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# The Alkaloid Profiles of *Lupinus sulphureus*

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Lupines are common plants on the rangelands in the western United States. Lupines contain alkaloids that can be toxic and teratogenic causing congenital birth defects (crooked calf disease). One such lupine, *Lupinus sulphureus*, occurs in parts of Oregon, Washington, and British Columbia. Specimens of *L. sulphureus* from field collections and herbaria were evaluated taxonomically and by chemical means. A total of seven distinct alkaloid profiles and the individual alkaloids associated with each profile were identified. Each alkaloid profile was unique in its geographical distribution and its potential risk to livestock. In conclusion, taxonomic classification is not sufficient to determine risk, as chemical characterization of the alkaloids must also be performed.

**KEYWORDS: Lupine; *Lupinus sulphureus*; lupine alkaloids; crooked calf disease**

## INTRODUCTION

Lupines (*Lupinus spp.*) are common plant species found on western U.S. rangelands (*1*). Lupines are highly adaptable, occurring in desert to alpine ecosystems. There are approximately 150 lupine species in the Intermountain West and Great Basin, and these species may contain a variety of piperidine and/or quinolizidine alkaloids (*2*). These alkaloids have been implicated in plant−herbivore interactions and possibly plant−microbe interactions (*2*). Furthermore, many of these alkaloids can be toxic and/or teratogenic to livestock, thus causing losses to livestock producers (*1*).

Historically, lupines have caused large losses in sheep due to acute intoxication (*1*). In the latter part of the 19th century thousands of sheep died from lupine poisoning, and isolated cases of smaller losses of sheep continue today (*1, 3*). In addition, ingestion of lupine by cattle can cause congenital birth defects in calves termed "crooked calf disease" (*4*−*6*). Crooked calf disease is the result of reduced fetal movement during days 40−100 of gestation that causes the limbs and spine to develop in misaligned or contracted positions (*7*−*9*). The quinolizidine alkaloid anagyrine (*10*) and some piperidine alkaloids (*11, 12*) can reduce fetal movement during this critical period of gestation (*13, 14*). Lupine-induced crooked calf disease continues to pose a problem in several western states. For example, lupine-induced crooked calf disease has been associated with *Lupinus sulphureus* (sulfur lupine) in Umatilla County in Northeastern Oregon and possibly in Adams County in the Channel Scablands of East-central Washington (*15*).

Alkaloid profiles are generally constant within a given lupine species although some variation does exist between plant parts (*2, 16*−*19*). However, there are a few cases where the same species of lupine have multiple alkaloid profiles. Wink and Carey (*16*) showed that *Lupinus argenteus* had multiple alkaloid profiles in the region near Crested Butte, Colorado. More, recently it was shown that both *Lupinus sulphureus* and *Lupinus leucophyllus* have at least two alkaloid profiles (*15*). For example, the above-mentioned population of *L. sulphureus* in Oregon contains the quinolizidine alkaloid anagyrine and the population in Washington contains the piperidine alkaloid ammodendrine (*15*).

The *Lupinus* genus is notoriously complex and difficult to classify taxonomically. The general problem is that few species have received detailed study regarding alkaloid composition, taxonomic delineations, and/or phylogenetic relationships. The objective of this study was to identify the characteristic alkaloid profiles of a lupine species throughout its geographical distribution. *L. sulphureus* was selected because of its agricultural importance and relatively small geographical distribution. Samples from field collections as well as specimens from herbaria were analyzed by gas chromatography/flame ionization detection (GC/FID), and the major alkaloids were identified by gas chromatography/mass spectrometry (GC/MS). Alkaloid content and composition were correlated with teratogenic and toxic potential. The locations of the samples were mapped to show the distribution of the different chemical profiles.

## MATERIALS AND METHODS

**Chemicals and Reagents.** Ammonium hydroxide, sodium sulfate, and chloroform were purchased from Fisher Scientific (Pittsburgh, PA), Baker (Phillipsburg, NJ), and Mallinckrodt Baker (Paris, KY). Caffeine and sparteine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO and Milwaukee, WI). Lupanine was obtained from Biomedical Research Co. (Los Angeles, CA.). D-α-Isolupanine perchlorate was purchased from Koch-Light Laboratories Ltd. (Coinbrook Bucks England).

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**Plant Material.** *Lupinus sulphureus* Douglas ex. Hook ssp. *sulphureus* (Leguminosae Family) (sulfur lupine) specimens from the Marion Owneby Herbarium at Washington State University, the Oregon State University Herbarium, the University of Washington Herbarium, the University of British Columbia Herbarium, and the Intermountain Herbarium at Utah State University were sampled for subsequent alkaloid extraction. Vegetative and floral tissues were sampled. Specimens of question were verified to be authentic *L. sulphureus* specimens by staff at the Intermountain Herbarium at Utah State University. In addition, field collections were made throughout the geographical distribution (Oregon, Washington, and British Columbia) of *L. sulphureus*. A single flowering stem from 4−6 plants was collected at each location, from late May to early June, in 2007 and 2008, and was immediately frozen on dry ice. As well, a voucher specimen was pressed at each location which was subsequently classified by staff at the Intermountain Herbarium at Utah State University. Specimens are retained at the Poisonous Plant Research Laboratory Herbarium. Voucher numbers of specimens from herbarium specimens and field collections are located in the Supporting Information.

**Alkaloid Extraction.** Plant material was freeze-dried if necessary and ground to pass through a 2 mm screen. Herbarium specimens (50 mg) or field collections (100 mg) were weighed into a 16 mL screw-top glass test tube. Plant material was extracted using a previously reported procedure (*15*). In brief, the plant material was extracted by mechanical rotation using the Rugged Rotator (Glas Col, LLC) with a mixture of 1 N HCl (4.0 mL) and $CHCl_3$ (4.0 mL) for 15 min. The samples were centrifuged (5 min), and the aqueous layer was removed. An additional 2.0 mL of 1 N HCl was added to the test tube containing plant material and $CHCl_3$, extracted again by mechanical rotation (15 min), and centrifuged, and the aqueous layer was removed. The aqueous portions were combined into a clean 16 mL screw-top glass test tube. The pH of the aqueous layer was adjusted to 9.0−9.5 with concentrated $NH_4OH$. The basic solution was extracted twice with $CHCl_3$, first with 4.0 mL and then with 2.0 mL. The $CHCl_3$ solutions were combined and filtered through anhydrous $Na_2SO_4$ into a clean 16 mL screw-top glass test tubes, and the solvent was evaporated under $N_2$ at 60 °C. The alkaloid fraction extracted was reconstituted in 2 mL (herbarium samples) or 4 mL of methanol (field collections) containing 1.3 $\mu$g/mL caffeine (internal standard). A portion (∼1 mL) was transferred to 1.5 mL GC autosample vials for GC/FID or GC/MS analysis.

