



## Soil solarisation delivers near zero levels of *Fusarium pseudograminearum* in cereal crown rot reference sites

Prue Bottomley<sup>a</sup>, Mark W. Sutherland<sup>a</sup>, Bree A.L. Wilson<sup>a</sup>, Bethany Rognoni<sup>b</sup>, Alison Kelly<sup>b,c</sup>, Cassandra D. Percy<sup>a,\*</sup>

<sup>a</sup> Centre for Crop Health, Institute for Life Sciences and the Environment, University of Southern Queensland, Toowoomba, QLD, 4350, Australia

<sup>b</sup> Department of Agriculture and Fisheries, Leslie Research Facility, Toowoomba, Qld, 4350, Australia

<sup>c</sup> Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Toowoomba, QLD, 4350, Australia

### ARTICLE INFO

#### Keywords:

Crown rot  
Disease control  
Soil solarisation  
Wheat stubble  
Straw decomposition

### ABSTRACT

The development of commercial varieties that are resistant or tolerant to crown rot caused by *Fusarium* species is an important goal for cereal breeding programs internationally. Ideally, this research requires experimental sites that are initially free from *Fusarium* in order to establish treatment plots that compare growth in the presence and absence of these soil- and stubble-borne pathogens. Specifically, the assessment of tolerance requires control plots free of disease to determine the reduction in crop yield in plots where the disease is present. The ability of soil solarisation to reduce the background *Fusarium pseudograminearum* level occurring at experimental sites in comparison to current stubble management techniques was investigated across three field trials at Wellcamp in Queensland. Stubble from a susceptible durum (*Triticum turgidum* ssp. *durum*) cultivar inoculated with *F. pseudograminearum* was incorporated by cultivation into the trial sites to establish a significant background level of inoculum prior to the application of all subsequent treatment plots. In these trials, solarisation over a period of twelve weeks reduced the presence of *F. pseudograminearum* to low detection levels when compared to the traditional crown rot management techniques of cultivation or growth of the non-host cover crops mungbean (*Vigna radiata*) and soybean (*Glycine max*). No negative effects of solarisation were observed on a subsequent crop of bread wheat (*Triticum aestivum*), with significantly higher yields observed in the solarised treatments. Solarisation has the potential to deliver near zero level crown rot reference sites for experimental purposes in one short application between cropping seasons.

### 1. Introduction

Crown rot of cereal crops in Australia is primarily associated with two pathogens, *Fusarium pseudograminearum* (Fp) and *Fusarium culmorum* (Backhouse and Burgess, 2002). Crown rot is widespread, and is found in all major wheat growing regions of Australia (Swan et al., 2000) and across many international production zones (Smiley, 1996; Lamprecht et al., 2006; Tunali et al., 2008; Alahmad et al., 2018). It is a major limiting factor of crop production in the northern region of Australia in particular, with yield losses as high as 50–60% in susceptible cultivars (Daniel and Simpfendorfer, 2008; Graham et al., 2015). In 2009, yield losses due to crown rot were estimated at approximately A \$97 million in wheat and barley annually (Murray and Brennan, 2009, 2010). More recent surveys conducted from 2015 to 2017 estimate this number to be closer to A\$404 million annually due to an increase in

stubble retention cropping practices and an intensification of cereals within the Australian cropping system (Hollaway et al., 2022).

Crown rot inoculum survives as mycelium in the stubble remaining from previous crops and infects susceptible hosts through direct contact with the plant (Burgess et al., 2001). *Fusarium pseudograminearum* can persist in infected paddocks for up to eight years (Wildermuth et al., 1997). Subcrown tissues, coleoptiles and seedling leaf bases of the plant are important primary sites of infection for crown rot (Purs, 1966; Percy et al., 2012). Progress into the lower internodes of the elongated tillers is achieved by mycelium growth within both the tissues and lumen of infected culms (Knight and Sutherland, 2016). In mature plants, symptoms include basal discolouration on the internodes and leaf sheaths, which can be seen as high as the sixth internode on a single tiller (Purs, 1966). A wet start to the season, followed by a dry finish during flowering and grain fill, often leads to the premature senescence of heads in

\* Corresponding author.

E-mail address: [cassy.percy@unisq.edu.au](mailto:cassy.percy@unisq.edu.au) (C.D. Percy).

<https://doi.org/10.1016/j.cropro.2024.106887>

Received 8 February 2024; Received in revised form 2 August 2024; Accepted 8 August 2024

Available online 13 August 2024

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infected plants (Smiley, 2019). These ‘whiteheads’ do not contain viable grain and contribute to a significant yield loss (Mudge et al., 2006; Klein et al., 1991).

Minimal tillage practices have significantly reduced issues regarding poor water retention between seasons and long-term decline in soil structure, however repeated cropping of cereal hosts under these conditions has frequently led to a build-up of stubble-borne pathogens such as *Fp* (Summerell et al., 1989). Current management strategies to combat the effects of crown rot focus on stubble management, crop rotation and the use of partially resistant and tolerant cereal varieties. Some of these stubble management techniques include burning the excess stubble (Wildermuth et al., 1997), incorporating stubble into the soil through cultivation (Burgess et al., 1993), undertaking a long bare fallow (Kazan and Gardiner, 2018), rotating with non-host crops (Readford et al., 2015) or carrying out interrow sowing where possible (Verrell et al., 2017). However, these methods exhibit limitations, as they typically only postpone the onset of the disease to subsequent years.

A significant effort has been placed on developing both resistant and tolerant crown rot varieties for commercial use. Although partial resistance to crown rot has been identified in several wheat and barley germplasm, incorporating this resistance into profitable cultivars has proven difficult (Rahman et al., 2020; Collard et al., 2005; Bovill et al., 2010; Liu and Ogbonnaya, 2015). Resistance sources tend to be partial and have complex inheritance requiring the pyramiding of multiple alleles for them to be effective (Rahman et al., 2020; Martin et al., 2015). The success of these resistance genes relies on improved technology in pre-breeding programs which have resulted in a number of high-yielding adapted lines being available to growers in recent years (Rahman et al., 2020; Nicol et al., 2012; Wallwork et al., 2004).

Tolerance testing requires the growth of advanced breeding lines under plus and minus disease conditions in the field, with the plus plots being inoculated with *Fp* and the minus plots receiving no inoculum and having very low to zero pre-existing background levels of the pathogen (Kelly et al., 2021). However, as the disease is now so widespread, there is frequently a significant base level of the crown rot inoculum already evident within the minus plots (Long, 2015), preventing a true uninfected yield potential value being determined. Additional variation between yield trial plots can also occur when the background level of inoculum varies across the trial site. No above-mentioned stubble management technique has proved to be effective in removing this background inoculum load between seasons. This issue has become a significant area of concern among crown rot researchers and breeders as tolerance research intensifies due to resistance being difficult to harness in high yielding varieties (Rahman et al., 2020; Martin et al., 2015).

Soil solarisation was first described by Katan (Katan et al., 1976) for use as a disinfestation method for managing soilborne pathogens, arthropods, and weeds in tomato (*Solanum lycopersicum*). In its basic form, soil solarisation involves laying transparent polyethylene film over an area of soil. The plastic increases the trapping of solar radiation within the soil below, producing higher temperatures through repeated daily cycles (Katan and Gamliel, 2012a). In Australia, soil solarisation results in temperatures of 35–60 °C when applied over the summer months and mostly affects the upper 0–30 cm of soil (Katan and Gamliel, 2012b). As many fungal soil pathogens are killed at 40–70 °C, soil solarisation has the potential to control the carryover of soilborne fungal pathogens from year to year (McGovern and McSorley, 1997). Soil solarisation also has the ability to trap existing moisture within the soil profile leading to an increase in available soil water, providing a suitable environment for fungal growth and organic decomposition (Zribi et al., 2015) until lethal temperatures are reached (McGovern and McSorley, 1997). This increase in soil available water can be a leading factor in higher yields and avoiding plant stress under pathogen load, especially during a drier year where no irrigation is available (Abed Gatea Al-Shammary et al., 2020; Day and Intalap, 1970; Chekali et al., 2011; Ahmadi et al., 2022).

