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Variability in the Yield of Benzophenanthridine Alkaloids in Wildcrafted vs Cultivated Bloodroot (*Sanguinaria canadensis* L.)

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Populations of bloodroot [*Sanguinaria canadensis* L. (Papaveraceae)] are found throughout the eastern forests of North America, with particular abundance in the southern Appalachian Mountains. Increasingly, it is finding use in Europe as a nonantibiotic animal feed supplement to promote weight gain. As efforts to cultivate this herb are underway, there is a need to understand the effect of agronomic permutations on both the dry mass rhizome yield and the yield of benzophenanthridine alkaloids. Month-to-month variability of the concentration of the alkaloids sanguinarine and chelerythrine in both cultivated and wildcrafted bloodroot was examined. The alkaloid yield was consistently higher, but more variable, in wildcrafted plants. On average, cultivated rhizomes were both larger and more consistently sized than those that were wildcrafted. The concentration of a suite of trace elements was measured in soil that was collected concomitantly with each plant accession. Differences in element profiles were compared against alkaloid yields.

KEYWORDS: Bloodroot; *Sanguinaria canadensis*; sanguinarine; chelerythrine; heavy metals; wildcrafted

INTRODUCTION

Populations of bloodroot [*Sanguinaria canadensis* L. (Papaveraceae)] are found throughout the eastern forests of North America. Although its distribution is rather patchy, it grows well from Florida to Nova Scotia (zones 3−9) with a native range that extends as far west as the Rocky Mountains (*1*−*3*). The attractive, white flowers of bloodroot are among the earliest spring blooms of the forest floor (*2*−*5*), and it is finding growing popularity as an ornamental (*3*). Both the scientific term, *Sanguinaria*, and the modern common name, bloodroot, derive from the deep orange-red latex that seems to "bleed" from cut rhizomes (*3, 4*).

Native Americans were the first to utilize bloodroot, both as a medicinal agent and as a skin paint and clothing dye. Medical uses varied between tribes and ranged from adding bloodroot to maple sugar to make throat lozenges (*6*) to treating gynecological conditions. By the mid-nineteenth century, bloodroot was employed by the Cherokee to treat breast cancer (*7*, *8*). Bloodroot quickly found its way into the colonial pharmacopoeia, as noted in medical texts of early nineteenth century America (*9*). Empirical observations on side effects encountered via different doses and dosing regimens ranged from nausea, vomiting, and hallucinations when consuming large amounts, for treating typhoid pneumonia and the croup, to a stimulating tonic that increases appetite and promotes digestion via small, teaspoon-sized doses (*9*). Its topical application as an escharotic was also noted (*9*), a use still in vogue with some modern herbalists to remove warts (*10*). These broad medical uses stimulated the chemical evaluation of bloodroot, and there is a good probability that the main alkaloid was isolated as early as 1824 (*5*).

A few investigations have explored the requirements for growth of bloodroot in the wild and the influence of environmental and genetic factors on the production of sanguinarine (**1**) (**Figure 1**). Marino et al. (*2*) demonstrated that *S. canadensis* has a rapid vegetative response to increased levels of light and nutrients, suggesting that the patchy distribution of bloodroot in the wild may result from an opportunistic response to disturbances in the forest canopy (i.e., tree falls). Bennett et al. (*4*) sampled rhizomes from 100 bloodroot populations throughout the eastern United States. In general, they found that the concentration of **1** was highest in populations from mountain or mountainlike habitats, especially the southern Appalachians, and that the content of **1** varied over the growing season, being highest in early spring just after flowering. The same authors found a positive correlation with rhizome weight or volume and

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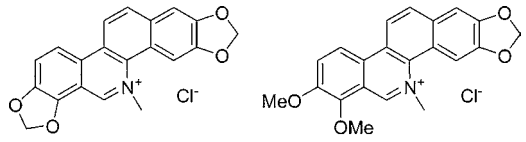

Figure 1. Structures of the benzophenanthridine alkaloids, sanguinarine (**1**) and chelerythrine (**2**).

the content of **1**, suggesting a net accumulation over time (*4*). Changes and trends in alkaloid content of bloodroot rhizomes over an elevation transect between Athens, GA, and Franklin, NC, were studied during the 1998 and 1999 growing seasons (*11*). In 1999, the content of **1** was greatest at lower elevations, thereby correlating with increased vegetative growth. However, the same trend did not seem to hold in the 1998 season, and in fact, the concentration of other alkaloids, including chelerythrine (**2**), did not vary over elevation for either season (*11*). The same researchers examined alkaloid content in *S. canadensis* in a garden plot over one growing season, where they were unable to elicit production of alkaloids via foliar application of jasmonic acid (*1*). However, they observed an increase in benzophenanthridine alkaloid production with decreasing light intensity (up to 80% shade) and decreased fertilizer levels; the authors note that the latter finding could be due to nutrient burn from high (400 ppm) fertilizer levels, thereby leaving the plants less vigorous (*1*).

