The distribution of tannins in lotus spp.: variations within plant and stage of maturity

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Summary. Two studies have been made of the distribution and variation of tannins within plant parts (Experiment 1) and within stage of maturity (Experiment 2) of *Lotus pedunculatus* cv. Maku and *L. corniculatus* cv. Dewey. Non-expanded and first leaves contained significantly higher tannins ($P<0.05$) than other plant parts of cv. Dewey, while in cv. Maku, non-expanded and first leaves, and ninth and tenth leaves accumulated most tannins. The first stage of maturity (plants containing two or three leaves) contained the highest tannins in cv. Maku. No significant difference in amounts of tannins was found between stages of maturity in cv. Dewey. The results indicated that young leaves and young plants of *Lotus* spp. contained the highest tannins.

Introduction

The occurrence of tannins in some forage legumes has attracted attention since they may have both beneficial and detrimental effects on ruminant performance. Low concentrations (2-4% DM) of tannins in plant material appear to be beneficial through protecting protein from rapid degradation by rumen bacteria and preventing bloat (11,16). On the other hand, at higher levels (>4% DM), tannins restrict voluntary intake and depress rumen digestion (1). *Lotus pedunculatus* and *L. corniculatus* are legumes which can grow well under infertile, droughty, acid and poorly drained soils, where other legumes fail (20). However, the occurrence of tannins in these legumes has sometimes caused them to be considered inferior to other crops (5,18).

In dicotyledoneous plants tannins occur in roots, stems, fruits, bark, wood, and leaves (8). However, the distribution of tannins is not uniform through the various parts of a plant or between different species of plants. Tannins in leaves of *Desmodium* were found to be significantly higher than in stems (10). In contrast, none of the *Medicago* spp. (annual or perennial) so far examined contain tannins in their leaves, but all produce tannins in their seed coats (14). In *Prunus domestics* extensive changes in tannin levels occur during fruit ripening, and during leaf development (9). Variation of tannins with stage of maturity has also been found in *Lespedeza sericea* and *Lespedeza cuneata (7).* It seems likely that much variation is general among tannin-containing plants.

In order to investigate the distribution of tannins in *Lotus* spp., two experiments were designed using *L. pedunculatus* cv. Maku and *L. corniculatus* cv. Dewey. The objectives of these experiments were to determine the distribution of tannins within plants and between two cultivars of *Lotus* and to determine tannin content at different stages of maturity.

Methods

Plant establishment

All seeds were obtain from CSIRO Division of Animal Production, Pastoral Research Laboratory, Chiswick, Armidale, NSW, where the experiments were conducted. Seeds were pre-germinated in petri dishes before placing into trays (consisting of 42, 4.5x4.5 cm pots), with two to three seeds per pot. All plants were inoculated five days after germination, using D Lotus Major and SU 343 CSIRO to *L. pedunculatus* cv. Maku and *L. corniculatus* cv .Dewey, respectively. Three days later Aquasol liquid fertilizer (N:P:K 23:4:18 + trace elements) was applied. Three month old plants were transplanted to polythene plastic pots (20 cm diameter) containing a mixture comprising 1:1:4 of peat, sand, and soil. The pH of the soil was 7.

Tannin determination

Tannin determination followed the method described by Broadhurst and Jones (4).

Experimental design

Experiment 1. Ten pots were used in this experiment, consisting of five pots of cv. Maku and five pots of cv. Dewey. There was a single plant per pot. Before starting the experiment, all plants were cut to 3 cm high. Pots were randomly arranged on tables in the glasshouse and every four days were re-randomized to minimise local micro-climate effects. The plants were watered once a day and fertilizer was applied once a week after the experiment started. Each plant received 150 ml of 5 g/L Aquasol solution. After six weeks all plants were harvested. Individual plants were separated into the top non-expanded leaf and first leaf (part 1), third and fourth leaf (part 2), sixth and seventh leaf (part 3), ninth and tenth leaf (part 4), upper stem (from the fifth leaf to the top plant, part 5), and lower stem (from the fifth leaf to the bottom, part 6). Each part was extracted and analyzed for tannins.

