The comparative toxicity of *Isocoma* species in calves

T. Zane Davis*, Benedict T. Green, Bryan L. Stegelmeier, Stephen T. Lee

U.S. Department of Agriculture-Agricultural Research Service, Poisonous Plant Research Laboratory, Logan, UT, USA

**ABSTRACT**

*Isocoma pluriflora* and *Isocoma acradenia* are toxic plants that contain the putative toxin tremetone. It is common for *I. pluriflora* to poison livestock in the southwestern United States. *I. acradenia* has been suspected of poisoning livestock but its toxicity has not been confirmed by association with clinical poisonings or experimental studies. Jersey calves dosed with *I. pluriflora* and *I. acradenia* for nine days developed “trembles” characterized by skeletal muscle degeneration and necrosis and large increases in serum creatine kinase activity. This is the first report of *I. acradenia* toxicity in an animal model. This study also demonstrates that *I. pluriflora* remains toxic even though tremetone concentrations in the plant were low due to storage of the plant for over five years. Thus, supporting recent research which indicates that another toxin in the plant may be responsible for, or at least contributes to causing “trembles” in livestock.

**Keywords:**
*Isocoma pluriflora*
*Isocoma acradenia*
Cattle
Toxicity
Tremetone

---

1. Introduction

*Isocoma* spp. are part of the Asteraceae family and are commonly found growing in riparian zones along river valleys, drainage areas, or dry plains in the southwestern United States and Mexico (Neesom, 1991). Of the 16 *Isocoma* spp. only *I. pluriflora* (rayless goldenrod) is widely reported to be toxic. *I. pluriflora* is a perennial shrub with yellow flowers at the top of woody stems reported to cause livestock losses in Arizona, Colorado, New Mexico, and Texas (Kingsbury, 1964). Tremetone has been suggested to be the toxin in rayless goldenrod (Couch, 1930; Bonner et al., 1961; Bonner and Degraw, 1962); however, purified tremetone has not been definitively demonstrated to be myotoxic in any animal or biologic model.

*I. acradenia* is commonly referred to in the southwestern United States as goldenbush and is commonly found in California, Nevada, Arizona, and Mexico. The plant has nearly the same appearance as *I. pluriflora* however the leaves are more serrated. Lee et al. (2015) reported that *I. acradenia* contained tremetone and other benzofuran ketones.

Original research to understand the toxicity of rayless goldenrod was undertaken in the early 1900s when “alkali disease”, also referred to as “trembles” was observed in cattle and horses in the Pecos Valley of southwestern Texas (Marsh and Roe, 1921). The cattle and horses became sick after ingesting *I. pluriflora* for several days to weeks. The disease was characterized by violent trembling when the animals were forced to move or when they became agitated. In 1930 Couch (1930) reported the isolation of a dark tar-like substance from rayless goldenrod and named it tremetol, as he had previously done for white snakeroot (Couch, 1927). Tremetol was later shown to be a mixture of many compounds including tremetone, dehydrotremetone, 6-hydroxytremetone and other compounds (Bonner et al., 1961; Bonner and Degraw, 1962).

Intoxications by rayless goldenrod usually occur when animals eat 1–2% of their body weight over a period of several days to weeks (Davis et al., 2013a, 2013b; Kingsbury, 1964). In arid regions, poisoning by rayless goldenrod usually occurs during fall when other forages have been consumed or in late winter and early spring when snow covers other forages and the taller rayless goldenrod is still readily available. Intoxications by rayless goldenrod are sporadic and difficult to predict. When livestock are poisoned, they become depressed, reluctant to eat, and inactive due to muscle soreness. Initial signs are followed by fine muscle tremors of the nose, flanks, and legs especially following exercise or when forced to move. Affected animals will often have tachycardia, tachycardia, a stiff gait, and altered or posted posture as they stand in an arched-back position. Nursing young often show signs of poisoning before the lactating dam as the toxin or its metabolites are excreted in milk (Panter and James, 1990).

