut height decreases.

Discussion

It is commonly accepted that the more susceptible a variety is to a particular disease, the greater the inoculum risk the stubble or volunteers pose for the following season. This remains true for diseases such as stripe rust, but the spore release data collected over the past 2 years in these experiments has shown that this might not be the case for STB and YLS. This has implications for crop rotation, and sequences and stubble management. In sNSW, the cropping sequence is dominated by cereal–canola–cereal rotations. As infected stubble is able to produce ascospores at an epidemic-inducing level for up to 2 years, it suggests that a single canola break might not be enough to reduce the risk of STB or YLS.

It is not known if the differences in ascospore release numbers between resistance ratings, although not significant, has an influence on epidemic severity (further experiments are currently testing this). What can be said, is that regardless of the resistance rating, the number of ascospores released from just 5 tillers for both *Z. tritici* and *P. tritici-repentis* are at a level that can initiate an epidemic up to 2 years later. Remembering that the number of ascospores counted were from 5 tillers only, once that is multiplied out over a square metre, hectare or paddock scale, the number of ascospores produced is at a level that can infect crops locally and regionally.

The distinction needs to be made between managing disease in the current wheat crop to minimise yield loss and inoculum risk from the stubble in subsequent seasons. Even though the number of ascospores released from the stubble does not significantly change with resistance rating, variety choice remains critical to managing STB and YLS within the current season. The NVT ratings show that a more resistant variety develops less disease compared with a more susceptible variety. Picking a variety with a higher resistance level will protect yield in the presence of disease, while reducing the number of fungicide sprays required which, in turn, reduces machinery, labour and input costs. Minimising fungicide use also reduces the risk of fungicide resistance developing within both on-target and off-target fungal pathogen populations.

Harvest cut height can have a big influence on the amount of standing stubble material left in the paddock for *Z. tritici* inoculum production. In turn, it also influences the number of ascospores released from the stubble in the following season. Lowering the harvest cut height, reduces the amount of inoculum left on the standing stubble. However, the excess material that is put through the harvester cannot be left in the paddock otherwise, the inoculum from the standing stubble is only relocated to the ground maintaining the same inoculum levels within the paddock. To achieve a net reduction in inoculum, the excess material from the lower cut height must be removed from the paddock. This can be done by bailing the straw or burning (narrow windrow or blanket burn). Some methods are less labour intensive than others, however the cost benefit risks of each must be considered before being undertaken.

Summary

Components of the research discussed above can be implemented into an integrated disease management plan to control STB and YLS. Acknowledging the risk, and duration of the risk i.e. up to 2 years, that any STB or YLS infected stubble can have on proceeding cereal crops can guide crop rotation decisions, or if growing wheat on wheat, allow a plan to be implemented that can appropriately manage the risk of these diseases. Variety choice, stubble cut height and stubble removal can all be implemented to reduce yield loss and inoculum levels of STB and YLS.

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Author contribution statement

Dr Andrew Milgate conceived of the study, coordinated its design and execution and analysed the data. Tony Goldthorpe and Merrin Spackman conducted the experiments. Brad Baxter and Dr Andrew Milgate wrote the manuscript.

Crop protection

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