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TERATOGENIC COMPOUNDS OF *VERATRUM CALIFORNICUM* AS A FUNCTION OF PLANT PART, STAGE, AND SITE OF GROWTH*

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Abstract—The level of the teratogen cyclopamine in *Veratrum californicum* varied considerably among plants from various collection sites. The variation was not correlated with differences in soil type, pH, soil nutrients, elevation, drainage, or sunlight. However, marked variation in both total alkaloid and percentage cyclopamine occurred as a function of stage of growth of the plant. The levels of both were highest in early growing season in the leaves, in midgrowing season in the stems and in late growing season in the root/rhizome system.

INTRODUCTION

THE PLANT *Veratrum californicum* (Durand), when ingested by pregnant sheep on day 14 of gestation, causes cyclopian and related cephalic malformations in the offspring.[1] We characterized the primary teratogen, which proved to be a steroidal alkaloid, and gave it the trivial name, cyclopamine.[2] Clinical and field observations[3] suggested a marked variation in teratogenic potential of the plant parts from various sites and growing periods suggesting a variability in the concentration of the teratogen. The work described herein was undertaken to examine the content of cyclopamine as a function of plant part, stage of growth, site of growth and as related to various natural environmental parameters. It was anticipated that these data would provide some insight into plant synthesis of teratogen. The information would also assist in determining the best site and time for plant collections to secure maximum teratogen yield and suggest the most hazardous grazing periods for livestock.

RESULTS

A number of *V. californicum* collections were made in areas of heavy stands of the plant in Utah and Idaho, U.S.A., when the plant at each site was at approximately the same growth stage—just before flowering. Cyclopamine was determined in the benzene extractable alkaloid fraction in leaves, stems and root/rhizome material from each collection site and considerable variability among sites was noted (Fig. 1). Percentage of cyclopamine in the root material was particularly high in the Muldoon collection with moderately high levels in the Franklin, Logan, Coulter, and Uintah collections. Percentage of cyclopamine in leaves was particularly high in the Logan, Uintah, and Raft collections, and percentage in stems high in the Kamas, Muldoon, and Logan collections. Total alkaloid concentration in the benzene extract in all collections was approximately the same (within 30%) and averaged near 0·35 g/100 g dry material.

* Part XII in the series "Teratogenic Compounds of *Veratrum Californicum*".

[1] W. BINNS, J. L. SHUPE, R. F. KEELER and L. F. JAMES, *J. Am. Vet. Med. Assoc.* **147**, 839 (1965).
[2] R. F. KEELER, *Phytochem.* **8**, 223 (1969).
[3] R. F. KEELER and W. BINNS, unpublished observations.

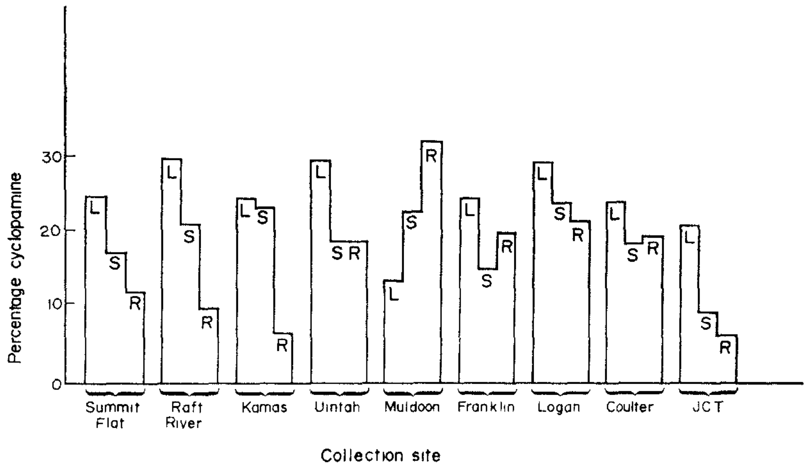

FIG. 1. CYCLOPAMINE AS A PERCENTAGE OF THE TOTAL ALKALOIDS (CYCLOPAMINE + VERATRAMINE + ALKALOID Q) IN THE LEAVES, STEMS AND ROOTS OF PLANT SAMPLES FROM VARIOUS COLLECTION SITES

No relationship was evident between the cyclopamine percentage in the root or in leaves and the soil type, pH, nutrient level, elevation, drainage, or sunlight (Table 1).

Successive collections during the growth period were made at two of the most accessible sites—Logan and Franklin to determine the seasonal fluctuation in total alkaloid and per cent cyclopamine. It is evident that a marked seasonal fluctuation in total alkaloids occurred Concentration of total alkaloids (Figs. 2 and 3) was highest in the leaf in late June, in the stem in early to middle July and in the root in middle August. The leaf peak was highest when the plant was 1·5–3 ft high, stem peak when the plant was 3·5–4 ft high, and the root peak when the plant was > 4 ft high and drying markedly. Figures 4 and 5 show that the percentage of the total alkaloid attributable to cyclopamine followed approximately this same general trend with leaf and stem highest in the early periods and root highest in August.