For more than two decades, *S. canadensis* has been exploited commercially, especially for its antiplaque properties for dental hygiene (*12*, *13*), via oral rinse and toothpaste products, first marketed in 1982, that contain extracts of *S. canadensis* (*14*). The use of these products grew steadily, until their consumption was first associated with precancerous lesions (*15*) and then shown to be a risk factor for oral leukoplakia via a series of case-controlled studies (*16*, *17*). Because of these findings, the sales of dental hygiene products containing bloodroot extracts have decreased significantly.

Recently, however, demand for *S. canadensis* has increased again, particularly in Europe as a nonantibiotic feed supplement to promote weight gain of livestock (*3*). This stems from the European Union's ban on the use of antibiotics for growth promotion (*18*), and this market void is being filled by "natural additives" to animal feed. Nearly all of the supply for this growing market, anticipated to be 120−150 metric tons of *S. canadensis* per year (*3*, *19*), comes via wildcrafting (*3*). As such, many botanists and conservationists are concerned that overharvesting may negatively impact wild populations, a problem compounded by the long growth cycle needed to produce a mature plant, which is estimated at 6 years from seed and 4−5 years from rhizome cuttings (*3*). In fact, to address illegal harvesting of medicinal plants from federal lands, the U.S. National Park Service is developing science-based law enforcement strategies for the protection of at-risk natural resources, including bloodroot (*20*).

Since there are probably less than 70 acres of bloodroot in cultivation, efforts are underway to develop protocols to produce this crop in a manner that is viable economically (*3*). This would not only serve to protect wild populations, but it may also present a new cash crop for rural farmers, especially those of Appalachia, who are phasing out production of tobacco and anticipate a competitive advantage to cultivating this plant in its native range (*19*). Before most farmers will produce a new crop, its marketability and profitability must be demonstrated. Recent farm enterprise budgets estimate that cultivation of bloodroot can net returns on the wholesale market of $1210 to $2035 per acre after 3−4 years of growth (*21*). Bloodroot requires little maintenance between planting and harvest, thereby enhancing its potential value. A recent report stated that demand factors from different markets, a lack of large-scale cultivation, diminishing natural populations, a current trading range of $12−16 per pound, and the possibility of a major demand surge in Europe all combine to make bloodroot a promising candidate for cultivation (*22*).

Developing methods to improve dry matter yields and increase the concentration of key alkaloids could enhance the value of bloodroot to both growers and buyers. Many biological effects have been ascribed to **1** and related alkaloids, indicative of their role as chemical defense agents against microorganisms and herbivores (*23*). Moreover, **1** and **2** have been reported to have appetite stimulation properties in livestock (*24*, *25*), suggesting that a higher yield of these could be beneficial for agribusiness applications (*3*). Toward this end, the differences in alkaloid production between wildcrafted and cultivated bloodroot rhizomes were compared systematically amid two sites over a 7 month period (one growing season). Soil samples from each site were collected concomitantly with rhizomes, and correlations between heavy metal and trace element content and alkaloid production were examined. With respect to the potential agronomic implications, the fresh and dry weight yields of rhizomes, a major economic determinant for farmers/harvesters of bloodroot, were determined and correlated to the relative production of **1** and **2**. To the best of our knowledge, although bloodroot has been an integral part of medicine and culture in North America since before colonization, studies on the cultivation of bloodroot and how various agronomic permutations affect the profile of alkaloids have not previously been examined.

MATERIALS AND METHODS

Chemicals and Materials. Sanguinarine was purchased from Chromadex (Santa Ana, CA); chelerythrine and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO); methanol (MeOH), acetonitrile (CH_3CN), and trace metal grade nitric (HNO_3) and hydrofluoric (HF) acids were obtained from Fisher Scientific (Fair Lawn, NJ); and hydrogen peroxide (H_2O_2) was purchased from J.T. Baker (Phillipsburg, NJ). Nominal 1000 μg/mL trace element standards [arsenic (As), calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), lead (Pb), strontium (Sr), yttrium (Y), and zinc (Zn)] were purchased from High Purity Standards (Charleston, SC). A soil standard reference material (SRM 2711, Montana Soil—moderately elevated trace element concentrations) was purchased from the National Institute of Standards and Technology (Gaithersburg, MD). Deionized (DI) water (~18 MΩ quality) was generated via a Pure Water Solutions system (Hillsborough, NC). Disposable 20 μm filters and SPE columns (HF MEGA BE-C18; 1 g of 120 μm C-18 sorbent) were purchased from Varian (Harbor City, CA), and disposable 0.45 μm filters for soil sample digests were purchased from Corning (Acton, MA).

