2016 ANNUAL REPORT

CENTRE FOR CROP AND DISEASE MANAGEMENT

BACKGROUND

The Centre for Crop and Disease Management (CCDM) is a national research centre co-supported by Curtin University and the Grains Research and Development Corporation (GRDC), collectively investing $100 million over a five-year period.

Launched in 2014, the centre's major aim is to reduce the economic impact of crop disease for Australian growers via three main research themes:

Fungicide resistance: by developing the necessary tools and strategies for industry to prevent and minimise its occurrence and impact.

Molecular genetics: by discovering and providing breeders with genetic tools to breed cereal, oilseed and pulse varieties with improved disease resistance.

Farming systems: by developing economical and adoptable Integrated Disease Management (IDM) strategies for managing the evolution of resistance to fungicides.

CCDM sits within Curtin's Department of Environment and Agriculture, and builds on the university's previous work through the Australian Centre for Necrotrophic Fungal Pathogens (ACNFP) by continuing to produce outcomes estimated to save industry more than $200 million per year.

Building on its molecular genetics and fungicide resistance research background, CCDM has grown from 20 to more than 60 research staff, and continues to build capacity in the agriculture research community with a large intake of post graduate students. The CCDM has nine research programs and one extension program, each delivering outcomes into one of the three main research themes.

Researchers of the CCDM

OUR 10 PROGRAMS

- Fungicide Resistance
- Septoria nodorum blotch of Wheat
- Yellow Spot of Wheat
- Net Type Net Blotch of Barley
- Powdery Mildew of Barley
- Sclerotinia Stem Rot of Canola
- Pulse Pathology and Genetics
- Bioinformatics
- Improved Farming Systems
- Extension and Engagement

THE CCDM TEAM
2016 PROGRAM LEADERS

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(Agronomy and Agribusiness)

Karam Singh
CCDM Co-Director
(Scientific Programs)

Richard Oliver
CCDM Chief Scientist

Amir Abadi
Program Leader
Improved Farming Systems

John Noonan
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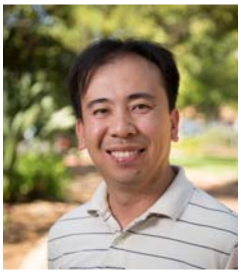
Extension and Engagement



Kar-Chun Tan
Program Leader
Septoria Nodorum Blotch of Wheat

Caroline Moffat
Program Leader
Yellow Spot of Wheat

Simon Ellwood
Program Leader
Net Blotch and Powdery Mildew of Barley

Lars Kamphuis
Program Leader
Sclerotinia Stem Rot of Canola

Robert Lee
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Pulse Pathology and Genetics

Fran Lopez-Ruiz
Program Leader
Fungicide Resistance Group

James Hane
Program Leader
Bioinformatics

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Vlad Piscetek, Research Assistant
Kim Riley, Research Assistant
Virginia Wainaina, Research Assistant
Blake Wood, Research Assistant

DIRECTOR'S REPORT
MARK GIBBERD

Looking back on the day of the Centre's launch, in April 2014, I remember thinking what a bright future our centre had, to have the privilege to focus on research areas so desperately needed by the Australian grains industry.

Fast forward three years, and I am so proud of what the centre has achieved in reducing the cost of crop disease for Australian grain growers.

As a testament to our success so far, three important things happened last year.

First we opened our new research facility. The $46 million building allows our researchers to safely work on pathogens that are a threat to Australian farming systems. With laboratories and plant growth facilities incorporating Physical and Quarantine Containment Levels PC2/QC2 and PC3/QC3, this research facility is the first in Australia to have such high containment standards dedicated to crop disease research.

Secondly, as the size and scope of CCDM research continues to grow, so has its leadership. We have recently welcomed Professor Karam Singh from CSIRO Agriculture and Food, in an inaugural joint leadership appointment between CCDM and CSIRO. Together Karam and I will co-direct the centre, leading research initiatives in plant pathology, agronomy and agribusiness as well as fungicide resistance, ensuring research outcomes deliver profitable solutions to Australian growers in all areas relating to crop disease. Karam has a background in soil borne fungal pathogens, insect pests and improving crop varieties through genomic techniques, and will be a valuable asset to the team.

