Capitalising on the discovery of messina for the pasture seeds industry

by Ross Ballard, Simon Crane, Jeff Hill and David Peck
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AgriFutures Australia is the new trading name for Rural Industries Research & Development Corporation (RIRDC), a statutory authority of the Federal Government established by the Primary Industries Research and Development Act 1989.
Foreword

Australia’s rural industries make a fundamental contribution to the Australian economy and way of life. In addition to the major industries, numerous new and smaller established rural industries bring opportunity, diversity and resilience to rural Australia. AgriFutures Australia invests in these industries on behalf of government and industry stakeholders. These industries provide opportunities to be captured by rural producers and investors. They also contribute to community resilience, regional development and provide a distinctive regional character in rural Australia.

Dryland salinity affects many pasture growers across Australia. Presently, 5.7 million ha is classified as being at high risk of salinity, with this area expected to increase threefold over the next 50 years. The productivity of salt-land pastures in these areas is limited by the lack of a suitable legume. After more than a decade of research funded by the Future Farm Industries CRC, a new legume with unprecedented levels of salt and waterlogging tolerance has been identified and progressed to the point of commercialisation. Messina (*Melilotus siculus*) is an annual legume new to Australian agriculture that provides an opportunity for growers and for the pasture seeds industry.

This report describes work funded by AgriFutures Australia, aimed at addressing key information gaps on the adaptation, establishment and management of messina, to support its commercial release in 2017 and its ongoing adoption. The information will help to build the confidence of the pasture seeds industry in adopting this promising but unfamiliar new plant.

This report is an addition to AgriFutures Australia’s diverse range of over 2000 research publications and it forms part of our Pasture Seeds RD&E program, which aims to support the sustainability of temperate pasture seeds production.

Most of AgriFutures Australia’s publications are available for viewing, free downloading or purchasing online at [www.agrifutures.com.au](http://www.agrifutures.com.au).

**John Harvey**  
Managing Director  
AgriFutures Australia
About the Author

Ross Ballard is a Senior Scientist with the South Australian Research and Development Institute. He provides rhizobial support to legume development programs and became involved with the development of messina when poor nodulation in regenerating messina pastures was recognised as a major barrier to its development. Ross led the development of a salt tolerant rhizobia strain for messina and has continued to be involved in its commercialisation.

Acknowledgments

The development of messina was funded by the Future Farm Industries CRC.

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Executive Summary

What the report is about

Messina (Melilotus siculus) is a new annual pasture legume with unprecedented tolerance of both waterlogging and salinity. Messina has the potential to transform salt-land pastures.

Work has been completed to address key information gaps on messina’s pH adaptation, herbicide tolerance and the impact of seed treatments on its establishment, in order to support its commercial release in 2017, as well as its ongoing adoption.

Who is the report targeted at?

The work is relevant to those in the messina supply chain and includes:

- researchers contributing to the plant’s ongoing development and promotion
- the commercial licensee (Seednet) and its seed growers
- agronomists and natural resource management (NRM) groups
- the rhizobial inoculant industry
- salt-land farmers

Where are the relevant industries located in Australia?

Messina is recommended for use in salt-land mixed pastures which are located in Western Australia (WA), the upper south-east, southern Yorke and lower Eyre Peninsulas of South Australia (SA) and north-west Victoria. Since this project has commenced, there has also been interest in using messina in saline seeps in the South Australian and Victorian Mallee.

The key seed production area is in the south-east of SA (Keith/Naracoorte).

The area estimated as suitable for messina is 600,000 ha. Current supply of seed is between 30 and 50 T per year (enough for 5000 ha).

Background

Messina (Melilotus siculus) is a new annual pasture legume with unprecedented tolerance of both waterlogging and salinity. It has the potential to transform salt-land pastures, which currently lack a suitable legume and therefore only sustain very low levels of production.

In tandem with the development of a salt tolerant rhizobia strain, it has been estimated that messina has the potential to deliver productivity increases across ~600,000 ha of agricultural land affected by dryland salinity in southern Australia. Gains will accrue from the feed value of messina itself and also from its contributions of fixed organic N for companion grass and shrub species.

Aspects of messina’s management and adaptation required clarification prior to its release in 2017, in order to provide the seed industry and growers with the confidence to adopt this exciting but unfamiliar new plant. The information generated by this project was needed to ensure reliable messina establishment, provide reliable pasture management practices to encourage legume dominant pastures and drive the demand for seed production.
**Aims/objectives**

The overall objective was to better understand the agronomy and management of messina, thus facilitating its greater adoption by the farming community and the pasture seeds industry.

The specific objectives of this project were to:

1. Determine the tolerance of messina to herbicides registered for pasture legumes.
2. Determine the sensitivity of the plant/rhizobia symbiosis to low pH conditions.
3. Examine the role of seed treatments in the reliable establishment of messina under low pH conditions.

**Methods used**

Six experiments were completed. They comprised:

- Two field experiments and one greenhouse experiment to understand the tolerance of messina to a range of herbicides commonly used in annual pastures.
- Two greenhouse experiments to provide detailed information on the pH sensitivity of messina symbiosis in order to define where it can be reliably grown.
- One greenhouse experiment to examine the impact of seed treatments on reliability of establishment.

The information was included in the Seednet information sheet produced for messina and has been extended to agronomists individually and via presentations at industry forums.

**Key findings**

*Herbicide tolerance*

Herbicides were identified that did not cause significant damage when applied to messina. Results from both 2016 and 2017 indicate that the broadleaf herbicides Broadstrike (Group B) and Igran (Group C) and Group A grass selective herbicides Verdict and Factor are safe to use on messina.

