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Chemistry of Toxic Range Plants. Variation in Pyrrolizidine Alkaloid Content of *Senecio*, *Amsinckia*, and *Crotalaria* Species

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The free base and *N*-oxide pyrrolizidine alkaloid contents of a number of *Senecio*, *Amsinckia*, and *Crotalaria* species, which occur as range plants in the United States, have been measured in order to study variations with season, location, and species. Sampling on a monthly basis during the growing season, carried out for a 3-year period, showed considerable inter- and intraspecies variation in alkaloid content. In most species the total alkaloid content reached a maximum at the preflower or early bud stage.

Range plants that contain hepatotoxic pyrrolizidine alkaloids (PAs) cause considerable economic loss to the livestock industry (Mathews, 1933; Snyder, 1972). Cattle and horses are most often affected but other animals are susceptible (Bull et al., 1968). Sheep, however, appear to be quite resistant to the toxic effects, and their use as a control for these plants by heavily grazing infested ranges has been proposed. The liver is the primary target of the alkaloids and is usually affected chronically, causing a cirrhosis-like condition. Lungs and other organs and tissues may also be insulted (McLean, 1970). The insidious nature of the toxicosis, whereby signs of poisoning and subsequent death of the animal may not be seen for many months after the plant is ingested (Johnson, 1978), results in a large proportion of deaths and illnesses being un-

diagnosed. The problem is therefore of much greater magnitude than is generally recognized.

Although sporadic episodes of human poisoning and loss of life from PA-contaminated grains have occurred in the past, recent deaths in the United States caused by using *Senecio* species as medicinals (Stillman et al., 1977) and the detection of PAs in certain herbal teas (Culvenor et al., 1980; Roitman, 1981) have focused attention on this problem. Such occurrences, coupled with the detection of PAs in honey (Deinzer et al., 1977; Culvenor et al., 1981) and the potential for PA contamination of milk (Dickinson et al., 1976; Deinzer et al., 1982) have caused increased concern in the PA content of plants and renewed interest in the mechanisms of action of these toxins.

PAs occur in numerous plant species and many genera, but they are found primarily in the families Compositae, Leguminosae, and Boraginaceae (Smith and Culvenor, 1981). The majority of PA-containing plants in the United States are *Senecio* species, and only a few of these are widespread in locations where they can present a problem to either livestock or man. Nevertheless, any plant producing toxic PAs is a potential hazard to animals that may graze it, to unsuspecting but well-meaning individuals who

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may use it or sell it for medicinal purposes, and to people or animals that may consume it unintentionally as a contaminant of their food. Plants that grow in the United States that have the ability to cause toxicity in animals due to their PA content, listed in probable order of adverse economic impact, are *Senecio jacobaea*, *Senecio douglasii*, *Senecio riddellii*, *Senecio vulgaris*, *Senecio plattensis*, *Senecio spartioides*, *Crotalaria spectabilis*, *Crotalaria saggitalis*, and *Amsinckia intermedia*.

S. jacobaea (tansy ragwort) is widely established in the world from western Europe to Australia, New Zealand, and North America, where it is an introduced species. In the United States, it occurs in the coastal Northwest from the Canadian border to northern California and in some areas of coastal New England. The plant is a tall, weedy biennial that inhabits marginal forest lands and has invaded pastures and hay fields. Tansy ragwort is difficult to control in many such areas because of annual reseeding from plants in nearby forested areas. It causes many cattle (Snyder, 1972) and horse losses, presents a continuing costly control problem, and is a constant threat as a possible source of contamination of the human food chain (Dickinson et al., 1976; Deinzer et al., 1977).

S. douglasii var. *longilobus*, most often referred to as *Senecio longilobus* (which will be used hereafter for brevity), is known by the common names of threadleaf and woolly groundsel. It is a low, woody perennial that grows throughout much of the southwestern United States. As with other toxic *Senecios*, livestock losses are sporadic and occur when animals are forced to eat the plant because of a lack of availability of more desirable forage. However, losses can be great when they occur, due to the large numbers of beef cattle on ranges where the plant grows (Mathews, 1933; Johnson and Molyneux, 1984). The economic impact of this plant is difficult to assess since many losses from it undoubtedly are not recognized. Similarly, human poisonings by this plant, which has been used medicinally in localized areas for many years, are extremely difficult to define. The recent documentation of two infant deaths in Arizona, caused by a medicinal tea made from threadleaf groundsel (Stillman et al., 1977), has drawn attention to the problem of PA toxicosis in humans due to this plant and other herbal remedies.