Soil solarisation as a tool in managing *Fusarium* spp. has focused on protecting high value horticulture crops like tomatoes (*Solanum*

*lycopersicum*) or lettuce (*Lactuca sativa*) (Matheron and Porchas, 2010; Minuto et al., 2000) from pathogens such as *F. oxysporum* (McGovern and McSorley, 2012). Broadacre field experiments have shown that soil solarisation has long-lasting effects on depressing pathogen populations in cotton (*Gossypium hirsutum* and *G. barbadense*). A decrease in cotton plant death was observed for up to 3 years after *F. oxysporum* was killed via solarisation (Katan et al., 1983). In this study, non-solarised plots infected with *F. oxysporum* showed a rapid increase in disease incidence (up to 60%) over the first 50 days of cotton growth, compared to solarised plots, which exhibited <1% disease incidence (Katan et al., 1983).

A survey of the most common *Fusarium* species and the effect of soil solarisation on their survival was conducted at four sites in Iran (Saremi and Saremi, 2013). *Fusarium pseudograminearum* was one of 17 *Fusarium* spp. isolated and after 6 weeks of solarisation, *Fp* inoculum was significantly reduced from 550 to 0 CFU g<sup>-1</sup> (colony forming units per gram of soil) (Saremi and Saremi, 2013).

In the work presented here, the feasibility of employing soil solarisation to reduce the background *Fp* load on experimental sites in comparison to other stubble management techniques was investigated across three field trials. The possible implementation of solarisation into a research system would allow researchers to effectively ‘clean’ their paddocks rapidly between seasons leading to better research outcomes. The management strategies tested included a series of cultivations to aid in stubble break down and the use of two different summer cover crops, mungbeans and soybeans. We hypothesised that solarisation would provide a practical tool in delivering near zero level crown rot reference sites for disease tolerance trials. These trials also investigated whether any detrimental effects would be observed in a subsequent bread wheat crop of cv. EGA Gregory by planting it over the treatments in the subsequent planting season.

## 2. Materials and methods

### 2.1. Field preparation

Two field trials were conducted in 2017 and 2018 to determine whether *Fp* can be reduced to below detectable levels (<0.1 log<sub>10</sub> (pg DNA/g soil + 1)) in a single season of solarisation. Field trials were conducted at the Queensland Department of Agriculture and Fisheries (QDAF) Wellcamp Research Station, Wellcamp, Queensland (Lat -27.566023, Long 151.861916). These trials are referred to throughout the text as 2017 FT1 and 2018 FT1. The site is fully described by Powell and Christianos (1985) with the soil type characterised as an alkaline vertisolic black soil. The soil is neutral to alkaline with good plant available soil water capacity and fertility (Powell and Christianos, 1985). Colonised millet inoculum was prepared with a mixture of known aggressive isolates of *Fp* (Table 1) following the method described by Dodman and Wildermuth (1987). To establish a high level of crown rot inoculum across the site to test the experimental treatments, durum wheat (*Triticum turgidum* ssp. *durum*) cv. Caparoi was planted with a Glen E Lee planter (Kingaroy Engineering Works, Australia) at a rate of 60 kg/ha in July of the preceding year. *Fusarium pseudograminearum* colonised millet inoculum was delivered into the furrow at planting

**Table 1**

*Fusarium pseudograminearum* isolates from wheat used in the field trials and their corresponding accession numbers in the Queensland Herbarium, Australia.

Accession number	Year	Isolate	Locality
BRIP 64947 a	2005	A05#37	Moree, NSW, Australia
BRIP 64948 a	2005	A05#54	Billa, QLD, Australia
BRIP 64949 a	2009	A09#04	Emerald, QLD, Australia
BRIP 64950 a	2011	A11#01	Goondiwindi, QLD, Australia
BRIP 64951 a	2011	A11#04	Yoothapina, QLD, Australia
BRIP 64952 a	2012	A12#02	Irvingdale, QLD, Australia

through a microband distributor at a rate of 2.2 g/m of row. At maturity (November), approximately 20 weeks after planting, the grain from the durum crop was machine-harvested, and the infected stubble was partly incorporated into the soil using a disc cultivator to a 30 cm depth.

Following the incorporation of the durum stubble, treatments were applied during the subsequent January to April period, coinciding with the midsummer to early autumn period of the year and the recommended planting dates for both the mungbean (cv. Jade-AU) and soybean (cv. Bunya) cultivars. Treatments were as follows and throughout the text will be referred by their allocated number: (1) no further treatment, essentially a fallow; (2) three passes of a disc cultivator at a depth of 30 cm with no subsequent solarisation; (3) three passes of disc cultivation immediately followed by twelve weeks of solarisation; (4) twelve weeks of solarisation only; (5) mungbean (*Vigna radiata*) cv. Jade-AU cover crop; and (6) soybean (*Glycine max*) cv. Bunya cover crop. Each field trial used a randomised block design with three replications of each of six treatments applied to 2 m × 6 m plots.

A third field trial (referred to as 2018 FT2) ran over both years with the treatments applied across consecutive summer/autumn periods separated by a winter fallow to determine if a greater reduction in *Fp* would be achieved by this approach. Each plot received the same treatment in both years.

## 2.2. Treatment application

In preparation for the solarisation, each plot was irrigated pre-treatment with approximately 5 mm of rainwater to aid heat conduction and to ensure all treatments began with similar moisture profiles (26–30% in 2017 and 19–22% in 2018 at 30 cm depth). To apply the solarisation treatment, furrows were mechanically dug on each side of the plot, and a 200 µm thick transparent polyethylene 2 × 6 m sheet was laid over the plot. Plastic was held in place by backfilling the furrows with soil, whilst pulling the plastic tight to reduce the likelihood of air pockets known to reduce temperatures and moisture retention (Abed Gatea Al-Shammari et al., 2020). After 12 weeks, the polyethylene sheet was removed from the solarisation plots. Weed control within the trial area was carried out over the 12 weeks by hand.

Mungbean and soybean plots were planted at a rate of 24 kg/ha and 40 kg/ha respectively using a cone delivery system on a Glen E Lee planter. Mungbean and soybean plots were dry inoculated at planting at the recommended rate with a commercial *Bradyrhizobium* spp. rhizobium (Group H for soybeans and Group I for mungbeans). In April, at approximately 12 weeks, the mature mungbean and soybean plants were harvested by hand.

In at least one replicate of each treatment, soil temperature was measured at a depth of 10 cm every 15 min with a HOBO Pro v2 external weatherproof datalogger. Ambient temperature was measured using a datalogger attached to a star picket approximately 1 m above the soil surface.

## 2.3. Data collection

### 2.3.1. Soil sampling

Soil samples were collected from each plot before (January) and following (May) treatment application in each year. A 30 cm long x 1 cm diameter core was collected using a Rimik soil sampling tube and a total of 15–20 cores (300–400 g combined total) were collected from each plot using a grid sampling pattern, ensuring that the locations for sampling differed during the second round of collection. Soil samples from the same plot were combined, air dried in a glasshouse and 300 g sent to the South Australian Research and Development Institute (SARDI) for PREDICTA® B testing, which quantifies the presence of *Fp*, *F. culmorum*, *Pyrenophora tritici-repentis*, *Bipolaris sorokiniana*, *Botryosphaeria* spp. and the root lesion nematodes *Pratylenchus thornei* and *P. neglectus*, based on species-specific DNA sequences (SARDI, 2018). No stubble was added to the samples.

Soil moisture was measured on subsamples from these same combined soil cores. Each sample was mixed thoroughly and weighed immediately after collection. The 100 g soil subsample was then dried at 105 °C for 48 h and soil moisture calculated as a percentage of the soil prior to drying. Soil moisture data for each of the plots was collected before and after the treatment period.

