## TECHNICAL NOTES

# Seasonal Concentration and Toxicity of Saponins in Alfombrilla

M. COBURN WILLIAMS AND LUIS C. FIERRO

#### Abstract

Alfombrilla collected monthly for 1 year in Chihuahua, Mexico, was analyzed for percentage of saponin and for toxicity to 1-week-old chicks. Saponin level ranged from 2.8% in dormant plants collected in January to 6.3% in mature fruiting plants collected in September. Saponin level was low in seeds. High saponin levels may persist in dormant plants into December. The amount of plant required to kill 50% or more of the chicks was inversely proportional to the saponin level of the plants.

Alfombrilla, Drymaria arenarioides H.B.K., is a highly poisonous species of the family Caryophyllaceae. The species is native to the Mexican states of Chihuahua, Sonora, Hidalgo, San Luis Potosi, and Zacatecas (Martinez 1960). The toxicological properties of alfombrilla are being investigated by the United States and Mexico because the species threatens to invade the United States from Chihuahua and Sonora.

Saponins were isolated as the poisonous compound by Williams (1978). Six saponins accounted for approximately 3% of the dry weight of the plant when collected in vegetative growth. Although the precise structure of the saponins is unknown they are highly toxic, and livestock may be poisoned by eating alfombrilla at 0.1% of body weight. Alfombrilla ingested at 0.5% of body weight is nearly always lethal (Jacoby and Morton 1974; Larios and Javalera 1976).

No information is presently available on the seasonal variation in the concentration of saponins in alfombrilla. Presumably, saponins in alfombrilla fluctuate during the growing season so that the species is more toxic at certain growth stages. The stability of saponins in dormant plants is also unknown. Many plant poisons, such as nitro compounds, virtually disappear at senescence (Williams and Norris 1969); others, such as oxalates in halogeton, (Halogeton glomeratus (Bieb.) C.A. Mey,), persist to render the plant poisonous far into the winter (Williams and Cronin 1966). A saponin level of 5 to 7% reported in the seed of corn cockle, (Agrostemma githago L.) (Kingsbury 1964) suggests that levels of saponins may be high at fruiting in some saponin-containing species of the Caryophyllaceae. Such data on alfombrilla would be of great value to Mexican ranchers who graze or trail livestock on alfombrilla-infested range.

This paper presents data on the seasonal variation in the concentration of saponins in alfombrilla during 1977 and the toxicity of monthly plant samples to I-week-old chicks.

### Materials and Methods

Alfombrilla was collected and dried monthly from January 1977

Authors are plant physiologist, Poisonous Plant Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Logan, Utah 84321; and assistant director, Rancho Experimental La Campana, Chihuahua, Chihuahua, Mexico. The research represents a cooperative investigation by the Science and Education Administration, U.S. Dep. Agr. Rancho Experimental La Campana and the Utah Agricultural Experiment Station, Logan 84322.

Manuscript received January 2, 1979.

through December 1977 at Rancho Experimental La Campana in Chihuahua, Mexico. Seed samples collected in October and December were combined for chemical analysis and feeding. Pland and seed were first shipped to the Animal and Plant Health Inspection Service Laboratory, U.S. Dep. Agr., in Los. Angeles for fumigation to destroy viable seed. The plant was then forwarded to the Poisonous Plant Research Laboratory at Logan, Utah. Each monthly plant sample and the pure seed were ground to pass a 40-mesh screen and then analyzed for percentage of saponins by the method of Wang (1969).

Fifty grams of alfombrilla was extracted with 70% ethanol for 24 hours on a Soxhlet extractor. The extract was reduced to dryness on a rotary evaporator, then redissolved in 75 ml of water. The water was extracted twice with 75-ml volumes of benzene. The benzene was discarded, and the water phase was reduced to 50 ml on a rotary evaporator so that 1 ml equalled the extract from 1 g of dried plant. Seed was processed as above except that 40 g was used rather than 50.

#### Chick Bioassy

One-week-old chicks (avg wt=70 g) were held overnight without food or water. At 8 a.m. they were dosed with alfombrilla extract that was introduced into the crop by a catheter. Two birds each were dosed with 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 ml of extract. After treatment, the birds were given food and water free choice. The chicks were observed for toxic signs, and the LD<sup>50</sup> (lethal dose for a 50% kill) was determined.

#### Results and Discussion

Saponin level in alfombrilla was greater than 3% each month except January (Table 1). Saponin level of alfombrilla increased throughout the growing season and peaked in September when seed was mature. An unusually high saponin level recorded in May

Table 1. Saponin level and toxicity of alfombrilla<sup>1</sup> collected during 1977 in Chihuahua, Mexico.

Month collected	% saponin (dry wt)	Stage of growth	LD <sup>50</sup> ml <sup>2</sup>
January	2.8	Dormant	4.0
February	3.1	Dormant-early vegetative	3.0
March	3.5	Vegetative	2.0
April	3.6	Flowering	2.0
May	5.3	Flowering	1.5
June	3.7	Seed formation	2.0
July	4.2 .	Seed formation	1.5
August	5.2	Seed mature or dispersed	1.5
September	6.3	Seed mature or dispersed	1.0
October	4.1	Seed mature or dispersed	1.5
October	1.3	<del></del>	4.0
November	6.0	Dormant	1.5
December	6.0	Dormant	1.5

<sup>1</sup>Aerial parts were tested each month except October, when both aerial parts and mature seed were tested.

<sup>21</sup> ml=saponin from 1 gram of dried plant.

was reflected in increased toxicity to chicks. Saponin level in alfombrilla collected in November and December remained high, and extracts from these collections were highly toxic to chicks. Seeds were low in saponins.

Alfombrilla was very toxic from early spring growth in February through December, when plants were dormant. Saponins were relatively stable in dormant alfombrilla so that plants remained poisonous well into the winter months.

The amount of alfombrilla required for LD<sup>50</sup> in chicks was inversely proportional to the saponin level in the plants. Chicks given a minimum lethal dose became depressed and stood with feathers ruffled and eyes closed within 1 hour of dosing. They usually became comatose within 2 to 4 hours and died shortly thereafter

If alfombrilla contains 3% saponins, sheep are acutely poisoned at 0.5% of body weight or at approximately 150 mg of saponins/kg of body weight. If chicks are acutely poisoned by alfombrilla at an equivalent of 2 grams of plant or less, the saponins in the plant exceed 3% (Table 1). Thus, the chick bioassay can be used to approximate the saponin content and the relative toxicity of the plant to livestock.

Alfombrilla poisoning of livestock in Chihuahua is particularly serious in late summer and early fall (Sánchez Muñooz 1978). Saponin levels are highest then and the plant becomes more attractive to cattle since grasses are largely dormant.

Since alfombrilla is toxic at all stages of growth, careful

management of livestock and range is necessary to avoid losses. Studies by Jacoby and Morton (1974) indicate that alfombrilla can be controlled by 2,4,5-T in 0.5 solution. Alfombrilla can be effectively controlled by deferred rotation grazing or resting infested pastures one or two productive growing seasons to allow for recovery of grasses.

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