Some of the herbicides tested (e.g. Treflan and MCPA) did cause damage to messina. Previously, Treflan was thought to be ‘safe’ on messina and should only be used with strict adherence to application rate (1.4 L/ha has been used safely), timing and method of application. MCPA is not recommended for use on messina.

Severe sensitivity of messina to the SU herbicide chlorsulfuron (Glean) highlights that seed growers are best to avoid fields sprayed with SU herbicides where possible, or if they have been used strictly observe plant back times (label will provide plant back periods and depends on soil pH, rainfall and the SU herbicide used).

*Sensitivity to low pH*

The messina symbiosis is sensitive to low pH. The results support the recommendation that messina should not be grown on soils where pH is less than 5.5\(\text{Ca}\) or 6.0\(\text{water}\).

Even this pH level may carry some risk, but this can be effectively eliminated with application of lime to the seed before sowing.
**Seed treatment**

The application of Apron to seed improved seedling emergence, but it is not currently registered for use on messina.

While satisfactory establishment of messina has been achieved in many previous field trials without the application of Apron, these results indicate that some level of seedling loss was likely. These losses are very easily overlooked in the field. A sowing rate of 10 kg/ha, similar to what was used in evaluation trials, is being recommended to growers and should provide an adequate buffer against seedling losses.

Lime pelleting of the seed is beneficial to the establishment, nodulation and growth of messina.

**Additional findings**

Messina was ineffectively nodulated (did not fix nitrogen) when inoculated with the lucerne inoculant strain. Using the recommended inoculant strain (SRDI-554) is critical to both the regeneration and N-fixation capacity of messina.

**Implications for relevant stakeholders**

The pH sensitivity of the messina symbiosis is unlikely to limit seed production in SA or elsewhere, so long as the limitation is recognised and the activity is undertaken on neutral or alkaline soils.

More broadly, messina’s pH sensitivity will limit its adoption because it is unlikely to be recommended for use on up to half the salt-land area in WA. This will limit its adoption and hence the demand for seed in the longer term.

With regard to the pasture seeds industry (certified production), its ability to capitalise on messina’s potential will be limited by the lack of Plant Breeders Rights (PBR) protection for the cultivar Neptune. Messina is easily harvested and we are already aware of on-farm seed harvesting and trading prior to the second year of Neptune’s release. Limited availability of the new inoculant strain (SRDI-554) and promotion of its importance to messina has no doubt provided some disincentive to the use of ‘over-the fence’ seed, but this strategy is not expected to be effective for more than a couple of years. Seed demand is likely to be strong for some time, however for the certified pasture seeds industry to capitalise it would need to produce and supply seed at a retail price point of about $8. The development of an improved messina cultivar with PBR protection could benefit the certified pasture seeds industry.

This project has identified a range of herbicides that are safe to use on messina, and some that are not. Neither the seed industry nor growers appear to be limited by the range of herbicides that could be used to establish and favour the growth of messina. However, no herbicides are specifically registered for use on messina and therefore they cannot be legitimately recommended or used by growers, other than where they are registered for general ‘legume seed crop establishment’ or use on ‘grass and legume pastures’.

For growers, the availability of affordable seed is likely to be the main constraint to the adoption of messina in the short to medium term (5 years). Current annual seed availability of 30-50 tonne is only sufficient for 5,000 ha.
Recommendations

Agronomists and growers need to be made aware that messina should not be grown on soils where pH is less than 5.5 (Ca) or 6.0 (water). Broader publication of the findings of this project, including a scientific paper on the pH tolerance, is needed. Where pH is below the critical level, growers should apply lime to increase soil pH.

The new rhizobia strain (SRDI554) should be applied close to sowing and the inoculated seed lime pelleted to ensure adequate nodulation. The medic rhizobia inoculant strain (WSM1115) or the lucerne strain (RRI128) should NOT be used on messina.

There are no pesticides registered for use on messina which means that, in general, they cannot be legitimately recommended or used. This issue affects other alternative pasture legumes and is a situation that requires discussion and resolution with the Australian Pesticides and Veterinary Medicines Authority (APVMA).

During the course of this project we have become aware of messina establishment issues linked to poor understanding of the seasonal variation in the salinity levels and information gaps around sowing strategies. Some monitoring of establishment practices and outcomes would be useful to further refine establishment recommendations.

Introduction

Further work on the agronomy of messina is required to maximise its adoption by the farming community and provide information for seed growers and farmers resulting in affordable seed production, reliable establishment and legume dominant pastures.

Messina (*Melilotus siculus*) is an exciting new annual pasture legume with unprecedented tolerance of both waterlogging and salinity (Teakle et al. 2012, Rogers et al. 2011, Nichols et al. 2012, Nichols et al. 2008).

It has the potential to transform salt land pastures, which currently lack a suitable legume and therefore only sustain very low levels of production (O'Connell et al. 2008). In tandem with the development and release of an elite rhizobial strain (Bonython et al. 2011), the messina cultivar Neptune was made available to growers in 2017. The licensee (Seednet) anticipated an initial domestic market of 100 tonnes/annum with excellent prospects for seed demand to increase several fold as farmers gain confidence in the variety. Approximately 35 tonne of seed along with the new rhizobia inoculant was sold to growers in 2017.

