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GEOGRAPHIC VARIATION IN ALKALOID CONTENT OF *SANGUINARIA CANADENSIS* (PAPAVERACEAE)

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ABSTRACT

We sampled *Sanguinaria canadensis* L. (bloodroot) rhizomes from 100 eastern U.S. populations to assess variation in the alkaloid sanguinarine. Sanguinarine content varied from 0.6% to 6.3% of rhizome dry weight (mean 2.7%). This variation correlates with latitude, longitude, slope, clay content, litter depth, rhizome size, and fruit size. A regression with latitude, pH, and percent humic matter accounted for 58% of the alkaloid variation among populations. Alkaloid concentrations also vary seasonally. Highest alkaloid levels occurred during flowering and fruiting stages.

Key Words: *Sanguinaria canadensis,* alkaloids, sanguinarine, bloodroot, geographic variation, rhizome, eastern U.S.

Alkaloids occur in more than 300 plant families (Li and Willaman, 1972) and are particularly abundant in Fabaceae, Papaveraceae, Ranunculaceae, and Solanaceae. The function of these secondary metabolites is uncertain. They may be metabolic wastes, nitrogen storage compounds, plant pigments, biochemical regulators, plant growth substances, or defense compounds (Robinson, 1974, 1979; Seigler, 1977; Waller and Nowacki, 1978; Rhoades, 1979). Despite their uncertain biological role, alkaloid synthesis requires substantial metabolic investments. Nicotine production in tobacco may require 10% of the plant's metabolic output (Robinson, 1974).

Sanguinaria canadensis L., a monotypic, alkaloid-bearing member of Papaveraceae, grows throughout the eastern and midwestern U.S. (Ernst, 1962). One of the earliest spring-flowering species, *Sanguinaria* is most abundant on cool, moist, well-drained, wooded slopes. Rhizomes produce one or two leaves, each borne on an elongate petiole. Both the generic name, *Sanguinaria,* and the common name, bloodroot, describe the deep orange-red latex found in the rhizome. The color is due to the water-soluble, benzophenanthridine alkaloid sanguinarine.

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Sanguinarine inhibits growth of *Phymatotrichum omnivorum* (Shear) Duggar, the fungus responsible for root rot, at concentrations as low as 2.5 ppm (Greathouse, 1939). Greathouse and Rigler (1940) found it the most effective of 62 alkaloids tested against *P. omnivorum.* Sanguinarine also may be effective against bacteria, human pathogenic fungi, parasitic protozoans, viruses, and cancerous tissue (*see* Howell et al., 1973).

Traditional bloodroot uses include stimulants, expectorants, and emetics (Martin et al., 1961; Lewis and Lewis, 1977). There is renewed interest in sanguinarine because of its bactericidal and bacteriostatic properties against oral plaque-forming organisms (Dzink and Socransky, 1985) Sanguinarine is the active, anti-plaque agent in an oral rinse and tooth paste produced by Vipont Laboratories.

Infraspecific variation of secondary metabolites is common and may correlate with geographic clines or environmental conditions. Levin and York (1978) discussed examples of clinical variation in alkaloid content and stated, "presumably these clines are the result of natural selection." Levin (1976) noted two distinct trends: more annual than perennial alkaloid-bearing plants, and a negative correlation between the incidence of alkaloids and latitude. Seasonal, diurnal and intrapopulational differences are also known (*see* Harborne and Turner, 1984). Here we describe variability of sanguinarine content in 100 populations of *S. canadensis* and discuss environmental and morphological correlations.

MATERIALS AND METHODS

We sampled one hundred *Sanguinaria canadensis* populations from North Carolina to southern Vermont, and west to Indiana. We collected plants from mid-spring to late summer of 1983, beginning in the southern Appalachians and working north. Though collection dates varied, we sampled all populations at equivalent phenological stages (late fruit). Subsequently, we re-sampled several populations during post-fruiting (mid-summer) and late season stages just before leaf senescence (early fall) and then in early flowering stages the following spring (1984).

We collected 10–40 bloodroot plants from each population and recorded rhizome, leaf, capsule dimensions, pedicel length, and rhizome weights for five specimens bearing fully-expanded leaves. To make a sample large enough for chemical analysis, we com-

bined the five measured rhizomes. We also analyzed the second sample from each population (5–35 rhizomes) but did not record leaf measurements for these.

