Foliar condensed tannin levels in lotus species growing on limed and unlimed soils in South-Eastern Australia

W.M. KELMAN and G.J. TANNER
CSIRO, Division of Plant Industry, Canberra, Australia

Abstract

Condensed tannin concentrations (% of dryweight) were measured in leaves of 22 accessions of *Lotus corniculatus*, 10 accessions of *L. pedunculatus*, 2 accessions of *L. tenuis*, and 1 accession of *L. subbiflorus* from plants growing on limed (pH = 5.2) and unlimed (pH = 4.3) soils near Canberra, A.C.T. There was a non-significant decrease in the mean condensed tannin concentration due to liming. The differences between species and between accessions within species were significant. For the *L. corniculatus* entries the range of tannin concentrations over the limed and unlimed sites was 0.15-7.28%, with a mean of 2.09%. The highest concentration was found in the otherwise promising accession CPI 66414. *L. pedunculatus* accessions ranged from 2.53-10.72% in tannin content, with a mean of 5.99%. Two Portuguese accessions that have promising agronomic features for coastal east Australian pastures had unacceptably high tannin levels - above 7.50%. Genotypic differences accounted for a large proportion of the total variability for tannin production, so there are good prospects for the improvement of these species by selection and breeding.

Keywords *Lotus corniculatus*, *L. pedunculatus*, *L. tenuis*, *L. subbiflorus*, condensed tannins

Introduction

Condensed tannins are polymeric flavanol compounds present in the leaf and stem tissue of some leguminous forages. An important property of these compounds is their capacity to bond with leaf proteins and thus prevent the formation of stable foams in the rumen of grazing animals, a condition causing bloat (Jones et al. 1973). Moreover, this protection given to protein in the rumen allows more protein to reach the abomasum and small intestine and thus increase protein utilisation (John & Lancashire 1981). The actual level of condensed

tannins in forage is important because, while beneficial effects may follow their presence, high levels of these compounds lower herbage intake and digestibility (Barry & Duncan 1984; Chiquette et al. 1988). Thus the production of condensed tannins and their concentrations are important attributes of forages.

The presence and levels of condensed tannins have been documented in several important leguminous forages (Jones et al. 1976; Sarkar et al. 1976; Marshall et al. 1979), but are notably absent in the leaves of lucerne and white clover, the predominant sown legumes of temperate pastures. The genus *Lotus* is a diverse group of species of wide adaptability. Cultivated forms are considered bloat-safe because of the presence of condensed tannins in the leaves. In New Zealand, *L. pedunculatus* is oversown on wet, acid pastures of the south island (Armstrong 1974), whereas the more drought tolerant *L. corniculatus* has been targeted for the high altitude, infertile tussock grassland (Scott & Charlton 1983). Extensive collections of germplasm have been evaluated more recently (Widdup et al. 1987; Keogh & Burgess 1987). The tetraploid *L. pedunculatus* cultivar 'Grasslands-Maku' is proving to be a valuable pasture legume in many parts of the world, including the coastal area of south-eastern Australia. In the United States and Canada *L. corniculatus* will grow on dry, infertile, acid soils and a number of cultivars have been developed (Grant & Marten 1985). The potential of *Lotus* species in a complementary role with the traditional legumes is now also under investigation in south-eastern Australia (Kelman & Oram 1989; Schachtman & Kelman 1991).

Lotus cultivation is thus projected for situations where nutrient shortages or toxicities are likely to occur. These conditions will certainly influence the levels of condensed tannins found in forage and thus affect both the nutrient metabolism and acceptability of stock. Barry & Forss (1983) have measured higher levels of condensed tannins in forage of Maku (8-11% of dry weight) growing on infertile soils than on well fertilised soils (2-3%). However, little is known of the condensed tannin levels in other accessions of this species or their reaction to infertile soils. The aim of this work was to document the variability for condensed tannin production in 4

species of *Lotus* growing on limed and unlimed soils at a site in south-eastern Australia. This information will complement studies on dry matter production (Kelman & Oram 1989) and on salt, aluminium and manganese tolerance (Schachtman & Kelman 1991) of these accessions to identify those that can be used in *Lotus* breeding programmes for south-eastern Australia.

