The Effect of Light and Moisture on Columbia Milkvetch Toxicity in Lodgepole Pine Forests

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Highlight: Variations in miserotoxin concentration of Columbia milkvetch located in pure lodgepole pine forests were compared to changes in rainfall patterns during the period 1973 to 1976. The substantial increase in precipitation for 1976 was reflected in soil and plant moisture changes and these conditions appeared to induce the formation of higher miserotoxin levels. In addition, a number of secondary miserotoxin peaks were generated during pod development in 1976. Understory light regimes at 12 lodgepole pine sites were determined by chemical actinometry, which expressed duration in direct sun at each plot as a percentage of "full sun" (FS) control. Sites with <15% FS exhibited lower miserotoxin levels than either the 15-35% or >35% FS groups. Miserotoxin levels above 6% predominated in the latter two categories. A positive relationship between light and toxicity was not apparent in the Douglasfir stands where miserotoxin levels remained low. A gas chromatography method was developed to speed up miserotoxin determinations and to screen Columbia milkvetch samples for the presence of free 3-nitropropanol.

Rangeland livestock poisoning resulting from the ingestion of Columbia milkvetch (*Astragalus miser* var. *serotinus*) could be reduced if danger zones were defined and avoided through a program of cattle movement. Recent surveys in British Columbia have pointed to specific rangeland areas that could be designated as hazardous. Seasonal growth patterns also reflected periodic elevated miserotoxin levels of Columbia milkvetch (Majak et al. 1974; Majak et al. 1976). For example, exceptionally high miserotoxin concentrations were observed with milkvetch plants in the rough fescue (*Festuca scabrella*) grassland zone during pre-bloom growth stages. A resurgence in toxicity during the pod stage on grassland sites was linked to a major rainfall in 1974. Concomitant increases in soil and plant moisture appeared to induce miserotoxin biosynthesis (Majak et al. 1976).

On the other hand, significantly lower miserotoxin levels were observed in Douglasfir (*Pseudotsuga menziesii*) stands, either pure or mixed with aspen (*Populus tremuloides*) and/or lodgepole pine (*Pinus contorta*) situated on Gray Luvisolic soils. A study of the variability of miserotoxin concentration (Majak and McLean 1975) corroborated the results of the earlier composite sampling experiments. The initial survey in 1973 pointed to other potential Columbia milkvetch problem areas including open lodgepole pine stands situated on Brunisolic soils. Pure lodgepole pine forests encompass approximately 50% of the 15 million acres of timbered rangeland in British Columbia.[1] A knowledge of the variation in Columbia milkvetch toxicity in these fire succession communities was required to develop guidelines for predicting hazardous zones.

The earlier study (Majak et al. 1974) suggested a relationship between available light and peak miserotoxin concentration. Miserotoxin maxima were lowest in medium-canopied montane forests, intermediate in semiopen areas such as parklands and savannahs, and highest on rough fescue grasslands devoid of any tree cover (Majak et al. 1974). To further test this relationship, variations in miserotoxin levels were assessed in relation to understory light regimes as determined by chemical actinometry and in relation to changes in rainfall patterns as reflected in soil and plant moisture levels.

Materials and Methods

Composite sampling of Columbia milkvetch was conducted sequentially at 15 experimental plots ranging in elevation from 960 to 1,260 m within a 20-km radius of Kamloops, B.C. (Table 1). These sites represented various successional levels within the Douglasfir zone (Tisdale and McLean 1957), with particular emphasis upon plant communities dominated by lodgepole pine. Plots 103 and 104 (Table 1) were located in pure stands of Douglasfir, plot 102 was in a mixed stand of lodgepole pine and Douglasfir, and the remaining sites were situated in pure lodgepole pine forests. Site indices were determined for lodgepole pine according to the method of Dodd et al. (1972). We confirmed the previous observations (Majak and Bose 1974) that oven-dried milkvetch samples yielded depressed miserotoxin values and, therefore, it was essential to extract fresh-frozen samples and to subsample for dry matter determinations.

