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A High-throughput Method for the Quantification of Proanthocyanidins in Forage Crops and its Application in Assessing Variation in Condensed Tannin Content in Breeding Programmes for *Lotus corniculatus* and *Lotus uliginosus*

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Lotus corniculatus and Lotus uliginosus are agronomically important forage crops used in ruminant livestock production. The condensed tannin (CT) content, dry matter (DM) production, and persistence of these species are key characteristics of interest for future exploitation of these crops. Here we present field data on 19 varieties of *L. corniculatus*, 2 varieties of *L. uliginosus* and, additionally, a glasshouse experiment using 6 varieties of *L. corniculatus* and 2 varieties of *L. uliginosus*. Current methods for the quantification of condensed tannins in crop species are slow and labor intensive and are generally based upon polymer hydrolysis following the extraction of chlorophyll in a liquid phase. Presented here is a high-throughput protocol for condensed tannin quantification suitable for microtiter plates based upon the precipitation of condensed tannin polymers in complex with bovine serum albumin (BSA) with subsequent hydrolysis of precipates using butan 1-ol/ hydrochloric acid.

KEYWORDS: ***Lotus*; condensed tannins; proanthocyanidins; protein precipitation; forage quality**

INTRODUCTION

Lotus corniculatus L. (bird's-foot trefoil) and *L. uliginosus.* syn *L. pedunculatus* (greater bird's-foot trefoil) are relatively minor perennial forage legume species within UK grassland. However, both species have important characteristics with potential benefits for UK grassland agriculture. The herbage contains proanthocyanidins, also known as condensed tannins (CTs), which help to reduce bloat, have anthelminthic properties (*1*), and can protect protein in the rumen (*2*) while reducing the rate of protein degradation and nitrogenous losses to the environment. In the widely used forage legumes, white clover (*Trifolium repens* L.) and red clover (*T. pratense* L), CTs are present in the inflorescences but not in leaf tissues and are therefore in insufficient quantities within the sward to reduce bloat or protect dietary protein.

The varieties of both *Lotus* species that are commercially available have not been bred for United Kingdom conditions and tend to lack persistence in mixed swards (*3*). The basis of this lack of persistence within United Kingdom grassland systems is unknown; however, there is evidence from studies in other countries that the longevity of *L. corniculatus* within the sward depends upon a combination of individual plant persistence and natural reseeding following successful seed set in the field (*4*). There is no published information on the traits that improve the persistence of *Lotus* in mixed swards, and identifying these characters is essential if genetic improvement programs are to develop improved varieties and enable the dietary, health, and environmental benefits of tanniferous *Lotus* species is to be realized.

CTs are flavonoid polymers that complex with soluble proteins and render them insoluble in the rumen, yet release them under the acidic conditions found in the small intestine, thereby reducing bloat and increasing amino acid absorption (*5, 6*). The levels of CTs in the herbage of *Lotus* species are critical as they can have beneficial or detrimental effects on ruminant livestock, depending upon their concentration. The CT content required to control bloat is considered to be 5 mg CT/g DM (*7*) whereas 20–40 mg CT/g DM may be regarded as being optimal for improved ruminant production (*8*). CT levels greater than 60 mg/g DM can reduce voluntary intake and have been reported to depress digestion efficiency (*9*). Another complicating factor is that the CT content of herbage from *Lotus* is influenced by a number of environmental and developmental factors including elevated CO_2, temperature, and drought (*18*).

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Table 1. Varieties Included in the Glasshouse Experiment with Their Origin and Description (*25*) Where Available

variety	species	origin	description
Inia Draco	*L. corniculatus*	Uruguay	erect type
Gran San Gabrielle	*L. corniculatus*	Italy	
Grasslands Goldie	*L. corniculatus*	New Zealand	
Leo	*L. corniculatus*	Canada	
Highgrove	*L. corniculatus*	United Kingdom	rhizomatous
Steadfast	*L. corniculatus*	USA	rhizomatous
Grasslands Maku	*L. uliginosus*	New Zealand	rhizomatous
Grasslands Sunrise	*L. uliginosus*	New Zealand	

There is evidence that the CT content of *L. corniculatus* is lower than *L.uliginosus* (*10, 11*) and that levels in stems are lower than in leaves and flowers (*12*). CT content has been reported as being higher in rhizomatous material, and in these experiments CT levels were influenced by the presence of a companion grass and fluctuated during spring and autumn seasons (*13*).

Recent field experiments at IGER have confirmed significant variety differences in CT content in 13 *L. corniculatus* cultivars (*14*). *Lotus* species grown for forage such as *L. corniculatus* and *L.uliginosus* are outbreeders and as such there is no published information on the variation in levels of CTs present within existing varieties under United Kingdom climatic conditions. Such variation within cultivars could be further exploited in future plant breeding programs. This lack of knowledge regarding within-cultivar variation is to some extent a consequence of the laborious methods used to quantify CT and also perhaps an oversight in previous breeding programs. Rapid techniques for measuring CT content are necessary to quantify the genetic variation in this trait, which when combined with information on variation in persistence and forage quality should enable identification of germplasm for use in future genetic improvement programs.

