

# CROP SCIENCE SOCIETY OF S.A. INCORPORATED

C/- WAITE CAMPUS

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INCORPORATING THE WEED SCIENCE SOCIETY

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## Next Meeting

## **'Industry Crop Protection Night'**

## Venue

**Richardson Theatre, Roseworthy Campus**

## Date

**WEDNESDAY 17<sup>th</sup> June**

## Time

**7.30 pm**

Come along to hear from industry representatives about what is new in crop protection, new products, applications and registrations. This meeting will not only provide an opportunity to learn about what is new, but also discuss with other crop science members and key industry people about your experiences using crop protection products.

## **Speakers will include:**

- John Both (Nufarm & Crop Care)
- Ashley Pilkington (Adama)
- Lyndon May (BASF)
- Mick Lines (Dow AgroSciences)
- Graham Hatcher (Bayer)

*Our 300<sup>th</sup> Newsletter doesn't disappoint – a 'scoop' report on the strategy for controlling BWYV and exciting developments from the prestigious zero- toxin vetch program....Happy reading!*

## **FINAL REPORT: GRDC DAS00151: Project Information, analysis and strategy for the *Beet western yellows virus* (BWYV) and its aphid vector outbreak in canola and pulses**

### **SAGIT S1114: Coordinator for survey of BWYV epidemic in canola**

By Jenny Davidson, Greg Baker, Ken Henry, Bill Kimber, Helen de Graaf, Kym Perry (SARDI), Frank Henry, Mohammad Aftab, Angela Freeman, Katherine Hollaway (VIC DEDJTR), Kurt Lindbeck, Joop van Leur, Don McCaffery (NSW DPI), Sam Holmes (Holmes Farm Consulting Pty Ltd), Paul Umina (**cesar**), Murray Sharman (QDAFF), Ray Correll (Rho Environmetrics).

### **Executive Summary**

A widespread outbreak of *Beet western yellows virus* (BWYV) (synonym: *Turnip yellows virus* [TuYV]) vectored by green peach aphid (GPA) in canola across the southern cropping region of Australia resulted in severe crop damage. Environmental conditions including widespread substantial rainfall in summer and autumn and mean air temperatures 2 °C above the long term average in May 2014 were considered to be the drivers for this outbreak. An antibody based virus assay for BWYV was used to identify virus infection but this assay does not distinguish between a number of luteoviruses, including BWYV and TuYV, both of which can infect canola and pulse crops. Molecular assays conducted by QDAFF confirmed the outbreak was caused by TuYV, but the virus is referred to as BWYV in the remainder of the document to avoid confusion within the industry.

618 canola crops were tested for BWYV by September 2014 of which 57 % were positive; 86.6 % of sampled crops were positive in South Australia and 50 % in Victoria. In New South Wales high infection rates were recorded in the southern region compared to the central and northern regions. Numerous weed species were also infected with BWYV including wild turnip (*Brassica tournefortii*) and marshmallow (*Malva parviflora*). Very limited infection by BWYV was recorded in pulse crops.

The effects of crop management practices on the severity of BWYV in commercial canola crops and the effect on grain yield were investigated through an online survey using Survey Monkey. Responses for 159 crops across the southern region were attained; 99 from South Australia, 57 from Victoria and 3 from New South Wales. The few replies from New South Wales limited analysis of the data for this state. It was not feasible to quantify any potential interactions between the different stresses such as frost, diamondback moth and drought, but possible interactions must be borne in mind when interpreting the results from the survey.

Crop losses from BWYV were estimated as 71,000 t in South Australia for the 302,000 ha sown and 75,000 t loss for the 460,000 ha sown in Victoria. It should be noted that the data were based on expert opinion rather than on an objective measure, this being the only way to estimate losses from the virus outbreak. There was insufficient data to estimate losses in New South Wales. The relationship between crop loss and percent plants infected with BWYV found 0.69 t/ha loss when all plants were infected which equates to 43 % of total yield, similar to the maximum 46 % yield loss identified by Jones et al. (2007) in experimental trials had all plants been infected.

Early sightings of virus symptoms were low where there was thick stubble or weeds, and highest in areas of poor nutrition, borders and bare earth (eg. wheel tracks, no stubble) as well as over entire paddocks. Aphid sightings were similarly distributed.