CCDM Co-Director (Agronomy and Agribusiness) Mark Gibberd at Curtin's Field Trail Area

We have also greatly expanded our profile. As an example, the second Crop Protection Forum - this time held in Wagga Wagga - allowed us to share our research outcomes with more than 70 agronomists from the northern and southern grain growing regions. The event, held in conjunction with the Australian Herbicide Resistance Initiative (AHRI), combined leading experts in weeds, disease and pests to explore the implications and solutions for chemical resistance in southern cropping systems.

It's for certain that not one of these activities would have happened without CCDM building on its strong history in crop disease research, particularly in molecular genetics and fungicide resistance benchmarking. We know that crop disease will never be a completely solvable research problem, as diseases will always continue to mutate and present new challenges. However, with our talented research team, along with their collaborative network and modern facilities at hand, we have the ability to stay in front of those challenges, and rapidly share important messages with the agricultural community. The future is exciting at the CCDM, and I look forward to what the next three years bring for delivering better bottom line solutions for Australian grain growers.

Mark Gibberd
CCDM Co-Director (Agronomy and Agribusiness)

The opening of Building 304 with Dr Steve Jefferies, Managing Director GRDC; Mr Colin Beckett, Chancellor Curtin University; Professor Deborah Terry, Vice-Chancellor Curtin University; The Hon. Mark Lewis MLC, Former Minister for Agriculture and Food, WA; Professor Chris Moran, Deputy Vice-Chancellor Research, Curtin University; and Mr John Woods, Chairman GRDC.

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THEME 1
FUNGICIDE RESISTANCE

Objective

To develop the necessary tools that will allow growers to prevent, mitigate and minimise the occurrence and impact of fungicide resistance in their crops by:

- Establishing an early warning monitoring system
- Developing new molecular tools for the fast analysis of field samples
- Testing new chemistry in both field trials and laboratory tests
- Unraveling the mechanisms by which pathogens become resistant to fungicides.

Some key achievements

- With the baiting trial network and Digital PCR (read more in the case study), the Fungicide Resistance Group have confirmed 3 cases of fungicide resistance in 2016:
  - The gateway mutation Y136F for group 3 fungicides resistance in wheat powdery mildew.
  - Net type net blotch mutation CYP51A to tebuconazole, with results published in *Frontiers in Microbiology*.
  - The first confirmed case of strobilurin (group 11 fungicides) resistance in wheat powdery mildew, from mutation G143A.
- A computational model of NTNB's CYP51A was developed to improve knowledge around the impact of mutations in the binding of the antifungal compounds. This has allowed the team to confirm that mutation F489L found in the target site of NTNB plays an important role in the binding of the antifungal compounds.
- The team has also been investigating the mechanism behind the fluquinconazole tolerance found in canola blackleg. Analysis of the tolerant isolates did not reveal any mutations at the target sites in the resistant isolates, therefore it was supposed that the mechanism had to be related to the target expression.

In the pipeline for 2017

- The Fungicide Resistance Group aims to develop high through-put methods for every resistance case detected, using Digital PCR and LAMP (Loop Mediated Isothermal Amplification) technologies.
- Hotspots from resistant isolates will continue to be analysed. Wheat powdery mildew strobilurin resistant strains carrying mutation G143A will be phenotyped to confirm resistance levels.
- The team will also continue the collection and analysis of resistance in samples, with the help of an Australia-wide trial program.
- Chickpea crops will also be monitored to determine the current fungicide resistance status, where chickpea production and fungicide application is currently high.

Virginia Wainaina and Fran Lopez Ruiz in Tasmania

CASE STUDY: BAITING TRIALS

For the first time in Australia, the Fungicide Resistance Group identified the first confirmed case of strobilurin (group 11 fungicides) resistance in wheat powdery mildew.

This finding started with CCDM's baiting trials – a national fungicide resistance monitoring system involving a partnership with the Foundation for Arable Research (FAR), who carry out a number of crop disease trials to monitor the efficacy of fungicides.

In the 2016 season, agronomists from FAR reported fungicide strobilurin was not working as well as it should in reducing powdery mildew in wheat, and rapidly sent samples to the CCDM laboratories for further testing.

