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Soil moisture influences low larkspur and death camas alkaloid levels

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Abstract

It has long been known that alkaloid composition and concentration in plants are affected by the stage of growth and by factors at the growing site of the plant. There is, however, a lack of knowledge on the environmental factors that elicit the physiological response of alkaloid-containing plants. A 3-year survey (1992 to 1994) was conducted on the levels of zygacine and methyllycaconitine, the major neurotoxic alkaloids of death camas (*Zigadenus venenosus*) and low larkspur (*Delphinium nuttallianum*), respectively. The alkaloid levels of both species do not exhibit diurnal fluctuations, so precise sampling times during the day were not required. Both poisonous species grew in overlapping communities at 2 of the 7 sampling sites. The levels of both types of alkaloids showed similar contrasts at both sites. Lower alkaloid accumulations were associated with site conditions that reduced soil moisture stress and zygacine levels were negatively correlated with soil moisture levels at 6.5 and 14 cm sampling depths. There were no significant correlations or obvious associations between soil temperature and alkaloid levels in either death camas or low larkspur. As expected, higher alkaloid levels were associated with earlier stages of growth in both plants.

Key Words: poisonous plants, *Delphinium nuttallianum*, *Zigadenus venenosus*, moisture stress

Resumen

Es bien conocido que la composición y concentración de alcaloides en las plantas son afectadas por el estado de crecimiento y factores del sitio donde estas crecen. Sin embargo, hay una falta de conocimiento sobre los factores ambientales que inducen la repuesta fisiológica de las plantas que contienen alcaloides. Durante 3 años (1992 a 1994) se condujó un estudio acerca de los niveles de zigacina y metilicaconitina, los principales alcaloides neurotóxicos de "Death cama" (*Zigademus venenosus*) y "Low larkespur" (*Delphinim nuttallianum*). Los niveles de alcaloides de ambas especies no muestran fluctuaciones diurnas por lo que durante el día no se requirieron tiempos precisos de muestreo. En 2 de los 7 sitios de muestreo las dos especies tóxicas crecen en comunidades traslapadas. Los niveles de los dos tipos de alcaloides mostraron contrastes similares en ambos sitios. Las bajas acumulaciones de alcaloides se asociaron con las condiciones del sitio que reducen el estres de humedad del suelo. Los niveles de zigacina fueron correlacionados negativamente con los niveles de humedad del suelo a 6.5 y 14 cm de profundidad de muestreo. Tanto en "Death cama" como "Low larkspur" no hubo correlaciones significativas o asociaciones obvias entre la temperatura del suelo y los niveles de alcaloides. Como se esperaba, en ambas especies, los niveles altos de alcaloides fueron asociados con los primeros estados de crecimiento.

Low larkspur (*Delphinium nuttallianum* Pritz.) and meadow death camas (*Zigadenus venenosus* Wats. var. *gramineus* [Rydb.] Walsh) are widely distributed in the southern Interior of British Columbia occurring on grasslands and in montane forest meadows at low to mid elevations (Brayshaw 1989; Parish et al. 1996). In the western U.S., the plants are found east of the Cascades. The 2 plants are often found growing together in wetter habitats such as swales. Both plants contain neurotoxic alkaloids. The diterpenoid alkaloid methyllycaconitine is the primary toxin in low larkspur (Majak et al. 1987) and in other native species of *Delphinium* (Majak 1993; Manners and Pfister 1993) and it mainly affects cattle. The steroidal alkaloid zygacine is the primary toxin in meadow death camas (Majak et al. 1992). It mainly affects sheep but humans and all types of livestock are susceptible (Panter and James 1989; Collett et al. 1996; Wagstaff and Case 1987). Death camas also contains other toxic esters of zygadenine (Kupchan and Deliwala 1953; Majak et al. 1992) but our studies indicate that they only become prominent when pods begin to develop (Makeiff et al. 1997). Both species are perennial and shallow rooted, typically penetrating less than 15 cm of soil.

In *Delphinium* spp. there is usually a decline in methyllycaconitine levels with advancing stages of growth (Pfister et al. 1994; Majak 1993). Methyllycaconitine levels in low larkspur can also vary from site to site (Majak and Engelsjord 1988; Majak 1993). There is less data on

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zygacine levels in death camas but differences were detected between stages of growth (Majak et al. 1992). The objectives of this study were 1) to compare levels of methyllycaconitine and zygacine at different sampling sites, 2) concurrently, to determine the relationship of soil moisture and temperature to the alkaloid levels of the two plants, and 3) to determine whether the levels of methyllycaconitine in low larkspur undergo diurnal fluctuations.

