Fiddleneck (*Amsinckia intermedia* Lehmann Boraginaceae): Toxicity in Cattle Potentiated by Burrow Weed (*Isocoma acradenia*)

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Abstract

All *Amsinckia* species contain pro-toxic dehydropyrrolizidine alkaloids; however, toxicoses of livestock have only been reported from four (i.e., *A. lycopsoides*, Lehm; *A. menziesii*, Lehm; *A. intermedia*, Fisch. & C.A. Mey; and *A. tessellata*, Gray). A recent case of poisoning and death of 15 out of approximately 150 pregnant cows on an Arizona rangeland is reported. Field investigation of the poisoning site implicated fiddleneck (*Amsinckia intermedia*) and burrow weed (*Isocoma acradenia*) as both were present with evidence of being grazed. Necropsy and subsequent histopathology of two dead cows supported the diagnosis of fiddleneck poisoning, likely potentiated by burrow weed ingestion, as the cows had hepatic necrosis with hemorrhage and minimal biliary hyperplasia indicative of pyrrolizidine alkaloid (fiddleneck) poisoning combined with both mycocardial and skeletal muscle necrosis indicative of burrow weed poisoning. Analysis of whole blood of two additional cows for pyrrolizidine alkaloid pyrrole adducts was positive. Chemical characterization of the dehydropyrrolizidine alkaloids identified in the fiddleneck samples collected included the monoester retronecines, lycopsamine and intermedine along with their 7- and 13- acetyl derivatives, and their respective *N*-oxides which collectively comprised 2% of the plant dry weight. While tremetone and other benzofuran ketones totaled 1.25 µg/mg dry plant in the burrow weed.

Key words: *Amsinckia*, burrow weed, *Isocoma*, fiddleneck, hepatotoxicity, myotoxicity

Introduction

Commonly referred to as “tarweeds” or “fiddlenecks” because of their distinctive scorpioid cymes (figure 1), the *Amsinckia* spp. are adapted to a variety of soil types and are common invasive weeds on many western U.S. rangelands (Kingsbury 1964, Burrows and Tyrl 2013). *Amsinckia*-related poisoning involves hepatotoxic effects, including fibrosis of the liver, and associated secondary effects such as jaundice, ascites, and photosensitization. *Amsinckia*-related poisoning has been reported to occur when animals are fed grain screenings contaminated with *Amsinckia* seed (McCulloch 1940a). Clinically, animals have severe jaundice and ascites and may develop photosensitization. Reported microscopic lesions include hepatic necrosis with biliary hyperplasia and fibrosis. If animals survive these changes, they can develop cirrhosis and ultimately liver failure.

Poisoning of pigs, horses, and cattle have been reported, with horses being extremely sensitive, while fowl, sheep, and mules are reported to be quite resistant

Figure 1. *Amsinckia intermedia* (original photo by K.E. Panter) and line drawing of *Amsinckia* showing the scorpioid cymes (original line drawing by Holly Broom-Hyer).

to poisoning from pyrrolizidine alkaloids in general and *Amsinckia* seeds specifically (Cheeke and Shull 1985, Burrows and Tyrl 2013). The disease was first described in horses as "walking disease," characterized by aimless wandering, and reported in the Pacific Northwest in 1925 (Kalkus et al. 1925), although farmers and ranchers had recognized the disease some 30 years or more before that (Kingsbury 1964). Other descriptive terms for the hepatic disease include hard liver disease, Walla Walla hard liver disease, protein poisoning, and winter wheat poisoning (Burrows and Tyrl 2013). The poisoning disease is linked to pyrrolizidine alkaloid-induced hepatotoxicity.

Broadly speaking, the pyrrolizidine alkaloids (PAs) are a diverse class of secondary metabolites comprising two fused 5-membered rings with a nitrogen at a bridgehead. The pro-toxic 1,2-dehydropyrrolizidine alkaloid esters (DHPAs) are a specific subclass that, following in vivo metabolic activation (especially by hepatocytes), are potent alkylators of biomacromolecules, thereby leading to the toxic sequelae. Colegate et al. (2013) reported on the phytochemical analysis of the *Amsinckia intermedia* collected in Arizona and Washington and screened plants beginning at the time of poisoning in this case and monthly thereafter. They determined that the alkaloid levels changed over time, and as the plants matured, the ratio of lycopsamine to its *N*-oxide varied from a low of 0.01 in February to a high of 1.06 in early May.

Until the mid-1900s, when herbicide technology emerged and application practices controlled broad leaf weeds, poisoning in livestock from contaminated grains was common (Cheeke and Shull 1985). With the advent of modernized cultivation and harvesting techniques, as well as current research advances, poisoning in pigs is now rare, and poisoning in horses and cattle is significantly reduced. Nonetheless, poisoning cases continue to occasionally occur and are usually associated with contaminated hay or lack of good-quality forage on the rangeland as presented in this case report.

