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Wild Goldenseal (*Hydrastis canadensis*) Rhizome/Root Alkaloid Content in Relation to Colony and Harvest Stage

Eric P. Burkhart[a,b] and Grady H. Zuiderveen[b]

[a]Shaver's Creek Environmental Center, The Pennsylvania State University, Petersburg, PA, USA; [b]Department of Ecosystem Science and Management, The Pennsylvania State University, University Park, PA, USA

ABSTRACT

This study examined the three major alkaloids (berberine, hydrastine, and canadine) in wild goldenseal (*Hydrastis canadensis)* roots and rhizomes in relation to plant colony and harvest stage. Goldenseal colonies in central Pennsylvania, USA, were sampled on four dates (July 2, August 7, September 8, and October 12) corresponding with observable phenological stages between fruit maturity and senescence. Variation was observed for all three alkaloids with berberine and hydrastine present in all colonies and samples, while canadine was not detected during some late season sample dates. Nineteen root samples (53%) met the established United States Pharmacopeia (USP) standards for berberine content, while only one sample (2.8%) met USP standards for hydrastine. All colonies and samples showed an increase in alkaloid levels at the time of senescence, which corroborated the industry guidance that rhizomes/roots should be harvested at senescence (typically during the Fall season). Harvesting at senescence also permits fruit to mature and thereby facilitates sexual reproduction. However, alkaloid levels averaged the second highest at fruit maturity (July 2) which suggested that alkaloids may fluctuate during the growing season in response to, or as a function of, key reproductive events.

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Received 4 December 2017

KEYWORDS
Alkaloids; berberine; medicinal plant chemistry; phytochemistry; secondary metabolites

Introduction

Goldenseal (*Hydrastis canadensis*) is an herbaceous perennial indigenous to eastern North American forestlands. The rhizomes and roots of this species are used medicinally for antimicrobial and digestive purposes (1). Significant medicinal chemical constituents identified to date in rhizomes and roots include the isoquinaline alkaloids berberine, berberastine, tetrahydroberberastine, hydrastine, hydrastinine, canadine, and canalidine (2,3). Of these, berberine, hydrastine, and canadine have received the most attention in research and have confirmed antimicrobial properties (4). Berberine is often credited with many of the healthful benefits associated with goldenseal (1). Although berberine can be obtained from other plant species such as *Berberis* spp. (3), the additional alkaloids hydrastine

CONTACT Eric P. Burkhart epb6@psu.edu Shaver's Creek Environmental Center, The Pennsylvania State University, Petersburg, PA 16669, USA

and canadine in goldenseal are also considered to be therapeutically important (4). This combined ("holistic") chemistry drives continued interest and demand for goldenseal despite alternative, and in many cases, cheaper and more readily available (e.g., *Berberis* spp., *Mahonia* spp.) berberine containing plants.

Goldenseal was introduced into cultivation in the United States more than a century ago (5), but the adoption of goldenseal as a specialty crop has been limited due to volatile prices and demand and profitability constraints (6,7). The available trade survey data suggest that most goldenseal in the herbal market today originates from wild harvesting in the Appalachian region of the eastern United States (8,9). It is the practice for some commercial root diggers and herb collectors in Appalachia to harvest plants at any time they are requested by buyers do so and/or plants are discovered during forest searches, with little if any attention paid to the influence of timing on constituent levels (10). Harvesting without consideration of phytochemistry raises questions regarding the quality and/or potency of wild collected medicinal products. Studies of other wild-collected Appalachian medicinal forest plants such as bloodroot, *Sanguinaria canadensis* (11), American mayapple, *Podophyllum peltatum* (12) and American ginseng, *Panax quinquefolius* (13), for example, have shown that there are often differences in chemistry resulting from when and where plants are harvested and that these can have important qualitative consequences for herb buyers and consumers.

The present study examined alkaloid content in goldenseal rhizomes and roots. In particular, berberine, hydrastine, and canadine were studied in wild-harvested rhizomes and roots in three plant colonies across four harvest dates and stages to examine (1): alkaloid variation in wild-harvested rhizomes and roots between colonies occurring in a single forested area and (2) the preferred post-reproductive phenological stage to harvest for purposes of maximizing root alkaloid content. The study sought to test the hypothesis that harvest location and timing could influence constituent levels in wild goldenseal. Given that the species is still largely harvested from the wild, the results may have implications for the collection and trade of this wild species and contribute to improved understanding of the timing of medicinal root collection in general. The results of this study can also be useful for the determination of harvest timing in agroforestry cultivation systems using a forest farming approach.

