Turning sand into feed: developing a perennial legume (*Lebeckia ambigua*) for low-profitable soils

Northern Agricultural Catchment Council (NACC) report 2018

This project was delivered by Murdoch University, Landskills and the Cripps Family. It was supported by NACC through funding from the Australian Government's National Landcare Program.

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1.0 Introduction

1.1. The need for perennial legumes in WA agriculture

Perennial plants live for two or more years. They can enhance ley-farming systems because they may close the "feed gap", which refers to the period of time (usually summer and autumn) where there is normally little green feed for animals (Moore et al. 2009). Perennial legumes with deep and extensive root systems may reach the water table and remain green through summer (Dear et al. 2003). They may also increase water use efficiency in their vicinity and reduce the incidence of dryland salinity in low lying areas (Ridley and Pannell 2005). Perennial legumes can deliver highly nutritious foliage rich in protein and it is also thought that perennial plants are more resilient to changing rainfall patterns (Howieson et al. 2013).

Native perennial legumes that exist in the south west of WA are not suitable for agriculture, due to their defence mechanisms to deter grazing, such as low-palatability, accumulation of toxins and pungent foliage (Cocks 2001). Therefore, researchers have examined other global Mediterranean climates, such as in South Africa, to find suitable plants (Howieson et al. 2013). The fynbos region of South Africa was identified as a region worthy of examination because of its low rainfall and deep acid sands that resemble closely the Wheatbelt of south west WA (Beard et al. 2000). In the past, research into potential perennial cultivars has focused on the legumes suited to wetter areas (Cocks 2001). However, developing perennial plants suited to dry areas with nutrient depleted sands is likely to become important for WA agriculture as cropping these regions becomes riskier and meat and wool become more profitable.

1.2. *Lebeckia ambigua* - a suitable perennial legume for WA agriculture.

Lebeckia is a perennial suffrutescent (herbaceous above ground, woody below) legume from the western cape of South Africa (fynbos region). It is found in regions of low rainfall (<300mm) with soils of poor soil fertility and moderately low pH (4.5-6.5) (Le Roux and Van Wyk 2007, Howieson et al. 2008). These challenging environments are consistent with the eastern Wheatbelt of WA (Hobbs et al. 1995). The discovery of Lebeckia surviving in the challenging edaphic conditions of the fynbos made it appealing to researchers on expeditions there between 2002-2007 (Howieson et al. 2008). Adaptations such as needle like leaves and an extensive tap root have aided Lebeckia to survive in such extreme environments.

In order to obtain its nitrogen, Lebeckia forms a symbiosis with a special group of rhizobia that have only recently been discovered to be symbionts (within the last 10 years). Several of the bacterial clade (termed *Burkholderia)* have been described as new species and shown to have high nitrogen fixation efficiency with Lebeckia, which enables the plant to grow without artificial nitrogen fertiliser (De Meyer et al. 2013, Howieson et al. 2013).

Since Lebeckia and its symbiotic rhizobia have never previously been investigated for agriculture, there are more questions than answers in this challenging phase of legume and rhizobial domestication.

1.3. Cultivation of Lebeckia

Lebeckia is sown in early spring (a date that varies with the region of the state) because soil temperatures of above 20°C seem to be necessary for its germination and early vigour. The late sowing also allows for maximum weed control. Hence, there is some variability in optimal seeding times around WA: the northern Wheatbelt temperatures increase after winter before those in the south. Across all regions, however, this is an awkward time for sowing legumes, as the following rainfall diminishes in reliability. Lebeckia also has a small seed (Le Roux and Van Wyk 2007, Edwards 2015) so it has to be sown in the top 10mm of the surface for successful emergence, which exposes them to drying conditions (Howieson et al. 1995, Loi et al. 2005). Combining the required seeding depth with the optimal time of year means there is little soil moisture available for sowing Lebeckia seeds into, and for keeping rhizobia inoculants alive. At this stage, we have not been able to develop a granular carrier for the Lebeckia rhizobia.