Burrow weed, jimmy weed, or rayless goldenrod (*Isocoma* spp.) intermittently poison livestock in many areas of the southwestern United States. Poisoning is characterized by muscle weakness that is most often seen as reluctance to move and trembling with forced exertion. Some poisoned animals may develop tachypnea and tachycardia with ascites and hydrothorax. Poisoning generally occurs in late fall or winter when other forages are limited. The burrow weed toxin or one of its metabolites is excreted in the milk causing secondary poisoning of nursing neonates often without apparent maternal toxicity (Davis et al. 2013a,b). Poisoning has been reproduced experimentally, and historically the toxins have been identified as benzofuran ketones, tremetone, hydroxytremetone, and 3-oxyangeloyl-tremetone, and several additional minor compounds (Lee et al. 2009, 2010). However, poisoning is sporadic and often does not seem to be related to the dose of the proposed toxins where tremetone has been present in toxic plant populations of *Isocoma* spp. and *Ageratina altissima* (white snakeroot) (Stegelmeier et al. 2010; Davis et al. 2013a,b, 2016).

In this case report, we describe (1) a recent case of *Amsinckia intermedia*-associated poisoning that may be potentiated by *Isocoma acradenia* of cattle in Arizona, (2) a phytochemical analysis of the *Amsinckia* and *Isocoma* species collected at the site and time of the poisoning outbreak, and (3) a chemical comparison of a second *Amsinckia intermedia* collection in Washington State where the plant is utilized by cattle but not associated with poisoning.

Materials and Methods

Case Overview

A case of 15 dead cows from a herd of approximately 150 was reported to the USDA-ARS Poisonous Plant Research Laboratory (PPRL), Logan, UT, in February 2012. The local veterinarian necropsied two cows, and blood samples were taken from two additional cows.

Cattle were moved to other pastures when the dead cows were discovered, and further losses were not reported. Tissue samples and blood serum samples were submitted to PPRL for histopathological and chemical analysis. Personnel from PPRL went to the range site approximately 25 miles north of Kingman, AZ, to evaluate the pastures. The rangeland where the cows died was surveyed for plant species that could be responsible for the death losses. The range was in poor condition with multiple forb species emerging but very little grass available for grazing. Immature fiddleneck and burrow weed with green leaves had been extensively grazed. Forbs were identified, recorded, and plant specimens collected for voucher filing and chemical analysis. All voucher specimens were filed at the Poisonous Plant Research Laboratory Herbarium for future reference.

Phytochemical Analysis

Amsinckia plant samples collected for chemical analysis were air dried, ground to pass a 5 mm screen, extracted with methanol, and analyzed according to Colegate et al. (2013). The *Isocoma acradenia* samples were also dried, ground, and analyzed as previously described (Lee et al. 2009).

Taxonomic Summary Revision

Plant voucher specimens were sent to Stanley Welsh at the Brigham Young University (BYU) Herbarium for identification. Welsh reported that there is much confusion and misinformation about the classification of the *Amsinckia* species in general. He suggested that a summary revision of the *Amsinckia* species would be a good companion paper to this case report. Therefore, a revision of the taxonomic classification of *Amsinckia* spp. is published as a companion paper to this case report.

Results and Discussion

Case Overview

Fifteen pregnant cows out of a herd of approximately 150 died while grazing on pastures north of Kingman, AZ, in February 2012. The local veterinarian necropsied two cows (case #12-029 and case #12-049) and found yellow discoloration of many serosal and mucosal epithelia and fatty tissues (severe icterus). The liver was firm and yellow, and on cut sections, it had an accentuated lobular pattern. The gall bladder was enlarged and bile filled. These lesions were consistent with DHPA-induced liver disease and provided the impetus for further investigation. Liver and blood serum samples were sent to the USDA-ARS Poisonous Plant Research Laboratory (PPRL) for further analysis. Field investigation revealed poor range conditions and an abundance of fiddleneck (*Amsinckia intermedia*) plants 3 to 6 inches tall. Earlier rains had caused a flush of fiddleneck growth, and there was extensive evidence of grazing of the new lush plants (figure 2). Admixed within the fiddleneck were numerous clusters of burrow weed with young green leaves that also had been grazed.

Figure 2. Grazed *Amsinckia* plants shortly after the cattle had been removed from the range.

Other plants present included cheese weed (*Hymenoclea salsola*), fourwing saltbush (*Atriplex canescens*), Big Galleta (*Hilaria regida*), Arizona popcornflower (*Plagiobothrys arizonicus*), and a few unidentifiable forbs. There was very little grass growing, and what was there had been heavily grazed. It was determined that poor range conditions and green growing fiddleneck was probably the cause of the poisoning. Fiddleneck and burrow weed samples were taken for voucher specimens at the time of the investigation and subsequently by the extension agent. Fiddleneck voucher specimens were collected, pressed, and filed in the Poisonous Plant Research Laboratory Herbarium (voucher #4378 collected February 16, 2012; voucher #4360 collected March 28, 2012; and voucher #4394 collected April 12, 2012). Burrow weed voucher specimens were also collected, identified as *Isocoma acradenia*, and filed with the Poisonous Plant Research Laboratory Herbarium (voucher #s 4426-4428 collected on February 16, 2012). Vouchers were sent to BYU Herbarium in Provo, UT, and Dr. Stanley Welsh confirmed that all were *Amsinckia intermedia* or *Isocoma acradenia*.

