

Expanding Pulse Production

Lentil herbicide management in low rainfall environments

Sarah Day¹

Helena Oakey², Penny Roberts¹ and Larn McMurray^{3,4}

¹ SARDI

² University of Adelaide

³ South Australian Research and Development Institute

⁴ The University of Adelaide

Break crops continue to occupy a small percentage of arable land across the southern low rainfall zone, despite extensive research demonstrating their role and value in farming systems. This is generally thought to be due to the perception that break crops have an increased risk and production cost, compared to cereals. There is also a lack of confidence about correct break crop management required to reduce production risk and minimise inputs in a low rainfall system. There is little information in this area, as break crop development has largely occurred in medium and high rainfall zones, and often these strategies are inappropriate for low rainfall environments. Herbicide damage in lentil commonly occurs in low rainfall environments and often the damage that occurs is not consistent across low rainfall environments. This study aims to identify crop safety levels and economic risk of pre- and post-emergent herbicide use on lentil across different soil types and environments in the southern low rainfall zone. To address the knowledge gap, a lentil herbicide management trial was established at Willowie, upper Mid North South Australia, in 2017 and extended to the upper Eyre Peninsula and South Australian Mallee in 2018. There were 24 herbicide treatments applied to PBA Hurricane XT lentil, consisting of six herbicides (Group B and C), each was applied at two timings and at two rates for each timing. Metribuzin applied at a low rate incorporated by sowing was included as a control treatment. Results were variable across environments with few similarities observed between environments. An interaction was observed between herbicide, application timing, and application rate for gross margin at Minnipa 2018, and indicated an increased level of economic risk for some treatments compared to the control. Terbutylazine expressed a lower safety level and higher economic risk compared to the control, Metribuzin, at Minnipa in 2018. Herbicide choice and application timing was shown to be important to reduce risk associated with lentil production

in low rainfall environments, particularly as lentil is sensitive to herbicide use in dry condition. Addressing the knowledge gap in low rainfall environments will provide growers with local information on lentil production and management and increase grower confidence in utilising lentil in their rotation.

Development of a DNA test to quantify the number of *Rhizobium leguminosarum* bv. *viciae* in soil

Ross Ballard¹

Daniele Giblot-Ducray², Herdina Herdina¹, Kelly Hill², Stephen Barnett¹, Elizabeth Farquharson³ and Alan Mckay²

¹ South Australian Research and Development Institute

² SARDI

³ South Australian Research and Development Institute - SARDI

DNA tests to quantify rhizobia in soil are being developed. These tests will help growers determine if rhizobia inoculation is required. They will also provide researchers with an alternative test to the time consuming Most Probable Number plant nodulation bioassay, to help better understand how agronomic practices affect rhizobia populations. They may have application in the quality control of rhizobial inoculants, particularly of granular products where more efficient tests are needed to support independent quality control.

The most advanced test targets the rhizobia that nodulate pea, bean, lentil and vetch; *Rhizobium leguminosarum* bv. *viciae* (*Rlv*). A quantitative real time PCR assay for the specific detection of *Rlv* was designed and a calibration standard prepared to estimate *Rlv* DNA copy number/g soil.

The specificity of the test was verified using pure cultures of rhizobia. The test detected various strains of *Rlv*, but did not detect strains of the closely related clover nodulating rhizobia, *Rhizobium leguminosarum* bv. *trifolii*.

Sensitivity of the DNA test was determined using moist peat cultures of *Rlv*, similar to commercial inoculants. Number of rhizobia was determined in eight 10-fold dilutions of peat cultures in sterile water, using the DNA test and by counting colony forming units (CFU) on agar. The DNA and CFU results were highly correlated ($R^2 = 0.97$, $n=48$) and showed high precision above 1000 CFU/g.

The DNA assay detected *Rlv* in 19 of 42 soils collected from grower paddocks. In comparison, *Rlv* was detected in 28 of the soils using a plant nodulation bioassay. However, there was no instance where *Rlv* was detected by the DNA test, but not by the plant nodulation test. The results indicate that the DNA assay is specific and unlikely to detect organisms other than *Rlv*, but is less sensitive than the plant nodulation test, which is able to detect 10 rhizobia/g soil.

When used to assess a field trial examining the colonisation of an acidic soil by strains of *Rlv*, the DNA test clearly showed that colonisation by the rhizobia was limited and that the extent of colonisation varied according to the rhizobia strain. In general, the DNA results were consistent with nodule numbers measured in parallel plant bioassays, but improved the levels of significance and provided results within days of sampling.

Further validation and calibration of the test to Most Probable Number counts of rhizobia will be completed using field soils representative of major soil types, to ensure its reliability in a field setting. Confidence is needed to ensure that where the DNA test result indicates a large rhizobia population, there is no possibility of nodulation failure occurring if growers decide not to inoculate.

A test for chickpea rhizobia is currently under development. If successful, similar tests could be developed for other crop and pasture legumes.

Powdery mildew species infecting mungbean in Australian paddocks

Lisa Kelly^{1,2}

Niloofer Vaghefi² and Levente Kiss²

¹ Department of Agriculture and Fisheries

² University of Southern Queensland

Powdery mildew affects mungbean (*Vigna radiata*) in all areas of production across Australia. Field trials in Queensland have demonstrated that early disease infection can reduce yields by up to 40% in conducive seasons. Despite spending significant time and resources attempting to breed cultivars with improved resistance, the Australian National Mungbean Improvement Program has only released cultivars that are rated as moderately susceptible to the powdery mildew pathogen(s). Consequently, disease management relies solely on multiple fungicide applications. In Australia, mungbean powdery mildew is thought to be caused by *Podosphaera xanthii*. This species used to be known as *Sphaerotheca fuliginea* or *S. fusca* in the earlier literature. Research outside of Australia, however, refers to the pathogen of mungbean as *Erysiphe polygoni*. This project was designed to validate the identification and taxonomy of the species causing powdery mildew in Australia, and improve our understanding of the pathogens host range, virulence and potential resistance to DMI fungicides. Results thus far have indicated that *P. xanthii* and a second species, preliminary identified as *Erysiphe* sp., are responsible for causing powdery mildew of mungbean in Australia. Future research will focus on accurately identifying the *Erysiphe* sp., and determining the host range, virulence, fungicide resistance and yield losses caused by both pathogens. The outcomes of this project will significantly improve our understanding of the pathogens infecting mungbean, their life cycles, and provide the necessary foundations for the development of integrated disease management strategies.

Outcrossing in Australian faba bean is less than expected

Kedar Adhikari¹

Lucy Burrows¹ and Abdus Sadeque¹

¹ The University of Sydney, Faculty of Science, Plant Breeding Institute, Narrabri, NSW

Faba bean is a partially outcrossing species. An isolation distance is needed to maintain genetic purity when more than one variety is grown in the field conditions. This information is crucial for seed growers and faba bean breeders. A study was conducted at the University of Sydney's Plant Breeding Institute, Narrabri over two years to examine the extent of natural outcrossing using the pure white flower characteristic as a morphological marker, which is controlled by a homozygous single recessive gene. The white flower genotype (IX225c) was grown in a paired row of 150 m long in four directions from a central 480 m² plot of normal flower genotype PBA Warda. Two bee-hives were placed in the central plot at flowering time and natural pollination was allowed. At maturity, seed samples were taken from the white flower genotype at designated intervals and 100 seeds from each sample were grown in the glasshouse/birdcage to the 4-5 leaf stage and the proportion of plants displaying the stipule spot pigmentation was used to determine the percentage of outcrossing (white flower and colourless stipule are linked in this genotype).

Maximum outcrossing of 2.5% occurred where both genotypes were grown side by side (0 m) and the degree of outcrossing decreased as the distance from the central plot increased. At 6 m distance the outcrossing was less than 1%, however it increased randomly reaching about 1% even at 130 m distance. This is probably an indication of random nature of bee flights. Directions, years and their interaction had no effect and the distance alone was the contributing factor indicating wind had no effect on the faba bean pollination. Because there was only about 0.3% outcrossing at the maximum distance of 150 m, it can be concluded that the safest isolation distance for maintaining genetic purity should not be more than 200 m. Maximum outcrossing of 2.5% suggested that honey bees do not take long flights while foraging among faba bean plants and/or Australian faba bean genotypes are almost self-fertile, and a narrow isolation distance would be enough while handling many genotypes in faba bean breeding programs.

Chickpea and lentil emergence and establishment is resilient to changes in sowing depth and timing.

Sarah Rich¹

Nathan Craig² and Roger Lawes¹

¹ CSIRO

² West Midlands Group

Impact of sowing timing and depth on chickpea and lentil emergence and establishment are being measured in two WA sites; Dandaragan, a medium rainfall sandy site and Merredin a low rainfall site on sandy loam.

Lentil and chickpea are somewhat unique amongst our common cropping plants in that they have hypogeal germination, where their cotyledons stay below ground and the seed sends up a thin epicotyl. This allows them to successfully germinate and emerge from extreme depths. Sowing depth, esp. depths greater than 10 cm have received very little attention in the literature, however farmers in Queensland are successfully experimenting with this. Under dryland conditions, especially those experiencing declining autumn rainfall, deep sowing could offer benefits in chasing deep soil moisture. Deep sowing could also be a key tool in farmer uptake of these crops as farmers can potentially sow high value legumes prior to their current sowing programs.

This season, both mid-April and mid-May sowing of lentil (PBA Bolt) and chickpea (PBA Striker) was undertaken at standard practice depth (~ 5 cm) and a very deep sowing treatment of 20 cm at both WA sites. Deep sown treatments had a 2-3 day delay in emergence time for chickpeas and up to a week in lentil. The deep and shallow treatments showed no differences in emergence number, aside from May sown lentils in Merredin where deep sown plots showed lower emergence. The effect on establishment will be discussed also.

Pulses have previously been grown extensively in WA, however the cropping area of many of these, including lentil and chickpea, declined following disease outbreaks. New cultivars have addressed these issues. We hope to stack these genetic technologies with the latest sowing techniques to create a resilient, profitable legume management package for the WA farming system.

Light quality parameters affecting flowering and morphology responses differ among pulse species

Rick Bennett¹

Federico Ribalta¹, Maria Pasos-Navarro² and Janine Croser¹

¹ The University of Western Australia

² Centre for Plant Genetics and Breeding, School of Agriculture and Environment, The University of Western Australia

Rapid progression to flowering is a key component in the accelerated single seed descent (aSSD) platform developed at The University of Western Australia in partnership with The Grains Research and Development Corporation (GRDC). This rapid flowering response is achieved through manipulation of *in vivo* growth conditions, including photoperiod, light quality and temperature, and has enabled the substantial shortening of time to maturity in long-day pulse species. Our previous research demonstrated that the ratio of red to far-red (R:FR) light was a significant contributor to a rapid flowering response (Croser et al 2016), possibly via a mechanism related to the shade-avoidance response (Runkle and Heins 2001). However, flowering and morphology responses to other parts of the spectrum have not been well explored in the pulse legumes, and the blue (B) portion of the spectrum is also known to contribute to flowering time responses. We set out to explore the effect on pulses of these other aspects of light quality with the aim of further improving our light environments for rapid flowering time and plant health.

A single, late-flowering cultivar was selected of lentil (Northfield), lupin (Tanjil), chickpea (Rupali), faba bean (Icarus) and field pea (Kaspa) and grown in four environments with identical temperature (24/20°C), light intensity (340 mol m⁻² s⁻¹) and photoperiod (20 h), but differing in light quality. Light sources were Valoya LED growth lamps (AP67 or AP673 spectra, B-series, Helsinki, Finland) with some modifications using filters to alter the proportion of far-red light (Lee filters, Hampshire SP10 5AN, England). The environments had B:G:R:FR profiles of 11:19:63:7 (AP673), 11:16:56:17 (1st generation AP67), 13:14:58:15 (2nd gen. AP67) and 9:10:57:24 (filtered AP67). Light measurements were made with a Sekonic C7000 SpectroMaster spectrometer (Sekonic Corp., Tokyo, Japan). Days to flower, flowering node, and height of plants at flowering were measured and analysed for response to the proportion of B, R and FR light, and the ratios of B:R, B:FR and R:FR by multiple linear regression (MLR) and principal component analysis.

Our results clearly indicate that responses to light quality differed among the species. Chickpea, lentil, faba bean and field pea had small but significant time to flower responses between approx. 5 and 1.5 days, indicating that the light spectra in tested environments may be close to optimal for these varieties at 20h photoperiod and the tested intensity. Lupin displayed the largest difference in flowering time (fastest slowest >12 days) and was also the most responsive species to FR light. MLR revealed that B:R or B:FR ratios had the largest effect on flowering time in all species compared to R:FR ratio. Comparing individual portions of the spectra, FR light contributed most to flowering time in lentil, lupin and field pea. Unexpectedly, B light contributed highly to flowering time in chickpea, lupin and faba bean, likely because the environments had sufficient photon load in the FR region.

Recently acquired Heliospectra lights will allow accurate adjustment of spectral composition in our efforts to further optimise the flowering time of pulse legumes progressing through the aSSD platform.

Group B Herbicide Tolerance Discovery in Chickpea

Dylan Bruce¹

Dili Mao¹, Simon Michelmore¹, Jeffrey Paul², Chris Preston² and Tim Sutton¹

¹ SARDI

² The University of Adelaide

To make full use of in-crop rainfall, stored soil moisture and nutrients, and prevent weed seed contamination, the control of weeds during a chickpea rotation phase is essential. Currently, there are limited pre- and post-emergent control options for weeds in chickpea, particularly broadleaf weeds. The primary chemical control options for broadleaf weeds in chickpea are the active ingredient herbicides terbuthylazine (Terbyne), isoxaflutole (Balance or Palmero) and simazine. Although these herbicides provide effective control options for a wide range of broadleaf weeds, there are a number of common weeds that are not effectively controlled. When this issue is coupled with the poor competitive ability of chickpea, these common weeds may turn problematic, resulting in major management issues. Herbicides are the primary method of weed control in broad-acre cropping systems and limited control options for broadleaf weeds in chickpea and the presence of possible herbicide residues from previous crops are major reasons that producers do not include the crop in their rotations. Since no major new mode of action herbicides have been introduced into the marketplace for decades, the development of tolerance to existing herbicide chemistries has become an attractive method to overcome these issues and expand broadleaf weed control options. To advance this concept in chickpea, mutagenesis techniques were initiated to include tolerance to acetohydroxyacid synthase (AHAS) inhibitor herbicides (Group B), a highly effective and relevant broad spectrum herbicide group to key weed species in Australian chickpea production systems where there are currently limited in-crop options available. Mass field screenings of M₃ populations of desi and kabuli chickpeas occurred in 2015 to identify germplasm with improved Group B tolerance. Significant levels of improved tolerance were confirmed in one desi and seven kabuli lines to imazapyr and sulfometuron methyl (Oust), respectively. Dose response and cross tolerance studies conducted in 2017/2018 confirmed the desi selection had a high level of tolerance to imidazolinone and the kabuli selections had high levels of tolerance to sulfonyleurea herbicides. Sequencing of the AHAS gene in the chickpea genome has identified target site tolerance in all lines. Preliminary field studies conducted at Pinery, SA in 2018 compared the IMI tolerant desi selection D15PAHI002 with control cultivar PBA HatTrick for tolerance to the imidazolinone herbicide Intervix

(imazamox/imazapyr), and sulfonylurea herbicides Ally (metsulfuron) and Glean (chlorsulfuron). D15PAHI002 demonstrated a high level of tolerance to post emergent applications of Intervix (including four-fold the recommended field rate). Improved tolerance to residue levels of the sulfonylurea herbicides were also found in the tolerant selection. While these results indicate a favourable level of tolerance to imidazolinone herbicides in chickpea, further field evaluation studies will be required to determine the agronomic and commercial potential of this trait.

