Variability of Miserotoxin Concentration in Timber Milkvetch

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Highlight: *The variability in miserotoxin concentration of 120 individual timber milkvetch plants was determined in the bud, flower, and pod stages of growth on rough fescue grassland, parkland, and Douglasfir zone locations. Although a broad dispersion in miserotoxin levels was evident within each sampling unit of ten plants, the grassland samples exhibited the greatest toxicity with an exceptional level (10.17 ± 1.13%) occurring during the bud stage. The bud stage of the parkland samples yielded intermediate concentrations (5.22 ± 1.18%) while forest plants contained lower miserotoxin levels (4.08 ± 0.95% to 2.49 ± 0.47%). A decline in miserotoxin levels occurred during the bud-to-pod interval at the grassland and parkland sites, but significant differences were not apparent between the progressive stages of growth at the forest locations. The timber milkvetch toxicity patterns based on the variability of individual plants confirmed previously described trends derived from composite sampling.*

Forests, which comprise approximately 86% of the rangeland in British Columbia, serve both as cattle range and wildlife domain and, with the exception of restricted areas on the grassland fringes, ecology and economics dictate against a general chemical control of timber milkvetch (*Astragalus* miser var. *serotinus*). It would be feasible, however, to control localized, lethal areas through cultivation and seeding to grass and/or with applications of either 2,4-D, silvex or 2,4,5-T, which were shown to effectively reduce the miserotoxin (3-nitro-1-propyl-β-D-glucopyranoside) (Stermitz et al., 1972) concentration of timber milkvetch in field experiments (Parker and Williams, 1974; Williams, 1970). Alternatively, reliable predictability criteria of timber milkvetch toxicity under various rangeland conditions and subsequent controlled cattle movement could serve as a means of preventing livestock losses.

Previously we described a survey of the fluctuations in miserotoxin concentration of timber milkvetch at representative rangeland sites throughout British Columbia (Majak et al., 1974). The data were derived from composite timber milkvetch samples and showed general trends in toxicity levels that could contribute towards range management planning. A comparison of seasonal, peak miserotoxin concentrations indicated that cattle poisoning resulting from timber milkvetch in-

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gestion could be controlled in part by avoiding the rough fescue grasslands in spring and minimizing grazing in open forests of the Douglasfir zone during the summer. A relationship was apparent between available light and toxicity; but physical features such as elevation, slope, exposure, latitude, and soil order did not correlate with miserotoxin concentration. The sampling procedure used in the initial study (Majak et al., 1974) gave an estimate of the average miserotoxin concentration (percent dry weight aerial shoots) at a given site and time. The present study was undertaken to determine the variation in miserotoxin concentration among individual timber milkvetch plants at various stages of growth on rough fescue grassland, parkland, and Douglasfir forest ranges. Individual variations in plant toxicity were assessed in relation to previous estimates based on composite sampling and thus an indication of the reliability of composite sampling was obtained. Relative site differences in miserotoxin concentration were compared with previously reported distributions of toxicity for rangelands in British Columbia.

Materials and Methods

Experimental Plots

Table 1 describes the physical features of the four sampling sites (at least 30 × 60 m) on rangelands near Kamloops. The Lac du Bois plot was located in the rough fescue (*Festuca scabrella*)-eriogonum (*Eriogonum heracleoides*) community of the rough fescue zone, the parkland plot (Pemberton Hill) on a grassland opening in a Douglasfir (*Pseudotsuga menziesii*)-rough fescue ecotone, and the montane forest sites (Watching Creek and Cannell Creek) in the Douglasfir-pinegrass (*Calamagrostis rubescens*) community of the Douglasfir zone.

Plant Samples

At each of the four sites, ten individual plants were harvested 1 inch above the crown at the bud, flower, and pod growth stages to provide 120 samples. Each fresh sample was placed in a plastic bag which was sealed, transported on ice, and stored at −10°C. Prior to analysis for miserotoxin, each plant sample was diced and weighed in a coldroom to yield three subsamples, two of which were used for duplicate dry weight determinations and the third was tared for extraction and maintained at −10°C.

Extraction of Miserotoxin

The procedures used for the extraction of miserotoxin from fresh-frozen samples of timber milkvetch were similar to those described previously (Majak and Bose, 1974; Majak et al., 1974) with the following modifications: one-half of the initial ethanolic filtrate was used for the preparation of Extract 1 adjusted to 100 ml. Extract 1, 0.10 ml, was chromatographed in duplicate on stratified TLC plates and the miserotoxin band was eluted into a 25 ml volumetric flask with 20 ml water prior to colorimetric determination in a darkened room. The colorimetric reagent, diazotized para-nitroaniline, was prepared by combining 0.65 ml 2.5% $NaNO_2$ (in H_2O) with 10 ml 0.3% para-nitroaniline (in N HCl) immediately before use.

Results and Discussion

The dry weight estimates differed from the sample means by less than ± 1.5% for the Lac du Bois and Pemberton Hill sites and by less than ± 2.5% for the Watching Creek and Cannell Creek sites. The total dry weights were at least two to three times greater for the timber milkvetch individuals from the grassland and parkland sites as compared with the forest samples, which increases the comparative toxicities of the various plots.

