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FINAL REPORT 2013

PROJECT CODE :	S1310R

PROJECT TITLE
Field evaluation and development of advanced multi-trait strand medic lines

PROJECT DURATION

Project Start date	1 July 2010
Project End date	30 June 2013

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PROJECT REPORT

Executive Summary

This project commenced in 2010 with the agronomic evaluation of a group of early generation strand medic hybrids with new traits of powdery mildew resistance, root-lesion nematode tolerance and improved rhizobial promiscuity. We have identified a small group of material which has excellent agronomic performance with respect to dry matter production and seed yield, exceeding our benchmark strand medic cultivars (Herald, Angel and Jaguar) in 2012 by up to 35%.

- The lines were derived from a cross made between Angel strand medic and a new line originally selected for powdery mildew resistance. They have powdery mildew resistance, SU herbicide tolerance, aphid resistance, and larger seed size.
- Further selections have been made on this material to stabilise traits and if the level of agronomic improvement can be confirmed in larger scale field trials (including grazing) at additional sites, there are excellent prospects for a future commercial release.
- Further improvements in medic production have been measured in response to rhizobial inoculation on several farms in the low rainfall Mallee. The work highlights the importance of inoculation and confirms that frequent grower reports of poor nodulation in the Mallee should be taken seriously. Inoculation will be strongly advocated for the new medic cultivars being developed.
- New evidence gained by this project again confirms that medics are appropriately classified as moderately resistant (MR) to *Pratylenchus neglectus*.

Project Objectives

To assess the potential of advanced multi-trait strand medics for commercial development.

More specifically this project will:

- Evaluate the agronomic performance of 25 advanced medic lines possessing various combinations of important new traits;
- Determine the benefit *Pratylenchus neglectus* root lesion nematode (RLN) tolerance has on medic production and measure the change in nematode populations after growing these medic lines;
- Make *in-situ* field selections from segregating medic lines under evaluation at the field sites;
- Screen lines possessing elite field performance for traits that are still segregating;
- Initiate seed increase of most promising line(s) for commercial release.

Overall Performance

Project objectives:

As a result of this project we have been able to short-list six lines for further cultivar development. Over the three years of the project the germplasm contributing to these six lines have consistently excelled in agronomic performance with respect to dry matter production and seed yield, exceeding our benchmark strand medic cultivars (Herald, Angel and Jaguar) in 2012 by up to 35%.

The lines were derived from a cross made with Angel strand medic and a line originally selected for powdery mildew resistance. Rapid progress has been made in stabilising the traits of interest and ongoing re-selection has resulted in these six non-segregating lines which now possess the traits of: powdery mildew resistance, SU herbicide tolerance, aphid resistance, and larger seed size.

Ongoing seed multiplication has enabled them to be further evaluated in larger scale field trials in 2013 at three sites (Rudall, Netherton and Karoonda) under a new SAGIT project (S1213). The larger plot size is to enable agronomic performance to be monitored under grazing (for the first time) in the year of regeneration (2014). We believe there are excellent prospects for one of these lines to be commercialised as a new cultivar, targeting especially the lighter soil types typical of the lower rainfall Eyre Peninsula and Murray Mallee dune swale land forms.

Nematode tolerant line RH1 and several bred lines have shown improved root health in the presence of *Pratylenchus neglectus* in the field. However, the correlation between root damage, herbage production and nematode level was low. Other factors may be over-riding the benefits of nematode tolerance in the field and need to be better understood before further development of the nematode tolerant material can be justified. However, we were able to show conclusively that medics are moderately resistant to *Pratylenchus neglectus* root lesion nematode (RLN) based on consistent reductions of nematode number after the growth of medic.

Participants in project:

SARDI: Scientists directly involved in the project include Jake Howie, Ross Ballard, David Peck and Barbara Morgan, and we especially acknowledge the highly competent technical support provided by Jeff Hill.

Collaborators: We are indebted to the following people for allowing us access to their farms and for their ready cooperation: Neville Rowe, Arthurton; Lester & Kay Cattle, Netherton; Peter & Hannah Loller, Karoonda; Trevor & Cath Pocock, Lameroo; Andrew & Helen Barr, Pinery; Roy Latta/Ian Richter, Minnipa Agricultural Centre.