### 2.3.2. *Fusarium pseudograminearum* incidence in stubble

Stubble from the previously planted durum crop was collected before and after the subsequent treatments were applied in each field trial. From each plot, twenty stubble samples were collected, which consisted of four stubble pieces from five sites per plot. Stubble pieces 3 cm in length were sampled from the lower tiller or crown region of the plant. After the removal of adhering soil, the stubble samples were aseptically surface sterilised for 20 s each in 70% ethanol, 2% sodium hypochlorite, followed by two washes with sterile distilled water. Four stubble pieces from the same treatment were air dried on sterile blotting paper, plated onto Czapek-dox agar plates (90 mm) containing streptomycin and chlortetracycline hydrochloride and incubated at 25 °C in the dark. Five plates were prepared per plot for a total of 20 stubble pieces. After 3–4 days incubation, plates were scored visually by noting the presence of *Fp* characterised as pink mycelial growth and values were expressed as an incidence of infected stubble per plot.

### 2.3.3. Stubble load

The stubble load in each plot was estimated visually by placing 50 cm × 50 cm quadrats within the sample plot, before and after the treatment period. Three quadrats were observed per plot, with the quadrats always placed in the same position before and after the treatment. The percentage of stubble cover was estimated per quadrat and images were taken using an Apple iPhone 8 with a 12-megapixel camera to provide a visual indicator of the stubble breakdown over the experimental period. The 2018 FT2 was only sampled for stubble load and stubble incidence in 2017 (pre-treatment) and 2018 (post-treatment).

### 2.3.4. Disease and yield measurements of post-treatment crop

At the end of the treatment period for each experiment, a yield crop was planted over the experimental plots in the following July. A susceptible bread wheat (*Triticum aestivum*), cv. EGA Gregory, was planted directly into the plots at a rate of 60 kg/ha and urea (50 kg/ha) was applied at planting. Two irrigation events of approximately 40 mm were applied using a sprinkler irrigation system to the crop directly following planting and before flowering at approximately 15 weeks after planting.

At maturity, the plants growing in 1 m length of a single row in the middle of each plot were hand-harvested. The total plant number per 1 m of row was counted and 10 plants were randomly selected for further processing. The total tiller number and the crown rot disease severity on the 10 plants were determined. Three tillers from the 10 plants were used for disease scoring: the main stem and two primary tillers. Crown rot disease severity was measured by the percentage of lesioning on the lower 15 cm of each tiller recorded in 10% increments.

The plots were cut out to 6 m × 2 m using a tractor slasher and the grain from the remaining plants in each plot was harvested using a small-plot harvester (Kingaroy Engineering Works, Australia). A 400 g sub-sample of seed was put through a Grintec Scientific Agitator sieve shaker to calculate the percentage of screenings per sample (set to 40 shakes per minute). Thousand grain weights (TGW) were also obtained from each plot using a Contador seed counter.

## 2.4. Statistical analysis

Traits with one response value per plot were yield (t/ha), thousand grain weight (g), screenings (g in 400 g subsample), pre- and post-treatment *Fp* DNA quantification in soil, pre- and post-treatment stubble crown rot incidence (%), pre- and post-treatment soil moisture content (%), pre- and post-treatment stubble cover (% of area

measured), and crown rot disease severity ratings. The pre- and post-treatment *Fp* DNA quantification data were transformed using a  $\log_{10}(x+1)$  transformation prior to analysis to keep in line with industry standard risk categories (SARDI, 2018), and to satisfy the model assumptions of normality and homogeneity of variance of residuals across the range of fitted values. Additionally, the crown rot disease severity data were transformed using a square root transformation, to satisfy the same model assumptions.

The analyses were conducted in a linear mixed model (LMM) framework. The LMMs for each of the across-trials analyses included fixed effects for the main effect of treatment with six levels, the main effect of trial with three levels, and the interaction between treatment and trial. For the traits measured on a plot basis, random terms for replicate block effects at each trial were included, and the plots represented the residual level at each trial. For the stubble cover traits with multiple samples per plot, random terms for both the replicate block and plot effects at each trial were included. For the crown rot disease severity trait, additional terms were also included to account for the three tiller types measured on each plant.

All LMMs were fitted using the ASReml-R statistical package v4.1.0.106 (Butler et al., 2018) in the R computing environment (R Core Team, 2021). Variance components were estimated using residual maximum likelihood (Patterson and Thompson, 1971). The fixed effects were tested using a Wald test (Kenward and Roger, 1997) and empirical Best Linear Unbiased Estimates (eBLUEs) were generated from the model for significant effects. Significant differences between pairs of treatments and/or trials were determined using Fisher's least significant difference (LSD) test (Welham et al., 2015). The level of significance was set at the 5% level for all testing. Graphics of results were produced using the ggplot2 package v3.3.2 (Wickham, 2016).

### 3. Results

#### 3.1. *Fusarium pseudograminearum* DNA quantification in soil

The mean pre-treatment background *Fp* levels achieved across each trial were 2.7, 2.8 and 2.4  $\log_{10}$  (pg DNA/g soil + 1) for 2017 FT1, 2018 FT1 and 2018 FT2 respectively (Fig. 1). There was a significant interaction between trial and treatment on the post-treatment *Fp* DNA detected in soil samples ( $p = 0.016$ ). In all trials, at least one of the solarisation treatments resulted in a significantly lower post-treatment level of *Fp* than all the other non-solarisation treatments, and there was no significant difference between the two solarisation treatments (3 and 4) in any of the trials. In the one-year trials (2017 FT1 and 2018 FT1), both solarisation treatments had significantly lower *Fp* than all other treatments. In 2018 FT2, the solarisation (4) treatment had significantly lower *Fp* than all other non-solarisation treatments, while the *Fp* for the cultivation + solarisation (3) treatment was not significantly different from the cultivation (2) treatment but was significantly lower than the *Fp* for the rest of the non-solarisation treatments.

#### 3.2. Pathogen in stubble

A significant treatment by trial interaction was detected for the incidence of *Fp* in stubble ( $p = 0.008$ ) (Fig. 2). There was a significantly lower *Fp* incidence in stubble after the two solarisation treatments (3 and 4) when compared to the non-solarised plots. In almost all cases, the mungbean (5) and soybean (6) treatments were not significantly different from the no treatment (1) and cultivation (2) treatments with the exception of the mungbean (5) treatment in 2018 FT2.

Pre-treatment stubble load levels over the three trials ranged between 28.8% and 38.1%. No significant difference in stubble load was identified between treatments in any of the trials (data not shown). A natural stubble breakdown occurred across the site over time with