A reliable method of CT quantification is the method of Terrill et al. (*15*), which provides an accurate linear determination of CT in both the soluble and the insoluble fraction; however, it is a slow and labor intensive procedure using macro quantities of volatile organic solvents. Other studies have used elegant methods based upon near-infrared reflectance spectroscopy (NIRS) to quantify tannin content (*13, 14*) using calibrations based on the vanillin-HCl method. As an alternative approach, we have used a simplified high-throughput system by essentially combining tannin protein precipitation together with butanol/HCl oxidative depolymerisation to quantify variation in tannin content. We describe field experiments and also a more detailed glasshouse study to quantify variation in tannin content, forage quality, and a range of morphological traits within varieties of *L. corniculatus* and *L. uliginosus*.

MATERIALS AND METHODS

Plant material. For glasshouse experiments, 100 seeds of 6 varieties of *Lotus corniculatus* L. and 2 varieties of *L. uliginosis* L. (**Table 1**) were sown in multicompartment trays containing John Innes No. 2 compost on January 12, 2004. The trays were kept in a glasshouse maintained at a day/night temperature of 20/15 °C until the plants were established; then, forty plants of each variety were transplanted into 15 cm diameter pots containing the same compost and transferred to an unheated glasshouse. The plants were laid out as a randomized block with 10 plants of each variety in each block.

Dry Matter Yield. On May 26, 2004, five plants per block of each variety (the "cut" plants) were defoliated with hand-held shears to a height of 3 cm, and the DM yield was quantified after drying in a preheated forced-draft oven at 80 °C for 24 h. The remaining five plants were left uncut (the "uncut" plants). After a further 45 days growth, the cut and the uncut plants were cut to a height of 3 cm, and DM yield was recorded as described. Three stems were removed from each plant prior to this cut and freeze-dried for later tannin assay of leaf and stem (the methodology is described later). The plants were kept in an unheated glasshouse, where temperatures were rarely below freezing, over the winter, and on June 25, 2005 plant persistence survival was assessed; the DM yield of each plant was determined by cutting with hand-held shears to a height of 3 cm as previously described.

Table 2. Varieties Included in the Field Experiment with Their Origin and Description (*25*) Where Available

variety	species	origin	description
inbred lines	*L. corniculatus*	United Kingdom	
Georgia	*L. corniculatus*	USA	erect type
Lotar	*L. corniculatus*	Czeck Republic	
Viking	*L. corniculatus*	USA	erect type
Leo	*L. corniculatus*	Canada	
Au Dewey	*L. corniculatus*	USA	prostrate habit
Norcen	*L. corniculatus*	USA	intermediate
Gran San Gabrielle	*L. corniculatus*	Italy	
Emlyn	*L. corniculatus*	United Kingdom	
Upstart	*L. corniculatus*	USA	
Grasslands Goldie	*L. corniculatus*	New Zealand	
A1 0528	*L. corniculatus*	Unknown	
Dawn	*L. corniculatus*	USA	semierect
Terre	*L. corniculatus*	UK	
Ober (Oberhaunstadter)	*L. corniculatus*	Germany	
Empire	*L. corniculatus*	USA	semierect
Inia Draco	*L. corniculatus*	Uruguay	erect type
Highgrove	*L. corniculatus*	United Kingdom	rhizomatous
Steadfast	*L. corniculatus*	USA	rhizomatous
Grasslands Maku	*L. uliginosus*	New Zealand	rhizomatous
Grasslands Sunrise	*L. uliginosus*	New Zealand	

Biological Measurements on Glasshouse-grown Plants. In 2004, only the following were measured on each plant: (a) the flowering date of each plant was recorded as defined when at least one open flower was observed; (b) on the date of first flower, the length and diameter (at the third node) of the longest stem was measured; (c) on June 28, the number of nodes per stem and pods per node was measured on three randomly selected stems per plant.

Forage Quality. Oven-dried vegetation samples were milled through a 1-mm sieve. In vitro dry matter digestibility (DMD) was measured according to the methods described in Jones and Hayward (*16*). Concentrations of nitrogen (N) and water soluble carbohydrate (WSC) were also measured according to the standard Kjeldhal method for total N (*26*), and the methods for WSC analysis were described in Humphreys (*17*). Crude protein (CP) concentration was then calculated by multiplying the N concentration by 6.25.

Field-grown Plant Material. Twenty seeds of each of 19 varieties of *Lotus corniculatus* and 2 varieties of *Lotus uliginosus* (**Table 2**) were sown in John Innes No.2 compost in an unheated glasshouse on June 15, 2003. On September 2, 2003, plants were transplanted into the field in bare ground in rows of 10 with 1 m between plants and between rows. The experiment was carried out at IGER, Aberystwyth (52°4′N, 4°0′W) on soil of the Nercwys series. Plants were exposed to typical winter conditions at the Institute of Grassland and Environmental Research (IGER) with occasional freezing temperatures.