Materials and Methods

Study Population and Associated Habitat Conditions

Goldenseal exhibits modular growth through asexual reproduction. Specifically, it is a clonal, colonial plant that forms "patches" over time which may be composed of many physically connected, interdependent individuals (i.e., ramets) (14,15). Accordingly, it is difficult to distinguish between genets and ramets in populations.

In this study, no attempt was made to distinguish between the two; instead, three spatially separate and distinct clonal "patches" (hereafter referred to as 'colonies') were sampled within a forested drainage covering an area of approximately six hectares. Colony-1 was situated at the lowermost topographic position in the forested drainage (elevation 305 m), colony-2 at a middle-upper location, and colony-3 occurred at the upper end of the drainage (elevation 370 m). Each colony was spatially distinct with approximately 450 m between colonies 1 and 3. The size of each colony within the population, root weight, and associated soil chemistry are provided in Table 1.

Owing to conservation concerns surrounding goldenseal, the exact location of this study is withheld from this paper but Global Positioning System (GPS) coordinates are available from the authors and are on-file with the Pennsylvania Department of Conservation and Natural Resources (DCNR) Wild Plant Management Program (Harrisburg, PA). Voucher specimens for the study were deposited in the following herbaria: the Pennsylvania State University in State College, PA (PAC); the Carnegie Museum of Natural History in Pittsburgh, PA (CM); and the Morris Arboretum of the University of Pennsylvania in Philadelphia, PA (MOAR).

Rhizome/Root Sampling and Processing

Goldenseal produces short, stout rhizomes covered with irregularly spaced fine roots, along with more slender rhizomes produced for colonial expansion. In this study, the entire rhizome and roots were gathered at sampling and were prepared and analyzed collectively. Hence, the term "rhizome/root" is used throughout. This was done to remain consistent with post-harvest handling practices in the trade where there is generally little effort to differentiate rhizomes from roots amongst collectors or buyers.

Goldenseal rhizomes/roots were harvested during the summer of 2012 from mature, reproductive stems (i.e., stems bearing 2–3 leaves) on four dates corresponding with the following phenological stages (1): July 2: fruit present and fully mature, foliage green (2); August 7: post-fruit bearing, foliage green (3); September 8: post-fruit bearing, some foliage beginning to yellow but not senescing; and (4) October 12: 90–100% yellow foliage in each colony, around 50% of each colony already senesced. For each sample date, three rhizome/root samples were collected from each of the three colonies for a total of nine samples per date and 36 samples total. Rhizomes/roots were washed and dried at 35°C for 24–36 h until they were dry enough to break cleanly. Dried rhizomes/roots were weighed and shipped overnight from Pennsylvania to North Carolina where they were prepared for analysis.

Table 1. Wild goldenseal (*Hydrastis canadensis* L.) colony characteristics and associated soil conditions for sample plots included in this study.

	Colony characteristics			Associated soil conditions (n = 5)[a]						
	Number of ramets		Dry root weights[a] (n = 50)	pH	Phosphate kg ha^{-1}	Potash kg ha^{-1}	Magnesium kg ha^{-1}	Calcium kg ha^{-1}	Cation Exchange Capacity	Acidity
	per m^2	total								
Colony-1	28–53	1,000–1,500	1.8 g (0.7)	6.3 (0.2)	83 (20)	202 (47)	536 (62)	7364 (1026)	17 (1.8)	3.8 (1.0)
Colony-2	24–45	1,500–3,000	1.7 g (0.4)	7.0 (0.3)	155 (9)	259 (52)	615 (164)	9518 (1451)	16.4 (1.7)	0.6 (1.3)
Colony-3	19–31	100–150	0.9 g (0.2)	6.6 (0.1)	89 (10)	298 (27)	492 (91)	6064 (1884)	13.5 (2.7)	2.4 (1.3)

[a]Standard deviations are included in parentheses.