Key Performance Indicators (KPI)		
KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
1. Field sites selected & established Three experiments were established, managed and monitored at Minnipa (EP), Arthurton (YP) and Karoonda (MM).	Y	01/07/2010
2. Root assessments completed at nematode site Root damage assessment completed at Arthurton (five medic genotypes +/- nematicide). NB Detailed root assessments were also completed at Pinery in 2013.	Y	31/12/2010
3. 1st year agronomic performance data monitored, recorded and analysed Dry matter production and analysis of seed yield has been assessed at all sites and analysed.	Y	28/02/2011
4. Nematode multiplication quantified Measurement of initial nematode number completed. Sampling of the 2011 sown trial was also undertaken in Jan 2012 to provide additional data on nematode multiplication under medic. Multiplication at Pinery was also assessed in Feb 2013.	Y	31/05/2011
5. Additional field sites selected & established Sites were established at Lameroo, Netherton and Karoonda.	Y	01/07/2011
6. 2nd year agronomic performance data recorded and analysed. Review of all data - Stop/Go event All data, including seed yields, have been analysed and after review, entries have been shortlisted for sowing in 2012 trials. A recommendation regarding the stop/go event was made in the 2011 Progress Statement and to the SAGIT board at the 2011 spring review of projects.	Y	15/03/2012
7. Most promising lines seed increased A final shortlist was developed and non-segregating PM lines were seed increased at the Waite in 2012 for future development. These have been harvested and are now being processed.	Y	31/12/2012
8. 3rd year agronomic performance data (regeneration) recorded and analysed All data, including seed yields, have been analysed and after review, six entries have been shortlisted for sowing in larger scale trials in 2013 for the new SAGIT project due to start July 1 2013.	Y	28/02/2013

Technical Information

Background – agronomic evaluation

As part of this project, *in-situ* field selections and glasshouse screening have been regularly undertaken of medic lines demonstrating elite field performance in order to stabilise several traits that were still segregating. Based on the excellent performance at multiple sites in 2010 and 2011, a set of 17 non-segregating strand medic hybrids with various combinations of powdery mildew (PM) resistance, SU tolerance, aphid resistance and large seeds, was shortlisted for sowing in 2012. This included eight homozygous aphid resistant daughter lines for field testing to ensure they perform as well agronomically as the segregating PM parent lines from which they were selected. Also included were five benchmark cultivars and parents and, in response to farmer feedback at field days and measures of poor nodulation in 2010 & 2011 field trials, we also included some rhizobial treatments.

2012 sown trials – agronomic evaluation

Two experiments were successfully established in the Murray Mallee at Netherton and Lameroo, enabling further evaluation of dry matter production, disease tolerance and seed yield. Two further sites consisting of un-replicated larger scale demonstration plots were also sown at Karoonda and Minnipa Agricultural Centre, adjacent to existing regenerating trials, providing a useful talking point for field days held at both sites.

A feature of the promising hybrids was again increased early season vigour, possibly a benefit of the larger seed size of the original PM resistant donor parent. We were very encouraged with the agronomic performance of the PM lines with respect to growing season dry matter (DM) production. At Lameroo the top five PM lines (range: 84–95 of % maximum site yield (MSY); average 89%) significantly out-yielded the benchmark strand medic cultivars, Herald, Angel and Jaguar (range: 55–71% MSY; avg. 66%). At Netherton the top five PM lines (88–95% MSY; avg. 91%) out-yielded the strand medic cultivars (70-81% MSY; avg. 76%).

Pod and seed yields, which provide a critical measure of potential pasture persistence and future productivity, were also measured. The harsh spring finish in 2012 provided a rigorous test of their ability to produce seed and persist. In previous years they have been excellent; eg at Netherton 2011 the PM resistant lines averaged 1100 kg/ha of seed, 30% greater than Herald and Angel. Again at Netherton in 2012 they were excellent: the top five PM lines (avg. 1120 kg/ha of seed) and significantly out-yielded the strand medic cultivars (avg. 822 kg/ha); conditions were harsher at Lameroo but the top five PM lines still out-yielded their cultivar checks (720 cf 592 kg/ha).

