Seasonal Changes in an Enzyme Inhibitor and Tannin Content in Sericea Lespedeza[1]

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ABSTRACT

We sampled 10 high- and two low-tannin plants of sericea (*Lespedeza cuneata* (Dumont) G. Don) at 2-week intervals from May 17 to October 18 by taking two or three stems from the numerous original spring crown shoots. Aqueous leaf extracts of all samples were tested for concentration of an enzyme-inhibitor substance (a fraction of the total tannin) by measuring its inhibition of one pectinase and two cellulases. Most of the samples were also tested for concentrations of two other tannin fractions, astringent tannin and leucoanthocyanidin. In the common high-tannin sericea plants all tannin fractions rose from low initial levels to high levels in July, with a gradual return to low levels in October. The low-tannin plants were quite low for all tannin fractions throughout the season. Although the inhibitor concentration followed the other tannins in a general way during the season, it was not closely correlated with the astringent tannin or the leucoanthocyanidin, neither for sampling date nor over the high-tannin plants. The inhibitor concentration for a given date or genotype appears to be relatively independent of the accumulation of other tannins. The implications of tannin concentration in the utilization of sericea forage were discussed briefly.

Additional index words: Cellulase, Pectinase, Rumen fermentation.

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THE high tannin content of common sericea, *Lespedeza cuneata* (Dumont) G. Don, has long been associated with low intake of sericea forage by livestock (9, 14, 20). Recent *in vitro* and intra-rumenal nylon bag digestibility studies (8, 11, 12) have shown superior quality for low-tannin forage. Tannin in sericea varies seasonally, with highest concentration occurring in midsummer (6, 10, 19), and in relation to light intensity, temperature, and plant maturity (2, 10).

Total tannin consists of several classes of related compounds. Burns (5) summarized analytical methods for total tannin and various classes. A water-soluble substance that inhibits both pectinolytic and cellulolytic enzymes (3, 4, 13, 17) is present in common sericea. This inhibitor substance is a part of the tannin fraction (15, 16), and has been shown to be a polymer of the basic unit delphinidin (4, 7). In the digestibility studies noted above (8, 11, 12) it was hypothesized that the high-tannin forage inhibited action of cellulases and possibly other enzymes produced by the rumen microflora. A purified inhibitor was effective in reducing *in vitro* dry-matter disappearance of forage from both low- and high-tannin sources (8). A pectinase has been demonstrated in rumen fluid (18), and logically may be expected to contribute to the efficiency of rumen fermentation.

The study was conducted to determine seasonal variation of the inhibitor and related tannin fractions, as measured periodically in individual low-tannin and high-tannin sericea plants.

MATERIALS AND METHODS

We selected 12 sericea plants to represent the range of tannin content available in a plant breeding nursery of 2-year-old spaced plants at the Central Crops Research Station, Clayton, N. C. Ten plants were medium to high in tannin, as indicated by a quick screening test, and two were genetic recessives for a low-tannin character. Each plant was sampled on May 17 by cutting two to three stems from the numerous original spring crown shoots. The sericea plants were not cut back during the season except for the periodic removal of the small samples. Samples were taken every 2 weeks thereafter until the middle of October. Aqueous extracts were prepared by blending 5 g of fresh leaves with 100 ml of distilled water for 10 min. The extract was tested for inhibitor content against crude enzyme preparations from three sources: cucumber flower pectinase, cucumber flower cellulase, and bovine rumen cellulase. The enzyme preparations and tests for enzyme inhibition were made as previously described (1, 13, 17) and values were recorded as units of inhibition. The inhibitor units were calculated from a standard curve semi-log plot of inhibitor concentration *vs* percent enzyme inhibition. The approximate astringent tannin content of each sample was determined for the sample collected on June 27 and thereafter by the vanillin-HCl method described by Burns (5). Beginning with the sample of July 12, the leucoanthocyanidin content was determined by the method of Bate-Smith (1). Values were recorded in terms of absorbence at 520 mμ. Five of the high-tannin plants were transplanted to the greenhouse, grown with supplemental light, and leaf samples from vigorously growing plants were tested twice in January for cellulase inhibition.

RESULTS AND DISCUSSION

The seasonal distributions of astringent tannin, pectinase inhibition, and rumen cellulase inhibition are shown in Fig. 1. The plotted values represent the means of the 10 high-tannin plants for each date. There was a general increase in all values until mid- or late July, followed by a gradual decline to rather low values in mid-October. The seasonal distribution of leucoanthocyanidin (not shown) was similar. The temporary decline of all values in late June was not associated with unusual rainfall or temperature patterns, and, consequently, is not readily explained.

Enzyme inhibition followed the tannin content in a general way, but continued increasing for 2 weeks or more after tannin began to decrease. Simple correlations over dates of the means of the 10 high-tannin plants (Table 1) show that the concentration of the inhibitor was not dependent on the levels of other tannin fractions. The astringent tannin and leucoanthocyanidin values were closely correlated over sampling dates (r = 0.90**), but neither was closely correlated with any of the enzyme inhibition values. The

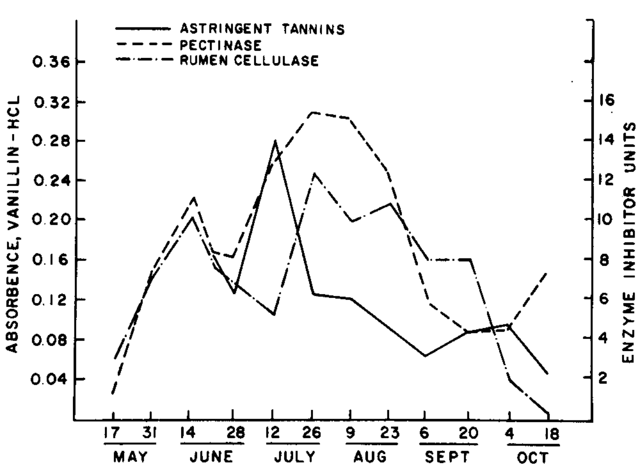

Fig. 1. Sampling date mean values of 10 high-tannin sericea plants for astringent tannin, cucumber flower pectinase, and rumen cellulase concentrations.

values for the three enzyme inhibition systems were rather highly correlated, each with the other two.

