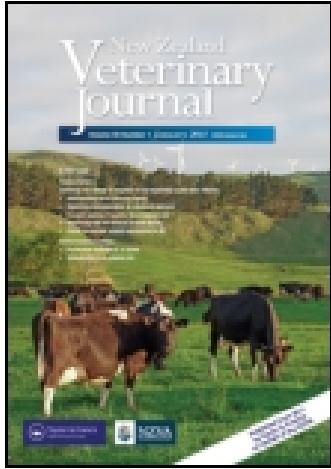


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K. Parton^a, D. Gardner^b & N.B. Williamson^a

^a Department of Veterinary Clinical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand

^b USDA-ARS Poisonous Plant Research Laboratory, Logan 84341, Utah, United States

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Short Communications

Isocupressic acid, an abortifacient component of *Cupressus macrocarpa*

K. Parton*‡, D. Gardner† and N.B. Williamson*

Abstract

Anecdotal reports suggest that abortion occurs in New Zealand cattle after ingestion of macrocarpa (*Cupressus macrocarpa*). The clinical signs observed are similar to those seen in abortions caused by ponderosa pine (*Pinus ponderosa* Laws). Isocupressic acid has been identified as an abortifacient component of the needles of ponderosa pine. Macrocarpa collected from the Palmerston North area contained 0.89% and 1.24% isocupressic acid, compared to 1.70% isocupressic acid in Ponderosa pine needles from Oregon in the United States as analysed by the USDA-ARS Poisonous Plant Research Laboratory. These findings support observations that macrocarpa causes abortion in late gestation cattle in New Zealand and suggest the causal agent is probably isocupressic acid. (New Zealand Veterinary Journal 44, 109–111, 1996.)

Introduction

Cupressus macrocarpa, the Monterey cypress, is a tree which was brought to New Zealand for use as shade and shelter belts. Planting has been widespread and accidental ingestion has been linked with abortions in livestock. In New Zealand, the abortifacient properties of macrocarpa have been reported since the 1950s⁽¹⁾⁽²⁾⁽³⁾. Macrocarpa consumption by cattle in the last trimester is associated with premature parturition of dead or weak calves within a week or two of ingestion⁽¹⁾⁽⁴⁾⁽⁵⁾. Retained placentas, often characterised by swollen cotyledons and associated straining, are common sequelae⁽¹⁾. Fatal poisoning of pregnant and dry cattle has been reported after large quantities of macrocarpa and *Cupressus sempervirens* had been ingested⁽⁴⁾⁽⁵⁾⁽⁶⁾. Macrocarpa toxicity resembles a condition caused by ponderosa pine (*Pinus ponderosa* Laws), which is from the closely related family Pinaceae. Third trimester abortions reported in cattle after pine needle ingestion are characterised by weak or dead calves, dystocia, retained fetal membranes and secondary complications like septic metritis, peritonitis or toxæmia which often result in the death of the cow⁽⁷⁾⁽⁸⁾. After parturition, the uterus is often atonic and filled with placental debris and blood⁽⁷⁾. Cows may retain fetal membranes for 10–15 days⁽⁷⁾.

Pine needle abortion in cattle appears to have a pharmacological rather than a pathological basis⁽⁹⁾. Studies on uterine blood flow (UBF) and receptor dynamics indicate that premature parturition follows a progressive decrease in UBF caused by vasoconstrictive effects of the substance(s) found in pine needles⁽¹⁰⁾⁽¹¹⁾⁽¹²⁾.

Initial work⁽¹³⁾ determined that methylene chloride extraction of pine needles contained the abortifacient compound(s). Bark, needles and new growth tips of branches were abortifacient and sometimes produced systemic toxicity as well⁽¹⁴⁾. The principal active abortifacient component of pine needles was identified as isocupressic acid (ICA)⁽¹⁵⁾. An assay of the ponderosa pine needles was reported to contain an isocupressic acid concentration of 1.7% (dry weight)⁽¹⁵⁾. A mixture of abietane-type acids was also isolated, but these compounds did not cause premature parturition; however, they were associated with depression, decreased appetite and death in cattle at doses greater than the 200 mg/kg fed twice daily⁽¹⁵⁾.