S. riddellii (Riddell's groundsel) has not been evaluated as to its economic impact but was incriminated over 50 years ago as the plant responsible for "walking horse disease" in Nebraska (Van Es et al., 1929) and was also shown to be toxic to cattle. Apart from its bright green color, it is similar in appearance to *S. longilobus*. The plant grows in waste areas of sandy soil in a belt that extends from northwest and north central Nebraska southward through western Kansas and eastern Colorado into Oklahoma, western Texas, and New Mexico. The very high PA content of this plant (Molyneux et al., 1979) gives it great potential for toxicity even though at present it is thought to be a serious problem to livestock in only a few localized areas. Stockmen now recognize the problem, and their control efforts have resulted in *S. riddellii* becoming scarce in many parts of its range (Barkley, 1978).

Qualitative analyses of PAs in the above *Senecios*, and in many *Crotalaria* and *Amsinckia* species, have been made (Bull et al., 1968), and levels of PAs in leaves, stems, flowers, and seeds of a number of *Senecio* species have been measured (Molyneux et al., 1979; Roitman et al., 1979). However, there are no reports of periodic quantitative analyses on the fluctuation of PA content in the various plants that would indicate when they are most or least toxic. Such information is essential for those investigating

various aspects of PA toxicosis, especially animal feeding studies, and to provide rangeland management recommendations to livestock producers with animals at risk from exposure to these plants.

We have therefore measured the PA content of *S. jacobaea*, *S. longilobus*, and *S. riddellii* on a monthly basis over a 3-year period from selected sites in order to determine the variation throughout the growth cycle and with location. In addition, other PA-containing plants were analyzed on an irregular basis, when obtainable, in an attempt to define their potential as a toxicity hazard for livestock and humans.

EXPERIMENTAL SECTION

Sample Collection and Preparation. The collection sites were visited, and the identification and presence, in sufficient quantity for purposes of the experiment, of the desired species was confirmed. Collectors were then selected who consisted primarily of university faculty or students in range science or botany or federal professionals in botany-related fields. Nonprofessionals were used as collectors in certain remote areas.

Each collector was supplied with shipping cartons and instructions to either completely or partially air-dry each sample in a shady protected place before shipment. Data sheets were provided for the collectors to indicate the approximate number of plants sampled, the general atmospheric conditions, plant growth stage, plant condition, and any other information that they deemed pertinent. Collections were made as near the same time each month as possible (generally at the first of each month), and individual plants were not sampled twice in the same season. Voucher specimens of the species from each location were collected and filed in the Intermountain Herbarium at Utah State University, Logan, UT.

Plant samples were given an accession number when received at the Poisonous Plant Research Laboratory and, if necessary, were further dried at room temperature. Leaves were stripped from the stems and ground in a Wiley mill to pass a 1-mm screen. Other plant parts taken at selected times were also ground to 1 mm for PA analysis. Limited PA analyses of green *S. longilobus* and *S. riddellii* were made to determine if there were differences in alkaloid level between green and dried plant.

Analysis for Alkaloids. Ground plant material was extracted with MeOH in a Soxhlet apparatus and the total PA and free base PA content determined for each sample by the previously described NMR method (Molyneux et al., 1979). PA N-oxide content was calculated from the difference between total PA and free base PA content.

RESULTS AND DISCUSSION

As originally designed, the intent of this study was to sample four *Senecio* species (*jacobaea*, *longilobus*, *riddellii*, and *spartioides*), together with *C. spectabilis* and *A. intermedia*, each month throughout three growth cycles at five widely scattered locations across the range of each species. Figure 1 shows the location of the collecting sites established for each species at the commencement of the study. Due to the logistics of collecting plants from so many locations throughout the United States and the vagaries of climatic conditions, the original plan to sample the above species for three complete growing seasons was modified, as the experiment progressed, so that some were sampled for shorter periods. Thus three species, *S. jacobaea*, *S. longilobus*, and *S. riddellii*, considered to be the most important, were concentrated upon for continued study in 1979, 1980, and 1981.

