Locating and Documenting Black Cohosh Populations in Western Maryland

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The population analysis of black cohosh, Actaea racemosa L. in Western Maryland was begun by Dr. Jim Howell and Dr. Sunshine Brosi starting in October 2007 and research is ongoing. The primary goal was to compare three State Forest Systems of Western Maryland: Green Ridge State Forest, Savage River State Forest, and Potomac State Forest. Collection of black cohosh plant population, community data, associated soil and other environmental data continued through the 2008, 2009 field seasons. Because of previous associations we were able to create close partnerships with land managers at all three of these forests. The creation of partnerships will allow information gained in this research project to be directly used in the forest management on public lands. Our research may potentially allow creation of cultivation and management zones specific to non-timber forest products. These partnerships also are extremely valuable for future ACES research project capabilities including research access.

Comparisons were made of black cohosh populations at Green Ridge State Forest, Savage River State Forest, and Potomac State Forest. One important aspect of the research is differentiation of black cohosh from other species. Blue cohosh, Caulophyllum thalictroides (L.) Michx and giant blue cohosh, Caulophyllum giganteum (Farw.) Loconte & Blackwell are unrelated species that are vegetatively distinct. However various species within the genus Actaea are extremely similar except when reproductive structures are present. Dollseye or white baneberry, Actaea pachypoda Ell., is vegetatively similar with distinctive flowers present in late-May and fruits present in mid-September. Appalachian bugbane or red baneberry Actaea rubifolia (Kearney) Kartesz (rubra) is another species that is similar to black cohosh until the red flowers and fruit are present. A. rubifolia is threatened in Illinois, Kentucky, Tennessee and considered endangered in Indiana, however the species range is further south than Maryland. Mountain bugbane or American bugbane, Actaea podocarpa DC. (formally Cimicifuga americana Michx), is endangered in Illinois, rare in Pennsylvania, and may have significant population declines in Maryland. During the 2008 and 2009 Summer and Fall field seasons we observed A. racemosa in flower as well as A. pachypoda and A. podocarpa. In order to separate these species it is important to visit field sites in late-May when A. pachypoda is flowering, mid-July when A. racemosa is in flower as well as in mid-September to mid-October when A. podocarpa is in flower and A. pachypoda and A. racemosa are in fruit. Separation can be made prior to this date by plant morphology characteristics. However, fall visits are needed for additional verification and collection of seed. All populations were visited in 2008 and 2009 during theses dates for verification and collection. In 2010 we have visited the sites in late-May to document A. pachypoda.

Black cohosh sites were analyzed for population and community dynamics. Black cohosh populations were measured for abundance, health, and reproduction. Rhizomes were collected from flowering black cohosh plants for chemical analysis in the fall of 2008 and 2009. Only
coyohs with racemes were collected as to verify species identity. In the fall of 2008 and 2009 seeds were collected for seed propagation techniques.

The vegetation on black cohosh sites included analysis of dominant tree species, saplings, and herbaceous species. Each population was marked using a GPS unit and various environmental factors that were measured at each site including: aspect, slope, and light environment using a densitometer. Soils were also collected from cohosh and non-cohosh sites for analysis of texture, pH, organic layer depth, % moisture. Mineral analysis includes calcium, nitrogen, phosphorous, magnesium, potassium, and other minerals.

Results to date include identifying over 83 black cohosh population sites in 3 forest zones (Green Ridge, Potomac, Savage River). We have collected 54 accessions for chemical analysis with representatives from each zone. We have documented associated community structure on 83 black cohosh population sites in 3 forest zones (Green Ridge, Potomac, Savage River): which includes 747 herbaceous vegetation plots, 249 sapling plots, 83 overstory plots, and 498 soil samples. For the 2008 and 2009 seasons collectively we have put in over 2000 total hours: 600 undergraduate hours and 1400 faculty hours.

In the summer of 2010 we expanded our research to include State Parks in Maryland and adjacent states. Surveys were completed in May on the following Maryland State Parks: Big Run, Harrington Manner State Park, Dan’s Mountain, and Ohiopyle (PA). Surveys to date have added an additional 40 locations. In the summer of 2010 they will expand to additional state parks. Organism analysis of soil biota and collection of samples for fungal analysis are also part of the future research plans.

Multivariate procedures were used to determine the environmental or community factors needed for optimal black cohosh growth and reproduction. Future work will also include analysis of black cohosh sites in order to determine which forest management zones are more likely to contain black cohosh.

The outcomes of this project include: determining management protocols for existing black cohosh sites on public lands, producing suggestions for potential black cohosh cultivation in state managed areas, and continuing collaboration with state forest systems for additional native medicinal plants. In addition this project is used as a teaching tool for classes at multiple institutions. Students from Allegany College’s Medicinal Botany class and have participated in the research during these semesters: Fall 2007, Spring 2008, Fall 2008, Spring 2009, and Fall 2009. Frostburg State University students in Introduction to Ethnobotany have also participated in the research during the Fall 2008 and 2009 semesters. Students in these classes learn plant and tree identification, habitat concepts, associated plant species, GPS navigation, plant usage, an understanding of research tools, and gain valuable field experiences.

