AusCanola2018

20th Australian Research Assembly on Brassicas **Perth**

GIWA
Grain Industry Association of Western Australia

AOF
Australian Oilseeds Federation Inc.

AusCanola2018
20th Australian Research Assembly on Brassicas, Perth

AusCanola 2018 Co-hosts

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Note: this E-Book is sorted in alphabetical order by surname of author.

Foreword, Professor Wallace Cowling[1]

Chair, AusCanola 2018 Steering Committee

[1] The UWA Institute of Agriculture, The University of Western Australia, Perth, WA 6009, Australia

Welcome to AusCanola 2018, the 20th Australian Research Assembly on Brassicas, held in Perth, Western Australia. We have a full programme of interesting talks, a valuable canola field day in the Western Australian cropping belt, and over 120 delegates from each of the canola growing states in Australia plus several international delegates.

The canola research community in Australia is a vibrant and globally-connected community which leads the way in developing and extending new canola research, with industry supporting, collaborating and benefiting from this research. Grains Industry Western Australia (GIWA) and Australian Oilseeds Federation (AOF) are co-hosting this conference, and you will witness the value they have generated by promoting industry involvement throughout this conference. We thank several sponsors, and especially the Grains Research and Development Corporation as Platinum Sponsor. This 20th Australian Research Assembly on Brassicas represents a strengthening of the research-industry nexus in canola.

We are proud of the fact this conference is returning to its roots in Perth, Western Australia. The first "ARAB" conference was held in Perth in 1977, when 11 Brassica researchers met at The University of Western Australia to create the 1st Australian Rapeseed Agronomists and Breeders Research Workshop. Greg Buzza was one of the "founders", as was Martin Barbetti, and both are registered delegates at this conference. Greg describes the aims of the original organisers:

"We decided that ARAB should have no rules as an organization but just be a forum for rapeseed breeding, agronomy and other rapeseed research. At each meeting there would be a decision as to which state would hold the next meeting in two years time and it was up to that state to run the meeting as they saw fit – a bit like awarding the Olympic games to a city – but without any stipulations." (*Buzza, 2007*)

Among the other highly respected researchers attending that conference included Noel Thurling, Richard Richards, Sarah Ryan, Lorelle Cargeeg and Vas from the The University of WA; Mick Poole and Narendra Roy from WA Department of Agriculture; Greg Buzza from VIC Department of Agriculture; Neville Mendham from University of Tasmania; Neil Wratten from NSW Department of Agriculture; and Terry Heard from SA Department of Agriculture (Buzza, 2007).

There have been twenty ARAB conferences including the inaugural conference in 1977 (Table 1). The name was changed to "Australian Research Assembly on Brassicas" after the 7th conference in Toowoomba in 1989. The conference was changed from "odd" to "even" years at the 18th conference in Tanunda in 2014. This avoided a clash with the International Rapeseed Congress (IRC), which is held every four years (14th IRC in Saskatoon, Canada in 2015; 15th IRC in Berlin, Germany in 2019; and 16th IRC in Sydney in 2023). The International Consultative Group of Research on Rapeseed, GCIRC, which is the governing body of the IRC, also holds Technical Meetings half-way between each IRC. The last Technical Meeting was in Alnarp, Sweden, in 2015. There is a strong connection between the Australian rapeseed research community and GCIRC, with at least eight Australian researchers elected as GCIRC members.

Committee members of the 20th Australian Research Assembly on Brassicas decided to use the brand name "AusCanola2018" for this conference. This makes it clear to industry that the conference focuses on canola. It also avoids any potential negative ramifications from using the acronym "ARAB" in publicity for the conference. The strong connections and traditions among ARAB researchers and agronomists, however, will not be diminished by this change!

The conferences have grown in size from 11 Brassica researchers in 1977 to more than 120 delegates in 2018, including representatives from research, industry and growers.

I thank colleagues from several WA institutions, industry groups and companies who served on the committees to organize this conference (Table 2). Most people contributed to most committees. The Steering Committee took overall responsibility for budget and sponsorship. The tireless work of GIWA representatives Ian Longson and Rachel Nash must be highlighted – their high level of organization over 18 months has brought this conference smoothly to its fruition.

Table 1. Locations of the first 20 Australian Research Assembly on Brassicas.

	Year	State	Location	Name
ARAB1	1977	WA	Perth	1st Australian Rapeseed Agronomists and Breeders Research Workshop
ARAB2	1979	VIC	Horsham	2nd Australian Rapeseed Agronomists and Breeders Research Workshop
ARAB3	1981	NSW	Wagga Wagga	3rd Australian Rapeseed Agronomists and Breeders Research Workshop
ARAB4	1983	SA	Tanunda	4th Australian Rapeseed Agronomists and Breeders Research Workshop
ARAB5	1985	WA	Perth	5th Australian Rapeseed Agronomists and Breeders Research Workshop
ARAB6	1987	ACT	Canberra	6th Australian Rapeseed Agronomists and Breeders Research Workshop
ARAB7	1989	QLD	Toowoomba	7th Australian Rapeseed Agronomists and Breeders Research Workshop
ARAB8	1991	VIC	Horsham	8th Australian Research Assembly on Brassicas
ARAB9	1993	NSW	Wagga Wagga	9th Australian Research Assembly on Brassicas
ARAB10	1995	SA	Struan	10th Australian Research Assembly on Brassicas
ARAB11	1997	WA	Perth	11th Australian Research Assembly on Brassicas
	1999		*Canberra*	*10th International Rapeseed Congress*
ARAB12	2001	VIC	Geelong	12th Australian Research Assembly on Brassicas
ARAB13	2003	NSW	Tamworth	13th Australian Research Assembly on Brassicas
ARAB14	2005	SA	Port Lincoln	14th Australian Research Assembly on Brassicas
ARAB15	2007	WA	Geraldton	15th Australian Research Assembly on Brassicas
ARAB16	2009	VIC	Ballarat	16th Australian Research Assembly on Brassicas
ARAB17	2011	NSW	Wagga Wagga	17th Australian Research Assembly on Brassicas
ARAB18	2014	SA	Tanunda	18th Australian Research Assembly on Brassicas
ARAB19	2016	VIC	Melbourne	19th Australian Research Assembly on Brassicas (CGW20/Brassica 2016)
AusCanola2018	2018	WA	Perth	20th Australian Research Assembly on Brassicas

Table 2. Committee members

Name, Institution	Name, Institution
Wallace Cowling, UWA, Chair Steering Committee	Peter Bostock, Gentech Seeds
Jacqueline Batley, UWA, Chair Program Committee	Sheng Chen, UWA
Jackie Bucat, DPIRD, Chair Field Day Committee	Mark Seymour, DPIRD
Ian Longson, GIWA - AusCanola2018 Coordinator	Jens Berger, CSIRO
Rachel Nash, GIWA – Sponsorship, Promotion	Bob French, DPIRD
Larissa Taylor, GIWA (CEO GIWA)	Martin Harries, DPIRD
Martin Barbetti, UWA	Phil Salisbury, University of Melbourne
Heping Zhang, CSIRO	Nick Goddard, AOF
Lars Kamphuis, Curtin / CSIRO	Michael Lamond, GIWA Oilseeds Chair
Ravjit Khangura, DPIRD	David Peake, BASF
Dave Minkey, WANTFA	

ACKNOWLEDGEMENTS

The Australian Oilseeds Federation have provided registration and web support for this AusCanola 2018 conference, and continues to host the Proceedings from previous conferences on its website. Grains Industry Western Australia managed the conference and helped to make the meeting a great success.

REFERENCES

Buzza, G., 2007: Canola breeding in the seventies – a personal look back. 15th Australian Research Assembly on Brassicas, Geraldton, Western Australia. Available at: http://www.australianoilseeds.com/__data/assets/pdf_file/0018/4617/Canola_breeding_in_the_seventies.pdf

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Opening Address, Dr Mark Sweetingham

Managing Director of Research Development & Innovation, Department of Primary Industries and Regional Development

Bachelor of Agricultural Science (Hons.) and PhD (Plant Pathology) from the University of Tasmania.

Dr Mark Sweetingham joined the Department of Agriculture - WA in 1983 as a plant pathologist where his pioneering research led to a comprehensive understanding and control strategies for a range of lupin diseases and Rhizoctonia pathology of grain crops.

His career in the Department was notable through his effective and diverse collaboration with plant breeders, agronomists and food & health professionals leading a wide range of statewide and national grains research projects and developing research alliances including the Australian Centre for Necrotrophic Fungal Pathogens, the Centre for Grain Food Innovation and supporting the success of Pulse Breeding Australia, the Centre for Legumes in Mediterranean Agriculture and the Centre for Food and Genomic Medicine.

In 2005 Mark was awarded the Medal of Agriculture by the Australian Institute of Agricultural Science and Technology.

More recently Mark has held a number of senior and Executive positions in the Department of Agriculture and Food. He was a key driver of the creation of the Australian Export Grains Innovation Centre and remains the co-chair of the National Grains Industry RD&E Strategy.

He has an extensive scientific publication record and a passion for mentoring and building applied R&D teams that work closely with industry participants.

GOVERNMENT OF WESTERN AUSTRALIA
Department of **Primary Industries and Regional Development**

AusCanola 2018 Program - 5th to 6th September 2018

Day 1: Wednesday 5th September 2018

8.00 - 8.30 am	**Registration and coffee**
8.30 - 8.55am	**Welcome and housekeeping** Wallace Cowling, Chairman, AusCanola 2018 Steering Committee **Opening** Mark Sweetingham, Department of Primary Industries and Regional Development
8.55 - 9.15am	**Where is canola genomics heading in the future?** Jacqui Batley, University of Western Australia and Chairman, AusCanola 2018 Program Committee
Session 1: New Germplasm Chair: Martin Barbetti	
9.15 - 9.30am	**An overview of the Australia India China Project** Dr Phil Salisbury, University of Melbourne
9.30 - 10.00am	**Mobilising alternative sources of genetic diversity for germplasm enhancement in Brassica oilseeds** Surinder Banga - Punjab Agricultural University
10.00 - 10.15am	**Evaluation of heat and drought tolerance at anthesis in novel canola germplasm from India and China** Sheng Chen, University of Western Australia
10.15 - 10.30am	**Review of the benefits of the India-China Exchange Project with particular reference to the identification of genomic regions and candidate genes for resistance to pod shatter in Brassica** Harsh Raman, NSW Department of Primary Industries
10.30 - 10.45am	***Brassica carinata*: Coming to a farm near you** Anthony van Herqaarden, University of Queensland
10.45 - 10.50am Poster 3 min	**New traits for Australian canola via TILLING and gene editing** Chris Helliwell, CSIRO
10.50 - 10.55am Poster 3 min	**Estimation of linkage disequilibrium and population structure among *Brassica Napus* genotypes for association mapping** Huma Qamar, Ayub Agriculture Research Institute, Faislabad
11.00 - 11.30am	**Morning Tea**

Session 2: Impact of Climate Change Chair: Trent Potter	
11.30 - 12.00pm	**Crop modelling: Physiological context for improved canola agronomy** Julianne Lilley, CSIRO
12.00 - 12.15pm	**A model for pre-breeding canola for heat and drought tolerance during global climate change** Wallace Cowling, University of Western Australia
12.15 - 12.30pm	**Effect of water stress on transpiration efficiency in canola** Rajneet Uppal, NSW Department of Primary Industries
12.30 - 12.45 pm	**Predicting canola phenology in warm environments** Jeremy Whish, CSIRO
12.45 - 12.50pm Poster 3 min	**Phi thickenings in Brassica roots - an adaptation to water stress?** David Collings, University of Newcastle
12.50 - 2.00pm	**Lunch**
Session 3: Pests and Diseases Chair: Lars Kamphuis	
2.00 - 2.30pm	**Sclerotinia Stem Rot in canola: tales from the executioner's handbook** Dwayne Hegedus, Agriculture and Agri-Food Canada
2.30 - 2.40pm	**Effective management of Sclerotinia stem rot (*Sclerotinia sclerotiorum*) in canola (*Brassica Napus*) through exploring host resistance** Muhammad Azam, University of Western Australia
2.40 - 2.50pm	**New avenues for breeding resistance to Sclerotinia stem rot into Australian canola** Mark Derbyshire, Centre for Crop and Disease Management, Curtin University
2.50 - 3.00pm	**Reassessing aphid thresholds in canola** Svetlana Micic, WA Department of Primary Industries and Regional Development
3.00 - 3.05pm Poster 3 mins	**Decision Apps for managing blackleg and Sclerotinia in canola** Art Diggle, WA Department of Primary Industries and Regional Development
3.05 - 3.10pm Poster 3 mins	**Survey of Sclerotinia stem rot sclerotia post harvest** Andrea Hills, WA Department of Primary Industries and Regional Development
3.10 - 3.15pm Poster 3 mins	**Environmental drivers of white leaf spot disease (*Pseudocercosporella capsellae*) on canola** Tamsal Murtza, University of Western Australia
3.15 - 3.20pm Poster 3 mins	**Alternaria spp. Leaf Spot Incidence associated with canola in Australia** Hebba Al-Lami, University of Western Australia

3.20 - 3.25pm Poster 3 mins	**Pathotypes of *Hyaloperonospora brassicae* on canola and other Brassicaceae** Akeel Mohammed, University of Western Australia
3.30 - 4.00pm	**Afternoon Tea**
Session 4: Breeding Chair: Wallace Cowling	
4.00 - 4.15pm	**GRDC Investment in Pre-breeding** Francis Ogbonnaya, Grains Research and Development Corporation
4.15 - 4.30pm	**National Variety Trials PV-Plus App** Lauren Borg, University of Wollongong
4.30 - 4.45pm	**Overview, the risk analysis framework used and public attitudes to GMOs** Alison Wardrop, Office of Gene Technology Regulator
4.45 - 5.00pm	**Hybrid vs. OP canola: which one wins where?** Heping Zhang, CSIRO
5.00 - 5.15pm	**Towards functional characterization of pod shatter resistant genes in *Brassica Napus*** Qiong Hu, Chinese Academy of Agricultural Sciences
5.15 - 5.30pm	**Establishing a diverse multi-year genomic selection reference population for key traits to underpin canola pre-breeding** Hans Daetwyler, Victorian Department of Economic Development, Jobs, Transport and Resources
6.30 - 9.30pm	**Conference Dinner**

Day 2: Thursday 6th September 2018

8.00 - 8.30am	**Registration and coffee**
Session 5: Growing canola in low rainfall environments (WA) Chair: Michael Lamond	
8.30- 9.30am	**Canola growing in WA** Michael Lamond to provide an overview then a panel of 3 growers will talk about their experiences growing canola in WA, where it fits in the rotation and issues they have growing canola
9.30 - 9.40am	**Retaining canola seed** Mark Seymour, WA Department of Primary Industries and Regional Development
9.40 - 9.55am	**Plant geometry and density for management of canola crops in low rainfall environments** Martin Harries, WA Department of Primary Industries and Regional Development

9.55am - 10.05am	**Canola sowing time to maximise yield in Western Australia** Imma Farre, WA Department of Primary Industries and Regional Development
10.05 - 10.15am	**Canola: Pathways -to profitability** Elizabeth Meier, CSIRO
10.15 - 10.30am	**Over twenty years of commercial canola crops show the place of canola in the southern Mallee of South Australia** Trent Potter, Yeruga Research Station
10.30 - 11.00am	**Morning Tea**
Session 6: Agronomy Chair: Peter Bostock	
11.00 - 11.30am	**Canola's rise to #1 on the Canadian Prairies – Innovation and "Out-o-vation"** Murray Hartman, Alberta Agriculture and Forestry
11.30 - 11.45am	**Determining the critical period for yield and quality in canola** John Kirkegaard, CSIRO
11.45 - 12.00pm	**Match varietal phenology with sowing date to drive canola productivity** Rohan Brill, NSW Department of Primary Industries
12.00 - 12.10pm	**How does the partitioning of yield, seed colour change and other determinants of windrow timing, influence seed yield and quality parameters in canola** Rick Graham, NSW Department of Primary Industries
12.10 - 12.20pm	**Canola establishment across central NSW -how to get it up** Colin McMaster, NSW Department of Primary Industries
12.20 - 1.30pm	**Lunch**
Session 7: Pests and diseases Chair: Jacqui Batley	
1.30 - 1.50 pm	**Dual herbicide tolerant canola for better weed management** Christopher Preston, University of Adelaide
1.50 - 2.10pm	**Creation of a hypervirulent isolate of the blackleg pathogen using CRISPR/Cas9 and Identification of *Brassica Napus* - *Leptosphaeria maculans* interactions through dual RNAseq** Dilantha Fernando, University of Manitoba
2.10 - 2.20pm	**Infection of *Brassica Napus* after stem elongation by *Leptosphaeria maculans* (blackleg): disease development and yield loss** Susan Sprague, CSIRO
2.20 - 2.30pm	**Fungicide resistance in Australian *Leptosphaeria maculans* populations** Angela Van de Wouw, University of Melbourne

2.30 - 2.35pm Poster 3 min	**Examining variety by environment (VxE) interaction in canola blackleg expression experiments** Lauren Borg, University of Wollongong
2.35 - 2.40pm Poster 3 min	**Inert materials are critical long-term carriers and disseminators of viable blackleg ascospores** Papori Barua, University of Western Australia
2.40 - 2.45pm Poster 3 min	**Imputation to whole-genome sequence increases the power of genome wide association studies for blackleg resistance in canola** Denise Barbulescu, Victorian Department of Economic Development, Jobs, Transport and Resources
2.45 - 2.50pm Poster 3 min	**Natural variation for interference traits against annual ryegrass in canola** Nawar Shamaya, NSW Department of Primary Industries
3.00 - 3.30pm	**Afternoon Tea**
Session 8: Product Quality, Marketing and Processing Chair: Nick Goddard	
3.30 - 3.50pm	**Greenhouse gas values for canola** Sandra Eady
3.50 - 4.10pm	**Development of an omega-3 long-chain fatty acid oil crop: Innovation in canola** Surinder Singh, CSIRO
4.10 - 4.25pm	**Key canola quality attributes in the new millennium – what are the trends over the past 18 years?** Donna Seberry, NSW Department of Primary Industries
4.25 - 4.30pm Poster 3 min	**Computational and biological characterization of 2S albumin proteins from *Brassica rapa*** Mahmudur Rahman, Southern Cross University
4.30 - 4.45pm	**The use of canola oil for deep frying in the food industry** Luke Peet, Chef, and AUSTRALIA Manager of Oil2u
4.45 - 5.00pm	**Closing Remarks** Nick Goddard, CEO, Australian Oilseeds Federation Wallace Cowling, Chairman Steering Committee
5.00pm	**Conference concludes**

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Thermal stability of Australian canola oil varieties

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ABSTRACT

The objective of this study was to investigate the frying performance of canola oil types processed by various processors in Australia. The scope of oils tested were: refined canola oils obtained after cold-mechanical-pressing (CmpCO); hot-mechanical-pressing of canola seeds (HmpCO-I and HmpCO-II); and a generic canola oil (GenCO – blend of canola oil from other extraction techniques). Samples were selected from the 2016/2017 production season to ensure limited variation in the fatty acids composition of the oils. The canola oil samples were used to fry fresh cut potato chips, and the oil degradation was monitored by measuring the total polar materials (TPM), free fatty acids (FFA) and tocopherols content. It was found that the HmpCO-I sample exhibited better thermal stability than other oils tested. Tocopherols retention during frying also improved the thermal stability of canola oils. Overall, the oils exhibited different degrees of thermal stability, which highlighted inherent differences in canola oils – as a function of the crude oil processing method. The outcome of this study will provide insight to processors on the thermal stability of their canola oils and could serve as a platform for further optimisation of their processes to produce the best quality products for domestic and international markets.

Key words: Extraction technique; Frying life; Canola oil; Total polar material; Tocopherols.

INTRODUCTION

Frying is a popular method of food preparation and it is applied on small to large scales in various industries (Rudzińska et al., 2018). Various types of oils, including soybean, palm and palm kernel, olive, coconut and other modified and blended oils are used to fry food (Aladedunye & Przybylski, 2014; Przybylski, Gruczynska, & Aladedunye, 2013). The attractiveness of fried foods owes to the array of chemical reactions that occur in the frying medium (oil and food), leading to attributes such as crispiness, flavour, taste, and golden colour of fried foods, which are desirable to consumers.

The selection of oil type for frying is influenced by the economics of the process, mainly the frying life and the cost of purchasing the oil. The frying life of the oil is determined by the source of oil, fatty acids composition, presence of minor bioactive components (e.g. tocopherols, polyphenolic compounds) and reactions that occur in the oil during extraction and refining (e.g. Maillard reactions, polymerization, hydrolysis etc). Frying practices and the food type being fried also affect oil stability and frying life. Whereas processors will be focused on the frying life of the oil and associated oil costs, the physicochemical and sensorial attributes of the fried food is important to end-users and consumers. Therefore, oil manufacturers and fried food producers must combine these factors into their processes to produce products that can satisfy adequately, all of these factors.

Canola oil is an economically important oil owing to its unique fatty acids profile (Ghazani, García-Llatas, & Marangoni, 2014) – saturated fatty acids <8%, monounsaturated fatty acids of 55 - 67% and polyunsaturated fatty acids of 23-31%. Over the past 4 decades, canola has grown in popularity globally as the third edible oil by volume after palm and soybean oil (Lin et al., 2013). The refined oil has significant quantities of tocopherols and phytosterols, which together with the high monounsaturated fatty acids are believed to affect cardiovascular health – by regulation of plasm lipids and lipoprotein, susceptibility of low-density lipoproteins oxidation and insulin sensitivity (Lin et al., 2013).

Extensive research has been conducted on canola oil frying qualities, looking at fatty acid compositions (Przybylski et al., 2013), frying temperature and quality changes in oil during frying (Aladedunye & Przybylski, 2009). However, little has been done to investigate the effects of the crude oil extraction techniques on the thermal stability of the refined oils, although anecdotal evidence exists of differences in the functionalities (Warner & Dunlap, 2006). Thus, more research is needed to understand the compositional and functional differences between refined canola oils obtained by the different extraction techniques.

In Australia, canola oil processing involves the crude oil extraction (cold-mechanical pressing, hot-mechanical pressing, pre-pressing followed by solvent extraction and 100% solvent extraction), followed by refining, either chemically, physically or both. Differences exist in oil yield and the crude oil quality indices between the different extraction techniques (Ghazani et al., 2014). Generally, solvent extracted oils are darker in colour, high in free fatty acids, gums, and chlorophyll. The proportions of minor components also varies considerably between the different extraction techniques. For example, solvent-extracted crude canola oils has been shown to have higher tocopherols and phytosterols content than mechanical-pressed crude canola oil (Ghazani et al., 2014; Van Hoed, Ali, Slah, & Verhé, 2010).

In this study, the thermal stabilities of refined bleached and deodorised cold-mechanical-pressed, hot-mechanical-pressed, and a generic canola oils were investigated. The behaviour of the oils with heating when frying provides insight into the thermal stability and frying life of the different oil variants.

MATERIALS AND METHODS

Four canola oil types were obtained from Australian processors. Canola oils were drawn from the 2016/2017 production season and included a cold-mechanical-press (CmpCO), two hot-mechanical-pressed (HmpCO-I and HmpCO-II) and a generic refined canola oil (GenCO). Fresh potato chips were purchased from a local processor. All other chemicals and reagents were of analytical and research grade.

Frying exercise: Frying was conducted in a 5 L capacity stainless steel double pan deep fryer (Model FFA2002, Anvil Double Basket Benchtop Fryer, Anvil Axis, South Africa). Canola oil (4 L) was heated at 180 ± 5°C for 9 hours daily (1 hour preheating + 8 hours of frying). Each frying cycle had 400 g potato chips fried for 8 minutes, every hour for 8 hours each day – for 7 days. The oil was filtered before frying on day 3 and 5, and oil volume was not topped-up. At the end of frying each day, 50 mL of oil was collected in a 50 mL centrifuge tube, wrapped in aluminium foil and kept at -20 °C for further analyses.

Monitoring oil quality: Peroxide value (AOCS Cd 8b-90, 2011) and free fatty acids content (AOCS Ca 5a-40, 2011) of unfried and fried canola oils were determined as per AOCS official methods of analysis. The total polar material (TPM) of the oils was measured before frying and after each frying cycle for 8 hours each day for the 7 days. TPM was measured with the Frying Oil Monitor DOM-24 (ATAGO Co., Ltd, Tokyo, Japan). Tocopherols content, oxidative rancidity index (OSI), and fatty acids composition were determined by in-house methods of the NSW Department of Primary Industries Oils Research Laboratory, which are based on ISO methods.

RESULTS

HmpCO-I, HmpCO-II and GenCO had low initial FFA content, ~0.05%. The CmpCO had very high initial FFA (0.52%) and peroxide value (~8.8 mEq/kg oil), as well as the lowest initial oxidative stability index (~5.8 hours) and total tocopherols content (~548 mg/kg)) when compared to the other three canola oil types (Table 1). The two hot-mechanical-pressed canola oils (HmpCO-I and HmpCO-II) had similar tocopherols content, however, the HmpCO-I recorded a higher initial peroxide value (1.8 mEq/kg Oil) than HmpCO-II (0.8 mEq/kg Oil). The GenCO had the highest tocopherols content of 707 mg/Kg. The GenCO is typically an aggregated blend of oils obtained by the different extraction techniques which, depending on the proportions of different oils blended, can affect the levels of minor components such as tocopherols, as well as the overall quality indices. The HmpCO-I, HmpCO-II and GenCO had comparable oxidative stability index (Table 1). The fatty acids composition of all the four canola oils were typical and were very similar for HmpCO-I, HmpCO-II and GenCO. The CmpCO sample had higher oleic acid and lower linoleic and linolenic acid contents of all the oils. This trend in CmpCO fatty acids composition is also reflected by a higher MUFA and lower PUFA and iodine value.

Table 1: Pre-frying oil quality and fatty acids composition of canola oils.

Parameters	HmpCO-I	HmpCO-II	CmpCO	GenCO
FFA as Oleic (%)	0.051±0.02	0.048±0.01	0.51±0.00	0.052±0.00
Peroxide Value (mEq/Kg Oil)	1.8±0.07	0.8±0.03	8.8±0.12	1.13±0.08
Oxidative Stability (h)	8.0±0.09	8.6±0.05	5.8±0.07	8.7±0.07
Tocopherols (mg/kg)	668±0.6	647±2.7	548±1.5	707±1.9
Fatty Acid composition (% total fatty acids)				
Palmitic acid (C16:0)	4.0±0.00	4.0±0.00	4.1±0.01	4.1±0.01
Stearic acid (C18:0)	1.9±0.00	1.9±0.00	2.0±0.00	1.9±0.00
Oleic acid (C18:1)	61.7±0.03	62.4±0.00	64.5±0.01	62.1±0.02
Linoleic acid (C18:2)	19.4±0.00	19.2±0.00	17.9±0.00	19.4±0.00
Linolenic acid (C18:3)	10.3±0.03	9.9±0.01	8.8±0.00	9.8±0.01
SFA	7.0±0.00	7.0±0.00	7.3±0.01	7.2±0.01
MUFA	63.3±0.03	63.9±0.00	66.0±0.01	63.7±0.02
PUFA	29.7±0.03	29.1±0.01	26.7±0.00	29.1±0.01
Iodine Value	114.7±0.10	113.8±0.02	110.6±0.02	113.6±0.03

Figure 1 shows the changes in TPM of the four canola oil types during frying. The TPM correlated with frying time and increased as the frying cycle / time increased. TPM increased in the order, HmpCO-II (3.0 – 19.5%) > HmpCO-I (3.5 – 24.3%) = GenCO (4.0 – 22.5%) > CmpCO (3.5 – 28.0%).

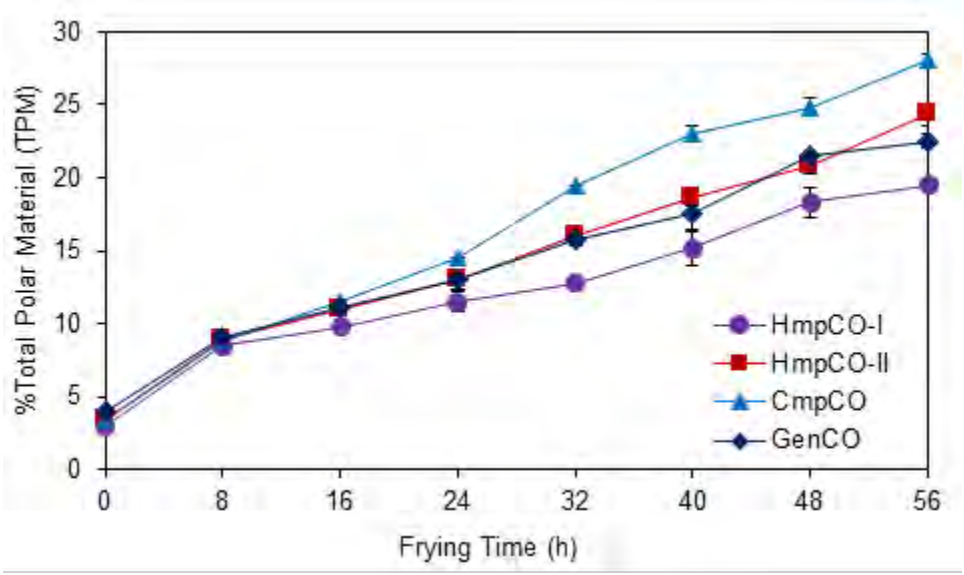

Figure 1: Changes in total polar materials of different canola oil types during frying. Legends: HmpCO-I and HmpCO-II = hot-mechanical-pressed; CmpCO = cold-mechanical-pressed GenCO = generic refined canola oil.

The formation of FFA during frying is presented in Figure 2. Again, FFA was well correlated with frying time and increased in a 2nd order polynomial fashion. The three canola oils HmpCO-I, HmpCO-II and GenCO displayed similar trends in data, however as shown in Figure 2, the CmpCO was well distinguished from the rest of the oils due to the unusually high initial FFA content.

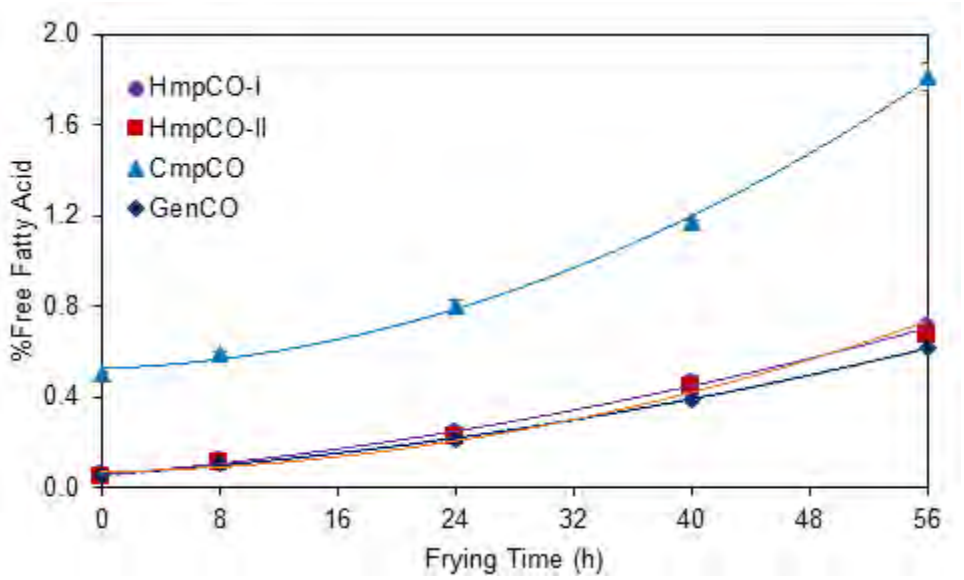

Figure 2: Free fatty acids evolution during frying. Legends: HmpCO-I and HmpCO-II = hot-mechanical-pressed; CmpCO = cold-mechanical-pressed GenCO = generic refined canola oil.

The percent loss in tocopherols during frying is shown in Figure 3. At the end of the 7th day, tocopherol losses were 68.5%, 82.5%, 92.6% and 78.8% for the HmpCO-I, HmpCO-II, CmpCO and GenCO, respectively. The CmpCO had the greatest loss in tocopherols over the frying period.

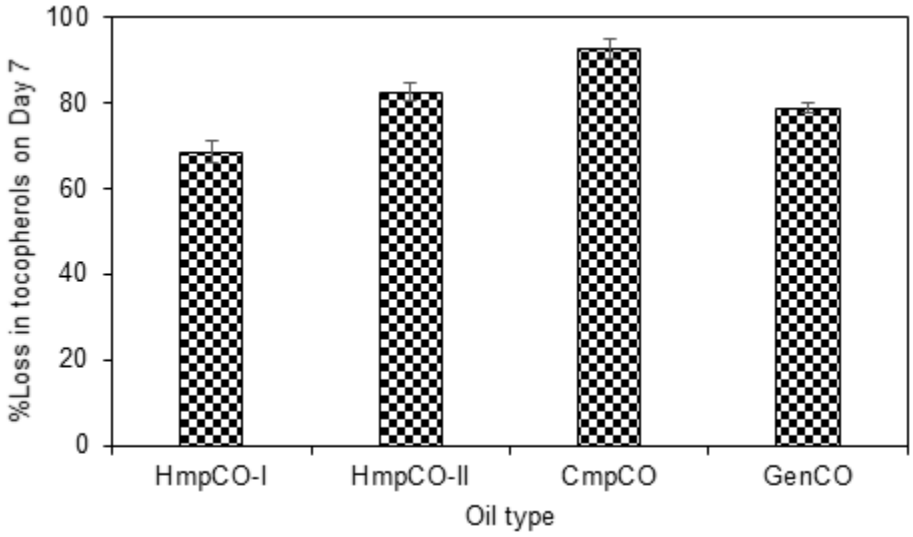

Figure 3: Tocopherols loss end of day 7 of frying. Legends: HmpCO-I and HmpCO-II = hot-mechanical-pressed; CmpCO = cold-mechanical-pressed GenCO = generic refined canola oil.

DISCUSSION

The current study focused on the effect of various processing techniques as applied to crude oils on the frying performance of four canola oil types. The fatty acids composition of the unfried canola oils was typical of the 2016/2017 canola oil production season. The CmpCO had the least amount of tocopherols, which can be attributed to the cold-pressing technique used for the crude oil extraction, which tends to extract less tocopherols into the oil (Ghazani et al., 2014), whereas the very high initial FFA resulted from the refining process applied to the oil. Most likely, the refining process did not reduce the initial FFA content in the crude oil especially during the neutralisation and deodorisation steps.

Canola oil deterioration during frying was monitored by measuring the TPM, FFA and tocopherol loss. TPM measurement is an accurate index to evaluate oil deterioration during frying (Przybylski et al., 2013) and, together with the FFA and tocopherols, can provide estimations on oil performance and frying life. Generally, oil is considered degraded when the TPM value reaches 24%. The effect of frying on TPM and FFA evolution correlated with frying time (Figures 1 and 2) – increasing with number of frying cycles. As shown in Figure 1, the CmpCO presented the lowest frying life and reached the TPM cut-off for frying oils by the end of day 6. The HmpCO-I recorded the best stability followed by the GenCO and the HmpCO-II. This underscores the intrinsic and performance differences between canola oils obtained by the different extraction methods and by different processors.

The evolution in the FFA was similar for the HmpCO-I, HmpCO-II and the GenCO, however the CmpCO was markedly distinct from the rest (Figure 2). This resulted from the unusually high initial FFA of the CmpCO compared to the other oils (Table 1). It is worth mentioning that the CmpCO, however, recorded the least relative change in FFA of 0.67 at the end of frying compared to the other oil which had increased by 1.12 – 1.13. This implied that, the high initial FFA of the CmpCO did not play any significant role in the thermal stability of this canola oil and might not be important to the overall stability. This suggested that other degradation products including dimeric and higher polymeric triglycerides, monomeric oxidized products, as well as mono- and diglycerides (Farhoosh & Tavassoli-Kafrani, 2010) most likely contributed more to oil deterioration. Thus, FFA is not the best predictor of oil degradation but TPM is, since TPM as an index combines the contributions of all the degradation products listed including FFA.

The stability of the oils with frying also correlated with tocopherols loss with greater tocopherols loss correlating with higher TPM values (Figures 1 and 3). Thus, tocopherol being a natural antioxidant protected the oils against oxidation and thermal degradation. The HmpCO-I samples although showed highest initial peroxide value and lowest initial oxidative stability index when compared to the HmpCO-II and the GenCO, had the longest frying life of the three canola oils (Figure 1 and 3). Thus, an initially high peroxide value may not necessary influence the frying life adversely and that the ability to retain tocopherols by protecting tocopherols against oxidation and thermal degradation was more important to frying life of the canola oils. The presence of Maillard reaction products has been shown to inhibit tocopherols loss and improved the frying performance of expeller-pressed refined soybean oil over solvent-extracted refined soybean oil (Warner & Dunlap, 2006).

In conclusion, the current study has demonstrated that the seed preparation and crude oil extraction techniques can influence the functionality of the refined oil and that differences exist between the different canola oil variants during frying. Further research is however needed to better understand the sources of these variations. The outcome of this study could lead to further process optimisation leading to products with improved functionalities such as longer frying life – and show canola oil as a non-commodity oil product which can command a market premium.

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Alternaria spp. leaf spot Incidence associated with canola (*Brassica napus*) in Australia

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ABSTRACT

Studies were undertaken to identify *Alternaria* spp. associated with leaf spot symptoms on canola (*Brassica napus*) across southern Australia in two cropping seasons. Preliminary results from the first season showed 7 different species of *Alternaria* and this likely increased to 12 different species in the second season. Studies to determine their pathogenicity on both *B. napus* and *B. juncea* under controlled environmental conditions further supported the identities of ten different species of *Alternaria* as being pathogenic on both *Brassica* species. Results so far suggest that three species of *Alternaria* are likely first records for Australia on any host including *B. napus* and five species are likely first records on both species across various Australian states. Species of *Alternaria* included *A. alternata* and *A. brassicae*, both worldwide pathogens across different *Brassica* hosts, including *B. napus* and *B. juncea*. These studies are the first to highlight that there is significant diversity of *Alternaria* spp. leaf spot pathogens associated with Alternaria leaf spot of canola in Australia.

Key words:

Alternaria leaf spot, canola, Brassica napus, mustard, Brassica juncea

INTRODUCTION

Alternaria leaf spot diseases together constitute one of the most important disease groups and responsible for yield losses up to 47% of Indian mustard (Kolte 1984) and are a problem on >70% of *Brassica* spp. worldwide (Kumar *et al.* 2014). In Canada, *A. brassicae* is estimated to cause losses of >30% of rapeseed in 1987 (Conn *et al.* 1990). In Australia, total canola yield comes in third place after wheat and barley, and the area of canola in Australia has increased by 1.7-fold between 2009 to 2013 (Elliott *et al.* 2015). Therefore, studies were undertaken to identify the *Alternaria* spp. associated with canola leaf spot across southern Australia over two cropping seasons; and to determine their pathogenicity on both *B. napus* and *B. juncea* under controlled environmental conditions. These studies are the first to highlight the diversity of Alternaria leaf spot pathogens associated with canola in Australia, and the association of 9 other pathogenic *Alternaria* spp. along with *A. brassicae* with leaf spotting in southern Australia.

MATERIALS AND METHODS

Fungal isolation and identification

Alternaria spp. isolates were obtained from diseased leaves of canola surveyed and sampled across southern Australia during two cropping seasons. Leaves showing disease symptoms likely to be from

Alternaria were targeted, placed into polyethylene bags, maintained at approximately 10oC and transferred to the laboratory. 2-mm2 sections were removed from the leading edge of each leaf lesion and placed in 2% NaClO for 60 sec then washed three times with sterilized deionized (DI) water; plated onto 9 cm Petri dishes containing water agar medium (2% w/v) containing 25 ppm aureomycin hydrochloride and incubated for 2 d at 22 °C under cool white fluorescent light. Emerging fungal colonies were transferred onto other media as appropriate. Isolates were then hyphal tipped to produce pure cultures and preserved from PDA cultures into lyophilized ampoules until needed.

DNA was extracted from one-week-old cultures of two cropping season's isolates growing on PDA. The procedure of Cenis (1992) was used for DNA extraction. The concentration and quality of the extracted DNA were determined using a NanoDrop 1000 Spectrophotometer. DNA samples were held at 4°C until needed. A polymerase chain reaction (PCR)-based assay was conducted by standard sequencing methodologies as appropriate for *Alternaria* spp. PCR products were visualized by electrophoresis on a 1% (wt/vol) agarose gel containing GelRed 10000x stain under UV light. PCR products (20 µl of each) were sequenced by Macrogen Inc., Korea. The sequence manipulation and alignment were conducted by using GENEIOUS software v.9.1.4. Consensus sequences, derived from DeNovo assembly of the forward and reverse sequences of each isolate for each primer, were compared with available sequence data information for type or representative isolates in GenBank of National Center for Biotechnology Information (NCBI). For phylogenic analysis, sequences were aligned and a maximum Likelihood (ML) phylogeny was obtained by using Nearest-Neighbor-Interchange (NNI) method and Tamura-Nei model

Morphological characteristics

Morphological characteristics, colony color, conidial shape, length, width and the number of septa, and conidiophore length, were recorded.

Pathogenicity tests

For inoculum preparation, one or more isolates of each *Alternaria* spp. identified were grown on various media for 7 d at 23°C under cool white fluorescent light. Conidia were collected by adding 10 ml of sterile deionized water (SDW) to each plate, brushing the colony surface gently with a sterile glass rod, using muslin to filter any agar pieces from the conidia. The final concentration was adjusted to 5×10^5 ml^{-1}.

Isolates of Alternaria across the identified species that readily produced conidia were used; along with two host test species, B. napus and B. juncea. Seeds were sown, seedlings thinned to 2 plants per pot, and fully expanded leaves were drop-inoculated (10 µl) with the conidial inoculum of each test isolate (or distilled water as a negative control check). Plastic boxes were internally misted with DI water and lids closed for 48 h after the inoculation to maintain high humidity. Inoculated leaves were assessed from 14 d onwards for disease symptoms to confirm ± pathogenicity of tested Alternaria isolates. To complete Koch's postulates, Alternaria spp. are being reisolated from all leaf lesion symptoms and isolate identities reconfirmed as inoculated Alternaria spp. by again having each isolate re-sequenced by Macrogen Inc., South Korea, coupled with morphological observations.

RESULTS AND DISCUSSION

What appear to be twelve different species of *Alternaria*, including *A. alternata* and *A. brassicae,* were associated with canola leaf spots across two cropping seasons. Ten *Alternaria* spp. isolates were pathogenic on both *Brassica* species. The full results will be revealed once the identification of all *Alternaria* spp. isolates has been completed. However, it is evident that the species of *Alternaria* included *A. alternata* and *A. brassicae*, both worldwide pathogens across different *Brassica* hosts,

including canola and mustard. These studies are the first to highlight the diversity of *Alternaria* spp. leaf spot pathogens associated with canola in Australia. Preliminary findings suggest that three species of *Alternaria* are likely to be first records for Australia on any host including *B. napus*, while five species are likely to be first records on both species across various Australian states.

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Effective management of Sclerotinia stem rot (*Sclerotinia sclerotiorum*) in canola (*Brassica napus*) through exploring host resistance

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ABSTRACT

Canola (*Brassica napus*) has seen a rapid increase in area sown in Australia over the past two decades. This has been associated with increases in incidence and severity of fungal diseases, particularly, Sclerotinia stem rot (*Sclerotinia sclerotiorum*). Sclerotinia stem rot has become a serious threat for Australian canola growers. Key management strategies should focus on cost-effective, long term control through locating, developing and deploying new and effective host resistances in order to protect and ensure sustainability of canola yields into the future. However, while some high-level resistance against Sclerotinia stem rot has been located, particularly within diverse Brassicaceae species, currently there are no commercial canola cultivars in Australia targeting resistance to this disease. To address this, cohorts of canola genotypes consisting of parents with known resistance/susceptibility to Sclerotinia stem rot and their succeeding generations are being evaluated for resistance against *S. sclerotiorum*. Segregating populations derived from these parents expressed variation in phenotypic responses at both seedling and adult stages, ranging from highly resistant to susceptible types. In both controlled and field environments, close relationships were identified between phenotypic expressions of host resistance within and between the segregating populations. Phenotypic selection in F_2 and F_3, coupled with planned QTL mapping in F_2 genotypes, is being utilized to define the basis of genetic control of resistance against this pathogen in canola. These outcomes will be crucial to plant breeders in developing new cultivars with improved resistance against this major disease of canola and will benefit farming communities, both within Australia, in particular, and worldwide, in general.

Keywords: *Sclerotinia sclerotiorum*, Brassica, host resistance, breeding populations

INTRODUCTION

*C*anola quality *Brassica napus* is the major *Brassica* crop worldwide due to its low erucic acid and low glucosinolate content that makes it a highly desirable human food and animal feed. However, Sclerotinia stem rot (*Sclerotinia sclerotiorum*) can be a devastating disease of both canola (*B. napus)* and mustard (*B. juncea*) worldwide, including in Australia, China, Europe and North America (Garg *et al.* 2008). In Australia, it causes up to 24% yield loss (Garg *et al.* 2008), with an estimated value of the loss of up to A$10.1 million.

Strategies to mitigate Sclerotinia stem rot include cultural practices, chemical controls and varietal resistance. Cultural controls are more a precautionary measure rather than a direct control of this disease (Garg *et al.* 2008), largely involving avoidance of infested stubbles and remaining sclerotia from previous crops. However, a challenge is that the fungal sclerotia remain viable in the field as resting sclerotia for many years (Li *et al.* 2006). Fungicides, on the other hand, are widely applied for disease control but they are expensive and their efficacy varies widely. This highlights the need for a strong focus to locate, develop and deploy effective host resistances in order to protect and ensure economic

canola yields. Hence, studies were undertaken to define the genetic variation in relation to disease resistance in *B. napus* crossing populations against Sclerotinia.

MATERIALS AND METHODS

***Brassica* crossing populations:** Three crosses were used in this study along with their parents, backcrosses and F2 populations; i) (*B. napus* NC8 x *B. napus* RQ-001-NCA-8 NC2-7), ii) (*B. napus* CRN x B. napus RQ-001-NCA-8 NC2-7) and iii) (*B. napus* CRN *x B. napus* NC4-5).

***S. sclerotiorum* isolate:** A Single isolate of *S. sclerotiorum* (isolate MBRS1) was used in all studies. This is the same isolate as used in earlier studies to identify seedling (Garg *et al*. 2008) or stem (Li *et al.* 2006) resistances in *B. napus* and/or *B. juncea* types.

Test conditions*:* *B. napus* germplasm lines were grown in plastic trays containing 20 small pots and with a single plant in each pot. Plants were maintained in a controlled environment room with conditions similar as described by Garg *et al.* (2008).

Cotyledon and Leaf Inoculations: The method used for inoculum production was as previously described by Garg *et al.* (2008). Inoculations of cotyledons were made 10 d after sowing. A single droplet of mycelial suspension of 10 µL was deposited using micropipette onto each lobe of each cotyledon. High humidity was maintained by hand misting the inoculated plants and plants covered with a polythene cover to retain moisture for 4 dpi. Lesion diameters were recorded using a linear ruler to the nearest millimetre for lesion size on each lobe of cotyledon. The mean of four cotyledon lesions on each seedling was taken as the measure of the host response from each plant. After having recorded disease scores for cotyledon infection, the same plants were allowed to grow for a further four weeks. Then, all leaves of each plant were inoculated using the same protocol and conditions as described above for cotyledons. Leaf lesions were assessed in the same way as for cotyledon lesions.

Stem Inoculations: Three separate blocks (one for each of the three populations) were grown in the field. For inoculation, the methods used were that used by Li *et al.* (2006, 2007), Uloth *et al.* (2013, 2015) and You *et al*. (2016) for field stem resistance studies in *Brassicaceae*. The data for stem lesion size was recorded using a linear ruler three weeks post inoculation.

RESULTS AND DISCUSSION

For cotyledon and leaf inoculations, there was significant segregation of resistance in all three populations. Positive and negative heterosis for disease resistance was observed within these populations. Resistance responses of plants for cotyledon and leaf were different. Similarly, as to others looking at cotyledon stage expression of resistances, some genotypes were found to have resistance against the pathotype chosen for the current study, but it remains to be seen in relation to their resistance against one or more other different prevailing pathotypes, such as has been used in some other studies (e.g., Garg *et al.* 2010).

For field stem inoculations, there were significant genotype effects for disease resistances on stems. Again, there was positive and negative heterosis for disease resistance observed within these populations. Here, many genotypes were found to have varying levels of resistance against *S. sclerotiorum*, and findings support those of You *et al*. (2016) who demonstrated existence of new and more valuable sources of stem resistance.

The current studies highlight a range of differences in disease response from resistant to susceptible types, confirming that host resistance can be utilized to develop selectable markers (phenotypic and genotypic) in breeding for resistance, particularly against stem infections from *S. sclerotiorum*. Studies are ongoing to understand the inheritance of such resistances within these breeding populations. In particular, studies on leaf and stem resistance are now in progress for a second season. However, You *et al.* (2016) found no correlation between leaf and stem resistances in the field suggesting that genetic control of these resistances is independent of each other.

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Mobilizing alternate sources of genetic diversity for germplasm enhancement in Brassica oilseeds

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Oilseed Brassicas are important as these contribute over 12 % to the total edible oil supplies of the world. Their production needs to continuously increase to meet the growing demand of edible oils and biofuel industry. This is in spite of constantly deteriorating production conditions; especially in southern hemisphere. Consequences of climate change are already visible in form of increased productivity losses due to various environment constraints. Genetic variation is critical for enabling adaption to the environment stresses. Crop breeding activities, in the past, have led to manifold increases in oilseed Brassica productivity in most producing nations of the world.

However, much of these increases have resulted from enhanced capitalization of genetic diversity available in crop gene pools. Various studies in both *B.napus* and *B.juncea* have shown narrowing genetic base and consequently slower response to selection. This necessitates access to novel genetic variation not easily available to commercial plant breeders due to biological constraints that limit genetic exchanges between distantly related species. Increasingly, more diverse germplasm across the species barriers is being screened for beneficial allelic variation.

However, it is difficult to use such material due to the time required to introgress desirable alleles and to remove undesirable characteristics from progeny due to linkage drag. Our group has been successful to increase genetic variation in brassica oilseeds by resynthesis of alloploids, alien introgressions and random mating. Genome re-sequencing and genotyping by sequencing have helped to characterize genetic variation and permitted the linkage and association mapping for diverse agronomic and defensive phenotypes in natural resynthesized and introgressed *B.juncea*.

Collaborations with Australian colleagues have been very helpful in enhancing our ability to screen introgression lines and resynthesized alloploids for novel phenotypes, especially disease resistance. There have been some successes in Canola as well. These include incorporation of pod shatter resistance, resynthesis of *Brassica napus* and recurrent selection to enhance resistance to abiotic stresses. Synthetic Brassica allohexaploid is another example of our collaboration. Both the countries have benefitted from exchange of germplasm under the collaborative projects.

Such germplasm resources have been used extensively in hybridization programmes. GSC 7, the most productive Canola cultivar and Canola mustard variety, RLC 3 have Australian germplasm in their pedigree.

Imputation to Whole-Genome Sequence Increases the Power of Genome Wide Association Studies for Blackleg Resistance in Canola

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ABSTRACT

Blackleg disease resistance is a target trait for global canola (*Brassica napus* L.) improvement programs to minimize yield loss. Genome-wide association studies (GWAS) can aid in understanding trait architecture and in identifying genomic regions potentially affecting the trait. In this study GWAS were performed on 585 diverse winter and spring canola lines (Fikere et al., 2018 these proceedings) using imputed whole-genome sequence (WGS) and lower density skim genotype-by-sequencing transcriptomics (GBS).

Two blackleg field disease nurseries were carried out in 2015 on a core diversity set of 600 spring and winter lines. The trials had a complete randomised block, with single row and two replicate design, and were located near Green Lake and Wickliffe, Victoria. The parameters collected from the disease nurseries were: emergence counts, adult plant survival and internal infection levels. Field trial data were spatially adjusted using autocorrelation models to account field variability, and broad-sense heritability was estimated.

All 600 lines were genotyped with an in-house skim GBS protocol that resulted in 64,072 single nucleotide polymorphisms (SNP) and a reduction to 585 lines after stringent quality control. WGS was carried out to 10x fold coverage on a subset of 153 lines that captured the genetic diversity of the GBS genotyped set. Eagle2.3/Minimac3 software were used to impute WGS from GBS density and 1,234,708 million higher quality imputed SNP were retained for downstream analysis. GWAS were completed on GBS and WGS data using the software Effective Mixed Model Association eXpedited (EMMAX). Seasonality was fitted as a fixed effect and a genomic relationship matrix calculated from the SNP was used to control for population structure. The WGS GWAS resulted in a substantially larger number of significant SNP (P-value $1x10^{-4}$) with comparable false discovery rates. Additional regions were highlighted by using imputed WGS data.

A meta-analysis was completed which combined signed t-values from the single-trait GWAS in survival and internal infection at both sites. Combining our results in this way highlights regions that have pleiotropic effects on both blackleg resistance traits and it resulted in much lower false discovery rates indicating that it has more power. The meta-analysis GWAS identified 37 significant ($P<1x10^{-5}$) regions for blackleg traits (survival rate and internal infection) spread across both subgenomes. The most significant peaks were observed on A02 followed by A05 and C03.

All significant SNP were annotated and *B. napus* genes within 30kb up and downstream of significant SNP were mapped against the *Arabidopsis thaliana* resource (TAIR). A number of gene orthologs in *A. thaliana* were involved in disease resistance. This study has highlighted that many regions are involved in Blackleg disease resistance and it has provided insight into potential molecular mechanisms underlying canola *L. maculans* resistance.

Key words

Genome-wide association – whole-genome sequence – canola – blackleg – genotyping-by-sequencing

INTRODUCTION

Blackleg disease resistance is a target trait for global canola (*Brassica napus* L.) improvement programs to minimize yield loss. The fungus *L. maculans* causes blackleg disease (Wouw *et al.* 2016). Genome-wide association studies (GWAS) can aid in understanding trait architecture and in identifying genomic regions potentially affecting the trait. It has been shown in other species that the use of whole-genome sequence can increase the power of GWAS. However, sequencing full genomes of large populations is still cost prohibitive. An alternative is imputation, which refers to the prediction of missing genotypes in lines genotyped at lower SNP density using a set of whole-genome sequenced lines (Marchini & Howie 2010). Imputation is not perfect, and its accuracy will depend on many factors, including the number of the sequenced lines, allele frequency of missing genotypes, and reference assembly quality.

In this study, GWAS were performed on 585 diverse winter and spring canola lines (Fikere *et al.* 2018) using imputed whole-genome sequence (WGS) and lower density skim genotype-by-sequencing transcriptomics (GBS).

MATERIALS AND METHODS

Two blackleg field disease nurseries were carried out in 2015 on a core diversity set of 600 spring and winter lines. The trials had a complete randomised block, with single row and two replicate design, and were located near Green Lake and Wickliffe, Victoria. The parameters collected from the disease nurseries were: emergence counts, adult plant survival and internal infection levels. Field trial data were spatially adjusted using autocorrelation models to account field variability, and broad-sense heritability was estimated.

All 600 lines were genotyped with an in-house skim GBS protocol that resulted in 64,072 single nucleotide polymorphisms (SNP) and a reduction to 585 lines after stringent quality control. WGS was carried out to 10x fold coverage on a subset of 153 lines that captured the genetic diversity of the GBS genotyped set. Eagle2.3/Minimac3 software were used to impute WGS from GBS density and 1,234,708 million higher quality imputed SNP were retained for downstream analysis. GWAS were completed on GBS and WGS data using the software Effective Mixed Model Association eXpedited (EMMAX). Seasonality was fitted as a fixed effect and a genomic relationship matrix calculated from the SNP was used to control for population structure. The WGS GWAS resulted in a substantially larger number of significant SNP (P-value $1x10^{-4}$) with comparable false discovery rates. Additional regions were highlighted by using imputed WGS data.

RESULTS

Population structure was investigated with a heat plot of the genomic relationship matrix, which calculated the relatedness of the lines based on the SNP. Three populations are apparent in Figure 1, the largest is the winter group, followed by a spring group and a smaller group annotated to be spring lines with winter lines in their pedigree. Single-trait GWAS results on GBS and WGS data are shown in Table 1 and as a Manhattan plot in Figure 2. The WGS analysis discovered more significant SNP than the GBS analysis and a lower false discovery rate was observed for WGS.

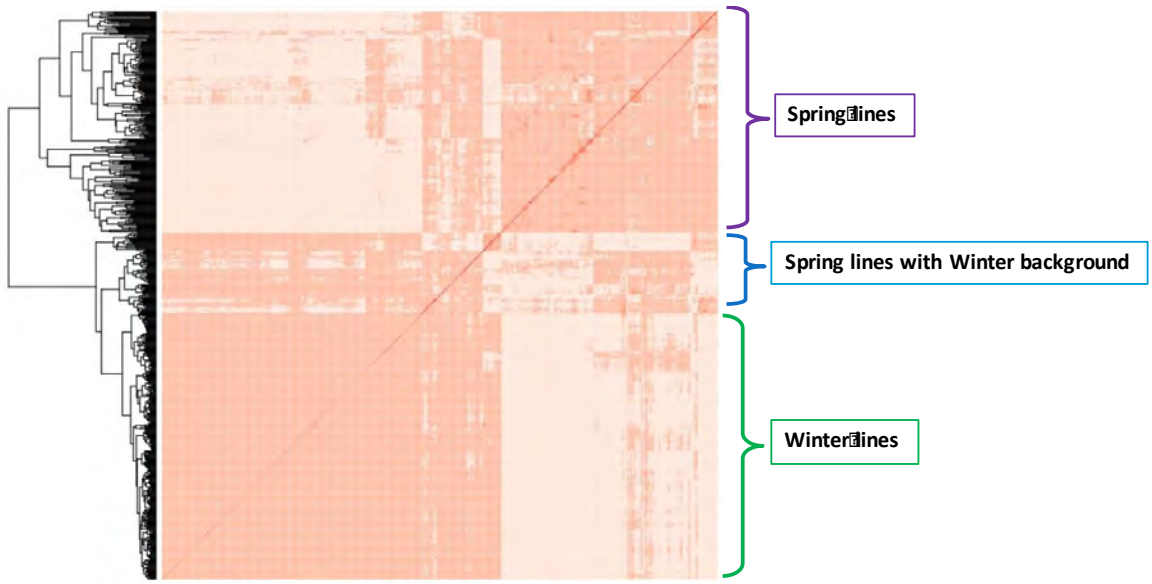


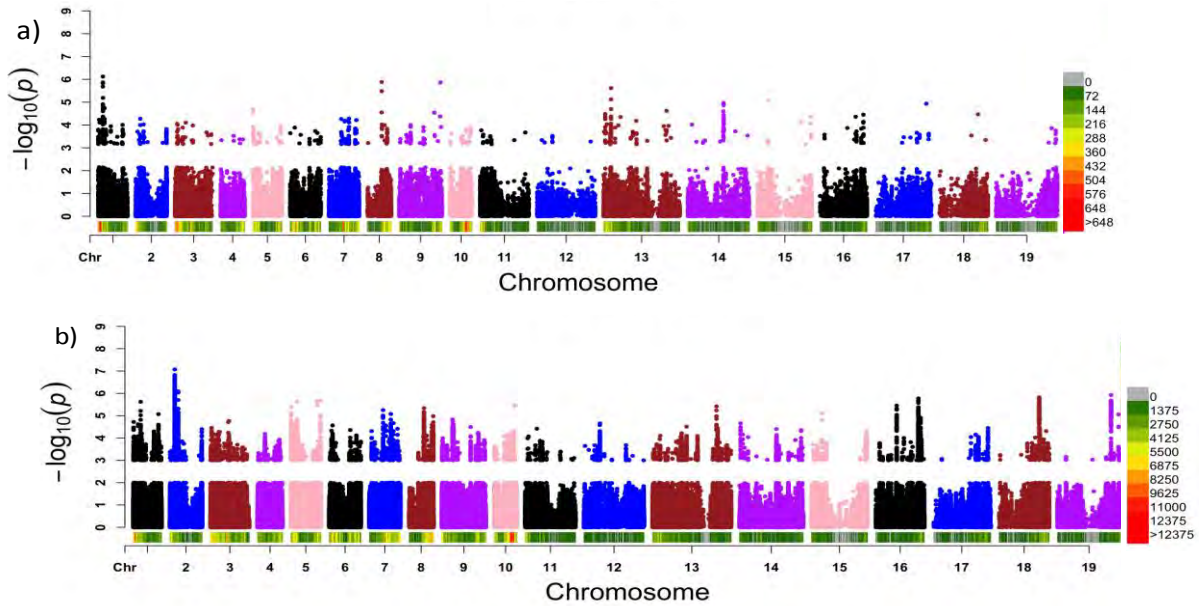
Figure 1. Heat map of the genomic relationship matrix for 585 diverse canola lines using the imputed 1,234,708 SNP markers. Darker colour indicates greater relatedness.

A meta-analysis was completed which combined signed t-values from the single-trait GWAS in survival and internal infection at both sites in 2015. Combining our results in this way highlights regions that have pleiotropic effects on both blackleg resistance traits and it resulted in much lower false discovery rates indicating that it has more power. The meta-analysis GWAS identified 37 significant ($P<1x10^{-5}$) regions for blackleg traits (survival rate and internal infection) spread across both subgenomes. The most significant peaks were observed on A02 followed by A05, C03 and C06.

All significant SNP were annotated and *B. napus* genes within 30kb up and downstream of significant SNP were mapped against the *Arabidopsis thaliana* resource (TAIR). A number of gene orthologs in *A. thaliana* were involved in disease resistance.

Table 1. Comparison of GBS versus WGS GWAS for emergence (EME), average internal infection (AvInf), and survival (Surv) at Wickliffe (WL) and Green Lake (GL) sites in 2015, where FDR is the false discovery rate .