Histopathology of the liver samples from case 12-029 revealed severe hepatocellular degeneration and necrosis. Most hepatocytes were swollen with extensive accumulations of intracellular lipid disruption

of hepatic cords. The degenerative hepatocytes were enlarged, and small numbers were fused and formed syncytia. The sinusoids and central veins were dilated and filled with blood. The myocardium had multiple small necrotic foci characterized by myocyte swelling, coagulation, and clumping of myofibers with focal monocytic and lymphocytic inflammation (figure 3). Skeletal muscles also had rare myofiber swelling and hypereosinophilia (figure 4). The second cow (case 12-049) was severely autolytic, which obscured many changes. The skeletal muscle had myofiber necrosis characterized by swelling and hypereosinophilia with myofiber clumping. There was minimal inflammation and increased numbers of nuclei suggestive of regeneration. Similar small foci of degeneration were present in the myocardium. The liver was congested, and the sinusoids were dilated and filled with blood (figure 5). These histologic changes are suggestive of both DHPA and *Isocoma* spp. intoxication.

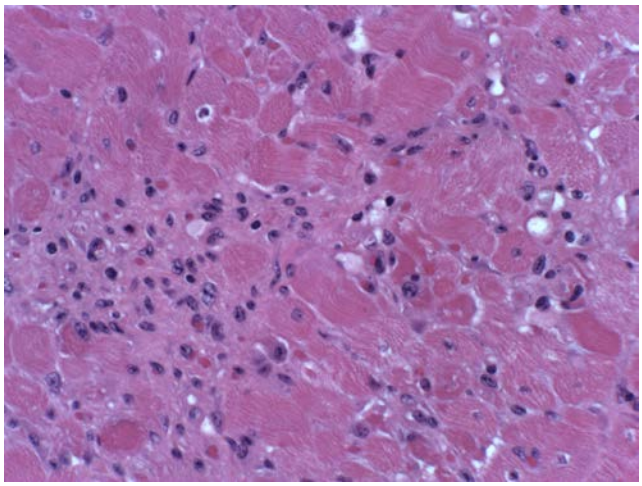


Figure 3. Myocardium from cow 12-029. Notice the focally extensive myocyte degeneration and necrosis seen as myofiber loss of striation, swelling, hypereosinophilia, and coagulation of proteins. The interstitium is expanded with fibrous connective tissue and monocytic inflammation.

Liver samples from both cows and blood samples from case #12-029 and two additional cows were submitted to PPRL for chemical analysis. Liver and blood samples were analyzed for pyrrolizidine alkaloid metabolites (pyrroles) by the method published in Brown et al. (2016) and were detected in all samples, confirming that fiddleneck was likely the primary cause of the poisoning and death losses reported.

Phytochemical Analysis

Previous chemical analyses of the fiddleneck plants from this Arizona case were compared with putatively non-toxic samples from Washington State (Colegate et al. 2013). These fiddleneck species contained the epimers lycopsamine (1) and intermedine (2) (figure 6) mainly present as their *N*-oxides. Minor components were the 7- and 13-acetyl derivatives of 1 and 2 (3 and 4, and 5 and 6, respectively) and the 7,13-diacetyl derivatives of 1 and 2 (7 and 8, respectively), and occasionally a C7 isomer of lycopsamine, tessellatine. As seen in other DHPA-containing plants, there were inconsistent *Amsinckia* alkaloid profiles between specimens purported to be of the same species, population, and phenotype (Molyneux and Johnson 1984). This inconsistency could be due to natural variation, variable degradation due to difference in collection and storage, or taxonomically misassigned specimens. The concentration of total DHPAs (and their *N*-oxides) varied from 1-4-000 µg/g plant leaf material (i.e. 0.0001-0.4% w/dw). As previously

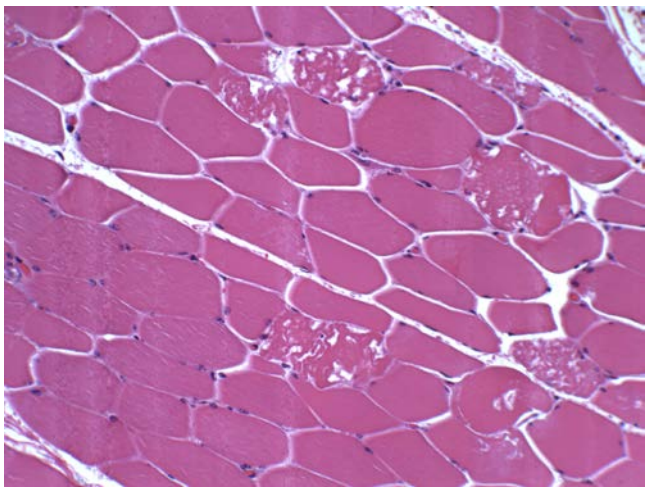


Figure 4. Skeletal muscle from cow 12-029. Notice the focal myofiber swelling hypereosinophilia with coagulation of sarcomere proteins.