**Objective #1: Characterization of Black Cohosh populations in Western Maryland.** In order to determine sustainability of black cohosh populations there are many crucial factors that need to be determined. Current research has found populations of cohosh and multivariate procedures will be used to determine the environmental or community factors needed for optimal black cohosh growth and reproduction. During the past two field seasons this project has identified and
characterized 83 locations of black cohosh on Savage River, Potomac, and Green Ridge State Forests. Habitat characterization, documentation of associated vegetation, and soil analysis has been completed on these known populations. Seed propagation trials have worked to determine methods for production of from seed. Black cohosh from the seed propagation trials have been outplanted in a forest and adjacent non-forested track under shade cloth and in various mixed species plantings. This study will help determine the optimal shade conditions and companion planting for growth and production of secondary metabolites. Increasing success rates of propagation and growth of Black Cohosh would allow this herb to be cultivated in the region to increase economic development. Future research for this project will include identification of additional black cohosh population sites on state lands in western Maryland as well as potential sites in southwestern Pennsylvania, northcentral West Virginia, and northwestern Virginia. This project will also continue collection of plants and seed for greenhouse studies. In the future we plan on expanding to include genetic studies, organism analysis of soil biota, and collection of samples for fungal analysis. One important aspect of the research is differentiation of black cohosh from other species. Blue cohosh, *Caulophyllum thalictroides* (L.) Michx and giant blue cohosh, *Caulophyllum giganteum* (Farw.) Locote & Blackwell are unrelated species that are vegetatively distinct. However various species within the genus *Actaea* are extremely similar except when reproductive structures are present. Dollseye or white baneberry, *Actaea pachypoda* Ell., is vegetatively similar with distinctive fruits present in mid-September. Appalachian bugbane or red baneberry, *Actaea rubifolia* (Kearney) Kartesz (rubra), is another species that is similar to black cohosh until the red flowers and fruit are present, with a current range not in western Maryland. *A. rubifolia* is threatened in Illinois, Kentucky, Tennessee and considered endangered in Indiana. Mountain bugbane or American bugbane, *Actaea podocarpa* DC. (previously *Cimicifuga americana* Michx), is endangered in Illinois, rare in Pennsylvania, and is rare in Maryland (S2: approximately 6-20 estimated occurrences). *A. podocarpa* may have significant population declines due to the loss of the eastern hemlock in state. During the 2008 and 2009 summer and fall field seasons *A. racemosa* was observed in flower as well as *A. pachypoda* and *A. podocarpa*. In order to separate these species it is important to visit field sites in mid-July when *A. racemosa* is in flower as well as in mid-September to mid-October when *A. podocarpa* is in flower and *A. pachypoda* and *A. racemosa* are in fruit. All plots have been visited to distinguish between the species.

Black cohosh sites were analyzed for population and community dynamics. The black cohosh plants on site were measured for abundance, health, and reproduction. Rhizomes were collected from flowering black cohosh plants for chemical analysis. This fall, germplasm will be collected for propagation and genetic analysis. The vegetation on black cohosh sites as well as sites without black cohosh included analysis of dominant tree species, saplings, and herbaceous species. Each population was marked using a GPS unit with various environmental factors that were measured at each site including: aspect, slope, and light environment using a densitometer. Soils were also collected from cohosh and non-cohosh sites for analysis of texture, pH, organic layer depth, and % moisture (Table 1). Mineral analysis includes calcium, nitrogen, phosphorous, magnesium, potassium, as well as other minerals (Table 2). Soil texture was determined for the 74 locations by combining the data from 3 soil samples near black cohosh plants at each location. Black cohosh was found primarily on sandy loam and sandy clay loam but was also found on clay loam, loam, and loamy sand (Figure 1).
In fall of 2008 and 2009, approximately 128,000 black cohosh seeds were collected from 156 parent plants in Savage River State Forest, Green Ridge State Forest, Potomac State Forest, and from Frostburg State University campus. In spring of 2009, seeds were separated according to buoyancy, cold-stratified, and subjected to a 30-day germination trial. Observed germination rates differed between individual parent plants, collection sites and between buoyant and non-buoyant seeds (Figure 2). A subsequent germination trial is underway in spring of 2010, in order to determine the heritability of germination rates. The outcome of these germination trials will be made available to the general public through the creation of a brochure on the cultivation of black cohosh and sustainability of wild harvesting.

Results to date include identifying over 150 black cohosh population sites in the 3 forest zones measured (Green Ridge, Potomac, Savage River). 82 accessions have been collected for chemical analysis with representatives from each zone. Associated community structure has been documented on 83 black cohosh population sites in the 3 forest zones measured: which includes 498 herbaceous vegetation plots, 249 sapling plots, 83 overstory plots, and 498 soil samples.

**Objective #2:** *Habitat modeling of Black Cohosh in Western Maryland.* Habitat models based on existing locations of Black Cohosh are being developed. This effort uses information on the values of incident solar radiation, canopy cover, potential soil wetness, soil reactivity, slope, distance to streams, etc. at known locations of Black Cohosh to inform habitat suitability models. The model(s) are then applied to large geographic regions using GIS to delineate areas most likely to have conditions suitable for Black Cohosh establishment. To date, a Mahalanobis Distance model has been successfully applied for Western Maryland. A second model, based on Maximum Entropy methods, is under development. Resultant mapping of habitat suitability by one or both models will aid in finding new populations and direct site selection for areas most suitable for restoration and cultivation. Future research plans include additional field verification of the models and expansion of the model to new public lands in western Maryland and surrounding states. The project could also be expanded to use information from field-verified habitat models and field studies to determine the abundance of black cohosh in the state of Maryland. This will allow assessments of the amount of black cohosh that can be sustainably harvested from the wild.