Single trait GWAS GBS				**Single trait GWAS WGS**		
No. of sig. SNPs and FDR at 4 p-values				No. of sig. SNPs and FDR at 4 p-values		
Traits	$p<10^{-4}$	$p<10^{-5}$	$p<10^{-6}$	$p<10^{-4}$	$p<10^{-5}$	$p<10^{-6}$
EMEWL	31	11	0	1396	269	4
FDR(%)	20.7	5.8	-	8.8	4.6	40.2
SurvWL	27	14	1	1197	101	3
FDR(%)	23.7	4.6	6.4	10.3	12.2	41.2
AvInfWL	32	18	1	1106	287	3
FDR(%)	20.1	3.6	6.4	11.2	4.3	41.2
EMEGL	38	4	0	954	220	1
FDR(%)	16.9	16.1	-	12.9	5.6	123.5
SurvGL	36	3	0	972	214	14
FDR(%)	17.8	21.4	-	12.7	5.8	8.8
AvInfGL	27	8	0	816	68	1
FDR(%)	23.7	8.1	-	15.1	18.2	123.5



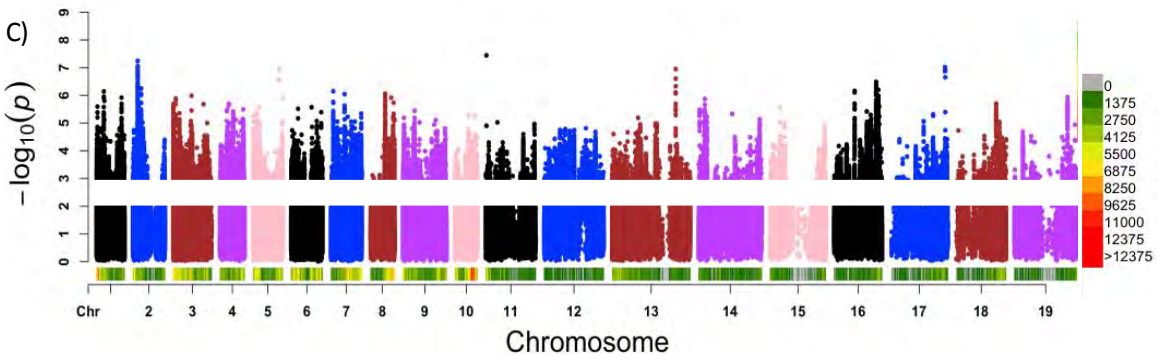


Figure 5. Increased power of WGS and meta-analysis of GWAS for internal infection as demonstrated by Manhattan plots for (a) transcriptomic genotyping-by-sequence (GBS) at Wickliffe and (b) imputed whole-genome sequence (WGS) at Wickliffe and (c) multi-trait meta-analysis of GWAS for internal infection and survival at the four 2015 blackleg trials. The colour bar shows SNP density per 1Mbp.

DISCUSSION

The GWAS results were improved by using imputed WGS rather than GBS. This was despite the imputation accuracy being approximately 0.75 across all WGS SNP on average. Nevertheless, the additional SNP from WGS would have increased the linkage disequilibrium between SNP and causative mutations, thereby leading to more powerful analyses. Imputation could be improved in several ways. First, a larger set of lines could be sequenced to ensure that the important haplotypes are observed at higher frequency. Second, the reference assembly could be improved, which is likely a major constraint to mapping sequence reads accurately to the genome. This is of course not a trivial task due to the allotetraploid nature of canola where significant homology exists between subgenomes (Chalhoub *et al.* 2014). Imputation could potentially also be improved by creating and exploiting family structures where longer haplotypes could be traced from ancestors to descendants. In this study, population-based algorithms were used as the lines were diverse.

The GWAS results confirmed most major resistance loci (Rlm genes) discovered to date. Except Rlm5, Rlm6 in *B. juncea* and Rlm8, Rlm11 in *B. rapa*, which do not have physical mapping information available (Larkan *et al.* 2016; Wouw *et al.* 2016). The significant regions in this study also overlapped with several quantitative trait loci observed previously. In addition, this study found a large number of new regions that harbor putative loci involved in resistance to blackleg disease. Thirteen of these regions were close to genes for which orthologs could be determined in *A. thaliana* with annotated functions related to disease (stress response, resistance, etc). This provides further information that underlines the validity of the newly discovered regions.

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Disseminators of viable *Leptosphaeria maculans* ascospores and wider implications for ascomycete pathogens

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ABSTRACT

A range of materials, including metals, fabrics, woods and plastics, were all effective carriers that maintain the long term viability of ascospores of the canola blackleg pathogen, *Leptosphaeria maculans* under three different temperature conditions. Selected sterile materials were inoculated and the ability of the test carrier materials to retain or release ascospores was assessed daily from day 1 until day 7, then weekly till day 30 (month 1), after which sampling was undertaken at regular 30 d intervals concluding at day 240 (month 8). At the lowest temperature regime day/night, ascospores remained viable for up to 240 days on wood while at highest temperature ascospores remained viable up to 60 days on jute. About 30% of intact ascospores recovered from inert carrier materials were able to germinate on artificial growth media within 48 hours of recovery. These findings confirm that *L. maculans* ascospores remain viable for a much longer time in the absence of a host than previously considered. Also demonstrates the importance of inert materials as long-term and long distance carriers of viable *L. maculans* ascospores, and highlights their potential role for spread of *L. maculans* races to new regions and countries via farming equipment, clothing and other associated materials.

Key words

Blackleg, *Leptosphaeria maculans*, ascospore viability, farm biosecurity

INTRODUCTION

Leptosphaeria maculans (blackleg stem canker, blackleg) is one of the most important disease of oilseed rape/canola (*Brassica napus*) worldwide (West et al. 2001; Fitt et al. 2006), including Australia (Barbetti and Khangura 1999; Li et al. 2006).

The primary source of *L. maculans* inoculum is wind-dispersed sexually produced ascospores released from pseudothecia on infested canola stubble (Li et al. 2006). Throughout the growing season ascospores are discharged from mature pseudothecia with each rainfall and infect canola crops (Savage et al. 2012). In the Mediterranean-type environment of south west and southern Australia, release of *L. maculans* ascospores can be diurnal (Savage et al. 2012).

Pseudothecia can remain viable for up to four years where dry summers predominate such as in Western Australia (Khangura and Barbetti, 1999). In mild/wet conditions as those in the UK it can approximately two years (West et al. 2001). Infested residues are the primary source of inoculum for spread of the disease, from season to season and between different areas (West et al. 2001; Huang et al. 2003). *L. maculans* can also be seedborne and both infected pods and seeds by *L. maculans* can play an important role in spreading the pathogen into fields of susceptible hosts (Wood and Barbetti, 1977). Temperature also plays an important role in development and establishment of stem cankers and consequent disease severity (Barbetti 1975).

Recent studies demonstrate that fungal spores can remain viable for even up to 12 months on inert materials depending upon the type of spores and the carrier material (Barua et al. 2018). Even though the spread of the pathogen is mainly through infected seed and/or infested crop debris (West et al. 2001), alternative means of entry and spread of *L. maculans* via attachment to different carrier materials used in daily life, farms and/or associated in the cargo and transport industry, or commonly used by travellers, also may play an important role.

In our studies we looked at the long-term viability of *L. maculans* ascospores on a range of different inert carrier materials and under three different temperature regimes to understand the potential risks from and role of long time viable *L. maculans* ascospores in blackleg stem canker spread by with movement of humans, farming equipment, clothing and commodities.

MATERIALS AND METHODS

***L. maculans* inoculum:** *L. maculans* ascospores were produced by collecting infested stubbles carrying pseudothecia from infested fields in early winter and stored dry at 25ºC. Ascospores were produced following the protocol of Li et al. (2004).

Selection of test materials: A range of carrier materials *Metals* - aluminium, brass, corrugated iron sheet, galvanised steel, painted steel, zinc; *Fabrics* - denim, fleece, silk, fibre polyester; *Woods- Pinus radiata* (pine), *Eucalyptus regnans* (Tasmanian oak); and *Miscellaneous* - glass, jute and plastic were selected.

Inoculation of carrier materials: The sterile test materials were organised in the sterile test culture plates and inoculated following the material and methods from Barua et al. (2017) and were placed under one of the three controlled environmental conditions: 23 ± 1ºC day/8 ± 1ºC night, 36 ± 1ºC day/14 ± 1ºC night, or 45 ± 1ºC day/15 ± 1ºC night, with a photoperiod of 14 h and 10 h for day/night, with light source LED cool white and incandescent light bulbs). There were six replicates for each carrier material, for every sampling time and each temperature treatment arranged in a fully randomized design to measure the effect of temperature and time on ascospore viability. The experiment was run over nine months with sampling to assess ascospore viability starting from day 1 until day 7, then weekly until day 30 (month 1), after which sampling was undertaken at regular intervals 30 day concluding at day 270 (month 9) until no viability was observed.

Assessment of ascospore viability: The ascospores were recovered from the carrier materials and the ascospore suspension was used to determine numbers of ascospores recovered, while the ascospore viability was assessed using a Alamar Blue (resazurin dye; 7-hydroxy-3H-phenoxazin-3-one 10-oxide) with an optimised incubation time of 2 h at 37ºC for testing the viability of *L. maculans* ascospores (Barua et al. 2017).

Germination of ascospores on test materials and on ½ PDA: Fresh ascospores (controls) were germinated on half-strength potato dextrose agar (½PDA) to observe the percentage of viability was assessed by using a compound microscope. In comparison with controls, the number of germinated ascospores in the recovered ascospore suspension from test material groups was also calculated until no further germinated spores were recovered from the inoculated materials. Non-germinated intact or broken ascospores recovered from materials were plated on ½ PDA to access their viability in terms of ability to germinate. The counting was conducted at regular time intervals until maximum germination rate of the control spores was observed on the ½ PDA.

RESULTS

Assessment of ascospore viability: The mean percentage of viable ascospores of recovered from all the carrier materials varied significantly with the time, temperature and the type of the carrier materials. At the coldest temperature, viable of ascospores were recovered for up to 8 months from jute, plastic, Tasmanian oak and pine; up to 7 months from aluminium, painted steel, glass, denim, silk and fibre polyester; up to 6 months from galvanised steel and zinc, 4 months from corrugated iron, and up to 3 months on brass and fleece. At the highest temperature, ascospores on jute remained viable for 4 months followed by denim and silk (1 month), fibre polyester (21 days), on glass, plastic, Tasmanian oak and pine (7 days) and 6 days on fleece, painted steel and zinc, 5 days on corrugated, 4 days on aluminium and 3 days on brass and galvanised sheet.

The different groups of materials varied in their capacity to retain viable spores and this also varied according to the temperature at which the materials were maintained. The percentage of viable spores recovered from materials from a particular group varied with the temperature. There was a significant (P<0.001) correlation between time, temperature and viability of spores over time.

Germination of ascospores on test materials and on ½ PDA: Mean maximum germination of the control ascospores (90%) were obtained within time period of 24 to 48 hrs on the ½ PDA. No change in the germination was observed after that time point. Some ascospores germinated directly on surface of test material groups. At least 1% ascospores germinated on metals, 5% on wood, 5% on fabric and 2% on miscellaneous materials through 48 hrs. Maximum germination rate of the non-germinated intact or broken ascospores suspension recovered after washing different material groups on the ½ PDA was at least up to 30% from metals, 50% from woods, 51% from fabrics and at least 41% from the miscellaneous materials within 48hrs.

DISCUSSION

Our findings confirm that *L. maculans* ascospores can remain viable for a much longer time in the absence of a host than previously considered, in some instances for up to 9 months. Infected debris of oilseed rape debris been the major mode of *L. maculans* pathogen survival, and source of inoculum for successive spread, between the seasons from one region to another (West et al. 2001, Huang et al. 2003) and wind borne ascospores of *L. maculans* produced on infected debris are the main source of inoculum for initiating infection of new oilseed rape crops (West et al. 2001). Huang et al. (2003) also showed that ascospores can survive longer than 1 month when exposed to dry air at 20°C, well short of the extended survival timeframe of the current study.

The results from the three controlled time and temperature regimes treatment experiments confirm the extended viability of *L. maculans* ascospores on various materials in absence of a host plant tissue after being discharged or released from pseudothecia. The differences observed between the temperature regimes in terms of survival of the ascospores and type of carrier materials on which the prolonged survival was important. For example, at 23°C/4°C day/night, ascospores remained viable on three test materials (jute, pine wood and Tasmanian oak) up to 8 months. However, at 45ºC/15ºC day/night, ascospores remained viable up to 4 months only on jute. As a minimum 1% ascospores were able to germinate on the inert materials and at least 30% of intact ascospores recovered from these materials were able to germinate on ½ PDA within 48 hrs highlights the viability of at least some surviving ascospores.

There were also significant differences between carrier materials in their abilities to retain ascospores. Some ascospores could be removed from the carrier materials even after washing the materials. This highlights the challenges in removing fungal spore contamination from farm equipment, clothes of farmers, travellers, wood and crafts etc.

Our results demonstrate the importance of inert materials as long-term and long-distance carriers of viable *L. maculans* ascospores and highlights their potential role for spread of *L. maculans* races to new regions and countries via farming equipment, clothing and other associated materials. The longer survival time period of ascospores suggests that there is a considerable risk of spreading of *L. maculans* into new areas through ascospores attached with these inert materials. These studies highlight the broader biosecurity implications in relation to the overall transport and movement of fungal spores through carrier materials.

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Examining Variety by Environment Interaction (VEI) in Canola Blackleg Expression Experiments

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ABSTRACT

Blackleg Expression Experiments (BEEs) assess canola varieties for resistance to blackleg disease. They are multi-environment trials (METs) grown over a number of sites and years in order to target a diverse range of canola growing environments. Variety by environment interaction (VEI), a result of not all varieties responding the same way to a change in environment, complicates the recommendation of varieties for commercial growing.

The trait of interest in BEEs is "blackleg expression" which is a measure of plant survival. The current analysis of BEE data involves an extension to the mixed model approach of Smith et al. (2001) which uses a Factor Analytic model to capture VEI. The modelling approach provides information on blackleg expression for the individual environmental conditions represented in the MET. At present, this information is averaged across environments for each variety and converted to a "resistance rating" for reporting in the Blackleg Management Guide. Reporting information in this way may mask important VEI. Furthermore, a key aim of the BEEs is to produce information to enable growers to choose varieties with "adequate blackleg resistance for [their] region" (Autumn Blackleg Management Guide, 2018). Here we explore the extent and nature of VEI within the 2011-2017 BEE data set.

Key words

Blackleg expression - Factor Analytic model - Multi-environment trials

INTRODUCTION

Blackleg Expression Experiments (BEEs) have been conducted annually in disease nurseries across Australia since 2007 in order to assess canola varieties for resistance to blackleg disease. They are multi-environment trials (METs), meaning that trials are grown at several locations over a number of years. An environment is defined to be the combination of the trial year and location. METs evaluate variety resistance across varying geographic locations and seasons, given that not all varieties respond the same way to a change in environment, an occurrence known as variety by environment interaction (VEI). In terms of making broad recommendations, varieties that have high overall mean resistance as well as consistent resistance across environments are desirable. It may also be of interest to make recommendations of varieties that are specifically adapted to certain environments.

There are two key types of VEI; non-crossover and crossover interaction. Non-crossover VEI occurs when the rankings of varieties in terms of resistance stays the same between environments; only the magnitude of difference between varieties changes across environments. For crossover interaction, the ranking of varieties changes between environments. This type of VEI is particularly important since it complicates making broad variety recommendations across environments.

In this paper we look at data from BEEs restricted to the 7 year period of 2011-2017. The dataset consists of 89 trials conducted in 78 environments; there are several environments in which multiple trials were grown. The number of varieties grown in each environment ranges from 23 to 138, with a total of 415 varieties grown across all environments.

The trait analysed, blackleg expression, is a measure of plant survival which is calculated for each experimental plot as the ratio of plants at a final plant count (taken at plant maturity) to an initial plant count (taken at plant emergence). It is common practice to treat canola seeds with a fungicide against blackleg called Jockey Stayer. Within a trial, varieties may be grown as either treated (+J), as bare seed (-J) or as both treated and untreated. Therefore, the treatments for the experiment consist of the factorial combination of fungicide treatments with canola varieties. The combination of fungicide treatments and canola varieties present across environments is sparse, which impacts the model used in the analysis. In 23 of the 78 environments in the dataset, varieties are tested using bare seed only.

The combined data across sites and years are analysed using an extension of the mixed model approach of Smith et al (2001) which employs a Factor Analytic model to capture VEI. The extension accommodates for the fact that a portion of varieties are treated with Jockey Stayer (Smith et al, 2018) and is a major development in the analysis. In years prior to 2016 a two-way model was used to model variety by environment (V×E) effects only, whereas the current analysis allows for the investigation of variety by treatment by environment (V×T×E) effects. Smith et al. (2018) showed that the extension to a three-way model for V×T×E effects resulted in substantial gains in accuracy of predictions, and thence information for growers. The output from this model includes a set of predicted effects for every variety within each environment in the MET data set for both the Jockey (+J) and the bare seed (-J) treatment. For the BEE dataset, there were 64740 predicted effects (415 varieties × 78 environments × 2 treatments).

A method of summarising this amount of information in a way that is informative and manageable for growers to utilise is then required. At present, the Blackleg Management Guide reports a "resistance rating", for varieties both with and without the Jockey fungicide application, for a subset of varieties. This rating is calculated as the average effect for that variety across environments and converted to a 1-9 scale which is defined in Table 1.

Table 1: Resistance rating classification for the 2018 Blackleg Management Guide.

Rating	Resistance classification
>8	Resistant (R)
7.5-7.9	Resistant to moderately resistant (R-MR)
6.5-7.49	Moderately resistant (MR)
6-6.49 5-5.9 4-4.9 3-3.9	Moderately resistant to moderately susceptible (MR-MS) Moderately susceptible (MS) Moderately susceptible to susceptible (MS-S) Susceptible (S)
2-2.9	Susceptible to very susceptible (S-VS)
0-1.9	Very susceptible (VS)

Averaging across environments may mask important VEI. Varieties may be highly resistant in some environments but perform ordinarily in others, for instance, and a simple mean is unable to distinguish between those varieties that are highly adapted to some regions/seasons and those varieties with a consistently average performance. Such distinctions are important, not only in making environment specific recommendations, but also in making broad recommendations of varieties that are stable across environments.

Here we examine the nature and extent of VEI in the 2011-2017 BEE data set, utilising the multiple regression form of the FA model to form graphical representations of the change in variety response across environments.

MATERIALS AND METHODS

Statistical model

Suppose there are p environments in which m varieties are tested in combination with s treatments. For the 2011-2017 BEE data, p = 78, m = 415 and s = 2 (+J/-J). The linear mixed model for the combined vector y of blackleg expression data across all trials is given by

$$y = X\tau + Z_g u_g + Z_p u_p + e \quad (1)$$

where τ is the vector of fixed effects with associated design matrix X. The fixed effects comprises an overall mean, the main effect for treatments and environments, the interaction of environments with treatments as well as any additional effects included to accommodate spatial trend within trials. The vector u_g is the smp-vector of random genetic effects, ordered as environments within varieties within treatments, with associated design matrix Z_g. The vector u_p is comprised of the non-genetic (peripheral) random effects for each trial and has the associated design matrix Z_p. The peripheral effects include effects associated with plot structures of individual trials. The vector of residuals is given by e.

We assume that u_g, u_p and e are mutually independent and distributed as multivariate Gaussian, with zero means. The variance for u_p is G_p, which is a block diagonal matrix of scaled identity matrices corresponding to sets of effects within trials. The variance of the residuals at each trial was assumed to follow a separable autoregressive process of order one, as proposed by Gilmour et al. (1997) for field trials.

The variance structure for u_g needs to encompass genetic variances and covariances between treatments and also between environments. We consider the vector of genetic effects to be separated into subvectors of genetic effects for each treatment such that $u^g = [\boldsymbol{u}_{g-}^T \ \boldsymbol{u}_{g+}^T]^T$, where u_{g-} and u_{g+} are the mp-vectors of genetic effects for each environment for the bare seed and Jockey treatments, respectively.

The variance structure is then formulated by assuming a factor analytic (FA) model (Smith et al., 2001) for each of the subvectors of u_g but with some additional constraints. For each treatment, the variety by environment effects are modelled as a series of multiplicative terms, where each term is a product of a variety effect (score) and an environment effect (loading), plus a residual. The aim of an FA model in this context is to explain variance and covariances of the genetic effects between environments in terms of a small number of common factors, where the number of factors is called the model order. We write,

$$u_{g-} = (I_m \otimes \boldsymbol{\Lambda}) f_- + \delta_-$$

$$u_{g+} = (I_m \otimes \boldsymbol{\Lambda}) f_+ + \delta_+ \quad (2)$$

where $\boldsymbol{\Lambda}$ is a $p \times k$ matrix of loadings for environments and k is the number of factors in the FA model. We assume the same environmental loadings for each sub-vector of u_g. The matrix I_m is an identity matrix of dimensions $m \times m$. The subvectors of $f = [\boldsymbol{f}_-^T, \ \boldsymbol{f}_+^T]^T$ correspond to treatments and are the mk-vectors of variety scores of each factor. Similarly the subvectors of $\delta = [\boldsymbol{\delta}_-^T \ \boldsymbol{\delta}_+^T]^T$ are the mp-vectors of genetic residuals for each treatment. The random effects f and δ are assumed to be mutually independent and distributed as multivariate Gaussian with zero means. We assume that,

$$\text{var}(f) = G_s \otimes I_m \otimes I_k$$

$$\text{var}(\delta) = G_s \otimes I_m \otimes \boldsymbol{\Psi} \quad (3)$$

where G_s is the 2 × 2 component matrix for treatments and **Ψ** is the $p \times p$ diagonal matrix of specific variances for each environment. Therefore,

$$\text{var}(u_g) = G_s \otimes I_m \otimes (\mathbf{\Lambda\Lambda}^T + \mathbf{\Psi}) \quad (4)$$

This so-called separable variance model was adopted as the data being modelled is unbalanced in terms of the factorial combinations of treatments with varieties. For our case, we assume an unstructured form for G_s such that there is a separate genetic variance for each treatment and a covariance between treatments. For the full details concerning the derivation and assumptions of this model see Smith et al. (2018).

Examining variety by environment interaction (VEI)

The expanded form of the model in equation (2) for variety *j* tested with treatment *i* is given as

$$ugij = f1ij\lambda1 + f2ij\lambda2 + \cdots + fkij\lambda k + \delta ij \quad (5)$$

This representation demonstrates how the FA model for either treatment can be written as a series of *m* multiple regressions on *k* hypothetical environmental factors. The loadings from this model are rotated to a principal component solution for interpretative purposes so that the rotated loadings for the first factor account for the maximum amount of genetic variance, the second factor accounts for the next largest amount of genetic variance and is independent of the first and so on (Cullis et al. 2010). We focus on the part of the predicted effects which ignore lack of fit, as the lack of fit represents the unexplained variation and is specific to a single environment. These are called the common variety by environment (CVE) effects and are given by,

$$CVEij = f1ij\lambda1 + f2ij\lambda2 + \cdots + fkij\lambda k \quad (6)$$

We can write the CVE effects for variety *i* and treatment *j* as

$$CVE_{ij} = f_{1ij}\boldsymbol{\lambda}_1 + \boldsymbol{\epsilon}_{1ij} \quad (7)$$

where $\epsilon_{1ij} = f_{2ij}\boldsymbol{\lambda}_2 + \cdots + f_{kij}\boldsymbol{\lambda}_k$. The model is written in this way as the first loading accounts for the maximum amount of genetic variance and so regressions on this factor have the greatest impact on the predicted genetic values. The FA model for variety *j* under treatment *i* can then be represented graphically by plotting the CVE effects against the environmental loadings for the first factor. The variety score for the first factor f_{1ij} is the slope of the regression line and $_{1ij}$ are the deviations from the regression. There is a separate regression for each variety by treatment combination. These so-called latent regression plots provide a visualisation of VEI, that is, the differential response of varieties to environments (Cullis et al., 2014).

Latent regression plots can also be formed for the remaining factors in the model by adjusting the *y*and *x*- axes for preceding factors. The regression equation for variety *j* under treatment *i* for the second factor is given by,

$$CVE_{ij} - f_{1ij}\boldsymbol{\lambda}_1 = f_{2ij}\boldsymbol{\lambda}_2 + \boldsymbol{\epsilon}_{2ij} \quad (8)$$

where $_{2ij} = f_{3ij}\lambda_3 + \cdots + f_{kij}\lambda_k$. The regression equation for the remaining factors continues in this manner up until the *k*th factor for which the regression equation is given by,

$$CVEij - \text{X}frij\lambda r = fkij\lambda k \quad (9)$$

$k-1$

$r=1$

RESULTS

Full details concerning the model fitting process for the 2011-2017 BEE dataset are described in Smith et al. (2018), including spatial modelling and the detection of outliers. A series of FA models of increasing order were fitted to the genetic effects within environments until the percentage of

genetic variance accounted for (%vaf) by all factors in the model was adequate. The final model fit was an FA model of order 4 (FA4) for which the combined %vaf by all model factors was high for both treatments (92.8% for the -J treatment and 89.4% for the +J treatment). The percentage of variance accounted for by the individual model factors is presented in Table 2.

Table 2: The percentage of genetic variance accounted for (%vaf) by individual factors in the FA4 model fit to the 2011-2017 BEE data for each of the Jockey and bareseed treatments. The overall %vaf by the combined factors of the model is also given.

Treatment	First factor	Second factor	Third factor	Fourth factor	Total
Bareseed (-J)	67.3	12.0	8.1	5.4	92.8
Jockey (+J)	64.8	11.6	7.8	5.2	89.4

In terms of genetic correlations between environments, we consider here the -J treatment since all environments used this treatment. Due to the separability assumption of the model, the genetic correlations between environments for the +J treatments (for those environments that used Jockey) are very similar to those for the -J treatment. The REML estimates of genetic correlations between environments for the -J treatment ranged from -0.26 to 0.96 with a mean of 0.60. For a pair of environments, a correlation of 1 corresponds to an identical rankings of varieties whereas a correlation of -1 corresponds to a reversal in ranking of varieties. Therefore, for the BEE dataset, there was a non-ignorable amount of crossover VEI.

In order to investigate this VEI, latent regression plots for varieties (as described in the methodology section) were produced, shown in Figure 1. For both treatments, a vast percentage of the genetic variance is accounted for by the first factor of the model (67.3% for the -J treatment and 64.8% for the +J treatment;

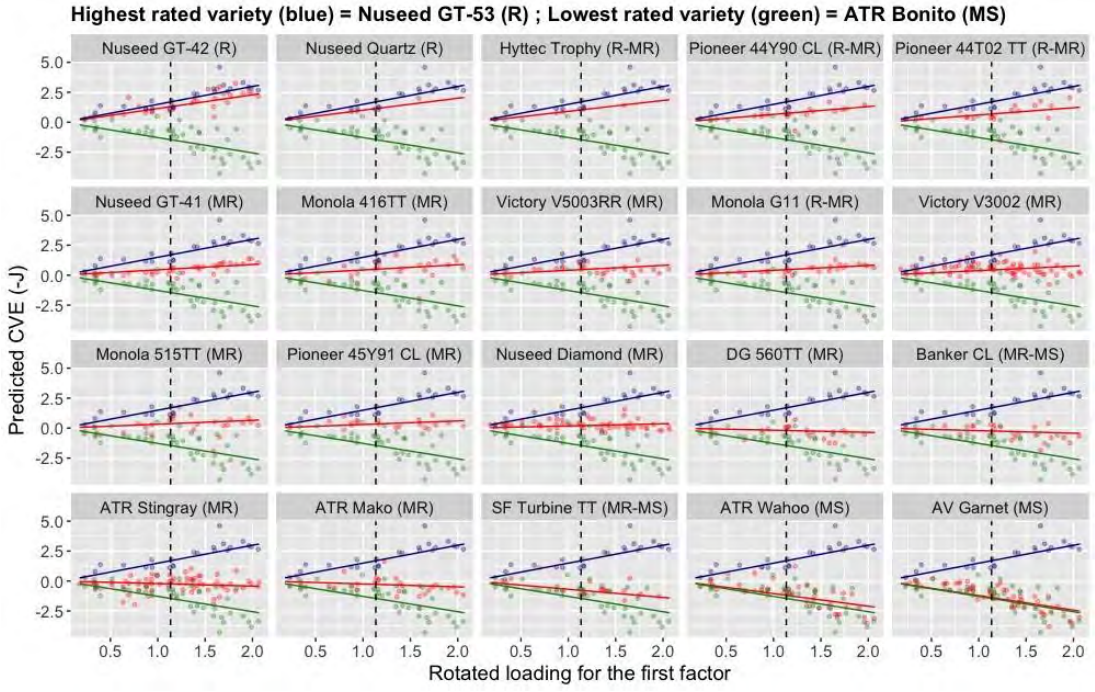

Figure 1: Latent regression plots for the first factor for a subset of varieties listed in the 2018 Blackleg

Management Guide for the bareseed (-J) treatment. The regression in blue corresponds to Nuseed GT-53 (the highest rated variety), the regression in green corresponds to ATR Bonito (the lowest rated variety) and the regression in red corresponds to the variety labelled at the top of each plot.

The resistance rating, as published in the management guide, is given after the variety name in parenthesis. The dashed vertical line is at the mean of the rotated environmental loadings for the first factor.

see Table 2). Therefore, regressions on the first factor have a substantial impact on variety predictions. For brevity, the plots shown in Figure 1 are the regressions for a subset of varieties which are listed in the 2018 Blackleg Management Guide for the bareseed treatment only.

There is a separate regression for each variety and a large positive slope for this factor for a variety is associated with high performance in terms of blackleg resistance. The regressions for the highest performing variety (Nuseed GT-53) as well as the lowest performing variety (ATR Bonito) are plotted together with each variety of interest in Figure 1 for comparison.

Given that the regression model in equation (7) has no intercept term (so that all regression lines for varieties pass through the origin) and the loadings for the first factor were all greater than zero, the regression lines for varieties do not intersect. This means that the ranking of varieties don't change across environments and the effects associated with the first factor of the model represent non-crossover interaction.

The spread of points around the regression line indicate the stability of a variety in terms of its responsiveness to the remaining factors in the model. A small spread are associated with a stable variety whereas a large spread is associated with an unstable variety whose ranking changes across environments. For instance, in Figure 1, Pioneer 44Y90 (CL) has a positive slope and so an above average resistance among the cohort of varieties as well as a tight spread of points around the regression line and so the variety is also fairly stable. Victory V3002, on the other hand, has a slope close to zero and so an average resistance for the first factor but a large spread of points around the regression and so the variety is not very stable.

The response of varieties to subsequent factors of the model can be investigated individually using latent regression plots. These plots for the second model factor for a selection of varieties are given in Figure 2. The regression for each variety is presented together on the same figure for direct comparison of their response across environments. There were a mixture of positive and negative environmental loadings for the second model factor such that the regression lines for varieties intersect. Therefore, effects associated with the second factor represent crossover interaction.

A large positive slope can be interpreted as the variety experiencing an increase in resistance in response to the environmental covariate associated with the second factor, while a large negative slope corresponds to a decrease in resistance. Latent regression plots can also be produced for the remaining factors in the FA model, but given the third and fourth factor account for less than 10% of the genetic variation for both the -J and +J treatments (see Table 2), these are not included here.

It was observed already from Figure 1 that Pioneer 44Y90 (CL) was a stable variety and this is confirmed in Figure 2 where the variety has a slope close to zero and so minimal response to the second factor. Victory V3002 was observed to be an unstable variety, and this is shown in Figure 2, where the variety has a large negative slope and so a decreased resistance in response to the second model factor. In Figure 1, Monola 416TT has a near zero slope and a large spread of points around the regression line and so the variety has an average performance and is fairly unstable. However, in Figure 2, Monola 416TT has a positive slope and so an increased resistance response to the second model factor. Therefore, although Monola 416TT has a fairly ordinary performance on average, its performance varies across environments and its resistance actually increases in response to the environmental covariate associated with the second factor. Therefore, the variety may be appropriate for recommendation to specific environments, particularly those affected by the covariate associated with the second model factor.

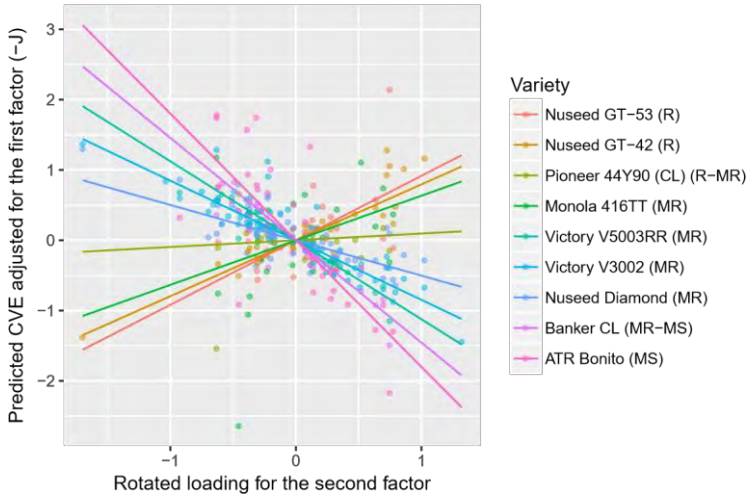

Figure 2: Latent regression plots for the second factor for a subset of varieties listed in the 2018 Blackleg Management Guide for the bareseed (-J) treatment. The resistance rating, as published in the management guide, is given after the variety name in parenthesis.

DISCUSSION

Appropriate modelling and reporting to growers of variety by environment interaction in Canola BEEs has the potential to improve industry productivity. BEEs are conducted at multiple sites over several years in order to capture blackleg resistance of canola varieties in a range of environmental conditions. The combined data across environments are then analysed together which allows for the provision of information to assist growers in choosing the best varieties for their conditions. The output from this analysis results in accurate predictions for every variety in every environment for both the Jockey and bareseed treatments. However, this is a large and impractical amount of information to present to growers and so a method of summarising this information is required. Current resistance ratings for varieties are calculated using the average effect for that variety across environments, which doesn't account for VEI.

In this paper we explored the VEI present in data from the 2011-2017 BEEs, using latent regression plots to provide a visual representation of the differential response of varieties across environments to key hypothetical environmental factors (or covariates) of the FA model. Although these plots are useful for pulling apart and examining VEI, it would be unrealistic to expect growers and advisers to examine these plots for every combination of variety and fungicide treatment.

Smith et al. (2018) present a method for producing measures of overall performance (OP) and stability for each variety, which can be used to provide concise yet informative summaries of VEI. These measures are derived from the regression structure of the FA model and take into account VEI. The OP for a variety is defined to be the value on the first factor regression line at the average of the environmental loadings. The square root of the mean of the squared deviations (RMSD) from the first factor regression line is then used as a measure of stability, where a small RMSD corresponds to a stable variety and a large RMSD corresponds to an unstable variety. Given the first model factor accounts for the maximum genetic variance and represents non-crossover interaction, it is the logical choice on which to base OP and stability measures. Varieties of interest for broad-environment recommendations are then those with high OP and a low RMSD value (high stability). Varieties with high OP but low stability may still be of interest, however, for environment-specific recommendations.

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Canola sowing can be shifted earlier in most areas of eastern Australia with the correct varietal phenology

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ABSTRACT

Changing rainfall patterns in eastern Australia has resulted in an increased frequency of late summer rainfall and declining autumn rainfall. Together with improved fallow management practices, there have been increasing opportunities to sow canola earlier than has been traditionally recommended. We report 24 field experiments examining the effect of early sowing of canola in environments from northern NSW, south to the Wimmera region of Victoria and west to the Eyre Peninsula in South Australia. We define 'early' as sowing dates of late March to early April for central and southern NSW and Victoria, and mid-April in northern NSW and South Australia.

Early sowing amplified the inherent phenological differences between canola varieties. Where there was only 5–7 days difference in the start of flowering of two contrasting varieties from a traditional early May sowing, there was often several weeks difference in the flowering dates of the same varieties sown in early April.

There were significant (*P<0.05*) interactions between variety and sowing date in 13 of the 24 experiments. A consistent finding was that early sowing of mid, mid–slow and slow developing varieties generally resulted in similar yield as faster developing varieties sown later (late April to early May). The main exception was in the high rainfall year of 2016 where fast varieties sown later yielded the highest overall. Of the experiments with no interaction between variety and sowing date, all had a significant main effect of sowing date. In six, grain yield was highest from early sowing and either declined at the second sowing date or at the third or fourth sowing date. Of five experiments where early sowing reduced yield, three were at Breeza in northern NSW. Overall varieties were ranked for yield in the order hybrid non-triazine tolerant (TT) = open-pollinated (OP) non-TT > hybrid TT > OP TT.

With the correct varietal phenology, growers can start sowing canola from late March/early April in southern and central NSW and Victoria; and mid-April in South Australia. The traditional sowing time of early May appears to remain the optimum for north-east NSW. Traditional sowing dates of around late April can still be successful in most regions provided a relatively fast cultivar is selected.

Key words

Canola, sowing date, phenology, yield, oil

INTRODUCTION

In eastern Australia, late April to early May has been the traditional target planting time for canola; however over the past decade there has been increased interest in sowing spring canola cultivars earlier in April and even into late March. This interest in early sowing has been driven by several factors including an attempt to avoid the dry spring conditions of the 'Millennium Drought' that severely reduced canola yields during that decade; an observed and projected increase in late summer rainfall coupled with a decline in autumn rainfall (Shi *et al.* 2008) that have provided earlier sowing opportunities; increased area of stubble retention retaining seedbed moisture into early autumn after summer rainfall; ability of early sown crops to access sub-soil water

from out-of-season rainfall; and success of early sowing in other crop species, especially wheat (Hochman *et al.* 2009). There are several potential disadvantages of sowing canola early, including increased frost risk (Robertson *et al.* 2002); increased risk of fungal disease infection, such as upper canopy blackleg (Sprague *et al.* 2017); higher evaporation rates from soil after sowing leading to patchy or failed crop establishment (Brill *et al.* 2016); a lower yield potential if flowering starts in mid-winter when photothermal quotient is low (Faraji 2014); and potentially a decline in the quality of light for reproductive growth due to greater vegetative biomass from early sowing (Smith and Whitelam 1997). To complicate canola sowing date decisions, growers are often required to order seed requirements months before sowing so there is little scope to adjust variety choice depending on the date of season-opening rainfall.

Despite canola being a mainstream crop in eastern Australia since the mid to late 1990s (Colton and Potter 1999), there has been little coordinated research identifying the optimum combination of sowing date and varietal phenology to optimise yield. Kirkegaard *et al.* (2016) reported on several individual sowing date experiments in eastern Australia from 2002–2012 and found that early to mid-April sowing of canola generated the highest yield in eight of the nine experiments conducted. Declines in seed yield (6.0–6.5%) per week delay in sowing were similar to yield decline rates from late April sowing reported by Robertson *et al.* (1999). An interaction between variety and sowing date for grain yield was observed in four of the nine experiments; however these experiments were not sown with varieties with diverse phenology.

We report here the phenology response and grain yield outcomes of 24 experiments conducted from 2014 to 2016 across environments from northern NSW to the Eyre Peninsula of South Australia. The experiments were conducted to investigate the feasibility of early sowing of canola in these diverse environments and the type of phenology required for early sowing. Early sowing is defined as late March/early April for central and southern NSW and Victoria; and mid-April for northern NSW and South Australia.

MATERIALS AND METHODS

Twenty four sowing date experiments were conducted across major canola growing regions of NSW, Victoria and South Australia with a minimum of three cultivars and a minimum of two sowing dates at each site (Table 1). Cultivars varied slightly across seasons as one of the aims was to use cultivars with diverse phenology. Each experiment was sown as a split-plot design with sowing date as main plot and cultivar randomised within sowing date blocks. Where the seedbed was dry at target planting dates, experiments were irrigated with drippers delivering (on average) 10 mm of water on a whole plot area basis concentrated in the seed furrow (except Condobolin which was irrigated by a lateral irrigator, and Breeza which was flood irrigated). Start of flowering (50% of plants with one open flower) dates were recorded at each site. In 2014 grain harvest was by plot harvester at five sites but at all other sites grain yield was taken by hand cuts conducted at maturity, which were then dried and mechanically threshed. Experiments were supplied with enough nutrition and crop protection so that generally yield was not limited by nutrient deficiencies or pest and diseases.

Table 1: Site description for 24 canola sowing date experimental sites from 2014 to 2016.

Year	Location	State	Varieties	Sowing dates	Fallow rain (mm)	In-crop rain (mm)
2014	Breeza*	NSW	6	10-4, 23-4, 8-5, 26-5	267	196
2015	Breeza*	NSW	6	17-4, 29-4, 19-5	327	306
2016	Breeza*	NSW	6	13-4, 16-5, 17-6	210	439
2014	Canowindra	NSW	6	2-4, 15-4, 28-4, 13-5	297	276
2015	Canowindra	NSW	12	2-4, 15-4, 1-5	224	405
2016	Canowindra	NSW	12	4-4, 14-4, 28-4	303	602
2014	Condobolin	NSW	3	1-4, 15-4, 28-4, 13-5	202	172
2015	Condobolin	NSW	6	17-4, 4-5, 19-5	195	265
2016	Condobolin	NSW	4	6-4, 20-4	202	502
2014	Ganmain	NSW	6	1-4, 15-4, 28-4, 13-5	131	251
2014	Hart	SA	6	14-4, 1-5, 16-5, 2-6	127	288
2015	Hart	SA	9	14-4, 30-4, 15-5	93	233
2015	Horsham	Vic	9	13-4, 29-4, 15-5	109	135
2014	Junee	NSW	6	15-4, 28-4, 21-5	130	266
2014	Lameroo	SA	6	14-4, 9-5, 5-6, 20-6	91	186
2015	Lameroo	SA	9	16-4, 1-5, 15-5	88	179
2016	Lameroo	SA	9	15-4, 2-5, 16-5	96	228
2016	Longerenong	Vic	12	4-4, 18-4, 2-5	95	385
2014	Trangie	NSW	3	1-4, 16-4, 1-5, 16-5	211	180
2015	Trangie	NSW	6	1-4, 14-4, 30-4	167	321
2016	Wagga	NSW	12	31-3, 13-4, 29-4	221	586
2014	Yeelanna	SA	6	14-4, 5-5, 2-6, 19-6	117	333
2015	Yeelanna	SA	12	16-4, 1-5, 15-5	67	307
2016	Yeelanna	SA	9	8-4, 20-4, 6-5	108	427

***Breeza was flood irrigated pre-sowing each year and was irrigated once in September 2014 only (each approximately 100 mm).**

RESULTS

Nuseed Diamond was overall the fastest cultivar from sowing to flowering with a median deviation [(days to flower - sowing date average days to flower) / (sowing date average days to flower)] of -0.20 from the sowing date mean for all sowing dates before 25 April. ATR Stingray, Hyola 575CL and IH30 RR were also fast to flower from early sowing with a deviation of <-0.075. The early flowering of these fast varieties from early sowing exposed them to high frost and disease risk as well as conditions of low sunlight during reproductive development. Eleven cultivars (Pioneer 44Y89 (CL), 43C80 CL, ATR Gem, AV Garnet, Hyola 559TT, 44Y84 (CL), GT50 RR, Pioneer 45Y86 (CL), Hyola 577CL, Pioneer 45Y88 CL, Hyola 600RR) had a flowering deviation of between -0.075 and 0.075. Five cultivars (Pioneer 44Y87 CL Hyola 750TT, ATR Wahoo, Hyola 725RT and Archer) were consistently slow to flower from early sowing with a flowering deviation of >0.075.

Early sowing amplified the inherent phenological differences between varieties. An example of this was at Wagga Wagga in 2016, where early sowing of fast varieties Nuseed Diamond and ATR Stingray in a warm autumn led to flowering starting in early June, compared to early August when sown in late April (Figure 1). From the same sowing dates, the slow variety Archer consistently started flowering in mid–late August.

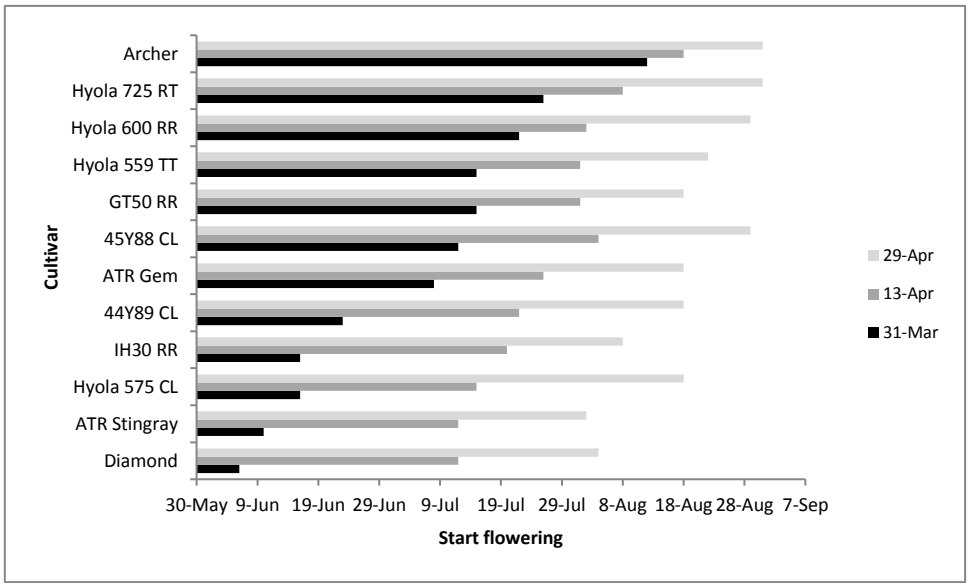

Figure 1: Effect of three sowing dates on flowering date (50% of plants with one open flower) of twelve cultivars at Wagga Wagga, 2016.

There was a significant interaction between cultivar and sowing date in 13 of 24 experiments (Table 2). In these experiments fast developing cultivars were consistently penalised by early sowing. In the high rainfall year of 2016 the fast cultivars sown in late April/early May often had higher yield than early sowing of slow cultivars as they had the highest harvest index (data not shown). Sowing mid or slow cultivars at later sowing dates generally resulted in a smaller yield penalty than sowing fast varieties at early sowing dates.

Table 2: Year, location, statistical significance and mean yield of 13 of 24 experiments with a significant interaction between cultivar and sowing date.

Year	Location	Mean yield (t/ha)	Significance
2015	Canowindra	3.0	*
2016	Canowindra	3.4	*
2014	Condobolin	0.6	**
2016	Condobolin	3.7	**
2014	Ganmain	1.5	**
2014	Hart	1.7	**
2015	Hart	2.1	*
2014	Junee	1.9	**
2016	Lameroo	1.8	*
2016	Longerenong	4.2	**
2016	Wagga Wagga	3.8	***
2015	Yeelanna	3.1	*
2016	Yeelanna	3.4	**

*****P<0.001, **P<0.01, *P<0.05**

There was a significant main effect of sowing date in the remaining 11 experiments (Table 3). In five of these experiments, yield increased with a delay in sowing from the first to second sowing date with Breeza in northern NSW responding this way in each year. In the remaining six experiments yield declined after the earliest sowing date. All experiments except Trangie in 2015 that had declining yield with later sowing were lower yielding than the mean of all experiments (2.5 t/ha).

Table 3: Year, location, mean yield and nature of the yield response (from first to second sowing date) with a significant (All *P*<0.001) main effect of sowing date.

Year	Location	Mean yield (t/ha)	Yield response
2014	Breeza	2.9	Increasing
2015	Breeza	4.4	Increasing
2016	Breeza	4	Increasing
2014	Canowindra	2.2	Increasing
2015	Condobolin	0.6	Decreasing
2015	Horsham	0.1	Increasing
2014	Lameroo	0.2	Decreasing
2015	Lameroo	0.5	Decreasing
2014	Trangie	1.1	Decreasing
2015	Trangie	3.4	Decreasing
2014	Yeelanna	1.7	Decreasing

Across all experiments yield of the non-triazine tolerant (TT) cultivars averaged 2.7 t/ha (both hybrid and open-pollinated (OP)). Yield of hybrid TT cultivars was 2.5 t/ha and yield of OP TT cultivars was 2.3 t/ha.

DISCUSSION

A clear finding of this research is that for most of the experimental area, the sowing window of canola opens much earlier than has been traditionally thought and with the correct phenology choice canola can be sown any time from late March (in central and southern NSW and Victoria) to early May with success. This finding is especially useful for environments that have an increasing amount of late summer rainfall e.g. southern NSW and in farming systems that maintain stubble close to the surface at least to the point of sowing. It is also especially useful in light of the general shift to earlier sowing of other major grain crops, especially wheat and barley as early sowing of canola will also allow cereals to be sown earlier. For growers who purchase hybrid canola seed there is little scope to adjust cultivar choice in reaction to the timing of the autumn break, so this research provides the confidence for growers to select mid or slow developing varieties that can be sown at any time from late March to early May (depending on seedbed moisture). For growers that retain their own canola seed for sowing they can afford to keep at least two varieties of contrasting phenology e.g. ATR Stingray and ATR Wahoo, which will allow them to adjust cultivar choice depending on the timing of the autumn break. In lower rainfall environments such as Trangie and Lameroo it may be wiser to select the best cultivar regardless of varietal phenology as there was little cultivar by sowing date interaction at these sites. The one location where early sowing was not successful was Breeza in northern NSW so traditional sowing dates of around early May are recommended for that environment.

In the two highest yielding experiments with an interaction between cultivar and sowing date (Wagga Wagga and Longerenong 2016), it was somewhat surprising that fast developing varieties sown later out-yielded early sowing of slow developing varieties. In these experiments the fast developing varieties had a higher harvest index but this was not related to water availability as both these sites had very high spring rainfall. Further research is required to better understand this outcome and determine if it is physiological or simply inherent differences between the cultivars used in these experiments.

With the interactions between canola phenology and sowing date reported here, it will be essential in future to ensure that the phenology of new canola varieties is well understood before sowing as the phenology findings in this research did not always relate well to the commercial maturity ratings provided by seed companies.

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Establishing a Diverse Multi-Year Genomic Selection Reference Population for Key Traits to Underpin Canola Pre-Breeding

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ABSTRACT

Genomic selection (GS) uses genome-wide markers, usually single nucleotide polymorphisms (SNP), genotyped on germplasm that also has field trial data to predict genomic estimated breeding values (GEBV) for lines that have only been genotyped. A diverse set of *B. napus* spring and winter lines was compiled which included pre-breeding lines, commercial varieties, and germplasm sourced from the Australian Grains Genebank. Field trials were carried out across three years. Blackleg disease resistance field trials with 600 spring and winter lines were planted with two replicates near Green Lake and Wickliffe Victoria in 2015. For 200 spring lines, agronomic and seed oil traits field trials were completed with two to three replicates per line near Horsham (irrigated) and Mininera (rainfed) in 2016, as well near Horsham (irrigated and rainfed) in 2017. Internal infection and emergence were recorded in each year, whereas other traits may only be available for subsets of years. The following traits were recorded: emergence, survival, internal blackleg infection, vigour, days to flowering, days to maturity, plant height, seed yield, seed moisture content. Seed composition was determined with NIR for seed oil and protein %, total seed glucosinolate, and the main fatty acids.

All lines were genotyped with an in-house transcriptome genotype-by-sequencing (GBS) protocol that resulted in up to 98,000 SNP after stringent quality control. A genomic relationship matrix was calculated using all quality-controlled SNP. Genomic best linear unbiased prediction (GBLUP) and BayesR GS methods were used. Genomic prediction accuracies were moderate to high for survival and internal infection (range 0.52 - 0.67) and reduced for emergence (range 0.19 – 0.45). Prediction accuracies across sites in 2015 were lower by approximately 0.1 when compared to within site, indicating genotype-by-environment effects. We compiled a list of known blackleg quantitative trait loci (QTL) regions and classified our SNP as either within (N=18,886) or outside (N=79,168) QTL regions. We then fit genomic relationship matrices for both SNP sets in a GBLUP model to determine the relative amount of genetic variance explained by the two SNP sets. Known blackleg QTL regions explained at most 33% of the genetic variance in survival or internal infection. It is clear from this analysis that selecting only on major known blackleg QTL using marker-assisted selection ensures sub-optimal genetic gain. However, as GS captures genome-wide marker effects it is much better placed to achieve genetic gain for blackleg resistance. GS accuracies for agronomic field traits ranged from 0.32 – 0.70 and from 0.33 – 0.62 in 2016 and 2017, respectively. NIR predicted seed composition traits were available for 2016 and prediction accuracies ranged from 0.30 – 0.64.

The level of accuracy achieved in all traits investigated to date is in the usable range that enables increased selection pressure to be placed in pre-breeding and breeding applications. Selection

based on GEBVs can now be used for all traits for any line that has been genotyped with genome-wide markers. Genomic selections can be done early in the breeding cycle to shorten breeding cycles significantly. Selection on all traits during pre-breeding ensures that germplasm is already higher yielding and adapted when it is accessed by breeding companies. GS will usher in a significant change to breeding and combined with advanced methods such as in-field phenomics, speed breeding, and doubled haploid production will deliver the genetic gain that is required by growers.

Key words

Canola – genomic selection – prediction accuracy – breeding

INTRODUCTION

Genomic selection (GS) uses genome-wide markers, usually single nucleotide polymorphisms (SNP), genotyped on germplasm that also has field trial data to predict genomic estimated breeding values (GEBV) for lines that have only been genotyped (Meuwissen *et al.* 2001). GS can accelerate genetic gain, as demonstrated in a range of livestock and crop species to date. Additionally, once a line is genotyped with genome-wide markers its future performance can be predicted for all traits with a prediction equation, thereby allowing for selection on all traits early in the breeding cycle.

The aim of this study was to establish a multi-year reference population of genotyped and phenotyped canola lines for all traits relevant to Australian production areas. In this data, genomic prediction accuracies were investigated to determine the feasibility of routinely applying GS in pre-breeding applications.

MATERIALS AND METHODS

A diverse set of *B. napus* spring and winter lines was compiled which included pre-breeding lines, commercial varieties, and germplasm sourced from the Australian Grains Genebank. Field trials were carried out across three years. Blackleg disease resistance field trials with 600 spring and winter lines were planted with two replicates near Green Lake and Wickliffe Victoria in 2015. For 200 spring lines, agronomic and seed oil traits field trials were completed with two to three replicates per line near Horsham (irrigated) and Mininera (rainfed) in 2016, as well near Horsham (irrigated and rainfed) in 2017. Internal infection and emergence were recorded in each year, whereas other traits may only be available for subsets of years. The following traits were recorded in agronomic trials: emergence, survival, internal blackleg infection, vigour, days to flowering, days to maturity, plant height, seed yield, seed moisture content. Seed composition was determined with NIR for seed oil and protein %, total seed glucosinolate, and the main fatty acids. The agronomic trials were protected for blackleg disease with Impact in-furrow and foliar fungicide (one application of prosaro at the 4-6 leave stage). Field trial data were spatially adjusted using autocorrelation (AR1) models to account for field variability, and broad-sense heritability was estimated.

All lines were genotyped with an in-house transcriptome genotype-by-sequencing (GBS) protocol that resulted in up to 98,000 SNP after stringent quality control. A genomic relationship matrix was calculated using all quality-controlled SNP. Genomic best linear unbiased prediction (GBLUP) and BayesR GS methods were used (Erbe *et al.* 2012; de los Campos *et al.* 2013).

RESULTS

Genetic correlations between traits were calculated using bivariate GBLUP models. Strong negative correlations were observed for Average Internal Infection with Yield and Lodging (-0.8 and -0.6, respectively). Linolenic and oleic acids were strongly negatively correlated (-0.54). Yield was negatively correlated with several fatty acids (Eicosanoic -0.31, Linoleic -0.37, Linolenic -0.36, Palmitic -0.38, Figure 1). Average Internal Infection was negatively correlated with Oil content (-0.59), Archidic acid (-0.53) and Stearic acid (-0.43), and its main positive correlation was with glucosinolate (0.50). Plant Height and Survival Rate were positively correlated with Oil content.

Phenotypic Correlation Coefficient										
Traits	AvInf	DTF	DTM	EMC	LOD	PLH	SHA	SurvRt	VIG	YIELD
ArA	-0.27	0.19	0.28	-0.13	0.21	0.26	0.13	0.24	-0.10	0.29
EiA	-0.18	0.12	0.14	0.15	0.20	0.20	0.17	0.19	0.14	-0.34
GCC	0.32	0.13	-0.13	-0.17	-0.19	-0.18	-0.15	-0.27	0.14	-0.26
LA	-0.14	-0.22	-0.21	0.18	-0.24	-0.13	-0.11	0.16	0.23	-0.37
LLA	0.16	-0.20	-0.18	0.14	-0.27	-0.20	-0.12	-0.11	0.11	-0.36
MC	0.11	-0.15	-0.22	0.24	-0.18	-0.12	-0.12	-0.24	0.15	-0.16
OA	-0.16	0.21	0.18	-0.19	-0.25	0.27	0.19	0.10	-0.11	-0.28
Oil	-0.35	0.15	0.32	-0.12	0.26	0.26	0.14	0.33	0.12	0.47
Other	0.18	-0.11	-0.26	0.11	-0.20	-0.21	-0.19	-0.27	-0.15	-0.31
PA	0.18	-0.11	-0.16	-0.17	0.21	-0.14	-0.16	-0.21	-0.15	-0.28
PC	0.18	-0.21	-0.18	0.17	-0.11	-0.23	-0.13	-0.18	0.14	-0.31
SA	-0.24	0.12	0.19	-0.15	0.26	0.20	0.12	0.25	-0.11	0.25
Genetic Correlation Coefficient										
Traits	AvInf	DTF	DTM	EMC	LOD	PLH	SHA	SurvRt	VIG	YIELD
ArA	-0.53	0.20	0.32	-0.14	0.50	0.57	0.16	0.52	-0.14	0.41
EiA	-0.23	-0.14	0.19	0.18	0.36	0.32	0.19	0.44	0.21	-0.31
GCC	0.50	-0.18	-0.26	-0.18	-0.38	-0.23	-0.18	-0.58	0.15	-0.36
LA	0.16	-0.37	-0.25	0.27	-0.31	-0.14	-0.24	0.18	0.54	-0.38
LLA	0.18	-0.25	0.19	0.13	-0.30	-0.40	-0.17	-0.21	0.19	-0.36
MC	0.43	-0.29	-0.35	0.30	-0.32	-0.16	-0.24	-0.49	0.22	-0.25
OA	-0.19	0.39	0.29	-0.33	0.12	0.48	0.22	0.23	-0.14	-0.30
Oil	-0.59	0.20	0.43	-0.15	-0.32	0.47	0.18	0.52	0.19	0.62
Other	0.27	-0.20	-0.36	0.12	-0.39	-0.39	-0.23	-0.46	-0.25	-0.59
PA	0.23	-0.16	-0.20	-0.26	0.34	-0.22	-0.18	-0.37	-0.31	-0.46
PC	0.17	-0.26	-0.20	0.18	-0.15	-0.34	-0.29	-0.29	-0.16	-0.48
SA	-0.43	0.17	0.21	-0.19	0.30	0.28	0.18	0.46	0.12	0.45

Figure 1. Phenotypic and genetic correlation between agronomic, disease, and quality traits.

* Colour range red (-1) to green (1). AvInf = Average internal infection, DTF = days to flowering, DTM = days to maturity, EMC = emergence count, LOD = lodging score, PLH = plant height (cm), SWP = seed weight per plot (g/plot), VIG = vigor score, MC = moisture content (%), Oil = oil content (%), GCC = glucosinolate (µmol/g seed), PC = seed protein content (%), PA = palmitic acid (C16:0), SA = stearic acid (C18:0), OA = oleic acid (C18:1), LA = linoleic acid (C18:2), LLA = linolenic acid (C18:3), ArA = arachidic acid (C20:0), EiA = eicosenoic acid (20:1), other.

Genomic prediction accuracies in spring lines (Figure 2) were moderate to high for survival and internal infection and reduced for emergence with similar trends in winter lines (data not shown). Prediction accuracies across sites in 2015 were lower by approximately 0.1 when compared to within site, indicating genotype-by-environment effects. GS accuracies for agronomic field traits ranged from 0.32 – 0.70 and from 0.33 – 0.62 in 2016 and 2017, respectively. NIR predicted seed composition traits were available for 2016 and prediction accuracies ranged from 0.30 – 0.64.

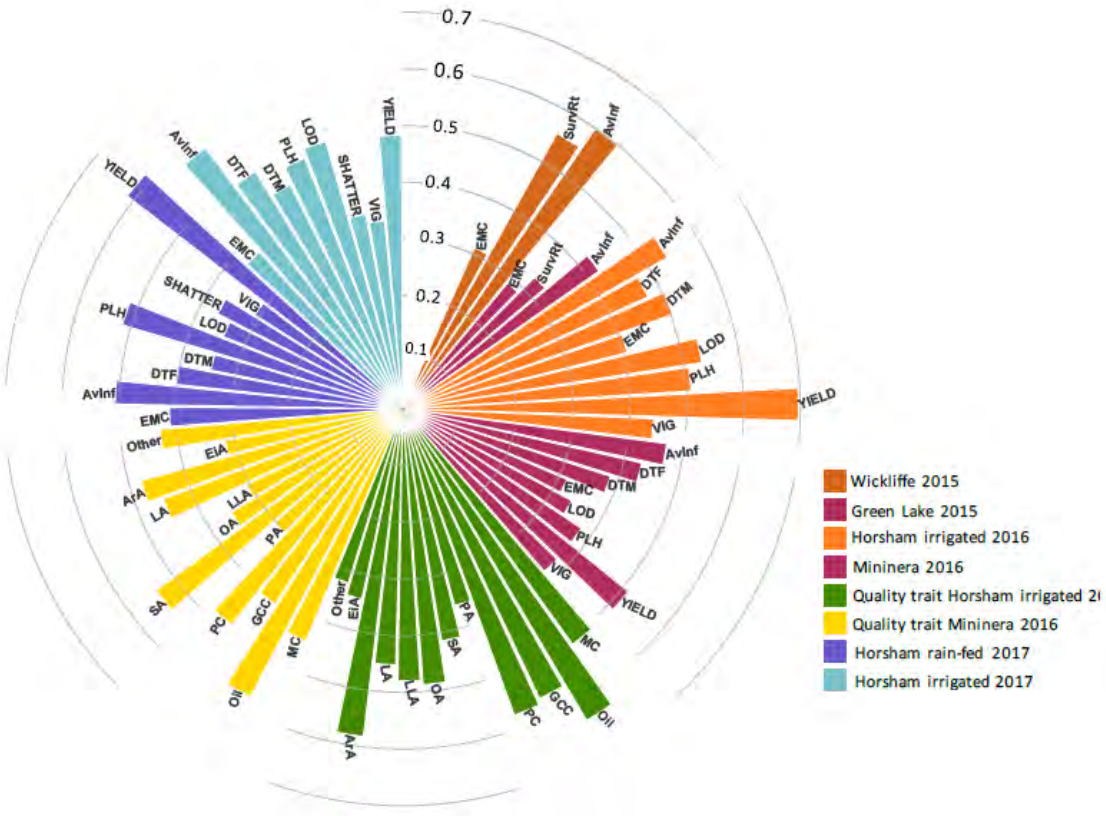

Figure 2. Summary of genomic prediction accuracies in spring lines for agronomic and quality traits within location using genomic best linear unbiased prediction (GBLUP). AvInf = Average internal infection, DTF = days to flowering, DTM = days to maturity, EMC = emergence count, LOD = lodging score, PLH = plant height (cm), SWP = seed weight per plot (g/plot), VIG = vigor score**,** MC = moisture content (%), Oil = oil content (%), GCC = glucosinolate (µmol/g seed), PC = seed protein content (%), PA = palmitic acid (C16:0), SA = stearic acid (C18:0), OA = oleic acid (C18:1), LA = linoleic acid (C18:2), LLA = linolenic acid (C18:3), ArA = arachidic acid (C20:0), EiA = eicosenoic acid (20:1), other.

We compiled a list of known blackleg quantitative trait loci (QTL) regions and classified our SNP as either within (N=18,886) or outside (N=79,168) QTL regions (Fikere *et al.* 2018). We then fit genomic relationship matrices for both SNP sets in a GBLUP model to determine the relative amount of genetic variance explained by the two SNP sets. Known blackleg QTL regions explained at most 33% of the genetic variance in survival or internal infection (Figure 3). It is clear from this analysis that selecting only on major known blackleg QTL using marker-assisted selection ensures sub-optimal genetic gain. However, as GS captures genome-wide marker effects it is much better placed to achieve genetic gain for blackleg resistance.

