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ELEVATIONAL TRENDS IN DEFENSE CHEMISTRY, VEGETATION, AND REPRODUCTION IN *Sanguinaria canadensis*

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Abstract—Evaluation of biotic interactions along geographic gradients reveals that pressure on plant populations by herbivores and pathogens increases as latitude decreases, and is accompanied by a parallel increase in the number and toxicity of alkaloid-bearing plants. We compared rhizome alkaloid content with plant reproductive and vegetative characters in *Sanguinaria canadensis* (Papaveraceae) along an elevational gradient over two growing seasons to ascertain 1) if alkaloid production in bloodroot varies among populations and systematically with elevation, and 2) if there exists a correlation between isoquinoline alkaloid, vegetative and reproductive production. In general, alkaloid content in bloodroot rhizomes declines with elevation, increases with rhizome water content, varies by site, and fluctuates seasonally with plant growth and reproduction. Alkaloid content was positively correlated with vegetative and reproductive effort with few exceptions. Analysis of total protopine and benzophenanthridine alkaloid concentrations revealed generally similar patterns as those of individual alkaloid concentrations, although significant differences did appear between individual alkaloid concentrations.

Key Words—*Sanguinaria canadensis*, bloodroot, isoquinoline alkaloids, elevation, plant defense, elaiosome.

INTRODUCTION

Abiotic and biotic environmental factors, as well as plant genotype, phenology and ontogeny can all influence the allocation of resources within plants (Coleman and Jones, 1991). Geographic gradients are useful in studying biotic interactions as

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they constitute natural experiments by providing variations in abiotic factors under which the biotic interactions can be evaluated. This has proven particularly effective for investigating facets of plant-herbivore interactions. There exists compelling evidence that pressures on plant populations by herbivores and pathogens increase with decreasing latitude (reviews by Levin, 1976; Vermeij and Veil, 1978; Jeanne, 1979; Gaines and Lubchenco, 1982); in a parallel fashion, there is an increase in the percent of alkaloid-bearing plants within and among plant families and an increase in toxicity of those alkaloids with decreasing latitude (Levin, 1976; Levin and York, 1978). These relationships are reflected in elevational gradients as well. Herbivory often declines with increasing elevation (Breulheide and Scheidel, 1999; Fuentes-Contreras et al., 1999 but see Reynolds and Crossley, 1997; Suzuki, 1998; Hengxiao et al., 1999), with evidence for decreasing alkaloid chemical defenses with increasing elevation (Chandra and Purohit, 1980; Carey and Wink, 1994).

Such patterns support hypotheses regarding selection on and costliness of plant defense. Considerable effort has been dedicated to determining the direct costs of chemical defense in terms of metabolism, growth, and reproduction, with the goal of illustrating a decrease in fitness with increasing defense in the absence of herbivores, but studies have shown a range of conflicting results (reviews by Gershenzon, 1994; Bergelson and Purrington, 1996). Mauricio and Rausher (1997) recently demonstrated that *Arabidopsis thaliana* plants released from insect and pathogen pressure experience a decrease in selection pressure on glucosinolate concentration and trichome density. It follows, then, that with decreasing herbivore and pathogen pressure toward higher latitudes or elevations, chemical defenses may similarly decline or show higher variation due to the release from selection pressure. Moreover, a relative increase in vegetative or reproductive effort in higher latitudes or elevations should be apparent if defenses are costly.

We present here a study of variation in isoquinoline alkaloid chemistry, vegetative growth, and reproduction among populations of *Sanguinaria canadensis* along an elevational gradient. The goals of our research are 1) to describe variation in isoquinoline alkaloid concentrations among populations and systematically with elevation in *S. canadensis* (bloodroot), and 2) to determine if there is a negative correlation between alkaloid production and efforts toward vegetative growth and reproduction.

