Agriculture **2015**, *5*, 456-474; doi:10.3390/agriculture5030456

OPEN ACCESS

agriculture

ISSN 2077-0472

www.mdpi.com/journal/agriculture

Review

Adverse Effects of Larkspur (*Delphinium* spp.) on Cattle

Kevin D. Welch [1,*], Daniel Cook [1], Benedict T. Green [1], Dale R. Gardner [1], James A. Pfister [1], Tara G. McDaneld [2] and Kip E. Panter [1]

[1] USDA-ARS Poisonous Plant Research Laboratory, Logan, UT 84341, USA;
E-Mails: Daniel.Cook@ars.usda.gov (D.C.); Ben.Green@ars.usda.gov (B.T.G.);
Dale.Gardner@ars.usda.gov (D.R.G.); Jim.Pfister@ars.usda.gov (J.A.P.);
Kip.Panter@ars.usda.gov (K.E.P.)

[2] USDA-ARS Meat Animal Research Center, Clay Center, NE 69833; USA;
E-Mail: Tara.McDaneld@ars.usda.gov

* Author to whom correspondence should be addressed; E-Mail: Kevin.Welch@ars.usda.gov;
Tel.: +1-435-752-2941; Fax: +1-435-753-5681.

Academic Editor: Wayne L. Bryden

Received: 14 May 2015 / Accepted: 10 July 2015 / Published: 16 July 2015

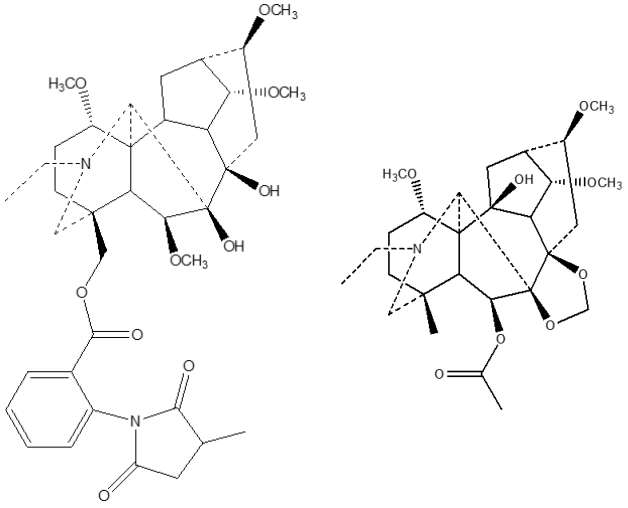
Abstract: There are numerous species of larkspur (*Delphinium* spp.) in North America. Larkspurs are a major cause of cattle losses on western ranges in the USA, especially on foothill and mountain rangelands. The toxicity of larkspur species is due to various norditerpenoid alkaloids. In this article, we review the current knowledge regarding larkspur ecology and distribution, analytical technologies to study and quantify the toxins in larkspur, the toxicology of the larkspur plants and their individual toxins, known genetic variations in larkspur susceptibility, and current management recommendations to mitigate losses from larkspur poisoning.

Keywords: larkspur; delphinium; methyllycaconitine; cattle

1. Introduction

There are over 60 wild species of larkspurs (*Delphinium* spp.) in North America [1] and a number of domestic horticultural varieties. Wild larkspurs are divided into three general categories based primarily on mature plant height and distribution: low, tall and plains larkspurs [2]. The larkspurs are a major cause of cattle losses on western ranges [3,4], especially on foothill and mountain rangelands [5].

Larkspurs have been shown to be toxic to horses, although horses will not voluntarily consume sufficient quantities of larkspurs to become poisoned [6,7]. Sheep have been shown to be quite resistant to larkspurs [6–8]. Consequently, cattle are the primary livestock species associated with larkspur toxicosis and thus the effect of larkspurs on cattle is the focus of this review. The toxicity of larkspurs is due to norditerpenoid alkaloids including two predominant types, the *N*-(methylsuccinimido) anthranoyllycoctonine (MSAL)-type and the non MSAL-type including the 7,8-methylenedioxylycoctonine (MDL)-type (Figure 1) [7,9]. In this article, we review the current knowledge regarding larkspurs, their toxicity, and current management strategies to minimize losses from livestock poisoning.



(**a**) (**b**)

Figure 1. (**a**) Methyllycaconitine (MLA) (MSAL-type alkaloid); (**b**) deltaline (non MSAL-type alkaloid). Structure of a MSAL-type alkaloid methyllycaconitine (MLA) and a non MSAL-type alkaloid deltaline.

2. Plant Ecology and Distribution

Taxonomic treatments of the genus *Delphinium* recognize greater than 60 species in North America [10–12]. Larkspurs have been divided into three categories based upon their height at maturity and their distribution: tall, low, and plains larkspur. The larkspur species in each category responsible for most of the reported livestock losses in the western United States are listed in Table 1. *Delphinium barbeyi*, *D. glaucescens*, *D. glaucum* and *D. occidentale* represent the dominant tall larkspur species [7]. Tall larkspurs are found typically at high elevation, snow-covered sites in varied plant communities (Figure 2). *Delphinium andersonii*, *D. bicolor*, *D. nuttallianum*, *D.parishii* and *D. scaposum* represent the dominant low larkspurs [7]. Low larkspurs are found in a variety of plant communities, for example *D. nuttallianum* may be found in mountain meadows while *D. andersonii* may be found in a desert shrub plant community. Lastly, the plains larkspur is represented by a single species, *D. geyeri*, and is intermediate in height compared to the low and tall larkspurs [7].

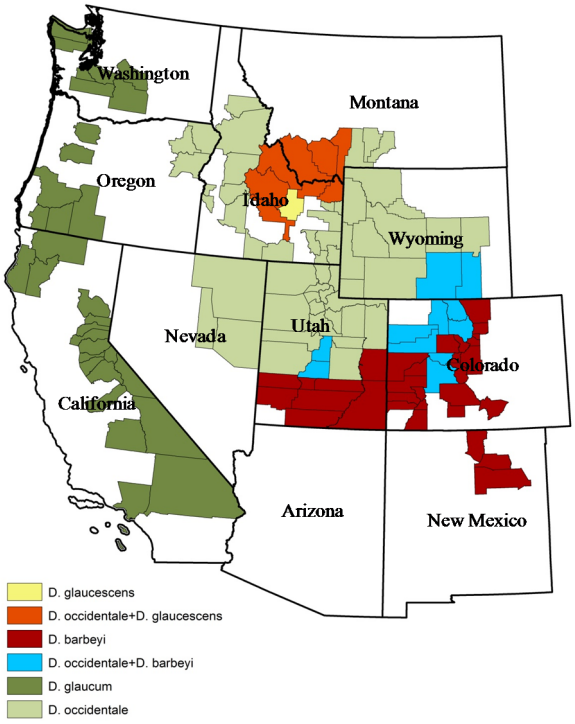


Figure 2. Distribution map of the major tall larkspur species in the western United States.

Table 1. Larkspur classes and major species causing livestock losses associated with each class, with associated plant communities and possible dominant alkaloids.