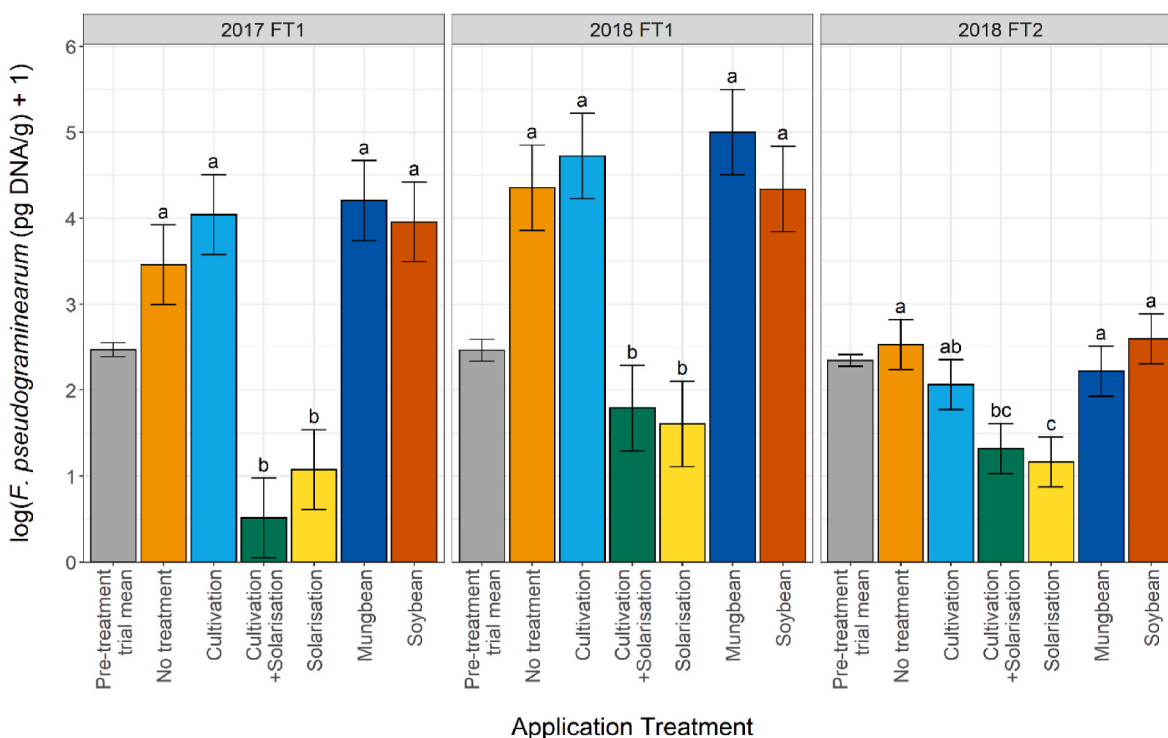
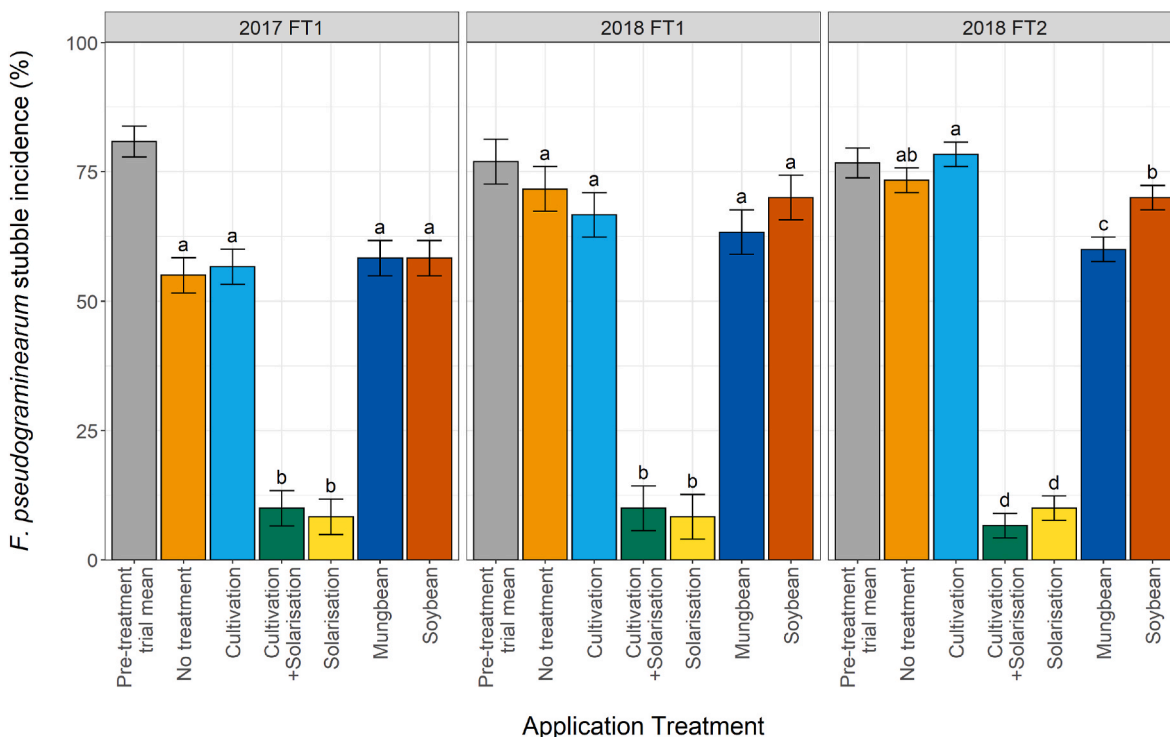


Fig. 1. *Fusarium pseudograminearum* ( $\log_{10}$  pg DNA copies/g + 1) in soil from the three field trials comparing the six application treatments, as well as the pre-treatment trial mean. The error bars represent the standard errors of the predictions. The letters above the bars indicate the results of the least significant difference (LSD) test for treatment comparison within each trial; treatments within the same trial bearing the same letter are not significantly different from each other, at the 5% level of significance.





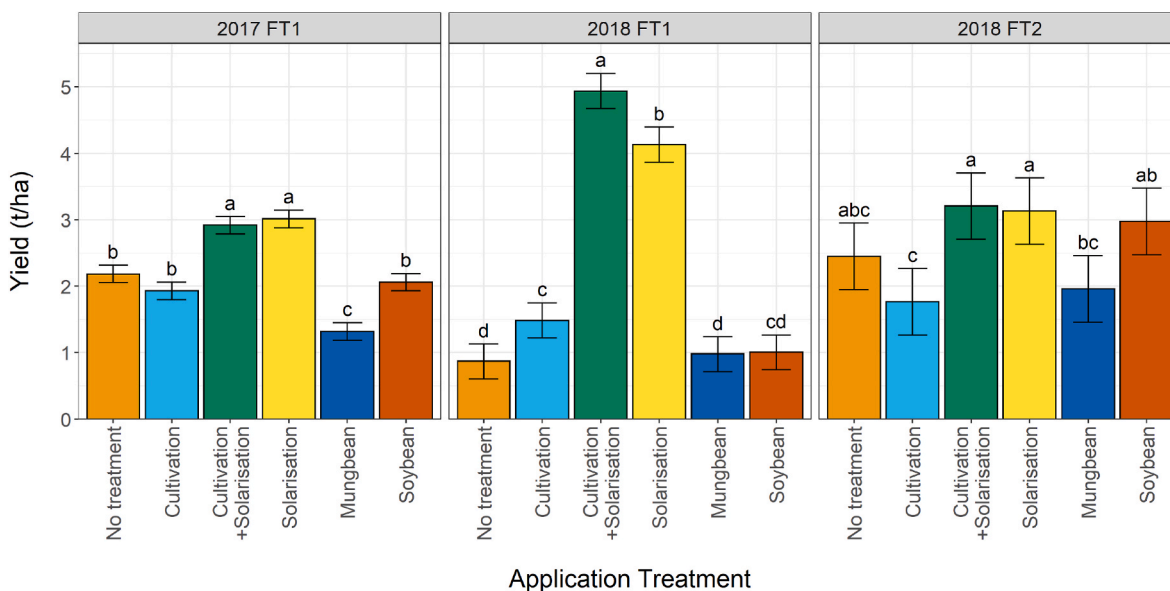
**Fig. 2.** *Fusarium pseudograminearum* incidence in durum stubble obtained from plated tillers, from the three field trials comparing the six application treatments, as well as the pre-treatment trial mean. The error bars represent the standard errors of the predictions. The letters above the bars indicate the results of the least significant difference (LSD) test for treatment comparison within each trial; treatments within the same trial bearing the same letter are not significantly different from each other, at the 5% level of significance.

significantly lower ( $p = 0.003$ ) levels of stubble load at the conclusion of 2018 FT2, compared to the conclusion of 2017 FT1 and 2018 FT1 (10.8% compared to 38.2% and 34.1%, respectively).

### 3.3. Post treatment grain yield and quality measurements

A significant treatment by trial interaction was detected for the grain yield of the bread wheat crop (cv. EGA Gregory) ( $p < 0.01$ ) (Fig. 3).

Yield was significantly higher in plots following solarisation than following all other treatments in 2017 FT1 and 2018 FT1 ( $p < 0.01$  and  $p < 0.001$ , respectively). The grain yield in the two-year trial (2018 FT2) following no treatment (1) and soybean (6) cover crop was not significantly different from the grain yield following the cultivation + solarisation (3) and solarisation (4) treatments, however the yield of the cultivation (2) and mungbean (5) treatments was significantly lower than that for the two solarisation treatments.



**Fig. 3.** Yield of the cv. EGA Gregory crop planted post-treatment and harvested at maturity, from the three field trials comparing the six application treatments. The error bars represent the standard errors of the predictions. The letters above the bars indicate the results of the least significant difference (LSD) test for treatment comparison within each trial; treatments within the same trial bearing the same letter are not significantly different from each other, at the 5% level of significance.