Limited research has been performed to determine the toxicity of most *Isocoma* spp. *I. coronopifolia* and *I. tenuisecta* were associated with an incident that was identified as “trembles” in cattle, but their toxicity was never confirmed in controlled animal studies (Buehrer et al., 1939). Several cases of suspected poisoning of cattle by *I. acradenia* in Arizona...
in 2014 and 2015 were reported to the USDA ARS Poisonous Plant Research Laboratory. After conducting investigations and chemical analyses of many plants, Lee et al. (2015) reported that *I. acradenia*, *I. tenuisecta*, *I. asteca*, and other *Isocoma* spp. contained tremetone, dehydrotremetone, 3-oxangeloyl-tremetone as well as, other structurally related compounds. The purpose of this study was to determine if *I. acradenia* collected in Arizona from a pasture with suspected intoxications of cattle could be demonstrated to be toxic to cattle. Toxic *I. pluriflora*, collected in Pecos, Texas, was also dosed to cattle as a comparison and as a positive control.

2. Materials and methods

2.1. Plant identification and material

*Isocoma pluriflora* was collected within the Pecos city limits, near Interstate 20 in Reeves County, Texas on May 17, 2010 at 06 42.656’ N/ 34’ 74.847’ E. *Isocoma acradenia* was collected near Mesa, Arizona in Final County on February 26, 2014 at 33’18.325′ N/111’35.301’ W. The plant material was taxonomically identified as *I. pluriflora* and *I. acradenia* by the staff at the Intermountain Herbarium at Utah State University. Voucher specimens, accession nos. 4191 and 4543, were retained at the Poisonous Plant Research Laboratory Herbarium, Logan, Utah. The plant material was air-dried and one day before beginning the study it was ground to pass through a 2.38 mm screen and mixed using a Gehl Mix-All, model 55°.

2.2. Plant analysis - benzofuran ketone extraction and analysis

Concentrations of the benzofuran ketone compounds (tremetone, dehydrotremetone, 6-hydroxytremetone, and 3-oxangeloyl-tremetone) were determined using the procedure reported in Lee et al. (2009). Briefly, dry ground aerial plant material was extracted for 16 h by mechanical rotation with hexane:ethyl acetate (70:30 v:v). The samples were centrifuged for 5 min and 1 mL was transferred into 1 mL autosampler vials for HPLC analysis.

Analytical scale reversed phase HPLC was performed on a Shimadzu LC-20AT equipped with an autosampler and PDA detector from the same vendor and a 100 mm x 2 mm i.d., 5 μm, Betasil C-18 column. Samples (10 μL) in hexane:ethyl acetate (70:30 v:v) were injected on to the column and eluted with a 20 mM ammonium acetate-acetonitrile mobile phase at a flow rate of 0.4 mL/min. The mobile phase program was 20 mM ammonium acetate-acetonitrile, 65:35 v:v for 4 min followed by a linear gradient to a composition of 65% acetonitrile at 20 min. At 21 min the composition was increased to 100% acetonitrile for 5 min. Detection of analytes in the eluant was performed at λ = 280 nm. Under these conditions tremetone, dehydrotremetone, and 3-oxangeloyl-tremetone eluted at 9.3, 12.7, and 15.3 min, respectively. These compounds were quantified against a seven-point standard curve using previously isolated compounds. The standard curve was prepared in hexane:ethyl acetate (70:30) over the range of 1.56 μg/mL – 100.0 μg/mL by serial dilution.

2.3. Animal studies

All animal work was performed under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee (IACUC #2442). Eighteen, weaned Jersey bull calves in average body condition with mean body weights of 99.8 ± 9.7 kg were obtained from the same herd in Twin Falls, Idaho. The calves were randomly divided into three groups with six animals per group. The calves were trained to move around a track that was approximately 40 m long and oval shaped for 10 min each day for three days before the start of the study and during (days 1, 3, 5, 7, and 9) the study approximately 30 min after collecting serum and blood samples. The day before the initial dosing, all animals were weighed and bled by jugular venipuncture. The calculated dose was split in half and administered at 0600 and 1600 h each day by intra-ruminal gavage BUD using a Frick speculum and a 1.5 cm plastic Tygon tube inserted through the pharynx and esophagus. The dried and finely ground treatment dose (2% of body weight (BW) in approximately 1.5 L water) was pumped with a bilge pump into the rumen of the calves for 9 days. The control group was given similar doses of ground, grass hay using the same method. During the entire study the calves had access to water and long stem alfalfa hay ad libitum.

