Relationship of Relative Total Alkaloid Concentration and Toxicity of Duncecap Larkspur during Growth

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Abstract

The toxicity of duncecap larkspur (*Delphinium occidentale* Wat.) was measured by a mouse bioassay and correlated relative to total alkaloid concentration for 5 samples during one season of plant growth. The concentration coefficient was -0.920 (standard error of estimate = 0.090) when this relationship was described by an exponential equation of the form $Y = ab^x$, where Y equals the total alkaloid concentration and X equals the LD_{50} for mice. The values a and b were estimated to be 5.410 and 0.978, respectively. It is predicted that over the entire growing season, toxicity of larkspur measured by mouse bioassay will be better correlated with toxicity to cattle than will be the correlation of relative total alkaloid content and toxicity to cattle.

Larkspur poisoning of cattle occurs frequently on mountain pastures of the western United States (James et al. 1980). Poisoned cattle appear clinically as having a neuro-muscular weakness syndrome, with episodes of tremors and falling, terminating with respiratory arrest, ventricular fibrillation, or regurgitation, with aspiration asphyxiation (Olsen 1978a). Poisoned cattle are most often found dead, occasionally resting on their sternum with hind legs partially extended behind the animal.

Larkspur plants are generally divided arbitrarily into low and tall groups. This study concerns the tall duncecap larkspur (*Delphinium occidentale* Wats.) which grows in Utah, Colorado, Wyoming, Montana, Idaho, Washington, and Nevada (Ewan 1945) where it often causes significant numbers of cattle deaths each year.

Toxicity of larkspur is due to alkaloids in the plant. It is known that relative total alkaloid concentration generally declines throughout the growing season and likewise, toxicity has been noted to decrease as the plant matures (Olsen 1978b); however, a quantitative relationship between toxicity and total alkaloid content has not been established. A knowledge of the relationship would be helpful as a reference for both laboratory and range studies.

This study correlates the relative total alkaloid content through the growing season of leaves and petioles with the toxicity of leaf and petiole extract as estimated by mouse bioassay.

Methods

The above-ground parts of duncecap larkspur plants were collected at approximately 2-week intervals during a single growing season. Forty individual plant stalks were carefully chosen for uniformity representing the predominant stage of growth in the larkspur community as judged by visual observation at each of 5 sampling times. The plants were collected from semiopen areas in an aspen *(Populus tremuloides)* tree community on the Logan River drainage in northern Utah, about 2 miles south of the Idaho border. The collection site covered an area of about 0.2 hectare. The larkspur plants were generally growing in small groups with some individual plants scattered throughout the site. Only one stalk was removed from a plant clump at one sampling time and an attempt was made to avoid resampling of the same plant at subsequent collection times. Immediately on return to the laboratory the leaves and attached petioles were separated from the stem and placed in a plant dryer. Each dried sample of combined leaves and petioles was ground, mixed, and divided into two aliquots. One plant aliquot was sampled for relative total alkaloid content (Williams and Cronin 1963). The other aliquot was extracted in 95% ethanol, filtered, and the filtrate was evaporated to dryness. The tar-like filtrate residue was then extracted with buffered saline, the resulting saline suspension was filtered (Olsen 1977), and the saline filtrate was subcutaneously injected into mice.

The median lethal doses (LD_{50}) for the leaf extracts were determined using 120 f_1 and f_2 progeny from Simonsen Swiss-Webster white mice.[1] These mice had a mean body weight of 31.7g ± 3.72 s.d. After preliminary testing of toxicity of each sample, four dosages were chosen increasing in a geometric progression. Six mice were used for each dosage (total of 24 mice per sample) and the LD_{50} was calculated according to the method of Weil (1952) from the mortality rate at 8 hours after injection.

[1]Test animals were raised at Poisonous Plant Research Laboratory from parent stock purchased from Simonsen Laboratories, Inc., Gilroy, California.