Dry Matter Yield and Persistence of Field-grown Plants. On May 26 and September 12, 2004, each plant was cut with hand-held shears to a height of 3 cm, and the DM yield was quantified after oven drying in a preheated forced-draft oven at 80 °C for 24 h. Immediately prior to the cut on May 26, three stems were collected at random from each plant and then freeze-dried for tannin assay of leaf and stem. Individual plant persistence was determined in May 2005 by visual assessment. A plant was scored as persistent if it was growing.

Determination of Tannin Content in Glasshouse-grown and Field-grown Plants. Between 3 and 4 mg of ground freeze-dried leaf samples were placed into a 2 mL well in an Eppendorf 96 well plate (VWR, UK) together with 250 μL of 0.2 M acetate buffer pH 4.8, containing 6 mg/ml bovine serum albumin, and the mixture was gently

Table 3. Dry Matter Yield (g/plant) in Year 1 (2004) and Year 2 (2005), Length and Width of the Longest Stem (cm) and Plant Survival of Six Varieties of *Lotus corniculatus* L. and Two Varieties of *L. uliginosus* L. Grown in the Glasshouse[a]

	dry matter yield (g/plant)			longest stem		% plant survival in 2nd ye	
	year 1						
variety and country of origin	1st cut	2nd cut	year 2	length (cm)	diameter (cm)	uncut	cut
Inia Draco (Uruguay)	62.0 ± 2.79	24.2 ± 2.37	9.1 ± 0.69	58.3 ± 3.34	2.0 ± 0.11	70	50
GSG (Uruguay)	81.4 ± 3.89	27.6 ± 4.06	13.8 ± 0.85	70.5 ± 2.97	1.9 ± 0.12	90	65
G. Goldie (NZ)	63.1 ± 3.55	28.4 ± 2.13	13.0 ± 0.86	72.6 ± 3.29	1.9 ± 0.28	90	100
Leo (Canada)	73.4 ± 3.45	29.7 ± 2.22	8.6 ± 0.49	50.1 ± 2.80	2.4 ± 0.21	85	100
Highgrove (UK)	56.9 ± 6.93	28.4 ± 2.26	12.6 ± 0.74	35.9 ± 3.55	1.2 ± 0.11	95	90
Steadfast (USA)	63.1 ± 3.55	30.3 ± 3.38	11.3 ± 0.08	58.8 ± 2.46	1.3 ± 0.11	95	95
G.Maku (NZ)[b]	56.7 ± 6.93	43.6 ± 11.25	17.6 ± 1.02	54.7 ± 5.42	1.6 ± 0.09	85	10
Sunrise (NZ)[b]	47.9 ± 3.32	20.9 ± 2.47	18.2 ± 0.63	63.4 ± 4.11	2.2 ± 0.13	100	100

[a] Data is presented as the mean of five replicate plants plus s.e.m. [b] *L. uliginosus* L.

agitated overnight at 4 °C. Then, 250 μL of cold methanol was added to each well, and the samples were vortexed gently for 2 min, centrifuged at 10 000*g* for 2 min, and then the supernatant was discarded without disturbing the pellet. Pellets were washed with 2 × 400 μL of methanol, and the cleaned pellets were dried at 50 °C in a hot block. CT-protein complexes were then oxidatively depolymerized by the addition of 500 μL of butan 1-ol/HCl (v/v 95:5) and heated in a water bath for 1 h at 100 °C, ensuring that the covering well mat was well sealed to prevent losses by evaporation. Reactions were terminated on ice for 10 min, briefly vortexed, and centrifuged at 10 000*g* for 2 min.

Following oxidative depolymerization, free anthocyanidin monomers absorb strongly at 550 nm. In these experiments, samples were scanned at 450–650 nm, correcting for baseline absorbance at 550 nm. The extinction coefficients ($E_{550}^{1\%}$) for purified condensed tannin of *L. corniculatus* and *L. uliginosus* were determined as 66 and 178, respectively, in 96 well plates and were used for the calculation of CT levels throughout this study. It should be noted that this value of 66 for CTs from the leaves of *Lotus corniculatus* corresponds to an $E_{550}^{1\%,1\text{cm}}$ of 150 as previously reported by this laboratory (*18*); the difference in values being explained by the shorter path-length when using this high-throughput method in Eppendorf 96 well plates.

Verification of High-throughput CT Analysis and Harvesting of Plants from Glasshouse and Field. CT values determined using this microtiter method were compared with values determined using the method of Terrill et al. (*15*), as modified by Carter et al. (*18*), and were statistically indistinguishable (data not shown).

For glasshouse studies, at the onset of flowering, three stems were removed from each plant, separated into leaf and stem fractions, and freeze-dried. The leaf and stem material was ground through a 1 mm sieve and CT content was determined. In field studies, prior to defoliation in May, three stems per plant were sampled per plant and separated into leaf and stem fractions and processed similarly to plants grown in the glasshouse.