Similar considerations applied to the choice of the plant part to be analyzed. Although the collection of a number

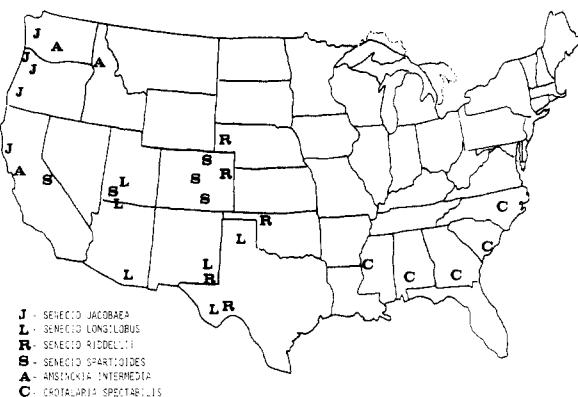


Figure 1. Collecting sites of plants analyzed for pyrrolizidine alkaloids.

of plant species presented an excellent opportunity to study alkaloid variation in the plant parts at the same time, the field portion of the experiment had to be kept as uncomplicated as possible because of the large area covered, numerous contacts to be maintained, long distances to be traveled in remote western areas, and extended wet periods in the South and Northwest that interfered with collecting and drying samples. Furthermore, handling and analyzing multiple samples from each collection were not economically or physically possible with the facilities and instrumentation available. For these reasons the leaves of the plants were chosen for analysis, since they are most likely to be eaten by range animals. However, *Crotalaria* seeds were also collected and analyzed since they are known to poison poultry (Bull et al., 1968).

The *Senecio* species examined in this study contain one or more of the PAs 1–8, all possessing 1,2-unsaturation in the heterocyclic ring, which is an essential structural fea-

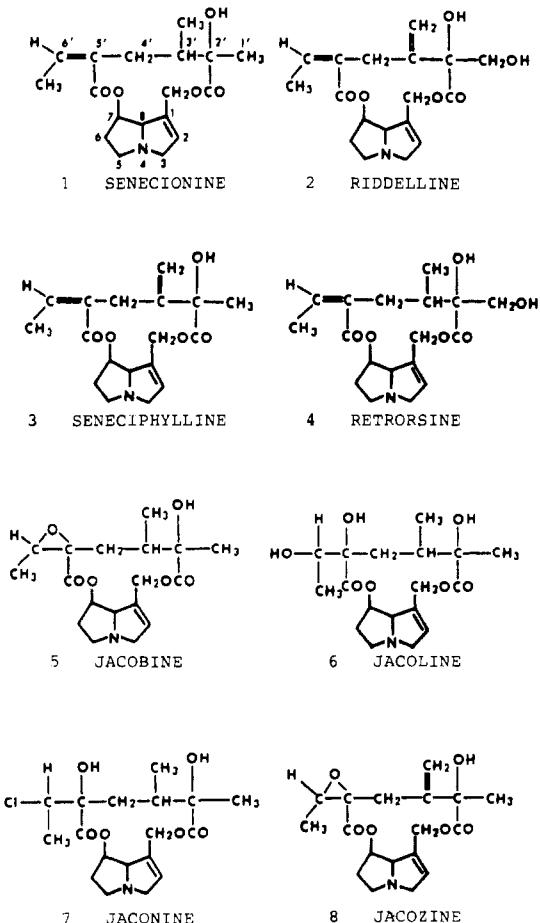


Table I. Pyrrolizidine Alkaloid Content and Composition in Leaves of *Senecio* Species Collected Monthly during 1979–1981.

species	collection site	PA content ^a		PA composition ^b	
		range	mean	free base	N-oxide
<i>S. jacobaea</i>	Canby, OR ^c	0.02–0.70	0.25	68	32
	Roseburg, OR	0.17–0.91	0.42	71	29
	Tillamook, OR ^c	0.16–0.46	0.29	79	21
	Tenino, WA ^c	0.19–0.54	0.39	77	23
	Fort Bragg, CA ^c	0.03–0.42	0.21	71	29
	mean	0.11–0.61	0.31	73	27
<i>S. longilobus</i>	Sonoita, AZ ^d	0.78–8.70	2.67	16	84
	Alpine, TX	0.40–5.54	2.62	20	80
	Canyon, TX	0.11–3.19	1.10	21	79
	Monroe, UT ^c	1.24–6.97	3.46	17	83
	Jal, NM ^e	0.25–5.25	1.98	22	78
	Colorado City, AZ ^e	0.40–2.81	1.33	18	82
<i>S. riddellii</i>	mean	0.53–5.41	2.19	19	81
	Woodward, OK ^f	2.98–17.99	7.90	21	79
	Scottsbluff, NE	2.14–12.37	6.30	12	88
	Alpine, TX	0.18–9.18	4.08	21	79
	Jal, NM ^e	0.45–10.83	4.58	25	75
	Burlington, CO	2.36–15.06	9.16	11	89
	mean	1.62–13.09	6.40	18	82