Plant Material. Rhizomes of *S. canadensis* L. (Papaveraceae) were collected from two sites in North Carolina that are separated by approximately 35 miles and that have a similar geography and climate. The wildcrafted samples were collected from the North Carolina Department of Agriculture and Consumer Services, Mountain Research Station (Waynesville, NC), from a wooded site that has been undisturbed since before the research station was established in 1944. The collection site (lat. 35°65′N, long. 82°97′W; elevation, 810 m) was populated by mixed hardwood trees that provided an average of 85% shade. The bloodroot population was natural and interspersed with a healthy population of poison ivy. The cultivated samples were harvested from the Mountain Horticultural Crops Research Station of North Carolina State University (Fletcher, NC) from a cultivated bed located under a lath-weave polypropylene shade cloth structure providing 85%

shade (lat. 35°43′N, long. 82°56′W; elevation, 631 m). The bloodroot had been brought into cultivation on another area of the research station prior to 1988. The population sampled for this study was transplanted to the test site in 1993 and had been divided and replanted many times. Representative voucher specimens for the wildcrafted (NCU 579762) and cultivated (NCU 579763) bloodroot samples are deposited in the Herbarium of the University of North Carolina at Chapel Hill. Each month, May−November, 2003, three wildcrafted and three cultivated rhizome samples, each from a different plant, were harvested at random from each site, and the above-ground plant parts were removed. The remaining rhizomes with roots were washed, air-dried to remove surface moisture, and weighed. They were then dried on screens at 35 °C in a forced air dryer until no measurable loss of weight was observed (typically 5 days). Dried samples were stored in paper bags in a controlled atmosphere until the time of extraction.

Chromatographic Instrumentation. Quantitative high-performance liquid chromatography (HPLC) analyses were performed using a Varian Prostar HPLC system (Walnut Creek, CA), equipped with Prostar 210 pumps, 330 photodiode array detector (PDA), and a 410 autosampler with a 100 μL sample loop, with data collected and analyzed using a Star Chromatography Workstation. A Sedex 55 (SEDERE, Inc., Lawrenceville, NJ) evaporative light-scattering detector (ELSD) was plumbed inline with the HPLC system immediately after the PDA. A 150 mm × 4.6 mm i.d., 5 μm, YMC ODS-A column (Waters, Milford, MA) was used for all chromatographic analyses.

Plant Extraction and Fractionation. Dried rhizome samples were chopped into chunks smaller than 1 cm with a Mini-mate Plus chopper/grinder (Cuisinart, East Windsor, NJ) followed by grinding to a fine powder with a MicroHammerMill IV milling machine (Glen Mills, Clifton, NJ). Aliquots of powdered rhizome (500 mg) were stirred for 16−18 h in MeOH (100 mL) using amber glassware. Solutions were filtered through 20 μm disposable filters and evaporated to dryness under nitrogen. Aliquots of the resultant extract (50 mg) were dissolved first in 0.5 mL of DMSO and then diluted in CH_3CN:H_2O containing 0.01% TFA (10:90, v/v; 10 mL). This solution was passed over a C-18 SPE column to remove sugars and to concentrate the alkaloids. For this, the column was flushed with CH_3CN (12 mL) and equilibrated with CH_3CN:H_2O containing 0.01% TFA (10:90, v/v; 18 mL), and then, the entire sample solution was loaded. The column was washed first with the same solvent (3 mL) under vacuum (18 mmHg). The eluent yielded a sugary mixture that was alkaloid-free, as shown by HPLC. Alkaloids were then concentrated by eluting the column with CH_3CN:H_2O containing 0.01% TFA (75:25, v/v; 28 mL) under vacuum (10 mmHg), and collected fractions were dried to calculate the alkaloid yield per gram of rhizome. All of the above operations were carried out in a low light environment, with overhead lights off and window shades drawn.

Quantitative Analysis. Aliquots of each of the concentrated alkaloid fractions were diluted to 1.00 mg/mL in DMSO in amber vials (National Scientific Co.) for 10 μL injections by autosampler. Quantitative analyses were carried out at 1 mL/min using a multistep gradient that increased from CH_3CN:H_2O containing 0.01% TFA (10:90, v/v) to 50:50 (v/v) over 20 min, then to 75:25 (v/v) over 5 min, remained isocratic for 5 min, then re-equilibrated to 10:90 (v/v) for 15 min. UV absorbance data were acquired via PDA at 335 nm. The entire sample set was analyzed consecutively, in triplicate.

Photosensitivity. One wildcrafted and one cultivated sample were prepared, and these were analyzed immediately by HPLC before exposure to light. Each sample was then divided equally into one amber and one clear vial, and the two vials from each sample were placed 15 cm from a pair of 30 W fluorescent light bulbs and exposed to light for 6 days. Placing the amber vials alongside the clear vials eliminated the chance of temperature or storage condition differences accounting for observed changes in the samples. Aliquots of each sample were analyzed using the aforementioned HPLC method on days 1, 3, and 6.

