FINAL REPORT 2014

PROJECT CODE : S1206

PROJECT TITLE
Strategies to reduce white grain on Eyre Peninsula

PROJECT DURATION

Project Start date	1 July 2012
Project End date	30 June 2014

PROJECT SUPERVISOR CONTACT DETAILS

Title:	**First Name:**	**Surname:**	
Dr	Hugh	Wallwork	
Organisation: South Australian Research and Development Institute (a group of Primary Industries and Regions SA)			
Mailing address:			
Telephone:	**Facsimile:**	**Mobile:**	**Email:**

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Project Code	
Project Type	

ADMINISTRATION CONTACT DETAILS

Title:	First Name:	Surname:
Mrs	Adrienne	Twisk

Organisation:

South Australian Research and Development Institute (a group of Primary Industries and Regions SA)

Mailing address:

Telephone:	Facsimile:	Mobile:	Email:

PROJECT REPORT

Executive Summary

Five variety screening and three fungicide efficacy field trials were undertaken on Eyre Peninsula during 2012 and 2013 to assess management options for white grain in bread wheat and barley. Dry spring conditions in 2012 and 2013 meant there was no white grain expression in any of the eight trials and the effectiveness of varietal resistance and fungicide efficacy for managing white grain could not be assessed.

Experience from the 2012 season suggested that variety screening and fungicide efficacy assessment trials relying on field infection alone would not be a reliable method for obtaining data. In 2013, methods for pot screening varieties were developed using artificial inoculation with white grain on the Terraces at the Plant Research Centre. These methods will be used, with on-going refinements, to screen varieties and fungicide application for managing white grain.

During the course of this project:

- White grain did not affect grain deliveries in South Australia in 2012 or 2013 due to dry conditions during flowering and grain fill.
- The fungi causing white grain were found to survive on infected cereal residues for at least 24 months.
- It became apparent that rain-splashed spores from infected stubble are likely to infect cereal plants (without causing symptoms in grain) and allow the white grain pathogens to persist in the farming system in the long-term.
- Visual symptoms of infection by the white grain fungi in stubble, green cereal heads and mature cereal heads were identified.
- The information sheet "White grain in cereals" was developed and has been updated yearly to describe the issue, show the symptoms and suggest management options. This sheet is available to industry personnel and growers on request.

Further funding to pursue this work was obtained from GRDC until June 2016.

Project Objectives

1. Identify varietal differences in reaction of wheat to white grain.
2. Determine whether fungicides can reduce the severity of white grain infection in wheat.

Overall Performance

Variety and fungicide trials were successfully sown, managed and harvested on Eyre Peninsula in 2012 and 2013 at 2-3 sites with significant levels of white grain inoculum.

However, as a result of dry spring conditions in 2012 and 2013, there were no obvious white grain symptoms in these trials, nor were there symptoms of white grain in any of the NVT trials sown in SA. As a result of the lack of white grain expression in the field, the project objectives were not met.

Despite the disappointing lack of results from field trials, this SAGIT project has been very helpful in providing support for:

1. Developing our knowledge of the white grain pathogens and their survival in current farming systems.
2. Identifying the symptoms produced in infected stubble and cereal plants.
3. Extension of information to the Grains Industry.
4. Travel to the Eyre Peninsula which contributed to stubble and spore trap studies.
5. Developing research directions.
6. Encouraging support from the GRDC (a summary of results from studies undertaken using complementary GRDC funding is provided in Appendix 1).

After the dry spring in 2012, it was noted in the progress report to SAGIT that dry spring conditions in 2013 were a risk to achieving project objectives. As a result, the potential of artificially inoculated trials was assessed using GRDC funding in 2013. Future variety and fungicide screening supported by GRDC will be undertaken using artificial inoculation and complementary, opportunistic assessment in field trials, particularly at NVT sites.

Project supervisor: Dr Hugh Wallwork, SARDI.
Scientific Officer: Dr Margaret Evans, SARDI.
Technical Officer: Mr Greg Naglis, SARDI.
Technical Officer: Ms Tijana Petrovic, SARDI, undertook stubble studies.
Dr Alan McKay and Dr Herdina, SARDI, undertook DNA analyses.
Mr Leigh Davis, Mr Brenton Spriggs, Mr Andrew Ware, SARDI, managed field trials.
Mr Graeme and Ms Heather Baldock, Buckleboo, provided trial sites 2012 and 2013.
Mr Rodney Quinn, Cleve, provided trial sites 2012 and 2013.
Mr Matt Micken, Coulta, provided 2012 trial site.
Ms Amy Murray (AgSave, Kimba) and Ms Cherylynn Dreckow (Elders, Cleve) assisted in site selection and stubble sample management in 2012 and 2013.

Key Performance Indicators (KPI)		
KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
Two field trials on Eyre Peninsula in 2012 to assess genetic differences in wheat to white grain infection.	Y	
A field trial on Eyre Peninsula in 2012 to assess efficacy of fungicides to reduce white grain levels.	Y	
Two field trials on Eyre Peninsula in 2013 to assess genetic differences in wheat to white grain infection.	Y	
A field trial on Eyre Peninsula in 2013 to assess efficacy of fungicides to reduce white grain levels.	Y	
Submit final report.	Y	

Technical Information

Assessing genetic differences in wheat

Five field trials (Buckleboo, Cleve and Coulta in 2012; Buckleboo and Cleve in 2013) were conducted at sites on Eyre Peninsula with a history of white grain issues. Stubble from the sites was collected and incidence of fungal infection was assessed to confirm the suitability of trial sites.