Powdery mildew resistance – field observations

Netherton 2011: a natural powdery mildew infection occurred which affected > 80% of leaves of most lines except the PM lines which displayed negligible powdery mildew symptoms (see attached paper). Premature leaf senescence resulting in severe defoliation is a typical expression of severe mildew infection and we observed significantly less leaf drop on the PM lines (12-24%) than Herald and Angel (54-70%).

Karoonda 2011: a natural powdery mildew infection occurred on the 2010/11 regeneration in early spring and the shortlisted PM lines showed much lower levels of powdery mildew infection (12-34% leaf infection; nb included background Harbinger) than Herald and

Angel (52-66%). Naturalised Harbinger medic at the site showed severe infection and defoliation and this may have implications for pasture rotations still reliant on old cultivars.

Karoonda 2012: Although experimentally quite variable, this site generally responded very well to winter rains with the best plots producing an estimated 4 t/ha DM. This provided a useful talking point at the Karoonda MSF Field Day where we were able to point out the PM lines looking fresh and showing no signs of powdery mildew infection whereas Herald and Angel, although also growing well, were developing heavy PM infection in the understory.

These were the first opportunities we have had to observe the impact of powdery mildew on the PM lines in the field and we were encouraged in that so far they support our results from greenhouse studies and field observations at the Waite Campus. However it is important to note that more fundamental research regarding the identification, pathogenicity and prevalence of different races of powdery mildew (if more than one) in SA is needed so that appropriate breeding strategies can be developed to ensure that the excellent levels of resistance in the current set of PM lines is maintained.

Ability to persist and regenerate

2012 regeneration of 2010 Minnipa Agricultural Centre site:

After growing very well in 2010, this site was sown to canola in 2011 and regenerated successfully in 2012 enabling two dry matter assessments to be made in August and September (MAC Field Day). As this has been our only site regenerating after a crop, it was pleasing to note the good performance, relative to the strand medic cultivars, of parental bulk lines which had subsequently been promoted (via their selected non-segregating progeny) to trials in 2011 and 2012.

Hardseed breakdown studies:

Pods of short-listed PM lines and both parents (Angel and PM2) were harvested from the Netherton 2011 site and taken back to the Waite Campus for hardseed breakdown studies conducted over 12 weeks from February to May 2012. At the end of the study Angel's hardseed content had declined from 99 to 88% and PM2 from 97 to 91%. The PM hybrid lines declined in hardseededness from 96-100% to 87-91%, very similar to both parents. This coupled with the Minnipa 2012 regeneration data, provides us with confidence that this material possesses an appropriate level of hardseededness for persistence in a ley farming system.

Coumestrol/isoflavone testing

As part of preliminary duty-of-care studies, senesced plant material from the Netherton site of two short-listed PM bulk lines (PM-250, PM-262) and their parents (Angel and PM2) was sent to the WA ChemCentre for testing the presence of phyto-estrogenic compounds such as coumestrol. Coumestrol is a coumarin derivative which accumulates in medic plants during the growing season with levels increasing in response to stress such as those induced by foliar fungal attack (Francis, 1965). Both oestrus and ovulation in sheep can be inhibited by very high coumestrol levels (> 1000 ppm) but at lower levels (< 400 ppm) only ovulation rate is affected (Collins and Cox, 1984).

In this study Angel plant matter contained 200 ppm coumestrol whereas PM2, 250 and 262 were below detection level (<20 ppm). As noted above, a natural powdery mildew infection

occurred in spring at Netherton which affected most lines, except the PM lines, which displayed negligible powdery mildew symptoms (Ballard *et al.* 2012). It is possible that the elevated coumestrol levels observed in Angel have been induced by the fungal infection and if so, suggests another significant benefit of targeting powdery mildew resistance in medics. It is possible that anecdotal reports of low fertility in Merinos on Upper Eyre Peninsula in 2011/12 (Ali Frischke, *pers. comm*.) could be linked to sheep grazing medic based pastures heavily infected with powdery mildew.