Larkspur Class	Species	Major Alkaloids	Plant Communities
Tall Larkspurs	*D. barbeyi* *D. glaucescens* *D. glaucum* *D. occidentale* *D. ramosum*	Deltaline 14-*O*-Acetyldictyocarpine Methyllycaconitine	Alpine, Aspen, Conifer, Mountain Brush, Mountain Meadows, Sagebrush
Low Larkspurs	*D. andersonii* *D. bicolor* *D. nuttallianum* *D. parishii* *D. scaposum*	Methyllycaconitine Geyerline Nudicauline 14-Deacetylnudicauline	Aspen, Conifer, Desert Shrub, Mountain Brush, Mountain Meadows, Pinion-Juniper, Sagebrush
Plains Larkspur	*D. geyeri*	Methyllycaconitine	Desert Shrub, Mountain Brush, Sagebrush, Shortgrass Prairie

Delphinium species contain numerous norditerpenoid alkaloids which represent two structural types the *N*-(methylsuccinimido) anthranoyllycoctonine (MSAL)-type and the non MSAL-type, which includes the 7,8-methylenedioxylycoctonine (MDL)-type alkaloids. Purified alkaloids of these two structural types differ significantly in toxicity based upon an LD_{50} in a mouse model [9,13–15]. In general, the non MSAL-type alkaloids are 20–30 times less toxic compared to the MSAL-type in the mouse model. The structural type of alkaloids and the individual alkaloids differ among species and populations of the same species. For example, *D. glaucescens* is composed predominantly of methyllycaconitine, an MSAL-type alkaloid with a small amount of MDL-type alkaloids, while many

populations of *D. barbeyi* are often found to contain a three-to-one ratio of non MSAL to MSAL-type alkaloids [16,17]. Additionally, another tall larkspur species, *D. stachydeum* only contains non MSAL-type alkaloids [18]. Likewise in *D. occidentale* there are two chemotypes, one that contains both MSAL and non MSAL-type alkaloids and one that does not contain MSAL-type alkaloids [19]. Each chemotype has a distinct geographic distribution shown in Figure 3. The low larkspurs in general contain a more diverse suite of MSAL-type alkaloids in addition to the non MSAL-type alkaloids [20]. These differences in the qualitative and quantitative composition of the MSAL and non MSAL-type alkaloids are implicated in differences in toxicity in cattle [16,18,21,22].

The accumulation of the norditerpenoid alkaloids varies seasonally and between plant parts among the different species of larkspur [23–26]. In individual parts, alkaloid concentrations are greatest in immature tissues and decrease as those tissues mature in tall and low larkspurs [27]. On a whole-plant basis, in tall larkspurs, alkaloids are the greatest early in the season and decrease as the plants matures while in low larkspurs alkaloid amounts are generally similar over the season [24–27]. As plants senesce, alkaloid concentrations continue to decrease.

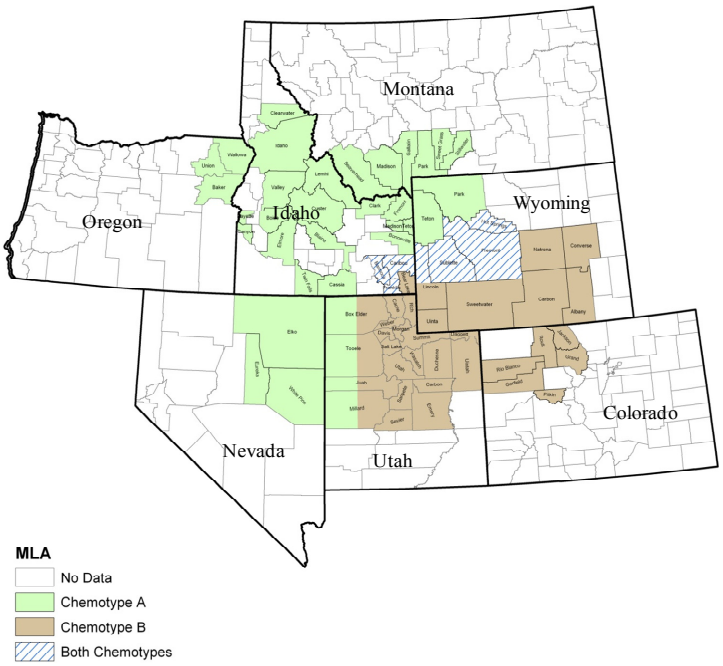


Figure 3. Distribution map of two chemotypes of *D. occidentale.* Chemotype A plants contain the MSAL-type alkaloids whereas chemotype B plants do not contain the MSAL-type alkaloids (modified from [19]).

The density of a given larkspur species on the range will influence consumption and thus its relative toxicity. Tall larkspur species are generally long-lived species where a similar number of plants are present on any given year [7]. In tall larkspur species weather factors such as temperature and precipitation will influence the abundance of flowers and pods. Anecdotal information suggests that on years where there are more flowers and pods, there is more relative consumption with subsequently increased cattle losses. In contrast, low larkspur species are also long-lived, but the number of plants can vary considerably from year to year based upon precipitation and temperature. Data suggests that increased precipitation in the preceding winter and spring months and cooler temperatures will

increase the density of low larkspur species [28,29]. As low larkspur species are small in stature, sufficient plant density must be present in order to have sufficient material available for consumption to impart toxicity.

3. Analytical Techniques

There have been a number of chemical methods used for the analysis of the toxic norditerpenoid alkaloids found in larkspurs. These methods have included classical wet chemical methods and various chromatographic and spectroscopic methods or a combination of chromatographic and spectroscopic methods. Earlier methods typically measured what was termed Total Alkaloid Concentration (TAC). This was measured initially by chemical titration methods [30,31], then gravimetrically [32–34], and then through calibrated Near Infrared Spectroscopy (NIRS) [35] and Infrared (IR) spectroscopy [36]. Methods for TAC are attractive in that they are typically less costly and can provide a relative rapid preliminary indicator of toxicity, especially if the individual alkaloid components of the larkspur being tested are previously known. Titration and gravimetric measurements require simple laboratory equipment but can be lengthy procedures. NIRS and IR methods can be rapid, but require initial investment in instrumentation and up front detailed calibrations. However, TAC methods do not distinguish between individual types of alkaloids (MSAL *vs.* non MSAL-type alkaloids). In order to partially address the need to selectively analyze for the MSAL-type alkaloids, a Fourier transform infrared (FT-IR) spectroscopy method was developed to measure the concentration of all MSAL-type alkaloids as well as the TAC in larkspurs [36]. This method has been used to analyze large number of samples obtained from rangeland investigations [37] and is still the method of choice for rapid evaluation of larkspur toxicity. The largest drawback of the FT-IR method is the extensive initial calibration of the instrument with a complete set of known samples for which the total MSAL and TAC has been measured using other standard methods.

For the analysis of specific individual larkspur alkaloids, a number of more detailed and sensitive methods have been developed based on modern chromatographic and spectroscopic methods. A relative simple method for the analysis of non MSAL-type alkaloids is based on the use of capillary gas chromatography [38]. Capillary gas chromatography can be an excellent method for the analysis of a number of individual alkaloids of the lycoctanine, or the MDL-type, alkaloids that are thermally stable and still volatile enough to be chromatographed in the gas phase. However, the non MSAL-type alkaloids tend to be the less toxic compounds and thus analysis of larkspur using solely this technique may not produce a complete evaluation of the potential toxicity of the larkspurs being analyzed.

The MSAL-type alkaloid methyllycaconitine (MLA) was initially indentified as one of the major and most toxic alkaloids found in larkspurs. MLA is not particularly amenable for analysis by capillary gas chromatography, but a number of different methods using liquid chromatography (HPLC) with UV detection have been developed that work well for these larger, thermally labile compounds. HPLC methods developed have used both normal phase [13] and reverse phase [39,40] type separations. When combined with atmospheric ionization and mass spectrometry (*i.e.*, HPLC-esi(+)MS) the methods can become extremely sensitive and selective [20,41–44]. Even in the absence of chromatography, a simple flow injection mass spectrometry method can be a valuable tool in assessing the alkaloid composition of larkspurs [42]. Such flow injection methods have been valuable in

chemo-typing the various larkspurs based on alkaloid profiles, geographical location and species identifications [17–19]. With development of fully hyphenated methods, such as LC-MS/MS, the sensitivity and selectivity have become sufficient for detection of very low concentration of alkaloids (*i.e.*, ng/mL) in such samples as sera or tissue (liver) for the purposes of defining toxicokinetics [22,45,46] or for diagnostic purposes in the analysis of contaminated feed supplies, tissues, rumen content or fluid samples of poisoned animals (Figure 4). The use of MS/MS techniques is also beneficial in the structure elucidation of new norditerpenoid alkaloids [23,42,44].