Seventy one bread wheat entries (commercial cultivars and breeders' lines) were assessed. Material for screening was acquired from across Australia and represented a broad range of genetic backgrounds, including resistance to fusarium head blight. Small numbers of commercial cultivars of other cereal types were also included up to – 10 barley; 2 durum wheat; 1 triticale; 1 oat and 2 cereal rye. See Appendix 2 for details of entries.

Two (2012, due to limited seed) or 3 replicates (2013) were used, with check plots of Axe and Yitpi (2102) or Axe alone (2013) gridded across trial areas to assess spatial variability in white grain infection. Plots were 3 rows x 7 m long and all grain was harvested and retained for assessment.

In both seasons, spring rainfall was very low, so grain from 16 check plots across each site in each season was visually assessed for white grain. In 1-2 plots per site in each season, 1-2 affected grains were found in the sample. Further assessment was not undertaken as varietal differences would not be detectable at this extremely low level of white grain expression.

Outcome – varietal responses to the white grain pathogens could not be assessed. Artificial inoculation under irrigated/misted conditions is most likely to provide this information in the short term.

Fungicide efficacy in reducing the severity of white grain infection in bread wheat

Four field trials, co-located with variety screening trials at Buckleboo and Cleve, were undertaken on Eyre Peninsula in 2012 and 2013. The bread wheat cultivars Axe (early maturity) and Yitpi (late maturity) were used in these trials to give the longest period of crop susceptibility to infection.

A trial design with 6 replicates and 2 times of spraying allowed easy spray application to achieve untreated, single spray and two spray combinations. Heading, flowering and early grain fill were targeted using Prosaro® @ 150 ml/ha. See Appendix 3 for treatment details.

In both seasons, spring rainfall was very low, so grain from 8 plots across each site in each season was visually assessed for white grain. In 1-2 plots per site in each season, 1-2 affected grains were found in the sample. Further assessment was not undertaken as fungicide efficacy could not be determined at this extremely low level of white grain expression.

Outcome – fungicide efficacy in reducing white grain levels in wheat could not be assessed. Artificial inoculation under irrigated/misted conditions is most likely to provide this information in the short term.

Identification and epidemiology

Knowledge about the pathogen complex and the expression of plant symptoms associated with white grain was gained during this project.

White grain infected stubble from 2011 cropping was collected from 2012 trial areas and was maintained in packets at the sites and at the Plant Research Centre. Stubble pieces from these packets were observed visually and microscopically over time to identify fruiting bodies and the length of time for which fruiting bodies were produced on the infected stubble. Most fruiting bodies produced pycnospores which would be rain-splash distributed and allow long-term survival of the pathogen but would not usually infect heads. Only a small proportion of fruiting bodies produced aerially distributed ascospores which would infect heads. Fruiting bodies and spores were still being produced on the 2011 stubble, although at lower levels, at the start of 2013. This suggests that the white grain pathogens are likely to survive over the long term, even if at low levels, in paddocks where white grain has occurred in cereals.

To identify white grain symptoms on green heads, rows of the bread wheat cultivar Axe were hand sown at the Plant Research Centre. Rows were inoculated with spores of the white grain pathogens at flowering and then twice after that at 10 day intervals. Humidity around inoculated plants was maintained by inoculating late in the day after misting the rows with water and then misting plants with water immediately after inoculation. Grain from heads and spikelets with suspected infection by the white grain pathogens were plated on agar, which confirmed the pathogens were present.

Symptoms of white grain in green bread wheat heads were grey spikelets on some heads, with a grey rachis behind infected spikelets. Despite high humidity levels, plant growth stages which were ideal for infection and the high numbers of spores applied, only 7% of grain exhibited visual symptoms. This suggests that very high spore concentrations as well as climatic conditions and plant growth stages conducive to infection are needed before significant levels of white grain will be seen in commercial cereal crops.

To validate the early symptoms of white grain in the field, EP trials and crops were checked during flowering/early grain fill. No symptoms were found in the trials, adjacent crops or local paddocks with a history of white grain issues due to the dry spring conditions. It became obvious that it would be difficult to detect white grain symptoms in green heads and that symptoms caused by minor frost damage and poor grain development were difficult to distinguish from white grain symptoms.

As symptoms are also difficult to distinguish in mature heads, visual identification of paddocks where the white grain pathogens are present will also be difficult and other risk assessment methods (e.g. DNA analysis as provided by the PredictaB service, Dr Alan McKay, SARDI) are needed. Soil and infected stubble from Eyre Peninsula trial sites were provided to Dr McKay's group for use in developing DNA tests for the white grain pathogens. Two tests for the white grain pathogens are now available within the PredictaB soil analysis service, but still require field calibration.

Photographs of white grain expression in grain, stems and heads are presented in the white grain information sheet attached to this report as Appendix 4.

Outcomes – improved knowledge base and ability to target future research. We can say that the white grain pathogens will survive for extended periods at low levels in affected paddocks without grain symptoms occurring will be difficult to detect visually prior to grain maturity and that two wet springs to build up spore levels will probably be needed before another outbreak of this problem occurs. Also that spore trapping combined with crop development and climatic information has potential to provide a pre-harvest risk indicator for white grain.

Conclusions Reached &/or Discoveries Made

We concluded that:

1. Variety screening and fungicide efficacy assessment trials relying on field infection alone would not be a reliable method for obtaining data.
2. Spore trapping combined with crop development and climatic information has potential to provide a pre-harvest risk indicator for white grain.

