

Spanning Spain for salt-tolerant rhizobia



By Laureta Wallace
Kondinin Group

Travelling to the other side of the world to search for elusive soil bacteria may not be everybody's ideal overseas trip. However, for Department of Agriculture and Food Western Australia (DAFWA) senior researcher Dr Phil Nichols and South Australian Research and Development Institute (SARDI) scientist Amanda Bonython it was all in the name of science.

The aim of the mission was to uncover and collect salt-tolerant rhizobia for the promising pasture legume *Melilotus siculus* and to collect seed of other waterlogging- and salt-tolerant species.

The pair is a part of the *Understorey: developing profitable pasture plants for saline environments* project, which is a joint initiative between the FFI CRC, DAFWA and SARDI.

Dr Nichols and Amanda successfully applied for two separate travel grants from the AW Howard Memorial Trust program. The trip was also partly funded by the FFI CRC.

Dr Nichols joined Amanda in London, en-route from a conference in France.

"I presented a paper at the EUCARPIA Fodder and turfgrass congress on *Melilotus siculus* and two posters summarising other work with sub-clover and other annual legumes," Dr Nichols said.

"While there were other parts of the world we could have chosen to collect rhizobia, visiting Spain took us back to the origins of the first *Melilotus siculus* sighting and allowed us to take advantage of our established networks there."

Background

Research to date has identified the annual legume *Melilotus siculus* as the only pasture legume able to persist on highly-saline, waterlogged sites in Australia's major agricultural regions.

In trials, the species has performed well in the first year but its potential is being profoundly hampered by nodulation failure in the years after establishment (see *Focus on Perennials* Issue 7). Seedlings have been regenerating, but the commercial rhizobia are failing to survive the high surface salinity levels during summer. This results in a high proportion of stunted seedlings that fail to grow properly.

To continue the species development researchers need to identify salt-tolerant rhizobium strains to complement those already under evaluation.

"SARDI is currently investigating rhizobia obtained in SA from other *Melilotus* and annual medic species – as well as rhizobia collected from *Melilotus siculus* growing in Western Australia and Israel," Amanda said.

"But our mission was to collect rhizobia from host plants of *Melilotus siculus* growing in saline and waterlogged sites to give this promising pasture plant every

ABOVE: DAFWA senior researcher Dr Nichols uncovers a patch of *Melilotus siculus* (Photo: Amanda Bonython) INSET: Dr Nichols and Amanda's research path

chance of further development for Australian farming systems."

Networking

The first stop for Dr Nichols and Amanda was Alicante University, about 400 kilometres, south east of Madrid. The university is the base of Botany Prof. Segunda Ríos, who is currently working with senior DAFWA plant breeder Dr Daniel Real.

"Dr Real and Prof. Ríos are collaborating on developing the perennial legume *Tedera* so it was useful to reacquaint with him and discuss the work we have been carrying out," Dr Nichols said.

Under the guidance of Prof. Ríos it was in the Oliva-Pego wetland that the first sighting of *Melilotus siculus* was made.

"The area had once been used for growing rice but had become too saline."

Dr Nichols said the site proved to be typical of where they would find *Melilotus siculus* in other parts of Spain – marshy areas on the transition between relatively fresh water and saltland.

"Where the plants were alive we collected live nodules and where they had dried off we took soil samples where the rhizobium cells had spread into the soil," Dr Nichols said.

Tedera trials

Amanda and Dr Nichols next stop was IMIDA—the agricultural research institute home of Enrique Correal, another researcher involved in developing *Tedera*, and Mercedes Dabauza

key points

- More salt-tolerant rhizobia are needed to further develop promising legume *Melilotus siculus*
- Rhizobia collected from *Melilotus siculus* by researchers in Spain could be the solution to the species second-year performance woes
- If the rhizobia problem is solved *Melilotus siculus* may be commercially available within 4-5 years.



Micó, who is currently on a three-month research visit to the University of Western Australia (UWA).

“We added value to the trip by investigating Tедера trials at IMIDA and collecting more Tедера rhizobia for Dr Real,” Dr Nichols said.

“That was an important part of the whole experience – it wasn’t just about collecting rhizobia and seeds for our own research.

“We networked and added value to other projects that our Australian colleagues are working on, while all the time keeping our eyes out for species that might warrant further investigation.”