That the precipitation patterns for Kamloops Airport (elevation 345 m) were applicable to the Columbia milkvetch experimental plots was substantiated by daily climatological reports at Cherry Creek (elevation 548 m) and Lornex mine (elevation 1,256 m) (Atmospheric Environment Service, AES, 1973–76; 1973a–1976a). The Kamloops Airport and Lornex mine weather stations were situated at the eastern and western ends of the experimental area and the Cherry Creek station was located centrally. Soil moisture was determined gravimetrically as the average of three samples taken from a depth of 10 cm. The system of the Canada Soil Survey Committee (1974) was used for soil classification.

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This report is Research Station Contribution No. 266.

Manuscript received January 18, 1977.

[1] Personal communication, A. McLean, Agriculture Canada, Kamloops.

Table 1. Physical features, site indices, and understory light regimes (% full sun) of Columbia milkvetch experimental plots.

Plot no.	Exposure	Slope (%)	Soil great group	Site[1] index (m)	Full[2] sun (%)
35	—	<1	Eutric Brunisol	—[3]	100 [a]
104	SSW	42	Gray Luvisol	—	60 [b]
100	SSE	6	Gray Luvisol	18	43 [c]
36	—	<1	Eutric Brunisol	14	42 [c]
102	SSW	35	Gray Luvisol	18	35 [d]
71	SSE	15	Gray Luvisol	17	33 [de]
101	S	7	Eutric Brunisol	17	30 [de]
30	—	<1	Eutric Brunisol	18	29 [e]
37	—	<1	Eutric Brunisol	15	28 [e]
29	—	<1	Gray Luvisol	20	22 [f]
103	SW	27	Gray Luvisol	—	18 [fg]
31	WNW	5	Gray Luvisol	23	13 [gh]
72	SSE	15	Eutric Brunisol	23	12 [h]
38	S	4	Gray Luvisol	21	11 [h]
70	SSW	4	Eutric Brunisol	18	10 [h]

[1] Height of lodgepole pine at age 100 years.
[2] Significance based on Duncan's multiple range test at 5% level. values sharing the same letter are not significantly different.
[3] Site index not determined.

Miserotoxin and 3-nitropropanol (3NPOH) Analyses

A rapid quantitative gas chromatography (GC) method was developed to speed up miserotoxin determinations and to screen Columbia milkvetch samples for the presence of 3NPOH, the aglycone of miserotoxin which has been reported as a constituent of other *Astragalus* species (Williams et al. 1975; Harlow et al. 1975).

Standard miserotoxin aqueous solution was obtained from the isolate described previously (Majak and Bose 1974). Synthetic 3NPOH was prepared by Garold Yost, Chemistry Department, Colorado State University, Fort Collins.

One-half of the filtrate from the ethanolic extraction of 15 to 20 g fresh-frozen milkvetch (Majak et al. 1974) was concentrated to dryness, re-suspended in 25 ml hot water, centrifuged at 27,000 × g for 5 min at 1°C, and the supernatant was decanted and stored at 2°C. The supernatant, 0.1 ml, was combined with 0.1 ml β-glucosidase (almond emulsin) solution (0.1% in 0.2*M* phosphate buffer, pH 6.2) and duplicate samples of the supernatant and standard miserotoxin were incubated at room temperature overnight. Beta-glucosidase liberated 3NPOH from miserotoxin in quantitative yield.

Quantitative GC was performed on a Microtek 220 gas chromatograph equipped with a flame ionization detector and 1 m × 4 mm (I.D.) glass columns containing 5% Carbowax 20M on Chromosorb W (HP), 80–100 mesh. The temperatures were adjusted as follows: inlet, 210°; oven, 185°; and detector, 210°C.

One-microliter aliquots of the incubated supernatant, the incubated miserotoxin standard, and the standard 3NPOH were injected with each set of analyses. The retention time for 3NPOH was 1.68 min, and the symmetrical peaks enabled one to use peak height for determinations. For these milkvetch samples it was sufficient to work in the 100–1,000 ppm 3NPOH range. Two microliter aliquots of the unincubated supernatant were injected to estimate the concentration of free 3NPOH, which, in all cases, was extremely low, occurring as <5% of the combined form of 3NPOH.