2.4. Serum analyses

Serum biochemistry and electrolyte analyses were performed on serum collected on dosing days 1, 3, 5, 7, and 9. The analytes including lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine kinase (CK) and other serum markers were analyzed using standard techniques with an automated biochemistry analyzer (Hitachi 7180, Hitachi High Technologies Inc., Pleasanton, CA). Manufacturer recommended reagents and methodology were used.

2.5. Collection of tissues

After dosing the calves for 9 days, all the animals were humanely euthanized and necropsied. At necropsy, samples of brain, heart, lung, liver, pancreas, kidney, thyroid, adrenal rumen, duodenum, jejunum, ileum, cecum, colon, urinary bladder and numerous skeletal muscles were collected. After the heart was fixed, small 1 x 0.5 x 2 cm samples of left ventricle free wall, left ventricular papillary muscle, interventricular septum, right ventricular free wall, right and left atrium were trimmed and processed for microscopic evaluation. At necropsy, similar samples of muscle from the right retro-ocular abductor, tongue, masseter, cricoid arytenoid, anterior superficial pectoral, intercostal, diaphragm, longissimus dorsi, psosas major, triceps brachii, biceps brachii, quadriceps femoris, biceps femoris, gluteus medius, adductor magnus, semitendinosus, semimembranosus (optimally 3 x 5 x 20 mm) were stapled to wooden tongue depressors and fixed by immersion in 10% neutral buffered formalin. Microscopic slides including both transverse and longitudinal sections of muscle were prepared and stained with hematoxylin and eosin and all slides were examined. The skeletal muscle lesions were scored by severity and distribution (percentage of tissue affected) and graded by the severity of lesions: 0 = none, 1 = mild (sarcoplasmic clumping with myocyte swelling affecting 1–5% of myocytes), 2 = moderate (sarcomere disruption with focal monoyctic inflammation and mild nuclear proliferation affecting 6–45% of myocytes), and 3 = severe (extensive inflammation, regeneration with fibrosis affecting greater than 45% of myocytes).

2.6. Statistical analyses

All results from the current study were analyzed as a completely random design with 3 treatments. Mean histology scores and select serum biochemistry analytes were compared using a comparison of variance and significance was determined to be 0.05. The significantly different means were identified using Tukey’s separation (proc GLM SAS, Cary NC USA).

3. Results

3.1. Dose determination

The concentrations of the benzofuran ketones; tremetone, dehydrotremetone, and 3-oxangeloyl-tremetone in *I. pluriflora* and *I. acradenia* are shown Table 1. The dosed *I. pluriflora* had been collected and stored in the dark for 5 years in a cool, dry room and over 90% of the tremetone had degraded and was no longer present in the plant dried plant.
Isocoma acradenia, or Isocoma pluriflora Jersey calves dosed intra-ruminally at 2% body weight with grass hay (control), groups were determined using Tukey significantly different means (P < 0.05). The calves were weak and exhibited fine trembling that was evident. Clinically, treated calves were reluctant to stand and move. When palpated, the appendicular muscles were swollen and firm and seemed to be painful to the touch. The I. acradenia treated calves were more severely affected than the I. acradenia treated calves.

3.2. Clinical signs

After 6 or 7 days of dosing, four of the six calves in both the I. acradenia and I. pluriflora treated groups became obviously intoxicated. Clinically, treated calves were reluctant to stand and move. When forced to move, the affected calves did so slowly and with a stilted, post-like gait. The calves were weak and exhibited fine trembling that was most pronounced when the large appendicular muscles of the legs flexed to carry weight. When palpated, the appendicular muscles were swollen and firm and seemed to be painful to the touch. The I. pluriflora treated calves were more severely affected than the I. acradenia treated calves.