2.0 Aim

The foremost aim of the recent experiments in the Northern Agricultural region (NAR) was to establish Lebeckia on deep sandy soils of the region and assess growth and persistence over three years, and thereby seek evidence of adaptation to the northern Wheatbelt climate. The region has a strong history of experimentation with perennial plants, including tagasaste, Rhode’s grass, Lucerne and native herbs such as Rhagodia, and farmers therein understand well the value of, and the challenges to, successfully implementing and managing perennial forages.

The second aim was to perform a survey of Lebeckia grown in the different soil types of the NAR, from coastal to inland areas. Comparison was made with establishment of Lucerne at all sites, and with Tedera at Buntine.

3.0 Demonstration site details

Table 1: Location and sowing details for Lebeckia in 2016. Full site data will be provided if requested.

FARMER/ LOCATION	SOIL TYPE	PH	SIZE (HECTARES)	SEEDING RATE (G/HA)	PRE SOWING OBSERVATIONS
Will Browne/ BADGINGARRA	Grey sand over gravel	5.3-6.4	1	350	Raddish problem
Boyd Carter/ BUNTINE	Wodgil	4.4-5.8	0.4	250	Huge weed and pest* burden
Jill Wilson/ KARAKIN 2016	Deep sand	5.9-6.0	0.8	300	Clean paddock
Jill Wilson/ KARAKIN 2017	Deep sand	5.9-6.0			Clean paddock
Cliff Harding/ MINGENEW* **	Deep sand	5.9-6.2	1	300	Clean paddock with pest* burden
Peter & Lloyd Cripps/ WEST BINNU	Deep sand	5.7-6.0	0.7	250	Clean paddock with pest* burden

*Pests refer to vermin; such as Kangaroos and rabbits

** Non NACC/NLP funded site

Prior to sowing Lebeckia, there were challenges identified by researchers and the farmers. Firstly, all paddock locations were subject to strong winds and therefore susceptible to furrow infill after sowing. Hence the best option was to add spaded tynes to the seeder, to make a shallow and wide furrow, for minimal infill. Secondly, vermin looked to be an issue at most sites due to the attractiveness of edible green pasture during summer where traditionally there is none. Hence where possible the sites were surrounded by rabbit proof fences, or baited for rabbits. Lastly, the abundance of weed seeds on the surface of the soil coupled with paddock weed history was a concern at Badgingarra and Buntine.

4.0 Methods

4.1. Examination of Lebeckia's adaptation to a variety of soils

Identification of the trial locations was left to the enthusiasm of farmers in different agricultural regions (i.e. Liebe Group, MIG, WMG, Landskills and Lloyd & Peter Cripps). Before sowing, soil sampling and analysis was undertaken. The soil was collected by a corer (10-20cm) and pogo (0-10cm) to ascertain the soil information. We were very interested in adaptation of Lebeckia to soils of low K and P status. Top soil was examined at Centre for Rhizobium Studies (CRS) Murdoch University to identify if any possible symbiotic rhizobia able to nodulate Lebeckia were resident.

Lebeckia seed was selected for the different areas and sown using an Aitchison mini seeder, with a split box (Figure 1 & 2) set to the configuration of three wide tynes 30cm apart followed by press wheels. One box contained Lebeckia seed inoculated with *Burkholderia dilworthii* (strain WSM4204) and Prima (gland clover) used as a filler (due to the low seeding rate desired).The other box contained wetter to maximise soil absorption of water. The seed was sown at a depth of 5cm. Potassium sulphate and magnesium sulphate were top dressed at a later date at some sites. The seeder was calibrated differently for each site as mentioned in section 4.2. Sowing times differed across the NAR as follows: Badgingarra- early September, Buntine- late August, Karakin- late September, Mingenew- mid September and West Binnu- late August.

Figure 1: Prof. Brad Nutt sowing Lebeckia with an Aitchison mini seeder at Karakin.

4.2. Identifying the optimal seeding rate for Lebeckia

A point to note is that Lebeckia is in a seed bulk-up stage and seed is not abundant, so consequently seeding rates in 2016 were quite conservative (Table 1). This allowed experiments to be sown at eight sites across WA, and two in South Australia.

