‘Neptune’, the world’s first messina (*Melilotus siculus*) cultivar: an annual pasture legume for saline soils prone to winter waterlogging

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Abstract
Saline soils are widespread across southern Australia. Such areas tend to be waterlogged in winter with traditional pasture legumes highly sensitive to the combined stresses, resulting in poor pasture productivity. A search for pasture legumes adapted to saline, waterlogged soils, has resulted in release of the annual pasture legume, ‘Neptune’, as the world’s first cultivar of messina (*Melilotus siculus*). This paper reports on the field performance of Neptune compared with balansa clover (*Trifolium michelianum*) cv. Frontier, burr medic (*Medicago polymorpha*) cv. Scimitar, and white melilot (*Melilotus albus*) cv. Jota over three years at five sites in South Australia and Western Australia. At the three most saline sites, Neptune had a dry matter yield that was 2.5, 4.2 and 57.2 times higher than Frontier, Scimitar and Jota, respectively. A salt-tolerant *Rhizobium* strain, SRDI554, has been released to facilitate nodulation of regenerating messina in saline soils.

Key Words
Melilotus siculus, pasture legumes, salinity, waterlogging, rhizobia

Introduction
Dryland salinity is a major issue in southern Australia. Such areas are often subject to winter waterlogging. The combination of salinity and waterlogging severely affects plant growth and survival and renders such areas unsuitable for cropping. Livestock enterprises are also detrimentally affected, since currently used pasture legumes, particularly subterranean clover (*Trifolium subterraneum*), are highly sensitive to salinity (Rogers *et al.* 2011). Consequently, pastures on saline areas tend to be unproductive and nitrogen (N) deficient with limited livestock carrying capacity.

A program to investigate the performance of a range of pasture legumes on saline land was initiated in 2003, as part of the former Cooperative Research Centre for Plant-based Management of Dryland Salinity. This involved existing commercial pasture legumes and some additional experimental species. In total, 42 annual and 24 perennial legumes from 53 species were evaluated over three years at seven saline sites across southern Australia. Included among these experimental species was the annual legume, messina (*Melilotus siculus*), a native of saline, marshy areas of the Mediterranean basin, western Atlantic coast of Europe and west Asia (Rogers *et al.* 2011). Of the commercial annual legumes, balansa clover (*Trifolium michelianum*) was productive on soils subject to waterlogging, but only where salinity levels were moderate, while burr medics (*Medicago polymorpha*) were productive on soils with higher salinity levels, but only in the absence of waterlogging (Nichols *et al.* 2008). Messina was the only species able to survive at sites with both winter waterlogging and high salinity levels over summer-early autumn. This observation first raised the possibility of developing messina as a new pasture legume species for saline soils prone to waterlogging.

Following the identification of a salt-tolerant *Rhizobium* strain, SRDI554, with capacity to nodulate regenerating messina plants in highly saline topsoils (Bonython *et al.* 2011), field evaluation trials were undertaken to determine the most productive and persistent messina accession in saline waterlogged environments. This paper describes the field work, undertaken as part of the former Future Farm Industries Cooperative Research Centre, that led to the selection and release of Neptune messina, an accession originally obtained from the Israel Plant Introduction Service in 1955 and tested as SA40002, as a new cultivar. Results are presented for Neptune compared with the three best existing commercial options for saline soil: balansa clover cv. Frontier, burr medic cv. Scimitar, and white melilot (*Melilotus albus*) cv. Jota.

Methods
Messina variety trials were sown at Laffer (36.1755°S, 140.0098°E), Petherick 36.3598°S, 140.0096°E)

and Coombe (35.9488°S,140.1284°E) in South Australia (SA) in 2010 and at Darkan (33.3764°E, 116.8117°S), and Tambellup (34.1397°S, 117.5048°E), in Western Australia (WA) in 2011. All sites were subject to waterlogging in winter (watertables < 0.6 m from the soil surface), and were inundated periodically following heavy rain. Table 1 shows soil texture, mean pH and electrical conductivity (EC_e) measurements in both summer-early autumn and in mid-winter of the top 10 cm of soil across transects of the five evaluation sites. As is typical of saline environments, the sites were spatially heterogeneous for salinity and their maximum and minimum values varied between years.