This study was conducted to determine if the same compounds were present in *C. macrocarpa* and could explain the signs reported after ingestion by cattle.

Materials and Methods

Macrocarpa foliage was collected in September 1994 from a farm in Linton. A second macrocarpa sample was collected in October of 1994 from trees in Tokomaru that had been previously associated with abortions. A dried sample of *Pinus radiata* from the Palmerston North area was submitted at the same time. In June 1995, a year after the initial samples were analysed, a sample of *Cupressocyparis leylandii* was submitted for analysis. The Linton and Tokomaru samples were dried at ambient and room temperature, respectively. Macrocarpa, Leyland cypress and radiata samples were coarsely ground to a 3 mm size, placed in plastic bags and sent via air courier to the USDA-ARS-Poisonous Plant Research Laboratory for analyses of isocupressic acid. The samples were split, half of the material was stored frozen, and the other half prepared for analysis by drying in a forced air oven at 48 °C for 48 hours and then grinding to pass a 40 mesh screen.

For each sample, 100 mg of the dry ground plant material was extracted with 4.0 ml of methylene chloride by rotation in a 10 ml sealed tube for 0.5 hours. Samples were quantitatively filtered and the solvent removed by evaporation under a stream of dry nitrogen at 70 °C. Samples were methylated by addition of 1.0 ml of dimethylformamide (containing 200 µg of diphenylacetic acid as an internal standard), 50 mg of potassium carbonate and 50 µl of

* Department of Veterinary Clinical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand.

† USDA-ARS Poisonous Plant Research Laboratory, Logan, Utah 84341, United States.

‡ Author for correspondence.

methyl iodide. The sealed tubes were then mixed by rotation for 0.5 hours. Chloroform (4.0 ml) was added to each sample and 1.0 μ l injected into a gas chromatograph for analysis of isocupressic acid.

Gas chromatography was performed using a Hewlett Packard 5890 GC equipped with a split/splitless injector (250 °C) and flame ionisation detector (325 °C). Samples (1.0 μ l) were injected splitless on to a DB-5 (J&W Scientific) column (30 m \times 0.32 mm id). The oven temperature was programmed at 100 °C for 1.0 min, 100–200 °C at 50 °C/min, 200–260 °C at 5 °C/min, 260–320 °C at 20 °C/min, and 320 °C for 5 min. The carrier gas was helium. The identification of isocupressic acid was verified by gas chromatography/mass spectroscopy analyses as previously reported⁽¹²⁾ and a direct comparison of retention times and mass spectrum to that previously isolated from *Pinus ponderosa* (Figure 1).

Results

The macrocarpa sampled from Linton and Tokomaru contained 0.89% and 1.24% isocupressic acid, respectively. The radiata sample contained less than 0.1% isocupressic acid. The 1995 sample of Leyland cypress contained 1.4% isocupressic acid.

A related labdane acid, tentatively identified as cummunic acid, was also found to be present in the macrocarpa samples. The abortifacient activity of this compound is unknown. No other diterpene acids were identified as major components of the macrocarpa samples.