Figure 2: Sowing Lebeckia at the most northern site in West Binnu on the property of Peter and Ann Cripps –discussion between Prof John Howieson, Prof Brad Nutt and Tom Edwards.

4.3. Developing a quality rhizobium inoculant for Lebeckia

Lebeckia is a wild plant and this makes it challenging to domesticate, especially with regards to the large amount of genetic variability in the species and the interaction of these types with rhizobial strains. We are seeking to ensure Lebeckia is optimally paired with its symbiotic rhizobia (*Burkholderia* spp.), another organism that has only been recently identified as a rhizobia (Howieson et al. 2013). Our pre-sowing glasshouse experiments indicated that there were no symbiotic *Burkholderia* spp. existing naturally in the target NAR soils. Experiments in the NAR were inoculated with a known high performing strain of *Burkholderia* (WSM4204). Inoculants were prepared in a sterile peat, where WSM4204 multiplied to more than 1 billion cells per gram of peat, and just prior to sowing that peat was adhered to the seed of Lebeckia.

4.4. Undertaking seed production studies and assessing differing hardseededness levels as affected by pod maturity

Seed production methodology was investigated by harvesting the stands of Lebeckia at sites that had been successfully established. Harvesting consisted of two methods; stripping pods by hand or with a mechanical reaper/ binder. Lebeckia pods that were brown or purple were picked and ripened in the glasshouse (many green pods were left to ripen and shatter onto the ground to increase the seed bank at all sites). Tests of mature seed in the laboratory followed a gentle drying phase (25°C for two weeks, in shade then two weeks at 40°C in a glasshouse), and consisted of scarification, germination and viability, comparing the different pod maturities at harvest (purple, green and brown). The results will be used to determine the correct time to harvest for maximum hardseededness and viability.

5.0 Results and Discussion

5.1. Lebeckia establishment at different sites

The table below summarises the problems that Lebeckia faced in its initial establishment at the various sites in the NAR. Plants grew at all sites, but unfortunately the final plant density varied according to challenges presented (Table 2). Pests and weeds, and soil infill after seeding, were the main problems identified at all five sites. Site management prior to the seeding of Lebeckia proved to be fundamental to successful establishment.

Table 2: Establishment counts of the first Lebeckia sowings in the NACC and challenges noted.

Farmer/ Location	***Establishment***	***Problems***	***Result***
Will Brown/ Badgingarra	4 plants/ 1m^2	Raddish and melon germination and paddock lease change.	Positive after minor setbacks
Boyd Carter/ Buntine	<1 plant/1m^2	Huge germination of raddish, afghan thistle and mulla mulla	Site abandoned
Jill Wilson/ Karakin 2016	5 plants/1m^2	Cockatoos and tagasaste moth	Re-sown in 2017 however old seed had poor germination
Cliff Harding/ Mingenew	1 plant/1m^2	Kangaroos and rabbits	Vermin decimated remaining plants
Peter & Lloyd Cripps/ West Binnu	4 plants/1m^2	Infill of furrows due to prevailing winds,	Positive with a successful

		rabbits and tagasaste moth	harvest of seed

Wild radish, ryegrass and melon germination in Badgingarra (Figure 3) smothered the Lebeckia seedlings and subsequently limited water available for the young plants. This weed burden decreased the success of the establishment operation. CRS staff hand weeded 2500 m^2 to remove some of this weed burden in December 2016. The site was effectively abandoned, however after it was grazed as part of the paddock throughout 2017 (without fencing), and the weeds were subsequently controlled by the sheep, surviving Lebeckia plants flourished (Figure 3, above).

Figure 3: Badgingarra weed competition after sowing of Lebeckia in November 2016 (above) and recovery after grazing in November 2017 (below).

At Binnu, establishment was very successful. Plant growth was vigorous through the summer of 2016/2017 and flowering followed by seed set was very impressive.

Through summer 2017/2018 the plants suffered a severe insect attack from Tagasaste grub, and were then lightly grazed, but the stand was very strong again by March 2018 (Figure 5a below). By comparison, the Lucerne had not flourished at this site –and had all but disappeared as at March 2018, 18 months after seeding (Figure 5b). The Cripps family undertook some P and K experiments in small quadrats.

