Western Juniper-Induced Abortions in Beef Cattle

Kevin D. Welch[1], Dale R. Gardner[1], Kip E. Panter[1], Bryan L. Stegelmeier[1], Cory Parsons[2], James A. Pfister[1], and Daniel Cook[1]

[1]USDA Agricultural Research Service, Poisonous Plant Research Laboratory, Logan, UT, USA
[2]Oregon State University, Baker County Extension Service, Baker City, OR, USA

Corresponding author: Kevin Welch, kevin.welch@ars.usda.gov

Abstract

It has been known for many years that ponderosa pine needles can induce late-term abortions in cattle. Labdane acids including isocupressic acid (ICA) and agathic acid are responsible for initiating abortions. Recent research demonstrated that a number of trees including many species of pine, juniper, and cedar contain either isocupressic acid or agathic acid at concentrations sufficient to be a risk for causing abortions in late-term cattle. The objective of this study was to determine if the bark from western juniper (*Juniperus occidentalis*) will induce late-term abortions in cattle. Pregnant cows were dosed starting on day 250 of gestation with 2.3 kg of ground plant material twice daily for 10 days or until abortions occurred. Western juniper bark used in this study contained approximately 0.7% labdane acids, on a dry weight basis, including isocupressic acid (0.025%), agathic acid (0.43%), imbricatoloic acid (0.15%), and dihydroagathic acid (0.05%). Two cows aborted 4-5 days after the start of the treatment. Both cows had retained placental membranes. The remaining 4 cows calved at full term (26-31 days after the start of treatment) and had no retained placental membranes. Results from this study indicated that western juniper trees contain compounds known to be abortifacient in cattle and that consumption of large amounts of bark in the third trimester of gestation may induce abortions. Although the risk of abortion from eating western juniper bark appears to be less than that from eating ponderosa pine needles, livestock producers should be aware of this potential.

Keywords: agathic acid, imbricatoloic acid, cattle, abortion, western juniper

Abbreviations

AA	Agathic Acid
DHAA	Dihydroagathic Acid
IMB	Imbricatoloic Acid
ICA	Isocupressic Acid
Rt	Retention Time
THAA	Tetrahydroagathic Acid

Introduction

Since the early 1900s, ponderosa pine (*Pinus ponderosa*) needles have a long history of causing abortions in cattle (MacDonald 1952, Stevenson et al. 1972, James et al. 1977, Panter et al. 1990). Pine needle related-abortions occur most frequently in the last trimester of gestation. Affected cattle often have incomplete cervical dilation and weak uterine contractions resulting in difficult calving (dystocia), followed by retained fetal membranes. Retained fetal membranes can result in endometritis and pyometria. The combination of early parturition, dystocia, and retained placental membranes forms the defining characteristics of pine needle-caused abortion (James et al. 1994). Calves are viable if the abortion is late in gestation; however, they are weak, often require assistance to suckle, and are prone to increased respiratory problems and disease.

Several years ago, isocupressic acid (ICA; figure 1), a labdane resin acid in the pine needles and bark of ponderosa pine (*Pinus ponderosa*), was identified as the major abortifacient agent in ponderosa pine needles (Gardner et al. 1994). Isocupressic acid was found to be metabolized in both the rumen and the liver through oxidative and reductive processes (Gardner et al. 1999). The major metabolites identified thus far include imbricatoloic acid (IMB), agathic acid (AA), dihydroagathic acid (DHAA), and tetrahydroagathic acid (THAA) (Lin et al. 1998, Gardner et al. 1999). Additional research has demonstrated that several other species of trees are also abortifacient (Panter et al. 2007). Current management recommendations indicate that any tree with a concentration greater than 0.5% ICA (on a dry weight basis) poses a risk for inducing abortions in late-term pregnant cattle. It recently was reported that the bark of Utah juniper (*Juniperus osteosperma*), which contains a high concentration of agathic acid (1.5% by dry weight) but no ICA, will induce abortions in cattle, suggesting that agathic acid is also abortifacient in cattle (Gardner et al. 2010). However, it remains uncertain as to whether the other metabolites, IMB, DHAA, or THAA, are biologically active as abortifacient compounds in late-term pregnant cattle.