Using high throughput technology, digital PCR, the team was able to quickly diagnose stobilurin resistance. The analysis of these strains revealed an important mutation, G143A, in the target of strobilurin fungicides that has been widely associated with total resistance in this and other fungal pathogens overseas.

Without the baiting trial system, along with high throughput diagnostic technology, this case of resistance would not have been detected this early. Messages around fungicide resistance in wheat powdery mildew has been communicated to growers, so they are able to make decisions about disease management in the coming 2017 season.


Alexandra Kay (back) and Belinda Cox (front) with the digital PCR machine

THEME 2
MOLECULAR GENETICS

Objectives

To provide plant breeders with the genetic tools needed to breed varieties that have a high level of disease resistance to common crop diseases including:

- Septoria nodorum blotch of wheat
- Yellow spot of wheat
- Net blotch of barley
- Powdery mildew of barley
- Sclerotinia stem rot of canola
- Ascochyta blight of pulses

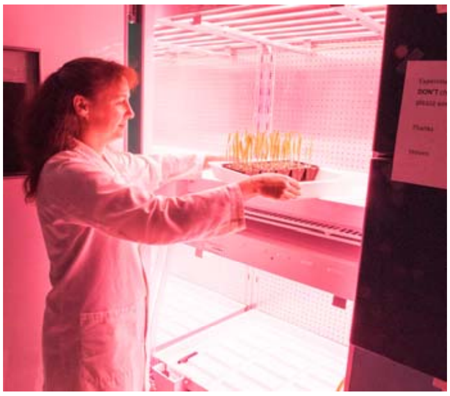
To use bioinformatics to analyse genomes of fungal pathogens for insight into host-pathogen interactions and how they evolve and adapt, and identify novel methods for computationally predicting genes that cause crop disease.

Some key achievements

- A transcription factor has been identified in *P. nodorum* and *P. tritici-repentis* that positively regulates the expression of effectors ToxA and Tox3. This transcription factor, or master gene, is hypothesised to regulate other effector genes. Read more in the case study below.
- Using a new high quality reference genome, CCDM have prioritised effector candidates for the pathotypes causing net form net blotch for further research and are currently screening or developing mapping populations to identify useful resistances for breeders.
- The *Sclerotinia sclerotiorum* genome has been sequenced and is one of the most complete fungal disease sequences produced so far. It will be used to identify weak points in pathogen biology for resistance breeding.
- About 1400 field pea, 1800 lentil and 450 chickpea lines acquired from Australian Grains Genebank (AGG), including landraces and cultivars collected from worldwide locations, were screened for resistance to ascochyta blight pathogens. For each plant species, a wide range of responses to infection was observed and the most resistant field pea, lentil and chickpea accessions were selected for further study.
- The discovery of a new resistance gene from an Ethiopian landrace was published in *Nature Scientific Reports*. The allele, a variant of the widely deployed mlo-11, is resistant to all forms of powdery mildew, without affecting yield.
- With the help of supercomputing resources, CCDM has narrowed down a number of genes that were laterally transferred some time in history from one species to another and have properties similar to other disease-causing genes. These genes will be further analysed to confirm their role in causing crop disease.

In the pipeline for 2017

- Genome annotations for *Parastagnonspora nordorum* as well as *Ascochyta rabiei, lentis, fabae* and *viciae-villosae* are almost complete, which will allow for evolutionary studies and effector discoveries.
- Effector candidates will continue to be screened to narrow down new effectors within pathogens causing yellow spot and septorian nodorum blotch of wheat and net form net blotch of barley that can be provided to breeders.
- CCDM will repeat an experiment from 2016, growing 100 spring canola genotypes sourced from a European consortium, to screen for genetic resistance to sclerotinia stem rot under controlled and field environments.


Julie Lawrence removes seedlings from a conviron in Building 304

CASE STUDY: DISCOVERY OF A 'MASTER' DISEASE-CAUSING GENE

Until recently, CCDM's main game has been to discover effector genes that produce toxic proteins that kill wheat cells, which the fungus then infects and feeds on.

But there are many effectors that take different forms in many diseases and the process of finding effectors can be extremely time consuming.