Table 1. Texture and mean pH (water) and electrical conductivity (EC_e) in both summer-early autumn and mid-winter of the top 10 cm of soil of the five evaluation sites. Samples were taken at regular intervals within each site and bulked. EC_e and pH values represent the maximum range at each site over three years.

	Darkan WA	Tambellup WA	Coombe SA	Laffer SA	Petherick SA
Annual average rainfall (mm)	525	450	460	460	460
Soil texture	Sandy loam	Sandy loam	Sandy clay loam	Sand	Sandy clay loam
$pH_{(water)}$	6.0	5.8	8.0-9.6	8.2-9.5	7.4-8.9
Summer-early autumn EC_e (dS/m)	31.9	25.2	25.1	18.3	14.8
Mid-winter EC_e (dS/m)	4.4	4.2	3.6	0.7	0.6

Entries consisted of 21 messina accessions obtained from the Australian Pastures Genebank. These were compared to balansa clover cv. Frontier, burr medic cv. Scimitar, and white melilot cv. Jota. The messina accessions were inoculated with the salt-tolerant *Rhizobium* strain, SRDI554, while Scimitar and Jota were inoculated with the Group AM (WSM1115) strain and Frontier with the Group C (WSM1325) strain. Entries were replicated four times in a row-column trial design. Measurements were conducted over three years and included seedling density, dry matter (DM) production, seed yield in Year 1 and seed bank in Years 2 and 3. Plots remained undefoliated throughout the growing season, apart from the Coombe site, where plots were grazed for two weeks following dry matter assessments on July 11 in 2012. The P values and least significant differences (LSD's) shown in Tables 2-7 are based on Analyses of Variance of all 24 trial entries.

Results

Neptune (SA40002) was the most productive and persistent of the 21 messina accessions (data not shown). It also produced more DM than the other pasture legumes across all sites and years. This was particularly apparent at the two WA sites. At Darkan, total DM produced over three seasons was 5.90 t/ha for Neptune, compared to 1.35 t/ha for Frontier, 0.64 t/ha for Scimitar and 0.27 t/ha for Jota (Table 2), while at Tambellup total DM produced in 2011 and 2013 was 3.65 t/ha for Neptune, compared to just 0.30 t/ha for Frontier, 0.36 t/ha for Scimitar and 0.02 t/ha for Jota (Table 3). Over three seasons at Coombe, the most saline of the SA sites, Neptune produced 12.71 t/ha of DM, compared to 11.78 t/ha for Frontier, 6.47 t/ha for Scimitar and 0.00 t/ha for Jota (Table 4). At Laffer, total DM of Neptune was less than Frontier and Scimitar in 2011, but greater in 2012 (Table 5). At the least saline site of Petherick, total DM for Neptune over the three seasons was 7.76 t/ha, compared to Frontier (12.52 t/ha), Scimitar (6.21 t/ha) and Jota (1.99 t/ha) (Table 6). The highest annual DM production of Neptune was 8.74 t/ha in a plot at Coombe in 2011, while 7.66 t/ha was measured in a Darkan plot in 2012 and 6.40 t/ha at Petherick in 2012.

Persistence of Neptune after three seasons was generally much greater than the other cultivars. At Darkan Neptune had 1,401 regenerating seedlings/m^2, compared to 567 for Frontier, 80 for Scimitar and 35 for Jota (Table 2). This was similar at Tambellup, where Neptune had 1,233 regenerating seedlings/m^2 in the third trial year, while Scimitar had only 18 seedlings/m^2 and Frontier and Jota had negligible seedlings (Table 3). At Laffer, Neptune also had much greater persistence than the other cultivars after three years. Here Neptune had 2,411 regenerating seedlings/m^2, while the densities for Jota, Scimitar and Frontier were only 924, 730 and 533 seedlings/m^2, respectively (Table 5). Neptune also persisted well at the other two SA sites. However, Frontier had higher seedling densities in the third year at Coombe (Table 4).