The North Carolina Department of Agriculture in Raleigh analyzed a composite soil sample from each population. In the field we estimated soil structure and clay content and determined pH colorimetrically. We also determined slope, aspect, elevation, depth of litter, parent rock, and species composition of the canopy for each site.

We dried rhizomes beneath low-temperature lights, then determined alkaloid concentrations at Vipont Laboratories in Ft. Collins, CO following analytical methods described by Boulware et al. (1984). Previous studies have shown that once dried, bloodroot's alkaloid constituents are stable (Boulware, unpubl. data). We performed analysis of variance and regressions using SAS (Goodnight et al., 1982) at the University of North Carolina at Chapel Hill.

RESULTS

Several alkaloids are identifiable in bloodroot rhizomes but sanguinarine is dominant, making up at least 50% of the total. Sanguinarine concentrations of sampled rhizomes ranged from .6% to 6.3% of rhizome dry weight. We recently found a population with 9% sanguinarine content but did not include it with this analysis. The average sanguinarine concentration was 2.7%, similar to that from commercial (foraged or wild collected) rhizomes sold in the pharmaceutical crude-drug markets. Collection sites are shown in the map (Figure 1).

We found significant correlations between sanguinarine levels and five of six continuous environmental and geographic variables (Table 1A). The strongest correlation was with longitude ($r = .60$). Latitude was negatively correlated with sanguinarine content ($r = -.40$). North of the Pennsylvania-West Virginia border there was a rather abrupt decline. *Sanguinaria* populations from Pennsylvania, New York and Vermont had very low concentrations (Figure 1). We detected no relationship between population size and vigor except that two of the highest alkaloid concentrations occurred in small, isolated populations. Clay content, slope, and litter depth also were correlated positively with sanguinarine concentrations ($r = .34–.36$).