Work to date has involved predicting habitat suitability across Garrett and Allegany Counties using a Mahalanobis Distance model and GIS data. Biophysical variables include elevation, slope, incident solar radiation, canopy cover, potential soil wetness and soil pH, and distance to streams. Specific focus has been on Green Ridge, Savage River and the Potomac-Garrett State Forests in western Maryland (Figure 3). Values for the aforementioned variables were extracted from GIS data layers (grids, 30m resolution) for the locations of known Black Cohosh populations (115 populations observed during the 2008 and 2009 field data collection) and compared to mean values from across all pixels in the State Forests (Table 3). Black Cohosh was generally found on areas of lower slopes, higher potential wetness (TCI) and a closer distance to streams than average for these state forests. GIS data for the observed locations was used as input to the Mahalanobis Distance model, which in turn yielded gridded GIS output for all of western Maryland in terms of habitat suitability. An example of the output for Savage River State Forest is shown (Figure 4). Habitat suitability for these State Forests was decomposed into binary suitable / not-suitable categories using a thresholding technique that
involved analysis of 1,000 randomly drawn points form the state forest data layers (see Figure 5). Transformation of the continuous data (e.g., Fig. 4) to a binary format is shown for Savage River State Forest (Fig. 6) for illustrative purposes. Results indicate approximately 30% of Savage River, 30% of the Potomac/Garrett and 10% of Green Ridge State Forests are highly suitable habitat for Black Cohosh. This information provides a baseline for potential carrying capacity and may be useful if Black Cohosh is considered as part of future forest management plans. Validation efforts were undertaken to estimate the predictive ability of the model and it was found that many sites considered “ideal” habitat for Black Cohosh had had recent timber harvests, highlighting the importance of disturbance history and land use characteristics on population abundance (potential or actual). Ongoing work includes expansion of the model to Washington County, MD, and establishment of an entirely new suitability model based on Maximum Entropy principles. The purpose of this second modeling effort is to determine which geographic areas are predicted to be ideal habitat by two independent models. Areas predicted as ideal by one of the 2 models would be considered to be less reliable while greater confidence would be assigned to areas predicted to be ideal by both models. Mapping suitability for black cohosh resulted in information to include in timber management plans. Future work will aid to assist in setting of harvest limits or number of collection permits issued. 7%-30% of western MD State Forests predicted to support black cohosh populations but actual coverage far less. We believe disturbance history plays a large part in suppression of black cohosh populations because our predictions are too optimistic. More research is needed including refining models by incorporating stand age (mapping in progress).

2010 Presentations

June 9, Stratification protocols and germination rates of black cohosh (Actaea racemosa L.) populations from western Maryland, Society for Economic Botany, Xalapa, Mexico. Natalie A. Walsh and Sunshine L. Brosi.

June 8, Sustainability of an Appalachian herb, black cohosh, Actaea racemosa L.: determining factors of habitat and abundance in western Maryland, Society for Economic Botany, Xalapa, Mexico. Sunshine L. Brosi.


June 9, Encouraging Community Involvement on the Grass Roots Level for Ethnobotanical Research and Outreach. Mimi Hernandez.

June 2, Ethnobotany and other majors at Frostburg State University, Fort Hill High School, Cumberland, MD. Sunshine L. Brosi

May 5, two undergraduate research posters, Biology Poster Session, Frostburg State University.
April 29, guest speaker, 2010 Richard A. Johnson Environmental Education Award, University of Maryland Center for Environmental Science Appalachian Laboratory, Frostburg, MD. Sunshine L. Brosi.

April 5-9, Habitat suitability for the medicinal plant black cohosh (Actaea racemosa, L.) in western Maryland - a baseline study for management of non-timber forest resources, 2010 International Association of Landscape Ecology, US-IALE Twenty-fifth Anniversary Symposium, University of Georgia, Athens, GA. Joseph R. Ferrari, Sunshine L. Brosi, and Jim Howell.

March 27, Green Jobs Panel, Fields of Green Internship Fair, Bethesda, MD, Sunshine Brosi

March 21-22, People and Plant Communities of the Appalachian Mountains: Ethnobotany in western Maryland, 33rd Annual Conference, Appalachian Studies Conference, Engaging Communities, North Georgia College and State University, Dahlonega, GA. Sunshine Brosi

March 21-22, The Last Forest Curriculum Guide, Appalachian Studies Conference, Engaging Communities, North Georgia College and State University, Dahlonega, GA. Sunshine Brosi.

March 21, An Herb Grows in Appalachia: Sustainability of Black Cohosh Populations in Western Maryland, 33rd Annual Conference, Appalachian Studies Conference, Engaging Communities, North Georgia College and State University, Dahlonega, GA. Natalie Walsh and Sunshine Brosi.