Seed yields of Neptune in the year of sowing were significantly higher than the other cultivars at Darkan (Table 2) and Petherick (Table 6) and similar to Scimitar at Tambellup (Table 3). Neptune maintained a higher seed bank than Frontier and Jota in the second year after sowing at Darkan (Table 2) and in both the second and third years after sowing at Laffer (Table 5).

Table 2. Field data of Neptune messina, Frontier balansa clover, Scimitar burr medic and Jota white melilot at Darkan, WA (sown 9/06/2011). Differences were highly significant (P <0.001) for all measurements.

Variety	Establishment (plants/m²) 3/08/11	DM (kg/ha) 2/11/11	Seed yield (kg/ha) 18/01/12	Regeneration (plants/m²) 19/07/12	DM (kg/ha) 30/10/12	Seed bank (kg/ha) 7/02/13	Regeneration (plants/m²) 7/08/13	DM (kg/ha) 31/10/13
Neptune	97	1580	492	170	2198	286	1401	2122
Frontier	50	929	2	157	200	86	567	222
Scimitar	13	278	32	19	180	116	80	184
Jota	9	55	0	8	134	17	35	85
LSD (P=0.05)	30	271	361	118	1032	198	603	823

Table 3. Field data of Neptune messina, Frontier balansa clover, Scimitar burr medic and Jota white melilot at Tambellup, WA (sown 8/06/2011).

Variety	Establishment (plants/m²) 2/08/11	DM (kg/ha) 4/11/11	Seed yield (kg/ha) 17/01/12	Regeneration (plants/m²) 20/07/12	% Ground cover 1/11/12	Regeneration (plants/m²) 6/08/13	DM (kg/ha) 29/10/13
Neptune	125	1521	123	95	11.9	1233	2133
Frontier	28	303	15	16	0.0	0.4	0
Scimitar	38	161	109	18	0.9	18.3	201
Jota	0.4	22	2	0	0.0	0.0	0
P value	P<0.001	P<0.01	P<0.01	P<0.05	P<0.01	P<0.001	P<0.001
LSD (P=0.05)	25	1032	104	53	5.5	514	1121

Table 4. Field data of Neptune messina, Frontier balansa clover, Scimitar burr medic and Jota white melilot at Coombe, SA (sown 4/06/10). Plots were grazed for 2 weeks after cutting for DM measurements on 11/07/12. Differences were highly significant (P <0.001) for all measurements.

Variety	Establishment (plants/m²) 1/09/10	DM (kg/ha) 27/10/10	% Ground cover 15/06/11	DM (kg/ha) 21/09/11	Regeneration (plants/m²) 1/05/12	DM (kg/ha) 11/07/12	DM (kg/ha) 18/09/12
Neptune	135	3033	72	6916	1547	1033	1729
Frontier	391	3420	67	5743	2693	634	1980
Scimitar	172	1663	81	3988	1293	273	541
Jota	129	0	5	0	1	0	0
LSD (P=0.05)	56	903	25	2607	537	464	783

Table 5. Field data of Neptune messina, Frontier balansa clover, Scimitar burr medic and Jota white melilot at Laffer, SA (sown 5/6/10). Differences were highly significant (P <0.001) for all measurements.

Variety	Establishment (plants/m²) 17/09/10	% Ground cover 27/06/11	% Ground cover 16/08/11	DM (kg/ha) 06/10/11	Seed bank (kg/ha) 19/12/11	Regeneration (plants/m²) 13/06/12	DM (kg/ha) 09/10/12	Seed bank (kg/ha)
Neptune	129	22	33	3609	2648	2411	4758	3181
Frontier	255	99	87	9354	1237	533	394	193
Scimitar	140	94	100	9272	1587	730	1949	1674
Jota	103	87	86	4075	*	924	807	385
LSD (P=0.05)	48	21.0		1756	755	982	1914	875

Table 6. Field data of Neptune messina, Frontier balansa clover, Scimitar burr medic and Jota white melilot at Petherick, SA (sown 7/06/10). Differences were highly significant (P <0.001) for all measurements.