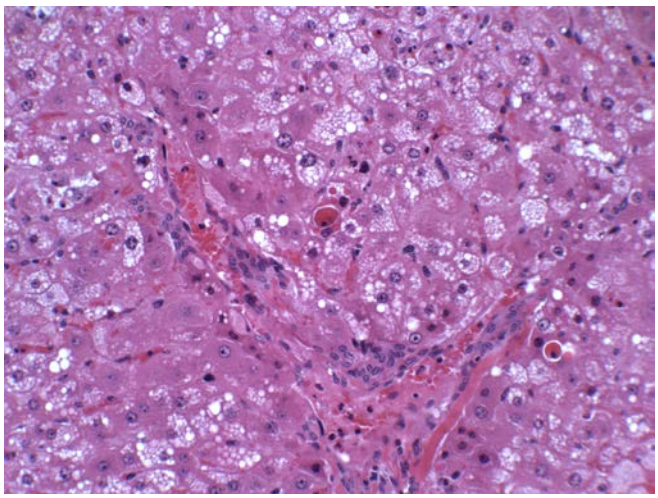
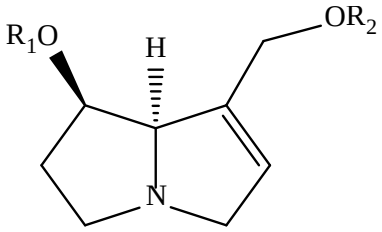
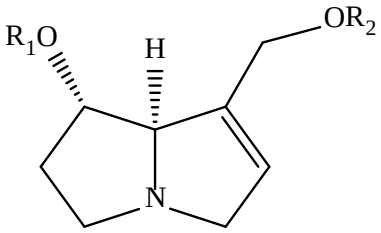


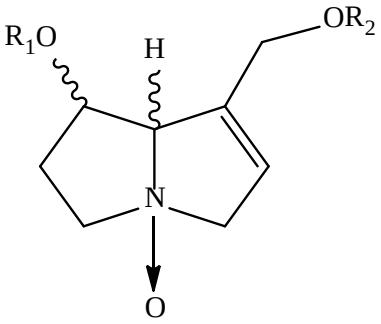
Figure 5. Liver of cow 12-049. Notice the marked hepatocellular swelling and vacuolation with foci of hepatocellular necrosis with residual cellular debris and apoptotic bodies. There is biliary and oval cell proliferation with mild periportal fibrosis with monocytic inflammation.



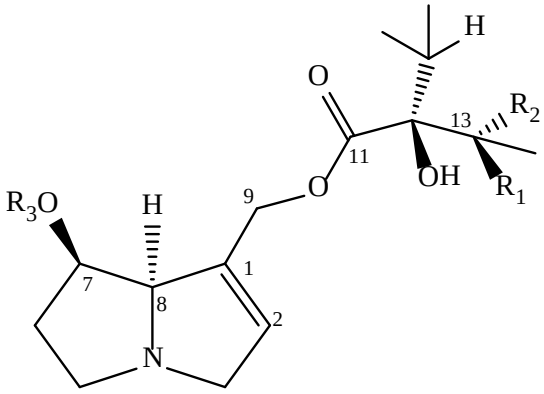
general retronecine



general heliotridine



general dehydropyrrolizidine-*N*-oxide



Compound	R_1	R_2	R_3
lycopsamine (**1**)	H	OH	H
intermedine (**2**)	OH	H	H
7-acetyllycopsamine (**3**)	H	OH	$COCH_3$
13-acetyllycopsamine (**4**)	H	$O(CO)CH_3$	H
7-acetylintermedine (**5**)	OH	H	$COCH_3$
13-acetylintermedine (**6**)	$O(CO)CH_3$	H	H
7,13-diacetyllcopsamine (**7**)	H	$O(CO)CH_3$	$COCH_3$
7,13-diacetyllcopsamine (**8**)	$O(CO)CH_3$	H	$COCH_3$

Figure 6. Chemical **s**tructures of the retronecine base, its N-oxide, and associated dehydropyrrolizidine alkaloids.