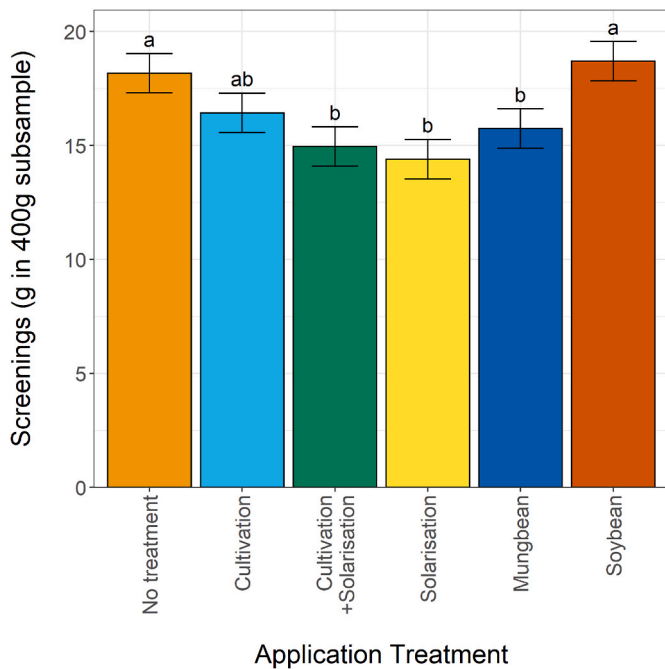


Fig. 4. Screenings from the cv. EGA Gregory crop planted post-treatment and harvested at maturity, from the three field trials comparing the six application treatments. The error bars represent the standard errors of the predictions. The letters above the bars indicate the results of the least significant difference (LSD) test for treatment comparison within each trial; treatments within the same trial bearing the same letter are not significantly different from each other, at the 5% level of significance.

The analysis of the grain screening data from the cv. EGA Gregory crop applied post-treatment (Fig. 4) demonstrated a treatment main effect ( $p = 0.02$ ) with the two solarisation treatments (3) and (4) reporting significantly lower grain screenings than soybean (6) and no treatment (1). The grain screenings for the mungbean (5) and cultivation (2) treatments, were not significantly different to the solarisation treatments (3 and 4). There was also a significant trial main effect ( $p <$

0.01). Screenings were significantly higher in the 2017 FT1 (29.2 g/400 g subsample) when compared to the other two trials: 12.3 g/400 g subsample and 7.8 g/400 g subsample in 2018 FT1 and 2018 FT2 respectively.

There was a significant interaction between trial and treatment on the TGW measured on the grain harvested from the cv. EGA Gregory crop ( $p < 0.01$ ) (Fig. 5). In the 2017 FT1 and the 2018 FT1, the effects of the treatments varied considerably; in both cases, at least one of the solarisation treatments resulted in a significantly higher TGW than the no treatment (1) and soybean (6) treatments. In 2018 FT1, the cultivation + solarisation (3) treatment resulted in a significantly higher TGW than all the non-solarisation treatments. However, in 2017 FT1, the mungbean (5) treatment had a significantly higher TGW than all other treatments. In the two-year experiment (2018 FT2), there were no significant differences in TGW between any of the treatments.

### 3.4. Post-treatment disease severity

There was a significant interaction between trial and treatment in their effect on the disease severity rating, the results for which are shown in Fig. 6 ( $p = 0.03$ ). There was no significant effect of tiller type either as a main effect or interaction effects with the other factors.

In 2017 FT1, there was very little differentiation in disease severity between the application treatments, with the only significant difference being between the mungbean (5) and soybean (6) treatments. In the 2018 FT1 and 2018 FT2 the solarisation treatments had a significant reductive effect on the disease severity. In 2018 FT1, both solarisation treatments resulted in a significantly lower disease severity than all other treatments, while in the 2018 FT2, the cultivation + solarisation treatment resulted in a significantly lower disease severity than all other treatments. The solarisation (4) treatment in 2018 FT2 resulted in a significantly lower disease severity than no treatment (1) and soybean (6), however was not significantly different from the cultivation (2) and mungbean (5) treatments.

### 3.5. Soil moisture

Post-treatment moisture in the top 30 cm of soil was different across the three trials, as indicated by a significant interaction between trial

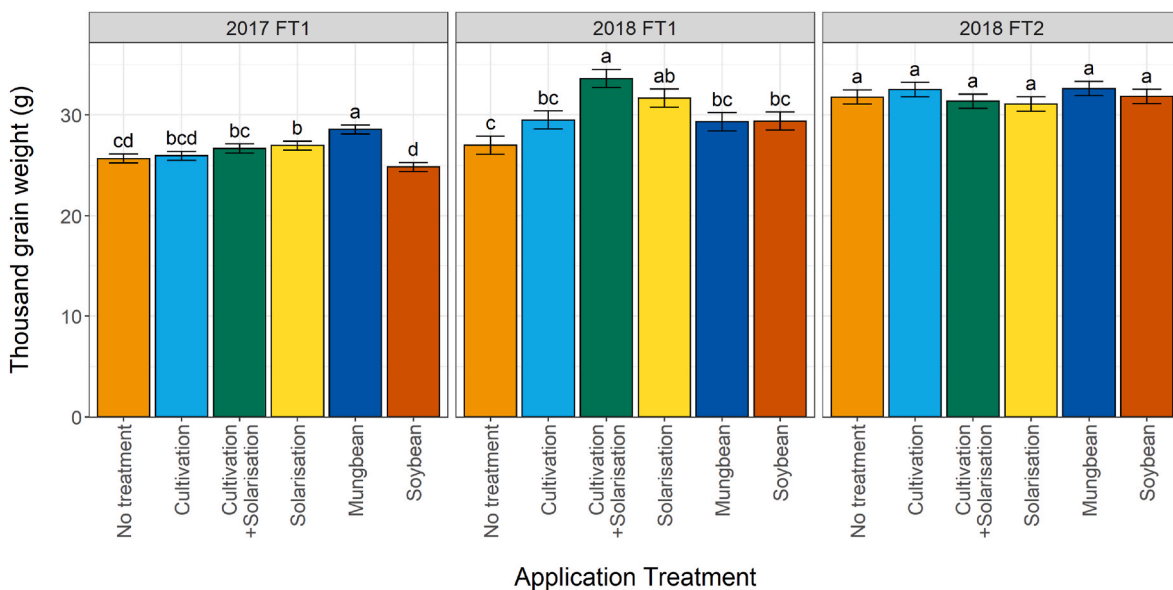
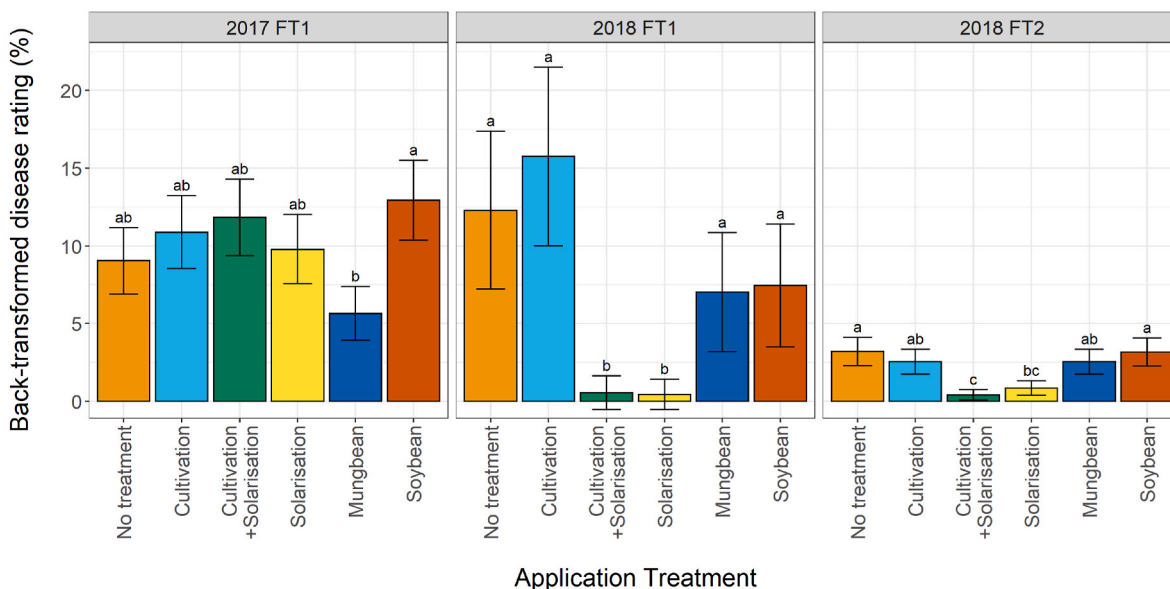


Fig. 5. Thousand grain weight (g) from the cv. EGA Gregory crop planted post-treatment and harvested at maturity, from the three field trials comparing the six application treatments. The error bars represent the standard errors of the predictions. The letters above the bars indicate the results of the least significant difference (LSD) test for treatment comparison within each trial; treatments within the same trial bearing the same letter are not significantly different from each other, at the 5% level of significance.



