FINAL REPORT 2015

PROJECT CODE :	S1208

PROJECT TITLE
Resistance monitoring of ascochyta blight in lentils

PROJECT DURATION

Project Start date	1 July 2012
Project End date	30 June 2015

PROJECT SUPERVISOR CONTACT DETAILS

Title:	First Name:	Surname:
Dr	Jenny	Davidson
Organisation:		
South Australian Research and Development Institute (a group of Primary Industries & Regions SA)		
Mailing address:		

Telephone:	Facsimile:	Mobile:	Email:

Office Use Only

Project Code	
Project Type	

ADMINISTRATION CONTACT DETAILS

Title:	First Name:	Surname:
Mrs	Adrienne	Twisk

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

Mailing address:

Telephone:	Facsimile:	Mobile:	Email:

PROJECT REPORT

Executive Summary

- Ascochyta blight of lentils (*Ascochyta lentis*) can now infect previously resistant cultivars Nipper and Northfield. PBA HeraldXT, PBA Hurricane, PBA Ace and PBA Bolt remain resistant in field conditions, although a few lesions can infect these lines in controlled experiments, indicating the potential for resistance breakdown for these cultivars.
- 85 of 139 isolates of *A. lentis* collected between 2010 and 2014 from commercial crops and trials were tested against a differential host set and compared to 17 isolates from earlier seasons (1987 to 2006). Nipper and Northfield were susceptible to 68% and 91%, respectively, of these isolates, compared to 24% from the earlier years.
- NVT lines were tested against a Nipper virulent isolate and against an isolate unable to infect Nipper. Field disease scores from Mallala 2013 and Horsham 2014 were highly correlated with disease scores from the Nipper virulent isolate, indicating that isolates able to infect Nipper are widespread across southern Australia.
- Spore release studies using ascochyta infested lentil stubble, conducted over 4 years and two sites, found wind-blown ascospores were consistently released from stubble through May to end of July. Hence manipulating sowing dates is unlikely to reduce infection from ascospores released from infested stubble.

Project Objectives

1. Identify resistance breaking isolates of *Ascochyta lentis* in South Australian and Victorian lentil growing regions which will be delivered to the national lentil breeding program and the national pulse germplasm enhancement program to assist with the development of resistant cultivars.
2. Identify timing of ascospore release from infested lentil stubble to improve timing of fungicide applications.

Overall Performance

This project tested a number of methods for rapid screening of *A. lentis* isolates in controlled conditions and devised a suitable method using a controlled environment room and humidity chambers. A large number of isolates collected over a wide variety of locations, host cultivars and years have been tested on a differential host set using this methodology. Results indicate that the resistance to ascochyta blight in Nipper and Northfield lentils is no longer effective across South Australia and Victoria. This is confirmed by disease scores in field trials and commercial crops. A small number of isolates are also able to cause restricted lesions on resistant cultivars such as PBA Herald XT and the resistant parent Indianhead suggesting this resistance may also be at risk of becoming ineffective in future years. Outcomes and isolates collected within this project have been shared with the national lentil breeder and pathology researchers in the National Pulse Germplasm Enhancement project and the Pulse Molecular Marker Project.

Spore release studies found that wind-blown spores from infested lentil stubble are released at the same time each year, irrespective of rainfall events during summer and early autumn. A thiram based seed dressing on lentils is recommended as the most effective means to minimize infection from these wind-blown spores. Manipulating sowing dates is unlikely to influence the infection rates from windborne spores. Previous research has demonstrated that later sowing reduces severity of ascochyta blight and this is most likely associated with added exposure to rainfall events which splashed spores onto further plants.

Outcomes of the project have been shared with industry via field days, GRDC updates, and industry and farmer meetings.