Sussing out Seville

Travelling south-west, the next stop was Doñana National Park, a world heritage wetland and bird nesting sanctuary – Spain’s equivalent to Kakadu.

“This was a key site, as research before the trip showed that *Melilotus siculus* was present in the park,” Dr Nichols said.

“We had to get written permission at a regional and national level for the whole trip and additional permission to collect in the park by providing authorities with details of our intended work.

“The south of the park is extremely saline and to get into it we had to drive 50 km along the beach to the mouth of the Guadalquivir River.

“At first we found no legumes, then a lot of other less-desirable *Melilotus* species, such as *Melilotus sulcatus* and *Melilotus indicus*. Then in the centre of the park, *Melilotus siculus* was discovered on saline soils next to fresh water lagoons and canals.

“It only grew in small patches and was actually a much rarer plant than we had originally expected. At one site we collected rhizobia and seed from burr medics *Medicago polymorpha* that were growing along a salt lake with fresh water flowing into it.

“In Australia, burr medics are known to have some salt tolerance, but are not known for their waterlogging tolerance, so maybe our discovery can help rectify that.”

The Seville collection proved to be a hectic, yet profitable ordeal for the pair.

“We were in the field all day until about 7.30pm and then at night we entered our data and sorted our samples in the ‘laboratory’ – the kitchenette of our accommodation,” Dr Nichols said.

“The Spanish don’t eat their evening meal until after 9pm so it fitted in well with us – however it made for some late nights!”

Bringing it all together

Armed with rhizobia, soil and seed samples Dr Nichols and Amanda arrived at the Spanish gene bank for pasture legumes at Badajoz in western Spain to process seed samples for entry into Australia.

“We needed to hand thresh seed from pods and remove any dust or plant contamination for quarantine purposes,” Amanda explained.

“This also involved heating the samples to 60°C for 24 hours to kill any insects.”

True to the laid-back nature of life in parts of Spain, Amanda said the research station only operated between 8.30am and 2.30pm.

“We only had limited time to process our samples and needed to stay until 8pm, which was a shock to our Spanish colleagues, but it was interesting to note the different cultural habits.”

The seed collection was split three ways between DAFWA, SARDI and the Spanish seed gene bank.

“It was only fair we shared the seed with Spain. The arrangement also acted as a security measure if some samples were to go astray or get caught up in quarantine,” Amanda said.

The results

The collected soil and root nodule samples have been given to rhizobiologists at both SARDI and Murdoch University who will isolate the rhizobia for future testing in the field.

After a salt-tolerant rhizobium has been found, the next step will be to test a range of *Melilotus siculus* genotypes on saline sites with the aim of selecting a new cultivar for commercial release.

“All going well *Melilotus siculus* could be commercially available within 4-5 years,” Dr Nichols said. ↘

More information

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Research – a global vocation

Amanda’s Spanish adventure has cemented her passion for research and opened her eyes to the benefits of international collaboration.

“It was amazing, we worked really long hours but it was great meeting the local people and having Spanish scientists show us around the environment,” Amanda said.

“Seeing *Melilotus siculus* growing in its natural environment was great too. It reassured us that our previous research had targeted the right species for saline environments in Australia.

“And going with Phil I knew I’d learn so much more about agriculture and the role plants play.”

Amanda holds a Bachelor of Applied Science in Environmental Management and has worked on *Melilotus siculus* for three years in her role as a researcher with SARDI.

“It has been an introduction to agriculture for me – I had the plant biology background but not agriculture.”

“I’ve really enjoyed the work we do with salinity and finding plants for saline areas.

“I love setting up the trials, recording the data and the pulling the story together.”

Under the guidance of senior SARDI researcher and *Understorey* project leader, Andy Craig, Amanda has taken on a leadership role with *Melilotus siculus*.

“I’m responsible for managing the field trials and am getting more involved with the budgeting and writing up of projects,” Amanda said.

Amanda describes the research environment in Spain as different to her base in Struan Research Centre, Naracoorte, SA.

“After a long day collecting seed and soil in the field, we often had to complete the soil analysis back in our accommodation. We would be working out EC and pH levels in the kitchen, using limited resources – so we had to be imaginative and it was a challenge.

“The whole experience opened my eyes to the benefits of international research collaboration and how it can help us get better results.

“On a personal level I have reassured myself that I love research and it is an exciting career with the opportunity to work not only interstate but across the world.” ↘

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