**GC/FID Analysis.** All samples were analyzed by GC/FID using a Shimadzu GC-2010 gas chromatograph equipped with a Shimadzu AOC-20i autosampler, a J&W DB-5 column (30 m × 0.32 mm, 0.25 $\mu$m film thickness) and a flame ionization detector (FID). Samples (1.0 uL) were injected splitless at 250 °C, and helium was used as the carrier gas at a constant flow rate of 2.0 mL/min. The column oven was temperature programmed starting at 100 °C for 1 min; increased to 200 at 50 °C/min; increased to 260 at 5 °C/min; increased to 320 at 50 °C/min; and held at 320 °C for 8.8 min for a total run time of 25 min. A table was constructed that contained relative retention times and peak area from the GC/FID analysis for each compound in the sample. A relative peak area of 90,000 units (approximately 30% of the caffeine peak) was used as a cutoff. Chemical profiles were classified into groups by visual analysis of the gas chromatogram based upon the presence and absence of major peaks and corresponding relative retention times from the GC/FID analysis.

**GC/MS Analysis.** GC/MS analysis was performed as previously reported by Lee and colleagues (*15*). In brief, six representative samples (2 $\mu$L) of each chemotype were analyzed by GC/MS using a Finnigan MAT GCQ equipped with a split/splitless injector and a DB-5MS (30 m × 0.25 mm; J&W Scientific) column. Injection port temperature was 250 °C and operated in the splitless mode. Split vent flow rate was 50 mL/min and purged after 0.80 min. Oven temperature was 100 °C for 1 min; 100−200 at 40 °C/min; 200−275 at 5 °C/min; and held at 275 °C for 1.5 min. Electron impact ionization (EI) at 70 eV was used with an ion source temperature of 200 °C.

**Alkaloid Identifications.** Alkaloid identification was performed as previously reported by Lee and colleagues (*15*). In brief, six individual alkaloids were identified from authenticated (MS, NMR) samples of ammodendrine, anagyrine, and thermopsine from the alkaloid collection of the Poisonous Plants Research Laboratory, USDA, ARS, Logan, UT, and from commercially obtained standards (sparteine, lupanine, and D-α-isolupanine). The yet to be identified alkaloids were determined from correlation of measured retention times to retention indices (RI) calculated by linear extrapolation from RI values generated from known standards and assigned RI numbers from the literature and their EI mass spectra (**Figure 1**) (*17*). In addition, alkaloid identification was further supported by correlation of measured relative retention times ($RR_t$) to lupanine and EI mass spectra to those reported in the literature (*20*).

**Data Analysis.** MANOVA and discriminant analysis of each predetermined group as a pairwise comparison was performed using BioNumerics 4.6 (Applied Maths, Inc.) Two parameters were reported: (1) *L* (Wilk's lambda likelihood ratio test) is the likelihood of the obtained discrimination with the assumption that the groups are drawn from the same population. A low *L* value infers that the groups are likely to be drawn from different populations. (2) *p* is the probability that a random grouping of the groups would yield the same degree of discrimination.

Global Positioning System (GPS) coordinates were determined from the location given on each herbarium specimen. These coordinates along with the coordinates from field collections were used to construct the maps showing the distribution of *L. sulphureus* using ArcGIS (ESRI, Inc.). In brief, a spreadsheet was created containing GPS points and chemotype of plants. The spreadsheet was converted to a feature class utilizing ArcCatalog in the ArcGIS program. The feature class was projected in ArcCatalog to match the projections of the state and county boundary shapefiles. Washington State and county boundary data were downloaded from http://fortress.wa.gov/dar/app1/datwed/dmmatrix.html (accessed September 2008). Oregon State and county boundary data were downloaded from http://www.oregon.gov/DAS/EISPD/alphalist.shtml (accessed September 2008). The feature class was opened in ArcMap and overlaid onto the state and county boundary shapefiles. A map was then created with the GPS points symbol color and type matching the corresponding chemotype.

## RESULTS AND DISCUSSION

**Identification of Chemotypes.** Samples from field collections and herbaria were analyzed by GC/FID analysis to obtain their alkaloid profiles. Chemical profiles were classified into groups by visual analysis of the gas chromatogram based upon the presence and absence of major peaks and corresponding relative retention times from the GC/FID analysis. Subsequently, 6 individual samples from each group were randomly selected for GC/MS analysis to identify the major peaks in each chemotype and confirm that the identifying peaks of each group were the same compounds. This resulted in the identification of 7 unique chemotypes (**Figure 2**). To confirm that each of these chemotypes was unique, multivariate statistical methods (MANOVA and discriminant analysis) were used to test for grouping. The results of the MANOVA are displayed in **Table 1**. An overall analysis found that there were differences between the defined groups ($p = 0.001\%$). A pairwise test was used to identify which groups were different. All groups were different from each other ($p = 0.001\%$). Discriminant analysis was also performed as a pairwise comparison or in groups of three to four profiles. In all cases, discriminant analysis showed clear separation of each group based upon one or more alkaloid. An example of the discriminant analysis is shown in **Figure 3**.

**Identification of Alkaloids.** Chemotype A contained a single GC/MS peak (**Figure 2A**, **Table 2**). This peak (peak 1) was confirmed to be ammodendrine (**1**) based on comparison with a standard. The distribution of this chemotype is denoted by an A in **Figure 4**. Plants with this chemotype grow in south central Washington extending up the middle of the State of Washington into Southern British Columbia.

