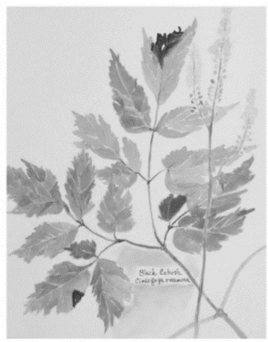
PROCEEDINGS

CONSERVATION | CULTIVATION | COMMERCE

The Future of Ginseng and Forest Botanicals Symposium



July 12-14, 2017

MORGANTOWN, WV

THIS EVENT IS MADE POSSIBLE THROUGH THE GENEOUS SUPPORT OF OUR SPONSORS

Edited by Alison Ormsby and Susan Leopold

Ormsby, A. and S. Leopold (eds.). 2018. Proceedings of The Future of Ginseng and Forest Botanicals Symposium. July 12-14, 2017. Morgantown, West Virginia.

Cover Art: American Ginseng (*Panax quinquefolius*) and Timber Rattlesnake (*Crotalus horridus*) by Shay Herring Clanton. Shay uses her art to bring awareness and build community support to protect our streams, mountains, and native plants.

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PREFACE

Knowledge is circular with regard to nature, or you could say it goes dormant and then like ginseng comes back on its own terms. Look back to the "Ginseng and Goldenseal Bulletin" that started its monthly subscription in 1914 by Penn Kirk and what you find is a network of woodland farmers growing medicinal plants and sharing trials and tribulations, along with advertisements and antidotes. In the early to mid-1900's, you can sense the impact of both WWI and WWII on the medicinal plant trade, and not so long ago herbal medicine faded from local stores and medical practices, thus the growers themselves were forgotten.

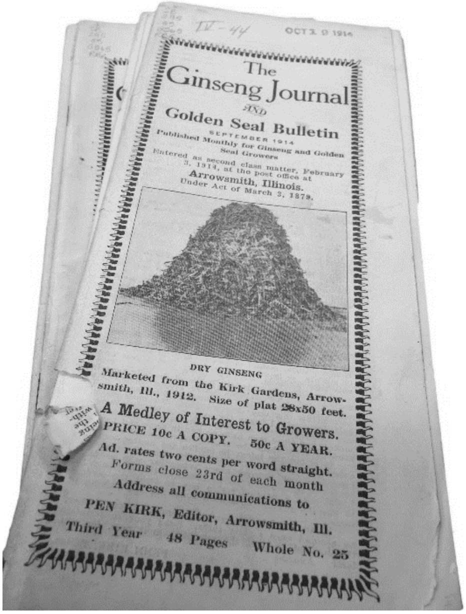

Photo courtesy of the Lloyd Library and Museum in Cincinnati, Ohio

Now that the trend in herbal medicine is once again on the rise, the wild harvest can no longer be sustained without the knowledge of growers to support the increased demand. The forests of Appalachia have survived clear-cutting, loss of habitat to fossil fuel extraction, unchecked development and irresponsible land use, which is sadly reflected in the region's decline in human health. Now it's up to the diverse stakeholders, such as those who came together for this symposium, who care about ginseng and forest botanicals, to share knowledge and reconnect a network of growers and thus advocate for the responsible harvest of Appalachian medicinal plants.

The ginseng and the rattlesnake motif seemed a fitting choice for the cover of this book. The story below, a short excerpt from Phyllis Light's book, tells of how the ginseng and the rattlesnake made a pact to protect each other and is iconic to the way ginseng inspires us to protect the forest. Folks who love ginseng are deeply protective of its home. This is demonstrated by all who presented and attended the symposium, including our generous sponsors and supporters who made the event possible. The proceedings provide a detailed overview of the current obstacles and opportunities in the medicinal plant trade and document a diverse series of papers summarizing the presentations.

We are now approaching the one-year mark since the symposium, so this cycle seems to be coming full circle with the published version of its proceedings. The future of ginseng and forest botanicals is in our hands, and how we define our relationship with these plants will define our future in Appalachia.

— Susan Leopold, June 28, 2018

Rattlesnakes make winter nests in the sides of mountains near ginseng patches but above wet ground. And they are looking for their winter's nests about the same time ginseng is ready to dig. Most ginseng hunters run up on at least one or two rattlesnakes during a season. According to legend, because rattlesnakes and ginseng live so close together and share the same land, they made a pact. If you injure one, the other extracts revenge; what you do to one, you do to the other. Killing a rattlesnake is always bad luck, the spirits don't like that. And even worse, if you harm a snake, the ginseng can stop working for you.

Excerpted from Phyllis' new book,
Southern Folk Medicine: Healing Traditions from the Appalachian Fields and Forests

Christine Laporte, Phyllis Light, and Kat Maier

Chip Carroll and Susan Leopold

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SPECIAL CROPS

New Series. APRIL, 1919. Vol. 18. No. 200

BUY VICTORY NOTES

LOGGING ON A CONCRETE ROAD

The natural home of Ginseng and Golden Seal is being pushed back year by year, and the time is not far in the future when we shall class these wild roots as practically a thing of the past. The market very soon must depend on cultivated root and the wise grower should be getting ready for that time.

BUY VICTORY NOTES

The Future of American Ginseng and Other Appalachian Forest Botanicals Symposium
Conservation, Cultivation, Commerce
July 12-14, 2017 | Waterfront Place Hotel | Morgantown, West Virginia

AGENDA

Wednesday: American Ginseng

8:00 - 9:00	CHECK-IN/REGISTRATION
9:00 - 9:15	WELCOME
9:15 - 10:15	OPENING KEYNOTE "Taking the Broad View: How Are Wild Ginseng Populations Faring and When Does Conservation Policy Need to Change?" – *Jim McGraw*

PRESENTATIONS: CURRENT RESEARCH ON AMERICAN GINSENG

10:15 - 10:30	Spreading the Ginseng Gospel: Case study from Watuagua County Cooperative Extension – *Jim Hamilton*
10:30 - 10:45	Supply and Regulation of Wild American Ginseng – *Greg Frey, James Chamberlain, Jeff Prestemon*
10:45 - 11:00	A Survey of the Genetic and Phytochemical Diversity of American Ginseng in Western North Carolina – *Jonathan Horton, H. David Clarke, Jennifer Rhode Ward, John Brock, Jess Burroughs, & Nicholas Freeman*
11:00 - 11:15	Break
11:15 - 11:30	American ginseng status assessment on four National Forests in the Mid-Atlantic U.S. – *John Young, David Smith, and Tim King*
11:30 - 12:30	PANEL DISCUSSION ON CONSERVATION, MANAGEMENT, AND POLICY *with Jim McGraw (WVU), Paul Hsu (Hsus Ginseng), Susan Leopold (United Plant Savers), Michael McGuffin (American Herbal Products Association) and David Cooke (Grow Appalachia). Moderated by Ed Fletcher, Herbal Ingenuity.*
12:30 - 1:30	Lunch (provided at hotel)
1:30 - 2:45	Can Wild Ginseng Regenerate New Plants from Replanted Rhizome? – *Robert Layton Beyfuss*
2:45 - 3:00	An examination of mycorrhizal symbiosis in forest grown American ginseng, and the influence of mycorrhizal infection on root ginsenoside content – *Tanner Filyaw and Sarah Davis*

3:00 - 3:15	Demographic response of American ginseng to three natural canopy disturbances common in mixed mesophytic forests – *Jennifer L. Chandler*
3:15 - 3:30	Assessing the Status of American Ginseng from Harvest and Monitoring Data – *JP Schmidt and Jenny Cruse- Sanders*
3:30 - 4:00	Questions for speakers/discussion
4:00 - 5:00	PANEL DISCUSSION ON LAW ENFORCEMENT w*ith Chad Taylor from NC Dept. of Agriculture, Brad Hadley from MO Dept. of Conservation, and Ron Ollis from OH Dept. of Natural Resources*
6:30 - 7:30	EVENING DINNER AT HOTEL AND SPEAKER: Ginseng in Appalachian Folk Medicine – *Phyllis Light*

Thursday: Appalachian Forest Botanicals

PRESENTATIONS: CURRENT RESEARCH ON APPALACHIAN FOREST BOTANICALS AND CONSERVATION/ MANAGEMENT OF WILD POPULATIONS

9:00 - 9:15	Recap and Announcements
9:15 - 9:30	Conservation status of forest botanicals: What do we know and why does it matter? – *Danna Leaman*
9:30 - 9:45	An Overview of NatureServe's Conservation of Native Plants - *Ann Frances*
9:45 - 10:00	An American ginseng story: NatureServe's work in Indiana and Illinois - *Leah Oliver*
10:00 - 10:15	Questions for speakers
10:15 - 10:30	Break
10:45 - 11:00	Black Cohosh: Harvest Impacts, Population Response and Implications for Sustainable Management of this and Other Medicinal Forest Products – *James Chamberlain and Christine Small*
11:00 - 11:15	Characteristics of Woodland Herbal Users in the United States: Summary from an Epidemiological Study - *Termeh Feinberg and Kim Innes*
11:15 - 11:30	Review of Effects of Growing Methods on Pharmacological profiles of Herbal Medicines – *Meghan Gonick*
11:30 - 12:00	Questions for speakers/discussion
12:00 - 1:00	Lunch (provided at hotel)
1:00 - 1:30	Producing wild leek in forest farming under northern climates - *Lapointe, L., Dion, P.-P., Denis, M.-P., Bussières, J. & Bernatchez, A.*

1:30 - 1:45	*Sanguinaria canadensis L.*, Bloodroot, historical and potential uses – *Meghan Gonick*
1:45 - 2:00	Root Report: Measuring the Market for Forest Medicinals – *Steve Kruger, John Munsell, James Chamberlain, Jeanine Davis, Ryan Huish, and Steve Prisley*
2:00 - 2:30	Questions and Discussion
2:30 - 2:45	Making Medicine: Sourcing and Sustainability in the Herbal Products Supply Chain – *Ann Armbrecht*
2:45 - 3:45	HERBALIST PANEL: PAST AND PRESENT USES AND ANALOGS AND SOURCING – *Kathleen Maier, Phyllis Light, Steven Yeager*
3:45 - 4:00	Wrap-Up/Conclusion
4:00 - 5:00	POSTER SESSION WITH MUSIC

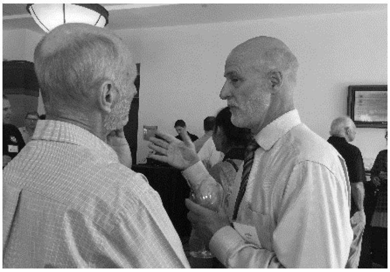
- Ginsenoside Profiles in American Ginseng (*Panax quinquefolius* L.) in Western North Carolina - *Jessica Burroughs, David Clarke, Jonathan Horton, Jennifer Rhode Ward, and John Brock*
- Connecting Appalachian Icons: The importance of conserving plant-animal mutualisms in a changing world - *Amy M. Hruska, Michael C. Elza, and James B. McGraw*
- Antidermatophytic Effect of Black Walnut hull, *Juglans nigra - Rosanna King, Andrea Lutac, Natalie Rubio, Jenna Yutzy, and Rebecca Rashid Achterman*
- Partial root harvest of *Panax quinquefolius* L. (American ginseng): a non-destructive method for harvesting root tissues for ginsenoside analysis - *Ian Sabo, Jonathan L. Horton, H. David Clarke, and Jennifer Rhode Ward*
- Flower Essences: Sustainable Supplements from Forest, Field, and Garden - *Katherine Ziff*
- Alkaloid Content in Forest Grown Goldenseal – *Grady Zuiderveen, Eric Burkhart, Josh Lambert, and Mike Jacobson*

Friday Farming – Growing the Network by Supporting the Cultivation

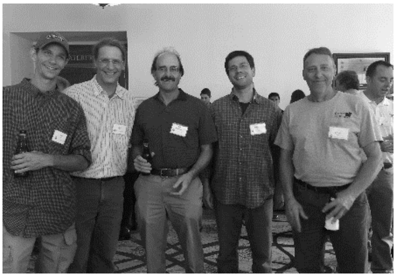
8:00 - 9:30	APPALACHIAN BEGINNERS FOREST FARMERS BREAKFAST MIXER
9:30 - 10:00	Overview of Appalachian Beginning Forest Farmers Coalition - *John Munsell*
10:00 - 10:30	American Ginseng Pharm Overview – *Anna Plattner*

10:30 - 11:00	Use of Natural Fungicides with Organic Ginseng Production — *Robert Eidus*
11:00 - 12:00	FOREST FARMING PANEL DISCUSSION CURRENT TRENDS AND OPPORTUNITIES – *Jennifer Gerrity from Mountain Rose Herbs, Chip Carroll from United Plant Savers, Marc Williams from Plants & Healers Internatinal, Tanner Filyaw from Rural Action, Stephen Gruget from Rareroot*
2:00-6:00	FOUNDATIONS OF FOREST FARMING AT HARDING'S GINSENG FARM *(NOTE: Must register separately as space is limited.) Class taught by Chip Carroll, Larry Harding, and Marc Williams.* Topics will cover basic botany and plant families of forest botanicals, growing ginseng and goldenseal, seed collection, and value-added products

Alison Ormsby and Marc Williams



Michaell McGuffin and James McGraw



Left to right: Chip Carroll, John Stock, Tom Redfern,Tanner Filyaw and Denny Colwell

Philippe Grenade, Karam Sheban and Jennifer Gerrity

July 12, 2017

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Dedicated to the conservation of native medicinal plants.

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PURPOSE

Welcome to the *Future of Ginseng and Forest Botanicals Symposium*. The goal of this gathering is to initiate a lasting collaboration across multiple stakeholders who care about the future of Appalachia's native medicinal legacy. Conservation, Cultivation, and Commerce are the three dynamic aspects that we will be focusing on as they are so deeply connected.

As you can see from the table below, each of you attending this symposium brings your unique insights and knowledge: we represent a wide range of stakeholders.

Attendees

Forest farmers/landowners (28%)
Industry (23%)
Members of United Plant Savers/Herbalists (21%)
Government agencies (15%)
Presenters/students (13%)

In your registration packet, we have included blank post-it notes. During these next three days, we encourage you to write your thoughts on a post-it as you think of concerns, challenges, and opportunities that you would like to share. Post-it thoughts will be placed on easels during the conference and at the end of Wednesday's talks in order to compile attendees' collective knowledge and feedback. This is your opportunity to contribute to United Plant Savers' working document on a Conservation Plan for Ginseng and Forest Botanicals. You can also email your thoughts to us directly at office@unitedplantsavers.org.

The outcome of the feedback we receive will be a document that will be published in our symposium proceedings that will be available after the conference. The proceedings will include all papers and summaries of the panel presentations from this historic symposium.

Our intention is that this document will help inform future policy, conservation programs, and provide support for forest farmers, and further development of a conscientious herbal industry. If we are to ensure the future of ginseng and forest botanicals, then we must work together to advocate for vibrant healthy forests. Thank you for participating and contributing.

Sincerely,

Susan Leopold, Executive Director
United Plant Savers

SUMMARY OF CONFERENCE

by Chip Carroll

Approximately 197 people, representing all stakeholder groups, attended this 3-day symposium in Morgantown, West Virginia.

During the conference, four flip charts were stationed in the exhibit area, with the following key topic headings: Conservation; Commerce; Policy/Management; and Cultivation. We asked symposium participants to take notes during sessions and post questions, ideas, concerns and thoughts on the appropriate flip chart to generate a more comprehensive view of stakeholder concerns and ideas.

The following is a summary of the points raised by symposium attendees in each of the four topic areas.

CONSERVATION

Under the Conservation heading, topics varied from environmental to scientific to policy-related issues. One theme that emerged under Conservation was concerns about habitat loss. Habitat loss was mentioned multiple times with some of the comments specific to effects of habitat loss due to surface mining and mountain top removal and climate change. Creating local seed banks and refugia for ginseng and other botanicals was another theme that came out of the comments. Concerns about genetic preservation and local seed sources were touched on in comments such as "Land Grant Universities should play a role in maintaining local seed sources and supplying growers like (they do for) other crops."

Several comments related to research needs and concerns with comments ranging from Citizen Science and Research Questions to questions and concerns about the timing of ginseng monitoring, e.g., population censusing needs to be done before June 15th to get reasonable demographic data, otherwise deer browse, etc. will skew census. Timing of monitoring efforts was a concern that was repeated along with the impacts that deer are having on ginseng populations.

Many of the comments were phrased as questions from participants such as, "What is our recovery goal for ginseng; we need to know not just how to reverse the loss, but what we are aiming for," and "Can a root size requirement help prevent LEGAL overharvesting / immature plant harvest?" Opinions in favor of developing a conservation plan for ginseng were mentioned with the suggestion of "modeling it on federally endangered species recovery plans." Questions surrounding issues of plant size-based harvesting or reproductive capacity were mentioned multiple times as discussions surrounding the idea of a root (thumb size or "slot" requirement as in fisheries) or leaf size based harvest criteria were mentioned and repeated. Comments in favor of a 10-year age requirement for ginseng were also noted.

Outreach, education and awareness raising were mentioned multiple times with ideas about creating "campaigns" to change public opinion and raise awareness. Comments about placing higher value and demand on cultivated botanical products (more than wild-harvested) were repeated throughout.

Themes that emerged under the conservation heading were **habitat loss/environmental concerns**, **needs for research** and identifying "gaps", **regulatory changes** (size and age-based) that would improve wild populations while protecting growers, **development of local refugia** for conservation and source for seeds/planting stock, **conservation plan for botanicals** and **evaluating methods** used to collect data on wild populations.

COMMERCE

Under the commerce heading, topics seemed to coalesce around education, marketing and regulation (i.e., digger licensing). Concerns over illegal trade and enforcement of current laws were mentioned along with interest in developing more local and domestic market opportunities for ginseng. Mention of "herbal medicine" reinforced the ideas and discussions around fungicide use and chemical inputs into traditional cultivated ginseng and concerns about residue left in roots being sold and consumed as medicine. "Marketing and Outreach" along with "Outreach and Education for Buyers" emphasized the need for a standard education to be provided to licensed dealers around the issues of illegal trade and current trends and issues. Throughout the event, discussions about increasing or requiring more education to dealers and diggers were common. There seems to be agreement on the need for requiring education or the passing of a test to become licensed as either a dealer or digger. Many state coordinators present seemed to be considering requiring a digger license in their states; currently only Wisconsin requires licensing of diggers. Other comments related to this issue included:

- "What if harvesting permits/licenses connected to a specific area? So the diggers would become stewards of their leased/local area. If they could keep the lease for years, and sell or pass on the lease, its long-term value would encourage long-term stewardship and connection & protection"
- "Digger licensing"
- "Educational component to licensing programs… pass the test"

- "How can we get all 19 states to enact a ginseng harvester's license? CHEAP! Like a fishing license type concept"
- "Stop illegal purchasing by non-licensed individuals in commerce"

Other creative concepts that were mentioned under the commerce heading included; "With lower amounts of harvesting why not limit sales to a USA market only until supplies return?" This comment revolves around lower availability of harvestable plants and harvestable areas in the wild and suggests limiting export until populations recover. Some comments revolved around commerce in the more traditional sense such as "Designing products around regenerative supply chains/forest farming into mainstream" and "Marketing regenerative supply chains/forest farms into mainstream" both of which are interested in placing more value on forest farmed and/or cultivated plant material. This is another theme that seemed to develop and continually be discussed throughout the event – a call for more companies to begin to place more/higher value on cultivated material.

Other ideas captured continued along the lines of the need for education and information sharing such as: "We need to keep lines of information flowing… how do we do that?"; "Menominee Tribe Message: -- Public Education & outreach meetings – Social Media Pages – Facebook – Brochures regularly annually"; and "Law enforcement: How does the Menominee tribe put the conservation message across? Could states use their message? And Methods?" These ideas seemed to involve the need for developing consistent educational materials across the board for the entire industry and using technology (social media) to better spread the messages.