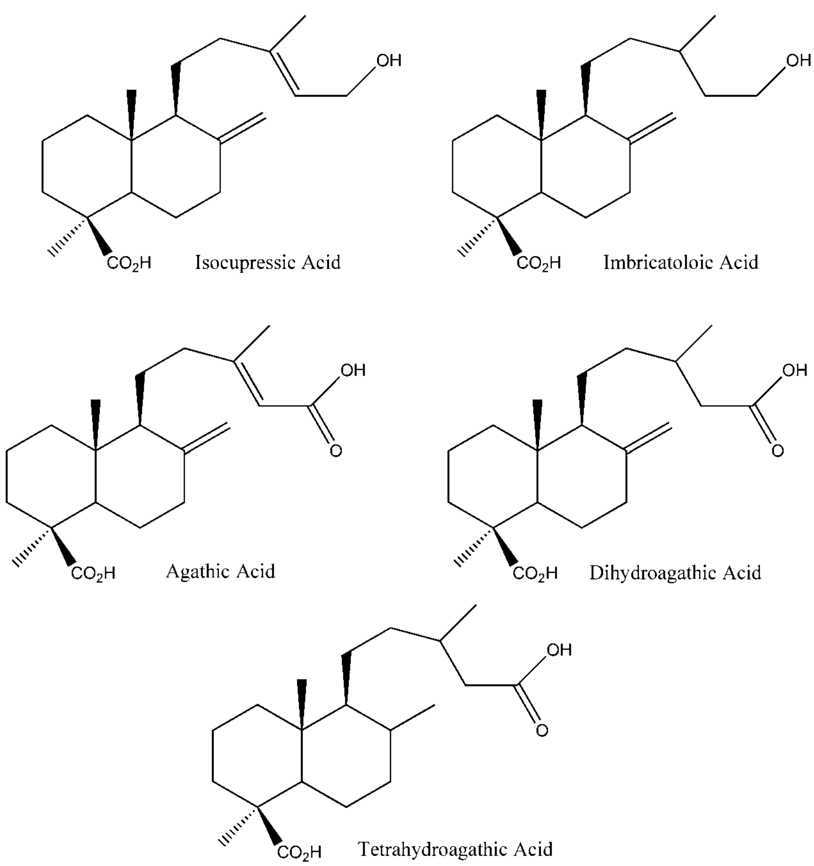

Figure 1. Chemical structures of several labdane acids.

During the late 1990s and early 2000s, many ranchers in Baker County, OR, were using western juniper trees in riparian restoration projects. These large, bushy trees were utilized as a source of large woody debris and placed in stream banks and beds to dissipate stream flow energy and to capture and store sediment within the stream channel. Shortly after the beginning of the riparian restoration project, several local ranchers reported atypical late-term abortions. There have been reports by several ranchers of 10 to 15 percent of their herds aborting after being pastured in these areas (personal communications). Ponderosa pine and other trees known to contain ICA were not found in the areas in which these abortions occurred. However, there was clear visual evidence that the cattle had been eating the bark of the downed western juniper trees. Therefore, samples of western juniper needles, berries, and bark were analyzed for labdane acid content. Both the needles and berries were found to contain low concentrations of labdane acids. However, preliminary analyses indicated that the bark of western juniper trees had a fairly high concentration of labdane acids (~ 1.0 %). Consequently, the objective of this study was to determine if the bark from western juniper trees could induce abortions in cattle.

Materials and Methods

Plant material—Bark from western juniper (*Juniperus occidentalis*) trees was collected in August 2009 from trees that had been cut down 1 and 2 years previously near Baker City, OR (N lat 44° 43. 281’, W long 117° 53. 009’). The bark was allowed to dry at ambient temperature and then stored on a canvas tarp in an enclosed, non-heated, un-insulated building at ambient temperature until treatment. Prior to treatment, enough plant material for 2 days of treatments was ground to pass a 2mm screen and stored in a plastic bag in the dark at 4°C until used.