Personnel:

1. Dr J Davidson supervisor (0.15 FTE)
2. Ms Michelle Russ. Technician (0.6FTE)
3. Dr Moin Salam DAFWA modelling of *A. lentis* spore release
4. Mr Sam Holmes, Holmes Farm Consulting, Paddock identification and incubation of stubble

Key Performance Indicators (KPI)

KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
Monitor ascospore release from infested lentil stubble	Y	
Collect *Ascochyta lentis* isolates in South Australian and Victorian sites during 2012 cropping season	Y	
Monitor ascospore release from infested lentil stubble and recalibrate Blackspot Manager model with 2011 data	Y	

Test *Ascochyta lentis* isolates from 2012 collection and forward selected isolates to breeding and germplasm enhancement programs	Y	
Collect *Ascochyta lentis* isolates in South Australian and Victorian during 2013 cropping season	Y	
Monitor ascospore release from infested lentil stubble and recalibrate spore release model with 2013 data	Y	
Collect *Ascochyta lentis* isolates in South Australian and Victorian during 2014 cropping season	Y	
Test *Ascochyta lentis* isolates from 2013 collection and forward selected isolates to breeding and germplasm enhancement programs	Y	
Test *Ascochyta lentis* isolates from 2014 collection and forward selected isolates to breeding and germplasm enhancement programs	Y	
Submit final report	Y	

Technical Information

A. **Ascochyta blight in commercial lentil crops and NVT trials.** Ascochyta blight was detected in commercial crops and trial plots of Nugget, PBA Flash and Nipper but not in PBA HeraldXT. In three NVT trials in 2013 *viz*. Minlaton, Maitland and Willamulka, the cultivar Nipper recorded a similar level of disease to Nugget (i.e. MR/MS), while PBAFlash rated the highest disease scores. PBAHeraldXT, PBAAce and PBABolt were free of infection while low to moderate infection occurred on PBABlitz and PBAJumbo, respectively. Disease was more likely to be present in rotations of 2-3 years than 6 year rotations of lentils.

B. **Isolate collections.** 139 isolates of *A. lentis* were collected from commercial crops and trials in South Australia, and from ascochyta infested lentil stubble incubated at SARDI. The latter included one isolate each on the highly resistant lines Indianhead, ILL7537 and PBAHeraldXT. 23 isolates were collected in Victoria by PhD candidate Saleem Khan (University of Melbourne) and Dr Matt Rodda (lentil breeder) and transferred to SARDI for testing in controlled conditions.

Isolates from stubble incubation: Lentil stubble infested with ascochyta blight was collected after harvest in December 2012 and 2013 from commercial crops (PBAFlash and Nipper) and incubated at SARDI in external conditions, next to pots containing seedlings of eight different lentil lines i.e. resistant sources Northfield, Indianhead and ILL7537; commercial cultivars Nipper, Nugget, PBAFlash and PBAHeraldXT; and a susceptible check, Cumra. Ascochyta lesions that developed on these seedlings were recorded and collected for storage and further research. While the majority of lesions developed on the susceptible lines Cumra and PBAFlash, a moderate number developed on Nipper and Northfield, and a small number also developed on each of the resistant sources and on PBAHeraldXT (Figure 1). These results demonstrate that virulent isolates able to overcome all resistant sources already exist in the *A. lentis* population in South Australia and the resistance in PBAHeraldXT and similar lines is at risk, particularly in where lentils are grown in tight rotations.

C. **Isolate testing in controlled conditions.** Experiments were undertaken to develop a rapid method of isolate screening in controlled conditions, including detached leaves, an irrigated shadehouse and numerous methods in a controlled environment room (CER). Two week old seedlings in a CER were the most efficient testing method for time and space, and this was used on all subsequent isolate tests. Testing was conducted on 102 isolates using the differential host set of Cumra (susceptible), PBAFlash (moderately susceptible), Nipper, Northfield, Indianhead (resistant parent) and breeding line ILL7537 (resistant parent). As expected, the majority were able to infect Cumra and PBA Flash. Nipper and Northfield were susceptible to a greater number of isolates in the more recent collections compared to the earliest collections. A few isolates were able to incite restricted lesions on Indianhead or ILL7537, although these were still recorded as resistant or moderately resistant (Table 1).