3.3. Serum enzyme changes

The collected serum was analyzed and compared statistically. The serum biochemical changes were most remarkable on Day 7 and those animals had remarkable changes suggestive of skeletal muscle degeneration and necrosis (marked increases in creatinine kinase). However, not all animals were affected, therefore there was considerable variation within groups. Consequently, except for serum creatine kinase activities, there were no differences between the mean values of serum markers of the different Isocoma treatments. However, individual, severely poisoned calves often had remarkable changes especially in creatinine kinase activity. For example, C17, a calf treated with I. pluriflora, had creatine kinase activities of 30,631 IU/L. This calf also had other changes of systemic disease including hypoglycemia and lipid changes with increases in alkaline phosphatase, lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase activities (Table 2).

3.4. Gross and histological lesions

At necropsy the clinically affected animals had skeletal muscle swelling and patches of palor in many muscles. Histologically the lesions and changes in muscles were scored and reported in Table 3. The primary microscopic change of Isocoma intoxication was necrotizing skeletal muscle degeneration and necrosis. This was characterized by multifocal to focally severe and extensive myocyte swelling, hyperesinophilic and loss of striation that progresses to coagulation of sarcoplasmic proteins often forming bands and clumps. There was nuclear proliferation with rowing of nuclei on the margins of damaged myocytes. The damaged proteins often clumped in the myocytes and when they broke up, they were ingested by focally intensive infiltrates of macrophages. In extensive lesions there was multifocal mineralization. There was some treatment related variation in the muscles affected (Figs. 1 and 2). The top three affected muscles for I. pluriflora treated calves were the 1) intercostal and diaphragm, 2) anterior superficial pectoral, and 3) gluteus medius, biceps brachii, and longissimus dorsi. The top three affected muscle groups for the I. acradenia treated calves were 1) anterior superficial pectoral, 2) biceps brachii, and 3) lateral cricoid arynoid.

The myonecrosis in both treated groups was polyphasic as the same muscles had lesions of differing duration. As seen in Fig. 2, lesions within the same anterior superficial pectoral muscle had areas of acute swelling and degeneration (panel A). Other adjacent muscle bundles had more acute zones of necrosis (panel B). And still others were more chronic with myocyte nuclear proliferation, extensive necrosis and inflammation and multifocal punctate myofibrile mineralization (panel C).

Both the I. acradenia and I. pluriflora treated calves had significantly

Table 1

| Plant Species Compound Concentration (μg/mg of dry weight) | Tremetone | Dehydrotremetone | 3-Oxyangelyol-tremetone |
|----------------------------------------------------------|-----------|------------------|------------------------|
| I. pluriflora                                             | 0.063 ± 0.009 | 0.063 ± 0.011 | 1.2 ± 0.2              |
| I. acradenia                                             | 1.5 ± 0.1     | 0.51 ± 0.04     | 0.84 ± 0.07            |

Table 2

Selected serum biochemistries (means and standard deviations) for 12-week-old Jersey calves dosed intra-ruminally at 2% body weight with grass hay (control), Isocoma acradenia, or Isocoma pluriflora for 7 days.

| Analyte                  | Control | I. acradenia | I. pluriflora | I. pluriflora C17* |
|--------------------------|---------|--------------|---------------|-------------------|
| Glucose (mg/dL)          | 61.5 ± 8.7 | 43.2 ± 20.5   | 38.0 ± 24.6   | 5                 |
| Creatinine (mg/dL)       | 0.6 ± 0.1 | 0.6 ± 0.1     | 0.6 ± 0.2     | 0.5               |
| Phosphorus (mg/dL)       | 6.5 ± 0.9 | 5.7 ± 0.8     | 5.9 ± 0.8     | 6.8               |
| Total Protein (g/dL)     | 6.9 ± 0.5 | 6.6 ± 0.4     | 6.6 ± 0.7     | 5.5               |
| Albumin (g/dL)           | 2.7 ± 0.1 | 2.5 ± 0.2     | 2.6 ± 0.3     | 2.2               |
| Cholesterol (mg/dL)      | 70.2 ± 7.9 | 72.4 ± 15.9   | 87.0 ± 18.9   | 63                |
| Bilirubin (mg/dL)        | 0.2 ± 0.1 | 0.4 ± 0.3     | 0.3 ± 0.2     | 0.6               |
| Alkaline phosphatase (IU/L) | 134 ± 13 | 157 ± 102    | 98 ± 30       | 80                |
| Lactate dehydrogenase (IU/L) | 1198 ± 13 | 1504 ± 381   | 1998 ± 1214   | 3325              |
| Alanine aminotransferase (IU/L) | 29 ± 13  | 45 ± 17     | 162 ± 182     | 461               |
| Aspartate aminotransferase (IU/L) | 95 ± 28  | 236 ± 155   | 1008 ± 1489   | 3519              |
| Creatinine Kinase (IU/L) | 447 ± 68 | 3386 ± 2337* | 11,523 ± 1163d | 30,631            |