Three species of aphids were considered as potential vectors for BWYV i.e. GPA, turnip aphid and cabbage aphid. GPA was significantly associated with symptomatic plants but turnip and cabbage aphid were not.

There was a regional effect as to when aphids were first sighted in crops, i.e. May in the Murray Mallee and Mid North of South Australia, and June in Yorke and Eyre Peninsula of South Australia. Victoria had equivalent sightings over May and June.

There was insufficient resolution in the data to detect a significant effect of sowing date although some regional effects were potentially attributed to sowing date. In a time of sowing trial at Hart Field Day site in South Australia, the last time of sowing (1<sup>st</sup> June) had least virus infection.

A four week period between weed control and sowing date was found to have a small but significant effect on reducing aphid numbers in the crop. Increased sowing rate decreased the percentage of infected plants, although actual numbers of both infected and uninfected plants increased with higher sowing rate. Yield was also increased by higher sowing rate. Sowing equipment had no effect on yield, aphid numbers or crop loss despite conflicting data from a trial conducted at Riverton in South Australia (Mick Faulkner, personal communication).

Neonicotinoid seed dressings (e.g. Cruiser®, Gaucho® and Senator®) reduced aphid numbers, reduced virus infection and increased yield. The foliar insecticide sulfoxaflor was effective against GPA in 88 % of cases whereas dimethoate, various synthetic pyrethroids and pirimicarb were largely ineffective.

The crop survey identified no varietal differences in incidence of BWYV, GPA nor yield associated responses. The large number of varieties, low degree of replication and the overall variability only gave low power for any comparison. Data from three National Variety Trials identified ATR Stingray had low infection rates as did two breeding lines. However ATR Stingray in commercial crops had up to 63 % infection.

It was anticipated that crops sown into standing stubble would benefit through few aphid landings and hence less virus infection. However crops sown into standing stubble had lower yield than crops without standing stubble, possibly due to widespread frosts that occurred in the southern growing region during the 2014 growing season. There was an indication that crops grown into slashed stubble had fewer aphids, fewer infected plants and higher yields, although this requires further validation.

Application of post emergent nitrogen increased yield of canola independent of virus infection.

A small survey of aphid and virus infection in weeds conducted in the lower north region of South Australia in autumn 2015 found very low numbers of virus infected plants and only one plant infested with GPA. Mean temperatures across the southern cropping region in March and April 2015 were 0 – 2 °C lower than the long term average. Overall this data indicates 2015 is low risk for an aphid and virus outbreak.

The project team developed a coordinated communication strategy allowing for a consistent message for industry. This message centred on -

- Stopping winter insecticide sprays aimed at GPA control as these measures were ineffective.
- Determining the need for insecticide sprays in canola and pulse crops in spring.
- Potential timing of spring insecticide sprays, if warranted.
- Identification of actives which are effective against GPA via **cesar** and results of GPA insecticide resistance testing.
- Adherence to label regulations (with assistance of PIRSA Rural Chemicals).
- Relay concerns of beekeepers regarding effect of insecticides (particularly neonicotinoids) on bees and the best strategies to minimise damage.
- A GRDC Tips and Tactics Fact sheet –‘Reducing aphid and virus risk’ was developed and released to industry in February 2015 following extensive rain in January 2015.

## Progress To-ward a Zero-Toxin Vetch, at the Waite (2015).

By Max Tate – The University of Adelaide, Waite Campus, Waite Rd. Glen Osmond 5064. (Email: [max.tate@adelaide.edu.au](mailto:max.tate@adelaide.edu.au) Tel 8313 7227)

### Background

Common vetch (*Vicia sativa* L.) is a leguminous, palatable, high protein feed source for livestock. Compared with peas and beans, common vetch is a low-input crop. It is widely known that anti-nutritional factors exist in common vetch seed, especially  $\beta$ -cyano-alanine and  $\gamma$ -glutamyl- $\beta$ -cyano-alanine, which have high toxicity to monogastric animals but are tolerated by ruminants. In the Eastern states, farmers have been using common vetch as a lucrative hay crop, sold into the dairy and beef industry, with the added benefits on-farm of nitrogen fixation, disease and weed control.

In 1992 I attempted to warn the local farming community through the Stock Journal (9<sup>th</sup> April 1992), that split red vetch with orange-red cotyledons (i.e. Blanche fleur cultivar of common vetch) should not be planted with an eye to selling the grain for human consumption, because it contained the known neurotoxin,  $\gamma$ -glutamyl- $\beta$ -cyano-L-alanine.