Rhizome/Root Sample Preparation and Chemical Analysis

Dried whole rhizome/root samples were ground to approximately 60 mesh with a Foss Cyclotec™ mill prior to extraction. For each sample, about 250 mg of ground material was weighed and placed into a 50 mL centrifuge tube to which 25.0 mL of extraction solvent (water–acetonitrile–phosphoric acid 70 + 30 + 0.1, v/v/v) was added. The sample was capped and mixed using a Vortex® mixer for 10 sec, sonicated in a Bransonic 2510 ultrasonic cleaner for 10 min, and then vortexed again following sonication. The solid material was settled by centrifuging the sample preparation for 5 min at 4000 rpm in a Sorvall® Legend tabletop centrifuge. An aliquot of the supernatant was then filtered with a 0.45 µm PTFE syringe filter. In an amber HPLC vial, 200 µL of filtered sample was added to 800 µL diluent (90% acetonitrile, 10% water) and vortexed prior to analysis.

Calibration curves were prepared from five mixed standard solutions containing hydrastine, berberine, palmatine, and canadine. Standards were prepared in low-actinic volumetric flasks and diluted with 90% acetonitrile/10% water. Concentrations of individual stock standards were calculated based on the reported purity and corrected for chloride content in the case of berberine and palmatine. The linear ranges of the calibration curves were as follows: hydrastine 10.3–155 µg mL^{-1}, palmatine 3.67–55.1 µg mL^{-1}, berberine 7.94–119 µg mL^{-1}, and canadine 5.64–84.6 µg mL^{-1}. Curves were all linear with an r^2 value of above 0.999.

High Pressure Liquid Chromatography (HPLC) analysis was performed using Chromeleon® chromatography management software on a Dionex ICS-3000 system equipped with dual pump, automatic sample injector, and variable wavelength detector. The column temperature was maintained at 40°C. The analytical column was an Agilent Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 3.5 µm). The mobile phase consisted of 25 mM ammonium formate, pH 3.8 (C) and 0.1% triethylamine in acetonitrile (D) at a flow rate of 0.75 mL min^{-1}. The isocratic eluting mobile phase was 70% C and 30% D, and injection volume was 10 µL. Detection was at 230 nm and total run time was 13 min per injection.

Reagents and standards: HPLC-grade acetonitrile, formic acid (≥98%) o-phosphoric acid (85%), triethylamine (99%), and ammonium formate (99%) were obtained from Fisher Scientific. HPLC-grade chemical reference standards berberine chloride (88.6%), (1R, 9S) -(-)-B-hydrastine (99.5%), palmatine chloride (tetramethoxyprotoberberine chloride, 79.2%), and DL-canadine (tetrahydroberberine, 97.2%) were purchased from Chromadex (Santa Ana, CA).

Statistical Analysis

In addition to basic statistical analyses, Pearson's correlation analysis was used to examine rhizome/root phytochemistry in relation to soil chemistry and colony. Linear regression was used to examine rhizome/root phytochemistry in relation to rhizome/root weight.

Analysis of variance was used to compare mean values of total alkaloid concentration, as well as the three individual alkaloids separately for each harvest time and location. Differences between individual harvest data and sites were analyzed using Bonferroni Pairwise Comparisons.

Statistical analyses were conducted using SPSS (version 24) and Minitab 17.3 (Minitab 17 Statistical Software, 2010).

Results

Variation was observed both within and between colonies for all three alkaloids (Table 2). Total rhizome/root alkaloid content varied from a low of 3.2% in August (represented by a single sample from Colony-1) to a high of 4.8% in July and October (samples both collected from Colony-3). Both total and individual alkaloid content was associated with harvest timing with the highest alkaloids observed at the senescent stage in October (Fig. 1). Berberine and hydrastine were detected in all samples and colonies. Canadine, however, was not detected in Colony-3 samples from two dates (Aug 7 and Sept 8). Since each sample was replicated (n = 3) on each date, and in each colony sampled, it is unlikely that the absence of canadine in colony 3 on two sample dates was the result of sample processing or analytical errors.