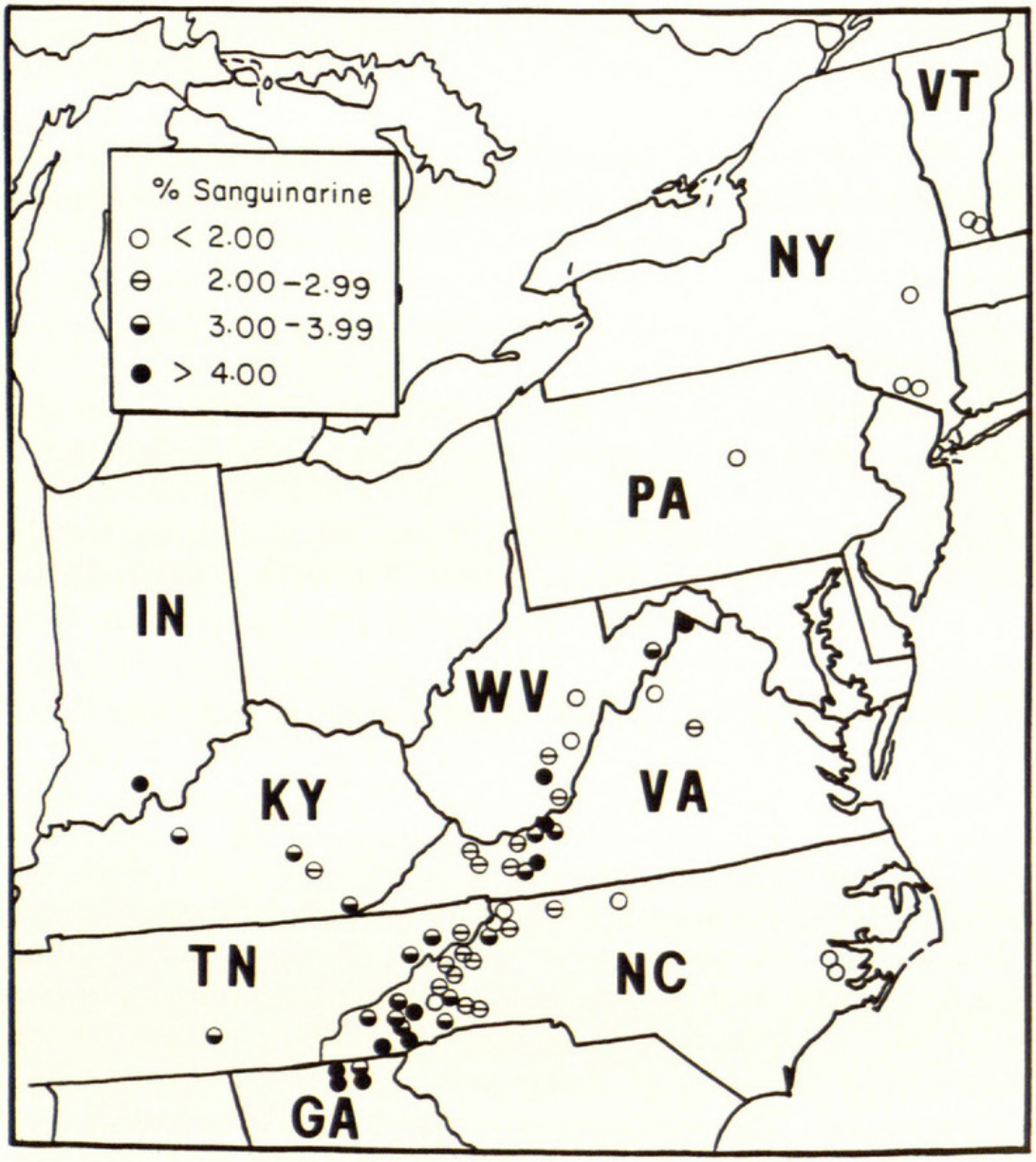


Figure 1. Sanguinarine concentration in *Sanguinaria canadensis* rhizomes from eastern U.S. sites.

Sanguinarine was correlated negatively with P and Cu but not with nine additional soil variables measured (Table 1B). We classified soils as structured (crumbly, blocky, or platy) or unstructured (massive) and included loamy soils with structured clays. Rhizomes from structured soils had a significantly greater mean sanguinarine content (Table 2).

No significant correlations occurred between leaf variables (length, width and petiole length) and sanguinarine content. However, fresh rhizome weight and volume (calculated from length

Table 1. Pearson Product Moment Correlation analysis. Rhizome sanguinarine content (% of dry weight) with all continuous variables. (n = number of observations, r = correlation coefficient, P = probability; CEC = cation exchange capacity, BSP = percent base saturation).

Variables	n	r	P
A. Environmental and Geographic			
Latitude	46	−.402	.001
Longitude	46	.598	<.001
Elevation	46	.161	ns
Slope	92	.360	.001
% Clay Content	92	.341	.001
Litter Depth	92	.337	<.001
B. Soil			
% Humic Matter (by Volume)	77	.201	ns
CEC (meq./100 cm^3)	77	.085	ns
BSP (% of CEC)	77	.044	ns
Acidity, (meq./100 cm^3)	77	−.098	ns
pH	77	.097	ns
P—index	77	−.335	.01
K—index	77	.131	ns
Ca—% of CEC	77	.0002	ns
Mg—% of CEC	77	.156	ns
Zn—index	77	−.185	ns
Cu—index	77	−.306	.01
C. Morphological			
Petiole Length	92	.077	ns
Leaf Width	92	−.180	ns
Leaf Length	87	−.173	ns
Capsule Length	29	−.373	.05
Capsule Width	28	−.504	.01
Peduncle Length	37	.390	.05
Rhizome Wet Weight	92	.332	.01
Rhizome Dry Weight	90	.123	ns
Rhizome Volume	92	.334	.01

and width measurements) were correlated positively and capsule size was correlated negatively with sanguinarine (Table 1C).

A stepwise multiple regression of sanguinarine content with the independent variables revealed only latitude, pH, and soil organic content accounted for significant variation (Table 3). All stepwise procedures selected latitude, pH and percent humic matter constitute as the best three-variable model even though single-variable regressions with each are significant only for latitude (Figure

Table 2. ANOVA table for % sanguinarine in structured and unstructured soils.

Soil Structure	% Sanguinarine $\bar{x} \pm$ SE (n)	F Value	P
Unstructured	2.33 .188 (44)	20.58	.0001
Structured	3.40 .145 (48)		

2). We excluded longitude from the analyses; its significance largely is due to low sanguinarine levels from North Carolina's coastal plain. Seasonal variation is due to higher alkaloid concentrations during flowering and fruiting periods early in the year (Figure 3).

DISCUSSION

Sanguinarine content is correlated negatively with latitude; highest-yielding populations were from the southern Appalachians and lowest from north of the West Virginia–Pennsylvania border and in North Carolina's coastal plain. Clinal variation, especially along north-south gradients occurs in many secondary metabolites including terpenes (Zavarin et al., 1975, 1978), flavonoids (Nicholls and Bohm, 1982; Levy and Fujii, 1978) and alkaloids (Levin and York, 1978).