Materials and methods

Ninety-five accessions of *Lotus* were included in 2-replicate experiments planted on limed and unlimed soils at Ginninderra Experiment Station, near Canberra, A.C.T. in November 1988. Soil pH (0.01M CaCl₂) of the limed site was 5.2 at 0-10 cm depth and 4.3 on the unlimed site. The mean soluble aluminium concentration (0.01M CaCl₂) on the limed site was 0.3 µg/g, and 4.2 µg/g on the unlimed site. Each accession was represented by 4 plants per plot in a single row. Thirty-five productive accessions were selected for analysis of condensed tannins and forage was sampled in December 1989. Six crown shoots were cut from each plant in each plot in one replicate on the limed and unlimed sites. The plant samples were oven-dried at 80°C for 24 hours. After drying, the leaves were separated from the stems and ground to pass through a 1 mm sieve in a cyclone type mill.

Measurement of condensed tannins

Assays for total condensed tannins were based on a method described by Stafford & Lester (1986). Condensed tannins were extracted from 50 mg of dried-leaf material in 5 ml of 70% methanol (v/v) for 2 hours at room temperature. With this material maximum tannin was extracted after 2-3 hours and began to decline after 4 hours. Duplicate extracts were made from leaf tissue of each plant. Ten and 20 µl aliquots of the extract were each pipetted into 13 x 100 mm culture tubes containing 1 ml butan-1-ol/hydrochloric acid (95:5 v/v) and 0.1 ml water. The volumes for the 10 and 20 µl aliquots were equalised with 70% methanol. After thorough mixing the tubes were sealed and placed in a waterbath at 95°C for 1 hour. The tubes were then cooled immediately in cold water and the absorbance read at 550 nm in a spectrophotometer. An average

absorbance was calculated from the 10 and 20 µl aliquots. Each batch of tubes assayed for condensed tannins at one time (25 samples each for the 10 and 20 µl aliquots) included 4 control samples. The control samples contained 5, 10, 15, or 20 µg of condensed tannin purified from leaves of *L. corniculatus* cv. 'Maitland' using the method of Jones *et al.* (1976). The amount of tannin in extracts was calculated from the linear standard curve.

Analysis of variance

The log-transformed values of condensed tannin concentrations per plant were averaged for each plot and the means analysed as an unreplicated nested factorial experiment. The lime x accessions interaction mean square was used to test the significance of all sources of variation (Table 1).

Results and discussion

Lime effect

Twenty-one of 33 accessions (*L. tenuis* excluded) had increased tannin levels on the unlimed compared to with the limed site and the mean condensed tannin level over the accessions on the unlimed site was 3.42 compared with 2.93 on the limed site. However, this difference was not significant (Table 1). Lowther *et al.* (1987) also found little difference between condensed tannin levels on fertile and infertile soils in 5 *L. corniculatus* cultivars.

Species effect

Species differences were very marked. The mean condensed tannin concentration of the *L. corniculatus* accessions over the limed and unlimed sites was 2.09%, that for *L. pedunculatus* 5.99% for *L. tenuis* 0.12%; the single *L. subbiflorus* accession had a mean of 3.16%.

The condensed tannin concentrations for accessions of *L. corniculatus* (Table 2) are generally in the range reported elsewhere for this species (Lowther *et al.* 1987; Roberts *et al.* 1989). Most of the accessions of this species tested here have average tannin levels close to or in the range of 2-4% of dry weight, which Barry & Forss (1983) found was the minimum concentration necessary to precipitate protein. The notable exception to this is the accession CPI 66414, in which condensed tannins were close to 7% on both limed and unlimed sites. Condensed tannin concentrations in the range of 6 to 11% of dry weight are uncommon in *L. corniculatus* but have been reported in three accessions from Ethiopia tested by Roberts *et al.* (1989). The accession CPI 66414 is also unusual in its morphology, differing from other *L. corniculatus* accessions in its more prostrate, dense growth habit and larger flowers. This type may prove to be better adapted than other *L. corniculatus* to grazing by sheep in the south-eastern Australian regions with modified Mediterranean climates. If so, selection of populations with lower tannin concentrations will be necessary.

Table 1 Analysis of variance of condensed tannin concentrations in leaves of 35 accessions of *Lotus* grown in limed and unlimed soils.

	df	MS	F prob.
Lime	1	0.0514	0.083
Species	3	0.9482	<0.001
Lime x species	3	0.0070	0.729
Accessions of <i>L. corniculatus</i>	21	0.0493	0.002
Accessions of <i>L. pedunculatus</i>	9	0.0458	0.014
Accessions of <i>L. tenuis</i>	1	0.0034	0.646
Residual	31	0.160	
Total	69		

Table 2 Plot means and variability for concentrations of condensed tannins (% of dwt) in leaves of 4 species of *Lotus* on limed and unlimed soils,