Animals—Six healthy, experimentally naïve, Angus heifer cows (mean ± SD weight, 599 ± 48 kg) were purchased from a herd with no history of abortion or reproductive disease. The cows were time bred and confirmed pregnant via palpation. The cows were fed alfalfa-grass hay and a dietary mineral supplement (standard diet provided to all cattle at the USDA-ARS Poisonous Plant Research Laboratory) and housed in outdoor paddocks. Pregnancies were monitored and verified via palpation prior to treatment. Ground plant material (2.3 kg) was administered twice each day (morning and afternoon) via stomach tube directly into the rumen starting on day 250 of gestation. This dose has been previously shown to be well tolerated and will consistently induce abortion with ponderosa pine needles containing 1.0% ICA or greater (James et al. 1994). Dosing continued daily until abortion or day 260 of gestation (maximum of 20 doses/animal). During treatment, the cattle were closely monitored for signs of parturition. In this study, if the placental membranes were not expelled within 24 hours after parturition, the animals were treated with uterine lavage and intrauterine antibiotic infusions.

Blood samples were collected via the jugular vein prior to treatment each morning. Blood samples were allowed to clot at room temperature for 30 min and then centrifuged at 1700 x g for 20 min to separate the serum from the cellular fractions. The serum was removed and stored in plastic vials at -20^{o}C until analysis. All protocols for animals used in this research were performed under veterinary supervision and reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Utah State University, Logan, UT.

Analytical methods—The concentration of labdane acids in the plant material was measured by gas chromatography. The dry ground bark (0.100 g) was extracted in duplicate using the published procedure for isocupressic acid (Gardner and James 1999) to give a crude organic acid extract. The dry extract was dissolved in 2.0 mL of methylene chloride. A 0.200 mL aliquot was transferred to a GC autosample vial and the solvent evaporated under flow of nitrogen. Pyridine (0.200 mL) containing 0.200 g/mL of heptadecanoic acid and 0.050 mL of BSTFA silyation reagent (Pierce Biotechnology, Rockford, IL) was added and the sample capped and heated for 30 min at 60°C. Before analysis, 0.75 mL of methylene chloride was added. Calibration standards (isocupressic acid) were prepared at 25, 50, 75, and 100 µg and were treated as above for silyation and dissolution. Gas chromatography was performed as previously described (Gardner and James 1999).

For analysis of sera samples for labdane acids, 1.0 mL aliquot of sera was placed into an 8 mL screw cap vial and 1.0 mL of saturated KH_2PO_4 was added. The samples were then extracted twice with chloroform (2.0 mL). After each extraction, the samples were centrifuged to aid layer separation and the chloroform solution was withdrawn with a

Pasteur pipette and passed through a filter of anhydrous sodium sulfate into a second 8 mL vial. The combined chloroform extracts were evaporated to dryness under a flow of nitrogen in a heat block at 60°C. The samples were dissolved in 0.25 mL of pyridine (containing 40 ppm heptadecanoic acid as a reference standard) and 0.050 mL of BSTFA silyation reagent and heated at 60°C for 30 min. Samples were then analyzed for metabolites of isocupressic acid (agathic acid, dihydroagathic acid, and tetrahydroagathic acid) by gas chromatography/ mass spectrometry. Peak areas of the detected metabolites were measured from selected ion chromatograms, referenced against the C17 standard and then plotted versus day collected to measure relative concentration of metabolites in the sera samples.

Data analysis—Data are expressed as the mean ± SD. Statistical comparisons of serum labdane acid concentrations between cattle that aborted and those that calved at full term were made by use of a two-way ANOVA with a Bonferroni post-hoc test. Correlations between serum labdane acid concentrations and the number of days to parturition were determined using Pearson's correlation.

Results

Analysis of the western juniper bark by gas chromatography found the major labdane acid to be agathic acid (AA; figure 2). The total AA concentration was measured at 0.43% (dry weight basis). The bark also contained low concentrations of other labdane acids including ICA (0.025%), IMB (0.15%), and DHAA (0.05%), as well as abietane and pimarane acids as the major components in the extract. The western juniper bark used for this study contained approximately 0.7% total labdane acids, by dry weight. This concentration of labdane acids was greater than the threshold concentration (0.5%) thought to be required to produce abortions in cattle (Gardner and James 1999). However, to date the only labdane acids that have been shown experimentally to induce abortions are ICA and AA. The concentration of those two labdane acids in this plant material was approximately 0.46%, which is slightly below the estimated threshold concentration that poses a risk for abortion.