Chemical Actinometry

Concurrent sunlight readings at the various experimental locations were accomplished with uranyl oxalate chemical light meters (actinometers) according to the method of Heinicke (1963), which was validated spectrophotometrically by Looney (1968). Freyman (1968), however, demonstrated light quality differences in the understory of seral communities of the Douglasfir zone; therefore, direct comparisons between the light regimes of lodgepole pine sites and Douglasfir sites should be qualified.

The actinometric technique was identical to that described by Heinicke (1963), and the exposure time was 24 hours. To facilitate fieldwork, duplicate actinometers (20 cm apart) were placed in racks (Fig. 1) and five racks were placed adjacent to five milkvetch plants selected at random at each plot. The actinometer aperture was 17 cm from the ground. The racks were oriented E-W and the movable support (Fig. 1, S) was aligned so that the actinometer aperture was normal to the direct rays of the sun at noon or the noon solar angle (Fig. 1, a). The noon solar angle for each experiment was computed from the equation $a = 90° - \Theta + \delta$ (List 1951), where Θ = degrees latitude and δ = degrees solar declination. The percent "full sun" value for each site (Table 1) was the mean from 10 actinometer readings. These readings were examined statistically by analysis of variance and Duncan's multiple range test.

Results and Discussion

Rainfall Patterns in Relation to Miserotoxin Concentration

The interval between 1973 and 1976 was characterized by two weather extremes during the summer grazing periods: exceptional drought in 1973 and record-breaking rainfalls in

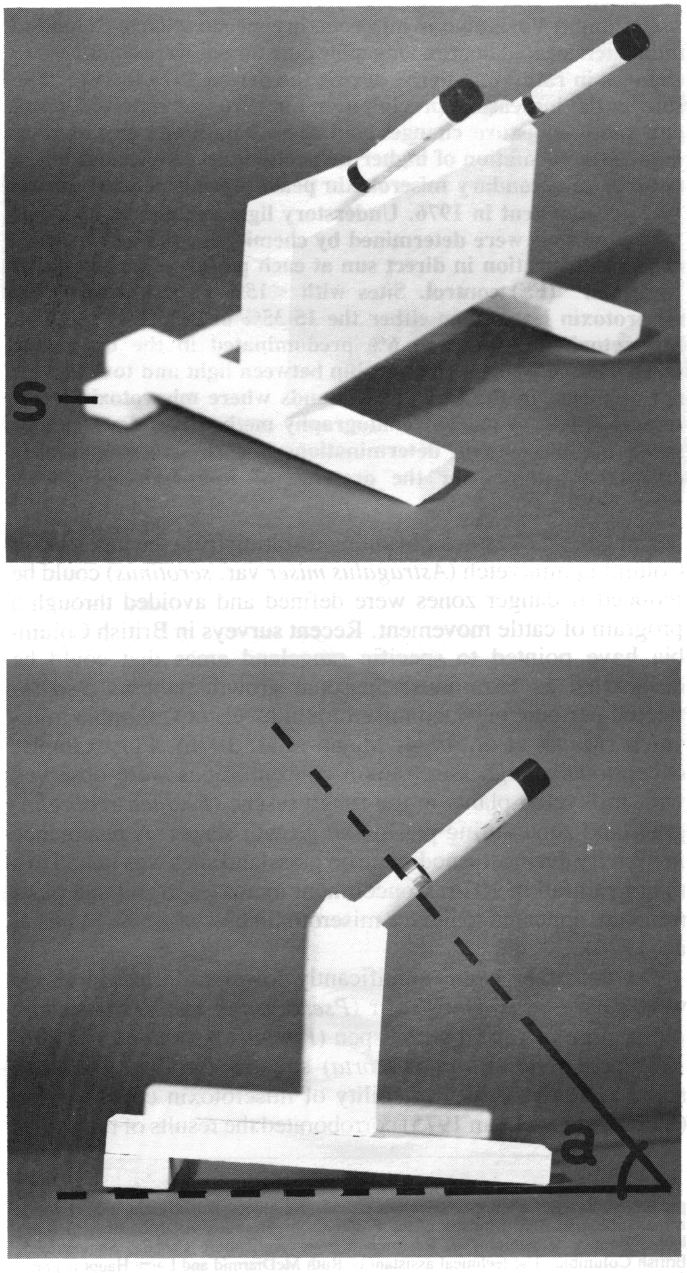

Fig. 1. *Two views of the chemical actinometer rack with the side view showing the movable support (S) set for noon solar angle (a) = 50°.*