RESULTS

Analysis of *Lotus* Varieties Grown in the Glasshouse. There was a significant difference between varieties in DM yield per plant and in length and diameter of the longest stem (**Table 3**). At the first cut, DM yield was greatest in Gran San Gabrielle (GSG) and lowest in Sunrise. At the second cut, DM yield was greatest in Maku and lowest in Sunrise. In the second harvest year, DM yields of all varieties were lower than at both the first and second cut in year 1. Yield was greatest in Sunrise and lowest in Leo. Goldie had a significantly longer stem than all varieties except GSG, and Highgrove had the shortest stem. Stem diameter was significantly greater in Leo than in all varieties except Sunrise and least in Highgrove than in all varieties except Steadfast. Stem length and stem diameter of Maku was in the mid range of the other varieties. All plants of Sunrise survived into the second harvest year irrespective of cutting management, but the persistence of Inia Draco and GSG was particularly reduced under the cutting management.

Table 4. Nodes and Pods Per Stem of Six Varieties of *L. corniculatus* L. and Two Varieties of *L. uliginosus* L. When Glasshouse Grown[a]

	nodes/stem		pods/stem	
variety	uncut	cut	uncut	cut
Inia Draco	12.7 ± 0.42	8.6 ± 0.32	1.5 ± 0.31	0.03 ± 0.03
GSG	14.5 ± 0.83	9.2 ± 0.21	1.2 ± 0.21	0
G. Goldie	11.5 ± 0.54	8.3 ± 0.22	0.7 ± 0.21	0
Leo	15.2 ± 0.53	9.6 ± 0.25	0.4 ± 0.14	0
Highgrove	11.3 ± 0.80	6.0 ± 0.42	1.4 ± 0.22	0.2 ± 0.11
Steadfast	11.3 ± 0.72	7.1 ± 0.33	1.6 ± 0.22	0.5 ± 0.21
G.Maku[b]	7.7 ± 0.52	9.7 ± 0.81	0.1 ± 0.12	0
Sunrise[b]	12.0 ± 0.72	8.1 ± 0.33	0.2 ± 0.11	0

[a] Data is presented as the mean of five replicate plants plus s.e.m. [b] *L. uliginosus* L.

Numbers of nodes per stem and pods were greater in the uncut than in the cut plants of all varieties except Maku (**Table 4**). In the uncut plants, nodes per stem was greatest in Leo and lowest in Maku, whereas in the cut plants they were significantly greater in Maku than in all varieties except GSG and were fewest in Highgrove. In the uncut plants, pod number was significantly greater in Steadfast than all varieties except Inia Draco and Highgrove and was the least in Maku. In the cut plants, Steadfast had more pods than Highgrove, and both had more than Inia Draco; a number of varieties yielded no pods (Sunrise, Leo, GSG, Goldie, and Maku).

Leaf CT content was greatest in Sunrise and Maku (*L.uliginosus* varieties) and lowest in Leo (**Table 5**). The range in CT content between individual plants of each variety was greatest in Maku and least in Steadfast and Leo. CT content of Maku was 45.7 mg/g DM with values ranging from 5.8 to 91.0 mg/g DM. Of the rhizomatous varieties, Highgrove (30 mg CT/g DM) had a significantly greater CT content than Steadfast (16.9 mgCT/g DM), and the variation between plants was also greater. Significant differences in DMD, WSC, and %N were also observed. DMD was greatest in Leo and lowest in Maku, %WSC was greatest in GSG and lowest in Maku, and %N was greatest in Leo and lowest in Maku. WSC and CP of the *L. uliginosus* varieties (Sunrise and Maku) were significantly lower than the other varieties.

Data from Field-grown *Lotus* Varieties. DM yield of the spaced plants was greater in June than in the recovery growth in September (**Figure 1**). DM yield in June was greatest in Emlyn and was lowest in A1 0528, and in September it was greatest in GSG, Ober, and Sunrise and was lowest in Terre, with the DM yield of Maku in the mid range of the other varieties at each date.

Tannin content of the leaf was greater than the stem, and there were significant variety differences in both stem and leaf

Table 5. Leaf Tannin Content (mg/g dry weight), DMD, WSC, and CP Concentration (g/kg DM) of Six Varieties of *Lotus corniculatus* L. and Two Varieties of *L. uliginosus* L. Grown in the Glasshouse[a]

	tannin content (mg CT/g dry weight)		(g/kg DM)		
variety and country of origin	mean	range	DMD	WSC	CP
Inia Draco	18.0 ± 1.99	5.4–41.6	0.71 ± 0.006	121 ± 2.7	143 ± 7.7
GSG	22.6 ± 1.85	11.7–35.1	0.73 ± 0.004	127 ± 2.7	151 ± 4.9
G. Goldie	24.3 ± 1.88	8.0–44.4	0.73 ± 0.004	123 ± 2.5	160 ± 4.6
Leo	8.5 ± 1.41	4.5–24.5	0.81 ± 0.004	111 ± 5.4	225 ± 5.3
Highgrove	30.4 ± 2.77	9.9–66.1	0.79 ± 0.004	100 ± 9.7	168 ± 19.0
Steadfast	14.2 ± 1.35	3.6–23.7	0.78 ± 0.005	103 ± 4.0	185 ± 7.2
G.Maku[b]	45.7 ± 11.68	5.8–91.0	0.58 ± 0.008	56 ± 6.7	62 ± 4.3
Sunrise[b]	58.8 ± 4.42	42.8–103.5	0.60 ± 0.005	82 ± 3.1	77 ± 2.8

[a] Data is presented as the mean of five replicate plants plus s.e.m. [b] *Lotus uliginosus* L.