Correlation results were insignificant for all soil chemistry parameters examined except for soil Ca and P, which were correlated with alkaloid content on two harvest dates (Table 3). These correlations were both positive (Ca, P, and canadine) and negative (Ca and hydrastine), however, revealed no clear trend. The most significant and perhaps interesting correlations were for total alkaloid content, root weight and colony at the final harvest date (October). In the case of root weight, there was a negative correlation between rhizome/root weight and total alkaloid content, indicating that smaller rhizomes/roots contained higher total alkaloid concentrations (Fig. 2). For the effect of colony, there was a positive correlation between colony sampled and total alkaloid content. Average root alkaloid content was the highest in colony-3 on all sample dates.

Discussion

The Influence of Harvest Stage and Timing on Quality

The World Health Organization (WHO) Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants provide the following guidance with respect to harvest timing of wild-collected medicinal plants (16):

"Medicinal plant materials should be collected during the appropriate season or time period to ensure the best possible quality of both source materials and finished products. It is well known that the quantitative

Table 2. Alkaloid levels in wild-harvested goldenseal roots/rhizomes in relation to colony and harvest stage (n = 3 for each plot on each harvest date, n = 36 total samples). Observations regarding phenological stage at the time of harvest are included below the harvest date.

	July 2, 2012	August 7, 2012	September 8, 2012	October 12, 2012
	Fruit present and fully mature; foliage green	Post fruit bearing; foliage green	Post fruit bearing; foliage beginning to yellow	Foliage yellow; plants senescing
Colony 1				
Berberine %w/w	2.5 σ 0.2 (2.3–2.7)	2.1 σ 0.2 (1.9–2.3)	2.3 σ 0.2 (2.1–2.6)	2.5 σ 0.5 (2.2–3.1)
Hydrastine %w/w	1.4 σ 0.2 (1.3–1.6)	1.6 σ 0.3 (1.3–1.9)	1.5 σ 0.0 (1.5–1.5)	1.5 σ 0.1 (1.4–1.6)
Canadine %w/w	0.08 σ 0.01 (.07-.09)	0.03 σ 0.01 (.03-.04)	0.11 σ 0.01 (.11-.12)	0.18 σ 0.03 (.14-.20)
Total alkaloids %w/w	3.9 σ 0.3 (3.6–4.1)	3.7 σ 0.5 (3.2–4.2)	3.8 σ 0.3 (3.5–4.1)	4.0 σ 0.6 (3.7–4.7)
Colony 2				
Berberine %w/w	2.3 σ 0.1 (2.2–2.5)	2.2 σ 0.1 (2.2–2.3)	2.5 σ 0.1 (2.3–2.6)	2.9 σ 0.1 (2.8–3.0)
Hydrastine %w/w	1.3 σ 0.1 (1.1–1.4)	1.4 σ 0.1 (1.3–1.5)	1.3 σ 0.1 (1.2–1.4)	1.5 σ 0.0 (1.5–1.6)
Canadine %w/w	0.09 σ .02 (.07-.09)	0.08 σ 0.04 (.03-.11)	0.09 σ 0.01 (ND-.09)	0.18 σ 0.02 (.17-.20)
Total alkaloids %w/w	3.6 σ 0.3 (3.4–3.9)	3.7 σ 0.1 (3.5–3.7)	3.8 σ 0.3 (3.5–3.9)	4.4 σ 0.1 (4.4–4.5)
Colony 3				
Berberine %w/w	2.5 σ 0.2 (2.3–2.7)	2.2 σ 0.3 (2.0–2.5)	2.6 σ 0.1 (2.5–2.7)	2.8 σ 0.1 (2.8–2.9)
Hydrastine %w/w	1.8 σ 0.3 (1.5–2.1)	1.6 σ 0.1 (1.5–1.6)	1.7 σ 0.1 (1.7–1.8)	1.9 σ 0.1 (1.8–2.0)
Canadine %w/w	0.07 σ 0.01 (.07-.08)	Not detected (ND)	Not detected (ND)	0.23 σ 0.04 (.20-.27)
Total alkaloids %w/w	4.3 σ 0.5 (3.8–4.8)	3.8 σ 0.3 (3.5–4.1)	4.3 σ 0.1 (4.2–4.5)	4.7 σ 0.1 (4.6–4.8)