Chickpea phenology and grain yield response to sowing date in southern and central western NSW

Lancelot Maphosa¹

Tony Napier¹, Leigh Jenkins¹, Aaron Preston¹ and Mark Richards¹

¹ NSW DPI

Chickpea (*Cicer arietinum* L.) growth is highly dependent on environmental conditions such as temperature and soil water availability experienced during the growing season. Chickpea research conducted under the Grains Agronomy and Pathology Partnership (GAPP) aims to understand the phenological drivers of crop development across three contrasting environments of central western and southern NSW. Experiments conducted in 2018 at Yanco, Wagga Wagga and Leeton in southern NSW, and Trangie in central western NSW, aimed to increase the understanding of phenological drivers of chickpea productivity in these regions. Sites were selected due to their different environmental characteristics. Four sowing date treatments were applied (SD1SD4), occurring fortnightly from mid-April to the end of May. Phenology, biomass accumulation, yield and yield components were measured to understand the drivers of crop growth and development and genotype \times environment interactions. The 2018 growing season was one of the most difficult and extreme on record with a high incidence of frost and below average growing season rainfall across the experimental sites.

Initial findings indicate that time of sowing did not affect establishment. As growing degree days (GDD) affect time to emergence, emergence was delayed in later sowing dates as temperatures decreased in late autumn and photoperiod decreased. The critical 115 GDD threshold for chickpea emergence was reached in 6 days (SD1) to 12 days (SD4) in the cooler southern sites and between 610 days in the warmer central western site. The mean accumulated thermal time to flowering ranged from 1049 to 1117 GDD at Leeton and Yanco respectively for SD4, and from 1227 to 1544 GDD at Yanco and Wagga Wagga respectively for SD1.

At Yanco, limited pre-sowing moisture and low (87mm) incrop rainfall shortened the duration of the growth season, causing varieties such as PBA Striker and GenesisTM 079 to flower and pod significantly earlier than normal. This resulted in low biomass accumulation and a mean yield of 1.23 t/ha averaged across sowing dates and varieties. As a comparison the Leeton site, 7km west of Yanco, had optimal water availability at sowing, widening the

sowing window, and showed significant interaction between sowing date and variety on grain yield.

Overall yield was influenced by a number of yield components such as the number of pods, filled and unfilled pods, seed number and seed weight. Grain yield was driven to a large extent by temperatures during flowering and podding, with lower temperatures resulting in flower and pod drop.

Across ecological zones, differing optimum sowing dates were observed for different varieties, with the second and third sowing dates (end of April to mid-May) producing higher yields at Yanco and Wagga Wagga, while there was no effect of sowing date at Leeton and Trangie. Late planting exposed seedlings to low temperatures which affected biomass accumulation, flower viability, pod drop and overall yield.

This research will improve chickpea profitability through better understanding the adaptation to different environments and agronomic management in the southern and central western NSW regions.

Lentil phenology and grain yield response to sowing date in southern and central western NSW

Lancelot Maphosa¹

Tony Napier¹, Leigh Jenkins¹, Aaron Preston¹ and Mark Richards¹

¹ NSW DPI

Lentil (*Lens culinaris* Medik.) growth is affected by genotype, management and environmental conditions such as temperature and soil water availability during the growing season. Lentil research conducted under the Grains Agronomy and Pathology Partnership (GAPP) aims to understand the phenological drivers of crop development across three contrasting environments of southern and central western NSW.

Experiments were conducted in 2018 at Yanco, Wagga Wagga and Leeton in southern NSW, and Trangie in central western NSW. A total of eight diverse varieties were sown on each of four sowing dates, occurring fortnightly from mid-April to the end of May. Phenology, biomass, yield and yield components were evaluated to understand the drivers of crop growth and development. The 2018 growing season was one of the most difficult and extreme on record with a high incidence of frost and below average growing season rainfall across the experimental sites.

At all sites, as sowing was delayed, lentil took longer to emerge, had accelerated development and flowered and podded quicker. Sowing time had no effect on establishment. Yield was largely driven by temperatures during flowering and podding, with lower temperatures resulting in flower and pod drop. In the southern sites, initial findings indicate that late April and mid-May are optimal sowing times. However, in the warmer central western site at Trangie, optimum yields were obtained in the mid-April and late April sowing dates. Results from the Leeton trial site demonstrated that when optimal water levels are available during sowing, the optimum sowing window can be lengthened to include late May sowing. This however would not be recommended as harvest efficiency may be decreased due to lower plant and pod heights.

High biomass levels in the early April sowings at the Leeton experiment combined with the dry spring conditions resulted in exposure to higher levels of moisture stress during pod fill. Limited pre-irrigation coupled with only 87 mm of in-crop rainfall affected overall plant biomass accumulation, and resulted in low mean yield as observed at the Yanco experiment. Severe frosts and moisture stress interacting with sowing date, variety and phasic

development had the most significant impact on grain yield. There were significantly higher numbers of unfilled pods in the mid-April sowing due to an interaction between flowering time and frost damage. This research will improve lentil profitability through better understanding the adaptation to different environments and agronomic management for lentil in the southern and central western NSW regions.

Chickpea phenology and grain yield response to surface residue in southern NSW

Mark Richards¹

Maheswaran Rohan¹, Aaron Preston¹ and Lancelot Maphosa¹

¹ NSW DPI

Chickpea (*Cicer arietinum* L.) growth is susceptible to low temperatures especially during the reproductive phase and require an average daily air temperature above 15C for viable pod development and retention. Stubble or surface residue insulates and affects the crop environmental factors such as moisture availability, canopy air and soil temperature. Air temperature is reduced above stubble due to a reduction in heat absorption in the soil, potentially leading to lower soil surface and canopy temperatures. This experiment aimed to determine the effect of surface residue (stubble load) on chickpea phenology and grain yield.

The experiment was conducted in 2018 at Wagga Wagga, NSW under dryland conditions using CICA1521 (breeding line), PBA Slasher and PBA HatTrick (varieties), under the Grains Agronomy and Pathology Partnership (GAPP). Surface residue treatments (0, 3, 6, 9 and 12 t/ha) were applied to a uniform site immediately post sowing to ensure there was no treatment effect on stored soil water at sowing. Stubble treatments simulated a flattened surface residue, not standing cereal stubble. The 2018 growing season was one of the most difficult and extreme on record with a high incidence of frost and below average growing season rainfall of 152.6 mm (April - October), significantly lower than the long term average of 276 mm.

High surface residues decreased plant establishment, lengthened the duration of growth phases and overall time to maturity. Grain yield did not increase as stubble load increased and no interaction between variety and stubble load treatments was observed. The higher residues treatments did produce greater pod number (filled and unfilled) and seed number, but also produced lower grain weight.

For the 12 t/ha surface residue treatment, 84, 11 and 5 frost days were recorded during vegetative, flowering and podding phases respectively. In contrast the 0 t/ha surface residue treatment recorded 60, 9 and 0 frost days during the vegetative, flowering and podding phases respectively. The low temperatures and severe frost damage resulted in decreased biomass accumulation, lower grain weight and grain yield observed at 12 t/h surface residue treatment. The overall growth duration was shorter for 0 t/ha (172 days) compared to 12 t/ha (189 days) treatment. The longer growth duration, including delayed flowering in the 12 t/ha

surface residue treatment was likely due to significant frost damage observed in this treatment. The consistently lower air temperatures with increasing surface residue delayed floral initiation and overall plant growth. In response to the severe main stem necrosis, new branches were produced below the necrotic tissue. The delayed development of these compensatory branches added significantly to the overall growth duration of the 12 t/ha treatment. Podding in the 12 t/ha treatment on the later developing compensatory branches occurred from late September to late October a time when the mean temperature was above 15C but approaching the heat and moisture stress period. As a result there was no significant response in grain yield.

This research will improve chickpea profitability through better understanding plant growth responses to variable levels of surface residues in southern NSW.

Conservative and profligate water use patterns found in *Cicer* and *Lupin* species

Pushpavalli Raju¹

¹ CSIRO

Conservative and profligate water use patterns found in *Cicer* and *Lupin* species

R. Pushpavalli^{1,*}, J. Berger¹, A. Fletcher¹, C. Ludwig¹, K. Whisson¹, S. Parsons¹

¹The Commonwealth Scientific and Industrial Research Organisation, Floreat-6014

Terminal drought constrains yield in chickpea and lupins. Both crops have limited genetic variation in the domesticated gene pool, while wild progenitors are highly diverse. Does this greater genetic diversity translate into wider adaptive potential? Water use strategies are closely related to drought tolerance. Previous studies showed different water use strategies under varying VPD conditions found to be linked to drought tolerance.

In this study, whole plant water use response was assessed gravimetrically in wild and domesticated *Cicer* and *Lupin* lines against changing vapor pressure deficit (VPD) conditions on a clear day in an uncooled glass house. *Cicer* species have a more profligate water use than *Lupin* species ($P < 0.001$). Domesticated chickpea has narrow, conservative water use patterns ($P = 0.148$), while wild species tend to be more profligate ($P = 0.001$). However, the range of VPD responses was much wider in wild compared to domesticated chickpea, and there were no differences between *C. echinospermum* and *C. reticulatum*. Wild counterparts had a wide range of response ranging from very conservative to highly profligate. This wide genetic variation in either species can be exploited by breeders to develop drought tolerant lines targeted at different regions. We anticipate that a conservative water use pattern may be advantageous in heavy soil, while profligate water use may be useful in light soils.

In Lupins, *L. angustifolius* has a more conservative water use than *L. luteus* ($P = 0.04$). Unlike *Cicer*, in both *Lupin* species there were no wild/domesticated differences in mean VPD response (*L. angustifolius* ($P = 0.392$) and *L. luteus* ($P = 0.365$)). In *L. angustifolius*, both wild and domesticated lines showed similar range of VPD responses, but in *L. luteus*, all domesticated lines were conservative, while wild lines had a wider range of VPD responses ranging from conservative to profligate.

Chickpea was domesticated for more than 10,000 years and adapted to grow in stored soil moisture. When limited amount of water available for whole plant growth season, it is advantageous for the crop to be conservative. Wild relatives of chickpea, on the other hand,

grow as winter annuals in in-season rainfall. In such scenario, before water is lost to competitors or environment, its advantageous to use water rapidly. Wild relatives are grown in different habitats as individuals/close to other species/population, and thus might have developed diverse adaptive mechanisms to use water resources. Lupin was domesticated only 100 years ago. Both wild and domesticated Lupins are grown in in-season rainfall. As a recently domesticated crop and grown in similar environments as its wild counterparts, Lupin had very less time to diverge for adaptive traits.

Pushing Pulse Yield Boundaries

Time of sowing impact on pulse crop grain yields.

Penny Roberts¹

¹ SARDI

The time of planting in crop production is known to be a key factor for many species to achieve the highest grain yield potential. The optimum time of sowing in cropping zones is well known for some species and varieties, with extensive research work undertaken to better match variety phenology with sowing time for wheat and canola. However, there is limited information regarding the effect of time of sowing on the grain yield of pulse crops in the key pulse production regions of South Australia.

The purpose of this study was to analyse the effect of time of sowing in the major pulse production environments: Yorke Peninsula, Mid-North, Eyre Peninsula and South-East. Research trials were undertaken from 2015-2018 focusing on lentil, chickpea and faba bean. For lentil and chickpea the first time of sowing was driven by the break in the season, generally early May. The second time of sowing was delayed by 3-4 weeks (late May-early June). Faba bean was generally dry sown in mid-April with two subsequent delays in sowing, 3-4 weeks apart.

The results from this work showed that there was large variation in the response to time of sowing for the different pulse species. For lentil, the response to time of sowing was complex, with location and environmental limiting factors having an impact on the yield response. The newer commercial varieties generally showed better adaptation to early time of sowing than the traditional varieties such as Nugget. There were differences in the time of sowing response in faba bean depending on the environment. In the medium rainfall environments there was a consistent positive response to sowing early, with yield reductions from delayed sowing. In contrast, in the high rainfall zone sites, time of sowing generally had no effect on grain yield for most varieties. There was a consistent trend for increased grain yield in chickpea, between 0 and 23%, with later sowing dates. While the trend was for higher yield from later sowing, there was variation in the variety response to early time of sowing. For example, PBA Slasher responded better to early sowing than other varieties.

While the results demonstrated clear trends for each species, an understanding of the differences in agronomic characteristics and how they interact with the environment and

potential constraints is important when making variety selection. Variety selection and an understanding the effect of time of sowing on pulse species and varieties allows growers to optimise their seeding program to achieve the highest yield potential for the crops grown.

Novel Targeted Genotyping by Sequencing (tGBS): A promising tool for lentil genetics research and breeding

Ruwani Dissanayake^{1,2}

Luke Pembleton³, Noel O.I. Cogan^{2,4}, Kevin Smith^{1,5} and Sukhjiwan Kaur⁶

¹ Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria 3052, Australia

² Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Department of Jobs, Precincts and Regions, 5 Ring Road, Bundoora, Victoria 3083, Australia

³ Department of Jobs, Precincts and Regions (DJPR), AgriBio, Centre for AgriBioscience, Bundoora, Victoria, Australia 3083

⁴ School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3086, Australia

⁵ Agriculture Victoria Research, Department of Jobs, Precincts and Regions, PB 105, Hamilton, Victoria, 3300, Australia

⁶ AgriBio, Centre for AgriBioscience, Agriculture Victoria, DEDJTR, 5 Ring Road, La Trobe University, Bundoora, VIC, Australia, 3083

Crop improvement is highly dependent on genetic variation being present and identified from within a breeding pool, often through the use of DNA markers. Currently, SNPs are the dominant form of molecular markers used for genetic and genomic analysis due to their abundance in genome, and relative ease of identification in a cost-effective manner. Although whole genome sequencing facilitates the identification of all SNPs within a genome, it is an expensive strategy when applied to plant species with large and complex genomes, including pulse crops. Therefore, development of low depth and cost-effective novel Genotyping by Sequencing (GBS) methods is required. For pulse crops, various complexity reduction-based GBS approaches have already been developed and applied. However narrow genetic diversity present within pulse cultivars, limits the number of SNPs detected, and the efficiency of the approach. Therefore to overcome this constraint, targeted GBS (tGBS) methods are needed that build from the genome sequences and known variant positions.