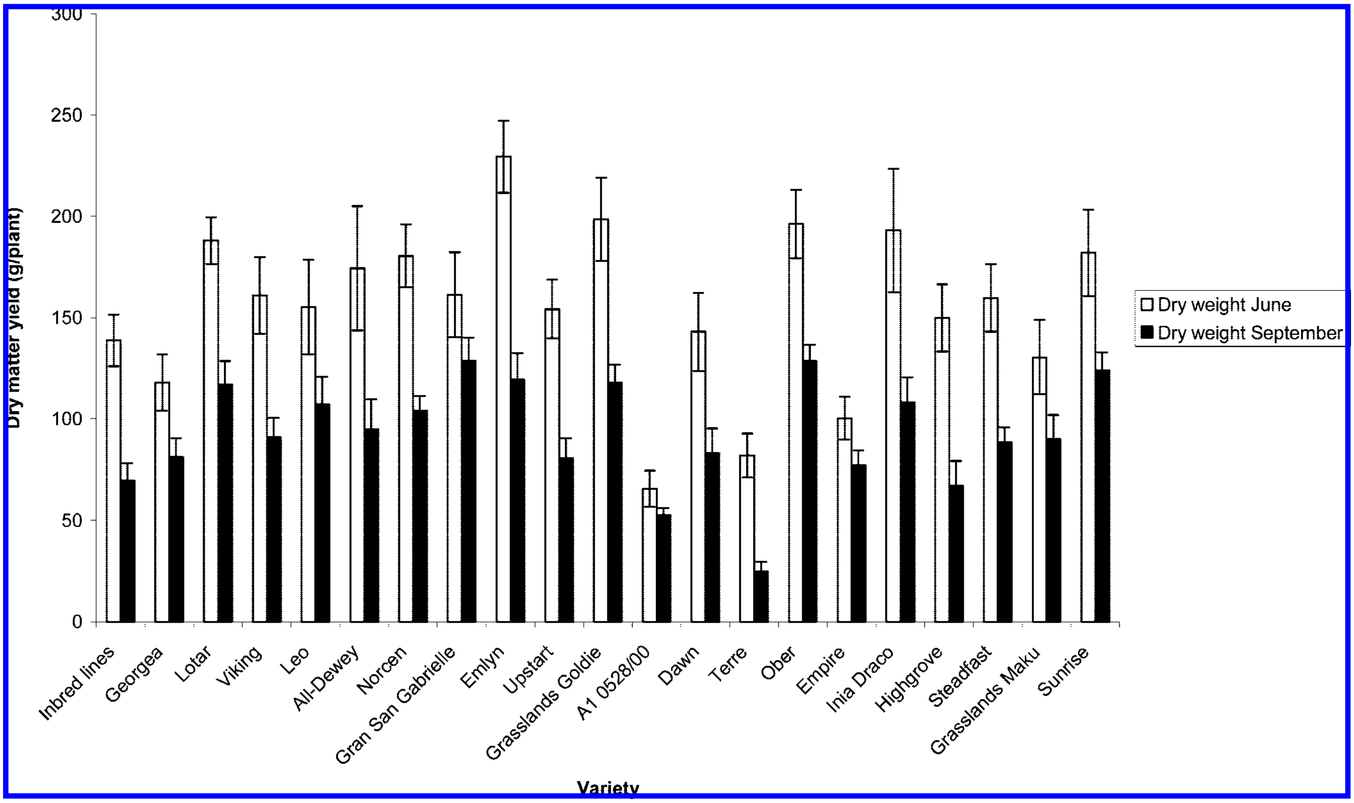

Figure 1. Dry matter yield (g/plant) of spaced plants of varieties of *Lotus corniculatus* and *L. uliginosus* in June and September 2004. Data is a mean of 20 plants per variety. Vertical bars represent the s.e.m.

tannin content (**Figure** , panels **a** and **b**, respectively). No significant differences were found in the tannin content of the leaf or stem fractions of the three stems sampled from the same plant nor any significant variety × stem interactions (**Table 6**). Stem tannin content was greatest in A1 0528 and was lowest in Upstart, which was significantly lower than all other varieties except Dawn, Maku, and Viking (**Figure a**). Leaf tannin content was greatest in A1 0528 and was significantly greater in this variety than in the others (**Figure b**). It was lowest in Dawn, though not significantly lower than the tannin content of Upstart, Inbred line, All-Dewey, Empire, and Norcen. There was considerable variation in both stem and leaf tannin content between spaced plants. In A1 0528, leaf tannin content ranged from 22 to 152 mg CT/g DM and from 3 to 29 mg CT/g DM in Dawn. The rhizomatous varieties Steadfast and Highgrove had a CT content of 30 and 39 mg CT/g DM, respectively, in leaf tissues, although the variation in Highgrove was considerably greater than in Steadfast.

