Final report

New Powdery Mildew Resistant and Spineless Barrel Medics for Temperate and Subtropical Australia

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Abstract

Barrel medics are widely grown annual pasture legume species with high nutritive value and protein levels. The leaf disease powdery mildew (PM) is the most widely recognised disease of barrel medics and all existing cultivars are susceptible. This project developed the PM resistant barrel medic cultivar Emperor by transferring PM resistance from the strand medics cultivar Seraph into barrel medic cultivar Paraggio by way of an inter-specific cross followed by backcrossing. Farmers are increasingly valuing spineless medic pods and often sow spineless burr medics when their soil type is better suited to barrel medics. We backcrossed the spineless trait into barrel medic cultivar Sultan-SU six time to develop the new cultivar Penfield. The project used speed breeding and achieved 3.9 and 5.1 generations per year for the mid-season cultivar Emperor and the early season cultivar Penfield respectively. Speed breeding will allow the new cultivars to be commercial in 6.5 years of starting the breeding, which is at least three years earlier than if traditional breeding methods were used. Emperor and Penfield are expected to be readily taken up by farmers. The spineless cultivar Penfield will enable barrel medic breeders to use accessions with long spines and high agronomic performance in their breeding. The two cultivars will be commercially released in autumn 2022.

Executive summary

Background

Powdery mildew (PM) infection of annual medics is the most recognised disease of annual medics. PM has increased in recent years and causes loss of livestock production due to reduction of dry matter combined with stock avoiding PM infected medics and increased levels of phytoestrogens which explains reports of reduced fertility from sheep grazed on PM infected pastures. Secondly, we have become aware that farmers are growing spineless burr medics as a way of reducing vegetable fault in their wool on soils that are better suited to barrel medics. The outcomes of this project are: 1) a PM resistant barrel medic cultivar; 2) a spineless barrel medic cultivar; 3) factsheets to assist the uptake of the new cultivars. The target audience is farmers and their advisors in low to medium rainfall areas with mildly acidic to alkaline soils. The results of the research will be two new cultivars which farmers can grow as high-quality pastures, being high in protein which is valuable to actively growing and lactating animals. The spineless cultivar will also allow barrel medics breeders to use accessions with long spines and high agronomic performance in their breeding programs.

Objectives

1. A barrel medic cultivar resistant to powdery mildew — Achieved (cv. Emperor)
2. A barrel medic cultivar with spineless pods — Achieved (cv. Penfield)
3. Fact sheets developed to assist the uptake of new varieties — Achieved

Methodology

Use speed breeding techniques to backcross traits into existing cultivars

PM resistance from strand medic cultivar Seraph into barrel medic cultivar Paraggio

Spineless trait from an old cultivar Cyfield (1969) into the modern cultivar Sultan-SU

Conduct pre-commercial seed build and PBR DUS trial

Produce fact sheets for the two new cultivars

Results/key findings

This project has developed a PM resistant barrel medic cultivar called Emperor and a spineless cultivar called Penfield. These are the first annual pasture legume cultivars developed using speed breeding methods with the first commercial sales in autumn 2022. This is 6.5 years from starting the breeding and at least three years earlier than if traditional breeding methods were used.

Benefits to industry

The PM resistant cultivar Emperor will avoid animal production losses of reduced liveweight gain and reduced fertility reported from PM infected medic pastures. The spineless cultivar Penfield will allow farmers who wish to minimise vegetable fault in their wool the option to grow a barrel medic cultivar with several valuable traits. The spineless trait is controlled by a single gene and Penfield will allow barrel medic breeders to use accessions with long spines and high agronomy in their breeding.

Future research and recommendations

This project has demonstrated that speed breeding is an efficient way of developing new cultivars and it is recommended that speed breeding be used to develop new cultivars for inbreeding species such as subclover, annual medics and serradellas. Speed breeding will also allow breeders to breed for increased agronomic performance and not just to overcome constraints. It is recommended that MLA, S&W seeds and SARDI meet to discuss if there are other breeding objectives to develop into a project.

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1. Background

Annual medics have been sown on over 24Mha and perform well on mildly acidic to alkaline soils (Nichols et al. 2012). These soils are widespread in the low and medium rainfall zone. Livestock numbers of the low and medium rainfall zone have increased in the 21st century due mainly to increased price of sheep meat. Annual medics have high quality and are a valuable source of protein for grazing animals which is particularly important for growing and lactating animals. Annual medics fix nitrogen and provide high quality pastures to livestock. In recent years farmers are increasingly planting annual medics instead of subclover due to the high levels of hardseed present in annual medics providing better long-term persistence. Climate change predictions are for increasing frequency of droughts (Moore and Ghahramani 2013) and high levels of hardseed will become increasingly important. The most widely sown annual medic species is barrel medic (Nichols et al. 2012).

S&W Seed is a large producer and seller of annual medic seed (and other pasture species) in Australia. S&W Seed have a large amount of market information as a result of contact with farmers either directly or via resellers across Australia. This market intelligence was reviewed along with changes in Australian farming systems, climate change predictions and developments in annual medic improvement. This review identified that:

1. Powdery mildew is a widely reported disease of annual medics which reduces animal production and farmers report reduced fertility in sheep grazing PM infected medics
2. The spineless trait in burr medics is highly valued by farmers and farmers frequently ask when will this trait will be available in barrel medics