Notes:
Significantly different means (P < 0.05) from the control group and the dosed groups were determined using Tukey’s separation and are noted with a lower case letter (a & b).

* C17 is a calf, treated with I. pluriflora, that had developed severe clinical myopathy.

Table 3

Means (±SD) of cumulative histologic scores for 12-week-old Jersey calves intraruminally dosed with 2% body weight (ground grass hay (control), Isocoma acradenia and Isocoma pluriflora) for 9 days.

| Animal  | Mean Histology Score | Affected Animal Score | Skeletal Muscles with Severe Myonecrosis |
|---------|----------------------|-----------------------|----------------------------------------|
| control | 9.0 ± 4.5            | NA                    | 0                                      |
| I. acradenia | 17.3 ± 4.5        | 20.0 ± 1.6             | 17                                     |
| I. pluriflora | 18.0 ± 8.6       | 23.0 ± 4.8             | 26                                     |

* Lesions were subjectively scored by severity and distribution (percentage of tissue affected): 0 = none, 1 = mild (sarcoplasmic clumping with myocyte swelling affecting 1–5% of myocytes), 2 = moderate (sarcosome disruption with focal monoyctic inflammation and mild nuclear proliferation affecting 6–45% of myocytes), and 3 = severe (extensive inflammation, regeneration with fibrosis affecting greater than 45% of myocytes). Many calves had mild to moderate pneumonia which likely contributed to background myonecrosis in the control calves.

Significantly different means (P < 0.05) from the controls were determined using Tukey’s separation.

The total number of skeletal muscles severely affected for the 6 calves in the group from which 17 different muscles from each animal were subjectively assessed.
higher skeletal muscle scores than controls (Table 1). However, there was no difference between the Isocoma dosed groups even though the I. acradenia treated calves tended to have higher scores and more muscles severely affected. Both the I. acradenia and I. pluriflora groups had two animals that did not respond to the treatment. These calves had small, rare background muscle lesions that were similar to those of the control calves.

One I. acradenia treated calf and one I. pluriflora treated calf had moderate myocardial degeneration (Fig. 3). These were characterized by myofiber swelling, hyper eosinophilia and loss of striation. Occasionally the affected myocytes were vacuolated with fragmentation and clumping of sarcomere proteins and pyknotic nuclei. Many of the calves in all three groups had background pulmonary lesions that varied from diffuse interstitial pneumonia to focal supplicative bronchopneumonia. There was no correlation between the pulmonary lesions and the severity of the skeletal muscle degeneration and necrosis. No other significant lesions were identified in any of the tissues that were collected or examined.

Fig. 1. Photomicrograph of the anterior superficial pectoral skeletal muscle from calves treated at 2% body weight with ground alfalfa (control), Isocoma acradenia and Isocoma pluriflora for 9 days. The control (A) had rare degenerative and swollen myofibers (arrowheads). These were characterized by focal myocyte swelling and hyper eosinophilia with loss of striation. The I. acradenia treated calf (B) had similar swelling (arrowheads) with clumping of sarcoplasmic proteins (arrows), myocyte disruption with digestion vacuoles and chronic inflammation and fibroblast proliferations (*) with minimal extracellular collagen deposition. The skeletal muscle from I. pluriflora treated calf (C) had similar but more extensive myocyte swelling and degeneration (arrowheads), Zenker’s like myonecrosis with myofiber clumping and coagulation (arrows) and chronic inflammation with fibroblast proliferation and increased numbers of myocyte nuclei (*). Note that the I. pluriflora induced lesion was more extensive involving nearly 90% of the myofibers.