Regarding the survival of *Lotus* cultivars grown in the field (**Figure 3**), values for survival after overwintering were highest for Grasslands Maku and Sunrise, both being *L. uliginosus* varieties. A1 0528 was the best performing *L. corniculatus* variety, and Highgrove was the best of the two rhizomatous *L. corniculatus* cultivars in this study.

DISCUSSION

Exploiting the potential of *Lotus* spp. in UK grassland systems depends upon developing genetic material that combines optimal CT content, enhanced persistence, and DM yield with management systems that enable it to grow and persist with other species in a mixed sward. Quantifying variation in CT content and in the morphological traits that may improve persistence within existing germplasm is the first step in determining the potential for genetic improvement of *Lotus* spp. In this study, significant variation was found between and within varieties for yield, persistence, and CT content, indicating the potential for genetic improvement of *Lotus corniculatus* and *L. uliginosus.*

Condensed Tannin Analysis. These experiments have confirmed the generally higher CT content of the leaf over stem

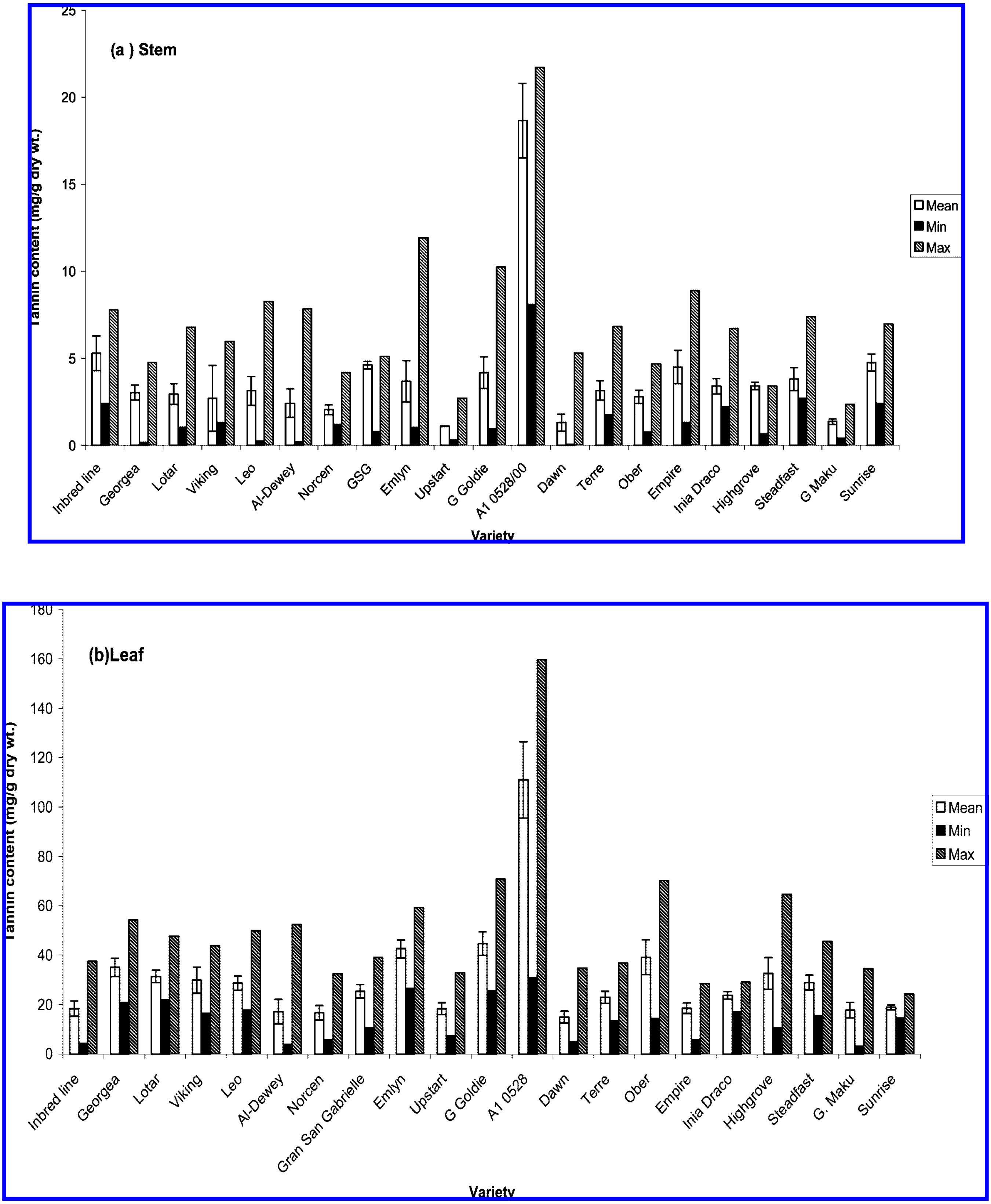

Figure 2. Tannin content (mg/g dry weight) of the stem (**a**) and leaf (**b**) of spaced plants of varieties of *Lotus corniculatus* and *L. uliginosus* in 2004. Mean, minimum, and maximum tannin content is derived from 20 plants per variety. Vertical bars represent the s.e.m.