Commerce topics related specifically to growers, and opportunities included concerns about availability of local seed, availability of local roots for local herbalists, start-up business opportunities and the need for an organized group or growers association. Comments included: "How can we build up growers and help growers start local/native ginseng seed banks"; "Finances of start-up companies"; "Local seed banks"; "Local roots for local practitioners"; and "States need ginseng growers associations or a viable National Ginseng growers network/association."

Remaining topics listed under the commerce heading were largely questions and concerns. Concerns about diversity and stakeholder inclusion were repeated several times. Comments such as, "Diversity, opportunities targeting African Americans and Native Americans" were repeated under all the headings as well as a concern over the lack of harvesters and diggers attending the meeting. Some key stakeholder groups were not well-represented at the event and finding ways to engage them will be an important part of any successful follow-up work.

A few other questions and comments included: "Is there any difference in medicinal value/price between Re and Rg genetic ginseng?"; "Are any diggers, growers & brokers considering offering ginseng leaf as a result of attending this symposium? Super sustainable product."

Themes that clearly emerged under the commerce heading include **the need for education around conservation, regulations and sustainability for all stakeholders** and interest in pursuing **digger licensing** (with "test" / education component) to combat illegal trade and get a grip on chain of custody issues. **Development of domestic market** and placing **greater value on cultivated materials** were two other topics that had a lot of interest. Overall the theme seemed to be driven by the need for more sharing and education throughout the industry and developing more opportunities that value the sustainable practices while discouraging poor practices through regulation and education.

POLICY/MANAGEMENT

Under the Policy/Management heading, topics ranged from ideas around using a new or additional metric to guide the harvest of ginseng (size-based or age-based) to concerns about deer and definitions. The policy/management topics cover a wide range of issues and concerns as well as ideas about how to better manage all facets of ginseng harvest and trade. Several presenters at the event discussed the idea of managing ginseng much like we manage our fisheries, with ideas about developing a "slot" system for ginseng harvest that would allow reproductive plants to be harvested but leave juvenile and "elderly" plants to grow, reducing the "high-grading" of ginseng in the wild. Some of these ideas require more discussion and evaluation before any new policies are developed.

One comment that deserves additional thought and discussion was "please suggest possible size criteria for harvest." Based on information presented by Jim McGraw, the idea is that it may make sense to explore other criteria besides age to base harvest on. Leaf width, stem size, root size and plant height were all possible alternatives. Administrating or enforcing another metric besides age will be difficult and requires additional thought and exploration. Another comment related to this idea was "Engage fishing policy makers, learn from them" – the idea of managing ginseng more like a fishery.

Some of the recurring themes under the policy/management heading revolved around ginseng theft (poaching); penalties; licensing of diggers, buyers and exporters; and illegal trade. Comments such as the following all encompass concerns about the education of those participating in the ginseng harvest and trade: “Digger licensing”; “Restrict first points of sale to fresh root only to cut down on early harvest”; “Education/Training, Buyers, Diggers. Licensing”; “Make sure that proposed tests built into any new regulations/licensing are fair and consider the education level of all involved”; “If we move in the direction of written tests for diggers permits…how can we address literacy concerns in the region that could disenfranchise people who have historically harvested this plant?” (Video instead of written?); “Educational Classes for dealers, diggers and buyers collectively instead of sending literature in the mail”; and “Ginseng training for agencies provided by industry & growers.” There seemed to be consensus around the idea of requiring educational components to any licensing programs as long as the educational components are equitable and fair to those who would be required to pass a test. Ideas about having diggers watch an educational video and take a brief test to qualify for a license were discussed as an alternative to written materials.

Another comment suggests administration with a point system that would penalize offenders: “Digger permits/Point System… Theft / Poaching = (x) points on permit. After (x) points or # offences, loss of digger permit, loss of dealer permit, loss of hunting/fishing licenses. Also have fines greater than $1000. Add Teeth to Regulations!” Some other concerns around this idea have to do with the ability of states to administer a ginseng licensing program because many of the state licensing agencies are separate from the agency tasked with managing ginseng. It is important to state that licensing would generate some income for ginseng management. Also there seemed to be consensus on the fact that any licensing requirement should be kept fairly priced with many discussing the idea of a $10 license fee. It is worth noting that the licensing discussion appeared under all the headings – conservation, commerce, policy, and cultivation.

Comments around the <u>theft issue and current laws</u> were also at the forefront of the policy/ management discussion. The following comments all revolve around the problems and concerns with the theft and illegal harvest of ginseng: “Law enforcement should develop A.) relationships with dealers and B.) better enforcement methods for theft/poaching/illegal harvest. Do it like regular FWS/Wildlife type law, e.g., Ability to search, etc.”; “Enforce current laws!!!”; “Fines for poaching need to be high enough that it’s not worth the risk. Price of ginseng is so high that most poachers feel it is worth it”; “Growers need real repercussions for ginseng thieves in order to do business & grow this valuable commodity crop – which in turn lessens market pressure on our beautiful wild ginseng populations”; and “Educate judges prosecuting cases – send $$$ (fines) back to species protection/research.” Theft and illegal harvesting were of major concern to all of the stakeholders present, suggesting that current regulations and laws addressing the issue may not be having the desired impact of reducing occurrences. In many states, the penalties for ginseng theft were much stiffer 100 years ago than they are today.

Other topics that came up under the policy management heading had to do with management of the resource, current policies, and a desire to develop consistent definitions and rules across all states. “Regulations across states”, “Consistency on definitions & reporting”, “Develop better interface/engagement”, “CITES listing concerns”, “Distinguishing wild from wild-simulated”, and “Monitoring” all capture the concerns around difficulties in managing ginseng when there are 19 states with differing agencies tasked with consistently managing this resource. Lack of standard definitions and policies across all states leads to confusion and difficulty in effectively managing ginseng harvest and trade. Inability to distinguish wild from wild-simulated roots creates the possibility of poor management decisions based on incomplete and inaccurate harvest data. Lack of regular interface and engagement between and amongst agencies and stakeholder groups can lead to confusion and distrust amongst stakeholders. Clearly there seems to be a desire for more interface amongst key groups such as what took place at this event. Bringing together stakeholders more often and regularly can provide the opportunity to “dig down” on some of these issues and come up with workable solutions for all involved. Other comments related to management included: “Beyond policy to prevent the exploitation of ginseng as a resource – what thoughts do people have here for using this resource/cultivation of it for preservation of our forests and watersheds…Keystone species”; “Deer”; “Monitoring”; “Forest management to consider plants/understory”; and “Identify gaps in existing research” all touched on the need for more research and better research methods i.e., timing of monitoring) in order to get a more accurate picture. Comments also touched on the correlation between non-timber forest products (NTFP’s), ginseng and overall forest health and management. New information about the interactions and relationships between ginseng and other species (e.g., wood thrush) and NTFP’s as an indicator of overall forest health were mentioned as was the need to consider NTFP’s in forest use planning and overall management decisions.

In summary of the policy/management heading, comments focused primarily on issues related to **Education** focusing on diggers, buyers and agencies, **Licensing** of diggers with a strong educational component, enforcement of existing **Laws** as well as development of more consistent rules and regulations across states. **Theft & Illegal Harvest** were front and center amongst the concerns mentioned as was **Deer Impacts** on both wild and wild-simulated ginseng populations. **Ginseng as a keystone species** encompasses the ideas around considering ginseng (and other botanicals) in our overall decision-making processes related to forest management.

CULTIVATION

Under the cultivation heading, most topics fell into three broad categories: grower verification; planting stock/seed sources; and theft or "poaching" issues. Other topics discussed relating to cultivation were about support and education for growers, and questions related to specific growing techniques.

Support for verification programs for growers was voiced in simple statements such as "verification" and "support for growers" and "Buyers/Consumers willing to pay premium prices for cultivated crops." The ideas about grower verification programs such as the one being administered by PCO (Pennsylvania Certified Organic) were discussed at the event. The need for this type of verification has been being discussed for at least the last decade and stems in large part from concerns ginseng growers had about the future ability for them to market their crops if an export ban were ever placed on American Ginseng. Since that time West Virginia legislatively established a ginseng grower certification/verification program, and PCO established their "Forest Grown Verified" program that includes other forest grown botanicals besides ginseng. In addition to potentially protecting a grower's ability to market ginseng, these programs have also placed value on raw materials that are verified and labeled under such a program.

The issue of ginseng theft was discussed repeatedly, with concerns about the lack of consistent and strong penalties for those who engage in such activity. Comments such as "Theft, stealing, poaching", "Fines for poaching need to be high enough that it's not worth the risk. Price of ginseng is so high that most poachers feel it is worth it", "Growers need real repercussions for ginseng thieves in order to do business & grow this valuable commodity crop – which in turn lessens market pressure on our beautiful wild ginseng populations" and "Educate judges prosecuting cases – send $$$ (fines) back to species protection/research" were repeated across headings.

Concerns over the lack of availability of local seed sources for ginseng were mentioned repeatedly as well. "Planting Stock Sources", "Seed Sources" and "How do I find local seed source for ginseng in my area?" were common concerns. Because so much attention and concern has been given/raised over the last decade in regard to genetic mixing of ginseng from cultivated gardens with that in the wild, sourcing "local" seed has become a hot topic. Although demand for local seed is high, producing it is difficult because of the intensity in which ginseng must be cultivated in order to produce any meaningful amount of seed. Comments related to this issue can be found throughout all four different headings discussed.

The importance of cultivation in general was expressed through comments such as: “Cultivation is of the upmost importance not just to take the pressure off of wild populations, but also to have easier methods of studying them. See the Chinese concept of Dao Di”; “Educate to encourage growing by private citizens”; “Create opportunities for African American and Native American partnerships/communities”; and “Polyculture/forest farming/ecological design/symbiotic species.” These comments share the idea that cultivation of these forest botanicals in their native habitats can provide benefits economically, environmentally, and socially if supported and encouraged properly.

Some comments and questions were broad and showcased the questions or “gaps” in information that people are seeking. Questions related to cultivation methods included: “Are there known companion plants to support ginseng production and/or pollinators and ways to bring them into ginseng populations”; “As a “would be” cultivator in PA, where is the best place to start? Education, Resources, Agencies. Is there a universal list for the entire US specifically for ginseng?”; “Is it possible to grow ginseng in the piedmont area of Virginia? East of Lynchburg, VA?” – “Yes”; and “Does canopy litter bioaccumulation act as a limiting factor in alkaloid content?” Other concerns listed under cultivation related to terminology and definitions and a concern over the use of fungicides and herbicides on ginseng crops: “Terminology problems – Woods grown, wild-simulated, wild”; and “No more poison in ginseng.”

In summary of the cultivation heading, clearly the big issues include issues around **Theft / Poaching**, **Planting Stock Sources** and **Grower Verification**. Comments indicate an understanding for the importance of cultivation and a recognition of the many benefits of forest cultivation of these botanicals. Comments also indicate a need for more information sharing and better communication amongst industry, regulators and growers to come up with workable solutions that can benefit everyone. Based on the questions asked, it appears that there is a need for more research and information gathering related to the cultivation of ginseng and many forest botanicals.

OVERALL SUMMARY OF INPUT

Interestingly, comments, concerns and discussions seemed to have focused most often on the need for greater education, educational resources and information sharing for all the stakeholder groups. This speaks to the need for stakeholders to come together more often and regularly to share current trends, information and research as well as to develop educational materials to be shared with the broader community. Lack of effective ways to reach folks on the ground (i.e., diggers, buyers and growers) who are often operating independently and in isolated rural communities will only exacerbate the many problems and issues surrounding the habitat loss and over-harvesting of these botanicals. Finding ways to engage all stakeholders more effectively and more often will go a long way to helping conserve these important species. Thinking creatively about new ideas and policies and working with the larger community to develop those ideas can potentially provide answers to some of the issues facing American Ginseng and other forest botanicals.

CONSERVATION PANEL DISCUSSION SUMMARY

<u>PANEL DISCUSSION ON CONSERVATION, MANAGEMENT, AND POLICY</u> *with Jim McGraw (WVU), Paul Hsu (Hsus Ginseng), Susan Leopold (United Plant Savers), Michael McGuffin (American Herbal Products Association) and David Cooke (Grow Appalachia). Moderated by Ed Fletcher, Herbal Ingenuity.*

The panel was opened with brief introductions and moved into discussion on current issues around ginseng conservation. Some of the ideas that were discussed during the panel included:

- Need for more education across the board. There was discussion about requiring education for buyers and requiring diggers to "pass a test" to obtain harvest license. Education should focus on stewardship and conservation. Universal licensing with mandatory educational component.
- Concerns were raised about deer browse and population issues (introduction of predators), out of season harvesting and climate change impacts on ginseng.
- It was noted that 6 of the 19 ginseng states already require digger licensing and that Wisconsin has required it since 1975.
- There were discussions around new harvest requirement ideas including a "staggered harvest" idea of only allowing wild harvesting every 3-5 years as well as discussion of a "slot system" harvest that would leave the most reproductive plants in the wild (similar to fisheries).

- There was good discussion around organizing stakeholders and the idea of "strength in numbers". Organizing politically so that stakeholders have a voice. Idea of National Ginseng Association.
- One idea that got some brief discussion was the idea of a fresh root only for sales and marketing. One benefit this idea could have would be to reduce the out of season harvesting.
- Need for a large network of nurseries who can cultivate and produce locally adapted planting stock and seed for sale to growers was shared by all.
- Development of more and stronger domestic marketing opportunities
- Importance and opportunities around grower verification and having the verified products bring a premium
- Development and transition into ginseng leaf market opportunities
- Expansion of Botanical Sanctuary Network to offer refugia of local ecotypes and locally adapted planting stock. Also, to preserve unique genetics.
- Noted that Menominee Tribe in Wisconsin already has a 10-year age requirement for ginseng harvest, talk about expanding that out across all states. Example was given that in Wisconsin the average number of roots in a pound was 125 while in West Virginia it was 1000.
- Need for industry to support these efforts and stand behind stakeholders.
- Talk of doing more restoration on public lands through stewardship activities including planting as local of seed as possible.
- It was noted that 2017 represents the 300th year ginseng exports with the roots first recorded export occurring in 1717.

SYMPOSIUM PAPERS
(alphabetically by author)

"The Sustainable Herbs Project: Sourcing and Sustainability in the Herbal Products Supply Chain"

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Abstract

I started the Sustainable Herbs Project (SHP) to create educational resources for consumers about the herbal products industry so that we can all become more informed about the issues involved in the industry and how to best support sustainable and ethical sourcing of high quality raw material. The SHP website will be a series of short videos following herbs through the supply chain to make visible the lives of the people and places involved in bringing these plants to market. In this presentation, I introduce the project by showing several brief videos and then discuss ways the SHP is working to raise awareness about issues relating to the conservation and commerce of forest botanicals.

Introduction

"As a nation, we are struggling with a profound lack of imagination," farmer and writer Wendell Berry (1996) once said. "We don't see the forests being cut down to build our homes, the lakes being drained as we fill our tub. We live on the far side of a broken connection." Not seeing the people and places on the other side, not seeing the moral and ecological consequences of producing these objects makes it easier to buy the wood or take a longer shower. Healing this broken connection, Berry concluded, begins with seeing beyond what the market wants us to see.

The Sustainable Herbs Project has been following herbs through the supply chain to explore much broader questions about our role as citizens of the world and how, through our choices about the objects we consume, we impact that world. Most broadly this project asks: How can we live more lightly on the earth? How can we treat each other, the earth, and ourselves with more care and respect? How can we create worlds that are healthier—physically, socially, emotionally and spiritually?

The multi-media website explores these questions by focusing on companies working to transform this industry so that the vision and values of herbal medicine apply to the entire supply chain, not just the end product. But my vision is much larger than changing this industry. My goal is to show how changing this particular industry is a way to change the world.

Aren't Herbs the Environmental Choice?

The global botanical medicine industry has grown significantly over the last few decades, reaching almost $100 billion in sales. Consumers of herbal supplements tend to believe that in buying products made with plants, they are making the environmentally responsible choice. Unlike the food industry where attention to traceability and sustainable and ethical sourcing is gaining traction, however, neither consumers, the media nor natural products companies pay much attention to the crucial connections between the quality of the raw material, traceability in the supply chain, and the efficacy of the finished product. Even for those seeking to know more, in an industry not known for transparency, it is very difficult to find accurate information about the supply chain or about the human and environmental costs.

I created the Sustainable Herbs Project because I was struck by the disconnect between the philosophy of herbal medicine and the reality of what it takes to produce herbal products on a large scale. This disconnect impacts the efficacy of these medicines. And it calls into question the promise of herbal medicine as safer, less expensive, and healthier for humans and the earth.

Plants can be a potent source of healing if and only if the principles at the heart of herbal medicine guide the entire supply chain. A handful of companies are leading the way in doing just this. I am focusing on their efforts not so that consumers rush to these companies. My goal is to outline standards we should demand of the industry overall and the steps to take to support companies working to make these changes.

What We Can Do

The Sustainable Herbs Project's goal is first to educate consumers about the supply chain and the issues involved. Secondly, I outline steps to encourage more companies to implement sustainability standards.

My primary focus has been on documenting the supply chain through videos so that the people, places and plants can speak for themselves. And so the rest of my presentation will walk viewers through the website. I will begin showing the overview, and then each of the pages focusing on different steps of the supply chain, including production, processing, manufacturing, quality control and purchasing. I will then show the sections exploring issues: Quality and Sustainability, Sustainability of Wild Collected Plants, Relationships through the Supply Chain, and Domestic Production. Finally, I will touch on the section: Building Healthy Worlds, what consumers, herbal practitioners, and herb companies can do to shift toward more sustainable practices.

Reference

Berry, Wendell. 1996. "Keynote talk," Watershed Gathering, Orion Magazine. Washington, DC: Library of Congress (in author's notes).

"Can Wild Ginseng Regenerate New Plants from Replanted Rhizome?"

Beyfuss, Robert Layton. Retired Agriculture Agent and American Ginseng Specialist for Cornell University Cooperative Extension, NY and Vice President American Ginseng Pharm LLC. rlb14@cornell.edu

Abstract

Wild American ginseng is a plant species of International Concern and is listed on CITES Appendix 2. Current conservation efforts are geared towards protecting existing wild populations by establishing regulations that, among other rules, are intended to prohibit harvest before plants have a chance to reproduce. This presentation will address the following questions: 1) Can wild ginseng regenerate new plants from replanted rhizome/neck fragments containing intact apical buds (vegetative reproduction)? If so, 2) Will plants that have regenerated from rhizome fragments become reproductive sooner than plants arising from seeds planted from harvested plants? and 3) What are the possible conservation/regulatory implications for this data?

In 2006, an experiment was performed to see if wild American ginseng plants could regenerate new root tissue and top growth from their severed rhizomes, with some root tissue left attached and a viable apical bud. The experiment was performed both in a greenhouse environment as well as in a forest environment. A significant percentage of the forest rootlets did survive and produced offspring within 5 years, whereas all the greenhouse plants perished.

Introduction

Wild American ginseng is a native North American herbaceous perennial that is highly valued as a herbal medicine, particularly by practitioners of traditional Chinese medicine. Its continued existence in the wild is of international concern, consequently it is listed on CITES Appendix 2.

Current conservation efforts are geared towards protecting existing wild populations by establishing regulations that prohibit harvest of the wild roots before plants have had a chance to reproduce. Harvested roots must display at least 5 abscission scars indicating that the plant has produced top growth for five seasons. However, age of ginseng plants, as determined by counting abscission scars on rhizomes, is not a reliable predictor of reproductive status for wild ginseng.