Soil Samples. Corresponding soil samples (∼100 g) were obtained each month from the immediate vicinity of the bloodroot rhizome collection sites and were dried at 50 °C in a type F6028C muffle furnace (Thermolyne, Dubuque, IA). Dried samples were ground manually and sifted through a 250 μm mesh, and nominal 0.5 g aliquots were transferred to acid-cleaned tubes for digestion in a MDS-2000 microwave oven (CEM, Matthews, NC). For each sample, the material captured on the mesh screen was retained in a plastic storage bag at ambient temperature for future evaluation, if necessary. Sifted samples were digested at atmospheric pressure in the presence of HNO_3 (5.00 mL), H_2O_2 (0.500 mL), and HF (1.00 mL). The microwave digestion parameters used during this investigation are presented in Supporting Information (**Table 1**). After digestion, samples were brought to 50.0 mL with DI water, filtered, and stored in acid-cleaned low-density polyethylene (LDPE) storage bottles until analysis.

The trace element content of the soil samples was determined using a Thermo Jarrell Ash (Franklin, MA) Atomscan-16 inductively coupled plasma optical emission spectrometer (ICP-OES). The employed ICP-OES instrumental parameters are presented in Supporting Information (**Table 2**). The instrument was calibrated with multielement standards (ranging from 0.0500 to 20.0 μg/mL for each analyte) in a matrix that approximated the acid content of the samples, and regression equations were generated. A midlevel calibration standard was analyzed immediately after instrument calibration, after a maximum of ten samples, and at the end of the analyses to monitor instrument stability. In order for bracketed data to be considered valid, the determined analyte concentration of this midlevel continuing calibration check standard was required to be within ±10% of its nominal concentration. An internal standard (Y) was utilized throughout this investigation. Aliquots of a 1000 μg/mL Y solution were added to the digested samples and standards so that the final nominal internal standard concentration was 5.00 μg/mL. Ratios of analyte emission response to internal standard emission response were used for all data processing.

Quality Control for Trace Element Soil Analyses. Several quality control samples were prepared with the soil samples to assess method performance. Method blanks ($n = 9$) were acids and oxidants that were digested to assess the trace element background contribution from the reagents and the procedure. A total of five low-level (nominal 1.00 μg/mL As, Cd, Cr, Cu, Pb, Sr, and Zn) and five high-level (nominal 10.0 μg/mL Ca, Fe, K, Mg, Mn, Na, and P) method control samples, which were method blanks fortified to assess analyte recovery in the absence of soil matrix, were prepared. Eight nominal 0.5 g aliquots of Montana Soil SRM 2711 were digested also to assess method accuracy. Overall, the recoveries for the method control and SRM samples, presented in Supporting Information **Table 3**, indicate a high-degree of method accuracy and precision.

For this investigation, the limit of quantitation (LOQ) for each element was conservatively defined as the concentration (in μg/mL) of the lowest standard included in its calibration curve. The LOQ for each element was expressed in μg/g by accounting for the 50.0 mL final sample volume and 0.5 g nominal sample mass. The determined trace element concentrations in the method blank samples were well below the LOQ for each analyte, indicating that contamination from the reagents and the procedure was not significant.

Statistical Analyses. All analyses were accomplished using SAS 9.1 (SAS Institute, Cary, NC). Paired *t* tests were performed using proc *t* test.

RESULTS AND DISCUSSION

The general goals of our study were to examine and correlate the agronomic output, literally the yield of plant material, and the variability of secondary metabolites, specifically the alkaloids sanguinarine (**1**) and chelerythrine (**2**) (**Figure 1**), in rhizomes of *S. canadensis* (bloodroot) over a growing season. Three rhizome samples were collected monthly from May to November, 2003, from two different research stations, one where bloodroot was growing naturally among trees and one where it was cultivated under polypropylene shade cloth. Differences between bloodroot grown under these conditions were observed, particularly with respect to both the content and the variability of **1** and **2**. Moreover, soil samples from each site were analyzed for a suite of trace elements, so as to assess differences and suggest possible amendments. These data form the baseline for a series of agronomic studies that are being conducted over the next 6 years.

