Diversity in the genus *Melilotus* for tolerance to salinity and waterlogging.

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Short title
Salt and waterlogging tolerance in *Melilotus* species

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Abstract

Identifying forage species that are productive in saline environments is an important research priority in many areas of the world affected by salinity. The salt and waterlogging tolerances of 19 species of *Melilotus* were evaluated in a series of glasshouse experiments. Measurements taken on each species included: dry matter (DM) production, root growth and development, shoot ion (Na^+, K^+ and Cl^-) concentrations, root porosity, and *in vitro* estimates of nutritive value. Research on several species was restricted because of their potential as weed risks. Of the remaining species, *M. siculus* (syn. *M. messanensis*), an annual species, showed high relative salt and waterlogging tolerances, good DM production under non-stressed and stressed (saline and hypoxic) conditions, a high level of root porosity under stagnant conditions, low tissue ion (Na^+, Cl^-) concentrations, and a reasonable dry matter digestibility content (range 66-69%) under highly saline conditions. *M. sulcatus* ssp. *segetalis* and *M. indicus* were also identified as species with good DM production and tolerance to salinity and waterlogging stresses. Further weed risk assessments and field trials on these species are required before they can be promoted for use as pasture forages on saline areas.

Introduction

The *Melilotus* genus, which originates from Eurasia, is closely related to the *Medicago* and *Trigonella* genera and includes approximately 25 species of annuals and biennials/perennials (Allen and Allen 1981). *Melilotus* species tend to be moderately winter-hardy, drought resistant and can be valued as pasture forage (Stevenson 1969). However, the genus is not widely grown in some parts of the world, for example, Australia, partly because of concerns relating to high coumarin levels and potential weediness of some species (Evans and Kearney 2003). Certain species (e.g. *M. wolgicus, M. elegans and M. neopolitanus)* are seen as potential weed risks that could possibly naturalise in the introduced environment affecting biodiversity and crop and pasture production (Bennet and Virtue 2003, Stone personal communications). Coumarin, a secondary plant compound, is associated with dicoumarol production, an anticoagulant, that can cause a haemorrhagic condition known as sweet clover disease (Evans and Kearney 2003, Nair et al. 2006). However, there is variation in the coumarin concentration in plants, both between and within species, and preliminary research also suggests that it is possible to undertake management practices to limit high concentrations of coumarin (Nair et al. 2006).

In some countries (e.g. Argentina, Spain, Canada and Russia), *Melilotus* species are grown in moderately saline areas where traditional forage legumes cannot be grown (Maddaloni, 1986) and variations in salt tolerance have been found between a limited number of *Melilotus* species. In a glasshouse study on salt tolerance of three *Melilotus* species, Marañón et al. (1989) found differences in tolerance to salinity at germination both between and within species and concluded that *M. siculus* (syn. *M. messanensis*) was the most salt tolerant of the species evaluated. Based on field evaluations in southern Australia, Evans and Kearney (2003) suggested that *M. alba* is a useful pasture legume to revegetate saline soils. Studies by Ashraf et al. (1994) using *M. indicus*, and Rogers and Evans (1996) on *M. alba*, revealed that populations collected from saline sites were more salt tolerant than populations collected from non saline sites and commercial cultivars.

In contrast to the limited research on salt tolerance, there have been no related studies examining the waterlogging tolerance of species within this genus. Information on plant tolerance to waterlogging is important since waterlogging is a frequent accompaniment to salinity in discharge sites in many parts of the world and in some regions winter-waterlogged, moderately saline land is greater in area than the severely salt-affected land (Evans and Kearney 2003).

This paper describes experiments that evaluated the salt and waterlogging tolerances of a range of *Melilotus* species. Selected physiological traits, such as shoot ion concentrations and root gas-filled porosity, as well as nutritive properties of the shoots, were also evaluated. The research was conducted in glasshouses at two sites in Australia.

Materials and Methods

Salinity tolerance assessment

Nineteen *Melilotus* species (listed in Table 1) plus 3 check species (strawberry clover -*Trifolium fragiferum* cv. Palestine, balansa clover - *Trifolium michelanium* cv. Paradana and lucerne - *Medicago sativa* cv. Sceptre) were sown into vermiculite in seedling trays in a naturally-lit glasshouse at Tatura, Victoria, Australia (36° 26' latitude, 145° 16' longitude, 114 m elevation) on 15th April 2004 and watered with non-saline water. The seeds of each species were first scarified by rubbing lightly, but thoroughly, between 2 sheets of very fine sandpaper. Each *Melilotus* species was represented by 5 accessions (selected from the South Australian Research and Development Institute's Genetic Resource Centre (GRC) in Adelaide) bulked together. Seed of the majority of these accessions had been collected on site by staff at the GRC at SARDI and the remainder of the accessions were acquired from existing collections at international genetic resource centres (Table 1). The check species were selected because these represented standard legume species grown over a range of environments in southern Australia.

Some species that were included in this study are now restricted from general importation into Australia under Australian Quarantine Inspection Service (AQIS). At the commencement of the evaluation program, these species were already held in Australian genetic resource centres and were available for evaluation under regulations existing at the time.