Messina has produced more than 4 t dry matter/ha/yr in evaluation trials and is therefore estimated to have the potential to achieve productivity increases of four Dry Sheep Equivalents per hectare (dse/ha) across 600,000 ha of agricultural land affected by dryland salinity in southern Australia. It is estimated that 5.7 million ha are at high risk of developing salinity (Barrett-Lennard et al, 2003, National Land and Water Resources Audit 2001). Gains will accrue from the feed value of messina itself, and also from its contributions of fixed organic N for companion grass and shrub species, which are presently acutely N deficient.

Aspects of messina’s management and adaptation still required clarification prior to its release, in order to provide the seed industry and growers with the confidence to adopt this exciting but unfamiliar new plant. The information continues to be important since the plant is still in the early stages of adoption and being grown for the first time by most growers.
Work has been completed to provide a basic knowledge of its herbicide tolerance (required by both seed growers and farmers); and to define the limits of plant and rhizobia adaptation to low soil pH. This is important because in the absence of accurate information, a conservative position has been taken not to recommend the legume for soils below pH 5.5_{(Ca)} or 6.0_{(water)}. This excludes or makes messina marginal for significant areas of salt affected lands in WA, but is a critical recommendation because establishment failures would undermine confidence in this new species. Studies have also been completed to understand the importance of seed treatments in the reliable establishment of messina.

**Objectives**

The overall objective is to better understand the agronomy and management of messina, thus facilitating its greater adoption by the farming community.

Avoiding failures in the first few years of a legume’s release is critical to generating farmer interest and creating demand for seed production.

Specific objectives of this project are to:

1. Determine the tolerance of messina to herbicides registered for pasture legumes
2. Determine the sensitivity of the plant/rhizobia symbiosis to low pH conditions.
3. Examine the role of seed treatments (lime and Apron fungicide) in the reliable establishment of messina under low pH conditions.

**Methodology**

1. **Messina herbicide tolerance (Objective 1)**

Two field experiments and one greenhouse experiment were completed to understand the tolerance of messina to a range of herbicides commonly used in annual pastures.

   *Experiment 1: 2016 herbicide tolerance field trial*

A search was completed of the APVMA website for herbicides that are registered for annual medics (very closely related to messina), annual clovers and lucerne. A review of the literature and herbicide labels was also completed to determine which herbicides to test and rates of application (Table 1). Low and mid rates of application were used where there was evidence in the literature of damage to annual medics or sub-clover at full label rates.

Messina seed was inoculated with the new rhizobia strain (SRDI-554) and sown at a rate of 15 kg/ha in the field at the Waite campus, University of Adelaide, on 18 May 2016.

Herbicide treatments were scored for early season herbage symptoms (1=no effect, 5=strong effect, 9=no plant growth) and late season dry matter production (0=no growth, 5=maximum growth).
Table 1: Herbicides (and Herbicide Group) applied (g a.i./ha) to messina in 2016, registration status for host crop in Australia (APVMA) and time of application (PS = pre-sowing, PE = pre-emergent, T3 = third trifoliate leaf).

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Experiment 2: 2017 herbicide tolerance field trial

Following an establishment failure associated with very low rainfall in June 2017 (6 mm compared to the average of 60 mm), a farmer sown messina paddock was identified at Mt Charles, South Australia and 15 post-emergent herbicide treatments applied on 29 and 30 August (Figure 1). Leaf symptoms were scored on 26 September and pasture growth assessed on 17 October.

Figure 1: Applying herbicide treatments to messina at Mt Charles, SA.
Experiment 3: Greenhouse test of sensitivity to Glean herbicide

A greenhouse experiment was completed to specifically examine the sensitivity of messina to the SU herbicide chlorsulfuron (Glean), a common residue in alkaline soils following cereal crops. Seed of messina and the barrel medic cultivars Caliph (intolerant control) and Sultan-SU (bred to be tolerant of SU herbicide residues) were sown into soil sprayed with 0, 0.06, 0.12, 0.25, 0.5 and 1 times the label rate of the SU herbicide chlorsulfuron (glean) and subsequent plant growth assessed.

2. Messina pH sensitivity and seed treatments (Objectives 2 & 3)

Three greenhouse experiments were completed to better define the sensitivity of the messina symbiosis to low pH and to examine the modifying effect of seed treatments on seedling establishment.

Experiment 4: Messina nodulation at low pH in a hydroponic system

The nodulation of messina (*Melilotus siculus* cv. Neptune) was compared with ‘acid-tolerant’ burr medic (*Medicago polymorpha* cv. Scimitar) seedlings grown in low pH nutrient solutions, in a greenhouse experiment. The experiment comprised 5 pH levels (4.9, 5.1, 5.3, 5.5 and 5.7).

At each pH level there were two rhizobia treatments comprising SRDI 554 (the new strain recommended for use on messina) or WSM1115 (the strain used on burr medic and reputed to be acid tolerant). Each treatment was replicated 3 times and the experimental units (pails – shown in Figure 2) arranged in a randomised block design in the greenhouse. Two extra control pails (not inoculated with rhizobia) were included in each replicate and maintained at pH 5.7. Absence of nodulation in these pails indicated that the transfer of rhizobia between all pails was negligible and therefore there was no confounding of the experimental treatments.