Visual identification of the presence of white grain in paddocks is difficult and that the presence of white grain inoculum is the best way to identify paddocks at risk of white grain expression in bread wheat and barley, with PredictaB being the best tool for determining this.

Intellectual Property

N/A

Application / Communication of Results

Main findings

- White grain did not affect grain deliveries in South Australia in 2012 or 2013 due to dry conditions during flowering and grain fill.
- The fungi causing white grain can survive on infected cereal residues for at least 24 months.
- Rain-splashed spores from infected stubble can infect cereal plants (without causing symptoms in grain) and allow the white grain pathogens to persist in the farming system in the long-term.
- Infected cereal residues will show visual symptoms by the beginning of the next cropping season.
- Visual symptoms of infection by the white grain fungi in green cereal heads are bleached or grey spikelets with the rachis behind the spikelets also being bleached/grey. Care should be taken as this symptom can be confused with those from frost damage.
- An information sheet "White grain in cereals" (updated annually) is available on request.
- Spore-trapping data combined with information about crop development and environmental conditions has the potential to provide a pre-harvest indicator of the risk of white grain in crops.
- A method for identifying white grain risk is needed. The PredictaB service is able to provide an inoculum detection service immediately but the levels havenot yet been calibrated to indicate risk levels.

Publications and extension articles

White grain in wheat in Australia. M. Evans. 2014. A paper compiled by Dr Evans to provide a summary of the history of white grain issues and research findings nationally. For use in planning future research by current participants in white grain research in Qld, NSW, Vic, SA and WA. Paper is attached in Appendix 5.

Extension and media articles and interviews:

- White grain information sheet 2012 and 2013.
- GRDC media release 2012
- 12 media articles and 1 radio interview 2012.
- Minnipa Agricultural Centre Field Day 2013.
- Eyre Peninsula Farming Systems results book 2013.
- Ground Cover Supplement (Issue 102) 2013.
- Crop Watch newsletter 2013.
- Cereal Variety Disease Guide 2014.

Presentations were made at:

- Agricultural Advisory Bureau field day (Buckleboo) 10th October 2012.
- Scientific seminar (Plant Research Centre), 18th April 2013.
- Farmer meeting (Buckleboo), 3rd September 2013.
- Briefing for Mr Bill Vandepeer (Plant Research Centre), 12th September 2013.
- Crossville Agricultural Bureau crop walk (Cleve), 4th October 2013.
- Mid-North High Rainfall Trial site field day (Riverton), 16th October 2013.
- Farmer field walk (Booleroo Centre), 18th October 2013.
- Independent Consultants Group meeting (PRC), 31st October 2013.
- GRDC Updates in Adelaide in February 2014 and at Kimba in August 2014

POSSIBLE FUTURE WORK

Possible areas for developing co-funded research with GRDC into management options for white grain include:

- Paddock preparation/stubble management
- Grain harvesting/handling
- Rotations

AUTHORISATION	
Name:	Dr Kathy Ophel Keller
Position:	Research Chief, Sustainable Systems
Signature:	
Date:	29 August 2014

Appendix 1 – findings from complementary studies funded by GRDC.

With GRDC funding, complementary studies were undertaken in 2013 which showed that:

1. Inoculated trials on the Terraces at the PRC can be used to screen cultivars for resistance to white grain. This implies artificial inoculation could also be used in field-based screening trials.
2. Visual symptoms of infection by the white grain fungi present in SA occur in green cereal heads as bleached or grey spikelets with the rachis behind the spikelets also being bleached/grey.
3. Even under ideal conditions with heavy spore loads applied to susceptible plants only some heads and a few spikelets within those heads developed symptoms. This makes it probable that reliably getting good infection in inoculated field screening trials could be difficult.
4. In collaboration with Alan McKay's group, DNA tests for the white grain fungi have been developed, validated and calibrated to allow conversion from pg of DNA per sample to spores per sample. This has allowed fungal DNA to be extracted from spore trap tapes and the results converted to spore numbers. Hence we have tracked air-borne spore numbers over time.
5. Air borne spores of the white grain fungi were present on upper EP from the first week of August to the first week of September in 2013 (see graph below). This is consistent with findings from stubble studies undertaken in 2012 and suggests spore trapping could be used to assess the risk of white grain prior to harvest.

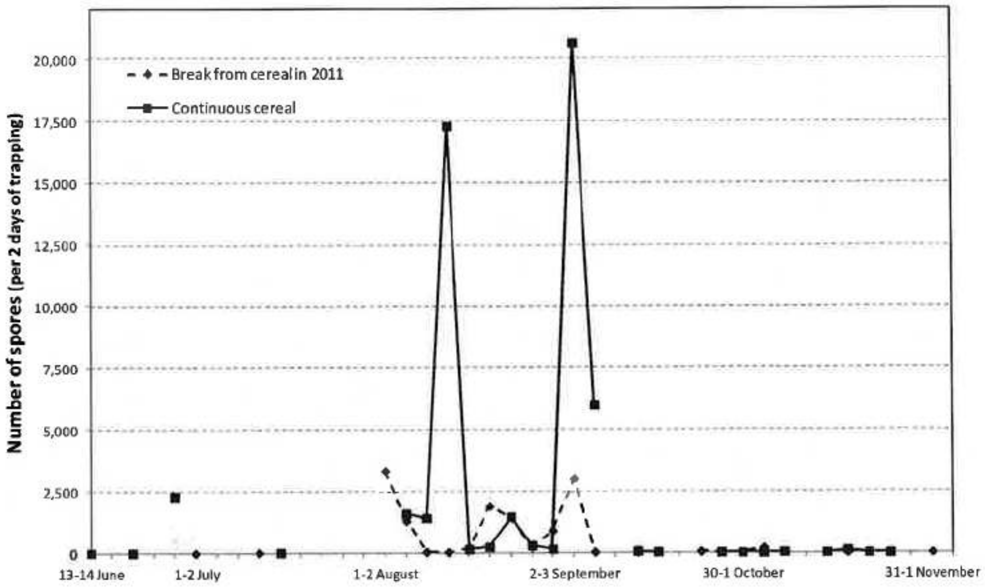

Figure 1. Presence of air-borne spores of the fungi associated with white grain in two paddocks at Buckleboo during 2013.