Fig. 2. Photomicrograph of severely affected anterior superficial pectoral muscle from a calf treated at 2% body weight with Isocoma acradenia for 9 days. Panel A is an acute lesion with degenerative and swollen myofibers (arrowheads) and early coagulation, clumping and banding of myocytes (arrows). This degeneration had early necrosis affecting most myocytes. Notice the characteristic focal myocyte swelling and hyper eosinophilia with loss of striation. Small numbers of more normal striated myocytes were also present. Panel B is older or more chronic myonecrosis with similar swelling (arrowheads), clumping of sarcoplasmic proteins (arrows), and with additional and more extensive myocyte disruption. There were also digestion vacuoles, inflammation and myocyte proliferation resulting in rowing of nuclei (*) along the myocyte margins. Numerous digestion vacuoles (#) with macrophages and fragments of sarcoplasmic fragments were also present. Panel C is from an area with chronic myonecrosis and mineralization. This section lacked sarcoplasmic clumping, but it had extensive nuclear proliferation and rowing (*) and numerous digestion vacuoles (#). The necrotic debris within residual myofiber tubules and digestion vacuoles was partially mineralized (m). Also notice several adjacent myocytes were striated and nearly normal (s).
Toxicon: X 5 (2020) 100022

4. Discussion

Rayless goldenrod (Isocoma pluriflora) like white snakeroot (Ageratina altissima) has been known to be toxic to livestock for nearly 100 years however, the toxicity of some Isocoma spp. has been suspected but not demonstrated. Recently we have developed a Spanish goat model for use in dosing studies with the purpose of definitively identifying the toxin(s) in rayless goldenrod and white snakeroot and to determine the dose required to cause disease so that risk assessments of different plant populations could be determined (Davis et al., 2013a). The development of clinical disease in the jersey calves dosed with I. pluriflora in this study was like the disease observed in Spanish goats using a plant collection from the same location in Pecos, Texas (Stegelmeier et al., 2010; Davis et al., 2013a). When calves were dosed at 2% of their BW “trembles” developed in four of the six calves in seven to nine days with large increases in serum creatine kinase activities in affected individuals as seen in other studies with goats (Davis et al., 2013a).

Histologic lesions were also like those observed in goats dosed with I. pluriflora except that the myonecrosis in the calves was polyphasic as the same muscle had lesions that were of differing durations. This contrasts with the goats where the myoskeletal lesions were primarily monophasic in each animal. In the goats, the lesions were most severe in the large appendicular muscles, diaphragm and longissimus dorsi and were multifocal to focally severe with extensive myocyte swelling, hypereosinophilia and loss of striation (arrowheads). Some myocytes were vacuolated with pyknotic nuclei.

Lesions were primarily monophasic in goats whereas the lesions in calves in this study were polyphasic. Monophasic necrosis is generally associated with toxic myopathies as we described previously in the goats. Polyphasic necrosis is usually nutritional myopathy (white muscle disease) as Se or Vit E deficiency occurs at different rates within muscles and between different muscle groups and types (Berridge et al., 2018). This has implications especially when we consider mechanisms, as polyphasic lesions are more likely to be a result of exhaustion of the oxidative protective mechanism. A logical explanation would be that calves have more local variability in oxidative protection within muscle groups. For example, arrhythmacyne induces myocardonecrosis in myocytes that have increased oxidative stress or a reduced oxidative stress status. This can be alleviated by antioxidants or therapy that increases cellular oxidative protective mechanisms (Vander Heide and L’Ecuyer, 2007). Such individual and subcellular changes in oxidative protection can be made by something as simple as changes in cellular estrogen receptors which have been linked to sex differences in many different toxicities (Steagall et al., 2017).