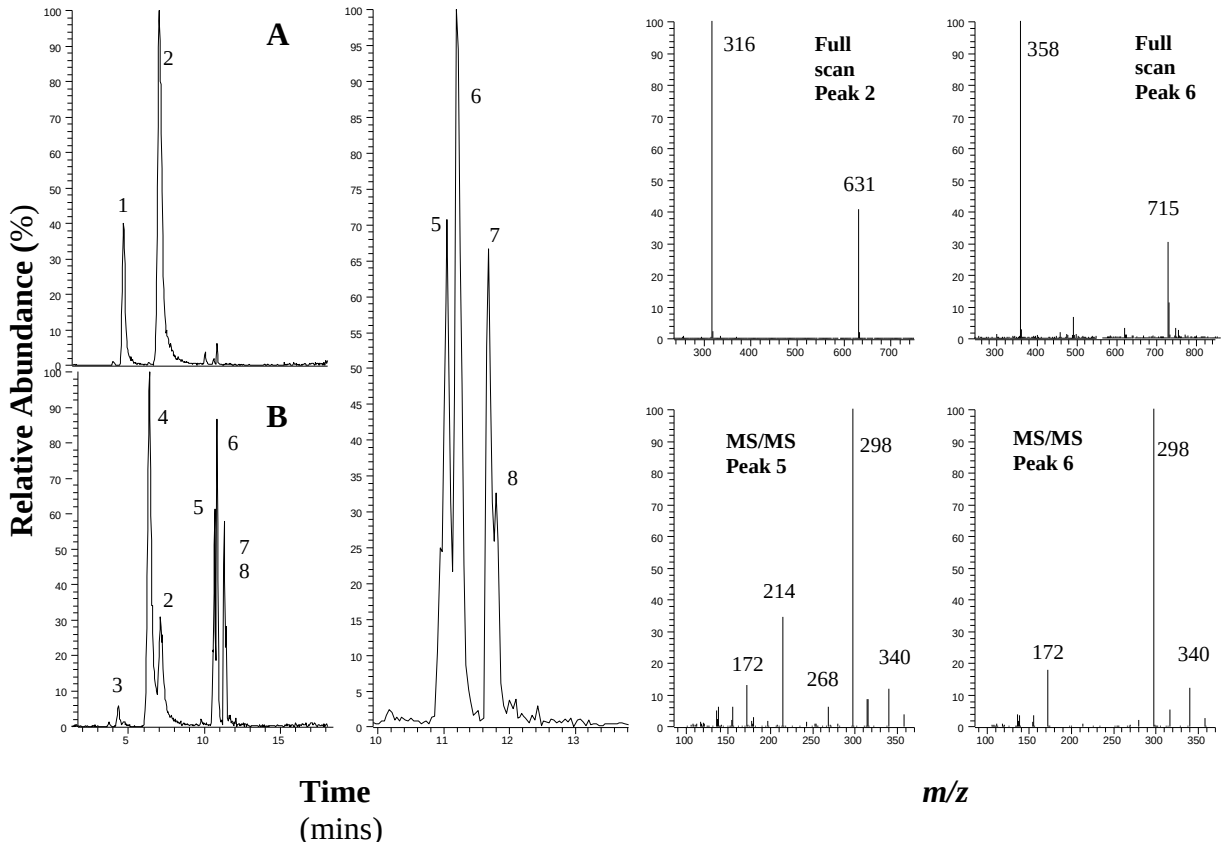


Figure 7. Reversed phase HPLC-esi(+)MS base ion chromatograms of crude methanol extracts of *Amsinckia intermedia* collected from (A) site of cattle poisoning in Kingman, AZ, and (B) one of three sites in Washington State with no record of poisoning. Peak 1 is lycopsamine; peak 2 is lycopsamine-*N*-oxide; peak 3 is intermedine; peak 4 is intermedine-*N*-oxide; peaks 5 and 6 are 7-acetyl and 13-acetylintermedine-*N*-oxides, respectively; and peaks 7 and 8 are the 7,13-diacetyl derivatives of intermedine-*N*-oxide and lycopsamine-*N*-oxide, respectively. Structures are shown in figure 6. Also shown are the full scan mass spectra of peaks 2 and 6 showing the protonated molecule and the significant dimer ion indicative of *N*-oxide structure, and the MS/MS spectra of peaks 5 and 6 showing the significant differences diagnostic of a 7-acetyl and a 13-acetyl derivative at peaks 5 and 6, respectively. *m/z*, mass to charge ratio; MS/MS, tandem mass spectrometry.

reported, *A. intermedia* seeds did not contain DHPAs (Colegate et al. 2013, Johnson et al. 1985). This Arizona *A. intermedia* differed from the other collections as it is primarily the *N*-oxide of lycopsamine (1) with a very minor amount of the 7-acetyl derivative (figures 7 and 8). When compared with other *A. intermedia* collections, the Kingman, AZ samples were about 3-4 times higher than other *A. intermedia* collections; at the time of the poisoning, DHPA concentrations were about 2% DM (Colegate et al. 2013).