Red meat producers require that their production system to be efficient and that their pastures are more productive and persistent than ever before. Powdery mildew is increasing in frequency and existing annual medic cultivars are susceptible to PM (Ballard et al. 2012). SARDI has identified a PM resistant strand medic, and when this project started they were well advanced in the development of PM resistant strand medic cultivar (subsequently released as the cultivar Seraph). However, barrel medics are more widely grown than strand medics and there is currently no work in the development of a PM resistant barrel medic. Barrel and strand medics can be crossed, and inter-specific problems of low fertility, abnormal plants and segregation distortions exist in F_2 population (Simon and Millington 1967). However, backcrossing into target species overcomes the inter-specific problems (Crawford et al. 1989, Peck and Howie 2012). Several traits have been transferred between barrel and strand medics with the barrel medics cultivars Mogul, Sultan-SU, Jester-SU, Cheetah and Lynx and the strand medic cultivar Herald starting with an inter-specific cross (IP Australia). We consider it highly likely that PM resistance can be transferred from strand medic into barrel medic. Paraggio will be used as the recurrent parent due to its ability to delay PM infection (Ballard et al. 2012) and it is the most widely grown barrel medic cultivar. Paraggio has the following traits: phoma resistance that enable it to maintain low phytoestrogen levels (Barbetti 2007) and therefore sheep fertility; bluegreen aphid resistance (Peck and Howie 2012); tolerant to high levels of boron (Bogacki et al 2013); low levels of hardseed for a medic (Latta and Quigley 1993, Peck and Howie 2012) that allows for high early feed; high persistence in a ley system (Latta and Quigley 1993).

S&W Seed market the spineless burr medic variety Cavalier and have become aware that farmers value the fact that the spines do not get caught in sheep wool. This results in farmers growing Cavalier when their soil type suggests that a barrel medic would be better suited. Barrel medics have had more breeding done on them and have more traits (Nichols et al. 2012). Cyfield was bred to be spineless and released in 1969 (Oram 1990) but was not widely grown. Brownlee and Denney (1985) showed that Cyfield and Tornafield (disc medic) spineless pods do not get caught in sheep wool, but the spiny cultivars do (figure 1). The spineless trait in annual medics is controlled by a single recessive gene. Sultan-SU is the most recently released barrel medic variety (released autumn 2014), it was bred to be tolerant of SU herbicide residues (also tolerant to intervix residues) and has resistance to bluegreen aphid and spotted alfalfa aphid, and boron tolerance (Peck and Howie 2012, tested as Z2438). Sultan-SU will be used as the recurrent parent and we will achieve backcross six which will provide >99% chance of maintaining each trait.

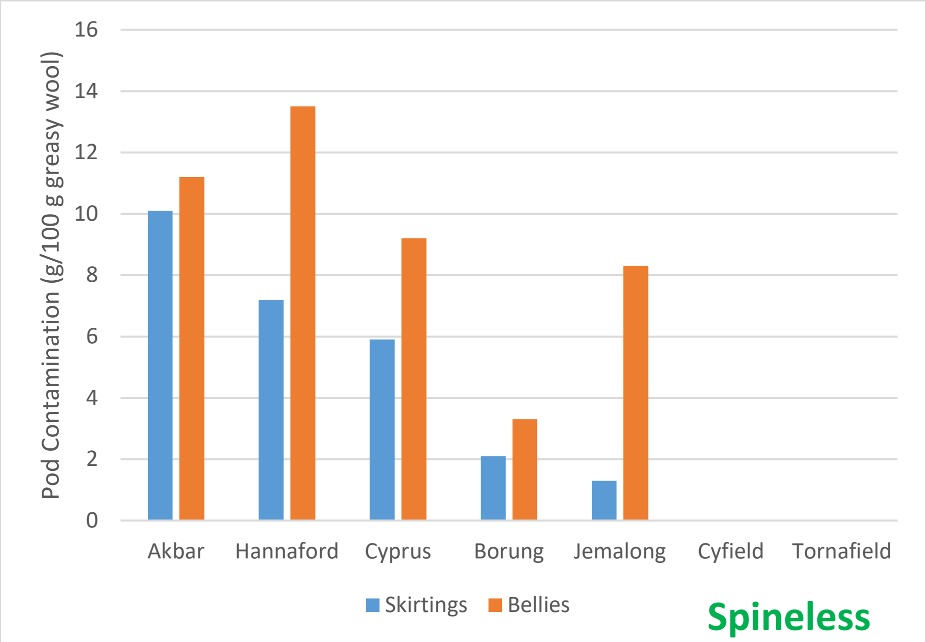

Figure 1: Pods from the spineless barrel medic cultivars Cyfield and disc medic Tornafield do not contaminate wool, whereas spiny barrel medic cultivars Akbar, Hannaford, Cyprus and Borung pods get caught in wool. Brownlee and Denney 1985.

Barrel medic cultivars have relatively short spines to minimize wool contamination and accessions with long spines are not used in breeding (Crawford et al. 1989). A modern spineless cultivar will allow accessions with long spines and high agronomic performance to be used in breeding. The spineless trait is controlled by a single recessive gene which means when crossed with accessions with long spines, 0.25 of F_2 plants will be spineless and 0.5 of F_2 plants will carry the spineless trait. The development of a modern spineless cultivar will be a breeding tool for the future.

Speed breeding techniques (Pazos-Navarro 2017) were developed in the MLA funded “pre-breeding in annual pasture legumes” project (P.PBE.0037) that can bring new cultivars to farmers quicker than if traditional breeding methods were used. The two cultivars developed will be the first known cultivars of annual pasture legumes developed using speed breeding methods in a pasture legume breeding program. Often in industry funded breeding program/s there is a delay between the completion of the breeding and the pre-commercial seed build up and ultimately a delay in new cultivars being commercially taken up. This project has the commercial seed company S&W Seed as part of the project from the start which will ensure there is no delay from when the breeding work is done, the pre-commercial seed production, and marketing of the new cultivars.

2. Objectives

This project will complete the breeding, selection, evaluation, PBR and pre-commercial seed build-up of a powdery mildew barrel medic cultivar. Objective met and cultivar Emperor will have first commercial sales in autumn 2022.

This project will complete the breeding, selection, evaluation, PBR and pre-commercial seed build-up of a spineless barrel medic cultivar. Objective met and cultivar Penfield will have first commercial sales in autumn 2022.