Chemotype B contained two GC/MS peaks (**Figure 2B**, **Table 2**). Peak 1 was confirmed to be ammodendrine (**1**) based

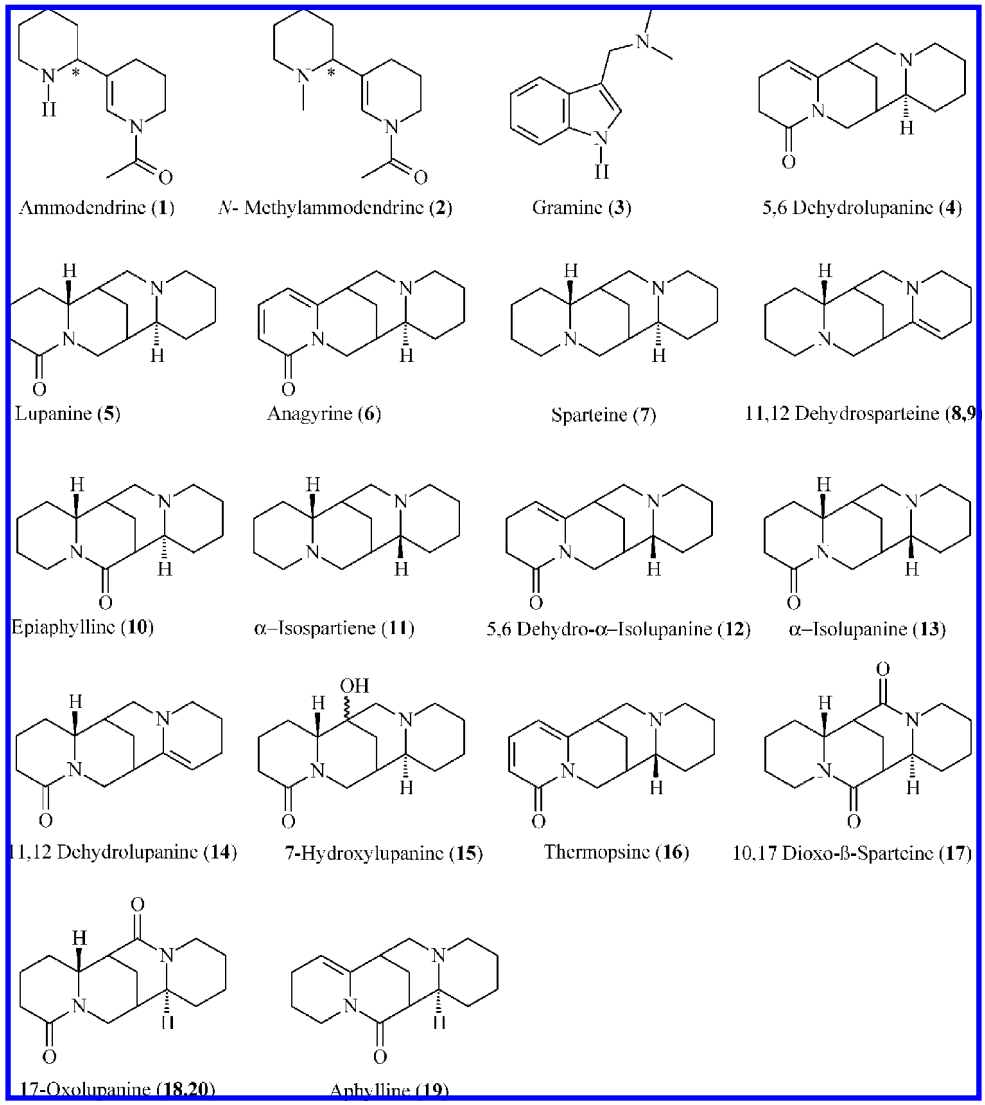


**Figure 1.** Structures of alkaloids identified by GC/MS in *L. sulphureus*.

upon comparison with a standard. Peak 2 was determined to be *N*-methylammodendrine (**2**) based on RI, $RR_t$, and EI mass spectra. The distribution of this chemotype is denoted by a B in **Figure 4**. Plants with this chemotype grow primarily in the southwest corner of Umatilla County and the eastern part of Morrow County in the State of Oregon. In addition a small number of plants of this chemotype are dispersed throughout the region that chemotype 1 covers. All plants of this chemotype contain ammodendrine (**1**) and *N*-methylammodendrine (**2**).

Chemotype C contained four GC/MS peaks (**Figure 2C**, **Table 2**). Peaks 3 and 4 were determined to be gramine (**3**) and 5,6-dehydrolupanine (**4**) based on RI, $RR_t$, and EI mass spectra. Peaks 5 and 6 were determined to be lupanine (**5**) and anagyrine (**6**), respectively, by comparison with standards. The distribution of this chemotype is denoted by a C in **Figure 4**. Plants with this chemotype grow only in Umatilla and Union Counties in the State of Oregon. All plants of this chemotype contain lupanine (**5**) and anagyrine (**6**). Gramine (**3**) and 5,6-dehydrolupanine (**4**) were detected in approximately 50% of the samples representing this chemotype.

Chemotype D contained 7 GC/MS peaks (**Figure 2D**, **Table 2**). Peaks 5, 6, and 7 were determined to be lupanine (**5**), anagyrine (**6**), and sparteine (**7**), respectively, by comparison with standards. Peaks 4, 9, and 10 were identified as 5,6-dehydrolupanine (**4**), 11,12 dehydrosparteine (**9**), and epiaphylline (**10**), repectively, based on RI, $RR_t$, and EI mass spectra. Peak 8 was given a tentative assignment of an 11,12-dehydrosparteine isomer (**8**) based on RI, $RR_t$, and EI mass spectra. The distribution of this chemotype is denoted by a D in **Figure 4**. Plants with this chemotype grow in the southeastern corner of Asotin County in the State of Washington. All plants of this chemotype contain lupanine (**5**), anagyrine (**6**), and sparteine (**7**). 5,6-Dehydrolupanine (**4**), a tentative assignment of 11,12-dehydrosparteine isomer (**8**), 11,12 dehydosparteine (**9**), and epiaphylline (**10**) were detected in greater than 80% of the samples representing this chemotype.