The present study has focused on the development and optimization of a GBS method applicable to lentil, based on target capture where 65,630 custom probes were designed to 47,367 targets that are distributed uniformly across the reference genome of lentil CDC Redberry (version 1.2). The experiment was performed using the NuGEN Allegro tGBS protocol. The approach was further optimized to enable miniaturization of the reaction

volume to reduce the costs further. The protocol was evaluated on 24 samples processed as a full reaction, and then 72 genotypes processed as half and quarter reaction, to a total of 168 samples. The sequence data for each reaction volume has revealed that there were no-significant differences in the number of targets detected (c. 47,000) or missing data percentages (6.82-7.83%), under data filtration on depth ($dp \leq 5$). Overall, the majority of probes successfully bound and were sequenced in all scales of the reaction. Further evaluation of data on optimal balance between number of mapping reads and missing data percentages has revealed that, c. 25% of targets were undetected ($dp \leq 5$), with one million of mapping reads. However, cost-effective bioinformatics tools can be applied to impute missing data rather than increasing the sequencing outputs. The developed tGBS method will facilitate a wide range of opportunities in different fields including, genome-wide association (GWAS) and genomic selection (GS). The optimised tGBS method will be exploited for c. 280 advanced lentil breeding lines targeting the genomic regulation of salt tolerance.

Foliar Fungicides Reduce, But Don't Eliminate, Stubble Borne Inoculum Of *Ascochyta* Blight In Lentils

Sara Blake¹

Marzena Kryszynska-Kaczmarek², Michelle Russ¹, Christine Walela³, Jade Rose³, Elizabeth Farquharson⁴, Larn McMurray^{5,6} and Jenny Davidson²

¹ South Australian Research & Development Institute (SARDI)

² PIRSA-SARDI

³ formerly of South Australian Research & Development Institute (SARDI)

⁴ South Australian Research and Development Institute - SARDI

⁵ South Australian Research and Development Institute

⁶ The University of Adelaide

Ascochyta blight (AB) (causal agent *Ascochyta lentis*) is a major foliar fungal disease of lentil that can be managed with foliar fungicides. To investigate the impact of foliar fungicides on the subsequent stubble borne inoculum load, a spore release experiment was conducted in 2017 and 2018. Lentil stubble infested with AB was sourced from field trials conducted in 2016 and 2017 at Maitland SA, which included two lentil cultivars with different AB resistance ratings, PBA Hurricane XT (formerly moderately resistant) and PBA Flash (moderately susceptible). Field trials were inoculated with AB infested lentil stubble and had two treatments *viz.* fortnightly sprays with foliar fungicide during the growing season or untreated. After harvest in December, stubble from individual plots was placed into nylon mesh bags (140 x 70 cms, 1 kg stubble per bag) and incubated in ambient conditions on the Waite Research Precinct campus to allow maturation of spores of *A. lentis*. Five months later seeds of nine lentil cultivars and elite lines were sown into pots (10 cm diameter) placed around bags of stubble from either treated or untreated PBA Hurricane XT or PBA Flash plots. Each pot contained five uniform plants and each cultivar was sown into five separate pots, totalling 45 pots around each bag of stubble. All bags of stubble with associated pots were separated from one another by at least 50 m to prevent cross-infection. Conidia and ascospores that developed on the infested stubbles were spread by rain and wind to the potted plants. Lesions of AB on the plants were counted and collected each week for 12-15 weeks. Data were square root transformed before analysis to normalise residuals. The Mann-Whitney U test found no significant difference between the data from the two years and split plot analysis was performed, with stubble type as main plots, cultivars as subplots and years as replicates. The interaction between stubble type and cultivar was

significant ($P < 0.001$). Highest lesion numbers developed on susceptible and moderately susceptible cultivars next to untreated stubble of PBA Flash. Lesion number was significantly reduced for cultivars next to the treated stubble of PBA Flash and generally similar to those from the untreated stubble of PBA Hurricane XT. Fewest lesions developed on all lines, irrespective of susceptibility, next to stubble of treated PBA Hurricane XT. The number of lesions was low on the moderately resistant cultivars PBA Hurricane XT and PBA Hallmark XT (tested as CIPAL1422). A small number of lesions also developed on the resistant lentil lines, Indianhead and ILL7537. This indicates there are isolates present in the pathogen population capable of overcoming the major sources of resistance in the lentil breeding program, albeit at a low frequency. In the presence of treated stubble, a significant number of lesions developed on a range of lentil hosts indicating that stubble from fungicide treated crops is still a major source of inoculum for future crops. Ongoing monitoring of the *A. lentis* pathogen population remains an important plant health surveillance activity each season.

Foliar fungicides in pulses during dry seasons

Jenny Davidson¹

Christine Walela², Penny Roberts³ and Larn McMurray^{4,5}

¹ PIRSA-SARDI

² formerly SARDI

³ SARDI

⁴ South Australian Research and Development Institute

⁵ The University of Adelaide

Foliar fungicide recommendations for pulse crops have been designed for the high disease pressure situations of intensive pulse cropping systems in medium to high rainfall regions and seasons. Low disease pressure occurred in field trials conducted in South Australia in 2017 and 2018 due to low in-season rainfall. Both years the trials were sown in traditionally medium to high rainfall areas with an additional field pea trial in a low rainfall zone in 2018. All trials included untreated plots and fortnightly sprays of chlorothalonil. Lentil trials had no additional treatments. Chickpea trials included a 4-spray strategy to compare efficacy of chlorothalonil with AviatorXPro. The 2017 field pea trial had two times of sowing, and compared mancozeb (2 sprays) with AviatorXPro (2 sprays) and AviatorXPro (2 sprays) plus mancozeb, all with P-Pickel T seed dressing. The 2018 field pea trials included 2 sprays of AviatorXPro or Veritas with or without P-Pickel T. Fungicide treatments significantly reduced AB severity in susceptible cultivars of all three crop types, and reduced seed staining in the lentil cultivar PBA Flash. However, associated yield gains were variable. No yield gains occurred in lentil and the incidence of seed staining was below the minimum receival standards. In chickpea, yield gains were associated with fungicide treatments applied to susceptible cultivars, Howzat and Sonali in 2017, and in fortnightly sprayed susceptible cultivars Howzat, PBAMonarch and PBAStriker in 2018. In field pea (2017), AviatorXPro (2 sprays) increased grain yields 17% while AviatorXpro plus mancozeb produced a 25% yield increase, similar to fortnightly chlorothalonil (mean yield = 3.1 t/ha). However, in 2018, there were no associated yield gains since yield and ongoing disease development was restricted by conditions (site mean yields; Hart = 1.42t/ha; Minnipa = 1.15t/ha). In the later sowing, there was no yield response to fungicides and a yield penalty of approximately 1 t/ha from the delayed sowing was observed. Previous research (McMurray et al. 2011) has shown that economic yield responses to fungicides for AB occur in early sown field pea crops in high disease with a yield potential greater than 1.5t/ha. This was confirmed in 2018 trials where

disease severity was low and grain yields less than 1.5t/ha at both sites. These results indicate that in dry seasons foliar fungicides are unnecessary in lentil crops. Yield was particularly compromised by drought in 2018 and in these conditions foliar fungicides are not economic, even in the most susceptible lines. Conversely, not spraying for AB is high risk in chickpea due to low levels of genetic resistance in all cultivars, and could result in losing the entire crop. In these circumstances, growers should intensively monitor crops for AB and, if disease is present, a foliar fungicide spray prior to rain fronts will reduce the risks of disease spread and losses in yield and quality, even in low rainfall environments.

McMurray et al. 2011, *Euphytica*, 180, 69-88.

Optimising fungicide timing to control botrytis diseases in pulse crops by monitoring canopy microclimate with Narrow Band IoT data telemetry

Mohsen Khani¹

Adam Hancock², Andrew Baker³, Rohan Kimber¹ and Jenny Davidson⁴

¹ SARDI

² Elders

³ SciOT

⁴ PIRSA-SARDI

Chocolate spot disease of faba beans and grey mould of lentils, caused by *Botrytis fabae* and *B. cinerea*, can be difficult to control in the south east region of South Australia (SA) due to extended growing seasons that require several fungicide spray applications. The requirement for more than two fungicide sprays has a major effect on the economics of growing these crops. The colder temperatures in south east regions also confound our understanding of botrytis diseases since the latent periods can be increased and the onset of disease symptoms can be delayed compared to more northerly cropping areas. This potentially renders the current strategy of applying fungicides at early flowering ineffective and may be an unnecessary expense. However, it could be possible to optimise fungicide application by combining field observations (germination of fungi sclerotes, sporulation and disease symptom initiation) with in situ, near real-time monitoring of the local environment conditions (in-canopy temperature, relative humidity and near-surface soil moisture). Recent advances in Internet of Things (IoT) connectivity, referred to as Low Power Wide Area Networks (LPWANs), presents opportunities to affordably acquire near real-time environmental field data that can be used to help mitigate the risks associated with agricultural pests. These networks are based on wireless technology that connects devices and sensors deployed in the field. The LPWAN technology that will be utilised for this project is called Narrow Band IoT (NB-IoT) and is currently operated in Australia by Telstra and Vodafone (deployed within the last 6 to 12 months). These networks are ideally suited to agricultural applications and currently provide blanket coverage to all regions in SA, south of the Goyder Line. Compared to traditional mobile networks (3G/4G), NB-IoT operates at a lower cost, has greater power efficiency (devices with years of battery life), transfer small packets of data (e.g. temperature, humidity and soil moisture for this project), and support more devices over a greater area (10s km²). By using Narrow Band data transmission technology we are going to remotely monitor these data in faba bean and lentil canopies at

several sites in south east, mid north and Yorke Peninsula of SA. This will provide a better understanding of microclimate elements and their effects on botrytis disease initiation and progression during the season. It seems that using NB-IoT data telemetry to monitor microclimate within crop canopies incorporated with monitoring agricultural pests and fungal diseases in the field can potentially be applied to many other crops and diseases. This can lead us to more precise application of the management options available to minimise the risk of these diseases and pests in many crops and areas.

Response of lentil genotypes to post emergence application of photosynthesis and carotenoid biosynthesis-inhibiting herbicides

Tim Nigussie¹

Jason Brand²

¹ **Agriculture Victoria**

² **Agriculture Victoria, Horsham Victoria**

Broadleaf weeds are one of the constraints of lentil production in the southern cropping region due to limited in-crop herbicide options. Herbicide resistance due to overreliance on group B herbicides in herbicide resistant crop varieties has also become a concern. Inhibitors of photosynthesis (Group C) and carotenoid biosynthesis (Group F) control many broad leaf weeds. However, some of these herbicides have low safety margin between phytotoxicity to weeds and to the lentil. Field experiments were conducted to evaluate the tolerance of a new genotype (SP1333) with improved metribuzin tolerance to post emergence (PE) application of various rates of Group C herbicides and Group C & F mixes in comparison with a commercial cultivar (PBA Jumbo2). SP133 had no significant injury from PE application of up to 720 gai/ha of diuron, 900 gai/ha of simazine, 376 gai/ha of terbuthylazine, 210 gai/ha of metribuzin and 400 gai/ha of terbutryn, while all the herbicide treatments except diuron and simazine caused significant injury to PBA Jumbo2. In both genotypes, adding 60 gai/ha of diflufenican to the low rates of atrazine, bromoxynil, cyanazine, diuron, metribuzin, terbuthylazine and terbutryn significantly increased the level of herbicide injury. Unlike PBA Jumbo2, SP1333 recovered well from significant injury caused by some of the herbicide treatments earlier in the season and produced dry matter equivalent to the nil treatment. The herbicide tolerance trait in SP133 will offer opportunity for breeding programs to develop group C herbicide tolerant varieties.

Bacterial resistance will further boost the productivity and reliability of mungbeans

Col Douglas

Valeria Paccapelo¹, Greg Platz¹, William Martin¹, Katie McIvor¹ and Renier Snyman¹

¹ DAF

Mungbean (*Vigna radiata* L. Wilczek var. *radiata*) is a short duration tropical pulse crop introduced to Australia in the late 1960s and grown predominantly for export but also for low-volume niche domestic markets such as sprouting. Mungbean is a valuable disease break and weed control options for our cereal based farming systems and highly competitive with cotton and coarse grains on both a gross margin and water efficiency basis.

The development of best management practice along with advances in grain yield, harvestability, and grain quality in later varieties such as Crystal and Jade-AU have positioned mungbean growers to take advantage of strong international demand for pulses and established mungbean as the main summer rotation for northern Australia.

The seed-borne bacterial diseases halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) and tan spot (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*) are significant production risks for mungbean and have been a key focus of research since inception of the National Mungbean Improvement Program in 2003. Bacterial resistance in current varieties Celera II-AU (small-seeded mungbean) and Onyx-AU (black gram) will soon be supported with the deployment of resistance in large-seeded varieties which are Australias key market.

Development of genomic resources for an important Australian grain legume, narrow-leafed lupin

Lars Kamphuis¹

Gagan Garg¹, Rhonda Foley¹ and Karam Singh¹

¹ CSIRO Agriculture and Food

Narrow-leafed lupin (NLL; *Lupinus angustifolius* L.) is the main grain legume grown in Western Australia and forms an important part of sustainable farming systems, reducing the need for nitrogenous fertilizer, providing valuable disease breaks and boosting cereal yields. Over the last years we have developed a number of genetic and genomic resources for NLL. These include a draft genome sequence [1], development of various transcriptome libraries [2,3] and a dense reference genetic map [1,2]. The use of these resources has led to the identification of candidate genes for key domestication traits. In addition, candidate genes involved in alkaloid biosynthesis and regulation have been identified [4]. A new four-year project has recently started to expand these resources, including the generation of a pan-genome and a TILLING resource for the species. These resources will significantly improve and accelerate NLL breeding programmes and an overview of the developed resources to date will be presented.

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Screening multiple pulse crops for resistance to root lesion nematodes in the field

Joshua Fanning¹

Katherine Linsell², Isabel Munoz-Santa^{3,4}, Jon Baker¹, Alan McKay² and Grant Hollaway¹

¹ **Agriculture Victoria**

² **SARDI**

³ **The University of Adelaide**

⁴ **University of Valencia**

The root lesion nematodes, *Pratylenchus thornei* and *P. neglectus*, cause grain yield losses internationally. Root lesion nematodes are controlled by planting resistant crops or varieties, which reduce nematode densities and therefore, the subsequent yield losses. In Australia, varieties of some pulse crops can vary in their resistance to specific species of root lesion nematodes. Therefore, it is important to screen and assign resistance classifications to new varieties, so growers can make informed decisions on variety selection to help manage root lesion nematodes. To compare the resistance to specific root lesion nematodes across pulses and cereals, a series of experiments were established with multiple crop species and varieties. Three field experiments for each of *P. thornei* and *P. neglectus* were sown in Victoria. The *P. thornei* experiments had a minimum of four current varieties of field pea, lentil and faba bean, along with several breeding lines from each crop type. For the *P. neglectus* experiments at least four chickpea varieties were also included. Each experiment for both *P. thornei* and *P. neglectus* also included a fallow treatment and resistant and susceptible cereal varieties, as controls to assist with standardising the ranking of varieties for resistance to root lesion nematodes across crops. Pre-sowing and post-harvest nematode densities were quantified using qPCR (PREDICTAB). The post-harvest nematode population densities were analysed using linear mixed models, incorporating spatial methods and residual maximum likelihood for variance parameter estimation. Nematode multiplication rates were derived from this analysis.