CCDM's septoria nodorum blotch and yellow spot programs recently discovered a "master gene"- a transcription factor that positively regulates the expression of effectors ToxA and Tox3 and perhaps other undiscovered effector genes.

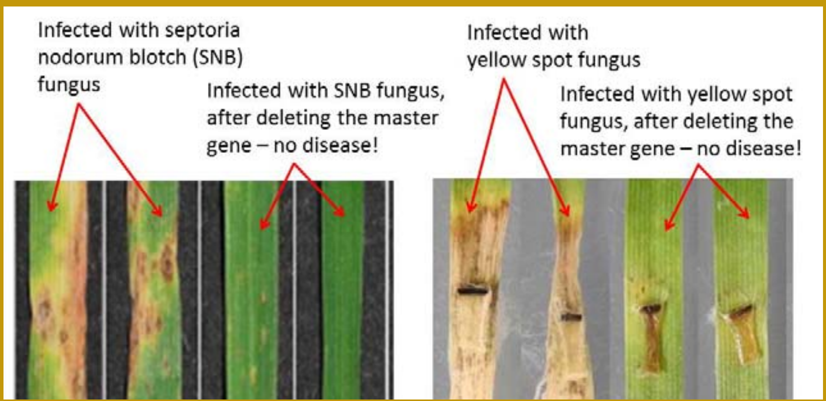
They got the idea from a Korean and US research team who found a master gene in the devastating cabbage fungal disease, dark leaf spot.

When this master gene was deleted from the pathogen in the lab, the pathogen was not able to cause disease symptoms on cabbage leaves any more.

The CCDM team set out to discover the same gene within septoria nodorum blotch and yellow spot. Once found and deleted in both pathogens, the SNB mutant was without the ability to produce toxic effectors ToxA and Tox3, while the Yellow Spot mutant was without the ability to produce ToxA, and necrosis was not visible on the leaves.

This discovery has potential to go beyond septoria nodorum blotch and yellow spot, to other necrotrophic diseases such as net blotches of barley.

CCDM will now try to discover the signal that activates the pathogen's master gene, to then formulate strategies to shut it down.



Wheat leaves infected with SNB and yellow spot, with and without the master gene

THEME 3
FARMING SYSTEMS AND EXTENSION

Objectives

- To minimise the impact of fungal pathogens through economical and adoptable IDM strategies and practices for managing evolution of resistance to fungicides.
- To ensure the adoptable outcomes from research activities undertaken to improve disease management are communicated and extended to growers and other stakeholders.

Some key achievements

- A GxE field trial has now completed its second year, with four WA locations involving six wheat varieties with short, medium and long maturity and various disease resistance characteristics, were evaluated against fungicide timing and treatment.

Leon Hodgson and Araz Abdullah in the field trial area

- Field pea yield response to different foliar fungicide treatments at different sowing times in the WA wheatbelt was studied, with initial results showing foliar fungicides applied after the initial seed dressing did not have an effect on yield, regardless of sowing date.
- High throughput sequencing was used to determine the fungal species that feed on common agricultural weed species in the WA wheatbelt. In 2016, 101 leaf samples were collected from 12 common weed species from 15 locations, and 20 of those samples have been sequenced.
- A stubble management trial has shown that windrow burning of wheat and canola stubble can be highly effective in reducing any pathogens that are captured within the windrow, and is a strategy that could help reduce the reliance on fungicides.
- Through research and industry consultation, a simple integrated disease management guide has been developed. The guide involves a spreadsheet that integrates agronomy, pathology, meteorology, economics and decision analysis principles.
- Canola Rot WAtch was launched, CCDM's second citizen science project involving four agriculture high schools planting canola, monitoring the local weather conditions, observing the crop for infection, and if infected sending samples to CCDM researchers for further analysis.
- CCDM once again teamed up with the Australian Herbicide Resistance Initiative (AHRI) to host the Crop Protection Forum, this time held in Wagga Wagga, targeting agronomists to upskill them in the most recent research and recommendations relevant to crop protection.
- CCDM citizen science project Mildew Mania celebrated five years of success, with more than 16,300 Western Australian students from years one through to 12 from more than 220 schools participating in the project so far.
- A masterclass pilot brought together 30 industry representatives including 18 key agronomists and chemical industry representatives to delve down into key disease management issues.
- 15 media releases, 8 Spotlight blogs, and more than 1000 tweets from @theCCDM and @FRGcurtin have been issued. Engagement from each of these tools has increased exponentially, as key messages are conveyed to CCDM's target audiences.