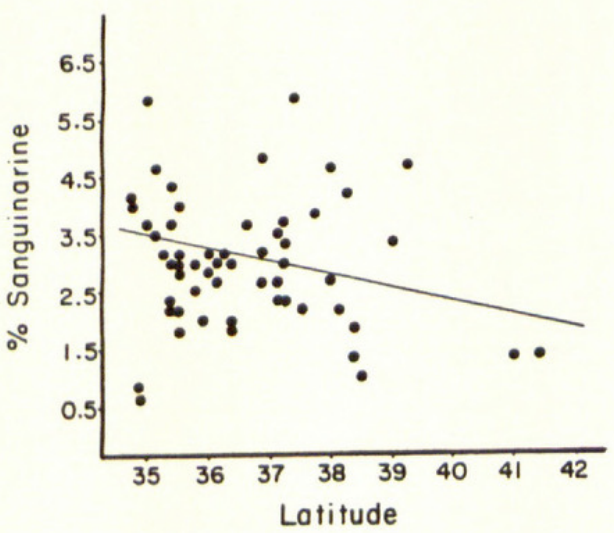
The correlation between sanguinarine content and longitude is spurious. The low-yielding populations in the north were also located farther east than most others. Sanguinarine concentrations peak in mountains or in mountain-like habitats; they are lower in the eastern piedmont and coastal plain in the central valley to the west.

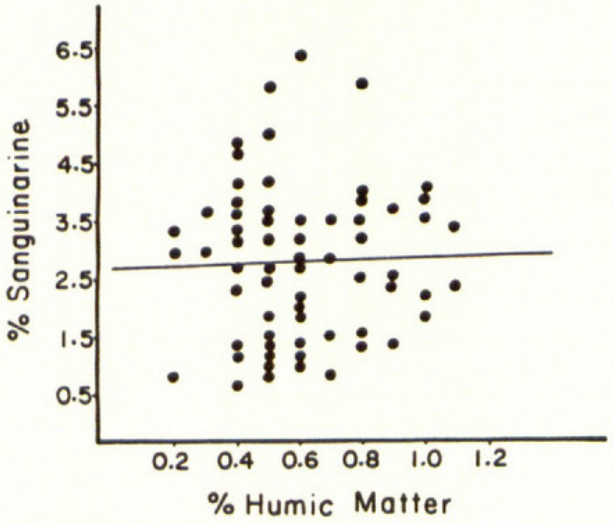
Among the environmental factors positively correlated with sanguinarine concentrations are litter depth, slope, clay content and soil structure. High-yielding populations grew in mature, undisturbed, deciduous forests often dominated by *Liriodendron tulipifera* L. or *Acer saccharum* Marshall and *Fagus grandifolia* Ehrhart. Other factors affecting secondary metabolites include light, pH, moisture, soil chemistry, temperature, altitude, successional status, geographical location, plant tissue, tissue age, cell age, and herbivory (*see* Harborne and Turner, 1984).

Production of secondary compounds varies considerably both within and between plant species. A general consensus is that conditions favoring optimal growth also favor the greatest pro-

Table 3. One, two and three-variable model results of the maximum R-square improvement stepwise regression procedure.

Variables	One-Variable Model			Two-Variable Model			Three-Variable Model		
	B-Value	F	*P*	B-Value	F	*P*	B-Value	F	*P*
Intercept	15.98			14.02			11.04		
Latitude	−.35	57.7	<.001	−.37	74.3	<.001	−.35	70.4	<.001
pH				.48	13.0	<.001	.73	24.3	<.001
% Humic Matter							.80	10.14	.01
R-Square		.453			.519			.578	





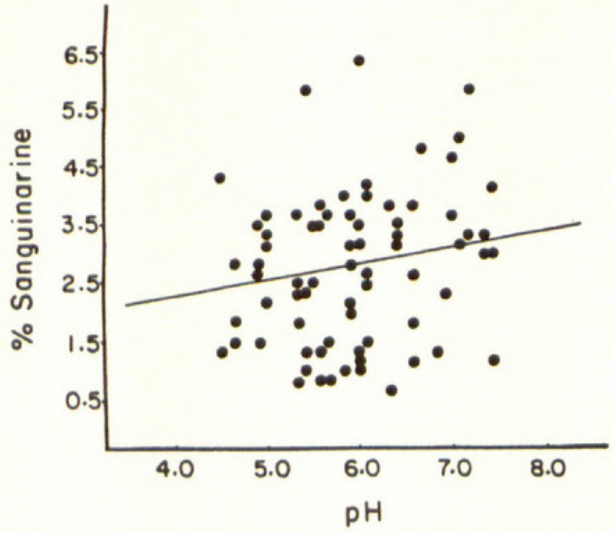


Figure 2. Single variable regressions of sanguinarine concentration and three environmental variables. (a) latitude: $r = -.40$, $P < .001$; (b) pH: $r = .19$, $P < .10$; and (c) % humic matter, $r = .017$, $P = .90$.