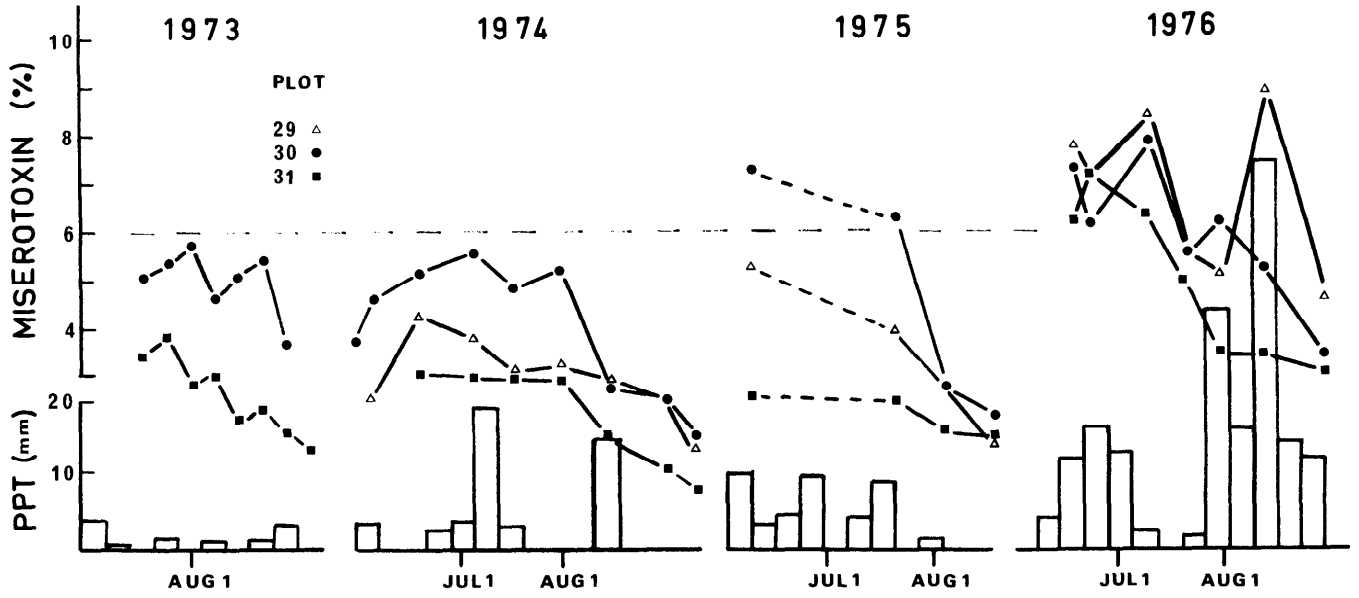

Fig. 2. *Variation in miserotoxin concentration (percent dry weight) of Columbia milkvetch at plots 29, 30, and 31 and weekly precipitation (PPT) for the Kamloops area during the summers 1973 to 1976.*