Chemotype E contained 12 GC/MS peaks (**Figure 2E**, **Table 2**). Peaks 5, 7, and 16 were determined to be lupanine (**5**), sparteine (**7**), and thermopsine (**16**) by comparison with standards. Peaks 9, 11, 12, 13, 14, 15, 17, and 18 were determined to be 11,12 dehydosparteine (**9**), α-isosparteine (**11**), 5,6-dehydro-α-isolupanine (**12**), α-isolupanine (**13**), 11,12-dehydrolupanine (**14**), 7-hydroxylupanine (**15**), 10,17-dioxo-*β*-sparteine (**17**), and 17-oxolupanine (**18**) based on RI, $RR_t$, and EI mass spectra. Peak 8 was given a tentative assignment of an 11,12-dehydrosparteine isomer (**8**) based on RI, $RR_t$, and EI mass spectra. The distribution of this chemotype is denoted by an E in **Figure 4**. Plants with this chemotype grow in Asotin, Garfield, Columbia, and Walla Walla Counties in the State of Washington. In addition, plants of this chemotype grow in

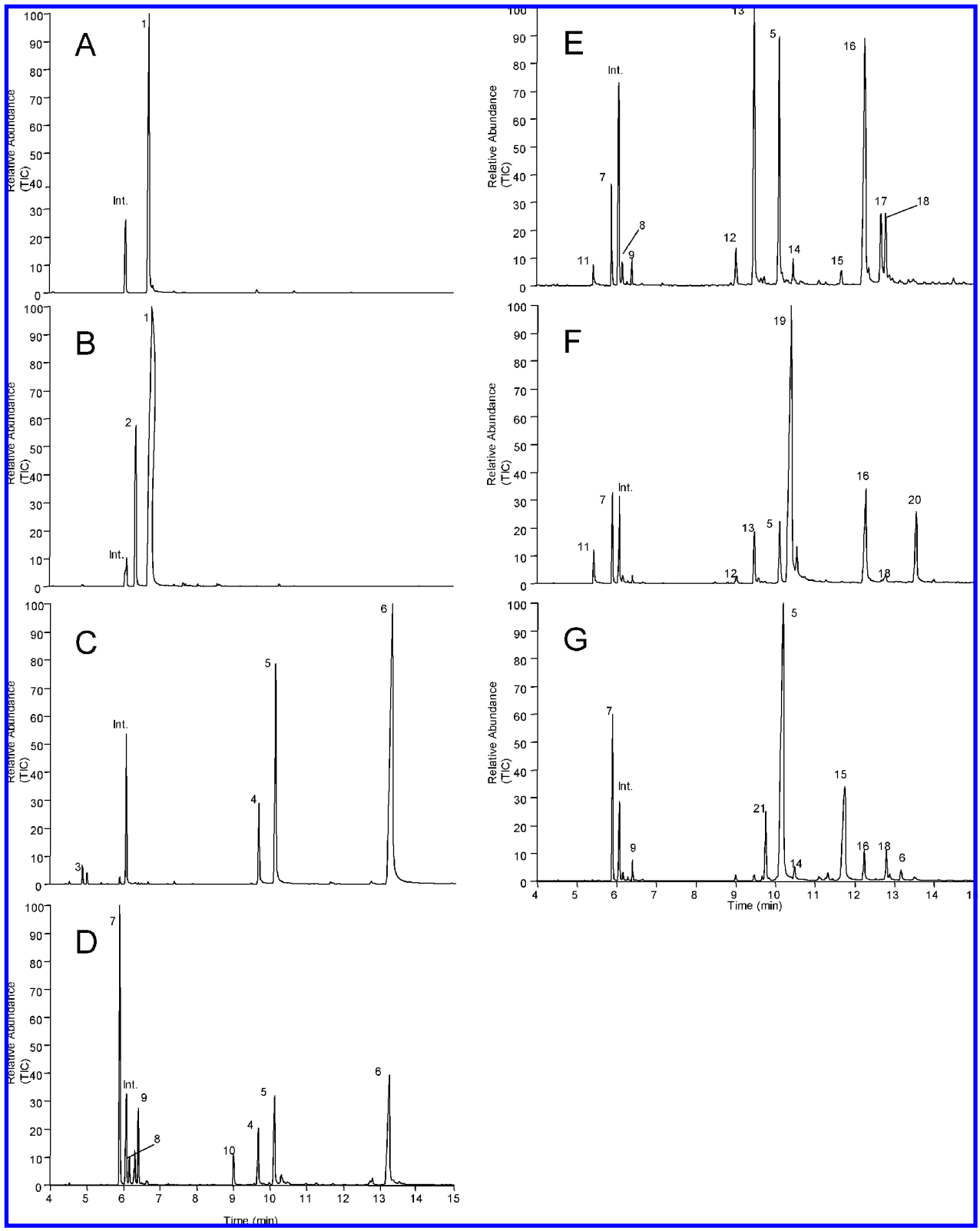


**Figure 2.** GC/MS total ion chromatograms of alkaloid profiles from *L. sulphureus* (**A**) chemotype A collected near Ritzville, WA, (**B**) chemotype B collected near Ukiah, OR, (**C**) chemotype C collected near Pendleton, OR, (**D**) chemotype D collected near Anatone, WA, (**E**) chemotype E collected near Pomeroy, WA, (**F**) chemotype F collected near Coppei, WA, and (**G**) chemotype G collected near Tollgate, OR.

Umatilla, Union and Wallowa Counties in the State of Oregon. α-Isolupanine (**13**) and thermopsine (**16**) were found in all plants of this chemotype. Lupanine (**5**), sparteine (**7**), and α-isosparteine (**11**) were detected in greater than 40% of samples representing this chemotype. A tentative assignment of 11,12-dehydrosparteine isomer (**8**), 11,12-dehydrosparteine (**9**), 5,6-dehydro-α-isolupanine (**12**), 11,12-dehydrolupanine (**14**), 7-hydroxylupanine (**15**), 10,17-dioxo-$\beta$-sparteine (**17**), and 17-oxolupanine (**18**) were detected in 10 to 30% of the samples representing this chemotype.