^a As percent of dry weight. ^b As percent of total PA. ^c Two seasons of collections. ^d Four seasons of collections. ^e One season of collection. ^f Five seasons of collections.

ture for hepatotoxicity. Since the NMR analysis method depends upon measurement of the H-2 proton intensity relative to the signal for an internal standard of *p*-dinitrobenzene (Molyneux et al., 1979), only PAs having 1,2-unsaturation can be detected. Saturated PAs are not detected by this method, but because they are not hepatotoxic their noninclusion in the measurement is advantageous for our purposes. *C. spectabilis* contains monocrotaline, which has an 11-membered macrocyclic ring rather than the 12-membered ring typical of the *Senecio* alkaloids (Adams and Rogers, 1939), while *A. intermedia* contains nonmacrocyclic PAs (Culvenor and Smith, 1966), all of which contain the 1,2-unsaturation requisite for detection.

The range and mean total PA contents for the three major *Senecio* species, collected over a 3-year period from 16 different sites, are summarized in Table I. *S. jacobaea* contained the lowest amount of total PA (free base plus *N*-oxide) with a mean 3-year value from five locations of only 0.31%. This contrasts with a 3-year mean PA content of 2.19% in *S. longilobus* from six locations and 6.40% in *S. riddellii* from five locations. In general, therefore, *S. riddellii* is the most potent PA producer, with a level 21 times that of *S. jacobaea*, whereas *S. longilobus* produces 7 times as much PA as *S. jacobaea*. However, these results are in direct contrast to the known toxicity data for cattle. Thus, while 2.5 mg/kg of body weight of *S. jacobaea* PAs was lethal to calves fed this plant for 20 days (Johnson, 1979), dose rates of 10–13 mg/kg *S. longilobus* PAs (Johnson and Molyneux, 1984) and 15–20 mg of *S. riddellii* PAs were required to produce the same effect (Johnson et al., 1984). The toxicity of the various *Senecio* species is therefore not dependent upon the total PA content alone. Nevertheless, the mean PA content of *S. riddellii* is so high that consumption of less than 250 g of green plant material/day over a 2–3-week period would be lethal to a 180-kg calf, whereas 750 g of green *S. jacobaea* would be necessary to kill the same size calf. Such quantities could easily be exceeded in range areas where these species predominate or are the only available forage.

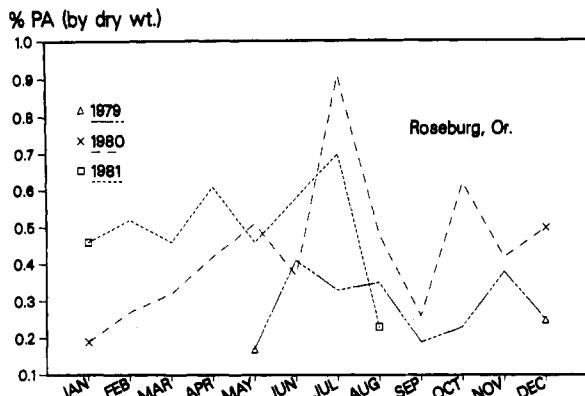


Figure 2. Pyrrolizidine alkaloid content of *S. jacobaea* leaves.

The values recorded in Table I also point out the large fluctuation in PA content over the various sites and collection periods. Thus, the ratio of maximum to minimum PA content is 46 for *S. jacobaea*, 79 for *S. longilobus*, and 100 for *S. riddellii*. It is apparent that with such variations the mere availability of a particular *Senecio* species does not define the extent of the hazard presented to animals grazing that particular area and that all factors influencing PA levels need to be considered in order to assess the threat to livestock.