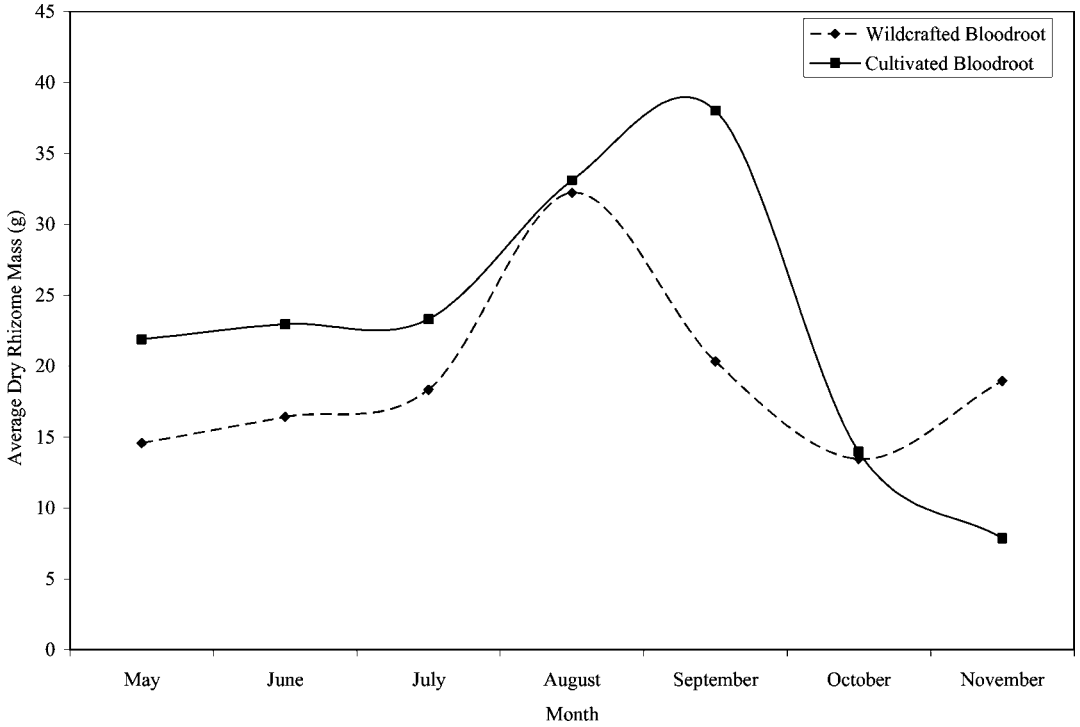

Figure 2. Average dry mass of bloodroot rhizomes from both wildcrafted and cultivated populations as a function of harvest month.

Agronomic Factors. Both the fresh and the dry weight of rhizomes from cultivated bloodroot tended to be larger (although not statistically different) than corresponding wildcrafted samples. Farmers/harvesters are compensated based on dry mass, and over the course of the study, the average dry mass for a cultivated rhizome was 23.0 vs 19.2 g for those for the wildcrafted setting ($p = 0.29$) (**Figure 2**).

Rhizome Alkaloid Yields. Extracts of the rhizomes of bloodroot were examined for the concentration of **1** and **2**, as identified by comparison of retention times and UV spectra to those of reference standards. To conduct such measurements, the quantitative analyses were facilitated greatly by removing sugars and other confounding compounds from the crude extract using an SPE column. Example chromatograms of the initial MeOH extract of a rhizome (**A**), the alkaloid-free fraction removed during SPE treatment (**B**), and the concentrated alkaloid fraction (**C**), analyzed by PDA at 335 nm and by ELSD, are shown in **Figure 3**. Although these benzophenanthridine alkaloids were observed readily at 335 nm, other non-UV-absorbing compounds made up a significant amount of the weight of the extract, as noted in the ELSD traces, and the sugary nature of these extraneous materials made it difficult to dry and solubilize extracts for quantitative HPLC. Importantly, neither **1** nor **2** was evident in the alkaloid-free sample. Regression equations for **1** and **2** were prepared over the range of 0.20−5.0 μg of alkaloid per 10 μL injection ($r^2 > 0.99$ for each equation; for **1**, $y = 2.13 \times 10^7x + 4.83 \times 10^5$; for **2**, $y = 1.59 \times 10^7x + -2.04 \times 10^5$). The levels of **1** and **2** in each rhizome sample were determined by analyzing the concentrated alkaloid fraction from each collected rhizome in triplicate.

Figure 4 displays the average trends for both **1** and **2** over the growing season. The concentration of **1** was always higher than the concentration of **2**, a finding that is consistent with the literature (*4*, *11*). Also, the wildcrafted rhizomes had a higher concentration of each of the alkaloids as compared to the cultivated rhizomes, statistically significant at the $p = 0.005$ level for **1** and $p = 0.007$ level for **2**. As evidenced by the magnitude of the error bars, however, the variability of the concentration of the alkaloids was much more pronounced in the wildcrafted samples, both within a single month and, perhaps more importantly for those interested in the commerce of bloodroot, between months. For example, over the growing season, the average amount of **1** ranged between 2.81 and 3.96% of the dry rhizome mass for wildcrafted samples, whereas it only varied from 2.25 to 2.75% of the dry rhizome mass for the cultivated samples. Of the months covered in this study, the yield of **1** appeared to be lowest for the wildcrafted rhizomes during the summer months, July−September; this trend held true for **2** to a lesser extent. Alternatively, the yield of **1** and **2** did not vary significantly over the study period in the cultivated samples.