Chemotype F contained 9 GC/MS peaks (**Figure 2F**, **Table 2**). Peaks 5, 7, and 16 were determined to be lupanine (**5**), sparteine (**7**) and thermopsine (**16**) by comparison with standards. Peaks 11, 12, 13, 18, and 19 were determined to be

**Table 1.** MANOVA Pairwise Test for Differences between *L. sulphureus* Chemotypes

| | | | *L*, *p* (%)[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| species | chemotype | *n* | B | C | D | E | F | G |
| *L. sulphureus* | A | 45 | 0.53, 0.001 | 0.10, 0.001 | 0.06, 0.001 | 0.05, 0.001 | 0.06, 0.001 | 0.06, 0.001 |
| | B | 39 | | 0.11, 0.001 | 0.10, 0.001 | 0.06, 0.001 | 0.08, 0.001 | 0.10, 0.001 |
| | C | 39 | | | 0.23, 0.001 | 0.07, 0.001 | 0.09, 0.001 | 0.18, 0.001 |
| | D | 17 | | | | 0.06, 0.001 | 0.05, 0.001 | 0.06, 0.001 |
| | E | 99 | | | | | 0.10, 0.001 | 0.09, 0.001 |
| | F | 21 | | | | | | 0.07, 0.001 |
| | G | 26 | | | | | | |

[a] *L* (Wilk's lambda likelihood ratio test): likelihood of the obtained discrimination with the assumption that the groups are drawn from the same population. *p* (%): probability that a random grouping of the groups would yield the same degree of discrimination.

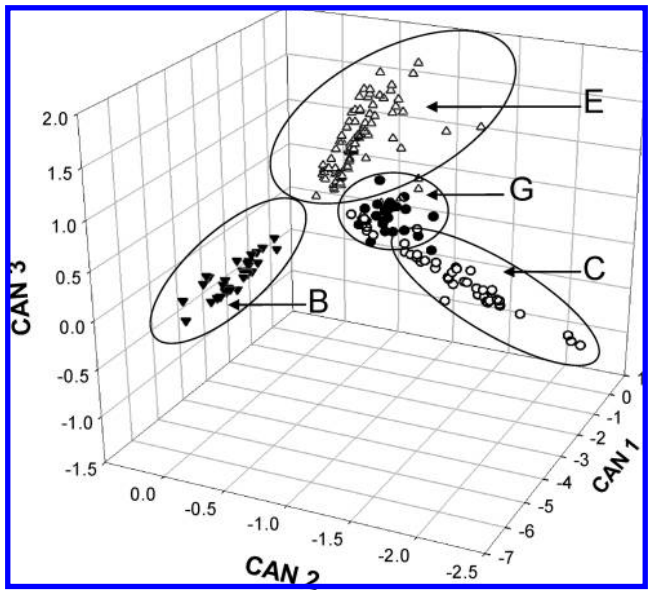


**Figure 3.** Plot of the first three canonical variables resulting from the discriminant analysis of the tabulated GC/FID data:▼ (chemotype B), ○ (chemotype C), Δ (chemotype E), and ● (chemotype G).

α-isosparteine (**11**), 5,6-dehydro-α-isolupanine (**12**), α-isolupanine (**13**), 17-oxolupanine (**18**), and aphylline (**19**) based on RI, $RR_t$, and EI mass spectra. Peak 20 was given a tentative assignment of a 17 oxo-lupanine isomer (**20**) based on RI, $RR_t$, and EI mass spectra. The distribution of this chemotype is denoted by an F in **Figure 4**. Plants with this chemotype grow in Walla Walla and Columbia Counties in the State of Washington. Aphylline (**19**) is found in all samples of this chemotype. α-Isolupanine (**13**), thermopsine (**16**), and a tentative assignment of a 17-oxolupanine isomer (**20**) were detected in greater than 90% of the samples representing this chemotype. Lupanine (**5**), sparteine (**7**), α-isosparteine (**11**), 5,6-dehydro-α-isolupanine (**12**), and 17-oxolupanine (**18**) were detected in 20 to 50% of the samples representing this chemotype.

Chemotype G contained 9 GC/MS peaks (**Figure 2G**, **Table 2**). Peaks 5, 6, 7, and 16 were determined to be lupanine (**5**), anagyrine (**6**), sparteine (**7**), and thermopsine (**16**) by comparison with standards. Peaks 9, 14, 15, 18, and 21 were determined to be 11,12-dehydrosparteine (**9**), 11,12-dehydrolupanine (**14**), 7-hydroxylupanine (**15**), 17-oxolupanine (**18**), and dehydrolupanine (**21**) based on RI, $RR_t$, and EI mass spectra. The geographical distribution of this chemotype is noted by a G in **Figure 4**. Plants with this chemotype grow in northern Umatilla County and are located between chemotype C, E, and F. Lupanine (**5**) and Sparteine (**7**) were detected in all samples of this chemotype. 7-Hydroxylupanine (**15**) was detected in greater than 65% of the samples. Thermopsine (**16**) and 17-oxolupanine (**18**) were detected in approximately 40% of the samples representing this chemotype. Anagyrine (**6**), 11,12-dehydrosparteine (**9**), 11,12-dehydrolupanine (**14**), and dehydrolupanine (**21**) were detected in approximately 10% of the samples representing this chemotype.

Some of the chemotypes have similar features, but others are completely different. Each chemotype was defined by the alkaloids that were always present or absent. Two chemotypes, A and B, are composed of piperidine alkaloids, and five chemotypes, C, D, E, F, and G, are composed of quinolizidine alkaloids (**Figure 2**). Chemotypes A and B contain ammodendrine (**1**), but chemotype B also contains *N*-methylammodendrine (**2**) (**Figure 2**). Likewise chemotypes C, D, and G as well as E, F, and G have many similar features. For example, chemotypes C and D both contain anagyrine (**6**) and lupanine (**5**), but chemotype D also contains a number of other alkaloids including sparteine (**7**) which is always present (**Figure 2**). In addition, chemotype C is composed of lupanine (**5**) and anagyrine (**6**) primarily while chemotype E is composed primarily of the stereoisomers of these compounds, α-isolupanine (**13**) and thermopsine (**16**) (**Figure 2**). Another interesting chemotype is G, which contains elements of chemotypes C and E (**Figure 2**). Chemotype G is located at the geographical interface between C and E suggesting that it may be the result of hybridization between plants with these two chemotypes (**Figure 4**).

This is the first complete investigation of the potential chemotypes of a lupine species throughout its geographical distribution. This research was part of systematic study to identify the characteristic alkaloid profiles of a lupine species, *L. sulphureus*, and the geographical distribution of those profiles. Previous to this work, two chemotypes of *L. sulphureus* had been identified, chemotype A from the area near Ritzville, Washington, and chemotype C from the area near Pendleton, Oregon (*15*). However, previous work provided no information regarding the distribution of these chemotypes. The results of this study provide a more thorough and systematic study of the alkaloid profiles of *L. sulphureus* throughout its natural geographical distribution. Five additional chemotypes were identified, showing that there is significant diversity in the chemical phenotypes of *L. sulphureus*. Furthermore, the geographical distribution of each chemotype was defined. Three important conclusions can be drawn from this data.