According to Professor James B. McGraw, WVU (pers. comm), "age in itself is a poor indicator of life stage generally, and reproductive capacity specifically. This has long been known in population biology as a general truth."

The regulation requiring visually intact rhizomes with 5 scars removes the most vigorous members of a population, with no real assurance that they have reproduced prior to their harvest. Removal of mature plants does not protect locally adapted gene pools. Most states require the replanting of ripe berries from the harvested roots in the immediate vicinity of where the roots were harvested, however there is no way to insure that the harvested roots had any ripe berries present at all, at the time of harvest. Even if the plants had berries and the berries were carefully replanted, the shortest period of time before the offspring become reproductive is at least 6 or 7 growing seasons.

My hypothesis was: Can wild ginseng plants regenerate new growth from fragments of rhizomes that are replanted on site at the time of harvest?

Surprisingly, there has been very little published research on this topic. The only recent citation I am able to offer is "Recovery of Populations of Goldenseal and American Ginseng Following Harvest" (Van Der Voort et al., 2003). From the abstract of Van Der Voort et al. (2003): "Both rhizomes and roots of goldenseal and ginseng are capable of regenerating plants, conferring a degree of short term resiliency following harvest."

Unfortunately, this observation is muddled by the inclusion of goldenseal into the research and it was a very small study for ginseng. It is well known that goldenseal easily regenerates from root fragments left in the ground, but no published data relating specifically to ginseng regeneration has been found.

Prior to the neck scar regulations, some ginseng harvesters routinely cut off the main body of the taproot and replanted the intact rhizome with a viable apical bud present. This procedure was used to shorten the time interval between root harvests, allowing root tissue to be harvested more than once from the same plant. As recently as 2015 I have been approached by ginseng diggers in NY wanting to sell roots that had their rhizomes removed and replanted with the explanation that "this is how I was taught to do by my Grandfather."

Methods

In fall of 2006 a total of 240 "presumed wild" ginseng roots were obtained from 3 sources. 106 of the roots were obtained from a local NY dealer who reported them as being dug in the Catskill mountain and Fingerlakes region of NY, 68 were obtained from a Pennsylvania dealer (including 18 rhizomes only with no shoulder root at all attached) and 66 were dug by this researcher, from 2 locations in NY (Adirondack region and South central NY). Each root was photographed, weighed intact, the cut off rhizome was weighed, rhizome length and age was noted (senescence scars counted), and origin of root was noted.

On November 3, 2006, 116 rhizomes with intact apical buds were re-planted in a beech/red maple forest (Siuslaw Model Forest, Greene County NY) in an area with no recorded presence of ginseng. 16 of these roots were "controls" i.e. entire, intact roots, not severed rhizomes.

Results: Recorded Data

The average uncut root weight was 5.78 grams. This translates into approximately 237 roots per pound dry weight. The average replanted neck weight was 1.04 grams (18% of the total root weight). The average root age based on neck scar counts was 10.9 years.

On November 1, 2006, 124 roots were potted up in a commercial potting soil (Metro Mix # 2) in 4.5 inch pots and placed in an unheated greenhouse. This included 14 uncut, intact "control" roots. All the potted roots in the greenhouse perished and were rotted by May 2007 (including all control plants).

Possible explanations why the greenhouse roots rotted are that the greenhouse heat was turned on 3 times during the coldest parts of winter to melt snow from the greenhouse (February 14, March 17, and April 12) and the inside greenhouse temperature rose to 70 degrees. These warm periods allowed pots to thaw. Potted greenhouse plants are not buffered by surrounding masses of forest soil. In addition, "Metro mix" is a sterilized commercial potting "soil less mix" comprised of chemical fertilizer, peat moss, perlite and vermiculite, not a forest soil. Potted plants were not continually monitored, i.e. watered when the pots thawed.

Lesson #1: Greenhouse studies of woodland plants need to be carefully designed, well simulated, and conducted to mimic natural conditions. Data derived from such greenhouse studies needs to be interpreted in proper context. Regulations based solely on greenhouse studies are not necessarily valid for plants growing in the wild.

Of the 100 experimental plants replanted in the forest, a total of 42 plants produced new top growth (42%) by May 15, 2007 (consistent with Van Der Voort study data). Only 9 of the 16 "control" plants emerged by May 15 (56%). Did they reproduce? Although all the emerged plants were 3 and 4 prongs, and some had flower stalks, they were tiny in stature. None produced berries in 2007 (not even the control plants).

2008 Data from the 2006 experiment: All of the 42 plants that had emerged in 2007 reemerged in 2008, as well as two additional experimental plants that did not emerge in 2007, but 2 of the control plants that did emerge in 2007 did not emerge in 2008. No plants produced berries in 2008.

Lesson #2: Not all forests are suitable sites for growing transplanted ginseng. Although the rhizomes replanted in the forested site provided slightly more of a "real world" simulation compared to the greenhouse phase, it still did not truly represent a "real world" situation in which freshly harvested roots are decapitated and immediately replanted in the exact same habitat they were growing in. It is also noteworthy that all the roots used for this experiment were all stored in a refrigerator for at least 6 weeks prior to replanting. In the winter of 2008, the experimental site was destroyed by a logging activity.

Lesson #3: Don't retire while a multi-year experiment is ongoing!

Discussion

Anecdotal data garnered by personal conversations with ginseng diggers over the past 30+ years has revealed that the practice of harvesting only a portion of a wild ginseng root, while replanting the rhizome, particularly if any adventitious roots were present, was practiced quite commonly prior to 1999. In situations where the roots were harvested for personal consumption, it was a preferred method to ensure that the patch could be revisited years later with a good chance of repeated harvest. Many small wild ginseng populations have no evidence of successful natural recruitment via seed, although mature 3 and 4 prong plants may be present. This may be partially due to the fact that seedling ginseng plants are far more susceptible to pests such as non-native snails and slugs that are capable of eating newly emerged seedling plants in a matter of a few days, or even less, whereas larger, 3 prong or larger plants are far more tolerant of this type of predation. The majority of "natural" ginseng mortality occurs during its first three years as the root struggles to become established. Once established in a suitable habitat, ginseng plants are capable of living 50 years or longer.

Markets for wild ginseng have changed, and the presence of intact rhizomes indicating roots that are at least 25 years old based on the neck scars have come to command a premium price in recent years. These roots are the most highly regarded by affluent Asian consumers since they are considered as being the most potent. In NY State these so called "long necked" roots, with at least 25 scars, have sold for upwards of $1,000 a pound, fresh weight as recently as 2014, whereas "average" roots with 10 or fewer neck scars sold for 1/3 of that price. The presence of 10 or fewer neck scars has no significant effect on prices whatsoever. In retail Chinese markets in NY City, wild roots with less than 25 scars often have the necks removed prior to sale since they confer no added value.

The five scar neck rule was implemented in 1999, effectively eliminating the practice of replanting rhizomes, as the export of roots without the prerequisite neck scars became illegal.

While the intention of the regulation was to prohibit root harvest before maturity and was partially based on the recorded increases in numbers of roots per dry pound, indicating that smaller and presumably younger roots were being harvested, the practice does target the most vigorous plants within a population. Removal of the “breeders” while allowing juvenile seedlings and two prong plants to survive, if any are even present, can seriously damage the genetic integrity of a small population.

If the goal of the overall ginseng conservation effort is to preserve locally adapted wild genotypes, the mandatory destruction of the established members of a population during harvest needs to be reevaluated. If wild ginseng roots can be partially harvested and then successfully replanted, with a much shorter wait for re-harvest or reliance on seed reproduction, the goal of genetic preservation will be enhanced.

Reference

Van der Voort, M.E., B. Bailey, D.E. Samuel, and J.B. McGraw. 2003. Recovery of populations of goldenseal (*Hydrastis canadesnsis L.*) and American ginseng (*Panax quinquefolius L.*) following harvest. *American Midland Naturalist*. 149: 282-292.

"Black Cohosh: Harvest Impacts, Population Response and Implications for Sustainable Management of this and Other Medicinal Forest Products"

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Abstract

Tens of thousands of pounds of black cohosh are harvested every year from Appalachian hardwood forests. The sale of this forest botanical contributes significantly to household incomes throughout the region. Unfortunately, few efforts have been made to understand the ecological impacts of harvesting and how to manage the resource sustainably. Without active management, the potential for negative impacts and population declines is tremendous. We have been studying the impacts of experimental harvests of black cohosh since 2004. After 3 years of harvesting at a rate of 66% we found significant reductions in foliage areas, stem production, mean and maximum height. Populations showed no evidence of recovery after 1 year. Results suggest that black cohosh is very responsive to harvest intensities and long recovery periods are needed to ensure long-term health of the populations. Subsequent analysis of population recovery after seven years indicates limited recovery and potential threats to long-term persistence of natural populations. Another challenge for sustainable management of this medicinal product is determining how much harvestable stock is available in a patch. Few methods exist to estimate below-ground biomass based on above-ground metrics. We developed a method to estimate marketable biomass of black cohosh which can be used to improve management activities. Our findings have significant implications on managing this medicinal plant which may be appropriate for other medicinal forest products.

For more information, see:

Chamberlain, J.L., G. Ness, C.J. Small, S.J. Bonner, E.B. Hiebert. 2013. Modeling below-ground biomass to improve sustainable management of *Actaea racemosa*, a globally important medicinal forest product. Forest Ecology and Management. 293:1-8

Small, C.J., J.L. Chamberlain and D.S. Mathews. 2011. Recovery of Black cohosh (*Actaea racemosa*) Following Experimental Harvests. American Midland Naturalist. 166(2):339-348

“Demographic response of American ginseng to three natural canopy disturbances common in mixed mesophytic forests”

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Abstract

An understory plant’s ability to exploit alterations to the light environment caused by canopy disturbance leads to changes in population dynamics. The purpose of this work was to determine if population growth of American ginseng increases in response to additional light inputs caused by canopy disturbance, or alternatively, declines due to long-term selection under low light conditions. We parameterized stage-based matrix models to quantify the demographic response of ginseng to three natural forest canopy disturbances. Asymptotic growth rates, stochastic growth rates, and simulations of transient dynamics were used to quantify population-level responses. Population growth rates at each disturbed site increased the transition period directly after canopy disturbance. Stochastic models revealed that growth rates increased in simulations that included disturbance matrices relative to those that excluded disturbance. Transient models indicated that population size was larger for each population when disturbances were modeled. American ginseng is likely pre-adapted to take advantage of canopy gaps and light influx to a degree, and this pre-adaptation may be due to long-term selection under dynamic old-growth canopies. This study provides evidence to aid our understanding of the population-level response of understory herbs to disturbances whose frequency and intensity are predicted to increase as global climates shift.

Please see full published paper:

Chandler, Jennifer L. and James B. McGraw. 2017. Demographic stimulation of the obligate understorey herb, *Panax quinquefolius L.*, in response to natural forest canopy disturbances. *Journal of Ecology* 105: 736–749. doi: 10.1111/1365-2745.12695

"Ginsenoside Profiles in American Ginseng (*Panax quinquefolius* L.) in Western North Carolina" (poster)

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Abstract

American ginseng (*Panax quinquefolius* L.) is a threatened perennial understory plant endemic to eastern deciduous forests. The plant is harvested and sold on the Asian markets for its secondary metabolites, ginsenosides, which give it its medicinal qualities. Information on phytochemical profiles of populations would give more insight on creating cultivars labeled for specific medicinal properties, ideally reducing the demand for wild harvested ginseng. Genetic diversity of ginseng is thought to be more widespread in the Appalachian region, due to the glacial refugia created during the Pleistocene epoch. Ginsenoside profile diversity may also be more widespread in the Appalachian region and may be linked to genetic diversity. We analyzed the ginsenoside profiles in 157 roots from 17 NC populations. Six ginsenosides (Rb1, Rb2, Rg1, Re, Rd, and Rc) were characterized and quantified using methanol-reflux extraction, followed by high performance liquid chromatography separation and ultraviolet detection (HPLC-UV). We found that most populations exhibited the RG chemotype (Re/Rg1<1), with populations HG, LS, and MC showing small variation in chemotypes. Ginsenoside Rg1 and Rb1 had the highest overall concentrations while Re had the lowest. Lack of chemotypic diversity suggests that if chemotypes are correlated to genetic factors, overharvesting has affected the presence of certain ginsenosides within these populations, or the Pleistocene refugia hypothesis does not apply to American ginseng.

Keywords: American ginseng, ginsenoside, chemotype, Panax quinquefolius, high performance liquid chromatography

Introduction

The perennial understory herb, *Panax quinquefolius* L. (American ginseng), is native to eastern North America and is currently a threatened species (Cruse-Sanders et al. 2005). American ginseng was wild harvested beginning in the 1800s to export to the Asian market as *Panax ginseng* (Asian ginseng) became too overharvested to meet demands (Carlson 1986, Glare 1968). Its medicinal qualities are found primarily in the root, but also in the shoot, in the form of secondary metabolites called ginsenosides. Ginsenosides are widely sought after on the Asian traditional medicine market for their wide range of curative effects (McGraw et al. 2013). They are the main bioactive component in ginseng and are saponins that naturally occur in many forms with Rb1, Rb2, Rc, Rd, Re, and Rg1 being the main types of ginsenosides present in American ginseng (Corbit et al. 2005, Schlag & McIntosh 2013). These compounds are purported to have positive effects on the immune, endocrine, cardiovascular, and central nervous systems, as well as cancer preventative effects and prevention of fatigue, oxidative damage, and mutagenicity, depending on the type of ginsenoside (Corbit et al. 2005).

While American ginseng has been commercially cultivated for over 200 years, wild harvesting has continued as non-cultivated roots earn higher prices on the Asian market (McGraw et al. 2013). This is due to phenotypic traits such as size, shape, and color, with the gnarled look of wild roots more comparable to Asian ginseng, and thus perceived to be more valuable (McGraw et al. 2013, Searels et al. 2013). In light of this, overharvesting has decreased the genetic diversity of American ginseng, seriously limiting the ability of the plant to withstand selection pressures (Cruse-Sanders et al. 2005, Mooney & McGraw 2008). *Panax quinquefolius* is now listed in Appendix II of the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES), and its harvest and commerce is regulated by the U.S. Fish and Wildlife Service (Schlag & McIntosh 2006).

Sengupta et al. (2004) and Qi et al. (2011) have shown that Asian ginseng and American ginseng have markedly different ginsenoside profiles, with Asian ginseng exhibiting higher Rg1:Rb1 and Rg1:Re ratios than American ginseng. Many studies have found that Rb1 and Re are the most common ginsenosides found in American ginseng roots (Li et al. 1996, Court et al. 1996). Ginsenoside composition among American ginseng plants has been shown to vary widely, with Schlag and McIntosh (2013) finding ginsenoside concentrations (mg ginsenoside/g root dry weight) of roots ranging from 0.06–1.18 for Rg1; 0.00–1.96 for Re; 0.19–2.82 for Rb1; 0.08–0.40 for Rc; and 0.04–0.17 for Rd. They found that the RG (Re/Rg1<1) chemotype was most common, with 54% of the plants exhibiting this chemotype, 39% of the plants demonstrating the RE (Re/Rg1>2) chemotype, and 7% of the plants exhibiting the I (1<Re/Rg1<2) chemotype (Schlag and McIntosh 2013). However, seven of the roots had no Re markers present at all, and earlier studies by the group showed significant differences in Rb1, Rc, and Rd concentrations among 44 plants (Schlag & McIntosh 2006).

The already marked variation in ginsenosides for populations in northern populations may be even more pronounced in western North Carolina (WNC) populations. In the Tertiary period (~65 Mya), plants were able to travel from Eurasia to North America via land bridges (Milne & Abbott 2002). Now these Tertiary relict floras are found in refugia within East Asia, west and southeast

North America, and southwest Eurasia, *P. quinquefolius* among them (Milne & Abbott 2002). During the last glaciation, the Appalachian Mountains may have served as a glacial refugia for many species including *Cypripedium parviflorum*, preserving genetic variation and preventing genetic drift (Wallace & Case 2000). Diminished seasonality of climate and prolonged post-glacial warming in the Appalachian Mountains allowed relict floras to persist along exposed cliff ledges, landslide scars, and in wet meadows at the basins of mountains (Delcourt & Delcourt 1998). Thus, WNC may contain populations of *P. quinquefolius* that are highly variable in genetics and/or ginsenosides. Previous research has shown that there is a strong correlation between genetic markers and distinct ginsenoside chemotypes.

This research aims to analyze ginsenoside profiles in roots of *P. quinquefolius* from 17 populations in the WNC region. Additional research will analyze microsatellite regions within the plants sampled to determine whether there is significant genetic variation among these populations. These two data sets will be combined to identify any correlations between genetic variability and ginsenoside variability among populations. If genotype is predictive of the chemotype, cultivars may be developed to select for specific phytochemical profiles. A population with a known high level of Rb1 ginsenosides could be specifically marketed for its potential to limit growth of cancer cells. As more research is done on the specific medicinal properties of ginsenosides, cultivated plants known for their specific uses could outcompete the need for wild-harvested ginseng. We hypothesize significant differences in ginsenoside profiles for these 17 populations of *P. quinquefolius* based on proposed correlations between genetic and chemotypic structures of the plant and the regions presumed high genetic diversity.

Methods

Sample Preparation

Root ginsenosides were the focus of this study, and plants were randomly chosen from 17 populations in WNC. A small portion of root was collected from a small subset of three-pronged, non-reproductive plants leaving most of the root intact. These root portions were harvested with minimal disturbance from 157 plants (Table 1). The root drying procedure was mimicked from commercial procedures, with wet root mass measured and samples placed in a drying oven at ~37° C for approximately 140 hours. Dry mass was also measured, and roots were ground in a Wiley Mill through a 40-mesh screen. The extraction procedure was adapted from the methanol reflux extraction used by Corbit et al. (2005). This method has shown the greatest concentration of ginsenosides after extraction over multiple other procedures.

Table 1. Populations within each county as well as number of plants sampled within each population.

County	Populations	Number of plants sampled
Buncombe	CF	6
	HG	15
	KF	10
	P001	2
	P049	4
	PC	12
	SC	16
Haywood	MP	11
Jackson	CB	16
	CH	5
	FG	9
	JC	4
	RB	6
Macon	MC	14
	DF	2
	HC	15
Madison	LS	10

For each sample, 100 mg of the powdered plant root was combined with 5 mL 100% HPLC-grade methanol. Samples were refluxed at ~63° C for an hour and then the methanol solution was filtered via vacuum filtration through Whatman 41 Ashless filter paper. Another 5 mL of 100% HPLC-grade methanol was added to the remaining root material and allowed to reflux for another hour. The methanol solution was filtered again through vacuum filtration and added to the previous extracted liquid. The vacuum flask was rinsed with another 5 mL of 100% HPLC-grade methanol and added to the liquid extraction. Samples were diluted to 20 mL with 100% HPLC-grade methanol and then filtered using a 0.45 µM filter and syringe.

Ginsenoside Analysis

Standards were prepared using ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd obtained from Indofine Chemical Company (Hillsborough, NJ). Ginsenosides in standards and plant extracts were separated by high performance liquid chromatography (HPLC, Thermo-Hypersil Gold, 150 x 3mm, C18 column 3µm particle size, Shimadzu Inc.) using gradient elution as follows: (% water/% acetonitrile) 0-22 min 95/5; 22-40 min 78/22; 40-50 min 55/45; 50-52 min 45/55; 52-58 min 35/65. The flow rate was 0.6 mL/min. The injection volume was 20 µL. The flow rate was 0.6 mL/min. The injection volume was 20 µL. The column temperature was held at 35° C and ultraviolet detection set at 205 nm. Each ginsenoside was identified by retention time, which remained constant throughout the analyses. The concentration of each ginsenoside was calculated using the peak area and a six-point external standard calibration curve (Figure 1a, 1b).