Natural History: S. canadensis (bloodroot) is a perennial herb that occurs in a widespread but patchy distribution in eastern North America from Florida to Nova Scotia, and produces several highly bioactive isoquinoline alkaloids in the rhizomes, stems, and leaves, noticeable as a bright orange-red latex. These broad-spectrum defense chemicals deter or kill bacteria, fungi, insects, nematodes, and protozoa (Miller and Feeny, 1983; review in Downum, 1992; Schmeller et al., 1997). Plants reproduce both by seeds and by vegetative propagation of the rhizome. Pods are produced from May through early July and may contain up to 50 seeds, each displaying an oil-rich seed appendage (elaiosome). The elaiosomes

contain fatty acids, amino acids, and diglycerides that attract and stimulate ants to act as dispersal agents (Marshall et al., 1979; Skidmore and Heithaus,1988; Gunther and Lanza, 1989). Germination rates in *S. canadensis* are greater after elaiosome removal by ants (Lobstein and Rockwood, 1993) and ant dispersal may influence genetic relatedness of local bloodroot populations (Pudlo et al., 1980).

METHODS AND MATERIALS

Field Sites. We monitored populations of bloodroot along an elevational transect between Athens, Georgia, and Franklin, North Carolina, during the 1998 and 1999 growing seasons. All populations were located within mesic coves in deciduous hardwood forest. Cover for these sites ranged from 68 to 92% after canopy leaf emergence, estimated using a densiometer estimation. Low-elevation populations ranged from 195 to 215 m in Clarke, Oglethorpe, and Oconee Counties,Georgia; mid-elevation populations between 340 and 550 m were located in Habersham and Rabun Counties, Georgia; high-elevation sites were found between 760-1280 m in Macon County, North Carolina within the Coweeta Hydrologic Laboratory LTER boundaries. Samples included 4 to 12 individuals per population (i.e., per site). Up to 14 sites were included per elevation. Spring 1999 samples ($n = 206$) included a second sampling of plants from November 1998 ($n = 120$), as well as newly sampled plants.

Buds and leaves emerged from the soil between February and April each year. After emergence, plants were tagged around the base of the leaf stem. In both years of study, field data were collected on rhizome width and length, flower and leaf phenology, leaf number and size at time of seed-set, and number of flowers and seeds produced. Sites were visited starting in February when plants emerged in the spring. Each site was visited weekly during flowering, twice monthly during early seed-set, and bi-weekly as seeds ripened. As the pods opened on the plant, seeds were collected, and diaspore and elaiosome weights were recorded. Elaiosome tissue was dissected from the seed and weighed. In November 1998, tissue samples were taken during plant dormancy for chemical analysis ("Fall 1998" samples). In 1999, March-May tissue samples were taken at the time of seed collection ("Spring 1999" samples). To take tissue samples, we first uncovered the rhizome and measured length and width; the roots were left undisturbed. We excised 0.5-1g of tissue from the middle of the rhizome equidistant from the base of the growing bud and the end of the living rhizome tissue. We then weighed the tissue sample using a portable digital balance, and placed it in a foil- covered glass vial with 10 ml of 100% HPLC-grade methanol. Vials were stored at $-20°$ C until processed for chemical analysis.

Chemical Analysis. Tissue samples for HPLC analysis were homogenized up to one minute in the original vial using an Omni homogenizer fitted with a

saw-tooth rotor. The resulting slurry was filtered using a 13 mm nylon syringe-tip filter, transferred to a clean vial, and stored at −20°C until HPLC analysis. The homogonizer was rinsed with 1 ml of methanol, adding to the total volume of the samples. No further pre-purification of the samples was performed. Due to the photosensitivity of sanguinarine, extraction procedures were conducted under low-light conditions, and vials were either amber glass or wrapped in aluminum foil. Filtered samples (40 μl each) were analyzed at 284 nm using an HPLC Separations Module 2690 and Waters 996 Photodiode Array Detector (Waters Assoc, Milford, MA). Alkaloids were separated using a Phenomenex Silica C18 column (5 μm, 1.5 × 46) with a mobile phase of 0.1 N tartaric acid (0.125% SDS) in 1:1 v:v in acetonitrile; the flow rate was 0.5 ml/min (Hashimioto et al.,1986; Mahady et al., 1993). External standards were obtained for sanguinarine (R_T = 11.87), berberine (R_T = 14.94), and chelerythrine (R_T = 16.13) from Sigma Chemical Co. (St. Louis, MO). Remaining peaks were tentatively identified as protopine (R_T = 8.59), allocryptopine (R_T = 9.42), chelirubine (R_T = 11.13), sanguirubine (R_T = approx. 14.79), chelilutine (R_T = 19.53), and sanguilutine (R_T = 21.49) by comparing elution times and relative peak areas from previous papers (Hashimoto et al., 1986; Thorne et al., 1986; Mahady et al., 1993). Because of extensive work on the pharmacological properties of bloodroot alkaloids, these identifications should be robust but we stress that we did not identify them ourselves. Consequently, we consider these identifications as tentative and their concentrations are given as sanguinarine equivalents. In some HPLC profiles, the peaks for sanguirubine coeluted with berberine and resolved with inconsistent clarity; we therefore excluded them from subsequent analyses.