When each *Melilotus* species had two true leaves (21st June - 67 Days After Sowing -DAS), the plants were transplanted into continuously aerated 120 L tanks (with a holding capacity of 25 x 18 plants) filled with non-saline tap water and modified Hoagland nutrient solution (Karmoker and Van Steveninck, 1978 viz. 0.5 mM KH_2PO_4, 3 mM KNO_3, 4 mM $Ca(NO_3)_2.4H_2O$, 1 mM $MgSO_4$, 37.5 µM FeEDTA (ethylenediaminetetraacetic acid iron III sodium salt), 23 µM H_3BO_3, 4.5 µM $MnCl_2.4H_2O$, 4 µM $ZnSO_4.7H_2O$, 1.5 µM $CuSO_4.5H_2O$ and 0.05 µM MoO_3.). The experiment was a split plot design with 4 salinity treatments and 4 replicates using 16 tanks in total. The experimental unit was a row of 18 plants. Tanks were emptied each week and a fresh supply of nutrient solution was added. The mean glasshouse temperatures for the duration of this experiment were 20.0 ± 4°C day/5.7 ± 2°C night.

The first harvest of 8 plants, selected randomly from each species, was taken on 19th July (95 DAS). The plants were destructively harvested and shoot and root fresh and dry weights (dried at

70° C for 48 hours) were measured. The NaCl treatments (0, 80, 160 and 240 mM NaCl) were then imposed on the 19th July 2004 in increments of 80 mM/day until full treatments had been reached (22nd July).

The second harvest occurred on 19th August (126 DAS and 28 days after the full salinity treatments had been imposed) with the fresh and dry weights of plant shoots and roots measured.

Samples of oven-dried shoots were ground and 0.1 g was weighed into a 10 ml vial and 10 ml of 0.5 M HNO_3 added and samples placed on a shaker at 20°C for 2 days. The extract was diluted appropriately and then K^+ and Na^+ were measured using a flame photometer (Jenway Ltd, model PFP7, Essex, UK). Chloride was determined using a Buchler-Cotlove chloridometer (Buchler Instruments, Model 4-2008, Fort Lee, USA). Plant tissue reference material was included in the analyses, with recovery being 109% for Na^+, 103% for K^+ and 96% for Cl^-. K^+ selectivity ratio, or ratio of tissue K^+ and Na^+ to that in the external solution (Pitman 1976), was calculated for each species.

Dried samples were ground to pass through a 1 mm sieve using a cyclone grinder (CYCLOTECH 1093 Sample Mill). Near infrared reflectance spectra for the dried samples were collected from 400-2500 nm with a scanning monochromator (model 6500 NIRSystems Inc. Silver Spring, MD USA).

Samples were analysed for Dry Matter Digestibility (DMD) (Klein and Baker 1993), Ash (Faichney and White 1983), Acid-Detergent Fibre (ADF) and lignin based on the method of ANKOM-TECHNOLOGY (1998) and Neutral-Detergent Fibre (NDF) using the Ankom filter-bag method (ANKOM-TECHNOLOGY 1998), with Cetyrimethylammonium bromide instead of Cetavlon. Hemicellulose was estimated by subtraction of ADF from NDF. A minimum of 10g of dried material was required for nutritive value analyses, consequently, analyses were only undertaken on species that produced enough dried material in the salt tolerance screening.

Waterlogging tolerance assessment

The same 19 species of *Melilotus* and three check species (same seed lots used for salt tolerance evaluations in the glasshouse) were also screened for waterlogging tolerance.

Because of space limitations, the experiment was conducted in two parts – composed of 9 and 10 species of *Melilotus* respectively in a controlled environment room ($20^oC/15^oC$ day/night temperature, with 12 hour photoperiod, irradiance of 375 – 490 µmol quanta m^{-2} s^{-1}, PAR). Scarified seeds (as in the glasshouse salinity experiment) were surface sterilised with 0.04% (w/v) bleach (sodium hypochlorite: $NaHClO_3$), thoroughly rinsed with deionized (DI) water and then imbibed in aerated 0.5 mM $CaSO_4$ for 3 hours in darkness.

Seeds were then placed on mesh screen floating over 10% aerated nutrient solution. The nutrient solution was the same as in the salinity glasshouse experiment but with the addition of 2.5 mM MES (2-[N-morpholino] ethanesulfonic acid). The pH of the solution was 6.3 and was adjusted by the addition of 1 M KOH.

The seeds were kept in darkness for 3 days, then exposed to light and transferred to 25% nutrient solution, still on floating mesh screen. One week after imbibition, seedlings were transplanted into 50% aerated nutrient solution in pots. Pots consisted of sealed 4.5 L buckets which were covered in aluminium foil to ensure roots were grown in darkness. There were 8 plants per pot inserted into holes in the lid and held there with polystyrene foam. After 1 more week, the solution was changed to full strength concentration (plants now 2 weeks old). Nutrient solutions were renewed weekly throughout the experiment.