*Figure 2: An example of the experimental set-up. Pail 14 (of 33) was maintained at pH 5.7, inoculated with rhizobia strain SRDI 554 and planted with 50 seedlings of both messina and burr medic.*
Each experimental unit (pH × rhizobia combination) comprised a plastic pail (330 mm diameter × 390 mm depth) containing 25 litres of ¼ strength N-free nutrient solution (Mc Knight, 1949) made to volume with reverse osmosis water. Each pail was painted black to minimise light flux to the legume roots and had been autoclaved for 15 min at 121 psi prior to the nutrient solution being added. The nutrient solution was added 3 days before planting to enable the adjustment and stabilisation of pH in each pail.

Messina and burr medic seed was surface sterilised in 4% NaOCl, rinsed in 10 aliquots of sterile water and stored as imbibed seed for two days at 4°C before being placed in an incubator at 27°C and allowed to germinate overnight. Germinated seedlings, with root radicals about 10 mm long, were sown into 1.6 mm diameter holes in a black plastic lid floating on the nutrient solution. Fifty seedlings of each plant type were planted in each pail.

Inoculation treatments (strain of rhizobia) were applied the day after the seedlings were sown. Rhizobial inoculants were prepared using pure cultures growing on yeast mannitol agar (YMA) media. Culture of each strain was suspended in sterile water and 25 ml added to each pail according to experimental treatment, to give a final concentration of approximately 10^5 cells ml⁻¹.

The pH of the nutrient solutions was monitored throughout the experiments and adjusted to the target pH as needed with the addition of 0.1M NaOH or 0.1M HCl.

Plants were harvested 16 days after inoculation. The number of nodules was counted on the roots of each plant. Plant shoots and roots were dried at 60°C and their dry weight determined.

**Experiment 5: Messina nodulation in low pH soils**

The factorial experiment was designed to examine the pH sensitivity of messina in different soils. The treatments comprised 3 soils × 6 soil pH levels × ± lime pelleting of the messina seed.

The three South Australian soils were collected from sites considered not to have any suitable background rhizobia, i.e. be responsive to inoculation. All three were confirmed has having no suitable rhizobia. The Keith soil was collected from near the core messina evaluation site. Soils from Tooperang and Pewsey Vale were collected from sites that had been well characterised and known to be acidic.

The pH of the three soils was amended with either CaCO₃ or 0.1M HCl to create six pH levels in the range of 4.3 to 5.5 (Table 2).

**Table 2: Final pH ranges of the three soils.**

<table>
<thead>
<tr>
<th>Soil pH (0.01 M CaCl₂)</th>
<th>Keith (K)</th>
<th>Pewsey Vale (PV)</th>
<th>Tooperang (TP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.69</td>
<td>4.66</td>
<td>4.26</td>
<td></td>
</tr>
<tr>
<td>4.80</td>
<td>4.90</td>
<td>4.44</td>
<td></td>
</tr>
<tr>
<td>5.01</td>
<td>5.25</td>
<td>4.62</td>
<td></td>
</tr>
<tr>
<td>5.15</td>
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<td>4.78</td>
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<td></td>
</tr>
<tr>
<td>5.54</td>
<td>5.49</td>
<td>5.16</td>
<td></td>
</tr>
</tbody>
</table>

Seed of messina cv. Neptune was surface sterilised in ethanol and air-dried. Seed was treated with Apron fungicide (1 ml per kg equivalent) prior to inoculation with rhizobia strain SRDI 554 (10 g seed inoculated with 0.25 g of peat slurry comprising 6.25 g peat inoculant in 25 ml of 1.5% methyl-cellulose sticker). The 10 g of messina seed allocated to the lime treatments was
coated with 2.5 g Omyacarb micro-fine lime. An uninoculated control treatment was also prepared as previously described, with the exception that no rhizobia were added.

Two hundred seed were removed from each inoculated batch (treatment) to estimate number of rhizobia. There were 11,000 and 9,000 rhizobia/seed recovered from the non-pelleted and pelleted seed treatments respectively.

Each experimental unit comprised a cardboard cup containing 275 g dry soil (Figure 3). The day before planting, 66 ml sterile RO water was added to each pot.

Seed was sown into the moistened soil within two hrs of inoculation. Five seeds were sown per pot and the soil surface covered with a layer of white polypropylene beads to reduce evaporation and minimise transfer of rhizobia between treatments. Treatments were randomised into 4 blocks.

Plant emergence was recorded and numbers thinned to 3 per pot. Plants were grown in greenhouse for 5 weeks. Each pot was watered to maintain a weight of approximately 355 g throughout the experiment.

Plants were harvested 35 days after sowing. The number of nodules was counted on the roots of each plant. Plant shoots and roots were dried at 60°C and their dry weight determined.

Figure 3: An example of the experimental set-up. Seed was inoculated with rhizobia strain SRDI 554 ± lime pellet and planted into one of three soils ranging in pHca from of 4.3 to 5.5.

Experiment 6: Seed treatments

A growth room experiment examined the impacts of seed treatment on messina establishment in two low pH soils. The experiment comprised 2 soils × 5 seed treatments detailed below:

Two soils

i) Keith SA, pH 5.2Ca
ii) Pewsey Vale SA, pH 4.7Ca

Five seed treatments

i) rhizobia SRDI 554 + Apron +lime
ii) rhizobia SRDI 554 +lime
iii) rhizobia SRDI 554 only
iv) rhizobia SRDI 736 + Apron + lime (a more acid tolerant rhizobia strain)
v) Apron+Lime

The Keith soil was collected from near the core messina evaluation site in SA. Soil from Pewsey Vale was collected from a site that had previously had a failed pasture establishment.
believed to be associated with root rot, was known to be acidic and therefore considered likely to provide a rigorous messina establishment test. Both were known not to contain suitable rhizobia.