Statistical AnalysIs. Data were analyzed using generalized linear model in SAS (PROC GENMOD; Anonymous, 1996) by eliminating variables and interaction terms sequentially from the model to provide the most simple and parsimonious models available (Agresti, 1996). Alkaloid concentrations were analyzed individually and as alkaloid group totals. The protopine group totals include protopine and allocryptopine; the benzophenanthridine group totals include chelirubine, sanguinarine, chelerythrine, chelilutine, and sanguilutine.

RESULTS

Variation in Alkaloid Characters. Alkaloid concentrations either declined or were unrelated to elevation. Total benzophenanthridine group concentrations decreased with increasing elevation for both Fall 1998 and Spring 1999, whereas total protopine group concentrations were unrelated to elevation for either date (Table 1; Figure 1). Individual concentrations of sanguinarine and chelerythrine were unrelated to elevation in Fall 1998, but the remaining individual alkaloid concentrations declined with increasing elevation. In Spring 1999, sanguinarine

TABLE 1. BETWEEN-YEAR VARIATION IN ALKALOID CONCENTRATIONS AND CORRELATION WITH ELEVATION AND SITE FOR 1998 AND 1999 IN *Sanguinaria canadensis*

	1998	1999	$\chi^2(p)$
Protopine			
Year effect[a]	1.48 ± 0.17	1.17 ± 0.06	4.44(.0351)
Elevation effect[b]	19.51(.0001) −	ns	
Site effect[b]	68.2(.0001)	52.69(.0004)	
Allocryptopine			
Year effect[a]	1.26 ± 0.14	1.07 ± 0.06	ns
Elevation effect[b]	12.24(.0005) −	ns	
Site effect[b]	49.02(.0005)	49.85(.0010)	
Total protopines			
Year effect[a]	2.27 ± 0.29	2.26 ± 0.11	ns
Elevation effect[b]	ns	ns	
Site effect[b]	47.43(.044)	51.38(.0006)	
Chelirubine			
Year effect[a]	1.78 ± 0.18	0.87 ± 0.04	35.24(.0001)
Elevation effect[b]	5.83(.0157) −	ns	
Site effect[b]	46.39(.0011)	ns	
Sanguinarine			
Year effect[a]	4.34 ± 0.27	4.1 ± 0.14	ns
Elevation effect[b]	ns	19.97(.0001)	
Site effect[b]	57.83(.0001)	48.77(.0013)	
Chelerythrine			
Year effect[a]	2.94 ± 0.19	2.29 ± 0.08	13.83(.0002)
Elevation effect[b]	ns	ns	
Site effect[b]	37.8(.0136)	37.14(.0314)	
Chelilutine			
Year effect[a]	1.99 ± 0.24	1.16 ± 0.06	21.41(.0001)
Elevation effect[b]	22.00(.0001) −	ns	
Site effect[b]	47.12(.0009)	39.58(.0171)	
Sanguilutine			
Year effect[a]	2.09 ± 0.25	1.25 ± 0.07	16.31(.0001)
Elevation effect[b]	26.08(.0001) −	ns	
Site effect[b]	44.23(.0022)	41.42(.005)	
Total benzophenanthridines			
Year effect[a]	13.65 ± 1.57	8.83 ± 0.30	13.65(.0001)
Elevation effect[b]	17.25(.0001) −	6.75(.0094)	
Site effect[b]	72.09(<.0001)	36.04(<.0408)	

Note: Alkaloid concentrations were measured over 3 consecutive days in November 1998 and as seeds set throughout Spring 1999. −: negative correlation; +: positive correlation. For 1998 site effect, $df = 25$, and for 1999 site effect, $df = 23$. For all other independent variables, $df = 1$.