1976 (AES, 1973a–1976a). These diversified weather patterns afforded a unique opportunity to observe interseasonal variations in Columbia milkvetch toxicity. The precipitation patterns for 1973 to 1976 and the toxicity trends at three milkvetch sites (plots 29, 30, and 31) located in pure lodgepole pine stands are illustrated in Figure 2. Declines in miserotoxin concentrations are evident for the mid-July to late-August interval for the years 1973 to 1975; but 1976 levels were substantially elevated during the same period. A previous comparison between 1973 and 1974 indicated that the additional moisture in 1974 extended and elevated miserotoxin levels on rough fescue grassland plots, but a minimal response to rainfall was observed in milkvetch samples from Douglasfir stands (Majak et al. 1976). The major rainfall (1.27 cm at Kamloops Airport) in July 1974, however, was deposited over a 24-hour period as compared with 19 days of precipitation (12.24 cm) in August 1976. The 1974 situation was a single, convective storm, while the 1976 case can best be described as a large-scale, slow-moving upper trough. These rainfall differences were also reflected in soil moisture differences with little or no change at forest sites in 1974 (Majak et al. 1976). In contrast, sharp increases in soil moisture were maintained at all experimental plots throughout August 1976. This abrupt change in soil moisture, illustrated in Figure 3 for four of the sites, was preceded by a period of water stress producing declines in soil moisture and plant moisture during July (Fig. 3). Recovery from this water deficit coincided with the major rainfalls, which began in late July, resulting in an immediate increase in plant moisture (Fig. 3) similar to that observed in 1974 for Columbia milkvetch on rough fescue grasslands. Three of the sites (plots 70, 71, and 72) in Figure 3 exhibited the most dramatic changes in plant moisture, and plot 29 showed the highest plant moisture levels during the period of August rain. This alleviation of water stress appeared to induce miserotoxin biosynthesis yielding pronounced peaks in mid-August at plots 70, 71, 72, and 29 (Fig. 4). At the remaining sites, the rapid decline in miserotoxin concentrations observed in other years (Fig. 2; Majak et al. 1974; Majak et al. 1976) was arrested. Recently it has been proposed that water stress suspends the aging process of physiologically active leaves (Begg and Turner 1976), and it is conceivable that a resurgence in miserotoxin biosynthesis could be the result of rapid development following recovery from water deficit. The toxicity trends for 1976 confirmed our previous observations that peak miserotoxin concentrations were associated with pre-bloom growth stages, with a decline occurring during bloom, and that the secondary miserotoxin maxima were generated during pod development.

Understory Light Regimes in Relation to Miserotoxin Values

The percentage full sun (FS) for each site (Table 1) indicates the ratio of time in direct sun to time in shade of any part of the foliar canopy (Looney 1968). The sites represented a tenfold change in light regimes, ranging from 100% FS for site 35

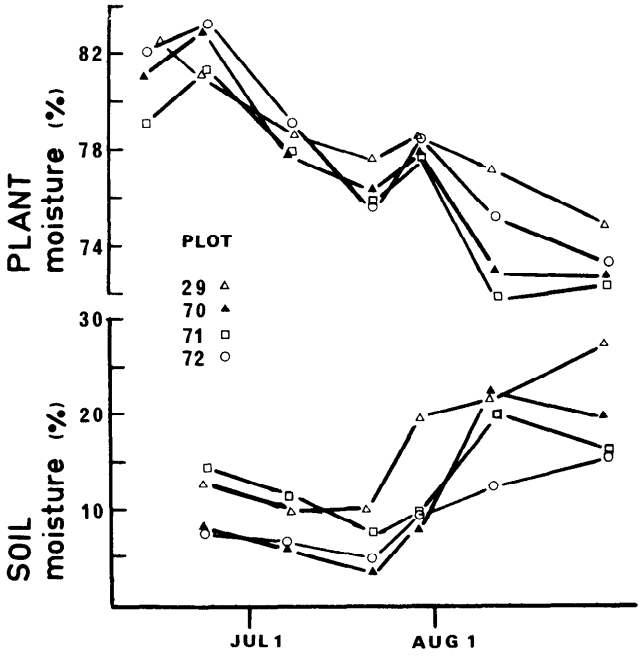

Fig. 3. *Changes in Columbia milkvetch moisture content and soil moisture content at plots 29, 70, 71, and 72 during the summer of 1976.*