The calves in this study were dosed intra-ruminally with ground plant at 2% of their body weight as were the goats in previous studies; however, due to degradation of tremetone in the plant over five years of storage the concentration of tremetone was approximately 10% of that administered to the goats. It should be pointed out, that the I. pluriflora remained toxic despite the relatively low concentrations of tremetone and dehydrotremetone of 0.062 ± 0.009, 0.062 ± 0.01, respectively. Lee et al. (2017) reported that in 5.8 years, tremetone concentrations in stored rayless goldenrod decreased by over 92%. The concentration of 3-oxyangeloyl-tremetone was relatively higher at 1.2 ± 0.021 but had also decreased by over 80% during storage.

The concentrations of the tremetone, dehydrotremetone, and 3-oxyangeloyl-tremetone concentrations were approximately 25×, 8×, and 1.5× higher in the I. acradenia than in the I. pluriflora, respectively (Table 1). This report demonstrated that I. acradenia caused a similar disease in calves as I. pluriflora. The I. pluriflora-dosed calves tended to have higher scores and had more severely affected muscles. This is remarkable because the tremetone concentrations in the I. acradenia was approximately 25 times greater than in the I. pluriflora. If tremetone is the toxin in rayless goldenrod, the clinical signs and associated lesions in I. acradenia poisoned calves would be expected to be more severe than in the I. pluriflora poisoned calves. Since this was not the case, this study suggests that tremetone is not the primary toxin or that there may be another unidentified toxin(s) that act in concert with tremetone producing rayless goldenrod toxicity. This hypothesis is supported by recent studies in Spanish goats treated with I. pluriflora and Ageratina altissima. These studies reported that there was no correlation between the relative toxicity of several different white snakeroot collections with tremetone concentrations in the plant (Davis et al., 2016). Additionally, in a previous study, tremetone extracts from white snakeroot and adsorbed onto dried alfalfa did not produce “trembles” when dosed in equimolar concentrations as dried, ground plant material from the same collection (Davis et al., 2015). Results from this study when considered with the data from the goat studies with white snakeroot supports the idea that it is likely that there is another toxin in white snakeroot and rayless goldenrod that contributes largely to toxicity or acts synergistically with tremetone in some manner to produce disease. Interestingly, each Isocoma spp. or white snakeroot collection that has been toxic contained some tremetone, therefore it is also possible that tremetone may be a chemical indicator for Isocoma spp. and white snakeroot toxicity.

In conclusion, we were able to produce and describe I. acradenia intoxication in cattle for the first time. Additionally, the lesions and serum biochemical changes produced in the I. acradenia dosed calves was the same as the disease produced in the I. pluriflora dosed calves with a few minor differences in the most affected muscles in the two groups as well as the lesions being more severe in the I. pluriflora treated group. Additional studies with extracts and other plant chemotypes should be conducted to definitively determine the toxin in Isocoma spp. and white snakeroot and to determine the specific pathogenic mechanism involved in intoxication.

Ethics statement

The authors declare that there are no conflicts of interest associated with the publication of the manuscript “The comparative toxicity of Isocoma species in Calves”. The research was performed under the supervision of a veterinarian following protocols that were approved by the Utah State University IACUC committee.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
CrediT author statement

T. Zane Davis: Conceptualization, Investigation, Writing-Original Draft. Benedict Green: Investigation, Writing-Review and Editing. Bryan L. Stegelmeier: Investigation, Validation, Writing-Review and Editing. Stephen T. Lee: Investigation, Methodology, Writing-Review and Editing.

Acknowledgements

The authors thank Ed Knoppel and Chuck Hailes for their assistance with this research. We would also like to thank M. B. Piep at the Intermountain Herbarium for his assistance in identifying the plants. This research was supported by USDA/ARS.