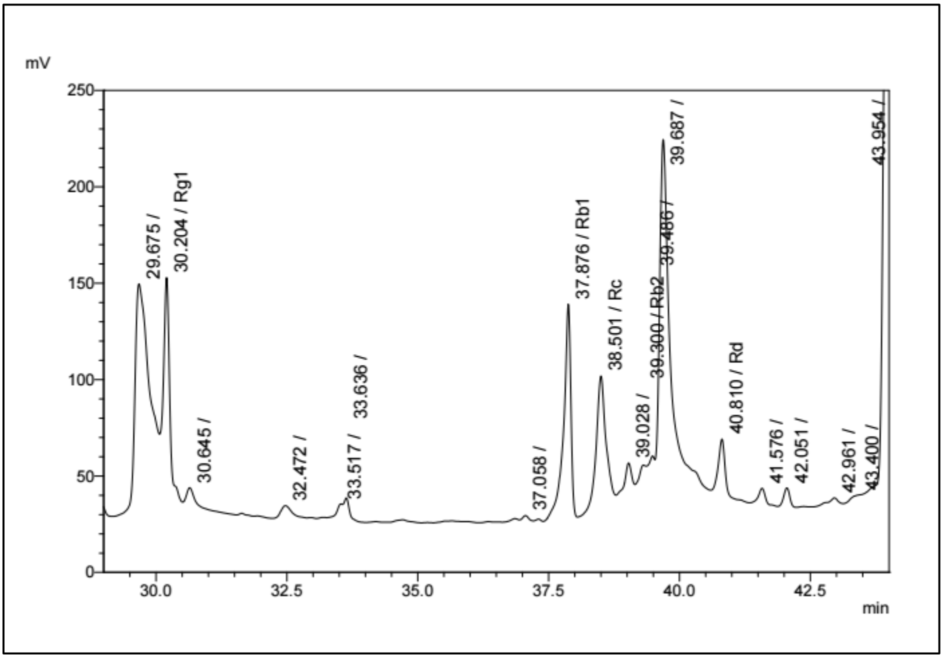

Figure 1a. Chromatogram of a root with an RG chemotype. Peaks identified by retention time (min)/name of ginsenoside.

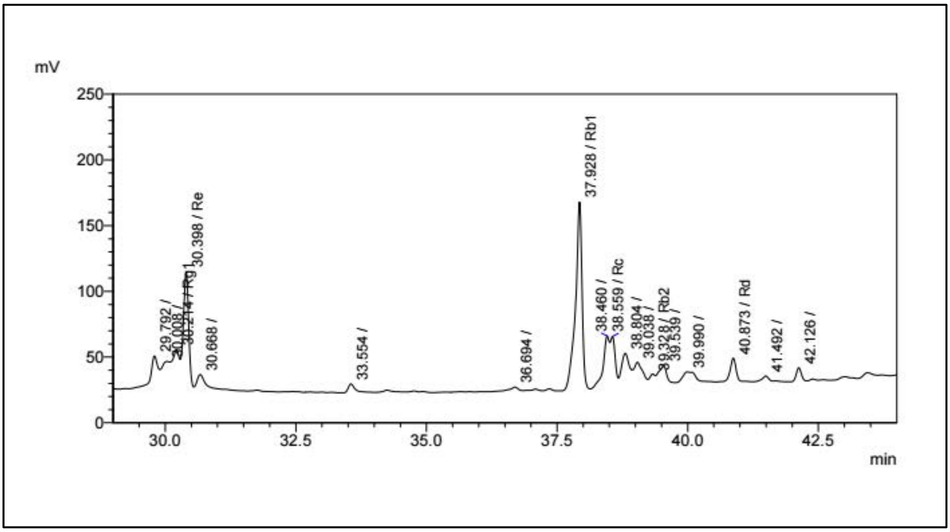

Figure 1b. Chromatogram of a root with an RE chemotype. Peaks identified by retention time (min)/name of ginsenoside.

Results

Descriptive statistics were examined across populations to evaluate trends in ginsenoside content. Concentrations for ginsenosides were measured in ginsenoside mg/dry root weight g. The most abundant ginsenoside across all populations was Rb1, with an overall average concentration of 8.64 mg/g; and the second most abundant ginsenoside was Rg1, with an average concentration of 5.76 mg/g across all populations (Table 2). The average overall ginsenoside content was 23.6 mg/g across all populations, with population CF having the highest average total ginsenoside concentration of 34.7 mg/g. The ginsenoside concentrations of individual roots ranged from 0.211-16.5 for Rg1; 0.008-15.6 for Re; 0.689-28.9 for Rb1; 0.50-12.4 for Rc; 0.584-5.93 for Rb2; and 0.727-7.96 for Rd (Table 2). Ginsenosides Re and Rb2 were the least abundant ginsenosides among all of the populations (Table 2).

Table 2. Mean ± 1 SE ginsenoside concentrations for all populations.

County	Population	Mean ± SE ginsenoside concentration (mg/g)						
		Rg1	Re	Rb2	Rb1	Rd	Rc	Total
Buncombe	CF	6.99 ±0.582	1.38 ±0.884	3.44 ±0.410	12.1 ±1.37	2.52 ±0.198	8.26 ±0.841	34.7 ±3.30
	HG	5.03 ±0.361	3.83 ±0.588	2.15 ±0.239	12.5 ±1.74	2.39 ±0.220	5.34 ±0.782	31.2 ±3.23
	KF	6.50 ±0.769	0.378 ±0.369	1.68 ±0.252	8.91 ±1.12	3.47 ±0.639	3.76 ±0.764	24.7 ±2.81
	P001	5.20 ±2.34	1.62 ±1.61	1.10 ±0.192	6.14 ±1.88	2.06 ±0.223	2.44 ±0.600	18.6 ±6.85
	P049	6.20 ±1.44	0.027 ±0.018	1.67 ±0.592	8.22 ±2.75	1.93 ±0.301	3.50 ±0.984	21.5 ±5.25
	PC	5.75 ±0.619	0.331 ±0.323	1.54 ±0.145	7.30 ±1.35	2.40 ±0.221	2.94 ±0.461	20.3 ±2.37
	SC	5.46 ±0.385	0.008 ±0	1.20 ±0.095	6.58 ±0.713	1.74 ±0.134	3.04 ±0.253	18.0 ±1.29
Haywood	MP	7.92 ±1.14	0.018 ±0.010	1.82 ±0.246	7.64 ±1.29	2.25 ±0.161	4.99 ±0.692	24.6 ±3.23
Jackson	CB	5.47 ±0.680	0.767 ±0.297	2.02 ±0.309	7.97 ±0.979	2.96 ±0.437	5.41 ±0.905	24.6 ±2.70
	CH	4.95 ±0.342	0.008 ±0	1.36 ±0.125	7.25 ±1.56	2.28 ±0.270	2.74 ±0.544	18.6 ±2.14
	FG	5.14 ±0.334	0.008 ±0	1.44 ±0.319	5.08 ±0.850	1.68 ±0.209	2.39 ±0.375	15.7 ±1.31
	JC	3.62 ±0.610	0.008 ±0	1.12 ±0.045	5.69 ±0.830	1.62 ±0.214	2.55 ±0.521	14.6 ±1.78
	RB	3.79 ±0.805	0.108 ±0.010	1.19 ±0.201	4.61 ±0.578	1.43 ±0.178	1.62 ±0.358	12.8 ±1.55
Macon	MC	4.75 ±0.838	5.83 ±1.20	1.90 ±0.343	14.2 ±1.99	2.24 ±0.243	4.26 ±0.602	33.2 ±3.29
	DF	8.87 ±1.37	0.008 ±0	1.19 ±0.038	16.2 ±0.226	2.02 ±0.247	2.86 ±0.206	31.2 ±1.60
	HC	7.85 ±0.971	0.008 ±0	1.41 ±0.164	8.62 ±1.04	2.05 ±0.219	4.46 ±0.593	24.4 ±2.40
Madison	LS	4.63 ±0.505	0.981 ±0.708	1.51 ±0.175	6.45 ±0.609	1.70 ±0.084	2.87 ±0.525	18.1 ±1.50
	Total	5.76 ±0.211	1.16 ±0.200	1.69 ±0.074	8.64 ±0.407	2.24 ±0.085	4.00 ±0.202	23.6 ±0.838

Population DF had the highest average concentration of Rg1 and Rb1, with concentrations of 8.87 mg/g and 16.2 mg/g, respectively (Table 2). Ginsenoside Re tended to have average concentrations around or below 1 mg/g across populations, but the MC population had the highest average concentration of 5.83 mg/g (Table 2). Population CF had the highest average concentration of Rb2 and Rc ginsenosides with concentrations of 3.44 mg/g and 8.26 mg/g, respectively (Table 2). Population KF had the highest average concentration of Rd, with 3.47 mg/g (Table 2).

The ratio of Re concentration/Rg1 concentration ranged from 0.0-26.9 mg/g. Most populations were comprised mainly of plants with the RG chemotype ($Re/Rg1<1$). Population HG had I chemotypes ($1<Re/Rg1<2$) present along with RG; population LS had one plant with an RE chemotype ($Re/Rg1>2$) with the rest of the plants exhibiting the RG chemotype. Population MC had the most chemotypic variation, with all chemotypes present. Among the plants in this study, 7%, 3%, and 90% were classified as I, RE, and RG chemotypes, respectively.

Discussion

While some previous studies (Li et al. 1996, Court et al. 1996) have claimed that Re and Rb1 are the most common ginsenosides in American ginseng, our results found Rb1 and Rg1 to be the most abundant ginsenosides in these populations. Re was actually the least abundant ginsenoside in this study.

Comparing percentages of chemotypes present between Maryland and WNC populations shows some discrepancies (Schlag & McIntosh 2013). Across 40 plants in a study by Schlag and McIntosh (2013), the RE, RG, and I chemotypes had frequencies of 39%, 54%, and 7%, respectively. In this study across 157 plants, the RE, RG, and I chemotypes had frequencies of 3%, 90%, and 7%, respectively (Table 3). This illustrates decreased chemotypic variation in WNC populations relative to Maryland populations. Lack of variation in the chemotypes may be due to higher rates of overharvesting in WNC relative to Maryland populations. However, previous studies in WNC had no I chemotypes identified among plants sampled (Searels et al. 2013). Although only 7% of plants sampled had the I chemotype, it is thought to be a distinct chemotype and may have significance in future studies on genetics and phytochemisty (Schlag & McIntosh 2013). While most populations exhibited the RG chemotype, the wide range of variation in the Re/Rg1 ratios (0-26.9 mg/g) indicates the importance of chemotypic differences among ginseng plants. This also illustrates that genetic markers, instead of chemotypic differences, are the ideal method to differentiate between Asian and American ginseng (Schlag & McIntosh 2013).

The abundance of Rg1 and lack of Re correlate with research by Schlag and McIntosh (2006) where north-central populations tend to produce more Re and southeastern populations tend to produce more Rg1. This reflects the geographic component of ginsenoside concentration, and statistical analysis will have to be done with this data to elucidate more of the geographic relationship.

Previous studies sampled five populations in WNC, and found ginsenoside concentrations about 2 times higher than those reported here; levels in Schlag and McIntosh (2013) were also about 3 times higher (Schlag & McIntosh 2006). The smaller ranges paired with the lack of chemotypic diversity suggest that unique chemotypes could have faced selection pressures due to overharvesting

(Schlag & McIntosh 2013). The Pleistocene glaciation may also not have been operative in this case; therefore, ginseng may not have had higher ginsenoside diversity in WNC to begin with. If there is a relationship between chemotype and genetic markers, more research needs to be done to see if genetic data show the same pattern.

Further studies sampling wider ranges of populations throughout WNC should be conducted to convey a more holistic view of ginsenoside diversity in the region. The results from this study will also be used in parallel with genetic data from these plants to identify molecular markers associated with the different chemotypes. This could eventually be used to create cultivars with specific ginsenoside profiles aimed to treat specific ailments, thus reducing the need for wild harvesting.

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"Use of Natural Fungicides with Organic Ginseng Production"

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Abstract

I present results of work from a 2000 SERA grant from the USDA about determining alternatives to chemicals to fight aboveground and soil fungus. I also discuss Davis's research study on goldenseal, and the role of goldenseal soil washes in soil-born funguses. The benefits of above-ground spraying with horsetail are presented, as the best over others tested. Additional fungicides such as plants (e.g. chamomile), hydrogen peroxide, bleach, and horticultural sulfur will also be discussed.

The Problem

Chemical fungicides have been sprayed on ginseng for decades. The growing and harvesting of ginseng is very labor intensive. Thus, once chemical spraying was made available and shown to be effective, ginseng growers quickly adopted the use of agricultural chemicals. Therefore, the chemical spraying reduced labor costs and as a result, increased profits. The use of chemical fungicides has altered the types of ginseng available in the world market.

Traditionally, ginseng was wild crafted (collected) from wild populations in the woods. Now almost all of the ginseng sold worldwide is cultivated, and 90% of all ginseng is sprayed. Ginseng is one of the most heavily sprayed crops worldwide; only tobacco and cotton are sprayed more (and we do not eat tobacco or cotton).

In North America, there are four main types of ginseng: wild, wild simulated, woods grown and cultivated. Both woods grown and cultivated are grown in a monoculture system under artificial shade and are usually sprayed with at least a fungicide in the summer and fall (Diathane M-45 is commonly used by many growers). Many growers spray every day from spring to fall.

Ginseng can become stressed and diseased in a mono-cultured environment, including some wild simulated settings. The goal of high-volume growers is to produce a marketable crop as quickly as possible. Therefore, the plants are spaced very close together and are pushed with fertilizers to speed the process. These agricultural practices, however, promote the development of fungal diseases that are able to destroy the plant and root. Thus, the grower under that agricultural mono-cultured system is forced to use chemicals to prevent or stop the spread of fungal disease(s).

The mono-cultured farming approach, with the heavy use of agri-chemicals, makes changes in the end product as compared with organic ginseng. In the long term, this is not a sustainable practice. In a short time, the soil can be depleted of nutrients and will be infested with diseases for years to come without intervention. Many plant species, including ginseng, may not be able to live in this polluted soil. Fungicide residues are making cultivated ginseng less desirable in the world market place. As a side effect, this heavy agricultural chemical spraying may also be affecting the drinking water in the area, along with other agricultural spraying of non-biodegradable elements.

Methods: The Site and Planting

We began with the preparation of the wooded site in the fall. On the north facing slope, a ten-foot high deer fence was constructed. Thirty beds in three rows were constructed within the natural forest environment. Each plot was 2.5 ft. by 3 ft. with an over-spray area between plots. All plant material was removed from the plots and a woodland soil mixture was spread over the beds.

Dr. Jeanine Davis, the Project Coordinator, participated in the planting from the North Carolina State University (NCSU) Agricultural Extension Service Research Station in Fletcher, NC. Nine hundred plants were used. Each plot had thirty roots planted in five rows with six plants to a row.

Spraying of Natural Fungicides

In early April, the heavy leaf mulch was removed. Some plants were replanted, and some were lost. A plant count was taken later in April and 674 plants had survived the winter. Dr Davis had conducted previous research that showed that goldenseal helped soil funguses in ginseng beds. This led to the possibility that goldenseal water drenches in beds used goldenseal anti-fungal properties to protect soil-borne funguses. For our study, a goldenseal wash was created by pouring boiling water into a jar with goldenseal rhizomes and placing the jar in the sun. Then that concentration was used in watering cans and washed the beds soil. The real problem was the aerial portions of the plant. These were sprayed with, (1) goldenseal, (2) horsetail, (3) Oxidate, (4) micronized compost tea or (5) water (as a control). This was a triple blind study.

Each application was scheduled to be sprayed on a weekly cycle. It should be mentioned for historical importance that the weather was considered to be in drought conditions for the entire study.

Horsetail E. was described by Rudolf Steiner (1924) as a wonderful anti-fungal. Horsetail is one of the oldest plants on our planet and has more silica than any other plant. This Bio Dynamic prep number 508, which can be used for ginseng, can be obtained from the Josephine Porter Institute in Virginia. It is prepared similar to the goldenseal spray but applied with a hand-held spray bottle or backpack spray.

Dr. Elaine Ingram, past Oregon State teacher, main author of Soil Biology Primer, USDA and now found on www.foodweb.com, lectures that the way to protect against funguses is to put good funguses on the plant so that bad funguses are not allowed to land on the leaves and stem. It should be noted that after each rain, especially in the summer and fall, the horsetail spray or other anti-fungal plants should be applied.

Results and Discussion

The control plants appeared to be the first to die off, which was anticipated. Goldenseal spray may not be as effective as the other three sprayed plots of horsetail, micronized compost tea, and Oxidate. Eagle Feather Farm uses goldenseal washes for the soil fungus, and horsetail hand sprayed on the parts of the plant that show yellowing around the edges of the leaves.

A recent article by Lee and Yu (2011) mentions four fungus species as follows: *Pythium ultimum, Alternaria alternata, Fusarium oxysporum* and *Rhizoctonia solani*. These four were investigated, noting the infection of these fungi effects the whole plant, noticing that the leaves become dry and die. They state, "The disease caused by *Pythium ultimum* can be prevented by using friendly environmental materials like *Chamaecyparis obtuse* essential oil and wormstop. *Alternaria alternata* and *Fusarium oxysporum* might be prevented by using wormstop extracted from the Neem tree, (*Azadirachta indica*)." (Lee and Yu, 2001, 11). Nothing that they tested could effectively prevent the growth of *Rhizoctonia solani*.

Therefore, we are learning more about plants and a tree that can help with the above ground parts of the ginseng plant. Note that chamomile is also anti-fungal.

Conclusion

Ginseng, both Asian and American, can be grown successfully without using chemicals. The health benefits should not have to be compromised by unwanted fungus resides, which need to be gotten out of the human body with anti-oxides, plus the consumer is not aware. There is no testing or labeling of fungicide build-up in this root crop. Our government needs to require testing coming into and out of the US; this would put a big dent in the current system. We require a "phyto" test, why not a residue level test? With Roundup being pushed worldwide it would not be unusual to find this carcinogenic in cultivated, woods grown and wild simulated ginseng.

Our universities could produce very useful information about this topic. I would hope that the Extension offices in the ginseng growing region stop recommending toxic substances as being acceptable for forest farming.

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“Characteristics of Woodland Herbal Users in the United States – Summary from an Epidemiological Study”

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Introduction

Botanicals (herbs) are grown, harvested, and used by many cultures worldwide for a variety of purposes, including the promotion of health or mitigation of disease. Although crude herbs are harvested within the U.S., consumer use of herbal preparations is largely relegated to dietary supplement status by the Federal Drug Administration. Population-based, epidemiological studies focusing on Nonvitamin, Nonmineral (NVNM) dietary supplement use have been conducted in nationally-representative populations in the U.S., and indicate approximately 17.9% of those in the U.S. consumed a NVNM in 2012 [1]. Limited studies have explored the patterns and correlates of supplement use on specific populations [2-4], while even fewer studies have been conducted to determine the characteristics of populations using specific botanicals [5, 6]. The Appalachian region of the U.S. is a woodland, ecological habitat responsible for a significant portion of U.S. botanical exports. Three woodland botanicals consistently harvested within the Appalachian region are Ginseng, Goldenseal, and Black Cohosh. In addition, Ginkgo grows across the U.S. The goal of this exploratory study was to determine the characteristics of Ginseng, Goldenseal, and Black Cohosh dietary supplement consumers across the U.S. in 2007 and 2012.

Methods

Data Sources and Study Population

Participants for this study were drawn from two nationally-representative samples of 23,501 and 34,525 U.S. adults (National Health Interview Surveys (NHIS), 2007 and 2012, respectively). The NHIS is an annual national, cross-sectional household survey of the non-institutionalized U.S. population and is administered by the Centers for Disease Control and Prevention’s National Center for Health Statistics. Survey details are described elsewhere [7, 8].