Materials and Methods

The 7 sampling sites were located between Kamloops and Pass Lake, 19 km north of Kamloops, British Columbia. They are described in terms of soil classification to the great group level (Agriculture Canada 1987), soil texture, slope, and elevation (Table 1). Death camas and low larkspur both occurred at Sites 4 (near Isobel Lake) and 5 (Opax Mountain) growing in overlapping communities in montane meadows. Low larkspur was also collected at Sites 1 and 8. Site 8 is 1 km east of Site 1 on an alluvial fan in the dry South Thompson River valley at Kamloops. Death camas was also collected at Sites 9, 10, and 11 in the rough fescue (*Festuca scabrella*) upper grasslands above Kamloops. Studies on low larkspur at Sites 1, 4, and 5 have been reported previously (Majak and Engelsjord 1988; Majak 1993).

Composite samples of death camas and low larkspur (100–200 g fresh weight, aerial portions) representing sequential stages of growth (vegetative, bud, flower, and pod) were collected randomly at each site during 3 growing seasons (1992–1994). Samples were usually collected once per week but the number of samples at each stage of growth varied with site and year depending on the duration of the phenological stage. The samples were frozen, freeze-dried and ground to pass through a 2 mm screen. We confirmed that freeze-drying is the preferred method of drying plant material prior to analysis for alkaloids in low larkspur. When the methyllycaconitine contents of freeze-dried and oven-dried samples of low larkspur were compared, the latter (60°C for 24 hours) yielded 18% less methyllycaconitine (range 13-28%, n = 6). Low larkspur samples were extracted and analyzed for methyllycaconitine by HPLC as described previously (Majak and Engelsjord 1988). Nudicauline, a closely related toxic congener of methyllycaconitine (Manners et al. 1995) was also detected but as a minor component (Majak et al. 1987). Death camas samples were extracted and analyzed for zygacine by image analysis on TLC (Makeiff et al. 1997). Methyllycaconitine and zygacine concentrations were expressed on the basis of freeze-dried sample weights. Soil moisture content was determined gravimetrically at depths of 6.5, 14, and 25 cm. Soil temperatures were also determined at those depths using bi-metallic thermocouples read with an Alltemp Sensor Model 900-706 thermocouple thermometer. Measurements of the soil variables did not always correspond with plant samplings, but soil data were usually collected within a week of the plant material.

The effects of year (1992–1994), site (4 sites for low larkspur, 5 sites for death camas) and stage of growth (3 stages for low larkspur, 4 stages for death camas) on alkaloid levels were evaluated by analysis of variance using the procedures of the SAS Institute Inc. (1989). Soil variables were analysed in the same way. Methyllycaconitine was transformed to logarithms for analysis to provide homogeneity of variance. The project design was completely randomized with 3 factors in a factorial arrange-

Table 1. Soil characteristics at death camas and low larkspur sites.

	Species		Soil Characteristics				
			Classification		Slope		
Site No.	Death camas	Low larkspur	Great Group/Order (U.S. soil nomenclature)[2]	Texture[1] (surface/subsurface)	% topography/shape	aspect	Elevation
							(m)
4	x	x	Dark Gray/Luvisol (Typic Eutroboralf)	20 cm fsl/1	5–30/convex-straight	S	975
9	x		Black/Chernozem (Udic Haploboroll)	20 cm cb sl/cb 1 co s	5/concave	SSW	900
1		x	Brown/Chernozem, lithic (Typic Haploboroll)	10–30 cm sl/bedrock	5/straight	S	350
11	x		Black/Chernozem (Udic Haploboroll)	40 cm sl/gr sl	5/concave	SE	850
8		x	Brown/Chernozem (Typic Haploboroll)	10 cm sl/cb co s	25/straight	S	350
10	x		Black/Chernozem (Udic Haploboroll)	30 cm sl/cb co sl	30/concave	N	825
5	x	x	Dark Gray/Chernozem, lithic (Boralfic Haploboroll)	30 cm sl/bedrock	25/straight	SSE	1,220

[1]Soil texture nomenclature: cb-cobbly, co-coarse, f-fine, gr-gravelly, 1-lloam(y), s-sand(y).
[2]U.S. Soil nomenclature in brackets as per Soil Survey Staff (1975).

ment. Sources of variation in the analyses of variance were: site, year, stage, site × year, site × stage, year × stage, and site × year × stage. All of these sources were tested against the residual error which was the variation among samples collected at different times at the same stage, site and year. Least square means of zygacine and methyllycaconitine concentrations and of soil temperatures and moistures were estimated for each year at each site. Only statistically significant terms were included in the models used for estimation. The model for estimating soil moisture at a depth of 25 cm at the zygacine sites employed 2 terms: site × year and year × stage. All other equations had the 2 terms: site × year and stage. The inclusion of growth stage in the equations provided estimates at a comparable but unspecified stage of growth. Correlations between alkaloid concentrations and soil variables were then calculated using these estimates. Means were compared by the L.S.D. test ($P < 0.05$).