March 12, Faculty Panel: The Curious Case of the Life of Academics, The 11th National Conference for McNair Scholars and Undergraduate Research, University of Maryland College Park, MD. Sunshine Brosi.

February 21, Winter Woods Wonderland, Maryland Association for Environmental and Outdoor Education, 10th Annual Conference, Rocky Gap, MD. Sunshine L. Brosi.


2009 Presentations

Aug. 5, Natalie A. Walsh and Sunshine L. Brosi, Stratification protocols and germination rates of open-pollinated families of black cohosh (Actaea racemosa L.) from three western Maryland state forests: Population dynamics of a medicinal herb native to the Appalachian Mountains (poster), 94th Ecological Society of America (ESA) Annual Meeting, Albuquerque, NM. Awarded the Traditional Ecological Knowledge Student Merit Award and Gira Campus to Community Fund.

July 7, Christopher Massimino and Sunshine Brosi, To increase our ethnobotanical knowledge of local medicinal plants (oral presentation, undergraduate student) University of Maryland, McNair Scholars Program, Summer Research Institute, awarded the McNair Ambassador Award. Also presented at the 2009 University at Buffalo (SUNY) McNair Research Conference at Niagara Falls, NY, July 18th.

June 10, Sunshine L. Brosi, Population Biology and habitat dynamics of black cohosh (Actaea racemosa) on state forests in western Maryland: Method development applicable to additional
Appalachian medicinal herbs (invited presentation), Progress and prospects of black cohosh as a sustainable medicinal plant, Appalachian Center for Ethnobotanical Studies Workshop, Rockville, MD.

June 4, Sunshine L. Brosi and Dr. James Howell, Prospects for sustainable management of black cohosh, Actaea racemosa L., in western Maryland (oral presentation); Natalie A. Walsh and Sunshine L. Brosi, June 4, 2009, Stratification protocols and germination rates of open-pollinated families of black cohosh (Actaea racemosa L.) from three western Maryland state forests: Population dynamics of a medicinal herb native to the Appalachian Mountains (oral presentation); Kathryn Schmelter, Sunshine L. Brosi, Mitra Karimian, Rebecca Shipe, Medicinal herb cultivation in pure and mixed plantings (student poster); Carson Sommerlatt, Sunshine L. Brosi, Thomas Berry, Rebecca L. Shipe, The effects of soil tilling in production of herbal species in the understory of forest in western Maryland (student poster), Society for Economic Botany (SEB) 50th Annual Meeting, Charleston, SC.

April 4, Sunshine L. Brosi, Healthy Women and Healthy Forests: Prospects for Sustainable Management of an Ethnobotanical Herb, Black Cohosh, in Western Maryland, Southeastern Women’s Studies Association (SEWSA) Annual Conference Appalachian State University, Boone, NC.

April 4, Natalie A. Walsh and Sunshine L. Brosi, 2009, Conserving Women’s Herbal Healing Wisdom: Protecting Black Cohosh in Eastern Forests, SEWSA Annual Conference, Appalachian State University, Boone, NC.

Tables

Table 1. General site characterization of populations of black cohosh in western Maryland.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of individuals per population</td>
<td>30</td>
<td>20-200</td>
</tr>
<tr>
<td>Soil pH</td>
<td>6.25</td>
<td>(5.5-7.5)</td>
</tr>
<tr>
<td>Organic matter (depth)</td>
<td>9.9</td>
<td>(3-20)</td>
</tr>
<tr>
<td>Organic matter (1-5 range, 1=low, 5=high)</td>
<td>4.47</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Preliminary soil analyzes for all black cohosh locations showing the percentage of each plots with the relative amounts of particular soil constituents.

<table>
<thead>
<tr>
<th>Amount</th>
<th>Calcium</th>
<th>Manganese</th>
<th>Carbohydrates</th>
<th>Sulfates</th>
</tr>
</thead>
<tbody>
<tr>
<td>None detected</td>
<td>12.5%</td>
<td>86%</td>
<td>88%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Low</td>
<td>39%</td>
<td>11%</td>
<td>4.7%</td>
<td>94%</td>
</tr>
</tbody>
</table>
Table 3. Spatial data associated with the 115 observed populations of black cohosh and average values for all area within the three state forests analyzed for western Maryland (Green Ridge, Savage River and Potomac/Garrett State Forests).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Observed Locations Mean</th>
<th>SD</th>
<th>Values across State Forests Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevation (m)</td>
<td>589.57</td>
<td>142.56</td>
<td>556.93</td>
<td>238.14</td>
</tr>
<tr>
<td>Slope</td>
<td>0.16</td>
<td>0.10</td>
<td>0.23</td>
<td>0.14</td>
</tr>
<tr>
<td>TCI (topographic convergence index)</td>
<td>9.33</td>
<td>3.34</td>
<td>6.91</td>
<td>2.46</td>
</tr>
<tr>
<td>DTS (distance to stream, m)</td>
<td>124.27</td>
<td>172.07</td>
<td>263.53</td>
<td>226.64</td>
</tr>
<tr>
<td>PRR (potential relative radiation)</td>
<td>12,360.00</td>
<td>778.90</td>
<td>12,621.00</td>
<td>861.15</td>
</tr>
<tr>
<td>pH</td>
<td>5.04</td>
<td>0.40</td>
<td>4.85</td>
<td>0.43</td>
</tr>
<tr>
<td>Canopy (%)</td>
<td>81.38</td>
<td>8.33</td>
<td>77.27</td>
<td>16.38</td>
</tr>
<tr>
<td>Mahalanobis Distance</td>
<td>6.94</td>
<td>4.47</td>
<td>18.06</td>
<td>18.90</td>
</tr>
</tbody>
</table>
Figure 1. Soil texture of known black cohosh sites in western Maryland. This spreadsheet w/pre-built USDA texture triangle was downloaded from http://nowlin.css.msu.edu/software/triangle_form.html.