Entries in white grain screening trials, SAGIT project S12/06

	2012 Season			2013 Season	
Cereal type	Buckleboo	Cleve	Coulta	Buckleboo	Cleve
Bread wheat	AGT01	AGT01	AGT01	AGT01	AGT01
Bread wheat	AGT02	AGT02	AGT02	AGT02	AGT02
Bread wheat	AGT03	AGT03	AGT03	AGT03	AGT03
Bread wheat	AGT04	AGT04	AGT04	AGT04	AGT04
Bread wheat	AGT05	AGT05	AGT05	AGT05	AGT05
Bread wheat	AGT06	AGT06	AGT06	AGT06	AGT06
Bread wheat	AGT07			AGT07	AGT07
Bread wheat	AGT08			AGT08	AGT08
Bread wheat	AGT09			AGT09	AGT09
Bread wheat	AGT10	AGT10	AGT10	AGT10	AGT10
Bread wheat	AUS29529	AUS29529	AUS29529	AUS29529	AUS29529
Bread wheat	AUS30323	AUS30323	AUS30323	AUS30323	AUS30323
Bread wheat	Axe	Axe	Axe	Axe	Axe
Bread wheat	Barham	Barham	Barham	Barham	Barham
Bread wheat	Binnu	Binnu	Binnu	Binnu	Binnu
Bread wheat	Bolac	Bolac	Bolac	Bolac	Bolac
Bread wheat	Bonnie Rock	Bonnie Rock	Bonnie Rock	Bonnie Rock	Bonnie Rock
Bread wheat	Bullaring	Bullaring	Bullaring	Bullaring	Bullaring
Bread wheat	Bumper	Bumper	Bumper	Bumper	Bumper
Bread wheat	Calingiri	Calingiri	Calingiri	Calingiri	Calingiri
Bread wheat	Carnamah	Carnamah	Carnamah	Carnamah	Carnamah
Bread wheat	Cascades	Cascades	Cascades	Cascades	Cascades
Bread wheat	Cobra	Cobra	Cobra	Cobra	Cobra
Bread wheat	Derrimut	Derrimut	Derrimut	Derrimut	Derrimut
Bread wheat	Eagle Rock	Eagle Rock	Eagle Rock	Eagle Rock	Eagle Rock
Bread wheat	EGA Blanco	EGA Blanco	EGA Blanco	EGA Blanco	EGA Blanco
Bread wheat	Emu Rock	Emu Rock	Emu Rock	Emu Rock	Emu Rock
Bread wheat	Espada	Espada		Espada	Espada
Bread wheat	Estoc	Estoc	Estoc	Estoc	Estoc
Bread wheat	Fortune	Fortune	Fortune	Fortune	Fortune
Bread wheat	Gladius	Gladius	Gladius	Gladius	Gladius
Bread wheat	Gregory	Gregory	Gregory	Gregory	Gregory
Bread wheat	Halberd	Halberd	Halberd	Halberd	Halberd
Bread wheat	IGW3073	IGW3073	IGW3073	IGW3073	IGW3073
Bread wheat	IGW3119	IGW3119	IGW3119	IGW3119	IGW3119
Bread wheat	IGW3424	IGW3424	IGW3424	IGW3424	IGW3424
Bread wheat	Impose CL Plus	Impose CL Plus	Impose CL Plus	Impose CL Plus	Impose CL Plus
Bread wheat	Janz CF1	Janz CF1	Janz CF1	Janz CF1	Janz CF1
Bread wheat	Justica CL Plus	Justica CL Plus	Justica CL Plus	Justica CL Plus	Justica CL Plus
Bread wheat	King Rock	King Rock	King Rock	King Rock	King Rock
Bread wheat	Kord	Kord	Kord	Kord	Kord
Bread wheat	Krichauff	Krichauff	Krichauff	Krichauff	Krichauff
Bread wheat	Kunjin	Kunjin	Kunjin	Kunjin	Kunjin
Bread wheat	Livingston	Livingston	Livingston	Livingston	Livingston
Bread wheat	LPB07-0548	LPB07-0548	LPB07-0548	LPB07-0548	LPB07-0548
Bread wheat	LPB07-0956	LPB07-0956	LPB07-0956	LPB07-0956	LPB07-0956
Bread wheat	LPB07-1325	LPB07-1325	LPB07-1325	LPB07-1325	LPB07-1325
Bread wheat	LPB08-0028	LPB08-0028	LPB08-0028	LPB08-0028	LPB08-0028
Bread wheat	LPB08-0360	LPB08-0360	LPB08-0360	LPB08-0360	LPB08-0360