A complicating factor in understanding pyrrolizidine alkaloid toxicosis is that each individual alkaloid may occur in the plant both as the free base and the *N*-oxide. Both forms are relatively nontoxic, but the free base is converted by liver microsomal enzymes into highly reactive alkylating pyrroles that are responsible for most of the pathologic changes recognized as being characteristic of pyrrolizidine alkaloidosis (Mattox, 1978). In grazing animals the potential exists for the *N*-oxides to be reduced to the corresponding free bases, particularly in the rumen, but possibly also in one of the other stomachs or elsewhere in the gastrointestinal tract, before exposure to the liver microsomal enzymes. Since the metabolism and excretion mechanisms of PAs have not been fully elucidated, and since there is a strong possibility that some or all of the *N*-oxides may be reduced to the free bases, it must be assumed that the total PA in the plant is available for conversion to pyrroles. Such total conversion may not occur in all cases, or indeed there may be no conversion of *N*-oxides to free bases at all, in which case only the free base content of the plant would need to be known to predict toxicity. Without full knowledge of the metabolism, excretion, and conversion of PAs to pyrroles, it becomes important to know both the *N*-oxide content and the free base content of the various plants so that one may at least hypothesize upon the variations in toxicity that are commonly encountered.

The mean free base PA and *N*-oxide contents for each location are expressed in Table I as a percentage of the total PA content. A striking contrast exists between *S. jacobaea* on one hand and *S. longilobus* and *S. riddellii* on the other. Whereas the former has a mean free base proportion over all locations of 73%, the latter species have free base proportions of only 19% and 18%, respectively. It is therefore possible to speculate that the higher toxicity demonstrated by *S. jacobaea* relative to the other two species is due to the larger percentage of free base. However, it should also be noted that the complexity of the alkaloid mixture may also contribute to relative toxicity. Thus, whereas *S. jacobaea* contains as many as nine individual PAs, *S. longilobus* contains four and *S. riddellii* contains only two (Smith and Culvenor, 1981). Slight variations in structure of closely related PAs may drasti-

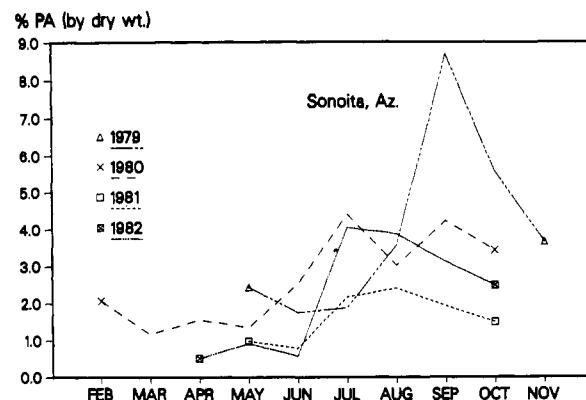


Figure 3. Pyrrolizidine alkaloid content of *S. longilobus* leaves.

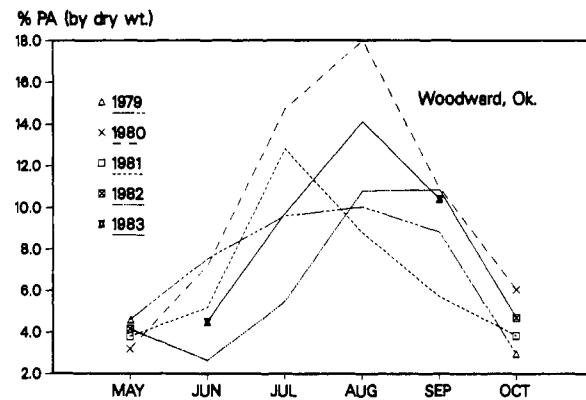


Figure 4. Pyrrolizidine alkaloid content of *S. riddellii* leaves.

cally alter the alkylating ability of the derived pyrroles or change the susceptibility of the alkaloids to elimination or detoxification.

The analysis of PAs in plant parts other than leaves, for the three *Senecio* species, was conducted on a limited basis. In general, stems contained 25–50% of the amount of PA found in the leaves of the corresponding plant, whereas flowers contained at least twice as much PA as the leaves. A single sample of root material, from *S. riddellii*, was analyzed and found to contain only trace amounts of alkaloids. There were no significant differences in PA content in samples divided and analyzed as fresh green plant and air-dried plant, indicating that there was no PA loss in the drying process.

Figures 2–4 show monthly PA fluctuation throughout the sampling period of plants from a single representative location for each of the three *Senecio* species under consideration. Each location selected for graphical display shows the typical full range of PA variation for these species.