Fig. 1. Delphinium occidentale *Wats. at the growth stage typical of sample one.*

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Fig. 2. Delphinium occidentale *Wats. at the growth stage typical of sample two.*

Results

Plant Growth Stage

Sample One: Individual plant stalks selected varied from 8 to 10 inches in height (Fig. 1). At this stage leaves and petioles comprised 84% of the dry weight of the sample.

Sample Two: Plant stalks selected varied from 18 to 20 inches in height. The crown of the plant was predominantly leaves with each stem being relatively large and succulent (Fig. 2). Leaves and petioles comprised 78% of the sample dry weight.

Sample Three: Individual stalks selected varied from 30 to 40 inches high, each having a very immature bud cluster about 1 to 2 1/2 inches long (Fig. 3). Leaves and petioles comprised 51% of the sample dry weight.

Sample Four: Plant stalks selected varied from 48 to 72 inches in height. The flowering racemes were about 3 to 8 inches long and contained mostly immature buds with only a slight amount of color in the most mature buds (Fig. 4). Leaves and petioles comprised 35% of the dry weight of the sample.

Sample Five: Stalks selected varied from 48 to 72 inches in height, each having a complement of green, turgid seed pods with occasional flowers at the top (Fig. 5). This particular plant sample had a lesser proportion of low basal leaves on each stalk because sheep had grazed through the collection area and consumed most of the lower leaves. Leaves and petioles comprised 19% of the dry weight of the sample.

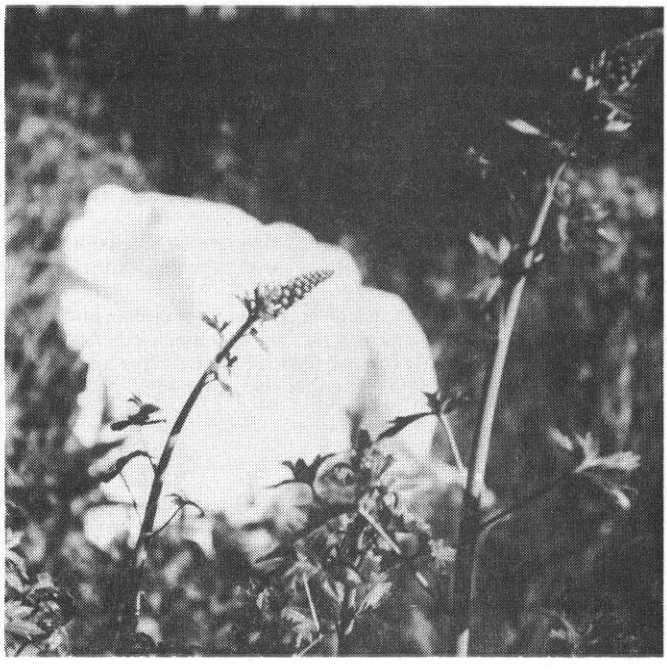

Fig. 3. Delphinium occidentale *Wats. at the growth stage typical of sample three.*

Fig. 4. Delphinium occidentale *Wats. at the growth stage typical of sample four.*

Relative Total Alkaloid Content

The percent total alkaloid content of the samples (Table 1) declined over the growing season in a slightly curvilinear fashion. The decline appeared to be relatively linear during the first 6 weeks, i.e., until the bud cluster began to elongate. Then the rate of decline in relative alkaloid content of the leaves and petioles seemed to lessen as the plant further matured.

Correlation of Relative Total Alkaloid Content and Toxicity

The relationship between relative total alkaloid content and toxicity for mice is illustrated in Figure 6. The toxicity of each sample for mice apparently did not change at the same rate as that of the relative total alkaloid content. The relatively large change in relative total alkaloid content among samples 1, 2, and 3 was proportionately greater than the change in toxicity when compared with the change noted among samples 3, 4, and 5. Thus, the

Fig. 5. Delphinium occidentale *Wats. at the growth stage typical of sample five.*

Table 1. Relative total alkaloid concentration of duncecap larkspur during growth[1].