Seed of messina cv. Neptune was surface sterilised in ethanol and air-dried. Each seed treatment was applied to a 10 g batch of seed. Apron fungicide (1 ml per kg equivalent) was applied prior to inoculation with rhizobia. Rhizobia strains SRDI 554 or SRDI 736 were then applied (10 g seed inoculated with 0.25 g of peat slurry comprising 6.25 g peat inoculant in 25 ml of 1.5% methyl-cellulose sticker). The 10 g batches of messina seed allocated to the lime treatments were coated with 2.5 g Omyacarb micro-fine lime. The uninoculated (no rhizobia) treatment was prepared as previously described, with the exception that the applied peat slurry contained no rhizobia.

Thirty seed for each treatment were sown (on the day of inoculation) into moistened soil that had been incubated at 15°C for 20 days to encourage the growth of root rot organisms. The soil was contained in a plastic trays (Fig. 4a, b, c) and after sowing the seed gently covered with soil and the trays watered to field capacity. The main ‘soil treatments’ (each containing five seed treatments) were randomised into 4 blocks.

Plant emergence was first recorded 5 d after sowing and then plant number recorded on a further 20 occasions until harvest at 46 d after sowing. At harvest, number of trifoliate leaves, number of nodules, root health score and root and shoot dry weight of the surviving plants were measured.

Statistical analysis

Data was subjected to analysis of variance. All references to differences imply statistical significance ($P<0.05$), unless otherwise stated.
Figure 4: A) An example of the experimental set-up. Thirty seeds from each of the five seed treatments were sown into the moistened soil. B) Plant survival in the soil from Pewsey Vale SA, 21 d after sowing. Note the reduced plant numbers in the minus-Apron treatment, second row from top. C) An example of plant growth at harvest, 46 d after sowing. Top row (SRDI/736 – an example of an incorrect inoculant strain) and the fourth row down (no rhizobia) are N deficient and demonstrate the importance of good inoculation practice to the successful establishment of Messina.
Results

1. Herbicide tolerance

2016 herbicide field trial

Herbicides that produced little or no foliage symptoms were Dual gold, Spinnaker, Broadstrike, Diuron, Igran, Kerb, Verdict, Select and Factor (Fig. 5a). The late dry matter production of messina was also unaffected by these herbicides, compared to the non-herbicide control (Fig. 5b). Although the Brodal options and Bromicide 200 caused moderate early damage symptoms to the foliage, they were not detrimental to overall dry matter production. Messina plants in the Stomp treatment emerged but failed to grow past the cotyledon stage, although it should be noted that this herbicide was inadvertently applied at higher than recommended rate. Treflan which is widely used pre-sowing and MCPA which is used for post establishment broadleaf weed control in medic, both caused early damage and reduced dry matter production.
Figure 5: Foliage symptom score (a; 1 no symptoms, 5 strong symptoms) and dry matter score (b; 0 no growth and 5 maximum growth) of messina following 19 herbicide treatments. Black bars indicate full label rate, mid grey bars medium rate, light grey bars low rate. The letter in parentheses indicates the Herbicide Group.

2017 herbicide tolerance field trial

Leaf damage symptoms approximately 4 weeks after post-emergent herbicide application is presented in Table 3. No leaf symptoms were recorded for Broadstrike, Igran, Bromicide 200, Factor or Verdict. A low level of leaf damage was recorded for the other herbicide treatments. Whilst lower rates of Simazine and Spinnaker are recommended for lighter textured soils, Simazine still caused a low but acceptable level of damage at the lower rate. Spinnaker also caused some low level damage at mid-rate, in contrast to the 2016 results where it did not cause damage. It indicates that on lighter textured soils, the low rate should be adhered to. Kerb applied at a high rate resulted in lighter coloured leaves whereas no symptoms were observed when previously applied at mid-rate. Brodal options resulted in leaf edge burn at mid and high rate which was reduced to slight edge burn at the low rate indicating only low rates should be used.

The site was inspected on 17 October. No differences between herbicide treatments on messina production were apparent. However, growth in the paddock was generally sub-optimal (less than 50% of potential based on better growth in some areas) which may have masked herbicide impacts on dry matter production.

An indication of weed control is also shown in Table 3. Kerb, Simazine, Spinnaker and Factor gave good control of silver grass. Kerb and Spinnaker effectively controlled ryegrass and are from group D, B respectively.

Good control of capeweed was provided by Igran (C) without damage to the messina. Although the other broadleaf herbicides were less effective at controlling capeweed in this experiment, their performance may be improved under grazing where sheep are used to selectively graze treated weeds.
Table 3: Effect of herbicide treatments on messina (leaf damage symptoms) and the occurrence of capeweed, radish, ryegrass, silver grass and crassula. Mt Charles, SA, 2017.