Entries in white grain screening trials, SAGIT project S12/06

	2012 Season			2013 Season	
Cereal type	**Buckleboo**	**Cleve**	**Coulta**	**Buckleboo**	**Cleve**
Bread wheat	LPB08-1224	LPB08-1224	LPB08-1224	LPB08-1224	LPB08-1224
Bread wheat	LPB09-0521	LPB09-0521	LPB09-0521	LPB09-0521	LPB09-0521
Bread wheat	LPB09-1041	LPB09-1041	LPB09-1041	LPB09-1041	LPB09-1041
Bread wheat	LPB09-2209	LPB09-2209	LPB09-2209	LPB09-2209	LPB09-2209
Bread wheat	LPB10-0034	LPB10-0034	LPB10-0034	LPB10-0034	LPB10-0034
Bread wheat	Mace	Mace	Mace	Mace	Mace
Bread wheat	Machete	Machete	Machete	Machete	Machete
Bread wheat	RAC1689R	RAC1689R	RAC1689R	RAC1689R	RAC1689R
Bread wheat	Scout	Scout	Scout	Scout	Scout
Bread wheat	Sokoll	Sokoll	Sokoll	Sokoll	Sokoll
Bread wheat	Stiletto CF1	Stiletto CF1	Stiletto CF1	Stiletto CF1	Stiletto CF1
Bread wheat	Sumai 3	Sumai 3		Sumai 3	Sumai 3
Bread wheat	Sun595b	Sun595b	Sun595b	Sun595b	Sun595b
Bread wheat	Sunguard	Sunguard	Sunguard	Sunguard	Sunguard
Bread wheat	Tincurrin	Tincurrin	Tincurrin	Tincurrin	Tincurrin
Bread wheat	Ventura	Ventura	Ventura	Ventura	Ventura
Bread wheat	Vorobey	Vorobey	Vorobey	Vorobey	Vorobey
Bread wheat	Wallup	Wallup	Wallup	Wallup	Wallup
Bread wheat	Wedin	Wedin	Wedin	Wedin	Wedin
Bread wheat	Westonia	Westonia	Westonia	Westonia	Westonia
Bread wheat	Wyalkatchem	Wyalkatchem	Wyalkatchem	Wyalkatchem	Wyalkatchem
Bread wheat	Yitpi	Yitpi	Yitpi	Yitpi	Yitpi
Barley	Buloke	Buloke		Buloke	Buloke
Barley	Fleet	Fleet		Fleet	Fleet
Barley	Gairdner	Gairdner			
Barley	Hindmarsh	Hindmarsh		Hindmarsh	Hindmarsh
Barley	Keel	Keel			
Barley	Maritime	Maritime			
Barley	Mundah	Mundah			
Barley	Schooner	Schooner			
Barley	Scope	Scope			
Barley	Sloop SA	Sloop SA			
Durum wheat	Tamaroi	Tamaroi	Tamaroi		
Durum wheat	Tjilkuri	Tjilkuri		Tjilkuri	Tjilkuri
Oat	Wintaroo	Wintaroo		Wintaroo	Wintaroo
Triticale	Tahara	Tahara	Tahara	Tahara	Tahara
Cereal rye	Bevy	Bevy	Bevy	Bevy	Bevy
Cereal rye		SA Rye			

White grain management with fungicides, generalised trial plan

	REP I		REP II		REP III		
	1	2	3	4	5	6	
	Buffer	Buffer	Buffer	Buffer	Buffer	Buffer	
1	Yitpi	**Yitpi**	**Yitpi**	Yitpi	Yitpi	**Yitpi**	1
2	Axe	**Axe**	**Axe**	Axe	Axe	**Axe**	2
3	Yitpi	**Yitpi**	**Yitpi**	Yitpi	Yitpi	**Yitpi**	3
4	Axe	**Axe**	**Axe**	Axe	Axe	**Axe**	4
5	Yitpi	**Yitpi**	**Yitpi**	Yitpi	Yitpi	**Yitpi**	5
6	Axe	**Axe**	**Axe**	Axe	Axe	**Axe**	6
7	Yitpi	**Yitpi**	**Yitpi**	Yitpi	Yitpi	**Yitpi**	7
8	Axe	**Axe**	**Axe**	Axe	Axe	**Axe**	8
	Buffer	Buffer	Buffer	Buffer	Buffer	Buffer	
	1	2	3	4	5	6	
	REP IV		REP V		REP VI		

Green cell colour = early fungicide spray (when Axe is flowering)

Yitpi Bold red writing = late fungicide spray (when Yitpi is flowering)

This trial design was chosen to facilitate ease of spraying (along one run, not randomised plot sprays) while allowing statistical analysis.

Fungicide = Prosaro® @ 150 ml/ha (prothioconazole + tebuconazole).
A prothioconazole + tebuconazole mix was chosen as this is most effective for reducing reducing infections by spores of fusarium head blight

With the gridded spray design in the above trial plan, this gives the following treatments:

1. Untreated - Axe
2. Untreated - Yitpi
3. Single application (flowering) - Axe
4. Single application (flowering) - Yitpi
5. Single application (early grainfill) - Axe
6. Single application (head emergence) - Yitpi
7. Two applications (flowering + early grain fill) - Axe
8. Two applications (heading + flowering) – Yitpi

White Grain in Wheat in South Australia

Margaret Evans (08) 8303 9379; marg.evans@sa.gov.au and Hugh Wallwork (08) 8303 9382; hugh.wallwork@sa.gov.au, SARDI
February 2014

White grain was first observed in bread wheat in South Australia and Victoria during the 2010 season harvest and caused rejection and down grading of deliveries in that year and also in 2011.

White grain has been detected across much of SA, with north eastern Eyre Peninsula and the Far North being most severely affected. White grain has not been detected on the West Coast of the Eyre Peninsula. Wet conditions after heading in 2010 and 2011 almost certainly contributed to the severe infections evident in crops in these seasons.

