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

Nichols P.G.H., Teakle N.L., Bonython A.L., Ballard R.A., Charman N., Craig A.D.

in

Acar Z. (ed.), López-Francos A. (ed.), Porqueddu C. (ed.).
New approaches for grassland research in a context of climate and socio-economic changes

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 102

2012
pages 155-159

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

P.G.H. Nichols[1,2,3,*], N.L. Teakle[3,4], A.L. Bonython[1,5], R.A. Ballard[1,6], N. Charman[1,6] and A.D. Craig[1,5]

[1]Cooperative Research Centre for Future Farm Industries, The University of Western Australia, Crawley WA 6009 (Australia)
[2]Department of Agriculture and Food Western Australia, South Perth WA 6151 (Australia)
[3]School of Plant Biology, The University of Western Australia, Crawley WA 6009 (Australia)
[4]Centre for Ecohydrology, The University of Western Australia, Crawley WA 6009 (Australia)
[5]South Australian Research and Development Institute, Naracoorte SA 5271 (Australia)
[6]South Australian Research and Development Institute, Adelaide SA 5001 (Australia)
*e-mail: phil.nichols@agric.wa.gov.au

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.

Keywords. Salinity – Waterlogging – Fodder – Cultivars – Plant breeding – Rhizobia – Nitrogen fixation.

Messina* (Melilotus Siculus) *– une nouvelle légumineuse fourragère annuelle pour de climats de type méditerranéen avec une grande tolérance à la salinité et l'engorgement

Résumé. *Messina* (Melilotus Siculus) *est une légumineuse fourragère annuelle tolérante à l'égorgement et ayant une tolérance à la salinité beaucoup plus élevé que d'autres légumineuses. Des études sous serre et en laboratoire ont identifié plusieurs mécanismes à la fois pour la tolérance à la salinité et à lengorgement qui expliquent son adaptation aux sols salins et détrempés. Essais sur le terrain sont en cours pour choisir la meilleure ligne adapté pour son lancement comme un nouveau cultivar. La sélection est également en cours pour une souche de* Rhizobium *adapté et tolérante à la sel. Cet article traite de l'écologie, la physiologie et l'agronomie de messina et des progrès vers sa sortie comme une nouvelle espèce fourragère pour des sols salins et gorgés d'eau en climats de type méditerranéen.*

Mots-clés. *Salinité – Saturation en eau – Fourrage – Cultivars – Amélioration des plantes – Rhizobia – Fixation d'azote.*

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_e) >8 dS/m in summer and persists on sites with EC_e >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_e >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_e 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.

Acknowledgments

We are grateful for the technical support of Brad Wintle, Darryl McClements, Stephen Biggins and Tammy Closter. We wish to thank John South, Twynam Cunningham, Bob and Craig Lubcke, Neville and Gordon Stopps, Duck Island Partners, Trevor Egel, Kym Herriot and Greg Vickers for their support and allowing us to conduct field trials on their properties.

References

Barrett-Lennard E.G., 2003. The interaction between waterlogging and salinity in higher plants: causes, consequences and implications. *Plant and Soil* 253, 35-54.

Bonython A.L., Ballard R.A., Charman N., Nichols P.G.H. and Craig A.D., 2011. New strains of rhizobia that nodulate regenerating messina (*Melilotus siculus*) plants in saline soils. *Crop and Pasture Science* 62, 427-436.

FAO, 2005. Global network on integrated soil management for sustainable use of salt-affected soils. Rome, Italy: FAO Land and Plant Nutrition Management Service. *http://www.fao.org.ag/agl/agll/spush*

Jeffery R., 2011. Mechanisms of salinity tolerance and avoidance at germination of 21 accessions of *Melilotus siculus* (messina). Honours thesis, Faculty of Natural and Agricultural Sciences, The University of Western Australia.

Marañón T., Garcia L.V. and Troncoso A., 1989. Salinity and germination of annual *Melilotus* from the Guadalquivir delta (SW Spain). *Plant and Soil* 119, 223-228.

Masters D.G., Norman H.C. and Dynes R.A., 2001. Opportunities and limitations for animal production from saline land. *Asian-Australian Journal of Animal Science* 14, 119-211.

National Land and Water Resources Audit, 2001. Australian dryland salinity assessment 2000. Extent, impacts, processes, monitoring and management options. National Land and Water Resources Audit, Canberra, Australia.

Nichols P.G.H., Craig A.D., Rogers M.E., Albertsen T.O., Miller S., McClements D.R., Hughes S.J., D'Antuono M.F. and Dear B.S., 2008. Production and persistence of annual legumes at five saline sites in southern Australia. *Australian Journal of Experimental Agriculture* 48, 518-535.

Nichols P.G.H., Loi A., Nutt B.J., Evans P.M., Craig A.D., Pengelly B.C., Dear B.S., Lloyd D.L., Revell C.K., Nair R.M., Ewing M.A., Howieson J.G., Auricht G.A., Howie J.H., Sandral G.A., Carr S.J., de Koning C.T., Hackney B.F., Crocker G.J., Snowball R., Hughes S.J., Hall E.J., Foster K.J., Skinner P.W., Barbetti M.J. and You M.P., 2007. New annual and short-lived perennial pasture legumes for Australian agriculture-15 years of revolution. *Field Crops Research* 104, 10-23.

Nichols P.G.H., Malik A.I., Stockdale M. and Colmer T.D., 2009. Salt tolerance and avoidance mechanisms at germination of annual pasture legumes and their importance for adaptation to saline environments. *Plant and Soil* 315, 241-255.

Rogers M.E., Colmer T.D., Frost K., Henry D., Cornwall D., Hulm E., Deretic J., Hughes S.R. and Craig A.D., 2008. Diversity in the genus *Melilotus* for tolerance to salinity and waterlogging. *Plant and Soil* 304, 89-101.

Rogers M.E., Colmer T.D., Nichols P.G.H., Hughes S.J., Frost K., Cornwall D., Chandra S., Miller S.M. and Craig A.D., 2011. Salinity and waterlogging tolerance amongst accessions of messina (*Melilotus siculus*). *Crop and Pasture Science* 62, 225-235.

Stevenson G.A., 1969. An agronomic and taxonomic review of the genus *Melilotus* Mill. *Canadian Journal of Plant Science* 49, 1-20.

Teakle N.L., Armstrong J., Barrett-Lennard E.G., Real D. and Colmer T.D., 2011. Aerenchymatous phellem in hypocotyl and roots enables O_2 transport in *Melilotus siculus*. *New Phytologist*, 190, 340-350.

Teakle N.L., Bowman S., Barrett-Lennard E.G., Real D. and Colmer T.D., 2012. Comparisons of annual pasture legumes in growth, ion regulation and root porosity demonstrate that *Melilotus siculus* has exceptional tolerance to combinations of salinity and waterlogging. *Environmental and Experimental Botany*, 77, 175-184.

Verboven P., Pedersen O., Herremans E., Ho Q.T., Nicolaï B.M., Colmer T.D. and Teakle N., 2011. Root aeration via aerenchymatous phellem: three-dimensional micro-imaging and radial O_2 profiles in *Melilotus siculus*. *New Phytologist*, 190, 1-12.