Primary outcome variables for this study were reported 30-day use of Ginkgo, Ginseng, Goldenseal, or Black Cohosh products labelled ‘dietary supplements’ and in the form of pills, capsules, tablets or liquids (including tinctures) (Yes/No for each botanical).

Exposure variables included demographics, lifestyle characteristics, health conditions, and medical care-related factors. Demographic factors included age, sex, race/ethnicity, education, employment, income, marital status, geographic region, and place of birth. Lifestyle factors included smoking status, alcohol use, exercise, BMI, and use of Complementary health approaches (CHA) other than NVNMs and prayer, other Natural Products (vitamins, chelation, probiotics, omegas) in the past year. Health conditions included self-reported history of physician-diagnosed diabetes, gastrointestinal disorders (inflammatory bowel disease, irritable bowel, severe constipation, ulcers), respiratory conditions (bronchitis (past year), emphysema, asthma), dyslipidemia, cardiovascular disease (coronary heart disease, angina, and/or heart attack), hypertension, migraine (past 3 months), mental health condition (depression, phobias, anxiety (past year), bipolar disorder), insomnia (past year), cancer, autoimmune condition (rheumatoid arthritis, lupus), and chronic pain condition (migraine or joint pain (previous 3 months), any arthritis). We also assessed number of health conditions (categorized as 0, 1, 2, and 3+ conditions). Medical care-related factors included self-reported health status, insurance status (overall, Medicaid, Medicare, private insurance), annual family out-of-pocket medical costs, and delayed access to care because “could not afford” or “worried about cost” (past year).

We conducted complete-case analyses using SAS 9.4 (Cary, NC, USA) and used sampling weights to account for complex survey procedures. We merged publicly-available NHIS files for each year and measured sample characteristics, including frequencies/prevalence rates of (each) botanical use for 2007 and 2012, respectively; we extrapolated estimates to generate U.S. population estimates using NHIS sampling weights. We considered trends between time points significant if there was no overlap in weighted percentage confidence intervals. In separate models, weighted logistic regressions were used to evaluate the independent associations of Ginkgo, Ginseng, Goldenseal, or Black Cohosh dietary supplement use to demographics, lifestyle factors, health conditions, and medical care-related factors using Rao-Scott Chi-square tests. Multivariate models adjusted for age and geographic region where sample sizes allowed (Ginkgo and Ginseng analyses).

Results

Prevalence and trends in the United States

The consumption of both Ginkgo and Ginseng declined significantly from 2007 to 2012. Ginkgo was consumed by an estimated 1,382,659 adults in 2007 (1.4%, (confidence interval (CI) 1.2, 1.6) vs. 828,340 adults in 2012 (0.8%, CI 0.7, 0.9). Ginseng was used by an estimated 1,559,834 adults in 2007 (1.6%, CI 1.4, 1.8) vs. 857,482 adults in 2012 (0.8%, CI 0.7, 0.9). While small sample sizes precluded statistical comparison, use of Goldenseal and Black Cohosh also appeared to decline from 2007 to 2012. Goldenseal was consumed by approximately 422,476 adults in 2007 (0.43%) vs. only 13,080 adults in 2012 (0.01%). Black Cohosh was used by an estimated 402,003 adults in 2007 (0.41%) vs. 38,757 adults (0.04%) in 2012.

Of participants who reported consuming a botanical, 65% (Ginseng, 2012) to 87% (Black Cohosh, 2007) were white. Across all botanicals, 4% (Black Cohosh, 2007) to 15% (Ginseng, 2012) were consumed by black participants. Twenty-seven (Black Cohosh, 2007) to 34% (Goldenseal, 2007) of consumers resided in the Western U.S. and 27% (Black Cohosh, 2007) to 35% (Ginseng, 2007) resided in the South, with only 9% (Ginseng, 2007) to 30% (Goldenseal, 2012) located in the Northeast. Middle-aged consumers (45-64 years) represented 43% (2007) to 46% (2012) of Ginkgo users, 35% (2007) to 44% (2012) of Ginseng users, 82% (2007) to 93% (2012) of Black Cohosh users, and 48% (2012) to 89% (2007) of Goldenseal users. In contrast, 13% (Black Cohosh) to 42% (Goldenseal) of consumers were aged 25-44 years. Ginseng users were predominantly male (55-57%; 2007 and 2012, respectively), while Ginkgo (51-54%), Goldenseal (59%), and Black Cohosh (92%) users were largely female.

Over 70% of all botanical consumers in both years were non-obese (BMI<30); 71% (Ginseng, 2007) to 80% (Goldenseal, 2007) reported at least some college education. Overall, 42% (Ginkgo, 2007) to 50% (Black Cohosh, 2007) of consumers were married/cohabitating, with 37% (Ginkgo, 2007) to 48% (Black Cohosh, 2007) indicating an annual income of under $45,000. The percentage of consumers reporting annual household out-of-pocket medical cost(s) under $2000 ranged from 64% (Ginkgo, 2007) to 71% (Black Cohosh, 2007). Approximately 90% of all botanical consumers reported positive health status (range: 88% (Black Cohosh) to 90% (all others)).

Between 46% (Ginseng, 2007) and 68% (Black Cohosh, 2007) of participants consuming botanicals reported at least one chronic pain condition, with 38% (Black Cohosh, 2007) to 45% (Goldenseal, 2007) indicating recent low back pain, 34% (Ginkgo, 2007) to 53% (Goldenseal, 2012) indicating recent joint pain, and 18% (Goldenseal, 2007) to 29% (Ginkgo, 2012) reporting arthritis. Twenty-five (Black Cohosh, 2007) to 29% (Ginseng, 2012) reported recent neck pain, 14% (Black Cohosh, 2012) to 28% (Black Cohosh, 2007) had recent migraine, and 8% (Goldenseal, 2012) to 25% (Black Cohosh, 2012) had headache in the past year. Further, 6% (Ginseng, 2007) to 15% (Black Cohosh, 2012) had ever received a cancer diagnosis, 13% (Goldenseal, 2007) to 32% (Ginkgo/Black Cohosh, 2012) had a respiratory condition, and 26% (Goldenseal, 2012) to 38% (Ginseng, 2012) reported mental illness. Those undergoing menopause within the previous year represented 5% (Ginseng, 2007) to 66% (Black Cohosh, 2012) of participants using a botanical.

Demographic Characteristics of Consumers in the United States

Relative to those 18-44 years, use of all botanicals (with the exception of Ginseng and Goldenseal, 2007) was increased with middle-age (45-64 years) (Table 1). Specifically, Black Cohosh use was increased by 9-fold among those middle-aged in 2007. Race was not associated with use of Ginseng or Goldenseal. Black participants were 74% less likely to use Black Cohosh than were non-Hispanic white participants, while participants of other minority status were about 40% less likely to use Ginkgo. Although Black Cohosh use did not differ by geographical region, participants living in the Western US were 1.5-2 times as likely to use Ginkgo or Ginseng compared to those living in the south after adjusting for age, and over 2 times as likely to use Goldenseal (not adjusted). Likelihood of botanical consumption increased significantly with rising educational attainment (p's for trend $\leq$0.02), with the exception of Ginseng in 2012, for which there was a threshold effect; this trend not apparent above the level of some college education (p for trend =.0003). Relative to those with a high school education or less, participants indicating at least some college were 1.7-2.6 times more likely to report use of Ginseng or Ginkgo. Those with at least a Bachelor's degree were nearly 4 times as likely to report the use of Goldenseal compared to those with a high school education or less.

While there were no significant differences in Ginkgo or Goldenseal use by sex, males were 1.4 (2007) to 1.6 (2012) times more likely to use Ginseng than were females. In contrast, males were 90% *less* likely to use Black Cohosh compared to women (Table 1). Those separated or formerly married were 2 times as likely to use Gingko, or nearly 3 times as likely to use Black Cohosh, compared to those who were single. Those born in the U.S. were 60% (Ginseng) to over 300% (Goldenseal) more likely to use botanicals compared to those not born in the U.S.

Table 1. Association of Woodland Herb and Ginkgo use to demographic, lifestyle, and health-related factors. National Health Interview Surveys 2007 and 2012, United States

	Ginkgo						Ginseng						Black Cohosh*			Goldenseal*		
	2007			2012			2007			2012			2007			2007		
	AOR	(95% CI)		AOR	(95% CI)		AOR	(95% CI)		AOR	(95% CI)		OR	(95% CI)		OR	(95% CI)	
Demographics																		
18-44 y (ref)*																		
45-64 y	**1.6**	1.2	2.2	**1.9**	1.4	2.6	0.9	0.7	1.2	**1.4**	1.0	1.8	**8.7**	**3.9**	**19.7**	1.5	0.9	2.4
65+ y	1.2	0.8	1.7	1.4	1.0	2.1	**0.4**	0.3	0.7	0.7	0.5	1.1	1.0	0.3	3.0	**0.4**	**0.2**	**0.8**
Male gender**	0.9	0.7	1.1	1.1	0.8	1.5	**1.4**	1.1	1.8	**1.6**	1.2	2.1	**0.1**	0.0	0.2	0.8	0.5	1.3
Race/Ethnicity**																		
Non-Hispanic white (ref)																		
Black	0.7	0.5	1.1	0.8	0.6	1.2	0.9	0.7	1.3	1.3	0.8	1.9	**0.3**	0.1	0.6	0.8	0.5	1.5
Other	0.8	0.6	1.2	**0.6**	0.4	0.9	0.9	0.6	1.2	1.1	0.8	1.5	0.5	0.2	1.0	0.6	0.3	1.1
Marital status**																		
Single (ref)																		
Married/Cohabiting	0.7	0.5	1.0	1.1	0.7	1.8	0.9	0.6	1.2	1.0	0.7	1.4	1.8	0.8	4.1	0.7	0.4	1.3
Separated/Divorced/Widowed	0.8	0.5	1.2	**2.0**	1.2	3.4	1.1	0.7	1.7	1.5	1.0	2.3	**2.7**	1.2	6.4	1.0	0.5	1.8
Education (vs. < High School/GED)**																		
Some college/Associate's/Technical	**2.5**	1.8	3.4	**2.1**	1.4	3.0	**2.0**	1.5	2.7	**1.7**	1.2	2.4	1.9	1.0	3.6	2.0	1.0	3.8
> Bachelor's degree	**2.5**	1.8	3.5	**2.6**	1.8	3.7	**1.6**	1.1	2.1	**1.9**	1.2	2.8	2.1	1.1	3.8	**3.7**	2.2	6.5
Family Income**																		
<$25,000 (ref)																		
$25,000-44,999	0.9	0.6	1.3	1.5	1.0	2.3	1.3	0.8	1.9	**1.8**	1.2	2.8	1.5	0.7	3.1	1.0	0.5	2.1
$45,000-74,999	1.0	0.7	1.5	**1.7**	1.0	2.8	**1.6**	1.1	2.4	1.5	1.0	2.4	0.7	0.3	1.8	**2.0**	1.1	3.7
$75,000+	1.1	0.7	1.8	1.4	0.9	2.2	1.1	0.6	1.9	1.5	0.9	2.6	1.7	0.7	4.2	2.1	0.8	5.0
Don't know/Missing	**0.5**	0.4	0.8	**0.6**	0.4	0.9	**0.6**	0.4	0.9	0.8	0.5	1.3	0.6	0.3	1.1	**0.5**	0.3	0.9
Employment Status**																		
Unemployed (ref)																		
Working (for pay or not for pay)	**1.6**	1.2	2.1	**2.0**	1.4	3.0	**1.6**	1.2	2.1	**2.0**	1.4	2.9	1.4	0.8	2.5	**1.6**	0.9	2.6
Born in the U.S.**	1.3	0.9	1.8	**2.0**	1.2	3.3	**1.6**	1.1	2.3	1.3	0.9	1.9	2.1	1.0	4.4	**3.1**	1.4	7.1
U.S region of residence**																		
Midwest	0.9	0.6	1.3	1.5	0.9	2.4	1.0	0.7	1.4	1.3	0.8	2.0	1.6	0.8	3.1	1.1	0.6	2.1
Northeast	1.0	0.7	1.4	0.7	0.4	1.3	0.9	0.6	1.3	0.6	0.4	1.1	1.3	0.6	2.6	1.4	0.7	2.7
West	**1.8**	1.3	2.4	**2.0**	1.3	3.1	**1.5**	1.1	2.0	**2.0**	1.3	3.1	1.7	1.0	3.1	**2.1**	1.2	3.7
South (ref)																		
Insurance, Medical Care/Costs																		
Insured (vs. not insured)**	0.9	0.6	1.2	1.0	0.7	1.5	0.8	0.6	1.0	0.7	0.5	1.1	0.6	0.3	1.0	0.8	0.4	1.3
Medical out of pocket costs**																		
no costs (ref)																		
<$500	1.0	0.7	1.6	1.3	0.8	2.1	1.3	0.8	1.9	0.9	0.6	1.4	**5.8**	1.4	24.7	2.1	0.9	5.4
$500-1,999	1.1	0.7	1.6	1.8	1.0	3.0	1.4	0.9	2.2	1.0	0.6	1.7	**6.7**	1.6	28.6	2.3	0.9	5.8
>$2000	1.5	1.0	2.4	**2.1**	1.2	3.7	**1.8**	1.1	2.7	1.1	0.7	1.7	**9.4**	2.2	40.2	2.9	1.1	7.9
Delayed Medical care (financial reasons)**	**2.3**	1.6	3.3	**2.1**	1.5	2.9	**2.1**	1.5	2.9	**2.2**	1.5	3.1	**3.1**	1.8	5.3	**2.4**	1.5	3.9
Health Behaviors																		
Smoking status (vs. never smoked)**																		
Former smoker	**1.6**	1.1	2.2	**1.6**	1.2	2.2	**1.9**	1.4	2.7	**1.6**	1.1	2.2	1.1	0.6	1.9	1.3	0.8	2.2

$500-1,999	1.1	0.7	1.6	1.8	1.0	3.0	1.4	0.9	2.2	1.0	0.6	1.7	**6.7**	1.6	28.6	2.3	0.9	5.8
>$2000	1.5	1.0	2.4	**2.1**	1.2	3.7	**1.8**	1.1	2.7	1.1	0.7	1.7	**9.4**	2.2	40.2	2.9	1.1	7.9
Delayed Medical care (financial reasons)**	**2.3**	1.6	3.3	**2.1**	1.5	2.9	**2.1**	1.5	2.9	**2.2**	1.5	3.1	**3.1**	1.8	5.3	**2.4**	1.5	3.9
Health Behaviors																		
Smoking status (vs. never smoked)**																		
Former smoker	**1.6**	1.1	2.2	**1.6**	1.2	2.2	**1.9**	1.4	2.7	**1.6**	1.1	2.2	1.1	0.6	1.9	1.3	0.8	2.2
Current smoker	**1.2**	0.9	1.6	1.1	0.7	1.6	**1.8**	1.3	2.3	**1.7**	1.2	2.3	0.9	0.4	1.8	1.2	0.7	2.0
Physical activity at least 10 min/Week**	**2.2**	1.7	2.9	**1.9**	1.3	2.7	**1.6**	1.3	2.1	**2.0**	1.4	2.7	**2.4**	1.4	4.3	**1.9**	1.2	3.2
Alcohol consumption (vs. none)**																		
Low	**1.8**	1.3	2.4	**2.4**	1.6	3.7	1.5	1.1	1.9	**2.2**	1.5	3.2	1.9	1.1	3.4	1.5	0.9	2.4
Moderate to high	**2.6**	1.8	3.6	**2.3**	1.5	3.6	**2.3**	1.7	3.2	**2.5**	1.6	4.0	1.6	0.8	3.5	**2.5**	1.4	4.4
Use of other Complementary Health Approaches**																		
Any (except prayer or herbs)	**18.4**	8.7	38.9	**35.8**	8.5	150.9	**11.1**	5.4	22.6	**9.6**	4.7	19.8	**8.3**	2.9	23.9	**32.1**	4.4	234.0
Other natural products	**10.8**	6.3	18.5	**20.7**	8.0	54.0	**9.1**	5.5	14.8	**7.2**	4.1	12.5	**3.1**	1.4	6.6	**32.4**	7.6	138.7
Anthropometrics, Health Status, and Reproductive History*																		
BMI**	0.9	0.8	1.1	1.1	0.9	1.2	1.0	0.9	1.1	1.0	0.9	1.1	0.8	0.6	1.1	1.0	0.8	1.3
Chronic pain†	**1.4**	1.1	1.9	**1.5**	1.2	2.0	**1.5**	1.2	2.0	**1.6**	1.1	2.4	**3.4**	2.0	5.9	**1.5**	1.0	2.3
Headache[a]	1.2	0.8	1.7	1.5	1.0	2.2	1.2	0.9	1.6	**1.5**	1.1	2.2	**1.8**	1.1	3.0	0.5	0.2	1.1
Migraine[b]	1.1	0.8	1.6	**1.9**	1.3	2.6	**1.5**	1.1	2.0	**1.6**	1.2	2.3	**2.8**	1.6	4.8	0.8	0.4	1.7
Diabetes (ever)	0.8	0.5	1.3	**0.5**	0.3	0.9	1.0	0.6	1.6	0.7	0.4	1.1	0.8	0.3	2.1	---		
Insomnia[a]	**1.4**	1.0	1.8	**2.2**	1.5	3.1	**1.8**	1.3	2.4	**2.0**	1.5	2.7	**1.9**	1.2	3.2	**1.9**	1.2	3.2
Cancer (ever)	1.2	0.8	1.8	1.1	0.7	1.8	1.0	0.6	1.6	0.7	0.4	1.2	0.5	0.2	1.8	---		
Mental health condition††	**1.9**	1.5	2.5	**1.4**	1.1	1.9	**1.8**	1.4	2.4	**1.9**	1.4	2.5	**2.2**	1.3	3.7	**2.0**	1.2	3.3
Dental problems‡	**2.0**	1.4	2.9	1.4	1.0	2.2	**1.9**	1.3	2.6	**1.9**	1.4	2.7	1.3	0.7	2.7	**1.7**	1.1	2.8
Respiratory Issues	1.2	0.9	1.7	1.1	0.8	1.6	1.3	1.0	1.7	1.0	0.7	1.5	1.1	0.5	2.2	0.9	0.5	1.7
Cholesterol (Ever)	1.3	1.0	1.7	1.0	0.7	1.5	1.2	0.9	1.6	0.9	0.6	1.4	0.9	0.5	1.5	0.8	0.4	1.4
Number of Health Conditions†††																		
None (ref)																		
One	1.7	1.2	2.5	1.3	0.8	1.9	1.9	1.4	2.8	1.4	1.0	1.9	**3.2**	1.6	6.2	1.6	0.9	2.9
Two	1.7	1.1	2.6	1.5	1.0	2.4	2.1	1.4	3.2	1.0	0.6	1.6	1.4	0.6	3.6	1.2	0.6	2.2
Three or more	1.9	1.2	2.9	1.6	1.0	2.7	2.0	1.4	3.0	**1.7**	1.2	2.6	2.7	1.3	5.7	1.4	0.8	2.6
Good-excellent health status (vs. fair/poor)	1.3	0.8	1.9	**1.7**	1.2	2.5	1.0	0.7	1.5	1.0	0.6	1.6	1.2	0.5	2.6	1.2	0.6	2.2
Menopause within past year (Women only)	**1.7**	1.1	2.7	**2.2**	1.3	3.7	1.7	0.9	2.9	**2.0**	1.2	3.5	**11.6**	7.0	19.3	**3.0**	1.5	6.1

Bold indicates significance at $p < 0.05$

Abbreviations:

‡ Pain in past year

† Including rheumatoid arthritis, arthritis, gout (ever); joint pain, and/or migraine (past 3 months)

††Including phobia, anxiety, and/or depression (past year); and/or bipolar disorder (ever)

††† Including diabetes, gastrointestinal condition, respiratory condition, rheumatoid arthritis, coronary vascular disease, hypertension, arthritis, gout (ever); kidney disease, liver disease (past year); migraine (past 3 months): and/or mental health condition

[a] Past year

[b] Past 3 months

* Crude Odds Ratio; not adjusted

**Adjusted for Age

*** Adjusted for Age and Region

Those with an annual income of \$45,000-\$74,999 were 1.6-2 times as likely to use Ginseng or Goldenseal in 2007 as those with an income of <\$25,000, while those with an annual income of <\$25,000 were nearly 2 times as likely to use Ginseng in 2012 (Table 1). Additionally, those unemployed were less likely to use any botanical, with the exception of Ginseng in 2012 or Black Cohosh (2007), for which there were no differences by employment status.