The issues

White grain of wheat caused by *Botryosphaeria zeae* was first detected in Queensland and northern New South Wales in 1999, where it has since been reported sporadically. In addition to *B. zeae*, there is at least one other *Botryosphaeria* species causing white grain in SA. Little is known about the epidemiology and management of the fungi causing white grain in wheat in Australia.

Wheat and barley can be affected by this disease, although only bread wheat has shown symptoms in SA. The fungus survives in cereal residues and is likely to remain a concern for the future, but severe problems are only likely to occur where wet springs promote infection late in the season.

There is no evidence to suggest that white grain in SA is associated with toxins. However, white grains are also a symptom of infection by Fusarium head blight/head scab, where toxins are present in the affected grain. This means buyers may perceive that the white grain that occurs in SA is associated with myco-toxins. This is a particular issue for export markets.

Symptoms

Usually only some grains in a head will be affected. Severely affected grain is very light grey to white and sometimes pinched when compared with normal grain (photo on right). Less severe symptoms can be difficult to detect as infected grains can look similar in size and colour to normal grain. The germ of infected grain is often shrivelled and just a shell. White grains will not germinate and germination may also be reduced in affected grains which do not show severe symptoms.

Stubble symptoms (photo below left) include "scabby" nodes (photo below middle) with black, slightly raised structures on them. Similar structures can be seen on internodes and leaf sheaths. As these fruiting structures mature, they erupt through the stem/leaf sheath surface (photo below right).

For positive identification, laboratory examination is needed as other fungi can cause similar symptoms on crop residues.

Leaf symptoms have not been seen in the field and even at crop maturity it is difficult to detect white grain symptoms on plants. Green heads (photo on left) may show bleaching or grey discolouration of infected spikelets and awns and the rachis behind the spikelet. Not all spikelets will be affected. More mature plants may have darkened stems below the head (photo above).

In field crops, rubbing out grain is the best assessment method when checking crops for white grain. Infected spikelets can be detected while heads are green but these symptoms can easily be confused with frost or poor grain development. Infected mature heads may show some greyish discolouration but are very difficult to detect without examining the grain. White grain affected plants are likely to be unevenly distributed, so check grain from a number of places in each paddock when assessing disease status.

Management

White grain may be an issue in any season where inoculum is present in the area and wet spring weather favors infection by the fungus. Management options for white grain are limited and symptoms are difficult to detect, so it is important to check grain prior to harvest if there has been moisture during flowering and grainfill. At least 24 hours of high humidity is optimal for infection.

Where significant levels of white grain are found it may be possible to adjust harvester settings to reduce the affected grain going into the bin, as white grain is lighter than normal grain. Occurrence of affected heads is often patchy within a paddock, so it may also be possible to harvest badly affected areas separately.

There is no evidence to suggest that variety choice or fungicide application consistently affect white grain expression. At least in part, this is because infection can occur over a range of temperatures (15-24° C) and crop growth stages (head emergence to soft dough). Axe, Yitpi, CF Justica, CF Stiletto, Gladius, Mace, Scout and Wyalkatchem were equally susceptible to this disease in controlled environment studies. White grain has been seen in commercial crops where fungicide has been applied for rust control.

Infected seed will not germinate and will not contribute significantly to inoculum levels unless the seed is transported to previously uninfected regions. Where seed is retained on-farm, check germination before use as seeding rates may need to be lifted where infection is severe.

Infected cereal stubble can produce spores for at least 24 months. Spores dispersed by rain-splash are likely to be present over most of the growing season. Air-borne spores are likely to be present mid- to late-season and these spores have the potential to travel long distances. In 2013, air-borne spores were present in high numbers during August and early September on upper Eyre Peninsula.

Research supported by SAGIT and GRDC funding in 2013 included fungicide efficacy trials, variety screening and spore trap studies. Variety screening, spore trapping and stubble studies funded by GRDC will continue in 2014.

WHITE GRAIN IN WHEAT IN AUSTRALIA

Compiled by Dr Margaret Evans, SARDI

The issue

White grain of wheat in Australia is associated with fungal species in the Botryosphaeriaceae family but the symptoms are similar in some respects to those produced by Fusarium head blight (FHB). As FHB infected grain contains toxins harmful to stock and humans, there are concerns that white grain may be perceived to contain toxins and that this may have an impact on Australia's grain export markets. An additional complication has been identifying the fungal species associated with white grain. Initially *Botryosphaeria zeae* was identified as being associated with white grain, but it is now known that four fungi

White grain

Photo: Margaret Evans, SARDI

Fusarium head blight

Photo: Dep't of Agriculture & Aquaculture, New Brunswick, Canada

are associated with white grain and these fungi are considered to belong to the genus *Tiarosporella*.

Expertise

ACT - 2011 on: toxin assessments; molecular identification
Peter Solomon, ANU Elisha Thynne, ANU

New South Wales - 1999 on: identification; extension of information to industry; management options.
Steven Simpfendorfer, NSW DPI Many others have also been associated with white grain work in NSW.