Experiment 2. Five pots of cv. Maku and five pots of cv. Dewey were used, as in the first experiment. All plants were cut to 3 cm high. The same amount of fertilizer as in Experiment 1 was given two days after the experiment began. The plants were watered and randomized as for Experiment 1. Harvests were conducted three times at two week intervals. The first plant samples were obtained at random from stems which contained two to three leaves (Stage 1). The second plant samples were obtained at random from stems which contained five to seven leaves (Stage 2). The final plant samples were obtained at random from stems which contained seven leaves or more (Stage 3).

Statistical analysis

All data were analyzed using repeated measures (BMDP 2V). Data were square-root transformed, where necessary, to stabilize the variance. All treatment means were compared using Duncan's Multiple Range Test (19).

Results and discussion

Experiment 1

Results for both *Lotus* species showed similar trends in the distribution of tannins between plant parts (Fig.1). Non--expanded and first leaves contained the highest tannin concentration, 2.57% DM and 1.32% DM in cv. Maku and cv. Dewey, respectively. The sixth and seventh leaves contains the lowest tannins, 1.85% and 0.74% in cv. Maku and cv. Dewey, respectively. No significance difference ($P>0.05$) in condensed tannin concentration was found between upper stem and lower stem in either species. However, in both cultivars, upper stems contained higher tannins than lower stems. Leaves from any part contained higher tannins than stems. Condensed tannin levels from any part of cv. Maku contained higher tannins than cv. Dewey (Fig. 1).

The results clearly showed variation of tannin levels among plant parts. A similar phenomenon has been noted in *Cecropia peltata* L. (Moraceae) (6). Some variation of tannin levels among parts of leaves also occurred in *Prunus domestica* where changes in tannin levels occurred during leaf development. The separation of leaves into four parts, and stems into two parts, according to their order of formation, may be assumed to represent the different ages of plant parts. Hence, the results generally indicated that young parts of the plants contained higher tannins than old ones. These results agree with findings that tannins were higher in the young tissues of leaves of slash pine, *Pinus elliotti* and that accumulation occurs within active metabolizing cells (3). In Sainfoin, *Onobrychis viciifolia,* separation of leaves into young and mature stages showed that young leaves contained higher tannins than mature ones (2); our results showed a similar trend. At the cell level, the sites of accumulation of tannins are in vacuoles and extracytoplasmic space (12). The relatively higher level of tannins in ninth and tenth leaves may be attributable to the fact that vacuoles are the biggest component of the cell volume in older tissues (17).

In this experiment, leaves were generally found to have higher tannin levels than stems. Similar results have been found in *Desmodium* spp. and *Trifolium* spp. (10,15).

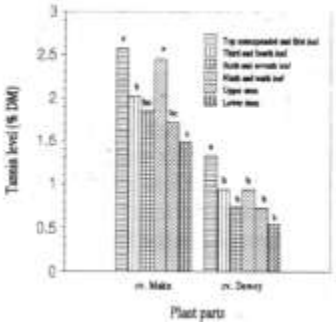

Figure 1. The distribution of tannins within plant parts of *Lotus* spp.

Experiment 2

There was a significant difference (P<0.05) in levels of tannins in cv. Maku with stage of growth. Stage 1 (plant containing two to three leaves on the stems) contained significantly higher tannin than stage 2 (plant containing five to six leaves and stage 3 (plant containing seven or more leaves on the stems). No significant difference (P>0.05) in condensed tannin concentration was found among stages of maturity in cv. Dewey, Table 1.

Table 1 The concentration of condensed tannins (% DM) in shoots of *Lotus* spp. at three stages of maturity.

	Tannin content (% DM)	
Stage of maturity	*L. pedunculatus* cv. Maku	*L. corniculatus* cv. Dewey
Stage 1	3.09[a]	1.02[a]
Stage 2	2.00[c]	1.11[a]
Stage 3	2.36[b]	1.02[a]

Values in the same column with the same superscripts were not statistically different using Duncan's Multiple Range Test (P>0.05)

In this experiment, the number of leaves per stem was used to classify the stage of maturity of the plant. Plants containing two or three leaves per stem were the youngest, while plants containing seven or more leaves per stems were the oldest. As in Experiment I, the results of this experiment indicated that younger plants tended to accumulate higher tannins than older plants.

From both experiments, it can be concluded that young plant parts as well as young plants contained higher tannins than old ones, and that leaves accumulated higher tannins than stems. Cultivar Maku generally contained higher tannins than did cv. Dewey.

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