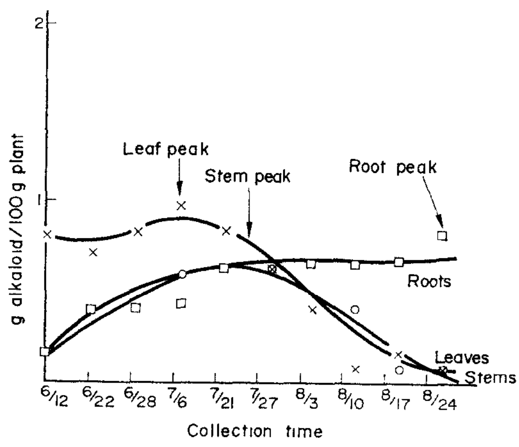

FIG. 2. TOTAL BENZENE SOLUBLE ALKALOIDS IN *V. californicum* COLLECTED AT THE FRANKLIN SITE AS A FUNCTION OF COLLECTION TIME.

TABLE 1. RELATIONSHIP BETWEEN THE % CYCLOPAMINE IN THE LEAVES AND IN THE ROOTS TO VARIOUS ENVIRONMENTAL PARAMETERS

Site	% Cyclop. in leaves	% Cyclop. in roots	Soil type			pH	Buffered	Available major nutrients (lbs/acre)			Elevation (ft)	Drainage	Sunlight
			Sand	Silt	Clay			N	P	K			
Summit	25·0	12·5	68	20	12	5·0	5·8	91	270	7400	7600	fair	fair
Raft	30·0	9·5	42	34	24	5·7	6·5	75	71	7400	6650	fair	good
Kamas	25·0	8	44	30	26	6·4	7·0	86	53	385	6200	poor	good
Uintah	31·0	19·5	36	30	34	5·6	6·0	152	33	267	8550	poor	fair
Muldoon	14·0	30	66	20	14	5·4	6·1	77	89	242	8500	good	good
Coulter	25·0	19·5	66	20	14	5·8	6·2	119	122	7400	7200	excellent	good
Jct.	22·0	6·5	68	16	16	6·0	6·6	75	315	7400	6000	good	good

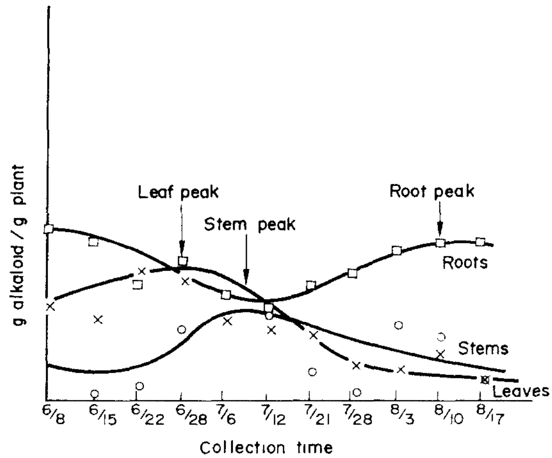

FIG. 3. TOTAL BENZENE SOLUBLE ALKALOIDS IN *V. californicum* COLLECTED AT THE LOGAN SITE AS A FUNCTION OF COLLECTION TIME.

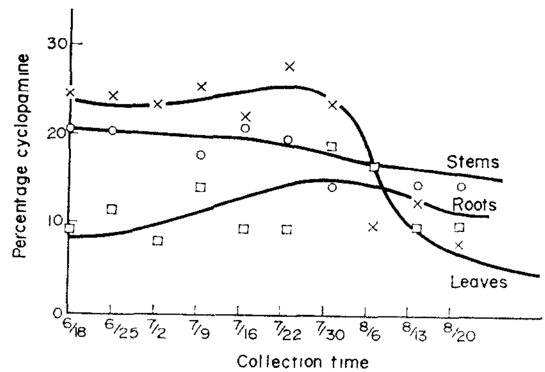

FIG. 4. CYCLOPAMINE PERCENTAGE OF THE TOTAL ALKALOIDS (CYCLOPAMINE + VERATRAMINE + ALKALOID Q AS A FUNCTION OF COLLECTION TIME ON PLANTS FROM THE FRANKLIN SITE

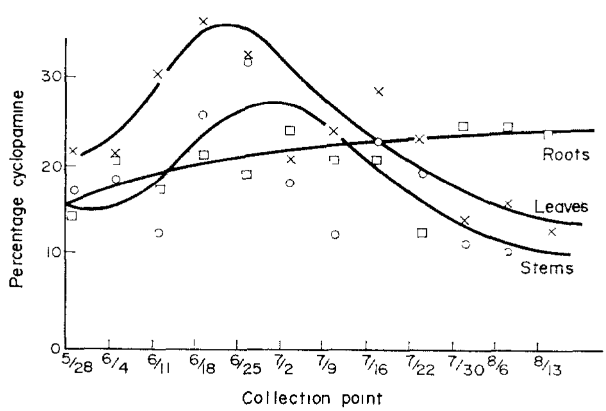

FIG. 5. CYCLOPAMINE PERCENTAGE OF THE TOTAL ALKALOIDS (CYCLOPAMINE + VERATRAMINE + ALKALOID Q) AS A FUNCTION OF COLLECTION TIME IN PLANTS FROM THE LOGAN SITE.