4. Toxicology

The primary result of larkspur toxicosis is neuromuscular paralysis from inhibition of normal function of the post-synaptic neuromuscular junction [47,48]. The toxicity of larkspurs is primarily due to the norditerpenoid alkaloids [7,22]. Larkspur alkaloids are competitive inhibitors of acetylcholine, particularly at nicotinic acetylcholine receptors (nAChRs) [49]. The binding of larkspur alkaloids to nAChRs in various tissues is correlated to toxicity [50], and may explain the tolerance of sheep to larkspur, in that larkspur toxins appear to bind less avidly to sheep nAChRs [51,52].

Clinical signs of larkspur intoxication include muscular weakness and trembling, straddled stance, periodic collapse into sternal recumbency, respiratory difficulty, and finally death while in lateral recumbency [53]. A non-lethal dose of larkspur causes labored breathing, rapid and irregular heartbeat, and muscle weakness to the point of collapse [54]. A lethal dose tall larkspur in Hereford steers (one of the more susceptible breeds of steers to tall larkspur toxicity) has been shown to be approximately 470 g of plant material (dry weight of a population of *D. barbeyi* containing 4 mg/g MSAL-type alkaloids and 16 mg/g total alkaloids) for a 500 kg animal, or 3.1 kg wet weight [22]. However, it should be kept in mind that due to the fact that the larkspur alkaloids are neuromuscular toxins, anything that causes additional stress and/or exertion can reduce the toxic/lethal dose.

The majority of dose-dependent research studies that have been performed followed a dosing regimen that consisted of a single bolus dose of finely ground plant material. These studies were extremely informative, but they lacked the multiple exposure scenario that likely occurs to cattle in a grazing environment [55]. Therefore a recent study was conducted to evaluate the acute toxicity of tall larkspur (*D. barbeyi*) administered in multiple doses, with the intent of identifying a No Observable Adverse Effect Level (NOAEL). The adverse effect was muscle weakness to the point the cattle could no longer walk, as would be required in a grazing environment. The research demonstrated that the overall daily intake is critical, and should be maintained under 2 mg MSAL-type alkaloids/kg BW/day [56]. Consequently, based upon a population of tall larkspur that contains 4 mg MSAL-type alkaloids/g plant material (16 mg total alkaloid/g plant material), a dose of 2 mg/kg/day would correspond to a daily intake of 250 g of tall larkspur on a dry weight basis for a 500 kg animal, which, assuming that tall larkspur is approximately 85% water, would correspond to 1.67 kg of fresh plant material each day. Therefore, based upon the results of that study, a 500 kg cow should be able to consume up to 1.67 kg of fresh tall larkspur per day without becoming severely poisoned.

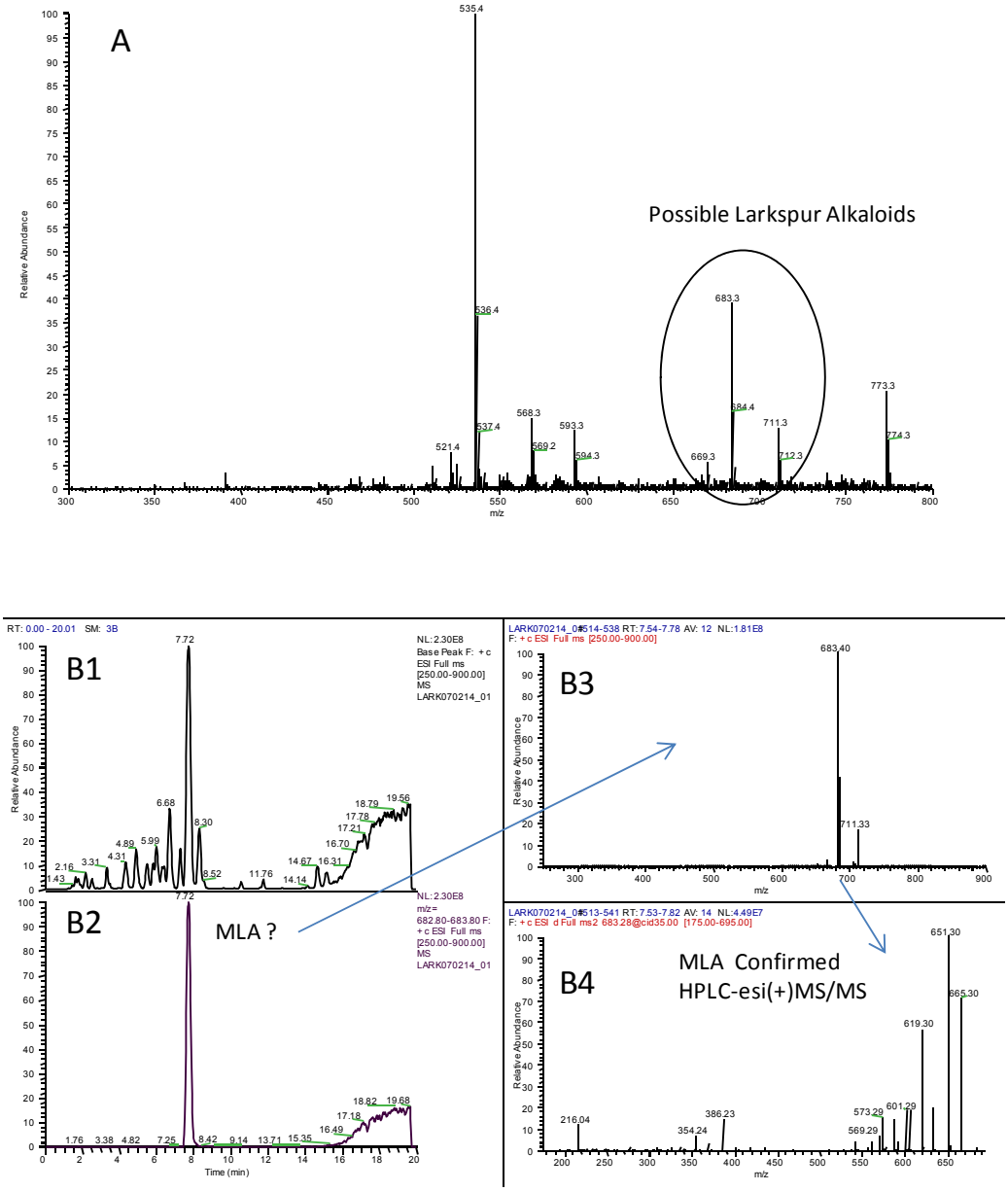


Figure 4. (**A**) MS spectrum of crude rumen extract obtained from a dead cow involved in possible rangeland plant poisoning case and analyzed by flow-injection esi(+)MS. Possible larkspur alkaloids in the rumen were indicated by ions at *m*/*z* 669, 683 and 711 that are consistent with those found in the low larkspurs (**B1**); HPLC-esi(+)MS/MS ion chromatogram obtained after isolation of the crude alkaloid fraction by acid/base extraction (**B2**); extracted ion chromatogram for *m*/*z* 683 and possible detection of the alkaloid methyllycaconitine (MLA) (**B3**); and confirmed by its MS/MS spectrum (**B4**).

Due to the fact that the MSAL-type alkaloids are much more toxic than the MDL-type alkaloids [13,14], management recommendations for grazing cattle on larkspur-containing ranges are based primarily on the concentration of MSAL-type alkaloids in larkspur [5,37]. However, in many species of tall larkspur the non MSAL-type alkaloids can be more abundant [7,17]. Research work using a mouse model suggested that MDL-type alkaloids enhance the overall acute toxicity of MLA in an additive manner [15]. In subsequent studies with cattle, the MSAL-type alkaloids such as MLA were the

primary factors responsible for the toxicity of larkspurs [16,21,22]. However, populations of larkspurs that contained large amounts of MDL-type alkaloids, in addition to high MSAL-type alkaloid content, were found to pose a greater risk to cattle than plants with only high MSAL-type alkaloids [16]. Consequently, in general for larkspurs to be toxic to livestock, a sufficient quantity of MSAL-type alkaloids is required. However, MDL-type alkaloids appear to potentiate the overall toxicity of the MSAL-type alkaloids and should be considered when predicting potential toxicity of larkspur populations [22]. However, recent investigations into the toxicity of *D. stachydeum* populations demonstrated that some populations could potentially have adverse effects in cattle even though they do not contain MSAL-type alkaloids [18]. Based upon the results of the last few years of research, it has become evident that the toxicity of tall larkspur populations is greatly dependent upon the alkaloid profile of each species and even specific populations within a species [16,18,19,22]. Consequently, it is impossible to provide a general statement as to how much larkspur a cow can eat without dying, as a lethal dose of larkspur is completely dependent upon the alkaloid profile of the plant and the genetic susceptibility of the animal.