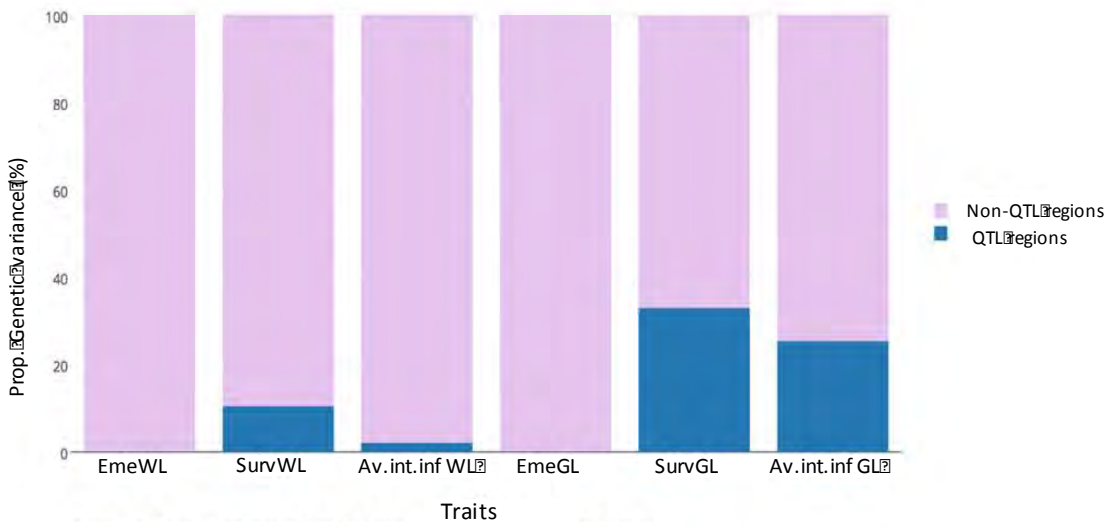

Figure 3. Proportion of genetic variance explained by known major blackleg resistance QTL and non-QTL Regions in *B. napus.*

DISCUSSION

Many observed genetic correlation followed trends documented in the literature between agronomic traits with oil content as well as fatty acid composition. Lodging was negatively correlated with oil content, protein content, and with fatty acids, which was thought to be due to interruption of biosynthesis (Kendall *et al.* 2017). The negative correlation of Glucosinolate content with yield had also been found by Wuerschum et al. (2012). We explored the genetic correlation between fatty acids and blackleg disease in canola. We found that average internal infection was weakly positively correlated with Palmitic acid, and negatively correlated with Arachidic acid and Stearic acid. A similar correlation trend was reported in eggplant (Xing & Chin 2000), where increases in palmitoleic acid production enhanced resistance to *Verticillium dahlia* disease. In addition, we found a negative correlation between oleic acid and linolenic acid with Average Internal Infection, suggesting a connection between these fatty acids with blackleg infection in canola. Our finding agreed with Xue et al. (2008) who found a similar relationship between oleic acid and linolenic acid with the fungal pathogen *Cercospora kikuchii* in soybean.

The level of prediction accuracy achieved in all traits investigated to date is in the usable range that enables increased selection pressure to be placed in pre-breeding and breeding applications. Selection based on GEBVs can now be used for all traits for any line that has been genotyped with genome-wide markers. Genomic selections can be done early in the breeding cycle to shorten breeding cycles significantly. Selection on all traits during pre-breeding ensures that germplasm is already higher yielding and adapted when it is accessed by breeding companies. GS will usher in a significant change to breeding and combined with advanced methods such as in-field phenomics, speed breeding, and doubled haploid production will deliver the genetic gain that is required by growers.

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Decision Apps for Managing Blackleg and Sclerotinia in Canola

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ABSTRACT

New decision apps have been developed for managing blackleg and sclerotinia of canola. The apps include seasonal risk factors, expected yield, and economics. They give the decision maker the range of possible outcomes that can arise from any management decision, customised for each paddock and season. The apps are designed for quick and efficient use with clients in the field. They will allow users to make the most profitable decisions about variety choice and fungicide use in canola.

Key words

Target yield – grain price – cropping history – disease history – varieties – Monté Carlo analysis – fungicide – uncertainty – risk preference

INTRODUCTION

Blackleg crown canker (*Leptosphaeria maculans*) and sclerotinia stem rot (*Sclerotinia sclerotiorum*) are serious diseases of canola across Australia. The best approach to manage these diseases in any situation depends on many factors. For blackleg, these factors include variety choice, yield potential, grain price, proximity to canola stubble from previous years, and several fungicide options. For sclerotinia, important factors are yield potential, grain price, history of broadleaf crops, past occurrence of sclerotinia, weather conditions and also fungicide options. All of these factors have implications for costs and returns, and many of these factors are uncertain at the time when management decisions need to be made. To assist with managing these diseases we have produced two tablet apps. BlacklegCM, for managing blackleg, is available for iPads or Android tablets from the Apple App Store or from Google Play. SclerotiniaCM, for managing sclerotinia, is being tested in the field this season and will be released in early 2019.

These apps share several characteristics:

They will work in the field with or without a network connection

They can quickly and easily be set to represent conditions in any paddock

They present implications of management decisions in dollar terms

They present the range of possible outcomes as minimum, maximum and most likely values

Users can choose to view results in tabular or graphic formats.

Using these apps, canola growers can apply their own risk preferences to their management choices, with improved confidence about the likely benefits and the best and worst-case outcomes.

For all canola varieties in the Blackleg Management Guide (GRDC, 2018), BlacklegCM also presents the blackleg resistance rating, blackleg resistance group, seed price, herbicide tolerance type, and end point royalty cost if any. This information is continually updated, so managers can always get the latest information about varieties from the app.

MATERIALS AND METHODS

The BlacklegCM and SclerotiniaCM apps produce a distribution of the difference of net return, in $/hectare, that can be expected as a result of a management decision. For blackleg, management comparisons can be made for any combination of variety, fungicide, proximity to past canola crops, grain price and target crop yield. For sclerotinia, comparisons are made for plus or minus application of foliar fungicide.

The app uses Monté Carlo simulation to generate the expected result from distributions of expected yield in the absence of disease, grain price, loss of yield in the absence of fungicide and efficacy of fungicide. Correlations between these factors are included in the calculation.

The app predictions and level of uncertainty were initially set based on expert opinion from leading Australian pathologists, and subsequently recalibrated based on available field data. It is intended that the apps will continually be updated to reflect new experimental results as they become available. This semi-quantitative approach to calibration of the app recognises the fact that management decisions for these diseases are, and must be, made each year with whatever information is available to each manager. Bringing the best available information to bear in an easily understood form can only assist in this process.

RESULTS

Table 1 lists input values for BlacklegCM for two situations, which differ only in the target crop yield. In both cases, the difference in net return resulting from application of the foliar fungicide Prosaro at the 4-6 leaf stage is estimated. For situation 1, where the most likely crop yield in the absence of disease is 1.5 t/ha, a foliar application of fungicide would result in the mean net loss of $5/ha. In 90% of years the net return would be expected to be less than $15/ha, and in 90% of years the net loss would be expected to be greater than $24/ha (Fig.1). For situation 2, where the most likely crop yield in the absence of disease is 2.5 t/ha, the mean net return is $56/ha. In 90% of years the net return would be expected to be less than $113/ha, and in 90% of years the net return would be expected to be greater than $4/ha (Fig.2). Additional examples of use cases for BlacklegCM can be found in Diggle et.al. (2018), Marcroft et.al. (2018 a and b), and Sprague et.al (2018).

Table 1. Settings for BlacklegCM for situations 1 and 2.

App setting	Value	App setting	Value
Target yield for situation 1 (t/ha)	1.5	Distance to 2 year old stubble	Sown in
Target yield for situation 2 (t/ha)	2.5	2 year old stubble standing?	Yes
Grain price ($/ha)	600	Canola variety	ATR-Bonito
Production cost ($/ha)	350	Blackleg resistance rating	MS
Canola in district (% of cropping area)	15	Seeding rate (kg/ha)	2
Spore maturity risk	High	Fungicide seed dressing?	Yes
Distance to 1 year old stubble (m)	100		

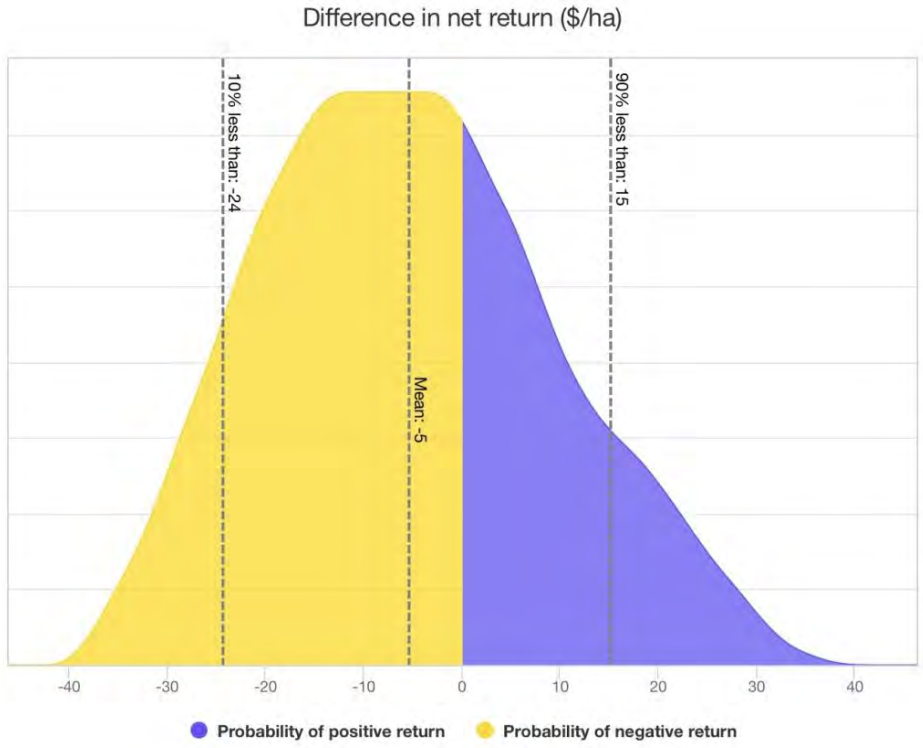

Fig.1. Probability density from BlacklegCM for net return from foliar spray of Prosaro® at 4-6 leaf stage for situation 1 where target yield is 1.5 t/ha and all other settings are as shown in Table 1.

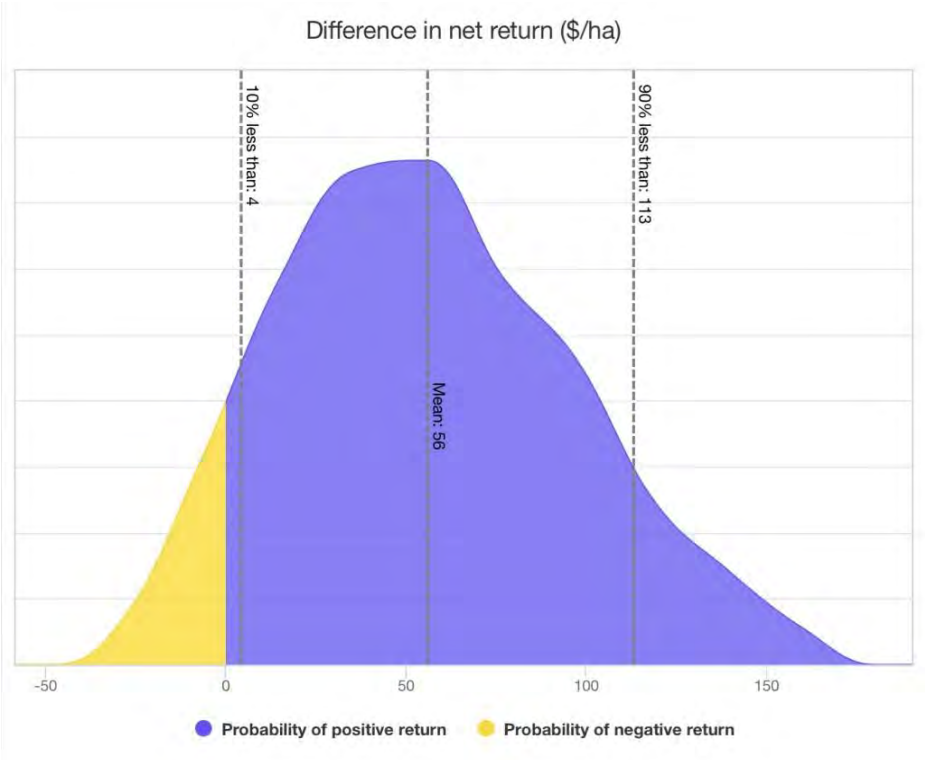

Fig.2. Probability density from BlacklegCM for net return from foliar spray of Prosaro® at 4-6 leaf stage for situation 2 where target yield is 2.5 t/ha and all other settings are as shown in Table 1.

DISCUSSION

The BlacklegCM and SclerotiniaCM apps have been designed to provide site and season- specific information to grain growers to inform their management decisions. The apps provide information in terms of the mean, minimum and maximum change in net return that can be expected from a management option that is being considered. The apps allow canola growers to apply their own risk preferences in making decisions, rather than recommending particular management options.

The apps are delivered on tablets and have a straight-forward user interface that asks for inputs that can be readily estimated by agronomic specialists. We envisage that the main use case for these apps will be as an aid to conversations about disease management between growers and their advisors, and that these conversations will typically occur in the field.

Preliminary indication is that these apps will be adopted and used. De-personalised electronic feedback from BlacklegCM illustrates that in the first five months after release, the app was downloaded 176 times and used in 848 session with 290 hours of total engagement time.

ACKNOWLEDGEMENTS

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Canola sowing time to maximise yield in Western Australia

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ABSTRACT

In the last decade there has been a trend towards earlier sowing of canola by Western Australian farmers. Sowing canola in mid-April has become standard practice in most of the WA cropping zone. This simulation study was designed to determine the optimum sowing window to maximise canola yield for different locations, soil types and canola cultivars in Western Australia. The optimum sowing windows for 24 locations, 3 soil types and 3 canola cultivars were obtained from a computer simulation analysis using the APSIM-Canola model. As a rule of thumb, in low and medium rainfall locations, the optimum sowing period for a medium maturity cultivar is April; and in the high rainfall locations the optimum sowing window spans from early April to mid or late May, depending on location.

Key words

APSIM, crop modelling, optimum sowing window, simulation

INTRODUCTION

In the last decade there has been a trend towards earlier sowing of canola by farmers. Sowing canola in mid-April has become standard practice in most of the north of the Western Australia (WA) cropping zone (Fletcher *et al.,* 2016). Yield declines with sowing date ranging from 5% to 12% per week delay in sowing have been found in several Australian studies with sowing dates mainly in May and June (Farre *et al*., 2002).

It is well documented that the potential grain yield is related to the biomass at flowering (McCormick *et al*., 2012). Typically, there is a yield penalty for very early sowings, when temperatures are high, the crop grows rapidly and biomass at flowering is low. Similarly, late sowing shortens the period from sowing to flowering, and thus reduces the biomass at flowering and decreases the yield potential.

The interaction of the crop with abiotic stresses of frost and heat are also dependant on sowing date. Sowing very early increases frost risk with greater potential yield penalty due to frost stress during early grain-filling. Sowing late increases the risk of greater yield penalty due to heat stress.

However, there is a lack of experimental data on very early sowings before mid-April. This simulation study was designed to obtain yield relationships across a wide range of sowing dates (March to June) and to establish the optimum sowing window to maximise grain yield accounting for frost and heat stress for different locations in Western Australia.

MATERIAL AND METHODS

The validated crop simulation model APSIM-Canola (v.7.9) (Farre *et al*., 2002; Keating *et al*., 2003) was used to run a series of crop simulation experiments to explore the effect of time of sowing and cultivar length on canola yields.

Long-term simulations for the period 1976-2016 were run for 24 locations (Table 1) in the Wheatbelt of Western Australia, with eight times of sowing from mid-March to end-June at 15 day intervals, three canola cultivars and three soil types (sand, duplex, clay). The locations were chosen to represent low (L), medium (M) and high (H) rainfall zones in the Wheatbelt of WA. The canola cultivars were generic long (e.g. Hyola 650TT), medium (e.g. ATR Bonito) and short (e.g. ATR Stingray) season cultivars, equivalent to series 6 to 7, 5, and 3 to 4 of the current cultivars, respectively. The three generic soil

types, differing mainly in the plant available water content (PAWC), were a sand (PAWC = 57 mm), duplex (PAWC = 90 mm) and clay soil (PAWC = 135 mm).

For each simulation, 10 mm irrigation was applied at sowing to ensure that the crop was successfully established for all sowing dates. Crop emergence occurred 9 to 13 days after sowing. In this study sowing date refers to sowing into moist soil, with immediate germination. It is different to dry sowing with an uncertain germination and emergence date.

Nitrogen fertiliser was applied to prevent nitrogen limiting yield. Crop management was simulated to reproduce best management practices in each rainfall zone.

A yield reduction to account for frost and heat damage was calculated for minimum air temperatures below 2 °C and maximum temperatures above 30 °C during a period of approximately 6 weeks around flowering and early grain filling, using the method of Lilley *et al*. (2015).

RESULTS

Peak yield

Simulated average yields increased from very early sowing up to a peak or maximum yield at the optimum sowing window and decreased with later sowing (Figure 1). Yields were higher for high rainfall locations. Peak simulated yields for ATR Bonito (medium maturity cultivar) on a duplex soil at Kellerberrin (L), Mingenew (M) and Kojonup (H) were 1.8, 2.0 and 2.8 t/ha, respectively (Figure 1).

Once passed the optimum sowing window (peak yield) there is a marked yield decline with sowing date. For example, in Kellerberrin, delayed sowing of ATR Bonito on duplex soil from mid-May to mid-June would incur an average yield penalty of 23 kg/ha/day (160 kg/ha/week) (Figure 1).

Different maturity varieties have slightly different optimum sowing times. The biggest difference in cultivar performance is for early sowing times, where long maturity cultivars out-yielded short and mid maturity cultivars (Figure 1). With late sowings, yield differences between cultivars decreased and short maturity cultivars out-yielded the other cultivars in general.

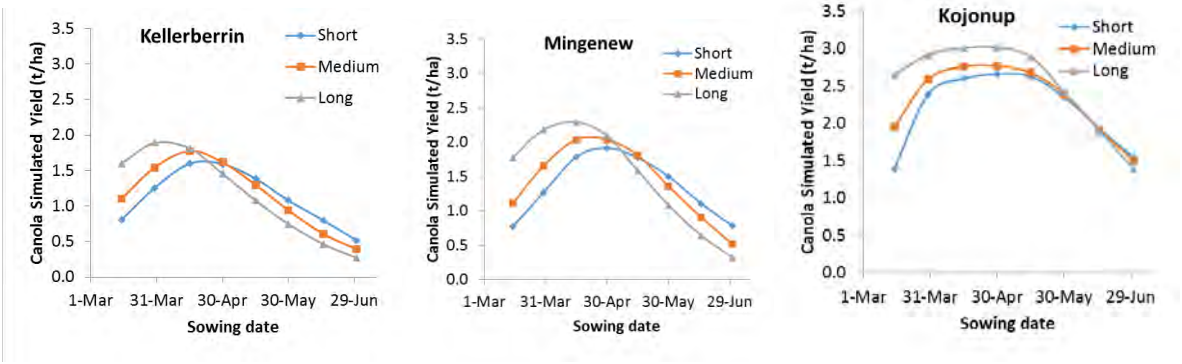

Figure 1. Yield response to sowing date for Kellerberrin (L), Mingenew (M) and Kojonup (H), for a short, medium and long season cultivar, on a duplex soil. Average simulated yields for the last 41 years of climate data.

Optimum sowing window

The optimum sowing window for each location was defined as the sowing period when average simulated yield was within 95% of the maximum yield (Table 1). The optimum sowing window varied with location, soil type and cultivar. As a rule of thumb, the optimum sowing window for a medium maturity cultivar in low and medium rainfall locations is April, and for high rainfall locations is from early or mid-April to mid-May or late-May.

Table 1. Canola optimum sowing window for 24 WA locations for a mid-variety (ATR Bonito) and mid-soil (duplex). Locations grouped according to AgZones (1 to 4, from North to South) and Rainfall zones (L=Low, M=Medium, H= High). Based on 41 years of climate data.

AgZone	Location	Optimal sowing window	AgZone	Location	Optimal sowing window	AgZone	Location	Optimal sowing window
L1/M1	Mullewa	7 Apr – 1 May	**M1**	Mingenew	9 Apr – 9 May	**H1**	Geraldton	13 Apr – 15 May
L2/M2	Dalwallinu	8 – 30 Apr	**M1/2**	Carnamah	8 Apr – 2 May	**H2**	Badgingarra	12 Apr – 18 May
L3	Kellerberrin	7 – 27 Apr	**M2**	Wongan Hills	7 Apr – 6 May	**H4**	Wandering	13 Apr – 15 May
L3	Merredin	3 – 26 Apr	**M3**	Cunderdin	5 – 30 Apr	**H5W**	Kojonup	31 Mar – 18 May
L3	Southern Cross	26 Mar – 23 Apr	**M3/H3**	Northam	8 Apr – 4 May	**H5W**	Frankland	29 Mar – 7 Jun
L4	Hyden	5 – 28 Apr	**M3/4**	Corrigin	7- 26 April	**H5W**	Mount Barker	29 Mar – 9 Jun
L5	Salmon Gums	26 Mar – 26 Apr	**M4**	Lake Grace	3- 26 Apr	**H5E**	Gibson	1 Apr – 13 May
			M4/H4	Wagin	2 Apr – 5 May			
			M5W	Ongerup	3 – 30 Apr			
			M5E	Ravensthorpe	3 Apr – 8 May			

The optimum sowing window began earlier and had shorter duration in low rainfall locations and had longer duration in high rainfall locations (Table 1). For example, the optimal sowing window for ATR Bonito on a duplex soil was 7 to 27 April in Kellerberrin (20 days duration) and 31 March to 18 May in Kojonup (48 days duration) (Table 1).

Sowing windows in Table 1 are for a duplex soil or soil with a medium water holding capacity. In general, light soils have earlier and shorter optimum sowing windows than heavy soils (about one week earlier). Also the duration of the optimum sowing window is shorter in light soils than in heavy soils. The duration of the sowing windows were 3-15 days shorter on the light (sand) soil than on the duplex soil, and it was up to 13 days shorter on the duplex soil than on the heavy (clay).

Risk

If the sowing opportunity is late, it is important to assess the chances of achieving a target yield or break-even yield (Figure 2). This information can help growers to make an informed decision regarding when is too late to sow canola. For example, there is only a 15% chance of achieving at least 1.5 t/ha with end-May sowing at Kellerberrin, but 45% at Mingenew and 95% at Kojonup (Figure 2), based on the last 41 years of climate data.

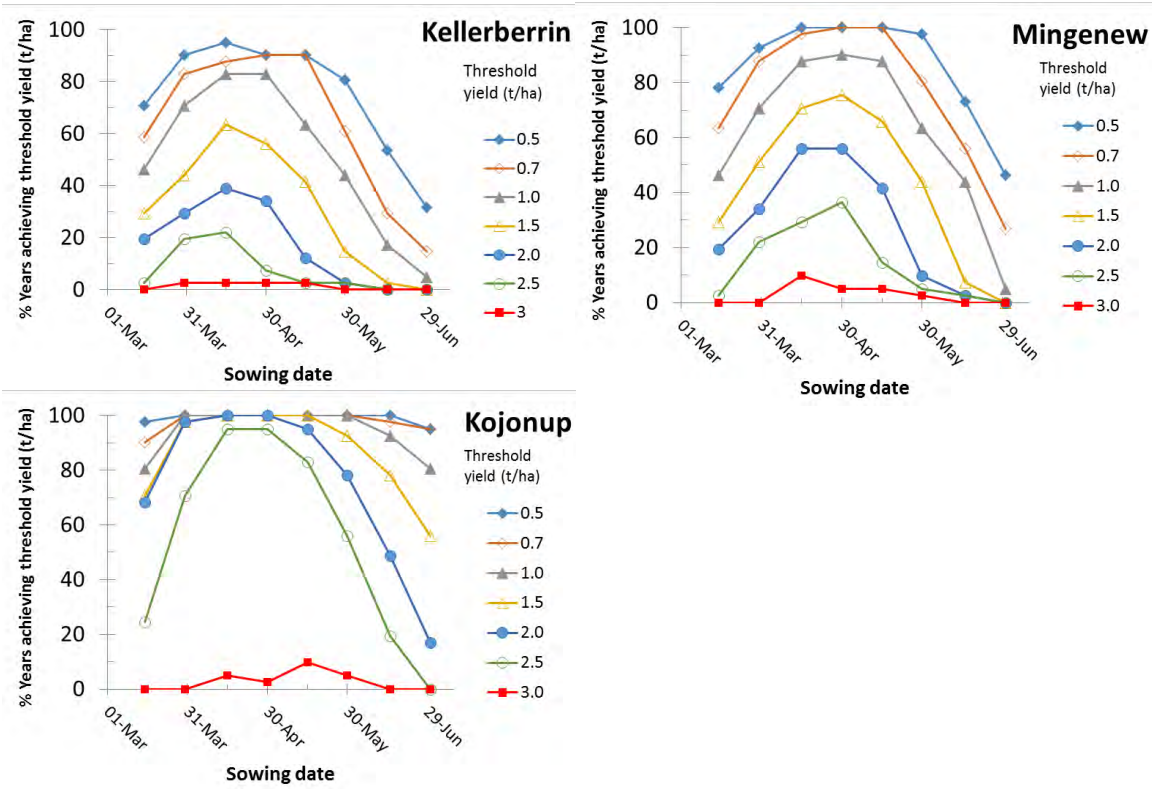

Figure 2. Percentage of years (%) with yield above certain thresholds for different sowing dates, at Kellerberrin (L), Mingenew (M) and Kojonup (H), on a duplex soil, sowing ATR Bonito (mid maturity cultivar). Yield thresholds were 0.5, 0.7, 1, 1.5, 2.0, 2.5 and 3.0 t/ha. Based on 41 years of climate data.

DISCUSSION

Very early sowings, such as mid-March, have a lower yield than sowings at the optimum time due to both low yield potential and greater yield penalty due to frost. More field trials with March sowings are necessary to validate the simulation results.

Weighing up the risk of not taking an early sowing opportunity, sometimes there may be good reasons for sowing earlier than the optimum sowing period. The early autumn rains have a high probability of being followed by long dry periods, so if a late March or early April sowing opportunity is not taken there is a risk a second opportunity will not occur until after the optimal sowing window has passed. This is especially the case in low rainfall short-growing season environments such as Mullewa or Merredin where it may be better to sow earlier than the optimum and risk some frost damage rather than risk missing an opportunity and having to sow after the optimal sowing window or not at all. This will only be advisable when there is sufficient stored soil moisture from summer rain to ensure an early emerging crop will survive until reliable winter rains begin.

CONCLUSION

Early sowing is the key to maximise canola yield in Western Australia. As a rule of thumb sowing in April will achieve the maximum canola yield in most locations in the WA cropping region, especially in low and medium rainfall locations. For long season environments and/or mild conditions (high rainfall locations) this period extends to mid-May or end-May.

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Characterization of *FLOWERING LOCUS C* genes in leafy *Brassica rapa* vegetables

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ABSTRACT

Many plant species require prolonged cold exposure, generally encountered during the course of winter, before flowering and setting seed. Without exposure to a prolonged cold period, flowering is blocked. This process is known as vernalization. A requirement for vernalization is evolutionary adaptation to temperate climates, preventing flowering before encountering a winter season and ensuring flowering under more the favorable weather conditions of spring. The vernalization requirement is also important for the quantity and quality of crop production. In vegetative crops, early bolting and flowering caused by a low vernalization requirement can limit the potential for yield increases or devalue the products. Therefore, understanding the mechanism that regulates flowering time is important for breeding of late-bolting lines in the *Brassica* vegetables.

In this study, we identified two *FRIGIDA* (*FRI*) genes of *Brassica rapa* and showed that BrFRIb functions as an activator of *FLOWERING LOCUS C* (*FLC*). There is a positive correlation between the steady state expression levels of the sum of *BrFLC* paralogs and days to flowering after four weeks of cold treatment, suggesting that this is an indicator of the vernalization requirement. We also showed that histone H3 lysine 27 tri-methylation (H3K27me3) accumulated after cold treatment around exon 1 of *BrFLC* paralogs. This H3K27me3 mark then spreads across the entire length of all *BrFLC* genes upon returning to warm conditions following cold exposure, indicating that *BrFLC*s are repressed by the accumulation of H3K27me3 and that the spreading of H3K27me3 promotes stable *FLC* repression. Based on these results, we consider that epigenetic modifications are involved in the vernalization requirement of *B. rapa*. We also identified long non-coding RNAs (lncRNAs) by RNA-sequencing in non-vernalized and vernalized plants, and compared the expression levels of lncRNAs between before and after cold treatments. We identified differentially expressed lncRNAs at the whole genome levels and three natural antisense transcripts (NATs) from *BrFLC2*, and two *BrMAF* loci, which were upregulated by short-term cold treatments. We discuss the biological function of lncRNA in *B. rapa* including whether lncRNA contributes to epigenetic regulation of *FLC* by prolonged cold treatment.

Key words: vernalization – epigenetics – histone H3 lysine 27 tri-methylation – *FLOWERING LOCUS C* – non-coding RNAs

Numerous plant species require prolonged cold exposure, generally encountered during the course of winter, before flowering and setting seed. Without exposure to a prolonged cold period, flowering is blocked. This process is known as vernalization, which is derived from the Latin word *vernalis*, meaning 'of, relating to, or occurring in the spring'. In *Arabidopsis thaliana*, *FLOWERING LOCUS C* (*FLC*) is one of the key determinants of vernalization, and encodes a MADS box DNA binding protein. FLC acts as a floral repressor by repressing expression of the floral integrator genes *SUPPRESSOR OF CONSTANS OVEREXPRESSION 1* (*SOC1*) and *FLOWERING LOCUS T* (*FT*). Before prolonged cold exposure, *FLC* is expressed and its chromatin contains active histone marks such as histone H3 lysine 36 tri-methylation (H3K36me3). Chromatin structure remodeling from

the active (euchromatic) to a repressed (heterochromatic) state is associated with an increase of H3K27me3 following vernalization, and *FLC* expression is reduced (Groszmann et al., 2011). In *FLC* silencing or maintenance of the *FLC* repression state by vernalization, three cold-responsive noncoding RNAs (COOLAIR, COLDAIR, and COLDWRAP) derived from different loci within the *FLC* locus, are considered to be involved in *A. thaliana* (Itabashi et al., 2018; Shea et al., 2018)

Brassica rapa L. comprises commercially important vegetable crops consumed worldwide and is related to the model plant *A. thaliana*. *B. rapa* is vernalization-sensitive and has four *FLC* paralogs (*BrFLC-1*, *BrFLC-2*, *BrFLC-3*, and *BrFLC-5*), repressed by vernalization (Kawanabe et al., 2016; Shea et al., 2018). Variation in the requirements for vernalization exists in many plant species and this is important for the quantity and quality of crop production. In vegetative crops, early bolting and flowering caused by a low vernalization requirement can limit the potential for yield increases or devalue the products.

MATERIALS AND METHODS

Six Chinese cabbage inbred lines (R29, S27, RJKB-T02, -17, -23, -24), four commercial *B. rapa* cultivars ('Harunosaiten', 'Harusakari', 'Natsumaki50nichi' (Watanabe Seed Co, Ltd.), and 'Yellow sarson'), and three doubled haploid lines (BRA2209, Homei, and Osome) were used as plant materials. Seeds were surface sterilized and grown on agar solidified Murashige and Skoog (MS) plates with 1 % (w/v) sucrose under long day (LD) condition (16 h light) at 22 °C. For vernalizing cold treatments, 14-day seedlings on MS plates were treated for four weeks at 4 °C under LD condition (16 h light) or four weeks at 4 °C and then seven days in normal growth condition.

BrFRIb or *BrFLC1*, *2*, or *3* cDNA fragments was amplified by RT-PCR using primers designed to add *Bam* HI and *Sac* I restriction sites to the 5'- and 3'-ends, and PCR products were cloned into the pGEM®-T Easy vector (Promega). DNA fragments of *BrFRIb* or *BrFLC1*, *2*, or *3* cDNA was inserted into *Bam* HI and *Sac* I restriction sites of pBI121. These constructs were transformed into *Agrobacterium tumefaciens* strain EHA105, and transformation of Col accession in *A. thaliana* was carried out by the floral dip procedure. Transgenic seedlings were selected through resistance to kanamycin and carbenicillin on a selection medium.

Chromatin immunoprecipitation (ChIP) experiments were performed as described by Buzas et al., (2011). One gram of cotyledons or first and second leaves of RJKB-T24 was used for ChIP analysis, and anti-H3K27me3 (Millipore, 04-449) antibodies was used. Purified immunoprecipitated DNAs and input DNA were sequenced by Hiseq2000 (36bp single-end). Low quality reads or adapter sequences were purged from the ChIP-seq reads using cutadapt version 1.7.1 and Trim Galore! version 0.3.7. The reads were mapped to the *B. rapa* reference genome v.1.5 using Bowtie2 version 2.2.3.

Total RNA from the first and second leaves, with and without cold treatments, were isolated by SV Total RNA Isolation System (Promega Co., WI, USA). We prepared two sequence libraries for RNA-seq with two replicates and they were sequenced using an Illumina HiSeqTM 2000 (100 bp, PE). Non-coding RNAs were identified from RNA-seq data.

RESULTS

We transformed *BrFRIb* into Col accession of *A. thaliana*, which lacks FRI function, and 14 independent T_1 plants were obtained. The flowering time segregated in T_2 plants that derived from three independent T_1 plants, and the flowering times of T_2 plants with transgene were later than the T_2 plants without the transgene or wild type Col. We confirmed induction of *AtFLC* in these late flowering transgenic plants, indicating that BrFRIb had a function like AtFRI.

Transformation of a 35S promoter::*BrFLC1*cDNA, 35S promoter::*BrFLC2*cDNA, or 35S promoter::*BrFLC3*cDNA construct into Col accession of *A. thaliana*, whose own *FLC* was not expressed because of loss of function of AtFRI, revealed that transgenic plants with overexpressed *BrFLC1*, *BrFLC2*, or *BrFLC3* showed late flowering, confirming that all three BrFLCs function as floral repressors like AtFLC.

We examined H3K27me3 states in non-vernalized (2-day cotyledons; 2d-C, 14-day leaves; 14d-L), vernalized (BrV1), and following return to warm conditions after vernalization (BrV2) by

ChIP-seq in *B. rapa*. Between non-vernalized and vernalized samples at similar developmental stages, H3K27me3 levels at the whole genome level had correlation coefficients of 0.97 and 0.99, indicating that vernalization does not lead to a genome wide change of H3K27me3 levels. Following return to 22 °C after vernalization, an increase of H3K27me3 was observed in all *FLC* paralogues, which started at a nucleation site and spread 5' to 3' along the gene (Fig. 1).

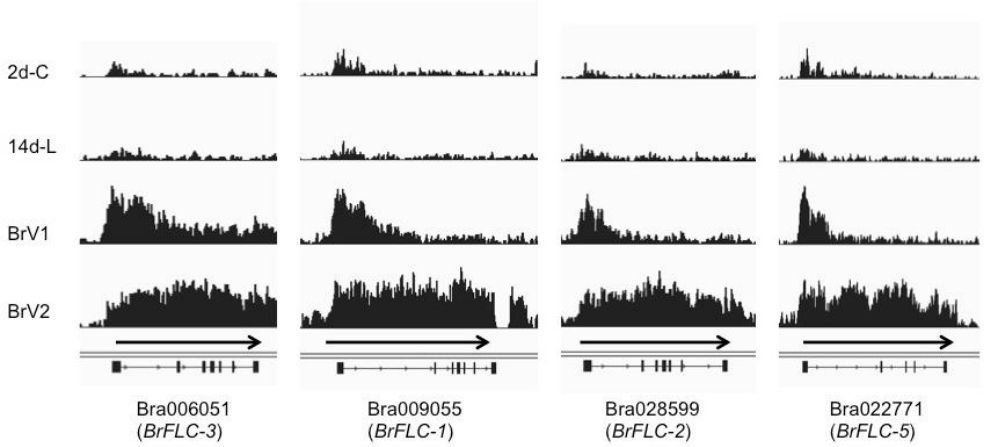

Fig.1. Visualization of H3K27me3 peaks by Integrative Genomics Viewer (IGV) in *BrFLC* loci. 2d-C and 14d-L are non-vernalized samples and BrV1 and BrV2 are vernalized samples. 2d-C, 2-day cotyledons; 14d-L,14-day leaves; BrV1, seeds were treated for 4 weeks at 4°C (vernalization); BrV2, seeds were treated for 4 weeks at 4°C and plants were transferred to the normal growth conditions for 12 days after vernalization.

To identify the relationship between expression levels of *BrFLC*s and level of vernalization requirement, we examined the days to flowering (scores) after four weeks of cold treatment in nine lines whose expression levels before cold treatment were examined. Scores were used for the evaluation of flowering time, because some late flowering lines did not flower within 100 days. 'Yellow Sarson' and Homei were early flowering, while Osome and BRA2209 were late flowering (Fig. 2). There was a high correlation between *BrFLC*s expression level and flowering time (r = 0.73, p < 0.05) (Fig. 2), suggesting that the expression levels of *BrFLC*s before cold treatment is associated with vernalization requirement.

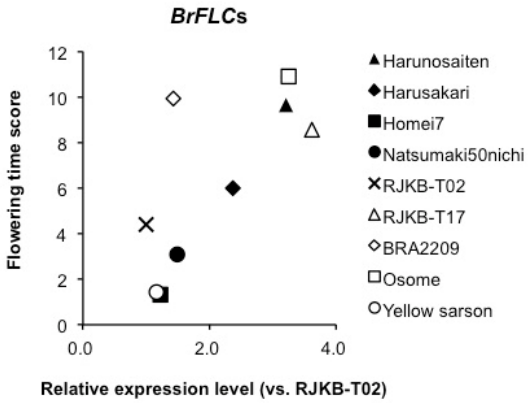

Fig. 2. The steady state expression level of *BrFLC*s is associated with days to flower after four weeks of cold treatment. The relationship between *BrFLC*s expression level and flowering time score are shown. Relative expression level to RJKB-T02 is shown.

Whole genome transcriptome analysis was carried out using RNA-seq of 14-day first and second leaves with and without four weeks of cold treatments to identify the protein-coding genes

(mRNAs) or lncRNAs that have changed their expression levels by vernalization in *B. rapa*. Of the *BrFLC* loci (*BrFLC1*, *BrFLC2*, and *BrFLC3*), only *BrFLC2* contained a natural antisense transcript (NAT), termed BrFLC2as (Fig. 3). Interestingly, no transcripts homologous to the *A. thaliana* lncRNA sequences COLDAIR and COLDWRAP were found at any of the *FLC* loci in *B. rapa* (Fig. 3). Examination of the BrFLC2as during differential expression analysis of the RNA-seq data showed that it was not differentially expressed at the FDR < 0.05 level. On the other hand, qPCR analysis of BrFLC2as revealed that BrFLC2as transcription is induced during at the initial onset of cold treatment (Fig. 3).

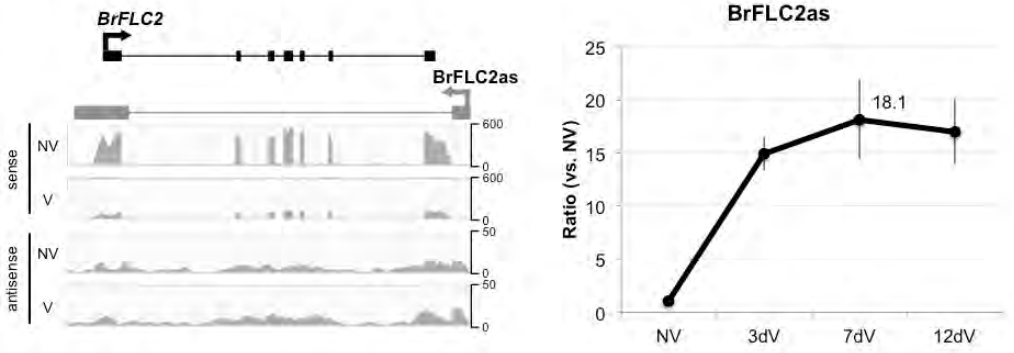

Fig. 3. Strand-specific read coverage plots for *BrFLC2*. Above each coverage plot, the mRNA transcripts are shown in black and the antisense transcripts (NATs) are shown in gray, with exons represented as thick bars and an arrow showing the 5' to 3' sense, and thin lines illustrating the introns. Expression level of BrFLC2as before and after cold treatment is shown in the right panel. qPCR results showing expression ratios normalized to NV for three NATs prior to cold treatment (NV), three, seven, and twelve days into cold treatments (3dV, 7dV, and 12dV, respectively).

DISCUSSION

The transformation of BrFRIb into Col delayed flowering time. In addition, BrFRIb induced *AtFLC* transcription, and this induced *AtFLC* transcript was suppressed by four weeks of cold treatment, indicating that BrFRIb and AtFRI have the same function. We confirmed all three FLCs function as floral repressors by overexpressing each *BrFLC* gene in Col accession of *A. thaliana*, suggesting that the sum of the three paralogous *FLC* transcripts is important for the contribution of *BrFLC*s to vernalization requirements.

We examined the relationship between the expression levels of *BrFLC* paralogs before cold treatment and the days to flowering after four weeks of cold treatment, and a positive correlation was observed between them. This suggests that the difference of the expression levels before cold treatment may be an indicator of the duration of cold required for vernalization.

One of the well-known environmental changes of H3K27me3 at *FLC* is an increase during vernalization and concomitant repression of the *FLC* gene (Helliwell et al., 2015; Itabashi et al., 2018). In *B. rapa* and *A. thaliana*, there is little change to genome-wide H3K27me3 levels by vernalization. Only *SOC1* and *FLC* showed similar H3K27me3 changes in *B. rapa* and *A. thaliana* following vernalization, suggesting that changes in H3K27me3 levels by vernalization are limited to decreased level of H3K27me3 in *SOC1* (consistent with the activation of its expression after vernalization) and increased H3K27me3 levels in *FLC* (consistent with a repression of its expression after vernalization) in both species.

In *A. thaliana*, H3K27me3 was observed in a specific region of *FLC*, downstream of the transcription start site, which is termed the nucleation region. Upon returning to warm conditions following vernalization, H3K27me3 spreads across the entire *FLC* locus. In this study, we showed that all four *BrFLC* paralogs had H3K27me3 in a limited region (putative nucleation region) after four weeks of vernalization, and that the H3K27me3 spreads across all of the *FLC* genes upon returning to warm conditions after vernalization. These results suggest that a mechanism similar to that in *A. thaliana* controls *FLC* repression by vernalization in *B. rapa*. In *A. thaliana*, noncoding RNA COLDAIR derived from the first intron plays a role in the recruitment of PRC2 to *FLC* (Itabashi et al., 2018). However, there is only weak sequence similarity in the first introns of the *FLC* paralogs in *B. rapa*

and *FLC* in *A. thaliana* (Shea et al., 2018). We could not find COLDAIR-like transcripts from the first intron of *FLC* in *B. rapa* during prolonged cold treatment. Without COLDAIR and COLDWRAP transcripts, it is not clear how H3K27me3 accumulation is mediated in *B. rapa* and raises questions regarding the role of non-coding RNAs in the vernalization of *B. rapa*.

ACKNOWLEDGEMENTS

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Plant geometry and density for management of canola crops in the Northern Agricultural Region of Western Australia

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ABSTRACT

Field survey and plot trials conducted in the northern agricultural region of Western Australia in 2015 and 2016 found that there is scope to improve canola (*Brassica napus*) yield by increasing the uniformity of spatial distribution of plants. Canola plant density in commercial fields ranged widely from 9 to 63 plants/m^2 averaging 23 plants/m^2. At the average density plants were spaced +/- 12 cm apart within the crop row. In a plot trial at densities below 40 plants/m^2 there was a yield improvement of 5% when plants were spaced evenly compared to unevenly. In this trial uneven treatments were analogous to the spatial distribution measured in the surveyed fields. The yield increase was due to an increase in pod number per plant and square metre from uniform distribution.

Key words

Canola, Brassica napus, density, uniformity, competition

INTRODUCTION

Plant density and spatial heterogeneity are important determinants of the degree of intracrop competition and seed yield within agricultural ecosystems. There are many studies of plant density relationships to yield for canola across varied environments. In Western Australia a recent trial series found an asymptotic relationship between plant densities of 10 to 80 plants/m^2 and seed yield (French, Seymour & Malik 2016). Studies of the relationship between spatial heterogeneity of canola (*Brassica napus*) and seed yield are much less common and limited to a few studies conducted in Canada (Angadi et al. 2003; Yang et al. 2014). From these studies yield improvements of up to 32% were reported from uniform plant geometry compared to uneven plant geometry. The greatest yield differences between the differing geometric configurations occurred at populations of 40 plants/m^2 or less and where yields were low by Canadian standards, ~1.0 t/ha. While these plant densities are far lower than the optimum advised for the Canadian environment they compare favorably with economic optimum densities recommended in Western Australia; ~25 plants/m^2 for low and medium rainfall sites (growing season rainfall <300 mm) and ~35 plants/m^2 for high rainfall sites (growing season rainfall 300-375 mm) (French, Seymour & Malik 2016).

These results present two means by which farmers may be able to increase profitability. Firstly at the plant densities commonly utilized in Western Australia yield may be greater if plants are uniformly spaced. Secondly seed costs for canola production have increased for growers who have switched from open pollinated to hybrid varieties by around $30/kg. Using better seed

placement methods and minimising intraspecies plant competition may enable seed rates to be lowered without compromising yield.

MATERIALS AND METHODS

One field survey and three field trials are presented. All studies were conducted in the Northern Agricultural Region of Western Australia.

A road-side survey of forty two commercial canola fields was undertaken from Geraldton to Merredin. Distribution of canola plants along 6 metres of row was recorded. Row spacing was also recorded to enable calculation of density. The results from this survey were used to inform treatments in a trial (Trial 3 below) comparing plants grown in the uneven spatial pattern, observed in fields, with a more uniform spatial arrangement.

In 2015 we conducted two seeding rate experiments using a precision seeder, which were located at Binnu (Trial 1) and Ogilvie (Trial 2), approximately 100 km north of Geraldton. An Agricola Italiana K series pneumatic precision drill was modified to seed canola Cv. Hyola404RR at four seed rates; 0.3, 0.5, 1.0 and 2.5 kg/ha. This type of seeder is commonly used to plant coarse grains such as corn or soybeans and for horticultural production. It distributes seed at equal distances along the crop row such that the spatial distribution of the plants is even along the row. The machine used had seven planter boxes spaced 50 cm apart (50 cm row spacing) hence plots were 3.5 m wide and they were 25 m long. The trial was arranged in four replicates of randomised complete blocks. The Binnu site was sown on April 16 and Ogilvie April 15. Soils at both sites were deep yellow sands (Orthic Tenosols). Measurements included plant population, biomass, yield component analysis and seed yield and quality. Yield components were measured from one plant per plot. Plants for yield component analysis were selected by measuring the diameter of 20 stems 3 cm from ground level in each plot, average stem diameter calculated and a representative plant selected.

In 2016 we conducted a uniformity experiment (Trial 3) at the Department of Primary Industries and Regional Development research station at Wongan Hills, Western Australia. It was sown on April 16 into a deep yellow sand. Plots were 20 m long by 2.0 m wide; a split plot design was used, 10 m of evenly spaced plants and 10 m of unevenly spaced plants. Plots were sown at a high seed rate and soon after establishment seedlings were hand thinned to achieve the desired plant densities and geometry. Plant densities were 80, 40, 20 and 10 plants/m². Plant geometry was spaced unevenly, simulating a commercial crop, and evenly, such that all seedlings were in line with each other across the rows of the plot. Measurements included Normalised Difference Vegetation Index (NDVI) at regular intervals, stem weight, grain yield components, grain yield and grain quality.

RESULTS

Field survey

Plant density of commercial crops ranged from 63 plants/m² to 9 plants/m² averaging 23 plants/m². As expected when plant density declined the variability in plant distribution along the row increased (Figure 2a). The variability in distribution observed was similar to what was achieved with uneven the hand thinned treatment in trial 3 at Wongan Hills (Figure 2b). For example at 20 plants/m² the standard deviation of the distance between plants in the row was approximately (+/-) 12 cm while this reduced to (+/-) 3 cm at 80 plants/m².

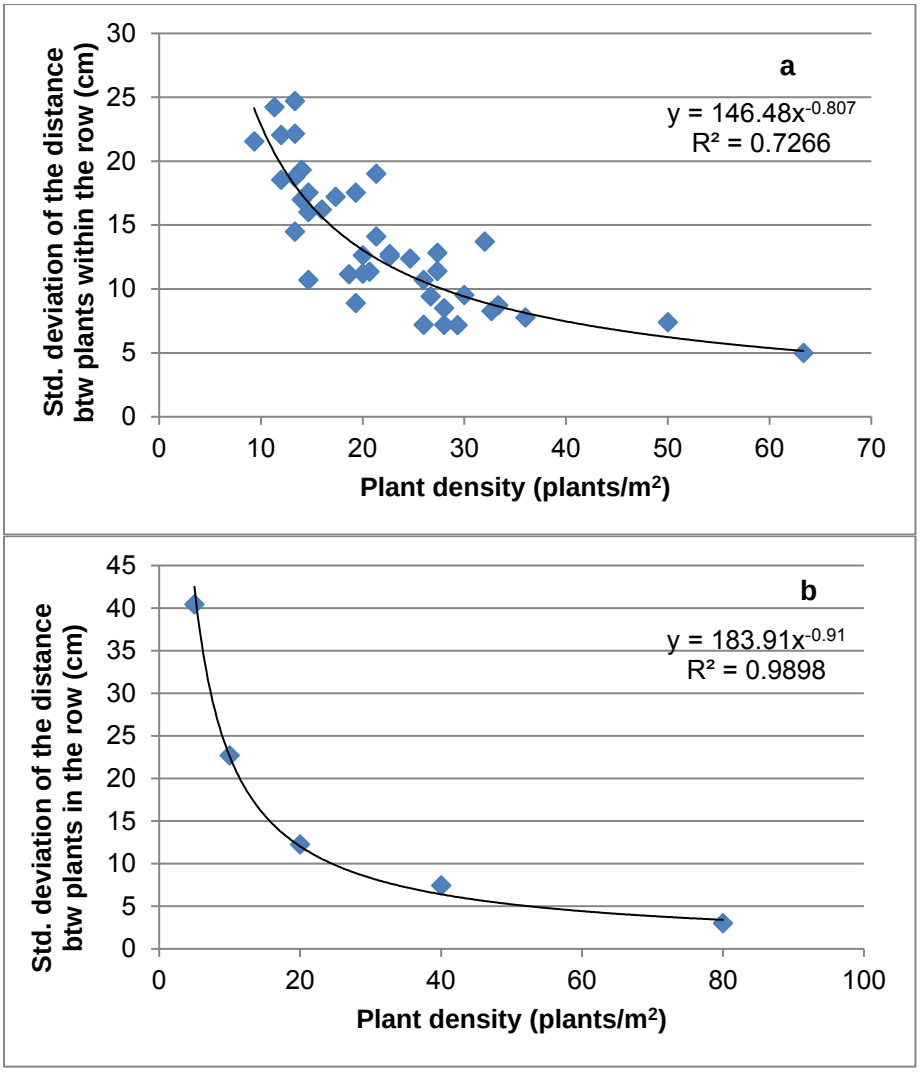

Fig. 2 (a) Relationship of plant density to uniformity of plants within the row from commercial canola crops (b) Plant density and uniformity of plants within the row of small plots hand thinned to represent a commercial crop.

Trials 1 and 2, 2015 precision seeder by plant density (Binnu and Ogilvie).

Annual and growing season rainfall were 373 mm and 248 mm and 398 mm and 286 mm for Binnu and Ogilvie respectively. Establishment varied between sites (Table 1). This occurred because conditions at seeding were not ideal with temperatures close to 30°C causing the seed bed to dry rapidly. Biomass measured on August 8 did not differ between the treatments at either site (Table 1). Plants in the lowest seed density treatments were 6-7 times heavier than those in the highest density treatment. The same response was observed for stem diameter, with lowest density treatments approximately 3 times wider than highest density treatments (Table 1).

Mean seed yield was 1814 kg/ha at Binnu and 2322 kg/ha at Ogilvie. At both sites there was a trend of decreasing yield from high to low seeding rate. At Binnu this was a significant response (Table 1). It should be noted that the 0.31 and 0.54 kg/ha treatments had very low plant density of 2.5 and 6.0 plants/m^2 respectively at this site. At Ogilvie the trend of lower yield at lower seeding rate was not statistically significant (Table 1).

The number of pods per plant increased with reduced plant density (P<0.001) with over ten times as many pods per plant in the 0.31 t/ha treatment compared to the 2.5 t/ha treatment (Table 1). Seed quality was unaffected by the seed rate (Table 1). At both sites seed oil% was

high, as would be expected from Cv. Hyola404RR, with no significant difference between treatments. Average seed weight was also unaffected by seed rate.

Table 1. Precision seeder density experiments at Binnu and Ogilvie in 2015 - Trial 1 and 2; plant density p/m2, Dry matter production (g/m2), single plant weight (g/plant), stem diameter (mm), pods per plant, seed per pod, 1000 seed weight (g), seed oil content (%), machine harvested yield (kg/ha).

Binnu									
Seed rate (kg/ha)	p/m^2	DM 10/8 (g/m^2)	Pt. wt. 10/8 (g/plant)	Stem dia. 17/9 (mm)	Pods /plant	Seeds /pod	1000 seed wt (g)	Oil (%)	Yield (kg/ha)
0.31	2.5	690	117	33	1369	na	4.10	48.2	1490
0.54	6.0	823	146	25	993	na	4.13	47.8	1697
1.01	15.1	843	104	17	396	na	4.12	47.9	2014
2.50	35.9	706	20	11	133	na	4.12	47.5	2057
Lsd	9.0		47	4	327		0.19	0.7	299
F Prob	HS	NS	HS	HS	HS		NS	NS	S
Ogilvie									
0.31	4.7	730	157	37	1622	20	3.87	47.6	2198
0.54	8.0	833	118	26	790	18	3.78	47.6	2315
1.01	15.4	751	59	20	357	15	3.80	47.8	2312
2.50	39.7	733	22	12	136	14	3.87	47.5	2463
Lsd	5.2		36	4	258	6.1	0.18	1.4	280
F Prob	HS	NS	HS	HS	HS	NS	NS	NS	NS

[1]NS = not significant, S = *P*<0.05, HS = *P*<0.001

Trial 3, 2016 Uniformity experiment (Wongan Hills).

Annual and growing season rainfall was 597 mm and 382 mm respectively. Higher plant density treatments increased ground cover (NDVI) on May 17 (*P*<0.001), May 30 (*P*<0.001) and July 5 (*P*<0.05) (data not presented) and reduced stem diameter (*P*<0.001) (data not presented), stem weight (*P*<0.001) and pods per plant (*P*<0.001) (Figure 1a). Plant uniformity did not affect NDVI (data not presented). Increased plant uniformity increased stem weight by 27% (*P*<0.05) and number of pods per plant averaged across density treatments from 180 to 230 (*P*=0.071), (Figure 1a) but had no significant effect on stem diameter, +4%. Seed oil concentration was also not affected by plant density or plant uniformity. Seed size increased with increased plant density (*P*<0.05), but there was no relationship between plant uniformity and seed size (data not presented). Seed yields were high, 3.0 t/ha average (Figure 1b), such that even at the lowest density of 10 plants/m² yields were above 2.5 t/ha. However, yield increased at plant densities higher than 10 plants/m² (*P*<0.001). Yield was not significantly altered by the uniformity of plant distribution however there was a consistent trend (*P*<0.1) of increased yield at even plant distribution.

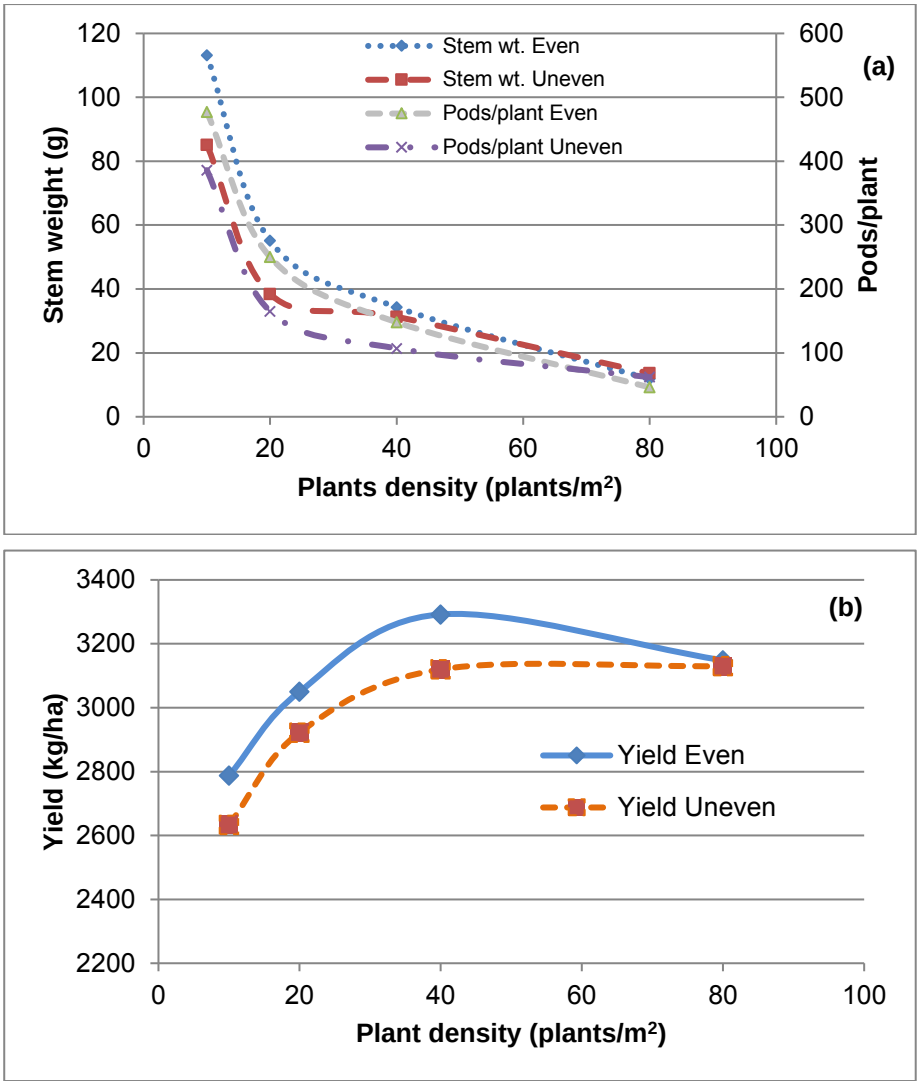

Fig. 1. Effect of plant density and spacing on **(a)** individual plant stem weight (g), pods/plant and **(b)** yield (kg/ha) at Wongan Hills in 2016

DISCUSSION

Plant distributions measured in canola stands in the northern region of Western Australia were quite uneven. Lower density stands were more uneven. Hence plant stands in lower rainfall zones are likely to have greater variability in plant distribution than high rainfall zones, because target (economic optimum) density is lower.

The results from Wongan Hills in 2016 (Trial 3) and Canadian trials (Yang et al. 2014) indicate that uniform plant distribution has a greater effect on yield at low plant density compared to high plant density. This is to be expected due to greater variability of spatial distribution at lower plant density. Considering this response Wongan Hills yield data was reanalysed excluding the 80 plant/m^2 treatment. Analysis of variance indicated uniform seed placement increased yield significantly (P<0.05) 2892 kg/ha to 3039 kg/ha or 5%. This yield difference occurred because at equivalent plant densities there were a greater number of pods per plant and per square metre (P<0.05) with even plant distribution compared to uneven.

Hence it would appear that even plant spacing is reducing intraspecific competition for resources at plant densities below 40 plants/m^2. Because the low rainfall zone of South Western Western Australia has a Mediterranean type climate in which terminal drought is a major abiotic constraint

water is often the most limiting resource. Studies of narrow-leafed lupin (*Lupinus angustifolius* L.) in this environment have shown that at wide row spacings soil water use is delayed until later in spring resulting in reduced plant water stress during grain filling as measured by leaf water potential (French & Harries 2006; French 2004). Interactions of canola to heat stress and terminal drought in the northern agricultural region of Western Australia were investigated by (Harries et al. 2018) who found plants sown on April 18 were exposed to lower temperatures at and after flowering, resulting in yield increases of 25% compared to plants sown on May 18. They also found spring irrigation increased stem sap flow in October and decreased pod abortion by 16%. These results suggest that the yield response observed from altering plant geometry and density could be linked to the amount of soil water available during the reproductive period.

The likelihood of achieving more uniform plant stands from a precision seeder is another aspect for consideration. Despite placing the seed accurately at the Binnu and Ogilvie trials there was a variation in plant density between the sites. This demonstrates that the proportion of seeds that emerge, and spatial variability, is dependent on the soil environment. An economic comparison of conventional and precision seeders for the production of hybrid canola found a precision seeder may be more cost effective in medium to high rainfall zones of Western Australia, where seed rates can be adjusted down from current levels to a greater extent (Cichy 2017). However, the yield density response curves using the precision seeder at Binnu and Ogilvie were similar to (French, Seymour & Malik 2016) using conventional seeders. If this is the case any reductions in seed rate using a precision seeder would need to be achieved through a higher field establishment rate. Comparisons of the two seeder types would be required to test this.

In conclusion these studies indicate that if plant uniformity can be improved from what is currently achieved yield is likely to be improved. Further field trials with narrow-leafed lupin and canola have been implemented in 2018 to better quantify this.

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A survey of Sclerotinia stem rot sclerotia in canola stems post-harvest

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ABSTRACT

Shortly after harvest, canola stems showing symptoms of sclerotinia stem rot were collected from ten sites across the Esperance, Western Australia district. Even in what was considered a low disease year, sclerotia were produced within canola crops. Sclerotia were found on the roots of plants at three sites. Most sclerotia were less than 5 mm in size closely followed by those of 5-10 mm. At the Scaddan focus site, most sclerotia were located within the first 10 cm of stem which reduces the potential effectiveness of lower harvest or swathing height to remove sclerotia and decrease inoculum levels. As an alternative method of categorizing sclerotia, those from the Scaddan site were also weighed to assess the variation in weight by size.

Key words

Sclerotinia sclerotiorum – sclerotia – canola – stem location – size

INTRODUCTION

Sclerotinia stem rot (SSR) of canola is a disease caused by Sclerotinia sclerotiorum which forms sclerotia in (or occasionally on) the affected plant; most are found within the stem where lesions form. The sclerotia are the resting bodies of the disease which can survive for a number of years in the soil. Sclerote size is correlated to apothecia production (Hao et al, 2003; Young et al, 2014) and survival over time (Harvey et al, 1994). SSR incidence is primarily the result of inoculum produced within a paddock (Boland and Hall, 1988; Young et al, 2014). Hence the number of sclerotia present in a paddock and their size contributes to overall inoculum pressure in subsequent broadleaf crop. So from an epidemiological point of view the population structure or size of sclerotia produced in affected crops is of interest and a small survey was undertaken to assess the situation in Esperance in the south eastern grain belt of Western Australia.

