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Messina (*Melilotus siculus*) – a new annual pasture legume for Mediterranean-type climates with high tolerance of salinity and waterlogging

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Abstract. Messina (*Melilotus siculus*) is a new annual pasture legume with high waterlogging tolerance and much higher salt tolerance than other legumes. Glasshouse and laboratory studies have identified several mechanisms for both salt and waterlogging tolerance that explain its adaptation to saline, waterlogged soils. Field trials are underway to select the best adapted line for release as a new cultivar. Selection is also being conducted for an adapted salt tolerant *Rhizobium* strain. This paper discusses the ecology, physiology and agronomy of messina and progress towards its release as a new pasture species for saline, waterlogged soils in Mediterranean-type climates.


I – Introduction

Approximately 400 million ha throughout the world are affected by salinity (FAO, 2005). In southern Australia around 1.5 million ha of agricultural land in Australia is currently affected by dryland salinity, with this area predicted to increase to 1.7-2.3 million ha by 2020 (National Land and Water Resources Audit, 2001). Such areas are often subject to winter waterlogging. The combination of these stresses severely affects plant growth and survival (Barrett-Lennard, 2003).
Self-regenerating annual pasture legumes are widely used in the farming systems of southern Australia (Nichols et al., 2007), which has a Mediterranean-type climate. However, currently used pasture legumes, particularly subterranean clover (*Trifolium subterraneum* L.), are very sensitive to salinity, particularly as germinating seedlings (Nichols et al., 2009; Rogers et al., 2011). This is a problem in the years following sowing, as soil surface salinity levels are generally highest at this time (Nichols et al., 2008). There is clearly a need to find an annual legume adapted to the combined stresses of salinity and waterlogging on saline soils to provide nitrogen for salt tolerant grasses and improve pasture quality.

*Melilotus siculus* (Turra) Vitman ex B.D. Jacks (syn. *M. messanensis* (L.) Mill.), given the common name of “messina” (Rogers et al., 2011), is a very promising annual pasture legume for saline, waterlogged soils. This paper summarises the results of studies on messina and its rhizobia and prospects for their commercialisation.

II – Adaptation of messina to saline, waterlogged soils

Messina is native to saline, marshy areas of the Mediterranean basin, Iberian peninsula and east Asia (Marañón et al., 1989). Initial interest in messina came from a series of trials conducted across southern Australia, in which herbage production and persistence of 42 annual pasture legumes were measured over three years at five sites that varied in extent of both salinity and waterlogging (Nichols et al., 2008). Messina is the only species that regenerates on waterlogged sites with surface (0-10 cm) electrical conductivity (*EC*<sub>e</sub>) >8 dS/m in summer and persists on sites with *EC*<sub>e</sub> >30 dS/m (Nichols et al., 2008; Nichols and Craig, unpublished data). This compares with burr medic (*Medicago polymorpha* L.), which can be productive on soils with summer surface *EC*<sub>e</sub> >8 dS/m, but only when they are well-drained, while balansa clover (*Trifolium michelianum* Savi) is productive on soils subject to waterlogging, but will not persist when summer surface *EC*<sub>e</sub> is <8 dS/m.

1. Salinity tolerance and avoidance mechanisms at germination

Messina has a range of salinity tolerance and avoidance mechanisms at germination. It has higher salinity tolerance *per se* as a germinating seedling than other pasture legumes. Nichols et al. (2009) showed messina germination was not reduced by 300 mM NaCl (equivalent to 30 dS/m), while significant reductions occurred for burr medic cv. Scimitar at 240 mM and balansa clover cv. Frontier at 120 mM NaCl. Significant variation for salinity tolerance at germination was also found amongst messina accessions by Rogers et al. (2011). Messina also has an ability to recover germinability after exposure to high levels of salinity. Nichols et al. (2009) showed messina was able to recover 31% of its potential germinability upon transfer to non-saline solution after 21 days in 600 mM NaCl, while Jeffery (2011) found variation amongst 21 messina accessions for germination recovery following 14 days at 600 mM NaCl, with four recovering full germinability and seven with >70% germination. This compared with no germination of burr medic cv. Scimitar or *Melilotus albus* cv. Jota.