Based upon the 3-year survey span, Figure 2 indicates that *S. jacobaea* PA content can be high during any month of the year and thus no safe period for grazing this plant can be recommended. Most of the higher PA values for all locations, however, were attained in the months of May through September, and the fact that a level of almost 1% was measured for one collection at Roseburg, OR, in July 1980 indicates that on rare occasions the PA content can exceed twice its usual value. The lack of a definitive PA pattern is perhaps explained in part by the fact that *S. jacobaea* is a biennial and is stimulated by cutting, which may interrupt or change its growth cycle. Thus, in some areas, several stages of growth, varying from rosette to seed stage, may occur simultaneously in the same location. Since some stages are difficult to recognize, collectors in the various locations were not always cognizant of the

particular growth stage that they gathered. This factor may also have contributed to the lack of a readily apparent pattern of PA occurrence in this plant.

S. longilobus flowers in early June and again in September, but some plants may be found in flower at any time between these months. In this study the plant showed an approximately 10-fold variation in mean PA content over its growing season but attained a maximum value of 8.7%, a very high alkaloid level for leafy plant material. The level of PA was highest in the months of July, August, and September (Figure 3), with the majority of peak values occurring in July when all locations in this study were considered. The period of maximum hazard for grazing *S. longilobus* therefore appears to be midsummer or when individual plants are flowering. However, the PA content, though lowest in the winter and early spring, is nevertheless sufficient to present a significant hazard (Figure 3).

Of the three *Senecio* species sampled for the full 3-year period, *S. riddellii* had the most consistent PA pattern through its growing season but the greatest fluctuation in the PA content, varying from 0.18 to 17.99% (Table I). The almost 18% total PA content is exceptionally high for leaf material and appears to be the highest level recorded in the literature for any class of alkaloid from any plant part, including seed, where alkaloids are often found to be concentrated. The highest recorded PA value previous to this study was a level of 9.28% in the seed of a strain of *Crotalaria retusa* from India, especially selected for its production of alkaloid (Kumari et al., 1966). The maximum PA value recorded for each of the five *S. riddellii* locations equaled or exceeded this high *Crotalaria* seed value (Table I).

S. riddellii is a perennial that dies to the crown in winter after fall seeding. Depending on temperature and moisture conditions, regrowth starts from the crown about the following May. At this stage leaf PA content is usually at its lowest or about the same as when the above-ground parts died in the fall. Under normal conditions the PA content then rises steadily and reaches its peak just before flowering in late August or early September (Figure 4). After flowering the PA content of the leaves drops rapidly to its low level. Therefore, grazing of this plant should be prevented especially during the bud and flower stage.

The data obtained in this study provided only limited information with respect to the other species examined. *S. vulgaris*, and introduced species, was collected for a 4-month period from February to May at Albany, CA. The plant has mean total PA content of 0.25%, and the level remained essentially constant over the growing season, consisting of approximately equal parts of free base and N-oxide. *S. vulgaris* contains senecionine (1), seneciphylline (3), retrorsine (4), and riddelliine (2). It infests pasturelands in the Central Valley of California, particularly in dry years when it presents a potential problem as a significant contaminant of forage.

S. spartioides, in areas where it could be found unhybridized with other *Senecio* species, occurred at elevations above 8000 ft where its growing season was only 3–4 months. Plants collected for 2 years at Cedar Mountain, UT, and Big Pine, CA, had a mean overall total PA content of 2.31% and a range of 0.28–7.88%. First collections were made in July, and these leaves contained the highest amount of PA, generally about 5% but approaching 8%. After flowering in August or September the leaf PA content dropped sharply and the plant usually froze in October.

Table II. Mean Pyrrolizidine Alkaloid Content and Composition of Leaves and Seeds from *Crotalaria spectabilis* Collected Monthly in 1979–1980

collection site	PA content ^a		PA composition ^b	
	range	mean	free base	mean
leaves				
Camden, AL	0.03–0.51	0.32	45	55
Tifton, GA	0.00–0.61	0.13	77	23
Charleston, SC	0.01–1.52	0.37	73	27
mean	0.01–0.88	0.24	67	37
seeds				
Camden, AL	3.53–3.98	3.75	79	21
Tifton, GA	2.41–4.79	3.98	92	8
Charleston, SC	2.32–5.35	4.51	91	9
mean	2.75–4.70	4.08	87	13

^a As percent of dry weight. ^b As percent of total PA.