Mechanisms to reduce inoculum levels in a paddock include crop rotation, burning (Brooks et al, 2018) and burying (Duncan et al, 2006). Potentially, sclerotia produced in infected crops could be prevented from increasing the soil borne inoculum load by decreasing crop cutting heights, whether by swathing or direct harvest, so that a greater length of stem material is processed during harvest in order to remove the sclerotia that have formed within them. Sclerotia can then be destroyed through the use of a Harrington Weed Seed Destructor or removed post-harvest during grain cleaning. However the effectiveness of lowering harvest heights has had limited study. Therefore a secondary question is where sclerotia are formed in the stem and whether decreasing cutting heights could significantly decrease sclerotia numbers returning to the soil.

While the value of a post-harvest study may initially appear of limited value, another Western Australian study also done in 2016 using whole plants, by Brooks et al (2017), showed that over

90% of sclerotia formed in open pollinated varieties (the dominant type grown in the south-east) were found below the typical harvest height of 40cm.

MATERIALS AND METHODS

Thirteen survey sites were randomly selected and ten were infected; four in the high rainfall area (Coomalbidgup, Dalyup 2, Hopetoun and Howick), four in the medium rainfall area (Dalyup, Munglinup, Neridup and Scaddan) and two in the low rainfall (Beaumont and Grass Patch). The collection of SSR infected stems was based on their appearance; after harvest, all stems dry down but those affected by SSR are stark white and often have a shredded look from stem lesions. At each site affected stems were pulled from the ground, placed into sacks and taken back for processing. The collection was ad hoc since the initial aim was only to gather sclerotia from different environments for epidemiological work. Hence disease incidence of the sites was not assessed.

In the laboratory, stems were split longitudinally using a knife and the sclerotia inside were removed and measured on their longest side. Sclerotia size was recorded as falling into the categories <5 mm, 5-10mm, 10-15 mm and >15 mm. Very small sclerotia (<2mm) were assessed for all sites apart from Scaddan. One hundred stems from the Scaddan site were assessed for sclerote location within the stem. Location categories from the base of the stem were 0-10 cm, 10-25 cm, 25-35cm and >35cm with sclerotia from beneath the base on underground plant parts designated 'root' sclerotia. Loose sclerotia where the original location was unknown, were counted separately. Additional stems from Scaddan had sclerotia removed, sorted into the same size fractions as above and individually weighed, but due to low numbers, the size fractions of 10-15 mm and >15 mm were combined into >10 mm. Sclerotia from Scaddan (n=1,725) of different size groups were also put over a 2mm sieve and those above and below counted.

RESULTS

Ten of the 13 sites visited were affected by SSR while three showed no signs of disease. At nine of the ten sites, sclerotia of <5 mm were the most common, averaging 52% (Fig. 1). Very small sclerotia (<2 mm) comprised 5% of the <5 mm category. Sclerotia of 5-10 mm were the second most prevalent.

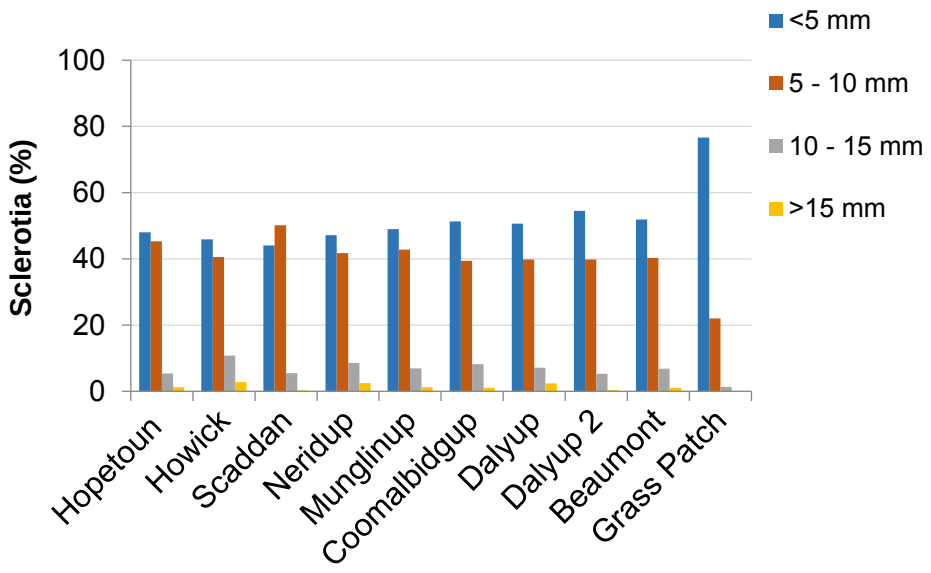

Fig. 1. Size distribution (mm) of Sclerotinia sclerotia from ten sites in 2016.

At Scaddan (Fig. 2), most sclerotia were found in the 0-10 cm portion of stem (57%), followed by root sclerotia and those at 10-25 cm (both 19%). Most of the sclerotia formed were 5-10 mm in size (51%). Of the <5 mm and 5-10 mm size categories, 12% and 2% passed through a 2 mm sieve but none over 10mm did. The average number of sclerotia formed was 11.4 per stem (median 11.0 per stem; standard deviation = 5.9). Less than one per cent of sclerotia were loose in the stem (Fig. 2), most were adhered to the inner sides or held in place by the pith.

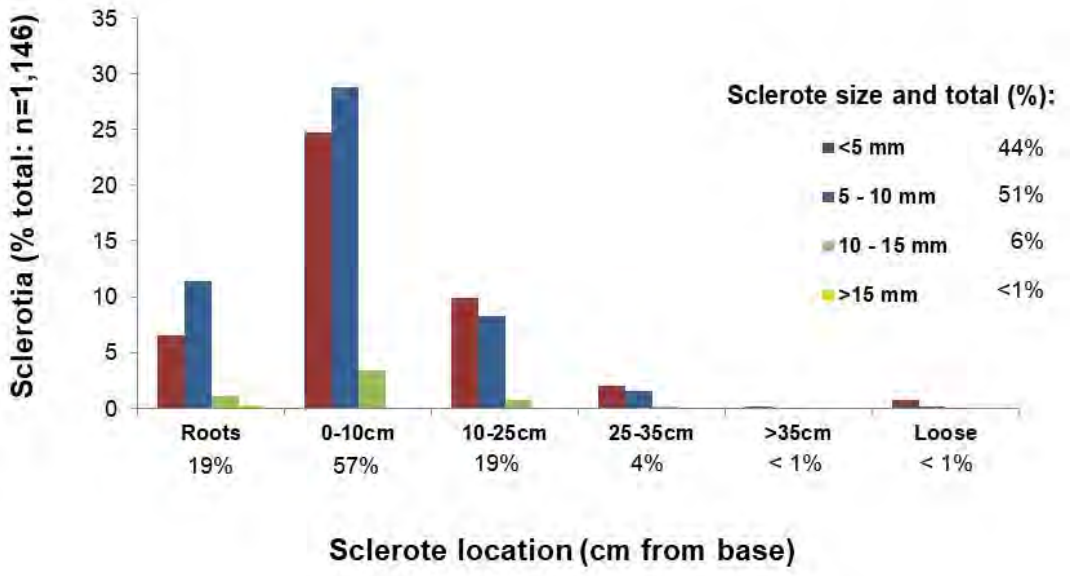

Fig. 2. Sclerote location (cm from stem base) and size (mm) with total percentage for each group for sclerotia from 100 stems at Scaddan in 2016.

The weight of sclerotia within each size group was assessed; in total 1,612 sclerotia were weighed. Fig. 3 shows how weights ranged from 0.5mg (<5 mm) to 292 mg (>10 mm) with overlap between the different size groups. Table 1 shows the variation in weight of the different groups; sclerotia became more variable in weight as their size increased.

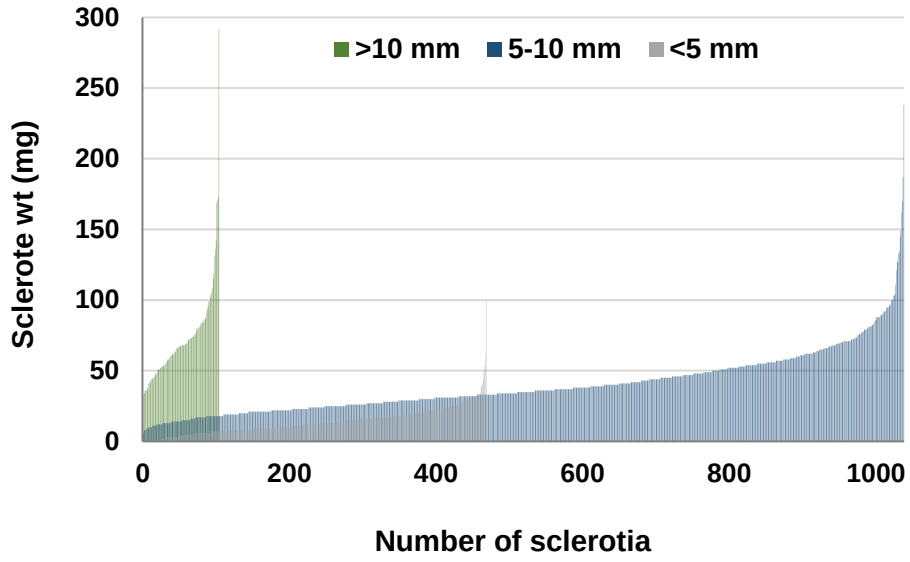

Fig.3. Sclerote weights of different size categories.

Table 1. Average and median weight and standard deviation of different sclerote size fractions at Scaddan.

		Weight (mg)		
Fraction (mm)	Number	Average	Median	Std dev
<5	469	14	12	9.6
5 - 10	1039	40	35	22.3
>10	104	73	68	35.7
All	1612	34	29	25.9

Table 2. Average number of sclerotia per stem sample based on size (mm) and type (root or stem) for samples where root sclerotia were Present or Absent.

Root sclerotia		<5 mm	5-10 mm	10-15 mm	<15 mm	Total
Present	- all sclerotia	5	7	1	0	13
	- root sclerotia only	1	2	0	0	4
	- stem sclerotia only	4	4	1	0	9
Absent		5	4	0	0	9

Table 3. Percentage of sclerotia at each location within stem samples from Scaddan where root sclerotia are Present or Absent.

Root sclerotia		Roots	0-10cm	10-25cm	25-35cm	>35cm
Present	- all sclerotia	28	57	13	2	0
	- stem sclerotia only	-	79	18	3	0
Absent[1]		-	60	30	7	1

[1] Loose sclerotia comprised 2% in samples where root sclerotia were absent

At three sites (Scaddan, Grass Patch and Howick) sclerotia had formed on below ground parts of the plant. These were flattened and followed the contours of the roots closely. At Scaddan 58% of stem samples had root sclerotia and these varied in length from less than 5 mm (34%) to greater than 15 mm (1%) but most (58%) were of 5-10 mm, the average size of sclerotia at Scaddan. Root sclerotia were nearly always (97%) present with sclerotia in the lower stem (0-10cm).

Similar numbers of sclerotia formed in the stems of samples regardless of whether they had root sclerotia or not (Table 2). However samples where root sclerotia were absent had more sclerotia form higher in the stem (Table 3); 19% had no sclerotia within 10cm of the base compared to 3% for those with root sclerotia present.

DISCUSSION

Although the incidence of Sclerotinia stem rot (SSR) in the south-east was considered minimal in 2016, infected stems were readily found at most of the sites visited. The site Dalyup 2 had a fungicide spray at 20% bloom but sclerotia still formed, presumably a result of late infections. These observations suggest that the soil borne inoculum levels in many canola paddocks are regularly being supplemented when canola is grown.

Surprisingly, given differences in rainfall and presumably crop management, the size distribution of sclerotia was similar across most sites, with the <5 mm portion most prevalent followed by 5-10 mm. While the small <5 mm sclerotia are likely to degrade before larger ones, the remaining proportion of larger sclerotia (47%) is considerable and would be expected to survive an even longer period, although overall degradation rates in WA soils are unknown.

Given that most sclerotia formed in the first 10cm of stem, the data from this survey indicate that reducing harvest or swathing height from the typical 40–50 cm currently used to as low as 10cm, to increase the proportion of sclerotia collected would still result in 77% of the sclerotia escaping collection. This result is a much higher proportion than that found by Brooks et al (2017) partly because that survey, while gathering and dissecting taproots, did not report any root sclerotia on them. However, even if root sclerotia are excluded, at this site only 29% were formed more than 10cm above the base of the stem.

While sclerotia readily form underground on the roots of other crops (eg. carrots), there is no mention in the literature of their occurrence on canola. The relatively high proportion of sclerotia that formed on roots at the Scaddan site means that they are worthy of further investigation to determine their incidence and viability. Although root sclerotia were not found at all sites, this does not preclude their formation as pulling stems from the ground may have dislodged the sclerotia so that only stems from the sandiest topsoils retained them. Whether the root sclerotia are a product of a stem lesion or a second separate point of direct (mycelial) infection underground is unknown, but as they were nearly always associated with the presence of lower stem (0-10 cm) sclerotia, they may be the result of the same low lesion.

In order to define and monitor paddock inoculum loads we need better ways of describing sclerote populations in studies such as these so that the proportions most likely to survive and propagate disease in coming years can be identified. While sieving sclerotia into different size fractions is the quickest, the weight of sclerotia can be quite variable given the variety of shapes they take. However a combination of sieving (for speed) and weighing will eliminate extremely light sclerotia to identify the different components of the sclerote population.

Grass Patch had exceptionally high levels of small <5 mm sclerotia which was a result of the thinner stems from that site which was primarily due to the low rainfall environment they were growing in. Stem diameter has been associated with decreased sclerote size in other studies,

too (Brooks et al, 2017). Could stem diameter be manipulated, through higher sowing rates, to reduce sclerote size and increase the turnover of the inoculum pool? Or does the increased density result in higher disease levels and inoculum?

To better understand how inoculum levels fluctuate, surveys that build on this one would need to be done across a wider area of the WA environment and ideally over a range of seasons.

ACKNOWLEDGEMENTS

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Determining the critical period for yield and quality in canola

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ABSTRACT

We conducted field experiments at two locations in southern Australia at Wagga Wagga (NSW), and Riverton (SA) to identify the critical period in canola when the crop was most sensitive to stress. We applied successive 100 °Cd shading periods (15% PAR transmitted) from early vegetative growth until maturity. The components of yield, its distribution on the plant, and the impact on seed quality (oil and protein) were assessed on hand-harvested areas from each shaded treatment and compared with the unshaded control. Despite the significant difference between the two sites for yield in the unshaded control (450 g m^{-2} at Wagga Wagga, and 340 g m^{-2} at Riverton), the critical period was consistent at both sites extending from 100 to 500 °Cd after the start of flowering (BBCH60), and centered 300 °Cd after BBCH60. Seed number (seed m^{-2}) was reduced by an average of 48% in the critical period, generated in equal parts by reduced pod m^{-2} in the early part of the period, and reduced seed pod^{-1} in the latter part. Reduced seed number was partially compensated by an increase in seed size of 29%. Seed oil content declined and protein content increased under shading in the critical period, while both oil and protein yield (kg m^{-2}) were reduced by 40-50% and 30-40% respectively. The critical period is coincident with the greatest number of near-open buds and newly opened flowers, which are highly sensitive to assimilate supply for ovule development. Both pod abortion and restricted capacity for compensatory growth of surviving pods are consequences of assimilate restriction on developing ovules. Identification of the critical period in canola provides a useful target for breeding and management strategies to maximize productivity.

Key words

Stress, yield components, seed number, seed size, rapeseed

INTRODUCTION

Canola (*Brassica napus* L.) or edible oilseed rape is the third most important oilseed produced globally with annual production increasing 2-4-fold in many of the major producing countries in the last 20 years. Canola has expanded from relatively reliable temperate growing areas in which it is well adapted, into more marginal and drier areas which combined with the predicted impacts of climate change will increase the future exposure of canola to abiotic stress such as temperature extremes and water deficit. As a result, there is an increasing need to understand the effects of the intensity, timing and duration of stress on yield determination to target breeding and management strategies to increase canola productivity.

The critical period for yield determination is defined as the physiological stage in which abiotic stresses have the largest impact on yield determination. Critical periods are typically determined using successive and discrete periods of shading to reduce the photosynthetic assimilates available for growth, mimicking the effects of abiotic stresses. The critical period for yield determination has

been defined in this way for numerous crops including cereals, grain legumes and sunflower. Yet despite its global importance as an oilseed crop, the critical period for canola has not been similarly determined. Previous studies have used either shading (Tayo and Morgan 1979; Habekotte 1993; Iglesias and Miralles 2014; Labra et al., 2017) or defoliation and targeted irrigation (Tayo and Morgan 1979; Zhang and Flottman 2018) to investigate source-sink relationships in canola and to investigate the plasticity of yield components. Defoliation and irrigation are likely to have confounding effects on yield (Lake and Sadras, 2014), and the shading experiments reported to date have used different intensity, timing and durations of shading. In most cases the shading extended for the entirety of the flowering period during which overlapping physiological processes including the growth and/or abortion of branches, flowers, pods and seeds are occurring simultaneously. Thus the existence of a discrete critical period most sensitive to stress, and the key physiological mechanisms involved remain unknown.

We report two field experiments in diverse environments in southern Australia in which successive 100 ^{o}Cd shading treatments were used to determine the critical period for yield determination in field-grown spring canola. The components of yield, its distribution on the plant, and the impact on seed quality (oil and protein) were also assessed.

MATERIALS AND METHODS

Field experiments were carried out in 2016 at two sites in south-eastern Australia: 25 km north of Wagga Wagga (-34.96; 147.31) in southern New South Wales (NSW); and Riverton in South Australia (-34.12; 138.76). Detailed description of the field experiments can be found in Kirkegaard et al., (2018). At both sites, the fast-mid spring hybrid variety Pioneer 44Y89 (CL) was sown on 2nd and 3rd of May at Wagga Wagga and Riverton, respectively, in plots 4m to 6m in length and comprising 6 rows spaced 0.25m apart. The crops were managed using recommended agronomy to manage weeds, pests and diseases and were fertilised to avoid nutrient limitations to growth. At both sites, crops received N and P as starter fertilser at sowing and were top-dressed with urea in winter. The crop at Wagga Wagga had 133 kg N ha^{-1} (133N) in the soil pre-sowing and received 211N in-crop. At Riverton the crop had 124N in the soil pre-sowing and received 100N in crop.

The shading treatments commenced around 30 days after sowing (das) at Wagga Wagga and 48 days at Riverton which corresponded to the 4-6 leaf stage at both sites and continued to physiological maturity (15 shade timings at Wagga Wagga and 14 at Riverton). Treatments were arranged in a randomised complete block design at each site with four blocks, and the shaded areas (2m x 3m Wagga Wagga; 2m x 1.5m Riverton) were established within the randomised plots in each block. Shading was applied with stabilised nylon net set onto steel frames that were mobile, and adjustable so that the height could be adjusted as the crop grew. The reduction in incoming photosynthetically active radiation was 85% at both sites.

Crop phenology was recorded weekly using the BBCH development code (Meier, 2003). The start of flowering was taken as the point when 50% of plants had one open flower (BBCH60), and this was used as the point of origin for the consideration of the timing of the critical period. The end of flowering corresponded to BBCH69. The phenology was recorded in thermal time (^{o}Cd), using a base temperature of 0^{o}C, and defined as SUM (Average Daily T – 0^{o}C base temperature). Bordered quadrats comprising the central 4 rows (1m^2) were sampled from each shaded area of the plots at maturity and oven dried to determine seed yield and yield components. Shoot biomass, yield, harvest index, pod number, seeds per pod, seed number, and individual seed size were determined. A subsample of seed was also analysed for oil content and protein using Near Infrared Reflectometry (NIR) (Foss Infratec 1241) with local calibrations derived for mass spectrometry (protein) and NMR (oil). Weather data (rainfall and

temperature) was collected from each site using automatic weather stations while radiation data was sourced from patched point data from the Bureau of Meteorology.

The effect of timing of shading was tested using one-way analysis of variance in GENSTAT16 separately for each environment as there was unequal numbers of shade treatments at the two sites. Dunnett's test was used for the pairwise comparison of all shade treatments with the unshaded control to reduce the family-wise error rates at P<0.05 (95% confidence interval), and to reduce the likelihood of false positives associated with multiple comparisons. The yield, its various components along with seed oil and protein were expressed as a ratio of the unshaded control for each timing of shading.

RESULTS

At Wagga Wagga flowering occurred from 17 August to 26 September and the crop was harvested on 8 November, 11 d after the last shading period was completed. The growing season rainfall (625mm) was double the long-term average and evenly distributed, while the temperatures were relatively mild throughout the growing period with no significant frost (< 0 ^{o}C) or heat (> 30 ^{o}C) events during the reproductive period. There were few yield-limiting abiotic stresses at the site during the experiment. At Riverton, flowering occurred from 25 August to 5 October and the crop was harvested on 7 November. The growing season rainfall (519mm) was above the long-term average of 461mm and evenly distributed with a dry period in spring from early October to early November. There were numerous frost events (<0 ^{o}C in canopy) from 2 August to October 23 coinciding with the reproductive period, which were the main potential yield-limiting factor at the site. There was only one period of heat (32.6 ^{o}C) on October 9.

The yield of the unshaded controls was 453 g m^{-2} at Wagga Wagga and 340 g m^{-2} at Riverton (Table 1). These yields were relatively high compared with National Variety Trials (NVT) average yields for Clearfield hybrids (2005-2014) in the same areas of 281 (39) g m^{-2} at Wagga Wagga and 247 (38) g m^{-2} at Riverton (Kirkegaard et al., 2016), reflecting the above average rainfall in 2016, favourable growing conditions and timely agronomy at the sites. The shade treatments had no impact on biomass but highly significant impacts on all yield components at both sites (Table 1). Oil content at both sites was above the industry standard of 42% oil, but was higher at Wagga Wagga than Riverton presumably reflecting the more favourable conditions during the seed filling period. Protein content showed the reverse trend with higher protein at Riverton than Wagga Wagga which is consistent with previous observations that oil and protein often trade-off in canola seed (Rondanine et al., 2014)

Table 1. Average (SE) canola yield and yield components for the unshaded controls in two at Wagga Wagga, NSW and Riverton SA in 2017. The significance for ANOVA on the effect of the shade treatments on yield and its components at both sites is also shown (* P<0.001, ** P<0.01, ns = not significant P<0.05).**

Site		Grain yield (g m^{-2})	Biomass (g m^{-2})	Harvest Index	Pods (m^{-2})	Seeds (pod^{-1})	Seeds (m^{-2})	Seed size (mg)	Seed oil (%)	Seed protein (%)
Wagga Wagga	**Mean**	453	1347	0.34	6388	21	133552	3.76	48.8	19.7
	SE	(5)	(22)	(0.01)	(410)	(1)	(2866)	(0.03)	(0.4)	(0.7)
	***P* (Shading)**	***	ns	***	***	***	***	***	***	***
Riverton	**Mean**	340	1252	0.27	5858	16.6	92774	3.65	42.6	21.1
	SE	(20)	(62)	(0.01)	(745)	(1.9)	(2779)	(0.10)	(0.2)	(0.2)

***P* (Shading)**	***	ns	***	**	***	***	***	*	***

Yield was not significantly reduced by shading at either site until the period commencing around 100 °Cd after BBCH60 and continuing up to around 500 °Cd after BBCH60 with the greatest impact at both sites (40-50% yield penalty) centred around 300 °Cd after BBCH60 (Fig 1A). At both sites, this critical period was approximately a 20 day period, commencing 10 days after BBCH60, and centred 20 days after BBCH60. Shading had no significant impact on biomass production at any stage (Fig 1B), including the critical period, so that the impact of shading on harvest index matched those for yield (Fig 1C).

The reductions in yield were almost fully accounted for by the reduction in seed number (Fig 1D) which peaked at 50 to 55% at both sites within the critical period. At Wagga Wagga this was driven in equal part by significant reductions in both pod number (Fig 1E) and seed per pod (Fig 1F), while at Riverton it was dominated more clearly by seed per pod, as the impacts on pod number were more variable. At Wagga Wagga, the impact on seeds per pod persisted beyond the impacts on yield, suggesting other compensatory processes were occurring. Indeed seed size increased by 20 to 30% at both sites during the same period that seed number was reduced Fig 1G).

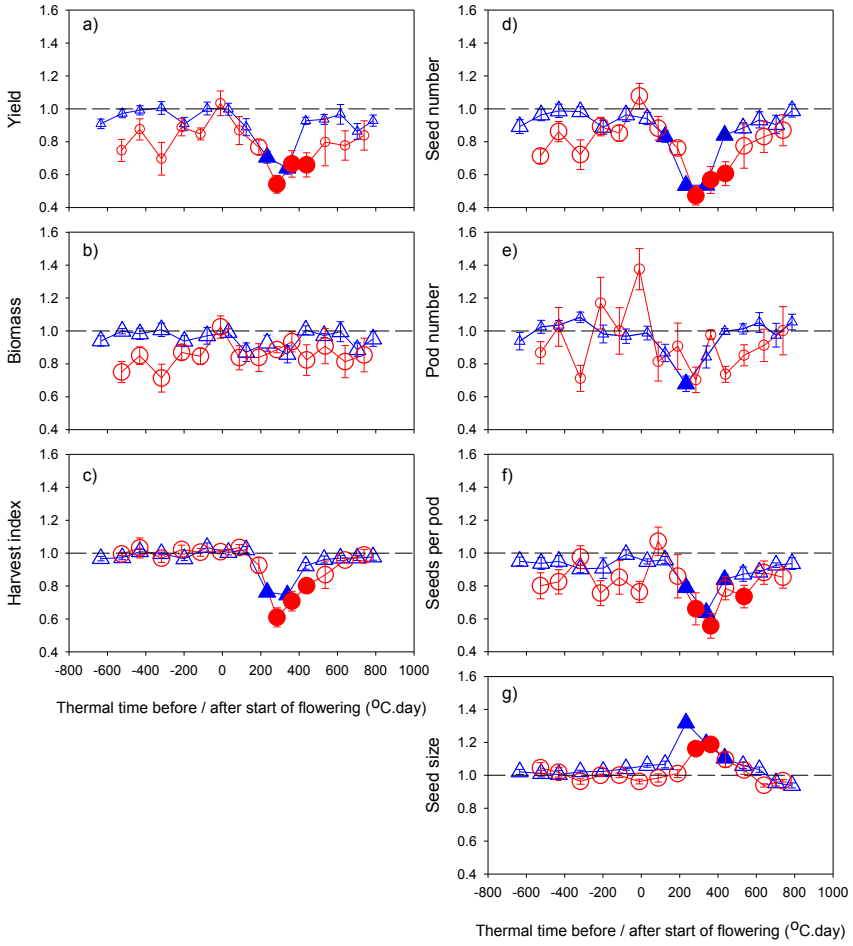

Figure 1. Effect of timing of shading on a) yield, b) biomass at maturity, c) harvest index, d) seed number, e) pod number, f) seeds per pod, and g) seed size of canola at Wagga Wagga (triangles) and Riverton (circles) compared to unshaded controls. Open symbols are not significantly different from control, while closed symbols are significantly different. Error bars are ±SE. Phenology scale is based on the unshaded controls. (from Kirkegaard et al., 2018)

At both sites, shading only affected the seed oil concentration within the same critical period as seed yield (200 and 400 °Cd after BBCH60) and oil content was unaffected at any other time (Figure 2A). The combination of reduced oil concentration and seed yield caused by shade reduced the oil yield by 40% to 50% in the critical period (Fig 2B). The combination of non-significant oil and seed yield impacts later in the season generated a significant reduction in oil yield of 18% at Wagga Wagga when shade occurred 700 °Cd after flowering. Seed protein content increased under shade during the same period that oil content decreased (200 to 300°Cd after BBCH60) at both sites, although the effect was not significant at Wagga Wagga (Fig 2C). This reflects the negative correlation between seed oil and protein content observed at both sites (Table 2). The combined effect of shade on seed yield and seed protein content reduced protein yield significantly at both sites (30 to 40%) during the critical period (Fig 2D). In summary, shading during the critical period centred around 300 °Cd after BBCH60, reduced oil yield by 40 to 50% and protein yield by 30 to 40% but had little impact outside the critical period.

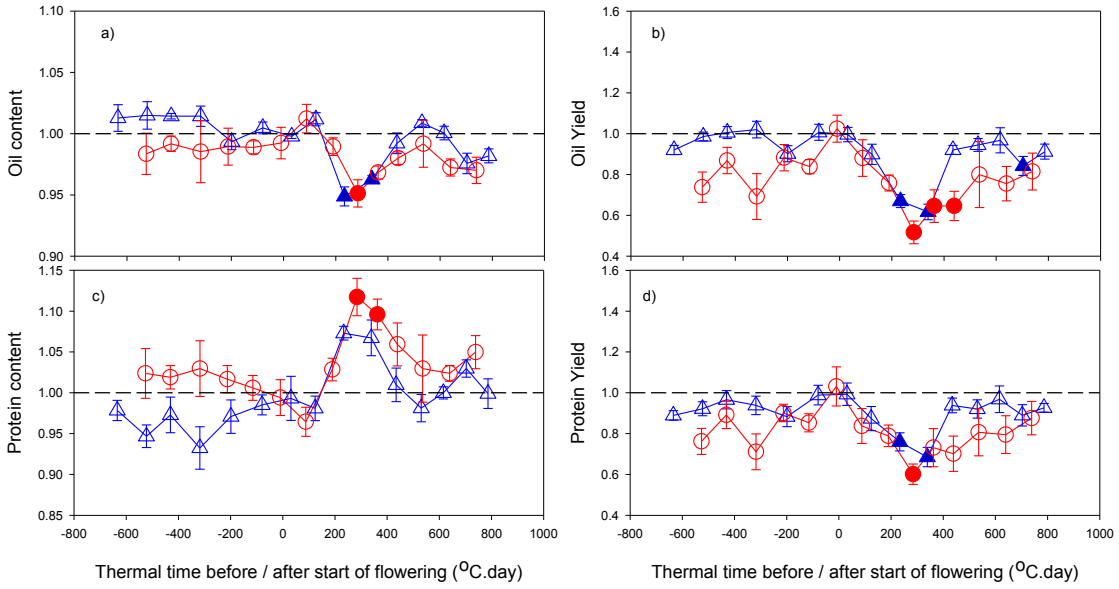

Figure 2. Effect of timing of shading on a) oil content, b) oil yield, c) protein content and d) protein yield of canola at Wagga Wagga (blue triangles) and Riverton (red circles) compared to the unshaded controls (from Kirkegaard et al., 2018).

DISCUSSION

The favourable growing conditions at both sites were ideal to investigate critical periods without confounding by additional stresses caused by water deficit or extreme temperatures. We have clearly identified a relatively discrete critical period for canola yield lasting from around 100 to 500 °Cd after BBCH60, and centred around 300 °Cd after BBCH60 at both sites. This concurs with the general consensus that the flowering period is the most critical for yield formation (Habekotte, 1993; Iglesias and Miralles, 2014; Zhang and Flottman, 2018). Comparisons with other field studies are somewhat difficult due to the different timings, durations and intensity of shading that were used,

the definitions of the flowering period, and the conditions under which the plants were grown. In one of the only previous studies using multiple (3) discrete successive shading treatments in a glasshouse, Tayo and Morgan (1979) identified a similar critical period. Most other published field experiments have not used sufficiently discrete periods of shading to identify critical periods. For example Habekotte (1993) and Iglesias and Miralles (2014) both used shade (60% and 50% respectively) for the entire anthesis to maturity period, generating 50% and 15% reductions in yield respectively, but a specific critical period could not be identified. Zhang and Flottmann (2018) used 60% shading at two periods (5 weeks during flowering; pod-fill to maturity) and both treatments reduced yield by 25%, with no evidence of increased sensitivity of one or the other. In the most recent study, Labra et al., (2017) surprisingly found no impact on seed yield from 70% shade imposed during the entire flowering period (BBCH61 to BBCH69) for field-grown canola in Chile, as a result of full compensation through a doubling of seed size. Thus, to our knowledge, this is the first study of field-grown canola that has clearly identified a relatively discrete critical period.

The results of two previous studies provide a possible explanation for the critical period identified. Tayo and Morgan (1979) found that pods that have experienced a shortage of assimilates when the flowers from which they developed were at the bud or early opening stage of development, had a limited capacity for compensatory growth – they had reduced husk and mean seed weights, and fewer seeds per pod. Thus, a lack of carbon assimilates on flowers and buds at that stage is particularly harmful as it not only reduces pod number directly, but reduces the capacity for compensatory growth in the pods and seeds when assimilate supply returns to normal. Habekotte (1993) has shown in field-grown rapeseed crops that the weight of flower buds and flowers (i.e. the sensitive stage in question) peaks around 350 to 400 ^{o}Cd after flowering, which coincides with the critical period identified in our study. Put simply, the large impact on yield at that critical time is generated by the direct impact of assimilate on pod number, and by the limitation imposed on the capacity of surviving pods and seeds for compensatory growth.

Our study found a reduction in oil content that coincided with the critical period for yield, well before oil biosynthesis at the crop level would be at its peak. Since 90% of the oil in the seed is located in the embryo, factors influencing embryo growth may influence seed oil content. Thus lack of assimilates to flower buds and newly-opened flowers may influence the capacity of the embryo to accumulate oil at a later stage. Seed oil content and protein content are often negatively correlated in canola (Rondanini et al., 2014). We found protein content increased under shade in the critical period when seed oil content decreased, but overall our results were consistent with those of Labra et al., (2017) in which protein content was quite conserved. In stark contrast to Labra et al., (2017) we found significant reductions in both oil and protein yield in the critical period, driven largely by the reduction in seed yield, but also by reduced oil content.

We hypothesize that the critical period coincides with the point in time when the maximum number of sensitive flower buds and recently opened flowers are present on the plant. A reduction in assimilate supply to these organs not only reduces pod number, but also the capacity for the surviving pods derived from them to compensate by restricting both seeds per pod and seed weight. Reduced assimilate in the critical period also appears to reduce the capacity for the surviving seeds to accumulate oil, possibly due to impacts on the developing embryos. Identification of this critical period provides a target to investigate the physiological processes involved, genotypic variability in sensitivity to stress at that time, and management strategies to avoid it.

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discussions on the methodology for the experiments. Table 1 and Figures 1 and 2 have been published in Kirkegaard et al., (2018). This study was funded by CSIRO, NSW DPI, SARDI and the GRDC (Project code CSP00187).

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APSIM-Canola: A physiological context to improve canola agronomy

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ABSTRACT

The APSIM-canola model has recently been updated to better represent the effect of crop phenology and frost and heat stress on growth and yield responses of modern commercial cultivars across a range of different environments. These enhancements underlie recent research initiatives in yield gap analysis and improved agronomy. Analysis of yield gaps across the Australian cropping zone has shown that on average farm canola yields are around 50% of the potential. Characterisation of frost, heat and water stress risks has led to the identification of the optimal time to start flowering (OSF) for canola which varied across the Australian cropping zone. OSF was earliest in the low rainfall areas of Western Australia (late Jun to late Jul) and latest in high rainfall environments in Victoria and Tasmania (Aug-Sep). This has provided recommendations to growers to match sowing dates with varieties of suitable phenology to optimise potential yield, especially in novel early sowing systems. Simulation studies of earlier sowing systems have identified that early sowing (from early April) and increased nitrogen (N) application (e.g. >200kgN/ha at Wagga Wagga, NSW in average to wet seasons) optimise profitability and reduce risk. Other studies have demonstrated the economic potential of dual-purpose canola from early sowing of long-season cultivars through increased returns from livestock and grain in high and medium rainfall environments. These studies also highlighted that increasing rates of N application above the industry standard often increased profitability.

Key words

Phenology – APSIM – simulation – yield gap – dual-purpose canola

INTRODUCTION

Agricultural simulation models can be utilised to analyse complex problems in farming systems, address food security and climate adaptation, and scale to farm or region. They offer the possibility to increase our understanding by combining many individual processes and interactions with climate and soil resources. The APSIM-Canola model was developed in the late 1990's and has been well validated and utilised in many farming system studies since that time (Robertson and Lilley 2016). Such studies have included assessment of production risks and constraints, sowing date guidelines (summarised by Robertson and Lilley 2016, Kirkegaard et al. 2016a, Brill et al. 2016), and developed guidelines for grazing dual purpose crops (Lilley et al. 2015).

Recent research has improved the capacity of the APSIM-Canola model to simulate flowering time, growth and yield of more recently released canola cultivars. The effects of frost and heat stress on grain yield have also been considered. In the past decade we have used simulation studies to demonstrate the economic potential of dual-purpose and grain only canola in the high rainfall zone (Lilley et al. 2015), investigate earlier sowing systems in medium and low rainfall zones (Kirkegaard et al. 2016a), identify the optimal time to start flowering for canola (Lilley et al. 2017) and identify yield gaps between actual and potential canola yield across the Australian cropping zone (Lilley et al. 2016). In this paper we present key findings of those studies and consider the next challenges to improve canola productivity and profitability

RECENT MODEL IMPROVEMENTS

Validation of phenology parameters for new cultivars

Adapting crop phenology to specific environments is an important determinant of yield. Incorporating the physiological drivers of canola phenology into crop simulation models is essential to assist in agronomic and farming systems research. Recent expansion of canola production into new regions, changes in climatic conditions, diversification of farming systems and the rapid turnover of canola cultivars within the industry require improved understanding of phenological response to the environment and ongoing validation of cultivar phenology to maintain the relevance of simulation models and grower targeted tools such as YieldProphet® and ProductionWise®.

Whish et al. (2018) studied phenological response of 36 recently released canola cultivars to a range of photoperiod and temperature combinations in the field at Gatton and Canberra between 2015 and 2017. From the data we developed parameters for the APSIM Canola phenology model to simulate flowering date of those cultivars. The new parameters were independently validated against flowering dates recorded for 596 combinations of cultivar, site, year and sowing date across the Eastern Australian cropping zone from Southern Queensland to the Eyre Peninsula in South Australia (Brill et al. 2018). In that dataset, the start of flowering (50% of plants with one open flower) was predicted within 3 to 8 days of the observed date, depending on the cultivar with an overall error of 5.4 days. Whish et al. (2018) showed that further improvements to the functions accounting for vernalisation could improve flowering date predictions in warmer environments further which is critical for earlier sowing.

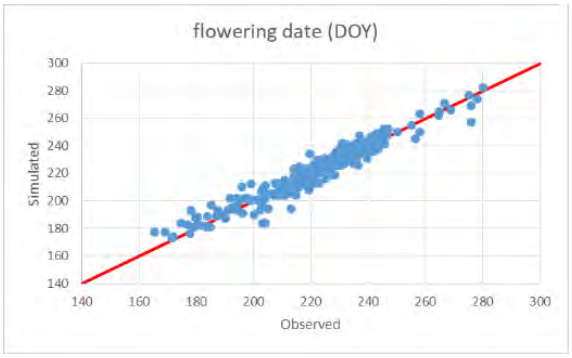

Figure 1. Simulated date compared to observed date for the start of flowering of 16 canola cultivars. Overall root mean square error is 5.4 days

Incorporating frost and heat damage in simulations

Sowing crops in the wheat belt of Australia in the traditional sowing window (May) minimised exposure of crops to frost and heat stress, however expansion of crops into new environments and across a broader range of sowing dates on larger farms and changing climate means APSIM must account for the effects of frost and heat stress on yield development. This is especially true of crops sown early to allow grazing. In a study of dual-purpose canola (Lilley et al. 2015), frost and heat indices were developed and incorporated into simulation predictions based on limited published data on the impacts of temperature extremes on yield in the field. A study on sowing time effects in NSW provided an opportunity for validation and showed that yield predictions were improved (Kirkegaard et al. 2016a). Recent work by Kirkegaard et al. (2018) defined the critical period for yield development using shading to restrict assimilate supply at different stages of crop growth, thus defining where yield development is most sensitive to stress. Based on this work, we have further refined the frost and heat stress indices in two ways. Firstly, the step-wise scale has been amended to a more continuous relationship and secondly, the period during which the crop yield is sensitive to the effects of frost and heat stress has been adjusted from an on/off relationship to one that more closely matches the critical period described by Kirkegaard et al. (2018) (Figure 2).

We have also validated these modified indices against the field data reported in Brill et al. (2018). Simulations of potential yield range from 0.1 to 6.0 t/ha and produced an overall root mean square error (RMSE) of 0.8 t/ha. Application of the frost and heat indices of Lilley et al. (2015) reduced the RMSE to 0.7 t/ha and the recent modified indices reduced the RMSE further to 0.6 t/ha.

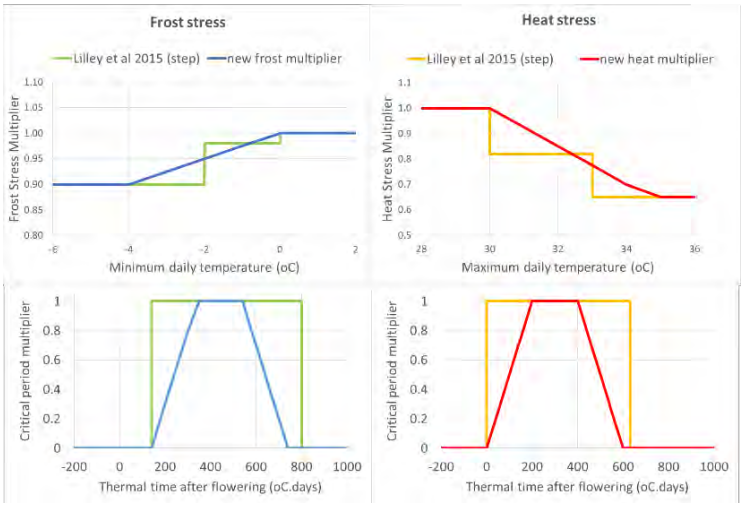

Figure 2: Original and new frost and heat stress indices (upper panels) and scalars for sensitivity to stress relative to thermal time after flowering (lower panels).

MODEL APPLICATIONS

Yield Gap Analysis

We used APSIM-Canola to conduct a yield gap analysis which is the first comprehensive national assessment of water-limited yield potential of canola in Australia (Figure 3; Lilley et al. 2016). The Yield Gap Australia website (yieldgapaustralia.com.au) is an interactive map-based tool to visualise the extent and geographic distribution of the gap between actual and potential production of field crops in Australia. Actual canola yields achieved by farmers (Ya) for each year from 1996 to 2012 were aggregated for 164 statistical local areas (SLA) where canola production covered more than 1000 ha. Using APSIM simulations we determined water-limited yield potential (Yw) at 4,043 weather stations using up to three dominant soil types per weather station, and management rules ensured yield was only limited by climate and water availability. The independently estimated annual Ya and Yw values were compared and the yield gap (Yw-Ya) and relative yield (Y% = 100 x Ya/Yw) were mapped. The interactive website enables growers and advisers to visualise and benchmark their own farm against local yields and yield gaps. The average farm yield (Ya) in Australia from 1996 to 2012 was 1.16 t/ha, while average potential yield (Yw) was 2.23 t/ha, resulting in a yield gap of 1.07 t/ha. Average site mean yields across a range of National Variety Trial sites in the period 2005 to 2014 confirmed that for elite varieties under experimental conditions, average yields of 2.5 to 3.0 t/ha were achieved (Kirkegaard et al 2016b). On average, grain-growers are achieving 52% of the water-limited yield potential. The canola yield gap analysis forms a basis for discussion about the causes of sub-optimal yields on farm, and allows research funders and policy makers to define the current extent and distribution of canola yield gaps in Australia.

Optimal Start of Flowering

Recent trends in agronomic practice towards earlier sowing systems (Flohr et al. 2018) highlight the need to better define the optimal start of flowering (OSF) for canola. We define the OSF as the range of dates in which it is optimal to start flowering to maximise yield. Crops which flower too early may have insufficient biomass or frost damage, while late flowering increases the risk of heat and water stress. We conducted a simulation analysis following the method of Flohr et al. (2017) where we determined the long-term average canola yield associated with a range of flowering dates at each location. The analysis showed the average frost, heat and water stress index associated with a range of flowering dates (Figure 4a). The OSF is a period when the combined effects of frost, heat and water stress on yield are minimised and is represented by simulated grain yield. The OSF was

defined as the range of flowering dates where long-term average yield fell within 5% of the maximum long-term average yield (Lilley et al. 2017). We have defined OSF for canola at 76 sites, comprehensively covering Australia's cropping zone (Figure 4b). The OSF is especially important for crops sown prior to the traditional sowing window (late April to early May) and is a first step to determine appropriate variety by sowing date combinations

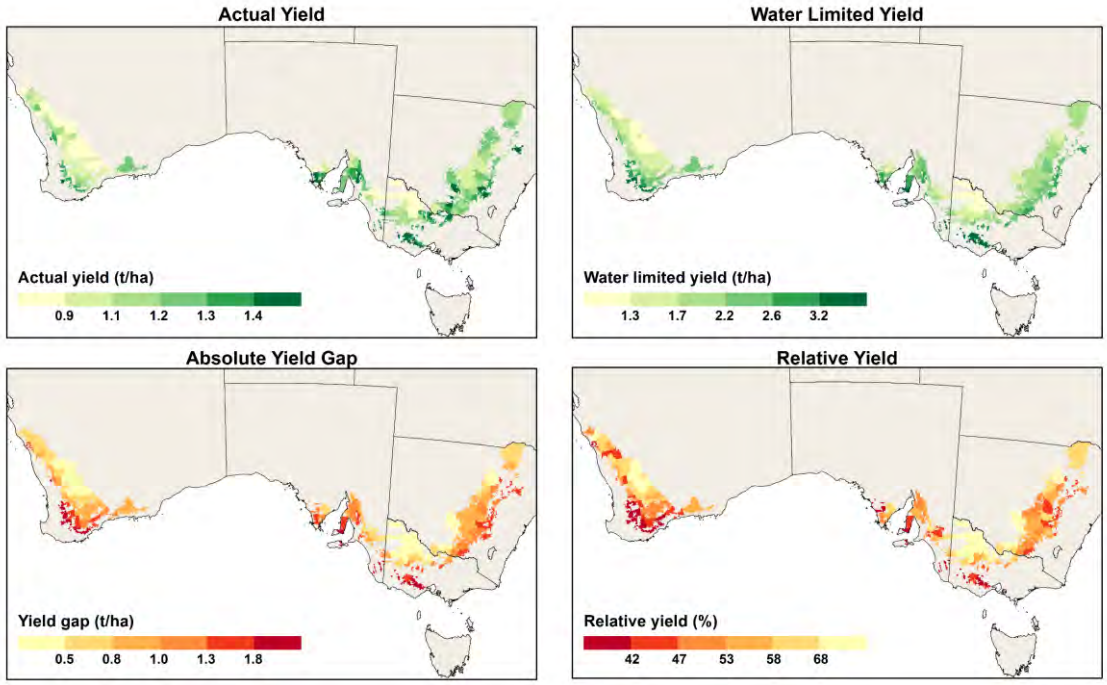

Figure 3: Actual and potential Australian dryland canola yield and yield gaps. 17-year averages (1996 to 2012) are shown for each statistical local area (SLA). The large spatial and temporal variability has been mapped at SLA scale and is available at www.yieldgapaustralia.com.au

to optimise yield in different environments. While this analysis accounts for abiotic stresses around flowering, the interaction of timing and severity of biotic stresses such as Blackleg and Sclerotinia in relation to flowering time will also impact the crop yield (Sprague et al. 2018).

The analysis provides recommendations to growers to allow them to match sowing dates with varieties of suitable phenology so that they can optimise potential yield. (https://grdc.com.au/10TipsEarlySownCanola). To assist grower decisions on sowing date and cultivar combinations at any location, we are developing an interactive App which incorporates historical weather record and also a seasonal forecast. Validated cultivar parameters are fundamental to the accuracy of the App.

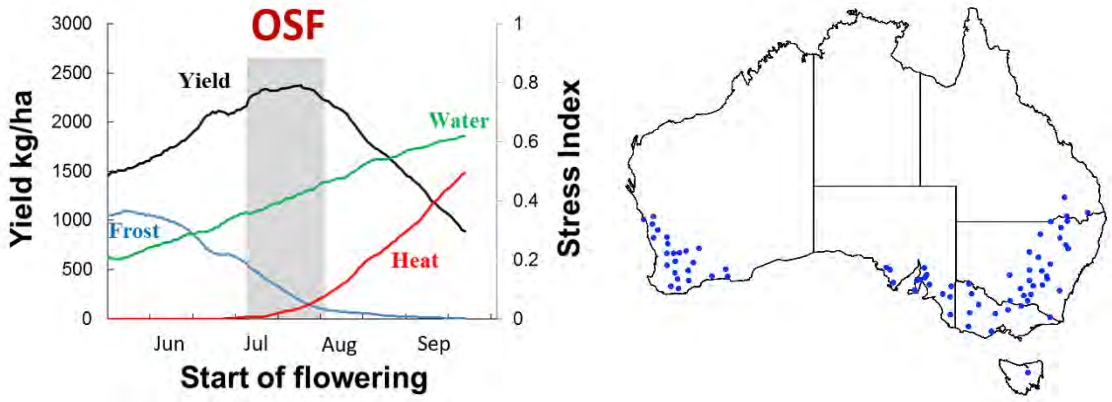

Figure 4: a) Relationship between average level of frost stress, heat stress and water stress and the simulated long-term average yield for a range of start of flowering dates at Condobolin, NSW. Optimal Start of Flowering (OSF, shaded) is defined as the period with the greatest 5% of long-term average yields. b) Location of 76 sites across Australian cropping zone where the OSF for canola was determined.

Early Sowing

Kirkegaard et al. (2016a) summarised previous experiments (2002-2012) investigating early sowing dates in NSW and proposed a re-evaluation of early-mid April sowing was warranted. Recent experiments investigated novel agronomy to optimise the opportunity to sow canola earlier than the traditional late-April to early May window across south-eastern Australia are presented by Brill et al. (2018). A comprehensive validation of APSIM-Canola to simulate these early sowing strategies, has been undertaken with several innovations used in the simulation approach.

New phenology parameters were used along with updated frost and heat stress functions. Growth parameters for hybrids were adjusted to improve radiation-use-efficiency and water-use-efficiency during the vegetative period by 10% based on evidence of Zhang and Flottmann (2016). The validation exercise covered 756 site x year x sowing date x cultivar x N or water management combinations. A comparison of observed and simulated yields showed that for each site, yield could be simulated with a RMSE between 0.4 and 0.8 t/ha, depending on the site and quality of the soil characterisation. Previous validation studies reported RMSE ranging between 0.3 and 1.1 t/ha (Robertson and Lilley 2016)

Based on this validation, an extrapolation of these strategies across 50 years of climate variability at a subset of low, medium and high rainfall sites has been undertaken using simulation and an economic study of farm gross margins (Meier et al. 2018). Briefly, these studies highlight the benefit of early sowing and the importance of adequate N application. The studies have shown that canola is highly responsive to N supply, with significant economic returns gained from the application of higher than typical rates of N-fertiliser across all rainfall environments.

Dual-purpose cropping

The development of dual-purpose canola systems, where the crops are sown earlier than normal and grazed prior to bud elongation and then managed for grain production (Kirkegaard et al. 2008, 2012; Sprague et al. 2015) has provided novel options to adapt canola to new production environments. Simulation studies into dual-purpose canola have demonstrated the economic potential of the grazed fodder as well as the harvestable grain in high and medium rainfall environments (Lilley et al. 2015). Figure 5 shows the grazing opportunity is greatest for earlier sown winter cultivars while fast developing spring cultivars provide a much smaller, but potentially beneficial opportunity. Season to season variability in grain yield was much greater than for fodder production. Maximum and least variable grain yield was derived from March sowing dates for the winter cultivar and April to May sowing for the spring cultivar at Young, NSW (Figure 5). In the study we also considered the likelihood of receiving a sowing opportunity from February to June at the 13 locations included in the study. Analysis of a range of N application rates showed that higher N application significantly increased fodder availability, as well as grain yield and the benefit was greater for longer season cultivars. Early sowing of winter or long-season cultivars produced significant yield increases over many seasons throughout the cropping zone.

THE FUTURE

As cultivars turn over rapidly in the canola industry it is necessary to continually provide parameters to describe newly released cultivars. Such knowledge is necessary for growers to match sowing date and cultivar phenology type to optimise yield, and requires knowledge of cultivar performance and particularly phenology characteristics as cultivars are released. Phenological characteristics can be derived through relevant experiments at carefully selected sites to cover a range of sowing dates and temperature regimes. Further research into genetic control of flowering to link genes to plant phenotype would provide a fast avenue to predict phenology performance of a cultivar by the time of

release. This physiological understanding of phenology responses would underpin agronomic advice in grower sowing guides and for use in tools such as Yield Prophet®, ProductionWise®and APSIM.

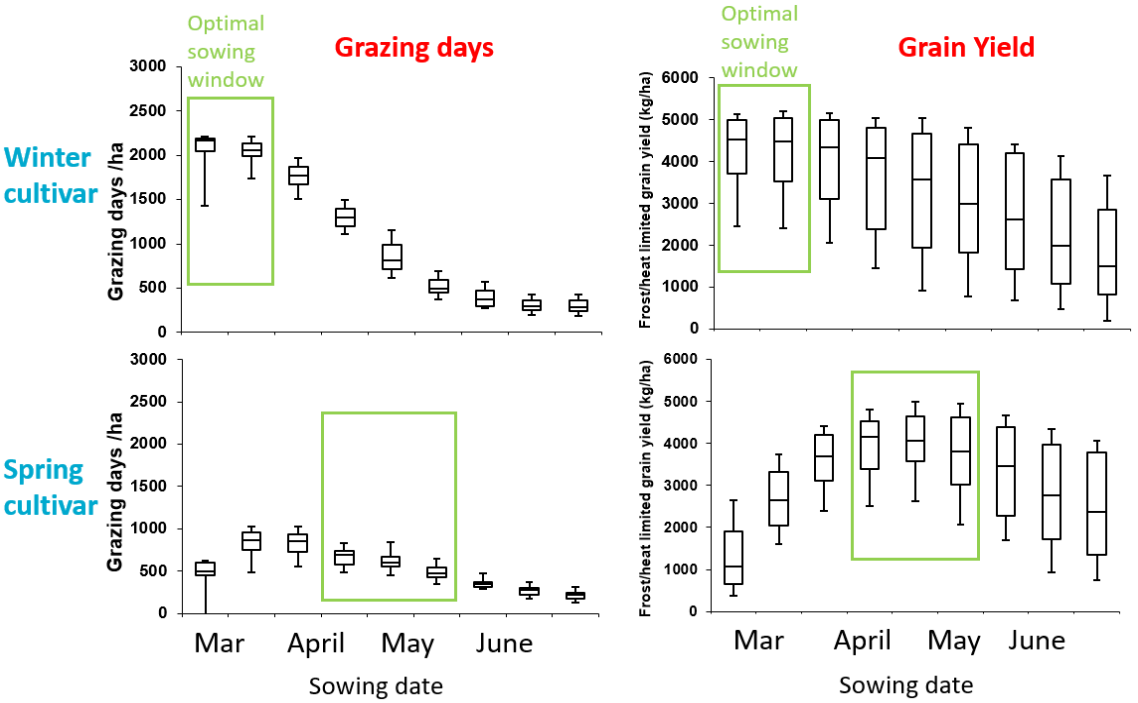

Figure 5: Simulated forage and grain yield over 50 years from dual-purpose canola across a range of sowing dates at Young, NSW. Box plots show seasonal variability and risk.

CONCLUSION

Simulated yield gaps across the Australian cropping zone have shown that on average farm canola yields in Australia are around 50% of the potential yield. Simulation has accompanied targeted agronomy to assist in development of novel, profitable canola production systems. Characterisation of frost, heat and water stress risks has led to the identification of the optimal time to start flowering for canola across the Australian cropping zone. Simulation studies have accompanied field experiments to identify crop management strategies including cultivar choice, fertiliser rate, sowing date and density, and timing of grazing which optimise profitability and reduce risk for different production environments in Australia.

ACKNOWLEDGEMENTS

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Canola establishment across central NSW – how to get it up?

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ABSTRACT

Canola establishment and uniformity was measured across 95 commercial paddocks within the low, medium and high rainfall zone of central NSW in 2017. Paddocks were selected to include various combinations of stubble management (burnt, retained and cultivated) and seeding system (knife point/press wheel, disc or scatter-plate). The survey design allowed for 10 paddocks with each combination of stubble management and seeding system from each rainfall zone. Plant establishment was measured across 15 x 1m^2 quadrants per paddock, and from this an establishment percentage was determined through knowledge of seed size and seeding rate. Plant population and uniformity was measured via the development of the "vacancy %" method, which used a 1m^2 section of mesh with 10 cm squares to count both plants, as well as total number of vacant squares (i.e. with no plants). Other data collected from the survey included: sowing date, seeding depth, fertiliser rate/placement/type, variety, seeding rate, GPS coordinates, sowing speed, soil type, seedbed moisture and crusting events that occurred post sowing.

The average canola establishment achieved across the 95 paddocks was 48%, and ranged from 17% to 86%. Establishment improved from low to medium to the higher rainfall zone, with the low, medium and high rainfall zone achieving a respective 43%, 47% and 55% establishment. Establishment improved as seed size increased, but this trend was not linear. There was an establishment increase by 6 percentage points (45% to 51%) from selecting hybrid seed over open pollinated seed. Reducing stubble loads via either burning or cultivation improved canola establishment by 10% (44% to 54%), and the main benefit was from the physical removal of the stubble, rather than the cultivated seed bed. Establishment varied across the three seeding systems, with the highest average establishment of 58% achieved with the scatter-plate seeding system, and then reduced to 49% and 41% with the respective knifepoint and disc machine seeding systems. It is likely that the main benefit of the scatter-plate seeding system was due to shallow seed placement combined with moist sowing conditions. Sowing speed was also critical for establishment, with a 16% (52% to 35%) reduction if speed increased from 6–8 km/hr to 13–17 km/hr. There was also a 7% (51% to 44%) reduction in establishment if P fertiliser was not separated from seed.

In summary, seed size, stubble removal, shallow seed placement, sowing speed and placement of fertiliser were key agronomic factors for improved canola establishment.

Key words

Canola establishment, fertiliser placement, sowing speed, stubble management

INTRODUCTION

Canola establishment has become an emerging issue within central NSW over the past decade, due to increased seed costs, reduced seeding rates, un-reliable autumn rainfall and sowing into marginal seedbed conditions.

Canola seed costs from 1990 to 2010 were relatively stable at 4% of total input costs, and have since increased to 14% of total input costs in 2018 (NSW DPI crop budgets). The increased seed cost is largely related to the dominance of hybrid (H) over open-pollinated (OP) varieties since 2011 to 2018 (Fig. 1). Target plant density during the era that OP varieties dominated the market place (pre-2010) was 50–80 plants m^2 (Wurst et al, 1997), and seeding rates between 3–5 kg/ha. However, since the adoption of hybrids the target plant density has been reduced to approximately 20–50 plants m^2 (Zhang et al 2016, Matthews et al, 2018) depending on rainfall zone. Currently, best management practice is to firstly determine target plant density (plants m^2) for your rainfall region, and then determine seeding rates via knowledge of seed size, germination % and estimate of establishment %.

Recent developments in understanding variety phenology, sowing time, and the adoption of slower developing spring varieties has bought forward the sowing window from 25 April to early April (Brill et al, 2018) for slower developing spring types. This broader, more flexible sowing window enables canola establishment to occur when seasonal conditions allow (i.e. rainfall events), rather than wait for the traditional Anzac Day trigger point to initiate sowing. This greatly improves the flexibility of the farming system, however establishing canola in early April has the disadvantage of high seedbed moisture dry back due to greater evaporation demands caused by higher temperatures. For example, at Parkes (medium rainfall zone) the average daily evaporation reduces from 5.9 mm, 3.7 mm to 2.2 mm across the respective months of March, April and May (Fig.2). This means that a shallow planted canola seed is at higher risk of seedbed moisture dry back if sown in early April compared to May, particularly in the warmer regions of NSW (i.e. Condobolin).

In summary, the margin for error in establishing canola is small, we are now sowing less seeds, they are costing more money, and we are placing those seeds in higher moisture dry-back conditions. Successful canola establishment is a significant factor, and risk in canola production. The primary purpose of this survey was to evaluate current canola establishment rates and uniformity of plant spacings. The secondary purpose of the survey is to evaluate management practices that affect canola establishment, such as stubble management, seeding systems, fertiliser and seed quality.

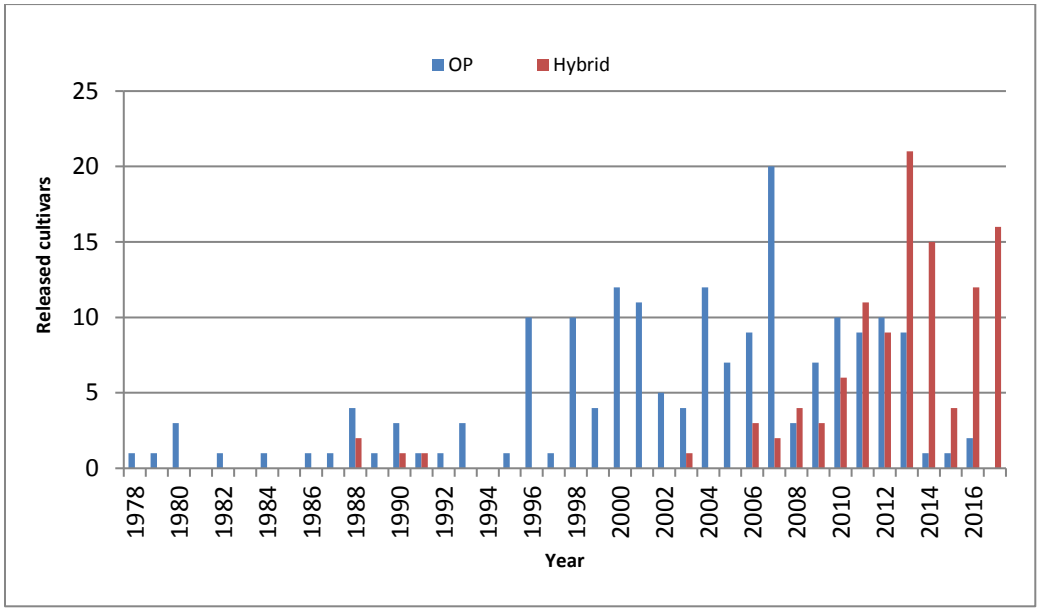

Fig.1. Year release of hybrid or open-pollinated (OP) canola varieties in Australia from 1978 to 2017 (Source: Steve Marcroft)

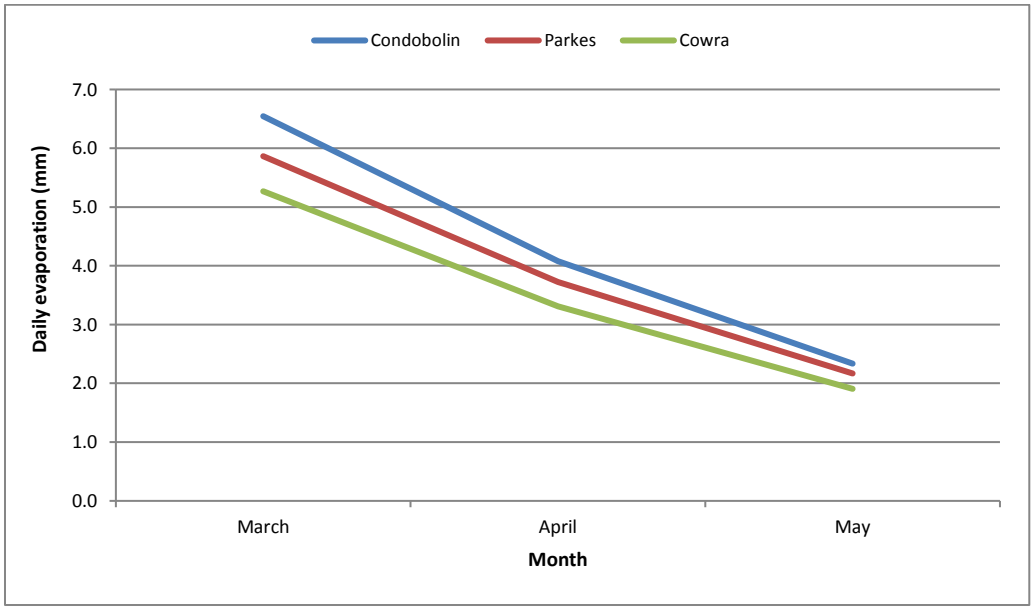

Fig.2. Average daily evaporation rates across central NSW during March, April and May (Source: CliMate)

MATERIALS AND METHODS

Field survey was conducted in 2017 across 95 commercial paddocks within the low, medium and high rainfall zone of central NSW (~30 paddocks from each rainfall zone). Paddocks were selected from the following localities; Tottenham, Tullamore, Trundle, Condobolin, Bogan Gate, Parkes, Forbes, Marsden, Manildra, Cowra, Young, Boorowa and Jugiong.