[a] Values are mean % dry weight ± S.E. $\chi^2(p)$.

[b] Values are $\chi^2(p)$.

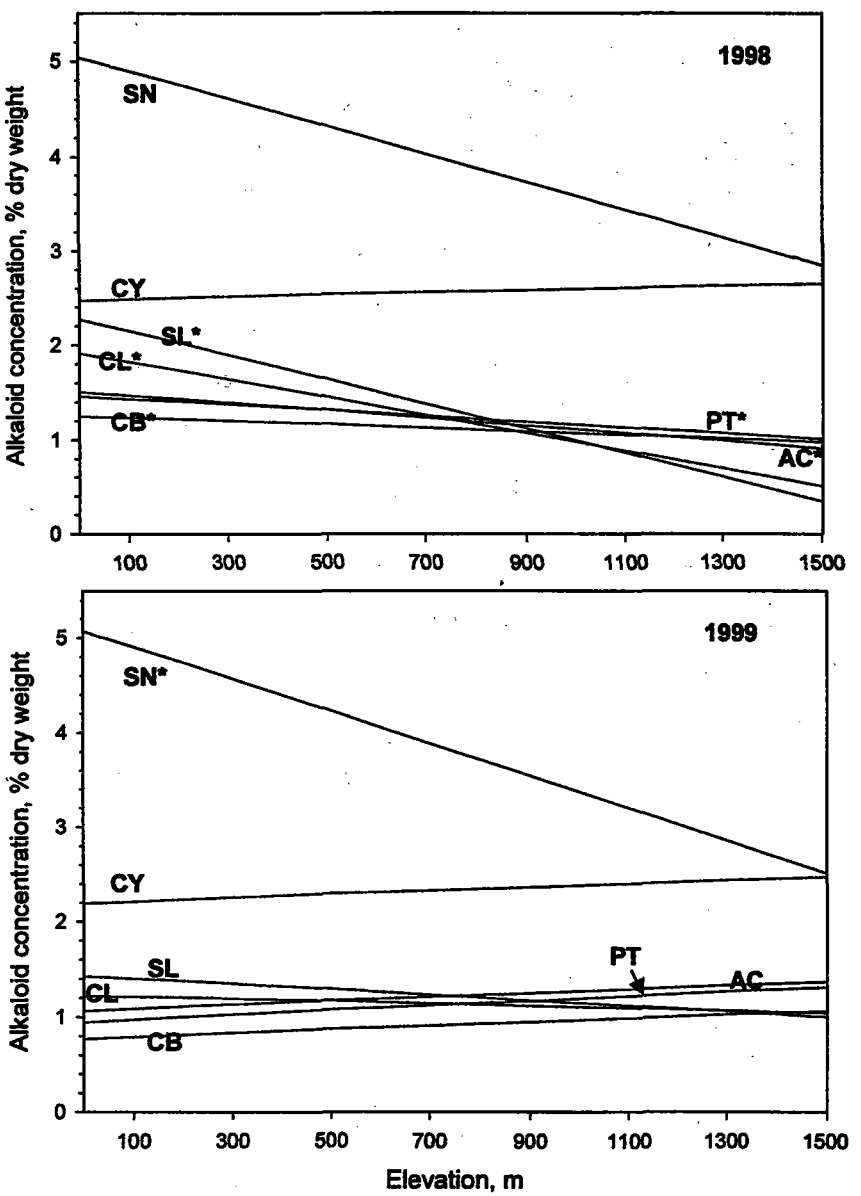

FIG. 1. Comparison of trends in alkaloid concentrations of *Sanguinaria canadensis* over an elevational gradient between November 1998 and Spring 1999. PT: protopine, AC: allocryptopine, CB: chelirubine, SN: sanguinarine, CY: chelerythrine, CL: chelilutine, SL: sanguilutine. * indicates significant relationships.

was the only individual alkaloid that significantly declined with elevation; the concentrations of all other alkaloids were unrelated to elevation (Table 1; Figure 1). Total benzophenanthridine group and total protopine group concentrations differed among sites in both 1998 and 1999. Individual alkaloid concentrations, with the exception of Spring 1999 chelirubine concentrations, showed variation by site for both dates (Table 1). Alkaloid content of rhizomes declined from dormancy (Fall 1998) to active growth (Spring 1999), with the exception of total protopine and sanguinarine concentrations (Table 1).