A fact sheet will be developed for both cultivars which will provide farmers with key details about the cultivars. Objective met.

3. Methodology

3.1 Speed Breeding

Speed breeding has been developed for sub clover (Pazos-Navarro 2017) which was also shown to be suitable to annual medics. Time to flowering is minimised by growing plants in a controlled environment room (CER) set at ideal temperatures (24 °C day and 18 °C night) for maximum growth combined with 20 hours of light and for long season cultivars placing geminating seed into a refrigerator for up to seven days. Freshly ripened subclover and annual medic seed have embryo dormancy (do not germinate when scarified and allowed to imbibe). Pazos-Navarro (2017) avoided embryo dormancy by rescuing immature seeds into tissue culture before embryo dormancy has developed. To avoid the laborious tissue culture method, we harvested mature pods and overcome embryo dormancy by placing pods in oven overnight at 45 °C, imbibing seed and placing in refrigerator for four days (Garcia et al. 2006) followed by placing them at 15 °C in the dark (Bolingue et al. 2010). This is expected to add ~ ten days per generation but save a lot of labour and allow for spare seed to be harvested as insurance for each breeding stage. Plants were grown in a controlled environment room supplied by The Plant Accelerator, Australian Plant Phenomics Facility.

3.1.1 Choice of parents

For each cultivar target, a single cultivar was chosen as the recurrent parent as this will minimise the field evaluation required. Paraggio was chosen as the recurrent parent for the PM cultivar as Paraggio: has an ability to slow PM infection (Ballard et al. 2012); is the most widely grown barrel medic; resistant to phoma which maintains low phytoestrogen levels (Barbetti 2007); resistant to bluegreen aphids (Peck and Howie 2012); tolerant to high levels of boron (Bogacki et al 2013); low levels of hardseed for a medic (Peck and Howie 2012) that allows for high early feed; high persistence (Latta and Quigley 1993). Paraggio is a mid-season cultivar. The new strand medic cultivar Seraph was bred to be PM resistant and was the PM donor parent.

Sultan-SU was chosen as the recurrent parent for the spineless cultivar as it is the most recent barrel medic cultivar released. It was bred to be tolerant of SU herbicide residues (also tolerant to intervix residues) and has resistance to bluegreen aphid and spotted alfalfa aphid, and boron tolerance (Peck and Howie 2012, tested as Z2438). Sultan-SU is an early season cultivar. The Australian Pasture Genebank has multiple spineless accessions in their collection. However, we used the old cultivar Cyfield due to it being a cultivar that had spineless trait (and large seed size) backcrossed into Cyprus and that our recurrent parent Sultan-SU contains a large amount of Cyprus in its breeding. Sultan-SU is a BC4 Caliph (Peck and Howie 2012, tested as Z23438) which in turn had aphid resistance backcrossed two times into Cyprus (IP Australia). The spineless trait is controlled by a recessive gene and spineless pods can be readily distinguished 5-7 days after flowering.

3.1.2 Inter-specific problems.

Barrel and strand medics can be crossed but have inter-specific problems of abnormal plants, low fertility, and segregation distortions (Simon and Millington 1967). However inter-specific problems can be overcome by backcrossing and commercial cultivars Mogul, Sultan-SU, Jester-SU, Cheetah, Lynx and Herald have been released. To allow us to select against inter-specific problems we will achieve multiple F1 plants for each backcross. To minimise unobserved inter-specific problems occurring we want to achieve BC4 which is two more then where inter-specific problems are expected to be observed.

When making inter-specific crosses between barrel and strand medics we have observed that some individual genotypes when crossed have less inter-specific problems than others. For this reason, we chose the cultivars Parragio, Sephi and the male sterile line tap as our barrel medic parents for the inter-specific cross. BC1 is the hardest to achieve due to low fertility in inter-specific plants. Achieving multiple BC1 plants is required due to potential segregation distortions and to allow selection amongst F1 BC1 plants for low levels of inter-specific problems. As we are using speed breeding, we are unable to conduct a PM screen of bred lines before making our crosses. This means we have to achieve many more F1 BC# plants than if we knew if the pollen parent was PM resistant or susceptible. We conducted PM screen of bred lines after we completed the next BC and from this, we knew which crossed pods have the potential to produce PM resistant offspring. When making crosses we historically used method three from Pathipanawat (1994) and achieve ~20% success rate. We tested the new crossing method of Veerappan et al. (2014) and we achieved ~80% success rate and subsequently used this method.

3.2 Powdery Mildew Screen

Powdery mildew infected annual medic plants were collected from the upper Eyre Peninsula by S&W Seed. A population was maintained in a glasshouse by regularly planting new pots of cultivar Jester. When we needed to conduct a screen, we established controls pots of Jester (very susceptible), Paraggio (susceptible) and Seraph (Resistant). Infected leaves were wiped over plants. PM screens were scored when all Paraggio plants had PM clearly visible. PM resistance was confirmed by scoring again one week later. When backcrossing was completed, we grew 704 F2 BC4 plants and selected 106 plants on dry matter and flowering time. We progeny tested (14 plants) these to find which F2 plants are homozygous PM resistant.

3.3 Seed Increase

Thirteen lines of each cultivar target were seed increased on weedmat at the Waite campus Adelaide. The recurrent parents Paraggio and Sultan-SU, mid-season barrel medics cultivars Jester and Lynx, early cultivars Caliph and Parabinga, strand medics cultivars Seraph and Angel, and spineless burr medic cultivar Cavalier were also seed increased. Field performance of each of the lines and cultivars were evaluated and measured, with seed harvested from all of the cultivars and eight lines for each cultivar target.