Paddocks were selected to include various combinations of stubble management (burnt, retained and cultivated) and seeding system (knifepoint /presswheel, disc or scatterplate). The survey design allowed for 10 paddocks with each combination of stubble management and seeding system from each rainfall zone (low, medium and high).

Plant establishment was measured across 15 x 1m^2 quadrants per paddock, and from this an establishment percentage (%) was determined via knowledge of seed size and sowing rate. If the seed size was un-known a sample was taken for seed size determination. Plant population and uniformity was measured via the development of the "vacancy %" method. This method relies on using a 1m^2 section of mesh with 10 cm squares. The mesh is used as the quadrant to count plants across 4 x 1 m linear rows, as well as to count the total number of vacant squares within 4 linear metres. From this plant population and a vacancy % was determined.

Other data collected from each paddock includes; sowing date, seeding depth, fertiliser rate/placement/source, variety, seeding rate, GPS coordinates, seed treatment, sowing speed, soil type, seedbed moisture conditions at sowing, crusting events post sowing.

RESULTS

A wet March and some timely rainfall events in April (Fig.3) allowed canola to be sown into favorable seedbed conditions across the low, medium and high rainfall zone of central NSW in 2017. March rainfall was above the long term average (LTA) at Condobolin, Parkes and Cowra, with an additional 41, 56 and 49 mm above the LTA, respectively. Seedbed moisture conditions were favorable at the start of April, and then started to decline from mid-April onwards. Additional rainfall around Anzac Day ensured favorable crop establishment for most of central NSW. In the paddock survey the earliest, median and last sowing date was 10 April, 22 April and 10 May, respectively.

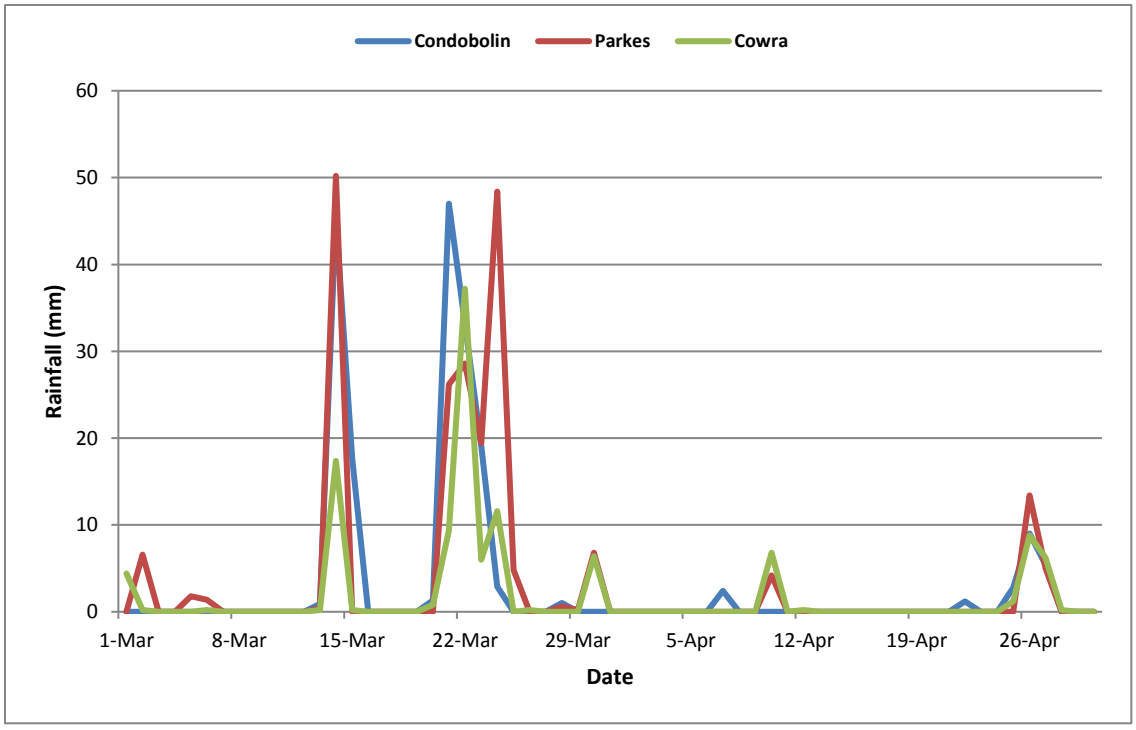

Fig.3. Daily autumn rainfall events across Condobolin, Parkes and Cowra in 2017

Across the 95 survey paddocks, 44 were hybrid and 51 were OP (Tab.1). Breeding type (H or OP) was largely influenced by growing season rainfall and length of growing season, with hybrids dominating the high rainfall zone (22 H, 1 OP), OP dominating the low rainfall zone (4 H, 28 OP), and even split between H and OP in the medium rainfall zone (H 18, OP 22). Refer to table 1 for further details

Table 1. Breeding type and herbicide tolerance of paddocks surveyed across the low, medium and high rainfall zone of central NSW in 2017

Breeding type	Rainfall region			Total
	Low	Med	High	
Hybrid	***4***	***18***	***22***	44
Clearfield	1	9	7	17
Conventional	3	4		7
Roundup Ready		2	2	4
Roundup Ready + Triazine Tolerant			2	2
Triazine Tolerant		3	11	14
OP	***28***	***22***	***1***	51
Triazine Tolerant	28	22	1	51
	32	40	23	95

Across the 51 survey paddocks that were OP, 16 paddocks were purchased seed and 35 paddocks were grower retained seed. Interestingly, only 4 of the 35 grower retained seed paddocks were not graded to seed size. Seed size grading ranged from 1.6 mm to 2 mm sieve size, however the sieve size was determined by the ratio of total seed graded to how much seed was required for the following sowing.

Interestingly, across all paddocks the average seeding rate was 2.5 kg/ha for OP (1.6–4 kg/ha), and 2.4 kg/ha for H (0.9–3.2 kg/ha). The average seed size from the hybrids was 4.9 g/1000 seed (203,610 seeds/kg), and 3.9 g/1000 seeds (257,106 seeds/kg) for the OP.

Table 2 illustrates a summary of results for establishment %, plant density and vacancy %. The average establishment was 48%, and the majority (between 1st and 3rd Quartile) of paddocks ranged between 38% and 58%. Establishment improved from low to medium to the higher rainfall zone, with the low, medium and high rainfall zone achieving a respective 43, 47 and 55% establishment.

Table 2. Establishment, plant density and vacancy % on 95 paddocks surveyed in central NSW in 2017

Frequency	Establishment (%)	Plants density (m^2)	Vacancy (%)
Min	17%	10	76%
1st Quartile	38%	19	56%
Mean	48%	26	47%
3rd Quartile	58%	32	39%
Max	86%	64	18%

Whilst each paddock had 36 pieces of information recorded, the main factor that differentiated establishment % was seed size. The mean seed size was 4.3 g/1000 seeds, and ranged from 3.3 to 6.6 g/1000 seeds.

Fig. 4 shows that establishment improved as seed size increased, however this trend was not linear and establishment decreased between the seed size of 4 and 4.5 g/1000. In addition to seed size,

there was an average increase in establishment by 6% (points) from selecting a hybrid seed over an OP seed (51% establishment for hybrid, and 45% establishment for OP).

After seed size, the top four agronomic practices that influenced canola establishment was seeding system (P=0.01), stubble management (P=0.02), sowing speed (P=0.02) and P fertiliser placement (P=0.05).

On average, reducing stubble loads via either burning or cultivation improved canola establishment by 10% (Figure 5). The main benefit appears to be from the physical removal of the stubble, rather than cultivated seedbed.

Table 2 shows that the average vacancy % was 47%, and ranged from 18% to 76%. Further research trials are being undertaken to develop calibration relationships between vacancy % and grain yield.

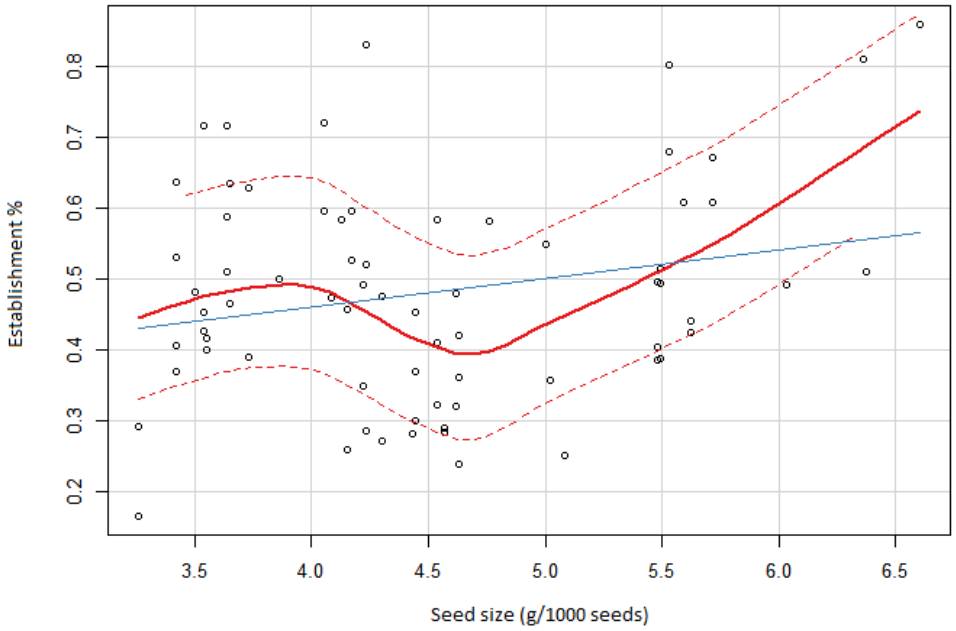

Fig.4. Fitted and observed relationship between seed size (g/1000 seeds) and canola establishment % with 95% confidence intervals.

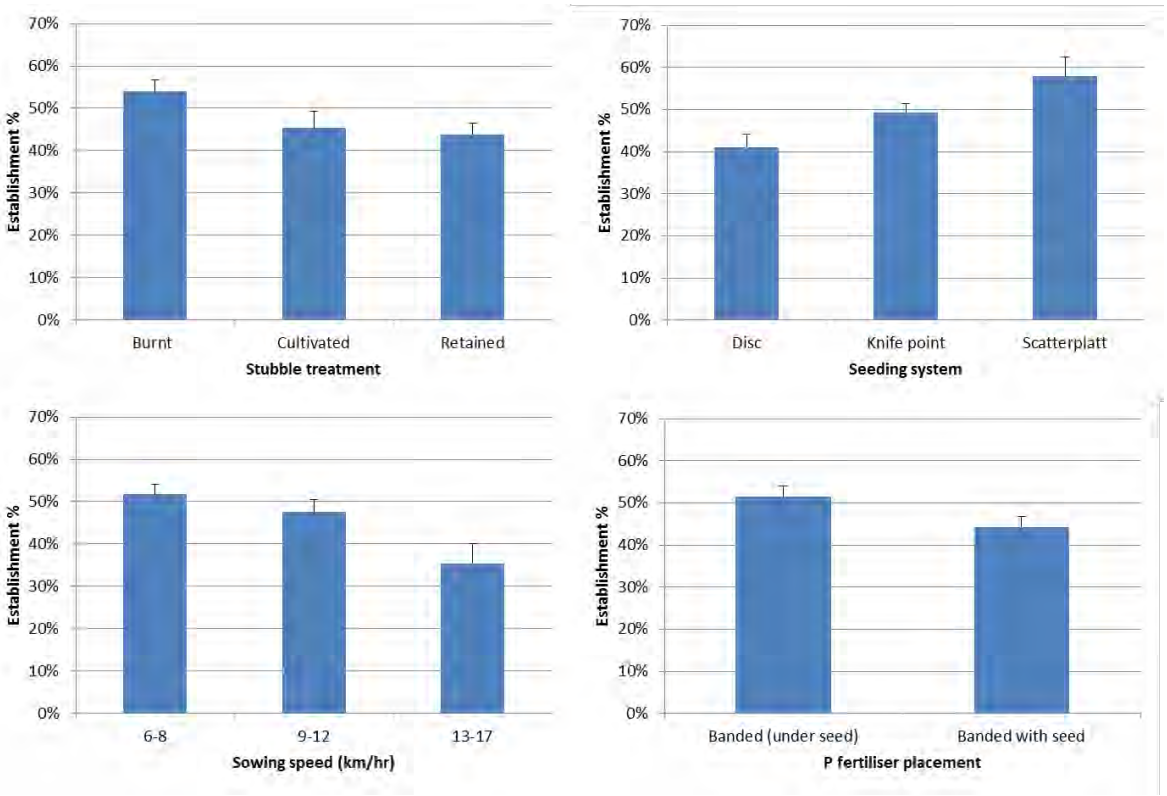

Fig.5. Effect of stubble management, seeding system, sowing speed and fertiliser placement on canola crop establishment. Standard error bars shown

Interestingly, old seeding system technology such as “scatter-plates” performed well in this survey. On average, the highest establishment of 58% was achieved with scatter-plates, and then reduced to 49% and 41% with the respective knifepoint and disc machine seeding systems. It is likely that the main benefits of the scatter-plate seeding system are due to shallow seed placement and favourable autumn conditions in 2017.

Establishment decreased as sowing speed increased, with a 16% establishment reduction if speed increased from 6–8 km/hr to 13–17 km/hr.

On average, there was a 7% reduction in establishment if P fertiliser was not separated from seed. There were two main groups of P fertiliser rates; 40% of paddocks had between 50–75 kg/ha MAP and another 40% had between 75–100 kg/ha MAP.

DISCUSSION AND CONCLUSION

Despite favourable sowing conditions in 2017, these results suggest there is an opportunity for improved canola establishment in central NSW. Effectively, growers are only establishing half of what they purchase, and if the autumn break was less favourable it’s likely to be much less. Traditionally growers would apply an extra 1–1.5 kg/ha of seed to compensate for poor sowing conditions, however this is no longer an option given the associated higher costs with hybrid seed.

Seed size was the main differentiating factor that improved canola establishment, and the other key agronomic practices were stubble removal, reduced sowing speed, shallow seed placement and P fertiliser separated from the seed. Hybrids were generally larger in size, and establishment was better than OP varieties. Further research is required to evaluate why the relationship between seed size and establishment was not linear.

The benefits of the scatter-plate seeding system in 2017 were likely to be associated with shallow seed placement combined with weather conditions that provided moist conditions for the canola

seedling to germinate and establish. Canola establishment results are likely to be different if moisture seeking was required. These results highlight the importance of taking time to set up seeding equipment, particularly with the disc seeding machine as they are typically used in high stubble load paddocks, sowing speeds are higher and have limited fertiliser separation from the seed.

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Canola: Pathways to profitability

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ABSTRACT

Recent modelling has established the time of sowing needed to achieve the optimal time of flowering in canola varieties at different locations to maximize yield by minimizing the combined risk of frost, heat and drought. However, managing canola production to achieve high profitability at lower risk in different environments involves a number of other important agronomic decisions regarding different inputs. In this study, canola crops were simulated with the APSIM v7.9 model at seven locations from a range of high, medium and low rainfall zones in New South Wales and South Australia. Crop production was simulated in response to a factorial combination of different agronomic decisions including cultivar type, cultivar phenology, sowing date, planting density and nitrogen (N) fertilizer rate. Cultivars included combinations of different phenology (fast, medium, slow) and vigor (hybrid, conventional, Triazine Tolerant). Sixteen weekly sowing dates were grouped into half-monthly sowing windows from 15-31 March to 1-15 July. Plant density was set to three different rates (15 to 75 pl m^{-2}) that spanned the range typically used by producers. Nitrogen fertilizer rates covered a range from deficient to more than adequate tailored for each growing region (from 50 to 500 kg N ha^{-1}). Crops were simulated using the historical weather record of the past 50 years in order to capture yield responses to weather variability. The combination of management practices resulted in approximately 200,000 crops simulated per location over the 50-year period. Location-specific gross margin data were obtained from industry advisors and published data, and were applied to simulated yield and management practices. Gross margin results were organised into the most profitable combination of management decisions for sowing window-rainfall decile combinations in each location using R studio. Sowing date, decile, cultivar type and nitrogen rate consistently contributed to highest average gross margins across locations, while cultivar phenology and planting density were important at fewer locations. To exemplify the straightforward decision framework for canola growers and advisers arising from the approach, we present the agronomic management predicted to maximize average profit for progressive sowing window-decile combinations at Wagga Wagga.

Key words

Gross margins – regression tree – sowing window – rain decile – APSIM

INTRODUCTION

The area sown to canola, and the productivity of canola crops, has increased in Australia since commercial production began in 1969 (Kirkegaard et al., 2016a). Within the past 10-15 years, this has included a five-fold increase in area to 2.5 million ha, and annual increases in grain yield of 34 kg ha^{-1}. These gains are attributed to substantial improvements in both cultivars and agronomy. Despite these gains, present canola yields are estimated to be ~50% of waterlimited potential

(Kirkegaard et al., 2016a). A possible contributor to this yield gap may be the timing and pattern of extreme temperature (frost and heat) experienced by crops in some years (Kirkegaard et al., 2016a). This climate driven variability in yield provides a source of risk that is often managed by moderating the level of crop inputs or accepting reduced yields to avoid the chance of catastrophic loss. An approach to assess the response to inputs and timing of management across a range of climate scenarios in terms of profitability and risk is likely to be beneficial to canola growers.

The purpose of this study was to evaluate the profitability of different management practices when used to grow canola crops in response to the diverse weather patterns that are experienced over many years. To achieve this objective, we adopted a simulation approach to grow crops at contrasting locations in response to long-term historical weather records, and collaborated with industry advisors to attribute gross margin data relevant to each location.

MATERIALS AND METHODS

Seven case study locations from a range of high, medium and low rainfall zones (HRZ,

MRZ and LRZ, respectively) were characterized for this study: Breeza and Young (HRZ),

Wagga Wagga (MRZ) and Condobolin (LRZ) in New South Wales, and Yeelanna and Brinkworth (MRZ), and Minnipa (LRZ) in South Australia. Soil properties were obtained from past characterization activities at the locations. Weather data for the locations was obtained from the SILO record (Jeffrey et al., 2001). Rainfall was highly variable, with a standard deviation of >50% relative to the average at all sites.

Crops were managed in response to a complete factorial combination of selected agronomic decisions that included choices for cultivar type and phenology, sowing date, planting density and nitrogen (N) fertilizer rate (Table 1). The same factors were used to manage crops at all locations except Minnipa where low rainfall reduced potential yields and hence income. This decreased the potential for costs to be recovered at this location, and so reduced the options available for crop management (Table 1). Cultivars used consisted of Triazine Tolerant Open Pollinated (TT OP), non-TT hybrid, and conventional open pollinated (CONV OP) types, each with fast, medium and slow phenology. Sixteen sowing dates were selected from 15 March to 12 July at weekly intervals. The sowing dates were subsequently grouped into eight sowing windows for the half-month periods from 16-31 March to 1-15 July. Plant density was set to three different rates that spanned the range typically used by producers in the different locations. Nitrogen fertilizer rates (principally applied as urea) covered a range from deficient to more than adequate (from 50 to 500 kg N ha^{-1}).

Table 1. Factors applied in combinations to manage crops at the case study locations

Factor	Locations excluding Minnipa	Minnipa
Cultivar type[1]	TT OP, CONV OP, non-TT hybrid	TT OP
Cultivar phenology	Fast, medium, slow	Fast, medium, slow
Sowing date	15/3 to 12/7 in 7d increments	15/3 to 12/7 in 7d increments
Plant density (m^{-2})	15, 45, 75	15, 30, 45
Target N fertilizer at 50 DAS[2] (kg N ha^{-1})	50, 100, 150, 200, 250, 300, 400, 500 (minimum 50)	5, 10, 20, 30, 40, 50, 70, 100 (minimum 5)

[1]TT OP, Triazine Tolerant Open Pollinated; CONV OP, Conventional Open Pollinated; DAS, days after sowing.

Canola crop production in response to the different combinations of management practices (Table 1) was simulated with the Agricultural Production Systems sIMulator (APSIM) v7.9 (Holzworth et al., 2014), which has been validated over a wide range of Australian environments for canola (Kirkegaard et al, 2016a; Robertson and Lilley, 2016). Canola cultivars can have a short commercial life, so generic cultivars were developed to simulate the TT OP, CONV OP and hybrid non-TT cultivar choices with fast, medium and slow phenologies. All crops received a fixed amount of N at sowing as

mono-ammonium phosphate (5 kg N ha^{-1} at Minnipa; 20 kg N ha^{-1} elsewhere), plus an amount of N at 50 days after sowing equal to the target N rate (Table 1) reduced by the amount of soil mineral N in the surface 0.3 m of soil. Crops were assumed to have failed if they did not germinate within two weeks of sowing. Frost and heat stress factors decreased simulated grain yield when the maximum temperature was greater than 30□C during anthesis, and when the minimum temperature fell below 2□C during pod filling (after Lilley et al., 2017). On 1 February each year, soil nitrogen and surface crop residues were reset to initial values (including 50 kg ammonium-N ha^{-1}) to prevent confounding nutritional effects from stubble carryover. Crops were simulated using the historical weather record of the 50 year-period 1967-2016 in order to capture yield responses to weather variability. Yield simulated from each combination of management decisions therefore reflected the response of crops to these decisions and in-crop climate alone. In order to elicit the management practices that were most profitable in response to seasonal climate, individual years were grouped as those with low, medium and high growing season (April to October) rainfall based on the respective decile groupings 1-3, 4-7 and 8-10. The combinations of management practices with the 50-year period resulted in approximately 200,000 sowings simulated per location.

Gross margin data for the three cultivar types (hybrid costed as Clearfield ®, TT OP, CONV OP) at each location was obtained from industry advisors and published data. The grain price was based on a 10-year average value of \$503 t^{-1} and minimum harvestable yields of 200 and 440 kg ha^{-1} at Minnipa and elsewhere, respectively (pers. comms., Andrew Ware and Andrew Zull, respectively). Across locations, the proportion of total costs represented by herbicide use was 27-29%, N fertilizer (14-16%), harvest and grain transport (21-23%) and seed (9-16%). Other costs (insecticide, fungicide, phosphorus fertilizer, insurance, levies, lime and gypsum) were each ≤ 10% of total costs. Costings were applied to simulated yields and associated management practices using R studio (R Core Team, 2016). Costs for N fertilizer and seed varied in proportion to the amount applied or sowing density used. Levies and transport costs varied in proportion to crop yield. All other costs were assumed to be incurred at the same rate for all crops within the same cultivar type.

Results for the gross margin points associated with the different combinations of management decisions were interpreted using a regression tree classification approach in RStudio. Following this approach, the gross margins for each combination of sowing window and rainfall decile grouping at a location was analysed. Gross margin values were successively split into groups with an average gross margin based on the most important management practice contributing to the split. The management practices contributing to the most profitable grouping within each sowing window and rainfall decile grouping form the focus of this study. While these practices contribute to the most profitable average, they include a range of values that may include losses in some years.

RESULTS

Regression classification trees were prepared for all combinations of rainfall decile and sowing window at each location (e,g. for the 1-15 May sowing window with high rainfall outlook at Wagga, Fig. 1). In the Wagga example, the average gross margin of the whole population for all management combinations and years was \$764 ha^{-1} (white shape). Applied N fertilizer was the most important management criterion to subdivide the gross margin grouping, with crops receiving more than 159 kg N ha^{-1} having a higher average gross margin (\$888 ha^{-1}) than those receiving less (\$549 ha^{-1}; orange shapes). Crops that received less than 108 kg N fertilizer ha^{-1} were the least profitable (\$475 ha^{-1}) compared to those fertilized with between 108 and 159 kg N ha^{-1} (\$691 ha^{-1}; blue shapes). For crops fertilized with more than 159 kg N ha^{-1}, the type of cultivar was the next most important management decision to identify the most profitable grouping (green shapes). This final split resulted in a large difference between average gross margins based on the use of TT OP cultivars (less profitable with average gross margin of \$641 ha^{-1}) or the use of other (hybrid or CONV OP) cultivars (most profitable grouping with average gross margin of \$1,010 ha^{-1}). While this represented the average most profitable grouping of results, it included a range of values from -478

to 1,743 $ ha^{-1} and 170 values (6%) of $0 ha^{-1} or less (data not shown). For the combination of a 1-15 May sowing window and high rainfall outlook at Wagga presented in Fig. 1, cultivar phenology and sowing density were less important influences on gross margins and did not form part of the main regression classification tree.

The management decisions identified with the regression tree approach that were used to describe the most profitable gross margin values in each combination of sowing window and rainfall decile grouping at Wagga are summarized in Table 2. Trends in these outcomes are described in terms of environmental conditions and management practices in the following sections.

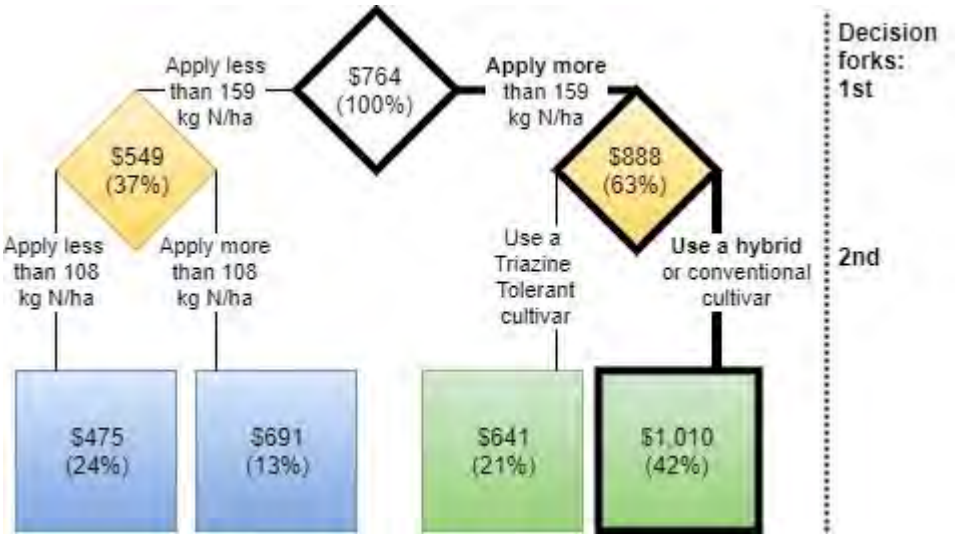

Fig.1. Regression tree classification of gross margin values based on the management decisions (i.e. cultivar type, cultivar phenology, planting density and N fertilizer rate) used to derive the gross margin values for the 1-15 May sowing window given a high rainfall decile at Wagga. Dollar values at each decision (diamonds) and end point (squares) represent the average gross margin value per hectare for the crops belonging to each group. The percentage values shown for each decision and end point represent the percentage of the total population of gross margin points for the sowing window-rainfall outlook combination at Wagga that contribute to the average gross margin value. The decision pathway leading to the highest profit grouping is outlined in bold.

Most profitable environmental conditions

Within any sowing window, the average gross margin could be doubled or tripled in response to a high rainfall decile grouping compared to a low one, highlighting the substantial impact of rainfall upon profitability (Table 2). The average gross margin for each rainfall decile grouping declined as the cropping season progressed, indicating that early sowing was on average most profitable regardless of rainfall decile experienced in that year. However, as the season progressed, sowings after May in low decile years resulted in low ($53 ha^{-1}) average gross margins or the incidence of losses ($166-264 ha^{-1}), indicating that sowing in these conditions was risky and unlikely to be profitable. Further, the percentage of gross margin points included within the most profitable grouping for the low rainfall grouping tended to increase with later sowing. This indicated that the regression tree approach was able to identify fewer groupings of profit outcomes as the season progressed and that fewer management practices could be identified to influence gross margins later in the season in years with low rainfall deciles.

Most profitable management decisions

In general, the most profitable grouping of gross margins was associated with the use of a CONV OP or hybrid cultivar type regardless of sowing window or rainfall decile grouping (Table 2). This was due to lower revenues arising from the lower-yielding TT OP cultivar which were not compensated by lower production expenses based on current costing information (data not presented). For crops sown before ~mid-April, the regression tree identified that a slowmaturing cultivar was more profitable, particularly in years of medium to higher rainfall deciles. For crops sown after this point, the most profitable grouping of results was associated with an increase in planting density, especially for medium to higher rainfall deciles. The rate of N fertilizer associated with the most profitable grouping of gross margin points varied between seasons and deciles, although some trends were apparent. These were that it was more profitable to: (1) increase the N rate for higher decile groupings in any sowing window, and (2) reduce the N rate in any decile grouping as sowing was delayed.

Table 2. Management decisions (N fertilizer rate, cultivar phenology, cultivar type, planting density) used to derive the most profitable grouping of gross margins and the percentage of the gross margins falling in the most profitable grouping. Results are shown for sowing window rainfall decile grouping at Wagga and include failed crops in the analysis.

Sowing window	Apr-Oct decile grouping	N rate (kg ha)	Cultivar phenology (S,M,F)[1]	Cultivar type (H,C,T)	Density (plants m^{-2})	Gross margin ($ ha^{-1} yr^{-1})	Pop'n (%)
16-31	1-3	118-407 >217	M,S S	H,C		760	26
March	4-7			H,C		1375	11
	8-10	>235	S	H,C		1364	11
1-15 April	1-3	>113		H,C		650	50
	4-7	>230		H,C		1202	31
	8-10	>245	S	H,C		1331	10
16-30 April	1-3			H,C		480	67
	4-7	>171		H,C	>30	1121	28
	8-10	>232		H,C		1168	32
1-15 May	1-3			H,C		328	67
	4-7	>159		H,C	>30	960	28
	8-10	>159		H,C		1010	42
16-31 May	1-3	<376		H,C		198	53
	4-7	>103		H,C	>30	688	34
	8-10	>151		H,C	>30	918	28
1-15 June	1-3	<373		H,C		53	53
	4-7			H,C		374	67
	8-10	117-500		H,C	>30	747	33
16-30 June	1-3					-166	100
	4-7			H,C		226	67
	8-10	>102		H,C	>30	585	33
1-15 July	1-3					-264	100
	4-7	<313		H,C		126	50
	8-10	115-488		H,C	>30	482	30

[1] slow (S), medium (M), fast (F) phenology; [2] hybrid (H), conventional (C), Triazine Tolerant (T) cultivar type

Simulation results for other locations

The degree of profitability varied between locations, but some consistency occurred in the response to management practices leading to the most profitable average gross margin groupings. These included greater profitability for: earlier rather than later sowings, higher rather than lower rainfall deciles, cultivars with slower phenology when sown early, and use of a hybrid or CONV OP cultivar.

DISCUSSION

The regression tree approach identified simple decisions (cultivar type, cultivar phenology, plant density and N fertilizer rate) that contributed to the class of practices that produced best gross margins for a sowing window-rainfall decile grouping combination for results from the 50-year time period that was simulated. These decisions are within the control of managers, and provide some guidance for the decisions that contribute to more profitable outcomes on average.

The yield for canola has varied historically due to inter-annual variability in seasonal conditions (Kirkegaard et al., 2016a). Similarly, annual rainfall within this period was highly variable within each location, and so contributed to the variability of gross margin results within the grouping. Since climate can have an over-riding effect on crop yields, it can be difficult to evaluate the effect of different crop management practices on yield over the short term. The approach used in this study took account of this variability by considering management within half-month and rainfall decile combinations, and thus highlights the value of the management decisions identified with the regression tree approach. In particular, this approach identified the effect of the low rainfall decile grouping in substantially reducing gross margins relative to higher deciles, and in reducing the availability of impactful management options available for this decile as the growing season progressed.

The management factors that contributed to the most profitable gross margin classification were consistent with other work. Earlier rather than later time of sowing in particular consistently contributed to obtaining the highest gross margin classification with any rainfall decile grouping (Table 2). Optimal time of sowing in canola is critical to ensure that yield is maximized: flowering too early limits yield through smaller crop biomass and exposure to frost risk; flowering too late limits yield through heat and water stress (Lilley et al., 2017). Time of sowing therefore has a strong effect on crop revenue through its effect on crop yield, which was the only source of income in calculation of crop gross margins. Similarly, the time of sowing has been shown to have a greater influence on yield than planting density (Brill et al., 2016) or cultivar phenology (Kirkegaard et al., 2016b), which were important only for some sowing window-decile combinations (Table 2). Since the time of sowing is critical to maximize crop yield, but was assumed to involve no expense, it was not surprising that this management decision was an important factor contributing to higher gross margins. By comparison, other decisions identified by the approach included choice of cultivar, planting density and rate of N fertilizer (Table 2). These decisions incur different costs, and may be expected to vary in their contribution to average profitability in response to changing yield (and hence, revenue) potential over successive sowing windows.

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Pathotypes of Hyaloperonospora brassicae on canola and other Brassicaceae

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ABSTRACT

Hyaloperonospora brassicae, the cause of downy mildew disease on brassicas, is important and endemic in canola growing areas of Australia, particularly in Western Australia. While it is most severe at the seedling and pre-flowering stages, particularly on more susceptible cultivars, it can infect and reduce crop productivity even when plants are infected at later stages of growth. A set of 30 isolates of *H. brassicae* collected, mainly from Western Australia but including some from across four southern Australian states, were inoculated onto seven days old seedlings of 28 canola and other miscellaneous *Brassica* hosts. Host responses ranged from no visible symptom or a hypersensitive response, to systemic spread and abundant pathogen sporulation in highly susceptible genotypes. Six host genotypes were identified as suitable for use as host differentials to distinguish and characterise *H. brassicae* pathotypes. Using an octal classification system based on the virulence of the isolates and their interaction with each host differential, eight distinct pathotypes of *H. brassicae* were characterised. This research provides the first characterisation of pathotypes in Australia and sets a benchmark for monitoring future changes in pathotype types and their distribution. Importantly, it allows breeders to incorporate effective resistance against specific, particularly prevailing, pathotypes of downy mildew into new canola varieties. Further, it will allow breeders to utilise the most appropriate resistances as pathotype distributions change in future. Of particular value will be pathotype-independent host resistances identified.

Key words: Downy mildew, Pathotypes, *Brassicaceae*, *Brassica napus*, *Brassica oleracea*

INTRODUCTION

Downy mildew on oilseed brassicas (canola, *Brassica napus*; mustard *B. juncea*) is a serious disease worldwide (Kaur *et al.*, 2011). For many plant diseases, different pathogen pathotypes and/or races are common and, while similar morphologically, they can have different potential to infect different species within a particular genus of plant host (Lebeda & Cohen, 2011). Host specificities can be utilised as indicators for different *H. brassicae* pathotypes (Sherriff & Lucas, 1990). While isolates of *H. brassicae* obtained from different *Brassica* species are generally compatible with and most virulent on their species of origin, they were still able to infect related species (Lucas *et al.*, 1988). Defining pathotypes is a critical requirement if control of plant diseases is to be successfully achieved by deployment of resistant cultivars. Furthermore, understanding host and non-host resistances to *H. brassicae* could lead to better identification of physiological forms and pathotype markers for developing host resistance against prevailing pathogen races.

Studies were conducted using canola and a range of important broad-acre and/or vegetable and/or weedy Brassicaceae. The objective was to identify and select suitable *Brassica* spp. differentials to enable characterization of *H. brassicae* pathotypes, and to use the octal code system of Goodwin *et al.* (1990) to define these pathotypes. These studies provide both a basis for standardizing phenotypic

characterization of pathotypes of *H. brassicae* worldwide and for monitoring and understanding changes in *H. brassicae* populations over time and between locations.

MATERIALS AND METHODS

Genotypes

A set of 28 Brassicaceae genotypes were used in this study, comprising 13 B. *napus*, two *B. juncea*, five *B. oleracea*, two *E. vesicaria* and one each of *B. nigra*, *B. carinata*, *B. rapa*, *Crambe abyssinica*, *R. sativus* and *R. raphanistrum*.

H. brassicae isolates

Thirty isolates of *H. brassicae* were collected, in particular from canola (*B. napus*) but also from, *B. oleracea* and *R. raphanistrum* from geographical locations across southern Australia. All *H. brassicae* isolates had been obtained from the original field samples by removing hyphal tips using fine tweezers and a dissection microscope and then transferring them to cotyledons of 7-day-old seedlings of highly susceptible *B. napus* cultivars.

Each *H. brassicae* isolate was maintained in isolation on cotyledons of 7-day-old seedlings of susceptible *B. napus* cotyledons. Equal numbers of cotyledons that supported abundant sporulation by each of the different pathogen isolates were collected separately for each *H. brassicae* isolate and then combined with the concentration adjusted to 10^5 sporangia mL^{-1}.

Inoculation

At 7 days after seedling emergence, seedlings were placed in clear plastic boxes with lids using the methodologies described earlier by Mohammed *et al*. (2017). High humidity was maintained for 24 h post-inoculation by misting the walls and lids of each plastic box with deionized water using a hand-held spray and then keeping the clear plastic lid on.

Disease assessment

The procedure of Williams (1985) was used to assess disease severity on plants at 7 days post-inoculation (dpi) using a 0–9 scale: 0 = no symptoms or sign of downy mildew disease; 1 = minute scattered necrotic flecks under the inoculum drop, no sporulation; 2 = larger necrotic flecks under the inoculum drop, no sporulation; 3 = very sparse sporulation, one to a few conidiophores, necrotic flecking but often with tissue necrosis evident; 5 = sparse sporulation, tissue necrosis; 7 = abundant sporulation, tissue necrosis and chlorosis may be present; and 9 = heavy sporulation, cotyledon collapse.

Pathotype characterization and nomenclature

Octal nomenclature, as developed by Goodwin *et al.* (1990), was used to code *H. brassicae* isolates according to their virulence on six differential *Brassica* genotypes selected from the 28 host genotypes.

RESULTS

There were significant differences between pathogen isolates, across the diverse host genotypes and a significant interaction between the two. Each of the selected host differentials showed clear bimodal distribution. Analyses of histograms based on disease severity scores of *H. brassicae* on these differential hosts identified clear resistance versus susceptibility separation points for each differential as follows: disease severity rating 5 for *B. napus* accession Cresor-770 B; 4 for Tranby; 4.5 for *B. nigra* Introduce P.23845; 6 for *B. napus* Thunder TT; 3 for CB Trilogy and 2.5 for *B. oleracea* Brussels sprout. Disease severity less than the separation point was considered a resistant response, while disease severity greater than and including the separation point was considered as a susceptible response. On this basis, eight distinct pathotypes were differentiated from among the isolates collected from Western Australia.

Among the agrogeographical locations sampled, the predominant and most widely distributed pathotypes were pathotypes 40 and 47, while the remaining pathotypes were only found at a single agrogeographical location.

DISCUSSION

This is the first study to define the phylogenetic relationships of *H. brassicae* isolates in Australia and the first anywhere to use octal nomenclature to characterize pathotypes of *H. brassicae*. The latter provides a novel basis for standardizing phenotypic characterization of pathotypes of *H. brassicae* worldwide and monitoring and understanding changes in *H. brassicae* populations over time and between locations. There were significant differences between pathogen isolates, across the diverse host genotypes and a significant interaction between the two. Host responses ranged from nil, to a hypersensitive response, to systemic spread and abundant pathogen sporulation. Isolates were generally most virulent on their host of origin. Six suitable host differentials and eight distinct *H. brassicae* pathotypes were identified**.**

While there were fewer different pathotypes amongst most recent isolates of *H. brassicae* than those collected a decade ago, recent isolates were overall more virulent. *H. brassicae* isolates can be either homo or heterothallic. Recombination of pathogenicity/virulence attributes has been suggested among progeny from outcrosses involving homothallic isolates and probably plays an important role in increasing variation for pathogenicity/virulence.

Downy mildew remains a significant threat to the oilseed *Brassica* industries in Australia and elsewhere (Barbetti & Khangura, 2000). Resistance identified in this study and elsewhere across various Brassicaceae × *H. brassicae* combinations will be critical for breeding effective disease resistance. Furthermore, studies involving pathotypes provide an evolutionary perspective on this oomycete pathogen; for example, as has been carried out for lettuce downy mildew (*Bremia lactucae*) by testing genetically uniform resistant hosts with different isolates of the pathogen sampled across the key vegetable production areas to identify the resistance to a wide collection of those particular pathogen isolates (Crute & Norwood, 1981). The host differentials developed provide the basis for monitoring changes in *H. brassicae* populations and allow early warning of new pathotypes able to overcome any resistances currently deployed commercially in canola and other oilseed *Brassica* industries.

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Understanding white leaf spot (*Pseudocercosporella capsellae)* epidemics on canola

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ABSTRACT

White leaf spot (*Pseudocercosporella capsellae*) is an underestimated pathogen severely impacting oilseed and horticultural *Brassica* yields worldwide. Among oilseed *Brassicas*, canola (*Brassica napus)* is the most important *Brassica* crop worldwide and in Australia it causes losses up to 30%, especially in highly susceptible varieties. Due to global climate change, canola in Australia is facing an increasingly unpredictable rainfall, variable and increasing temperatures and elevated periods of inadequate rainfall and drought that together have been associated with shifts in virulence and increased diversity within the *P. capsellae* population. A cotyledon based screening assay has been used to study the Australia-wide populations of *P. capsellae* in relation to their pathogenicity and virulence across different canola genotypes and their genetic differences have also been assessed. Increased incidence and severity of white leaf spot appears associated with increased climatic variability, likely due to more disease conducive conditions for severe epidemics. In south west Western Australia, the variable Mediterranean climate makes it an attractive location to study and model current and future climatic effects, and also to predict future risks at various spatio-temporal scales, of white leaf spot epidemics on canola. Studies are being undertaken to explain the basic environmental drivers of white leaf spot epidemics. Further, within the *P. capsellae* population significant genetic and phenotypic variation has been identified and this is also helping to explain the increasing adverse impact and importance of white leaf spot disease over recent decades.

Key words: white leaf spot, Brassica, temperature, pathogenicity

INTRODUCTION

White leaf spot is an important oilseed brassica disease across many countries on both oilseed and horticultural brassicas. In Australia it results in losses up to 30%, especially in highly susceptible varieties (Hamblin *et al.* 2004). Leaf fall can be caused by severe infection (Barbetti and Khangura 2000). However, the leaf symptoms on different *Brassica* species like turnip, mustard and Chinese cabbage can be different from each other (Crossan 1954). In Western Australia, *P.capsellae* was recorded on *B. juncea* by (Eshraghi *et al.* 2005) and remains problematic across both canola and mustard. *B. juncea* and *B. carinata* largely represent susceptible and resistant genotypes, respectively, and variation in resistance is also region based (Gunasinghe *et al.* 2014). In southern Australia, canola experiences the particularly severe epidemics, possibly in association with the variable Mediterranean climate that occurs in that region (Barbetti *et al.* 2012). There have been extensive studies on how change in climatic factors might affect fungal pathogens; such as environmental variables like temperature cause shifts in the virulence of pathogen isolates. For example, temperature adaptation in isolates of Sclerotinia stem rot to warmer or cooler regions can determine their virulence on *Brassica carinata* (Uloth *et al.* 2015). Also a previous study by (Gunasinghe *et al.* 2016a) highlights that differences in virulence of the isolates can be attributed to variation within the pathogen population including their pathogenicity, virulence and their genetic makeup. Hence, to understand pathogen population variation is an essential criterion towards implementing the sustainable agricultural practices by providing the foundation both for breeding resistant genotypes and for development and implementation of effective integrated management practices under current and future climate conditions.

MATERIALS AND METHODS

Single conidial isolates of *P. capsellae* collected from canola crops across southern Australia were used. All the isolates were preserved as lyophilized ampoules containing living pathogen. Each isolate was subcultured on freshly prepared plates of malt extract agar to revive the fungus. Pathogenicity and virulence tests were conducted against three different genotypes of canola. Experiments were conducted in a control environment room set at 15 °C with a 12 hour day/night photoperiod and a light intensity of 380 umol^{-2} s^{-1}.Plants were fertilised weekly with a complete fertilizer (Thrive). Plants were spot inoculated by depositing a 10 µL droplet of inoculum to each cotyledon lobe. Controls were inoculated with deionized water.

RESULTS

Lesions started to develop 8-9 days post inoculation. All tested isolates of *P. capsellae* were pathogenic, producing typical disease symptoms. Disease data was recorded 14 days post inoculation. All isolates were pathogenic. However, the virulence of isolates and susceptibility of host genotypes varied significantly depending on the tested isolate. In addition, there was significant and phylogenetic variation identified within the Australia-wide population of *P. capsellae* isolates

DISCUSSION

The pathogenicity test conducted with *P. capsellae* isolates against different *Brassica napus* genotypes confirmed that all isolates were pathogenic against all test hosts. However, there was huge variation in virulence among the Australia wide isolates of this pathogen. Also there were clear differences in the level of susceptibility of the host. This relates well with the findings of (Gunasinghe *et al.* 2016a) in which *B.napus* was found as susceptible host with a wide variation among different isolates of *P.capsellae.* There were also clear variations in virulence associated to other factors like the different locations and/or environments across the different canola growing regions of Australia from where the isolates being collected. This is not surprising, as factors such as temperature variation influences the production of secondary metabolites, making them more pathogenic, like in studies by (Amadioha 1993; Uloth *et al.* 2015) that revealed that temperature changes affect the production of oxalic acid by *Rhizoctinia bataticola* and *S. sclerotiorum.* Similarly, temperature and moisture may encourage development of new pathotypes of *P. capsellae,* against which new host resistances need to be identified if we are to significantly improve white leaf spot management in *Brassica* crops (Gunasinghe *et al.* 2016b). Currently, experiments are underway to quantify the extent of environmental effects on this pathogen as such effects have never been investigated previously for *P. capsellae*. Ongoing studies should define how these factors can alter the disease severity and incidence on canola crops Australia-wide from white leaf spot. The project is offering opportunity to understand the white leaf spot disease epidemic, providing basis for breeding resistant Canola genotypes and importantly helping to predict the disease under future weather conditions across Australia.

In summary, these studies to date have highlighted how *P. capsellae* pathogen populations show significant pathogenic and phylogenetic diversity and how this pathogen is well placed to adapt to any future changes in cropping practices. Current studies now underway investigating environmental drivers of epidemics should enable development of forecasting of the adverse impact of this disease with respect to changing climate conditions across southern Australia.

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How Brassica oleracea FLOWERING LOCUS C 2 works in Brassica rapa genetic background

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ABSTRACT

Many plant species require cold exposure for flowering. This process is called vernalization. Regulation of flowering is an important trait not only for seed crops such as canola but also for vegetables in the genus *Brassica*. We harvested reproductive tissue of canola (*Brassica napus*), broccoli and cauliflower (*Brassica oleracea*), or vegetative tissue of cabbage and kale (*B. oleracea*) and Chinese cabbage (*Brassica rapa*). *B. napus* is derived from the interspecific hybridization of the A and C genomes of *B. rapa* and *B. oleracea*, respectively. How introgressed genes work in the genetic backgrounds of other species is of interest. We used an introgression line (IL) derived from an interspecific crossing between Chinese cabbage (*B. rapa*) and cabbage (*B. oleracea*). This line contains an introgressed region from the upper-arm of chromosome 2 derived from the *B. oleracea* chromosomal fragment covering *B. oleracea FLOWERING LOCUS C 2* (*BoFLC2*), in a *B. rapa* genetic background. The floral repressor *FLC* associated with vernalization in *Arabidopsis thaliana* is present in the *Brassica* genus and *FLC* is epigenetically silenced by prolonged cold exposure, leading to flowering. *B. rapa* is seed-vernalization-responsive, responding to cold exposure during the juvenile vegetative growth phase, while *B. oleracea* is plant-vernalization-responsive and does not respond to vernalization during juvenile vegetative growth phase. The IL exhibits an increased vernalization requirement with respect to the duration of cold when compared with the parental *B. rapa* line, though the IL remains seed-vernalization-responsive. We confirmed the introgressed regions from *B. oleracea* in the IL by DNA markers and examined the expression levels of the *FLC*s (using a primer set that amplifies the three functional *FLC* paralogs) and *FLC2* in the IL, *B. rapa* and *B. oleracea* at the juvenile vegetative growth phase by RT-qPCR. We found that the expression pattern of *FLC*s in the IL is similar to that of *B. rapa* and different from that of *B. oleracea.* By contrast, the expression pattern of *BoFLC2* in the IL is not similar to that of either *BrFLC2* in *B. rapa* or *BoFLC2* in *B. oleracea*. This result indicates that the regulation of *BoFLC2* by cold exposure in the IL is regulated by the combination of the *cis* (*BoFLC2* sequence) and *trans* (*B. rapa* genetic background) effects.

Key words: *Brassica rapa* – *Brassica oleracea* – vernalization – *FLOWERING LOCUS C* – introgression line

INTRODUCTION

Flowering is a major transition in the developmental program for plants. Plants have evolved a network to regulate flowering time, which includes many endogenous factors and environmental factors including temperature and photoperiod. Many plant species require cold exposure for flowering, and this process is called vernalization. Regulation of flowering is important not only for seed crops such as canola but also for vegetables in the genus *Brassica*.

The genus *Brassica* includes numerous agronomically important crops including vegetables, vegetable oil, forage, condiments, and ornamental plants. *Brassica rapa* L. (2n=20, AA genome) includes Chinese cabbage (var. *pekinensis*), pak choi (var. *chinensis*), and komatsuna (var. *perviridis*), root vegetables including turnip (var. *rapa*), and oilseed (var. *oleifera*). *Brassica oleracea* L. (2n=18, CC) includes cabbage (var. *capitata*), broccoli (var. *italica*), kale (var. *acephala*), kohlrabi (var. *gongylodes*), and cauliflower (var. *botrytis*). *Brassica napus* L. (2n=38, AACC) is an important oilseed crop and is derived from the interspecific hybridization of the A and C genomes of *B. rapa* and *B. oleracea*, respectively (U, 1935). *B. rapa* is seed-vernalization-responsive, responding to cold

exposure during the juvenile vegetative growth phase, while *B. oleracea* is plant-vernalization-responsive and does not respond to vernalization during juvenile vegetative growth phase.

Vernalization in both *B. rapa* and *B. oleracea* is controlled by the MADS-box DNA-binding protein FLOWERING LOCUS C (FLC), first identified as a floral repressor in *Arabidopsis thaliana* (Itabashi et al., 2018; Shea et al., 2018a). *FLC* is epigenetically silenced by prolonged cold exposure, which leads to flowering (Kawanabe et al., 2016; Itabashi et al., 2018; Shea et al., 2018a). There are four homologs (*BrFLC1, 2, 3, 5*) in *B. rapa*, and four (*BoFLC1, 2, 3, 5*) in *B. oleracea* (Shea et al., 2018a). Among them, *BrFLC5* and *BoFLC5* are thought to be pseudogenes (Shea et al., 2018a).

How introgressed genes work in the genetic background of other species is of interest. In this study, we used an introgression line (IL) derived from an interspecific crossing between Chinese cabbage (*B. rapa*) and cabbage (*B. oleracea*). This line contains an introgressed region from the upper-arm of chromosome 2 derived from the *B. oleracea* chromosomal fragment covering *BoFLC2*, in a *B. rapa* genetic background (Shea et al., 2017). The IL exhibits an increased vernalization requirement with respect to the duration of cold when compared with the parental *B. rapa* line, though the IL remains seed-vernalization-responsive (Shea et al., 2018b). Previous studies have shown that one of the quantitative traits locus (QTL) controlling flowering time links to the region covering *BoFLC2* (Irwin *et al*., 2016; Okazaki *et al*., 2007). The objective of this study is to examine the vernalization response of *BoFLC2* in the IL, which is almost entirely comprised of the *B. rapa* genomic background, and compare *BoFLC2* to the original parental line of *B. oleracea*.

MATERIALS AND METHODS

The IL was derived from interspecific crossing between 'CR Kanki' (Nihon Norin Seed Co. Ltd., Japan) of Chinese cabbage (*B. rapa* var. *pekinensis*) and a DH line (P01) of 'Reiho' (Ishii seed company, Japan) of cabbage (*B. oleracea* var. *capitata*). The line used in this study is BC_3F_3 and contains an introgressed region from the upper-arm of chromosome 2 derived from the *B. oleracea* chromosomal fragment covering *BoFLC2*, in a *B. rapa* genetic background. Two Chinese cabbage commercial cultivars, 'Munbichi' (Syngenta Japan Co, Ltd) and 'CR Kanki' and a Chinese cabbage inbred line (Nou 7 gou) were used. Three cabbage commercial cultivars, 'Reiho', 'Matsunami' (Ishii seed company), and 'Kinkei No. 201' (Sakata seed Corp.), were used.

Seeds were surface sterilized and grown on agar solidified Murashige and Skoog (MS) plates with 1 % (w/v) sucrose under long day (LD) condition (16h light) at 21 °C. Plants were harvested at 14 or 19 days after sowing as non-vernalized samples (NV). For vernalizing cold treatments, 14 or 19-day seedlings were treated for 6 weeks at 4 °C under LD condition (16h light) as 6-weeks vernalized samples (6wksV) or 6 weeks of cold treatment followed by 12 days under normal growth condition (6wksV+12).

For DNA marker analysis, 14-day first and second leaves of *B. rapa* and 19-day first and second leaves of *B. oleracea* were harvested for isolation of genomic DNA. The DNA markers were developed using the sequence information of the reference genome in *Brassica* database (BRAD, http://brassicadb.org/brad/).

For gene expression analysis, total RNA of first and second leaves of NV, 6wksV, and 6wksV+12 were isolated and cDNA was synthesized from 500 ng total RNA. RT-qPCR was performed using the cDNA as a template.

RESULTS

To identify the introgressed regions from *B. oleracea,* we conducted DNA marker analysis. We made dominant or co-dominant DNA markers, which can distinguish the *B. rapa* and *B. oleracea* synthetic sequences on chromosome A02 and C02, respectively (Fig. 1). The markers covering the 0 Mb to 3.1 Mb regions indicated that the chromosomal segment was derived from the *B. oleracea* genome. From 3.1 Mb to 9.5 Mb, the IL is heterozygous for the two species, and the region from 9.5 Mb was from the *B. rapa* genome. The markers based on the *B. oleracea* genome, the region from 0 Mb to 4.1 Mb was from the *B. oleracea* genome. From 4.1 Mb to 12.4 Mb, the IL was heterozygous for the two genomes and the region from 12.4 Mb was derived from the *B. rapa* genome. The *BoFLC2* locus is located at

2.7 Mb in the C02 chromosome in *B. oleracea*. Therefore, the IL contains an introgressed region from the upper-arm of chromosome C02 derived from the *B. oleracea* chromosomal fragment covering *BoFLC2*, in a *B. rapa* genetic background (Fig. 1).

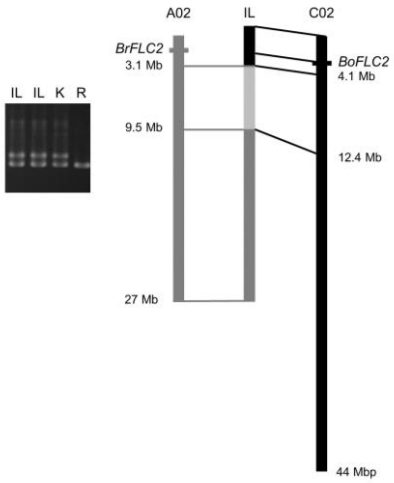

Fig. 1. Schematic diagram of chromosome 2 in the introgression line (IL). The location of *BrFLC2* in *B. rapa* and *BoFLC2* in *B. oleracea* are shown. Grey lines and black lines indicate the *B. rapa* and the *B. oleracea* genome, respectively. The light grey line in the IL genome indicates heterozygotes of *B. rapa* and *B. oleracea*. The picture on the left side is an example of genotyping using the DNA marker. K, 'CR Kanki' (*B. rapa*); R, 'Reiho' (*B. oleracea*).

The IL exhibits an increased vernalization requirement with respect to the duration of cold when compared with 'CR Kanki', though the IL remains seed-vernalization-responsive (Shea at al., 2018b). The introgressed *BoFLC2* in the IL may cause an increased vernalization requirement. Therefore, we investigated the expression levels of the *FLC*s (using a primer set that amplifies the three functional *FLC* paralogs) or *FLC2* in the IL, *B. rapa* and *B. oleracea* lines at the juvenile vegetative growth phase by RT-qPCR. In 3 *B. rapa* lines, the expression of *FLC*s decreased by 6 weeks of cold exposure and recovered after the plants returned to warm conditions, but the expression levels didn't fully return to the expression levels seen in the non-vernalized plants (Fig. 2). The expression of *FLC*s in the IL also decreased after 6 weeks of cold exposure, whereas the expression levels returned to the expression levels recorded in non-vernalized plants (Fig. 2). In contrast, the expression of *FLC*s in 3 *B. oleracea* lines were not silenced by cold treatment (Fig. 2).

The expression of *BrFLC2* in *B. rapa* and *BoFLC2* in *B. oleracea* were silenced by 6 weeks of cold exposure and didn't fully return to the expression levels of the non-vernalized plants upon returning to warm conditions following cold (Fig. 2). However, *BoFLC2* in the IL was not silenced by 6 weeks of cold exposure (Fig. 2).

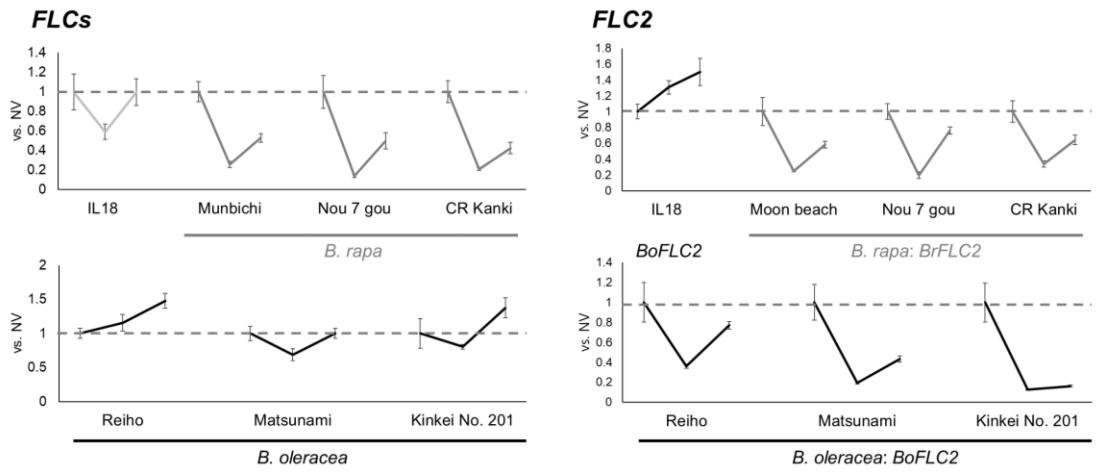

Fig. 2. The expression pattern of *FLC*s and *FLC2* before and after vernalization. Expression levels were normalized using expression levels of non-vernalized samples (NV). In each graph, the first point is the expression level of NV, the second point is that of 6-weeks vernalized samples, and the third point is that of 6 weeks of cold treatment followed by 12 days under normal growth condition.

Discussion

We showed that the IL contains a 4.1 Mb *B. oleracea* fragment while the synthetic *B. rapa* region was 3.1 Mb, and that the IL contains homozygotes of *BoFLC2* in a *B. rapa* genetic background.

The expression of *FLC*s in *B. rapa* and the IL were silenced by cold exposure. However, *FLC*s in *B. rapa* didn't reactivate to the levels seen in non-vernalized plants once returned to warm conditions, whereas *FLC*s in the IL reactivated. This result suggests that the vernalization requirement in the IL was increased by the introgressed chromosome fragments. Moreover, the expression of *BrFLC2* in *B. rapa* and *BoFLC2* in *B. oleracea* were silenced by cold exposure, whereas *BoFLC2* in the IL was not silenced. The expression pattern of *BoFLC2* in the IL was not like that of either *BrFLC2* in *B. rapa* or *BoFLC2* in *B. oleracea*, indicating that the regulation of *BoFLC2* by cold exposure in the IL is regulated by the combination of the *cis* (*BoFLC2* sequence) and *trans* (*B. rapa* genetic background) effects.

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Over twenty years of commercial canola crops show the place of canola in the southern Mallee of South Australia

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ABSTRACT

Yield data from a range of farm canola crops grown on one farm near Lameroo in the southern Mallee of South Australia since the early 1990s is presented to show the variability of production over more than twenty years. Actual farm yields were compared to the water-limited potential yield estimated by modelling with APSIM and yields in nearby research trials. Of 36 canola crops that were grown between 1990 and 2013 only 8 crops produced more than 1 t/ha, and 18 out of 36 crops exceeded 0.7 t/ha. Eleven canola crops produced less than 0.4 t/ha, generally due to drought or late sowing.

While initial crops used conventional cultivars, the farmers switched to triazine tolerant cultivars when they became available and later grew Clearfield canola in most years to increase grain yields.

Initially, low rates of nitrogen were applied to the canola crops even though the canola followed a cereal in the rotation. In later years nitrogen application rates were increased to 25 to 43 kgN/ha.

Blackleg internal infection data from yield trials conducted in 2004-2006 showed little blackleg occurred when only a limited number of canola crops were grown in the district. However, a paddock survey undertaken in 2011 showed that much higher levels of internal infection were present following the larger area of canola crops grown in 2010, suggesting that cultivars with improved blackleg resistance would be needed if the area sown to canola in the low rainfall zone significantly increased.