5. Genetic Susceptibility

Recently, there has been a great deal of research effort put into genomic-based selection methods for the improvement of economically important traits such as milk yield and meat tenderness in cattle [57]. However, the genetic basis of livestock responses to plant toxins, such as larkspur alkaloids, has received little attention. The identification of larkspur-resistant animals would be beneficial for animal welfare, thus potentially improving the economics of livestock production of cattle on larkspur-containing ranges. Initial research on the selection of animals for resistance to larkspur toxins at this laboratory used inbred mouse strains as a model to determine if there was a potential to select for differences in larkspur toxicity. Results from this research showed that there was a difference in susceptibility of nine mouse strains to MLA toxicosis with an approximate two-fold difference between the A/J and 129 strains (3.3 mg/kg *vs.* 5.8 mg/kg respectively) [58]. The difference in the LD_{50} values between the two strains was attributed to the amount of nAChR subunits expressed by the two strains. The 129 strain of mice had roughly twice the amount of alpha 7 nAChR subunit expression as the A/J strain, as well as higher expression of the alpha 3 and alpha 5 nAChR subunits. The presence of an alpha 7 nAChR reserve in the 129 strain could explain the resistance to MLA toxicity, as MLA is a competitive antagonist of the nAChRs. With more nAChRs, the 129 strain would be able to tolerate a higher dose of MLA before the percentage of blocked receptors would reach toxic concentrations compared to the A/J mice. However, more recent studies have demonstrated that there is no difference in the susceptibility of mice lacking the alpha 7 nAChR to MLA toxicosis compared to wild type mice [59]. The results of that study suggest that either the alpha 7 subunit does not play an integral role in the acute toxicosis of MLA or that alpha 7 nAChR knockout mice developed a compensatory response to the lack of the alpha 7 subunit. Consequently, other nAChR subunits found in the neuromuscular junction are likely the primary target for MLA, resulting in acute toxicosis. The success of these experiments provided the basis for ongoing experiments to identify genetic factors that correlate with susceptibility to larkspur toxicity in cattle.

Recently, we reported the phenotyping of cattle with a standardized dose of tall larkspur, and the associated muscle weakness (Figure 5) [60]. The measurement of phenotypic differences between cattle is the first step in the identification of genetic markers associated with resistance to larkspur poisoning. Based on the results of that experiment, cattle were classified as susceptible, intermediate or resistant in each breed of cattle. We have identified at least one individual resistant to larkspur poisoning in all breeds tested to date. The number of larkspur resistant animals may vary in each individual herd/breed due to the genetic background of that herd/breed. These differences are likely due to each individual animal's genetic predisposition for larkspur poisoning, which may be predictable from its DNA sequence and genomic analysis.

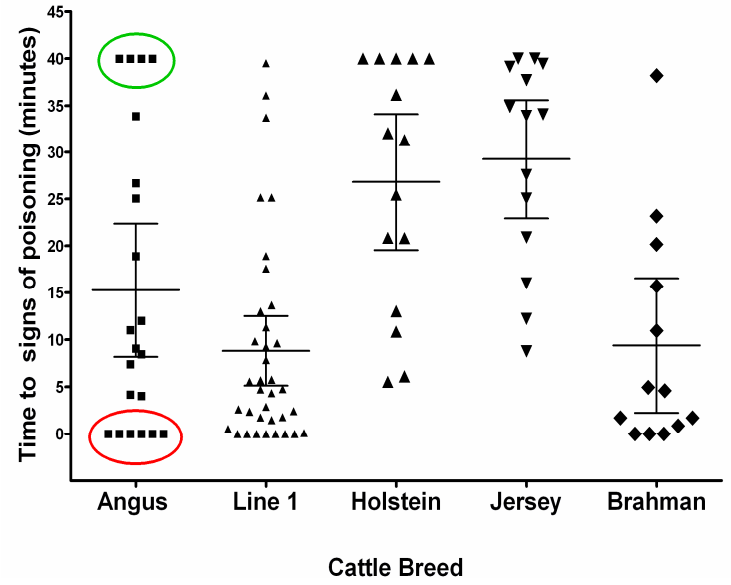


Figure 5. A comparison of different breeds of cattle to larkspur toxicosis. Data represent a scatter plot with the mean and 95% confidence interval for each breed. The data depict the cattle responses to a standardized dose of tall larkspur (8 mg/kg total toxic alkaloid) at 24 h after oral dosing. Line 1 represents Line 1 Herefords from Fort Keogh Livestock and Range Research Laboratory, Miles City, Montana. To determine the time to signs of poisoning, the cattle were exercised behind a tractor at 5–6 kph on a dirt track for 40 min or until they exhibited larkspur-induced muscle weakness. Examples of resistant (**green circle**) and susceptible (**red circle**) animals are depicted for Angus (From [60]).

Genomic analyses allow for the capture of information from larkspur-challenged animals that can be subsequently applied to select for larkspur-resistant cattle. The calves from these selected animals would also be expected to inherit tolerance to larkspur. In order to capture this genetic information, the first aim was to identify regions of the genome harboring genetic variation in larkspur resistance. To accomplish this, we have initially applied a genome wide association study approach based on a large DNA marker assay (BovineHD beadchip assay, over 770,000 markers, Illumina Inc., San Diego, CA, USA). Sixty-eight cattle (21 Angus, 16 Line 1 Hereford, 3 Brahman, 26 Holstein, and 2 Jersey) with divergent phenotypes for larkspur resistance were genotyped with the BovineHD beadchip assay. From these genotyping results, regions across the genome have been identified as significantly associated with larkspur resistance. To further define these regions of interest across the genome, an exome

sequence (gene coding regions of the genome; ~50 million bases) was generated for 32 Angus cattle (21 with BovineHD genotypes). The combination of the genotyping and sequence data allows one to evaluate every base of DNA in the genomic regions of interest and identify possible candidate genes and DNA markers that can be used by the industry to select for larkspur tolerant cattle. These experiments will allow us to work towards development of commercially available DNA markers for larkspur tolerance in cattle.

6. Food Safety

Studies have been performed to characterize the kinetic profile of larkspur alkaloids in cattle [46,61–63]. In these studies, the elimination half-life of MLA from cattle dosed once with dried and finely ground larkspur via oral gavage was approximately 20 h. Additionally, the T_{max}, or time to maximal toxin concentration, was approximately 10 h. These data indicate that cattle that have consumed larkspur will be most susceptible to poisoning 10 h after consumption and that after the cattle have stopped consuming larkspur >99% of the toxins will be eliminated after 6 days. Consequently no food products should be obtained from cattle for at least 7 days after they have been exposed to larkspurs. It is important to note here that these data are for serum only, and not samples such as meat, milk, liver or other biological samples. More complete kinetic studies have been performed in mice wherein the elimination rate of MLA from numerous tissues was determined [15,45]. The results from those studies indicate that serum MLA concentrations are a good representation of body burden for MLA, and that once serum larkspur alkaloid concentrations are zero the alkaloid concentrations in the rest of the tissues are close to zero as well.