<table>
<thead>
<tr>
<th>Trt #</th>
<th>Group</th>
<th>Product</th>
<th>rate</th>
<th>Messina leaf symptoms</th>
<th>Capeweed</th>
<th>Radish</th>
<th>Ryegrass</th>
<th>Silver Grass</th>
<th>Crassula</th>
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<tbody>
<tr>
<td>1</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Broadstrike</td>
<td>high</td>
<td>none</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>stunted</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Brodal options</td>
<td>low</td>
<td>slight leaf edge burn</td>
<td>present</td>
<td>infrequent</td>
<td>present</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Brodal options</td>
<td>mid</td>
<td>leaf edge burn</td>
<td>present</td>
<td>infrequent</td>
<td>present</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>Brodal options</td>
<td>high</td>
<td>leaf edge burn</td>
<td>present</td>
<td>infrequent</td>
<td>present</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
<td>Bromicide 200</td>
<td>low</td>
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<td>present</td>
<td>present</td>
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<td>present</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>Igran</td>
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<td>present</td>
<td>absent</td>
</tr>
<tr>
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<td>present</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>9</td>
<td>C</td>
<td>Igran</td>
<td>high</td>
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<td>absent</td>
<td>absent</td>
<td>present</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>10</td>
<td>D</td>
<td>Kerb</td>
<td>high</td>
<td>slightly lighter colour</td>
<td>present</td>
<td>absent</td>
<td>absent</td>
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<td>present</td>
</tr>
<tr>
<td>11</td>
<td>C</td>
<td>Simazine</td>
<td>low</td>
<td>slightly lighter colour</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>infrequent</td>
<td>stunted</td>
</tr>
<tr>
<td>12</td>
<td>C</td>
<td>Simazine</td>
<td>mid</td>
<td>leaf edge burn</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>13</td>
<td>B</td>
<td>Spinnaker</td>
<td>mid</td>
<td>slight stunting and yellow</td>
<td>present</td>
<td>absent</td>
<td>absent</td>
<td>infrequent</td>
<td>stunted</td>
</tr>
<tr>
<td>14</td>
<td>B</td>
<td>Spinnaker</td>
<td>high</td>
<td>slight stunting and yellow</td>
<td>present</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>stunted</td>
</tr>
<tr>
<td>15</td>
<td>A</td>
<td>Factor</td>
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<td>absent</td>
<td>infrequent</td>
<td>present</td>
</tr>
<tr>
<td>16</td>
<td>A</td>
<td>Verdict</td>
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<td>present</td>
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<td>present</td>
<td>present</td>
</tr>
</tbody>
</table>

Greenhouse test of sensitivity to Glean herbicide

Messina was highly sensitive to the SU herbicide chlorsulfuron (Glean). At the lowest applied rate (1/16\(^{th}\) of label rate) messina and the barrel medic cultivar Caliph had very stunted growth whereas Sultan-SU (bred to be tolerant of SU residues) maintained high growth (Figure 6).

![Figure 6: Plant growth score of messina and barrel medic cultivars Caliph and Sultan-SU (tolerant of SU residues).](image)
2. **Acidity tolerance of the messina symbiosis**

*Messina nodulation at low pH in a hydroponic system*

The messina symbiosis was shown to be sensitive to low pH in a hydroponic experiment. The percentage of messina plants that formed nodules declined from 53% at pH 5.7 to less than 1% at pH 4.9 (Figure 7a). The reduction was not linear, it was most rapid between pH 5.7 (53%) and 5.5 (20%).

As the percentage of plants with nodules decreased, the number of nodules on individual plants similarly declined (Figure 7b), from 2.7 nodules/plant at pH 5.7 down to ~1 nodule/plant at pH 5.3.

The results show that the strain of rhizobia (strain SRDI 554) selected for messina is slightly more sensitive to acidity than the plant itself, because when rhizobia strain WSM 1115 was substituted for SRDI 554 the nodulation of messina was improved. For example, at pH 5.5 messina nodulation with WSM 1115 was increased to 36%, from 3% with SRDI 554.

At the highest pH (5.7) messina produced more nodules than Scimitar burr medic, regardless of the inoculant strain.

Shoot dry weight of messina decreased by 24% as pH decreased from 5.5 to 4.9 (data not shown). By comparison the shoot dry weight of burr medic remained stable across the same pH range. Root dry weight of both messina and burr medic was not affected by pH or strain of rhizobia.
Messina nodulation in low pH soils

In soil, there were no significant treatment effects on plant germination, but beyond that growth stage all treatment factors (soil, pH and lime pellet) produced highly significant ($P<0.01$) effects on nodule number, nodule weight and shoot and root dry weight.

Nodulation was reduced as soil pH declined below 5.5 and below this critical level lime pelleting was beneficial.
The general relationship between soil pH and nodule number per plant is shown in Figure 8. Without lime pelleting, nodule number decreased rapidly below pH\(_{Ca}\) 5.5. Pelleting the seed with lime increased nodule number to the extent that nodule number was maintained above 6/plant below pH 5. Regardless of treatment, most nodules (97%) were confined to the tap root.

![Figure 8: Effect soil pH and seed pelleting on the number of nodules per messina plant.](image)

The relationship between soil pH and shoot weight is shown in Figure 9. Without lime pelleting, shoot weight decreased in a similar manner to nodulation below pH\(_{Ca}\) 5.5. Pelleting the seed with lime decreased the rate of shoot weight decline.

![Figure 9: Effect soil pH and lime application to seed on the shoot weight (mg DM/plant) of messina.](image)
There were significant soil effects. In the absence of a lime pellet, plant nodulation and growth responses were greatest in the Keith soil (Figs 10 & 11). As pH declined in the Keith soil from 5.5 to 4.7, nodulation decreased from 14 nodules per plant to zero (Fig. 10). The critical pH level in that soil was between 5.47 and 5.25 with both nodule number and nodule weight (data not shown) decreasing rapidly between these two levels.

Figure 10: Effect soil type and pH in the absence of a lime pellet on the nodulation (number/plant) of messina. PV = Pewsey Vale, K = Keith, TP = Tooperang.