Latest research into canola cropping in the low rainfall zone shows the need to sow early, to use cultivars that are early flowering and have herbicide tolerance, and to apply adequate rates of nitrogen fertiliser up front. This is the system that the Maynards have developed for their farm over a wide range of seasonal conditions.

Key words

Canola – low rainfall – farm yield – simulated yield – agronomy - blackleg

INTRODUCTION

Canola has been grown in the low rainfall zone of SA since the early 1990s but production was often restricted by a lack of adapted cultivars, competition by broad leaf weeds resulting in a need for herbicide tolerance, variable timing of seasonal breaks, variable spring conditions of drought and/or frost and initially by a lack of grower experience and confidence in the crop. Canola has been shown to act as a very effective cereal disease break crop (Angus et al. 2015) by increasing wheat yield in the following year by about 0.8 t/ha compared to 1.0 t/ha for wheat after pulses. In low rainfall areas such as the southern Mallee, wheat grown after canola produced similar yields to wheat grown after pulse crops provided additional nitrogen was applied following the canola crop (Potter et al. 1997).

However, while the break crop effect is important, the canola crop also needs to produce adequate returns in its own right.

To determine the success or failure of canola in crop rotations in this zone we decided to evaluate commercial canola production over a wide range of seasons on a farm where growers had developed a high degree of experience in growing the crop.

MATERIALS AND METHODS

Data including previous crop, sowing date, canola cultivar, herbicide tolerance type, nitrogen application rate and rainfall from 36 canola crops grown between 1990 and 2013 by the Maynard family near Lameroo in the southern Mallee of South Australia was used to provide "farm yields". These were compared to water-limited potential yield that was simulated using the APSIM (Agricultural Production SIMulation) model version 7.9 (Holzworth et al. 2014; www.apsim.info) to simulate canola yield. APSIM-Canola has been widely validated across a broad range of environments (Robertson and Lilley 2016). Climatic data were obtained from the SILO Patched Point Dataset (Jeffrey et al. (2001); https://silo.longpaddock.qld.gov.au/) and farm rainfall records. The soil selected to represent the site was APSoil No. 385 (www.apsim.info/Products/APSoil.aspx), a loam over constrained clay from the region with a plant available water content of 113 mm. To estimate soil water and N content at sowing of the canola crop, the simulations were run without soil resetting for 2.5 years before the canola crop was sown. Simulations began on 15 November, initialised with 10% plant available water and soil mineral N of 7 kg N/ha. Two years of barley and wheat crops were sown on the autumn break to match the paddock history. The autumn break was considered to be when at least 15mm of rain fell over 3 days. Soil water and N content at sowing of the canola crop were a consequence of climatic conditions and typical crop management during the fallow and cereal cropping seasons. After harvest of the crop preceding the canola crop, stubbles were grazed and the fallow periods were assumed to be weed free. Canola was sown and managed based on the grower reported sowing date, cultivar and fertiliser management. One canola crop was sown after a hay crop with all other canola crops being grown after a cereal (17 barley crops, 16 wheat crops and 2 triticale crops). All cereal stubbles were kept relatively weed free over summer. While the first three canola crops (1990 and 1994) were sown in June, 9 crops were sown in April (1998, 2000, 2006 and 2012). All other canola crops were sown in May (11 in the first half of May and 13 in the second half of May).

APSIM does not account for biotic stresses, therefore reported yields assume complete pest and disease control. Yield of the canola crop was adjusted using the frost and heat stress factors reported by Lilley et al. (2015).

Canola cultivar trials were conducted at Maynard's farm near Lameroo from 1995 onwards. Plot size was 8 m long by 8 rows at 15 cm row spacing. Various rates of nitrogen were applied at seeding but always at higher rates than for the commercial crops. Additional nitrogen was top dressed during the season, also at higher rates than for the commercial crop. Trials were harvested in all years except 2002, 2007 and 2008 (Figure 1).

Blackleg internal infection data was measured in the years 2004-2006 when 20 plants per plot were cut at ground level and the % internal infection was scored. We also conducted a survey of 14 canola crops in the southern Mallee in October 2011 to investigate the levels of blackleg in that district. A range of crops was sampled (Table 2) and 50 plants were taken randomly across the field (approximately 1 plant every 10 m travelled). These plants were cut at ground level and the amount of internal infection was scored per plant. The mean % internal infection was calculated for each paddock.

RESULTS AND DISCUSSION

Of 36 canola crops that were grown between 1990 and 2013 only 8 crops produced more than 1 t/ha, most of these being in 2011 and 2013. If a break-even yield is considered to be above 0.7 t/ha, then 18 out of 36 crops exceeded the break-even yield. Drought occurred in several years, notably 1994, 1997, 2002, 2006 and 2007 while September to October rainfall was also very low in 2008. Nine canola crops grown in these years produced less than 0.4 t/ha, however, another 2 crops also produced less than 0.4 t/ha, the first canola crop sown in late June 1990 and one sown in 2008.

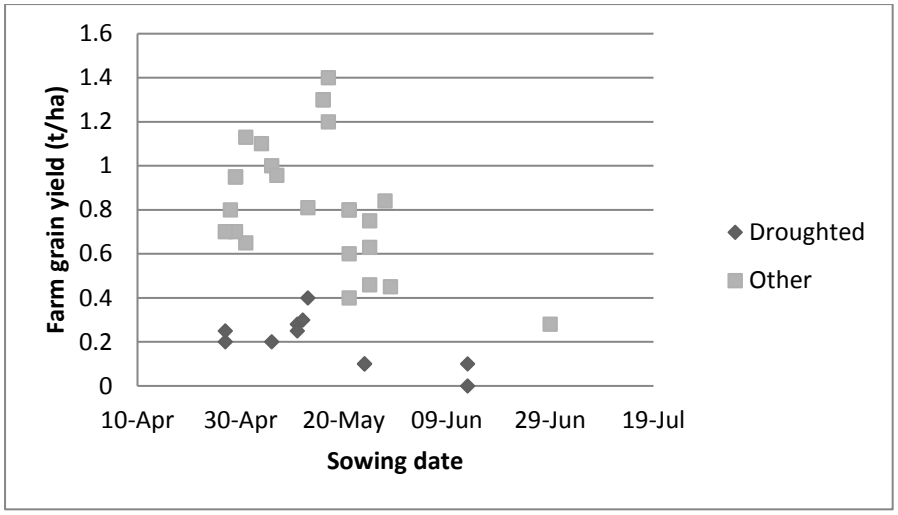

Figure 1. Effect of sowing date on farm grain yield (t/ha) for droughted and other years at Lameroo between 1990 and 2013

Canola crops were sown after sufficient rain had occurred to enable crop germination and emergence, however, due to the logistics of sowing a large crop programme sometimes up to a week or so may have occurred between sufficient rainfall occurring and sowing date. There was a trend for decreasing grain yield as sowing was delayed (Figure 1).

Between 1990 and 1994 conventional canola cultivars were sown with the poorly adapted, long season triazine tolerant cultivar Siren being sown in 1995. Between 1997 and 2007 only triazine tolerant cultivars were sown, usually early to mid-season types such as Karoo, Drum, Pinnacle and Surpass 501TT. While triazine tolerant canola cultivars have a lower yield potential than other herbicide tolerant types their use gave the ability to manage *Brassica* weeds that can compete with canola plants and also contaminate the grain sample at harvest (Potter and Salisbury, 1993). From 2008 until 2013 Maynards sowed the Clearfield cultivar 44C79 (7 crops) and the TT cultivar ATR-Stingray (4 crops). Most cultivar decisions were made based on results from trials sown on Maynard's farm in the previous year. Simulated yields show that changes in crop management practices over time led to an increase in potential yield despite variability in climate (Figure 2). On average, farm yield was 68% of simulated yield and generally farm yield improved over the two decades (Figure 2).

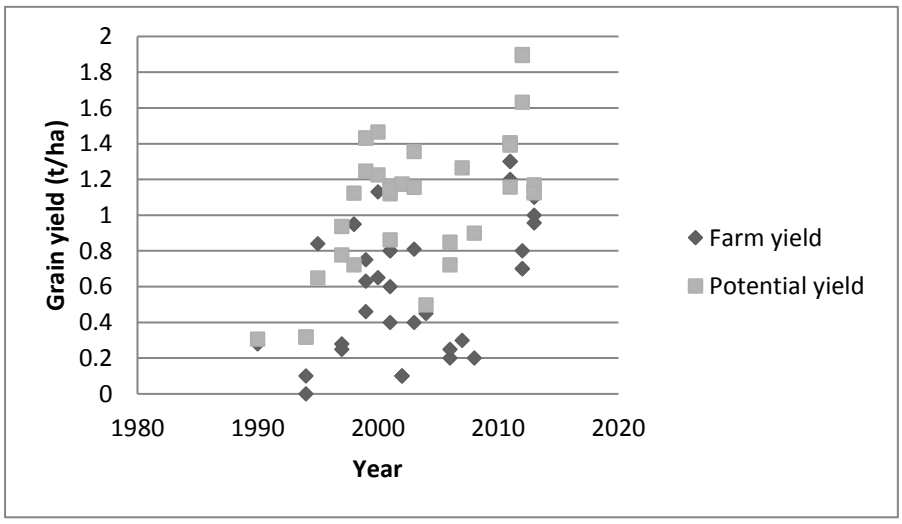

Figure 2. Farm yield and modelled potential yield (t/ha) for 36 canola crops grown in the southern Mallee of South Australia between 1991 and 2013

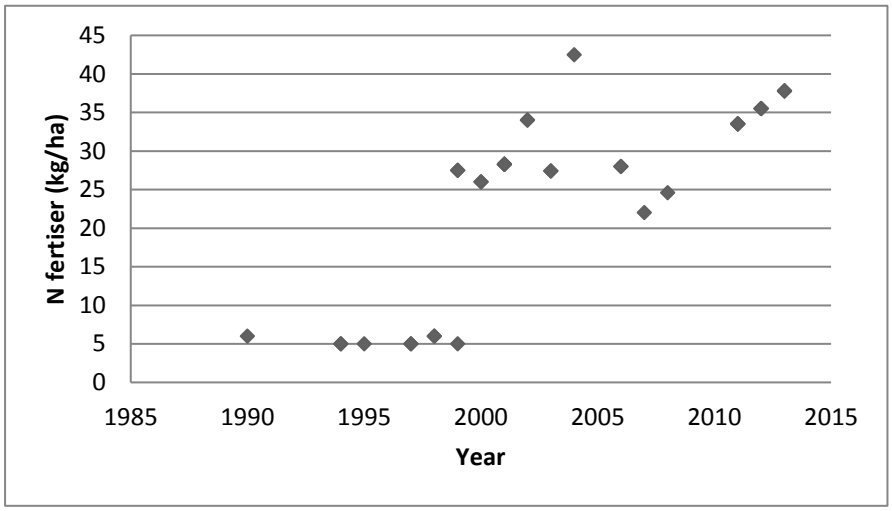

Figure 3. Total Nitrogen fertilizer (kg/ha) applied at sowing and topdressed later during growth in 36 canola crops grown in the southern Mallee of South Australia between 1991 and 2013

While cultivar choice changed as new cultivars were released, N management also changed significantly over the two decades. The first canola crops grown at Maynard's farm received very little nitrogen fertilizer even though they followed a cereal crop (Figure 3). However, later crops were given a total of 25 to 43 kgN/ha.

Comparisons were also made between farm yields and plot yields of the same cultivars sown in experimental variety trials (Figure 4). Plot grain yields for the same cultivars as used commercially each year were taken from cultivar trials. Generally plot grain yields were similar to the farm yields except for 2003 when plot yields were much greater than the farm yields (Figure 4).

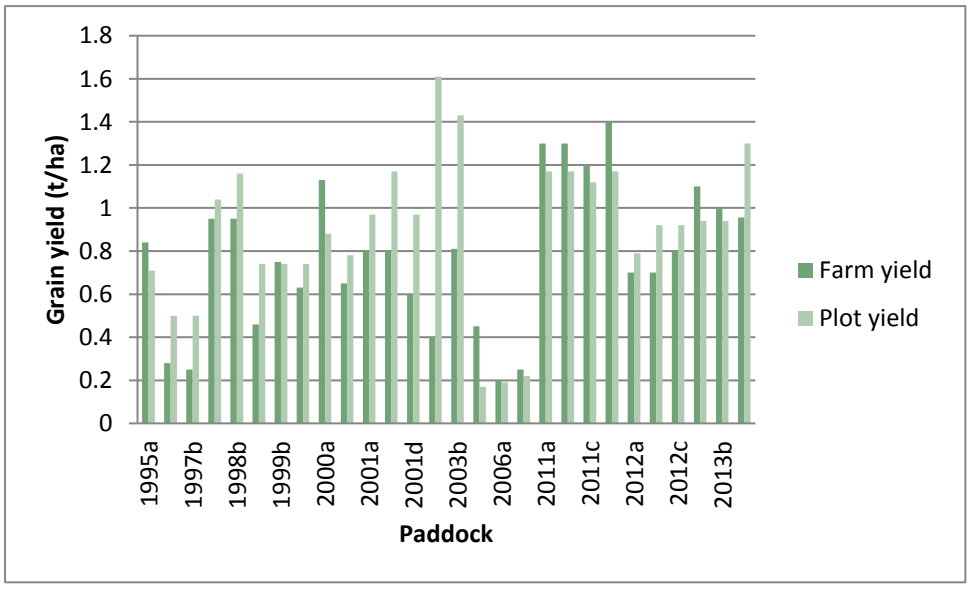

Figure 4. Grain yield (t/ha) for the same cultivar grown in farm crop and in plots in the same year

Water use efficiency was calculated using the simulated water use (transpiration) from the crop model (Figure 5). WUE tended to increase over time and this may be attributed to greater agronomic experience, the continual upgrading to improved cultivars, particularly to Clearfield cultivars rather than triazine tolerant types, and also the increased nitrogen rates applied to the farm crops.

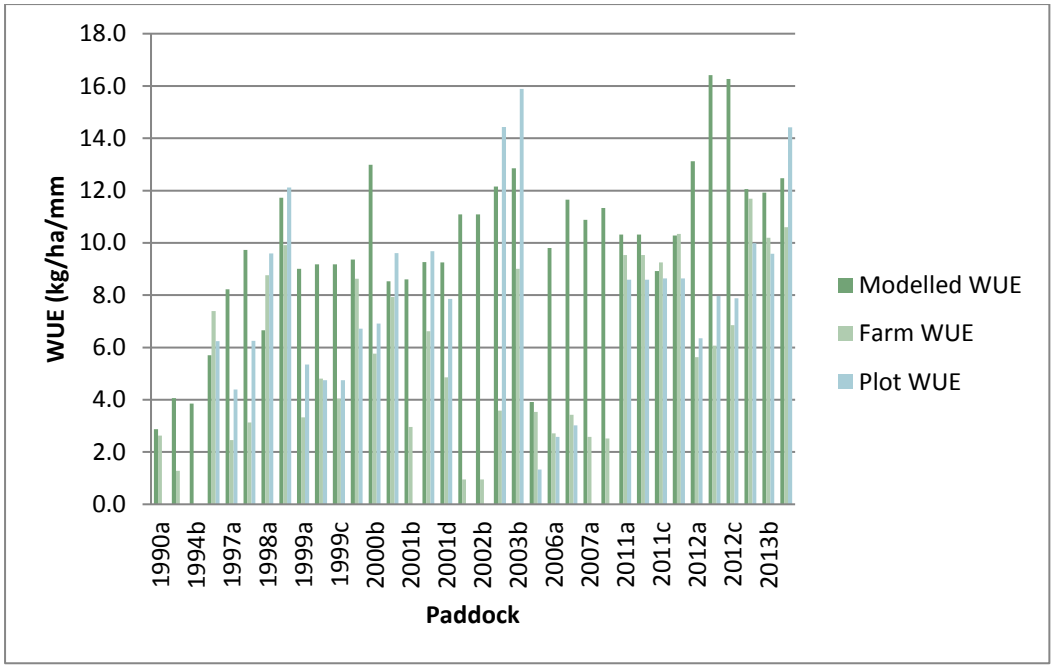

Figure 5. Farm Water Use Efficiency (WUE) of 36 simulated, commercially farmed and experimental canola crops grown in the southern Mallee of South Australia between 1990 and 2013. Crop water use was extracted from the simulations and then applied to farm, plot and modelled crop yields

Blackleg disease in canola has traditionally been regarded as a minor issue in the low rainfall zone when only a low intensity of canola cropping occurs. When little canola was grown in the southern Mallee very low levels of blackleg infection were noted at Lameroo in variety trials between 2004 and 2006 (Table 1). Similar low levels of blackleg infection were also noted in other low rainfall areas where canola was grown at low intensity (S. Marcroft pers.com.).

High levels of internal infection caused by blackleg were present in 2011 in the southern Mallee and many of the crops were sown to cultivars that had a low level of blackleg resistance (Table 2). The increase in blackleg can be attributed to a greater area being sown to canola in 2010 and 2011 than in previous years, as well as the summer and autumn rain in 2010-2011 that resulted in a likely more rapid and greater release of blackleg spores throughout the district. If the area sown to canola in the southern Mallee increases further then farmers would be advised to use cultivars with improved blackleg resistance as well as targeting early flowering cultivars with phenology that suited the low rainfall conditions.

Table 1. Mean internal infection and % of plants sown in trials near Lameroo in 2004-2006

Year	Mean internal infection (%)	
	ATR-Beacon	Surpass 501TT
2004	0.25	1.67
2005	5.0	6.5
2006	1.75	0.75

Table 2. Mean internal infection and % of plants with more than 50% internal infection at 14 paddocks in the southern Mallee 2011.

Location	Blackleg Rating 2011	Variety	Mean % Internal infection.	% plants with more than 50% internal infection.
Parilla	MS-S	Tanami	17.5	12
Pinnaroo	MS	43C80	22.1	12
Pinnaroo	MS	43C80	13.9	6
Pinnaroo	MS	44C79	26.7	16
Pinnaroo	MS	44C79	21.7	10
Lameroo south west	MS	44C79	18.7	14
Lameroo south west	MS	44C79	5.3	0
Lameroo south west	MS	44C79	11.0	6
Parilla north	MS	44C79	19.4	18
Parilla north	MS	Scaddan	2.6	0
Lameroo west	MR-MS	44Y84	11.8	8
Lameroo west	MR-MS	45Y82	18.2	12
Lameroo south west	MR	FighterTT	5.1	6
Parilla	MR	HurricaneTT	3.2	0

Latest research into canola cropping in the low rainfall zone shows the need to sow early, to use cultivars that are early flowering and have herbicide tolerance, and to apply adequate rates of nitrogen fertiliser up front. This is the system that the Maynards have developed for their farm over a wide range of seasonal conditions.

ACKNOWLEDGEMENTS

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Dual herbicide tolerant canola for better weed management

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ABSTRACT

Herbicide-resistant annual ryegrass (*Lolium rigidum*) is becoming a major issue for canola production. Across southern Australia, annual ryegrass has increasing levels of resistance to most herbicides registered for its control in cereals. Canola has provided an opportunity to obtain better management of annual ryegrass in the rotation, largely through the use of post-emergent herbicides. However, resistance to clethodim, the main post-emergent used in canola for annual ryegrass, is also increasing. Canola with tolerance to two herbicides offers opportunities to combine herbicide modes of action for better grass weed control. We have conducted field trials at Roseworthy, South Australia of canola with dual tolerance to imidazolinone and triazine herbicides (CT canola) to determine strategies for improved annual ryegrass control. In 2017 Hyola 580 CT canola (experimental T6001) was sown into a field with annual ryegrass known to be resistant to trifluralin, the Group A herbicides and the Group B herbicides. A total of 6 herbicide strategies were used: no herbicide (nil), atrazine incorporated by seeding (IBS) and followed with either clethodim alone (AC), atrazine plus clethodim (AAC), or Intervix followed by clethodim (AIC) post-emergent; or propyzamide IBS followed by atrazine plus clethodim (PAC) or Intervix + clethodim (PIC) post-emergent. Propyzamide was more effective as a pre-emergent herbicide reducing annual ryegrass emergence by 75% compared to the nil. Atrazine as a pre-emergent treatment only reduced annual ryegrass emergence by an average of 42%. In early August, the most effective treatment was PIC, reducing annual ryegrass populations by 91% compared to the nil. All other treatments reduced annual ryegrass populations by only 78%. By harvest, PIC, PAC and AIC were the most effective treatments at reducing annual ryegrass seed production and had all reduced annual ryegrass seed heads by more than 97%. Grain yield in the nil treatment was 0.57 T ha^{-1} reflecting the impact of weed competition on yield. The better early control provided by the PIC compared with all other treatments resulted in this treatment having the highest canola yield of 2.0 T ha^{-1}. Dual tolerant canola with well-structured herbicide programs provides opportunities to improve control of herbicide resistant annual ryegrass, as a single herbicide is not relied on. There will be further benefits from dual tolerant canola. There will be rotational benefits as this technology will allow canola to be planted back on imidazolinone herbicide residues and then using alternative herbicides, including the triazines, for weed control.

Key words: Dual tolerant canola, weed management, triazines, imidazolinones

INTRODUCTION

Herbicide tolerance in canola has been an important agronomic trait for the expansion of canola production in Australia (Salisbury et al. 2016). Initially, herbicide tolerance was required to manage Brassica weeds, such as wild radish (*Raphanus raphanistrum*), which would both compete with canola and also reduce the quality of the crop (Blackshaw et al. 2002). The introduction of triazine-tolerant (TT) canola in the 1990s resulted in a considerable expansion of the area of canola sown in Australia (Salisbury et al. 2016). Since then two other herbicide tolerant canola types have been marketed: Clearfield canola resistant to the imidazolinone (IMI) herbicides and Roundup Ready canola resistant to glyphosate.

In south-eastern Australia, the main weed problem in canola production has become annual ryegrass (*Lolium rigidum*). This is primarily because annual ryegrass has evolved resistance to most of the herbicides registered for its control (Boutsalis et al. 2012). In particular, it has

become difficult to control annual ryegrass adequately in wheat, making break crops important in the integrated management of herbicide resistant annual ryegrass (Swan et al. 2015; Kleemann et al. 2016). Herbicide tolerance in canola allows increased herbicide options for the control of annual ryegrass.

The increasing incidence of resistance to clethodim in annual ryegrass makes control of annual ryegrass in canola more difficult (Saini et al. 2015). For example, the use of pre-emergent herbicides alone is insufficient to provide effective control and imidazolinone herbicides can be ineffective if resistance is present (Saini et al. 2016). Dual tolerant canola allows multiple opportunities to control weeds within the crop. For example, canola resistant to both glyphosate and triazine herbicides (RT canola) can be used to better manage herbicide resistant annual ryegrass by allowing multiple controls to be included (Preston et al. 2016).

As Roundup Ready canola cannot be grown in all states of Australia, other dual tolerant canola types will be useful in canola production. One such option is canola with tolerance to imidazolinone and triazine herbicides (CT canola). This trial investigated herbicide strategies for the control of herbicide resistant annual ryegrass in a dual tolerant canola CT canola.

MATERIALS AND METHODS

A trial was conducted at Roseworthy, SA to investigate annual ryegrass control options in dual tolerant canola. Hyola 580CT (experimental canola line T61001) from Advanta Seeds, tolerant to both triazine and imidazolinone herbicides, was sown on 12 May 2017 at 3.5 kg ha^{-1}. A standard knife-point press wheel system was used to sow on 22.5 cm row spacing. Fertiliser rates were 100 kg ha^{-1} diammonium phosphate (DAP) applied at sowing followed by 50 kg ha^{-1} urea when the crop was had reached 7 true-leaf growth stage.

Pre-emergent herbicides were applied in 100 L ha^{-1} of water on the day prior to sowing. Post-emergent atrazine and imazamox + imazapyr were applied on 23 June 2017, when annual ryegrass was at the early tillering stage. Clethodim was applied on 5 July 2017. The various herbicide strategies used are listed in Table 1. Chlorpyrifos at 150 g ha^{-1} was applied directly after sowing for insect control.

Table 1. Herbicide treatments applied for the management of annual ryegrass in Hyola 580CT canola in 2017. IBS, Incorporated by sowing; POST, post-emergent.

Treatment	Herbicides used	Rates (g ha^{-1})
Nil	-	-
PIC	Propyzamide IBS, imazamox + imazapyr POST, clethodim POST	500, 25.75 + 11.25, 120
PAC	Propyzamide IBS, atrazine POST, Clethodim POST	500, 1980, 120
AC	Atrazine IBS, clethodim POST	1980, 120
AAC	Atrazine IBS, atrazine POST, clethodim POST	990, 990, 120
AIC	Atrazine IBS, imazamox + imazapyr POST, clethodim POST	1980, 25.75 + 11.25, 120

The trial was established as a randomized complete block design (RCBD) with 4 replicates. Assessments made were: annual ryegrass numbers in crop, measured 4 weeks after sowing and 4 weeks after clethodim application, annual ryegrass spike numbers at harvest, canola emergence 4 weeks after sowing and canola yield. Data were analysed by ANOVA with transformation of data to stabilize variances if required. Means separated by Fisher's protected lsd.

RESULTS

Seasonal conditions in 2017 in South Australia were challenging for canola production and herbicide efficacy. Following above average April rainfall, there was well below average June rainfall. Rainfall was then above average for July and August. April to October rainfall at Roseworthy in 2017 was 318 mm slightly higher than the long-term average of 287 mm. Frost was common during June 2017, potentially influencing the performance of post-emergent herbicides in this period.

There were no adverse effects of pre-emergent herbicides on CT canola establishment (P=0.964), with crop density similar to the Nil (62 plants m^{-2}) for all herbicide treatments (data not shown). All IBS herbicide treatments reduced annual ryegrass establishment compared to the Nil (P<0.001); however, propyzamide was more effective than atrazine (Table 2). Atrazine as a pre-emergent herbicide reduced annual ryegrass establishment by about 40%, whereas propyzamide reduced establishment by about 75%.

Table 2. Effect of different herbicide treatments on ryegrass plant density and spike production, and grain yield of Hyola 580CT canola at Roseworthy in 2017.

Treatment		Annual ryegrass			Grain yield
		IBS density[1]	IBS + POST density[2]	Spike density[3]	
		plants m^{-2}		spikes m^{-2}	kg ha^{-1}
Nil		216a	331a	663a	573a
	PIC	54c	29c	3d	1995c
	PAC	52c	59b	14d	1580b
	AC	143b	89b	86b	1470b
	AAC	135b	80b	34c	1362b
	AIC	95b	69b	2d	1467b

Mean values within column followed by different letters are significantly different (Fisher's protected LSD test P ≤ 0.01).
[1]Density 4 weeks after sowing
[2]Density 4 weeks after clethodim application
[3]Density of spikes at harvest

The post-emergent herbicide applications were able to further reduce annual ryegrass numbers with the exception of the PAC treatment (Table 2). The PIC treatment was most effective, reducing annual ryegrass numbers by more than 90% compared with the nil. All other treatments were slightly less effective, reducing annual ryegrass numbers by an average of 75%.

At harvest, the number of annual ryegrass spikes present were much reduced compared to the Nil for all the herbicide treatments (P<0.001). The AC treatment was the least effective treatment, reducing annual ryegrass spike numbers by 87%. In contrast, the PIC, PAC and AIC treatments were highly effective and all reduced annual ryegrass spikes by more than 97% (Table 2).

The high annual ryegrass population present had a significant effect on crop yield (P<0.001), with yield in the Nil treatment reduced by more than 70% compared to the highest yield (Table 2). The highest canola yield was achieved with PIC treatment with nearly 2 T ha^{-1}. The PAC treatment had a significantly higher yield than the AIC treatment, despite both treatments reducing annual ryegrass spikes to very low levels. This shows the importance of pre-emergent herbicides providing early annual ryegrass control in canola that protects yield.

DISCUSSION

In this work, CT canola provided effective management of herbicide resistant annual ryegrass by allowing a combination of treatments to be used. The best treatment combinations included an effective pre-emergent herbicide and two post-emergent herbicide applications. These multi-

treatment strategies allowed a reduction of more than 97% in annual ryegrass seed production in the canola crop. Control of herbicide resistant annual ryegrass can be difficult in cereals due to herbicide resistance and rotations with break crops improve management by allowing additional control practices to be used (Swan et al. 2015; Kleemann et al. 2016). Dual tolerant canola has the advantage of allowing multiple in-crop treatments to be employed (Preston et al. 2016).

Annual ryegrass has extensive resistance to both imidazolinone herbicides and clethodim in southern Australia (Boutsalis et al. 2012), but little resistance to atrazine. Despite this resistance, combinations of treatments can be effective at controlling herbicide resistant annual ryegrass in canola production, and dual tolerant canola facilitates this by providing additional herbicide options. While pre-emergent herbicides can be ineffective on their own for the control of annual ryegrass in canola (Saini et al. 2015; 2016), their use is essential for reducing early weed competitive effects on the crop and allowing yield to be maximized.

In addition to providing better integrated management of herbicide resistant annual ryegrass, CT canola offers the additional benefit of tolerance to imidazolinone residues in the soil. Imidazolinone herbicides can be persistent under dry conditions and can limit the subsequent cropping options to crop cultivars with tolerance to imidazolinone herbicides (Ramezani et al. 2008). CT canola offers the opportunity to sow a crop safely, but to avoid the further use of imidazolinone herbicides.

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Genomic selection in global diversity pools - a new paradigm for pre-breeding in canola

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ABSTRACT

We propose a new paradigm for pre-breeding in canola based on genomic selection in a globally diverse pool of *Brassica napus* germplasm. The base population comprising over 400 diverse canola lines representing global genetic diversity in the crop was developed at NSW DPI, Wagga Wagga. In this study, we assessed genetic diversity among 368 canola lines representing Australian *Brassica napus* Homozygous Diversity Set (ABnHDS) by Illumina SNP markers. These lines were evaluated for early vigour, flowering time, grain yield, and resistance to blackleg under field conditions as well as for tolerance to Mn^{2+} under controlled environment conditions. A subset of 196 lines was also analysed for structural variations (INDELS) and SNPs using whole genome resequencing approach. Cluster and population structure analyses of ABnHDS (368 lines) grouped canola lines based on their growth habit and geographic origin. Using both molecular and phenotypic data, individual lines were assigned genomic predicted breeding values (GPBV) by genomic best linear unbiased prediction (GBLUP). Genomic prediction accuracies ranged from 47 to 80% across traits (except for 37% for flowering time under short day conditions) indicating that genomic prediction can be used quite confidently to predict the performance for some canola lines with missing phenotypes. We further employed mating design based on optimal contributions selection (OCS) to manage long-term genetic gain and genetic diversity in the global diversity pool. Canola breeders will have the accelerated access of lines with potential value in their commercial programs based on their ranking for economic index and GPBV for individual traits. The global diversity pool will also be useful to identify the role of individual genes that underlie traits of economic importance.

Key words: Canola diversity panel – Genomic selection – Resequencing – SNP – Phenotyping – Optimal cross design

INTRODUCTION

Traditional varieties (landraces) are invaluable resources of genetic diversity and have been exploited to develop improved varieties for range of traits in various crops (Tanksley and McCouch, 1977). However, no such source is available in canola which has a very short history of domestication. Genetic bottlenecks posed by intensive selection, domestication and linkage drag, largely contributed to a narrow genetic base of canola germplasm. As a result, there is a limited genetic variation for several traits of agronomic importance such as resistance to pod shatter and sclerotinia stem rot, tolerance to drought, radiation use efficiency, and harvest index. Molecular marker- and pedigree-based analyses have also revealed the narrow genetic base of *B. napus* varieties (Cowling, 2007; Bus et al., 2011). Maintenance of genetic diversity in pre-breeding germplasm is the key to the future

breeding of improved varieties for meeting the current and future energy demands. Recently optimal contributions selection (OCS) was proposed to maintain genetic diversity while making genetic gains during pre-breeding of crop plants (Cowling et al., 2017), and genomic selection methods are available to support such procedures (Gaynor et al., 2017; Hickey et al., 2017). In this study, we assessed genetic diversity among 368 *B. napus* genotypes representing National Brassica Germplasm Improvement Program germplasm collected at the Wagga Wagga Agricultural Institute using Illumina SNP markers. The same set of germplasm was evaluated for various traits of agronomic importance across environments. Finally, we combined genomic predicted breeding values (GPBV) in an economic index to optimize a mating design based on OCS to maximize genomic diversity and make long-term genetic gain in the Australian *Brassica napus* Homozygous Diversity Set (ABnHDS) base population, using technology developed for animal breeding at University of New England, Armidale, NSW, Australia.

MATERIALS AND METHODS

Plant materials and evaluation of agronomic traits: 368 canola lines representing ABnHDS were used for molecular genetic diversity analysis. These lines were evaluated for early vigour, flowering time, harvest index, grain yield, and resistance to blackleg in two replicates at two field sites, as well as for response to photoperiod and tolerance to Mn^{2+} under controlled environment conditions. For field evaluation, three trials at Wagga Wagga and Condobolin were conducted in plots (2 x 10 m). Tolerance to Mn^{2+} toxicity was evaluated using nutrient solution culture (Raman et al., 2017). Photoperiodic response was assessed by growing plants under short day (8 h of light/16h of dark) and long day (16 h of light/8 h of dark) at 20°C (Raman et al., 2018).

Genotyping and population structure: All 368 ABnHDS lines were genotyped with 12,197 Infinium Illumina SNP markers (Clarke et al., 2016). A subset of 196 lines was also analysed for structural variations (INDELS) and SNPs using whole genome resequencing approach. The genomic relationship matrix (GRM) was built by using the method described previously (Dodds et al., 2015). In order to make the GRM positive definite, the diagonal was augmented by adding small positive values.

Statistical analysis and genomic prediction: GBLUP was used to estimate both the variance components and to predict direct genomic breeding values in the univariate analyses for each trait. The univariate analysis model was:

$$\mathbf{Y} = \mathbf{1}\mu + \mathbf{Z}\mathbf{g} + \mathbf{e}$$

Where, **y** is the data vector of the adjusted mean of genotyped varieties, μ is the overall mean, **1** is a vector of ones, **Z** is a design matrix that matches data to breeding values, **g** is a vector of breeding values to be estimated, and **e** is a vector of residuals. It was assumed that $\mathbf{g} \sim \mathbf{N}(\mathbf{0}, \mathbf{G}\sigma^2_g)$ where σ^2_g is the additive genetic variance, and **G** is the marker-based GRM and $\mathbf{e} \sim \mathbf{N}(\mathbf{0},\mathbf{I}\sigma^2_e)$ where σ^2_e is the residual variance. Narrow-sense heritability was calculated as $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_e)$. Bivariate analyses with the same fixed and random effects as the univariate analyses were completed to estimate genetic correlations for all combinations of the traits. All analyses were performed with the ASReml package (Gilmour et al., 2015).

Optimal mating design: The optimal set of parents was selected from the ABnHDS base population, which represented global genetic diversity in the crop. The selection and mate allocation method used previously (Cowling et al., 2017) involves a function whose key components relate to genetic gain and genetic diversity. The optimization of this function results in OCS which is based on the breeding program implementation platform "Matesel" (Kinghorn & Kinghorn, 2018). The practical implementation of this method is based on an evolutionary algorithm, with constraints invoked to ensure practical relevance and precise control of each trait (Kinghorn et al., 2009; Kinghorn, 2011). Matesel determines which individuals to select and the actual mating allocations. Very conservative settings for genetic diversity were used in the weighting of genetic diversity and response to selection for the index, based on grain yield at Wagga and Condobolin, blackleg resistance at Wagga (low scores for internal canker infection and high scores for plant survival selected), and Mn^{2+} tolerance.

Accuracy of Genomic prediction

A replicated cross-validation method was conducted to estimate the predictive ability of genomic prediction. The data was randomly split into five groups with one of the five groups as the validation set and the other four groups as training sets. This was repeated for five times so each line was used for testing in one group and for training in other four groups. The accuracy of genomic prediction was measured as the Pearson correlations between the predicted genomic breeding values and the observed values in the validation set, divided by the square root of the heritability. This 5-step process was replicated 100 times. The mean and standard deviation of the Pearson correlation was calculated across the 100 replicates.

RESULTS

Phenotypic means, narrow-sense heritability values and accuracy of genomic prediction are presented in Table 1. Levels of variability between canola lines varied between traits, with high variation found in blackleg internal infection rate, moderate variation in grain yield-related traits varying in extent across sites (46 to 56%) and relatively low variation in other traits (0 to 24%). We found higher heritability values for flowering time (55 to 64%) and Mn^{2+} tolerance (58%) compared to shoot biomass, plant survival, NDVI and grain yield (15 to 21%), and all estimates were significantly greater than zero (Table 1). The genomic prediction accuracies ranged from 47 to 80% (except for 37% for flowering time under short day conditions) indicating that the genomic prediction can be used quite confidently to predict the performances for some canola lines with missing phenotypes (as well as for lines with phenotype data). There is evidence for significant G x E effects, with the estimate of genetic correlation between flowering time and grain yield in the 2017 trial at Wagga as 0.3 (+/-), but -0.26 (+/-) at the Condobolin site in 2017. The genetic correlation estimated between NDVI and flowering time scored under field conditions at 3 sites across Wagga Wagga and Condobolin was r = 0.3 to 0.4, and r = 0.7 under long- day conditions in a controlled environment cabinet.

Table 1. Phenotypic means, narrow-sense heritability values (h^2) and accuracy of genomic prediction (Acc) of different traits evaluated among 368 diverse lines of *B. napus*.

Trait	Phenotyping Year	Environment	Site	Population Mean	h^2 (%)	Acc (%)
Manganese tolerance (0-5 scale)	2017	Controlled conditions	Wagga Wagga	3.45	58	63
Grain yield (g/row)	2017	Field	Condobolin	55.81	32	47
Grain yield (g/plot)	2017	Field	Wagga Wagga	819.96	27	54
Flowering time (days)	2017	Short day conditions	Wagga Wagga	131.01	26	37
Flowering time (days)	2017	Long day conditions	Wagga Wagga	44.23	55	66
Flowering time (days)	2017	Field	Wagga Wagga	117.18	64	77
Flowering time (days)	2017	Field	Condobolin	110.39	59	77
Flowering time (days)	2017	Field	Wagga Wagga	115.93	59	80
Yield g/plant	2017	Field	Wagga Wagga	1.23	18	59

Shoot biomass (g)	2017	Field	Condobolin	8.17	15	67
Harvest Index	2017	Field	Condobolin	16.09	40	61
Blackleg (Internal infection %)	2017	Field	Wagga Wagga	8.83	27	61
Blackleg (Plant survival %)	2017	Field	Wagga Wagga	82.37	21	69
Plant maturity (0-9 scale)	2017	Field	Wagga Wagga	8.22	48	64
NDVI[1]	2018	Field	Wagga Wagga	0.62	19	63

[1]**Normalised Difference Vegetative Index measured with GreenSeeker**

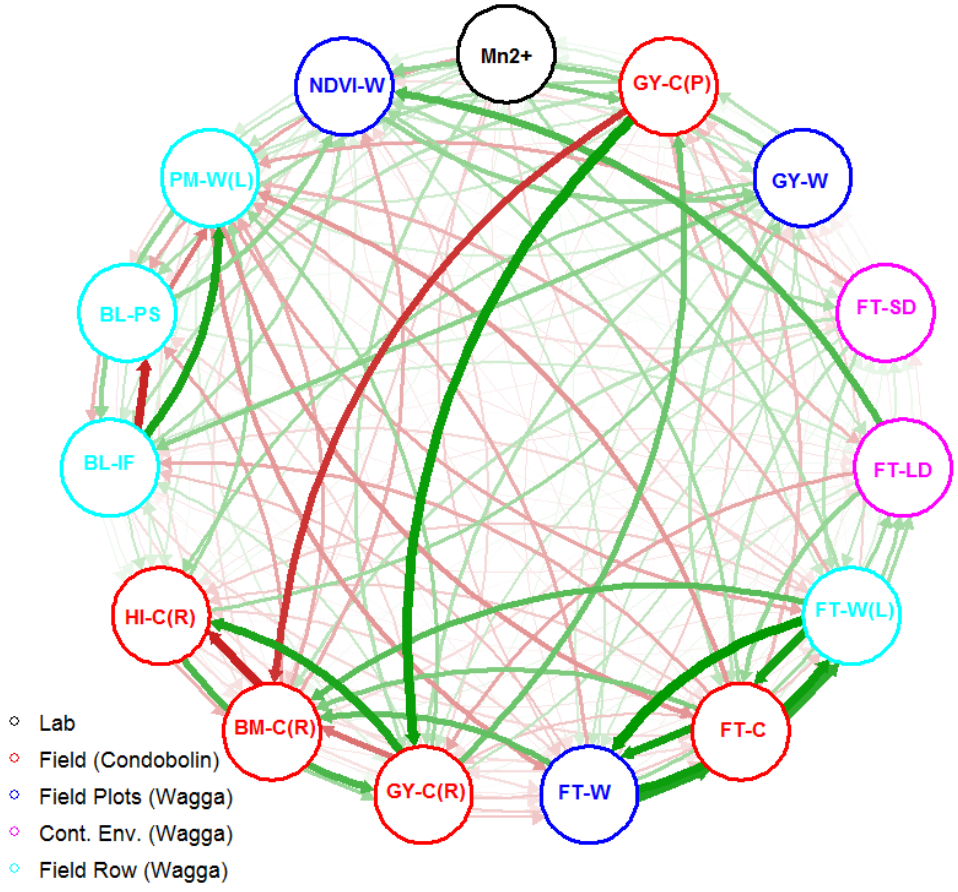

Figure 2: Estimated genetic correlations among different trait measurements made in the Australian *Brassica napus* Homozygous Diversity Set evaluated under laboratory, field and controlled environment conditions, Mn^{2+}: tolerance to Mn^{2+} toxicity, GY-C(P): Grain yield at Condobolin (in plots); GY-W: grain yield at Wagga (in plots), FT-SD: flowering time under short day conditions; FT-LD: flowering time under long day conditions; FT-W(L): flowering time at Wagga Wagga (lateral move); FT-C: flowering time at Condobolin (in plots); FT-W: flowering time at Wagga Wagga (in plots), FY-C(R): flowering time at Condobolin (in rows); BM-C(R): shoot biomass at

Condobolin (in row); HI-C(R): harvest index at Condobolin (in rows), BL-IF: blackleg resistance scored as an internal infection (%); BL-PS: blackleg resistance scored as plant survival (%); PM-W(L): plant maturity at Wagga Wagga (lateral move). Thick lines indicate strong correlation while thin lines indicate weak correlation.

A total of 12,197 high quality Illumina SNPs were used for determining genomic relationships among *B. napus* genotypes. The same marker set was used for cluster and population structure analyses which enabled grouping of the canola lines; largely based on their growth habit and/or geographic origin. In order to determine genomic diversity among founder lines to be used for optimal crossing, we performed genomic resequencing from DNA isolated from ABnHDS lines. More than 3.7 million high-confidence sequence variants were identified which could be anchored on the reference *B. napus* genome cv. Darmor (Figure 1). The marker density/chromosome ranged from 105,388 (A08) to 322,402 (C03), with the average marker density of 195,168.

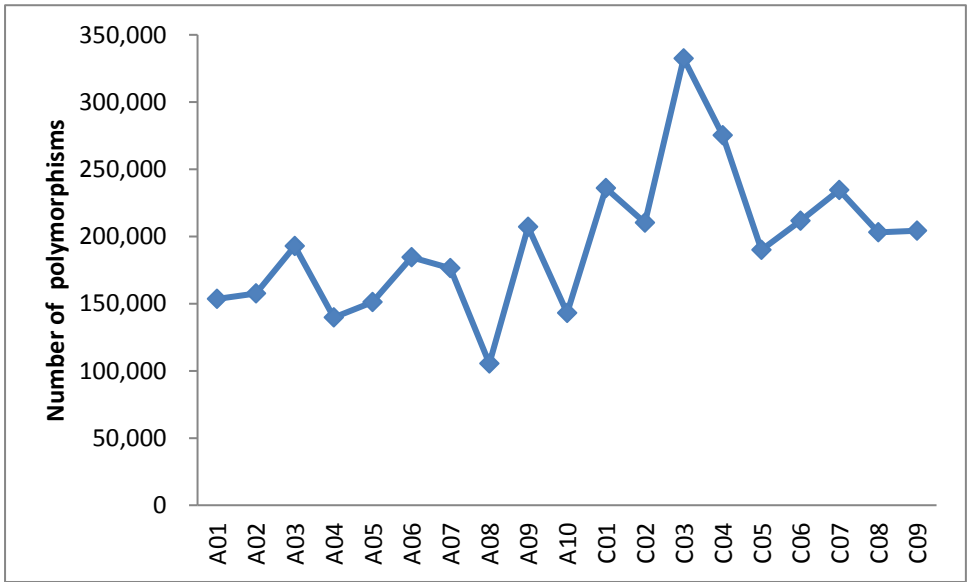

Figure 2: Genome wide distribution of SNP, identified via whole genome resequencing approach, across 19 chromosomes of canola

Optimal Mating Design

An optimal mating design was derived using MATESEL, with 100 matings, based on OCS, used 75 parent genotypes and targeted moderate increase in economic index and high genetic diversity maintained. This mating design resulted in a predicted increase in economic index of $139/ha at Wagga Wagga and $95/ha at Condobolin, with a low predicted increase in population coancestry of 0.057, and there were parallel improvements in blackleg resistance and Mn^{2+} tolerance. Predicted average flowering time in progeny was two days earlier than the original population (ABnHDS) at Wagga Wagga at three days earlier at Condobolin.

DISCUSSION

Thousands of molecular marker-trait associations for various agronomic traits in QTL and genome-wide association analyses studies have been identified in canola. Using this marker information in conjunction with phenotypic data can improve trait selection, for both traits impacting quality and productivity traits. However, a limited number of markers have been widely applied for selection of quantitative traits such as grain yield. While genomic selection provides scope for rapid genetic gains, it can also increase the risk of reducing genetic diversity by more precisely selecting superior allelic combinations. OCS can maintain genetic diversity while making genetic gains. In this study, we explored the application of OCS in this program for pre-breeding context for improvement of economically important traits sought by canola breeding companies such as grain yield and its related traits (flowering time, early vigour, and harvest index), tolerance to abiotic and biotic stresses such as

tolerance to drought, heat stress and toxicity to Mn^{2+}, and resistance to blackleg disease. In conclusion, we developed a genomic resource: a pre-breeding population in canola (ABnHDS) which was genotyped with over 12,000 Illumina SNPs (368 lines) and over 3.7 million high confidence SNPs (196 lines) identified on the basis of whole genome resequencing. The phenotypic dataset based on field and controlled-environment conditions generated in 2017 and 2018 wasused in estimating genomic breeding values for traits of agronomic importance to the Australian canola breeding programs. Parents were chosen for crossing based using OCS which permitted genetic improvement for an economic index (including grain yield, blackleg resistance, Mn^{2+} tolerance), as well as retention of high levels of genetic diversity present in ABnHDS. Future progenies, resulting from crossing and selection in this population will retain valuable alleles for genetic advancement for target traits and other traits of interest to commercial breeders such as grain quality, pod shatter resistance, and heat, drought or frost tolerance. Breeders will have the accelerated access to pre-breeding lines with potential value in their commercial programs based on their ranking for economic index and GPBV for individual traits.

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Identification of genomic regions and candidate genes for resistance to pod shatter in Brassica

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ABSTRACT

Pod shatter causes significant yield loss in various members of the *Brassicaceae* family including *Brassica napus*. Under the National Brassica Germplasm Improvement Program (NBGIP), we have (i) determined the extent of genetic variation and localised loci for resistance to pod shatter using linkage and genome wide association studies and (ii) developed promising pod shatter resistant lines for Australian canola breeding programs. Phenotypic analyses across multiple environments revealed that there is a limited variation for pod shatter resistance in canola. Utilising natural variation available to NBGIP, we delineated multiple loci on chromosomes A01, A03, A06, A07, A09, C02, C03, C04, C05, C06 and C08 accounting for pod shatter resistance in diverse accessions of *B. napus*. In order to enhance the level of pod shatter resistance in Australian canola, we also characterised genetic variation for pod shatter resistance in *B. rapa* and *B. carinata,* and identified accessions that had more than 10 times shatter resistance (pod rupture energy) as compared to canola. Two intercross populations of *B. rapa* and *B. carinata* were developed and whole-genome based bulk segregant analysis was performed to identify the genomic regions for resistance to pod shatter. We are also developing resynthesis *B. napus* lines to combine resistance loci from *B. rapa* and *B. carinata* accessions. In addition, resynthesis *B. napus* lines derived from *B. rapa*/*B. carinata* (accessed from China) and *B. napus*/*B.carinata* (accessed from UM0045 project) for pod shatter resistance using delayed harvest/pendulum test at Wagga Wagga. Three accessions were found to be promising and are being progressed for canola genetic improvement. To pinpoint loci for pod shatter resistance in resynthesised *B. napus,* two F_2 populations derived from *Brassica rapa*/*B. napus* x *B. napus,* and *B. napus/B. carinata* x *B. napus* were developed. Mapping of loci for pod shatter resistance in resynthesis lines is in progress.

Key words: Pod shatter – resistance – genetic mapping – Evaluation – Resequencing – comparative mapping

INTRODUCTION

Pod shattering is a negative trait affecting canola productivity worldwide. Upon maturity, pod valves open, dehiscing seeds for dispersal. These lost seed not only become 'volunteer weed' in the next crop, which makes crop management difficult, but also cause significant economic loss

to farmers by reducing their gross margin. The extent of yield loss varies year to year; under hot and windy conditions, up to 25% yield loss can occur and is a major issue for canola production. In recent years, mechanical harvesting is being preferred over wind-rowing, therefore varieties having improved shatter resistance are highly sought to reduce the yield losses.

Pod structure of canola and other diploid (*B. rapa*, *B. nigra*, *B. oleracea*) and allopolyploid oilseed species (*B. carinata* and *B. juncea*) of the family Brassicaceae is similar to its close relative, *Arabidopsis thaliana*. In these species, ovary is formed from two carpels that are believed to arise congenitally fused along their margins from the center of the floral meristem and grows to a pod (Bowman et al., 1999; Ferrándiz et al., 1999; Ferrandiz et al., 2000). Both carpels contain seeds which are attached to septum with funiculus. Pod valve separate from replum via formation of dehiscence zone, characterized by separation layer and lignification layer. Mechanism of pod shattering (seed dehiscence) is well understood in *A. thaliana*. Several genes involved in valve (*FUL*), valve margin identity/dehiscence zone (S*HP1*, *SHP2*, IND, and ALC) and replum differentiation (*RPL*) have been cloned and characterized (Ferrandiz et al., 2000; Rajani and Sundaresan, 2001; Liljegren et al., 2004; Sorefan et al., 2009; Arnaud et al., 2010; Girin et al., 2010). Besides, *CRABS CLAW*, *SPATUA* and *ETTIN* were also shown to alter ovary morphogenesis (Sessions et al., 1997; Bowman and Smyth, 1999; Heisler et al., 2001). However, genetic basis of natural variation in pod shatter resistance is not well understood in *B. napus* and its related species.

Under the DAN00117 (2008-15), DAN00208 (2015-16) and Project 9176589 (2017-2020) projects, one of the foci has been on the understanding the genetic basis for pod shatter resistance in *B. napus*. We determined the extent of natural variation in *B. napus*, *B. rapa* and *B. carinata* accessions and identified loci associated with pod shatter resistance in collaboration with research partners at the OCRI, Wuhan, China (Prof. Qiong Hu) and Huazhong Agricultural University, Wuhan, China (Prof. Jinling Meng and Dr Jun Zou). We also accessed and evaluated resynthesis germplasm developed from the *B. napus*/*B. carinata* developed under the UM0045 project (Dr. Phil Salisbury and Prof S.S. Banga) and *B. rapa*/*B. carinata* germplasm developed at the HAU, China, and determined the genetic variation in pod shatter resistance. The potential of improvement in pod shatter resistance in *B. napus* via introgression from related species was explored in *B. napus*. Finally, we assessed the germplasm for pod shatter resistance under field conditions using delayed harvesting.

MATERIALS AND METHODS

Several populations of *B. napus*, *B. rapa*, *B. carinata* and resynthesis lines were evaluated for pod shatter resistance and subsequently analyzed for marker-trait associations (Table 1). Resistance to pod shatter was evaluated either using pendulum test, measured as rupture energy, RE (Kadkol et al., 1985; Liu et al., 1994) and/or random impact test (Peng et al., 2011). Delayed harvest method was also employed to test the pod shatter resistance under field conditions at the Wagga Wagga Agricultural Institute.

Table 1. Details of mapping populations used for genetic analyses of pod shatter resistance in canola and related species.

Species	Population	Population type (size)	Phenotyping Method	Genotyping	Reference
B. napus	BLN2762/Surpass400	DH (126 lines)	Pendulum test	17,420 Genotyping-by sequencing markers (DArTseq)	Raman et al. (2014)
	Diversity Panel	GWAS (200 lines)	Pendulum test	89,618 DArTseq markers	
	R1/R2	DH (96 lines)	Random impact test	7728 Illumina SNP markers	Liu et al. (2016)
	Diversity Panel	Diverse lines (143 lines)	Random impact test		
		Intercross population (144 lines)	Random impact test		
	Z11/R1	DH	Random impact test	16341 SNP markers	Wang et al (unpublished)
B. rapa	BC11229/ BC80012	F_2	Pendulum test	DNA Resequecing DArTseq	Raman et al (unpublished)
	GWAS panel	Diverse lines	Pendulum test	DArTseq	Raman et al (unpublished)
B. carinata	BC73526/BC73524	F_2	Pendulum test	6464 DArTseq markers	Raman et al. (2017)
	GWAS panel	Diverse lines	Pendulum test	DArTseq	Raman et al. (2017)
	BcDH64/BcDH76 (YW population)	DH	Pendulum test	DArTseq	Raman et al (unpublished)
Resynthesis lines *B.rapa/B. napus* *B. napus/B. carinata*	08-6702P/BLN3343)	F_2	Pendulum test	15,500 DArTseq markers Resequencing	Raman et al (unpublished)
B. napus/B. carinata/B. napus	GSC/1772	F_2	Pendulum test	DArTseq	Banga et al (unpublished)

RESULTS

Genetic dissection of pod shatter resistance in *B. napus:* We determined the extent of genetic variation for pod shatter resistance (pod strength based on rupture energy measured using pendulum test) of diversity panel comprising 200 accessions of *B. napus*, *B. rapa*, *B. juncea,* and *B. carinata* (Raman et al 2014). Pod strength varied from 2.09 mJ to 5.58 mJ in birdcage and field experiments. To determine loci controlling pod shatter resistance in *B. napus*, we conducted both genome-wide association mapping (GWAS) and quantitative trait loci (QTL) studies in germplasm being utilized in Australia (specially under National Brassica Germplasm Improvement Program at the NSW Department of Primary Industries, Wagga Wagga) and China (OCRI, Wuhan). We delineated multiple loci on chromosomes A01, A03, A06, A07, A09, C02, C03, C04, C05, C06 and C08, accounting for pod shatter resistance in diverse accessions of *B. napus* (Raman et al., 2014; Liu et al., 2016). We further aligned the physical positions of QTL/markers underlying pod shatter resistance with the *priori* candidate genes involved in pod shatter resistance in *A. thaliana* such as *FULL*, *CLAVATA, AGL15, SHP, RPL*, *IND*, *CELLULASE*, *AP2-like ethylene-responsive transcription factor* and *PG, and* organ identity genes such as, *AGAMOUS, CLAVATA, CRAB CLAW, DELLA,* and *KANANDI* and identified 'putative' candidate genes for pod shatter resistance in the canola germplasm. For example, QTLs: *Qrps.wwai-A09b*, *Qrps.wwai-C08b*, *Qrps.wwai-A07* and *Qrps.wwai-C06* were detected near the *SHP1* gene in *B. napus* (Raman et al 2014). We further mapped *SHP1* gene- specific markers within the QTL regions associated with pod shatter resistance, suggesting that *SHP1* may likely control genetic

variation in a DH population from BLN2762/Surpass400. In an independent study, Liu et al (2016) identified six QTL for resistance to pod shatter on chromosomes A01, A06, A07, A09, C02 and C05. Two of the QTL, *qSRI.A09* (A09) and *qSRI.A06* (A06) were detected across environments in a GWAS panel, DH and IF_2 populations, suggesting that at least two loci on chromosomes A06 and A09 were the main contributors to pod shatter resistance in germplasm of Chinese origin. Both studies described above show that at least one QTL present on chromosome A09 which map in the vicinity of *SHP1* gene control pod shatter resistance in *B. napus* germplasm (Raman et al., 2014; Liu et al., 2016). This QTL accounted for 16.5 to 17.6% and 9.8 to 16.9% variance for pod shatter resistance in the Australian and Chinese germplasm, respectively. Under the collaborative project, DAN00208 with the OCRI-CAAS, China, we identified three quantitative QTLs for pod shatter resistance in a mapping population derived from Z11/R11 (282 lines); which was mapped with markers based on whole genome resequencing approach (Wang el al, unpublished).

Structural variation in pod shatter resistance loci: Under the DAN00208 project, we resequenced 21 accessions of *B. napus* (~100x coverage), 174 accessions (6-10x), five accessions of *B. carinata* (~100x) and two accessions of *B. rapa* (100x) which showed segregation for pod shatter resistance and other priority traits identified for NBGIP research (water use efficiency, blackleg resistance, flowering time and grain yield). We are currently analyzing sequence variation in coding, intronic and promotor regions to understand the mechanism of pod shatter resistance in canola and its close relatives. Sequence analyses have shown that multiple paralogs exist in *B. napus* genome. For example, at least five gene models of *SHP1* and three of *SHP2* are described in the reference genome of *B. napus* cv Darmor (Chalhoub et al., 2014). Extensive structural variation in *SHP1* paralogs on chromosomes A07 and A09 suggests that it could account for functional variation in pod shatter resistance in canola.

Accessing genetic variation for pod shatter resistance from related species of *B. napus:* Our previous study showed that *B. rapa (AC-Sunshine, B46 and DST17D), B. juncea* (CBJ001, SaharaCL, Seetha and Urvashi) and *B.carinata* (ATC93184-1, ATC94044-1) accessions were more resistant to pod shatter as compared to *B. napus* (Raman et al 2014). These results suggested that 'superior alleles' for pod shatter resistance which exist in related species may have been lost during intensive selection, domestication, or due to linkage drag of undesirable alleles in canola (Raman et al 2014). In order to enhance the level of pod shatter resistance in Australian canola, we further characterized genetic variation for pod shatter resistance among 200 accessions of *B. rapa* and *B. carinata,* and identified five accessions that had more than 10 times pod rupture energy as compared to canola (Raman et al., 2014; Raman et al., 2017). Three populations of *B. rapa* and *B. carinata* (Table 1) were developed and investigated to identify the genomic regions for resistance to pod shatter.

Genetic mapping of pod shatter resistance loci in *B. rapa*

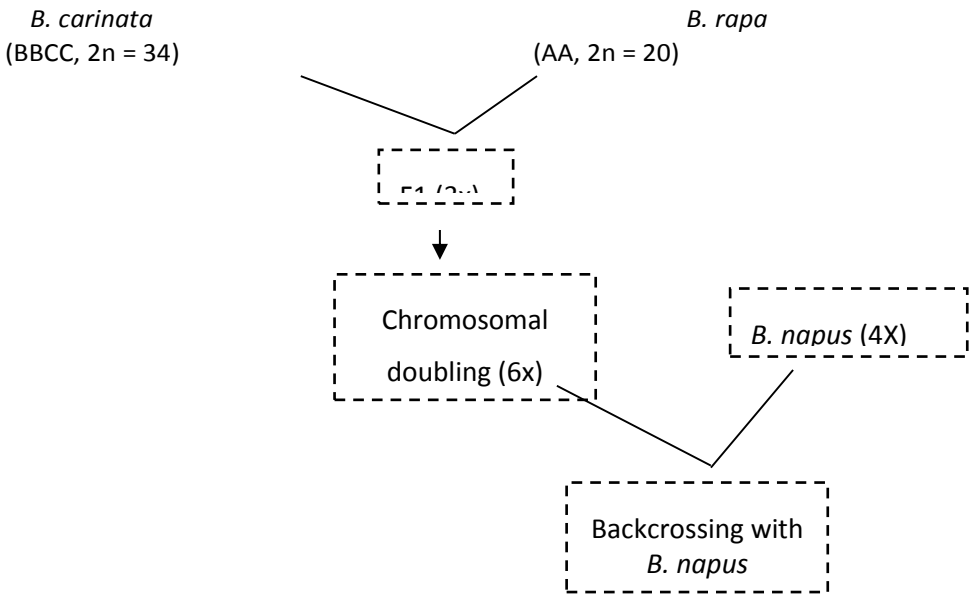
We evaluated an F_2 derived population (200 lines) derived from the BC11229/BC80012 for pod shatter resistance in 2015 and $F_{2:3}$ lines in 2016 under birdcage conditions. A linkage map based on DArT seq markers was constructed and utilized for QTL detection. We identified at least one major QTL for pod shatter resistance. We further used whole-genome sequencing-based bulk segregant approach and identified polymorphisms that showed significant association with pod shatter resistance (Raman et al, unpublished).

Genetic mapping of pod shatter resistance loci in *B. carinata*

Two populations were utilized for genetic analyses to identify loci associated with pod shatter resistance (Table 1). QTL analysis of an F_2 population of *B. carinata* (300 lines) derived from BC73526 (shatter resistant with high RE) and BC73524 (shatter prone with low RE) revealed five QTL for pod shatter resistance on chromosomes B01, B03, B08, and C05 (Raman et al., 2017). Shatter resistance was evaluated using the pendulum test. Genetic analysis showed that shatter resistance is controlled by recessive genes. Besides, we evaluated a DH population derived from a cross: BcDH64 (yellow petal)/BcDH76 (white petal), designated as (YWDH) at Wagga Wagga for two years in 2013, and 2014 to reveal loci for pod shatter resistance. The genetic map covering 2,048 cM of YWDH population (Zou et al., 2014; Zhang et al., 2017) was employed for QTL analysis. Three significant QTL were detected in this population (Raman et al, unpublished).Overall QTL mapping studies on *B. napus*, *B. rapa* and *B. carinata* populations revealed that limited number of loci control pod shatter resistance across environments. Research on characterizing identified genomic regions and underlying candidate genes contributing natural variation in pod shatter resistance is in progress.

Development of new resynthesis lines derived from *B. rapa* and *B. carinata*

To develop new source of germplasm with improved level of pod shatter resistance and capture subgenomic diversity from *B. carinata* (BBCC genome), we are developing resynthesis lines (Figure 1). Five interspecific crosses were made between two accessions of *B. rapa* and three accessions of *B. carinata* with shatter resistance (Table 1). Triploid plants were subjected to chromosome doubling and we obtained 44 hexaploid plants from the five cross combinations. These hexaploid plants were crossed with new-type *B. napus* with good combining ability, and will be further backcrossed to new-type *B. napus* and screened for shatter resistance.



Characterisation and genetic mapping of pod shatter resistance in resynthesis lines

In addition, we evaluated resynthesis *B. napus* lines derived from *B. rapa*/*B. carinata* (China) and *B. napus*/*B. carinata* (accessed from UM0045 project) for pod shatter resistance using delayed harvest/pendulum test methods at Wagga Wagga. Three accessions were found to be promising and are being progressed for canola genetic improvement. To pinpoint loci for pod shatter resistance in resynthesised *B. napus,* we developed two F_2 populations derived from *B. rapa*/*B. napus* x *B. napus,* and *B. napus/B. carinata* x *B. napus* crosses. Mapping of loci for pod shatter resistance in resynthesis sources is underway.