in both species and in all varieties (*12*), that the mean leaf tannin content differed significantly between varieties, and that all but one variety was generally within the range reported as optimal for good animal health and nutrition (*19*). Considerable variation in CT content has previously been shown within spaced plants of *L. corniculatus* (*20, 21*). Of the eight varieties grown in both experiments, CT content in six were greater in the field, and the field content was lower for two *L. uliginosus* varieties in this study, Sunrise and Maku. There was however significant variation within all varieties in CT content in both experiments, including both of the rhizomatous *L. corniculatus* varieties (Steadfast and Highgrove), although the extent of this variation

Table 6. Analysis of Variance Table of Leaf and Stem Tannin Content in the Field

stem	variance ratio	F-probability
samples	0.13	0.879 ns
variety	50.92	<0.001***
stems × variety	0.77	0.835 ns

leaf	variance ratio	F-probability
samples	0.29	0.750 ns
variety	47.84	<0.001***
leaf × variety	0.18	1.000 ns

differed between varieties. A CT content of up to 35 mg/g DM was common among plants in both experiments, with some plants showing values of 70 mg/g DM. One variety (A1 0528) had a CT content of 150 mg/g DM, which is at a level that could have a negative effect on animal performance; this was also one of the most persistent varieties in the field (see later).

Levels of 20–40 mg CT/g DM are regarded as being optimal for improved ruminant production (*8*), whereas levels of >60 mg CT/g DM can reduce voluntary intake and depress digestion efficiency (*9*). However, the extent of variation in CT content and the reported ease of breeding for CT (*22*) indicate the potential for developing *Lotus* germplasm with CT content appropriate for UK grassland. CT content has been reported to vary over the growing season (*12*) and is influenced by companion grass (*13*) and soil fertility (*23*). Developing germplasm with a CT content that is relatively stable is therefore desirable. Future studies on a limited number of varieties will investigate the stability of CT content over the growing season and in a range of environments. This will be more feasible using the high throughput technique used in this study. Variation between the three stem and leaf samples per plant was not significant, suggesting that analysis of a single stem from each plant would be appropriate for future tannin assays. Other studies have used NIRS to measure CT content (*12*), but the NIRS methodology can give an overestimate for CT values when compared with methods based upon the oxidative depolymerisation of CT polymers (*15, 12*).

In this study and under the environmental conditions in Aberystwyth, the two accessions of *L. uliginosus* did not accumulate CTs at levels predicted to decrease animal performance. By contrast, *Lotus uliginosus* grown in New Zealand (*10*) accumulated high levels of CT, 6–12% DM, and this may reflect environmental modulation of this pathway by high levels of light etc. However, even though CT levels in *L. uliginosus* in this study were below 6%, it is still unclear whether or not the unusual structure of CTs in this species can result in the overprotection of plant proteins and no overall increase in the absorption of essential amino acids from the small intestine when consumed by ruminant livestock (*24*).

Other Aspects of Forage Quality. Species differences in forage quality were also found, with *L. corniculatus* having a higher WSC content than *L. uliginosus.* This concurs with the findings of Marley (*14*) who suggested that the higher WSC content of *L. corniculatus* would make it more appropriate for ensiling than *L. uliginosus*. We also note from these experiments higher values for DMD and CP in varieties of *L. corniculatus* when compared with *L. uliginosus*. Both DMD and CP are quality characteristics for forage crops when grazed by ruminant livestock, and in this context, *L. corniculatus* appears to be superior to the two varieties of *L. uliginosus* used in this study.

Improving persistence and maintaining a high DM yield is also essential if *Lotus* spp are to have a role in UK grassland systems. Both experiments identified significant variety differences in DM yield and persistence, although variety performance was not consistent throughout the growing season, with differ-