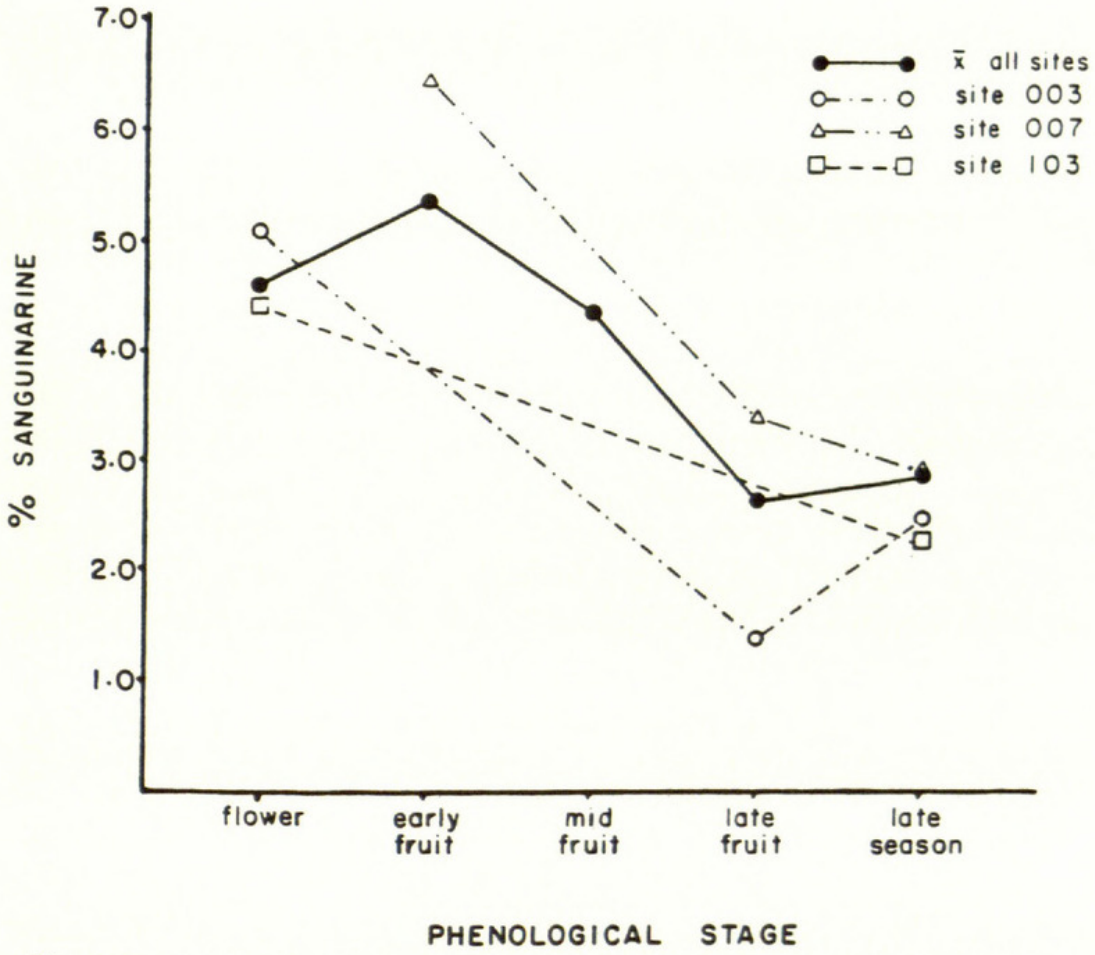


Figure 3. Seasonal variation of rhizome sanguinarine content in *Sanguinaria canadensis*.

duction of secondary metabolites. Levin (1976) suggested that optimal growth conditions for a species create optimal conditions for its predators and pathogens. If this connection is true, then selection should favor greater production of protective compounds in such habitats. *Sanguinaria* grows best in cool, shaded, forest understories on circumbasic soils. We usually found higher alkaloid concentrations in these habitats. Nevertheless, we found one of the highest sanguinarine concentrations in a small, exposed population growing under marginal conditions.