For *P. thornei* generalised crop effects were evident, even though there was variation within each crop type. Faba bean was the crop most susceptible to *P. thornei*, with four faba bean lines more susceptible than the susceptible wheat control, and all lines were more susceptible than the moderately resistant to moderately susceptible (MRMS) cereal control. Lentil and field pea varieties ranked more resistant than the MRMS cereal. The most resistant variety

tested was the field pea var. PBA Percy, with several other field pea lines being more resistant than the fallow treatment.

For *P. neglectus*, all pulse crops were more resistant than the susceptible wheat varieties, and field pea was the most resistant crop. Lentil varieties were less resistant than the field pea varieties, but more resistant than the fallow treatment. Faba bean varieties tended to be more susceptible than the fallow treatment, and chickpea varieties were the most susceptible to *P. neglectus* of the pulses, with most varieties being slightly more susceptible than the moderately resistant (MR) wheat.

Excluding faba beans for *P. thornei*, pulses are an ideal option for managing high population densities of root lesion nematodes in south-eastern Australian paddocks. The methods used in this study with multiple crops in the same trial provide a viable option for testing the resistance to root lesion nematodes and ranking varieties across crop types.

Identification of Quantitative Trait Loci (QTLs) for seedling resistance to ascochyta blight in the ILL6002 x ILL7537 lentil mapping population

Robert Lee¹

Ido Bar², Mahsa Khorramdelazad³, Bernadette Henares⁴, Christina Grime⁴, Lina Farfan-Caceres⁴ and Rebecca Ford²

¹ Centre for Crop and Disease Management - Curtin University

² Environmental Futures Research Institute, Griffith University, QLD, Australia, 4111

³ Environmental Futures Research Institute, School of Environment and Sciences, Griffith University, QLD, Australia

⁴ Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, WA, Australia

The lentil cultivar ILL7537 is widely recognised as highly resistant to infection by *Ascochyta lentis*¹. A recombinant inbred line (RIL) population of 155 individuals was generated between the resistant ILL7537 and susceptible ILL6002 lentil accessions through *in vitro*-assisted single seed descent (aSSD)². A genetic linkage map was constructed derived from 3,071 high quality ddRADseq SNP markers producing a final map comprising 10 linkage groups (LGs) with an average marker density of 0.9 markers per centiMorgan (cM). This includes 7 major LGs with between 766 to 45 markers and 3 smaller LGs with fewer markers.

Up to 148 RILs were screened at Curtin University in seedling assays with the *A. lentis* isolates AL4, FT16299-2 and FT14069. In addition, RILs were screened for response to *A. lentis* AL4 at Griffith University. Two week-old seedlings were inoculated with spore suspensions and stem and leaf lesion % coverage were scored at 14 days after inoculation. QTL Mapping was performed using R/qtl2³. Co-located QTL peaks were identified for an 80 cM region on LG4 with significant LOD scores ($P < 0.005$) for AL4 (two independent phenotyping assessments) and FT16299-2. Isolate FT14069 QTLs were identified at a similar location but were not significant ($P < 0.005$). The isolates tested fall into two provisional pathotype groups, being either virulent on PBA Hurricane and avirulent on Nipper lentil (AL4 and FT16299-2), or the converse (FT14069). Our results suggest that the genetic resistance conferred by ILL7537 likely specifies a gene-for-gene interaction with different responses for different isolate types. FT14069 is PBA-Hurricane avirulent and Nipper-virulent, and displays a weak interaction with the QTL. Key gene differences at the QTL will likely

provide markers to assist plant breeders in improving resistance towards *A. lentis* isolates that are virulent on PBA Hurricane and similar cultivars such as PBA Bolt and PBA Hallmark.

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Genome sequencing and characterisation of diverse ascochyta blight species isolated from legumes

Robert Lee¹

Lina Farfan-Caceres², Johannes Debler², Christina Grime², Bernadette Henares² and Robert Syme²

¹ Centre for Crop and Disease Management - Curtin University

² Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, WA, Australia

Ascochyta blight disease in legume species is caused by a number of closely related ascomycete fungal pathogens from the class Dothideomycetes. *Ascochyta lentis* is a host specific pathogen of lentil and *A. fabae* specifically infects faba beans. *Ascochyta rabiei* (Syn: *Phoma rabiei*) is the sole ascochyta pathogen of chickpeas. In field peas ascochyta blight, or pea blackspot as the disease is commonly known, can be caused by a number of pathogens from several genera, including *Peyronellaea pinodes*, *Peyronellaea pinodella*, *Phoma koolunga* and *Ascochyta pisi*. *P. pinodes* and *P. koolunga* are the most serious and most commonly found organisms in Australian field pea blackspot epidemics. Other ascochyta species from legumes have been reported but there is a lack of taxonomic description and genomic sequence information available with which to identify fungi that may be present on legume crops and related species.

In this study we have isolated ascochyta blight fungi from field peas, from a *Vicia villosa* crop, and from *Vicia sativa* and *Lathyrus tingitanus* weeds along roadsides in Western Australia. Isolates have been characterised by growth on plates, and by spore size and morphology. We have assessed the capacity of ascochyta from lathyrus and vetch to cause disease symptoms on field peas and lentils and also in the host species from which they were isolated. One representative cultivar for each of the host species was used in seedling assays with the collected isolates. To classify the isolates we have carried out Illumina genome sequencing and have constructed a phylogenetic tree of these ascochyta blight species. Species include a novel *Phoma* species, *Ascochyta viciae-villosae*, *Ascochyta viciae*, *Phoma koolunga* and *P. pinodes* from field peas, and *Peyronellaea lethalis* from lathyrus. Genome assembly sizes ranged from 33 Mb to 48 Mb and can be accessed from the NCBI BioProject archive. Additional genomic sequences for *A. lentis*, *A. rabiei* and *A. fabae* were obtained from other CCDM projects. A Neighbour-Joining tree shows three clades of species. Clade 1: *Ascochyta* includes *A. lentis*, *A. fabae*, *A. viciae* and *A. viciae-villosae*. Clade 2: *Phoma*

includes *A. rabiei*, *P. koolunga* and an unknown *Phoma* sp. from vetch. Clade 3: *Peyronellaea* includes *P. pinodes*, *P. pinodella* and *P. lethalis*. Additional characterisation of the species described in the study was achieved by microscopic evaluation of diseased leaves from *in vitro* seedling infections. We observed two main types of pathogen-host interactions; a compatible reaction in which extensive mycelial colonisation of host tissues and brown, spreading necrosis were evident, and what appeared to be an incompatible reaction with red-brown discoloration of discrete host cells at the site of penetration attempts and restricted hyphal development.

Among the closely related *Ascochyta*, *Phoma* and *Peyronellaea* species there is a mix of host-specific and generalist fungi. The host response and susceptibility towards different pathogen species can be measured in traditional seedling assays but further details of the plant response can be revealed through microscopy. Genome sequences will assist future studies to identify virulence genes and effectors that underlie the molecular mechanisms that determine host specificity.

Agronomic approaches in grain legumes to management of stress induced by climate change.

David McNeil¹

¹ Department of Primary Industries and Regional Development, Western Australia

Increased global atmospheric CO₂ (eCO₂) has a wide range of environmental effects (IPCC, 2018). These effects express themselves in a mix that varies from region to region. The primary driver is eCO₂ creating a greenhouse effect. Secondary effects include; increased global temperatures, increased global rainfall, locally reduced rainfall, increased evaporative demand, increased climate and weather variability, increased water demand, changes in climate boundaries and cloud cover affecting frost frequencies. Tertiary changes will also arise from complex interactions of these environmental changes. Examples include altered pest and disease pressures and ranges, altered manure breakdown rates and complex interactions of diseases, pests, CO₂ levels and water and nutrient availability. Complex cropping adaptation strategies will be required involving government, social, varietal and agronomic changes. Agronomic adaptation (including breeding) for making legumes suited to the new conditions, will be a major means of meeting these future complex interacting environmental challenges.

Grain legumes generally have characteristics that set them apart as crops including; large seeds, high seed protein, nitrogen fixation increasing limitations on carbohydrate availability and symbiosis stresses, often they have had less breeding and are less improved than cereals, high nitrogen content which suits pests and diseases, often they are less determinate, have C₃ physiology and are often directly consumed by people stressing need for high seed quality. Agronomic management decisions thus need to be made within these constraints

A potential mechanism for screening future environmental challenges and future agronomic adaptation responses (FAARs) is to evaluate their effects in locations offering future climate conditions today. These data can then be fed into existing, or new, growth models to extend their range to include the test locations and future climates of existing regions. The Frank Wise Research Station (FWRS) in the Ord Irrigation Area offers mean field temperatures reproducibly ramping from a mean of >35⁰C to <20⁰C providing ability to test temperature effects and agronomic (genetic) response effects on field establishment, flowering and other specific growth periods. The environment allows full control of water availability, solar radiation and soil type (clay, silt, sand) to isolate effects and assess interactions. Trials are

presently underway across a range of species assessing germination responses of small seeds, flower fertility effects on chickpeas and mangoes plus whole of season effects on legume and non-legume crops.

This paper reviews the main stresses induced by future climates for legumes and adaptation methods that have been proposed and evaluated to date. It then considers some of the trials underway, or proposed, to evaluate various FAARs at the FWRS in Australia's tropical north. As an example seed (particularly small) germination can be adversely affected by elevated temperatures. Possible agronomic management methods presently being tested include; 1) genetics, 2) weather forecast based modification of; planting date, sowing rate, seedbed preparation, sowing depth and water management, 3) pre-sowing treatments. Using the FWRS climate these could be field tested for a range of legumes along a clear and reproducible temperature range with separated soil type, water availability and solar radiation interactions.

The Australian Grains Genebank - what is involved in managing the germplasm collection that underpins the development of new, more resilient pulse varieties for Australia.

Sal Norton¹

Shane King¹, Kath Whitehouse¹, Giao Nguyen¹, Erica Steadman¹, Nicole Sawyer¹, Virginia McQueen¹ and Tony Slater¹

¹ Agriculture Victoria

The Australian Grains Genebank (AGG) is the national genebank for grain crops for Australia that provides a wide diversity of crop species, their progenitors and wild relatives to the Australian plant research and breeding communities. The AGG is a partnership between Agriculture Victoria and the Grains Research and Development Corporation, with a mandate for the acquisition, conservation and distribution of grain crop genetic resources for all of Australias agroecological growing environments. Sounds straight forward and simple, but what is really involved? Firstly, the AGG imports new germplasm from all around the world on behalf of Australian research and breeding programs for priority traits/crop species. The AGG processes this material through Post Entry Quarantine, a process that can take 12-18 months before daughter seed is released to the genebank. AGG then curates data/information about each line in the database and conserves the seed for the long-term in -20°C freezer vaults. Even under long term storage conditions, seed will still lose its ability to grow, so the AGG routinely monitors seed viability and quantity in storage for each line. Seed regeneration and characterisation of prioritised lines is undertaken each year to produce pure seed of each line to add back into storage. The AGG applies high-throughput phenotyping technology using sensors and camera for evaluating germplasm for useful agronomic traits during the seed regeneration trials. Along with the basic passport data, these phenotypic data can be of primarily invaluable information for further research. This process ensures that seed for priority germplasm and its comprehensive data are available for Australian research and breeding programs to evaluate and develop into the new pulse varieties needed to meet the changing conditions expected into the future.

Assessment and Application of Chinese faba bean & broad bean germplasm to accelerate Australian faba bean breeding program

SHI YING YANG¹

Samuel Catt¹ and Jeffrey Paull¹

¹ The University of Adelaide

China is the world's largest producer of faba bean and broad bean, and Chinese germplasm is generally very distinct from germplasm from other regions. A significant amount of Chinese germplasm has been assembled in Australia, but has not been widely used in the breeding program other than as a valuable source of resistance to Bean Leafroll Virus (BLRV). Greater use of this germplasm would increase the overall genetic diversity within the Australian faba bean program and also introduce novel traits, such as Chinese quality characteristics. A total of 109 lines of Chinese faba and broad bean germplasm from six provinces, plus 8 Australian faba bean varieties and breeding lines, were assessed at the Waite Campus in 2018-2019 for a range of morphological, reproductive and seed traits. These traits include: emergence, number of branches, plant height, leaf area, days to flower, flower colour, days to maturity; pod traits maximum pods per node, shape, length, width, indehiscence, pods per plant; seed traits colour, seeds per pod, seeds per plant and 100 seed weight. Crosses were made between Australian elite lines and random lines from each province in 2018 to capture the overall genetic variation in the Chinese germplasm. More targeted crosses will be made in 2019 taking the data collected in 2018 into account for selection of the parents.

The first report on identification of faba bean host differentials for assessing variation on *Uromyces viciae-fabae* populations and pathotype nomenclature system in Australia
Kedar Adhikari¹

Usman Ijaz², Richard Trethowan³, Harbans Bariana³ and Urmil Bansal³

¹ **The University of Sydney, Faculty of Science, Plant Breeding Institute, Narrabri, NSW 2390**

² **Australian Grain Technologies 100 Byfield Street Northam WA 6401**

³ **The University of Sydney, Faculty of Science, Plant Breeding Institute, 107 Cobbitty Road Cobbitty, NSW, 2570, Australia**

Faba bean rust caused by *Uromyces viciae-fabae* (Pers.) J. Schrt.) is an important disease limiting faba bean production globally. The development of rust resistant cultivars provides economical and viable solution to overcome this disease. Different sources of resistance have been identified, but there is a lack of understanding on the pathotype variation. Faba bean rust is an obligate parasite exhibiting all five spore forms in its life cycle on a single host allowing sexual recombination and variation in the pathogen population. However, neither continuous surveillance for the pathogen variation has been conducted nor a host differential set to assess the variation has been developed. This study examined differences in the virulence of Australian *U. viciae-fabae* isolates by developing a differential set of faba bean genotypes collecting isolates from major faba bean growing states in Australia; South Australia (SA), New South Wales (NSW) and Queensland (Qld). A single spore culture was generated from each isolate and multiplied in isolation on susceptible cultivar Fiord, under greenhouse conditions. In the absence of a differential set, these pathogen isolates were used for assessing virulence on 40 diverse faba bean genotypes. Based on the host-pathogen interactions, 12 putative host genotypes were identified that displayed variation in the rust isolates. These genotypes have been proposed as a differential set for assessing pathotype variability in the future.

We found a variation in the pathogen, but there was no system of naming the pathogen isolates. For the first time, a nomenclature system was developed using the binary pathotype naming system similar to developed for common bean rust. Each isolate was given a two-digit code separated by a hyphen. The differential set genotypes were ranked (1 to 12) on the basis of resistance; genotypes showing resistance against most isolates were arranged first followed by the genotypes with resistance on fewer isolates. Each susceptible reaction of a differential genotype was attributed a binary value. In this arrangement, the genotypes were

separated in two groups (genotype 1 to 6 and 7 to 12) and the name of each pathotype was assigned by the concatenation of the corresponding binary values attributed to the susceptible reaction of the differential lines within each group. Nine virulence patterns on the differential set were detected and the isolates were named using the new nomenclature system.

SA pathotypes were the least virulent, NSW moderately virulent and Qld highly virulent. This fits well with the development of rust in these regions. The pathotype 63-63 from the southern Qld was highly virulent and overcame all host differentials posing a risk of rust outbreak in the region. This is the first report of the well characterised *U. viciae-fabae* pathotypes based on differential set in Australia. The differential set will allow to detect any new variation in pathotypes before it becomes epidemic in the region. This information will facilitate breeding for rust resistance in faba bean according to the prevalence of pathotypes in the target region and monitor the breakdown of resistant genes.