First, each chemotype identified poses a different risk to livestock due to its alkaloid composition (*15, 21*). For example, chemotypes C and D contain the teratogen anagyrine (**6**), and chemotypes A and B contain the suspected teratogen ammodendrine (**1**). In addition, chemotypes E, F, and G contain thermopsine (**16**), which induces myopathy in livestock (*22*). This clearly demonstrates that taxonomic identification of a lupine species is not sufficient to determine risk and that alkaloid

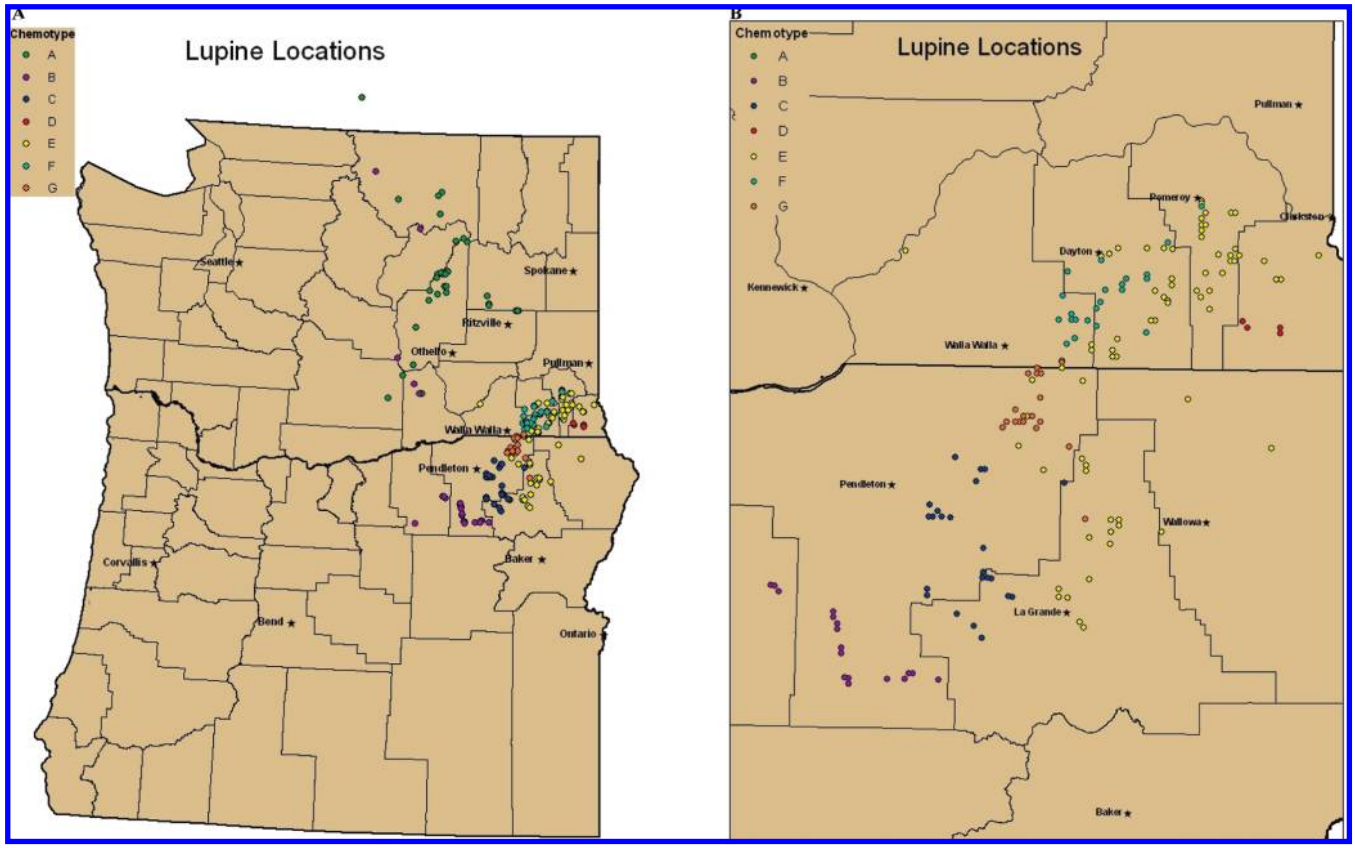


**Figure 4.** Map showing the distribution of the 7 chemotypes of *L. sulphureus*. (**A**) Map of the total distribution of *L. sulphureus* in the States of Washington and Oregon, and the province of British Columbia. (**B**) Higher resolution map of *L. sulphureus* population in the Blue Mountains overlapping the States of Washington and Oregon.

