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Pyrrolizidine alkaloid content of houndstongue (*Cynoglossum officinale* L.)

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Abstract

Houndstongue (*Cynoglossum officinale* L.) is a biennial weed infesting pasture, hayfields, and disturbed areas throughout North America. Houndstongue contains pyrrolizidine alkaloids (PAs) that are hepatotoxic. First and second year's growth of houndstongue were harvested from emergence to maturity. Nuclear magnetic resonance was used to determine the levels of total PAs, free base, and N-oxide forms of the alkaloids in leaves, stems, buds, flowers, and pods. PA levels generally were highest (1.5 to 2.0% dry weight) in immature plant tissue, with a gradual decline during maturation. Most plant parts contained greater quantities of the N-oxide form of PAs (60–90%) compared to the free base form. Leaves and pods of mature houndstongue contained sufficient PAs to be potentially toxic if ingested by livestock.

Key Words: plant alkaloids, poisonous plants, livestock feeding

Houndstongue (*Cynoglossum officinale* L.) is a poisonous weed native to Europe and Asia, that has been introduced into much of North America (Dickerson and Fay 1982, Welsh et al. 1987, Upadhyaya et al. 1988, Upadhyaya and Cranston 1991). It has been implicated in the deaths of cattle (Greatorex 1966, Baker et al. 1989) and horses (Knight et al. 1984) in England, Russia, and in the western U.S. Houndstongue is biennial and grows from 0.3 to 1.2 m tall in pastures, disturbed areas, and along roadsides (Whitson 1987). The first year's growth typically forms a robust rosette, with large, rough leaves (15 to 20-cm long, and 2 to 7-cm wide) resembling a canine tongue, hence the common name. Although the green plant is generally considered to be unpalatable to livestock, the plant is readily consumed when dry or harvested with hay (Baker et al. 1989).

The toxins in houndstongue are pyrrolizidine alkaloids (PAs) (Mattocks 1986). These toxins are found in other plant species worldwide (e.g., *Senecio, Crotalaria, Eupatorium, Amsinckia, Heliotropium*). Many PA-containing plants are cytotoxic, with liver (hepatic) toxicity of primary concern (Mattocks 1986). Ingested PAs are converted by the liver to toxic pyrrolic metabolites in the mixed function oxidase (cytochrome P-450) system of the endoplasmic reticulum. Pyrrolic metabolites are toxic, accumulate in the liver, cause damage to nuclear DNA, and inhibit cytoplasmic protein synthesis (Mattocks 1986). Houndstongue contains 4 individual alkaloids: 7-angelylheliotridine, echinatine, heliosupine, and acetylheliosupine (Mattocks 1986). All except the latter have been tested for toxicity in their purified form in rats (Bull et al. 1968). Heliosupine, the predominant alkaloid in the plant, was found to be 4–6 times more toxic than the other alkaloids (ca. LD_{50} for heliosupine is 60 mg/kg, Mattocks 1986).

Information on PA content of houndstongue is important for predicting potential toxicity problems in livestock consuming the plant. There is little information available on seasonal levels of PAs in various plant parts of houndstongue. Knight et al. (1984) measured PA levels in immature and mature houndstongue, but beyond this no information is available. The purpose of this study was to characterize the PA level (N-oxide and free base forms) of houndstongue plant parts over the growing season. Both free base and N-oxide content were determined because of the potential differences in toxicity.

Materials and Methods

Plant Collection and Preparation

Individual plant parts (i.e., leaves, stems, buds, flowers, and pods) were collected periodically from first and second year's growth of at least 10 individual houndstongue plants, near Logan, Utah, from April to late June, 1990. The collection site had been severely overgrazed in past years, but was not being grazed when our collections occurred. Soils in the collection area are moderately well drained with a loam to a silty clay subsoil; the collection site is level or gently sloping (0–3%) at elevations of 1,300 to 1,400 m with annual precipitation of 35–43 cm (SCS 1974). The vegetation on undisturbed areas often consists of western wheatgrass (*Elymus smithii* [Rydb.] Gould), Great Basin wildrye (*Elymus cinereus* Scribn. & Merr.), and saltgrass (*Distichlis spicata* [L.] Greene).