7. Management Recommendations

Two critical elements in risk management to reduce cattle losses from larkspur are the amount of alkaloid consumed (*i.e.*, alkaloid concentration and amount of plant eaten) and the rate of ingestion. Both of these factors are encompassed in two major conclusions from numerous field studies: (1) cattle typically eat most larkspur after the plants have begun to flower [55]; and (2) weather conditions (e.g., short-lived storms) may promote rapid consumption of relatively large amounts of larkspur [7,64]. Cattle generally begin consuming tall larkspur after flowering racemes are elongated, and consumption peaks during the pod stage of growth in late summer, when cattle may eat large quantities (25% to 30% of diet as herd average; >60% on some days by individual animals). Toxicity of tall larkspurs declines with maturation, but acceptability to cattle, and the potential rate of ingestion, typically increases as the grazing season advances [32,65]. These conclusions led to the development of the "toxic window" as a management scheme to reduce losses (Figure 6). Grazing during spring before larkspur flowers engenders relatively low risk because consumption is low. Further, later in summer once pods begin to shatter, larkspur-infested rangelands can usually be grazed with impunity because pod toxicity declines rapidly, and leaf toxicity is low.

There is one exception to the observation that cattle begin consuming tall larkspurs after flowering racemes elongate—*Delphinium glaucescens*, which grows in more xeric habitats compared to other tall larkspur species—and this may explain why cattle consume this species before flowering [66]. In

addition, low larkspurs do not show a large decline in toxic alkaloid concentrations over time [24], and acceptability after flowering remains mostly static until the plants senesce [28,67].

Previous studies showed no relationship between concentration of alkaloids and larkspur palatability to cattle [68]. Thus, higher alkaloid concentrations alone appear to be unimportant in altering flavor and reducing short-term consumption by cattle. Although short-term larkspur consumption by cattle is not negatively influenced by high alkaloid concentrations, sub-lethal intoxication and multi-day consumption patterns are partially dependent on toxic alkaloid concentrations. Larkspur ingestion by cattle above a toxic threshold is regulated by post-ingestive consequences in a dose-dependent manner [55,69]. Cattle limit ingestion of larkspur (*i.e.*, dose of toxic alkaloids) so that periods of high consumption (1–2 days) are followed by periods of reduced consumption (3–4 days). This cyclic consumption pattern allows for detoxification after episodes of intoxication [55,69]. Cattle apparently increase consumption because larkspur is very nutritious (e.g., 15%–20% crude protein; [32]), but then decrease consumption due to toxic feedback in a dose-dependent fashion. Cyclic consumption generally enables cattle to regulate larkspur consumption below a subclinical toxic threshold (~8 mg alkaloid/kg; [54,60]), and allows cattle to periodically graze this toxic but nutritious plant. On larkspur-infested rangelands during periods when cattle are eating larkspur, all grazing cattle will consume some quantity of larkspur, yet over 85%–95% of the typical herd will not be fatally poisoned. Our observations also indicate that many cattle deaths occur after brief (~90 min) periods of over-ingestion [32,64], and death likely occurs before negative post-ingestive feedback can act to temporarily reduce larkspur consumption. Further, consumption of smaller amounts of larkspur for several consecutive days may keep animals from exceeding a toxic threshold such that overt symptoms are not evident, but only brief exercise (e.g., running to consume water) is sufficient to push animals over a toxic threshold such that they become recumbent [70]. Thus, cattle may quickly be fatally intoxicated when intensely exercised even in the absence of overt clinical signs [28,67,70]. The observations regarding exercise have been consistent for cattle grazing either tall or low larkspurs.

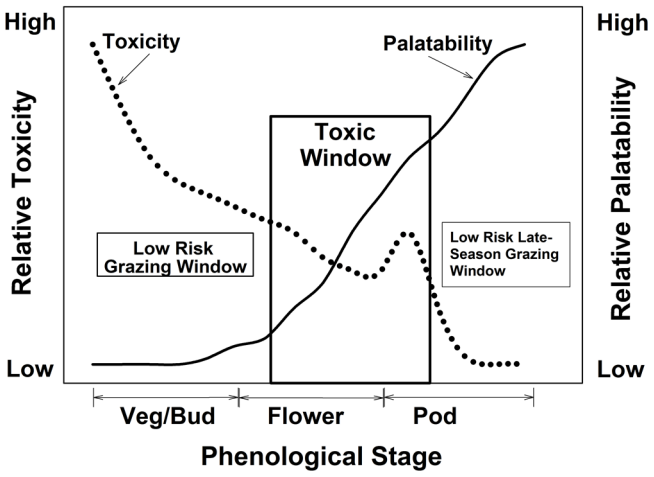


Figure 6. Relationship between toxicity and palatability in tall larkspurs. Most cattle deaths are predicted to occur during the toxic window when the concentration of norditerpenoid alkaloids is substantial and consumption by cattle increases after flowering racemes elongate.

Diagnosis of larkspur poisoning may be difficult particularly under extensive rangeland circumstances; grazing animals are often found dead and the only link to larkspur as the cause may be the presence of the plants in the pasture or analysis of tissues or rumen contents from dead animals (Figure 4). Recently, polymerase chain reaction (PCR) has been used to detect larkspur remnants within the partially-digested rumen matrix [71]; the PCR-based technique appears promising to diagnose larkspur poisoning.

8. Grazing and other Management Options

Delphinium glaucum is the most toxic of the tall larkspurs in western North America, containing the highest levels of the toxic alkaloids MLA and 14-deacetylnudicauline (DAN; [17]. The mean concentration of MLA in *D. glaucum* from California was 19.5 mg/g, compared to 9 mg/g in *D. barbeyi*, and 3.5 mg/g in *D. occidentale* over the summer growing season. Even though the MSAL alkaloid concentration declined throughout the growing season, toxic alkaloid concentrations in *D. glaucum* remained relatively high (*i.e.*, above 3 mg/g) until after seed shatter [72], and ripe pods contained 4.9 mg/g [27]. Therefore, *D. glaucum* appears to remain highly toxic throughout most of the grazing season. However, no grazing studies have been conducted with *D. glaucum* to determine when it is preferred by cattle. Nonetheless, historical losses of cattle have been large where it grows in thick patches [6]. Furthermore, heavy winter snow in the Sierra Mountains may retard summer flowering in *D. glaucum* until late summer, thus extending the toxic window to late August and September [73]. After pods ripen and shatter, toxic alkaloid concentrations decrease rapidly and subsequent risk to cattle is low [23]. Our recommendation is to restrict cattle access to this species throughout the growing season until seed shatter, which typically occurs in late August or September.

Delphinium barbeyi makes up 2%–5% of total plant cover throughout much of its range, but may constitute up to 50% of the total plant cover in areas with large patches. *D. barbeyi* (along with *D. occidentale*) is also one of the most conspicuous species of the tall forb community in snow drift sites, at the edge of wet meadows, and along streams in various mountain habitats (Figure 2). *Delphinium barbeyi* is also a dominant component of the tall forb plant community growing in aspen groves throughout its distribution. Grazing studies with *D. barbeyi* indicate that cattle will not graze it until flowering begins [55]. Thus, we recommend grazing these mountain pastures during the early/late season to avoid the high risk of poisoning during the toxic window from bud elongation to the mature pod stage.

Delphinium occidentale occurs in the northern Rocky Mountains from northern Utah and Colorado into western Wyoming, Idaho, Montana, and throughout the basin and range mountains of Nevada (Figure 2). It grows mostly under aspen and in more mesic areas in sagebrush communities. *Delphinium occidentale* grows in areas where summer drought is common, thus it typically matures and senesces by mid to late summer. However, there are extensive populations of *D. occidentale* in the western U.S. that contain little or no MSAL alkaloids (Figure 3; [19]). The *D. occidentale* populations that contain little or no MSAL alkaloids appear to provide safe and nutritious forage at any stage of growth. However, MLA concentrations in populations of *D. occidentale* in central and southern Idaho generally remain above 4 mg/g until the pods mature and shatter [27,72], and thus pose a moderate risk until late in the grazing season.

Low larkspurs contain varying concentrations of toxic alkaloids, depending on location [24]. Concentrations of MLA in *D. nuttallianum* ranged from 0.8–4.5 mg/g plant, compared to a range of 3–6 mg/g plant for *D. andersonii* [24]. Management to reduce losses due to low larkspur begins with recognition of the plant during spring. Low larkspurs will typically begin growth before other forage. Low larkspur populations (*i.e.*, density) fluctuate with environmental conditions, with cool, wet late winter and spring climatic conditions favoring the growth of dense populations [28]. Risk of losing cattle is much higher during years with dense populations, as cattle typically consume low larkspurs in proportion to the availability of the plant [7]. During periods of high density, deferring grazing for 4–6 weeks until the low larkspur has senesced will virtually eliminate cattle losses.