**Table 2.** Identification of alkaloids in *L. sulphureus* by GC/MS

| peak | RI DB-5 | $M^+$ | other fragments (relative abundance) |
|---|---|---|---|
| ammodendrine (**1**) | 1865 | 208 | 80(30), 94(50), 108(25), 109(41), 110(34), 122(31), 123(30), 136(43), 165(58), 191(100) |
| *N*-methylammodendrine (**2**) | 1840 | 222(73) | 94(54), 98(44), 108(54), 109(42), 122(47), 136(100), 137(66), 150(74), 179(34) |
| gramine (**3**) | 1718 | 174(12) | 51(5), 77(12), 102(11), 103(10), 128(12), 129(14), 130(100), 131(42), 132(6) |
| 5,6-dehydrolupanine (**4**) | 2104 | 246(56) | 70(29), 82(19), 96(23), 97(37), 98(100), 120(13), 134(22), 148(15), 245(20) |
| lupanine (**5**) | 2165 | 248(31) | 94(14), 98(17), 110(15), 134(33), 136(100), 148(34), 149(51), 150(33), 247(24) |
| anagyrine (**6**) | 2390 | 244(52) | 70(30), 96(14), 98(100), 134(11), 136(25), 146(32), 160(19) 229(12), 243(12) |
| sparteine (**7**) | 1785 | 234 | 70(13), 96(16), 98(51), 110(15), 122(33), 134(19), 136(58), 137(100), 138(13), 193(38) |
| 11,12-dehydrosparteine isomer (**8**) | 1822 | 232(88) | 96(22), 98(41), 122(22), 134(100), 135(31), 136(23), 148(43), 175(56), 189(21) |
| 11,12-dehydrosparteine (**9**) | 1841 | 232(54) | 94(15), 96(28), 97(22), 98(18), 134(100), 135(27), 136(18), 148(28), 175(46) |
| epiaphylline (**10**) | 2050 | 248(47) | 96(37), 97(32), 98(63), 110(57), 122(30), 136(60), 137(32), 191(43), 220(100) |
| α-isospartiene (**11**) | 1758 | 234(67) | 96(23), 98(100), 110(27), 122(41), 134(34), 136(73), 137(86), 150(26), 193(41) |
| 5,6-dehydro-α-isolupanine (**12**) | 2052 | 246(62) | 70(27), 82(26), 96(30), 97(45), 98(100), 134(26), 136(20), 220(17), 245(23) |
| α-isolupanine (**13**) | 2091 | 248(40) | 98(19), 110(14), 134(29), 136(100), 137(13), 148(33), 149(52), 150(29), 247(30) |
| 11,12-dehydrolupanine (**14**) | 2174 | 246(97) | 106(17), 132(21), 134(100), 136(21), 146(22), 148(39), 231(38), 245(31), 247(20) |
| 7-hydroxylupanine (**15**) | 2265 | 264(83) | 96(44), 98(72), 110(50), 122(47), 124(56), 150(62), 152(100), 246(75), 236(47) |
| thermopsine (**16**) | 2310 | 244(62) | 70(27), 96(18), 98(100), 134(12), 136(33), 146(33), 160(22), 229(15), 243(16) |
| 10,17-dioxo-β-spartiene (**17**) | 2346 | 262(100) | 84(73), 97(33), 110(37), 136(30), 150(93), 151(25), 152(56), 164(25), 234(40) |
| 17-oxolupanine (**18**) | 2356 | 262(41) | 84(17), 96(14), 97(20), 98(13), 110(28), 136(14), 150(100), 151(18), 234(26) |
| aphylline (**19**) | 2163 | 248(41) | 96(36), 97(33), 98(64), 110(55), 134(33), 136(73), 137(34), 191(44), 220(100) |
| 17-oxolupanine isomer (**20**) | 2413 | 262(37) | 84(14), 110(16), 111(12), 124(15), 150(100), 151(13), 152(10), 219(9), 234(9) |
| dehydrolupanine (**21**) | 2110 | 246(48) | 80(23), 110(28), 134(43), 136(73), 148(30), 149(22), 150(100), 151(22), 245(55) |

analysis must be performed on each lupine population to determine risk.

Second, in considering potential risk to livestock, distribution and density of the poisonous plant must be considered (*1, 21*). Each chemotype for the most part has a distinct distribution with defined boundaries. Interestingly, none of the chemotypes appear to follow notable geographical features such as watersheds. It is interesting to note the broad geographical range of chemotypes A and E in contrast to the narrower geographical range of chemotypes F and G. Also notable is the fact that all field collections of *L. sulphureus* at a particular location in this survey have the same chemical phenotype. This is in contrast to *L. argenteus*, where the same location had multiple chemical phenotypes (*16*).

Third, this work suggests that the qualitative nature of the alkaloid profile in *L. sulphureus* remains constant and is not significantly modified by the environment. This conclusion is supported by the fact that the field collections have the same chemical phenotypes as the herbarium specimens from identical locations that were collected over 100 years apart. Furthermore, this suggests the alkaloid composition of herbarium specimens is not modified as a result of long-term storage at room temperature. This does not establish however that the quantitative amounts of these alkaloids do not vary between years; quantitative assessment of the alkaloids over time merits further investigation.

We are currently not able to explain why there is such a large diversity in the alkaloid composition between populations of *L. sulphureus*, although some possibilities merit consideration and discussion:

(1) These lupines may represent distinct varieties or species. For example, the same lupine species may have similar alkaloid profiles as is the case for *L. polyphyllus* from North America or the *L. linearis*−*L. gibertianus* complex from South America (*17*,*23*). Alternatively, the same lupine species may have multiple alkaloid profiles as is the case for *L. argenteus*, *L. formosus*, *L. leucophyllus*, and *L. sulphureus* (*15*, *16*, *24*).

(2) These alkaloid profiles may be a result of chemical warfare between the plant and herbivores. In certain instances, one chemotype is more susceptible to herbivores than another chemotoype (*25*, *26*). Furthermore isomers of the same compound can have differential toxicity to herbivores (*27*). We propose that the stereoisomer split in chemotypes C and E is one such example.

(3) The individual populations may be a result of hybridization between another population and/or another lupine species. For example, *L. polyphyllus* var. *polyphyllus* and *L. arcticus* var. *subalpinus* intergrade in terms of their alkaloid profiles where the two species overlap (*28*). We suggest that chemotype G is one such example. It has characteristics of both chemotypes C and E and is at the overlapping geographical boundary of chemotypes C and E.

(4) Due to the great diversity of chemical profiles in the Blue Mountains of Oregon and Washington we propose that the Blue Mountains is a center of chemical diversity and possible genetic diversity of *L. sulphureus* and that one of these chemotypes, chemotype B, escaped and now represents chemotype A further north in the State of Washington.

To address these possibilities we plan to pursue phylogenetic analysis of the field collections representing collections of each of the distinct alkaloid profiles. In addition, we are pursuing taxonomic studies to identify morphological characters that may separate some of these groups based upon alkaloid composition. In conclusion, this study clearly demonstrates that taxonomic identification of a lupine species is not sufficient to determine risk and that alkaloid analysis must be performed on each lupine population to determine risk.

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**Supporting Information Available:** Spreadsheet containing voucher numbers of specimens from herbarium specimens and field collections. This material is available free of charge via the Internet at http://pubs.acs.org.