Chemical analysis of the burrow weed by high pressure liquid chromatography (HPLC) using the method reported by Lee et al. (2009) showed that the plant material contained 0.32 µg tremetone/mg plant and 0.93 µg dehydrotremetone/mg plant. These concentrations are comparable to those reported (Lee et al. 2015) for other *Isocoma* spp. in the southwestern United States. Historically, tremetone has been reported to be the toxin in *Isocoma* spp., but it has never been experimentally proven. Recent research has demonstrated that the relative toxicity of tremetone-containing plants (*Isocoma* spp. and *Ageratina altissima* known as white snakeroot) does not correlate with tremetone concentrations in the plant (Davis et al. 2016); nor do extracts containing tremetone produce toxicosis (Davis et al. 2015), which has raised some doubt about tremetone being the toxin as previously thought. However, it should be noted that every plant population that has been dosed at PPRL and reported (Davis et al. 2013a,b, 2016), as well as several other species that have been dosed and not yet reported, have contained a significant amount of tremetone.

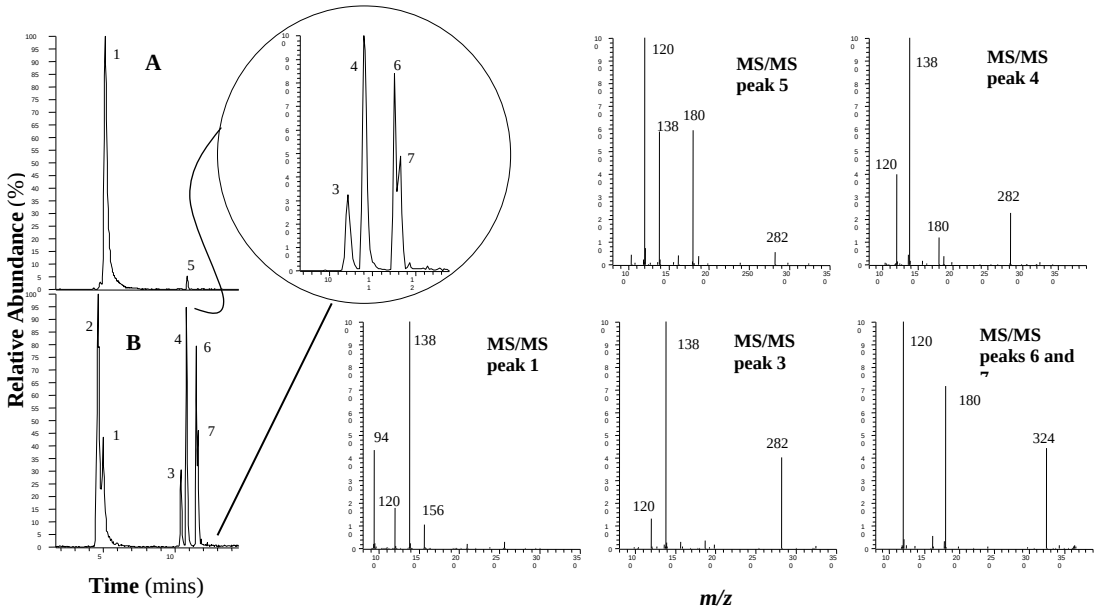


Figure 8. Reversed phase HPLC-esi(+)MS base ion chromatograms of the reduced crude methanol extracts of *Amsinckia intermedia* collected from (A) site of cattle poisoning in Kingman, AZ, and (B) one of three sites in Washington State with no record of poisoning. Peak 1 is lycopsamine; peak 2 is intermedine; peaks 3 and 4 are 7-acetyl and 13-acetylintermedine, respectively; peak 5 is 7-acetyllycopsamine; and peaks 6 and 7 are the 7,13-diacetyl derivatives of intermedine and lycopsamine, respectively. Structures are shown in figure 6. Also shown are the MS/MS spectra of selected peaks. *m/z*, mass to charge ratio; MS/MS, tandem mass spectrometry.

Discussion

The clinical findings, field investigation, and gross and microscopic lesions and chemistry indicate that these cattle were poisoned with both fiddleneck and burrow weed. The DHPA-induced liver disease was the most severe and likely the primary cause of death; however, there are also significant clinical signs and lesions indicative of burrow weed poisoning. As both toxins likely deplete cellular anti-oxidative systems, it is likely that the burrow weed potentiated DHPA toxicity by depleting oxidative preserves such as glutathione and superoxide dismutase. More work is needed to determine if this is the case and better define current management to avoid such potentiated intoxication.

This is the first report of *A. intermedia* poisoning in free-ranging cattle. Previous poisonings were commonly associated with seeds and contaminated grain (McCulloch 1940a,b; Kennedy 1957). Those early reports indicated that seeds contained 0.5% total DHPA and were much more toxic than other plant parts (Fowler and Schoenthal 1967). Neither the *A. intermedia* assay done in 1985 nor that done in 2013 detected DHPAs in the seeds, and the other plant parts had higher but variable DHPA concentrations (Johnson et al. 1985, Colegate et al. 2013). The lack of seed toxicity lends question to earlier studies and the etiology of those previously described diseases such as "walking disease" or "Walla Walla hard liver disease." There are several additional references to *A. intermedia*-contaminated hay poisoning dairy calves. However, this was also a combined intoxication as the hay was reported to contain about 10% *A. intermedia* and 10% *Senecio vulgaris* (Fowler 1968). As *S. vulgaris* contains several DHPAs that are much more toxic than the *Amsinckia* toxins, the *Senecio* probably played a larger role in those poisonings. Both intermedine and lycopsamine have reported LD_{50}s of about 1,500 mg/kg in rats (Fowler and Schoenthal 1967), although cows are more sensitive than rats. Also, these studies were done using a single oral dose, and the toxicity is likely to be higher in more susceptible species such as horses or cattle exposed for longer durations. The *A. intermedia* populations thus far studied have total DHPA concentrations of around 0.5%. Therefore, if poisoning in cattle were to occur, fiddleneck would need to be the bulk of the diet in cattle, and this appeared to be the situation in this case. Furthermore, it may be that *A. intermedia* is only toxic under certain conditions. Similar sporadic poisoning