After recognising the extensive area that had been planted, it was clear to me that my warning had been in vain. Subsequently our paper "(Tate, M.E. and Enneking, D. (1992) A mess of red pottage, *Nature* 359, 357-358.), was published.

In response to publication in the most important biological journal available, the market for split red vetch as a cheap substitute for genuine red-lentils, and as a food legume collapsed. And as might be expected, my popularity was somewhat lower than that of a skunk in the proverbial scent factory.

The last time I spoke about this matter to a Crop Science Society meeting was just before the 1992 *Nature* article appeared and we know that article effectively destroyed most of the vetch/lentil substitution racket, right up until I theoretically retired in 1998. A second attempt to market it after my retirement was again demolished by a second article (Tate, M.E. *et al.*, 1999). Covert trade in toxic vetch continues. *Nature* 400 207. However, this time, it was recognised that the sale of red split vetch was becoming a genuine threat to Australia's, fledgling and very profitable, genuine red lentil (*Lens culinaris* Medik) market.

Tim Fischer Deputy Prime Minister and Minister for Trade at that time, intervened, with the ultimate result, that Legislative amendments were made (Commonwealth Gazette, 18<sup>th</sup> June 2003) which absolutely prohibit the export of split vetch from Australia, making it a criminal offence with a maximum of five years imprisonment. As far as I am aware the vetch/lentil substitution racket ceased to exist from that time on.

Now (June 2015), with the prospect of a zero cyano-alanine toxin vetch within our grasp, I hope that we will ultimately be able to market the new **Lo-vet** (N) vetch, as a well-adapted, safe, high protein legume. I expect that initially, this will take place by producing a cultivar which is suitable as poultry feed, but with the definite aim to do what the Canadians did in the 1960s by removing the anti-nutritional factors from the toxic Rapeseed to produce Canola, and produce an Australian, low cost, food quality vetch.

The new low toxin vetch: "**Lo-vet**" cultivar N, where the number N has yet to be determined, will undoubtedly have to be given close scrutiny for all its agronomic properties in addition to its highly beneficial, if not near zero toxin content. So we are probably still looking at

several years of breeding work before it will be generally available for the farming community as a whole. It may also need a special export licence to discriminate it, from the earlier neurotoxic line; the dark testa, with orange cotyledons of the “Blanche fleur” cultivar.

In order to develop a zero toxin vetch which could readily be distinguished “just by looking over the back of the truck”, as I was asked to do, at that Crop Science Society gathering at the Waite, Charles Hawker Centre, back in 1992, we also investigated the way that vetch colour traits were inherited.

This work was subsequently published (Doza M.S. Chowdhury *et al.*, 2004). An important feature of this study was the fact that the crossing of the two white-flowered cultivars, Blanche fleur and Jericho white gave a purple flowered F1, but another white-flowered cultivar cross (Blanche fleur x Cummins) yielded a white flowered F1. This example of the complementation of two different defective loci in a pigment gene by restoration with their functional counterparts paralleled the classic work by Bateson and Punnett in 1906 with commercial white-flowered lines of the sweet pea (*Lathyrus odoratus*) (W. Bateson and R.C. Punnett. 1906).

Using this understanding of vetch colour traits, we have now obtained a segregate (**Lo-vet 8**) with orange-cotyledons and a readily recognisable (“Over the back of the truck.”) white testa (hull). This unusual testa was incorporated into **Lo-vet 8** from the white testa trait, of the highly toxic field mutant, now called Jericho white. Jericho white, was named after Laurence Jericho, the observant Cummins farmer, who around 1986 first identified, both it and Cummins, (a dark-testa, white-flowered mutant) that were present in a single bag of the purple-flowered Languedoc cultivar. At present the **Lo-vet 8** toxin content is still too high (0.60% +/- 0.02%), for poultry feed, but with these distinctive colour traits for both the cotyledon and testa, coupled to a lower (<0.4% toxin) or even a zero toxin vetch, it could be marketed as a low toxin, whole vetch, even under the current guidelines.

### Progress (1993-2015)

It should be noted that progress in the figure below has been produced at the Waite, by recurrent selection, and analyzing the toxin content of traditional Mendelian crosses of low and high toxin lines produced by Dr. Jane Rathjen, the late Dr. Doza Chowdhury and latterly by Dr. Shi Yang.