An initial harvest of 4 plants per pot, selected at random, was carried out 4 weeks after imbibition. The shoot was cut from the root and the lateral roots were separated from the main root. Shoot fresh and dry weights were measured. The roots were oven dried (70^o C) and weighed. A stagnant treatment was imposed on the same day as the initial harvest. The stagnant treatment pots were bubbled with N_2 gas until the O_2 concentration in the solution was approximately 10% of that in air-saturated solution. The pots were then left stagnant for 24 hours, and then the solution was replaced with stagnant agar nutrient solution (0.1% w/v dissolved agar added to the standard nutrient solution to prevent convective movements). Prior to adding to the pots, the solution was bubbled with N_2 overnight to displace the O_2 out of solution. A set of pots also continued to be aerated (i.e. controls).

In total, there were 2 treatments, aerated or stagnant, and there were 3 replicate pots for each species and treatment combination. Pots were arranged randomly within each replicate block and were repositioned every second day.

A final harvest of 4 plants per pot was carried out after 4 weeks of treatments. The shoot was cut from the root and the lateral roots were separated from the main root. Root porosity (proportion of gas volume per root volume) was measured in both main and lateral roots, following the method of Raskin (1983) and with the equations as modified by Thomson et al. (1990). Roots and shoots were oven dried (70^{o} C) and weighed.

Statistical analyses

All glasshouse measurements (viz. shoot and root DM production, relative production, tissue ion data, nutritive value, root porosity, relative growth rates) were analysed by ANOVA with a randomised block design with salinity and waterlogging levels fitted as orthogonal polynomials (Genstat 8.1. Lawes Agricultural Trust, Rothamsted Experimental Station). Residuals were checked for normality and homogeneity. Where there had been incomplete data from some treatments because of a limited amount of material e.g. for nutritive value, this data was analysed by REML (Restricted Maximal Likelihood) analyses (Genstat 8.1, Lawes Agricultural Trust, Rothamsted Experimental Station). Regression analyses were performed to evaluate possible relationships between various growth parameters and root porosity.

Results

Salinity tolerance assessment

The growth of most species of *Melilotus* was significantly affected by the salinity treatments (Table 2) – however there were differences amongst species in the degree of growth reduction and therefore in relative salt tolerance. For example, at 240 mM NaCl, the growth of *M. tauricus* and *M. wolgicus* was still over 90% compared with their growth under non-saline conditions. By comparison, DM production in plants of *M. speciosus* and *T. michelianum* at 240 mM NaCl was only approximately 30% of that of the control. There was a large amount of variation in absolute DM production under non-saline conditions amongst the *Melilotus* species with some species (e.g. *M. speciosus* and *M. siculus*) producing more DM under control conditions compared with slower growing perennial species such as *M. suaveolens* and *M. polonicus* (Table 2).

Concentrations of Cl^- (Table 3) and Na^+ (Table 4) in the shoot tissues increased ($P<0.001$) with increasing NaCl concentrations in all species, but there were also differences ($P<0.001$) between species. At 240 mM NaCl, some species (e.g. *M. speciosus* and *M. italicus*) had more than twice the concentrations of Na^+ and Cl^- in their shoots compared with other species (e.g. *M. wolgicus* and *M. indicus*). Similarly, some species maintained relatively low concentrations of Na^+, in particular, and Cl^-, at 80 mM NaCl compared with other species. Concentrations of K^+ decreased ($P<0.001$) with increasing levels of NaCl (Table 5). The K:Na selectivity (Table 5) showed some species – *M. indicus, M. wolgicus, M. siculus*, had selectivity ratios that were more than twice those of the species with highest concentrations of Na^+ in their shoots (*M. infestus, M. italicus, M. speciosus*). There were negative correlations ($P<0.001$) between shoot Na^+ and Cl^- concentrations and relative salt tolerance (viz. $r=-0.51$ and -0.52, respectively), but no association between shoot K^+ concentration and relative salt tolerance ($r=0.222$).

Nutritive value assessments were limited by the amount of DM available for each species. There were differences in DMD levels between *Melilotus* species ($P<0.001$ Table 6). When the DMD results were adjusted for the soluble salt content, there was a downward trend in DMD as an effect of increasing NaCl concentrations. This effect was lower for some species (e.g. *M. siculus*) than it was for other species (e.g. *M. speciosus*). The relationship (correlation) between DMD (soluble salts removed) and relative salt tolerance was significant ($r=0.658$, $P<0.001$). There was some variation amongst species in the response of the fibre analyses (NDF and ADF) to increasing levels of NaCl. In *M. albus, M. elegans* and *M. indicus*, levels tended to increase with increasing salinity,

however, this trend was reversed for the four other species (*M. italicus, M. siculus, M. speciosus* and *T. michelianum*).

Waterlogging tolerance

There were differences ($P<0.05$) amongst *Melilotus* species in the effect of waterlogging on plant production (Table 7), with several species - including *M. dentatus, M. indicus, M. infestus, M. siculus, M. sulcatus* ssp. *segetalis* and the check species *M. fragiferum* and *M. michelianum*, showing good tolerance to the hypoxic conditions in terms of both shoot and root growth. In other species, e.g. *M. albus, M. italicus, M. neapolitanus, M. speciosus, M. wolgicus, M. sulcatus ssp brachystachys* and *Medicago sativa*, the growth of the shoots, main and lateral roots were significantly reduced ($P<0.05$) by the stagnant treatment. Under stagnant conditions, relative growth rates were highest in *M. indicus, M. altissimus, T. fragiferum* and *M. siculus* and were lowest in *M. wolgicus* and *M. neapolitanus* (data not presented).