Variation in Vegetative and Reproductive Characters. Elevational trends observed in vegetative and reproductive characters varied with character and sample date. Leaf size increased with elevation in 1998, but decreased with elevation in 1999. Rhizome length and width were unrelated to elevation for either date (Table 2). Mean rhizome width was greater in 1999. Notably, water content of rhizomes declined with elevation for both dates. In general, the majority of vegetative characters did not vary according to site, although in some instances, weak correlations were evident for rhizome width, rhizome length, and leaf size (Table 2).

In 1998, the number of flowers per plant and elaiosome weight were unrelated to elevation, whereas they both declined with increasing elevation in 1999 (Table 2). The number of seeds per plant increased with elevation in both years. Reproductive characters varied more strongly by site in 1999 than in 1998, although seed weight variation was unrelated to among-site differences (Table 2). Elaiosome weight was higher and average flowers per plant lower in 1999. Reproductive characters were unrelated to percent water in the rhizome in both 1998 and 1999.

Alkaloids vs Vegetative and Reproductive Characters. Alkaloid content generally increased with, or was unrelated to, vegetative and reproductive effort, with a few exceptions. Total Fall 1998 benzophenanthridine and protopine group concentrations increased with rhizome size, and total protopine group concentrations decreased with leaf size in 1998 (Table 3). Total protopine concentrations sampled in Spring 1999 were unrelated to vegetative characters, whereas total benzophenanthridine concentrations increased with rhizome size and decreased with leaf size. Total benzophenanthridine and protopine group concentrations increased with rhizome water content in both fall 1998 and Spring 1999. With the exception of chelirubine, individual alkaloid concentrations reflected the same correlation for both sample dates (Table 3).

Total alkaloid concentrations in Fall 1998 were higher in plants that had produced more flowers and heavier seeds during the 1998 growing season. Individually, all alkaloids increased with seed weight, allocryptopine increased with number of flowers per plant, and chelerythrine increased with number of flowers and number of seeds per plant in 1998 (Table 4). In contrast, protopine, allocryptopine, and sanguilutine concentrations decreased with an increase in the number of seeds per plant in 1998. No relationships were found between either total benzophenanthridine or total protopine group concentrations and Spring 1999

TABLE 2. BETWEEN-YEAR VARIATION IN REPRODUCTIVE AND VEGETATIVE CHARACTERS AND CORRELATION WITH ELEVATION AND SITE FOR 1998 AND 1999 IN *Sanguinaria canadensis*

	1998	1999	$\chi^2(p)$
Elaiosome weight			
Year effect, mean g ± S.E., $\chi^2(p)$	0.0018 ± .0001	.0036 ± .0001	78.75(.0001)
Elevation effect[a]	ns	26.90(.0001) −	
Site effect[a]	56.46(.0001)	112.8(.0001)	
Seed weight, g			
Year effect, g ± S.E., $\chi^2(p)$	0.012 ± .0004	.012 ± .0001	ns
Elevation effect[a]	ns	21.99(.0001) +	
Site effect[a]	152.75(.0001)	ns	
No. of seeds/plant			
Year effect, No. ± S.E., $\chi^2(p)$	11.8 ± .92	13.8 ± .75	ns
Elevation effect[a]	9.40(.0022) +	24.18(.0001) +	
Site effect[a]	32.13(.0212)	43.25(.0065)	
No. of flowers/plant			
Year effect, No. ± S.E., $\chi^2(p)$	2.08 ± .25	1.35 ± .04	18.54(.0001)
Elevation effect[a]	ns	5.75(.0165) −	
Site effect[a]	ns	39.26(.0256)	
Leaf width, cm			
Year effect, cm ± S.E., $\chi^2(p)$	12.56 ± .45	11.77 ± .26	ns
Elevation effect[a]	7.79(.0053) +	35.10(.0001) −	
Site effect[a]	ns	ns	
Rhizome width, cm			
Year effect, cm ± S.E., $\chi^2(p)$	1.25 ± .03	1.17 ± .02	5.69(.0171)
Elevation effect[a]	ns	ns	
Site effect[a]	34.32(.0335)	ns	
Rhizome length, cm			
Year effect, cm ± S.E., $\chi^2(p)$	7.6 ± .74	6.55 ± .25	ns
Elevation effect[a]	ns	ns	
Site effect[a]	ns	5.09(.0241)	
% Water of total rhizome weight			
Year effect, % total weight ± S.E., $\chi^2(p)$	79.5 ± .8	78.5 ± .43	ns
Elevation effect[a]	10.39(.0013) −	37.14(.0001) −	
Site effect[a]	40.11(.0072)	ns	