The absolutely essential pure reference standard of the known neurotoxin for the Infrared toxin analysis was isolated from common vetch, purified by crystallization and its structure unequivocally determined, by X-ray crystallography (I.M. Delaere *et al.*, 1995). Availability of this compound at the Waite is undoubtedly responsible for all of our progress.

There are no cheap and easily applied post-harvest treatments for detoxification of vetch to enable safe human consumption, therefore plant-breeding efforts at the Waite Institute (University of Adelaide) have been targeted at radically, and permanently reducing the toxin level of common vetch seeds. Using conventional breeding methods, At the Waite, we have reduced the toxin concentrations from ~1.6% in the 1990s to ~0.35% (2015) (Fig.).

In the latest step forward, the total seed-toxin for the whole pod has been estimated from analysis of the toxin content of the first and last seeds, and this value has then been normalised in terms of the mass (mg) of the empty pod to provide the total seed toxin for the pod in micrograms per mg of empty pod. Traditional attempts, to reduce the toxin content, by measuring % toxin in bulk seed samples instead of the pod, e.g. (“A selection

Strategy for Low Toxin Vetches (*Vicia sativa* spp.) or Toxin (mg) per seed were much slower. This was probably due to the fact that in a normal plant, the pods at the bottom of the plant are much larger than those near the top. Single seed analysis has shown that pods with less than three seeds have much higher % toxin in their seeds, than pods with four or more seeds. However by normalizing the data to account for environmentally induced alterations in the % toxin of seeds from different sized pods, these problems can be substantially overcome and the lowering of the pod toxin content appears to be a much better selection procedure.

However, it should be noted that no matter what measure of toxin is used, (% Toxin, Toxin (mg)/ seed, or  $\mu\text{g}$  of toxin per mg of the empty pod.), they will all reach zero simultaneously.

The lowest % Toxin in the seed achieved (Lo13R F01 2014/2015 Pot3) to date contains 0.35%  $\pm$  0.01% w/w. At this level and at the usual legume concentration (20%) of the diet (i.e. 0.07% w/w dietary Toxin) Dr Jane Rathjen's study (PhD Thesis, University of Adelaide, 1997) indicated (p. 115-117) that above 0.2% w/w it was lethal to week old chicks and implied that below 0.15% w/w dietary toxin, it would probably be tolerated by week old chicks. In which case, at 0.07% toxin in the diet, the initial aim of a common vetch suitable for poultry feed has already been achieved. The field behavior of this most recent screen-house segregate (Lo13R F01 2014/2015 Pot3), awaits examination. Dr. Rathjen's thesis also identified the presence of tannins (p.232, 242) in vetch hulls, reduced the feed intake of vetch by both pigs and poultry. Thus the tannin free white testa lines should improve the digestibility to poultry as well as providing a unique visual identifier for certain Waite lines.