3.4 Field Evaluation

Field evaluation trials were established in small plots at Penfield (S&W Seed research farm) and Roseworthy. Penfield was a key site as it can be irrigated and hence protects us from a dry year which is important as only one field evaluation year was planned. The Penfield site was irrigated in spring to approximate an average rainfall year. Seed was sown at 10 kg/ha and four replicates used. Measurements are winter and spring dry matter production, seed yield and percent hardseed in late autumn. The hardseed study was done using standard method of placing pods inside pockets made of insect screen and pinning to the soils over summer-autumn (figure 3). Pods collected late April, wet up and allowed to germinate for 2 weeks, dried, hardseed extracted and percent hardseed determined relative to amount of seed present in a duplicate sample at time zero.


Figure 2: Harvesting medic pods.


Figure 3: Field hardseed study

3.5 Pre commercial seed build-up

3.5.1 Seed Increase short listed lines

At S&W Seed, research farm at Penfield, plots targeting ~ 2kg seed increase were hand sown in a paddock that had been medic free for many years. Barriers were erected between each plot. Pods were swept up and stored until field evaluation and hardseed studies were completed and final line chosen for release. Pods were aspirated, checked to ensure all pods are true to type, seed extracted, scarified and germination tested.

3.5.2 Seed Increase Emperor and Penfield

In 2020, 2 kg of seed of each variety was planted in a paddock located on the edge of a commercial medic seed increase paddock at Roseworthy. The paddock was regularly inspected to ensure plants were true to type and weed free. 200kg of clean seed of Emperor and 250kg of clean seed of Penfield was obtained. This was classified as breeders seed.

4. Results

4.1 Breeding

This is the first known example of using speed breeding to develop cultivars of a pasture legume species. Commercial seed will be available within 6.5 years of breeding commencing. This is at least three years earlier than if traditional breeding was used. We used the method that Pazos-Navarro (2017) developed in the MLA funded “pre-breeding in annual pasture legumes” project (P.PBE.0037). They saw the method as being suitable for single seed descent work. For this project we used it a backcrossing breeding project. We modified the method of Pazos-Navarro (2017) by harvesting seed rather than rescuing immature seed into tissue culture tubes.

4.1.1 PM resistant cultivar Emperor

Figure 4 provides an overview of each generation to create Emperor.

Generation	Activity	Description
1.	Inter-specific cross	Sephi x Seraph
2.	Grow F_1 plant	F_1
3.	Cross into Paraggio	Paraggio x F_2
4.	BC_2	Paraggio x F_1
5.	BC_3	Paraggio x F_1
6.	BC_4	Paraggio x F_1
7.	Grow F_1	F_1
8.	Select F_2	F_2
9.	Select F_3	F_3
10.	Seed Increase 2018	F_4
11.	Seed Increase 2019	F_5

Figure 4: Overview of each generation that created the PM resistant cultivar Emperor. Note $BC_{\#}$ refers to backcross into barrel medic.

Pods from the inter-specific F1 plants were harvested, threshed and the pods contained 1-2 seeds per pod compared to 5-7 in the parents. This low level of fertility is typical of inter-specific crosses between barrel and strand medics. F2 seeds were sown and as expected from inter-specific cross (Simon and Millington 1967, Peck and Howie 2012), many plants were chlorophyll deficient and had low vigour and fertility. However about 10% had reasonable growth and pod set. The first backcross was difficult to achieve due to the poor pollen fertility in interspecific plants. We were unable to achieve any BC1 using the crossing method of Pathipanawat et al. (1994) but readily obtained pods with the method of Veerappan et al. (2014). We subsequently adopted Veerappan et al. (2014) method and achieved ~80% success compared to ~ 20% with Pathipanawat et al. (1994).

Generations 1-9 were conducted using speed breeding methods at a rate of 3.9 generations per year, compared to 2 generations per year typical in backcrossing program for a mid-season barrel medics cultivar. For generation 1 we also crossed Seraph into Paraggio and tap. Sephi x Seraph had lower inter-specific problems than the other two genotypes which continued into generation 3. From generation 4 onwards we only used the backcrosses that started with Sephi x Seraph. Inter-specific problems of chlorophyll deficient leaves (i.e. yellow) or reduced fertility (low pods per plant and/or low seeds per pod) were selected against and were not observed from generation five (BC2) onwards. PM resistance is controlled by a single dominant gene and each backcross was conducted such that a minimum chance of 99% of being successful (i.e. achieve minimum of seven F1 seeds from seven different male parents). For generation 8 we grew 704 plants and selected 106 plants which we progeny tested to find plants homozygous for PM. 13 lines were selected to go into seed increase and field observation (generation 10).

4.1.2 Spineless cultivar Penfield

Figure 5 provides an overview of each generation to create the spineless cultivar Penfield.

Generation	Activity	Description
1.	Initial cross	Sultan-SU x Cyfield
2.		F_1
3.	BC_1	F_2 x Sultan-SU
4.	BC_2	Sultan-SU x F_1
5.		F_1
6.	BC_3	F_2 x Sultan-SU
7.	BC_4	Sultan-SU x F1

8.	BC_5	Sultan-SU x F1
9.		F_1
10.	BC_6	F_2 x Sultan-SU
11.		F_1
12.	select plants	F_2
13.	SI field 2018	F_3
14.	SI field 2019	F_4
15.	SI field 2020	F_5

Figure 5: Overview of each generation that created the PM resistant cultivar Emperor. SI = seed increase

Spineless pods do not get caught in sheep's wool (Figure 1) and easily distinguishable from pods of current cultivars that have short spines (Figure 6). Generations 1-12 were conducted using speed breeding methods at a rate of 5.1 generations per year, compared to 2.5 generations per year achieved for early season cultivar using traditional breeding. When we used spineless F2 plants to make our crosses we had to wait a week before we could classify plants as spineless or spiny. This is an illustration of the need to factor in some delays when planning a speed breeding project. However growing many female plants and the adoption of efficient crossing method allowed us to achieve enough crosses on the first day of crosses.