The correlation between rhizome weight or volume and sanguinarine content suggests a net accumulation of the alkaloid with time. Alkaloid concentrations are a function of synthesis, degradation, and translocation rates. In rhizomes, these processes occur over the life of the plant, in leaves they occur in a single season. We found foliage levels of .5% sanguinarine but Hegnauer (cited in Levin and York, 1978) reported levels of .08%. Cates and Rhoades (1977) noted that toxins, including alkaloids, are

usually characteristic of ephemeral tissues and that concentrations decrease rapidly in longer-lived plant tissues. This finding is not so for bloodroot.

The allocation of protective compounds in *Sanguinaria* is also counter to other hypotheses of plant defense. Feeny (1976) and Rhoades and Cates (1976) suggested that alkaloids should be found in "unapparent plants" (in Feeny's terminology) or those of young successional stages. These species can potentially escape from predators in both space and time. On the other hand, "apparent plants," characteristic of mature communities, should be defended by substances that reduce digestibility (e.g., tannins) and are metabolically more costly. Perennial herbs should have intermediate strategies according to Rhoades and Cates (1976). *Sanguinaria canadensis* has foliage-defense allocations characteristic of "unapparent plants" to their potential predators, soil-borne pathogens. Although rhizomes contain high levels of secondary metabolites, these products are toxic alkaloids which should be characteristic of "unapparent plants."

The high sanguinarine concentration in bloodroot rhizomes is an anomaly, especially considering the alkaloid's toxicity to soil pathogens at very low concentrations (Greathouse, 1939). Cates and Rhoades (1977) noted that toxins are often found in small quantities, sometimes less than 2% by dry weight. Levin and York (1978) found that alkaloids made up an average of .62% dry weight of leaf tissues in plants with these compounds. We found similar foliage concentrations in *Sanguinaria* but considerably more in rhizomes. Allelochemical effects of the water-soluble sanguinarine are unknown.

Leaf width, length, and petiole length were not significantly correlated with sanguinarine content, perhaps because alkaloid content of rhizomes is a function of several years of accumulation and does not necessarily represent present conditions. On the other hand, capsule length and width were positively correlated with sanguinarine levels. Both are measures of reproductive effort. Harris (1910) found that seed and ovule number per fruit and fruit length were significantly correlated. The negative relation between capsule length and alkaloid concentration may represent an energy tradeoff between reproduction and defense.

Seasonal variation in secondary metabolite concentrations occur in *Pteridium aquilinum* (L.) Kuhn flavonoids (Cooper-Driver et al., 1977), *Dentaria* spp. glucosinolates (Feeny and Rosenberry,

1982) and *Chelidonium majus* L. alkaloids (Kustrak et al., 1982). Our preliminary data show that rhizome sanguinarine concentrations peak early then decrease continually through the season. A similar pattern occurs in *P. aquilinum* flavonoids (Cooper-Driver et al., 1977) and some *Sanguinaria* populations (Farwell, 1915; Homerberg and Beringer, 1913). The two latter papers refuted a suggestion in the U.S. Pharmacopeia that *Sanguinaria* should be harvested just before leaf senescence for maximum alkaloid yield.

Some plants translocate alkaloids into above-ground tissue very early in the season. This translocation may be a cause of seasonal variation. Alternatively, the continuous decrease in concentration through the season may simply result from carbohydrate accumulation at a greater rate than alkaloid synthesis. Our data suggest that older bloodroot rhizomes have higher alkaloid concentrations than younger ones so that there must be a net accumulation each year. In contrast to *Sanguinaria* alkaloid concentrations in *Chelidonium majus,* a closely related member of the Papaveraceae, show the opposite seasonal trend (Kustrak et al., 1982).

If alkaloids are metabolically expensive to produce, then allocation to different tissues should be influenced by selection. The greater allocation to the perennial rhizome than to the ephemeral above-ground tissue should increase fitness. As a corollary of Levin's (1976) pest-pressure hypothesis, we suggest that increasing pest, predator, or disease pressure along a north-south gradient in the southern Appalachians may be responsible for higher alkaloid concentrations within southern bloodroot populations. We know of no data to refute or corroborate this hypothesis at present. Within a geographic region, however, high alkaloid concentration occur under optimal growth conditions for *Sanguinaria.*

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