Root lesion nematode studies

Assessment of medic lines selected for tolerance to root lesion nematode:

Assessments of the field tolerance (reductions in root damage) and agronomic fitness (growth) of medic lines with putative tolerance to *Pratylenchus neglectus* was undertaken at three field sites with high levels of the nematode. RH1 (tolerant parent) showed consistent reductions in root damage and increased root weight, compared to Herald. However, this did not translate to improved shoot growth, because RH1 was generally less well adapted than Herald. The evaluation of various bred lines (Herald × RH1) showed lines Z2365, Z2371 and Z2463 to be most productive and the best source of nematode tolerance to transfer the trait into new strand medics.

Quantifying the impact of Pratylenchus neglectus on medic growth:

The relationship between nematode number in soil and medic production remains ambiguous. On the one hand, positive medic growth responses were measured on several occasions when Temik nematicide was applied to 32 multi-trait medic lines at Arthurton in 2010. Spring medic production was increased by 15%, associated with a 50% decrease in nematode number (from 30 to 15 per g soil), and small but significant reductions in root damage. On the other hand, there was no evidence of relationship between medic spring herbage production and nematode number at a second site sown at Pinery in 2012. At that site, nematode levels had been successfully manipulated to range between 2 and 66 nematodes per g soil following the growth of 19 cereals or fallow the previous year in collaboration with Dr Alan McKay's GRDC nematode program. Herald and RH1 medics were sown across the range of nematode levels. The lack of medic production response was consistent with the prior assessment of root damage which was similar across the nematode levels, despite the putatively tolerant line RH1 having much less root damage overall (Figure 1) and greater root weight (+35%), when compared to Herald.

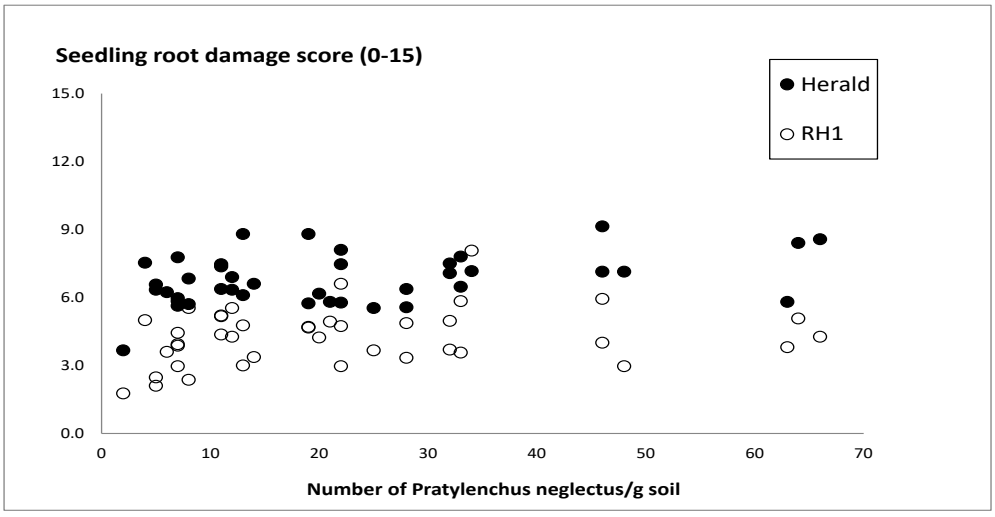

Figure 1. Relationship between root damage to Herald (closed circles) or RH1 (open circles) and number of root lesion nematodes (*Pratylenchus neglectus*) per g soil, at Pinery in 2012.

There have been a number of indications, based on root damage symptoms and DNA analyses of roots and soil that other soil microbes (both pathogens and beneficials) are negating the expected production responses. While growth room studies leave no doubt that the nematode has the potential to severely damage medic roots and that tolerant medic selections including RH1 have good levels of tolerance, these benefits are unlikely to be realised until the impact of other soil microbes are understood and can be managed.

Changes in Pratylenchus neglectus number under medic pasture (resistance)

Root lesion nematode number was reduced by medic pasture in three trials, by between 33 and 59%.This assessment was completed at three trial sites. At the first site, nematode number under eight medic lines was reduced on average by 33%, from 31 to 21 nematodes per g soil. At two other sites, where nematode levels had been manipulated using different wheat cultivars in the year prior to sowing medic, nematode number was similarly reduced under the medic plots (Fig. 2). Where nematode number was initially moderate (19 per g soil after EGA Wylie wheat), medic pasture reduced the number by 47%, down to 10 per g soil. Where nematode number was initially high (39 per g soil after Brookton wheat), medic pasture reduced the number by 59%, down to 16 per g soil. Herald and RH1 medics resulted in similar reductions in nematode number.

