Miserotoxin Levels in Fertilized *Astragalus miser* var. *serotinus*

W. MAJAK AND B.M. WIKEEM

Abstract

The effect of fall application of urea fertilizer on toxicity of timber milkvetch was examined in 2 growing seasons at 2 rangeland sites in southern British Columbia. On the grassland site, aerial application of urea at 100 kg N/ha did not affect levels of miserotoxin in timber milkvetch. At the forest clearcut site, 200 kg N/ha reduced toxin levels at later stages of growth in the first growing season. In the second year, however, an increase in the level of miserotoxin was detected at the clearcut.

Timber milkvetch (*Astragalus miser* Dougl. ex Hook. var. *serotinus* (Gray) Barneby) is found mainly in the Douglas-fir *(Pseudotsuga menziesii)* forest and rough fescue *(Festuca scabrella)* grassland zones of southern British Columbia. A large part of this area accommodates grazing for 0.3 million beef cows and calves (B.C. Ministry of Agr. 1982). Timber milkvetch and other species of *Astragalus* synthesize large quantities of miserotoxin (Williams 1982, Williams and Davis 1982), a glycoside (3-nitro-1-propyl-β-D-glucopyranoside) that causes acute and chronic poisoning in ruminants (James et al. 1980). In 1981, the Research Branch of the B.C. Ministry of Forests initiated a study to determine the feasibility and biological consequences of aerial rangeland fertilization. Timber milkvetch was present at 2 of the 5 experimental sites and this provided an opportunity to examine the toxicity of the native legume after fall application of urea fertilizer. The objective of this study was to determine the effects of N fertilizer on miserotoxin levels in timber milkvetch.

Materials and Methods

The 2 sites were located at Beaverdam Lake, 16 km southwest of 70 Mile House, B.C., and at Lac du Bois, 11 km north of Kamloops, B.C. The 75-ha site at Beaverdam Lake (51° 16′ N lat.; 121° 35′ W long; elevation 1,067 m) was a lodgepole pine *(Pinus contorta)* clearcut. It was logged in 1977, drag scarified and seeded to domestic grasses in 1978. At Beaverdam Lake, the site was stratified into 3 vegetation units based upon cover of understory grasses and regenerating trees: lodgepole pine regeneration with native grasses, lodgepole pine regeneration with domestic grasses, and trembling aspen *(Populus tremuloides)* with native grasses. Pinegrass *(Calamagrostis rubescens)* and timothy *(Phleum pratense)* were the principal native and seeded grasses, respectively. The soil at Beaverdam Lake was a Degraded Eutric Brunisol (Eutrochrept). The 100-ha site at Lac du Bois (50° 48′ N lat.; 120° 26′ W long.; elevation 900 m), located in the rough fescue grass land was stratified into 3 vegetation units based upon sub-communities of native grasses dominated by either rough fescue, Kentucky bluegrass *(Poa pratensis)* or bluebunch wheatgrass *(Agropyron spicatum)* growing on a Black Chernozemic soil (Udic Boroll). Both sites were grazed by cows and calves, the grassland for 3 weeks in September and the clearcut continuously from May to mid-September, 1982. Grazed plants which showed residual stubble or delayed phenology were excluded from sample collections.

Forest grade urea was chosen because of its availability, low cost, and pellet size which was suitable for aerial dispersal by helicopter. The fertilizer was applied aerially during 22-24 September 1981 at 100 kg N/ha on the grassland site and at 200 kg N/ha on the clearcut. The higher rate at the clearcut was derived from previous fertilizer studies in the Douglas-fir zone of British Columbia (Freyman and van Ryswyk 1969). The lower rate on the grassland was based on studies which showed that, under less favourable moisture conditions, 100 kg N/ha is sufficient to produce a moderate forage dry matter response (A.L. van Ryswyk, unpublished data). Field traps placed at both sites determined that ground application rates were 99.5 ± 1.61 kg N/ha (n = 45) at the grassland site and 218 ± 1.55 kg N/ha (n = 45) at the clearcut. Fertilizer was applied once, in a single strip, so that each vegetation unit had fertilized and unfertilized treatment.

For miserotoxin determination, composite samples consisting of 10 timber milkvetch plants were collected at random from each strip in each vegetation unit from May to July 1982 at the following growth stages: bud, early bloom, full bloom, early pod, mature pod, and late pod. In 1983, samples were collected at the full bloom and mature pod stages to determine if residual fertilizer affected miserotoxin levels. All samples were stored in a freezer before analysis for miserotoxin by gas chromatography (Majak et al. 1977, 1983).

Toxin levels at each experimental site were examined separately using analysis of variance with a randomized block design. Vegetation units served as blocks for the source of experimental error and duplicated determinations were considered subsamples. Phenological stages and levels of fertilizer were factorially arranged within blocks. Initial tests indicated that the two-way interaction terms were not significantly different from the three-way interactions. Therefore the vegetation unit × fertilizer interaction (V × F) was a reasonable estimate for the error term used to test the effects of fertilizer (F) or vegetation unit (V). A pooled error term (V × P plus V × F × P) was used to test the effects of phenological stage (P) and the F × P interaction. Single degree of freedom contrasts were used to compare toxin levels at different stages of growth. Analysis of variance was also used to determine toxin differences among years, fertilizer treatments, and stages of growth. Means shown in the text are with standard errors.