Exploring genetic variation to improve salinity tolerance in faba bean

Ahsan Asif¹

Jeffrey G Paull¹

¹ School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia

Faba bean (*Vicia faba* L.) is one of the most important high protein cool-season grain legumes; however, it is sensitive to salinity stress. Breeding for improved salinity tolerance of faba bean is needed to improve its productivity on saline soils. This study was designed to assess the level of genetic variation for salt tolerance among 22 faba bean genotypes, including 17 Australian varieties, and identification of new genetic resources with desirable combinations of salt tolerance sub-traits. Faba bean genotypes were grown in soil and treated with 0 mM (control) and 100 mM NaCl (salt treated) for 21 days. Salinity tolerance sub-traits related to plant growth (plant height, shoot and root dry biomass), ion accumulation (Na^+ , K^+ , Cl^- contents in leaf, shoot and root) and photosynthesis (leaf chlorophyll contents, stomatal conductance and leaf chlorosis) were measured in control and salt treated plants. A significant variation was noted amongst the varieties, with PBA Nasma, PBA Zahra, Doza and PBA Samira identified as tolerant varieties and Manafest, Nura and Fiesta as sensitive ones. The most tolerant varieties (PBA Nasma and PBA Zahra) possessed better ability to maintain higher leaf K^+/Na^+ , low leaf Cl^- accumulation, lower root K^+/Na^+ and less leaf chlorosis, resulting in higher total (shoot and root) dry biomass production. Leaf and shoot Na^+ and Cl^- contents, considered as major salt tolerance sub-traits, exhibited significant positive correlation with leaf chlorosis, but not with total dry biomass (shoot and root). However, the root Na^+ contents were positively correlated with total dry biomass and negatively correlated with leaf Na^+ level. The ability of plants to accumulate higher Na^+ contents in roots and restrict its transportation to leaves was associated with salt tolerance. These results also suggest that different salt tolerance sub-traits varied independently, and higher salt tolerance could be achieved through different combinations of salt tolerance sub-traits.

Trait-based approaches to support genomic selection strategies in the Australian lentil breeding program

Arun S.K Shunmugam¹

Hossein V Kahrood², Laura James³, German C Spangenberg^{2,4}, Sukhjiwan Kaur⁵ and Garry M Rosewarne³

¹ Agriculture Victoria Research

² Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Department of Jobs, Precincts and Regions, Bundoora, Victoria 3083, Australia.

³ Agriculture Victoria Research, Grains Innovation Park, Department of Jobs, Precincts and Regions, Horsham, Victoria 3400, Australia.

⁴ School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3086, Australia.

⁵ Agriculture Victoria, AgriBio

The Australian lentil breeding program focusses on delivering new lentil varieties that are better adapted for Australian environments. It had developed and released several high yielding lentil varieties that are disease resistant, herbicide tolerant and abiotic stress tolerant. The program has investigated the use of genomic selection (GS) methods which have the potential to significantly improve selection intensity and selection accuracy and dramatically reduce the length of the breeding cycle. Significant success has been achieved in developing GS prediction equations for yield and several biotic and abiotic stress tolerant traits with moderate to high accuracies. The next step is to derive genomic selection indices (GSI) to effectively select for multiple traits simultaneously. Analysis of selection indices versus predicted responses will be utilised to iteratively adjust trait weights to ensure that GSI matches specific breeding targets. Through correlation and regression analysis we identified traits that are significantly affecting yield in individual environments. Initial selection indices will be designed with knowledge of agronomic and economic importance of traits. This study will detail the progress made in defining economic values for these traits and the efforts to develop selection indices for independent traits to support GS based strategies in lentil.

Keywords: lentil breeding, economical traits, genomic selection, selection indices.

Fine mapping of a locus controlling vigour in chickpea

Duong Nguyen¹

Julie Hayes², Judith Atieno³, yongle Li², Ute Baumann², Angela Pattison⁴, Helen Bramley⁴, Kristy Hobson⁵, Tim Colmer⁶ and Tim Sutton¹

¹ SARDI

² University of Adelaide

³ PIRSA-SARDI

⁴ University of Sydney

⁵ Department of Primary Industries

⁶ University of Western Australia

Early vigour is considered a valuable trait for chickpea, and is identified as a key breeding target for Australia's central, southern and western chickpea growing regions. These regions are characterised as having very cold winters, short growing seasons, and low rainfall, meaning that a good early start to growth is imperative. Seedling vigour promotes strong establishment and overcomes early weed competition which is particularly relevant in pulses. Early vigour also results in early canopy closure, minimising evaporative loss, maximising water use efficiency and conserving soil moisture in the profile for later in the season. Recent studies have identified a locus on chromosome 4 that is known to control several vigour-related traits (plant height, water use, projected shoot area) and agronomic traits (100 seed weight, harvest index, seed number) under both glasshouse and Australian field conditions. A range of complimentary approaches are now being used to determine the molecular control of vigour at this locus. The genetic size of the region has been delimited using populations derived from residual heterozygosity found in recombinant inbred lines. Sequence capture technology is now under way to obtain a 2Mb sequence across the QTL locus from a collection of 100 Australian chickpea breeding lines. Analysis of sequence haplotypes observed at the locus with field and glasshouse phenotypes relating to vigour is being investigated to identify association of genetic changes and the vigour phenotype. This information will provide knowledge on the extent of genetic variation for genes controlling vigour at this locus in chickpea breeding program and could provide molecular tools to select vigour alleles targeted to specific environments.

Loop- mediated Isothermal Amplification (LAMP) - A new diagnostic tool for *Pseudomonas syringae* pathovars *syringae* and *pisi* that causes bacterial blight in field pea

Pragya Kant¹

Mario Fruzangohar², Rachel Mann¹, Brendan Rodoni¹, Grant Hollaway¹ and Garry Rosewarne³

¹ **Agriculture Victoria**

² **Biometry Hub, University of Adelaide, waite campus, Urrbrae, SA 5064**

³ **Agriculture Victoria, Grains Innovation Park, 110 Natimuk Road, Horsham, Vic 3400, Australia**

Bacterial blight (BB) is a major disease of field pea caused by the pathogen, *Pseudomonas syringae* pathovars *syringae* and *pisi*. Both pathovars cause similar symptoms and can be isolated from a single plant. Several races have been identified in the *pisi* pathovar, however there is no race specificity in the *syringae* pathovar. Due to frequent frost events, BB epidemics occurred in two consecutive years, 2017 and 2018. To understand the pathovar populations, survey samples were collected from Victoria and other states. The isolates were characterised using traditional polymerase chain reaction (PCR) to identify the pathovar specificity which was time consuming and laborious. Here we demonstrated the development of sequence based diagnostic markers for pathovar identification by utilizing whole genome sequencing of 16 *Pseudomonas syringae* pathovars including *syringae*, *pisi* and two outgroups. Through comparative analysis, a unique genomic region specific for the *pisi* pathovar was identified and used to develop a loop-mediated isothermal amplification (LAMP) assay. Our collaborators previously have developed a LAMP assay for pathovar *syringae* diagnostics. The LAMP assay has been validated on several *Pseudomonas* species and unrelated outgroup bacteria obtained from VPRI (Victorian herbarium). The results showed high accuracy towards *pisi* pathovars. Both LAMP assays were successfully utilised on the survey work in 2018 and historical isolates from the Horsham collection for high throughput diagnostics.

Development of a control strategy for Faba bean gall: A devastating faba bean disease in Ethiopia

Asnakech Beyene¹

Martin Barbetti², Beyene Bitew¹, Gemechu Keneni¹, Musa Jarso¹, Seid Kemal³ and Joop van Leur⁴

¹ Ethiopian Institute of Agricultural Research

² School of Agriculture and Environment and the UWA Institute of Agriculture

³ International Center for Agricultural Research in the Dry Areas

⁴ NSW DPI

Faba bean cultivation is an essential part of the Ethiopian highlands farming systems with over 450,000 ha sowed annually, making it the most important winter pulse in the country and Ethiopia the second largest (after China) faba bean producer in the world. Faba bean (and other pulses) form a vital source of protein for the rapidly growing Ethiopian population, particularly for low-income households, and also plays an important role for maintaining soil fertility. Rainfall in the Ethiopian highlands is variable, but seasons with high and frequent rainfall are common. The highly disease conducive environment in wet years result in frequent and severe epidemics of a range of faba bean diseases like chocolate spot, rust, *Ascochyta* blight and root rots.

A decade ago an unknown disease was noted in a number of faba bean fields in the northern highlands. The disease caused numerous galls on leaves and stems and could cause complete crop failure. Based on these symptoms the disease is called Faba bean gall (FBG) or Qormid in the local Amharic language. The disease is clearly favoured by high rainfall and can be transmitted by contaminated soil. It has spread rapidly through Ethiopia and is now already reported in all faba bean growing regions. The causal agent not yet established, but the presence of zoospores and sporangia in leaves point to a Chytrid species. The only reports of a similar disease on faba beans are from spring sown crops in the high altitudes of western China and is attributed to *Olpidium viciae*. However, while *Olpidium* species are well known vectors of virus diseases, they are not known to cause severe damage to foliage of host plants. A collaborative project, involving Ethiopian and Australian research institutes and supported by the Australian Centre for International Agricultural Research, commenced in 2019 to develop control strategies for this disease. Its first objective is to properly identify the causal agent. Fungal DNA will be isolated in Ethiopia and brought under permit into Australia for

in-depth analysis at the University of Western Australia. Chemical control methods will be evaluated as a stopgap option, but long lasting control is expected to come from incorporating resistance into locally adapted germplasm. In order to identify sources of resistance, testing methods will be developed to evaluate faba bean germplasm from diverse origins in field and greenhouse tests.

The project will be of great value to Ethiopian farming families, but also has substantial benefits for the Australian faba bean industry: Knowing the cause of FBG and its epidemiology will allow for the development of a sound containment strategy in case this disease ever enters the country. It also provides opportunities to test Australian faba bean breeding material in Ethiopia for disease resistance, not only for FBG but also for a range of diseases that are of importance in Australian fields.

Association mapping of root-lesion nematode *Pratylenchus thornei* resistance in *Cicer reticulatum*, the wild progenitor of chickpea.

Rebecca Zwart¹

Roslyn Reen¹ and John Thompson¹

¹ Centre for Crop Health, University of Southern Queensland

Root-lesion nematodes (*Pratylenchus thornei*) are migratory endoparasites that cause substantial damage to a wide range of cereal and pulse crops. Damage caused to roots during feeding and movement of the nematodes through the root cortex leads to poor uptake of water and nutrients by the plant, ultimately reducing plant growth and yield. In the sub-tropical northern grains region of Australia, where 90% of Australia's chickpea (*Cicer arietinum*) production occurs, yield losses of up to 25% have been reported for *P. thornei* intolerant chickpea cultivars. Moreover, high nematode populations build up in the soil to infect and cause yield loss to subsequent susceptible crops. Integration of resistant crops in farming systems is recognised as the most practical, effective and economical management strategy for plant-parasitic nematodes. However, plant breeding for resistance to *P. thornei* is hindered by the lack of genetic diversity in *C. arietinum*. The immediate wild progenitor of chickpea, *C. reticulatum*, offers a means to broaden genetic diversity and reintroduce traits to the *C. arietinum* gene pool. Using replicated glasshouse experiments, sources of resistance to *P. thornei* were recently identified in a collection of *C. reticulatum* accessions originating from 15 sites in SE Turkey, spanning the species natural geographic range (Reen *et al.* 2019). From this collection, 125 *C. reticulatum* accessions were used in a preliminary genome-wide association study (GWAS) to identify markers associated with *P. thornei* resistance. Single nucleotide polymorphisms (SNPs) generated using genotyping-by-sequencing on the wild *Cicer* collection were obtained from the NCBI database (von Wettberg *et al.* 2018). After stringent filtering, a dataset of 22,802 high quality SNPs across the 125 *C. reticulatum* accessions was used for association mapping. To minimize the occurrence of false positive marker-trait associations, two statistical models were tested (i) a general linear model (GLM) taking into account population structure, and (ii) a mixed linear model (MLM) taking into account population structure and genetic relatedness. This exploration into the potential of GWAS to identify markers associated with *P. thornei* resistance has revealed the first insights into the chromosomal regions determining *P. thornei* resistance in chickpea. The number of accessions in the association panel is currently being expanded to increase the statistical power of GWAS to identify significant marker-trait associations. The SNPs associated with

P. thornei resistance identified by GWAS are candidates for genomics-assisted breeding to introgress *P. thornei* resistance from wild *Cicer* into commercial chickpea cultivars.

Genomic selection in Chickpea using Whole Genome re-sequencing data

yongle Li¹

Kristy Hobson², Nicole Dron³, Dave Edwards⁴, Pradeep Ruperao⁵ and Tim Sutton⁶

¹ University of Adelaide

² Department of Primary Industries

³ NSW DPI

⁴ University of Western Australia

⁵ University of Queensland

⁶ SARDI

Chickpea production in Australia has increased dramatically in the recent years, mainly due to the fast growing demand from the Indian subcontinent. Additionally, chickpea plays an important role in the Australia farming system, serving as a disease break crop and nitrogen fixer. Achieving yield potential in chickpea is limited by many yield constraints such as biotic and abiotic stresses. Phytophthora root rot (PRR) is one of the major root diseases of chickpea in NSW and QLD which are the major chickpea production areas in Australia. Next-generation sequencing technology together with advanced statistical modelling have promising potential to increase genetic gain more efficiently.

We re-sequenced 310 PBA chickpea advanced breeding lines using Illumina next-generation sequencing technology. More than half million SNPs were discovered with a genome coverage of 5-10X. There was a big difference among genomic regions in term of SNP density and linkage disequilibrium. Analysis of population structure revealed a distinct group of ~70 breeding lines with many unique alleles difference from the PBA varieties released recently. Genome-wide association studies (GWAS) identified several SNPs significantly associated with Phytophthora root rot resistance and grain yield evaluated in seven field locations. Reduced level of nucleotide diversity and the long extent level of linkage disequilibrium suggested some regions in the chickpea genome may have gone through selective sweeps probably caused by selective breeding of PRR. We further investigated the effect of genotype by environment interaction on genomic prediction, which is another approach to deal with complex traits. We found that the training set should be phenotyped in the environments relevant to the targeted environments. We also investigated the effect of SNP function on prediction accuracy using different subsets of SNPs based on their annotation such as SNP located in regulatory, exon, and alternative splice site regions.

These results have important implication for implementing genomic selection into plant breeding programs.

Molecular genetics and genomics approaches to improve phytophthora root rot resistance in chickpea (*Cicer arietinum* L.)