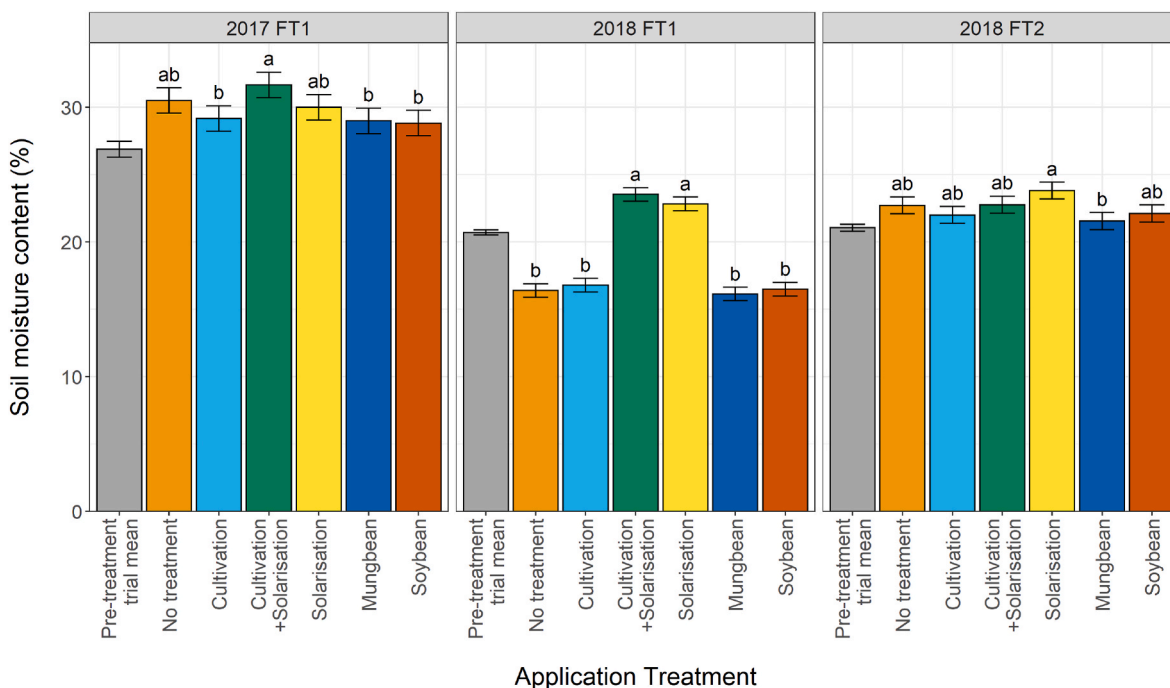
**Fig. 6.** Predictions for crown rot disease severity, from the three field trials comparing the six application treatments. The error bars represent the standard errors of the predictions. The letters above the bars indicate the results of the least significant difference (LSD) test for treatment comparison within each trial; treatments within the same trial bearing the same letter are not significantly different from each other, at the 5% level of significance.

and treatment ( $p < 0.01$ ) (Fig. 7). While the solarisation treatments were mostly not significantly different from other treatments in the 2017 FT1 and the 2018 FT2, the significant differences in the 2018 FT1 were very pronounced, with the cultivation + solarisation (3) and solarisation (4) treatments having significantly higher moisture levels than the non-solarised treatments. In 2017, rainfall during the 12-week treatment period totalled 448.8 mm compared to the total rainfall of 272.4 mm in the same period of 2018 (data obtained from Australian Bureau of Meteorology, from Toowoomba Airport Weather Station located 4 km away). In-crop rainfall during the crop of cv. EGA Gregory totalled

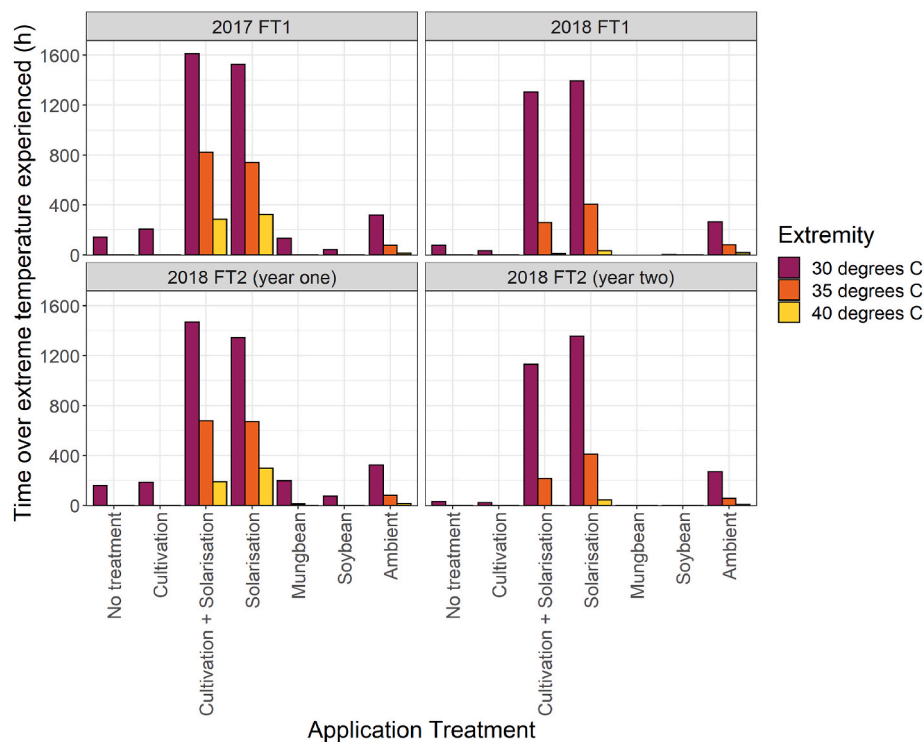
205.8 mm in 2017 and 206.6 mm in 2018. Detailed rainfall data is provided in Appendix A.

### 3.6. Soil temperature

Average soil temperature in the top 0–10 cm soil across the three trials tended to be 10–20 °C higher in the solarised plots compared to the other treatments (Appendix B). Maximum soil temperatures reached 49.9 °C (2018 FT2) and 48.5 °C (2017 FT1) in the solarisation (4) and cultivation + solarisation (3) plots respectively (Appendix B). This is



**Fig. 7.** Soil moisture measurements post-treatment (top 30 cm), from the three field trials comparing the six application treatments, as well as the pre-treatment trial mean. The error bars represent the standard errors of the predictions. The letters above the bars indicate the results of the least significant difference (LSD) test for treatment comparison within each trial; treatments within the same trial bearing the same letter are not significantly different from each other, at the 5% level of significance.