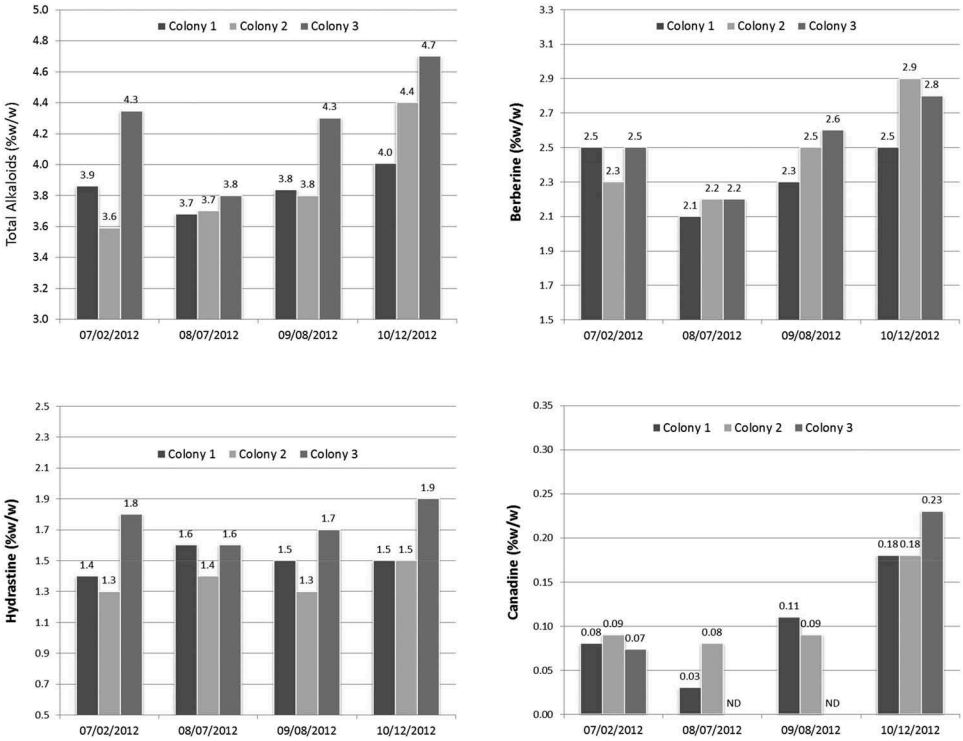

Figure 1. Alkaloid levels in wild-harvested goldenseal rhizomes/roots in relation to colony and harvest date. Clockwise from top-left: total alkaloid content (berberine, hydrastine and canadine), berberine, canadine and hydrastine. Note the different scales on the vertical axes. Harvest dates corresponded with the following phenological stages: 07/02 = fruit present and fully ripe, foliage green; 08/07 = fruit gone, foliage green; 09/08 = foliage beginning to yellow; 10/12 = foliage yellow, plants senescing.

concentration of biologically active constituents varies with the stage of plant growth and development […] The best time for collection (quality peak season or time of day) should be determined **according to the quality and quantity of biologically active constituents** rather than the total vegetative yield of the targeted medicinal plant parts." [bold emphasis authors']

Goldenseal is included in the United States Pharmacopeia (USP) and USP standards require a minimum alkaloid content of 2.5% berberine and 2.0% hydrastine for dried goldenseal rhizomes and roots (17). Out of the 36 wild-harvested goldenseal rhizome/root samples analyzed in this study, only about half (53%, n = 19) met this threshold for berberine content while only one sample (2.8%) met the hydrastine threshold (Fig. 3). Nearly half of wild rhizome/roots samples harvested before senescence, and nearly all harvested in July and August, failed to meet USP minimums for alkaloid content.

Table 3. Pearson's correlation coefficient (*r*) between soil nutrient elements, root weight, colony, and root/rhizome alkaloid levels in wild goldenseal in relation to harvest date ($n = 9$).