Results and Discussion

Diurnal fluctuations in methyllycaconitine levels in low larkspur did not occur (Table 2). A similar finding has been made for zygacine in death camas (Makeiff et al. 1997). This contrasts with the noticeable fluctuation in the alkaloid levels of *Lupinus albus* where increases of 2- to 3-fold were reported during the day for the quinolizidine alkaloid lupanine (Wink and Witte 1984). The absence of diurnal shifts in methyllycaconitine or zygacine levels suggests that the biosynthesis of these alkaloids is not light-dependent. It also indicates that precise sampling times during the day are not required when plants are collected.

Zygacine and methyllycaconitine levels decreased with advancing stages of growth of death camas and low larkspur, respectively (Table 3). As expected, higher alkaloid levels were associated with earlier stages of development. In this study, there was insufficient data on the vegetative stage of growth of low larkspur but previously it was shown to have the highest levels of methyllycaconitine (Majak 1993).

Table 3. Levels of zygacine in death camas and methyllycaconitine in low larkspur at different stages of growth averaged over years.

Stage[1]	Death camas[2]	Low Larkspur[2]
	(%)	(%)
Vegetative	0.50a	—
Bud	0.40 ab	0.16a
Bloom	0.36 b	0.17 a
Pod	0.32 b	0.14 b
SE	0.03	0.01

[1]Refers to predominant stage of growth as determined by field observations.
[2]Means within columns followed by the same letter are not significantly different ($P < 0.05$) by L.S.D. test.

Table 2. Diurnal levels of methyllycaconitine in low larkspur.

Site	Date of collection 1993	Time of collection	Methyllycaconitine level
		(Hour)	(%)
1	May 4	0700	0.18
		1300	0.17
		1900	0.17
	May 5	0700	0.16
		1400	0.17
		1800	0.16
SE			0.003
4	May 18	0700	0.36
		1400	0.28
		2100	0.27
	May 19	0730	0.31
SE			0.02

The data in Table 4 indicate that average levels of methyllycaconitine at Site 4 were almost 2-fold greater than at Site 1, which is consistent with earlier reports for low larkspur at the 2 locations (Majak 1993, Majak and Engelsjord 1988). Differences related to site have also been reported for zygacine levels in death camas. The average levels of zygacine were previously found to be 1.5-fold greater at Site 9 than at Site 10 (Makeiff et al. 1997). Comparable differences in alkaloid levels between the sites were also detected when data from the 3-year survey were analyzed. The survey showed that zygacine levels at Site 9 were 1.4-fold higher than at Site 10 (Table 4).

Table 4. Levels of zygacine in death camas and methyllycaconitine in low larkspur at different sites averaged over years and growth stages.

Zygacine[1]		Methyllcaconitine[1]	
Site	Level	Site	Level
	(%)		(%)
4	0.47a	4	0.27 a
9	0.47a	8	0.15 b
11	0.42 ab	1	0.16 b
10	0.34 bc	5	0.10 c
5	0.28 c		
SE	0.04		0.01
n	87		72

[1]Means within columns followed by the same letter are not significantly different ($P < 0.05$) by L.S.D. test.

Most notable in Table 4 are the differences between Sites 4 and 5, in the montane meadows, where both low larkspur and death camas are found. Zygacine levels are almost 2-fold higher and methyllycaconitine levels are almost 3-fold higher at Site 4 than at Site 5. Alkaloid accumulations in both plants showed a similar physiological response to differences in edaphic or climatic conditions. Elevation at Site 5 is 245 m higher than at Site 4 (Table 1) but it is unlikely that differences in elevation or changes in climate due to elevation affect alkaloid accumulations in low larkspur (Majak 1993) or death camas (Makeiff et al. 1997).

More likely, differences among sites are related to the local influence of moisture stress, which has been implicated extensively in the physiological response of plants that contain sec-

Table 5. Average soil moisture content at death camas and low larkspur sampling sites averaged over years.