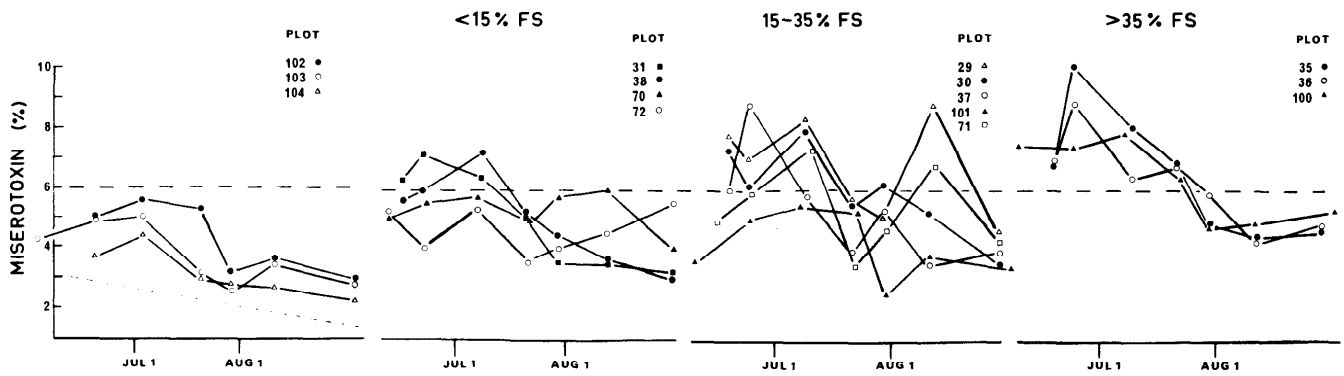

Fig. 4. *Variation in miserotoxin concentration (percent dry weight) of Columbia milkvetch collected in 1976 and located in Douglasfir and lodgepole pine forests. The regression line for Douglasfir sites was developed from data for 1973 and 1974. The lodgepole pine sites are divided into three groups with respect to light regimes as demonstrated by percent full sun (FS) determinations.*

(located on a cleared, hydroelectric right-of-way between sites 30 and 36) to 10% FS for site 70. The miserotoxin levels of Columbia milkvetch growing at three lodgepole pine sites (plots 30, 29, and 31) which had different light regimes (29, 22, and 13% FS, respectively) were determined over the 4-year period 1973–1976 (Fig. 2). The miserotoxin profiles during 1973 to 1975 pointed to a positive relationship between light and toxicity, the higher miserotoxin maxima being associated with site 30 (29% FS) while site 31 (13% FS) showed lower levels and site 29 (22% FS) intermediate values. The pattern for 1976 was somewhat obscured by the additional effects of rainfall (see above), but site 31 continued to maintain a lower position (Fig. 2).

The 12 lodgepole pine sites were divided into three groups with respect to their light regimes (<15%, 15–35%, and >35% FS); and the results of the miserotoxin determinations for 1976 are illustrated in Figure 4. Miserotoxin maxima above 6% predominate in the 15–35% and >35% categories. On the other hand, miserotoxin minima below 4% are found in the <15% and 15–35% FS categories but these two groups yielded secondary miserotoxin peaks as well. In spite of significant differences in light regimes at the Douglasfir plots (60, 35, and 18% FS for plots 104, 102, and 103, respectively) these sites continue to show lower miserotoxin values (Fig. 4). The miserotoxin levels at three Douglasfir sites (plots 13, 20, and 21) which we described previously (Majak et al. 1976) also varied considerably in their percent FS (55, 25, and 30%, respectively); but again, miserotoxin values were depressed during the 1973–74 study, which yielded the regression line indicated in Figure 4. Therefore, a relationship between light and Columbia milkvetch toxicity is not apparent in Douglasfir forests, and the factors which appear to inhibit miserotoxin accumulation at these sites remain to be uncovered. The increased rainfall for June 1976 (91% of normal) and August 1976 (455% of normal), however, could be a contributing factor which resulted in higher miserotoxin levels at Douglasfir sites in 1976 (Fig. 4).

The "% FS" value for each site (Table 1) is really a measure of the relative incoming solar radiation for that site (Heinicke 1963). This energy is used in (a) photosynthesis, (b) heating (air, plant, and soil), and (c) evapotranspiration (Rosenberg 1974). The interaction of these factors in relation to miserotoxin turnover remains to be unravelled. Growth chamber experiments, however, have shown that miserotoxin concentration was greater in *A. miser* plants grown at higher day temperatures and that excluding light from *A. miser* var. *hylophilus* for 2 weeks significantly lowered miserotoxin levels (Parker and Williams 1974).