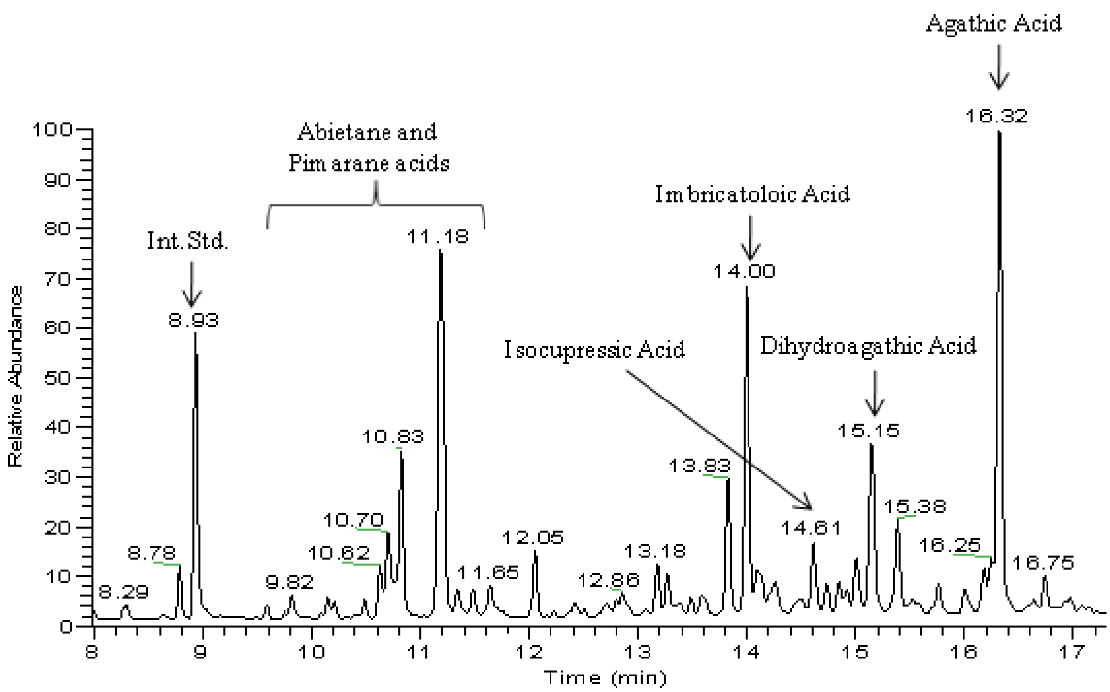

Figure 2. Chemical analysis of western juniper bark. Gas chromatography/mass spectrometry chromatograms and the major diterpene acids identified from the plant material. Specific peaks include Imbricatoloic Acid (Rt, 14.00 min), Isocupressic Acid (Rt, 14.61 min), Dihydroagathic Acid (Rt, 15.16 min), and Agathic Acid (Rt, 16.32 min).

Table 1. The amount of plant material dosed daily, the corresponding daily dose of labdane acids, and the number of days to parturition after start of treatment

Animal	Weight (kg)	Daily Plant Dose (g/kg)	Daily Labdane Acid Dose (mg/kg)	Daily Agathic Acid Dose (mg/kg)	Daily Imbricatoloic Acid Dose (mg/kg)	Days to Parturition
1	623	7.4	48.4	31.7	11.1	26
2	532	8.6	56.6	37.2	13.0	5
3	632	7.3	47.7	31.3	10.9	4
4	545	8.4	55.3	36.3	12.7	26
5	640	7.2	47.1	30.9	10.8	31
6	623	7.4	48.4	31.7	11.1	26

Two of the 6 cows aborted after 4 to 5 days of treatment (table 1). Both abortions were typical of pine needle-induced abortions, including parturition 4 to 5 days after treatment started as well as typical clinical signs including dystocia and retained placental membranes (James et al. 1994). The remaining 4 cows calved normally 26 to 31 days after the start of treatments on gestation days 276 to 281. There were no complications observed during parturition and no retained placental membranes in these four cows.

In this study, there was not a dose-response relationship observed between the amount of labdane acids dosed and the number of days to parturition (table 1). One of the cows that aborted received the highest daily dose of labdane acids (56.6 mg/kg). However, the other cow that aborted received one of the lowest daily doses of labdane acids (47.7 mg/kg). The cows that did not abort received a daily dose of labdane acids that was in between the range to those that did abort (47.1 – 55.3 mg/kg). Additionally, there was no correlation between the dose of labdane acids received and the number of days to parturition (P=0.54).