Amritha Amalraj¹

Julian Taylor¹, Sean Bithell², Yongle Li³, Kevin Moore², Kristy Hobson⁴ and Tim Sutton⁵

¹ The University of Adelaide

² NSW DPI

³ University of Adelaide

⁴ Department of Primary Industries

⁵ SARDI

Phytophthora root rot (PRR) caused by *Phytophthora medicaginis* is a major soil borne disease of chickpea in the northern region of Australia. With no economical in-crop control available, a genetic approach is the most practical management option. To date, moderate field resistance has been identified and incorporated in the cultivated *Cicer arietinum* variety Yorker, and an alternative source of resistance identified in *C. echinospermum* has also been incorporated in interspecific hybrids. Using these sources of resistance as parents, three recombinant inbred line (RIL) mapping populations were previously generated. We genotyped them using a Genotype-by-Sequencing approach and phenotyped for PRR across three field environments using a mixture of ten *P. medicaginis* isolates. Whole genome QTL analysis identified major QTL on chromosomes 3 and 6 with the resistance source derived from *C. echinospermum*. Major QTL were also identified on chromosomes 5 and 6 with the resistance source derived from *C. arietinum*. The PRR resistance loci from the cultivated and wild species were found to be distinct.

This research also aimed at developing a high-throughput, controlled environment PRR infection system that can be used as a phenotyping tool in breeding. Current PRR phenotyping methods in chickpea are field based, rely on the application of mycelium slurries or oospore inoculum solutions and are impacted significantly by environmental variation. A procedure for *P. medicaginis* zoospore production was standardized and was used to produce the inoculum for a hydroponics based infection method. The system was validated qualitatively based on observations of PRR symptom development and quantitatively based on pathogen DNA quantification in roots. Two of the RIL populations for which resistance QTL from field experiments are known were screened in the hydroponics system. Two traits (plant survival time and canker length) were measured and

genetic analysis identified QTL associated with PRR resistance. A model-based correlation analysis demonstrated a high genetic correlation between the field and hydroponics traits, indicating the expression of genetic factors involved in PRR resistance under field and controlled environment is similar.

The findings from this research provide tools for marker assisted selection in chickpea and information to assist the development of populations suitable for fine-mapping of loci associated with PRR resistance. Implementation of the developed method in breeding could facilitate high-throughput screening of new genotypes and elite breeding lines, whilst also assisting with molecular-focussed studies aimed at identifying resistance genes in chickpea and virulence factors in *P. medicaginis*.

A platform for the rapid gene introgression of wild alleles into chickpea

Maria Pazos Navarro¹

Simone Wells¹, Judith Lichtenzveig¹ and Janine Croser¹

¹ The University of Western Australia

Chickpea (*Cicer arietinum* L.) would benefit from access to a wider genetic variability than currently available within the species. The expansion of genebank entries of the wild chickpea progenitor, *Cicer reticulatum* L., offers new opportunities to identify and introgress traits of value into the domestic species.

To accelerate introgression of genes from wild *Cicer* relatives to domestic, we report the development of a platform for rapid gene introgression (RGI) between divergent taxa with distinctive phenology: *e.g.* chickpea flowers about two months earlier than *C. reticulatum* during the cropping season. The RGI platform (1) shortens the gap in time to flowering under controlled-environment conditions to allow interspecific crosses, (2) permits year-round crossing, (3) yields high numbers of F2 seed through cloning of F1 hybrid material and (4) utilises the chickpea accelerated single seed descent (aSSD) process to rapidly return interspecific progeny to homozygosity.

To shorten the time-to-flowering gap, we evaluated 20 *C. reticulatum* accessions and four chickpea cultivars. Plants were grown in two controlled-environments (CEs) at 24/20°C day/night with 20 h photoperiod provided by AP67 LED arrays (Valoya, Finland). Growth under CEs compressed time to flower across all wild accessions and cultivars by 40-80% when compared to in-season growth. The flowering gap was reduced to one week *i.e.* PBA HatTrick 23 1 days after imbibition (DAI) and *C. reticulatum* CudiB_022C 31.50.8 DAI. Artificial hybridisations between PBA HatTrick x *C. reticulatum* CudiB_022C were performed under CE. Putative F1 seeds were harvested at maturity. Confirmed F1s were grown in the glasshouse under natural light and cloned up to 10 x per hybrid to produce > 1000 F2 seeds.

To refine the growth conditions for SSD, we grew two sets of 110-F2 individuals in two environments. Environment 1 (E1) had a 20 h photoperiod supplied by natural light with supplementation with AP67 LED arrays (Valoya, Finland) and time to flowering of 22-47 d. Environment 2 = 20 h supplied by AP67 LED arrays and time to flower of 25-45 d. At 28 days after flowering (DAF) seed was harvested, nicked and imbibed prior to germination to

the following generation. As time to flowering was faster in E1 ($P > 0.01$), it was adopted to repeat this process until F6.

Using the RGI platform, the breadth of genetic variation is maintained. This is evident from the range of time to flower at F6, 24-47 days (median= 25 days) and demonstrates late genotypes are not selected against. Generation turnover time ranged from 54-77 days (DAF + 28 d until pod maturity), with 89.4% of the population under 60 days from seed to seed.

We validated the effectiveness of the RGI platform by developing a F6 RIL population derived from a domestic x wild genotype in less than 18 months from crossing to F6. The platform represents a successful mechanism to rapidly harness traits from wild germplasm and has potential to be adapted to wild relatives from other species including lentil.

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Lentil and partial metribuzin tolerance

Jasmine Marsh¹

Garry Rosewarne², James Nuttall³, Sukhjiwan Kaur⁴ and Hans Daetwyler⁴

¹ Agriculture Victoria and La Trobe Univesity

² Agriculture Victoria, Grains Innovation Park, 110 Natimuk Road, Horsham, Vic 3400, Australia

³ Agriculture Victoria - Horsham

⁴ Agriculture Victoria, AgriBio

Lentil are important in southern Australias cropping systems as they provide benefits including a break to limit disease build up in cereals and increasing soil nitrogen through fixation. Weed control in lentil, especially for broadleaf weeds, can be challenging as there are limited in-crop herbicide options. Current herbicides can be damaging to the plants if applied outside the narrow crop safety window that lentil possess. Metribuzin is part of the Group C herbicides and works by inhibiting the flow of electrons in photosystem II in the chloroplasts of plants. It is used widely in Australia as a selective herbicide for suppression and control of broadleaf and grass weeds. A recent study has produced mutant metribuzin tolerant lines, however it has been found that these mutants cause a yield penalty. Partial tolerance has also been identified in the lentil line, SP1333 without obvious yield penalty. Genomic selection is a new molecular plant breeding technique that can efficiently combine partial tolerances. This technique uses a reference population that has been phenotyped and genotyped to develop prediction equations. Recent work in lentil has developed prediction equations for a number of traits with accuracies ranging from 0.2-0.75. In this study, field and controlled environment assays will be used to phenotype advanced lentil breeding germplasm and combined with existing genotypic data to develop prediction equations for metribuzin tolerance. For imposed herbicide treatments, damage will be assessed using a combination of visual scores, normalised differentiation vegetation index and yield data. The methodologies and initial results from these screenings will be discussed and the results from this study will be used to calculate prediction equations for genomic selection for partial tolerance of metribuzin in lentils. If genetic variation in lentil tolerance to metribuzin can be identified, this will be incorporated into current lentil breeding programs and ultimately provide industry with greater flexibility in herbicide options.

Genomic selection of Phytophthora root rot resistance in the PBA chickpea breeding program

yongle Li¹

Amritha Amalraj², Kristy Hobson³, Sean Bithell⁴, Kevin Moore⁴ and Tim Sutton⁵

¹ University of Adelaide

² The University of Adelaide

³ Department of Primary Industries

⁴ NSW DPI

⁵ SARDI

Phytophthora root rot (PRR) is one of the major fungal diseases of chickpea in NSW and Queensland which are the major chickpea production area in Australia. we have identified more than 10 QTLs for PRR resistance using three RIL mapping populations. Subsequently, we have developed and validated nine KASP markers flanking seven major QTL. The KASP markers were used to screen two F2 populations and ~500 advanced chickpea lines for recurrent selection.

Although we have successfully implement marker-assisted selection (MAS) in the project, we noticed many QTL have relatively small effect size which limited the efficacy of MAS. Therefore, we also investigated the value of an emerging alternative method called genomic selection (GS). GS used all marker across the genome to estimate the breeding value of individual plants and thus shift the focus of marker identification to parent selection. This approach is more relevant to breeding programs as it can select the best parents for crossing, reduce the cost and time of a breeding cycle.

We used the three RIL mapping population to predict the PRR resistance of a set of chickpea germplasm with divers genetic background and the result was promising with a prediction accuracy of up to 0.7. The work is still in progress with the aims of assisting parent selection from F2 populations and advanced lines selection with the purpose of releasing new varieties.

Identification and characterisation of potential novel sources of resistance to ascochyta blight within the exotic germplasm of lentil

Rama Harinath Reddy Dadu¹

Rebecca Ford², Prabhakaran Sambasivam², Ido Bar², Janine Croser³, Sukhjiwan Kaur⁴, Federico Ribalta³ and Dorin Gupta⁵

¹ **School of Agriculture and Food, Faculty of Veterinary and Agriculture Sciences, The University of Melbourne, Dookie College, VIC, Australia, 3647**

² **Environmental Futures Research Institute, Griffith University, QLD, Australia, 4111**

³ **The University of Western Australia**

⁴ **AgriBio, Centre for AgriBioscience, Agriculture Victoria, DEDJTR, 5 Ring Road, La Trobe University, Bundoora, VIC, Australia, 3083**

⁵ **The University of Melbourne**

Ascochyta blight (AB) caused by *Ascochyta lentis* affects gross profits and yield stability of lentil in Australia. The susceptibility of previously released resistant cultivars (Northfield and Nipper) and a future uncertainty over the resistance status of the few remaining available resistance sources, has necessitated an immediate influx of novel and diverse resistance sources into the Australian lentil breeding program. To aid in this, the potential of exotic germplasm including thirty accessions from five-wild species of lentil collected from Australian grain genebank (AGG), Horsham were screened against two highly aggressive *A. lentis* isolates (FT13037 and FT13038). The bioassay revealed two highly resistant *L. orientalis* accessions (ILWL 180 and ILWL 7) and a ten (*L. nigricans* (6), *L. odomensis* (1), *L. ervoides* (1), *L. lamottei* (1), and *L. orientalis* (1)) moderately resistant accessions. Of these, accession ILWL 180 of *L. orientalis* was the most resistant compared to the control ILL 7537. Apart from validating the symptomatic results, replicated histopathological studies was later carried out to understand the resistant mechanisms underlying lentils defence to *A. lentis* including the assessment of spore germination percentage, germ tube length and timing of appressoria formation of highly aggressive isolate FT13037. This indicates early and rapid recognition of *A. lentis* invasion is likely a major contributor for superior resistance as observed in ILWL 180. Additional evidence of faster accumulation and notably higher levels of reactive oxygen species (ROS; hydrogen peroxide and superoxide) and phenolic compounds in response to *A. lentis* penetration at 12 hours post inoculation (hpi) compared to ILL 7537 confirmed ILWL 180 could be a potential resistant source for future lentil breeding program to develop cultivars with more durable *A. lentis* resistance.

To better understand the genetic basis of resistance, a F₅ recombinant inbred line (RIL) population (N = 140) was constructed from an interspecific cross between ILWL 180 and AB susceptible cultivar ILL 6002 using accelerated single seed descent technology. The RILs and parents were then sequenced through transcriptome sequencing and using 815 high quality single nucleotide polymorphism (SNP) markers generated, a linkage map was constructed. The map stretched 488.02 centiMorgan (cM) along eight linkage groups (LGs) with an 0.66 cM average marker-marker distance. Genetic dissection of the RIL population detected a quantitative trait loci (QTL) on LG5. The identified QTL region stretched 4.93 cM and harboured nine putative candidate genes linked to AB resistance. Of these, five candidate genes were directly related to plant defence responses. Overall, the disease symptomatology, physiological and biochemical responses, and genetic evidence of resistance against AB infection support the conclusion that a stable and novel AB resistance was identified and characterised from ILWL 180. This offers significant potential to improve AB resistance of the existing cultivars within the Australian lentil breeding program.

Genetic regions controlling *Ascochyta* blight resistance in chickpea

Judith Atieno¹

Jenny Davidson¹, Marzena Kryszynska-Kaczmarek¹, Yongle Li², Kristy Hobson³ and Tim Sutton⁴

¹ PIRSA-SARDI

² University of Adelaide

³ Department of Primary Industries

⁴ SARDI

Ascochyta blight caused by the fungus *Phoma rabiei* is a major disease of chickpeas in Australia resulting in significant crop loss and management cost for growers. The disease severely impacted the growth of chickpea industry in the late 1990s necessitating the need to find sources of resistance to be incorporated into the breeding program. However, there has been an emergence of aggressive isolates in the recent years that has led to severe disease pressure on the formerly known resistant cultivars.

Progress has been made in identifying new accessions resistant to the two most recently identified aggressive *Phoma rabiei* isolates in Australia from the southern region (Pt Broughton, SA i.e., isolate F17191-1) and the northern region (Gurley, NSW i.e., isolate TR9571) belonging to pathotype group 4. Screening of lines from ICARDA nurseries and a wild *Cicer* collection has identified a number of lines that are significantly more resistant compared to the resistant check line Genesis 090. Genetic analysis using phenotypic data and whole-genome resequencing SNP data of wild *Cicer* germplasm identified three marker-trait associations on chromosomes one, seven and eight for *ascochyta* blight resistance. The SNP on chromosome 8 is near a region that has previously been reported to control *ascochyta* blight resistance in chickpea and is in close proximity to the physical location of a NBS resistance gene. KASP markers will be provided to the breeding program for validation and thereafter used in marker-assisted breeding. The results of this study will provide information on potentially new sources of resistance present in wild *Cicer* collections and to make informed choices to aid in pyramiding resistance loci into breeding lines within the Australian breeding program.

Cicer inter-specific barriers (and gates)

Judith Lichtenzweig¹

Maria Pazos Navarro¹, Theo T. Pfaff¹, Simone Wells¹ and Janine Croser¹

¹ The University of Western Australia

The primary gene pool of chickpea (*Cicer arietinum* L., Ca), including closely related wild species, was once a single interbreeding population. About 95-127 thousand years ago [1], a series of barriers to genetic exchange arose resulting in the partial isolation of two populations and subsequent speciation into *C. reticulatum* Ladiz. (Cr, the wild chickpea ancestor) and *C. echinospermum* Davis (Ce) [2]. Ca*Ce and Cr*Ce hybrids are partially sterile [2]. Evidence indicates chromosomal rearrangements are primary contributors to hybrids fertility [2]. A recent report hypothesised about an incompatibility locus underlying pollen viability in Ca*Ce hybrids [3]. Through cross-pollination, only some of the genetic information is transferable from Ce to chickpea. Considering the Ce germplasm bears several traits of interest and benefit to breeders, we aim to identify and map transferrable loci in wild accessions.

The extended collection of wild material by Cook and colleagues [1] provides new opportunities. We generated 46 hybrids for 14 combinations between cultivars PBA Hattrick or PBA Slasher and representative Ce accessions from six different sites in Turkey described in [1]. Another 72 crosses, 18 Ca*Cr combinations, were done for comparison and research into complex traits. Hybrids were assessed for pollen viability and frequency of seed formation in reproductive nodes. F2 populations were evaluated for plant fertility and genotyped to identify chromosomal rearrangements in linkage maps. The loci will be further evaluated using *in-situ* hybridisation.