This data provides compelling evidence that medics do not multiply root lesion nematode (*Pratylenchus neglectus*) under field conditions. Medics are appropriately classified as moderately resistant.

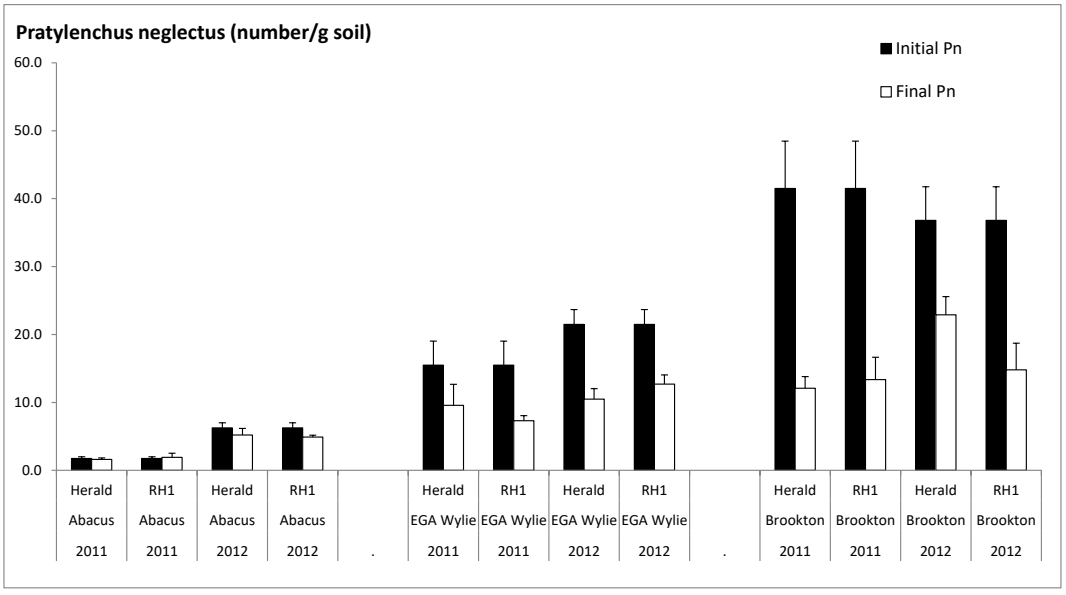

Figure 2. Number of root lesion nematodes (*Pratylenchus neglectus*) in soil, before (closed bars) and after (open bars) medic pasture in the field at Arthurton SA (in 2011) and Pinery SA (in 2012). Initial nematode numbers varied according to the cereal host (Abacus, EGA Wylie or Brookton) grown the previous year.

Conclusions Reached &/or Discoveries Made

We have identified a small group of material which has excellent agronomic performance with respect to dry matter production and seed yield, exceeding our benchmark strand medic cultivars (Herald, Angel and Jaguar) in 2012 by up to 35% for dry matter and seed yield. The lines were derived from a cross made with a line originally selected for powdery mildew resistance and Angel strand medic. They have powdery mildew resistance, SU herbicide tolerance, aphid resistance, and larger seed size. Further selections have been

made on this material to stabilise traits and if the level of agronomic improvement can be confirmed in larger scale field trials (including grazing) at additional sites, there are good prospects for a future commercial release.

Nematode tolerant line RH1 and several bred lines have shown improved root health in the presence of *Pratylenchus neglectus* in the field. However, the correlation between root damage, herbage production and nematode level was low. Other factors may be over-riding the benefits of nematode tolerance in the field and need to be better understood before further development of the nematode tolerant material can be justified.

Further evidence gained by this project shows conclusively that medics are appropriately classified as moderately resistant (MR).