Figure 5. Peter Cripps inspects the Lebeckia in March 2018, 18 months after sowing (above) and compares Lebeckia with the weak growth and survival of Lucerne (below).

At Karakin, a late decision was made to change site to avoid a possible carryover of a heavy rate of Diuron/MCPA which had been sprayed several months earlier. There was a very good level of establishment at the new site, however the weed burden soon became an issue. Strips sown across the site sprayed with Diuron/MCPA applied at 2L in the previous winter grew well, and gave valuable information on the resistance of Lebeckia to this chemical (Figure 6, left).

Figure 6. Early growth at Karakin in December 2016 (left on the Diuron/MCPA treated soil) and right, the damage by birds after establishment.

As the season progressed into 2017, many of the young plants that survived competition from the weeds were removed by white cockies which broke off the young plants at ground level (Figure 6, right).

At Buntine and Mingenew, weed burden, soil infill and animal predation proved too great a set of obstacles for Lebeckia, Lucerne and Tedera. Surviving plans of Lebeckia flourished at both sites however, indicating tolerance of the conditions.

Despite the wide range of challenges, Lebeckia proved to be able to grow on a variety of soils of poor fertility in the NAR, some with very low nutrient status, as presented in Table 3.

Table 3: Soil analysis results from sites through the soil profile of 0-20cm.

Farmer/ Location	Colwell P (mg/kg)	Colwell K (mg/kg)	Sulphur (mg/kg)	Organic C (%)	Conductivity (dS/m)	Exc. Aluminium (Meq/100g)
Will Browne/ Badgingarra	16.6	23.3	7.1	<1	0.051	0.1
Boyd Carter/ Buntine	15.2	15.3	11.7	<1	0.022	0.6
Jill Wilson/ Karakin 2016	6	22	3.1	1.03	0.035	0.08
Cliff Harding/ Mingenew	6	>15	2.6	>1	0.017	0.1
Peter & Lloyd Cripps/ West Binnu	4.5	>15	2.8	>1	0.03	0.09

5.3. Inoculant quality and strain for Lebeckia

To obtain a successful stand of Lebeckia an essential factor is to identify an adapted strain of rhizobia that can tolerate desiccation and fix nitrogen abundantly. Figure 4 illustrates the importance of rhizobia on Lebeckia growth. The poor nodulation that was identified at Badgingarra (below left) indicated that rhizobia strain WSM4204 (*Burkholderia dilworthii*) was not as effective as the strain *Burkholderia sprentiae* (WSM4184) which was used in the Great Southern at Harrismith. When plants were excavated from Harrismith (below right), excellent nodulation was noted. To take advantage of this information, West Binnu was top dressed with Margurita pod coated in *Burkholderia sprentiae* strain (WSM346) in winter 2017. The nodulation of the NAR sites will be monitored in 2018.

Research is continuing on revising and identifying the best possible rhizobia to be coupled with Lebeckia in the glasshouse at the CRS (Murdoch University).

Figure 4: Lebeckia plants that failed to nodulate at Badgingarra (left) vs Lebeckia plants that nodulated at Harrismith (right).

5.4. Lebeckia seed production and quality at various sites

Lebeckia seed was successfully harvested from West Binnu (4kg) and small amounts were harvested from Badgingarra and Mingenew (that were too little for seed quality experiments). Seed germination experiments to determine hardseed were carried out on three different pod types (purple, green, brown) from West Binnu as well as the combined sample. The amalgamated sample of West Binnu (Binnu 2017) had a 100 seed weight of 186mg which is impressive considering the rainfall for West Binnu in 2017 was 196mm and 39.8mm was recorded from first flower to harvest (BOM 2018).

All samples indicated to have a high amount of hardseed except for the purple coloured pods after 20 days of germination (Figure 5). This result is transferred across to the combined Binnu 2017 seed as majority of pods harvested were purple which caused a lot of seed death (>50%). However, generally the hard seed of a legume is viable and rarely non-germinable after hardseed breakdown or mechanical scarification (Nutt 2010). Harvesting experiments were undertaken by hand and machine in November 2017 (Figure 4).