References

Berridge, B.R., Bolon, B., Herman, E., 2018. Fundamentals of Toxicologic Pathology, third ed., pp. 195–212
Bonner, W.A., Degraw Jr., J.I., 1962. Ketones from “white snakeroot” Eupatorium urticaefolium. Tetrahedron Lett. 18, 1295–1309.
Bonner, W.A., Degraw Jr., J.I., Bowen, D.M., Shah, V.R., 1961. Toxic constituents of white snakeroot. Tetrahedron Lett. 12, 417–420.
Buehrer, T.F., Mason, C.M., Crowder, J.A., 1939. The chemical composition of rayless goldenrod (Isocoma hartwegi). Am. J. Pharm. 111, 105–112.
Couch, J.F., 1927. The toxic constituent of richweed or white snakeroot (Eupatorium urticaefolium). J. Agric. Res. 35, 547-576.
Couch, J.F., 1930. The toxic constituent of rayless goldenrod. J. Agric. Res. 40, 649–658.
Davis, T.Z., Green, B.T., Stegelmeier, B.L., Lee, S.T., Welch, K.D., Pfister, J.A., 2013a. Physiological and serum biochemical changes associated with rayless goldenrod (Isocoma pluriflora) poisoning in goats. Toxicon 76, 247-254.
Davis, T.Z., Lee, S.T., Collett, M.G., Stegelmeier, B.L., Green, B.T., Buck, S., Pfister, J.A., 2015. Toxicity of white snakeroot (Ageratina altissima) and chemical extracts of white snakeroot in goats. J. Agric. Food Chem. 63, 2062-2077.
Davis, T.Z., Stegelmeier, B.L., Lee, S.T., Collett, M.G., Green, B.T., Pfister, J.A., Evans, T.J., Grum, D.S., Buck, S., 2016. White snakeroot poisoning in goats: variations in toxicity with different plant chemotypes. Res. Vet. Sci. 106, 29-36.
Davis, T.Z., Stegelmeier, B.L., Lee, S.T., Green, B.T., Hall, J.O., 2013b. Experimental rayless goldenrod (Isocoma pluriflora) toxicity in horses. Toxicon 73, 88-95.
Kingsbury, J.M., 1964. Poisonous Plant of the United States and Canada. Prentice Hall, Englewood Cliffs, NJ.
Lee, S.T., Cook, D., Davis, T.Z., Gardner, D.R., Johnson, R.L., Stoneciper, C.A., 2015. A survey of tremetone, dehydrotremetone, and structurally related compounds in isocoma spp. (goldenbush) in the southwestern United States. J. Agric. Food Chem. 205, 872-879.
Lee, S.T., Davis, T.Z., Cook, D., 2017. Evaluation of the stability of benzofuran ketones in rayless goldenrod (Isocoma pluriflora) and white snakeroot (Ageratina altissima) under different storage conditions. Int. J. Poisonous Plant Res. 4, 36-42.
Lee, S.T., Davis, T.Z., Gardner, D.R., Stegelmeier, B.L., Evans, T.J., 2009. A quantitative method for the measurement of three benzofuran ketones in rayless goldenrod (Isocoma pluriflora) and white snakeroot (Ageratina altissima) by HPLC. J. Agric. Food Chem. 57 (12), 5639-5642.
Marsh, C.D., Roe, G.C., 1921. The “Alkali Disease” of Livestock in the Pecos Valley, vol 180. US Dept Agriculture, pp. 3-8.
Neesom, G.L., 1991. Taxonomy of isocoma (Asteraceae: astereae). Phytologia 70, 69-114.
Panter, K.E., James, L.F., 1990. Natural plant toxicants in milk: a review. J. Anim. Sci. 68, 892-904.
Steagall, R.J., Yao, F., Shaikh, S.R., Abdel-Rahman, A.A., 2017. Estrogen receptor activation enhances its cell surface localization and improves myocardial redox status in ovariectomized rats. Life Sci. 182, 41-49.
Stegelmeier, B.L., Davis, T.Z., Green, B.T., Lee, S.T., Hall, J.O., 2010. Experimental rayless goldenrod (Isocoma pluriflora) toxicity in goats. J. Vet. Diagn. Invest. 22 (4), 570-577.
Vander Heide, R.S., L’Ecuyer, T.J., 2007. Molecular basis of anthracycline-induced cardiotoxicity. Heat Metabol. 35, 1-4.