has been attributed to other DHPA-containing plants. *Echium plantagineum* is known as "Salvation Jane" in parts of Australia and is the only available forage for livestock in some areas. In other areas, it is known as "Patterson's curse" due to its poisoning of animals. More work is needed to determine if *A. intermedia* similarly has two faces. Many different components contribute to toxicity, and those components need to be identified and understood before the underlying risk can be determined and strategies to avoid poisoning can be devised. In this report, we propose yet another factor as combined intoxications seem to be important. Concurrent burrow weed poisoning seems likely to have potentiated fiddleneck poisoning in these cattle.

Conclusion and Implications

The evidence and subsequent research confirmed that the cattle poisoning in this case was caused by *Amsinckia intermedia*-induced liver failure, potentiated by *Isocoma acradenia*-induced myocardial and skeletal muscle necrosis. This is the first documented incident of *A. intermedia* poisoning in free-ranging cattle. Certainly, the underlying cause was poor range conditions and lack of adequate quality forage for these cattle. This was partially due to drought conditions, but cattle cannot be expected to thrive or even survive under such range conditions without a poor outcome. Too often this is a common pattern in large livestock losses from poisonous plants. Having said this, and notwithstanding any differences in rangeland character or grazing/livestock management practices, the quantitative difference or the difference in profiles of the DHPA suites for the Arizona and Washington State plant collections (figure 9) could both play a part in apparent differential toxicity. Additional research on *Amsinckia* toxicoses is needed, but perhaps more importantly is the lack of research on the effects of ingestion of multiple plants and the combined intoxications. More research is definitely needed in this area to better define risk, avoid poisoning, and ensure animal health.

Acknowledgments

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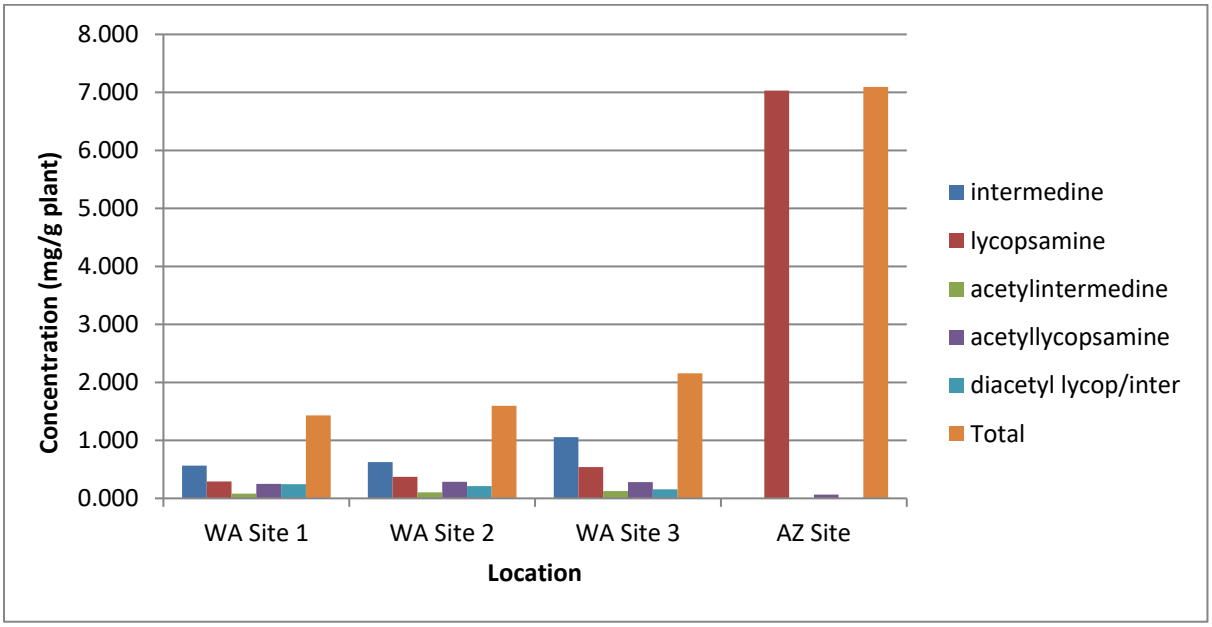

Figure 9. Relative concentrations of dehydropyrrolizidine alkaloids from extracts of *Amsinckia intermedia* collected from three sites in Washington State (WA), USA, where livestock poisonings have not been reported, and the range in Arizona (AZ), USA, where the cattle poisoning in this case occurred. The phenological stage of plant development from the Washington State plants and the Arizona plants was not the same.