S. plattensis is also a short-lived plant that may hybridize with other *Senecio* species. Following growth in early May, the plant sends up a flowering stalk and dies by early July, depending on moisture and temperature. *S. plattensis* is small, with few leaves, and for this reason attempts to obtain leaf samples from Kansas and Colorado were largely unsuccessful. The few samples that were analyzed revealed no PA, or less than 0.1% when present. Flower samples, however, obtained from eastern Kansas, contained 0.54% PA, and those from central and southern Colorado contained 0.22 and 0.56% PA, respectively. Contacts with veterinarians and livestockmen indicated that this plant causes sporadic poisoning but seems to be more of a problem in drier spring seasons when other plants and grasses may not grow as well as the well as the *S. plattensis*.

A. intermedia seeds have been incriminated as a cause of pyrrolizidine alkaloidosis in pigs (McCulloch, 1940). Efforts to collect this plant throughout its growing season were successful at only one location, in central Washington. The leaves contained a mean total PA content of 0.24%, with a range of 0–0.38% for the 3-month collection period. No appreciable amounts of PA were detectable in the seeds.

C. spectabilis, a legume introduced into a southern United States for soil improvement, has long been known to produce highly toxic seeds that contain the PA, monocrotaline (Adams and Rogers, 1939). Seeds cause toxicity in poultry and wild fowl but their greatest economic damage is to soybean production, where even a few *Crotalaria* seeds contaminating the crop reduces its value. Plant parts other than seeds may also poison livestock under certain conditions (Sipple, 1964). PA analyses of *C. spectabilis* collected from Alabama for 1 year and from Georgia and South Carolina for 2 years are summarized in Table II. The overall mean total PA content for all three locations is 0.24% for leaves and 4.08% for seeds, with an absolute range of 0.01–1.52% in leaves and 2.41–5.35% in seeds. The results obtained in this study indicate that leaf PA is greatest in young plants, in June and early July, with the level declining greatly as flowers form. Flowers, which usually appear in July, may contain up to 1% PA and early seed pods up to 2.5% PA. The total PA in leaves consists of 67% free base and 33% N-oxide, while in the seeds the proportions are 88% free base and 12% N-oxide. The very high alkaloid levels found in the seed, in combination with the large proportion of free base, indicates that *C. spectabilis* presents a considerable health hazard, both to animals that eat the seeds directly and to humans through entry into the food chain as a contaminant of soybeans. The latter possibility ap-

pears to be well recognized and adequately controlled by means of the inspection process.

The information obtained in this study shows that plants can vary greatly in their PA content. PA levels probably rise in response to stress conditions such as drought and infertile soils. These data indicate that grazing of *Senecio* species should be particularly avoided during the bud and flower stages and that *Crotalaria* is most hazardous during seed stages. The insidious nature of pyrrolizidine alkaloid toxicosis, the great variability of alkaloid content, and the poor understanding of the relative toxicities and metabolism of the free base and *N*-oxide forms dictate that the utmost effort should be made to control access of grazing animals to PA-producing plants at all times and to prevent entry of PAs into the human food chain.

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Studies on Seed Protein of Pearl Millets. 1. Amino Acid Composition of Protein Fractions of Early and Late Maturing Varieties

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Grain proteins of seven varieties of early and late season pearl millets (*Pennisetum typhoides*) were separated into five soluble fractions by the Landry-Moureaux method. The distribution of protein among the five fractions was identical in all the varieties in spite of some differences in their nitrogen content. The true prolamin fraction (fraction 11) constitutes the largest percentage (40%) of total nitrogen for all the millets. The amino acid composition of the five protein fractions is independent of genotype.

Seed protein constitutes a major source of protein for food in Nigeria where millet is consumed to a large extent by adults and infants in the form of a dough called fura (Okoh and Eka, 1978).

Loosli (1974) showed that 70-90% of the 59 g of protein that are available per capita each day in Nigeria is from plant sources. These proteins have lower biological value than those originating from animal sources (eggs, milk, and meat) because they contain less of the essential amino acids

needed for growth and maintenance. Howe et al. (1965) indicated that for a consumption of cereal satisfying caloric requirements, the ingested protein would be quantitatively adequate if its quality was comparable to that of animal protein. The poor biological value of cereals cultivated in the subtropical and tropical regions, namely, pearl millet, corn, and sorghum, is due to their deficiency in lysine and tryptophan (Nwasike et al., 1979). Jambunathan and Mertz (1973) have shown from their work on sorghum that in order to improve the quality of cereal grains, it is desirable to know the distribution and amino acid composition of protein fractions isolated by selective extraction. Some studies on protein of pearl millet (Swaminathan et

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