Figure 6: Penfield has no spines whereas existing barrel medic cultivars have short spines.

The spineless trait is recessive, and we used spineless F2 plants as the female when we made BC1, BC3 and BC6. For BC1, we planted many F2 seeds into soil treated with low level of SU herbicide and selected early flowering spineless plants with tolerance of SU herbicide residues. The spineless donor parent is later flowering than Sultan-SU and we selected early flowering plants as parent to make BC1. For the subsequent generations, flowering time of all plants were similar to recurrent parent Sultan-SU.

4.2 Field Evaluation

4.2.1 Seed Increase and field evaluation

The PM cohort dry matter production and flowering time were similar to each other and the recurrent parent Paraggio. A natural PM infection occurred and Paraggio developed PM much later than other barrel medic cultivars which supports the finding of Ballard et al. (2012). Late in the season we had a week of showery humid weather and PM developed on Paraggio and several of the PM-lines. However, five PM-lines did not develop any PM and are therefore PM resistant. This suggests that the PM progeny screen of generation 8 was not severe enough, but the previous ones were. For progeny screen of generation 8, we had many more plants growing per pot, much higher percentage of PM resistant plants and greater percentage of Paraggio in their breeding which is likely to contribute to the false identification of homozygous PM resistant plants. Waite seed increase plots always get infected with PM and was important to rescreen PM status of lines. Five PM infected lines had seed collected, processed, and prepared for 2019 field evaluation trials. PM resistance of the chosen line Emperor was confirmed in 2020 and 2021

The spineless cohort differed in DM scores. Plots were un-replicated so it may be due to field position. We selected the lines with the highest DM scores to progress to the field evaluation.

4.2.2 Field Evaluation

Both field sites established well. However, the Roseworthy site suffered unexpected herbicide damage when sprayed with a grass herbicide while the Penfield site suffered limited herbicide damage that it rapidly recovered from. The damage at Roseworthy was due to the wetter bringing residues of the herbicide hammer (last herbicide used) into solution during the transport of the spray equipment from Penfield to Roseworthy. The Roseworthy site did not recover and was abandoned. 2019 was a dry year and the ability to irrigate the Penfield site to an average spring was vital in getting field evaluation results.

Table 1 presents the winter dry matter scores, spring dry matter score, seed yield and mean % max of the bred lines and the comparator cultivars. Paraggio outperformed the other mid- season cultivars Jester and Lynx. PG08 was the top performing PM resistant line and had similar performance as its recurrent parent Paraggio. PG08 was chosen as the PM resistant cultivar Emperor. The recurrent parent Sultan-SU provided higher performance than the early season barrel medic cultivars Caliph and Parabinga. The bred lines performed well with the best performing line being PG16, which was chosen as the spineless cultivar.

Barrel medic cultivars are required to have suitable hardseed levels to persist through 2-3 years of cropping while providing adequate early season growth. Annual medic breeders typically target hardseed levels of 70-90%; cultivars Paraggio, Parabinga, Jester and Sultan-SU have suitable levels of hardseed while Caliph is considered too high (Peck and Howie 2012). Hardseed levels are affected by the environment of the growing season, summer, and autumn. Hardseed levels were higher than expected due to cool wet (irrigated) spring combined with cool February and March. For each barrel medic cultivar, we plotted hardseed levels against hardseed levels recorded in 2008-09 by Peck and Howie (2012), (Figure 7). The hardseed levels in 2019-20 are higher than in 2008-09 but there is a strong linear relationship between the two years. The new cultivars Penfield and Emperor had similar hardseed levels as their recurrent parents Sultan-SU and Paraggio respectively and hence considered to be a suitable level. Soft seed of Penfield of 95 kg/ha and Emperor 230 kg/ha, this level of soft seed will provide high amount of early dry matter (Silsbury 1979).

Table 1: Winter dry matter score (0-100), spring dry matter score (0-100), seed yield (kg/ha), mean percent maximum (winter DM, spring DM and seed yield) and percent hardseed for PM cohort, spineless cohort, recurrent parents Paraggio and Sultan-SU and other medic cultivars.

line	Winter score	Spring score	Seed Yield	Mean % max
PM cohort				

Paraggio	84	83	1534	87
PG08	82	80	1633	87
PG09	78	79	1327	81
PG11	76	76	1486	80
PG07	68	71	1442	75
PG06	70	61	1546	73
PG05	73	61	1470	73
PG04	68	67	1299	71
PG10	72	51	1321	67
Jester	75	65	1707	79
Lynx	83	57	1240	72
Seraph	55	50	1075	56
Angel	55	36	1315	54
Spineless cohort				
Sultan-SU	83	62	2011	85
PG16	86	72	2384	96
PG25	85	74	2246	94
PG24	82	72	2196	92
PG19	86	68	2108	90
PG26	81	72	1943	87
PG17	72	73	2112	87
PG14	77	67	2116	86
PG21	67	70	1747	78
Cavalier	83	71	1935	88
Parabinga	84	67	1529	81
Caliph	74	47	1757	72
lsd	17	19	517	