Figure 2. 2009 Germination rates for black cohosh seeds across four collection sites in western Maryland. Differences in seed viability were greatest between Savage River State Forest and all other sites.
Figure 3. Location of Green Ridge, Savage River and Potomac State Forests in Western Maryland.
Figure 4. Habitat suitability based on Mahalanobis Distance ($D^2$) for Savage River State Forest, western Maryland. Low values indicate high suitability, high values correspond to low suitability.
Figure 5. Mahalanobis distance ($D^2$) cumulative frequency distribution for observed black cohosh locations and randomly chosen locations within the western Maryland study area. Dashed line represents the difference between the Mahalanobis distances (observed – random) with the peak indicated at a value of 7.9.
Figure 6. Habitat within Savage River State Forest partitioned into ideal habitat ($D^2$) and less than ideal habitat ($D^2 > 7.9$).
Figure 7. Suitable Habitat in Green Ridge State Forest, Allegany County, MD
Figure 8. Black cohosh populations from 2008 and 2009 surveys.
The impact of light, environment and companion plants on the cultivation of *Actaea racemosa* L. in Western Maryland

*Robert Kutchman, Graduate Research Assistant, Frostburg State University Department of Biology*

**Introduction:**

In recent years, the global demand for black cohosh (*Actaea racemosa* L.) has grown considerably due to the potential efficacy of plant parts used to treat a variety of medical conditions. The native range of *A. racemosa* includes the eastern woodlands of North America, stretching from Southern Ontario to Georgia and west to Arkansas and Wisconsin (Greenfield et al. 2006). Most of the black cohosh that is collected for use is done so in the wild. A survey conducted between 1997-2001 indicated that 97.6% of *A. racemosa* sold by herb dealers were wild harvested, resulting in 1.4 million pounds of the plant (American Herbal Products Association 2003). Limited information exists about the abundance and reproduction of black cohosh in the wild; therefore, an increase in demand for the species may result in unsustainable harvesting and the loss of unique, locally adapted populations. This is already evident in Massachusetts and Illinois, where black cohosh has officially been listed as an endangered species (Endangered Species Protection Board 2004, Natural Heritage and Endangered Species Program 2008). Traditionally, the rhizome and the root of black cohosh are used medicinally for a variety of ailments including kidney troubles, rheumatic pain and tuberculosis (Moerman 1998). The collection of the rhizome and root for medicinal use results in the mortality of the plant when collected. Black cohosh is ecologically important as the host plant for the Appalachian azure butterfly (Lonner 2007) and culturally important to many groups as a source of medicine (Moerman 1998). Increases in cultivation would reduce the demand on wild populations and may result in sustainable harvesting. However, limited information exists on effective methods of cultivation (Fischer et al. 2006). This research project is focused on determining optimal cultivation techniques for black cohosh; a beneficial and potential economically important plant.

**Location:**

Research will be conducted at the Evergreen Heritage Center in Mount Savage, MD which has a rich local history and dedication to environmental education and sustainability. I also hope to duplicate my experiment at another location close to the area.

**Estimated Time Table:**

- **January – March ’10** Start plants from seeds in the greenhouse on campus, care for established plants to prepare for transplanting in the spring.
- **March- April ’10** Start work preparing the grounds for planting, including building a shade cloth structure and erecting a fence to keep wildlife out. Transplant from the greenhouse to the shade cloth structure and the surrounding woods.
- **April- September ’10** Care for the plants, collect data involved with the experiment including height, diameter, number of leaves, and health measures. Defend research proposal, complete oral examinations and be admitted to candidacy.
• **September- October ’10** Harvest 5% of the plants for chemical composition and above- and below-ground measurements. Pull the shade cloth back to prepare for the winter months and snow cover. Present first-year data at the Western Maryland Native Plant Society Meeting.

• **March’11- September’11** Re-cover the plants with shade cloth and care for plants, collecting the second year of data for the site. Present second-year data at the Ecological Society of America meeting.

• **September ’11 – December ’11** Harvest 5% of the plants for chemical composition and above- and below-ground measurements. Analyze and publish results of research, graduate.