Figure 11: Effect soil type and pH in the absence of a lime pellet on the shoot weight (mg DM/plant) of messina. PV = Pewsey Vale, K = Keith, TP = Tooperang.
The nature of the pH response in the Keith soil is further illustrated in Figure 12. The reduction in plant growth and nodulation is obvious below pH 5.47, as is the effectiveness of the lime pelleting to overcome the pH constraint even at the lowest pH level (4.66).

Figure 12: Effect of decreasing soil pH (left to right) and lime pelleting (bottom row) on the nodulation and growth of messina in the Keith soil.

3. Seed treatments

The beneficial effect of Apron on seedling number throughout the experiment is shown in Figure 13. At harvest, the addition of Apron had increased seedling number by 45%, compared to inoculation with rhizobia alone. Differences between treatments developed between 7 and 14 days after sowing (DAS).

The root health of the surviving plants was excellent, with only two of the 453 seedlings at harvest showing any sign of root damage. There were no significant differences in root dry weight of the remaining seedlings.

Nodulation of seedlings in the acidic Pewsey Vale soil was unsatisfactory (1 nodule per plant) where Apron and rhizobia were applied to the seed in the absence of lime. The addition of lime increased nodulation from 1 to 6.3 nodules per plant (Figure 14).

The overall impact on total shoot dry weight is shown in Figure 15. There was a trend ($P=0.09$) of increasing shoot dry weight with the application of both Apron and lime. Differences in shoot dry weight were mostly determined by number of plants rather than differences in the weight of individual plants.
Figure 13: Effect of seed treatment on the number of established messina seedlings. Three of five treatments shown. Data are means of the two soil treatments (which were not significantly different). Rh554 indicates rhizobia applied.

Figure 14: Effect of seed treatment on the nodulation of messina seedlings. Three of five treatments shown. Bars indicate standard error of the treatment mean.
Discussion

**Herbicide tolerance**

Herbicides were identified that can be applied to messina based pastures without causing significant damage to the messina. Safe herbicides were identified belonging to Groups A, B, C, D, K and if accepting of some damage symptoms Group F. Where registered for ‘legume seed crop establishment’ or use on ‘grass and legume pastures’, the diverse range of ‘safe’ herbicides provides options for the control of a wide range of grass and broadleaf weeds whilst minimising the risk of developing herbicide resistance.

Results from both 2016 and 2017 indicate that the broadleaf herbicides Broadstrike (Gp B) and Igran (Gp C) and Group A grass selective herbicides Verdict and Factor appear safe to use on messina. Although the other broadleaf herbicides were less effective at controlling capeweed, their performance may be improved under grazing where sheep are used to selectively graze treated weeds.

Some of the herbicides tested (e.g. Treflan and MCPA) did cause damage to messina. Previously, Treflan was thought to be ‘safe’ on messina and should only be used with strict adherence to application rate (1.4 L/ha has been used safely), timing and method of application. MCPA is not recommended for use on messina.

The severe sensitivity of messina to be the SU herbicide chlorsulfuron (Glean) highlights that seed growers are best to avoid fields sprayed with SU herbicides where possible, or if they have been used strictly observe plant back times (label will provide plant back periods and depends on soil pH, rainfall and the SU herbicide used).
Sensitivity of messina to low pH

This work has improved our understanding of the pH sensitivity of the messina symbiosis and clearly shows that messina should not be grown on soils where pH is less than 5.5 (Ca).

Even this level may carry some risk, given that the level of nodulation in the hydroponic system was less than expected (53% at pH 5.7) and the decline in nodulation was rapid between pH 5.7 and 5.5. However, since the hydroponic system is known to provide a harsh test of acidity tolerance, compared to soil that is heterogeneous and therefore likely to contain micro sites of higher pH, we believe pH 5.5 is a sensible recommendation for growers based on the results of the soil experiment and the ability to greatly reduce the risk with the application of lime to the seed before sowing.

Having advocated the application of fine lime on seed, we caution that lime pelleting should not be used to push the pH boundary for messina below pH 5.5. We state this because the benefits of lime pelleting are transient and limited to the establishment year. Hence, nodulation of the regenerating messina pasture would likely be poor where soil pH is less than 5.5 (Ca).

Nodulation at low pH was more limited by strain of rhizobia (SRDI 554) than the plant itself. In general, the messina root systems remained healthy across the pH range indicating that the impacts of reduced pH were probably limited to the processes of nodule formation rather than causing more general damage to the plant roots. The implication of this finding is that future selection efforts would be best focused on the rhizobia, but we point out this could require several years of work to find a strain with acidity tolerance and comparable levels of salt tolerance. Practically, growers could achieve similar benefit through lime application to increase soil pH.

Seed treatment

The application of Apron to seed improved seedling emergence by 45% and resulted in a trend of increased total shoot weight (+31%), noting that Apron XL 350 (Syngenta) is presently not registered for use on messina.

While satisfactory establishment of messina has been achieved in many previous field trials without the application of Apron, these results indicate that some level of seedling loss was likely. These losses are very easily overlooked in the field. A sowing rate of 10 kg/ha, similar to what was used in evaluation trials, is being recommended to growers and should provide an adequate buffer against seedling losses.

A further consideration in the potential application of Apron is that it was shown to significantly reduce the nodulation of seedlings in the acidic (4.7) Pewsey Vale soil in absence of lime pelleting. This interaction is almost certainly the result of Apron reducing the number of rhizobia on seed, which is a critical limitation to nodulation under acid stress conditions. When lime was also added to the seed, nodulation was restored to a satisfactory level.