Concentrations of miserotoxin differed between sites and growth stages, as well as for the interaction of these two factors (Table 2). A particular site × stage combination could significantly modify the expected toxicity level. The coefficients of variability (CV) for mixerotoxin concentrations (Table 3) reflected a significant dispersion within each group of ten plants as they varied from 15% (bud stage, Lac du Bois) to 35% (flower stage, Watching Creek). A relationship between the CV's, however, was not evident.

Table 1. Physical features of experimental plots and timber milkvetch collection dates in 1974.

Plot location	Collection date: Bud	Flower	Pod	Elev. (m)	Slope (%)	Exposure	Soil order
Lac du Bois	May 22	June 20	July 11	910	12	West	Chernozem
Pemberton Hill	May 24	June 14	June 28	960	11	South	Chernozem
Watching Creek	June 20	July 11	Aug. 1	1100	20	South	Luvisol
Cannell Creek	June 20	July 25	Aug. 1	1160	30	South	Luvisol

Table 2. Analysis of variance for the concentration of miserotoxin in timber milkvetch plants at various growth stages and sites near Kamloops, B.C.

Source	df	Mean square	F-value
Site	3	156.94	115.97*
Stage	2	38.27	27.79*
Site × stage	6	6.83	4.96*
Subtotal	11		
Residual	108	1.38	
Total	119		

*Significant at .99 level of confidence.

Fescue Grassland

Higher miserotoxin levels were present at all stages of growth in timber milkvetch plants located on the grassland site. The bud stage on the grassland site (Lac du Bois) showed the greatest interaction response in miserotoxin concentration (10.17 ± 1.13%) (Table 3). Thus at the bud stage of the Lac du Bois site, the mean miserotoxin level was 2 to 4 times as great as the means of plants from the other sites regardless of stage. Unusually high miserotoxin levels, over 6.0%, have been reported by Parker (1973) for *A. miser* var. *serotinus* at the late bud (¼ flower) stage, and he postulated that the exceptionally high concentrations found in 1971 were produced by a combination of environmental events, including elevated temperatures and light intensity and increased xeric conditions. The plots in Parker's study which showed high miserotoxin levels were located in an open area (elevation 870 m) within a coniferous forest. We reported previously (Majak et al., 1974) that timber milkvetch in the fescue grassland plots in British Columbia had the highest miserotoxin levels (5.8 to 7.3%) as compared with composite samples from the Douglasfir forest (3.1 to 4.3%) and semiopen areas (4.3 to 5.8%) and that the peak concentrations were associated with prebud and bud stages of growth. The present variability study on the grassland site corroborates the previous findings based on composite sampling.

Table 3. Mean miserotoxin levels* (percent dry weight), 95% confidence intervals of the mean, and coefficients of variability (CV) for timber milkvetch sampling units.

	Experimental plots							
	Lac du Bois (grassland)		Pemberton Hill (parkland)		Watching Creek (forest)		Cannell Creek (forest)	
Stage	Mean level	CV	Mean level	CV	Mean level	CV	Mean level	CV
Bud	10.17 ± 1.13[a]	15	5.22 ± 1.18[c]	30	3.31 ± 0.41[de]	17	4.08 ± 0.95[d]	31.
Flower	7.10 ± 1.27[b]	24	4.06 ± 0.61[d]	20	2.63 ± 0.66[e]	35	3.84 ± 0.57[d]	19
Pod	6.65 ± 1.08[b]	22	2.44 ± 0.36[e]	19	2.49 ± 0.47[e]	25	3.53 ± 0.70[de]	27

*Means sharing the same letter did not differ significantly according to Duncan's New Multiple Range Test ($P = .05$) over all sites and stages. Each mean is derived from the miserotoxin determinations of ten individual palnts.

Unlike the data for 1973, however, the current study reveals significantly higher relative levels at the three stages of growth and no significant difference between the flower and pod intervals. Research is in progress on the climatic implications of this interseasonal variation.

Parkland Ecotone

This site is identical to Plot 5 sampled during the 1973 survey (Majak et al., 1974). The Pemberton site (Table 1) is a grassy, south-facing slope resulting from conditions of inadequate moisture, and is surrounded on three sides by trees. The resulting ecotone typifies the parkland situation of many south-facing river valley slopes of southern British Columbia. Progressive growth stages indicate a concomitant and significant decline in the miserotoxin levels of timber milkvetch. The bud stage at this site exhibits an intermediate miserotoxin concentration (5.22 ± 1.18%) as compared with similar growth stages in the grassland (10.17 ± 1.13%) and forest areas (4.08 ± 0.95% and 3.31 ± 0.41%). The miserotoxin levels at the bud and flower stage were higher in 1974 (Table 3) than the readings (3.8 and 3.0%, respectively) for Plot 5 in 1973, based on composite samples.

Montane Forest

The Cannell Creek site was situated in a Douglasfir stand, whereas the Watching Creek plot was located in a mixed forest of lodgepole pine (*Pinus contorta*), Douglasfir and, to a lesser degree, aspen (*Populus tremuloides*). Although the statistical analyses do not indicate significant differences between the various stages, a declining trend in miserotoxin levels is evident. The timber milkvetch plants of the Douglasfir-pinegrass community had the lowest miserotoxin levels, and this agrees with the toxicity trends described earlier (Majak et al., 1974), which demonstrated miserotoxin maxima between 3.1 and 4.3% on the basis of composite sampling.

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