**Project:**
Sustainability, which is the main goal of this research can involve many different factors. One key factor in sustainability is increasing the supply of a plant which is in high demand. Specific information on optimal growing conditions for black cohosh will aid growers and increase cultivated production. I plan to look at two main aspects regarding the cultivation of black cohosh:

*Goal 1)* A recent study by McCoy et al. (2007) showed a significantly higher level of growth and seed production of *A. racemosa* under a 78% shade cloth structure as compared to two separate forested areas of 90% shade, indicating that it may be beneficial to cultivate black cohosh with less shade than originally predicted. I plan to compare cultivation of black cohosh plants under 6 different light treatments: full sun, forest edge, forest interior, and 4 different levels of shade cloth (50%, 60%, 70%, and 80%). All light level plots will be 15 feet square and each light treatment will have two replications at each of the two planting sites. Plants grown under shade cloths will be in the same area with cloths arranged in a random order. This structure will be accomplished by the use of tall logs firmly planted in the ground to allow for the shade cloth to be stretched over the planting bed at a height of at least 5 feet. Full sun and forested plots will be adjacent to the area with the shade cloth. Organic weedguard will be laid down before planting under the shade cloth but not in the forested plots. Black cohosh will be evaluated base on size (height, number of leaves, and diameter at the base), and health (based on nutrient levels in tissue samples and chlorophyll-level readings). In addition, prior to leaf senescence at the end of growing season 2010 and 2011 a 5% sample will be harvested. Above-ground biomass will be measured using a leaf area meter. Below-ground biomass will be weighed. Leaves will be measured for levels of macro- and micronutrients. Differences in growth rate, health, and survival will be used to determine optimal growing conditions for this important medicinal herb.
Light

<table>
<thead>
<tr>
<th></th>
<th>Forest</th>
<th>Shade cloth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Sun</td>
<td>Edge</td>
<td>Interior</td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>Number of Leaves</td>
<td></td>
<td>60%</td>
</tr>
<tr>
<td>Diameter At Base</td>
<td></td>
<td>70%</td>
</tr>
<tr>
<td>Nutrient Levels</td>
<td></td>
<td>80%</td>
</tr>
<tr>
<td>Chlorophyll Levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount of Active Constituents</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Goal 2) Because of the current limited economic value of black cohosh, production may be more profitable if grown in companion with other valuable medicinal herbs such as bloodroot, goldenseal and ginseng. All of these herbs are thought to require shade for successful growth, although a study by Marino et al. (1997) actually found increased growth rates in bloodroot under higher light conditions. Companion planting of ginseng and other shade-dependent herbs has shown to reduce attack by fungal and other antagonistic agents (Cech 2002). The large size of black cohosh plants may also allow for the cultivation of ginseng in full sunlight. Ginseng, goldenseal, and bloodroot plants will be grown in various combinations with black cohosh to determine impacts on growth, health, and survival. This part of the project will be accomplished in the interior forest at the evergreen heritage center and in full sunlight to allow for more uniform light conditions.
Goal 3) Various environmental factors influence the production of secondary metabolites in individual plants. Light environments have been shown to impact the amount of particular compounds in various species which may result in impacts to potential effectiveness. Companion plants may also impact chemical constituents in plants. Rhizomes will be sampled at three different time periods for differences in the amount of active constituents, prior to planting, after year one, and after year two. Differences in growth rate, health, survival, and potential effectiveness will be used to determine optimal growing conditions for this important medicinal herb.

Goal 4) Conduct a population study of black cohosh and common companion plants. Gathering data involving distance between and abundance of black cohosh and neighboring plants will provide me with a natural model to compare my studies with.

Materials:

Materials provided by the Appalachian Center for Ethnobotanical Studies:

- Auger digger for posts 1.5’ deep
- Rope to secure the shade cloth in place with equal tension
- 50 Ginseng Plants
- 50 Black cohosh plants
- Measuring tapes
- Shade cloth (4) 20’x20’ 50%,60%,70%,80% Shade- $1,114.60
- Organic biodegradable planting cloth 20 Rolls 23”x250’- $785.00
- Labor-$2500/semester x 4 semesters = $10,000
- Total Provided by FSU/ACES grant = $11,899.60

Materials provided by Evergreen Heritage Center:

- Fence posts (30) 6’ = $150.00 and (5) 8’ = $50.00
- Deer fence and gate - $500.00
- Labor- $500.00
- Total Provided by Evergreen Heritage Center= $ 1,200.00

Additional Materials requested from Evergreen Heritage Center for spring 2010:

- Gravel to hold logs in place for the shade cloth structure $50.00
- 300 additional Black Cohosh Plants- $39.00/15 Rhizomes- $780.00
- 300 Bloodroot Plants- $48.17/1lb.-$192.68
- 300 Goldenseal Plants- $.75/rootlet- $225.00
- 300 Ginseng Plants- $69.95/100 rootlets- $209.85
  Total requested -$1,456.68

Justification:

The consistent depletion of black cohosh in the wild and the inversely proportionate demand for the plant make the issue of sustainability especially important. Discovering techniques that result in more cost effective cultivation is the key to sustainability of these populations. This research project could serve as a model for organic farms in the Appalachian Mountains looking to diversify crop production and also for an education experience for K-12 students. With a substantial history of surface mining in Western Maryland, this could provide for a viable alternative as well. This integration of science and outreach to the community will make this a very beneficial research project. Frostburg State University is a small teaching institution with limited resources for chemical analysis. The chemistry department is undergraduate only and to date has been unable to analyze samples for active constituents. Chromadex offers relatively inexpensive analysis of constituents which would be a very valuable first-step in screening.