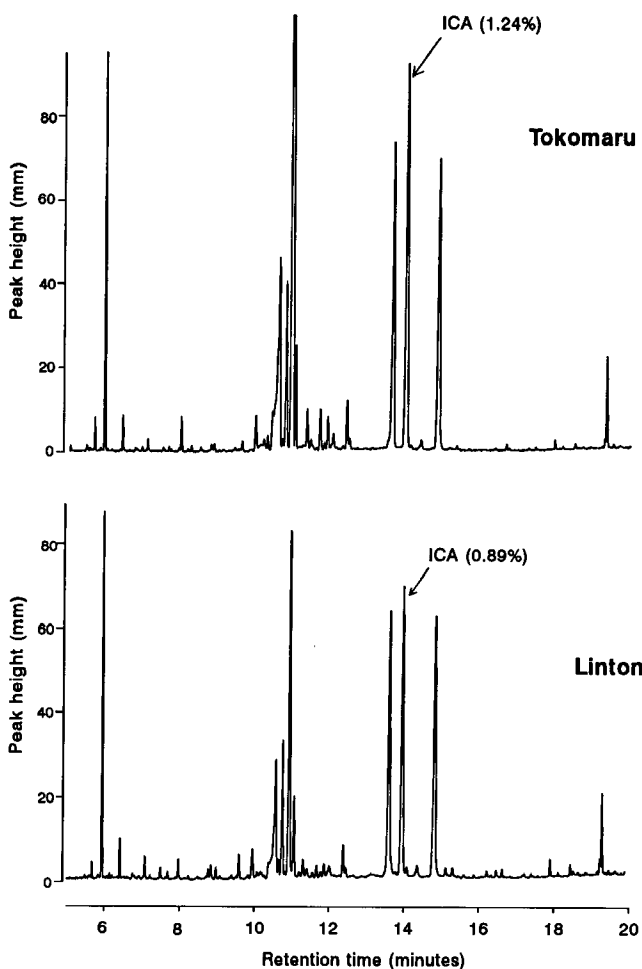


Figure 1. Gas chromatograms of extracts from *Cupressus macrocarpa* from two localities in New Zealand, analysed for ICA.

Discussion

The similarity of abortions resulting from pine needle and macrocarpa ingestion suggests that isocupressic acid could be the abortifacient component of macrocarpa. The identification of isocupressic acid as the major active compound in ponderosa pine associated with late term abortions in cattle has made it possible to analyse similar plants for this compound. The presence of isocupressic acid in macrocarpa suggests that both conditions have the same aetiology.

Pine needle abortion appears to result from a decrease in uterine blood flow in the caruncular arteries. The exact mechanism is unknown, but researchers are studying α_2 -adrenergic receptors responsible for the vasoconstriction in the caruncular arteries⁽¹²⁾. Christenson *et al.*⁽¹²⁾ believe that pine needles contain a compound that interferes with oestrogen metabolites, namely catechol oestrogens, which are responsible for increasing uterine blood flow via their action at potential sensitive Ca^{2+} channels. Although ICA from pine needles has been demonstrated to be the abortifacient in cows⁽¹⁵⁾, the association between ICA and inhibition of uterine blood flow through effects on potential sensitive Ca^{2+} channels needs to be studied.

The ingestion of *Pinus radiata* has been similarly linked as a cause of late trimester abortions in cattle⁽¹⁶⁾. A sample of radiata collected in September 1994 from Palmerston North was found to have only a small amount of ICA (<0.1%). While preliminary findings do not support a linkage between radiata and ICA-related abortions, further study is warranted.

The Leyland cypress is a hybrid with macrocarpa as one of the parent stock. Questions about its abortifacient potential were expressed in 1975 by the New Zealand Forest Service⁽¹⁷⁾. The planting of Leyland cypress for shelter belts on farms increases the likelihood of cattle ingestion. The findings of 1.4% isocupressic acid indicate that the ingestion of a sufficient quantity of Leyland cypress could induce abortion.

Gardner *et al.*⁽¹⁵⁾ determined that an oral dose of about 100 mg/kg of isocupressic acid fed twice daily for several days was required to induce an abortion. Thus a pregnant cow averaging 430 kg would need to ingest 7–8 kg of macrocarpa containing 1.24% isocupressic acid daily to cause an abortion.

The isocupressic acid composition of *Pinus ponderosa* varies considerably depending on provenance⁽¹⁸⁾. Macrocarpa, radiata and Leyland cypress probably follow the same pattern. Variation in concentration of the two macrocarpa samples analysed also may be due to other factors such as the small sample size, differences in trees, time of year, tree parts sampled and storage conditions for the samples. Further work is required to determine the effect of season, stage of growth, provenance and storage conditions on the ICA content in macrocarpa and other cypress or cypress hybrids.

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