Sample no.	% total alkaloid
1	5.26
2	4.05
3	2.60
4	2.51
5	1.60

[1]Samples were collected at approximately 2-week intervals during a single growing season. See text for details.

relationship of the data can be described by an exponential equation of the form $Y = ab^x$; where Y equals the relative total alkaloid concentration and X equals the extract LD_{50} for mice. The correlation coefficient for this exponential relationship was $r = -0.920$ with a standard error of estimate of 0.090 (Palm and Hill 1974). The values of a and b were estimated to be 5.410 and 0.978, respectively.

Discussion

An exponential or curvilinear relationship between alkaloid content and toxicity was established by our study for duncecap larkspur. A similar relationship probably applies to most larkspur species when one considers what is known thus far about larkspur alkaloids.

A particular species of larkspur usually contains a mixture of alkaloids (Keith and Pelletier 1970). Indeed, duncecap larkspur has been shown to contain at least 12 alkaloids (Mayo 1959). Furthermore, it is likely that the relative concentration (and absolute amount) of a particular alkaloid changes with the stage of growth (Kreps 1969); and the relative toxicity of each individual alkaloid can vary. So, one must keep in mind that the relative total alkaloid concentration is likely not to be the most suitable indicator of toxicity.

The extrapolation of larkspur toxicity as measured by the mouse bioassay to that of larkspur toxicity for cattle is subject to reservation at this time but some reasonable correlation can be expected, based on limited studies to date. It is our experience that the relative toxicity for cattle of different batches of extract from *Delphinium barbeyi* prepared in our laboratory for rumen infusion can generally be predicted by determining the toxicity of the extract for mice. Also, studies in our laboratory have shown large differences in toxicity of different collections of duncecap larkspur as measured by mouse bioassay and these differences in toxicity were later confirmed by feeding studies to be about the same magnitude for sheep (Olsen 1979). The toxicity of larkspur for sheep can be related to toxicity for cattle (Olsen 1978a).

The conclusion is made that if one knows the relative total alkaloid content of duncecap larkspur, the LD_{50} for mice can be approximated by use of the equation $Y = ab^x$. Because of expected differences in toxicity and content of particular individual alkaloids found in different larkspur species, we would predict that over the entire growing season toxicity of larkspur measured by mouse bioassay will be better correlated with toxicity to cattle than will be the correlation of relative total alkaloid content and toxicity to cattle.

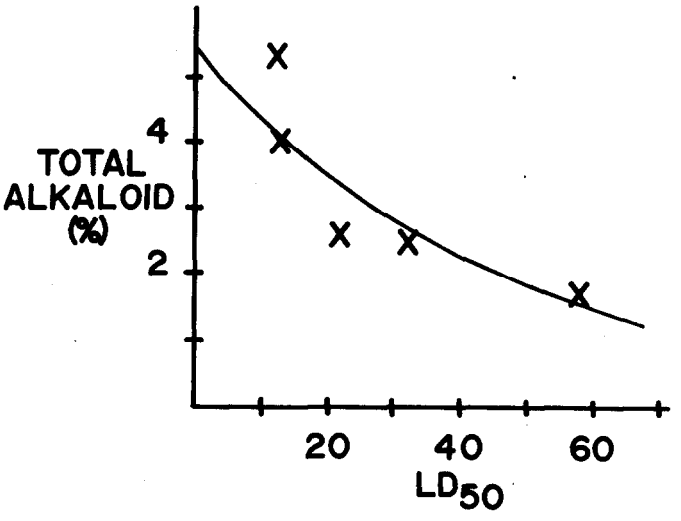

Fig. 6. *Relationship of relative total alkaloid content of* Delphinium occidentale *Wats. and (LD_{50}) for mice during a growing season. Each data point (X) represents the relationship of alkaloid content and toxicity for each biweekly sample collected. The continuous curve was constructed from an exponential regression analysis of the data using the equation $Y = ab^x$, where $a = 5.410$ and $b = 0.978$.*

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