Photosensitivity. In a purified state, it has been established that **1** undergoes photochemical conversion to oxysanguinarine (*26*). To demonstrate that **1** and **2** are photosensitive in crude extracts of the rhizome and that special handling precautions could be taken to avoid such photodegradations, concentrated alkaloid fractions of both wildcrafted and cultivated samples were examined with and without exposure to light. Chromatographic data from these samples were markedly different, especially for **1**. As noted in **Figure 5**, **1** decomposed by approximately 20% after 24 h, and after 6 days, it was nearly absent (approximately 1% of initial value). Similar results were observed for **2**, although to a much lesser extent (approximately 40% of initial value after 6 days; data not shown). Importantly, the use of amber vials seemed to eliminate photodegradation.

Trace Element Content of Soil Samples. Trace element content data for the soil study samples collected from the August time point are presented in **Table 1**. These data are representative of the relative differences observed between soil collected from the cultivated and wildcrafted sites. However, data for all of the collection time points are available for review in Supporting Information **Table 4a**,**b**. The presented concentration data are the average of three soil samples from each site, and percent relative standard deviation data are provided in parentheses. When the determined analyte concentration for one or

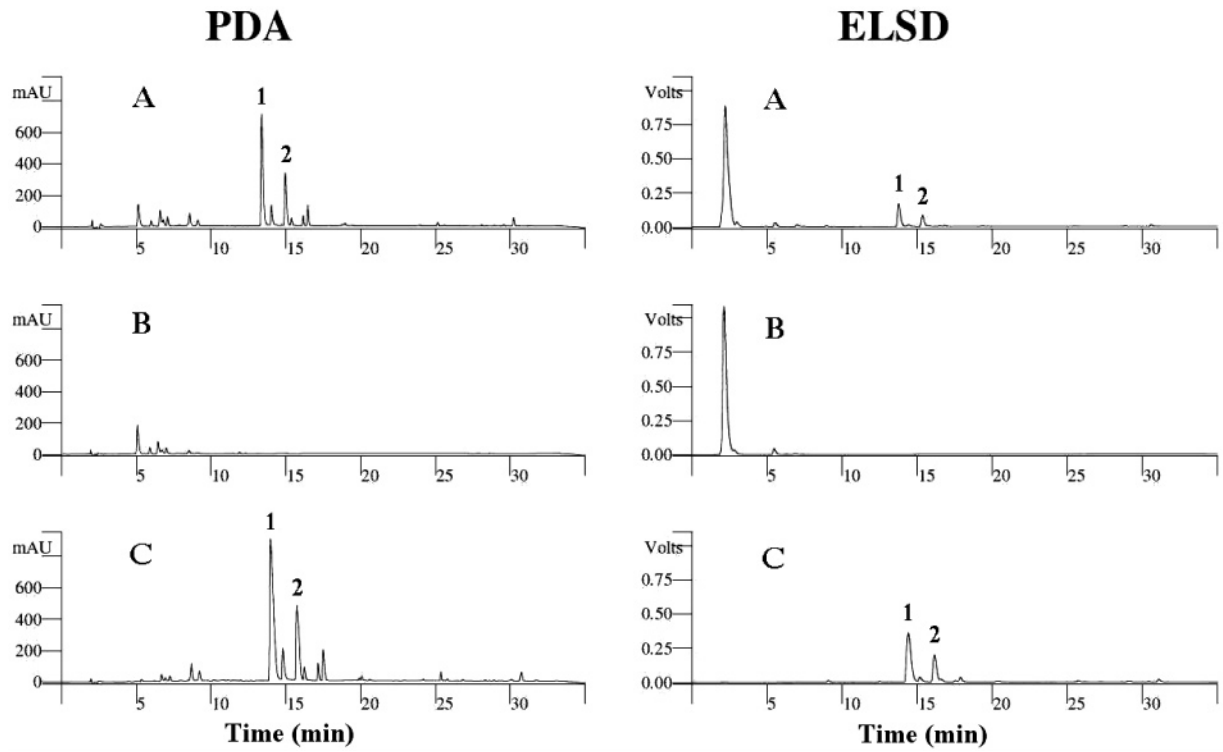

Figure 3. Chromatograms of the initial MeOH extract of a rhizome (**A**), the alkaloid-free fraction removed during SPE treatment (**B**), and the concentrated alkaloid fraction (**C**) showing the elution of sanguinarine (**1**) and chelerythrine (**2**) as detected by either PDA at 335 nm or ELSD.