## LITERATURE CITED

(1) Kingsbury, J. M. *Poisonous Plants of the United States and Canada*; Prentice-Hall: Englewood Cliffs, NJ, 1964.
(2) Wink, M. The role of quinolizidine alkaloids in plant-insect interactions. In *Insect-Plant Interactions*; Bernays, E. A., Ed.; CRC Press: Boca Raton, FL, 1992; pp 131−166.
(3) Chesnut, V. K.; Wilcox, E. V. The stock poisoning plants of Montana: A preliminary report U.S. Department of Agriculture: Washington DC, 1901; Bulletin No. 26, pp100−110.
(4) Palotay, J. L. Crooked Calves. *West. Vet.* **1959**, *6*, 16–20.
(5) Wagnon, K. A. Lupine poisoning as a possible factor in congenital deformities in cattle. *J. Range Manage.* **1960**, *13*, 89–91.
(6) Binns, W.; James, L. F. A congenital deformity in calves, similar to "crooked calf disease" has been experimentally produced by feeding heifers lupine and lead. *Proc. West. Sect. Am. Soc. Anim. Prod.* **1961**, *12*, 1–3.
(7) Shupe, J.; James, L. F.; Binns, W. Observations on crooked calf disease. *J. Am. Vet. Med. Assoc.* **1967**, *151*, 191–197.
(8) Shupe, J.; Binns, W.; James, L.; Keeler, R. Lupine, a cause of crooked calf disease. *J. Am. Vet. Med. Assoc.* **1967**, *151*, 198–203.
(9) Shupe, J.; Binns, W.; James, L.; Keeler, R. A congenital deformity in calves induced by the maternal consumption of lupin. *Aust. J. Agric. Res* **1968**, *19*, 335–340.
(10) Keeler, R. F. Lupin alkaloids from teratogenic and nonteratogenic lupins. III. Identification of anagyrine as the probable teratogen by feeding trials. *J. Toxicol. Environ. Health* **1976**, *1*, 878–889.
(11) Keeler, R. F.; Panter, K. E. Piperidine alkaloid composition and relation to crooked calf disease-inducing potential of *Lupinus formosus*. *Teratology* **1989**, *40*, 423–432.
(12) Panter, K. E.; Gardner, D. R.; Molyneux, R. J. Teratogenic and fetotoxic effects of two piperidine alkaloid-containing lupines (*L. formosus* and *L. arbustus*) in cows. *J. Nat. Tox.* **1998**, *7*, 131–140.
(13) Panter, K. E.; Bunch, T. D.; Keeler, R. F.; Sisson, D. V.; Callan, R. J. Multiple congenital contractures (MCC) and cleft palate induced in goats by ingestion of piperidine alkaloid-containing plants: Reduction in fetal movement as the probable cause. *Clin. Toxicol.* **1990**, *28*, 69–83.
(14) Panter, K. E.; Keeler, R. F. Induction of cleft palate in goats by *Nicotiana glauca* during a narrow gestational period and the relation to reduction in fetal movement. *J. Nat. Toxins* **1992**, *1*, 25–32.
(15) Lee, S. T.; Cook, D.; Panter, K. E.; Gardner, D. R.; Ralphs, M. H.; Motteram, E. S.; Pfister, J. A.; Gay, C. C. Lupine induced "Crooked Calf Disease" in Washington and Oregon: identification of the alkaloid profile in *Lupinus sulfureus*, *Lupinus leucophyllus*, and *Lupinus sericeus*. *J. Agric. Food Chem.* **2007**, *55*, 10649–10655.
(16) Wink, M.; Carey, D. B. Variability of quinolizidine alkaloid profiles of *Lupinus argenteus* (Fabaceae) from North America. *Biochem. Syst. Ecol.* **1994**, *22*, 663–669.
(17) Wink, M.; Meiβner, C.; Witte, L. Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. *Phytochemistry* **1995**, *38*, 139–153.
(18) Dolinger, P. M.; Ehrlich, P. R.; Fitch, W. L.; Breedlove, D. E. Alkaloid and predation patterns in Colorado lupine populations. *Oecologia* **1973**, *13*, 191–204.
(19) Wink, M. Quinolizidine Alkaloids In *Methods in Plant Biochemistry, Alkaloids and Sulphur Compounds*; Waterman, P., Ed.; Academic Press: London, 1993; Vol. 8, pp 197−239.
(20) Kinghorn, A. D.; Balandrin, M. F. Quinolizidine alkaloids of the leguminosae: Structural types, analysis, chemotaxonomy, and biological activities In *Alkaloids: Chemical and Biological Per-*

*spectives*; Pelletier, S. W., Ed.; John Wiley & Sons, Inc.: New York, 1984; Vol. 4.

(21) Panter, K. E.; Gardner, D. R.; Gay, C. C.; James, L. F.; Mills, R.; Gay, J. M.; Baldwin, T. J. Observations of *Lupinus sulphureus*-induced "crooked calf disease". *J. Range Manage.* **1997**, *50*, 587–592.

(22) Keeler, R. F.; Baker, D. C. Myopathy in cattle induced by alkaloid extracts from *Thermopsis montana*, *Laburnum anagyroides*, and a *Lupinus sp.* *J. Comp. Pathol.* **1990**, *103*, 169–182.

(23) Planchuelo-Ravelo, A. M.; Witte, L.; Wink, M. Quinolizidine alkaloid profiles of South American Lupins: *Lupinus linearis* and the *Lupinus gibertianus* complex. *Z. Naturforsch.* **1993**, *48c*, 702–706.

(24) Lee, S. T.; Molyneux, R. J.; Panter, K. E.; Chang, C. W. T.; Gardner, D. R.; Pfister, J. A.; Garrossian, M. Ammodendrine and *N*-methylammodendrine enantiomers: isolation, optical rotation, and toxicity. *J. Nat. Prod.* **2005**, *68*, 681–685.

(25) Berenbaum, M. R.; Zangerl, A. R. Stalemates in the coevolutionary arms race: syntheses, synergisms, and sundry other sins In *Chemical Mediation of Coevolution*, *Spenser*, K. C., Ed.; Academic Press: San Diego, CA, 1988; pp 113−132.

(26) Bowers, M. D.; Puttick, G. M. Response of generalist and specialist insects to qualitative allelochemical variation. *J. Chem. Ecol.* **1988**, *14*, 319–351.

(27) Berenbaum, M. R.; Zangerl, A. R.; Lee, L. Chemical barriers to adaptation by a specialist herbivore. *Oecologia* **1989**, *80*, 501–506.

(28) Majak, W.; Keller, W. J.; Duan, Z.; Munro, D.; Smith, R. A.; Davis, A. M.; Ogilvie, R. T. Alkaloid distribution in two species of *Lupinus* in Central British Columbia. *Phytochemistry* **1994**, *36*, 883–885.

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