Queensland - 1999 on: molecular identification; identification; management options; extension of information to industry
Stephen Neate, DAFF Yu Pei Tan, DAFF
Others associated with this work include - Barry Blaney, Victor Galea, Stephen Neate, Greg Platz, Roger Shivas, Yu Pei Tan, Graham Wildermuth, Peter Wilkinson and Peter Williamson

South Australia - 2010 on: identification; epidemiology; biology; management options; inoculum quantification; distribution; extension of information to industry.
Hugh Wallwork, SARDI Margaret Evans, SARDI Alan McKay, SARDI

Western Australia - 2013 on: molecular identification (future plans include: epidemiology; biology; management options; distribution; extension of information to industry).
Kithisri Jayasena, DAFWA Geoff Thomas, DAFWA Kirsty Bayliss, Murdoch University

Overview

White grain was first recorded in Australia in 1999 in Queensland and the causal agent was identified as *Botryosphaeria zeae* (Wildermuth *et al.*, 2001). Since that time, white grain (known as Botryosphaeria Head Blight or BHB) occurred sporadically in Queensland and northern New South Wales, often at infection rates of 50% or more. White grain was detected in South Australia and Victoria in 2010 and in Western Australia in 2013.

In 2011 174,370 tonnes of white grain affected wheat were delivered to SA silos, with infection rates as high as 27%, but with most of the loads assessed having less than 7% infected grain. Loads were downgraded and rejected because Viterra had concerns that export markets might be compromised due to perceptions that white grain was associated with toxins similar to those produced by Fusarium Head Blight (FHB).

Initially, *B. zeae* was considered to be the causal agent of white grain in southern Australia and the concerns about toxins were limited as a feeding study in pigs (Kopinski and Blaney, 2010) demonstrated they fed and grew well on affected grain without any signs of being affected by toxins. Concerns about grain quality for bread making were allayed when white grain affected wheat from SA was used to make good quality test loaves. When it was realised *B. zeae* was not the only fungus associated with white grain, the toxin issue became more pressing.

The fungal complex associated with white grain is now known to consist of four closely related fungi (*B. zeae*, Clade 2, Clade 1a and Clade 1b). *B. zeae* and Clade 2 occur in Qld and northern NSW. All fungi occur in SA, WA and Vic, with Clade 2 most common in WA, Qld and NSW and Clade 1a most common in SA.

In a manuscript prepared for submission to Australasian Plant Pathology by Elisha Thynne in 2014, it is being recommended that the fungi associated with white grain be placed in the genus *Tiarosporella*. Specifically - *Tiarosporella tritici-australis* (= Clade 1), *Tiarosporella darliae* (=*B. zeae*), and *Tiarosporella pseudodarliae* (=Clade 2). As yet there is not enough information to separate Clade 1a and Clade 1b. Molecular work undertaken in Qld, SA, NSW and WA support placing these fungi in the genus *Tiarosporella*.

While it is difficult to prove the fungi associated with white grain do not produce toxins, evidence to date strongly suggests they do not. Assays of affected grain showed toxin levels well below those allowed for human consumption and the levels were consistent with the low levels of *Fusarium pseudograminearum* contamination associated with the samples. Mapping of the genomes of Clade 1a, Clade 1b, Clade 2 and *B. zeae* did not detect significant homology matches for the genes involved in trichothecene synthesis. This gives us confidence that the fungi do not produce any of the toxins produced by FHB (i.e. Deoxynivalenol, nivalenol and similar derivatives).

PreDicta B now offers tests for Botryosphaeria Clade 1 (=Clade 1a + Clade 1b) and Clade 2 (=Clade 2 + *B. zeae*) which are being used to assess distribution, soil inoculum levels (which still need calibrating) and spore numbers collected on field-based spore traps. This test is based on real time qPCR and is very sensitive to the DNA of the white grain fungi. Results from soil samples already suggest that white grain inoculum is present in paddocks over a wide area of Australia.

White grain expression in wheat crops in the future is likely to be sporadic and related to wet springs with extended periods of head wetness during flowering and grain fill. However, the implications of white grain for our export markets remains a significant issue and warrants further work.

Recommendations

1. Confirm identification of the fungi associated with white grain in Australia including specific names for Clade 1a and Clade 1b.
2. Notify the National Consultative Committee of re-designation of *Botryosphaeria* spp. as *Tiaro-sporella* spp. (??SARDI to undertake) and its association with white grain in Australia.
3. If required, develop a rapid toxin test for assessing infected grain.
4. If required, develop specific tests within PreDicta B for Clade 1a and Clade 1b (currently jointly in Clade 1), Clade 2 and *B. zeae* (currently jointly in Clade 2).
5. Define the national distribution and prevalence of the pathogens.
6. Continue assessing management options (e.g. fungicides; varieties; rotations; paddock prepa-ration; spore trapping; calibrating PreDicta B soil analyses; grain harvesting/handling).
7. Ensure all data and findings are readily available to all for future use.

Detailed chronology

1999. First Australian record of white grain of wheat from Queensland.

- The causal pathogen was identified as *Botryosphaeria zeae* (Wildermuth *et al.* 2001) and white grain was given the common name of Botryosphaeria Head Blight (FHB).

1999-2009. Sporadic detection of white grain in crops in Queensland and northern New South Wales.

- White grain affected heads were found to have a white/grey rachis (not brown as in FHB).
- Affected crops were known to have up to 50%-60% infection rates.
- A great deal of work was done on *B. zeae* in Qld and NSW during this period but much of the infor-mation and data are not readily accessible as they exist only in hard copy. Stephen Neate is in the pro-cess of gathering an overview of that information.
- Information sheets, overhead presentations and a range of other extension material were produced and made available to industry.

2010. First record of white grain in wheat from South Australia and Victoria.

- Rejection and downgrading of white grain affected wheat deliveries to silos in SA due to concerns for export markets if white grain is perceived to be "tombstone" grain as produced by FHB.
- *B. zeae*-infected wheat used in a feeding study with weaner pigs (Kopinski and Blaney, 2010) and found not to affect palatability or pig growth rates. No suggestion of toxic effects were found.

