Pasture legumes — a test of tolerance

LEFT: The difference between well nodulated (dark green, background) and poorly nodulated plants (yellow, foreground) is profound. (Photo: A Bonython)

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The search for suitable pasture legumes for Australia's harsh saline environment is always going to prove a challenge, but FFI CRC through the experiences of the South Australian Research and Development Institute (SARDI) pasture legume team has proven that its not only the pastures that need to be highly salt tolerant.

In their quest for a hardy legume species well adapted to Australia's more extreme conditions, researchers have searched far and wide. They have sought out genera with reputed tolerance to salt and waterlogging from around the world and put them to the test. One particular species of *Melilotus* — *Melilotus siculus* — has proven a tempting possibility for widespread adoption.

While farmers view the local species of *Melilotus* as little more than a weed, which can taint milk and meat and contaminate grain, FFI CRC researcher, Andy Craig (SARDI), believes *Melilotus siculus* may overcome these challenges and prove a valuable addition to the pasture legume toolbox.

"To date we have field tested 17 *Melilotus* species and *Melilotus siculus* in particular sparked our interest," Andy said.

"Not only does it tolerate salt and waterlogging but it has low coumarin levels."

Coumarins are the compounds researchers believe are responsible for the tainting qualities of the local *Melilotus* species.

"*Melilotus siculus* is the same genera, but a different species altogether," Andy explained.

"Lab tests revealed this species' levels of coumarins were very low — no higher than many other commercially-acceptable plants and indeed lower than many.

"We also investigated other potential nasty attributes, including the weed risk potential, and with a clean bill of health, it still looked well worth pursuing."

Passing the lab test

With promising field results Andy and his team took a range of legume species into the lab for further analysis.

"We did some lab testing to see how the species fared within the larger salt-plant story," Andy said.

Andy explained the characteristics needed for success in a saline environment include:

- A significant level of salt tolerance as a germinating seedling
- Tolerance to waterlogging
- Ability to tolerant a high level of salt not only as a seedling, but also as a maturing plant.

Melilotus siculus ticked all the boxes.

Murphy's law

Having sailed through initial phases with ease, Murphy's law intervened.

"We established the plant in the field and in the first year all went well, but in the second year something quite odd happened," Andy explained.

"The first-generation plants were setting seed and regenerating, but the second-generation seedlings were small, unthrifty, yellow and generally disappointing."

"Regenerating plants lacked vigour to the point where they were overrun by other species — basically they failed when stressed."

"There was much preliminary head scratching and we shared our experience with colleagues, in particular Dr Phil Nichols (DAFWA) who had shared almost identical experiences in trials in Western Australia."

Both Andy and Phil described similar observations, which validated the SA experience across different geographical areas, with different soil types and conditions — the problem was manifest in two different environments.

BELOW: The answers lay beneath the graveyard plots. (Photo: R Ballard)

key points

- *Melilotus siculus* is set to provide a powerful tool to manage salinity and waterlogging if researchers can just solve the puzzle of rhizobial persistence
- Researchers have a hunch the answer lies in finding a suitable rhizobia to match the plant and the harsh saline environments in which it could be used
- Trials of rhizobial strains have been disappointing to date, but a glimmer of hope has appeared in SRDI 554.

Response of *Melilotus siculus* to rhizobial inocula in regenerating saline pasture field trial

ABOVE: A ray of hope - SRDI 554 (top) may provide the solution. (Photo: N Charman)

Enlisting the experts

Andy had a hunch and enlisted the help of SARDI rhizobia experts, Ross Ballard and Nigel Charman, to carry out some preliminary testing.

Together the team investigated the field trial, which showed inconsistencies within the second-generation seedlings — 95 per cent were visibly unhealthy, but patches totalling about 5% remained healthy.

Rhizobial measurements revealed the healthy patches contained about 500 rhizobia per gram of soil and the poor patches contained an average of six rhizobia per gram of soil.