References

Brown AW, Stegelmeier BL, Colegate SM, et al. 2016. The comparative toxicity of a reduced, crude comfrey (*Symphytum officinale*) alkaloid extract and the pure comfrey derived pyrrolizidine alkaloids lycopsamine and intermedine in chicks (*Gallus gallus domestica*). *Journal of Applied Toxicology* 36(5):716-725.

Burrows GC and Tyrl RJ. 2013. *Toxic Plants of North America*, 2nd edition, pp. 267-269. John Wiley and Sons, Inc., Ames, IA.

Cheeke PR and Shull LR. 1985. *Toxicants in Feeds and Poisonous Plants*, pp. 100-106. AVI Publishing Company, Inc., Westport, CT.

Colegate SM, Gardner DR, Davis TZ, et al. 2013. Identification of a lycopsamine-*N*-oxide chemotype of *Amsinckia intermedia*. *Biochemical Systematics and Ecology* 48:132-135.

Davis TZ, Green BT, Stegelmeier BL, et al. 2013a. Physiological and serum biochemical changes associated with rayless goldenrod (*Isocoma pluriflora*) poisoning in goats. *Toxicon* 76:247-254.

Davis TZ, Lee ST, Collett MG, et al. 2015. Toxicity of white snakeroot (*Ageratina altissima*) and chemical extracts of white snakeroot in goats. *Journal of Agricultural Food and Chemistry* 63(7):2092-2097.

Davis TZ, Stegelmeier BL, Lee ST, et al. 2013b. Experimental rayless goldenrod (*Isocoma pluriflora*) toxicosis in horses. *Toxicon* 73:88-95.

Davis TZ, Stegelmeier BL, Lee ST, et al. 2016. White snakeroot poisoning in goats: variations in toxicity with different plant chemotypes. *Research in Veterinary Science* 106:29-36.

Fowler ME. 1968. Pyrrolizidine alkaloid poisoning in calves. *Journal of the American Veterinary Medical Association* 152:1131-1137.

Fowler ME and Schoenthal R. 1967. Toxicity of pyrrolizidine alkaloids in *Amsinckia intermedia*. *Journal of the American Veterinary Medical Association* 150:1305-1306.

Johnson AE, Molyneux RJ, and Merrill GB. 1985. Chemistry of toxic range plants. Variation in pyrrolizidine alkaloid content of *Senecio, Amsinckia,* and *Crotalaria* species. *Journal of Agricultural and Food Chemistry* 33:50-55.

Kalkus JW, Trippere HA, and Fuller KR. 1925. Enzootic hepatic cirrhosis of horses (walking disease) in the Pacific Northwest. *Journal of the American Veterinary Medical Association* 68:285-298.

Kennedy PC. 1957. Symposium of Poisoning-Part 2. Case 16-Tarweed poisoning in swine. *Journal of the American Veterinary Medical Association* 130:305-306.

Kingsbury JM. 1964. *Poisonous Plants of the United States and Canada*, pp. 271-274. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Lee ST, Cook D, Davis TZ, et al. 2015. A survey of tremetone, dehydrotremetone, and structurally related compounds in *Isocoma* spp. (Goldenbush) in the southwestern United States. *Journal of Agricultural and Food Chemistry* 63:872-879.

Lee ST, Davis TZ, Gardner DR, et al. 2009. A qualitative method for the measurement of three benzofuran ketones in rayless goldenrod (*Isocoma pluriflora*) and white snakeroot (*Ageratina altissima*) by high-performance liquid chromatography (HPLC). *Journal of Agricultural Food and Chemistry* 57(12):5639-5642.

Lee ST, Davis TZ, Gardner DR, et al. 2010. Tremetone and structurally related compounds in white snakeroot (*Ageratina altissima*): A plant associated with trembles and mild sickness. *Journal of Agricultural Food and Chemistry* 58(15):8560-8565.

McCulloch EC. 1940a. Hepatic cirrhosis of horses, swine, and cattle due to the ingestion of seeds of the tarweed, *Amsinckia intermedia*. *Journal of the American Veterinary Medical Association* 96:5-18.

McCulloch EC. 1940b. Nutlets of *Amsinckia intermedia* toxic to swine, horses and cattle. *Madrono* 5:202-203.

Molyneux RJ and Johnson AE. 1984. Extraordinary levels of production of pyrrolizidine alkaloids in *Senecio riddellii*. *Journal of Natural Products* 47:1030-1032.

Stegelmeier BL, Davis TZ, Green BT, et al. 2010. Experimental rayless goldenrod (*Isocoma pluriflora*) toxicoses in goats. *Journal of Veterinary Diagnostic Investigation* 22:570-577.

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