All species appeared to acclimate to stagnant conditions by increasing root porosity (% gas volume per unit root volume) in the main (data not presented) and lateral roots (Figure 1). Again there were differences ($P<0.05$) amongst species in the development of root porosity. In stagnant conditions, in *M. siculus* nearly 23% of the volume of the main root and 14% of the volume of the lateral root was gas spaces, whereas for *M. italicus* the gas volume in the lateral roots was less than 4%.

Root dry mass as a percentage of total plant dry mass varied between 1 and 46%. In the two waterlogging experiments, shoot dry mass was positively correlated with lateral root porosity (r^2 = 0.62 and 0.33), but although the correlation with main root porosity was also positive, this was significant only in the second experiment (r^2=0.61). Similarly, in both experiments, total root dry mass was positively correlated with the porosity of the lateral roots (r^2 = 0.38 and 0.57), whereas there was no relationship with main root porosity. Thus, the higher lateral root porosity was associated with larger root systems and in turn, larger shoots.

Discussion

In order to assess a plant or species suitability for saline field conditions, it is valuable to evaluate its tolerance to both salinity and waterlogging stresses – since these stresses often occur in combination in discharge areas throughout the world. Plant growth is usually more depressed by a combined salt and waterlogging stress than by either stress alone (Barrett-Lennard 2003). This is because waterlogging interacts with salinity to increase the concentrations of Na^+ and Cl^- in plant shoots, and these increased concentrations have adverse effects on plant growth and survival (Barrett-Lennard 2003, Rogers and West 1993). In this large screening study, it was difficult to combine salinity and waterlogging stresses, partly because of the large numbers of plants required for such a complete design. Nevertheless, our study has documented the diversity that exists within the *Melilotus* genus with regard to tolerance to both hypoxic and saline conditions in the glasshouse and such variation is consistent with, but extends, other studies that evaluated the salt tolerance of much smaller sets of *Melilotus* species (Marañón et al. 1989, Rogers and Evans 1996). It is also the first time that the degree and variation of waterlogging tolerance within this genus has been described.

Despite showing good responses to saline and waterlogged conditions, several of the species that were evaluated in glasshouse experiments (viz. *M. dentatus, M. elegans, M. neopolitanus, M. polonicus, M. suaveolens* and *M. wolgicus*) are considered potential weed risk – at least in Australia- and research on these species will not be continuing. Some of these species, in particular the perennial and biennial *M. dentatus, M. polonicus, M. suaveolens* and *M. wolgicus*, were also very slow growing so that the poor performance of plants under control conditions may have had a masking effect on the stress response (Tables 2 and 7). It also appears that slow growing species may not be as suited to glasshouse studies because any small differences in dry matter production are manifested as large differences in relative growth responses and therefore clear interpretation of results may be difficult. Of the remaining *Melilotus* species, *M. siculus* showed the greatest potential under both saline and hypoxic stresses (Tables 2 and 7, Figure 1). This annual species showed a high relative salt and waterlogging tolerance, good DM production under non-stressed and stressed (saline and hypoxic) conditions, a high level of root porosity under stagnant conditions, low shoot tissue ion concentrations and reasonable DM digestibility levels under highly saline conditions. *M. siculus* is known to have naturalised in some areas of Australia, including south western Victoria, on marshy or seasonally inundated areas and in the presence of salt (Jeanes 1996). In Spain, *M. siculus* has also been found to inhabit saline areas with an EC_e of up to 26 dS/m (equivalent to approximately 260 mM NaCl) and is more salt tolerant at germination than both *M. sulcatus* ssp. *segetalis* and *M. indicus* (Marañón et al. 1989). Recent field studies in

southern Australia (Nichols personal communication) have also shown that this species has potential for use on saline soils prone to waterlogging. Additional advantages of this species are that it is relatively free from coumarins and it produces large seeds on upright stems (Nichols personal communication). Australian research is continuing to identify a suitable strain of *Rhizobium* to improve its production under saline field conditions (Charman et al. 2006).

M. sulcatus ssp. *segetalis*, *M. albus* and *M. indicus* were also identified in the present work as species with good productivity and with tolerance to the salinity and waterlogging stresses. *M. indicus* and *M. albus* are also known to colonise moderately saline areas in southern Australia (Jeanes 1996) and research in Pakistan (Ashraf et al. 1994) has found that *M. indicus* performed better than *Medicago sativa* (lucerne) when grown at NaCl levels up to 240 mM (24.8 dS/m). However, *M. indicus* contains moderate to high levels of coumarins (Craig, personal communication) which may restrict its usefulness as an agricultural species. *M. sulcatus* ssp *segetalis* has been found to grow naturally in saline soils in the Guadalquivir delta of Spain although its shoot biomass is significantly reduced at soil EC_e levels of greater than 15 dS/m (Romero and Marañón 1994).