Sheep can be herded into or bedded onto discreet tall larkspur patches to reduce availability or acceptability to cattle [74]. In other areas where tall larkspur is uniformly spaced over a pasture, sheep must eat immature larkspur and leave sufficient feed for subsequent grazing by cattle. This can be problematic, since early growth tall larkspur may not be palatable to sheep. Our observations [75] indicate that sheep eat little low larkspur (*D. nuttallianum*) unless a high stock density is used.

A variety of remedies have been applied in the field when ranchers find intoxicated animals (e.g., bleeding by cutting the tail), but most are without a solid scientific rationale [76]. Any imagined success with these treatments was probably related to the dose. If a sub-lethal dose was ingested, the animal would likely recover in spite of any treatment, unless bloat or aspiration pneumonia occurred during recumbency. Drugs that increase the acetylcholine concentration in the neuromuscular junction have potential for reversing larkspur toxicity. The cholinergic drugs, physostigmine and neostigmine (0.08 mg/kg i.v.), have been successfully used under field and pen conditions to reverse clinical larkspur intoxication [54,77,78]. This reversal lasts about 2 h, and thus repeated injections of the drug may be required. Under field conditions, physostigmine temporarily abates clinical signs and animals quickly (~15 min) become ambulatory. Depending on the larkspur dose, the intoxication may recur. Consequently, the use of drug therapy may aggravate losses in the absence of further treatment if suddenly ambulatory animals later develop increased muscular fatigue, dyspnea, and death.

Larkspur losses can be reduced greatly if dense larkspur populations are reduced by herbicides. Picloram, metsulfuron, and glyphosate have proven to be effective in killing tall larkspurs when applied at specific growth stages [79]. Such treatments may be efficacious for >15 years. However, caution should be used as these herbicides do not reduce toxic alkaloid concentrations in treated larkspurs, and in fact metsulfuron may increase toxicity. Additionally herbicide application may increase the palatability of larkspurs. Therefore, sprayed areas should not be grazed until the following growing season. Herbicide use as a technique to control low larkspur species should be used with some caution as the number of plants in a population that may be flowering on a given year can vary significantly.

9. Conclusions

Larkspur poisoning in cattle is a serious problem for many livestock producers in the western ranges of North America. Years of research efforts have resulted in the development of numerous management strategies to reduce the number of cattle deaths from larkspurs. Future research efforts will continue to evaluate the role of individual alkaloids and the effects of various mixtures of alkaloids on the potency of the larkspurs. Studies will continue in order to better understand the

distribution of toxic and non-toxic larkspur populations. Additionally, considerable efforts will be made to identify genetic markers of susceptibility and/or resistance to larkspur toxicosis.

Acknowledgments

This work was supported by direct congressional appropriations via the United State Department of Agriculture to the Poisonous Plant Research Laboratory, which is part of the Agricultural Research Service.

Author Contributions

All authors contributed to the writing of this review article.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Kingsbury, J.M. *Poisonous Plants of the United States and Canada*; Prentice-Hall, Inc.: Englewood Cliffs, NJ, USA, 1964.
2. Burrows, G.E.; Tyrl, R.J. *Toxic Plants of North America*; Iowa State University Press: Ames, IA, USA, 2001.
3. Nielsen, D.B.; Ralphs, M.R. Larkspur: Ecconomic considerations. In *The Ecology and Ecconomic Impact of Poisonous Plants on Livestock Production*; James, L.F., Ralphs, M.H., Nielsen, D.B., Eds.; Westview Press: Boulder and London, UK, 1988; pp. 119–129.
4. Nielsen, D.B.; Ralphs, M.H.; Evans, J.O.; Call, C.A. Economic feasibility of controlling tall larkspur on rangelands. *J. Range Manag.* **1994**, *47*, 369–372.
5. Pfister, J.A.; Ralphs, M.H.; Gardner, D.R.; Stegelmeier, B.L.; Manners, G.D.; Panter, K.E.; Lee, S.T. Management of three toxic *Delphinium* species based on alkaloid concentrations. *Biochem. Syst. Ecol.* **2002**, *30*, 129–138.
6. Marsh, C.D.; Clawson, A.B. Larkspur poisoning of livestock. *USDA Bull.* **1916**, *365*, 1−91.
7. Pfister, J.A.; Gardner, D.R.; Panter, K.E.; Manners, G.D.; Ralphs, M.H.; Stegelmeier, B.L.; Schoch, T.K. Larkspur (*Delphinium* spp.) poisoning in livestock. *J. Nat. Toxins* **1999**, *8*, 81–94.
8. Olsen, J.D. Tall larkspur poisoning in cattle and sheep. *J. Am. Vet. Med. Assoc.* **1978**, *173*, 762−765.
9. Panter, K.E.; Manners, G.D.; Stegelmeier, B.L.; Lee, S.; Gardner, D.R.; Ralphs, M.H.; Pfister, J.A.; James, L.F. Larkspur poisoning: Toxicology and alkaloid structure—Activity relationships. *Biochem. Syst. Ecol.* **2002**, *30*, 113–128.
10. Ewan, J. *Synopsis of the North American Species of Delphinium*; University of Colorado: Boulder, CO, USA, 1945.
11. Warnock, M. A taxonomic conspectus of North American Delphinium. *Phytologia* **1995**, *78*, 73–101.
12. Warnock, M. Delphinium. *Flora N. Am. North Mex.* **1997**, *3*, 196–240.

13. Manners, G.D.; Panter, K.E.; Ralphs, M.H.; Pfister, J.A.; Olsen, J.D.; James, L.F. Toxicity and chemical phenology of norditerpenoid alkaloids in the tall larkspurs (*Delphinium* species). *J. Agric. Food Chem.* **1993**, *41*, 96–100.
14. Manners, G.D.; Panter, K.E.; Pelletier, S.W. Structure-activity relationships of norditerpenoid alkaloids occurring in toxic larkspur (*Delphinium*) species. *J. Nat. Prod.* **1995**, *58*, 863–869.
15. Welch, K.D.; Panter, K.E.; Gardner, D.R.; Green, B.T.; Pfister, J.A.; Cook, D.; Stegelmeier, B.L. The effect of 7,8-methylenedioxylycoctonine-type diterpenoid alkaloids on the toxicity of methyllycaconitine in mice. *J. Anim. Sci.* **2008**, *86*, 2761–2770.
16. Welch, K.D.; Green, B.T.; Gardner, D.R.; Cook, D.; Pfister, J.A.; Stegelmeier, B.L.; Panter, K.E.; Davis, T.Z. Influence of 7,8-methylenedioxylycoctonine-type alkaloids on the toxic effects associated with ingestion of tall larkspur (*Delphinium* spp.) in cattle. *Am. J. Vet. Res.* **2010**, *71*, 487–492.
17. Gardner, D.R.; Ralphs, M.H.; Turner, D.L.; Welsh, S.L. Taxonomic implications of diterpene alkaloids in three toxic tall larkspur species (*Delphinium* spp.). *Biochem. Syst. Ecol.* **2002**, *30*, 77–90.
18. Cook, D.; Welch, K.D.; Green, B.T.; Gardner, D.R.; Pfister, J.A.; Constantino, J.R.; Stonecipher, C.A. The relative toxicity of *Delphinium stachydeum* in mice and cattle. *Toxicon* **2015**, *99*, 36–43.
19. Cook, D.; Gardner, D.R.; Pfister, J.A.; Welch, K.D.; Green, B.T.; Lee, S.T. The biogeographical distribution of duncecap larkspur (*Delphinium occidentale*) chemotypes and their potential toxicity. *J. Chem. Ecol.* **2009**, *35*, 643–652.
20. Gardner, D.R.; Pfister, J.A. HPLC-MS analysis of toxic norditerpenoid alkaloids: Refinement of toxicity assessment of low larkspurs (*Delphinium* spp.). *Phytochem. Anal.* **2009**, *20*, 104–113.
21. Cook, D.; Green, B.T.; Welch, K.D.; Gardner, D.R.; Pfister, J.A.; Panter, K.E. Comparison of the toxic effects of two duncecap larkspur (*Delphinium occidentale*) chemotypes in mice and cattle. *Am. J. Vet. Res.* **2011**, *72*, 706–714.
22. Welch, K.D.; Green, B.T.; Gardner, D.R.; Cook, D.; Pfister, J.A.; Panter, K.E. The effect of 7,8-methylenedioxylycoctonine-type diterpenoid alkaloids on the toxicity of tall larkspur (*Delphinium* spp.) in cattle. *J. Anim. Sci.* **2012**, *90*, 2394–2401.
23. Gardner, D.R.; Manners, G.D.; Panter, K.E.; Lee, S.T.; Pfister, J.A. Three new toxic norditerpenoid alkaloids from the low larkspur *Delphinium nuttallianum*. *J. Nat. Prod.* **2000**, *63*, 1127–1130.
24. Gardner, D.R.; Pfister, J.A. Toxic alkaloid concentrations in *Delphinium nuttallianum*, *Delphinium andersonii*, and *Delphinium geyeri* in the intermountain region. *Rangel. Ecol. Manag.* **2007**, *60*, 441–446.
25. Ralphs, M.H.; Gardner, D.R. Alkaloid levels in duncecap (*Delphinium occidentale*) and tall larkspur (*D. barbeyi*) grown in reciprocal gardens: Separating genetic from environmental influences. *Biochem. Syst. Ecol.* **2001**, *29*, 117–124.
26. Ralphs, M.H.; Gardner, D.R. Distribution of norditerpene alkaloids in tall larkspur plant parts through the growing season. *J. Chem. Ecol.* **2003**, *29*, 2013–2021.
27. Gardner, D.R.; Pfister, J.A. Late season toxic alkaloid concentrations in tall larkspur (*Delphinium* spp.). *J. Range Manag.* **2000**, *53*, 329–334.