Figure 7. Harvesting Lebeckia pods with the binder / reaper machine at Binnu in November 2017.

Lebeckia pods mature over a period of six weeks, which makes the harvest window difficult to predict. If left too late, the pods shatter. If harvested too early, the seeds are immature. To examine this, the harvested pods were separated into maturity grades of green, purple or brown pod colouration to examine the effect of harvest time on seed viability.

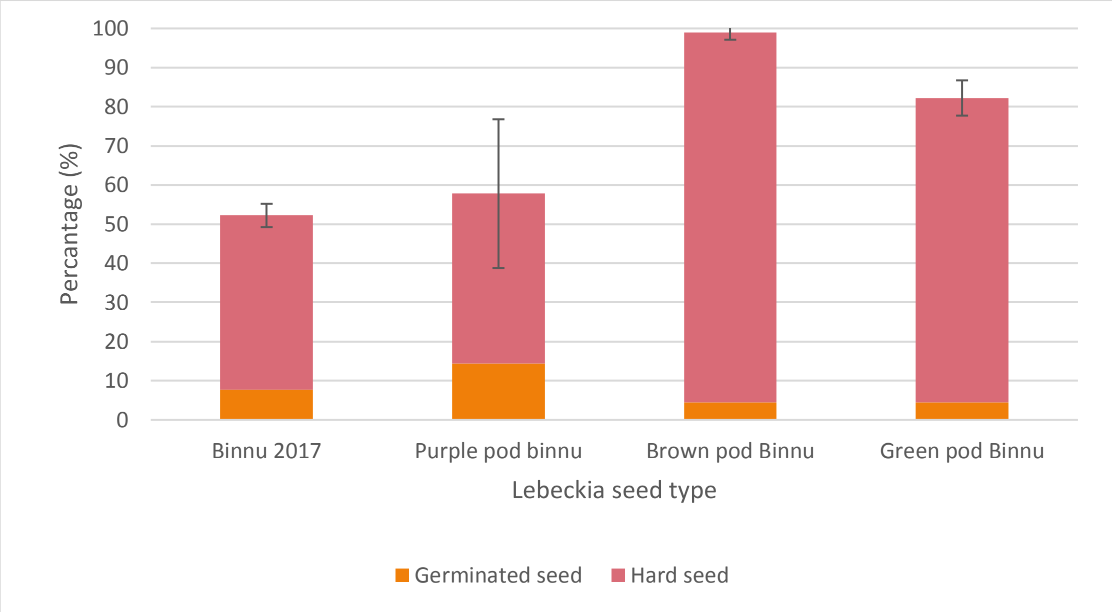

Figure 4: Quality of Lebeckia seed from West Binnu after 20 days germination testing. Missing values that add to 100% indicate dead seed. Binnu 2017 portrays a sample taken from the 4kg of harvested seed from the area. Standard error is only presented for hardseed.

Seeds from brown (mature) pods were nearly 100% viable, and had hardened, whereas up to 40% of seed from the less mature pods was damaged. This will be followed up with trials to assess windrowing options in 2018.

6.0 Conclusion and Future work

Lebeckia was recognised as a perennial pasture legume potentially suited for WA agriculture because of a range of promising physiological and reproductive features, and its adaptation to a similar environment (Howieson et al. 2013). Noticeably, its most positive ability is using it as a pioneer species on deep sandy and disturbed soils to combat soil erosion and poor fertility. Along with its ability to provide year round green feed, it can also provide a foot hold for a diverse range of secondary succession species to colonise (e.g. subclover and serradella). West Binnu, as indicated above, was the most difficult environment for Lebeckia to be sown into with respect to rainfall, soil fertility and prevailing costal winds. However, Lebeckia overcame these conditions to prove to be suitable for even the harshest Wheatbelt environments. This was largely due to the ideal site management and pest control from the Cripps'. Regrettably, other sites were not as successful but this was due to poor site management and vermin grazing. Moreover, for Lebeckia to be successful, site preparation and management is essential for success especially once the right rhizobia is delivered.