The lentil entries in the 2013 National Variety Trials were tested against an isolate virulent on Nipper as well as an isolate avirulent on Nipper. Results of the Nipper virulent isolate were highly correlated with disease assessments in the PBA breeding trial at Mallala in 2013 and the PBA breeding trial at Horsham in 2014. The correlation coefficient for comparison of the Nipper virulent isolate (FT12013) with Mallala = 0.82 ($P<0.001$) and with Horsham raw data = 0.87 ($P<0.001$); the correlation coefficient for comparison of the Nipper avirulent isolate (FT10002) with Mallala = 0.53 ($P<0.02$) and with Horsham raw data = 0.68 ($P<0.001$). Results were comparable between the two field sites i.e. correlation coefficient = 0.92 ($P<0.001$). Data for Horsham PBA trial in 2014 were supplied by Dr Matthew Rodda Vic DEPI.

D. **Release of ascochyta blight ascospores from infested lentil stubble.** Experiments were conducted to investigate the timing of release of ascospores of *Didymella lentis* (the sexual phase of *A. lentis*) from lentil stubble. Stubble was was collected from commercial lentil crops (PBA Flash, Nugget and Nipper) on Yorke Peninsula and lower north region after harvest for four season (Dec 2010 to 2013). Pouches of the stubble were incubated at Maitland SA in 2011 to 2014 (assisted by Sam Holmes) and also at Bute in 2014 (assisted by Sam Trengove). Each fortnight from early April to July a pouch was sent to DAFWA laboratory at Northam WA for spore counts. In all years the ascospores were released from the beginning of May, continuing until mid-June or the end of July (Figure 2). This timing identifies that delayed sowing is unsuitable for reducing infection from ascospores. However a fungicide seed dressing would minimise infection from these airborne spores and is recommended for all lentil crops. Data from this work have been analysed by DAFWA scientist Dr. Moin Salam to develop an ascospore release model. Data indicate that spore maturation is triggered by cold temperature (<10C) and spore release by rainfall.

Table 1: Summary of *A. lentis* isolate testing on 5 differential hosts in controlled conditions

Host	Resistance category	1989-2006 isolates	2010-2013 isolates	2014 isolates
Cumra	R-MR	0 (0.0%)	17 (27.0%)	1 (4.8%)
	S-MS	17 (100.0%)	46 (73.0%)	21 (95.5%)
Northfield	R-MR	13 (76.5%)	50 (79.4%)	2 (9.1%)
	S-MS	4 (23.5%)	13 (20.6%)	20 (90.9%)
Nipper	R-MR	13 (76.5%)	55 (87.3%)	7 (31.8%)
	S-MS	4 (23.5%)	8 (12.7%)	15 (68.2%)
ILL7537	R-MR	17 (100.0%)	63 (100.0%)	22 (100.0%)

	S-MS	0 (0.0%)	0 (0.0%)	0 (0.0%)
Indianhead	R-MR	17 (100.0%)	63 (100%)	22 (100.0%)
	S-MS	0 (0.0%)	0 (0.0%)	0 (0.0%)
	TOTAL	17	63	22

Figure 1. Number of lesions on lentil seedlings when incubated adjacent to commercial lentil stubble.