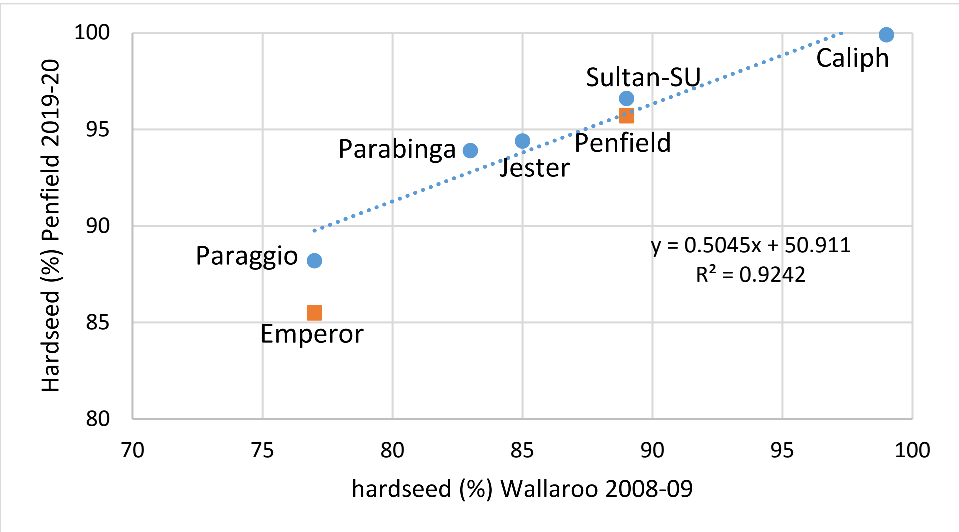

Figure 7: Hardseed levels of barrel medic cultivars in 2019-20 plotted against hardseed levels 2008-09 reported by Peck and Howie (2012). The new cultivars Penfield and Paraggio are plotted against their recurrent parents Sultan-SU and Paraggio.

4.3 Pre-commercial seed increase

4.3.1 Target 2kg

Pods were collected from all lines and stored until data was reviewed and lines to make cultivar decided. It was decided to limit the amount of seed for long term storage and most seed was scarified and planted in the 2020 seed increase. Typically seed increase targeting 5kg is done the first growing season after the decision is made to release a cultivar. By seed increasing 2 kg of seed of all shortlisted lines we were able to bring the cultivar to market one year earlier. Using most of the seed for the 2020 seed increase maximises the pre-commercial seed increase. Usually more seed is kept back as insurance in case of some disaster such as a drought or a bushfire (e.g. 2015 Pinery bushfire burnt > 86,000 ha of farmland including medic seed increase paddocks) destroying the crop before seeds are harvested. The insurance was another 2kg of seeds at S&W Seed research farm which could be irrigated.

4.3.2 Breeders Seed increase 2020

The first stage of breeders seed production for any seed variety is critical to it's success. Importantly with medic varieties, paddock history and hygiene must be adequate to ensure high yield of pure breeders seed. 2kg of each variety was sown in 0.4ha at Roseworthy nearby to the S&W Penfield research site at Virginia, South Australia, with a target production of 100-200kg. Planting and harvest was completed using research scale equipment due to the relatively small production area. Inspections on the growing crop were conducted regularly to agronomically manage the production, monitor growth to ensure purity and meet or exceed the required tolerances for pure seed production.

4.3.3 Pre-commercial Seed Increase 2020/21

Emperor

- 44 kg produced in 2020
- 6 hectares planted for 2021 production
- Estimate 900 kg/ha production however has sustained some hail damage

Penfield

- 151 kg produced in 2020
- 20 hectares planted for 2021 production
- Estimate 1,000 kg/ha production however has sustained some hail

4.4 Commercial Seed Production and Marketing

S&W Seed Company has a network of medic seed growers with the capacity to produce several hundred tonnes of certified medic seed every year under seed certification. Penfield and Emperor will be classified as leading varieties within the S&W portfolio and pushed to scale production as quickly as possible. Given the suitability and potential of both Penfield and Emperor as varieties, total production will be targeted to reach 80t and 50t of clean seed a year respectively by 2023 as these products mature in the portfolio.
S&W Seed Company has a national sales network with 13 territory sales managers covering all key areas for temperate legume varieties. Combined with a national distribution network of depots to supply retailers, access to Penfield and Emperor for growers will be exceptional. S&W Seed Company

supplies all retailers, including independent and corporate businesses. There will be equitable access for all retailers to procure Penfield and Emperor.
In 2021 multiple trial sites were sown including both Penfield and Emperor in NSW, South Australia and Victoria. These sites demonstrate performance across a range of geographical conditions and a range of enterprises, such as large-scale dryland grazing operations, over sowing subtropical grasses, under-sown into cereal crops and traditional mixed faming rotation with broadacre grain and pulse crops. The aim of this demonstration series is to showcase how the improved varieties with novel traits can perform locally and in a wide variety of situations.

4.4.1 Seed Production

Penfield and Emperor will be released for sale in 2022, available in both straight run product as well as customised seed blends. Estimates of available seed for 2022 is 20t and 5t for Penfield and Emperor respectively, subject to harvest and cleaning. Similar to previous variety releases (e.g. Seraph) production is planned to increase to accommodate the demand from known and current medic markets but also to explore and develop potential areas of agriculture that can benefit from the agronomic packages of Emperor and Penfield.
Planned production estimates for 2023 include 52t of Penfield and 18t of Emperor. Export opportunities will be reviewed to best promote sales growth and maintain critical mass to ensure the latest genetic material is readily accessible to all Australian producers.
From 2023 onwards projected production is 50 tonnes of Emperor and 80 tonnes of Penfield to be produced each year. Options are available to increase production volumes should demand be forecast to outstrip supply.

4.4.2 Licence Agreement

The IP of this project is the generation of a new PM resistant barrel medic and a spineless barrel medic cultivar. The IP split was agreed in the development of the project. Draft licence agreements were developed in the first six months of the project and set aside until breeding and evaluation was completed and line chosen for cultivar release. This was to allow for cultivars to be described and referred to by name. The cultivars were developed by backcrossing program and sister lines have the same attributes, similar performance and not distinguishable from the lines chosen for cultivar release. This means they have no value going forward.