Lifestyle and Medical-related Characteristics of Botanical Users in the United States

Relative to never smokers, former smokers had 60-90% higher odds of using Ginkgo and Ginseng (2007); current smoking was also positively associated with Ginseng use, but was unrelated to consumption of other botanicals (Table 1). Those exercising over 10 minutes per week were 1.9-2.4 times more likely to consume Ginkgo, Ginseng, Black Cohosh, or Goldenseal compared to those who engaged in no weekly exercise in 2007. Likewise, those exercising in 2012 were 2 times as likely to use Ginkgo or Ginseng. In both 2007 and 2012, those consuming moderate to heavy amounts of alcohol had 2.3 to 2.6 fold higher likelihood of using Gingko, Ginseng, or Goldenseal compared to alcohol abstainers.

There were no differences in overall insurance status for Ginkgo, Ginseng, and Goldenseal (p range $\geq$0.08). However, there was a borderline-significant decrease in Black Cohosh use among those uninsured compared to participants who were insured (Table 1). Those spending over \$2000 per year were over 9 times as likely as those with no out-of-pocket costs to use Black Cohosh, and those delaying medical care due to cost were 3 times as likely to use Black Cohosh. Comparatively, those delaying medical care due to cost were 2.1-2.4 times as likely to use Ginkgo, Ginseng, or Goldenseal and were only about 2 times as likely to spend over \$2000 per year on out-of-pocket medical costs (Ginkgo 2012, Ginseng 2007). Participants who had ever used any other Complementary Health Approaches (CHAs) besides herbs and prayer were over 8 times as likely to use Black Cohosh, 10-11 times as likely to use Ginseng, 18-36 times as likely to use Ginkgo, and over 30 times more likely to use Goldenseal compared to those not using these approaches; estimates remained unchanged after additional adjustment for variations in use by region.

Reproductive History, Obesity, and Health Status

After controlling for geographical region and age, chronic pain was significantly and positively associated with use of all botanicals. As illustrated in Table 1, this association was most pronounced with consumption of Black Cohosh;

participants who reported a chronic pain condition were more than 3 times as likely to use this herb relative to those without chronic pain. Participants who reported recent migraine were approximately 1.5-3 times more likely to use Ginkgo (2012), Ginseng (2007, 2012), and Black Cohosh (2007). Likewise, those indicating headache in the past year were also more likely to use Ginseng and Black Cohosh (ORs 1.5-1.8).

As indicated in Table 1, participants who reported insomnia in the past year were more likely to indicate using Ginkgo (AOR's 1.4-2.2), Ginseng (AOR's 1.8-2.0), Black Cohosh (OR 1.9), and Goldenseal (OR 1.9). Similarly, those indicating a history of mental illness were 1.4 to 2.2 times as likely to use Ginseng (AOR's 1.8-1.9), Ginkgo (AOR's 1.4-1.9), Black Cohosh (OR 2.2) and Goldenseal (OR 2.0).

After adjusting for age and region, participants who reported three or more health conditions were nearly twice as likely to use Ginseng (2012), and 3 times as likely to use Black Cohosh (2007; no additional adjustment) related to those with no medical conditions (Table 1). Among female participants, those experiencing menopause in the previous year were 1.7-2.2 times as likely to use Ginkgo (AOR's 1.7-2.2), Ginseng (2012 AOR 2.0), and Goldenseal (2007 OR 3.0). Alternately, those experiencing menopause in the previous year were nearly 12 times as likely to use Black Cohosh (2007), compared to those not experiencing relatively recent menopause.

Discussion

In our study, a number of demographic, lifestyle, and health-related factors were associated with the use of Ginkgo, Ginseng, Goldenseal, and Black Cohosh. The average botanical consumer in our samples largely mirrored factors associated with the use of (overall) natural product consumption in the U.S., particularly higher supplement use among white, middle-aged consumers with higher levels of education. All botanicals were used more often by those with chronic pain, insomnia, or mental health conditions. Further, those using Black Cohosh had higher out-of-pocket medical costs, and presence of chronic pain, headache, migraine, and mental health conditions compared to Ginkgo, Ginseng, and Goldenseal. Yet, those using Black Cohosh were more likely to report no health conditions, comparatively, but had much higher rates of menopause. Ginkgo was not associated with the presence of headache and was only positively associated with recent migraine in 2012.

It is possible that all botanicals measured in this study reflected only a piece of the potentially health-protective behaviors utilized by participants for general health, as all botanical users had an increased, positive association with weekly exercise and former smoking. Alternately, the use of these botanicals may reflect a behavior associated with riskier health behaviors, as current smokers also maintained positive (but decreased) rates of botanical use, as did those consuming moderate to heavy amounts of alcohol. Thus, botanical use in the context of factors associated with coping mechanisms in the presence of health conditions should be further explored.

The major strength of this study is its use of large datasets to explore patterns and correlates of botanicals previously unexamined in epidemiological studies. There were, however, some limitations. First, there was no geographic harvest origin data available for dietary supplements in the dataset; thus, we analyzed data with the assumption that at least some of it derived from the U.S. In addition, there were no Latin names available in the NHIS dataset. Our reliance on common names may have increased the chances of outcome misclassification. Despite our use of the largest US dataset containing information on a variety of NVNMs, our sample sizes were quite small for outcomes Goldenseal and Black Cohosh, demonstrated by wide confidence intervals. Replication of these analyses in larger sample sizes also including the use of crude herbs for health purposes are needed to confirm characteristics of specific botanical use and to lay the foundation for efficacy studies related to the use of woodland and other botanicals for specific conditions.

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"Mycorrhizal Symbiosis in Forest-Grown American ginseng (*Panax quinquefolius*) and the Relationship Between Mycorrhizal Colonization and Root Ginsenoside Content"

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Abstract

American ginseng (Panax quinquefolius L.) is a valuable medicinal plant that has been harvested from the forests of eastern North America for over 300 years, and commercially cultivated since the late 1800's. Arbuscular Mycorrhizal Fungi (AMF) are symbiotic soil organisms that colonize plant roots, and often contribute to enhanced growth by increasing the uptake of water and nutrients. The role of AMF in the production of American ginseng has become a topic of increasing interest, but forest-based research on this subject is limited. This study quantified AMF colonization in six-year-old forest-grown ginseng roots, resolved the relationship between AMF colonization and root ginsenoside content, and identified species of AMF present in forest production sites. Roots from four production sites were measured for AMF colonization, and ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd were quantified by High Performance Liquid Chromatography (HPLC). AMF spores were extracted from soil samples by wet-sieving, and identified morphologically. Results indicate that AMF colonization varied significantly between sites ($p < 0.05$), but no significant differences in ginsenoside content were resolved between sites ($p = 0.104$). Furthermore, ginsenoside content was determined to not be significantly influenced by AMF colonization ($p = 0.0823$). Significant inverse relationships between AMF colonization and Rg1 ($p = 9.826e-05$) were detected, and there was a positive correlation between AMF colonization and Re ($p = 0.007$). Due to high spore degradation, Rhizophagus intraradices (formerly Glomus intraradices) was the only species of AMF identified between production sites.

Introduction

American ginseng is a long-lived herbaceous perennial herb belonging to the Araliaceae family that is typically found growing in the deeply shaded understory of mature hardwood forests (Chandler & McGraw, 2015), ranging from southern Canada to northern Georgia, and west to states along the Mississippi River (Burkhart, 2013). Ginseng is highly valued as a medicinal plant species and has been harvested from the forests of eastern North America for over 300 years (Burkhart, 2013). Concerns about overharvesting, loss of natural habitats, and observed declines in wild populations have increased the need and demand for high-quality wild-simulated (WS) roots that are intentionally produced on private forestlands. WS roots are grown with the goal of producing a root that is wild in appearance, and is virtually indistinguishable from truly wild ginseng, thus enabling producers to capture premium prices typically paid for wild roots (Carroll & Apsley, 2013).

Symbiotic mycorrhizal fungi have the potential to improve the health, productivity, and quality of commercially produced medicinal plants. Mycorrhizal fungi are symbiotic soil organisms that form partnerships with the root systems of approximately 80% of all terrestrial plant species (Whigham, 2004). Mycorrhizae function as an extension of the plant root systems and facilitate the uptake of water and nutrients, particularly phosphorus and nitrogen, in exchange for a supply of carbohydrates (*e.g.* glucose) (Hodge et al., 2010). Ginseng, as well as most herbaceous plant species, form partnerships with Arbuscular Mycorrhizal Fungi (AMF), a class of endo-mycorrhizal species that penetrate and colonize the internal cortex of fibrous secondary and tertiary roots (McGonigle et al., 1999). Previously observed benefits of mycorrhizae in the production of ginseng roots include increased yields and biomass production (Li, 1995), and enhanced production of secondary metabolites (e.g. ginsenosides) that are attributed to increased medicinal potency (Zeng et al., 2013; Fournier et al., 2003).

Ginsenosides are chemically classified as triterpenoid saponins and are considered the major active constituents of American ginseng. More than 60 unique compounds have been isolated from the roots, shoots, leaves, flower buds, and berries, with additional novel ginsenoside compounds found to be produced through metabolic processes and biotransformation (Qi et al., 2011). Ginsenosides typically account for 3-6 % of total root mass (Robbins, 1998), with ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd being the six most abundant by weight (Lim et al., 2005). Previous research has also shown that ginsenoside concentrations are typically correlated with root mass (Robbins, 1998; Smith et al., 1996) and plant age (Court et al. 1996), with higher concentrations in larger and older roots.

Thus far, studies examining mycorrhizal symbiosis in WS ginseng have been under-represented relative to more widely researched production methods (*e.g.* field cultivation). Growing conditions in these production systems are significantly different from those required to produce ginseng roots with wild characteristics (Carroll & Apsley, 2004). Key differences in habitat conditions, production practices, and harvest cycles raise questions about how ginseng-mycorrhizae interactions may differ in WS ginseng. Research sites and sources of root material for this study represent the variation in habitat conditions found within the central portion of the natural range for American ginseng, and the diversity of production practices currently used in the forest-farming community to produce "wild-simulated" ginseng.

Methods

Objectives

1) To quantify rates of mycorrhizal colonization observed in WS ginseng roots.
2) To determine if there is a statistical correlation between mycorrhizal colonization and root ginsenoside concentrations.
3) To characterize the community of mycorrhizal species present in forested ginseng production sites.
4) To determine the infectivity potential of forest soils at each production site.

Study Sites

Roots were collected from four commercial ginseng production sites located in Ohio, Pennsylvania, and Maryland. These sites are representative of two commonly used WS production systems that vary in scale, intensity of production, and cultivation/management practices (Table 1). Sites A and B are distinguished from sites C and D by four main differences: (1) the use of moderate soil tillage to prepare planting sites, (2) the removal of competitive understory vegetation prior to planting, (3) higher planting density, and (4) the application of fungicides as needed to prevent and control disease during the cropping cycle.

Table 1. Description of sampling site habitat characteristics and management practices

Site/State	Soil pH	Fungicide	Vegetation Management	Soil Type	Aspect	Elevation (m)
A (PA)	5.5	Yes	Understory cleared	Silt Loam	S	485
B (MD)	6.3	Yes	Understory cleared	Stony Loam	NE	609
C (OH)	5.4	No	Moderate thinning	Silt Loam	N	225
D (OH)	5.5	No	Moderate thinning	Silt Loam	N	274

Root and Soil Sampling

Sixty six-year-old roots were collected using a randomized sampling design (15 roots per site). Each sampling unit was represented by a 1.5 m x 6 m grid, and roots were harvested using randomly selected coordinates. Roots were harvested between August 27, 2016 and September 10, 2016 when mycorrhizal colonization (Whitebread et al., 1996) and ginsenoside concentrations are typically at peak levels (Li et al, 1996). After harvesting, roots were gently washed, then weighed in order to resolve relationships between AMF colonization, ginsenoside concentrations, and root mass. To quantify mycorrhizal colonization the fibrous roots were removed from the rhizome, weighed, and half of the fibrous root mass was randomly selected for mycorrhizal analysis. The remaining fibrous roots were dried with the tuberous portion of the root for ginsenoside analysis. Ten soil samples were randomly collected from each sampling grid for mean infectivity assays, and AMF spore identification. Samples were collected at a depth of 10 cm where AMF spore density is greatest (Egerton-Warburton & Allen, 2000).

Mycorrhizal Analysis: Root Clearing and Staining

The fibrous roots selected for mycorrhizal analysis were cut into 2 cm segments (McGonigle et al., 1999), placed in labeled tissue cassettes and cleared (*e.g.* cellular contents removed) in pre-boiled 10% potassium hydroxide (KOH) for 30 minutes. Once cleared the tissue cassettes were rinsed in distilled water, and submerged in 2% hydrochloric acid (HCl) for 15 minutes prior to staining (INVAM, 2014). Tissue cassettes were then submerged in a pre-boiled solution of 0.05% direct blue histological stain (1:1:1 distilled water, glycerin, and lactic acid (v/v/v)) and soaked for 5-7 minutes (INVAM, 2014). Samples were then rinsed in distilled water to remove excess stain, and stored in distilled water until the colonization analysis was conducted (INVAM, 2014).

Measuring Mycorrhizal Colonization

The percentage of root length colonized by AMF was determined using the gridline-intersect method (Giovanetti & Mosse, 1980). Stained roots were placed in 10 cm diameter petri dish marked with a 1.3 x 1.3 centimeter grid, then examined using a dissecting microscope (Giovanetti & Mosse, 1980). The number of horizontal and vertical intersections where mycorrhizal structures were present were tallied, and the number of positive intersections was divided by the total number of intersections to determine percent colonization. Roots were quantified for Total Percent Colonization (TPC) by counting all mycorrhizal structures (*e.g.* internal and external hyphae, intra-radical spores, external spores, vesicles, and arbuscules), and for Percent Arbuscule Content (PAC), by solely counting intersections where arbuscules were present.

Ginsenoside Sample Preparation and Analysis

A subsample of 32 roots (8 from each site) were randomly selected for ginsenoside analysis. Ginsenoside concentrations were quantified using High Performance Liquid Chromatography (HPLC). Ginsenoside samples were prepared using a combination of methods previously described by Lim et al. (2005) and Corbit et al. (2005). Roots were dried at 35°C (95°F) in a forced-air dehydrator (Nesco Gardenmaster), then ground to powder. Extracts were prepared by combining 300 mg of root with 10 mL of 70% HPLC-grade methanol in 15 mL centrifuge tubes. Sample slurries were extracted in a water bath sonicator (Fischer Scientific, model FS20H) at 40°C for 30 minutes, centrifuged (Eppendorf Model 5810 R) for 2 minutes at 3500 rpms, and the supernatants collected. The pellet was re-extracted using the same process, and the supernatants were combined. Supernatants were roto-evaporated (IKA® RV 10) to remove the methanol fraction, and the residues re-dissolved in 2 mL of 100% HPLC-grade methanol (Fischer Scientific, Pittsburgh PA). The 2 mL solution was lyophilized (Virtis Genesis 25 ES) to reduce to dryness, then dissolved in 500 µl of 73% acetonitrile and filtered with 0.02 µm nylon filters prior to HPLC injection (Lim et al., 2005; Corbit et al., 2005).

Extracts were analyzed against standards for ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd (Indofine Chemical Co, Hillsborough NJ, and Sigma Aldrich, St. Louis MO), and were quantified based on peak height. Calibration curves were developed for each standard in concentrations ranging from 10 µl/ml – 2 mg/ml. Samples were analyzed with a Shimadzu Prominence HPLC system (Shimadzu Corporation, Kyoto Japan), with a LC-20AD pump, degasser, SIL-20AD HT autosampler, SPD-M20A diode array detector, and a Phenomenex C18 250 mm x 4.6 mm analytical column (5 µm pore size). The data was collected and analyzed with Shimadzu LC Solutions software. The mobile phase was a binary gradient of acetonitrile (A) and water (B) at a flow rate of 1.3 ml/min. The gradient was as follows: 0-15 min., 21% A; 16-38 min., 30% A; 39-55 min., 42% A; 56-65 min., 90% A; and 66-80 min., back to 21% A (Corbit et al., 2005).

Mean Infectivity Percentage and Species Identification

Mean Infectivity Percentage (MIP) was used to determine the infectivity potential of mycorrhizal populations at each production site (Moorman & Reeves, 1979; INVAM, 2014). Soils from each site were used to inoculate a sterile growing medium, planted with corn (*Zea mays*), and grown for 21 days (INVAM, 2014). Corn roots were then measured for percent colonization using the methods previously described.

AMF spores were extracted from soil samples by wet-sieving (Smith & Skipper, 1979), and identified by morphological characteristics. Mean infectivity analysis and species identification was conducted at the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi by Dr. Joseph Morton (INVAM).

Statistical Analysis

One-way analysis of variance (ANOVA) was used to test for site-based differences in root mass, fibrous root mass, AMF colonization, and ginsenoside concentrations. Two-way ANOVA was used to resolve site-level differences in the effect of AMF colonization on ginsenoside concentrations, the effect of AMF colonization on concentrations of individual ginsenosides, and the effect of root mass on total ginsenoside concentrations. Where site differences were not observed, regression analysis was used to quantify the overall relationship across all sites between AMF colonization, root mass, and ginsenoside concentrations. For ginsenoside variables that could not be rendered normally distributed, Welch's One-way ANOVA and Spearman rank order correlations were used. Pearson product moment correlations were used to test for significant correlations between fungal colonization and total ginsenoside concentrations.

Results

Mycorrhizal Colonization

One-way analysis of variance (ANOVA) determined that Total Percent Colonization (TPC) ($p = 9.835e-07$) and Percent Arbuscule Content (PAC) ($p = 0.0008$) were significantly different between sites, with differences between sites A and C, and A and D accounting for most of the observed variation in both TPC and PAC. The highest mean TPC was recorded at Site C (66.15%), followed by Sites D (63.72%), B (53.97%), and A (48.87%), and the highest mean PAC was recorded at Site D (15.86%), followed by Sites B (13.69%), A (11.88%), and C (6.70%). The distribution of TPC and PAC across all sites is illustrated in Figures 1 and 2 respectively.

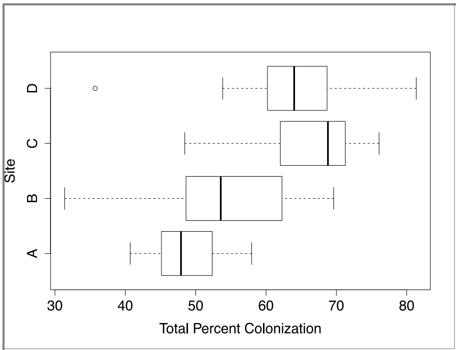

Fig 1. Mean (n=15) and distribution of total percent colonization (TPC) of AMF in roots by site.