	Root/rhizome alkaloid															
	Berberine				Hydrastine				Canadine				Total alkaloids[a]			
	July	Aug	Sept	Oct	July	Aug	Sept	Oct	July	Aug	Sept	Oct	July	Aug	Sept	Oct
Soil pH	−0.359	0.372	−0.072	0.390	−0.179	−0.108	−0.492	0.047	0.478	0.479	−0.073	−0.107	−0.282	0.128	−0.276	0.328
Soil P	−0.625	0.300	−0.021	0.310	−0.374	−0.340	−0.627	−0.229	0.435	**0.730***	−0.263	−0.224	−0.457	−0.081	−0.388	0.141
Soil K	−0.629	−0.030	0.333	0.253	−0.085	−0.619	0.063	0.376	0.018	0.241	−0.631	0.454	−0.269	−0.447	0.231	0.335
Soil Ca	−0.580	0.022	−0.110	0.342	−0.410	−0.436	**−0.745***	−0.317	0.541	**0.782***	0.121	−0.452	−0.507	−0.253	−0.484	0.114
Soil Mg	−0.476	0.000	−0.132	0.258	−0.298	−0.393	−0.393	−0.140	0.164	0.590	0.101	−0.109	−0.422	−0.225	−0.316	0.115
Root weight	−0.325	−0.034	−0.632	−0.396	−0.383	0.096	−0.635	**−0.903****	0.256	0.651	0.505	**−0.857****	−0.371	0.929	**−0.825****	**−0.688***
Colony	.072	0.327	0.480	0.426	0.566	−0.080	0.514	**0.820****	−0.195	−0.351	**−0.892****	0.632	0.469	0.095	0.616	**0.696***

[a]Total alkaloids is the sum of the three individual alkaloids.
Bolded values indicate the correlation is significant at the 0.05 level (*) or 0.01 level (**), respectively.

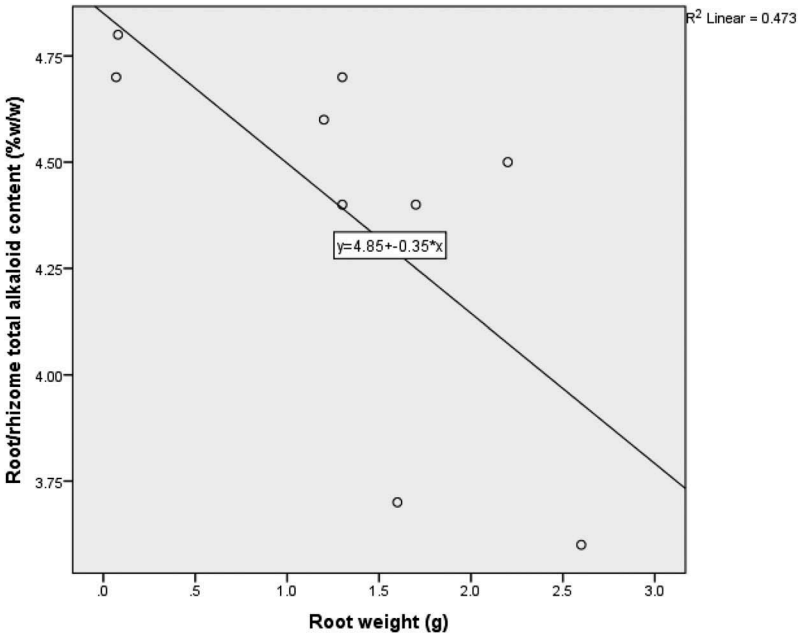

Figure 2. Goldenseal rhizome and root total alkaloid content for October harvest date in relation to root weight.