4.4.3 Marketing

S&W’s commercial marketing strategy has been in place since 2019 to promote the release of these new medic varieties. In 2019, S&W’s 14 national district representatives met at Penfield, SA and reviewed the two new cultivars and discussed medic agronomy. They then inspected the evaluation trial seeing Emperor and Penfield performance relative to existing cultivars. This early awareness of the products will assist the early uptake of the new cultivars. Further demonstrations in-person were reduced through 2020 and 2021 due to COVID-19, however more than 10 demonstration sites are planned for the 2022 season.

Fact sheets have been developed for both cultivars of which extracted information will be entered into S&W’s 2022 autumn Emerge edition. 250,000 copies of Emerge will be dispatched nationally into rural newspapers and dropped via mail distribution to S&W’s farmer mail database and key agricultural consultants. Fact sheets will be readily accessible via the S&W website with a focus on the promotion of these new varieties. In addition to the initial release of information, there will be distribution of fact sheets via field days, local focus style info days with retailers and farmer cooperatives and finally targeted marketing distribution via regional newspapers.

Social media campaigns across various platforms will be undertaken to promote these new varieties. From February 2022 onwards, S&W will be highlighting the novel traits and benefits of these varieties to farmers. S&W's goal will be to communicate the agronomic packages, clearly describing the points of differentiation including improved forage production, increased end product quality (reduced vegetable matter in wool) and improved livestock forage quality/animal health benefits.

In 2021, tactical trial sites were initiated planting current commercial cultivars as a comparison to Penfield and Emperor. Due to travel limitations (COVID-19) these trials were located in Mallee SA, Eyre Peninsula and the Mid North of SA. Comparative yield/forage data was collected for regional sales promotion and marketing activities. Given seed supply timing and the volume was restrictive, 2022 will present an opportunity to increase the number of localised forage demonstration and evaluation sites. Typically, sites are used purely as a demonstration purpose however additional seed stocks will make available opportunities for replicated sites demonstrating the productive value to farmers and improve management tools to maximise pasture production and return on investment. This will likely be in the form of sowing rates, sowing depth, seed applied insect management, best means establishment, weed management, grazing management, blend formulation and any other localised issues or areas of interest. The outcomes of these trials will be to draw together marketable data for future years as well as a series of field days throughout the course of the year at different stages noting differences between early performance, winter production, spring grazing/hay performance, maturity and seed set.

Key markets for the broadly adapted Penfield and Emperor range across Australia. Regions such as the northern, eastern and southern wheatbelt in WA, Eyre and Yorke peninsula, mid north, Riverland and mallee in SA, western Vic, mallee, Sunraysia and northern Victoria, all of NSW, southern and possibly central west Queensland are all potential target markets for these products. We believe the demand for these products will steadily increase over time as the diverse use and benefits within each region and agricultural production system becomes better understood.

4.5 Agronomy Conference Paper

A paper has been accepted by the agronomy conference and will be presented in February 2022. The paper will be published online after the conference. The focus of the paper is: 1) introduce agronomists to the two new cultivars; 2) use the two new cultivars as an example of what can be achieved with speed breeding and modifications made to speed breeding methods to make it more practical.

4.6 PBR protection

Penfield and Emperor are both bred lines. DUS trials have been completed and part 2 application submitted to IP Australia. The PBR part 2 examination fee invoice has not yet been generated or processed as the DUS trial was delayed until 2021. The invoicing by IP Australia, and payment by SARDI is expected to be finalised in the coming weeks.

Emperor is PM resistant and is distinguishable from its recurrent parent on this basis (figure 8). Penfield is spineless and is distinguishable from its recurrent parent Sultan-SU on this basis (figure 9).

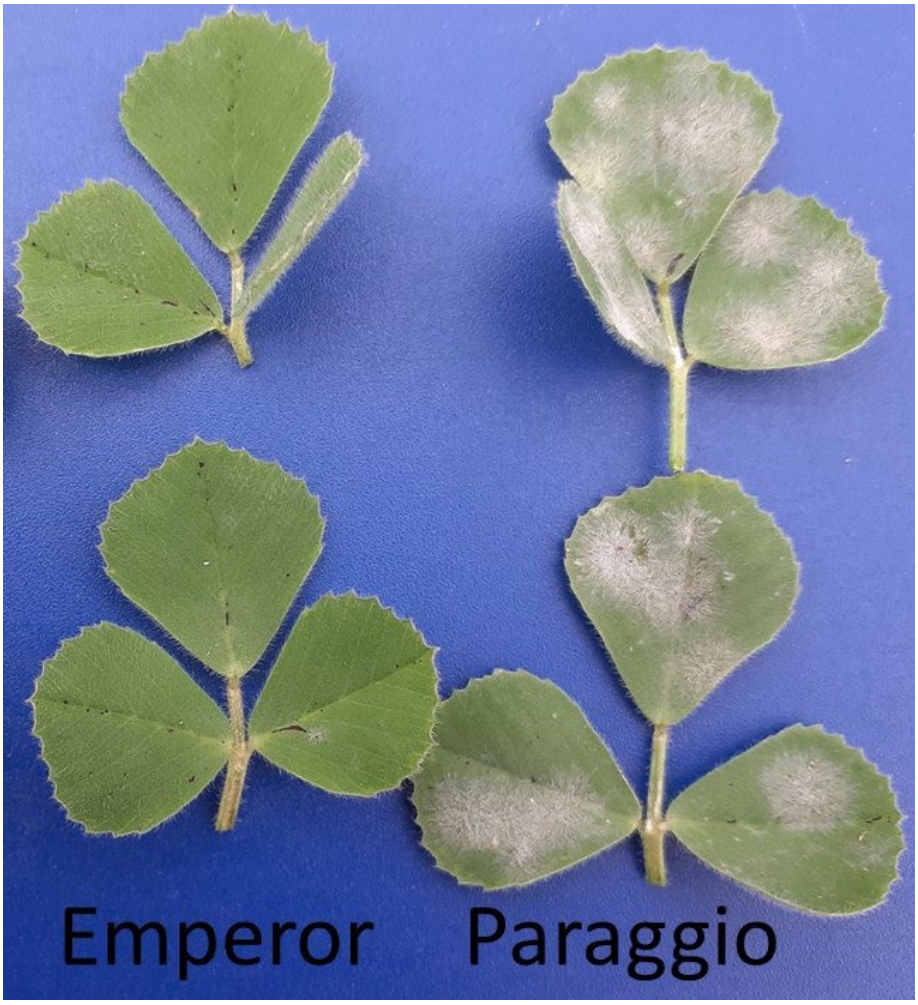

Figure 8: PBR distinguishing photograph. Barrel medic – ‘Emperor’ is resistant to powdery mildew whereas ‘Paraggio” is susceptible