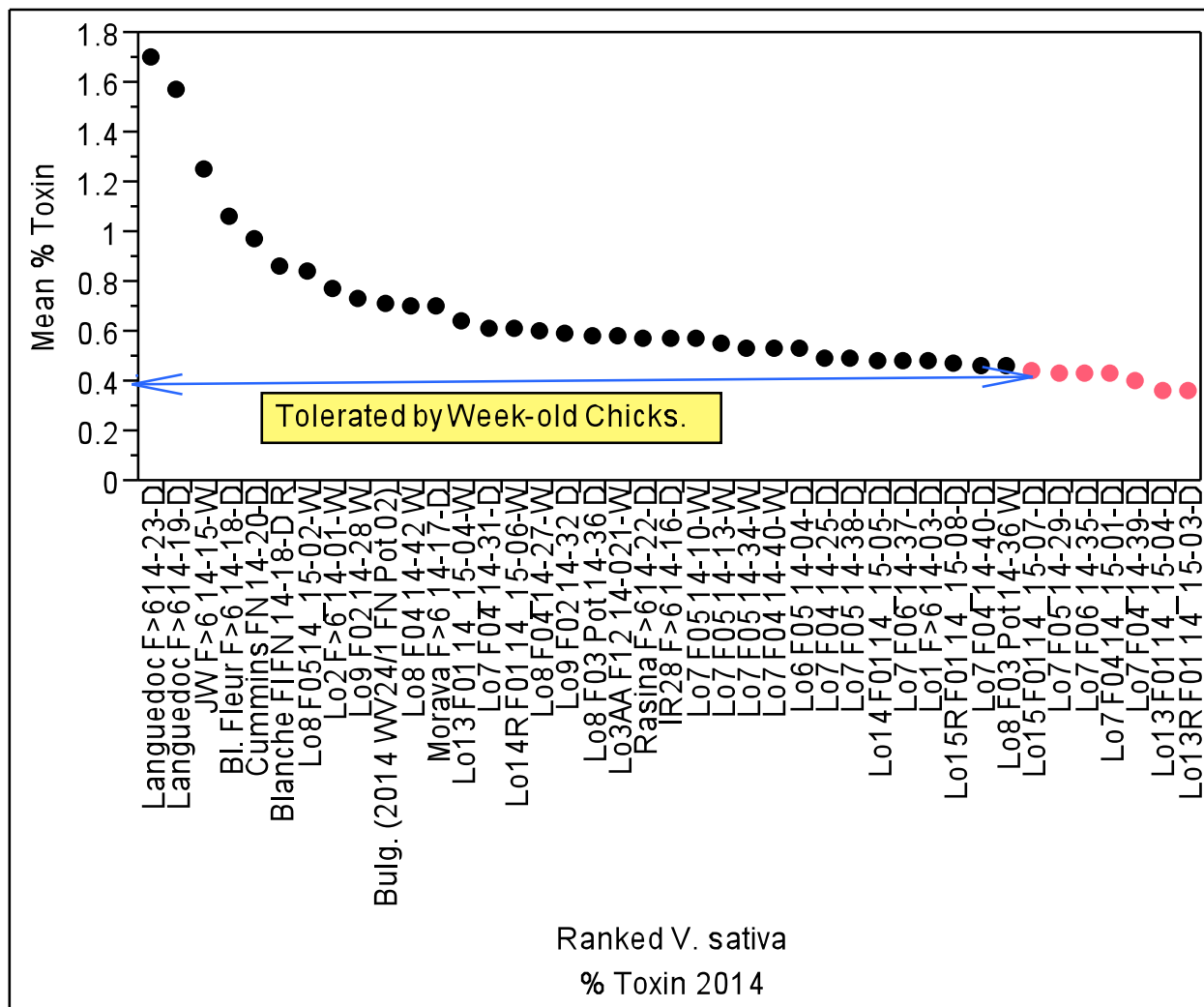
## The Future

We believe that a non-toxic vetch has the potential to provide a much-needed robust and reliable alternative legume, for many farming systems. For the first time, after initial few years of invaluable SAGIT funding for the late Dr. Chowdhury as a plant breeder and more than two decades of self-funded research, we now have convincing quantitative evidence, in the Figure below, that with further effort, the seed toxin level can ultimately be reduced to zero.

Just as the Canadians in the 1960s produced Canola from the toxic rapeseed by lowering both erucic acid and glucosinolate anti-nutritional factors to market standards, now here at the Waite, we are now quietly confident that we can produce a zero-toxin common vetch cultivar. A low toxin product such as this zero cyano-alanine-toxin vetch would provide a significant new protein resource for humanity. A low toxin vetch (**Lo-vet**) or preferably the zero toxin vetch would also compete well as a cheap replacement for Soybean meal in the poultry feed market of Indonesia, which both SA and WA are geographically well-placed to service.

The next question which remains to be examined is: “What is the molecular reason for the successful reduction in the toxin levels of common vetch which have been achieved so far?” To this end Professor Diane Mather, Dr. Melissa Garcia and students have commenced an examination of the DNA sequences in our highest and lowest toxin lines.

#### Oneway Analysis of Mean % Toxin By Ranked *V. sativa* % Toxin 2014



**Conclusion:** If seed of the **Lo13R F01 Cross**, is heterozygous, it may already herald the key to a zero-toxin vetch. Let us see what the F02 progeny, toxin content is in November/December this year (2015). A tannin free (white hull) line has also been identified. So that at this time, two major limiting factors in common vetch genome (toxin and tannin) have been minimized. An exciting time for common vetch research lies ahead!



WGD was first observed in bread wheat in South Australia during harvest in 2010, when it caused rejection and down grading of grain deliveries. In 2011 174,370 tonnes of affected wheat were delivered to SA silos, with infection rates between 1% and 7% being most common. In 2012 there were only 2 deliveries confirmed as being affected by WGD in SA. No report of WGD expression in grain in SA was made in 2013 or 2014.