Species/cultivar or accession number	Origin	Condensed tannin conc.		Relative* variability
		Limed	Unlimed	
<i>L. corniculatus</i>				
CPI 66414	Portugal	7.28	7.22	0.052
AU Dewey	USA	3.80	2.94	0.097
CPI 114291	Italy	2.48	4.41	0.111
CPI 114294	Israel	3.00	3.71	0.197
Quimey	Chile	2.57	2.37	0.120
Oberhaunstaedter	Germany	1.29	3.23	0.124
HAM298		2.75	1.79	0.123
CPI 114293	Italy	1.82	1.96	0.162
Vega	USA	1.23	2.45	0.086
AT-I	USA	1.06	2.48	0.121
CPI 28612	Spain	1.23	2.16	0.087
AT-P	USA	1.80	1.66	0.082
CPI 115190	Brazil	0.89	2.91	0.039
HAM299	Portugal	1.41	1.56	0.150
GA-I	USA	0.79	2.13	0.101
Ginestrino	Italy	0.33	2.59	0.115
Vega II	USA	1.06	1.83	0.064
CPI 114301	Ukraine	1.42	1.25	0.117
CPI 36764	Belgium	1.68	0.21	0.233
CPI 115191	Yugoslavia	1.26	1.35	0.168
CPI 114302	USSR	0.53	1.32	0.091
Cree	Canada	1.04	0.15	0.137
Species mean		1.85	2.35	
<i>L. pedunculatus</i>				
CPI 67677	Portugal	8.59	9.98	0.062
CPI 67678	Portugal	7.82	10.72	0.088
P15302		8.75	8.21	0.081
CPI 67676	Portugal	7.20	7.73	0.084
Grasslands Maku	New Zealand	4.88	5.57	0.118
Marshfield	USA	3.37	6.25	0.093
CPI 114248	Chile	5.80	2.92	0.194
CPI 114246	Chile	5.42	2.53	0.083
G4704	New Zealand	2.57	5.32	0.050
Columbia	USA-	3.75	2.63	0.198
Species mean		5.82	6.19	
<i>L. tenuis</i>				
Tresur Chaja	Argentina	0.07	0.00	0.035
CPI 44854		0.07	0.32	0.091
Species		0.07	0.16	
<i>L. subbiflorus</i>				
CPI 35903	Denmark	2.37	3.95	0.110

* Relative variability was obtained as the square root of the pooled variance of log-transformed values for individual plants within accessions about their respective means on limed and unlimed treatments.

Within *L. pedunculatus* the highest condensed tannin concentrations were present in CPI 67677 and CPI 67678 and were almost twice the level previously reported to reduce the voluntary intake of Maku (Barry & Duncan 1984). These two lines, which originate in the Algarve region of southern Portugal, are regarded as promising agronomically for the northern NSW and southern Queensland coastal belt (A. O'Brien, pers. comm.). The Algarve accessions are earlier flowering than Maku and, unlike Maku, can produce seeds in latitudes as low as 26° But outcrossing and selection of low tannin genotypes will be essential. In contrast to these entries, condensed tannins of cv. 'Columbia', the New

Zealand breeding lines G4704 and CPI 114246 were in the range of 3 to 4% of total dryweight.

Between-plant variation

Plant to plant variability within *L. corniculatus* accessions was generally greater than that of *L. pedunculatus* (Table 2). The variability in *L. corniculatus*, however, is unlikely to affect animal intake or rumen function. Although dry matter digestibility of *L. corniculatus* forage with condensed tannins near 5% is significantly reduced compared to digestibility at 1-2% (Chiquette *et al.* 1988), a low frequency of high tannin plants in a population of predominantly low tannin plants is unlikely to affect

rumen function or intake. In contrast, the variability within the higher tannin producing *L. pedunculatus* accessions may influence condensed tannin levels in variable populations of this species by inducing selective grazing of more palatable low tannin plants.

It was possible, using variance components estimated from the mean squares in Table 1, and the within-plot variance, to quantify the proportion of the total variance attributable to differences between accessions. This value, 0.47, is not strictly a heritability estimate, but it does indicate the considerable influence that genotype has on the levels of condensed tannins at this site. If confirmed in later studies, the prospects for the genetic alteration of mean leaf tannin concentrations are good. Strong genetic control of condensed tannin production is also evident in the results of Dalrymple *et al.* (1984) who proposed a single dominant gene for tannin production (T) to account for F_2 and backcross ratios of high and low tannin segregants in crosses within *L. corniculatus*. This study prompted the production of isosynthetic strains of this species that differ by 5% in condensed tannins (Chiquette *et al.* 1988).