28. Pfister, J.; Cook, D. Influence of weather on low larkspur (*Delphinium nuttallianum*) density. *J. Agric. Sci.* **2011**, *3*, 36, doi:10.5539/jas.v3n1p36.
29. Saavedra, F.; Inouye, D.W.; Price, M.V.; Harte, J. Changes in flowering and abundance of *Delphinium nuttallianum* (ranunculaceae) in response to a subalpine climate warming experiment. *Glob. Chang. Biol.* **2003**, *9*, 885–894.
30. Cundiff, R.H.; Markunas, P.C. Determination of nicotine, nornicotine, and total alkaloids in tobacco. *Anal. Chem.* **1955**, *27*, 1650–1653.
31. Williams, M.C.; Cronin, E.H. Effect of silvex and 2,4,5-T on alkaloid content of tall larkspur. *Weeds* **1963**, *11*, 317–319.
32. Pfister, J.A.; Manners, G.D.; Ralphs, M.H.; Hong, Z.X.; Lane, M.A. Effects of phenology, site, and rumen fill on tall larkspur consumption by cattle. *J. Range Manag.* **1988**, *41*, 509–514.
33. Manners, G.D.; Pfister, J.A.; Ralphs, M.H.; Panter, K.E.; Olsen, J.D. Larkspur chemistry: Toxic alkaloids in tall larkspurs. *J. Range Manag.* **1992**, *45*, 63–67.
34. Pelletier, S.W.; Dailey, O.D., Jr.; Mody, N.V.; Olsen, J.D. Isolation and structure elucidation of the alkaloids of *Delphinium glaucescens* rybd. *J. Org. Chem.* **1981**, *46*, 3284–3293.
35. Clark, D.; Ralphs, M.; Lamb, R. Total alkaloid determinations in larkspur and lupine with near infrared reflectance spectroscopy. *Agron. J.* **1987**, *79*, 481–485.
36. Gardner, D.R.; Manners, G.D.; Ralphs, M.H.; Pfister, J.A. Quantitative analysis of norditerpenoid alkaloids in larkspur (*Delphinium* spp.) by fourier transform infrared spectroscopy. *Phytochem. Anal.* **1997**, *8*, 55–62.
37. Ralphs, M.H.; Gardner, D.R.; Turner, D.L.; Pfister, J.A.; Thacker, E. Predicting toxicity of tall larkspur (*Delphinium barbeyi*): Measurement of the variation in alkaloid concentration among plants and among years. *J. Chem. Ecol.* **2002**, *28*, 2327–2341.
38. Manners, G.D.; Ralphs, M.H. Capillary gas chromatography of *Delphinium* diterpenoid alkaloids. *J. Chromatogr.* **1989**, *466*, 427–432.
39. Majak, W.; McDiarmid, R.E.; Benn, M.H. Isolation and HPLC determination of methyllycaconitine in a species of low larkspur (*Delphinium nuttallianum*). *J. Agric. Food Chem.* **1987**, *35*, 800–802.
40. Coates, P.A.; Blagbrough, I.S.; Lewis, T.; Potter, B.V.; Rowan, M.G. An HPLC assay for the norditerpenoid alkaloid methyllycaconitine, a potent nicotinic acetylcholine receptor antagonist. *J. Pharm. Biomed. Anal.* **1995**, *13*, 1541–1544.
41. Turek, J.W.; Kang, C.H.; Campbell, J.E.; Arneric, S.P.; Sullivan, J.P. A sensitive technique for the detection of the alpha 7 neuronal nicotinic acetylcholine receptor antagonist, methyllycaconitine, in rat plasma and brain. *J. Neurosci. Methods* **1995**, *61*, 113–118.
42. Gardner, D.R.; Panter, K.E.; Pfister, J.A.; Knight, A.P. Analysis of toxic norditerpenoid alkaloids in *Delphinium* species by electrospray, atmospheric pressure chemical ionization, and sequential tandem mass spectrometry. *J. Agric. Food Chem.* **1999**, *47*, 5049–5058.
43. Wada, K.; Bando, H.; Kawahara, N.; Mori, T.; Murayama, M. Determination and quantitative analysis of alkaloids in *Aconitum japonicum* by liquid chromatography atmospheric pressure chemical ionization mass spectrometry. *Biol. Mass Spectrom.* **1994**, *23*, 97–102.