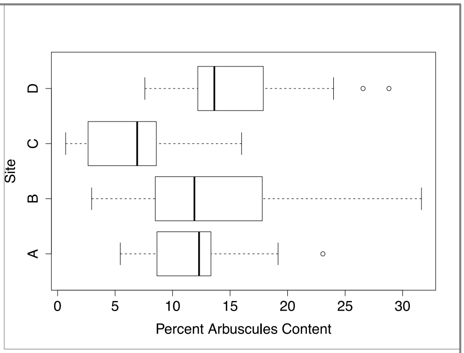

Fig 2. Mean (n=15) and distribution of percent arbuscule content (PAC) in roots by site.

One-way ANOVA was also used to compare total root mass across production sites, and indicated that root masses ($p = 5.143e-08$) were significantly different across sites. The distribution total root mass is illustrated in Figure 3. Pearson product moment correlations were used to test for relationships between AMF colonization and measurements of root mass and fibrous root mass, and determined that PAC was inversely correlated with both total root mass ($p = 0.021$, $rho = -0.2978$) and fibrous root mass ($p = 3.233e-05$, $rho = -0.5093$), and this relationship was consistent across sites. The relationship between PAC and root mass are illustrated in Figure 4. Results showed no significant correlations between TPC and either root or fiber mass. Data for TPC, PAC, total root mass, and fibrous root mass for all sites is summarized in **Table 2**.

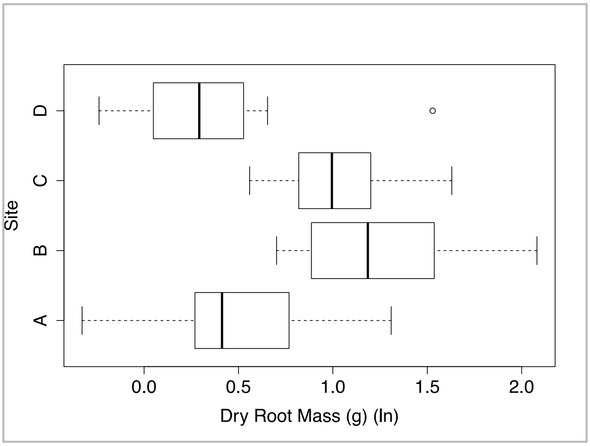

Fig 3. Mean (n=15) and distribution of root mass by site. Data was log transformed to achieve normality.

Table 2. Mean values for total percent colonization (TPC), percent arbuscule content (PAC), total root mass, and fibrous root mass (± STDEV)

Site	TPC	PAC	Root Mass (g)	Fiber Mass (g)
A	48.87 (± 5.49)	11.88 (± 4.74)	1.81 (± 0.82)	0.36 (± 0.16)
B	53.97 (± 9.98)	13.69 (± 7.65)	3.72 (± 1.64)	0.48 (± 0.28)
C	66.15 (± 7.75)	6.70 (± 4.65)	2.96 (± 0.93)	0.61 (± 0.17)
D	63.72 (± 10.22)	15.86 (± 6.32)	1.56 (± 0.91)	0.26 (± 0.15)

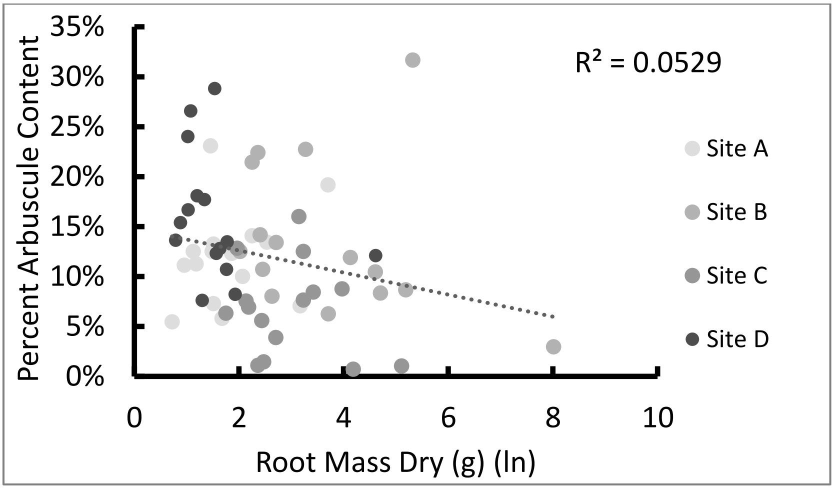

Fig 4*.* Inverse significant relationship ($p < 0.05$) between arbuscule content (PAC) and root mass.

Ginsenoside Analysis

One-way ANOVA was used to compare root ginsenoside concentrations across all production sites, and there were no significant differences between sites ($p = 0.104$). Mean total ginsenoside concentrations were highest in roots sampled from Site B (108.73 mg/g), followed by sites C (107.51 mg/g), A (96.83 mg/g), and D (65.09 mg/g), representing 10.87%, 10.75%, 9.68%, and 6.51% of mean root mass respectively. Mean ginsenoside concentrations for each site are illustrated in Figure 5, and the percentage of root mass represented by ginsenosides is summarized in Table 3.

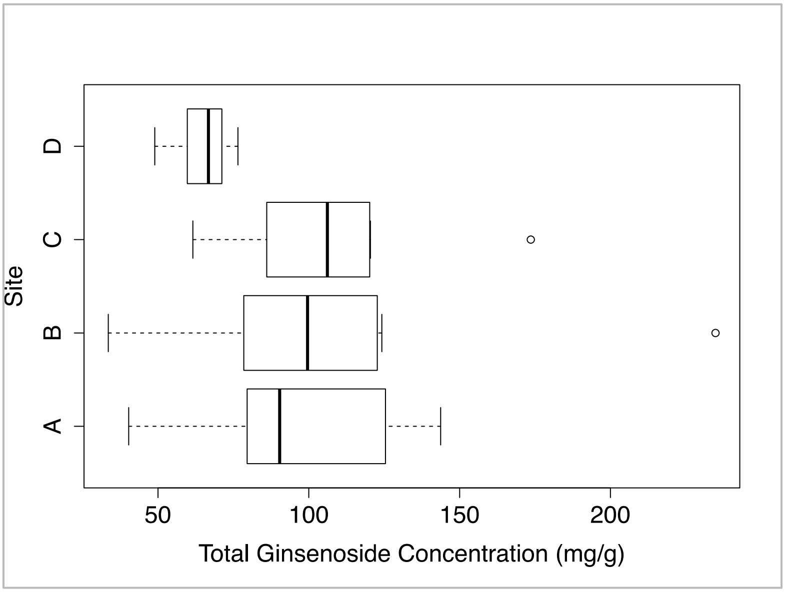

Fig 5. Mean (n = 8) and distribution of total root ginsenoside concentrations (mg/g) for each site.

Table 3. Mean values for total ginsenoside concentration, and percent of root mass represented by ginsenosides (± STDEV)

Site	Percent Ginsenoside Content (%)
A	9.68 (± 3.34)
B	10.87 (± 5.91)
C	10.75 (± 4.31)
D	6.51 (± 0.88)

One-way ANOVA's were also used to compare the levels of individual ginsenosides present in roots across sites, and determined that the amount of ginsenosides Rg1 ($p = 0.002$) and Re ($p = 0.006$) were significantly different across sites, with higher amounts of Rg1 present in roots from sites A and B, and higher amounts of Re present in roots from sites C and D.

Two-way ANOVA was used to test for an interactive effect of root mass and site on ginsenoside concentrations. The results indicated that total ginsenoside concentrations varied significantly with root mass ($p = 0.031$), but there was no interactive effect of root mass and site ($p = 0.930$). The relationship between ginsenoside concentrations and root mass are illustrated in Figure 6. Regression analyses support this finding, and suggest that approximately 12.46% of the variation in ginsenoside concentrations can be explained by root mass (p = 0.0269). When examined individually, no significant relationships between individual ginsenosides and root mass were detected.

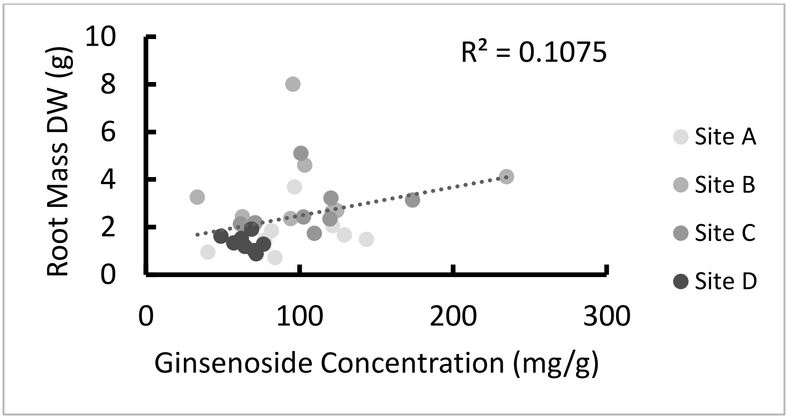

Fig 6. Positive correlation (p <0.05) between root mass (g) and ginsenoside concentrations (mg/g).

Two-way ANOVA was also used to test for interactive effects of AMF colonization and sites on ginsenosides. There was no significant interactive effect of TPC (p = 0.56706) or PAC (p = 0.4471) and there were no significant main effects of TPC (p = 0.0823) or PAC (p = 0.182) on ginsenosides, although regression analyses suggest that TPC may have a minor effect on ginsenoside concentrations (p = 0.1058), with approximately 5.4% of the variation in explained by TPC.

When examined individually, Spearman's rank order correlation test showed that Rg1 was inversely correlated with TPC (p = 9.826e-05, rho = -0.6338) (Figure 7), while Re was positively correlated with TPC (p = 0.007, rho = 0.4691) (Figure 8).

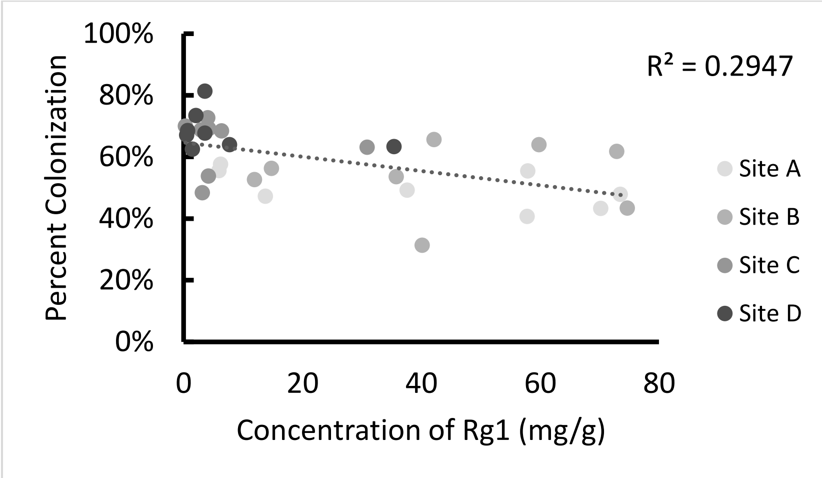

Fig 7. Inverse relationship ($p < 0.05$) between total percent colonization (TPC) and concentrations of Rg1 (mg/g).

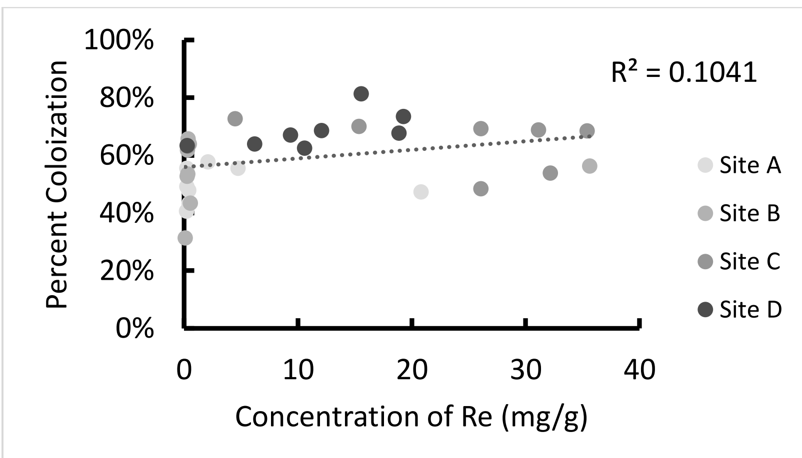

Fig 8. Positive correlation ($p < 0.05$) between total percent colonization (TPC) and concentrations of Re (mg/g).

AMF Species Identification and Mean Infectivity Potential

Rhizophagus intraradices (formerly *Glomus intraradices*) was the only species of AMF identified across all four production sites. AMF spores extracted from soil samples were heavily degraded, thus limiting the ability to identify species based on morphological characteristics. Mean infectivity analyses showed no mycorrhizal colonization in the root systems of trap plants after 21 days of growth.

Discussion

The colonization of WS ginseng roots by AMF were determined to be significantly different between production sites, with greater PAC and TPC observed in sites that are less intensively managed, suggesting that AMF may be influenced by management interventions in WS production. Measurements of PAC in WS roots (6.70%-15.86%) were lower than values previously reported by Whitebread et al. (1996) and McGonigle et al. (1999), which ranged from 23% to 57% in one to three-year-old field-cultivated roots. Hyphal colonization in WS roots (48%-66%) was substantially higher than values reported by Whitebread et al. (1996) and McGonigle et al. (1999), which ranged from 8%-33% in field-cultivated roots.

The determination of significant differences in total ginsenoside concentrations between sites is supported by findings previously reported by Lim et al. (2005) and Schlag and McIntosh (2006). Differences observed in concentrations of Rg1 and Re between production sites were previously reported by Schlag and McIntosh (2006), who suggest that chemotypic differences are likely attributed to plant genotype (e.g. seed source). This is supported by differences in seed origin between production sites utilized in this study. Among individual ginsenosides, Re was the only ginsenoside determined to be positively correlated with TPC. The correlation between Re and mycorrhizal colonization is supported by the results of Fournier et al. (2003), who also determined that Re is significantly influenced by mycorrhizal colonization. Total ginsenoside concentrations measured in WS roots during this study (6.51% - 10.87% of root mass) were consistent with previously reported values. Li and Fitzloff (2002) determined that commercially available ginseng powders and capsules contained between 5.1% and 10.9% ginsenosides by weight. Concentrations measured in WS roots were higher than those previously reported for field-cultivated (Court et al.,1996; Li et al., 1996) and young wild roots (Assinewe et al., 2003), with concentrations ranging between 3.0% and 5.0% of total root mass.

Differences in total ginsenoside concentrations were determined to not be based on differences in mycorrhizal colonization, plant genotype/chemotype (*e.g.* Rg1/Re ratios), or management intensity (e.g. low vs. high-intensity). Rather, ginsenoside concentrations were determined to be more significantly influenced by root mass, with the highest concentrations observed in sites B and C where the highest mean root masses were recorded. The relationship between root mass and ginsenoside concentrations is supported by results previously reported by Smith et al. (1996) and Court et al. (1996). Additionally, root mass was not shown to be significantly influenced by the extent of AMF colonization in

WS roots, with results actually indicating an inverse relationship between root mass and PAC, suggesting that smaller ginseng roots may rely more heavily on fungal partnerships for nutrient acquisition and resource foraging. These results differ from those previously reported by Li (1995), which showed that inoculated one and two-year old roots had higher root masses compared to un-inoculated controls.

The identification of *Rhizophagus intraradices*, although limited in scope, provides new insight into the AMF associates of American ginseng in WS production. No previous reports of AMF associates in wild or WS American ginseng were identified during the course of this study. *Glomus intraradices* (e.g. *R. intraradices*) was previously identified by Seok-Cho et al. (2007) in field-cultivated Korean ginseng roots, and has also been used as an inoculant in studies conducted by Fournier et al. (2003) and Li (1995). The use of more advanced identification techniques, such as DNA analysis, is recommended to help resolve the identify of fungal partners associating with American ginseng in forested production sites.

In conclusion, the results of this study may further underscore the importance of site selection and habitat quality as key factors contributing to the success of WS ginseng production. Production sites with opposing production practices and management intensity, varying levels of mycorrhizal colonization, and with differing plant genotypes and chemotypes, were observed to produce roots with the highest ginsenoside concentrations, as well as being the most productive based on root mass. Although the results of this study provide new insights regarding ginseng-mycorrhizae interactions in forest-based production systems, additional research is needed to better resolve these relationships, particularly in regards to identification of mycorrhizal associates of American ginseng, and how different AMF species may influence root development and plant phytochemistry.

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“NatureServe and Native Plant Conservation in North America”

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Abstract

The conservation of native plants, especially those experiencing threats and population declines, is dependent on accurate information about each species location, population health, and protection needs. In cooperation with Natural Heritage Programs in each U.S. state, Canadian Conservation Data Centres, and other collaborators, NatureServe uses a long-standing, standardized, and vetted methodology to evaluate each plant species for its risk of imperilment and conservation priorities. To carryout species-specific assessments at the state/provincial, national, and global levels, the Botany Department develops and maintains the taxonomic, geographic, ecological, and conservation data that determine priorities that support the protection and management of the rarest and most vulnerable plant species. Information on species native to the U.S. and Canada can be accessed on NatureServe Explorer (explorer.natureserve.org).

Keywords: Plant Conservation, Status Assessments, Extinction Risk

Life on Earth depends on plants: they are the foundations of ecosystems and important habitats, and critical sources of food, oxygen, and medicine. Globally, about one in five plants is estimated to be at risk of extinction (Kew 2016). In the United States and Canada, more than 30% of vascular plant species are currently vulnerable to extinction (NatureServe 2017). Plants continue to face threats such as habitat loss and degradation, invasive species, overpopulation, and climate change (Kew 2017, Hernández-Yáñez et al. 2016). Without an increased focus on plant conservation, we risk losing plant diversity.

Despite their importance, and ongoing decline, plants are consistently underrepresented—and sometimes completely absent—in conservation plans and associated funding streams. For example, even though nearly 60% of species on the US Endangered Species List are plants, they consistently receive less than 5% of State and Federal funding (Negron-Ortiz 2014). At the state level, species conservation is often implemented through State Wildlife Action Plans, which establish a framework for protecting species before they become endangered (Stein and Gravuer 2008). However, only 15 U.S. states and territories have included plants in their lists of Species of Greatest Conservation Need, and were prioritized using conservation tools.

One tool used to prioritize plant conservation efforts is the conservation status assessment, which evaluates a species' relative risk of extinction globally or of extirpation locally (Collen et al. 2016, Master 1991). Because of the recognized importance of status assessments to conservation, several international policy initiatives and strategies include status assessments as part of their strategic goals. For example, Target 2 of the Convention on Biological Diversity's Global Strategy for Plant Conservation calls for "an assessment of the conservation status of all known plant species, as far as possible, to guide conservation action" by 2020 (Convention on Biological Diversity 2012). Similarly, the North American Botanic Garden Strategy for Plant Conservation calls on botanic gardens to review and contribute to conservation status assessments of plants using criteria and standards developed by NatureServe and the IUCN (BGCI 2016).

The two most widely used platforms for assessing conservation status of species in North America are NatureServe's Conservation Status Assessments and the IUCN Red List. NatureServe's conservation status assessments, or ranks, developed independently from the IUCN Red List and other conservation status assessments. NatureServe is a non-profit that provides scientific information, expertise, and information technology tools that connect science with conservation action. The NatureServe Network is a public-private partnership that includes more than 80 independent member programs, commonly known as Natural Heritage Programs or Conservation Data Centres. NatureServe works collaboratively with the Natural Heritage Network to provide conservation information on rare plants, animals, and ecosystems in the Western Hemisphere. This partnership allows NatureServe to work cooperatively and efficiently with all jurisdictions in North American to assess the conservation status of species facing emerging threats.