The success rate in crossing cultivars to wild germplasm was higher than reported so far, 0.07-0.92 and 0.22-0.50 for Ca*Cr and Ca*Ce, respectively. As expected, Ca*Ce hybrids had lower percentage of fully developed pollen grains with differences depending on the Ce accession (range 0.00-33.98%) compared to Ca*Cr ones (99.00- 97.00%). Plant fertility was also genotype specific for Ca*Ce hybrids (<0.001-0.154). Our observations point to intra-specific karyotype diversity; the existence of incompatibility loci is yet to be evaluated. The identification and validation of loci associated with the chromosomal rearrangement(s), (*i.e.* difficult to introduce into cultivars) are underway. All F2 populations are being advanced to

F5:6 recombinant inbred lines (RILs); seeds will be released through the AGG as soon as ready and genotypes will be available upon request.

This project is co-funded by GRDC and UWA (UWA00175-var2). The projects expected outputs are (i) characterisation of a diverse range of Ce germplasm and gene introgression into locally adapted cultivated backgrounds, (ii) determination of Ces genetic potential in conventional chickpea breeding and production of a catalogue of transferable (gates) and non-transferable loci (barriers), (iii) genotyped RILs and near isogenic line (NIL) to determine the genetic basis of traits affecting yield. The significance of this project is broader; it contributes to our understanding of the crops evolution and our ability to expand its adaptation to a range of changing climates.

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1-von Wettberg et al., 2018 Nature Communications

2- Ladizinsky & Adler, 1976 Euphytica

3- Kahraman et al., 2017 Crop Science

Growth of pea (*Pisum sativum*) under conditions for accelerated maturity shifts the expression pattern of key hormones related to embryo physiological maturity

Federico Ribalta¹

Maria Pazos Navarro¹, Kylie Edwards¹, John Ross², Sergio Ochatt³ and Janine Croser¹

¹ The University of Western Australia

² School of Biological Sciences, University of Tasmania

³ Agroecologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comte

The University of Western Australia (UWA) has partnered with The Grains Research and Development Corporation (GRDC) to undertake research aimed at delivering rapid genetic gain in pulses through the deployment of an accelerated single seed descent (aSSD) platform. Manipulation of key growth conditions, including photoperiod, light quality and temperature, has enabled the substantial shortening of time to maturity across the five major cool-season pulse species. A key aspect of the aSSD platform research has been determining the timing at which robust precocious germination of the immature seed is achievable. We have previously established sugar and moisture content as indicators of physiological maturity in pea. In the research reported herein, we now address the question of how hormone profiles are regulated by plant growth environment and the relevance of these fluctuations to precocious *in vitro* germination.

Three pea cultivars (PBA Twilight, PBA Pearl and Kaspera) defined as early, mid and late based on field flowering time were grown under two environments. Environment 1 (E1) was a controlled environment room set at a 20 h photoperiod provided by LED AP67 arrays (Valoya, Finland) to promote rapid maturity. Environment 2 (E2) was a glasshouse with a 13-14 h photoperiod provided by natural light. Temperature of 24/20C (day/ night) was constant in both environments. The profiles of key embryo development hormones indolacetic acid (IAA), chlorinated IAA (4Cl-IAA), gibberellins (GA₂₀ and GA₁) and abscisic acid (ABA) were measured in the developing seed in a period between the end of embryo morphogenesis (10 days after pollination, DAP) and the attainment of embryo physiological maturity (22 DAP).

Growing plants under E1 conditions altered the seed hormone content by advancing the auxin, GAs and ABA profiles by 4-8 days compared to those of seeds grown under E2 conditions. In E1, there was a synchronisation of auxin peaks across the different pea genotypes. GA₁ was only detected in seeds harvested from the less intensive conditions. The

results point to an acceleration of embryo physiological maturity by up to four days in the intensive environment and the utility of auxin and GA profiles as reliable indicators of seed maturation. A second experiment aimed to *in vitro* germinate seeds harvested at time-points 12-22 DAP, with and without exogenous hormones. The results demonstrated the extent of sensitivity of developing seeds to exogenous ABA was strongly genotype-dependent. Concentrations between 5-10 M inhibited germination of seeds harvested 18 DAP. Germination of seeds harvested 12 DAP was enhanced up to three fold with the addition of 125 M GA₃. These results show for the first time the fluctuations of seed hormone levels across genotypes, stage of seed development and environmental conditions and the influence of these changes on precocious germination competence in pea.

Flavonoids are plant-derived compounds that regulate positive and negative interactions between chickpea with its natural symbiont *Mesorhizobium ciceri* and the plant-parasitic nematode *Meloidogyne javanica*

Jason Ng¹

Haniza Zainal Abidin¹ and Ulrike Mathesius¹

¹ The Australian National University

Legumes are unique due to their ability to form beneficial interactions with rhizobia. Nitrogen fixation during legume-rhizobia symbiosis allows legumes to thrive in nitrogen (N)-poor soil environments, enriches the rhizosphere with N compounds for future crops, and circumvents the application of costly fertilisers. In the field, legumes are also exposed to multiple pathogens, including plant-parasitic nematodes (PPNs). PPNs are soil-borne, microscopic roundworms that collectively account for >USD100 billion (~15%) of global agricultural yield loss. Root-knot nematodes (RKNs; *Meloidogyne spp.*) are the most damaging group of nematodes that parasitise almost every vascular plant, including legumes. RKNs induce the formation of galls in infected roots, which are essentially nutrient sinks where female RKNs grow and reproduce.

Flavonoids are plant-derived compounds that play multiple roles in rhizospheric interactions. During the legume-*Rhizobium* symbiosis, flavonoids are exuded from host roots, which will then be recognised by the compatible *Rhizobium* species and induce *Rhizobium* nodulation (nod) gene expression. Rhizobia produce nodulation factors that are recognised by the compatible legume hosts to initiate nodule formation. Flavonoids have also been shown to affect PPN behaviour, including their motility, chemotaxis (repellent activity) and mortality. However, little is known about which flavonoid compounds are effective nod gene inducers for different legume-*Rhizobium* partners. Much is also unexplored about which are the most potent flavonoid compounds in reducing nematode parasitism.

Our work focusses on the interaction between chickpea (*Cicer arietinum*) with *Mesorhizobium ciceri* and *Meloidogyne javanica*. We hypothesised that distinct sets of flavonoid compounds are optimal for positive and negative interactions with rhizobia and nematodes, respectively. A selection of 20 chickpea varieties common to Australian growers were cultivated in individual sand pots in the glasshouse. We showed that nematode infection reduced chickpea yield by an average of two-fold while rhizobia inoculation increased yield by an average of more than 2.5-fold, compared to control plants. Root flavonoid exudate was

extracted from the rhizospheric sand. We analysed flavonoid exudates using liquid chromatography-tandem mass spectrometry (LC-MS/MS) by targeting 40 flavonoid compounds. These compounds were chosen based on their potential to act as nod gene inducers/nematode repellents by assessing their known activities in other legume-*Rhizobium*/nematode combinations. We found a very significant variation in flavonoid exudate concentration across the 20 varieties under control, nematode-infected and rhizobia-inoculated conditions. Two flavonoids - kaempferol and genistein were strongly correlated with gall numbers in nematode-infected plants, whereas 2-hydroxyflavone, chrysin, isoliquiritigenin and taxifolin were strongly correlated with nodule numbers in rhizobia-inoculated plants. We performed motility tests to investigate if certain flavonoids could affect nematode movement and showed that several flavonoids, including kaempferol, genistein, medicarpin, isoliquiritigenin and coumestrol could affect (accelerate or reduce) nematode movement in a concentration-dependent manner. Next, we also performed chemotaxis assays and demonstrated that some of these flavonoids (medicarpin, coumestrol and isoliquiritigenin) could repel nematodes to varying degrees. Some of these flavonoids could also potentially be strong nod gene inducers. Our goal is to establish flavonoid markers in chickpea that is indicative of high nodulation and tolerance/resistance towards PPNs for use in breeding programs and seed priming technologies.

Progress in Non-Chemical Weed Management in Pulses in western Canada

Eric Johnson¹

Oleksandr Alba², Lena Syrový³, Jessica Weber⁴, Breanne Tidemann⁵ and Steven Shirtliffe¹

¹ Dept. of Plant Sciences, University of Saskatchewan

² AeroGrow Manufacturing Ltd.

³ Pure Roots Farms

⁴ Western Applied Research Corporation

⁵ Agriculture and Agri-Food Canada

Weed management in pulse crops is challenging due to their poor competitive ability, limited herbicide options, and increasing incidence of herbicide resistant weeds. There is approximately 700,000 hectares of organic field crop production on the Canadian Prairies. Since the use of synthetic herbicides is prohibited in organic production, growers rely heavily on mechanical and cultural weed control methods. Results from two studies will be presented: 1) the effect of post-emergence tillage (rotary hoeing, harrowing and inter-row cultivation) integrated with crop seeding rate on weed control in field pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) and, 2) the effect of clipping *Brassica* weeds above a lentil crop canopy on weed seed production. The first study was conducted in Saskatoon, SK, Canada in 2016 and 2017. Mechanical weed control methods including rotary hoeing, harrowing and inter-row cultivation were applied in a factorial arrangement with two seeding rates in organically grown field pea and lentil (targeted plant densities of 90 and 135, and 130 and 260 plants m⁻², respectively). All mechanical treatments resulted in similar field pea yield increases ranging from 38% to 50%. Paired and multiple treatments reduced weed biomass in field pea by 73% to 86%. Increasing the seeding rate of field pea did not improve weed control, but it increased field pea yield by 13%. The combination of post-emergence rotary hoeing followed by inter-row cultivation resulted in 40% higher lentil grain yield than the untreated check. Doubling the seeding rate of lentil resulted in a 23% increase in yield, while weed biomass was reduced by 16%. Sequential treatments of rotary hoeing and inter-row cultivation in lentil resulted in a 76% decrease in weed biomass. Treatments including rotary hoeing provided the greatest spectrum of weed control in both crops as they controlled more than 80%, 60%, and 86% of green foxtail (*Setaria viridis* L.), wild mustard (*Sinapis arvensis* L.), and common lambsquarters (*Chenopodium album* L.), respectively. The second study was conducted at Lacombe, AB, and Saskatoon, SK, in 2017 and 2018 to determine the

optimum timing, frequency, and height of weed clipping for reducing *Brassica* seed production in imidazolinone-tolerant lentil. All clipping treatments in lentil were conducted above the crop canopy to avoid crop damage, but included multiple timings of clipping (Weeks 1-5 after the initiation of *Brassica* flowering), and conducted at different frequencies ranging from 1 to 5 times. Clipping conducted in the first week of weed flowering reduced seed production by only 14%. A single, delayed clipping in the fourth week of flowering reduced seed production by 67%, however delaying clipping beyond the fourth week resulted in dispersal of viable weed seeds at the time of clipping. Performing multiple clippings resulted in the lowest weed seed production, with clipping two and three times resulting in reductions of 74 and 90%. Combining mechanical and cultural practices can reduce weed interference and improve crop yield in organic pulse production; however, some of these practices may have application in managing herbicide resistant weeds in conventional cropping systems.

Identifying the plant contribution to efficient nitrogen fixation in chickpea

Frank Bedon^{1,2}

Penelope Smith^{1,2}

¹ La Trobe University

² Legumes for Sustainable Agriculture

Legumes form a symbiotic interaction with rhizobia that can fix atmospheric nitrogen, allowing them to access fixed nitrogen and reducing their reliance on nitrogenous fertilizers. The interaction plays an important role in sustainable agricultural systems. In the past there has been a lot of research to identify rhizobia that allow efficient nitrogen fixation in crop plants but the contribution of the plant to this process has rarely been a focus. In our research program we aim to identify chickpea genotypes that are efficient at accessing and utilising fixed nitrogen. We are screening a range of genotypes from the ICRISAT core collection, as well as commercially released chickpea varieties. Our initial work is assessing rhizobial strains obtained from the collection of Rosalind Deaker at University of Sydney to identify those that can interact with a wide range of chickpea genotypes. A mixture of these rhizobia, including the current chickpea inoculant CC1192, will be used to screen the 260 chickpea genotypes. We will assess the ability of inoculated plants to generate biomass and compare this to biomass production in plants supplied with nitrogen containing fertilizers. Results will be used in a genome wide association study to identify plant genes that contribute to effective fixation of nitrogen and utilisation of this nitrogen. Genotypes with traits that are superior to current cultivars can be used in prebreeding to transfer the traits into more commercially acceptable germplasm.

Fast Faba - delivering rapid homozygosity

Christine Munday¹

Federico Ribalta¹ and Janine Croser¹

¹ The University of Western Australia

Faba bean (*Vicia faba* L.) is established as a high-value, high-protein pulse crop option for Southern Australia. In 2018, prices were double that of lentil and up to \$200/ T more than chickpea. Faba bean breeding has led to substantial genetic improvement over the past decade, resulting in improved crop resilience and reliability. We propose herein a new tool for plant breeding in faba an accelerated single seed descent (aSSD) platform for year-round growth under fully artificial conditions. It is expected the aSSD platform will provide the basis for rapid delivery of improved traits such as herbicide resistance to the farm gate.

Faba bean is well known to be difficult to grow indoors due to a reliance on insect vectors for pollination and the resulting low seed set when grown under controlled environment conditions. We have been researching methods to achieve both a rapid onset of flowering and robust pod set in the absence of insects. Our efforts have resulted in the turnover of up to five generations per year in segregating populations. These segregating populations represent a wide phenotypic spectrum within the breeding program.

An 18 h photoperiod, 22/ 18 C temperature regime and optimised Red:Far Red light ratio provided by AP67 LED light fixtures (Valoya, Finland) is optimal for acceleration of faba flowering and successful seed set. We mimic bee pollination using vibration and individual tripping of flowers. We manipulate plant height to facilitate multi-tier controlled environment growth through application of the anti-gibberellin agent Topflor (SePro Corporation) enabling a density of up to 95 plants/ m². A rigorous regime of pest control, watering and weekly liquid fertiliser maintains plant health. At time of writing, the Faba aSSD platform has delivered >550 F6/ F7 lines to the breeding program, with a further 1960 lines under development.

How the seasons change: the influence of environment on mungbean yields

Kylie Wenham¹

Marisa Collins²

¹ The University of Queensland

² LaTrobe University

In Australia, the inclusion of pulses in crop sequences as break crops within cereal based cropping systems has increased in popularity through additional yield benefits provided as a result of water, nitrogen and disease dynamics. Mungbeans (*Vigna radiata* L. Wilczek) are the only summer pulse grown in Australia with >95% of crops planted in northern New South Wales and Queensland. Grain yield for mungbeans has been historically variable and considered high-risk for growers due to the factors dictating crop performance and yield being poorly understood, providing an ongoing challenge for the industry. Over the past decade, the total area of production of mungbeans has increased in response to rising commodity prices and the opportunity to utilise mungbeans as a break crop or a short-duration crop in double cropping situations, highlighting the need to better understand the physiology of the crop and how to grow it better. The relationship between biomass accumulation, flowering and yield components as influenced by seasonal variability and water dynamics is crucial for understanding what is driving grain yields in mungbean crops in the northern region and how risk can be managed. Field trials across spring and summer seasons aim to examine how these key drivers may influence biomass accumulation and understand those factors critical to maximising flower number and most importantly grain number and overall yield. Understanding the fundamental physiological relationships that determine yields in mungbean have the potential to increase overall yields both in the paddock through better understanding of environment and agronomic management, as well in breeding programs that will gain clear targets for improving yields in upcoming varieties.