Sera samples were analyzed to determine if the variation in results observed in this study was due to the amount of bioavailable labdane acids in the cattle. Serum concentrations of ICA and IMB were essentially zero at all time points (figure 3A). Agathic acid, DHAA, and THAA were detected in blood serum samples of cows dosed with western juniper bark (figure 3). However, the animals that aborted did not have a higher concentration of any of the labdane acids in their sera; in fact, there was a trend for the two cows that aborted to have a lower sera concentration of agathic acid (figure 3A).

Discussion

It has been known for many years that pine needle-induced abortion is a problem, estimated at $4.5 million annually (Lacey et al. 1988), for ranchers that are forced to pasture their cattle in the last trimester of pregnancy in rangelands that contain ponderosa pine trees (Bruce 1927). Additionally, it has been demonstrated that labdane acids including ICA and AA are the compounds responsible for initiating the abortions (Gardner et al. 1994, Gardner et al. 2010). Recent research efforts have demonstrated that a large number of trees including many species of pine, juniper and cedar trees, contain either ICA or AA at concentrations sufficient to be a risk for causing abortions in late-term cattle (Panter et al. 2007). The results from the study presented here demonstrate that the bark from western juniper trees can also induce abortions in cattle.

Although western juniper bark is not likely a normal part of cattle's diet, large winter snow storms often force cattle to graze on non-typical forages, which could include slash piles of downed western junipers. The State of Oregon is currently clearing large areas of western juniper trees in an attempt to recover critical grasses in mountain ranges used by wildlife and livestock for grazing. As a result of these efforts, large piles of downed western juniper trees are available to cattle in many Oregon rangelands.

The results observed in this study were varied, in that only two of the six cattle aborted. Additionally, the variation cannot be explained by differences in the daily dose of labdane acids administered to the cattle. The variation may stem from the fact that the concentrations of ICA and AA in this plant material were too low to consistently cause abortions. Previous research in our laboratory has demonstrated that an ICA concentration of 0.5% or greater is required to induce abortions in cattle (Gardner and James 1999), with the incidence increasing with higher concentrations of ICA, with concentrations greater than 1.0% being significant risk (personal observations). The bark used in this study contained 0.7% labdane acids; however, IMB and DHAA have not yet been shown to be abortifacient. Consequently, the concentration of known abortifacient labdane acids in this plant material was 0.46 percent, which is below the putative threshold.