In the pipeline for 2017

- CCDM will continue the analysis of pathogens on common agricultural weed species and analyse data to find correlations between weed hosts and disease-causing pathogens and the impact of genotype and climate on fungal community composition.
- The masterclass and Crop Protection Forum will likely happen again, this time rolled out in another Australian grain growing region.

CASE STUDY: CANOLA STUBBLE MANAGEMENT

With no resistant varieties and only two active ingredients available to control sclerotinia stem rot on canola, it is now more important than ever to identify diverse, non-fungicidal integrated disease management (IDM) strategies.

To improve growers' options for sclerotinia management, CCDM researchers have looked into stubble management as an alternative method for reducing the sclerotinia inoculum load in the soil.

They found that narrow windrow burning of canola stubble produced very hot temperatures, hot enough to stop fungal growth from sclerotia that have been concentrated in the windrow.

They did this by placing different sizes of sclerotes in the windrow and measured the heat within the burning windrow using temperature probes, where temperatures above 200 degrees were maintained for almost 1000 seconds.

They then worked out exactly how much heat was required to inhibit fungal growth from large sclerotia on agar plates, and found that only 40 seconds at 200°C was enough to stop fungal growth from big sclerotia.

This information fits in well with a no-till system, as burning narrow windrows will leave most of the paddock unaffected and reduce sclerotia without tillage. It may also take pressure off important fungicides, adding to the growers' tools in the toolbox for sclerotinia management. However, further investigation is needed to confirm these outcomes.