Note: Characters were measured at seed set in the 1998 and 1999 growing seasons; measures of rhizome size and sampling of tissue to determine % water in the rhizomes were completed over 3 consecutive days in November 1998 and as seeds set throughout Spring 1999. −: negative correlation; +: positive correlation. For 1998 site effect, $df = 25$, and for 1999 site effect, $df = 23$. For all other independent variables, $df = 1$.

[a] Values are $\chi^2(p)$.

reproductive characters. Individually, sanguinarine concentration increased with elaiosome weight in 1999 although no other relationships between individual alkaloid concentration and reproductive characters were apparent for that sample date (Table 4).

TABLE 3. CORRELATION OF ALKALOID CONCENTRATIONS WITH VEGETATIVE CHARACTERS IN *Sanguinaria canadensis*

	PT	AC	Total PR	CB	SN	CY	CL	SL	Total BZ
No. of leaves/plant									
1998	ns	ns	ns	ns	ns	ns	ns	ns	ns
1999	ns	ns	ns	**6.58(.0103)**	ns	ns	ns	ns	ns
Average leaf size									
1998	**10.47(.0012)**	ns	**7.8(.0052)**	ns	ns	ns	ns	ns	**8.51(.0035)**
1999	ns	ns	ns	ns	ns	ns	ns	ns	**9.17(.0025)**
Rhizome width									
1998	ns	ns	ns	ns	ns	ns	ns	ns	ns
1999	9.40(.0022)	.98(.0017)	ns	24.30(.0001)	ns	9.66(.0019)	29.23(.0001)	154.8(.0001)	6.24(.0125)
Rhizome length									
1998	6.55(.0105)	10.01(.0016)	ns	ns	ns	ns	ns	ns	7.89(.005)
1999	**4.67(.0306)**	ns	ns	ns	ns	ns	ns	ns	ns
% Water weight of rhizome									
1998	ns	ns	40.93(.0001)	29.96(.0001)	178.6(.0001)	16.78(.0001)	23.45(.0001)	16.86(.0001)	95.6(.0001)
1999	7.11(.0077)	7.14(.0075)	20.11(.0021)	9.49(.0021)	44.24(.0001)	27.74(.0001)	27.74(.0001)	4.49(.0341)	4.49(.0001)

Note: PT: protopine, AC: allocryptopine, Total PR: Total protopine group, CB: chelirubine, SN: sanguinarine, CY: chelerythrine, CL: chelilutine, SL: sanguilutine, Total BZ: Total Benzophenanthridine group. Negative correlations are indicated in bold text. For all independent variables, $df = 1$.