Lebeckia is one of the only perennial plants (apart from Tagasaste) that is able to grow and be productive on the non-alkaline deep sandy soils of WA. Furthermore, Lebeckia persists for three –four years, which compared to Tagasaste is a short life cycle. This short life cycle coupled with its high production of hardseed suggests the possibility for Lebeckia to be used in a perennial ley system and in turn give more flexibility to farming systems.

Soil tests will be taken in the future at successful sites to quantify the benefits of Lebeckia as a perennial pasture and ability to transform low productive non-alkaline soils into cropping land.

7.0 Acknowledgements

Murdoch University would like to recognise the support from the Northern Agricultural Catchments Council through funding from the Australian Government National Landcare Program. We also acknowledge the enthusiasm and help from the grower groups and farmers involved; Peter and Lloyd Cripps, Liebe Group, Boyd Carter, Mingenew Irwin Group, Cliff Harding, West Midlands Group, Will Browne, Landskills - Jill Wilson and John Reymond.

References

Beard, J. S., A. R. Chapman and P. Gioia (2000). "Species richness and endemism in the Western Australian flora." Journal of Biogeography **27**(6): 1257-1268.

Cocks, P. S. (2001). "Ecology of herbaceous perennial legumes: a review of characteristics that may provide management options for the control of salinity and waterlogging in dryland cropping systems." Crop and Pasture Science **52**(2): 137-151.

De Meyer, S., M. Cnockaert, J. Ardley, G. Maker, R. Yates, J. Howieson and P. Vandamme (2013). "*Burkholderia sprentiae* sp. nov., isolated from *Lebeckia ambigua* root nodules." International journal of systematic and evolutionary microbiology **63**(11): 3950-3957.

Dear, B. S., G. A. Moore and S. J. Hughes (2003). "Adaptation and potential contribution of temperate perennial legumes to the southern Australian wheatbelt: a review." Australian Journal of Experimental Agriculture **43**(1): 1-18.

Edwards, T. (2015). The seed ecology of *Lebeckia ambigua*. Unpublished honours. J. G. Howieson and B. J. Nutt. Murdoch University. **1:** 1-122.

Hobbs, R. J., R. H. Groves, S. D. Hopper, R. J. Lambeck, B. B. Lamont, S. Lavorel, A. R. Main, J. D. Majer and D. A. Saunders (1995). Function of biodiversity in the mediterranean-type ecosystems of southwestern Australia. Mediterranean-Type Ecosystems, Springer**:** 233-284.

Howieson, J. G., S. E. De Meyer, A. Vivas-Marfisi, S. Ratnayakeb, J. K. Ardley and R. J. Yates (2013). "Novel *Burkholderia* bacteria isolated from *Lebeckia ambigua* – A perennial suffrutescent legume of the fynbos." Soil Biology and Biochemistry **60**: 55-64.

Howieson, J. G., A. Loi and S. J. Carr (1995). "*Biserrula pelecinus* L.-a legume pasture species with potential for acid, duplex soils which is nodulated by unique root-nodule bacteria." Crop and Pasture Science **46**(5): 997-1009.

Howieson, J. G., R. J. Yates, K. Foster, D. Real and B. Besier (2008). Prospects for the future use of legumes. London, Elsevier.

Le Roux, M. M. and B.-E. Van Wyk (2007). "A revision of *Lebeckia* sect. *Lebeckia*: The *L. sepiaria* group." South African Journal of Botany **73**(1): 118-130.

Loi, A., J. G. Howieson, B. J. Nutt and S. J. Carr (2005). "A second generation of annual pasture legumes and their potential for inclusion in Mediterranean-type farming systems." Australian Journal of Experimental Agriculture **45**(3): 289-299.

Moore, A., L. Bell and D. Revell (2009). "Feed gaps in mixed-farming systems: insights from the Grain & Graze program." Animal Production Science **49**(10): 736-748.

Nutt, B. J. (2010). Incidence and inheritance of hard-seededness and early maturity in *Ornithopus sativus.* J. G. Howieson, Murdoch University. **PhD thesis**.

Ridley, A. M. and D. J. Pannell (2005). "The role of plants and plant-based research and development in managing dryland salinity in Australia." Australian Journal of Experimental Agriculture **45**(11): 1341-1355.