The five high-tannin plants sampled during January in the greenhouse averaged only 0.2 inhibition unit when tested against rumen cellulase, indicating that plants contained essentially no inhibitor. The low value is in agreement with unpublished results by the author that indicate only traces of tannin in sericea grown during the winter in the greenhouse.

Throughout the season the two low-tannin plants were low for astringent tannin and inhibition, and relatively low for leucoanthocyanidin (Table 2). The 10 high-tannin plants varied widely for each character except leucoanthocyanidin, probably reflecting previously reported genotypic differences (19), and, because of single-plant samples, considerable environmental effects.

Simple correlations among mean values for the 10 high-tannin plants are all positive (Table 2). The astringent tannin-leucoanthocyanidin correlation of 0.52 was slightly below the 5% significance level. Five of the six correlations for the astringent tannins and leucoanthocyanidins with the three enzyme inhibition systems were significant at the 5% level. Correlations among the three enzyme inhibition systems were quite high and significant at the 1% level. Such correlations, in conjunction with similar correlations over sampling date (Table 1), would be expected on the assumption that one inhibitor substance is effective against the three enzyme systems. They are thus in agreement with previous reports (3, 4, 13, 17) on the distinct activity of the inhibitor.

Seasonal distribution of astringent tannin, leucoanthocyanidin, and the enzyme inhibitor were similar

Table 1. Correlations over dates among tannin and inhibitor mean date values of 10 high-tannin sericea plants.

Trait	Leucoantho-cyanidin (HCl)	Cucumber flower pectinase	Cucumber flower cellulase	Rumen cellulase
Astringent tannin (Vanillin HCl)	.898** (8)†	.393 (10)	.221 (10)	.079 (10)
Leucoanthocyanidin (HCl)		.471 (8)	.196 (8)	-.156 (8)
Cucumber flower pectinase			.835** (13)	.608* (13)
Cucumber flower cellulase				.776** (13)

*, ** Significant at the .05 and .01 levels, respectively. † Numbers in parenthesis indicate number of common dates for correlation calculations.

Table 2. Seasonal means of 12 sericea plants for astringent tannin, leucoanthocyanidin, and units of inhibition against three enzymes, with correlations among traits.

	Absorbance (x 10)		Inhibition units		
				Cellulase	
	Astringent tannin	Leucoantho-cyanidin	Pectinase (CF)†	CF†	Rumen
High-tannin plants					
1	1.20	2.10	6.49	1.07	2.01
2	1.43	1.91	9.70	2.79	8.28
3	1.45	2.03	8.94	2.98	8.74
4	1.33	2.19	11.91	5.38	10.63
5	0.83	2.09	8.04	1.06	5.48
6	1.18	2.06	9.48	3.64	7.30
7	1.19	2.00	9.00	3.41	8.49
8	0.83	1.67	7.24	1.40	4.69
9	0.82	1.68	4.95	0.43	2.29
10	1.23	1.88	7.75	1.45	7.21
LSD, .05	0.39	ns	2.81	1.76	2.78
Low-tannin-plants					
11	0.13	0.84	0.18	0.02	0.02
12	0.15	0.89	0.19	0.04	0.00
Correlations, 10 high-tannin plants					
Astringent tannin	--	0.52	0.64*	0.64*	0.67*
Leucoanthocyanidin			0.64*	0.59*	0.46
Pectinase, CF				0.93**	0.92**
Cellulase, CF					0.91**

*, ** Significant at the .05 and .01 levels, respectively. † From cucumber flower.

to that previously reported for total tannin (6, 10, 19). A sharp rise with advancing season and plant maturity was followed by a gradual reduction to quite low levels in early fall. However, the inhibitor concentration did not vary directly with the other tannins, but continued to rise after the astringent tannin peak, and remained at a relatively high level for several weeks. The inhibitor as a proportion of the other tannin fractions obviously varies over the season. It apparently also varies among plants, as suggested by the lower correlations between the three enzyme inhibition systems and the two tannin fractions than those among the three enzyme inhibition systems.

There is no apparent explanation for the variation of the inhibitor as a proportion of the other tannins. According to the analytical method used, the inhibitor should be a component of the astringent tannin. Possibly the inhibitor represents relatively unstable, random polymer linkages of delphinidin as suggested by Cook et al. (7), while the other tannin fractions are more stable formulations.

The influence of seasonal tannin changes on forage quality can be modified to a limited extent by management practices. The first hay cut should be taken early in the spring to limit tannin accumulation. Later cuts should be taken at an early regrowth stage to restrict the plant maturity effect (10) on tannin accumulation. It is impractical to make hay from the low-tannin, late-summer growth, since harvest after mid-August may seriously reduce stands the following year. Because the inhibitor substance apparently is structurally modified during hay curing (15), the inhibitor content of sericea leaves might be expected to have its greatest effect on forage quality when sericea is grazed. Moderate late-season grazing can be done to utilize forage of lower tannin and inhibitor content. The low tannin genotype should be superior in forage quality to common sericea throughout the season.

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