The freshly sampled material was pooled by plant parts from the different individual plants, immediately frozen at –20° C, and freeze-dried. Dry plant material was ground to pass through a 1-mm screen in a Wiley mill.

Alkaloid Analysis

The general procedure for analysis was that used for PAs in *Senecio* spp. (Molyneux et al. 1979). Briefly, the method entailed exhaustive overnight extraction with methanol of a weighed amount of plant material using a Soxhlet apparatus and evaporation of the extract to dryness using a rotoevaporator. After dissolving in 2N hydrochloric acid and extraction with ether, the residue was divided into equal portions, and the first portion basified, then extracted with chloroform and evaporated to dryness. The second portion was reduced with zinc dust. The portion treated with zinc dust gives a measure of total PA content since the N-oxides are reduced to the corresponding PAs; the unreduced (first) portion provides a measure of free base alkaloids. The N-oxide content is calculated from the difference between the total and free base content. Concentrations of total and free base PAs were determined from nuclear magnetic resonance (NMR) spectra, using a known weight of p-dinitrobenzene as an internal standard. The total PA content was calculated as heliosupine, since it is the dominant alkaloid in houndstongue (Mattocks 1986, Knight 1984).

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Table 1. Total pyrrolizidine alkaloid content[1] (% of dry weight), free base alkaloid content, N-oxide content, and proportion (%) of N-oxide of total pyrrolizidine alkaloid composition in first year's growth of houndstongue during 1990 in Utah.

Date	Item	Total PAs	Free base	N-Oxide	N-Oxide- % of total PAs
		--------------	%	--------------	
5 Apr.	Leaves	1.73	0.32	1.41	82
18 Apr.	Leaves	1.31	0.58	0.73	56
3 May	Leaves	0.91	0.29	0.62	68
18 May	Leaves	1.08	0.52	0.56	52
1 Jun.	Leaves	0.96	0.35	0.61	63
16 Jun.	Leaves	0.45	0.16	0.29	64
27 Jun.	Leaves	0.89	0.36	0.53	60

[1]Analytical variation for duplicate samples was ± 0.01%.

Results and Discussion

Total PA levels were highest in immature leaves of houndstongue (Tables 1 and 2). Immature first and second year leaves contained PA levels at or near 2% of dry weight, and leaf PA levels declined by 50% or more with maturation. Generally, but not always, the majority (60 to 90%) of the leaf pyrrolizidine alkaloids were in the N-oxide form (Tables 1 and 2).

Table 2. Total pyrrolizidine alkaloid content[1] (% of dry weight), free base alkaloid content, N-oxide content, and proportion (%) of N-oxide of total pyrrolizidine alkaloid composition in second year's growth of houndstongue during 1990 in Utah.

Date	Item	Total PAs	Free base	N-Oxide	N-Oxide- % of total PAs
		--------------	%	--------------	
5 Apr.	Leaves	1.89	0.31	1.58	84
18 Apr.	Leaves	2.12	0.74	1.38	65
3 May	Leaves	1.05	0.71	0.34	32
18 May	Leaves	0.84	0.42	0.42	50
	Stems	1.29	0.32	0.97	75
	Buds	1.27	0.44	0.83	65
1 Jun.	Leaves	0.31	0.06	0.25	81
	Stems	0.56	0.21	0.35	63
	Flowers	1.36	0.30	1.06	78
16 Jun.	Leaves	1.20	0.17	1.03	85
	Stems	0.13	0.04	0.09	69
	Flowers[2]	0.12	0.05	0.07	58
27 Jun.	Leaves	0.68	0.02	0.66	97
	Stems	0.10	0.09	0.01	10
	Pods	1.00	0.29	0.71	71

[1]Analytical variation in duplicate samples was ± 0.01%.
[2]Flowers were in late bloom in transition to pod stage.

Stem material in the second year's growth showed a large decline in PA content with maturation, and by the late flower stage stems contained small amounts of PAs (Table 2). Most (>60%) of the stem PAs were in the N-oxide form until maturity, when virtually all PAs were in the free base form. Buds and flowers in full bloom contained substantial quantities of PAs (>1%), but late bloom flowers were low in PA content until the pods set. As with the other plant parts, buds and flowers also contained more N-oxide than free base alkaloids.