DISCUSSION

Observations over many years of feeding trials have shown considerable variability in teratogenic effects of *V. californicum*. The plant material fed varied in collection site and growth stage. The data herein suggest that some of the variability was no doubt due to site and to the stage of growth of the plant when the collection was made. Thus an early July collection of leaves and stems, when the plant is about 2·5–3 ft high, would probably be highest in teratogenicity because of the high cyclopamine content. Roots have the highest cyclopamine content after the middle to late August after the leaves have started to brown. The relative hazard of seeds was not determined; seed setting is irregular and did not occur the year these collections were made. The data of Figs. 2 and 3 also suggest that the alkaloids of this plant are synthesized in the leaves and translocated to the roots/rhizome system for storage, although tracer experiments are needed for verification.

EXPERIMENTAL

Composite plant collections of leaves, stems or root/rhizome material were air-dried, ground to a powder and stored at 3°. The collections were made at various sites in the following national forests: Cache (Franklin and Logan sites), Boise (Coulter, Jct. and Summit flat sites), Uintah (Kamas and Uintah sites), Challis (Muldoon site), Sawtooth (Raft River site). Portions were used to determine oven-dry weight and for extraction for total alkaloids and cyclopamine determination.

Total alkaloids were determined as follows. 10 g was extracted with 100 ml of a 5:1 emulsion of benzene–5% NH_4OH. The benzene was filtered off after 4 days whereupon an additional 100 ml of benzene was then allowed to percolate through the plant material. The combined benzene layers were evaporated to dryness, taken up in 5 ml $CHCl_3$ and 150 ml 1% H_2SO_4, swirled exhaustively and allowed to stand 24 hr. The acid layer was basified to pH 9·5 with NaOH and extracted with 10 ml and then 5 ml $CHCl_3$. 0·5 ml EtOH was added and the resultant aqueous layer discarded. The $CHCl_3$ layer was then evaporated and the solid taken up in exactly 1·5 ml of $CHCl_3$. An aliquot was partitioned into 5 ml of 1% H_2SO_4 to which two drops of Mayer reagent* was added. The alkaloid concentration was determined by measuring the turbidity at 530 nm against a standard curve. Cyclopamine is not stable in 1% H_2SO_4 and undergoes conversion to veratramine,[4] but this occurs on a 1 for 1 basis and both give the same total alkaloid value on a weight basis. Therefore, there is no effect on total alkloid level by the cyclopamine conversion.

Percentage cyclopamine was determined as follows. A portion of the plant material was extracted as above but without acid partition. An aliquot of the extract was dried, dissolved in 70% EtOH and extracted with light petroleum. The EtOH layer was dried and taken up in a 1:1 benzene–MeOH for TLC. TLC was carried out on activated silica gel layers of 0 6 mm thickness and developed in a 2:1 benzene–MeOH mixture. The separated alkaloids were quantitatively estimated by spraying the plate with 0·1% bromphenol blue in MeOH–acetone (1:1), followed by drying, spraying with 4% NaH_2PO_4 in 50% MeOH (1:1), followed again by drying and a final spray of 5% Na_2HPO_4. The spots were photographed (3·25 × 4 Polaroid transparency) and the concentration of separated alkaloids determined by densitometry on the photograph. The three primary alkaloids in the benzene extract were cyclopamine, veratramine, and alkaloid Q.[5] The variable total ($\cong$ 10%) of minor alkaloids was ignored.

Composite soil samples were oven-dried and tested for pH, soil type, and major nutrients as follows. Soil pH was determined on a 1:2 suspension of soil in H_2O, and buffered pH was determined by the method of Schoemaker *et al.*[6] Available P, K and N were determined by conventional techniques.[7] Soil type was determined by the hydrometer technique.[8]

Observations were made at each collection site on elevation, general drainage (terrain) and sunlight (proximity of trees, slopes, etc.).

* 1·36 gm $HgCl_2$ and 5 gm KI in 100 ml H_2O.

[4] R. F. Keeler, *Teratology* **3**, 175 (1970).
[5] R. F. Keeler, *Phytochem.* **7**, 303 (1968).
[6] H. E. Schoemaker, E. O. McLean, and P. F. Pratt, *Soil. Sci. Soc. Am. Proc.* **25**, 274 (1961).
[7] Anon, Soil testing methods, Iowa State University Soil Testing Laboratory. Sept. 1963.
[8] V. J. Kilmer and L. T. Alexander, *Soil Sci.* **68**, 15 (1949).