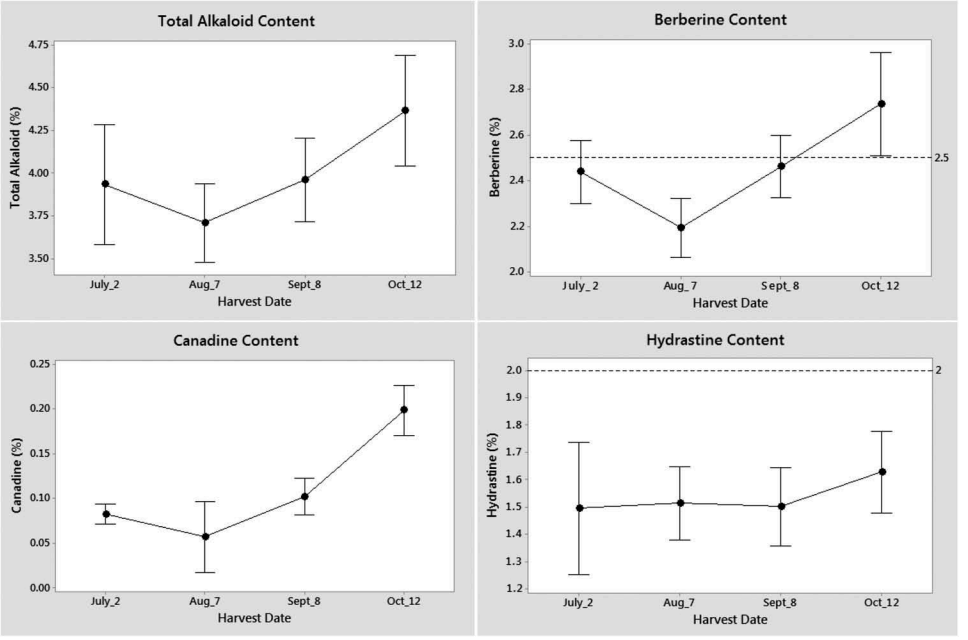

Figure 3. Effects of Harvest Date on alkaloid levels in goldenseal root/rhizome. Bars in each graph represent 95% confidence intervals, which were calculated using individual standard deviations. Dotted lines represent standards for minimum alkaloid content in goldenseal root/rhizome as listed by the United States Pharmacopeia.

The Influence of Environmental Factors on Phytochemistry

A variety of environmental factors influence wild plant phytochemistry in studies of other wild occurring North American medicinal forest plants (12,13). Of the factors examined in this study, rhizome/root size and colony were both correlated with total alkaloid content, suggesting that phytochemistry can vary both within and between populations. These differences may be due to environmental conditions and/or underlying genetic differences not measured or controlled for in this study. The smaller rhizome/root weight, and slightly higher alkaloid levels, associated with colony-3 may also reflect some underlying environmental influence resulting in higher phytochemical constituents. Environmental stress (e.g., drought, light intensity), for example, was important factor causing higher secondary metabolites in plants (18,19).

There was little support for any influence of soil chemistry on alkaloid levels in this study. However, the study sample size was small, and the methodology was limited to correlation rather than experimental manipulation. Additional studies of wild goldenseal phytochemistry with increased replication may provide better understanding of the influence of soil conditions, and other environmental factors, on rhizome/root chemistry. These should ideally be paired with experimental manipulation of environmental conditions (e.g., factorial plots) to provide a more complete understanding of any underlying interrelationship(s).

Although these findings were limited to a single location in Pennsylvania, these phytochemistry results are of potential significance throughout the wild goldenseal harvest region of the United States. Total alkaloid content was highest at plant senescence corroborating industry guidance (20) that root diggers should harvest roots and rhizomes at senescence (typically during late summer and fall months). However, rhizome/root collectors will frequently harvest wild goldenseal at any time during the growing season, a behavior that is increasingly visible in part because collectors post pictures of their harvests on social media platforms (e.g. Facebook) in "root digger" groups. Appalachian root buyers begin advertising prices as early as April, and many collectors begin harvesting goldenseal as soon as plants emerge in the spring. In many cases, no guidance appears to be offered from buyers to collectors as to when to harvest wild rhizomes/roots.

Results obtained here support a late season or Fall harvest of goldenseal rhizomes/roots at the stage when plants are senescing (i.e., turning yellow) or dormant. Findings suggest that scientifically based harvest timing guidance should be provided to buyers, and in turn goldenseal collectors, in order to maximize quality. In doing so, harvest timing could also be shifted to a plant stage (e.g., post-fruit maturation) that allows for plant sexual reproduction. The timing of harvests to permit fruit maturation, allowing an adequate

recovery interval, and attention to site influences are all important components to sustainable harvesting from wild goldenseal populations (14,15,21).

An unexpected finding in this study was that alkaloid levels were nearly as high at fruit maturity (early July) as they were at plant senescence (early October). This suggests further research is needed to examine early season alkaloid levels particularly at flowering and fruiting, and the period in between. It may be that alkaloid levels fluctuate during the growing season in relation to key reproductive phenological stages, as has been observed in other wild-collected medicinal plants (11,22,23). While early (pre-fruit maturation) season harvests would negatively affect sexual reproduction in wild populations, early season harvests from cultivated or forest-farmed populations would not necessarily present such an ethical dilemma.

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