44. Wada, K.; Mori, T.; Kawahara, N. Stereochemistry of norditerpenoid alkaloids by liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *J. Mass Spectrom.* **2000**, *35*, 432–439.
45. Stegelmeier, B.L.; Hall, J.O.; Gardner, D.R.; Panter, K.E. The toxicity and kinetics of larkspur alkaloid, methyllycaconitine, in mice. *J. Anim. Sci.* **2003**, *81*, 1237–1241.
46. Green, B.T.; Welch, K.D.; Gardner, D.R.; Stegelmeier, B.L.; Pfister, J.A.; Cook, D.; Davis, T.Z. A toxicokinetic comparison of norditerpenoid alkaloids from *Delphinium barbeyi* and *D. glaucescens* in cattle. *J. Appl. Toxicol.* **2011**, *31*, 20–26.
47. Aiyar, V.N.; Benn, M.H.; Hanna, T.; Jacyno, J.; Roth, S.H.; Wilkens, J.L. The principal toxin of *Delphinium brownii* Rydb., and its mode of action. *Experientia* **1979**, *35*, 1367–1368.
48. Benn, M.H.; Jacyno, J.M. The toxicology and pharmacology of diternpenoid alkaloids. In *Alkaloids: Chemical and Biological Perspective*; Pelletier, S.W., Ed.; John Wiley and Sons: New York, NY, USA, 1983; pp. 153–210.
49. Dobelis, P.; Madl, J.E.; Pfister, J.A.; Manners, G.D.; Walrond, J.P. Effects of *Delphinium* alkaloids on neuromuscular transmission. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 538–546.
50. Kukel, C.F.; Jennings, K.R. *Delphinium* alkaloids as inhibitors of alpha-bungarotoxin binding to rat and insect neural membranes. *Can. J. Physiol. Pharmacol.* **1994**, *72*, 104–107.
51. Ward, J.M.; Cockcroft, V.B.; Lunt, G.G.; Smillie, F.S.; Wonnacott, S. Methyllycaconitine: A selective probe for neuronal α-bungarotoxin binding sites. *FEBS Lett.* **1990**, *270*, 45–48.
52. Stegelmeier, B.L.; Panter, K.E.; Pfister, J.A.; James, L.F.; Manners, G.D.; Ralphs, M.R.; Olsen, J.D. Experimental modification of larkspur (*Delphinium* spp.) poisoning in livestock. In *Toxic Plants and Other Natural Toxicants*; Garland, T.R., Barr, A.C., Eds.; CABI: Wallingford, Oxforshire, UK, 1998; pp. 205–210.
53. Panter, K.E.; Welch, K.D.; Gardner, D.R.; Lee, S.T.; Green, B.T.; Pfister, J.A.; Cook, D.; Davis, T.Z.; Stegelmeier, B.L. Poisonous plants of the United States. In *Veterinary Toxicology, Basic and Clinical Principles*, 2nd ed.; Gupta, R.C., Ed.; Elsevier: New York, NY, USA, 2012; pp. 1031–1079.
54. Green, B.T.; Pfister, J.A.; Cook, D.; Welch, K.D.; Stegelmeier, B.L.; Lee, S.T.; Gardner, D.R.; Knoppel, E.L.; Panter, K.E. Effects of larkspur (*Delphinium barbeyi*) on heart rate and electrically evoked electromyographic response of the external anal sphincter in cattle. *Am. J. Vet. Res.* **2009**, *70*, 539–546.
55. Pfister, J.; Provenza, F.; Manners, G.; Gardner, D.; Ralphs, M. Tall larkspur ingestion: Can cattle regulate intake below toxic levels? *J. Chem. Ecol.* **1997**, *23*, 759–777.
56. Welch, K.D.; Green, B.T.; Gardner, D.R.; Cook, D.; Pfister, J.A. The effect of administering multiple doses of tall larkspur (*Delphinium barbeyi*) to cattle. *J. Anim. Sci.* **2015**, in press.
57. Goddard, M.E.; Hayes, B.J. Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nat. Rev. Genet.* **2009**, *10*, 381–391.
58. Welch, K.D.; Green, B.T.; Panter, K.E.; Gardner, D.R.; Pfister, J.A.; Cook, D.; Stegelmeier, B.L. Investigation of the susceptibility of various strains of mice to methyllycaconitine toxicosis. *J. Anim. Sci.* **2009**, *87*, 1558–1564.

59. Welch, K.D.; Green, B.T.; Panter, K.E.; Pfister, J.A.; Gardner, D.R. The role of the α7 subunit of the nicotinic acetylcholine receptor in the acute toxicosis of methyllycaconitine in mice. *J. Appl. Toxicol.* **2013**, *33*, 1011–1016.
60. Green, B.T.; Welch, K.D.; Pfister, J.A.; Chitko-McKown, C.G.; Gardner, D.R.; Panter, K.E. Mitigation of larkspur poisoning on rangelands through the selection of cattle. *Rangelands* **2014**, *36*, 10–15.
61. Green, B.T.; Welch, K.D.; Gardner, D.R.; Stegelmeier, B.L.; Davis, T.Z.; Cook, D.; Lee, S.T.; Pfister, J.A.; Panter, K.E. Serum elimination profiles of methyllycaconitine and deltaline in cattle following oral administration of larkspur (*Delphinium barbeyi*). *Am. J. Vet. Res.* **2009**, *70*, 926–931.
62. Green, B.T.; Welch, K.D.; Gardner, D.R.; Stegelmeier, B.L.; Pfister, J.A.; Cook, D.; Panter, K.E. Toxicokinetics of norditerpenoid alkaloids from low larkspur (*Delphinium andersonii*) orally administered to cattle. *Am. J. Vet. Res.* **2012**, *73*, 1318–1324.
63. Green, B.T.; Welch, K.D.; Gardner, D.R.; Stegelmeier, B.L.; Lee, S.T. A toxicokinetic comparison of two species of low larkspur (*Delphinium* spp.) in cattle. *Res. Vet. Sci.* **2013**, *95*, 612–615.
64. Ralphs, M.H.; Jensen, D.T.; Pfister, J.A.; Nielsen, D.B.; James, L.F. Storms influence cattle to graze larkspur: An observation. *J. Range Manag.* **1994**, *47*, 275–278.
65. Pfister, J.A.; Ralphs, M.H.; Manners, G.D. Cattle grazing tall larkspur on Utah mountain rangeland. *J. Range Manag.* **1988**, *41*, 118–122.
66. Pfister, J.A.; Cook, D.; Gardner, D.R.; Baker, S.D. Early season grazing by cattle of waxy larkspur (*Delphinium glaucescens*) in central Idaho. *Rangelands* **2013**, *35*, 2–5.
67. Pfister, J.A.; Gardner, D.R. Consumption of low larkspur (*Delphinium nuttallianum*) by cattle. *J. Range Manag.* **1999**, *63*, 378–383.
68. Pfister, J.A.; Manners, G.D.; Gardner, D.R.; Price, K.W.; Ralphs, M.H. Influence of alkaloid concentration on acceptability of tall larkspur (*Delphinium* spp.) to cattle and sheep. *J. Chem. Ecol.* **1996**, *22*, 1147–1168.
69. Pfister, J.A.; Provenza, F.D.; Manners, G.D. Ingestion of tall larkspur by cattle: Separating effects of flavor from postingestive consequences. *J. Chem. Ecol.* **1990**, *16*, 1697–1705.
70. Pfister, J.A.; Gardner, D.R.; Stegelmeier, B.L.; Hackett, K.; Secrist, G. Catastrophic cattle loss to low larkspur (*Delphinium nuttallianum*) in Idaho. *Vet. Hum. Toxicol.* **2003**, *45*, 137–139.
71. Cook, D.; Pfister, J.A.; Constantino, J.R.; Roper, J.M.; Gardner, D.R.; Welch, K.D.; Hammond, Z.J.; Green, B.T. Development of a PCR-based method for detection of *Delphinium* species in poisoned cattle. *J. Agric. Food Chem.* **2015**, doi:10.1021/jf5053496.
72. Ralphs, M.H. Toxic alkaloid concentration in tall larkspur species in the western U.S. *J. Range Manag.* **1997**, *50*, 497–502.
73. Chesnut, V.; Wilcox, E. The stock poisoning plants of Montana. *USDA Div. Bot. Bull.* **1901**, *26*, 1−150.
74. Ralphs, M.H.; Olsen, J.D. Comparison of larkspur alkaloid extract and lithium chloride in maintaining cattle aversion to larkspur in the field. *J. Anim. Sci.* **1992**, *70*, 1116–1120.
75. Pfister, J.A.; Gardner, D.R.; Cheney, C.C.; Panter, K.E.; Hall, J.O. The capability of several toxic plants to condition taste aversions in sheep. *Small Rumin. Res.* **2010**, *90*, 114–119.

76. Knight, A.P.; Pfister, J.A. Larkspur poisoning in livestock: Myths and misconceptions. *Rangelands* **1997**, *19*, 10–13.
77. Nation, P.N.; Benn, M.H.; Roth, S.H.; Wilkens, J.L. Clinical signs and studies of the site of action of purified larkspur alkaloid, methyllycaconitine, administered parenterally to calves. *Can. Vet. J.* **1982**, *23*, 264–266.
78. Pfister, J.A.; Panter, K.E.; Manners, G.D.; Cheney, C.D. Reversal of tall larkspur (*Delphinium barbeyi*) poisoning in cattle with physostigmine. *Vet. Hum. Toxicol.* **1994**, *36*, 511–514.
79. Ralphs, M.H.; Evans, J.O.; Dewey, S.A. Timing of herbicide applications for control of larkspurs (*Delphinium* spp.). *Weed Sci.* **1992**, *40*, 264–269.