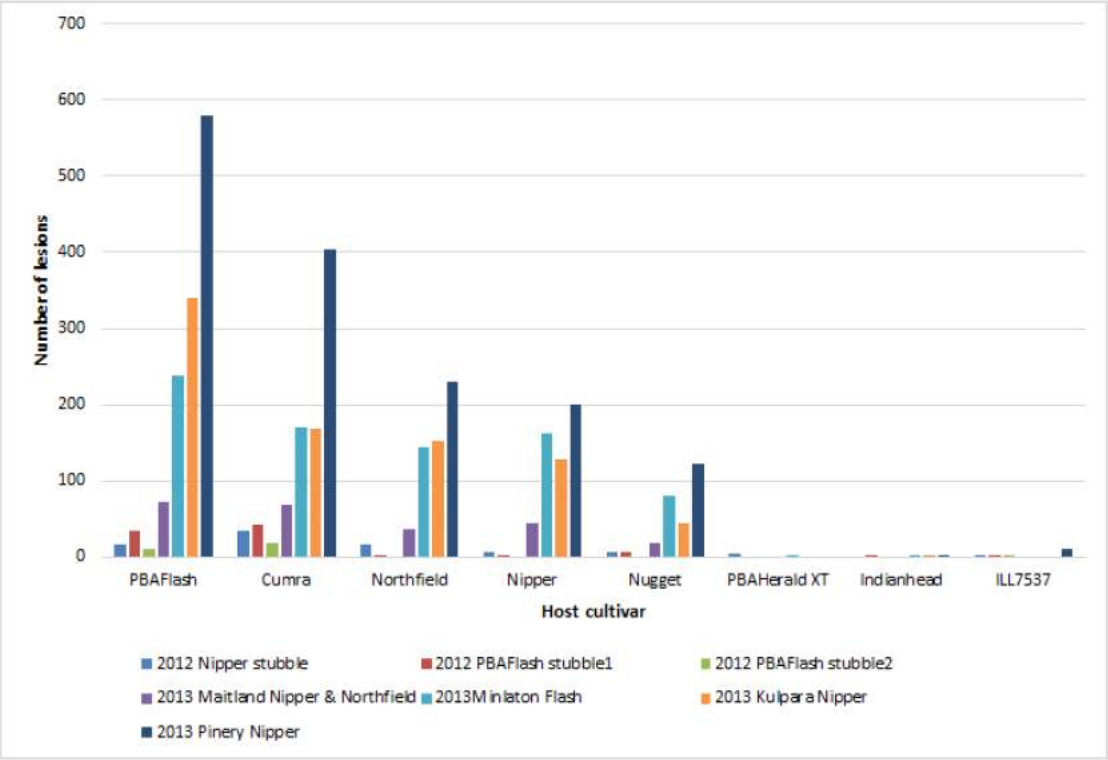
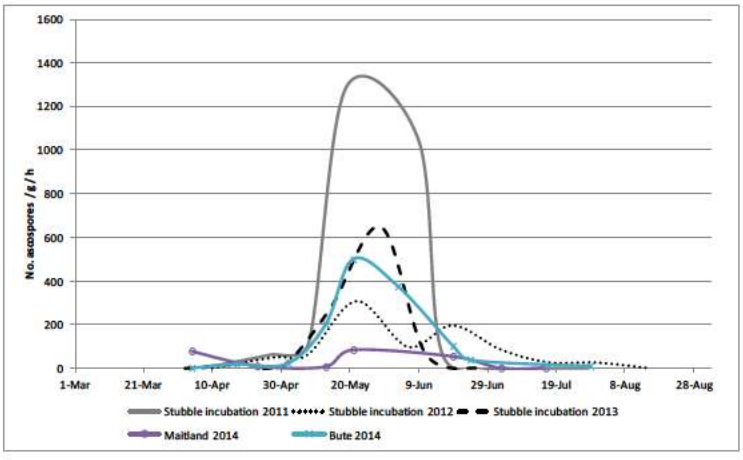


Figure 2. Numbers of ascospores per gram of stubble per hour released from lentil stubble naturally infested with *A. lentis* after incubation at Maitland or Bute in 2014, and at Maitland in 2011, 2012 and 2013. (Data generated by DAFWA Northam Laboratory)



Conclusions Reached &/or Discoveries Made

- Ascochyta blight of lentils (*A. lentis*) can infect the previously resistant cultivars Nipper and Northfield across South Australia and Victoria. These lines are now rated as Moderately Susceptible and will require foliar fungicides in wet seasons to prevent ascochyta blight infection on foliage as well as pod abortion and disease staining on seeds.
- Field disease scores of ascochyta blight on lentil NVT lines at Mallala in 2013 and Horsham in 2014 were highly correlated with disease scores from a Nipper virulent isolate in the controlled screening while an isolate not able to infect Nipper was less correlated with field scores. This indicates that isolates able to infect Nipper are widespread across southern Australia lentil growing regions.
- PBA HeraldXT, PBA Hurricane, PBA Ace and PBA Bolt remain resistant in field conditions.
- A few lesions can infect these resistant lines in controlled experiments, indicating that isolates with the potential to overcome their resistance are present in lentil growing areas. Selection for these aggressive isolates may occur in the future, especially if the cultivars are grown in tight rotations.
- Spore release studies using ascochyta infested lentil stubble, conducted over 4 years and two sites, found wind-blown spores were consistently released from stubble starting early May until the end of July. Variable rainfall patterns in summer and early autumn do not alter the timing of spore release. Seed dressings with thiram should help to reduce the infection from these airborne spores.