Previously we reported that low toxin levels in Douglasfir forests were associated with Gray Luvisolic soils. The results for the lodgepole pine sites, however, indicate that high levels of toxin can be produced on both Gray luvisols and Eutric Brunisols (Table 1, Fig. 4). Site indices, which reflect productivity differences between sites, appear to be higher in low toxin areas (Table 1). The corollary suggests, therefore, that the formation of high miserotoxin levels could be a response to moisture stress conditions and this would agree with the miserotoxin observations of Columbia milkvetch situated in the drier conditions of upper grasslands. More detailed soil moisture measurements involving tension as well as amount at various depths within the rooting zone could more clearly define the role of moisture in miserotoxin fluctuations.

The feasibility of predicting Columbia milkvetch toxicity from aerial photographs and forest cover maps remains to be tested but preliminary studies with the present sites indicate that high-toxin areas can be distinguished from low-toxin areas on the basis of tree species and the density of lodgepole pine crown cover.[2]

Literature Cited

Atmospheric Environment Service. 1973-76. Monthly record: meteorological observations in Canada (various months). Environment Canada, Downsview, Ontario.

Atmospheric Environment Service. 1973a-1976a. Monthly meteorological summaries at Kamloops Airport, B.C. Environment Canada, Kamloops Airport, B.C.

Begg, E. J., and N.C. Turner. 1976. Advances in Agronomy. Vol. 28. N. C. Brady (ed.). p. 180-181. Academic Press, New York.

Canada Soil Survey Committee. 1974. The system of soil survey for Canada. Information Canada, Ottawa.

Dodd, C. J. H., A. McLean, and V. C. Brink. 1972. Grazing values as related to tree-crown covers. Can. J. Forest Res. 2:185-189.

Freyman, S. 1968. Spectral distribution of light in forests of the Douglasfir zones of southern British Columbia. Can. J. Plant Sci. 48:326-328.

Harlow, M. C., F. R. Stermitz, and R. D. Thomas. 1975. Isolation of nitro compounds from *Astragalus* species. Phytochemistry 14:1421-1423.

Heinicke, D. R. 1963. The microclimate of fruit trees. I. Light measurements with uranyl oxalate actinometers. Can. J. Plant Sci. 43:561-568.

[2] Personal communication, D. Tjader, B.C. Forest Service, Kamloops.

List, R. J. 1951. Smithsonian Meteorological Tables, 6th Revised ed. p. 497. Smithsonian Institute, Washington.

Looney, N. E. 1968. Light regimes within standard apple trees as determined spectrophotometrically. Proc. Amer. Soc. Hort. Sci. 93:1-6.

Majak, W., and R. J. Bose. 1974. Chromatographic methods for the isolation of miserotoxin and for the detection of aliphatic nitro compounds. Phytochemistry 13:1005-1010.

Majak, W., A. McLean, T. P. Pringle, and A. L. van Ryswyk. 1974. Fluctuations in miserotoxin concentrations on rangelands in British Columbia. J. Range Manage. 27:363-366.

Majak, W., and A. McLean. 1975. Variability of miserotoxin concentrations in timber milkvetch. J. Range Manage. 28:362-364.

Majak, W., R. J. Williams, A. L. van Ryswyk, and B. M. Brooke. 1976. The effect of rainfall on Columbia milkvetch toxicity. J. Range Manage. 29:281-283.

Parker, R., and M. C. Williams. 1974. Factors affecting miserotoxin metabolism in timber milkvetch. Weed Sci. 22:552-556.

Rosenberg, N. J. 1974. Microclimate: The biological environment. p. 165. John Wiley & Sons, New York.

Tisdale, E. W., and A. McLean. 1957. The Douglasfir zone of southern British Columbia. Ecol. Monog. 27:247-266.

Williams, M. C., F. R. Stermitz, and R. D. Thomas. 1975. Nitro compounds in *Astragalus* species. Phytochemistry 14:2305-2306.