2011. 174,370 tonnes of wheat delivered to silos in SA were affected by white grain.

- Rejection and downgrading of white grain affected wheat deliveries to silos in SA.
- Infection levels in 78 affected deliveries: <2% in 25; 3-7% in 44; 8-16% in 8; 27% in 1 delivery.
- Toxin (Peter Solomon), management (Hugh Wallwork) and molecular (Stephen Neate, Yu Pei Tan) re-search begins.

2012. Only 2 records of white grain in SA.

- Molecular characterisation of fungal isolates associated with white grain (Stephen Neate, Yu Pei Tan). *B. zeae* and another pathogen (unidentified and designated as Clade 2) shown to be associated with white grain in the north. Clade 2 is also present at low levels in SA and Vic. Two other pathogens (unidentified and designated as Clade 1a and Clade 1b) shown to be associated with white grain in SA and Vic but not with white grain in the north. *Tiarosporella* spp. are present within the molecular schematics produced by Stephen and Yu Pei.

- White grain affected wheat from SA demonstrated to meet bread making standards.
- White grain affected wheat from SA was found to have low levels (well below those allowable in grain for human consumption) of the toxins associated with FHB. This grain could not be guaranteed free from *Fusarium pseudograminearum* and the toxin levels present were consistent with that type of contamination.
- SA research program includes controlled environment studies, field screening for resistance, assessment of fungicide efficacy, fruiting body development on stubble, identification of symptoms on stubble and spore trapping.
- SA produces an information sheet which is regularly updated to reflect most recent research observations and findings.
- The process of developing tests for and including white grain in the suite of pathogens detected by the PreDicta B service begins.

2013. First record of white grain in wheat from Western Australia. Only 1 record of white grain in SA.

- Nine affected grain samples confirmed from a range of sources in WA. Initial testing indicates Clade 2 is most common, but Clade 1 is also present.
- Visually affected white grain from wheat plants artificially inoculated in 2012 (Clade 1b) were sent for toxin analysis. This grain was known to have a low level of infection with a *Fusarium pseudograminearum*. Very low level of toxins (consistent with low level *Fusarium pseudograminearum* infection) were detected. Levels well below those allowable in grain for human consumption.
- Putative Botryosphaeriaceae genus (*Tiarosporella*) was one of the most commonly detected genera in stored wheat grain in WA (Bayliss 2013).
- Molecular and visual identifications undertaken by Elisha Thynne and Peter Solomon shows that the fungi associated with white grain should be placed in the genus *Tiarosporella* and preparation of a manuscript begins.
- Diana Hartley's (CSIRO) white grain schematic developed for PreDicta B became available and indicated the fungi associated with white grain are from or closely related to the *Tiarosporella* genus.
- Molecular schematic from WA shows the fungi associated with white grain would best be placed within the *Tiarosporella* genus.
- Development of PreDicta B tests for Clade 1 (does not differentiate between 1a and 1b) and Clade 2 (which detects both Clade 2 and *B. zeae*) were completed and calibrated against pycnospores of Clade 1a from SA cultures. This test is based on real time qPCR and is very sensitive to the DNA of the white grain fungi, with indications that DNA from a single spore can be detected.
- Spore tapes from 2013 for two traps on Eyre Peninsula (SA) were analysed using PreDicta B technology. Attempts to detect spores of the fungi associated with white grain visually on the tapes were not successful as the spores are too small and too clear to be detected. The DNA of these fungi must be very robust as some of the spore trap tapes were 12 months old as they were collected before the PreDicta B test was developed and calibrated. The tapes were kept dry over silica gel during storage.
- The genomes of single isolates of Clade 1a, Clade 1b, Clade 2 and *B. zeae* were mapped without finding any significant homology matches for the genes involved in trichothecene synthesis (Eli Thynne

and Peter Solomon). This gives us confidence that the fungi do not produce any of the toxins produced by FHB (i.e. Deoxynivalenol, nivalenol and similar derivatives). It is possible, but unlikely, that the white grain fungi might produce the toxins by another pathway.

- Findings from SA research of use to growers and advisors are summarised in the White Grain information sheet. Other points in addition to this:
  - ◊ Varietal screening and fungicide assessments need to be undertaken using artificial inoculation as field infections are unpredictable and usually at very low rates.
  - ◊ Even with artificial inoculation, infection rates can be low.
  - ◊ Spore trapping might be suitable as a tool for predicting seasons likely to have high levels of white grain in crops.

2014.

- PreDicta B offers tests for Botryosphaeria Clade 1 (=Clade 1a + Clade 1b) and Clade 2 (=Clade 2 + *B. zeae*) in the suite of tests available for soil samples. Distribution maps for the Clades are available based on 2014 pre-season sampling and these results already suggest that white grain inoculum is present in paddocks over a wide area of Australia, which is consistent with the recorded distribution of expression of white grain in crops.
- Based on the work by Eli Thynne and Peter Solomon, a manuscript is ready for submission to Australasian Plant Pathology. In this manuscript it is recommended that the fungi associated with white grain be placed into three species - *Tiarosporella tritici-australis* (= Clade 1), *Tiarosporella darliae* (*B. zeae*), and *Tiarosporella pseudodarliae* (Clade 2).
- SA and WA plan complementary research programs, with assessment of distribution of the white grain fungi being one of the priorities.
- Further quantities of white grain affected wheat will be provided for toxin analysis from SA after the 2014 harvest.