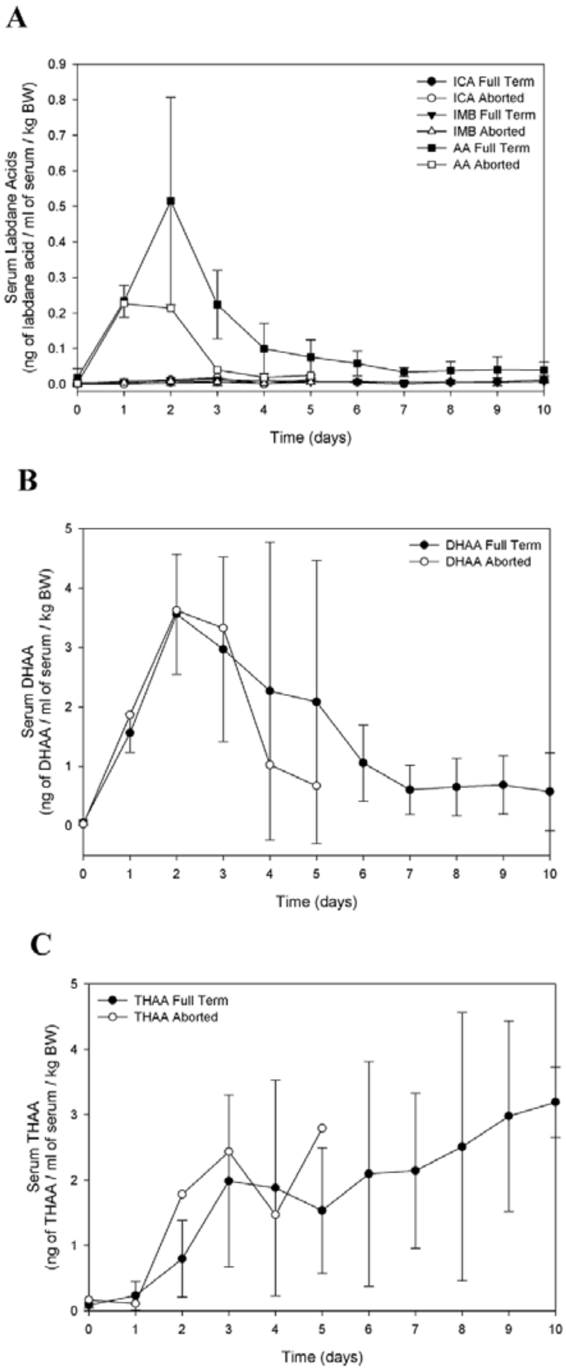

Figure 3. Comparison of labdane acid concentrations in sera of cows that aborted versus cows that calved at full term. A) Isocupressic (ICA), Imbricatoloic (IMB), and Agathic (AA) acids, B) Dihydroagathic Acid (DHAA), and C) Tetrahydroagathic Acid (THAA). Results represent the mean ± SD (n=4) for the full term group and the mean (n=2) for the aborted group.

Therefore, it is possible that the variation in response was due to the lack of abortifacient compounds in the bark and consequently only the two most susceptible animals aborted. These results are in accordance with most field observations wherein 10 to 20 percent of a herd will abort when they are exposed to pine needles during a large winter storm (personal communications), suggesting that normally only the most susceptible animals have a problem.

Future research efforts will include a study to sample bark from western juniper trees across Oregon and California to determine if there are areas that contain higher concentrations of ICA and/or AA. Areas where western juniper bark contains ICA and/or AA at 0.5 percent and greater would be considered a risk for causing abortions in cattle. Further, if samples of bark are found that contain ICA and/or AA at much higher concentrations, additional experiments could be performed to determine if abortions would occur at a higher incidence with western juniper bark that contains a higher ICA and/or AA concentration. There also remains a potential to find a sample of western juniper bark that would contain a high concentration of IMB and very little of the other labdane acids. This bark would be of high interest for determining if IMB is also abortifacient.

An additional interesting observation from this study was that after 3 to 4 days of continuous dosing, the serum AA concentrations dropped below the level of detection. Similar results have also been observed in previous studies (Gardner et al. 2010). Typically, when a compound is administered multiple times, it will lead to accumulation of the compound in the body (Shargel and Yu 1993, Shen 2008). For example, if a compound follows first-order elimination kinetics, the elimination rate increases as the body burden increases. Thus, with a fixed, continuous exposure, accumulation of a compound in the body reaches a point when the rate of intake equals the rate of elimination for that compound. From that point forward, the body burden remains constant, which is commonly referred to as a steady state (Shen 2008). However, with regard to the labdane acids ICA, AA, and IMB in cattle, a steady state does not appear to occur. One possible explanation for this observation is that multiple doses of these compounds could result in an induction of the enzymes/pathways that are involved in the elimination of these compounds from the body (Meyer and Gut 2002, Parkinson and Ogilvie 2008). Additionally, multiple dosing of these compounds orally may induce a change in the rumen microflora such that these compounds are subsequently metabolized in the rumen to an extent that they are not absorbed by the cattle (James et al. 1967, Van Kampen and James 1969, James and Cronin 1974).

Additional experiments are needed to study this phenomenon in more detail. If it is determined that a multiple-dosing regimen of these compounds does enhance their elimination from the body, or inhibit their absorption of the GI tract, changes to management recommendations could be made. These changes would reflect the possibility that naïve cattle may be more susceptible to pine needle-induced abortions and that the grazing of naïve cattle in pastures or rangelands abundant in ponderosa pine trees (or other ICA- and related labdane acid-containing trees) should be even more limited during the latter part of pregnancy.

In conclusion, the bark from western juniper trees contains labdane acids that have been associated with abortion in cattle. Consequently, livestock producers should be aware of the potential for western juniper trees to induce abortions in late-term pregnant cattle, especially if grazing conditions deteriorate so that cattle are compelled to eat juniper.

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