Currently, the legume options for saline land in southern Australia focus on *Medicago sativa* (well-drained soils), *Trifolium fragiferum* (high rainfall areas) and *T. michelianum* (waterlogged environments). However, these species have their limitations. *M. sativa* is susceptible to waterlogging (Rogers 1974), *T. fragiferum* lacks sufficient drought tolerance while *T. michelianum* persists poorly under moderate (around 10 dS/m) saline conditions (Rogers and Noble 1991). The particular advantages of some of the *Melilotus* species in this study *(M. siculus, M. indicus* and *M. sulcatus* ssp. *segetalis*), are that they are more waterlogging tolerant than *M. sativa*, more salt tolerant than *T. michelianum*, more productive than *T. fragiferum* and have adequate nutritive value levels (Agricultural Research Council 1984), and therefore may be suitable species to grow in saline areas.

The relationship between tissue ion concentrations (Na^+ and Cl^-) and salt tolerance was found to be negative (i.e. ion concentrations were lowest in shoots of the more tolerant species) and there were large differences amongst *Melilotus* species (Tables 3-5). Salt exclusion and the resultant high ratio of K^+/Na^+ in leaves is recognised as an important tolerance mechanism in many crop species (Läuchli 1984, Munns et al. 2005) and in this respect our results relate well to research on *M. indicus* (Romero and Maranon 1994), *M. alba* (Rogers and Evans 1996), *M. sativa* (Noble et al. 1984), *Trifolium repens* (Rogers et al. 1997) and *Lotus tenuis* (Teakle et al. 2006). By contrast, it

has also been suggested that salt tolerance of *M. indicus* is associated with ion (Na^+ and K^+) 'inclusion' rather than 'exclusion' (Ashraf et al. 1994), although this was found to be coupled with a favourable K:Na selectivity ratio. In our study, the K:Na selectivity ratios at 240 mM NaCl were high (range 15-57) and the species with the more favourable selectivity ratios also tended to be those with greater relative salt tolerance (Table 5).

The *Melilotus* species differed in the development of porosity in roots (Figure 1). In stagnant conditions, the highest porosity values for lateral roots were for *M. siculus* at 14%, with the lowest for *M. italicus* just below 4%. Higher lateral root porosity was associated with larger root systems and in turn, larger shoots. The value of 14% root porosity in *M. siculus* was similar to that in the waterlogging-tolerant check species *T. michelanium*, both of which were close to the most tolerant *Trifolium* species identified by Gibberd et al. (2001). All these porosity values for *Melilotus* and *Trifolium*, however, are significantly lower than root porosity in most wetland species (Colmer 2003).

Using the glasshouse to screen for tolerance has advantages over longer-term field experiments - such as avoiding the spatial variability in salinity and waterlogging that exist in the field, and generally a lower cost. Preliminary field results on salt and waterlogging tolerance in *Melilotus* species have shown a good relationship between glasshouse and field results (viz. Spearman's rank correlation coefficients were 0.89 and 0.85 for salinity and waterlogging respectively, Rogers personal communication). Nevertheless, it will also be essential to conduct longer-term field research on selected, priority species to enable other important plant attributes to be assessed, as well as to study plant response to prolonged saline and/or waterlogged conditions that may also vary with the seasons. Further weed risk assessment is also required before recommending many *Melilotus* species for use on saline areas. Many agricultural and environmental weeds have arisen as a direct result of past introductions escaping cultivation and naturalising in a wide range of environments (Bennet and Virtue 2004). The *Melilotus* species currently restricted from importation into Australia because their weed risk has yet to be assessed, are unlikely to have a role in the Australian agricultural landscape unless selection of significantly less weedy cultivars can be made. It is more likely that the *Melilotus* species that are permitted entry to Australia can be developed to have improved production on saline areas and be of low weed risk to agriculture and native ecosystems.

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Table 1. The origin of the *Melilotus* species used in this study. Genebank accession numbers can provide more information regarding collection environments.

Species	Common Name	Lifespan	Country of seed collection	Genebank Accession No.
M.albus Medik.	White sweet clover	Annual/biennial	Algeria, Argentina, Australia, Israel, Spain, Rhodesia, Turkey	SA 1991, 27773, 34659, 34666, 35627, 35628, 35629, 35635, 37094, 37421
M.altissimus Thuill.	Tall yellow sweet clover	Biennial/perennial	Ethiopia, Russia	SA 36956, 36957
M.dentatus (Waldst. & Kit.) Pers	Small flowered Melilot	Annual/biennial	USA, Mongolia, China	SA 36946, 36948, 36955
M.elegans Ser.	Elegant Melilot	Annual	Hungary, Iran, Ethiopia, Canada, Sardinia	SA 36958, 36960, 36961, 36962, 40032
M.hirsutus Lipsky		Perennial	Russia	SA 36963
M.indicus (L.) All.	King Island Melilot	Annual	India, Afghanistan, France, Israel, Peru	SA 36965, 36966, 36967, 36968, 36969
M.infestus Guss.	Rounded fruit Melilot	Annual	Hungary, Algeria, Tunisia, Italy	SA 34478, 34494, 36971, 36972, 39983
M.italicus (L.) Lam.	Italian Melilot	Annual	Morocco, Israel, Sicily, Spain, Czech republic	SA 36973, 36974, 39986, 39998, 40073
M.neapolitanus Ten.	European sweet clover	Annual	USA, Portugal, Greece	SA 36150, 36987, 36989, 37257, 40009
M.officinalis (L.) Pall.	Yellow sweet clover	Biennial	New Zealand, Canada, Turkey, Portugal, USA	SA 36494, 37399, 37401, 37403, 37419
M.polonicus (L.) Pall.	Caspian sweet clover	Perennial	Russia	SA 36976, 36977
M.segetalis (Brot.) Ser. (syn *M.sulcatus ssp. segetalis)*	Corn Melilot	Annual	USA, Spain, Israel, Morocco, Portugal	SA 36975, 36979, 39996, 40022, 40029
M. siculus (Turra) Vitman ex B. D. Jacks. (syn.*M.messanensis)*	Messina	Annual	Greece, Cyprus, Israel, Russia. Portugal	SA 36980, 36981, 36982, 36983, 40003
M.speciosus Durieu		Annual	Canada, USA	SA 36984, 36985, 36986, 37269, 40008
M.suaveolens Ledeb.		Annual/ biennial	USA	SA 36991, 37280, 37288, 37289, 40011
M.sulcatus Desf. (syn *M.sulcatus ssp. brachystachys)*	Furrowed Melilot	Annual	Morocco, Jordan, Algeria, Tunisia	SA 34470, 34477, 34488, 40018, 40061