Since Apron was often used in previous rhizobia trials during messina’s development without obvious impacts on nodulation, it should be safe to use so long as sowing recommendations that include the application of lime and sowing within 24 hrs of inoculation are followed.

The seed treatment experiment again demonstrates the benefit of lime pelleting to the establishment, nodulation and growth of messina.
Implications

The immediate benefit of the work is though the avoidance of establishment failures. With pasture renovation costs exceeding $120 per ha, successful establishment (rather than failure) on 5000 hectares would provide a benefit equivalent to five times the cost of this project. Avoiding failures in the first few years of a legume’s release is critical to generating strong farmer interest and the demand for seed production.

There will be longer term benefits as messina is adopted by the farming community over the next 20 years. Based on economic productivity gains of $60 per hectare, if the area of adoption is increased by 10% (~60,000 ha) the potential benefit is $3.6 million per annum and would require 600 t of seed.

The pH sensitivity of the messina symbiosis is unlikely to limit seed production in SA or elsewhere, so long as the limitation is recognised and the activity is undertaken on neutral or alkaline soils.

More broadly, messina’s pH sensitivity will limit its adoption because it is unlikely to be recommended for use on up to half the salt-land area in WA. This will limit the demand for seed in the longer term. Selection for improved acidity tolerance is possible, but would be difficult to justify until the plants potential is better understood. If the benefits from messina meet expectations, the liming of acid soils may be worthwhile.

With regard to the pasture seeds industry (certified production) its ability to capitalise on messina’s potential will be limited by the lack of Plant Breeders Rights (PBR) protection for the cultivar Neptune. Messina is easily harvested and we are already aware of on-farm seed harvesting and trading prior to the second year of Neptune’s release. Limited availability of the new inoculant strain (SRDI-554) and promotion of its importance to messina has no doubt provided some disincentive to the use of ‘over-the fence seed’, but this strategy is not expected to be effective for more than a couple of years. Having said that, growers using ‘over the fence seed’ may not have the same level agronomic support and access to information such has been generated by this project.

For the pasture seeds industry to fully benefit from the strong demand for messina it will either have to be able to produce seed at a retail price point of about $8, or wait for the development of a cultivar with PBR protection.

This project has identified a range of herbicides that are safe to use on messina, and some that are not. Neither the seed industry nor growers appear to be limited by the range herbicides that could be used to establish and favour the growth of messina. However, no herbicides are currently registered specifically for use on messina, and therefore they cannot be legitimately recommended to growers. This is the case for many alternative pasture legume species.

Similar to the issues outlined above for herbicides, Apron XL 350 (Syngenta) is presently registered for use on sub-clover and lucerne, but not for use on messina. There is no withholding period following its application to pasture seeds, the only caution being that treated seed should not be fed to livestock. These results of this project show that Apron is likely to be beneficial to the emergence of messina during pasture establishment and on that basis consideration should be given to the applying for a ‘minor use’ permit. In the absence of Apron, a seedling rate of 10 kg/ha should be used to allow for some seedling losses.
For growers, the availability of affordable seed is likely to be the main constraint to adoption in the short to medium term (5 years). Current annual seed availability of 30-50 tonne is only sufficient for up to 5000 ha if sown at 10 kg/ha.

**Recommendations**

Agronomists and growers need to be made aware that messina should not be grown on soils where pH is less than 5.5 (Ca) or 6.0 (water). Broader publication of the findings of this project, including a scientific paper on the pH tolerance, is planned. Where pH is below the critical level, growers should apply lime to increase soil pH.

The new rhizobia strain (SRDI-554) should be applied close to sowing and the inoculated seed lime pelleted to ensure adequate nodulation. Application of a lime pellet consistently improved nodulation and growth. Even where messina is sown on soils where pH is indicated to be above 5.5, spatial variation in pH across a paddock can be large and lime pelleting will be important to achieve even establishment.

The medic rhizobia inoculant strain (WSM1115) or the lucerne strain (RRI128) should NOT be used to inoculate messina. Although they can nodulate messina they will not persist in saline soil, resulting in poor performance of regenerating messina pastures. The lucerne rhizobia strain forms an ineffective symbiosis with messina.

The pH tolerance of messina could be improved with further rhizobia selection, but this would be a major undertaking and only justifiable upon demonstration that there is strong demand for messina, that the areas of limitation are large and that other options such as liming are not feasible.

There are no pesticides specifically registered for use on messina, which means they cannot be legitimately recommended or used, other than where pesticides are registered for general 'legume seed crop establishment' or use on 'grass and legume pastures'. This issue affects other alternative pasture legumes and is a situation that requires discussion and resolution with the APVMA.

During the course of this project we have become aware of messina establishment issues linked to poor understanding of the seasonal variation in the salinity levels and information gaps around sowing strategies. Messina is recommended for saline and waterlogging prone soils but its maximum salt tolerance is about 30 dS/m ECe (measured in summer). Farmers attempting to grow it at higher salt levels have experienced failures. Salt levels at establishment are critical to successful messina establishment and farmers would benefit from monitoring salt levels and delaying sowing until early rains have flushed salts from the soil surface, but before waterlogging occurs.

Some monitoring of establishment practices and outcomes would be useful to further refine sowing recommendations.

Future work to quantify the environmental benefits of messina (including the measurement of soil carbon, salt and water levels) would be useful for promoting its use. Its role in mixtures with other pasture species to minimise the development of saline seeps is an area that could be explored further.
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