"This fact put forward a compelling argument that the problem was one of nodulation and an unsuitable rhizobia," Andy said.

There was a glimmer of hope. The initial inoculum used was the commercial medic inoculum (Medicago and *Melilotus* are closely aligned genetically), a logical choice, but perhaps not the best option.

"Interestingly, the trial site is not a particularly hostile environment and sowing had taken place, during late May and early June, after most of the salt had gone," Andy said.

"But during the second year the rhizobia had to survive during summer when soil moisture was low and salt levels were higher."

Additional data supported this argument, so the team searched for strains of rhizobia that were better matched for the plant and the environment.

Change of focus

The current hurdle not only prolonged the search for a suitable pasture legume species, but also took the focus away from the plant and onto the rhizobia.

Seeing merit in the argument, FFI CRC provided funding to further investigate different strains of rhizobia.

"Time was of the essence, so Ross quickly isolated some potentially useful rhizobial strains, 20 in the first cut, to test in the field, in Keith, SA, sown during June 2007," Andy said.

A second more targeted batch of 25 strains of rhizobia were then collected from harsh (saline) environments, applied to seed and sown later (late July, early August 2007).

"Although sown late, our hopes lay in this second, more targeted batch," Andy said.

Disappointing performance

As winter 2008 came and the second-generation seedlings from the early-sown plots were emerging, the results were disappointing.

"We had improvements in a biological sense — there was some statistical improvement in seedling health and performance — but from an agronomic viewpoint, the improvement was not enough to see any commercial viability," Andy explained.

Even worse —the late-sown, targeted batch initially gave exactly the same response as the earlier sown plots.

"Yet we weren't totally despondent — an intriguing phenomenon had captured our interest," Andy said.

"Because we sowed the plots of the second batch late and the season had seen high wind levels during summer (after seed set), sand had blow across the plots creating a mounding effect of sterile sand across the top of the plots — eerily reminiscent of a graveyard.

"This became know as our graveyard trial."

Grave concerns

On first inspection, during August 2008, the trial had been a dismal failure, with no rhizobial strains having any greater effectiveness or persistence than the original commercial strain.

"However after a day of walking, talking and thinking in our graveyard, we noticed a higher percentage of 'happy' plants along the bottom edges of many graves at ground level," Andy said.

"Nigel speculated the 'unhappy' plants on the tops of the mounds were trying to nodulate in a heap of sterile soil, containing no suitable background rhizobia, whereas the 'happy' plants growing at the edges of the plot were nodulating reasonably well."

"Nigel's hypothesis was that the plants at the edges of the graves, which hadn't been covered with sterile soil, were tapping into the original rhizobia."

The team got down and dirty at the end of each grave scraping into the soil in which the initial rhizobia had been sown.

With time running out, they sowed sterile (un-inoculated seed) into the scraped foot of the graves (during late August 2008). If the hypothesis was right, they were sowing seed into soil which contained the original rhizobia and where first year establishment took place, the results would speak for themselves.

Visual assessment of seedlings after 4-6 weeks (happy versus unhappy) suggest the hypothesis seemed well founded.

"Plants in the scraped foot of the graves were generally healthy and nodulated and some strains were outperforming those in the control (nil inoculation) and commercial inoculant treatments," Andy said.

"In particular a rhizobial strain, SRDI 554, showed significant promise — much better than the current best practice rhizobium."

The hypothesis will be further tested during the coming season with 80 new, untested strains of rhizobia being trialled at sites in SA and WA.

"It looks promising, but there is still a lot of work to be done," Andy cautioned.

"Even if this does come through, and we are confident we do have a solution to the rhizobial problem, we still have to go back and refocus on the plant, re-evaluate it and ensure it is ready for commercial production." "To avoid disappointment in the commercial arena, we need to provide a rigorous package that delivers sound practices for establishment, growth and management for production and persistence."

More information

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