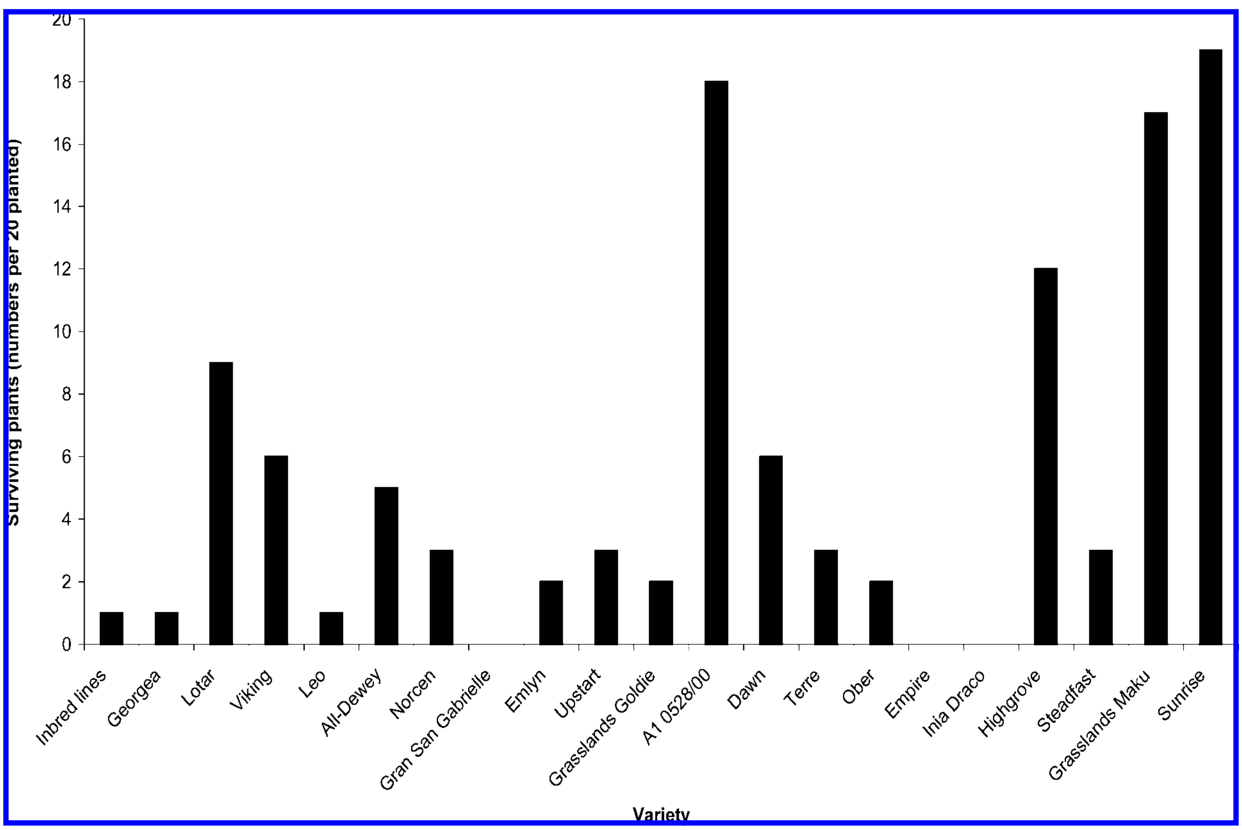

Figure 3. Numbers of plants of each variety surviving in May 2005. Data is derived from 20 plants per variety planted in 2003.

ences in variety ranking between cuts in both experiments. The glasshouse experiment also highlighted the extent to which DM yield declined in the second year, a factor that has limited the use of *Lotus* in UK grassland systems (*3*). Yield decline was greatest in Leo and least in Maku (*L. uliginosus*). In the field experiment, DM yield was greatest in Emlyn, Lotar, and Ober. Selection of plants with appropriate yields at the first and second cut and beyond the first harvest year will be important in future breeding programs.

The Ober variety has previously yielded and persisted well in plot experiments at IGER (*14*). It is likely that, in swards, persistence is dependent upon a combination of individual plant persistence along with some natural reseeding. The evidence from this study is that the extent to which these mechanisms influence persistence may be variety dependent. In the glasshouse experiment there was significant variation in pod number per stem, with high numbers in the rhizomatous types (Steadfast and Highgrove). Plants of these varieties also produced pods following defoliation while other varieties including the *L. uliginosus* varieties (Maku and Sunrise) produced relatively few pods. There was also some evidence that the rhizomatous growth habit improved persistence in the field, with the rhizomatous varieties (Steadfast and Highgrove) persisting better than some of the nonrhizomatous types. In the spaced plant field experiment, natural reseeding did not contribute to improved persistence; however, the capacity to produce pods following defoliation suggests that both rhizomotous varieties are likely to be highly persistent when grown in swards. Maku has also been reported to produce some rhizomes (*25*), which may explain its good persistence in the field; however, pod production was low, suggesting that persistence through rhizome production is critical in this cultivar.

In this study, spaced plants were cut twice per year and were grown without a companion grass and without grazing. Further work is clearly needed to quantify the persistence of rhizomatous types in swards under different management systems. Similarly, information from this and other studies on *Lotus*, on CT content, persistence, and DM production, will help identify genetic material that will form the basis of future germplasm improvement programmes.

SAFETY

Butan 1-ol is classified as a class 3 solvent with a low toxic potential (http://www.pharmainfo.net).

ABBREVIATIONS USED

CT, condensed tannin; DM, dry matter; BSA, bovine serum albumin; NIRS, near-infrared reflectance spectroscopy; HCl, hydrochloric acid; N, nitrogen; WSC, water soluble carbohydrate; DMD, dry matter digestibility; CP, crude protein.

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