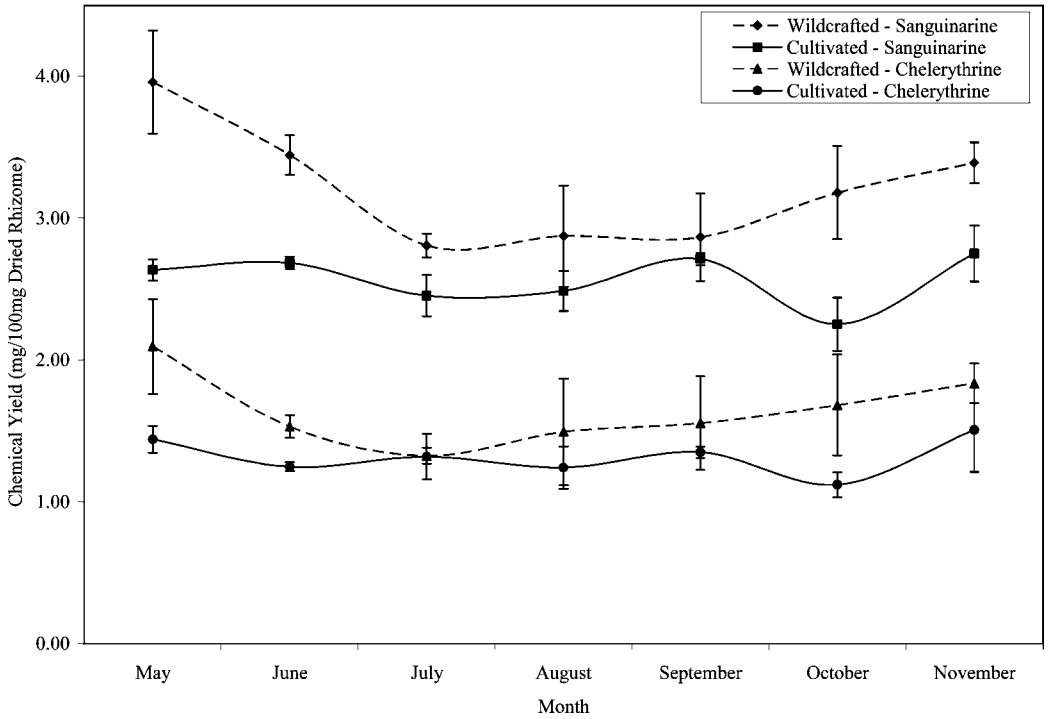

Figure 4. Sanguinarine (**1**) and chelerythrine (**2**) alkaloid yield for wildcrafted and cultivated bloodroot as a function of harvest month.

more of the three soil samples was less than LOQ, the data were flagged and the LOQ for the element was presented. For samples with a determined trace element concentration less than the LOQ, the LOQ was used to calculate average and percent relative standard deviation statistics.

The data in **Table 1** indicate that the determined concentrations of Ca, Cr, Cu, Fe, Mn, P, and Zn in the soil obtained from the wildcrafting area were higher than the observed levels for these same elements in the soil obtained from the cultivation site. In contrast, the measured levels of K and Sr were higher in the soil samples from the cultivation site. Although Pb was detected in the soil samples from the wildcrafted area, it was only measured above its LOQ for one of the cultivated soil samples. The determined concentrations of As and Cd were less than LOQ for all study soil samples, and the observed concentrations for Mg and Na were comparable between sites. In general, the percent relative deviations for the study elements were also lower for the soil samples obtained from the cultivated area, as one might expect given the more controlled environment.

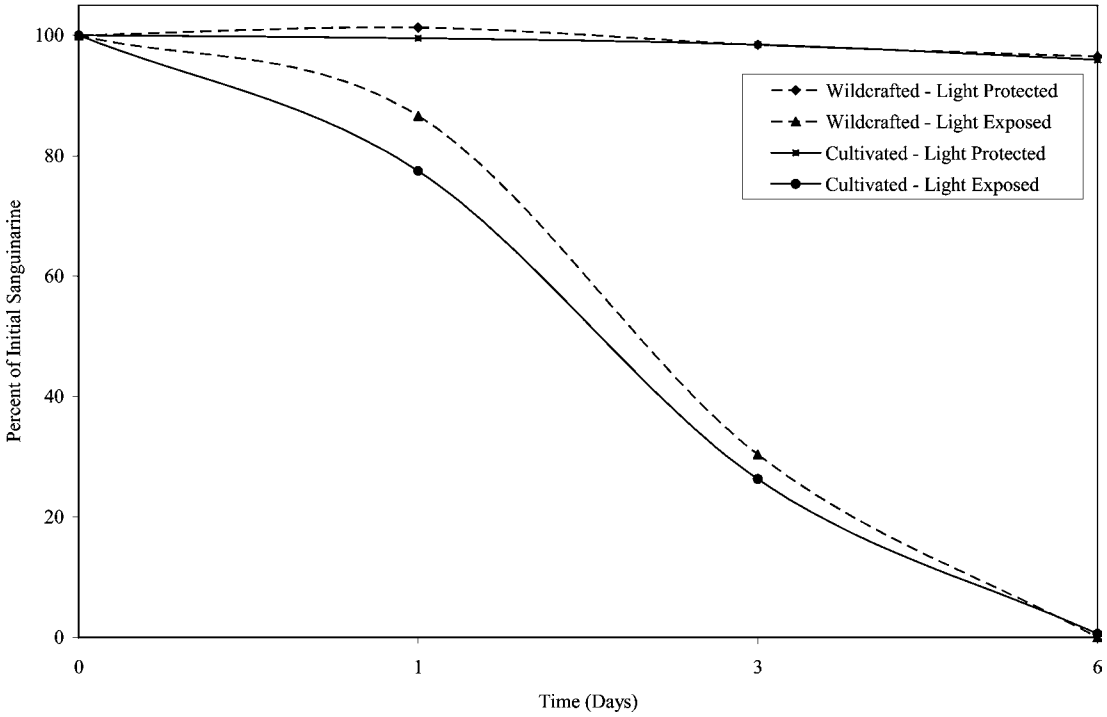

Figure 5. Photodegradation of sanguinarine (**1**) as a function of time for both wildcrafted and cultivated bloodroot.