References


Natural Heritage and Endangered Species Program. 2008. Massachusetts list of endangered, threatened, and special concern species. Massachusetts Division of Fisheries and Wildlife, Boston, MA, USA.
Stratification Protocols and Germination Rates of Black Cohosh Populations from Western Maryland
Natalie Walsh, Graduate Student, Frostburg State University Department of Biology

Black cohosh (Actaea racemosa L. fmr. Cimicifuga racemosa (L.) Nutt.) is a culturally and economically significant herb native to eastern forests of North America. Used historically by the Cherokee for many common ailments, it is employed today as a dietary supplement to reduce menopause symptoms, especially hot flashes. As the popularity of A. racemosa as an alternative to hormone therapy grows, plant populations face increased threats from wild harvesting and habitat loss. Black cohosh is endangered in Illinois and Massachusetts, listed as an —At-Risk‖ plant by United Plant Savers and as a top-ranked medicinal plant species of special concern by the Nature Conservancy. In addition, black cohosh is the host plant for the Appalachian Azure (Celastrina neglectamajor Opler and Krizek), a forest understory lepidopteran that is at risk of extinction in many parts of its native range.

The collection of relevant ecological information about this at-risk species is paramount to protecting populations before market forces overwhelm the sustainability of wild harvests, from which most black cohosh is obtained. Given the recent rise in popularity of black cohosh, it is important to consider conservation measures that can prevent possible further habitat loss, habitat degradation, and overharvesting, such as cultivation. Current cultivation practices de-emphasize the use of locally-adapted seed as a viable planting stock, instead focusing on methods of rhizome propagation and the use of cuttings. Rhizome propagation methods produce clonal plantings with diminished genetic diversity that are less conducive to conservation of the diversity of locally-adapted types.

In the fall of 2008 and 2009, approximately 128,000 seeds were collected from individual open-pollinated black cohosh plants in different physiographic regions of western Maryland, resulting in separate taxa. In 2008, seeds from 92 parent plants were winnowed, counted, weighed, and sorted according to buoyancy. In 2009, seeds from 65 parent plants were added to the study to compare yearly variation. Comparisons across years and populations detected significant variation in seed morphology, buoyancy, germination rate, and seedling characteristics. Seed stratification was consistent with published techniques to break double dormancy. From the 2008 collection, 327 seedlings emerged after 30 days, only 0.5% of all seeds. Bleach treatments showed increased germination when compared to controls.

Rigorous investigation of the stratification and germination requirements of black cohosh will be the first step in the domestication of a wild medicinal plant. Results will provide valuable insight into the utility of seed-based husbandry for growers, help to encourage cultivation of locally-adapted seed by growers of other medicinal herbs and specialty crops, and provide a potential method for cultivation of other Actaea L. species.
Ecological Relations of Black Cohosh
Dan Fiscus, Frostburg State University Department of Biology

1. Worked in collaboration with Dr. Phil Allen, FSU Geography Department, to prepare for pollen identification and quantification. Phil is developing methods to remove pollen from bees and to identify and count pollen grains under a light microscope. We will also collaborate to collect pollen from black cohosh (Actaea racemosa) plants and other plants flowering at the same time to develop a set of reference pollen slides, reference pollen images and a pollen ID key. See Figure 3 for a photograph of black cohosh pollen from prior work.

2. Conducted correspondence and received assistance from Dr. Sam Droege at USGS Patuxent Wildlife Research Center on bee identification and pollination ecology. Sam also sent Chris Waldrup (see below) a box of dried and pinned bee specimens, with species and capture locations noted for each, to aid learning the Discover Life online bee identification tools (see below). We also learned about and subscribed to the bee monitoring email list, a discussion list through which experts provide assistance with bee identification and survey protocols. Sam identified the bee in Figure 1 as most likely Bombus impatiens. Information on Dr. Droege and his work with bees is available here: http://www.pwrc.usgs.gov/staff/profiles/documents/droege.htm

3. Worked with a volunteer, Chris Waldrup, toward insect collection, storage, preservation and identification. Chris has experience with entomology, and he has led this work. Chris has also assisted by learning to use the online bee identification resources at Discover Life, many of which were developed by Sam Droege. This website is an excellent tool and is online here: http://www.discoverlife.org/

Chris has pinned, labeled and preserved all the insects and other species captured on black cohosh flowers in summer 2009. He has made initial identifications to orders, families and in some cases sub-families. See the photo in Figure 2 for a subset of these specimens.

4. Developed a list of species known to pollinate several plants closely related to black cohosh (Actaea racemosa), including mountain bugbane, Actaea podocarpa, a nectariferous species (Pellmyr, O. 1986a), and Actaea rubifolia, a nectarless species (Pellmyr, O. 1986b). These pollinators include:

Apis mellifera (L.) (honeybees)

Five species of bumblebees:
Bombus affinis Cresson (Now basically extinct, Sam Droege, personal communication)
Bombus impatiens Cresson
Bombus perplexus Cresson
Bombus vagans Smith
Bombus pensylvanicus
And flies from the family Syrphidae:
*Melanostoma mellium* (L.)
*Meliscaeva cinctella* (Z.)
*Epistrophe grossulariae* (Meigen)

5. Consultation and initial assistance from Dr. Sunshine Brosi and Dr. Jim Howell ( Allegany College of Maryland) on how to determine successful pollination of black cohosh by inspection of fruits and seeds on plants for which the pollinator visits have been observed, recorded and quantified.