**Fig. 8.** Number of hours of extreme temperatures experienced in the 12 weeks from the three field trials comparing the six application treatments. Data from 2018 FT2 is shown for both years (year one in 2017 and year two in 2018). Data was obtained using dataloggers recording every 15 min at a depth of 10 cm.

compared to maximum temperatures of 34.2 °C and 35.3 °C in the no treatment (1) and cultivation (2) plots, and 37.2 °C and 32.3 °C in the mungbean (5) and soybean (6) plots respectively. Temperatures remained higher overnight when solarisation was applied, despite ambient temperatures dropping considerably, with the lowest recorded ambient temperature across the trials being 3.2 °C in 2017 (Appendix B). Only the solarised treatments (3 and 4) reached temperatures over 40 °C and were able to sustain those temperatures for a long period of time, for example, in 2017 FT1 the solarised treatment (4) plots spent an average total of 320 h over 40 °C (Fig. 8).

### 3.7. Other pathogen levels

The Predicta B pathogen DNA quantification of the 2017 pre-treatment soil samples recorded a high level of *Pyrenophora tritici-repentis* (yellow leaf spot) with an average of 4.7 log<sub>10</sub> (pg DNA/g soil + 1) across the trial area (Appendix C). *Pyrenophora tritici-repentis* was also present in 2018 FT1 and FT2, at lower levels. *Bipolaris sorokiniana* (common root rot) was detected at low levels in all three trials where pre-treatment levels were recorded at an average of 1 log<sub>10</sub> (pg DNA/g soil + 1). Other crop pathogens detected in the pre-treatment soil samples included *Pratylenchus thornei* (root lesion nematodes), *Didymella pinodes/Phoma pinodella*, and *Macrophomina phaseolina* (which were found at low-risk background levels across the three trials). The level of these pathogens did reduce considerably after the application of all treatments. However, stubble plating of a sample of tillers across the treatments from the yield trial in the 2017 FT1 indicated that *B. sorokiniana* was found in some of the diseased tissue (data not shown). This suggests that *B. sorokiniana* was still present in the stubble for some treatments when the cv. EGA Gregory crop was planted and may have contributed to the higher disease ratings seen in 2017 FT1 for the solarisation treatments (Fig. 6). The detailed PREDICTA® B pathogen DNA results are presented in Appendix C, however this data shows only DNA from the soil, not stubble.

## 4. Discussion

This research has demonstrated that a 12-week summer solarisation period is effective in reducing *Fp* levels in both the soil and stubble in the upper 30 cm of soil. Pre-treatment soil levels of *Fp* across 2017 FT1, 2018 FT1 and 2018 FT2 averaged 2.5, 2.5, and 2.3 log<sub>10</sub> (pg DNA/g + 1) respectively, falling into the high and medium risk category according to the PREDICTA® B northern risk categories (SARDI, 2018). Following solarisation, *Fp* levels in the soil reduced significantly to the risk category rating of low (0.1–1.5 log<sub>10</sub> (pg DNA/g + 1)) while other treatments maintained high or medium risk levels. This is consistent with the findings of Saremi and Saremi (2013) where similar high pre-treatment *Fp* levels decreased to below detection level using a single solarisation treatment.

Notably, the solarisation trials reported here indicate that yield loss mitigation may be achieved by applying solarisation treatments over *Fp* affected areas for a relatively short period of time between consecutive seasons. A recent study into the impact of crown rot of wheat in Australia estimated that 42% of surveyed paddocks in the northern region contain *Fusarium* crown rot (Hollaway et al., 2022). Of these paddocks with crown rot incidence, 24% contained *Fp* levels categorised in the low risk category, 5% were medium risk and 14% were high risk (SARDI, 2018; Hollaway et al., 2022). Trials from the southern region have seen yield losses of 50% in the high risk category, which is reduced to 15% when *Fp* levels are only in the low risk category (in a season conducive to high crown rot conditions) (Hollaway et al., 2013, 2022). By applying solarisation to a research site with medium to high-risk background levels of *Fp* it can be clearly seen that yield losses in the following research trial could be significantly reduced, leading to more reliable results.

DNA tests (Appendix C) indicated that *Pyrenophora tritici-repentis*, *Pratylenchus thornei*, *Didymella pinodes/Phoma pinodella* (combined), and *Macrophomina phaseolina* were found in all experimental paddocks across the three trials. All treatments had a highly significant effect in reducing levels of these pathogens, with solarisation treatments perhaps being more effective than the other treatments for these pathogens.



*Bipolaris sorokiniana* DNA was found in all the trials pre-treatment at low levels (see Appendix C). *Bipolaris sorokiniana* can be particularly persistent in paddocks, surviving as spores that can withstand several years of fallow and rotations with non-host plants (Kumar et al., 2002; Acharya et al., 2011; Wildermuth et al., 1997). The persistence of *B. sorokiniana* in some plots post-treatment may have contributed to the absence of significant differences in the disease severity ratings on the cv. EGA Gregory tillers in 2017 FT1 since the disease symptoms caused by *B. sorokiniana* can be similar to those caused by *Fp*. Further research is needed to fully understand the impact of solarisation on these other pathogens.

Incidence of *Fp* in the stubble for each treatment in the two-year trial (2018 FT2) followed a similar pattern to that seen in the one-year trials. The results of 2018 FT2 suggest that there is no significant advantage in applying the solarisation treatment for a second consecutive year following an intervening fallow. The reduction in stubble load for 2018 FT2 was consistent for all treatments, indicating that a natural breakdown in stubble was occurring over the two years. Despite this stubble breakdown, similar disease severity levels were observed between 2018 FT1 and 2018 FT2 in the subsequent crop of cv. EGA Gregory and in the *Fp* DNA in soil samples. The soybean (6) and mungbean (5) cover crops showed slightly lower levels of *Fp* DNA after the second treatment year in 2018 FT2. Although this indicates that these cover crops could assist in the reduction of pathogen levels after two seasons, the *Fp* DNA levels achieved were still in the high-risk category for *Fp*.

The solarisation treatments from this study have shown a significant reduction in *Fp* inoculum in the soil after a single treatment. Several studies have demonstrated that crop rotations with various non-hosts of *Fp* reduce the amount of crown rot in subsequent wheat crops (Readford et al., 2015; Evans et al., 2010; Lamprecht et al., 2006). However a long-term study by Flower et al. (2019) suggests that several in-season fallows or alternative non-host crops may be required to achieve this outcome. In contrast, by using a combination of cultivation and solarisation (3) in 2017 FT1, *Fp* DNA levels were reduced by 99.8% over a significantly shorter period. This study highlights the persistence of the pathogen across several seasons and demonstrates that rotations need to be long and must only include non-host crops to achieve even small reductions in pathogen load.

Tillage has also been shown to reduce *Fp* levels when compared to no tillage in stubble management trials in southern Queensland conducted by Swan et al. (2000). Infection was 33–56% higher when stubble was retained, compared to when it was incorporated (Swan et al., 2000). In the research reported here, across all three trials, cultivation (2) when compared to no treatment (1) had no effect on pathogen load in soil, pathogen incidence in stubble, stubble load in soil, or disease severity in the following crop (Figs. 1–4, and 6). In addition to this, cultivating soil prior to solarisation (3) had no significant impact on the final result across all data compared to the solarisation only treatment (4). However, it should be noted that the infected durum stubble used to establish high background levels in the trials reported here was incorporated into the soil before the trial was carried out. Solarisation cannot be applied to plots with standing, retained stubble.

Soil physical and thermal properties greatly influence the effectiveness of soil solarisation in reducing pathogen load (Abed Gatea Al-Shammary et al., 2020). The soil type on which this trial was conducted was a black vertisol (Powell and Christianos, 1985), which would have excellent thermal conductivity due to its dark colour and high water holding capacity (Stapleton et al., 2000). Soils that have poor moisture holding capacity and are light coloured may not be suitable for solarisation (Stapleton and DeVay, 1986). In addition, excessive tillage of soils may result in a reduction in soil thermal conductivity due to diminished heat transfer through loosened soil particles and insufficient moisture (Blanco-Canqui and Ruis, 2018). However, with the limited cultivation used in these trials, no positive or negative effect on pathogen load was seen from cultivating the soil before solarisation. It has also been shown that a positive relationship can be seen between organic

matter content in the soil and heat flow (Abed Gatea Al-Shammary et al., 2020). As vertisols typically have a high organic matter content, this may also have contributed to the success of solarisation reported here.