TABLE 4. CORRELATION OF ALKALOID CONCENTRATIONS WITH REPRODUCTIVE CHARACTERS IN *Sanguinaria canadensis*

	PT	AC	Total PR	CB	SN	CY	CL	SL	Total BZ
Elaiosome weight									
1998	ns	ns	ns	ns	ns	ns	ns	ns	ns
1999	ns	ns	ns	ns	4.3(.0379)	ns	ns	ns	ns
Seed weight									
1998	22.96(.0001)	19.43(.0001)	6.57(.0104)	19.43(.0001)	6.97(.0083)	23.67(.0001)	31.99(.0001)	10.58(.0011)	16.21(.0001)
1999	ns	ns	ns	ns	ns	ns	ns	ns	ns
No. of flowers/plant									
1998	ns	3.96(.0466)	4.55(.0329)	ns	ns	5.22(.0223)	ns	4.29(.0382)	12.64(.0004)
1999	ns	ns	ns	ns	ns	ns	ns	ns	ns
No. of seeds/plant									
1998	**11.38(.0007)**	**23.22(.0001)**	ns	ns	ns	4.02(.0449)	ns	**5.66(.0173)**	ns
1999	ns	ns	ns	ns	ns	ns	ns	ns	ns
No. of flower × No. of seeds									
1998	ns	ns	ns	ns	ns	4.8(.0283)	ns	ns	ns
1999	ns	ns	ns	ns	ns	ns	ns	ns	ns
No. of seeds × seed weight									
1998	ns	18.69(.0001)	ns	ns	ns	ns	ns	ns	ns
1999	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: PT: protopine, AC: allocryptopine, Total PR: Total protopine group, CB: chelirubine, SN: sanguinarine, CY: chelerythrine, CL: chelilutine, SL: sanguilutine, Total BZ: Total Benzophenanthridine group. Negative correlations are indicated in bold text. For all independent variables, $df = 1$.

Overall Predictive Model. In a model assessing the relative contributions of elevation, site, reproductive, and vegetative characters to rhizome alkaloid content of bloodroot, rhizome water content and site explained most of the variance in both total benzophenanthridine and total protopine group concentrations for both 1998 and 1999 (Table 5). The strongest predictor of individual alkaloid concentrations in Fall 1998 was seed weight in most cases. Elevation and percent water in the rhizome were related to individual concentrations in Spring 1999 (Table 5).

DISCUSSION

Elevational Variation. Our results provide further evidence for an elevational cline in alkaloid production. Because we did not quantify herbivory or pathogen load, it cannot be determined if lower rhizome alkaloid content observed at higher elevations is a result of release from selective pressure, but our results are consistent with this hypothesis. Significant site effects suggest there are local influences on alkaloid production apart from larger climatic forces that accompany elevational gradients, or genetic variation in the alkaloid production of local populations.

The strong increase in alkaloid concentration with rhizome water content may result from nitrogen- or micronutrient-limitations. Water availability can constrain nutrient absorption because diffusion rate is the limiting step in the uptake of scarce nutrients (Landers et al., 1997) and soil nutrients influence alkaloid production (Waterman and Mole, 1989; Ohnmeiss and Baldwin, 1994; Baldwin et al., 1998; Salmore and Hunter, 2001). The observed increase in seed number and leaf size with elevation suggests that any nutrient limitation to alkaloid biosynthetic pathways may be a consequence of a within-plant allocation shift, rather than an overall nutrient limitation to the whole plant. Furthermore, the ability to generate secondary compounds under nutrient enrichment may be constrained genetically (reviews in Waller and Nowacki, 1978 and Roberts and Wink, 1998).

The decrease in rhizome alkaloid content between fall and spring sample dates supports the findings of Bennett et al. (1990), that showed the lowest concentration alkaloids in the rhizome at late seed-set. During active growth, alkaloid production may appear to decline due to an increase in biomass, even though total production may be equivalent or greater in the spring. Little change in rhizome size was observed between years to account for this. Translocation of alkaloids from the rhizome to fine roots or above-ground parts could cause this pattern, but currently no evidence exists for long-distance transport of alkaloids in *S. canadensis* (Kutchan et al., 1985). Alternatively, alkaloid production in fine roots or above-ground parts could increase at the expense of alkaloids produced in the rhizome.