A temperature probe measures the heat within the burning canola windrow

2016 PEER-REVIEWED PUBLICATIONS

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- De Wit, P., A. Testa, and R. P. Oliver. 2016. “Fungal plant pathogenesis mediated by effectors.” Microbiology Spectrum 4 (6).
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- Ge, X., W. Deng, Z. Lee, F. Lopez-ruiz, P. Schweizer, and S. R. Ellwood. 2016. “Tempered mlo broad-spectrum resistance to barley powdery mildew in an Ethiopian landrace.” Scientific Reports 6V.
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- John, E., F. Lopez-Ruiz, K. Rybak, C. Mousley, R. P. Oliver, and K. Tan. 2016. “Dissecting the role of histidine kinase and Hog1 mitogen-activated protein kinase signalling in stress tolerance and pathogenicity of Parastagonospora nodorum on wheat.” Microbiology - In press.
- Lu, X., B. Kracher, I. Saur, S. Bauer, S. R. Ellwood, R. Wise, T. Yaeno, T. Maekawa, and P. Schulze-Lefert. 2016. “Allelic barley MLA immune receptors recognize sequence-unrelated avirulence effectors of the powdery mildew pathogen.” Proceedings of the National Academy of Sciences of USA 113 (42): E6486-E6495.
- Mair, W., F. Lopez-ruiz, G. Stammler, W. Clark, F. Burnett, D. Hollomon, H. Ishii, T. Thind, J. Brown, B. Fraaije et al. 2016. “Proposal for a unified nomenclature for target-site mutations associated with resistance to fungicides.” Pest Management Science 72 (8): 1449-1459.
- Mair, W., W. Deng, J. Mullins, S. West, P. Wang, N. Besharat, S. R. Ellwood, R. P. Oliver, and F. Lopez-ruiz. 2016. “Demethylase Inhibitor Fungicide Resistance in Pyrenophora teres f. sp. teres Associated with Target Site Modification and Inducible Overexpression of Cyp51.” Frontiers in Microbiology 7.
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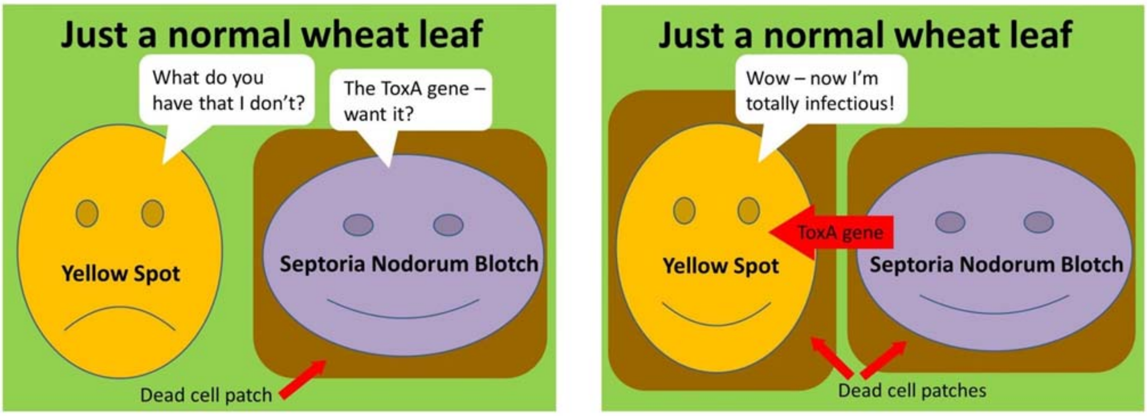
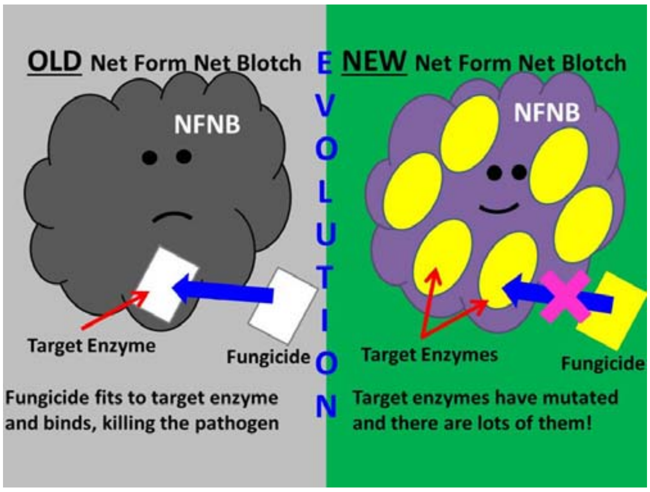
- Williams, A., M. Sharma, L. Thatcher, S. Azam, J. K. Hane, J. Sperschneider, B. Kidd, J. Anderson, R. Ghosh, G. Garg et al. 2016. “Comparative genomics and prediction of conditionally dispensable sequences in legume–infecting Fusarium oxysporum formae speciales facilitates identification of candidate effectors.” BMC Genomics 17 (191).

THE SPOTLIGHT - CCDM'S BLOG
POPULAR POSTS OF 2016

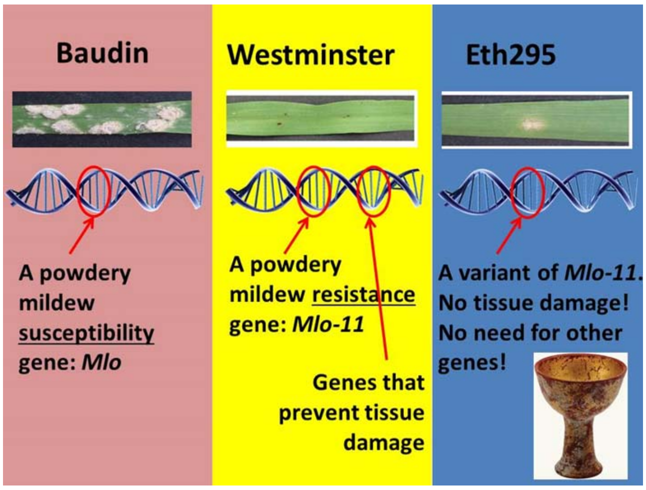


May 2016: Two cereal killers conspire against the grain



August 2016: Did Freddie Mercury predict today's fungicide resistance?

December 2016: The baby boomer conundrum for canola



July 2016: The holy grail for disease resistant barley

September 2016: Could a moustache or goatee reduce disease? Stubble management research

Centre for Crop and Disease Management

GRDC GRAINS RESEARCH & DEVELOPMENT CORPORATION

Curtin University