A lower rainfall year such as 2018 can cause drought stress to plants contributing significantly to plant stress and higher levels of disease, which would be avoided in the solarisation treatments due to the higher levels of available soil moisture (Ahmadi et al., 2022; Chekali et al., 2011). Post-treatment soil moisture levels varied between the treatments and between the years. This difference may have also contributed to the variability seen in grain yields recorded from the crop of EGA Gregory (Fig. 3), favouring the wetter year (2017) and also the solarisation treatments (3 and 4). To rectify this in future experiments, treatment plots could be irrigated before planting the subsequent grain crop to bring the experimental site up to field capacity and minimise the impact of soil moisture at planting time as a contributing factor to yield differences between treatments and trials.

There are a number of downsides of soil solarisation that should be addressed, including cost, environmental considerations, and the effect on non-target microorganisms. Pathogen control through solarisation can create a biological vacuum, making reinfestation possible under extreme disinfection procedures (Gullino et al., 2022). However, this scenario can also provide an opportunity to introduce beneficial organisms and biocontrol agents successfully.

Studies have shown that beneficial organisms generally recolonise the soil faster than the removed plant pathogens due to heat tolerant characteristics. These include *Trichoderma*, *Pseudomonas* and *Bacillus* (Desaeger et al., 2023). “Microbial flush” is also a common occurrence after solarisation whereby the higher temperatures have encouraged microbial growth of nutrient cyclers, increasing nutrient availability and other soil characteristics (Meng et al., 2019). Our current studies are investigating these microbial changes in the soil as a result of solarisation, the use of solarisation on a larger scale in a cereal research program, and the costs and logistics associated with this. Further solarisation trials across a range of soil types and climatic conditions are also required, particularly in cooler summer environments and in the presence of other *Fusarium* species, in particular *F. culmorum*.

## 5. Conclusion

These small-scale solarisation trials have demonstrated the significant benefits of using solarisation when compared to currently available crown rot management techniques aimed at lowering inoculum in soil and stubble. The potential to deliver near zero level crown rot reference sites in a single season will enable improved screening of cereal varieties for resistance and/or tolerance to *Fp*. These decreased background *Fp* levels in both the soil and stubble, and an increase in soil moisture availability, would be of great benefit to a crown rot research program. Most importantly, solarisation can result in significantly greater grain yields in subsequent variety trials, providing a realistic estimate of crop genetic potential in the absence of disease and a more sensitive estimation of genetic tolerance to disease in inoculated plots. Solarisation would remove the necessity of sampling and estimating pathogen levels in every individual plot prior to planting. This study has shown that this type of reliability and quick turn-around time in achieving minimal *Fp* levels in a trial site can provide an approach far superior to other crown rot minimisation techniques currently in use.

## CRedit authorship contribution statement

**Prue Bottomley:** Writing – original draft, Methodology, Investigation, Conceptualization. **Mark W. Sutherland:** Writing – review & editing. **Bree A.L. Wilson:** Writing – review & editing. **Bethany Rognoni:** Writing – review & editing, Writing – original draft, Formal analysis. **Alison Kelly:** Writing – review & editing, Methodology. **Cassandra D. Percy:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition.

### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Cassandra D. Percy reports financial support was provided by Grains Research and Development Corporation (US00075). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

### Acknowledgements

This work would not have been possible without the effort of the Queensland Department of Agriculture and Fisheries, Toowoomba farm staff who were instrumental in developing and carrying out methodologies to conduct these field trials. Special thanks also goes to the hard-working UniSQ Soilborne Diseases team who assisted with the setup and conduction of these field trials.

### Appendix A. Rainfall data (measured in mm) from Toowoomba Airport Weather Station, QLD in 2016–2018 obtained from the Bureau of Meteorology

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	TOTAL
2016	82.2	79.6	70.2	6.4	19	109.6	40.4	54.8	104	29	25	33.8	654
2017	93.4	21.2	313.6	20.6	14.4	35.8	26.4	2	1	150.4	26	104	808.8
2018	8.6	189.8	51	23	7.6	13.8	15.6	6.6	16.6	110	57.8	48.8	549.2

### Appendix B. Temperature data from the three trials obtained from dataloggers buried at 10 cm under the soil surface. Some data not available due to logger malfunction

Treatment	Trial	Average Temp (°C)	Min Temp (°C)	Max Temp (°C)
No treatment	2017 FT1	25.0	15.4	34.2
	2018 FT1	24.8	18.3	32.7
	2018 FT2 (year one)	24.9	15.3	34.2
	2018 FT2 (year two)	24.4	17.5	31.4
Cultivation	2017 FT1	25.6	16.1	35.0
	2018 FT1	25.0	19.2	31.2
	2018 FT2 (year one)	25.3	16.2	35.3
	2018 FT2 (year two)	24.3	17.0	31.5
Cultivation + Solarisation	2017 FT1	33.9	22.0	48.5
	2018 FT1	31.5	23.4	41.1
	2018 FT2 (year one)	32.9	22.2	45.8
	2018 FT2 (year two)	30.9	23.4	40.2
Solarisation	2017 FT1	33.7	22.0	49.8
	2018 FT1	32.1	23.8	41.4
	2018 FT2 (year one)	32.8	21.0	49.9
	2018 FT2 (year two)	32.0	23.2	45.6
Mungbean	2017 FT1	25.3	16.4	32.6
	2018 FT1	NA	NA	NA
	2018 FT2 (year one)	25.0	14.6	37.3
	2018 FT2 (year two)	24.2	16.0	28.9
Soybean	2017 FT1	25.2	17.7	31.8
	2018 FT1	24.6	19.8	30.1
	2018 FT2 (year one)	24.5	15.6	32.3
	2018 FT2 (year two)	23.8	20.0	29.1
Ambient	2017 FT1	23.7	3.2	43.3
	2018 FT1	23.0	8.2	45.8
	2018 FT2 (year one)	23.7	3.3	43.6
	2018 FT2 (year two)	22.9	8.1	43.9

### Appendix C. Pathogen (other than *F. pseudograminearum*) DNA levels detected in soil samples across the three field trials analysed by PREDICTA® B testing (SARDI, 2018). A $\log_{10} + 1$ has been applied to all data to make it comparable to *F. pseudograminearum* data presented throughout and the northern-Australian Predicta B risk categories. Data includes both pre- and post-treatment sampling times. N/A = test not available at time of sampling

Treatment	(1) No treatment						(2) Cultivation						(3) Cultivation + Solarisation						(4) Solarisation						(5) Mungbean						(6) Soybean						
Trial	2017 FT1		2018 FT1		2018 FT2		2017 FT1		2018 FT1		2018 FT2		2017 FT1		2018 FT1		2018 FT2		2017 FT1		2018 FT1		2018 FT2		2017 FT1		2018 FT1		2018 FT2								
Time of Sampling	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post			
<i>Gaeumannomyces graminis</i> var <i>tritici</i> pg DNA/g sample	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
<i>Pyrenophora tritici-repentis</i> (YLS) copies/g sample	5	3	1	1	5	4	5	3	1	0	5	4	5	0	0	0	5	3	5	0	0	0	5	0	5	4	1	0	5	4	5	3	1	1	5	4	
<i>Bipolaris</i> pg DNA/g sample	1	0	2	1	1	0	1	0	2	0	1	0	1	0	1	1	2	1	1	0	1	0	0	0	0	0	2	1	1	0	1	0	2	0	1	0	
<i>Eutiarosporella tritici-australis</i> copies/g sample	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0		
<i>Eutiarosporella darliae/pseudodarliae</i> copies/g sample	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
<i>Pratylenchus thornei</i> nematodes/g soil	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	
<i>Didymella pinodes/Phoma pinodella</i> pg DNA/g sample	2	1	0	0	1	1	1	0	0	0	2	1	1	0	0	0	1	0	1	0	0	0	1	0	1	2	1	2	1	1	1	1	0	1	1	1	
<i>Macrophomina phaseolina</i> copies/g soil	N/A	3	N/A	0	N/A	3	N/A	3	N/A	0	N/A	3	N/A	3	N/A	0	N/A	3	N/A	2	N/A	0	N/A	3	N/A	3	N/A	0	N/A	3	N/A	3	N/A	0	N/A	3	N/A

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