Results and Discussion

Miserotoxin levels at the grassland site were not significantly (P>0.05) affected by the urea fertilizer at 100 kg N/ha. This is in agreement with an earlier study in the U.S. (Parker and Williams 1974). The toxin levels at the grassland site were inversely related to timber milkvetch maturity (Table 1). Higher concentrations (P<0.01) of the glycoside were detected during the bloom and bud stages of growth than during pod development. Similar trends were reported previously (Majak et al. 1974, Parker and Williams 1974).

Toxin levels were not detectably different (P>0.05) among vege-

Table 1. Average percent miserotoxin in timber milkvetch by fertilizer treatment and growth stage during 1982, data expressed on a dry matter basis.

	Grassland site		Clearcut site	
	Fertilized 100 kg N/ha	Unfertilized	Fertilized 200 kg N/ha	Unfertilized
Bud	5.86	5.58	3.59	3.44
Early bloom	4.39	4.36	4.43	4.74
Full bloom	4.17	4.02	3.75	3.71
Early pod	3.13	3.15	3.63	3.79
Mature pod	2.67	3.54	2.15	3.31
Late pod	2.49	2.75	2.83	3.50
SE (df)	0.29 (20)		0.17 (20)	

Authors are biochemist, Agriculture Canada Research Station, and range scientist, B.C. Ministry of Forests, 3015 Ord Road, Kamloops, B.C. V2B 8A9 Canada.
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tation units at the grassland site. However, a small but statistically significant difference ($P<0.01$) in miserotoxin levels was detected among vegetation units at the clearcut where the average miserotoxin values were 3.66, 3.41, and 3.66%. The slightly lower value for one of the units would not have a practical consequence.

Tests on treated and untreated plants at the clearcut showed a rapid increase in miserotoxin levels during bud to early bloom (Table 1). Thereafter, the concentration in untreated plants did not change significantly. With advancing stages of growth miserotoxin levels decreased rapidly in fertilized plants compared to controls. The most rapid change ($P<0.01$) occurred from early to mature pod stages when concentrations declined by 1.48 in treated plants compared to 0.48 percentage points in untreated ones. This rapid change was not accompanied by a parallel drop in timber milkvetch moisture (W. Majak, unpublished data). Therefore, the lower toxin levels could not be attributed to a more rapid desiccation of the fertilized plants. Lower toxin levels could result from shading of timber milkvetch by the vigorous growth of grasses in response to applied N. The production of grass was significantly enhanced in 1982 and its yield (g/m^2) showed an average increase of 64% and 182% on fertilized areas at the grassland and forest clearcut, respectively, compared to the control but increases in timber milkvetch on fertilized areas were not detected (B. Wikeem unpublished data). In previous studies, miserotoxin levels in timber milkvetch were inversely related to shading (Majak et al. 1977) and complete cover for 2 weeks reduced the toxin levels by 28 to 44% in *A. miser* var. *hylophilus* (Parker and Williams 1974).

On the clearcut site, a significant increase ($P<0.05$) was detected in miserotoxin during the late pod stage when the average level of the glycoside was 3.17% as compared to 2.73% at mature pod. A larger increase in toxin levels (31%) appeared to occur on treated areas compared to untreated ones (6%) but the interaction between fertilizer levels and these stages of growth was not significant. Increases in miserotoxin levels could be induced by the effects of rainfall as reported earlier (Majak et al. 1976, 1977). Timber milkvetch samples at the late pod stage were collected on 19 July and the precipitation for the previous week amounted to 34.0 mm, the average normal rainfall for the entire month of July (Atmospheric Environment Service 1982). In response to the rainfall, the plant moisture content increased significantly ($P<0.05$) from an average of 69.0% at mature pod to 72.2% at the late pod stage.

Significant differences in toxin levels between years were not detected at the grassland site. Fertilized sites at the clearcut, however, showed higher ($P<0.01$) toxin levels in 1983 (4.69% $\pm$ 0.36, averaged over full bloom and mature pod growth stages) than in 1982 (2.95%). The clearcut site also experienced heavy rainfall (51 mm in 8 days) during July 1983 just before sample collection (Atmospheric Environment Service 1983). It appears that rainfall may interact with the fertilizer treatment to enhance miserotoxin levels in timber milkvetch and this effect can occur in the second growing season after urea application.

Except for the bud stage of growth, large differences in miserotoxin levels were not observed between sites in the first year (Table 1). Urea applied at 100 kg N/ha did not intensify timber milkvetch toxicity and it can be safely used to increase forage production on grasslands. At 200 kg N/ha applied to a clearcut, toxin concentrations were not elevated in the first year but they may be enhanced in the second year as the vigor of the plant improves under reduced interspecific competition. Whether this is due to residual N, favorable moisture conditions, or an interaction between the two is unknown; but the results indicate a more hazardous situation for livestock grazing fertilized clearcuts in the second year.

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