Variety	Establishment (plants/m²) 1/08/10	DM (kg/ha) 1/11/10	Seed yield (kg/ha) 20/12/10	% Ground cover 1/06/11	DM (kg/ha) 28/09/11	Regeneration (plants/m²) 1/05/12	DM (kg/ha) 10/10/12
Neptune	128	3154	1244	38	2130	475	2474
Frontier	520	4413	296	89	7407	158	701
Scimitar	305	2182	491	81	3651	361	381
Jota	203	677	657	89	315	569	1002
LSD (P=0.05)	73	1129	576	25.39	2723	452.6	1471

Discussion

This study confirmed that Neptune messina is well adapted to winter-waterlogged, saline soils. Its performance on soils with summer surface EC_e levels exceeding 8 dS/m was superior to balansa clover, burr medic and white melilot. Indeed, Neptune was able to persist on soils with summer surface EC_e levels up to 32 dS/m. These results accord with growth room studies that demonstrated the superior salinity tolerance of messina at germination (Nichols *et al.* 2009) and as 2-month old plants (Rogers *et al.* 2011), while having a similar waterlogging tolerance to balansa clover (Rogers *et al.* 2011). Teakle *et al.* (2012) also demonstrated the superior ability of messina to grow under the combined stresses of salinity and waterlogging than other pasture legumes. Messina has several physiological adaptations to salinity and waterlogging. It regulates the uptake and transport of Na^+, Cl^- and K^+ by the roots, and maintains a greater K^+/Na^+ ratio in the shoots (Rogers *et al.* 2011). Under waterlogged conditions it produces porous aerenchymatous phellem tissue in the hypocotyl and roots, which greatly enhances root internal aeration (Teakle *et al.* 2011).

Neptune grew well in this study on sands to sandy clay loams and has also grown well on clay soils (Nichols 2019). However, the messina symbiosis is sensitive to acidity. The sites in this experiment had soil $pH_{(water)}$ in the top 10 cm ≥ 6.0, but further glasshouse studies (RA Ballard, unpublished data) indicate that nodulation is significantly reduced if pH is below this level. Consequently, liming is recommended if the top 10 cm has a pH in water < 6.0 or in $CaCl_2$ of < 5.5 (Nichols 2019). Neptune had a suitable plant maturity for the growing seasons at the sites in this study, which had a mean annual rainfall of 450-525 mm. Neptune has a similar flowering time to Frontier balansa clover and further studies have led to a recommendation for its use in areas of southern Australia with ≥ 375 millimetres (mm) annual rainfall (Nichols 2019).

Conclusions

Neptune messina has the capacity to achieve landscape change across 600,000 ha of saline land in southern Australia. It provides landholders with a new option to improve livestock carrying capacity on saline soils prone to winter waterlogging, which are currently unproductive. The release of rhizobia strain SRDI554 will allow Neptune to persist on such soils. Neptune will complement salt-tolerant perennial grasses, such as puccinellia (*Puccinellia ciliata*) and tall wheatgrass (*Thinopyrum ponticum*), and act as an N source to increase their productivity and that of other grasses and herbs. It can also be used as an understorey legume in saltbush-based pastures. Neptune can be mixed with balansa clover and burr medic to counter the heterogeneous nature of saline landscapes. Grazing studies have shown that Neptune poses no animal health issues (Winslow *et al.* 2018) and weed risk assessments have shown it poses no weed risk threat. Further messina research should focus on identification of a salt-tolerant *Rhizobium* strain with greater acidity tolerance than SRDI554 to increase its range of adaptation.

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