M.tauricus (M. Bieb.) Ser.	Biennial	Russia, Canada, Czech republic	SA 36996, 36998, 38086, 40035, 40036
M.wolgicus Poir.	Biennial	Russia, Italy, USA. Denmark	SA 37000, 37001, 40037, 40038, 40039

Table 2. Effect of NaCl on the shoot dry matter production of 19 species of *Melilotus* and three check species (*M. sativa, T. fragiferum* and *T. michelianum*). Data in parenthesis show production (%) relative to nonsaline conditions. Species are listed in order of increasing dry matter production in non-saline conditions.

	Shoot dry mass (g/plant) at:			
Species	**0 mM**	**80 mM**	**160 mM**	**240 mM**
M. suaveolens	0.01	0.01 (111)	0.01 (101)	0.01 (89)
M. dentatus	0.02	0.01 (90)	0.01 (81)	0.01 (67)
M. polonicus	0.03	0.03 (100)	0.03 (88)	0.02 (80)
M. wolgicus	0.03	0.02 (97)	0.02 (96)	0.03 (101)
M. tauricus	0.04	0.03 (92)	0.04 (106)	0.04 (108)
M. hirsutus	0.05	0.07 (132)	0.05 (89)	0.04 (82)
M. officinalis	0.08	0.06 (73)	0.05 (63)	0.06 (77)
M. altissimus	0.09	0.07 (82)	0.05 (55)	0.04 (51)
M. neapolitanus	0.11	0.09 (80)	0.10 (84)	0.07 (62)
M. albus annual	0.20	0.19 (94)	0.13 (63)	0.12 (62)
M. sulcatus ssp. *segetalis*	0.21	0.18 (86)	0.17 (77)	0.16 (73)
M. sulcatus ssp. *brachystachys*	0.21	0.20 (92)	0.13 (59)	0.10 (47)
M. elegans	0.26	0.31 (121)	0.15 (58)	0.15 (60)
M. indicus	0.27	0.25 (92)	0.22 (79)	0.19 (69)
M. infestus	0.27	0.22 (81)	0.17 (62)	0.11 (42)
M. italicus	0.31	0.32 (103)	0.29 (72)	0.20 (65)
M. albus perennial	0.33	0.29 (88)	0.25 (74)	0.18 (55)
M. siculus	0.40	0.36 (89)	0.28 (71)	0.36 (89)
M. speciosus	0.87	0.72 (84)	0.37 (42)	0.27 (31)
T. fragiferum	0.11	0.13 (118)	0.09 (81)	0.07 (68)
T. michelanium	0.31	0.33 (107)	0.18 (60)	0.10 (31)
Medicago sativa	0.19	0.21 (114)	0.14 (75)	0.14 (75)

Salinity*species P<0.001
l.s.d. (P=0.05) absolute growth
salinity=0.03, species=0.04
salinity *species =0.09
l.s.d (P=0.05) relative growth
salinity=10.8, species=20.8
salinity * species =36.0

Table 3. The effect of NaCl on the concentrations of Cl^- in the shoots of 19 species of *Melilotus* and three check species (*M. sativa, T. fragiferum* and *T. michelianum*).

	Shoot Tissue Cl^- Concentration (mmol/g dry mass) at NaCl concentrations of:			
Species	**0 mM**	**80 mM**	**160 mM**	**240 mM**
M. albus annual	0.07	0.50	1.28	2.13
M. albus perennial	0.07	0.47	1.45	1.86
M. altissimus	0.09	0.48	1.20	2.02
M .dentatus	0.30	0.59	1.58	1.79
M. elegans	0.14	0.63	1.73	2.03
M. hirsutus	0.06	0.45	1.54	2.65
M. indicus	0.12	0.46	1.90	1.38
M. infestus	0.05	0.89	2.56	3.23
M. italicus	0.10	0.75	2.20	3.39
M. neapolitanus	0.10	0.80	2.53	2.97
M. officinalis	0.08	0.43	1.34	1.95
M. polonicus	0.15	0.46	1.27	2.19
M. siculus	0.16	0.62	1.33	1.49
M. speciosus	0.06	0.66	2.27	3.25
M. suaveolens	0.15	0.55	1.13	1.72
M.sulcatus ssp.brachystachys	0.10	0.67	1.67	2.10
M. sulcatus ssp. segetalis	0.12	0.66	1.44	2.23
M. tauricus	0.08	0.47	1.73	1.97
M. wolgicus	0.13	0.43	0.79	1.13
T. fragiferum	0.10	0.49	1.65	2.34
T. michelanium	0.21	0.73	1.94	3.09
Medicago sativa	0.07	0.46	1.39	2.35