CONCLUSIONS

Our data demonstrates limited natural genetic variation for pod shatter resistance in *B. napus* germplasm, drawing attention to the importance of introgressing superior alleles from related species such as *B. rapa*, *B. juncea* and *B. carinata*. The genetic analysis of segregating populations provides evidence that the genetic variation for resistance may be associated with the same gene network involved in Arabidopsis such as *SHP1/SHP2*, *FUL*, and *IND*. Sequence analyses showed that several paralogs may underpin genetic variation in pod shatter resistance.

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An overview of the Australia, India & China Brassica improvement projects

Phil Salisbury, Martin Barbetti, Surinder Banga, Sheng Chen, Allison Gurung

2004-2010:

Oilseed Brassica Improvement in China, India and Australia (CIM/1999/072)

Co-funded by ACIAR and GRDC

2011-2017:

Expanding the Brassica Germplasm Base through collaboration with China and India (UM00045)

Funded by GRDC

Thanks to our major project collaborators from the following institutes:

Australia

University of Melbourne

University of WA

DEDJTR, Vic

DPI, NSW

Dept of Agric & Food, WA

India:

Punjab Agricultural University

Indian Council of Agric Research

National Res Centre on Rapeseed-Mustard

Haryana Agricultural University

The Energy & Resources Institute

China:

Huazhong Agricultural University

Oil Crops Research Institute, CAAS

Institute of Economic Crops, XAAS

Summary

The overall aim of these trilateral projects was to utilise germplasm from China, India and Australia to enhance productivity of canola (*Brassica napus*). The projects sought to expand the canola germplasm base and incorporate traits such as disease resistance and shatter tolerance from closely related wild species into canola using conventional breeding techniques.

The GRDC funded project "Expanding the germplasm base through collaboration with China and India" involved several challenging, labour intensive methods for introducing new genetic variation into the *B. napus* genome.

These included:

1. incorporating genes from wild relative species into *B. napus* (through crossing, chromosome doubling and backcrossing),
2. incorporating genes from the B genome (*B. carinata*) into *B. napus* (through crossing, chromosome doubling and backcrossing),
3. resynthesising *B. napus* from progenitor species in the Triangle of U (synthetic *B. napus*).

The project also included screening of the new material for key traits including disease resistance, shatter tolerance and heat and drought tolerance.

Progress on expanding the Brassica germplasm base was significant.

Incorporation of genes from wild relatives:

Crossing programs to enhance the canola germplasm base through incorporation of genes from wild Brassicaceae species were initiated in the 2011/2012 cropping season in India. Thousands of hybrid ovules were cultured using embryo rescue techniques in an attempt to obtain wide hybrids (with a success rate of less than 1%). Amphiploidy was successfully induced in some of the hybrids and hybridity was confirmed with cytological and molecular analyses. Hybrids were recovered from crosses including *B. napus* and *B. juncea* by *Diplotaxis* and *Erucastrum*. Following successful backcrossing, seed of these lines was exchanged with Australia (7 *B. napus* & 14 *B. juncea* lines) and screened in Australia for disease resistance and agronomic traits.

Incorporation of genes from *B. carinata* (B genome):

Euploid lines were successfully selected from aneuploid lines with B genome introgressions that were developed in the previous ACIAR/GRDC project "CIM/1999/072"(Table 1). Further cycles of crossing of these euploid *B. napus* lines to *B. napus* were carried out to improve fertility & reduce linkage drag. New *B. napus* by *B. carinata* hybridisations were also produced and chromosome doubling of the hybrids was undertaken to produce amphiploids. B genome introgression lines of *B. napus* were exchanged and seed increased in Australia (Table 2). Screening in Australia for disease resistance and agronomic traits has identified significant variation. Data from the phenotype screenings has been published and was also used in association mapping.

Sixteen introgression lines selected for shatter tolerance were screened for disease resistance, agronomic traits and shatter tolerance in Australia in 2016. Promising variation for shatter tolerance compared to canola controls was observed in a delayed harvest field trial in 2016/17.

Resynthesis of *B. napus* from triangle of U progenitors:

Crosses between *B. napus* progenitor species were carried out in India and embryo rescue was initiated. Synthetic (derived) *B. napus* genotypes have been resynthesized from different combinations involving *B. carinata* x *B. juncea* & *B. juncea* x *B. oleracea*. Thirty eight of the resynthesised lines were exchanged in 2015 and seed increase was undertaken in Australia. In 2016, phenotype screening of the 38 lines was carried out in Australia. Significant variation for blackleg resistance, yield, heat tolerance and drought tolerance was observed in these lines.

Screening and recurrent selection for traits of interest:

Germplasm screening and selection for agronomic attributes and resistance to biotic and abiotic stresses has been carried out in Australia with the exchanged lines. The lines screened to date comprise 150 *B. napus* B genome introgression lines from India, 20 conventional lines from China, 21 *B. juncea* & *B. napus* lines with wild introgressions and lines from the previous ACIAR/GRDC project. Screening has identified lines with significant differences in blackleg resistance and Sclerotinia resistance. Drought and heat tolerance screening in Australia identified genotypes with good seed yield and less than 20% yield loss under drought conditions. Recurrent selection of lines identified to show drought and heat tolerance is continuing and seed selected from the recurrent selection experiments was exchanged in 2016.

When and how industry can benefit from the work

All germplasm created in the project and data from trials has been provided to NBGIP members for use in breeding programs. The traits will be of benefit to the yield of canola crop, leading to economic and social benefits.

Who can benefit from the results

This project has provided new germplasm resources with enhanced genetic diversity and scientific knowledge for breeders and researchers to use for further cultivar development. The project will result in improved and more stable productivity of canola in Australia due to the availability of new germplasm with enhanced levels of resistance to biotic and abiotic stresses. This expansion of the Brassica germplasm base has been highly beneficial for the breeders.

Table 1. Germplasm produced/exchanged during the ACIAR/GRDC funded project (2004-2010)

Type	Details	No. lines	Distribution status
B. napus	57 x Chinese lines 11 x Indian lines 56 x Australian lines	124	Project collaborators, NBGIP/CBG &/or AGG
B. napus / B. carinata hybrid	2 x Indian lines	2	"
B. juncea	30 x Chinese lines 54 x Indian lines 29 x Australian lines	113	"
B. juncea/B. carinata hybrid	2 Indian lines	2	"

Table 2. Germplasm produced/exchanged during the GRDC funded project (2011-2017)

Type	Details	No. lines	Distribution status
B. napus x weedy introgressions	*B. napus* x *Erucastrum cardaminoides* *B. napus* x *Diplotaxis erucoides*	5 2	Project collaborators, NBGIP/CBG
B. juncea x weedy introgressions	*B. juncea* x *Erucastrum abyssinicum* *B. juncea* x *Diplotaxis tenusiliquae*	4 10	"
B. napus B genome introgression lines	*B. napus* x *B. carinata*	151	"
Synthetic *B. napus*	*B. napus* 're-made' from Triangle of U species	38	"
Chinese lines		20	"
Recurrent selections (low seed quantity)	Crossing and selection among Australian cultivars selected for drought/high temperature tolerance	118	Project collaborators

Key canola quality attributes in the new millennium – what are the trends over the past 18 years?

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ABSTRACT

Canola (low erucic, high oleic rapeseed) production in Australia began in the late 1980's following the introduction of rapeseed in 1969 and targeted breeding from the mid-1970's. It is the major broadleaf winter crop and is the third largest crop in Australia behind wheat and barley. Canola is the dominant oilseed crop, comprising over half of total national oilseed production.

The Australian Oilseeds Federation (AOF) and NSW Department of Primary Industries (NSW DPI) have collaborated for the past 25 years to compile a snap-shot of the quality of the canola harvest each year. Samples representing each receival site are analysed for key canola quality attributes. Volumetric grain weight (test weight), oil, protein and glucosinolate content, fatty acid composition (FAC) and iodine value (IV) are recorded for each receival site and weighted averages are calculated at a regional, state and national level.

Seasonal factors such as frost and drought, as well as agronomic practices such as time of sowing and crop rotations, new cultivars, and advancements in agricultural technologies all influence the quality of the canola harvested each year. Identifying all of the individual factors that have contributed to quality of canola in a particular year is difficult. However, analysing trends in key quality traits can help the Australian canola industry by providing a snapshot of current quality levels, and allowing researchers, breeders, traders and producers to make informed decisions based on scientific data.
Key words: Canola – quality – oil content – protein content – glucosinolate content

INTRODUCTION

Canola producers, traders, processors and end users all use key quality indicators to make informed decisions. Canola breeders and researchers also use quality indicators to measure performance in breeding programs and influences of agronomic practises and seasonal conditions.

Each year canola samples representing the Australian national canola harvest are collected by GrainCorp Operations Limited (New South Wales and Victoria), Viterra Pty Ltd (South Australia) and CBH Group (Western Australia) and sent to NSW Department of Primary Industries (NSW DPI) in Wagga Wagga for laboratory analysis. Each sample is analysed for oil, protein and glucosinolate content; fatty acid composition (FAC), Iodine Value (IV) and test weight. These samples are representative of the seed collected at each of the receival points and have been taken to cumulatively represent the Australian harvest.

This paper illustrates the influences on, and trends in, the quality of Australian canola from the 2000 harvest until the 2017 harvest.

MATERIALS AND METHODS

Sample collection: Samples were collected per site from New South Wales, South Australia and Victoria. Sampling in Western Australia has varied over the years; initially samples were collected per site, however, in recent years, composite samples representing larger regions were created. NSW DPI has no control over sample collection and all data reported is derived from the analysis of the provided samples.

Sample analysis: Test weight is measured using a Franklin chrondrometer. A 500 mL volume brass vessel is filled with canola seed and weighed using a balance beam. Test weight is reported as kilograms/hectolitre.

Moisture, oil, protein and glucosinolate contents are determined on whole seed using a FOSS 6500 Near Infra-Red (NIR) spectrophotometer The NIR calibration equations were developed at NSW DPI Wagga Wagga using standard methods ISO 665 (moisture content), ISO 659 (oil content), AOF 4-3.3 (nitrogen × 6.25 = protein content) and AOF 4-1.22 (total glucosinolate content). The moisture contents are used to convert the raw data for oil, protein and glucosinolates to the appropriate moisture content for reporting. Oil contents are reported as a percentage of the whole seed at 6% moisture. Protein contents are reported as percent protein in oil-free meal at 10% moisture. Glucosinolate contents are reported as µmoles glucosinolates/gram in oil-free meal at 10% moisture

Fatty Acid Composition is determined by grinding the canola seed, drying the ground sample in the oven to remove excess moisture, followed by the addition of hexane and methylated sodium hydroxide to produce fatty acid methyl esters (FAMEs). The FAMEs are analysed by gas chromatography (GC) using an Agilent 7890A GC with a split/splitless injector, SGE BPX70 column (30 m) and a flame ionisation detector (FID). Fatty acid composition is calculated using peak areas and reported as a percentage of total fatty acids.

Iodine value is calculated from the fatty acid composition according to method AOCS Cd 1c-85: Calculated iodine value. Iodine value is the sum of individual monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) multiplied by specific coefficients (determined by number of carbon atoms in the fatty acid chain and level of unsaturation).

Data analysis: Regional, state and national averages are reported as weighted averages, based on the tonnage represented.

RESULTS

Table 1: Average canola production (tonnes) by state 2000 to 2017

Year	NSW	SA	Vic	WA	Australia
2000	700,000	230,000	400,000	350,000	1,680,000
2001	550,000	210,000	370,000	360,000	1,490,000
2002	100,000	180,000	180,000	330,000	790,000
2003	282,000	310,000	420,000	610,000	1,622,000
2004	420,000	226,000	395,000	490,000	1,531,000
2005	254,000	218,000	338,000	630,000	1,439,000
2006	30,000	42,000	75,000	365,000	512,000
2007	44,000	155,000	200,000	670,000	1,069,000
2008	262,000	227,000	251,000	1,138,000	1,878,000
2009	261,000	259,000	391,000	986,000	1,897,000
2010	610,000	360,000	440,000	705,000	2,115,000
2011	720,000	455,000	770,000	1,240,000	3,185,000
2012	1,495,000	415,000	866,000	1,364,000	4,140,000
2013	900,000	500,000	700,000	1,796,000	3,896,000
2014	835,000	314,000	647,000	1,635,000	3,431,000
2015	890,000	293,000	387,000	1,528,000	3,098,000
2016	842,000	400,000	700,000	2,200,000	4,142,000
2017	618,000	375,000	750,000	1,900,000	3,638,000

Source: Quality of Australian canola. Editions 2000–2017.

Canola production is shown in Table 1. Canola quality data is shown in Table 2. Samples from Western Australia were unavailable in 2014 (Seberry *et al*, 2014), however CBH Group provided average oil and protein contents for each grade at each Port. Hence, Australian averages were calculated on samples from NSW, SA and Victoria only for the remaining quality traits. Samples from Western Australia were again unavailable in 2015 (Seberry *et al*, 2015), therefore, Australian averages were calculated on samples from NSW, SA and Victoria only.

Table 2: Average Australian canola quality 2000 to 2017

Year	Oil[1]	Protein[2]	Glucs[3]	TW[4]	C18:1[5]	C18:2[6]	C18:3[7]	C22:1[8]	IV[9]
2000	42.1	39.2	14	68.7	60.8	20.1	11.3	0.3	117.2
2001[10]									
2002	41.3	40.7	14	66.2	61.5	20.1	9.6	<0.1	114.1
2003	41.5	39.2	17	67.7	61.5	20.2	9.1	0.1	112.9
2004	41.2	41.6	16	67.7	60.3	20.3	10.6	0.1	115.9
2005	42.2	36.3	12	64.1	60.9	19.9	10.8	0.1	116.2
2006	42.2	40.1	6	68.8	60.0	20.2	11.1	0.1	116.8
2007	44.0	40.0	13	66.7	59.7	20.4	11.0	<0.1	116.6
2008	41.8	41.0	16	67.8	60.0	20.3	10.7	<0.1	115.7
2009	41.6	40.1	14	66.8	61.6	19.3	9.9	<0.1	113.3
2010	42.9	39.9	17	65.5	61.2	19.4	10.4	0.1	114.6
2011	44.0	37.9	15	66.0	61.8	18.7	10.4	0.2	114.1
2012	43.4	39.3	14	66.8	63.0	18.2	9.8	<0.1	112.5
2013	45.7	37.3	10	66.8	62.8	18.2	10.2	<0.1	113.1
2014[11]	44.1	38.8	10	67.5	63.7	17.9	9.3	<0.1	111.3
2015[12]	42.0	39.9	10	66.3	64.3	17.9	8.6	<0.1	109.8
2016	47.2	37.5	10	65.3	60.5	19.9	10.9	<0.1	116.2
2017	46.4	39.4	15	66.1	61.2	20.3	10.3	<0.1	115.7

[1]Oil (% in whole seed @ 6% moisture), [2]Protein (% in oil-free meal @ 10% moisture), [3]Total glucosinolates (µmoles/g in oil-free meal @ 10% moisture), [4]Test Weight (kilograms/ hectolitre), [5]Oleic acid (% of total fatty acids in oil portion of seed), [6]Linoleic acid (% of total fatty acids in oil portion of seed), [7]Linolenic acid (% of total fatty acids in oil portion of seed), [8]Erucic acid (% of total fatty acids in oil portion of seed), [9]Iodine value, [10]2001 harvest was not analysed for quality traits, [11]Australian averages calculated on samples from NSW, SA and Victoria only for test weight, glucosinolates, fatty acids and iodine value, [12]Australian averages calculated on samples from NSW, SA and Victoria only

DISCUSSION

Australian canola production ranged from 512,000 tonnes in 2006 to 4,142,000 tonnes in 2016 (Table 1). Severe drought across the canola growing areas in 2002 and 2006 resulted in significantly reduced production in those years. From 2006 to 2012 there was a continual increase in the volume of canola produced across Australia. During this period Western Australia accounted for approximately 50% of the national harvest each year with the exception of 2010 and 2012. During these two years Western Australia experienced drought conditions in contrast to above average rainfall during the growing season in the other three states. As a result, in 2010 and 2012, Western Australia only produced a third of the national harvest.

After the record high of 1,495,000 tonnes of canola produced in New South Wales in 2012, state production fell to 900,000 in 2013, which was still the second highest on record. Canola production remained just under 900,000 tonnes for the following three years until a return to drought conditions and severe frosts during flowering in 2017 saw production fall to a seven year low of 618,000 tonnes.

Canola production reached a record high of 500,000 tonnes in South Australia in 2013 and remained between 300,000 and 400,000 tonnes in the following four years.

After a record high of 866,000 tonnes in 2012, canola production fell in Victoria for the subsequent three years. Canola production in Victoria increased again in 2016 and 2017, with the 750,000 tonnes produced in 2017 the third highest on record.

Unlike the other three states, canola production in Western Australia continued to increase until 2013, reaching a peak of 1,796,000 tonnes, before falling for the following two years. Canola production in Western Australia reached a record high of 2,200,000 tonnes in 2016. In comparison the national canola production was only 2,115,000 tonnes in 2010. Canola production in Western Australia fell to 1,900,000 tonnes in 2017 but was still the second highest on record.

The Australian average test weight ranged from 64.1 kg/hL in 2005 to 68.8 kg/hL in 2006. In 2005 a mild finish to the season resulted in large seeds with an oil content of 42.2%, reducing the test weight. The severe drought conditions in 2006 resulted in very small seed size; however, as the oil content remained at 42.2%, despite the drought conditions, the test weight was 4.7 kg/hL higher in 2006 compared to the 2005 harvest.

Australian average oil content ranged from 41.2% in 2004 to 47.2% in 2016 (Table 2). Up until 2001 the AOF base oil level for canola was 40%; it was raised to 42% in 2002. From 2000 to 2009 oil content remained between 41% and 42% with the exception of 2007. In 2007 Western Australia accounted for 63% of the national canola harvest and a very good season in the state resulted in a state average oil content of 44.7%. By contrast, the continuing drought conditions in New South Wales in 2007 resulted in an average oil content of 39.2%, however, as New South Wales only accounted for 4% of the national harvest this did not impact on the national average of 44.0%. Since 2010 canola breeding has resulted in increasing oil contents which has kept the national average well above 42%, with the exception of 2015 (Seberry *et al*, 2015). Western Australian samples were not provided for 2015 (Seberry *et al*, 2015). Grain Industry Association of Western Australia (GIWA) crop reports for the 2015 season (GIWA 2016) state that oil content ranged from 46% to 48%. As Western Australia produced 49% of the canola the national average Australian oil content was more realistically about 44% in 2015. The 2016 harvest resulted in a record high oil content of 47.2% and is the only year when all receival sites recorded oil contents above 42%.

Average Australian protein content ranged from 36.3% in 2005 to 41.6% in 2004 (Table 2). Protein content has generally been perceived as having an inverse relationship with oil content. However, data shows that small changes in oil content have resulted in larger changes in protein content. The oil content remained relatively stable from 2002 to 2004 (41.2% to 41.5% Table 2) and yet the protein content ranged from 39.2% to 41.6% (Table 2).

The Australian average total glucosinolate content ranged from 6 µmoles/g in 2006 to 17 µmoles/g in both 2003 and 2010 (Table 2). The AOF Standard Manual Commodity Standard for canola sets the limit for glucosinolates at 30 µmoles/g oil-free meal @ 10% moisture. Whilst the national average and even state averages have remained under this limit for the past 18 years localised drought conditions or severe frosts (such as in New South Wales in 2017) have resulted in some receival sites recording glucosinolate contents above 30 µmoles/g oil-free meal @ 10% moisture.

Fatty acid composition in canola oil is traditionally accepted as 60% oleic acid (C18:1), 20% linoleic acid (C18:2) and 10% linolenic acid (C18:3) which produces an iodine value over 114.

The Australian average fatty acid compositions reflected these traditional values for most of the first decade of the new millennium. Oleic acid ranged from 59.7% in 2007 to 61.6% in 2009 (Table 2); linoleic acid ranged from 19.3% in 2009 to 20.4% (second highest on record) in 2007; linolenic acid ranged from 9.1% (second lowest on record) in 2003 to 11.3% (second highest on record) in 2000. During this four year period the iodine value ranged from 112.9 in 2003 to 117.2 in 2000 (highest on record) (Table 2).

Fatty acid composition is influenced by both genetics and environmental factors and the influence of breeding has been observed in the analyses in the second decade of the millennium. The Australian average oleic acid contents rose steadily from 2010 to 2015, increasing from 61.2% to a record high of 64.3%. Correspondingly, the Australian average iodine values fell during this same period from 114.6 to a

record low of 109.8 in 2015. The Codex Standard for Named Vegetable Oils (CODEX-STAN 210 – 1999) sets the acceptable range for iodine value in canola oil as 105 to 126. In 2015 nine receival sites recorded iodine values below 105, the lowest being 101.8. The declining iodine value resulted in shipments of canola oil being rejected because they failed to meet CODEX STAN 210 – 1999. In 2016 canola fatty acid compositions and iodine values returned to more traditional quantities of oleic acid 60.5%, linoleic acid 19.9%, linolenic acid 10.9% and iodine value 116.2 (highest since 2007). The fatty acid compositions in 2017 were similar to 2016 with iodine value dropping 0.5 to 115.7.

Canola breeding programs have continued to release new canola varieties which have increased yield, disease and pest resistance and drought tolerance. This, together with advances in agronomic practices such as weed control, has increased canola yields and oil contents over the past 18 years. Both glucosinolate content and iodine value require ongoing attention in breeding programs, and genetics versus environment (G x E) field trials across a range of environments are needed to ensure commercial varieties continue to meet the trading standards and the requirements of end users.

Drought conditions across eastern Australia in the first half of 2018 has reduced the estimated total national production for the 2018 harvest to 2,660,000 tonnes (AOF July 2018). Should drought conditions persist through spring it will likely have a marked effect on the quality of canola harvested, as well as total production.

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Retaining canola seed

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ABSTRACT

In a series of 11 experiments conducted in the lower rainfall regions of south-western Western Australia we tested the performance of multiple generations of OP TT and hybrid TT seed. We found in most instances OP TT canola out performed hybrid TT canola. We found that compared to commercially supplied Generation 1 hybrid TT seed, Generation 2 and 3 hybrid TT seed produced earlier flowering plants and a higher proportion of male sterile plants. On average Generation 1 hybrid TT seed produced 3% higher yields than Generation 2 seed. The relatively small reduction in yield combined with higher seeding costs of Generation 1 hybrid TT seed resulted in lower financial returns compared to sowing Generation 2 or 3 hybrid TT seed or OP TT seed.

Key words: canola, retained seed, generations, hybrid

INTRODUCTION

Western Australian (WA) canola production is dominated by open-pollinated (OP) triazine tolerant (TT) varieties. OP TT varieties account for over 70% of the area sown to canola, with RoundupReady (RR) hybrid varieties being the next most widely grown type of canola at 23% of the area sown to canola in WA, whilst hybrid TT varieties are not widely grown (<3%).

Historically breeding companies regularly released OP TT varieties to farmers, but in recent years OP TT varieties have been released by only one company - Nuseed. Thus there are concerns amongst growers and the industry that the rate of release of OP varieties will slow down and farmers may be forced to keep the same variety for many years. In response to the lack of OP varieties high rainfall areas farmers that regularly produce canola yielding 1.5 t/ha or more may choose to grow hybrid TT varieties, which is supported by the analysis of Zhang et al 2016. In lower rainfall parts of WA expected yields in the range of 0.7 to 1.5 t/ha make the higher upfront costs of hybrid TT canola less attractive. But if no new OP TT varieties are available it is inevitable that TT growers will either keep their existing OP TT cultivar for as long as practicable or consider keeping seed from hybrid TT crops to use as seed in the following year.

This paper reports on a series of experiments conducted in the low and medium rainfall zones of WA to test if keeping stored OP TT seed, or using seed kept from multiple years of harvesting OP TT or hybrid TT canola results in reduced performance and/or financial returns.

MATERIALS AND METHODS

We conducted three field experiments comparing generations of OP TT canola and eight field experiments comparing generations of hybrid canola to each other and to commercial seed lots of OP TT canola.

Comparing generations of OP TT seed

In 2014 we purchased seed of the Nuseed OP TT variety ATR Bonito which we refer to as "Generation 1a" seed. We placed the majority of the seed in storage in our laboratory cupboards and grew some of the seed out at our Grass Patch field site in 2014 and at harvest retained seed– referred to as "Generation 2" seed. In 2015 we again sowed Generation 1a seed at Grass Patch to produce fresh "Generation 2" seed and also sowed Generation 2 seed to produce "Generation 3" seed. In 2016 we tested all generations of seed in two field experiments at Grass Patch and Wittenoom Hills. In 2016 at our Grass Patch site we repeated the procedure in order to produce Generations 2 and 3 seed, and also sowed Generation 3 seed to produce "Generation 4" seed. In 2017 we purchased fresh seed of ATR Bonito (seed lot FSBM7598COM) from a local reseller which we refer to as "Generation 1b" seed and this was tested against all other generations of ATR Bonito at Grass Patch in 2017. In all experiments all generations of seed were cleaned in the same manner. Some of the experiments had extra treatments of +/- graded seed (>1.85mm sieve), but we report here on comparisons between ungraded commercial seed (Generation's 1a and 1b) and graded Generation 2, 3 and/or 4 seed as that closely mirrors the way in which growers could manage their seed lots. The germination rate and seed size of all seed lots was determined each year and that information was used to adjust seeding rates to target plant densities of 40 plants/m².

Comparing Hybrid TT generations

In 2012 we obtained CB Junee HT from the seed company Canola Breeders Inc. This 'Hybrid Generation 1" seed was kept in storage at our office and some was grown out at Grass Patch. We retained seed at harvest in 2012 – referred to as "Hybrid Generation 2". We compared Hybrid generation 1 and 2 to the OP TT variety CB Telfer in a field experiment at Grass Patch in 2013. Additional treatments included combinations of +/- grading, seeding rates of 2 and 4 kg/ha, and mixes of Hybrid Generation 1 (25%) and Hybrid Generation 2 (75%), but here we report only on Ungraded Hybrid Generation 1 at 2 kg/ha, compared to Graded CB Telfer sown at 4 kg/ha and Graded Hybrid Generation 2 sown at 4 kg/ha which closely mirrors the way in which growers could manage their seed lots. The experiment was a randomized block design with three replications.

In 2014 we obtained Hyola 450TT from the seed company Pacific Seeds. This 'Hybrid Generation 1a" seed was kept in storage at our office and some was grown out at Grass Patch. We retained seed at harvest in 2014 – referred to as "Hybrid Generation 2". In 2015 we obtained a fresh supply of Hyola 450TT from the seed company which we refer to as 'Hybrid Generation 1b" We compared Hybrid generation 1 and 2 seed to the OP TT variety ATR Bonito at Ballidu, Grass Patch and Merredin in 2015. Additional treatments included combinations of +/- grading, seeding rates of 2 and 4 kg/ha, and mixes of Hybrid Generation 1 (25%) and Hybrid Generation 2 (75%), but we report here on Ungraded Hybrid Generation 1 at 2 kg/ha compared to Graded CB Telfer sown at 4 kg/ha and Graded Hybrid Generation 2 sown at 4 kg/ha which closely mirrors the way in which growers could manage their seed lots. The experiments were randomized block designs with three replications. At the Grass Patch site we retained the seed from each hybrid generation so as to produce new Hybrid Generation 2 and 3 for testing in 2016.

In 2016 we compared Hybrid Generation 1b sown at a target density of 25 plants/m² to Hybrid Generation 2 and 3 and ATR Bonito sown at a target density of 40 plants/m² at Dalwallinu, Grass Patch, Merredin and Wittenoom Hills. The experiments were randomized block designs with three replications.

All field experiments in this series were sown with a small plot seeder. Plots were 20 m long x 1.54m wide - sown at 2m centres. Measurements included plant establishment from 5 x 2m of row 2 to 4 weeks after sowing (WAS), NDVI at 8 and/or 12 WAS, date of 50% of flowers and % of male sterile flowers on the main stem, seed yield and quality. Canola was harvested with a small plot harvester and yields were calculated on harvested plot length x 2m. 2 to 5 kg of seed was collected from individual plots and these samples were hand cleaned. We then used CBH Infratec NIR machines to measure moisture, oil and protein content. Seed yield, oil and protein were adjusted to 6% moisture prior to data analysis. Gross margins per plot were calculated based on the following assumptions – seed costs of $2/kg for retained TT seed which included grading costs, $17/kg for new commercial OP TT seed, $24/kg for new commercial hybrid seed; grain value of $550/t for varieties without End-Point Royalty (EPR) and $545/t for varieties with EPR, grain value was adjusted for oil% as per industry practice of +/- 1.5% for every percentage point above or below 42% oil; other costs for operations, herbicides and fertilisers varied with location. All data was analysed using ANOVA or REML in Genstat.

RESULTS

Generations of OP TT seed produced similar seed yields (Fig 1) at the three sites in 2016 and 2017. Generation 4 seed at Grass Patch in 2017 produced 7% higher seed yields than the other generations. All generations of ATR Bonito produced similar oil% (P>0.05), averaging 49.7% oil. Gross margins of Generation 4 seed at Grass Patch in 2017 and Generation 3 seed at Wittenoom Hills in 2016 were higher than new commercial (Generation 1a or b) seed treatments. Generation 3 seed at Wittenoom Hills 2016 produced gross margins of $612/ha - significantly larger than other generations at $511 to $522/ha. We observed no differences in plant establishment, flowering time, or % of male sterile flowers (all nil), or date of maturity.

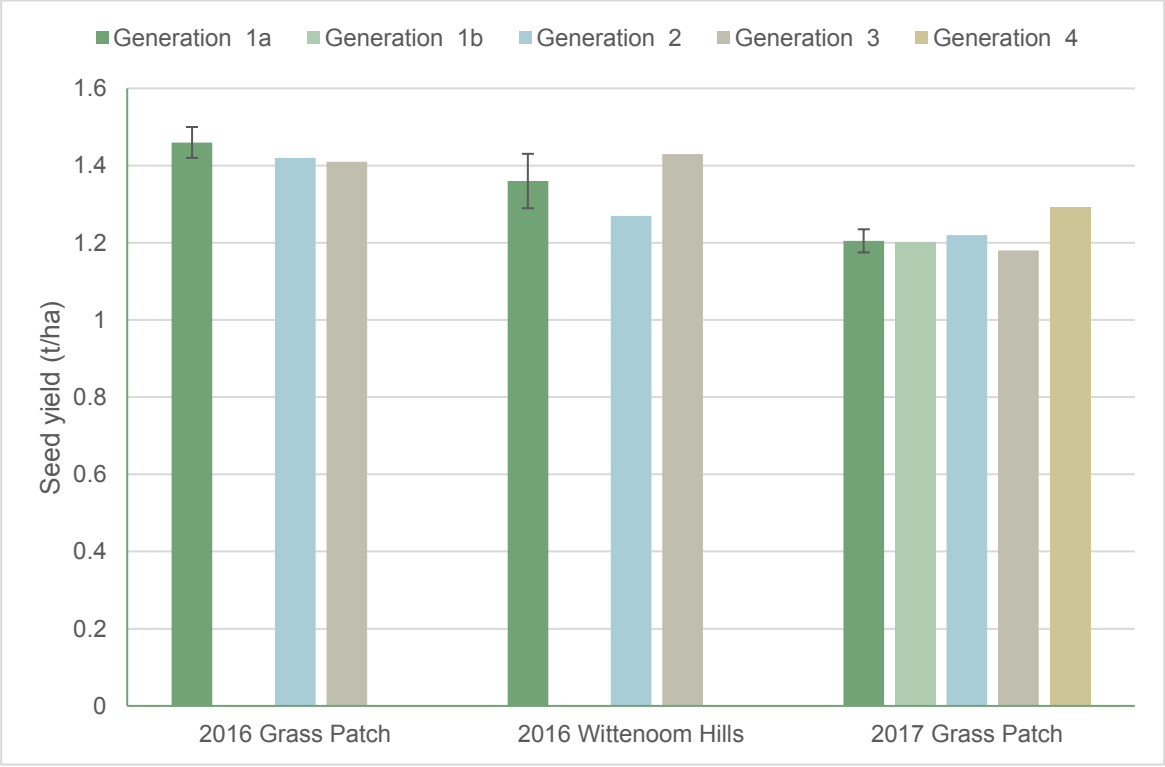

Fig.1. Seed yield of OP TT canola seed generations at three sites in Western Australia. Verticals bars denote LSD.

In the hybrid generation experiments we observed earlier flowering in Generation 2 and 3 than in Generation 1 treatments. We also observed male sterile flowers in Generation 2 and 3 hybrid plants - up to 9%. This on occasions resulted in no pods being formed at the base of the main flowering raceme, however we also observed that once nearby plants began flowering and providing pollen for outcrossing that pods were produced further up the raceme and the plants in those treatments compensated with larger seeds. There was very low disease pressure in our experiments – therefore we observed no breakdown in disease resistance in Generation 2 or 3 hybrids.

Seed yield of hybrid Generation 1 seed was higher than at least one of the later generations in 4 experiments, equal in two experiments and lower yielding in two experiments (Fig. 2). With average site mean yields of 1.3 t/ha the magnitude of the difference between hybrid generations 1 and 2 was relatively small, ranging from -0.2 t/ha to +0.15 t/ha, averaging –30 kg/ha. As a consequence of the relatively small differences in yield and oil% (not shown) we found no financial incentive in sowing hybrid Generation 1 seed compared to using retained hybrid Generation seed with Generation 2 treatments averaging $10/ha higher returns than Generation 1 treatments.

OP TT canola produced equal or higher seed yields and gross margins than all first Generation hybrid seed treatments. On average OP TT treatments produced gross margins of $515/ha compared to $419/ha for first Generation hybrid seed.

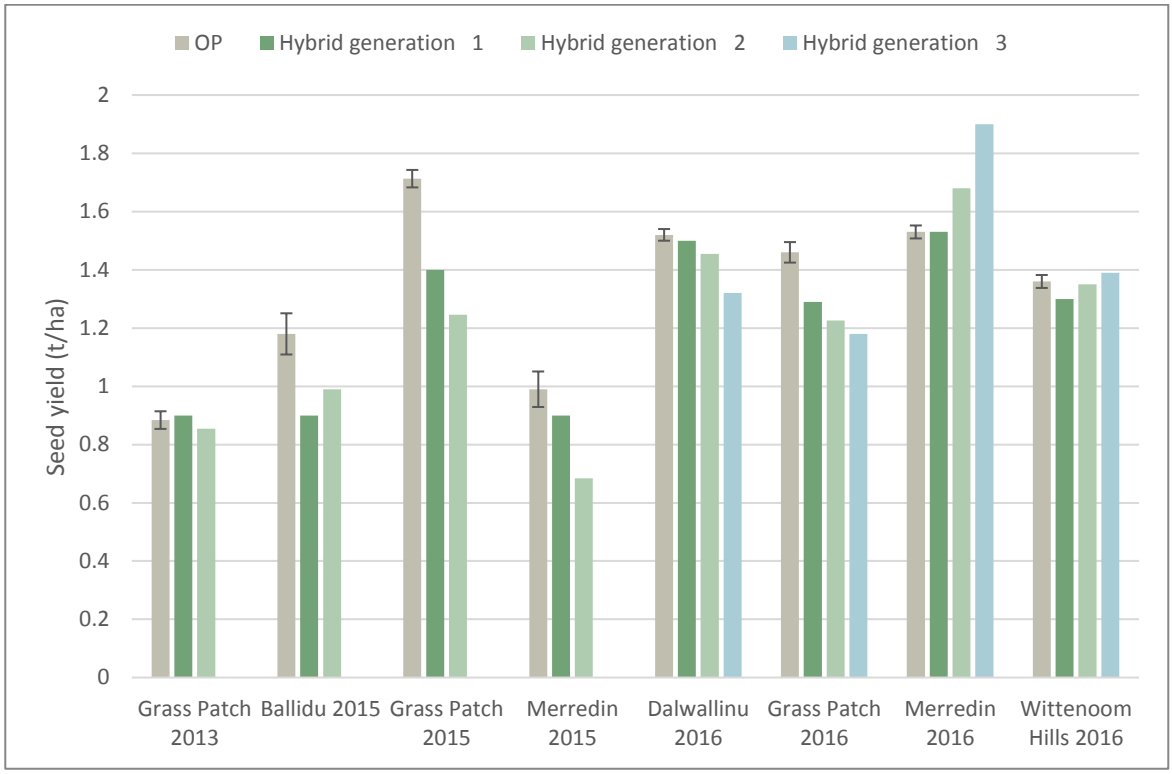

Fig.2. Seed yield of OP TT and hybrid TT canola seed generations at eight sites in Western Australia. Verticals bars denote LSD.

DISCUSSION

We found multiple generations of OP TT seed could be used in WA's lower rainfall environments with no deleterious effects. This is in agreement with other unpublished work by Paul Carmody and Graham Walton in WA dating back to 1995 to 2000.

Previous work has shown that keeping seed from hybrid canola can lead to variability in flowering, increase in the number of sterile flowers, reduced disease resistance, reduced vigour and reduced yield of 25-30% (Potter et al. 2009, Kudnig et al. 2010). We also observed variability in flowering, with retained hybrid seed often leading to earlier flowering. We also observed an increase in male sterile flowers. Disease pressures were very low in the locations we conducted our experiments, so we did not observe any variation in disease resistance in hybrid generations. Whilst we did on occasions measure yield losses they were small and infrequent, and the average loss in our experiments was 3% - much lower than the 25-30% reported by Potter et al. 2009 and Kudnig et al. 2010. It is feasible that in regions with lower yield potential such as those where we conducted our experiments that the benefits of hybrids are not realised. Similar observations have been made by Zhang et al. 2016. In most cases the reduction in seed cost due to using retained seed more than compensated for any loss in revenue from reduced yield.

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Natural variation for interference traits against annual ryegrass in canola

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ABSTRACT

Weeds are a major issue for canola (*Brassica napus* L.) growers because they cause significant reduction in grain yield, seed and oil quality. The prolonged and widespread use of herbicides to control weeds, especially after the introduction of herbicide-tolerant cultivars, has led to an increase in the risk of herbicide resistance evolution in weeds. Crop interference is an approach to tackle weed infestation along with other agronomic interventions for weed management. In Australia, studies have found genetic variation in the capability of canola for weed suppression in the field and in *vitro* conditions; however, the canola ideotype for interference against weeds has not been defined / validated under glasshouse conditions. In this study, we evaluated the competitive ability of 26 canola genotypes against annual ryegrass (*Lolium rigidum* Gaudin) in glasshouse conditions to study the phenotypic traits associated with weed competition. The results reveal that canola biomass was positively associated with suppression ability, suggesting that vigorous canola varieties would provide effective competitiveness to weeds.

Key words

Weed-crop competition - *Brassica napus* - Annual ryegrass - glasshouse conditions

INTRODUCTION

Weeds are major constrain for canola (*Brassica napus* L.) production worldwide as they cause significant reduction in grain yield and quality (Lemerle *et al.*, 2001). Annual ryegrass (*Lolium rigidum* Gaudin) was one of the most widespread weeds in various crops, including canola (Lemerle *et al.*, 2001). Although application of herbicides is an option to control weed infestation, the prolonged and widespread chemical use, especially after the introduction of herbicide-tolerant cultivars to triazine, imidazolinone and glyphosate, has led to an increase in the risk of resistance evolution in weeds (Pratley *et al.*, 2018). Crop interference is an environmental friendly approach to tackle weed infestation along with other agronomic interventions for weed management (Donald, 1963; Pratley, 1996). Allelopathy and crop-weed competition are two distinct mechanisms underlying crop interference. Previous studies based on laboratory and field experiments have shown that genetic variation for allelopathy and weed competition exists in canola (Asaduzzaman *et al.*, 2014a; Asaduzzaman *et al.*, 2014b; Lemerle *et al.*, 2014). However, no study has been conducted to reveal genetic variation of weed-crop competition under glasshouse conditions in Australia. Under field conditions, it is very difficult to achieve uniform weed-plant density across trial, and this may influence the differential response of canola genotype to weed competition/allelopathy (Coleman *et al.*, 2001; Worthington and Reberg-Horton, 2013). In this study, we determined weed suppression ability of 26 canola genotypes against annual ryegrass under glasshouse conditions while maintaining uniform density of annual ryegrass.

MATERIALS AND METHODS

A set of 26 diverse *Brassica* genotypes was used to test their competitive ability against annual ryegrass cv. Wimmera under glasshouse conditions. These genotypes are listed in Table 1. Seeds were accessed from the National Brassica Germplasm Improvement Program (Wagga Wagga).

Table 4: Phenology of canola genotypes.

Genotype	Phenology	Country of origin
PAK85388-502	Semi-winter	Pakistan
X6-06-3275-3	Semi-winter	China
Ningyou7	Semi-winter	China
Av-Opal	Spring	Australia
Barossa	Spring	Australia
ATR-409	Spring	Australia
SturtTT	Spring	Australia
Hurricane	Spring	Australia
Av-Garnet	Spring	Australia
CB-Argyle	Spring	Australia
RP04	Spring	Australia
Ag-Outback	Spring	Australia
Skipton	Spring	Australia
Ag-Spectrum	Spring	Australia
BLN3343C001401	Spring	Australia
Rainbow	Spring	Australia
Rivette	Spring	Australia
ROY98310	Spring	Australia
Tarcoola-141	Spring	Australia
Lantern	Spring	Australia
CB-Trigold	Spring	Australia
Gross-Luesewitzer	Winter	Germany
Tapidor	Winter	Germany/France
Licapo	Winter	Germany
Beluga	Winter	Italy
Akela	Winter	Germany

The experiment was conducted at Wagga Wagga Agricultural Institute, NSW Department of Primary Industries, NSW, Australia. The experiment was arranged in split plot design with three replicates; main plots were 26 canola genotypes and subplots were the weed and weed-free treatments. The target canola density was five plants per pot in each treatment. Ten annual ryegrass plants per pot were grown in the weed treatments. At flowering, shoots of canola and ryegrass from each experimental unit were cut at soil level and measurements on canola height, shoot biomass for canola and ryegrass, and number of canola leaves were taken. Shoot biomass samples from canola and annual ryegrass plants were dried at 70°C for 48 h, and weighed. Data were analysed using R software (R Core Team, 2017) and the ASReml package.

RESULTS and DISCUSSION

Both canola genotypes and weed treatment had significant ($p<0.001$) effects on all three traits measured; crop biomass, crop height and leaf number. However, the interaction between canola genotypes and weed treatments was not significant. As expected, canola shoot biomass (g plant^{-1}), plant height (cm plant^{-1}) and leaf number were higher in the weed-free treatment compared with the weed treatment. Among different genotypes, two winter varieties (Akela and Gross-Luesewizer), one semi-winter variety (Ningyou7) and two spring varieties (Av-Opal and Av-Garnet) significantly reduced the growth of ryegrass compared with varieties Ag-spectrum, Sturt TT and Lantern (Fig 1). It has been previously identified *in vitro* and in the field that Av-Opal and Av-Garnet suppress weeds through allelopathy and weed competition respectively; however, it is difficult to distinguish between allelopathy and weed competition under field conditions. In our study, we conducted another glasshouse experiment following the canola harvest using the pot soils where 10 varieties, included eight varieties listed in Fig 1, were grown to measure if there was a chemical residual effect of the weed competitive varieties on ryegrass growth. Ryegrass was sown in these pot soils at the same density as in the first glasshouse experiment. The shoot biomass and root biomass of ryegrass were measured a month after ryegrass sowing. We did not find any significant differences in allelopathic effects of canola varieties (data not shown).

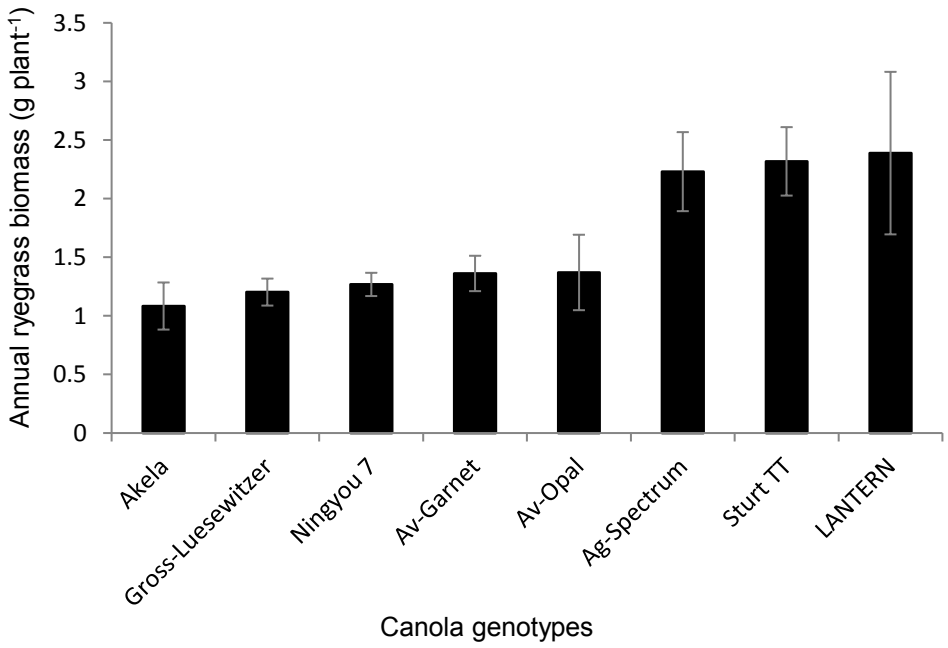

Figure 2: Genotypic effect on the weed biomass (g plant^{-1}) of annual ryegrass. For clarity, response of only selected varieties representing winter, semi-winter and spring types of canola is shown.

A highly negative correlation coefficient of - 0.78 (Fig 2a) was observed between canola biomass and annual ryegrass biomass. These results indicate that plant vigour, manifested with higher canola biomass is associated with suppression of annual ryegrass. This is in agreement with previous studies where different canola germplasm was compared under field conditions and showed that early-season crop biomass accumulation was associated with weed suppression (Daugovish *et al.*, 2002; Beckie *et al.*, 2008; Asaduzzaman *et al.*, 2014b; Lemerle *et al.*, 2014). Crop height and leaf number showed low positive correlation (0.28 to 0.21) with biomass of annual ryegrass (Fig 2b and c). We observed that winter type canola accumulated more biomass compared to spring type. We also found that leaf biomass was significantly correlated with weed suppression (r = - 0.50), whereas crop stem biomass did not show such correlation (r = - 0.05). These results indicate that larger leaves are likely to provide shade and thus interfere with light inception to ryegrass. In cereals, it has been demonstrated that extensive leaf display, leaf area index, long flag leaves and good ground cover are associated with superior competitive ability (Donald and Hamblin, 1976; Huel and Hucl, 1996; Johnson *et al.*, 1998).

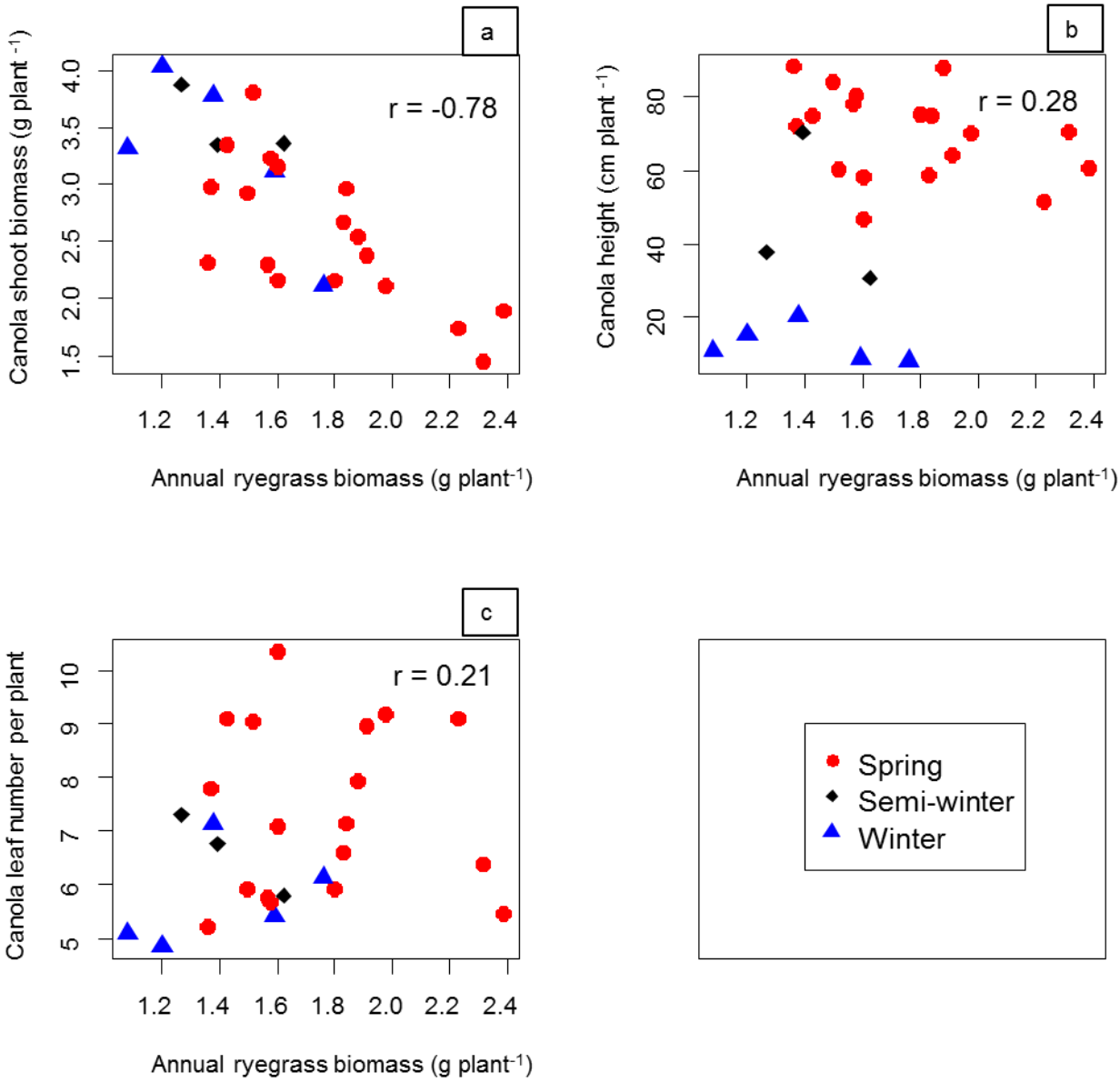

Figure 3: Correlation between annual ryegrass biomass (g plant^{-1}) and three component traits of canola: a) shoot biomass (g plant^{-1}), b) plant height (cm plant^{-1}) and c) leaf number per plant.

In conclusion, this study suggests that (i) vigorous canola varieties would provide competitiveness to ryegrass and (ii) glasshouse conditions can be used to evaluate weed suppression ability of a large number of canola accessions while maintaining uniform density of annual ryegrass.

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Infection of *Brassica napus* after stem elongation by *Leptosphaeria maculans* (blackleg): disease progression and yield loss

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ABSTRACT

Leptosphaeria maculans, the causal fungus of blackleg (phoma stem canker) is a serious threat to *Brassica napus* (canola, rapeseed) production worldwide. *L. maculans* can infect all plant tissues but the formation of cankers at the crown that result from leaf infection during vegetative growth, are considered the primary cause of yield loss. Infection of all reproductive plant parts post-stem elongation (peduncles, flowers, branches, upper stems and pods) has become prevalent across all canola-growing regions of Australia. Upper canopy infection (UCI) is the collective term to describe all symptoms. Field experiments were undertaken in 2016 and 2017 in NSW to determine the development of disease symptoms associated with UCI and to quantify the effect of UCI on grain yield. Multiple sowing times generated differences in disease exposure during flowering and fungicide treatments were imposed to determine the level of yield loss. Symptoms appeared during flowering, increasing in severity during grain fill with disease severity greatest in the earliest flowering crops. The maximum yield reduction in the earliest flowering crop was 0.99 (26%, 22 June) and 0.64 t/ha (28%, 5 August) in 2016 and 2017, respectively. In both years, there was no yield reduction in crops starting to flower after mid-August but the seasonal differences indicate an interaction with environment. Seed size was reduced by an average of 15% in pods with blackleg lesions >10mm but number of seeds/pod was generally unaffected. In contrast, severe stem lesions reduced the number of seeds/pod from 12.6 to 9.7 (23%) with a small reduction in seed size. These data suggest are consistent with blackleg disrupting vascular function to the developing pods and seeds. Although delay of flowering until mid-August minimizes disease and yield loss, potential yield gains from earlier flowering may not be captured. Future crop growth models (such as APSIM) that are based on abiotic factors to predict crop yield require inclusion of abiotic factors which will allow more robust investigation of crop management strategies. The apparently sudden appearance of UCI and resultant impact on canola production requires dissection to determine the physiological mechanisms of yield loss and control strategies. In addition, the epidemiological, environmental and management factors underlying UCI require investigation.

Key words: flowering time, upper canopy infection, canola, phoma, rapeseed

INTRODUCTION

Leptosphaeria maculans is ubiquitous in all canola-growing regions of Australia causing the disease blackleg. Crown cankers develop from leaf infections whereby the fungus grows asymptomatically within the plant to the crown, thereby limiting water and nutrient uptake. Blackleg crown canker is estimated to result in an average 15% yield loss across Australia annually (Murray and Brennan 2012). However, all above- and below-ground plant parts of *Brassica napus* are susceptible to infection by *L. maculans* (West *et al.* 2001, Sprague *et al.* 2007). Upper canopy infection is the collective term used throughout this paper for all symptoms caused by *L. maculans* on plants that

have undergone stem elongation. Although crown canker has been the primary cause of yield loss, observations across canola-growing regions of Australia since 2010 indicate that upper canopy blackleg infection is causing significant levels of yield loss equivalent to or greater than crown canker in some locations (Sprague *et al.* 2018). Sprague et al (2018) report a relationship between sowing and start of flowering dates, whereby the severity of upper canopy infection was greater with earlier sowing and flowering dates. Earlier commencement of flowering during a period in which spore release coincides with cool, moist conditions conducive for infection would likely increase disease severity. However, data around the onset and progression of UCI symptoms and ultimately their effect on crop yield is lacking. The aim of the experiments presented in this paper was to investigate the progression of UCI symptoms, association of disease with the start of flowering and determine any associated yield penalty.

MATERIALS AND METHODS

Cultivar and experimental design

Field experiments were conducted at two sites in 2016 (Wagga Wagga and Canowindra, NSW) and one site in 2017 (Marrar, NSW) in southern NSW with a history of blackleg. All treatments were sown with fungicide applied to seed and fertilizer to reduce crown canker infection. Untreated crops (no fungicides applied after stem elongation) of cultivars 44Y89CL (2016 and 2017, Blackleg Resistance Group BC, Blackleg Resistance Rating R-MR) and Archer (2017 only, Blackleg Resistance Group C, Blackleg Resistance Rating MS) were compared to a "Full control" treatment (multiple fungicide applications, Prosaro applied at 450 ml/ha) to ascertain the level of disease in crops starting to flower at different times and to determine any associated yield penalty by control with fungicides. Due to the unusually wet seasonal conditions in 2016, the fungicide regime applied did not fully control disease, particularly in the earliest flowering crops, and therefore the actual yield loss may be greater. Both cultivars are susceptible to upper canopy blackleg but differ in phenological development with Archer flowering later. Experiments were a split-plot design with three replicate blocks and two (2017) or three (2016) sowing times to generate differences in disease exposure during flowering. Treatment plots (12m x 1.5m, 6 rows at 25cm spacing) were randomised within each sowing time.

Table 1. Sowing date, in-crop rainfall (Apr-Oct), timing of fungicide applications, start of flowering and maturity dates for cvs 44Y89CL and Archer at three sites in NSW in 2016 and 2017.

Year	Site (in-crop rainfall)	Cultivar	Sowing date	Fungicide application dates	Start flowering	Maturity
2016	Wagga Wagga	44Y89CL	30 Mar	27 June, 28 July	22 June	12 Oct
	(625 mm)	44Y89CL	13 Apr	28 July, 23 Aug	17 July	21 Oct
		44Y89CL	2 May	23 Aug, 20 Sept	18 Aug	7 Nov
	Canowindra	44Y89CL	1 Apr	11 July, 1 Aug	10 July	18 Oct
	(628 mm)	44Y89CL	14 Apr	2 Aug, 23 Aug	1 Aug	25 Oct
		44Y89CL	28 Apr	23 Aug, 13 Sept	15 Aug	7 Nov
2017	Marrar	44Y89CL	8 Apr	28 Jul, 15 Aug, 13 Sep	5 Aug	27 Oct
	(232 mm)	44Y89CL	27 Apr	15 Aug, 13 Sept	26 Aug	4 Nov
		Archer	8 Apr	15 Aug, 13 Sept	24 Aug	4 Nov
		Archer	27 Apr	13 Sept	7 Sept	13 Nov

Disease assessments

The severity of infection on flowers, pods, branches and upper main stems was assessed on each plot at regular intervals from the start of flowering until maturity. Each plot was given a separate score for flower, branch and upper main stem infection using a 0 to 4 scale, whereby 0 = no disease symptom, 0.5 = small amount of symptom present, 1 = <10% tissue area affected, 2 = tissue area affected, 3 = 30-49% tissue area affected, 4 = ≥50% tissue area affected. The incidence of infected pods was determined by assessing 200 random pods and counting the number with lesions.

Yield assessments

Hand cuts (2 x 1m^2) were taken from each plot at a similar stage to when commercial crops would be windrowed, approximately 50% seed colour change. Samples were air-dried prior to threshing with a mechanical thresher. Grain yield was calculated from oven-dried (48h at 70ºC) weights.

In addition, grain was collected from individual pods and main racemes with varying levels of blackleg. Pods with varying sizes of blackleg lesions (none, 1-2 mm, 2-5 mm, 5-10 mm, >10 mm) were collected from cv 44Y89CL at Wagga Wagga and Canowindra in 2016 and from a commercial crop of cv ATR-Stingray at Horsham, VIC, in 2017. For each disease category, 50 pods were collected from three replicate plots (total 150 pods per disease category) and threshed to determine seed weight and seeds/pod. In 2017, pods were collected from the main racemes of cvs 44Y89CL, Archer and Nuseed Diamond at Wagga Wagga, NSW, with varying severity of upper main stem infection (none, low, moderate, severe). Approximately 15-20 pods were collected from individual plants at the top of the raceme above the infection. For each disease category, pods from 20 individual plants were collected to determine seed weight and seeds/pod.

Statistical analysis

Data from each site was analysed separately by ANOVA using appropriate models in Genstat v.16 to determine main effects of treatment (sowing time and fungicide application) and interactions. Fisher's protected l.s.d. ($P<0.05$) was used to determine differences between treatment means.

RESULTS

In 2016, ~625 mm of in-crop rainfall and mild temperatures during winter and spring were recorded at both sites (Table 1). In contrast, there was 232 mm of in-crop rainfall and extreme temperatures (frost and heat) in 2017 at Marrar (Table 1). Across both years, the start of flowering occurred over a wide period from 22 June to 26 Aug for the earlier maturing cv 44Y89CL and from 28 Aug to 7 Sept for the later maturing cv Archer (Table 1). The duration from start of flowering to harvest was shortest for the later sown crops and longest for the earliest sown crop, ranging from 82 to 112 d in 2016 and 70 to 83 d in 2017 (Table 1). Although infected flowers and branches were present in both seasons, infected pods and upper stems were the predominant symptoms in 2016 and infected upper stems in 2017 (Figure 1). The incidence of pod lesions and severity of upper stem lesions followed similar patterns of progression across all flowering times but disease incidence/severity at crop maturity was greatest in the earliest flowering crops (Figure 1). Stem lesion severity at maturity was similar in both seasons, but the incidence of pod lesions was low in 2017 (<5%) compared to 2016 (>60%) in the earliest flowering crops. In 2017, assessment of stem lesion severity was conducted after maturity when crops were harvested and showed a sharp increase (Figure 1B).

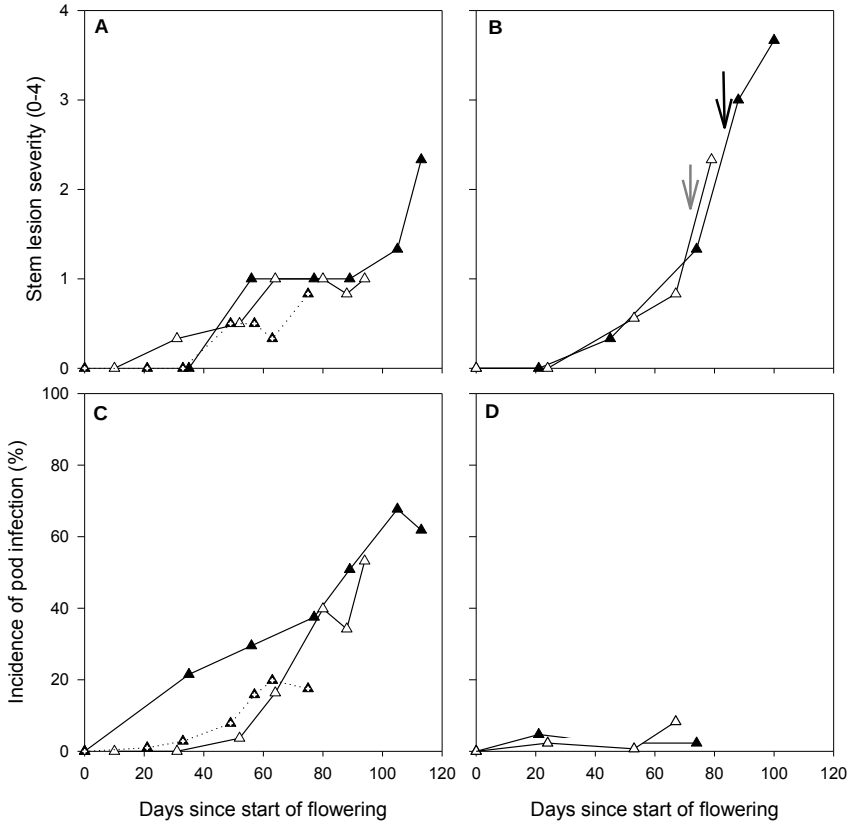

Figure 1. Severity of stem lesions (A, B) and incidence of pods (C, D) infected by *Leptosphaeria maculans* on cv. 44Y89CL in field trials at Wagga Wagga and Marrar in 2016 (A, C) and 2017 (B, D), respectively. Crops started flowering on 22 June (▲), 17 July (Δ) and 18 Aug ( ) in 2016 and on 5 Aug (▲) and 18 Aug (Δ) in 2017. In (B), arrows indicate dates for hand-harvest for crops starting to flower on 5 Aug (black) and 18 Aug (grey) in 2017.

In individual plants, infection of pods or upper stems significantly reduced yield potential. Across three experiments, the average thousand seed weight for pods with large lesions >10mm was 2.86 g compared to 3.37 g in pods with no lesions, an overall reduction of 15% with little effect on seeds/pod (data now shown). In contrast, severe infection of upper stems reduced the number of seeds/pod (12.6 to 9.7) and also seed weight (3.83 g to 3.59 g) which was consistent for the three cultivars tested (data not shown).