Large Responses to rhizobial inoculation were measured in the field. The reasons remain to be elucidated but in the interim all future trials will be "over inoculated" to ensure symbiotic constraints are minimised and inoculation of the new medic lines will be strongly advocated when they are commercialised. The work confirms that frequent grower reports of poor nodulation in the Mallee should be taken seriously and some effort is needed to elucidate why this is occurring.

As discussed with the SAGIT Project Manager and Scientific Officer in the 2012 spring review, we strongly believe the excellent agronomic performance of the PM hybrid lines to date merits potential commercialisation and that rapid progress has already been made in stabilising the traits of interest. A new SAGIT project seeks to complete this promising work.

Intellectual Property

This project has generated IP through the identification, re-selection and short-listing of an elite cohort of agronomically superior lines. There is a strong possibility that one of the short-listed lines will be commercialised and released as a new cultivar as an outcome of a subsequent SAGIT project (S1213). Assuming Plant Breeders Rights (PBR) are sought, SAGIT will have an equity share of royalties on the sale of seed, proportional to their investment and the investment of others.

Application / Communication of Results

Main findings

- We have identified a small group of material which has excellent agronomic performance with respect to dry matter production and seed yield, exceeding our benchmark strand medic cultivars (Herald, Angel and Jaguar) in 2012 by up to 35% for dry matter and seed yield.
- The lines were derived from a cross made with a line originally selected for powdery mildew resistance and Angel strand medic. They have powdery mildew resistance, SU herbicide tolerance, aphid resistance, and larger seed size.
- Further selections have been made on this material to stabilise traits and if the level of agronomic improvement can be confirmed in larger scale field trials (including grazing) at additional sites, there are good prospects for a future commercial release.
- Responses to rhizobial inoculation were measured in trial work at several field sites.

When combined with frequent grower reports of poor nodulation in the Mallee, our recommendation is that all medics sown in Mallee soils should be inoculated. Some effort is needed to elucidate why poor nodulation is occurring.

- Nematode tolerant line RH1 and several bred lines have shown improved root health in the presence of *Pratylenchus neglectus* in the field. However, the correlation between root damage, herbage production and nematode level was low. Other factors may be over-riding the benefits of nematode tolerance in the field and need to be better understood before further development of the nematode tolerant material can be justified.
- Additional evidence gained by this project confirms that medics are appropriately classified as moderately resistant (MR).

Potential Industry Impact

Target zone: The estimated area in South Australia of cropping soils with pH >6.5 and average annual rainfall below 350mm suited to strand medics is over 3.9 million hectares, mainly located in the Upper Eyre Peninsula, Murray Mallee and Mid/Upper North (*nb also large areas in Vic, WA & NSW*). In these low rainfall areas, medics are ideally suited to the relatively low input farming systems and provide a cost-effective means of adding biologically fixed nitrogen to the system. Other benefits include providing a disease break for following cereal crops and a reduction in economic risk compared to intensive cropping.

Based on the often poor state of existing medic pastures, we believe it is possible to improve the production of many pastures by at least 100% (eg from 1.8 t/ha to 3.8 t/ha, Latta and Crettenden, 2012) with the sowing of improved cultivars, higher inputs and improved management. On nitrogen contributions alone this could represent an annual return of $45,000 (eg 500 ha x 2 t/ha x 30 kg N/ha/t x $1.50/kg N) on an 'average' cropping property. In addition to this is the value of the increase in wheat yield arising from reduced cereal diseases (Davoren *et al*, 2011) as a result of more competitive, productive and persistent medic pastures. The resistance/tolerance to powdery mildew and SU herbicide residues bred into the putative new cultivar, as well as its improved agronomic performance, should make reaching this target even more feasible. One measure of adoption will be sales of seed of the new cultivar in which SAGIT will have an equity based share of the royalties.

Where powdery mildew is present, the benefit of the new lines is visually obvious and likely to promote uptake by growers.

Publications and extension activities

The scientists involved on this project have addressed multiple field days and written many articles outlining its progress and results to date. These are captured in detail in earlier progress reports but as an example the activities from 2012 are outlined below:

- Jake Howie and Ross Ballard addressed ~175 farmers and advisers (including the GRDC Southern Panel) at the Karoonda Mallee Sustainable Farming Trial Site Walk on 4th of September 2012. Adjacent to two experiments illustrating outcomes from the current SAGIT funded project they discussed the encouraging results from a group of powdery mildew resistant medic lines and significant responses to rhizobial inoculation on certain Mallee soils as well as other aspects of pasture improvement, medic agronomy, disease and nodulation issues.