Conclusions

Condensed tannin concentrations in leaves of most of the accessions of *L. corniculatus* examined are at levels adequate to prevent bloating, while not adversely affecting dry matter digestibility. However, some Mediterranean populations may contain too much tannin. *L. pedunculatus* has a wide range of variation within the accessions tested; two Portuguese accessions which are agronomically promising in eastern Australia will require crossing and selection for lower tannin levels.

ACKNOWLEDGEMENT

We thank Dr R.M. Oram for the supply of the *Lotus* accessions and his helpful discussions.

REFERENCES

- Armstrong, C.S. 1974. 'Grasslands Maku' tetraploid lotus (*Lotus pedunculatus* Cav.). *NZ journal of experimental agriculture* 2: 333-336.
- Barry, T.N.; Forss, D.A. 1983. The condensed tannin content of vegetative *Lotus pedunculatus*, its regulation by fertiliser application, and effect upon protein solubility. *Journal of the science of food and agriculture* 34: 1047-1056.
- Barry, T.N.; Duncan, S.J. 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 1. Voluntary intake. *British journal of nutrition* 51: 485-491.
- Chiquette, J.; Cheng, K.J.; Costerton, J.W.; Milligan, L.P. 1988. Effect of tannins on digestibility of two isosynthetic strains of birdsfoot trefoil (*Lotus corniculatus* L.) using in vitro and in sacco techniques. *Canadian journal of animal science* 68: 751-760.
- Dalrymple, E.J.; Goplen, B.P.; Howarth, R.E. 1984. Inheritance of, tannins in birdsfoot trefoil. *Crop science* 24: 921-923.
- Grant, W.F.; Marten, G.C. 1985. Birdsfoot trefoil. In 'Forages, the science of grassland agriculture' (4th ed.). M.E. Heath & R.F. Barnes (eds). Published by Iowa State University Press. pp. 98-108.
- John, A.; Lancashire, J.A. 1981. Aspects of the feeding and nutritive value of *Lotus* species. *Proceedings of the NZ Grassland Association* 42: 152-159.
- Jones, W.T.; Anderson, L.B.; Ross, M.D. 1973. Bloat in cattle. XXXIX. Detection of protein precipitants (flavolans) in legumes. *NZ journal of agricultural research* 16: 441-446.
- Jones, W.T.; Broadhurst, R.B.; Lyttleton, J.W. 1976. The condensed tannins of pasture legume species. *Phytochemistry* 15: 1407-1409.
- Kelman, W.M.; Oram, R.N. 1989. Responses of perennial *Lotus* and *Astragalus* accessions to acid soils. *Proceedings 5th Australian agronomy conference*, Perth. p.628.
- Keoghane, J.M.; Burgess, R.E. 1987. The search for an improved *Lotus pedunculatus* for the high country pastoral systems. *Proceedings of the NZ Grassland Association* 48: 125-129.
- Lowther, W.L.; Manley, T.R.; Barry, T.N. 1987. Condensed tannin concentrations in *Lotus corniculatus* and *L. pedunculatus* cultivars grown under low soil fertility conditions. *NZ journal of agricultural research* 30: 23-25.
- Marshall, D.R.; Broue, P.; Munday, J. 1979. Tannins in pasture legumes. *Australian journal of experimental agriculture and animal husbandry* 19: 192-197.
- Roberts, C.A.; Beuselinck, P.R.; Derks, J.A.; Marek, S.M. 1989. Tannins in *Lotus corniculatus* accessions. *Lotus newsletter* 20: 38-41.
- Sarkar, S.K.; Howarth, R.E.; Goplen, B.P. 1976. Condensed tannins in herbaceous legumes. *Crop Science* 16: 543-546.
- Schachtman, D.P.; Kelman, W.M. 1991. Potential of *Lotus* germplasm for the development of salt, aluminium and manganese tolerant pasture plants. *Australian journal of agricultural research* 42(1): (in press).
- Scott, D.; Charlton, J.F.L. 1983. Birdsfoot trefoil (*Lotus corniculatus*) as a potential dryland herbage legume in New Zealand. *Proceedings of the NZ Grassland Association* 44: 98-105.
- Stafford, H.A.; Lester, H.H. 1986. Proanthocyanidins in needles from six genera in the Taxodiaceae. *American journal of botany* 73: 1555-1562.
- Widdup, K.H.; Keoghane, J.M.; Ryan, D.L.; Chapman, H. 1987. Breeding *Lotus corniculatus* for south island tussock country. *Proceedings of the NZ Grassland Association* 48: 119-124.