The total PA content of houndstongue leaves was generally much higher than found for ragwort (*Senecio jacobaea* L.), about equal to threadleaf groundsel (*S. longilobus* Benth.), but much lower than Riddell's groundsel (*S. riddellii* T & G), as reported by Molyneux et al. (1979) and Johnson et al. (1985a). Unlike *Senecio* or *Crotalaria* spp. (Johnson et al. 1985a), houndstongue leaf PA content did not peak at the bud or flower stage, but was highest in early growth stages. Similar to the findings of Johnson et al. (1985a) and Molyneux et al. (1979), houndstongue leaves generally contained more alkaloid in the N-oxide than in the free base form. It is difficult to compare the PA content of houndstongue plant parts other than leaves. Other studies generally have examined alkaloids in whole plants or mixed plant parts (e.g., Molyneux et al. 1979, Johnson et al. 1985a). Knight et al. (1984) found that houndstongue contained 2.1% and 0.6% PAs in the rosette and mature stages, respectively.

Because much of the PAs in houndstongue occur in the N-oxide rather than free base form, the comparative toxicity of N-oxide vs. free base alkaloids must be considered. Mattocks (1986) has emphasized that PAs are toxic only after being metabolized by liver mixed function oxidases (MFOs) to pyrrolic intermediates. PAs in the form of N-oxides are not converted by MFOs to pyrrolic metabolites, and before N-oxides can be converted to toxic pyrroles, they must first be reduced to the free base form (Mattocks 1986). This conversion occurs in the gastrointestinal tract in animals, but it is unknown how much reduction of N-oxide to free base occurs in the rumen (Molyneux et al. 1988). Recent experiments in which pure riddelliine and riddelliine N-oxide isolated from *Senecio riddellii* were fed to calves, individually and in combination, indicated that both free base and N-oxide forms caused intoxication at equivalent dose rates (Molyneux et al. 1991). However, it is unclear how much of the toxicity of houndstongue can be ascribed to the N-oxide form of the alkaloids, and until further information is forthcoming, we must assume virtually complete conversion from the N-oxide to the free base form in livestock.

Although grazing studies have not determined consumption by free-grazing livestock, there is no question that houndstongue is at least as toxic as *Senecio* spp. to cattle and horses. Baker et al. (1991) conducted the first controlled feeding study with houndstongue to determine toxicity to calves. The houndstongue plant used in the study of Baker et al. (1991) contained 0.73% total PA. Ingestion of 60 mg PA/kg body weight (about 1 kg air-dry plant weight) was fatal to all calves within 48 hours due to massive liver failure. A chronic dose of 15 mg PA/kg (about 400 g of plant daily) for 21 days caused moderate, but eventually fatal hepatic damage. It seems reasonable to assume that cattle consuming hay contaminated with houndstongue could ingest from 400 to 1,000 g daily for a number of days, leading to hepatic failure and subsequent death (Baker et al. 1989).

Knight et al. (1984) fed ground houndstongue to a pony at the rate of 60 g/kg body weight for 20 days; when later euthanized, the pony had liver damage suggestive of PA toxicosis. Younger animals such as the calves in the study of Baker et al. (1991) may be much more susceptible compared to mature animals such as the pony in Knight's study (Mattocks 1986).

Intoxication from PAs has a cumulative effect on the liver over time (Mattocks 1986), and the threshold between no-effect level and a chronically intoxicating dose is unknown for houndstongue. In *Senecio* spp., the threshold level has been estimated at 2.5–3.0 mg PA/kg body weight/day for *S. jacobaea*, 10–13 mg/kg/day for *S. longilobus*, and 15–20 mg/kg/day for *S. riddellii*, when fed for 20 days (Johnson et al. 1985b). Therefore we assume that because houndstongue contains substantial levels of PAs during early growth and at maturity, there is no safe exposure level for livestock. Producers with houndstongue in pastures and hayfields should excercise due caution to ensure that livestock do not consume the plant. The greatest danger probably lies with mature, senescent houndstongue on rangelands, and from dried plant in hay (Baker et al. 1989). If animals begin to consume houndstongue in pastures, they should be quickly removed. Contaminated hay should not be fed to livestock.

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