- David Peck attended the Minnipa Field Day on 12 September 2012. He spoke to ~120 farmers in front of the 2010 regeneration and 2012 demonstration strips illustrating outcomes from their current South Australian Grains Industry Trust funded project.
- RA Ballard, DM Peck, DL Lloyd, JH Howie, SJ Hughes, RE Hutton and BA Morgan (2012). *Susceptibility of annual medics (Medicago spp.) to powdery mildew (Erysiphe trifolii).* Proceedings of 16th Agronomy Conference 2012. University of New England, Armidale, NSW 14-18th October 2012. http://www.regional.org.au/au/asa/2012/pests .
- Howie Jake, R Ballard and D Peck (2013). Powdery mildew resistant medics for the Mallee and Eyre Peninsula. Mallee Sustainable Farming Results compendium, 2012. http://www.msfp.org.au/docs/research_111.pdf
- Howie Jake, R Ballard and D Peck (2013). Powdery mildew resistant medics for the EP and Mallee. Eyre Peninsula Farming Systems Summary 2012, pp. 135-137.
- Howie Jake, R Ballard and D Peck (2013). Powdery mildew resistant medics for the EP and Mallee. Upper North Farming Systems, 2012 Harvest Report, pp. 23-26.
- Howie Jake, R Ballard, D Peck and J Hill (2012). *Multi-trait medics for the Mallee*. Karoonda Field Day Information Booklet 2012. Mallee Sustainable Farming Inc., pp. 27-30.
- Howie Jake, R Ballard, D Peck and R Latta (2012). *Multi-trait medics for the Mallee*. 2012 Minnipa Field Day, 12th September, pp. 28-30.

Suggested path to market

If the new project confirms the agronomic performance of the short-listed lines under grazing, seed of the new cultivar will be bulked up and made available to the seed company making a successful bid in an open tender process. It is proposed that SAGIT be an integral part of this selection process if deemed appropriate by SAGIT. SARDI Feed & Forage group has well established links with the pasture seeds industry to facilitate this process.

Barriers to adoption

The initial cost of pasture establishment (eg seed, fertiliser, machinery etc) will always be an impediment to pasture improvement and the uptake of new cultivars. However regular presentations at field days at Karoonda MSF (2010 – 12) and Minnipa Agricultural Centre (2010, 2012) indicate a high level of interest in this material, particularly from farmers in areas that have suffered significant damage to their pastures from powdery mildew and loss of livestock production. Responses are highly visible and therefore will encourage uptake. This coupled with a larger seed size which should help to keep the cost of seed down, suggests a ready market for this new variety.

POSSIBLE FUTURE WORK

Further research regarding the identification, pathogenicity and prevalence of different races of powdery mildew (if more than one) in SA is needed so that appropriate breeding strategies can be developed to ensure that the excellent level of resistance in the current set of PM lines is maintained. The current suite of tolerant medic lines provides a valuable resource to undertake such studies and also quantify broader impacts of the pathogen, for example on feed utilisation by livestock. One particular aspect worth more detailed study is the potential role of this pathogen in elevating levels of coumestrol, a phytoestrogen which is linked to reduced sheep fertility.

Further work is needed to elucidate the reasons for poor base level nodulation measured at Netherton, Lameroo and Karoonda when compared to nodulation in several rhizobia inoculation treatments incorporated into the trials. The durability of these inoculation responses and ability to top-up the rhizobia in existing pastures are also issues requiring clarification. Ballard and Howie are seeking to address some of these issues in a submission to the recent CFOC Innovation Grants round, seeking to demonstrate novel and low-cost methods to re-introduce effective rhizobial strains into an existing medic pasture base.

Finally, there have been a number of indications based on root damage symptoms and DNA analyses of roots and soil that other soil microbes (both pathogens and beneficials) negated the expected production responses to *Pratylenchus neglectus* tolerance. While there seems significant opportunity to further improve medic through reductions in root damage, better understanding of the pathogen complexes is needed before such studies are likely to be fruitful.

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