Figure 9: PBR distinguishing photograph. Barrel medic – 'Penfield' has spineless pods whereas comparator 'Sultan-SU' has spiny pods

5. Conclusion

5.1 Key findings

- The PM resistant barrel medic cultivar Emperor will be available to farmers 6.5 years after breeding commencing
- The spineless barrel medic cultivar Penfield will be available to farmers 6.5 years after breeding commencing
- Recurrent parents of both cultivars have a range of traits valued by red meat producers
- Modifications were made to the speed breeding method which makes it less labour intensive
- This project has demonstrated what speed breeding can achieve and speed breeding should be considered for other inbreeding annual pasture legumes

5.2 Benefits to industry

Barrel medics are widely grown in southern Australia and their high quality and protein content make them valuable pastures for actively growing livestock. This project will deliver two new barrel medic cultivars with a range of traits that farmers are seeking. Productive pasture legumes not only benefit red meat producers but also the yield of following cereal crops. In the current Dryland Legume Pasture Systems project, regenerating medic pasture benefits to subsequent wheat were in excess of 90% with up to 2.9 t/ha extra wheat yield (Flohr et al. 2020). Large farming systems benefits from growing medic on mixed farms will increase the percentage of a mixed farm allocated to red meat production. The new medic cultivars Penfield and Emperor will increase the supply of medic seed to meet the expected increase in demand.

The project has demonstrated what speed breeding can achieve and made modifications to speed breeding which makes speed breeding more efficient. For inbreeding annual legume pastures species such as sub clover, annual medics and serradella, speed breeding can deliver new cultivars quicker and more efficiently than ever before. Australia is the world leader in the main annual legume species used in Australia (Nichols et al 2012) and hence unlikely to be able to obtain new cultivars from overseas.

6. Future research and recommendations

This project delivered two new cultivars of barrel medics to farmers within 6.5 years from the project beginning. This is at least three years earlier than if traditional breeding was used. The speed breeding method developed in the MLA pre-breeding project was modified by harvesting seed and overcoming seed dormancy in a timely fashion. This allows us to avoid the tissue culture step and greatly reduce the labour input and for us to grow each generation as a batch. To maintain speed, we needed to achieve backcrosses before we were able to determine the PM status of plants, which means more crosses were required. We adopted a new crossing method that achieved ~ 80% success rate compared to ~ 20% of our prior method.

Speed breeding makes breeding more efficient and therefore more breeding solutions should be considered. This project used speed breeding for backcrossing traits into existing cultivars. It is also ideally suited to develop single seed descent (SSD) populations prior to evaluation. The spineless cultivar is considered a breeding tool as it will allow accessions with long spines and high agronomic performance to be used in breeding programs.

The history of barrel medic improvement in Australia largely consists of two stages. Stage one was largely finding well adapted material either by finding naturalised medics with high performance (e.g. Jemalong) or conducting overseas collection trips searching for high performing accessions (e.g. Cyprus, Borung, Paraggio, Parabinga, Sephi). Stage two is backcrossing new traits into existing cultivars to overcome constraints. Examples are: aphid resistant cultivars Jester, Caliph, Mogul; tolerant to SU herbicide cultivars Sultan-SU, Jester-SU; PM resistant Emperor; spineless Penfield. We suggest that speed breeding can be used to commence stage 3, which is to cross cultivars/accessions with diverse backgrounds and selection focused on increased dry matter production. Speed breeding will also allow stage 3 breeding to be done on other inbreeding annual legume species. The spineless cultivar will also allow barrel medic accessions with long spines and high agronomic performance to be used in stage 3 breeding.

Having a seed company as part of the project ensures that the new cultivars will be taken up without any delay between completion of the breeding and the pre-commercial seed increase. The seed company and their network of trials and resellers is the main way in which the project outputs will be adopted in a timely fashion. This project developed two cultivars with clear traits that farmers are searching for, which will ensure that they are readily taken up. Seed bags will be branded with the MLA logo (and the S&W Seed Company logo). This will ensure growers recognise the collaborative development of the product and MLA financially supporting research into pasture cultivar development. With growers being aware that MLA fund pasture research it may encourage suggestions for future breeding.

The best pasture cultivars combined with the best management results in the best pastures and maximum potential for livestock production. MLA and S&W Seed Company have communication divisions which can provide audiences awareness of the new cultivars, what they can deliver and the best way to make use of the cultivars. The new cultivars have received a bullish reception, however closer work between MLA and S&W communication teams will assist the pull through of the cultivars over the medium term. Potential exists for an MDC project being developed on communication of the two new cultivars and agronomy required to maximise pasture production. S&W seeds have a network of trial sites demonstrating pasture cultivars. Agronomy trials could be co-located at these sites. Farmers are often unsure on the best way to establish new pastures and often by minimising costs reduce the chances of successful pasture establishment. An example of agronomic variables on pastures establishment includes sowing rate, sowing date, weed control, and date required to stop grazing to ensure good seed set by first year annual pastures.

This project has demonstrated that the adoption of speed breeding methods can efficiently develop new cultivars in a short time frame. It should inspire other speed breeding activities in other pasture legume species.

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