Wet conditions after heading in 2010 and 2011 almost certainly contributed to the severe infections evident in crops in these seasons. PreDicta™ B results from soil samples taken in commercial paddocks show the fungi causing WGD are widely distributed in paddocks in SA. This makes WGD a continuing concern, with severe problems most likely to occur where wet springs promote infection late in the season.

In 2010 and 2011 grain affected by WGD was detected across much of SA, with north eastern Eyre Peninsula and the Far North being most severely affected. White grain disorder was not detected on the West Coast of the Eyre Peninsula. White grain disorder was first detected in 1999 in Queensland and northern New South Wales and has also been detected in Victoria (2010) and Western Australia (2013).

*Botryosphaeria zeae* was initially identified as the causal agent of WGD. This identification has now changed and it is known that at least three species of fungi in the genus *Tiarospora* are associated with WGD. The main species associated with WGD in SA is *Tiarospora tritici-australis* (= WGD Clade 1), which is also present in WA and Vic but not in Qld and northern NSW.

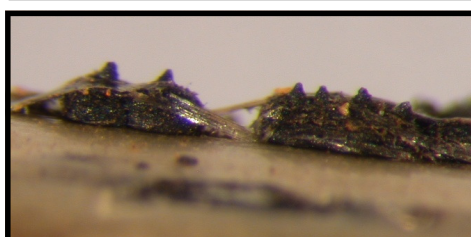
There is no evidence to suggest that WGD in SA is associated with toxins. However, white grains are also a symptom of infection by *Fusarium* head blight/head scab, where toxins are present in the affected grain. This means buyers may perceive that the WGD that occurs in SA is associated with myco-toxins.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

## Hosts and symptoms

Bread wheat, durum wheat, barley and triticale can be affected by this disease, although mainly commercial bread wheat crops have shown symptoms in SA.

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's below) include “scabby” nodes with black, slightly raised structures on them. Similar structures can be seen on internodes and leaf sheaths. As these 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.

Leaf symptoms have not been seen in the field and even at crop maturity it is difficult to detect WGD symptoms on plants. Green heads (photo on left) may show bleaching or grey discoloration 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 on right).



In field crops, rubbing out grain is the best assessment method when checking crops for

WGD. 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 discoloration but are very difficult to detect without examining the grain. Plants affected by WGD are likely to be unevenly distributed, so check grain from a number of places in each paddock when assessing disease status.

## Management

White grain disorder may be an issue in any season where inoculum is present and wet spring weather favors infection by the fungus. Management options for WGD 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 grain fill. At least 24 hours of high humidity is optimal for infection.

The PreDicta<sup>™</sup> B analytical service has developed tests for WGD pathogen levels in soil samples. These tests are available for research purposes and their commercial applicability is being investigated.

Where significant levels of WGD 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 consistent evidence from commercial paddocks to suggest that variety choice or fungicide application affected WGD expression in 2010 and 2011. 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). Preliminary data from variety screening using artificial inoculation in 2014 suggest there are differences in WGD expression in current commercial bread wheat cultivars. Variety screening will be continued in 2015 and resistance ratings for selected cultivars should be available in 2016.

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 for most of the growing season and this is likely to be the mechanism by which WGD pathogens survive in commercial paddocks even when grain symptoms are not present. Air-borne spores, infect the grain and are likely to be present mid- to late-season. These spores have the potential to travel long distances. On upper Eyre Peninsula in 2012, air-borne spores were present in high numbers during September and October, while in 2013 spore numbers were highest during August and early September.

## Funding

### South Australian Grains Industry Trust (2012-2014):

- S1206 “Strategies to reduce white grain on Eyre Peninsula”.

### Grains Research and Development Corporation (2012-2017):

- DAS00139 “Improving grower surveillance, management, epidemiology knowledge and tools to manage crop disease in South Australia”.
- DAS00154 “White grain disorder in wheat”
- DAS00137 “National improved molecular diagnostics for disease management.”

## Congratulations to Mark Hill OAM

Mark Hill has been a long-time member, past president and regular meeting attendee of the Crop Science Society. His extensive contribution both to the agricultural and livestock industries and to the community of Tarlee have culminated with recognition on the Queen's Birthday honours list. A well-deserved achievement!



Mark Hill OAM, with friends (photo supplied by *Barossa Herald*)