Smart Farms And Remote Sensing

Manipulation of sowing time and variety in lentil and faba bean to improve modelling capabilities and aid in management of abiotic stress

Lachlan Lake¹

Victor Sadras¹ and Yash Chauhan²

¹ SARDI

² DAF Queensland

Manipulation of sowing time and variety in lentil and faba bean to improve modelling capabilities and aid in management of abiotic stress

Australian lentil and faba bean production is constrained by extreme temperature and drought. Crop species, variety and sowing time are the main management options for limiting the impact of these stresses. Further insight into optimal management practices can also be provided by crop modelling accounting for management and climate variables. We measured phenology in 10 lentil and 10 faba bean varieties over three seasons (2016 - 2018) and three regions in southern Australia; Hart and Roseworthy in the mid north, Minnipa on the Eyre Peninsula and Bool Lagoon and Conmurra in the South East. Within each location we used six times of sowing, spaced two weeks apart beginning mid-April. For both crops across locations, time to flowering and pod set declined linearly with sowing date. Similarly and across locations and sowing times, flowering was advanced at 3 d d⁻¹ in faba bean and 5 d d⁻¹ in lentil. The phenology data was combined with weather records and then used to optimise prediction of flowering time in the Agricultural Production System Simulator (APSIM) modelling software. Data was optimised for both lentil and faba bean ($R^2 > 0.9$); accounting for soil moisture improved the predictive power but more so for lentil than faba bean. These results have been used to model optimal sowing time across selected environments and will aid in risk management decisions to minimise exposure to frost, heat and water stress across the southern region.

Do sensors explain the advantage of navy bean intercropped with maize under limited water and N environments?

Abeya Temesgen Tefera^{1,2}

¹ The University of Queensland, Queensland Alliance for Agriculture and Food Innovation (QAAFI)

² Agriculture Victoria Research

Sensor technology has been widely used under field conditions to improve our understanding of crop stress level to a wide range of production factors. Here we used active and passive sensors, such as infrared thermometer, SunScan canopy analysis system and neutron moisture meter to understand the physiological and environmental drivers of navy bean-maize intercropping advantage as the level of water and N input decreases. Field experiments were carried out at Gatton research station of the University of Queensland in 2011/12 and 2012/13 summer seasons. Navy bean maize intercropping systems and their sole crops were evaluated using automatic rainout shelters in three trials viz., under low water/nitrogen (W/N), medium W/N and high W/N conditions. Sensors were deployed to quantify the aboveground and below ground production factors, viz., plant available soil water, fraction of intercepted photosynthetic active radiation (fPAR), relative humidity and temperature both inside and outside canopy of sole and intercropping systems. Canopy stress index as well as productivity index viz., Land Equivalent Ratio (LER), were computed to assess the stress level of component crops and grain intercropping advantage gained over their sole crops. Resource use (i.e. water, nitrogen and light) and use efficiency were analysed in both intercropping and sole cropping systems. The result indicated that the higher the level of water and nitrogen, the lower the advantage of intercropping system in terms of LER. Under low water and N input, intercrop navy bean had lower canopy temperature and stress index values compared to sole crops. In addition, intercrop system intercepted significantly higher fPAR than both sole crops for long growing period under limited water and N availability. The intercropping advantage (in terms of LER) was directly related to the intercropping advantage on systems water use efficiency (not to water use) and systems N use (not to NUE). However, there was no clear relationship between the intercropping advantage (in terms of LER) and radiation intercepted or use efficiency. Overall, this research has demonstrated that sensors appear to be more applicable in explaining factors driving intercropping advantage as the level of water and N availability decreases. From this finding, it seems that intercropping of navy bean with maize might have maintained navy beans temperature in a water stressed environment.

Further research efforts should be needed to automate the sensors we used for applications at a larger scale in pulse based cropping system.

Automated phenotyping for abiotic stress tolerance in lentil

Sameer Joshi¹

Mohamad Javid¹ and Surya Kant¹

¹ Agriculture Victoria, Grains Innovation Park, 110 Natimuk Road, Horsham, Vic 3400, Australia

Lentil yield in low rainfall areas is limited due to several abiotic stresses. High-throughput phenotyping techniques could speed-up breeding varieties tolerant to abiotic stresses. Three phenotyping assays for water stress, boron toxicity and salt stress have been developed at the Plant Phenomics Victorias high-throughput plant phenotyping facility located at Horsham. Three different set of lentil genotypes were used for each assay, with three watering regimes, four boron levels, and four salt (NaCl) levels. Plants were imaged - top view and three side views (0, 120 and 240) - three times per week. Destructive harvesting was conducted during the vegetative growth stage. High correlations were observed between the projected plant area derived from visible images and manually harvested fresh and dry biomass. Significant varietal responses were also observed for each abiotic stress treatment. For water stress, PBA Ace showed higher projected area compared to PBA Jumbo2 and Northfield. For boron stress, ILL2024 had a higher projected area than PBA Flash and PBA Blitz. For salinity stress, CIPAL1504 showed a higher projected area than ILL2024 and Nugget. The phenotyping protocols established efficient identification of genotypes tolerant to abiotic stresses and will be useful to accelerate crop breeding efforts in the improvement of lentil.

Value Adding Pulses

Assessment Of Lentil Seed Quality; Inherent Longevity And Its Progression Through Development

Katherine Whitehouse¹

Sally Norton², Christine Best¹, Nicole Sawyer¹ and Tony Slater³

¹ Agriculture Victoria

² Australian Grains Genebank, Agriculture Victoria, Grains Innovation Park, 110 Natimuk Road, Horsham, Victoria 3400, Australia.

³ Agriculture Victoria, AgriBio, 5 Ring Road, Bundoora, Victoria 3083, Australia

There are many circumstances in which it is both beneficial, and important, to be able to predict the effect the environment has on the longevity of seeds whether they are being stored short-term, e.g. between growing seasons, or long-term, for hundreds of years in genebanks. The Australian Grains Genebank (AGG) is the national programme for preserving grain crop genetic resources for Australia and, like all active genebanks, it has a mandate to conserve and maintain viable seed for use in plant research and/or breeding programmes. However, for this to be a reality, we must be able to manage our seeds effectively (i.e. ensuring timely regeneration), therefore, understanding inter- and intra-species differences in seed longevity is critical as it underpins decisions on accession viability retest intervals, regeneration and/or recollection.

Equations for predicting seed longevity for a particular species under a constant set of temperature and humidity conditions were developed from the 1960s onwards and underpin all seed conservation practices. For orthodox seeds which can tolerate being dried down and stored at subzero temperatures, longevity increases in a predictable manor with a decrease in temperature and moisture content. However, the level of sensitivity to these environmental conditions differs between species resulting in inter-specific differences in longevity. These sensitivity parameters, more commonly known as seed viability constants, need to be estimated for each species. To date, these have only been determined for approximately 70 mostly crop species which is a very small proportion of the worlds plant species. The AGGs pulse collection contains over 28,300 accessions, with field pea (c. 6,332), chickpea (c. 9,114) and lentil (c. 5,328) the three largest crop groups. The seed viability constants for both chickpea and field pea were discovered in 1988, whereas, the inherent longevity of lentil is

still unknown. This poster will outline the experiment(s) which aim, firstly, to determine estimates for these species-specific parameters for lentil, and secondly, to broaden our understanding of lentil seed quality (physiological) development in relation to time. This will form the basis of several follow-up experiments where we plan to delve deeper into the genotype-by-environment interaction which, affects three important facets: the value of maximum seed quality; the time when it is first attained during seed development and maturation; and for how long it is maintained thereafter *in planta*. The outcome of this research will help shape our regeneration procedure to ensure seeds are at their maximum quality when placed into storage hence, optimising longevity, and limiting loss of genetic integrity. This will ensure that the AGG provides high quality seed to Australias research and breeding programmes for the development of more resilient grain crop varieties into the future.

Lupin Seed Storage Proteins and their Potential for Human Health

Rhonda Foley¹

Lars Kamphuis^{1,2,3} Jose Jimenez-Lopez^{3,4} and Karam Singh^{1,2,3}

¹ CSIRO Agriculture and Food, 147 Underwood Avenue, Floreat WA 6014, Australia

² Centre for Crop and Disease Management, Curtin University, Bentley, WA, 6102, Australia

³ UWA Institute of Agriculture, Perth, 6009, Australia

⁴ Department of Biochemistry, Cell and Molecular Biology of Plants, Estacion Experimental del Zaidin, Spanish National Research Council (CSIC), Granada 18008, Spain.

Narrow-leaved lupin (NLL; *Lupinus angustifolius* L.) is a major grain legume crop that is important for sustainable farming systems, particularly in Western Australia. It has been used traditionally as an animal feed, and is important for sustainable agriculture by acting as a disease break for cereals and replenishing soils with nitrogen and phosphorous. Recently, the grain has gained attention as a human health food for its high protein and fibre content and being gluten-free. It also has various nutraceutical benefits through influencing satiety and lowering blood pressure and cholesterol, benefiting those that suffer from lifestyle diseases such as obesity, diabetes and cardiovascular disease.

Our group has led the identification and characterisation of the major proteins in lupin seeds. These are called conglutins, and they have primary roles in supplying carbon, sulphur, nitrogen and energy for the germinating seedling. They fall into four families: α , β , γ , δ ; and interest in these conglutins is growing as family members have been shown to have beneficial nutritional and pharmaceutical properties. We analysed the conglutins in a range of lupin species in terms of their gene structure, phylogenetic relationships as well as their expression during seed development. The majority of the heterogeneity of conglutin polypeptides is likely to arise from post-translational modification from a limited number of precursor polypeptides rather than a large number of different genes. Overall, the results demonstrate a high degree of plasticity for conglutin expression during seed development, allowing the way to modification of the expression of conglutins through reverse genetics strategies. This could be achieved through a TILLING population currently under development or gene editing,

with the ultimate aim of reducing allergenicity and improving nutritional and pharmaceutical properties.

Influence of processing conditions on pulse flake quality

Stephen Cork^{1,2}

Asgar Farahnaky^{2,3}, Christopher Blanchard^{1,2} and John Mawson²

¹ School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW, 2650 Australia

² ARC Industrial Transformation Training Centre for Functional Grains(FGC) and Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, NSW, 2650 Australia

³ School of Science, RMIT University, Bundoora West Campus, Plenty Road, Melbourne, VIC, 3083, Australia

Pulses are a healthy and sustainable food source of global significance. Pulse crops like chickpeas and faba beans are gaining popularity with Australian farmers, however, pulse producers face volatile international markets and limited domestic processing markets. Pulse consumption is well below the recommended daily intake in developed countries such as Australia, and the traditional pulse dishes in developing nations are being replaced by ready-to-eat cereals. Therefore, new ways to increase pulse consumption are required, especially in the processed ready-to-eat market. The production of pulse flakes could be a means of increasing pulse consumption if we can address pulse processing challenges such as their structural weakness and long cooking times.

This research investigated the influence of processing conditions on the flaking quality of Australian chickpea and faba bean splits. The process consisted of first conditioning the pulse splits using different steam injection times (one, three or five minutes). Secondly, the softened splits were flaked between two rollers with gap sizes ranging from 0.6 to 1.9 mm. Finally, the rolled pulse flakes were rapidly dried using a fluidised bed drier at either 150C or 200C.

The results demonstrate that the quality parameters of flakes generated using Australian pulses including the degree of cooking, appearance, texture and durability are affected by processing parameters. By optimising processing conditions, it may be possible to produce ready-to-eat pulse flakes that are healthy and add value to pulse grains.

Breeding and selection for faba bean seed quality

SHI YING YANG¹

Samuel Catt¹ and Jeffrey Paull¹

¹ The University of Adelaide

Global food production will need to continue to increase to meet the demand of the world's growing population. Faba bean (*Vicia faba* L) is one of the most important grain legume crops, for food and feed, both worldwide and in Australia. It has the biggest seeds in the legume family, and its genome is the largest among legumes (13.4 Gb). Australian varieties occupy a significant share in international faba bean trade and the quality of the Australian varieties is generally well regarded. Usually, good quality faba bean seeds are consumed as food by humans and the lower quality seeds are used for animal feed or processing. The faba bean price is determined by both the seed quality and international supply and demand. With the faba bean price increasing dramatically last year to over \$800 per tonne, even lower quality faba bean seeds were a valuable product. Hydration capacity, seed colour and 100-seed weight are among the most important quality traits that are tested in the faba bean breeding program. The seed quality data is combined with disease resistance and yield data for selection of breeding lines to progress to release as new varieties. The quality tests and selection have provided a valuable opportunity to generate the optimal use of resources and accelerate the improvement of faba bean breeding strategies.

Post entry quarantine virus screening and its importance to the pulse industry

Virginia McQueen¹

Shane King¹ and Sally Norton¹

¹ Agriculture Victoria

Protecting the pulse industry from pest and diseases starts at the border. The Australian Grains Genebank (AGG) Post Entry Quarantine (PEQ) program in Horsham, has been operational for over twenty years. It is our duty of care to the industry to ensure all newly imported accessions are free from exotic diseases which have the potential to cause losses in production, yield and/or seed quality. When new varieties of temperate pulses arrive in the Australia, they are required to undertake a full growth cycle in a Department of Agriculture (DA) approved facility before they can be released and deposited in the Genebank. The plants are monitored under strict guidelines for fungal pathogens and are screened for exotic seedborne viruses. Approximately 600 lines of temperate pulses are screened for six viruses, using serological and molecular methods, annually. When all the screening requirements have been fulfilled, and harvest is complete, the seed is signed off and released by DA; with all seed being deposited into the Genebank.

Characterisation of frost affected lentils, post harvest, using sensor technologies

Cassandra Walker¹

**Linda McDonald², Audrey Delahunty¹, Joe Panozzo³, James Nuttall¹, Ashley Wallace¹,
Sahand Assadzadeh¹ and Alexander Clancy¹**

¹ Agriculture Victoria - Horsham

² Department of Jobs Precincts and Regions

³ The University of Melbourne, Parkville Victoria

Post-farm gate pulse value is based on visual characteristics, which can be affected by both abiotic and biotic stresses during the growing season. If segregation using spectral-based characterisation were available this would provide benefit through adding value post-harvest. Lentil (*Lens culinaris*) is well adapted to southern Australia and is important for diversity within cropping rotations and contributes significantly to farm profitability. One of the risks associated with growing lentil in the southern region is that it is highly susceptible to frost, particularly during flowering and pod-set. The likelihood of the occurrence of frost in this region is high; decreasing grain yield, reducing quality and devaluing the commodity.

The aim of this research is to develop and validate novel practices using spectral-based technologies to objectively quantify the impact of frost on the whole grain lentil samples. The development of algorithms that estimate the portion of non-frost affected grain within a frost affected crop offer opportunities to potentially segregate grain using rapid-objective measures.