Competition for substrate during the production of alkaloids derived from similar metabolic pathways may explain some of the variation in individual alkaloid concentrations. The isoquinoline alkaloids found in bloodroot are biosynthetically

TABLE 5. BEST PREDICTORS OF ALKALOID CONCENTRATIONS IN *Sanguinaria canadensis* AMONG ELEVATION, SITE, REPRODUCTIVE, AND VEGETATIVE CHARACTERS

	Fall 1998				Spring 1999			
	Variable	*df*	χ^2	*p*	Variable	*df*	χ^2	*p*
Protopine	+Seed weight	1	22.73	.0001	−Elevation	1	7.00	.0081
	+%H_2O	1	4.24	.0393	+%H_2O	1	12.62	.0004
Allocryptopine	+Seed weight	1	12.22	.0005	−Elevation	1	9.4	.0021
	+Rhizome Length	1	6.97	.0083	+%H_2O	1	14.98	.0001
Total protopines	Site	25	65.12	<.0001	Site	23	78.53	<.0001
	+%H_2O	1	14.24	.0002	+%H_2O	1	3.89	.0486
	+Rhizome Length	1	4.95	.0206	−Rhizome Length	1	20.11	<.0001
	−Leaf size	1	9.76	.0018	+Elaiosome weight	1	4.03	.0448
Sanguinarine	+Seed weight	1	3.90	.0481	−Elevation	1	5.56	.0184
	+%H_2O	1	4.31	.0378	+%H_2O	1	24.81	.0001
Chelerythrine	+Seed weight	1	34.44	.0001	−Elevation	1	10.12	.0015
	−Elevation	1	5.4	.0201	+%H_2O	1	28.39	.0001
Chelirubine	+Seed weight	1	19.43	.0001	−Elevation	1	8.29	.004
					+%H_2O	1	9.48	.0021
Chelilutine	+Seed weight	1	24.03	.0001	+%H_2O	1	19.85	.0001
	−Elevation	1	5.09	.0240				
Sanguilutine	+Seed weight	1	28.70	.0001	+%H_2O	1	12.36	.0004
	−Elevation	1	17.16	.0001				
Total benzophenanthridines	Site	25	63.49	<.0001	Site	23	37.06	.0320
	+%H_2O	1	81.37	<.0001	+%H_2O	1	34.4	<.0001
	+Rhizome length	1	4.17	.0412				

Note: %H_2O: Percent water content of rhizome; +: positive correlation between characters; −: negative correlation.

closely related; protopine is a key substrate for the more highly oxidized benzophenanthridine alkaloids, and these derived molecules may compete for substrate as well (Zenk, 1994; Roberts, 1998).

Correlations Between Defense, Growth, and Reproduction. Contrary to our prediction, sanguinarine was positively correlated with seed and elaiosome size, all alkaloids increased with seed weight, and higher alkaloid concentrations occured in larger rhizomes. If herbivory drives the level of defense investment (Feeney, 1976; Rhoades and Cates, 1976), then better defended plants escape herbivory and are able to produce higher quality seeds, or if resource availability determines the amount of nutrients available (Bryant et al., 1983; Coley et al.,1985), then bloodroot plants that are able to invest in higher quality seeds also are able to invest more in sanguinarine production. Quantification of herbivory and pathogen loads would provide insight into the mechanism driving patterns of alkaloid production. An increase in 1998 concentrations of protopine, allocryptopine, and sanguilutine with a decline in the number of seeds produced in the previous growing season may provide evidence for a trade-off, but this pattern is evident only in the 1998 sample date and for three of the seven alkaloids. A more likely cause for this correlation is the production of sanguinarine, which requires available protopine pools, and competes for a biosynthetic dihydro-intermediate with more oxidized alkaloids (Roberts, 1998) like sanguilutine.

Conclusion. Alkaloid concentrations in *Sanguinaria canadensis* provide further evidence for an elevational cline in the production of defensive compounds, although the results of this study demonstrate that rhizome alkaloid content varies with seasonal plant activity. The manifestation of trade-offs involving alkaloid production, in turn, is affected by these fluctuations and may lead to different conclusions based on the date that samples were taken. Total alkaloid group concentrations provide insight into main biosynthetic activities, while patterns seen in individual alkaloids illustrate complex within-organism metabolic allocation. Alkaloid content data resulting from growing clones or transplants of *S. canadensis* over several years under an experimental manipulation of herbivores and pathogens would better delineate trade-offs between defense, growth, and reproduction.

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