Intellectual Property

Isolates of *Ascochyta lentis* have been collected, tested for virulence patterns against a differential set and stored at SARDI. This information is made freely available to industry and other researchers and has no potential for commercialisation. A spore release model for ascochyta blight of lentil will be produced by DAFWA. Blackspot Manager model of field pea which is used as a basis for the *Ascochyta lentis* model is owned by DAFWA and GRDC. Outputs from these models are made freely available to industry and not intended for commercialisation.

Application / Communication of Results

Main findings

- Ascochyta blight of lentils (*A. lentis*) can infect the previously resistant cultivars Nipper and Northfield across South Australia and Victoria.
- Field disease scores of ascochyta blight on lentil NVT lines at Mallala in 2013 and Horsham in 2014 were highly correlated with disease scores from a Nipper virulent isolate in the controlled screening while an isolate not able to infect Nipper was less correlated with field scores. This indicates that isolates able to infect Nipper are widespread across southern Australia lentil growing regions.
- PBA HeraldXT, PBA Hurricane, PBA Ace and PBA Bolt remain resistant in field conditions.
- A few lesions can infect these resistant lines in controlled experiments, indicating that isolates with the potential to overcome their resistance are present in lentil growing areas
- Spore release studies using ascochyta infested lentil stubble, conducted over 4 years and two sites, found wind-blown spores were consistently released from stubble starting early May until the end of July. Variable rainfall patterns in summer and early

autumn do not alter the timing of spore release. Seed dressings with thiram should help to reduce the infection from these airborne spores.

Potential industry impact

- Nipper is now rated as Moderately Susceptible to ascochyta blight in southern Australia and will require foliar fungicides in wet seasons to prevent ascochyta blight infection on foliage as well as preventing pod abortion and disease staining on seeds.
- PBA HeraldXT, PBA Hurricane, PBA Ace and PBA Bolt all have a similar heritage for ascochyta blight resistance i.e. from Indianhead. Selection for aggressive isolates on these lines is likely to occur in the future if the cultivars are grown in tight rotations.
- The timing of spore release from lentil stubble indicates that thiram based seed dressings are important to reduce infection from this source.

Extension

- The results of this study have been communicated to farmers and industry in the enewsletter CropWatch, at Hart Field Days, GRDC updates in Adelaide, Crop Science Society, the Pulse Breeding Australia summer newsletter, and the Inaugural Pulse Breeding Australia Conference (October 21-23, Adelaide). A scientific paper of the findings of this study has been prepared for submission to the online journal Frontiers in Plant Science which is running a special topic on Ascochyta; guest editors Dr J Davidson SARDI, Dr S Fondevilla University of Cordoba and Dr W Chen USDA (draft manuscript is attached).

Path to market

- Results and isolates have been shared with the lentil breeder in Victoria (Dr Matt Rodda) and researchers in the GRDC funded project DAV00126 'Molecular Markers for Pulse Breeding Programs - Phase II' in VIC DEPI (Dr Sukhjiwan Kaur and Dr Muhammad Javid). Isolates of *A. lentis* collected in South Australia have been submitted to Melbourne University (Assoc. Professor Rebecca Ford) for molecular analyses and to Curtin University (Dr. Judith Lichtenzveig) for DNA sequencing and development of resistance effector assays.

POSSIBLE FUTURE WORK

- GRDC have provided funds via Curtin University to continue research on monitoring virulence of populations of *A. lentis* across Australia as well as the other ascochyta fungi that infect pulse crops. SARDI has a subcontract with Curtin as part of this project to collect isolates and conduct phenotyping against differential sets.
- Additional sources of resistance for *A. lentis* are vital for the lentil breeding program.
- Alternative, new actives for foliar fungicides are required for the pulse industry, including lentils, to manage ascochyta blight. Some of these fungicides (eg. active ingredients pyraclostrobin and azoxystrobin) are already registered in international markets where they are proven to be effective.

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