Salinity*species P<0.001
l.s.d. (P=0.05)
salinity=0.33, species=0.19
salinity*species=0.52

Table 4. The effect of NaCl on the concentrations of Na^+ in the shoots of 19 species of *Melilotus* and three check species (*M. sativa, T. fragiferum* and *T. michelianum*).

	Shoot Tissue Na^+ Concentration (mmol/g dry mass) at NaCl concentrations of:			
Species	**0 mM**	**80 mM**	**160 mM**	**240 mM**
M. albus annual	0.01	0.29	0.96	1.97
M .albus perennial	0.02	0.44	1.27	1.80
M. altissimus	0.02	0.30	1.15	2.07
M. dentatus	0.09	0.34	1.42	1.76
M. elegans	0.02	0.58	1.50	2.03
M. hirsutus	0.01	0.20	1.03	2.34
M. indicus	0.02	0.56	1.08	1.51
M. infestus	0.03	1.03	2.80	3.33
M. italicus	0.03	0.90	2.54	3.48
M. neapolitanus	0.02	0.73	2.02	2.70
M. officinalis	0.01	0.20	1.07	1.82
M. polonicus	0.03	0.26	1.04	2.06
M. siculus	0.03	0.75	1.51	1.86
M. speciosus	0.02	1.01	2.54	3.34
M. suaveolens	0.04	0.38	0.97	1.58
M. sulcatus ssp. *brachystachys*	0.02	0.65	1.63	2.30
M. sulcatus ssp. *segetalis*	0.02	0.71	1.41	2.27
M. tauricus	0.01	0.25	1.42	1.82
M. wolgicus	0.03	0.28	0.74	0.93
T. fragiferum	0.04	0.55	1.44	2.25
T. michelanium	0.03	1.16	2.19	2.80
Medicago sativa	0.01	0.28	1.17	2.18

Salinity*species P<0.001
l.s.d. (P=0.05)
salinity=0.33, species=0.19
salinity*species=0.49

Table 5. The effect of NaCl on the concentrations of K^+ in the shoots and the K^+/Na^+ Selectivity Ratio in the shoots of 19 species of *Melilotus* and three control species (*M. sativa*, *T.fragiferum* and *T. michelianum*)

	Shoot Tissue K^+ Concentration (mmol/g dry mass) at NaCl concentrations of:				**Selectivity S (K^+, Na^+) at:**
Species	**0 mM**	**80 mM**	**160 mM**	**240 mM**	**240 mM**
M.albus annual	1.26	1.11	0.96	0.95	36
M.albus perennial	1.45	1.38	1.03	1.01	44
M.altissimus	1.14	1.00	0.77	0.64	23
M.dentatus	0.97	0.96	0.80	0.78	35
M.elegans	1.52	1.38	0.92	0.92	34
M.hirsutus	1.24	1.11	0.98	0.96	33
M.indicus	1.55	1.31	1.11	1.03	49
M.infestus	1.39	0.94	0.97	0.70	15
M.italicus	1.25	0.95	0.64	0.78	15
M.neapolitanus	1.28	1.17	0.97	0.91	23
M.officinalis	1.06	1.10	0.98	0.88	39
M.polonicus	0.90	0.93	0.81	0.79	32
M. siculus	1.73	1.61	1.19	1.16	44
M.speciosus	1.51	1.13	0.73	0.86	18
M.suaveolens	0.77	0.73	0.63	0.68	31
M.sulcatus ssp.brachystachys	1.34	1.06	0.85	0.90	27
M.sulcatus ssp. segetalis	1.63	1.42	1.08	1.06	33
M.tauricus	1.14	1.08	0.95	0.88	34
M.wolgicus	1.03	0.97	0.86	0.87	57
T.fragiferum	1.54	1.30	1.10	1.08	35
T.michelanium	1.99	1.42	0.93	0.91	33
Medicago sativa	1.49	1.34	1.11	1.05	20
Salinity *species P<0.001 l.s.d.(P=0.05) salinity=0.10, species=0.06 salinity treatment*species=0.15					Species P<0.001 l.s.d. (P=0.05) =15

Table 6. The effect of NaCl on (a) dry matter digestibility (soluble salts subtracted), (b) neutral detergent fibre and (c) acid detergent fibre in selected species of *Melilotus* grown at a range of NaCl concentrations.