Field experiments conducted in 2016 and 2017 show that in the absence of sclerotinia stem rot or blackleg crown canker, UCI caused yield loss of up to 0.99 t/ha compared to where disease was fully controlled (Table 2). In both seasons, delaying the onset of flowering after mid-August reduced yield loss. However, in 2016 crops starting to flower in early August had minimal yield loss compared to those in 2017 that had 0.64 t/ha yield loss.

Table 2. Grain yield in crops flowering on different dates for cvs 44Y89CL and Archer in 2016 and 2017 in southern NSW. The 'Untreated' is compared to a 'Full control' at each flowering time whereby disease was controlled by fungicide applications to provide an estimate of yield loss associated with blackleg infection. *Indicates significantly different from Untreated treatment for data within each sowing date.

Year	Site	Cultivar	Start of flowering date	Grain yield (t/ha)		
				Untreated	Full control	Difference in grain yield
2016	Downside	44Y89CL	22 June	2.88	3.87*	0.99*
		44Y89CL	17 July	2.99	3.28*	0.29*
		44Y89CL	18 Aug	2.91	3.01	0.10
	Canowindra	44Y89CL	10 July	3.24	3.66*	0.42*
		44Y89CL	1 Aug	2.93	3.79*^	0.86*^
		44Y89CL	15 Aug	3.24	3.22	0.02
2017	Marrar	44Y89CL	5 Aug	1.71	2.35*	0.64*
		44Y89CL	26 Aug	2.00	2.12	0.12
		Archer	24 Aug	2.23	2.44	0.21
		Archer	7 Sept	1.63	1.70	0.07

^Yield effect cannot solely be attributed to control of blackleg due to high levels of Sclerotinia stem rot.

DISCUSSION AND CONCLUSION

Upper canopy infection (UCI) is the collective term for symptoms resulting from infection by *L. maculans* on a wide range of plant parts in canola crops that have undergone stem elongation. UCI symptoms were present on all plant parts in both seasons but the severity of symptoms differed between years. The severity of stem lesions at crop maturity was similar in the earliest flowering crops in both years despite the large differences in start of flowering date (22 June 2016 and 5 August 2017) and time for disease development prior to crop maturity (112 d in 2016 and 83 d in 2017). For pod infection, the incidence was high in 2016 and low in 2017, coincidental with seasonal rainfall. Disease severity at maturity was greatest in the earliest flowering crops but appearance and progression of symptoms on crops starting to flower at different times was generally similar. This suggests that the extended period from onset of flowering to maturity in earlier flowering crops allows greater proliferation of the fungus within the plant leading to higher disease severity but seasonal differences indicate an interaction between environmental conditions and disease development that requires further exploration.

The impact of UCI on yield development was significant at the plant and crop levels. In crops, delaying the onset of flowering until after mid-August reduced yield loss in both seasons. However, in 2016 crops starting to flower in early August had minimal yield loss compared to those in 2017 that had 0.64 t/ha yield loss. At the plant level, seed yield/pod was reduced by infection of pods and stems but the yield component affected was dependent on the plant part infected. Seed size was reduced by infection of pods, whilst seed number was reduced by infection of upper stems. At the petiole and crown, *L. maculans* is known to invade vascular tissue causing disruption to nutrient flow (Eckert et al. 2005, Sprague et al. 2007). While the cytology of UCI by *L. maculans* is unknown, it is likely that invasion of similar plant tissues occurs and the expected effects are consistent with our findings at both the plant and crop level. Infection of pods occurs after seed number is set with infection reducing nutrient supply to developing seeds, thereby limiting seed size. Infection of the stem affects nutrient supply to developing flowers and pods thereby reducing seeds/pod. At a crop

scale, disruption to vascular function is most likely to affect yield under water-limited conditions such as those in 2017. Indeed, this has been reported for the effect of crown canker on grain yield (Sprague et al. 2010). In 2016, the crop was unlikely to be water-limited and the large yield reduction in the crop flowering on 22 June (0.99 t/ha) is probably primarily due to severe pod infection causing premature loss of pods or shattering prior to harvest rather than disruption to vascular function caused by stem infection.

Earlier planting times for canola in south-eastern Australia can provide increased grain yield, oil and water-use efficiency (Kirkegaard et al. 2016). To achieve these yield gains, it is critical to commence flowering in the optimum window to avoid the impact of frost, heat and water stress (Lilley et al. 2016). Simulation analyses in APSIM applying a frost/heat index can account for these abiotic factors in predicting potential yield (Lilley et al. 2015; 2016), however, this study clearly shows that biotic stresses such as disease also require consideration if yield improvements gained through earlier flowering times are to be realised. These studies reveal the need to build and integrate disease models into APSIM to improve yield predictions from different sowing date x genotype scenarios.

Our research shows that UCI can cause significant yield loss and presents plausible physiological mechanisms. Although flowering time is one factor important in the development of UCI, seasonal conditions will determine the prevalence and severity of UCI. Spore development and release, as well as infection events and subsequent environmental conditions interact with crop development stage to produce varying severity of UCI and modifies the effects of disease on yield outcomes. Further research is required to understand and predict these interactions as well as determine the physiological processes affected by UCI to inform potential control strategies.

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Effect of water stress on transpiration efficiency in canola

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ABSTRACT

Canola (*Brassica napus* L.) is frequently exposed to terminal drought stress in the Australian environments which limits the crop productivity. This study investigates the response of diverse canola genotypes to water deficit during vegetative and reproductive growth stages. Two glasshouse experiments were conducted at Wagga Wagga Agricultural Institute, Wagga Wagga in 2017 to investigate the genotypic differences for transpiration rate (TR), transpiration efficiency (TE), grain yield (GY) and harvest index (HI). Twenty genotypes were grown under well-watered (WW) and water deficit (WS) in a randomized complete block design. In Experiment 1, transpiration response to water deficit during vegetative stage (in terms of TR and TE) was assessed daily for two weeks. Measurements for leaf area, specific leaf area, biomass were recorded. In Experiment 2, transpiration response to water deficit during reproductive stage was assessed at weekly intervals until physiological maturity and data on water use, TE, GY and HI were recorded. Significant differences were observed between genotypes, water treatment and genotype × water for leaf traits, biomass and grain yield. TE at vegetative and at maturity was significantly affected by genotypes; however genotype × water interaction for TE was significant only at maturity.

Key words

Drought stress-grain yield-leaf area-transpiration efficiency

INTRODUCTION

Canola is the third most important crop to Australian agricultural industry, mainly grown as rainfed crop. Abiotic stresses such as moisture shortage and elevated temperature, especially during flowering, result in significant yield loss (Morrison, 1993). Canola is frequently exposed to terminal drought stress at the end of the season in Australian environments. With the impending climate change, the frequency of extreme climatic events is expected to increase (BOM, 2007) which would pose a serious impact on winter season crops. Canola is notably susceptible to drought stress at meiosis, coincident with flowering and also during grain filling. Drought stress at anthesis lowers plant architecture, leaf area, biomass and grain yield (Gan 2004; Sinaki et al.,2007). Therefore, there is need to breed canola varieties which adapt better to the water-limited conditions and/or greater water use efficiency (WUE).

Transpiration efficiency (TE), grain yield per unit of water transpired, is the preferred measure for examining genetic variation in WUE in crop species. In water limited environments, yield is represented as function of: Y= Transpiration (T) × Transpiration efficiency (TE) × Harvest index (HI) (Passioura, 1977). Each component of this framework can be considered as a breeding target, however in water-limited environments, improving TE can lead to higher biomass and grain yield. Recent studies have identified genotypic differences in TE among various crops (maize, pearl millet, sorghum, and wheat (Rebetzke et al., 2006; Vadez et al., 2014).

Genotypic differences for water extraction at the critical key stages is important with the ability of some genotypes to extract less water during vegetative stages which could be made available during reproductive and grain filling period (Vadez et al., 2014), leading to higher grain yield and harvest index in durum wheat, legumes, pearl millet and chickpea (Vadez et al., 2014). In canola, the WUE to produce grain ranged from 11-15 kg/ha/mm in Mediterranean environments (Robertson and Kirkegaard, 2005) and WUE for grain yield could be twice as much for water used during grain filling. Having high TE could contribute to a slower rate of soil moisture depletion and accessing water during grain filling.

In *Brassica napus*, genetic variation in various traits involved in drought escape such as flowering time (Raman et al., 2011; Hou et al., 2012; Zou et al., 2012; Raman et al., 2013; Raman et al., 2016; Raman et al., 2016; Raman et al., 2018), drought avoidance such as canopy temperature depression bud temperature (Guo et al., 2015; Pandey et al., 2017) and drought tolerance such as early vigour, biomass accumulation, carbon isotope discrimination, water soluble carbohydrates, chlorophyll fluoresce, leaf osmotic adjustment and leaf proline concentration and grain yield has been investigated (Cowley and Luckett, 2011; Luckett et al., 2011 Cowley et al., 2014; Norouzi et al., 2008; Ma and Turner, 2006). In addition, low leaf and bud temperature associated with higher biomass and drought tolerance has also been investigated in Brassica rapa (Guo et al., 2015). Genomic regions associated with flowering time, early vigour, biomass accumulation, carbon isotope discrimination and grain yield have also been identified in ten biparental populations and two genome-wide association panels; BnASSYST and AHGDS (Raman et al 2018). Based on described field results and controlled environment experiments (rain-out shelters), twenty canola genotypes were selected to determine genetic variation in TE in canola genotypes under drought stress.

MATERIALS AND METHODS

Plant material and growth conditions

Twenty genotypes of *Brassica napus* which included commercial varieties, parents of DH and elite lines were selected for study based on the previous experiments. Plants were grown during May-August and August–November under near optimal conditions in pots with 20 cm diameter and 5 kg soil holding capacity. Each pot was filled with organic garden mix (consisting of loam, river sand and compost) and was mixed with peat moss.

Experiment 1

A pot experiment in a randomised complete block design was conducted with 20 genotypes under well-watered (WW) and drought stressed (DS) conditions. Ten plants per pot were sown and then thinned to three plants after two weeks of sowing. Each genotype was represented by nine replicates in each treatment. Transpiration response of the plant to water deficit was assessed in May-August 2017. The assessment was initiated at 50 days after sowing in experiment 1. The experiment was design to estimate whether fraction transpirable soil water (FTSW) threshold where the transpiration declines varied with the genotype. At the time of drought imposition, pots were saturated with water and allowed to drain overnight. The volumetric moisture content was 34% at full saturation. The following morning, soil surface was covered with polyethylene beads @ 250 g per pot to minimize soil evaporation, and pots were subsequently weighed. The pot weight was thereafter taken every day in the morning. WW plants were kept at close to field capacity by applying water daily and DS plants were exposed to drying down cycle for two weeks days. The experiment was harvested when the transpiration of DS plants was <10% of that of WW plants and leaf area and dry weights was measured. Three plants were harvested and TE was measured as dry weight biomass divided by water applied (Vadez et al., 2014).

Experiment 2

Same set of genotypes were used in a randomised complete block design under well-watered (WW) and drought stressed (DS) conditions. Plants were sown in trays on 4 May 2017 and were vernalized for 12 weeks to account for vernalisation requirement for some genotypes. Plants were transplanted to the pots and were fertilized when required. Drought stress treatment was initiated at the time of 50 % flowering by restricting water by 2.5% of the field capacity in DS pots. At the time of imposing the treatment, pots were saturated with water and allowed to drain overnight. The following morning pots were weighed for initial weight and then subsequently weighed at periodic intervals till physiological maturity. Grain yield was recorded on dry weight basis after drying at 35ºC for 72hrs and TE was calculated as described in experiment 1.

RESULTS AND DISCUSSION

There was significant effect of genotype and water ($P<0.05$) on total biomass, leaf weight, leaf area and transpiration during vegetative stage. Water stress significantly reduced leaf weight, total biomass, leaf area and grain yield in all canola genotypes (Fig 1). Interaction between genotype × water was significant for biomass and leaf traits but not for TE during vegetative stage. Grain yield, plant height and transpiration (water use) at

maturity was significantly affected by genotype, water and genotype × water. TE at maturity was significantly affected by genotype and genotype × water; however there was no effect of water.

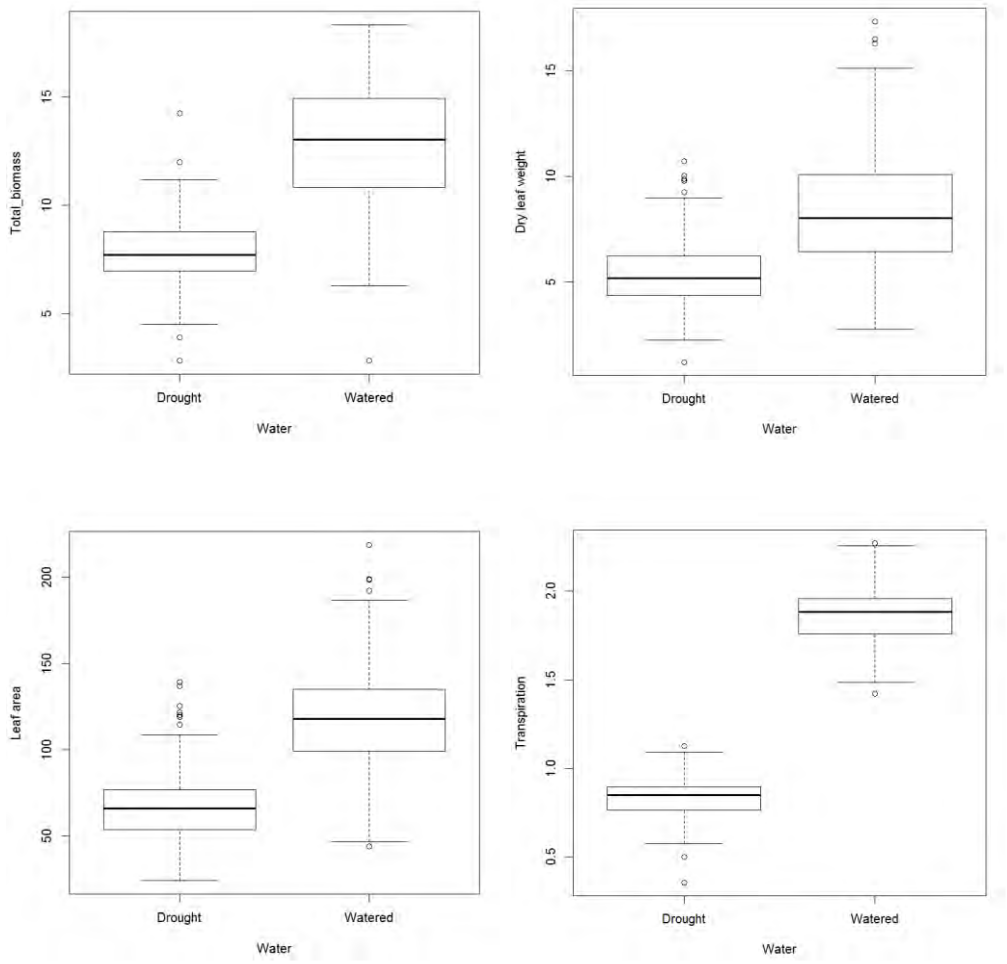

Figure 1: Effect of water stress during early vegetative stage on biomass (g), dry leaf weight (g), leaf area (cm²) and transpiration (kg) in canola

Significant G × E interaction for biomass traits during early vegetative stage, and transpiration and TE at maturity shows that genotypes had differential response to water availability. Differences between genotypes for transpiration might have resulted from differences in leaf area and stomatal regulation. Under well-watered conditions, plants transpired more water and resulted in high water use. However drought induced plant size reduction and alteration in leaf area and specific leaf area affects transpiration. TE, amount of dry matter produced per unit of water transpired is crucial for success of crop under water stresses environments. The ability of some genotypes to conserve water during vegetative stages and access more water during grain filling can lead to high TE in water stresses environments (Vadez et al., 2014)

ACKNOWLEDGEMENTS

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Fungicide resistance in Australian *Leptosphaeria maculans* populations

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ABSTRACT

The increase in canola production in Australia has been, in part, dependent on the use of fungicides for the control of blackleg disease caused by the fungus *Leptosphaeria maculans*. Seed treatment (Jockey® with active ingredient fluquinconazole) and fertiliser-applied (Impact® with active ingredient flutriafol) fungicides have been available for control of blackleg for over two decades in Australia. In 2013 the first foliar fungicide (Prosaro® with active ingredients prothioconazole and tebuconazole) was registered and provided the first in-crop control strategies for blackleg. All of these chemistries belong to the same fungicide group, the demethylation inhibitors (DMI) (Group 3 fungicides). Due to all these fungicides having active ingredients belonging to the same chemical class of fungicides, there is a risk of selecting for resistance. Recently, new fungicides have been released belonging to different chemical classes, the succinate dehydrogenase inhibitor (SDHI) (Group 7 fungicides) and the Strobulurins (Group 11 fungicides).

We developed an *in planta* assay for screening for fungicide resistance to each of these fungicides. Briefly, trays containing ten individual punnets were sown with cultivar ATR-Stingray; seeds sown in eight punnets were treated with fungicide and seeds sown in two punnets were not. Ten days after sowing, mature stubble (crop debris) from single sites was moistened and then evenly distributed above the plants and used to inoculate the seedlings. Twenty-one days after inoculation, plants were scored for the presence of lesions, indicative of the presence of fungicide resistant isolates. In 2015, using this *in planta* assay, we showed that resistance to fluquinconazole was present in 15% of 200 populations surveyed. In addition, we showed that this resistance is specific to fluquinconazole, with no cross resistance to flutriafol or the prothioconazole + tebuconazole mixture.

In the current study we have repeated the fungicide resistance survey using the *in planta* screen but extended the work to look for resistance to all fungicides commercially available for the control of blackleg.

Key words: Fungicide resistance – *Leptosphaeria maculans* – *in planta* screen

INTRODUCTION

The emergence of resistance to chemical agents is a well-known problem facing our ability around the world to treat infectious diseases, cancers, and particularly to protect agricultural crops, where an estimated $15 million USD is spent of fungicides worldwide each year (Leadbeater, 2015). The control of blackleg disease of canola is no exception. Blackleg disease of *Brassica napus*, caused by *Leptosphaeria maculans*, results in an average 10% yield loss, with epidemics resulting in 90% yield losses in specific regions (Van de Wouw et al., 2016; Sprague et al., 2006). The Australian canola industry is worth over AUD$2 billion annually, with production having increased rapidly over the past decade. The major drivers of this increase are the use of cultivars with disease resistance genes, management strategies to minimize disease and more recently, the use of fungicides. However, *L. maculans* is a high-risk pathogen, in that it can rapidly evolve to 'break down' disease resistance bred into canola (Van de Wouw et al., 2010; Van de Wouw et al., 2014). Until recently, all fungicides

used to treat blackleg of canola belonged to the same fungicide mode of action (azoles); these are often applied up to three times during a growing season (as a seed-treatment, as fungicide-amended fertilizer and as a foliar application), which puts severe selection pressure on fungal populations to develop fungicide resistance. Based on the evidence that fungal populations change to overcome resistance genes bred into cultivars and the outcomes from the use of fungicides to protect other crops from disease (Leadbeater, 2015), the continual use of azole fungicides means that the risk of fungicide resistance evolving in *L. maculans* is extremely high.

Recently, the authors developed an innovative *in planta* assay for screening for fungicide resistance (Van de Wouw et al., 2017). This assay involves using stubble (crop debris) which harbors the sexual fruiting bodies, to inoculate seedlings that have been treated with a fungicide. The benefits of this assay are two-fold. First, it allows for screening of millions of isolates as canola stubble, upon which the fungus undergoes sexual reproduction, is used as the source of the inoculum. Second, the exposure to fungicide occurs while the fungus grows *in planta* rather than under *in vitro* conditions; this simulates the selection pressure that the fungus is exposed to in the field. Using this *in planta* assay, the authors showed that fluquinconazole resistance was present in 15% of the 200 fungal populations sourced from stubble collected across Australia in 2015; this is the first-time azole resistance has been reported for *L. maculans* anywhere (Van de Wouw et al., 2017). However, as this was a single snap-shot in time, it is unclear whether the frequency of fungicide resistant isolates is increasing, stable or decreasing in Australian populations. Furthermore, only a single fungicide was assessed.

Recently, Syngenta Australia, Bayer Crop Sciences and Adama have released fungicides with different modes of action to the azoles for the control of blackleg. These fungicides are succinate dehydrogenase inhibitor (SDHI) (Group 7 fungicides) and the Strobulurins (Group 11 fungicides) (Table 1). Previous research and thus our understanding of resistance has historically been reactive, i.e. once a new chemical ceases to be effective, only then is research initiated to investigate the reasons behind this. The proportions of pre-existing populations with resistance and time frames for population changes, which translates into the effective life span of a chemical, are rarely known prior to use. Here, the imminent release of new chemistries to combat a major agricultural disease, blackleg of canola, in Australia provides the exciting opportunity to be proactive in understanding the evolution of fungicide resistance. In this study we use an *in planta* screen to assess Australian populations for fungicide resistance to all fungicides currently available to control blackleg, including the new chemistries recently released in Australia.

Table 1. Details of the fungicides currently available for control of blackleg disease in Australia.

Registered product	Company	Application type	Active ingredients	Fungicide class[1]	FRAC[2] classification
Jockey® plus generics	Multiple	Seed dressing	Fluquinconazole	DMI	3
Impact in Furrow® plus generis	Multiple	Fertilizer	Flutriafol	DMI	3
Prosaro®	Bayer Crop Science	Foliar	Tebaconazole	DMI	3
			Prothioconazole	DMI	3
Miravis	Syngenta Australia	Foliar	Pydiflumetofen (adepidyn™)	SDHI	7
Saltro	Syngenta Australia	Seed dressing	Pydiflumetofen (adepidyn™)	SDHI	7
Aviator®XPro®	Bayer Crop Science	Foliar	Bixafen	SDHI	7
			Prothioconazole	DMI	3
ILeVo®	Bayer Crop Science	Seed dressing	Fluopyram	SDHI	7
Veritas®	Adama	Foliar	Azoxystrobin	QoI	11
			Tebaconazole	DMI	3

[1] DMI = Demethylation Inhibitors also known as Azole fungicides. SDHI = Succinate-dehydrogenase inhibitors also known as carboxamides. QoI = Quinone outside inhibitors, also known as Strobulurins.

[2] Fungicide Resistance Action Committee (www.frac.info).

MATERIALS AND METHODS

A previously established *in planta* screening method for identifying fungicide resistance in *L. maculans* populations was used to screen for fungicide resistance in Australian populations. Over 100 stubble samples were collected from around Australia and then allowed to naturally mature by placing on the bare earth in a paddock, promoting the *L. maculans* growing saprotrophically in the stems to undergo sexual reproduction and for the pseudothecia to mature over summer. Once mature, the individual stubble samples were used to inoculate seedlings treated with the various fungicides (Figure 1).

Cultivar ATR-Stingray was used for the fungicide screens as although this cultivar contains resistance gene *Rlm3*, currently all Australian isolates are virulent towards this resistance (Marcroft et al., 2012; Van de Wouw et al., 2018). For screening of the foliar fungicides (Miravis®, Aviator®XPro®, Veritas®, Prosaro® and Flutriafol) bare seed was sown. Seven days post sowing (three days prior to inoculations), the seedlings were treated with the individual foliar fungicides at label rates. For screening of the seed dressing fungicides (Jockey®, ILeVO®, Saltro® and Maxim®), seed was treated with the individual fungicides at label rates prior to sowing. Untreated controls were also sown (as bare seed) and used in each experiment.

At ten days post sowing, punnets of each treatment (each punnet containing 8 seedlings), were placed in a tray. An individual tray (containing a single punnet of each of the ten treatments) were then placed in a plastic tub and the stubble was suspended 10cm above it (Figure 1a-c). The stubble was moistened to trigger spore release before the tub was sealed with a lid. After 30 hours, trays were removed from the plastic tubs and placed in the glasshouse to allow development of the

disease (Figure 1d-f). This process was replicated three times with stubble being dried for 24 hours before the next replicate was screened as to simulate a rainfall event in the field. For each replicate, the placement of each of the treatment punnets was randomized.

Lesion development was measured 14 days post inoculation. For each of the eight plants within each punnet, the number of cotyledons infected (incidence) and total number of lesions (severity) was determined). For each replicate, the ratio of lesions on each of the fungicide treatments was compared to the untreated controls to determine populations that have resistance to any of the various fungicides.

Fig.1. Methodology of the high throughput *in planta* assay used to screen for fungicide resistance in Australian populations. (a) Individual stubble samples are used to inoculate seedlings treated with the various fungicides. (b) Each stubble sample, representing an individual population, is screened in a plastic tub. (c) Each tray contains ten punnets, each with a different fungicide treatment, including an untreated control (highlighted with red box). Each punnet contains eight replicate plants. (d) Lesion development on the untreated control seedlings. (e) Lesion development on a Jockey® treated seedling indicative of a population with fungicide resistance present. (f) No lesions detected on the Miravis® treated seedlings suggesting no resistance present in the population screened.

RESULTS

A total of 110 stubble populations have been collected from around Australia representing much of the Australian canola growing region (Figure 2). These stubbles have been used to screen for resistance towards all commercially available fungicides for the control of blackleg disease in

Australia. Preliminary results suggest that resistance towards the azole fungicides is present in Australian populations whilst there is no resistance towards the new fungicide chemistries (SDHIs and QoIs).

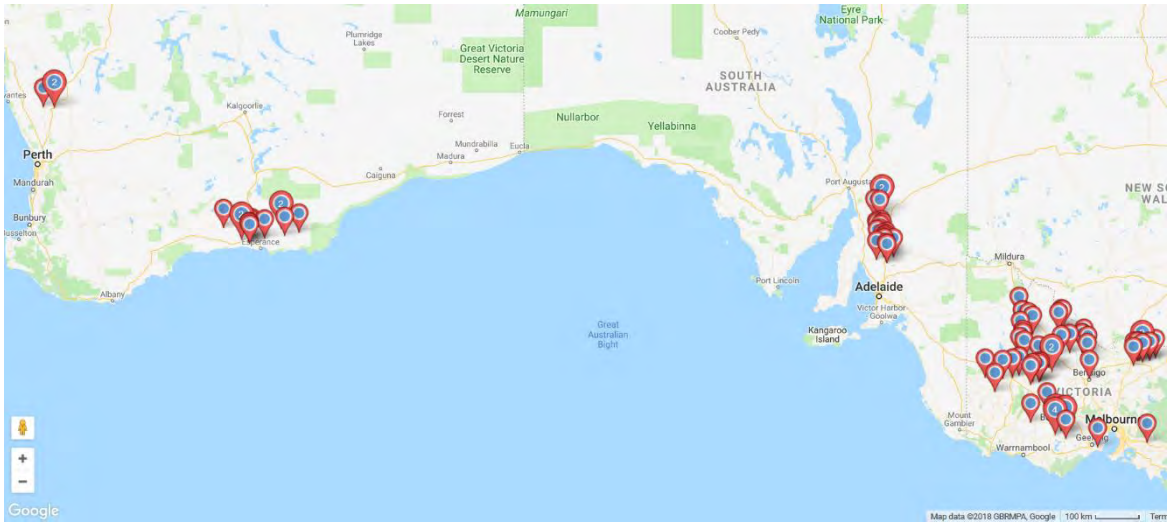

Fig. 2. Location of stubbles used for fungicide resistance survey.

DISCUSSION

Fungicide resistance is becoming a major threat to the Australian and worldwide grains industry with resistance developing in many of the plant pathogens and towards many of the current fungicides. In the current study we are taking a proactive approach and screening for fungicide resistance at the time of release of new fungicide chemistries, rather than once resistance has developed. The preliminary data suggest that no or extremely low levels of resistance are present for the new fungicide chemistries and provides a base line for monitoring changes in fungicide resistance as these chemistries are taken up by industry.

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Predicting canola phenology in warm environments

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ABSTRACT

The geographic and within-season range of Australian canola production is expanding and so crops are being sown earlier and in areas that experience warmer winters. Simulation models are being used to help match canola genetics to the local environment to identify opportunities for early sowing and to identify the optimal flowering time to avoid frost, heat and water stress. But there is uncertainty as to whether these models can accurately represent plant response in warmer environments?

As part of the GRDC Optimising Canola Profitability Project a detailed phenological study of Australian canola cultivars was undertaken. These studies used the diverse environments of Canberra ACT, and Gatton Qld, combined with lights to create varying photoperiods and vernal temperatures. The approach easily identified that many Australian commercial cultivars display significant vernal responses and only a few have significant photoperiod responses (day length).

The use of modelling to investigate new opportunities for novel canola production systems (early sowing, warmer regions), highlights the importance of accurately characterising phenological responses for Australian canola cultivars.

Key words: Thermal Time, Degree Days, Vernalisation, Simulation

INTRODUCTION

The geographic and within-season range of Australian canola production is expanding and so crops are being sown earlier and in areas that experience warmer winters. Simulation models are being used to help match canola genetics to the local environment to identify opportunities for early sowing and to identify the optimal flowering time to avoid frost, heat and water stress. The majority of Australian canola research occurs within the tradition canola regions, and the majority of vernalisation research occurs within cooled controlled environment facilities, responses under warmer conditions are uncertain.

The time to flowering in canola is measured by the accumulation of thermal time above a base to a measured target. In some cultivars, reduced temperatures between gemination and floral initiation can reduce the time taken to reach flowering - known as a vernal response. Three vernal response patterns have been characterised (Murphy and Scarth, 1994), however, only two dominate in Australian cultivars. These are, (1) the cumulative response that decreases the length of the vegetative period with an increased duration of cold and will be referred to as a facultative response and (2) the threshold response, which occurs with extended periods of cold and triggers a change in development once a set period of cold has been experienced. This form of vernalisation is common in winter type canola and will be referred to as obligate vernalisation (Hodgson, 1978).

Vernal time, based on the paper by Robertson and Lilley (2016), is calculated from the average daily temperature with specific cardinal numbers. If the average daily temperature was 2°C then one vernal day is accumulated, no vernal time is accumulated when the average temperature is below 0°C or above 15°C.

Thermal time in simulation models like APSIM (Holzworth et al., 2015) is calculated by fitting a circadian curve between the maximum and minimum temperatures to create eight sub-daily segments. These are used to calculate an average daily temperature. This method accounts for the cooler temperatures during the morning and milder temperatures in the evening that contribute to development. However, this same approach is not always applied when calculating vernal time. When vernal time is calculated using the eight-segment approach, small fractions of vernal time can be accumulated within the morning, resulting in accumulation of some vernal time in warmer environments, whereas none is accumulated when the average daily temperature approach is used in that environment.

Canola is a long day plant so in addition to cold temperatures, increasing photoperiods can reduce the time to reach a flowering thermal time target. Photoperiods between ~10 and 16 hours (Robertson et al., 2002) have been shown to reduce time to flowering. It has been reported that spring cultivars from Europe, Australia and Canada all responded to increasing daylengths beyond 10-12 hours with saturation occurring at around 16 hours daylength. However, values of saturation at 18 hours (King and Kondra, 1986) or 20 hours (He et al., 2017) for specific genotypes or locations have been reported. There is little data describing interactions between daylength and vernalisation (Robertson and Lilley, 2016) and as a result or for simplicity, these two processes are generally treated independently within crop models (Habekotte, 1997; Robertson et al., 2002).

As part of the GRDC Optimising Canola Profitability Project a detailed phenological study of Australian canola cultivars was undertaken. These studies focused on the extremes of the Australian canola production regions using the diverse environments of Canberra ACT and Gatton Qld, to achieve varying vernal temperatures. Lights were included to extend photoperiods to 14 and 16 hours. The aims were to identify the flowering responses within Australian commercial cultivars and to improve the prediction of flowering and therefore facilitate matching of genetics to the environment.

This paper will describe the results from a selection of cultivars that demonstrated different responses to vernalisation and photoperiod particularly in the warmer environment of Gatton.

MATERIALS AND METHODS

Two sites (Ginninderra, ACT) and (Gatton, QLD) were selected for this study because of their contrasting environments. Preliminary environmental assessments showed these sites bounded the Australian canola growing regions. Working at these extremes was considered the most efficient way to identify different phenological traits within current Australian canola germplasm.

The Ginninderra site was located near Canberra ACT (latitude -35.201°, longitude 149.082°) on a Yellow Chromosol (Isbell, 1996). The average rainfall at Ginninderra is 601 mm with 391mm falling during the winter growing season. The average daily temperatures for the 6 month winter growing season between April and October ranges from 6°C to 15°C.

The Gatton site was located in Queensland (Latitude -27.558590° Longitude 152.313924°) and was on a deep black weakly cracking Vertosol (Isbell, 1996). The average rainfall is 802mm with 239mm falling during the winter growing season April to October. The average daily temperatures for the 6 month winter growing season between April and October ranges from 13°C to 22°C.

Two replicates of up to 24 cultivars were sown at each site at 2-5 sowing dates over 3 years. Daylength treatments were included at specific sowing dates to ensure the greatest contrast between the natural light treatment and the extended light treatments. A total of 35 sowing date x daylength x site treatments were imposed. A total of 36 cultivars were studied. (Table 1).

An artificial lighting system was used to extend the daylength at both Canberra and Gatton. When applied, a full set of the tested cultivars experienced both light extensions and a natural light control (Table 1). The system consisted of 48 fluorescent grow lights (Sylvania Gro-Lux F36W/GRO-T8) mounted in waterproof housings and suspended between two crop rows at a height of 800mm. Lights were raised as the cultivars began to elongate to ensure even light distribution. A light intensity of 5µmol m^{-2} s^{-1} was measured at the soil surface within the crop row using a Ceptometer (AccuPAR model LP-80, Decagon Devices Inc.).

Both sites were fertilised and irrigated to a level that would ensure minimal stress, insect pests and diseases were controlled by additions of industry recommended chemicals and rates. Weed control was maintained by hand weeding to ensure no chemical stress to the plants.

Phenological stage identification was conducted biweekly using the BBCH key (Monograph, 2001) with a specific focus on stages that could be easily identified. Eight plants were randomly selected from each 3 m row at each assessment time. Data was analysed in the statistical package R, the date each growth stage was achieved for each cultivars was determined when 50% of the observations had achieved the growth stage.

Thermal time, vernal time and daylength were calculated from meteorological records, recorded at the site using the equations described in the crop simulation model APSIM (Holzworth et al., 2015; Robertson and Kirkegaard, 2005; Robertson and Lilley, 2016). Floral initiation was identified by dissecting two juvenile plants per rep at each assessment time. The vegetative apex was assessed following the methods described by Moncur (1981).

Temperature and rainfall were recorded at each site using a Tinytag® Logger (Hasting Dataloggers, Australia). Missed data were infilled from the local meteorological data record available from SILO (Jeffrey et al., 2001) using station 40082 for Gatton and 70014 Canberra.

Table 1: Dates for the time of sowing experiments conducted in Canberra and Gatton between 2015 and 2017

Time of Sowing	Canberra 2015	Canberra 2016	Canberra 2017	Gatton 2015	Gatton 2016	Gatton 2017
TOS 1	1/4/15	31/3/16	18/4/17†ß	31/3/15	19/4/16	4/5/17†ß
TOS 2	16/4/15	26/4/16†ß	15/5/17†ß	15/4/15	5/5/16	6/6/17†ß
TOS 3	29/4/15	19/5/16	16/6/17	7/5/15	10/5/16†ß	
TOS 4	13/5/15		11/09/17	15/5/15	20/5/16	
TOS 5				23/6/15†		

† Included a 16 hour Daylength treatment
ß Included a 14 hour Daylength treatment

RESULTS

Vernalisation was calculated using the eight segmented thermal time approach, because the additional vernal time accumulated early in the season, especially at Gatton reduced clumping of the data and improved the slope of the relationship between vernal time and thermal time to flowering. This was particularly important for fast maturing spring types that responded to small amounts of vernalisation and in cool environments can saturate their vernal requirement soon after emergence. In warm environments no vernal time was recorded when the average

day method was used. This caused the data to clump around zero masking vernal responses and reducing the position of the intercept for cultivars with a facultative response. The amount of accumulated vernal time to trigger an obligate response in winter canola was less when the average approach was used compared to the eight segment approach. This highlights the need to specify the vernal time calculation method used to develop cultivar descriptions.

We assessed accumulation of vernal time and daylength during different phenological phases (emergence, floral initiation, green bud and flowering) and determined which variable and phase best predicted / explained flowering time. Analysis of the data identified accumulated vernal time to floral initiation, and daylength at green bud, provided the best relationship for predicting the reduction in duration of the vegetative period caused by vernalisation. These calculated values are used for all results presented in this paper.

To identify the impact of vernalisation on flowering, the thermal time from emergence to flowering was plotted against the vernal time accumulated from sowing to floral initiation. A range of different responses were observed within the Australian canola cultivars examined. These included a linear facultative response, a segmented facultative response, facultative responses that interacted with daylength and finally the classic winter type obligate trigger, followed by a facultative response. Over the three years of data collection, a clear vernalisation base was not identified, but the trend supports the literature reports of >25 vernal days.

The commercial cultivar ATRWahoo demonstrated a facultative vernalisation response that was not influenced by daylengths up to 16 hours. The linear regression estimated a thermal time target of 844 C°d and a reduction rate of 14.1 C°d / Vernal day (Figure 1).

The short season spring type Stingray demonstrated a segmented facultative response with a steep decline in thermal time to flowering for about 5 vernal days, then a reduced slope reducing thermal time to flowering with increasing cold accumulation (Figure 2). A distinct response to daylength was observed for sowing dates that accumulated less than 10 vernal days and there was no significant effect of daylength when accumulated vernal days were greater than 10. The base thermal time target was 836°Cd when the daylength was less then 13 hours dropping significantly to 632°Cd when day lengths were longer than 13 hours. A vernal response was observed in both curves, but appears to plateau between 5 and 7 vernal days.

The mid-season cultivar Archer was similar to Wahoo in demonstrating a facultative response to increasing vernal days, however, a significant daylength response was observed for 16 hour daylengths (Figure 3). The inclusion of long daylengths significantly reduced non vernal response of Archer from a base of 904°Cd to 754°Cd.

No difference was observed between the four winter types tested (Hyola_970_CL, Hyola_971_CL, SF_Brazzil, SF_Edimax) and these cultivars all showed an obligate response that occurred around 15 vernal days and then followed a facultative response to 25 vernal days after which it appears to plateau (Figure 4). Increased daylength had no effect on the winter types we tested and Only the facultative response has been fitted. The boundary of the data identifying the trigger was used to estimate the obligate response.

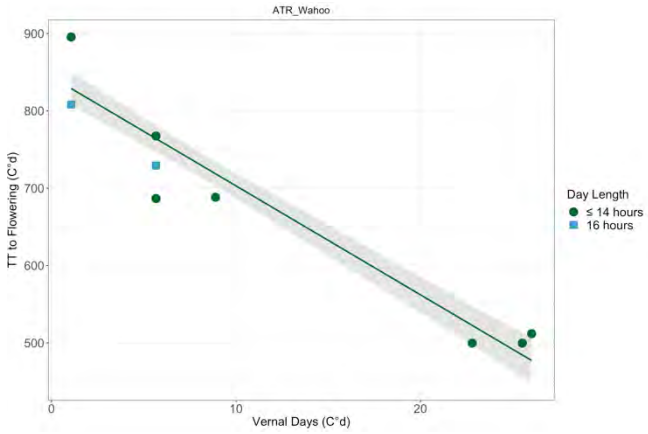

Figure 1.
A typical facultative vernalisation response where the vegetative period is reduced by increasing accumulation of cold. Demonstrated in the commercial cultivar ATR Wahoo. Symbols differ for data collected under different daylengths, the shaded area describes the standard error of the predicted regression line. Regression y = -14.1 (1.6) x + 844 (22.7), R^2=0.91, P<0.0001

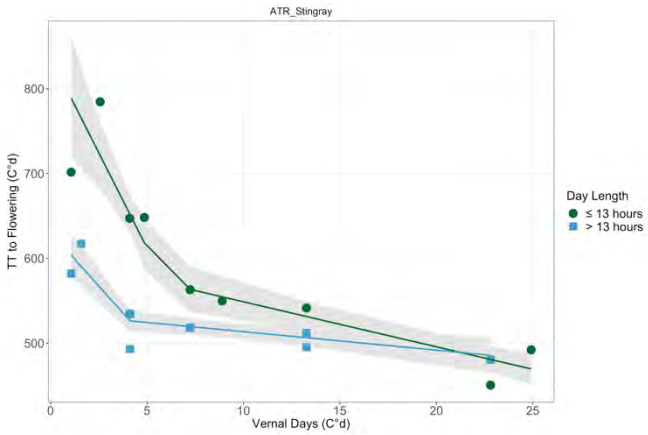

Figure 2: A segmented facultative vernalisation response with the vegetative period being reduced with increasing accumulation of cold and increasing daylength. Demonstrated in the commercial cultivar ATR Stingray. The segmented regression under the natural conditions had an Intercept of 837 (94) and a slope of -45 (23) to the breakpoint 5.9(1.5) to a slope of -5.3 (1.9) in the second segment. Under extended daylength the Intercept was 632.5 (90) and a slope of --26 (66) to the breakpoint 3.9 (7) to a slope of -2.2 (1.5) in the second segment.

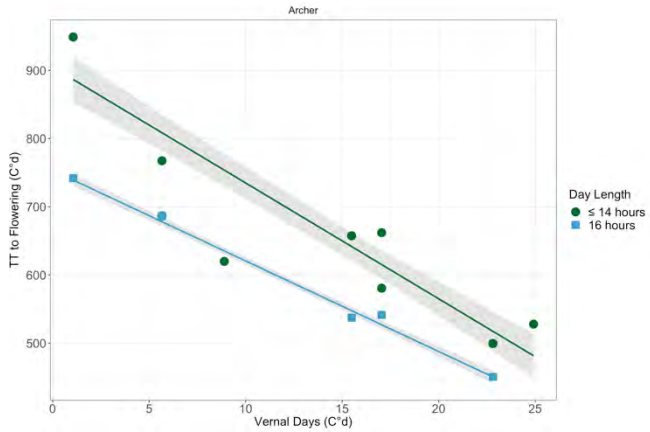

Figure 3: A facultative vernalisation response with the vegetative period being reduced by increasing accumulation of cold for both treatments experiencing daylengths of more and less than 16 hours. Demonstrated in the commercial cultivar Archer. The regression for daylengths of 14 hours or less is described by the equation y = -17 (2.2) x + 905 (36), R^2=0.88, P<0.0001 and for those of 16 hours was y = -13.3 (0.7) x + 753 (9.7), R^2=0.99, P<0.0001

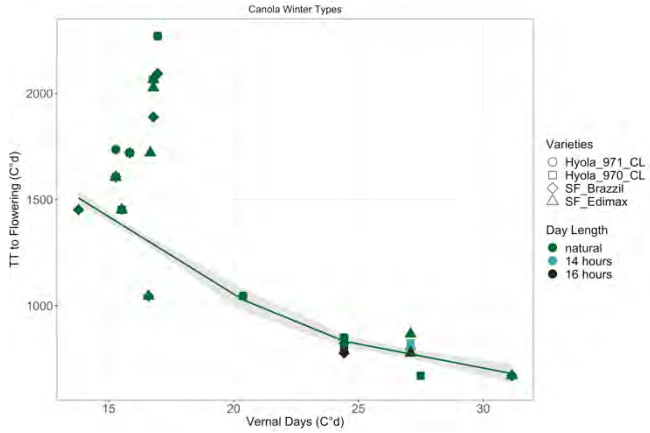

Figure 4: An obligate vernal response, with the obligate trigger occurring at 15 vernal days. A segmented facultative vernal response is observed once the obligate accumulated cold has been achieved. The response of the four winter types (Hyola_970_CL, Hyola_971_CL, SF_Brazzil, SF_Edimax) was the same and can be described by a segmented regression with an Intercept of 2512 (176) and a slope of -73 (11) to the breakpoint 22.4 (1.7) to a slope of -22.7 (8.5) in the second segment. No effect of daylength extension was observed.

DISCUSSION

Significant responses to vernalisation were observed in all of the cultivars presented. The approach of using sub-daily thermal time calculations to calculate vernal time, combined with the collection of data from contrasting environments, increased the values of vernal time and improved the fit. This elongation of the data extended the fitted response and reduced the error around the prediction of the intercept compared to the average day method.

In the majority of Australian canola growing areas, the small vernal response observed in the fast developing cultivar, Stingray would be satisfied very early on, possibly before emergence, so for all effective purposes it will behave as if there is no vernal response. However, when it is moved to the

warmer environments of northern NSW and Southern Queensland it can behave more like an medium-fast developing variety. Thus in these environments it could be planted earlier than cultivars traditionally known to have no vernal response.

The facultative response observed in the slow developing cultivar, Archer was consistent across sites and sowing dates, and can help explain its use in a broad range of environments. The distinct photoperiod response for daylengths over 16 hours was almost additive to the vernal response, significantly reducing the time to flowering. However, within the majority of Australian grain growing regions it is unlikely that 16 hours daylength will ever be experienced. This is a highly relevant issue when testing the phenology of new cultivars because in a field situation, the main driver reducing the time to flowering will be vernalisation.

No photoperiod effects were observed in the winter type canola that had both an obligate and facultative vernalisation response. This is good news for the practice of using canola as a dual purpose crop. The lack of a photoperiod response will ensure flowering does not occur too early when planted in early autumn or even late spring as has been trialled in the high rainfall zone of eastern Australia (Paridaen and Kirkegaard, 2015).

The use of field-based experiments in contrasting environments to assess the phenological responses of Australian canola varieties has proved to be a successful and efficient approach to understanding the drivers/timing of flowering in canola. Despite the difficulties of running this type of experiment in the field, the range of environments experienced were broader than those experienced in a controlled environment facility. The use of two environments that bookend the canola growing environments experienced within Australia provides confidence in the data and the temperature response functions derived from it. The application of this approach to newly released canola varieties would provide accurate characterisation of new varieties and assist canola growers to match the correct genetics to their individual environments.

ACKNOWLEDGEMENTS

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Hybrid vs. OP Canola: which one wins where?

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Abstract

Realising high yield potential of hybrid canola is strongly dependent on the environment and the profitability of hybrid technology must be assessed against open-pollinated (OP) canola. This study compared the yield and gross margins of hybrid and OP canola across a wide range of environments in Western Australia (WA), and in the National Variety Trial network (NVT) across southern Australia. Hybrid canola had yield advantages over OPs in favorable environments where rainfall was high (>300 mm) and the growing season was long. However, in areas of low rainfall where yield potential was low (< 260 mm), hybrids showed little yield advantages over OPs. The gross margin analysis suggested that hybrid triazine tolerant, conventional and Roundup Ready canola was profitable in the medium and high yielding environments, but not profitable in the low yielding environments because the cost associated with seed outweighed any small yield benefit.

Key words: hybrid, open-pollinated, gross margin.

Introduction

Canola is the third largest crop in Australia. It has expanded into lower rainfall areas from its initial high rainfall zone (HRZ) heartland, and playing different roles, from cash crop in the HRZ to break crop in the low rainfall area, depending on yield potential and farming system needs. Current canola cultivars offer a range of options to accommodate the needs of growers across the rainfall zones using hybrid (HB) and open-pollinated (OP) cultivars with four of herbicide tolerances (HT) groups (triazine tolerant: TT; Roundup Ready: RR; imidazoline tolerant or more commonly used Clearfield: CL, and conventional: CV). OP TT canola seed is cheap (ca. $2/ha), offers robust weed control and has been widely adopted, despite the acknowledged yield penalty associated with the technology. Conversely, hybrid canola is more vigorous and weed-competitive and can yield up to 20% more than OP cultivars in Australia and Canada (Brandt et al., 2007; Kirkegaard et al., 2012), but it is more expensive to grow as hybrid seed costs approximately $27-34/kg. Recently, canola breeding has gradually shifted from OP to hybrids because canola breeders value the income stream presented by hybrid production as farmers cannot retain seed. One of the important questions faced by growers is whether they should grow hybrid or OP canola, given the yield-cost trade-off and this is likely to depend upon yield potential of specific sites according to rainfall and growing season length. While hybrid canola might provide opportunities to achieve higher yield, is it more profitable in any given environment? This paper seeks to answer these questions and provide growers with guidelines to select the right varieties. It also serves to demonstrate to seed suppliers and breeders which varieties are likely to be relevant in different regions in the longer term and which direction breeding effort for canola should be focused.

Method and materials

A total of five field experiments using 19-20 canola varieties and two nitrogen (N) rates (0 and high, as appropriate for the rainfall zone) were conducted in the low (Merredin, 2014), medium (Cunderdin, 2013 and 2014), and high (Kojonup, 2013 and 2014) rainfall areas of Western Australia. Current cultivars were used, balanced by heterosis (OP and hybrid), herbicide group (TT, RR, CV, and CL) and phenology as much as possible. Experiments were laid-out in a split-plot design with herbicide group as main plots to facilitate the contrasting herbicide treatments and replicated three times at each location. The high N rate treatment increased with rainfall zone, from 80 kg N/ha at Merredin, 100 kg N/ha at Cunderdin, and 150-175 kg N/ha in Kojonup. At seeding, 20 kg N/ha was drilled as a base application, 50% of the remaining N was applied at the six-leaf stage and the other 50% at bud visible stage as urea. The seeding rates were set to achieve 40 plants m^{-2} based on seed weight and a *priori* germination rates. For each herbicide group, the corresponding herbicides were sprayed to control weeds. The plot size was 20 m by 1.54 m. The whole plot was harvested using a plot harvester, and 1 kg of seed sample from each plot was collected to analyze oil, protein and moisture content using a

calibrated FOSS Infratec. The yield was reported at 8% moisture and 42% oil content. In addition to these experiments, the NVT data from experiments in Western Australia, Victoria, New South Wales, and South Australia from 2010 to 2014 were provided by the Australian Crop Accreditation System Limited (ACAS). To minimize the effect of imbalance, only varieties tested at > 20 locations were included in the analysis. Finlay-Wilkinson (1963) analysis was used to quantify responsiveness to environment using the 2 N treatment means for each of the 5 trials to provide a total of 10 environment means from Merredin (2014), Cunderdin (2013, 1014) and Kojonup (2013, 1014) in regressions of varieties nested within heterosis and/or herbicide groups. The same Finlay-Wilkinson (1963) regression was performed on the NVT data. Residual plots were generated in both regression and ANOVA to detect errors and check for common, independent error variance. To estimate the gross margin of hybrid and OP systems, the yield of hybrid and OP canola was derived from the Finlay-Wilkinson linear regression equations from the NVT data. The N fertilizer input cost varied with yield while the other nutrients cost was set at $34/ha. The assumption was that 50 kg N/ha are required to produce 1 ton of canola grain. The cost of input for different herbicide systems and grain price for canola was based on current agronomic consultants' estimation. The grain price was set at $523 for CL, TT, and CV canola and $509 for RR canola. To make sure that breeders can recover their investment in OP canola varieties, we assumed $5/ton of endpoint royalty for OP canola and subtracted the endpoint royalty from the revenue for all OP canola.

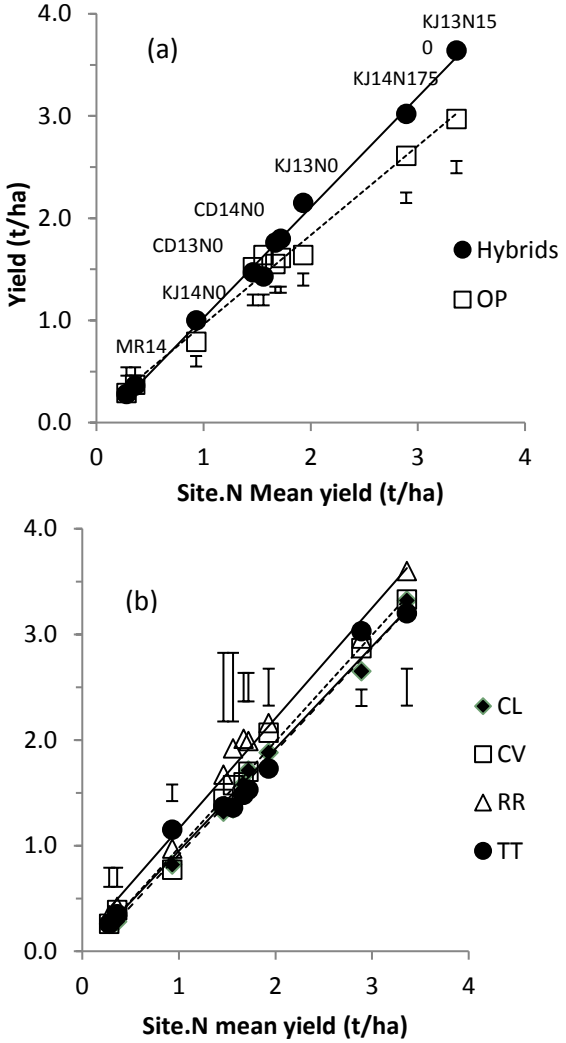

Fig. 1 The response of (a) hybrid and open-pollinated (OP) canola, and (b) four herbicide systems (Clearfield: CL, conventional: CV, Roundup Ready: RR, and triazine tolerant: TT) to yield potential represented by the site.N mean yield at five site and year combinations in 2013 and 2014. The letters followed by two digits and N treatment represent the site (Kojonup: KJ, Cunderdin; CD, Merredin: MR), year and nitrogen treatment.

Results

A Finlay- Wilkinson (1963) approach of regressing hybrid and OP means against each site by year N treatment mean (e.g., low and high N means per site year) was very effective, capturing 96% of the variance. The large slope differences were between hybrid (1.087±0.0018) and OP (0.883±0.03) canola (Fig.1a), with only minor cultivar differences within heterosis groups. This resulted in a fan-shaped response to site yield potential (Fig. 1a), where both heterosis groups emerge from a common yield at low yielding sites (0.32 for hybrid s and 0.33 for OPs at Merredin), and then separate as site mean yield > 1 t/ha. In contrast to the heterosis groups, there were no slope differences between the four herbicide tolerance groups, indicated by the parallel lines in Fig. 1b. However, RR canola had a consistent 0.2 t/ha ($P < 0.05$) yield advantage over the others (CL, CV and TT canola), captured by intercept differences in the regression.

Finlay- Wilkinson (1963) analysis was also very effective for showing national differences in canola responsiveness, capturing 94.4% of the variance of NVT trials. When the data from 4 HT groups in WA, NSW, Vic, and SA were pooled, the accumulated analysis of variance showed that the heterosis group and its interaction with site and HT groups had significant effects on yield and that no interaction was observed between the state, HT, and heterosis groups. Because the interaction between heterosis and HT groups was significant, we subdivided the dataset into TT, RR, CL, and CV groups for the Finlay-Wilkinson analysis. For TT and RR canola, hybrids had significantly ($P < 0.01$) greater slopes than their OPs (Fig. 2a, b). The slopes for hybrid and OP CL and hybrid and OP CV were not significantly different (Fig. 2c, d). Both hybrid TT and RR canola had similar yields to OP canola when the site mean yield was low (<0.7 t/ha). However, as the site mean yield increased, the advantage of hybrid canola become more apparent, and the responses of hybrids to the environment were greater than OPs for TT and RR canola (Fig.2a, b). Although the responsive slopes were similar, hybrid CV produced higher yields than OP CV because of a significantly greater intercept difference (0.28 t/ha) (Fig. 4d). However, there was no significant difference in yield between hybrid and OP CL canola.

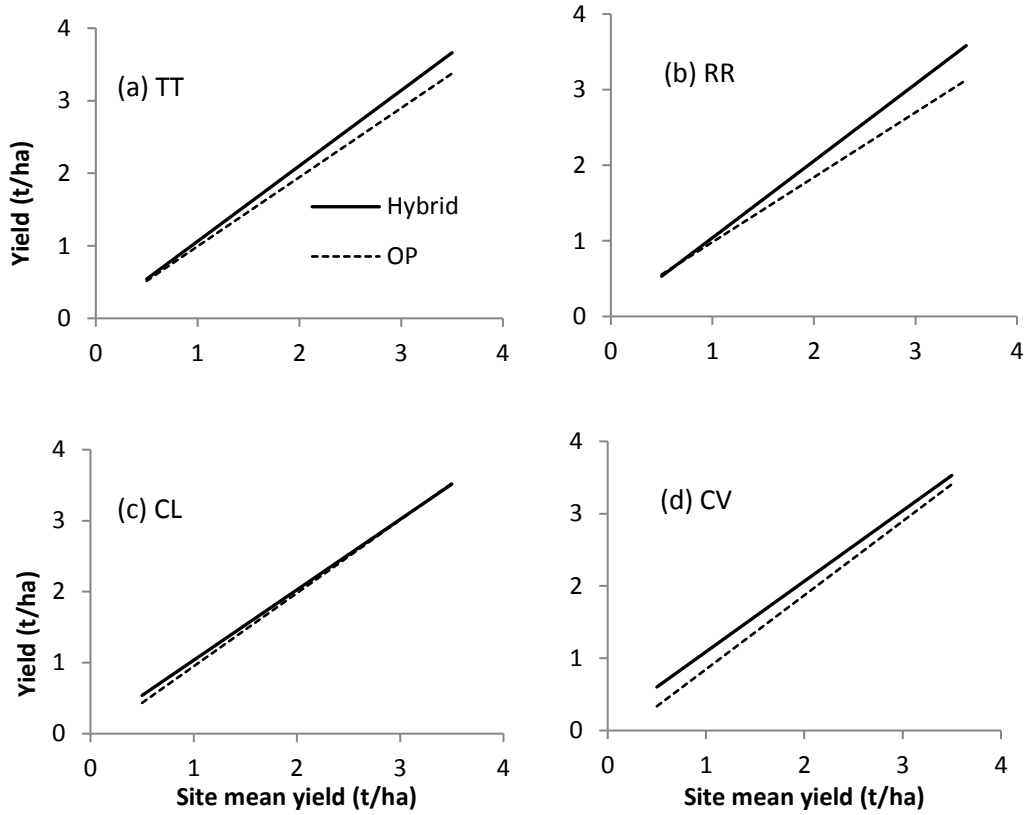

Fig. 2 Responses of hybrid and OP (a) TT, (b) RR, (c) CL and (d) CV canola to the environment in Western Australia, Victoria, New South Wales and South Australia from 2010 to 2014.

Given the fan-shaped yield responses of OP and hybrid canola, gross margins were strongly linked to yield potential: hybrid canola was profitable only when the gains from higher yield outweighed the

additional seed cost. Differences in costs, value and yield responsiveness of OP and hybrid canola among the four herbicide groups lead to different yield-gross margin relationships (Fig. 3). In RR canola, the break-even yield between OP and hybrid was 0.7 t/ha (Fig. 3a) because OP RR canola is considerably less yield responsive than H hybrid RR canola. Conversely, the break-even yield was 1.3 t/ha for hybrid versus OP TT canola (Fig. 3a). For CL canola, hybrids were less profitable than OP CL canola because of similar yields and high cost associated with HYBRID seeds. Compared with OP CV, it was more profitable for hybrid (Fig. 3a) because hybrid CV always produced a higher yield than OP CV (Fig. 2d). Given the prevalence of OP TT in Australian production, it is useful to set this as a standard in comparisons (Fig. 3b). Compared with OP TT canola, hybrid RR and hybrid CV become more profitable only when yield is greater than 2.4 t/ha and 1.0 t/ha (Fig. 3b), respectively, while hybrid CL is less profitable throughout the experimental yield range because of the most expensive herbicide cost among the four systems.

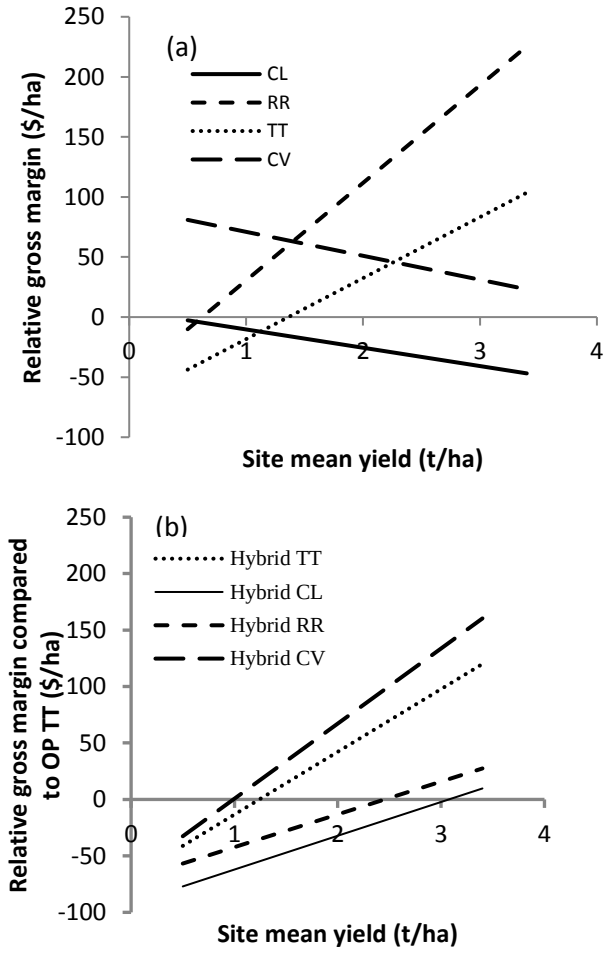

Fig. 3 The gross margin (GM) analysis of (a) hybrid canola for four herbicide tolerance groups (Clearfield: CL, conventional: CV, Triazine tolerant: TT, and Roundup Ready: RR) compared their open-pollinated counterparts and (b) hybrid canola compared to open-pollinated TT canola.

Discussion

Our study showed that the relative yield performance and profitability of hybrids over OPs strongly depend on site potential yields associated with growing season rainfall in Australia and the herbicide groups considered. This is in contrast to the consistently higher yield advantage and profitability of hybrids over OPs reported in Canada (Brandt et al., 2007). The greater yield and gross margin advantage of hybrids over OPs make hybrids the best option in favorable environments where rainfall is relatively high (> 300 mm) and the growing season is relatively long. However, in areas of low rainfall coupled with high temperatures during the seed filling period, hybrids showed little yield advantages over OPs. The gross margin analysis suggests that hybrid RR and TT canola were profitable in the medium and high yielding environments where potential yield was high. However, they were not

profitable in the low yielding environments because the cost associated with seeds outweighed the small yield benefit. This probably explains why around 80% of canola grown in Australia is still OP TT canola. This leads us to conclude that canola breeding in Australia must take into account this fact. The high potential yield and profitability of hybrid canola in favorable high rainfall areas will require canola breeding companies to use the advantage of heterosis to breed hybrid canola. However, the low profitability of hybrids in the low rainfall areas suggests there will be little market for hybrids and requires them to continue to embrace OP TT canola. The departure from breeding OP canola raises the necessity to restore the endpoint royalty system that allows breeders to recoup their investment. The endpoint royalty system has been very successful for wheat breeding. Whether an endpoint royalty system will work in canola is still in question because a much smaller area of canola (20% of wheat area) is grown. Furthermore, farmers can purchase a small amount of OP canola seed and grow them in the nursery to produce seeds for the next few seasons without the need to purchase seeds every year. This makes the endpoint royalty system much more attractive to breeding companies.

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