Seed coat impermeability (hard seeds) was shown by Nichols et al. (2009) to protect the seed against the toxic effects of salinity over summer. They also showed messina had a delay in the timing of hard seed breakdown (seed softening) over the summer-autumn period, compared to *T. subterraneum* and *T. michelianum*. This delay acts as a salinity avoidance mechanism to defer germination until late autumn-early winter, when reliable rainfall, capable of flushing salts from the surface, is more likely to occur. Jeffery (2011) found variation in the timing of seed softening amongst 21 messina accessions.
2. Tolerance to salinity and waterlogging in the vegetative stage

Glasshouse experiments have demonstrated the high salt tolerance of messina. In a study of 19 *Melilotus* species Rogers *et al.* (2008) found 2-month old messina plants subjected to 28 d in an aerated solution of 240 mM NaCl had 89% the shoot biomass of non-saline controls, compared to 31% for balansa clover cv. Paradana. Rogers *et al.* (2011) found variation for salinity tolerance amongst 29 messina accessions, with ten having >80% the shoot biomass of non-saline controls after 21 d at 300 mM; no plants of balansa clover cv. Frontier survived. In another experiment shoot biomass of messina was 30% that of non-saline controls after 21 d at 450 mM, compared to 15% for both balansa clover cv. Paradana and burr medic cv. Scimitar (Teakle *et al.* 2012).

The waterlogging tolerance of messina has been confirmed in glasshouse studies. Rogers *et al.* (2008) found messina shoot biomass after 28 d in stagnant solution (designed to emulate the hypoxic conditions of waterlogged soils) was 102% of aerated controls, compared with 99% for balansa clover cv. Paradana and only 29% for lucerne (*Medicago sativa* L) cv. Sceptre. Root biomass of messina in the stagnant solution was 119% that of the aerated solution, compared with 144% for balansa clover and only 32% for lucerne. Rogers *et al.* (2011) examined 23 messina accessions and found none had shoot growth reductions >20% in stagnant solution, compared with aerated controls, while root biomass increased by as much as 41%. Teakle *et al.* (2011) and Verboven *et al.* (2011) showed that waterlogging tolerance of messina is aided by a highly porous form of aerenchyma, termed “phellem”, which develops around the roots under stagnant conditions and enables O$_2$ transport from the hypocotyls.

A recent study by Teakle *et al.* (2012) showed that messina is very tolerant to the combined stresses of salinity and waterlogging. New leaves were produced in messina after 14 d in stagnant nutrient solution with 550 mM NaCl (~ sea water salinity), while both balansa clover cv. Paradana and burr medic cv. Scimitar died after 5 d in a 400 mM NaCl stagnant solution.

III – Commercialisation of messina and an adapted *Rhizobium*

Initial field trials on saline, waterlogged soils showed that while messina was able to set seed and regenerate, the vast majority of regenerating seedlings failed to nodulate and grow (Nichols *et al.*, 2008; Bonython *et al.*, 2011). This was shown to be due to the inability of the commercial *Rhizobium* strain (*Sinorhizobium medicae* strain WSM 1115), used to inoculate annual medic (*Medicago* spp.) seed, to persist over summer in the highly saline soil surface. Field screening has identified several *S. medicae* strains with much greater ability to nodulate regenerating messina plants on saline soils (Bonython *et al.*, 2011). This now paves the way for development of messina as a new species for agriculture.

Field trials are currently underway to evaluate 21 accessions of messina over a 3-year period on saline, waterlogged sites in southern Australia. Measurements include biomass production, seed production, seedling regeneration densities and nodulation ability. It is intended that the best adapted variety will be released to the seed industry in 2014, along with the best adapted salt-tolerant *Rhizobium* strain.

IV – Further research

Before messina can be released as a new species for agriculture, duty of care trials need to be conducted to ensure there are no risks to animal health. Messina has negligible levels of the chemical coumarin, found in other *Melilotus* species (Nair *et al.*, unpublished data; Stevenson, 1969), which can taint the flavour of meat, milk and flour and cause a haemorrhagic condition in livestock if fed mouldy hay (Masters *et al.*, 2001). Messina also has similar nutritive value to other...
pasture legumes (Rogers et al., 2008). However, animal feeding trials need to be conducted to confirm its lack of anti-nutritional factors and its value as a stock feed.

Once the best adapted accession has been selected as a new cultivar, agronomic and grazing management packages need to be developed to optimise pasture performance and animal production. Factors include establishment methods, mixtures with salt tolerant grasses, fertiliser rates, broadleaf herbicide options and grazing strategies. Seed production strategies also need to be devised. A preliminary seed harvesting trial produced seed yields of over 1,500 kg/ha (A.D. Craig, unpublished data), indicating the high yield potential of messina and its potential for seed harvesting with a conventional cereal harvester. This should make possible the provision of inexpensive seed for sowing.

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