(a) dry matter digestibility

Species	% Dry matter digestibility (soluble salts removed) at:			
	0 mM NaCl	80 mM NaCl	160 mM NaCl	240 mM NaCl
M. albus	73.5	71.4	66.2	66.3
M. elegans	74.6	70.2		
M. indicus	70.6	68.4		
M. italicus	73.3	72.0	65.1	
M. siculus	70.6	69.0	66.3	67.4
M. speciosus	69.8	69.9	61.7	60.1
T. michelianum	75.4	73.7	71.9	

Standard Error of Difference (SED) Average, Salinity=1.00, species=1.10 Salinity*Species=1.03
Chi pr, Species P<0.001, Salinity NS
Salinity*Species P<0.001

(b) neutral detergent fibre

Species	% Neutral detergent fibre (on a dry matter basis) at:			
	0 mM NaCl	80 mM NaCl	160 mM NaCl	240 mM NaCl
M. albus	31.3	27.1	29.9	31.8
M. elegans	29.2	32.3		
M. indicus	33.3	35.9		
M. italicus	31.6	30.1	28.3	
M. siculus	30.9	30.3	26.8	21.4
M. speciosus	31.9	28.7	25.3	23.9
T. michelianum	23.9	22.4	18.2	

Standard Error of Difference (SED)Average, Salinity=2.01, species=1.99, Salinity*Species=1.812
Chi pr, Salinity P<0.001, Species P<0.001
Salinity*Species P=0.144

(c) Acid detergent fibre

Species	% Acid detergent fibre (on a dry matter basis) at:			
	0 mM NaCl	80 mM NaCl	160 mM NaCl	240 mM NaCl
M. albus	23.2	20.1	22.3	23.8
M. elegans	21.3	23.7		
M. indicus	25.6	26.7		
M. italicus	23.3	22.0	18.8	
M. siculus	23.3	23.3	20.5	16.6
M. speciosus	24.3	20.7	18.5	16.4
T. michelianum	19.1	18.2	14.2	

Standard Error of Difference (SED) Average Salinity=1.49, species=1.48, Salinity*Species=1.33
Chi pr, Salinity P<0.001, Species P<0.001
Salinity treatment *Species P=0.020

Table 7 a and b. The effect of growth in stagnant nutrient solution on shoot and root dry mass in 19 species of *Melilotus* and 3 check species (*M. sativa, T. fragiferum* and *T. michelianum*). Data in parenthesis show production relative to aerated conditions.
Note. The experiment was conducted in two parts because of space restrictions in the controlled environment room.

Species	Shoot dry mass		Total root dry mass	
	Aerated (g/plant)	Stagnant (g/plant)	Aerated (g/plant)	Stagnant (g/plant)
M.albus annual	2.32	0.92 (39)	0.86	0.38 (44)
M.albus perennial	1.76	0.98(56)	0.85	0.46 (54)
M.altissimus	0.68	0.42 (62)	0.31	0.17 (55)
M.dentatus	0.25	0.20(80)	0.39	0.20(51)
M.elegans	1.72	0.63(37)	0.55	0.14 (25)
M.hirsutus	0.62	0.16 (26)	0.25	0.08 (32)
M.indicus	0.44	0.48 (109)	0.15	0.17 (113)
M.infestus	1.18	1.26 (107)	0.43	0.41 (95)
M.italicus	1.68	0.58 (35)	0.63	0.10 (16)
T. fragiferum	1.64	1.53 (93)	0.31	0.24 (77)
T. michelianum	5.33	4.00 (76)	0.55	0.73 (133)
M. sativa	6.20	2.57 (41)	1.17	0.38 (33)
l.s.d.(P=0.05)				
Waterlogging	0.22		0.04	
Species	0.63		0.09	
Waterlogging *species	0.83		0.24	

Species	Shoot dry mass		Total root dry mass	
	Aerated (g/plant)	Stagnant (g/plant)	Aerated (g/plant)	Stagnant (g/plant)
M. neapolitanus	1.03	0.18 (17)	0.30	0.03 (10)
M. officinalis	1.52	0.50 (33)	0.86	0.31 (36)
M. polonicus	0.59	0.30(51)	0.39	0.19 (49)
M. siculus	2.40	2.45 (102)	0.79	0.94 (119)
M. speciosus	3.91	1.38 (35)	1.15	0.37 (32)
M. suaveolens	0.55	0.37 (67)	0.59	0.35 (59)
*M. sulcatus (*ssp. *brachystachys)*	1.51	0.30 (20)	0.48	0.06 (13)
*M. sulcatus (*ssp. *segetalis)*	1.59	1.38 (87)	0.54	0.52 (96)
M. tauricus	0.61	0.27 (44)	0.40	0.14 (35)
M. wolgicus	0.75	0.16 (21)	0.61	0.15(25)
T. fragiferum	1.46	1.44 (99)	0.27	0.22 (81)
T. michelianum	3.92	3.90 (99)	0.50	0.72(144)
M. sativa	5.77	1.70(29)	1.20	0.38(32)
l.s.d.(P=0.05)				
Waterlogging	0.24		0.04	
Species	0.53		0.12	
Waterlogging *species	0.71		0.22	

Figure 1. The effect of growth in stagnant nutrient culture on root porosity in 19 species of *Melilotus* and three check species (*M. sativa, T.fragiferum* and *T. michelianum.)*. L.s.d. (P=0.05) are shown as vertical lines on the graphs.