6. Developed plans for summer field work to complete the first major portion of black cohosh pollination work and identify the main pollinators. This field work will be conducted at four sites in Western Maryland that represent different habitat types (forest interior and forest edge) and physiographic provinces (Appalachian Plateau and Ridge and Valley provinces). Plans include these major aspects:

   a. Photography of insect visitors to multiple black cohosh flowers over short (20 minute) and long (4-6 hour) time samples with later identification of the visitors from photographs with assistance of Sam Droege and others on the bee monitoring list.

   b. Capture of insect visitors over short (20 minute) and long (4-6 hour) time samples with later identification from the specimens. These specimens will also be sources of pollen for pollen identification and quantification.

   c. GPS and marking of plants for return (roughly 2 weeks later, as fruit and seeds develop).

   d. Quantification of the degree of successful pollination of the plants by examination of fruits and seeds at the marked plants.

   e. Correlation between the visitor species and frequencies with pollination success to determine the effective insect pollinators.

   f. Identification of the pollen and quantification of the relative proportions of black cohosh versus other species’ pollen in the corbicular loads of captured bees. This will help determine the degree of fidelity or plant specificity of each of the major pollinators and the interactions with other plants (native and non-native) in the area.

References:


Figure 1. Photo of bumblebee at work on black cohosh, July 2009, near Frostburg, MD. This bee is most likely *Bombus impatiens* (Sam Droege, personal communication). Note large pollen load.
Figure 2. Photo of some of the insect specimens captured on black cohosh flowers in summer of 2009. Insects have been pinned, preserved, labeled and identified to initial degree by Chris Waldrup. The yellow block is a preservative and pest repellent.
Figure 3. Scanning electron micrograph of black cohosh pollen grains. Photo by Dr. Kristen Lennon, FSU Biology Department. Pollen was removed from a bee captured near Frostburg, MD in summer 2008.
Chemical Analysis of Black Cohosh

Peggy Biser, Frostburg State University Department of Chemistry

The individuals working with Peggy Biser on the chemical analysis of Black Cohosh expanded and currently includes two undergraduate students and one postdoctoral fellow. The two undergraduates are Andrew Minnick and Marcus Carter. Andrew has been involved with the project since 2008. Marcus joined the lab in the fall of 2009. The laboratory welcomed Dr. Mitra Sadeghipour in the spring of 2009.

In 2009 we codified the methodology that used for the processing and storage of rhizomes. This more rigorous approach was applied to the processing of some 41 root samples supplied by Dr. Jim Chamberlain from sites in Virginia and 18 root samples supplied by Drs. Sunshine Brosi and Jim Howell from sites in Maryland.

Efforts to improve, expand and evaluate the extraction protocol continued through 2009 into 2010. Storage conditions were explored and determined to be problematic, leading to the establishment of conditions that minimize light and temperature. The extraction protocol was explored and modified. The Soxlet extraction process was abandoned in favor of a lower temperature process. Rotovaporation was replaced by vacuum drying. Additional solvents have been added to those used previously in an effort to determine the effect of solvents on the quantity and quality of phytochemicals extracted.


Minnick, A.J. and Biser, P.: Comparison of conventional Cimicifuga racemosa Soxhlet extraction technique to a low temperature alternative method. Poster session presented at: Undergraduate Research Poster Session: Analytical Chemistry. The 237th ACS National Meeting; 2009, March 22-26; Salt Lake City, UT.
Evaluation of Natural Insectiside Production by Black Cohosh and increasing levels of active ingredients within rhizomes

David Puthoff, Frostburg State University Department of Biology

The continued battle of plants and plant growers against insect induced predation will never end. Increasing the use of insecticides may not be the most environmental solution to combat these attacks. One method to prevent or hinder insect attack would be to identify more natural insecticides or feeding deterents from plants, especially plants that have a reputation as having insect immunity. Black cohosh and its close relatives represent one group of plants that may possess such compounds.

Investigations were started to extract a feeding deterrent from black cohosh. Leaf extracts were made from black cohosh using either methanol or isopropanol. These extracts were used in bioassay experiments designed to detect feeding deterents using several wild and commercially available insects (*Manduca sexta*, *Helicoverpa zea* and *Spodoptera frugiperda*). No differences in feeding behavior were identified when comparisons of extract treated plants were compared to control plants. Continued study in this area will consist of identifying insect species that may prefer not to feed on black cohosh.

Another project that has been initiated is to identify ways which will yield higher active ingredient levels in harvested black cohosh material. The increase in active ingredient levels will help conserve wild populations because fewer plants will need to be removed from the wild populations in order to meet the market demand for black cohosh.

It is well known that plants produce many secondary metabolites in response to being attacked by insect predators. These products range from phenolics to terpenoids. Given that not much information is available on the leaf, root and rhizome predators of black cohosh, laboratory simulations of insect attack will be used to induce the production of active ingredients within the black cohosh rhizome. Black cohosh rhizomes (both purchased and wild collected) will be subjected to simulated insect attack. Forty eight hours after this attack, the rhizomes will be dried, extracted and evaluated for increases in two active ingredients (actein and deoxyactein). High Performance Thin Layer Chromatography (HPTLC) will be used to quantify the active ingredients.