	Death camas				Low Larkspur		
	Soil depth (cm)				Soil depth (cm)		
Site	6.5	14	25	Site	6.5	14	25
	---------- (%) ----------				---------- (%) ----------		
4	30	20	23	4	28	21	23
9	30	22	21	8	8	—	4[1]
11	33	25	21	1	12	10	7[1]
10	28	19	21	5	58	40	32
5	54	39	35				
SE	4	1	1		5	2	2[2]
n	68	67	35		39	33	12

[1]Soil moisture measured at 50 cm.
[2]SE at 25 cm applies to sites 4 and 5.

ondary plant metabolites, including alkaloids (Saenz et al. 1993, Gershenzon 1984, Majak et al. 1979). In this study moisture stress, or the lack of it, could be indicated by differences in soil moisture which was compared among years, sites, and stages of growth using analysis of variance. The effect of site, presumably the effect of the soil features at the site, had the greatest and most consistent impact on soil moisture values and there were no meaningful interactions among the 3 factors. The highest soil moisture value was observed at Site 5 (Table 5) which also yielded the lowest levels of zygacine and methyllycaconitine (Table 4). Soil moisture at 6.5 and 14 cm was significantly correlated with zygacine levels ($r = -0.54$ and $r = -0.52$, $P < 0.05$). Thus, a 45% average decrease in water content between Sites 5 and 4 was associated with an increase of 40% in death camas zygacine. This suggests that decreasing soil water availability increases the alkaloid content of these species. Dark Gray Chernozem, lithic phase soil at Site 5 represents a special ecological niche because bedrock occurs at about 30 cm soil depth. Bedrock acts as a barrier to deep water-percolation and allows water draining from upslope to keep this soil profile wetter later into the growing season than on adjacent sites.

Other sites (Table 1) may be drier because of better drainage due to steep convex topography (Site 4), very shallow sandy loam over bedrock (Site 1), or cobbly coarse sand (Sites 8 and 9). Concave topography creates a water-receiving position as opposed to convex to straight topography which creates a water-shedding position and presumably drier conditions. The levels of zygacine at Site 10 were also lower than at Sites 4 or 9 (Table 4) but soil moisture values were not significantly different among the sites (Table 5). Site 10 has a northern exposure; the others have mostly southern aspects (Table 1). In the Northern Hemisphere, soil moisture conditions are optimized when the exposure is to the north and moisture stress should be consequently reduced. Site 10 also has concave topography that accumulates water.

Soil moisture was not significantly correlated with methyllycaconitine levels (Table 5). This can be partly attributed to the much lower soil moisture levels at Sites 1 and 8 (Table 5) that are situated in the dry valley bottom on Brown Chernozem soils. Except for Site 4, which is situated on a Luvisolic soil, the upland sites are all found on Black Chernozem soils. Whether the Luvisolic soil features at Site 4 contribute to moisture stress and alkaloid accumulation in low larkspur remains to be seen. Low larkspur samples obtained from other meadows near Site 4 also yielded relatively high levels of methyllycaconitine (mean 0.31%, range 0.24 – 0.36%, n = 6). The soils at these sites were also Luvisols on convex topographies that tended to shed water.

For death camas, zygacine levels did not vary among years (mean = 0.40%, SE = 0.03). For low larkspur there were significant differences in methyllycaconitine levels among years (means = 0.11, 0.17, and 0.21, SE = 0.01, for 1992, 1993, and 1994, respectively). The yearly means for 1993 and 1994 are similar to those reported earlier for low larkspur (Majak 1993; Majak and Engelsjord 1988). In 1992, the precipitation for March was almost double the long term normal (9.7 mm) and the highest for that month in the continuing ten-year study on low larkspur (Atmospheric Environment Service 1985–1994). The month of March is the beginning of the growing season, especially at lower elevations in the study area. The precipitation for the remainder of the low larkspur growing season (April to June, 1992) was also above normal. The adequate rainfall may have alleviated soil moisture stress with a resultant decline in methyllycaconitine levels in 1992. It has long been known that alkaloid-containing plants are less potent in wet periods than in dry periods (Gershenzon 1984). There was insufficient data on soil moisture in March to effectively compare years with respect to soil moisture.

There were no correlations or any obvious associations between soil temperature and alkaloid levels in either death camas or low larkpur. Soil temperatures were related to stage, site, and year in complicated ways. Soil temperatures had significant stage, year, site, site × stage, year × stage, and site × year effects and these effects varied with soil sampling depth and toxic plant community (W. Majak, unpublished data).

In summary, low alkaloid levels in this study appear to be associated with poisonous plants growing on topographies that enhance soil moisture conditions. Conversely, high alkaloid levels are promoted under conditions of soil moisture stress. These results indicate that, in addition to growth stage, it may be possible to predict the toxicity of alkaloid-containing plants by understanding the physical features of their growing site and how the habitat influences soil moisture availability.

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