Table 1. Soil Trace Element Content: August Collection

	average found concentration[a] (μg/L)	
element	cultivated harvest soil	wildcrafted harvest soil
As	<10.0 (NA)[b]	<10.0 (NA)[b]
Ca	3040 (33%)	594 (73%)[c]
Cd	<10.0 (NA)[b]	<10.0 (NA)[b]
Cr	32.6 (2.8%)	70.0 (3.6%)
Cu	12.1 (5.5%)	45.8 (9.5%)
Fe	24400 (5.6%)	58700 (3.3%)
K	10700 (2.6%)	3620 (27%)
Mg	1750 (8.6%)	1440 (110%)[c]
Mn	430 (6.5%)	5680 (2.6%)
Na	580 (4.9%)	762 (22%)[c]
P	730 (6.5%)	1630 (10%)
Pb	<20.0 (NA)	33.1 (4.9%)
Sr	47.3 (16%)	18.8 (45%)[c]
Zn	76.8 (7.0%)	139 (6.0%)

[a] Concentration data are averages of three samples from each harvest method for the August collection; percent relative standard deviation data are provided in parentheses. [b] Not applicable; all three collected samples less than LOQ. [c] One of three collected samples less than LOQ. LOQs were estimated as 10 μg/g for As, Cd, and Sr; 20 μg/g for Pb; and 100 μg/g for Ca, Mg, and Na.

Overall, these studies have established several parameters that suggest that cultivated bloodroot may be an attractive alternative to wildcrafting. Specifically, rhizomes from cultivated plants were 20% larger than those obtained from wildcrafted plants. These data should be encouraging to those considering the production of bloodroot commercially, since at present the market value is driven largely by the dry weight of the rhizomes. Moreover, the percent dry weight and the content of **1** and **2** in cultivated samples did not vary as much as in wildcrafted samples. This suggests that cultivated rhizomes can be harvested at anytime without sacrificing quality or yield, thereby allowing the grower, and perhaps the market price, to dictate the optimum time of harvest. In contrast, the content of these same alkaloids in wildcrafted samples was elevated in the early spring. Although this may be a convenient time for wildcrafting, since bloodroot is recognized easily through its early flowering, doing so could have a negative impact on native populations, as this may preclude maturation and dissemination of seeds. Indeed, bloodroot is already listed as "at risk", and excessive harvesting from the wild may accelerate the decline of native populations, especially since bloodroot rhizomes take nearly half a decade to reach maturity. Although at this time buyers may not be interested in the chemical composition of bloodroot and want only high dry mass yields, a competitive market advantage may be realized by optimizing agronomic variables to achieve a superior profile of secondary metabolites in the cultivated rhizomes. Presently, we have demonstrated that the cultivated samples produce a more consistent product, at least in this limited sample set. One or more of the described soil trace element profile differences may contribute to the higher alkaloid yields observed for the wildcrafted samples. In a previous investigation of this plant species, Bennett et al. (*4*) found no correlation between Ca, K, and Mg soil concentrations and the levels of **1** and a negative correlation with Cu and P soil concentrations. Other researchers have suggested that elevated fertilizer levels could result in nutrient burn, possibly impacting plant alkaloid production (*1*). Incorporation of one or more of the trace elements measured during this investigation into plant rhizomes or leaves could also impact alkaloid production. As a result, future experiments to determine rhizome and plant uptake of targeted trace elements from soils are planned. Overall, this study has catalogued what happens to bloodroot as it is transitioned from a wildcrafted to a cultivated setting. In general, although the absolute concentration of the major secondary metabolites in bloodroot, **1** and **2**, was slightly diminished in the cultivated samples, their quantity was much more consistent, suggesting that growers could harvest bloodroot as dictated by the market, not by plant stage of growth. The trace element analyses of soil presented here have established a baseline, and future studies will experiment with the addition and/or ablation of such elements in search of soil amendments that may drive

the concentration of the secondary metabolites up while maintaining a consistent profile throughout the growing season.

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Supporting Information Available: Tables of microwave digestion parameteres, ICP-OES instrumental parameters, trace element quality control sample recoveries, and soil trace element contents. This material is available free of charge via the Internet at http://pubs.acs.org.

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