NatureServe's Botany Department compiles and maintains extensive data on the taxonomy, distribution and conservation status of plants and selected fungi, lichens, and algae of the United States and Canada, with a focus on species that are most imperiled. Using a shared data structure, NatureServe network member programs collect and manage information on the location and conservation status of taxa in their jurisdictions. NatureServe Explorer (explorer.natureserve.org), an online encyclopedia, provides detailed information on more than 65,000 plants, animals, and ecosystems of the United States and Canada from our collective, multijurisdictional database.

Species information on NatureServe Explorer includes NatureServe's Conservation Status Assessments, or Ranks. These Ranks evaluate the potential extinction or extirpation risk of a species by systematically analyzing factors grouped into three factor groups: rarity, threats and trends (Faber-Langendoen et al., 2012; Master et al., 2012). Ranks are completed at three nested, geographic scales: Global (G), National (N), or Subnational (S). By indicating species imperilment at multiple scales, governments are better able to allocate resources for the most imperiled species in their respective jurisdictions while considering a species overall risk of extinction (Faber-Langendoen et al. 2012). For example, comparing the Subnational (state or provincial rank), or Sranks, to the Global, or Granks, provides information on jurisdictions within a species range that may be more vulnerable than others (Figure 1).

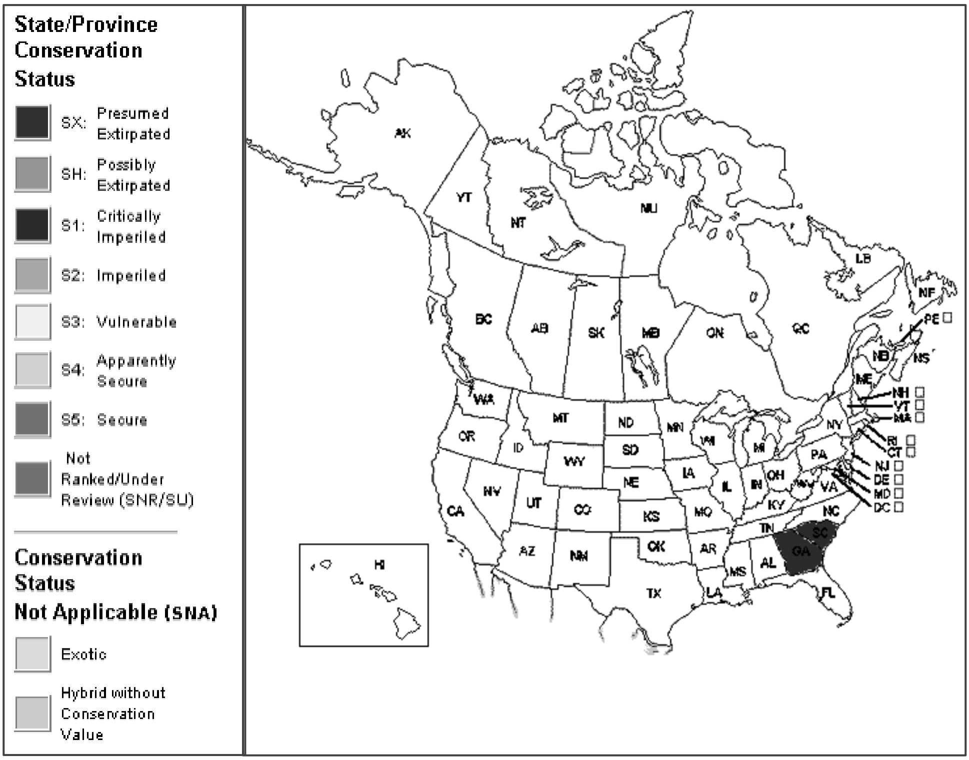

Figure 1. Distribution and Subnational Rank of *Trillium persistens*, a narrow endemic that is Critically Imperiled (S1) in Georgia and South Carolina, Critically Imperiled-Imperiled in the United States (N1N2) and Globally (G1G2). Data extracted from NatureServe Explorer (2017).

Species and infraspecific taxa (varieties and subspecies) are ranked from most to least imperiled on a scale of 1-5 (Table 1). NatureServe Global Ranks also include GX (Presumed Extinct) and GH (Possibly Extinct). Uncertainty in a global rank is expressed through variant ranks, rank qualifiers, and range ranks, such as the G1G2 in the *Trillium persistens* example (Table 1). Taxa with questionable taxonomy that would affect the conservation rank have a rank qualifier of "Q".

Table 1: Definitions of NatureServe's Global Ranks, including Variant Ranks and Rank Qualifiers

Global (G) Rank	**Definition**
GX	Presumed Extinct — Species not located despite intensive searches and virtually no likelihood of rediscovery.
GH	Possibly Extinct — Known from only historical occurrences but still some hope of rediscovery. There is evidence that the species may be extinct, but not enough to state this with certainty.
G1	Critically Imperiled—At very high risk of extinction due to extreme rarity (often 5 or fewer populations), very steep declines, or other factors.
G2	Imperiled—At high risk of extinction or elimination due to very restricted range, very few populations, steep declines, or other factors.
G3	Vulnerable—At moderate risk of extinction or elimination due to a restricted range, relatively few populations, recent and widespread declines, or other factors.
G4	Apparently Secure—Uncommon but not rare; some cause for long-term concern due to declines or other factors.
G5	Secure—Common; widespread and abundant.
Variant Global Ranks	**Definition**
G#G#	Range Rank — A numeric range rank (e.g., G2G3, G1G3) used to indicate uncertainty about the exact status of a taxon.
GU	Unrankable — Currently unrankable due to lack of information or due to substantially conflicting information about status or trends.
GNR	Unranked – Global rank not yet assessed.
GNA	Not Applicable — A conservation status rank is not applicable because the species is not a suitable target for conservation activities.
Rank Qualifiers	**Definition**
?	Inexact Numeric Rank — Denotes inexact numeric rank; this

	should not be used with any of the Variant Global Conservation Status Ranks or GX or GH.
Q	Questionable taxonomy that may reduce conservation priority— Distinctiveness of this entity as a taxon at the current level is questionable; resolution of this uncertainty may result in change from a species to a subspecies or hybrid, or inclusion of this taxon or type in another taxon or type, with the resulting taxon having a lower-priority (numerically higher) conservation.
C	Captive or Cultivated Only —At present presumed or possibly extinct in the wild across entire native range but extant in cultivation, in captivity, as naturalized populations outside their native range, or as a reintroduced population, not yet established. Possible ranks are GXC or GHC.

Conservation status assessments are completed by scoring up to ten rank factors categorized into rarity, threats, and trends (Figure 2). The rank factors are summarized in Master et al. (2012), while the methodology for assigning ranks is detailed in Faber-Langendoen et al. (2012). The Conservation Rank Calculator is an automated tool in Microsoft Excel that ensures the accurate application of the ranking methodology. Use of the tool improves the reliability, transparency, and consistency of the ranking process by applying an algorithm to implement the standard methodology. NatureServe Network's methodology for assigning conservation status is intended be transparent, consistent, rigorous, and scientific. The rank factors and rank reasons are behind each assessment are freely available on NatureServe Explorer (explorer.natureserve.org). For example, the reasons given for *Trillium persistens*' Global Rank of G1G2 are that it is a narrow endemic, known from a single drainage straddling the border of Georgia and western South Carolina. A large, contiguous population formerly extended along the river banks before major dams and reservoirs inundated former habitat and fragmented the range. The species account also includes information on the number of occurrences, population size, threats, and trends. In addition to gathering data necessary to assign a conservation status Rank, NatureServe also compiles information on the taxonomy, ecology, life history, management, and economic attributes of a species. This information is also freely available on NatureServe Explorer.

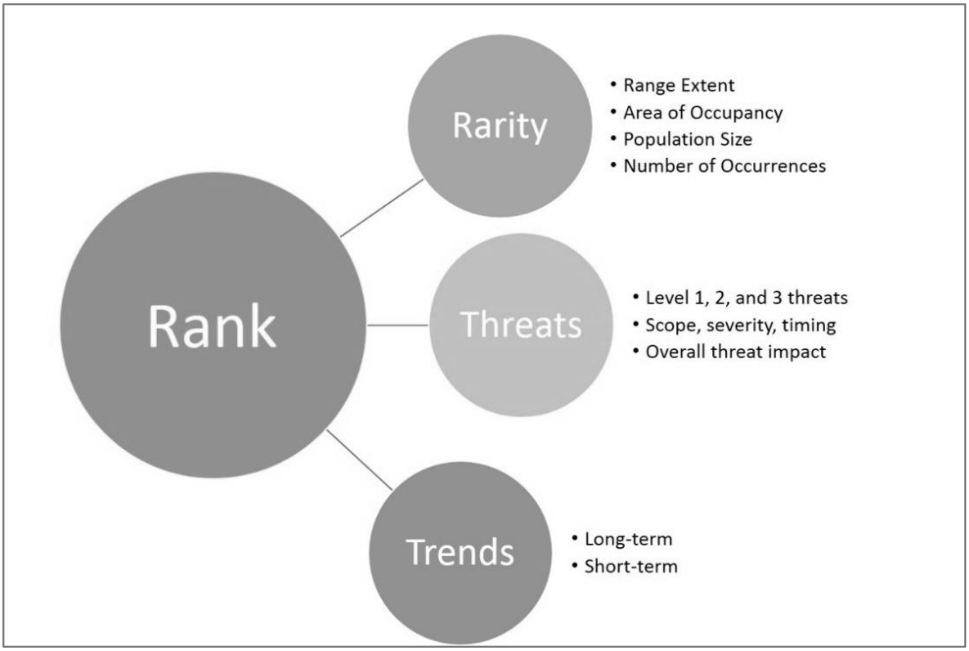

Figure 2. The three factor groups (rarity, threats, trends) and selected factors that are used to calculate the NatureServe's Conservation Status Rank (Faber-Langendoen et al. 2012; Master et al. 2012). See also http://www.natureserve.org/conservation-tools/conservation-status-assessment

The conservation of North American plants, including medicinal plants, is a core part of NatureServe's mission. Conservation status assessments provide integral information to guide conservation actions. NatureServe partners with other organizations like the IUCN and United Plant Savers to provide the most current information on medicinal plants and forest botanicals; this information is freely available on NatureServe Explorer. With increased promotion of conservation status assessments, NatureServe and its partners will continue to raise awareness of the need to conserve medicinal plants and their habitats.

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“Supply and Regulation of Wild American Ginseng”

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Abstract

American ginseng (*Panax quinquefolius*) root is highly valued as traditional medicine in Asia. High harvest levels for the export market led to concerns about the long-term sustainability of the plant which resulted in many states and the federal government implementing harvest regulations. Such regulations have the potential to affect income generation in rural communities in Appalachia and the Ohio valley. Using harvest data from the US Fish and Wildlife Service and price data, we estimated wild ginseng root economic supply and demand models that identify how quantity supplied responds to price, how economic crises affect harvests, and how regulations affect quantities supplied. We found evidence that quantities supplied are related negatively to price over a portion of the long-run supply curve, indicating that increasing harvest pressure in the short-run may be reducing inventories and reducing production possibilities in the long run. This finding is similar to open access resources such as fisheries in international waters. We uncovered limited evidence that increases in local unemployment rates increased harvest. Further, our analysis reveals that federal regulation banning exports of roots from plants under 5 years old has led to a shift in the long-run supply curve. This result could be due to the slow natural rate of population recovery from harvesting. We discuss implications of the shape of the supply curve for conservation and regulation.

“Indications for the Importance of Growing Methods on Pharmacological profiles of Herbal Medicines”

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Abstract

This presentation originated as a naturopathic doctorate thesis reviewing examining the research on the effects of growing conditions on qualities and quantities of constituent in medical herbs. Herb based medicines form a large portion of the *materia medica* and therapies currently used in Naturopathic practice and other alternative Medical practices. The international export value of pharmaceutical plants alone was estimated around $2.2 billion per year in 2011 (TRAFFIC). An estimated 50,000–70,000 medicinal and aromatic species are harvested from the wild (Schippmann, *et al*. 2006). This raises serious environmental and future supply concerns as the industry continues to grow. Some herbs valued for their medical properties are quite difficult to produce agriculturally and are usually wildcrafted. Other plants are wildcrafted because practitioners of many traditional medicine system regard the natural plants as more potent. There appears to be little research comparing the effects of growing herbs in their natural environments versus otherwise. However, there may in many cases be validity to regarding wildcrafted plants as more potent. Specifically, many of the medically effective compounds are secondary metabolites, which fluctuate in response to predation and other environmental stressors. This suggest that further research comparing growing methods effects on herbal constituents may be helpful in determining, which methods of growing medical herbs.

Keywords: naturopathy, herbs, secondary metabolites, constituents, wildcrafting

Introduction

One of the major modalities used for treating patients in Naturopathic medicine and other alternative medicine practices is through herbal medicine and phyto-pharmaceutical compounds. In recent years, increased consumer interest in plant-based medicine has increased demand for medical herbs. This has both increased opportunities in for those involved in the growth, collection, and processing of these plants as well as concerns about their availability and status in the wild. Additionally, with modern transportation, previously indigenous herbal medicines have become popularized worldwide, with 50,000-70,000 species used for medicine and cosmetics worldwide (Schippmann, *et al*. 2006). These plants can be threatened not only by over-collection, but by habitat destruction and disruption through deforestation, development, invasive plant species, overgrazing, and climate change. As early as 1930 the USDA commented on concerns that the increases in agriculture had led to a decrease in supply of medical herbs due to loss of habitat (USDA, 1930).

As medical herb growing methods change due to increased demand, it can alter the quality of the final product. From an economic point of view, research on growing methods for a plant may be concerned only with amount of final product, such as the study showing *Calendula officinalis* L. (Asteraceae) inflorescences size with changes in hydroponic nutrition (Stewart, Lovett-Doust, 2003). However, larger size doesn't necessary indicate a better therapeutic effect. In fact, one study showed that *Sanguinaria canadensis* L. (Papaveraceae) rhizomes were larger in cultivated plants, but contained lower levels of active constituents: sanguinarine and chelerythrine (Graf *et al*. 2007). Similarly, some cultures believe that plants that appear more standardized are less useful in medicine, as shown by the demand in China for wild-appearing ginseng, *Panax quinquefolius* L. (Araliaceae) radix, due to the belief that it has more of the active constituents, ginsenosides (Teel, Buck, 1998).

It is estimated by the World Health Organization that 80% of people internationally rely on herbs for primary healthcare (Bodeker et al., 2005). While herbal medicine is a growth industry, it is very fragmented, uses a wide variety of plants from many regions, and doesn't yet have the organization or resources to sponsor high levels of research. However, many researchers in fields such as botany, biochemistry, pharmacology, genetics, ethnobotany, and food chemistry have contributed individual studies.

Due to the scarcity of literature comparing wildcrafting vs cultivation, this paper reviews the chemical classes of medically active constituents and their biosynthesis, literature on any effects of growing conditions on these constituents, and finally different strategies for conservation.

Methods: Procedure of Literature Review

Databases searched included Google Scholar, Pubmed, and myEureka between March 10th and April 1st, 2014. Years searched were not limited. Search terms used included: (wildcrafting + constituents), (native + constituents), (agriculture, cultivation + native, wildcrafting). The first 20-30 hits for each were reviewed until the relevance seemed to have dropped. The initial search results were poor. A second set of searches using (secondary metabolite + wildcrafting, native, herb, cultivation) was more fruitful. The first 100 results on each database were checked for potential articles. Inclusion and exclusion criteria included whether or not full text was available, whether the article was a duplicate search result, whether there was comparison between different growing techniques or the article focused on environmental effects on secondary metabolites, with emphasis on any articles containing information about quantities of constituents. Articles that focused exclusively on molecular biology in model organisms to elucidate pathways were also excluded.

Using google scholar, 80 papers were deemed appropriate for review and 30 articles were downloaded and reviewed. Using myEureka, 63 articles were deemed appropriate for review and 36 articles were downloaded with 10 duplicates. In Pubmed, exclusion was easier; only 36 articles were reviewed with 27 downloaded.

Background

History of Herbal Medicine

It is important to understand the contexts of herbal medicine that has been adapted by current Naturopathic medicine. Naturopathic herbal medicine is primarily derived from American 19th century medicine's use of medical herbs and modern phyto-therapeutic research, although other traditional herbal medicines such as Native American, Chinese herbal, Ayurvedic, and other cultural/ethnic groups' herbal medical traditions have also contributed to the development of current Naturopathic materia medica. Advances in chemistry since the 1930's and increased interest in the pharmaceutical qualities of individual constituents have increased the use of constituents either extracted from herbs or produced from cultures, leading to constituents based supplements, which does not represent the complete medical profile of the herb.

American 19th century medicine combined knowledge of herbs used by the European medical tradition and the knowledge shared by Native Americans available at the time through: education, oral traditions, writing, and clinical experience with patients. Even through the knowledge of medical herbs was not based on large standardized studies, it was not without scientific and empirical methods. Research was based on experiments and evaluation of individual cases/experiences to determine how well a medicine worked. Plant extracts were used for medicine teas, decoctions, tinctures, oils, gums, juices, and salts.

Many of the comprehensive texts written on medical herbs between the late 1800's and the 1930's are still in use today, and they describe not only herbs used, what they are used for and how they are processed, but also where they are obtained in nature. The 1892 *Millspaugh's Medical Plants* describes 180 herbs with basic classes of chemical constituents, listings in the USP and a description of both processing and the final processed drug, while 1907 Potter's *New*

Cyclopaedia of Botanical Drugs and Preparations gives dosage and formulation used but less often a description of preparations (Millspaugh, 1974; Wren, 1975).

While these books provided physical descriptions of both the plant and its natural location, they did not describe methods of cultivation, since the majority of medical herbs were either widely available garden plants or collected from the wild (Millspaugh, 1974; Wren, 1975). In addition to descriptions and locations of plants in herbals, as late as 1966 the USDA published collection guides for the crude drug trade as a way of stimulating economic activity (Cavender, 2006), which provided locations, names, parts used, descriptions, and estimates of the demand for these herbs (USDA, 1930).

For over 150 years, one of the primary sources of medical herbs for pharmaceutical and other industries has been south central Appalachia, which includes western North Carolina, southeastern Virginia and eastern Tennessee and has around 1,100 species of plants with reported medical uses out of an estimated 2,500 total plant species, including native species such as, ginseng (*P. quinquefolius)*, goldenseal (*Hydrastis canadensis* L.) (Ranunculaceae), bloodroot (*S. canadensis)*, and black cohosh (*Cimicifuga racemosa* [L.] Nutt.) (Ranunculaceae) (Cavender, 2006). Interestingly, a study of the folk medicine of Appalachia shows that since at least the 1930's, these peoples' materia medica has focused on store-bought and gardened produce foods such as garlic and potatoes, suggesting that the collection of wildcrafted herbs was more for export profit than personal home use, and the author suggests this may be due to difficulty of identification, collection, and lack of seasonal availability (Cavender, 2006).

Modern Phytotherapies

In contrast to using traditional herbal medicine, phytopharmaceuticals use isolated plant constituents or herbal extracts standardized to one or two key constituent concentrations. These are usually based on modern research looking to confirm the activity described in traditional use or previous studies. These studies often focus on either showing the constituent can induce a specified activity *in vivo* or to elaborate the mechanisms of action *in vitro*. This research not only provides ideas for drug development, but evidence for the effectiveness of medical herbs and novel herbal treatments. The advantages of using isolated constituents or standardized products is that the dosage can be more standardized, growing conditions are not of therapeutic concern, and alternative plant sources (other species, or other parts) and culture-produced chemicals can be used.

The disadvantage of using isolated constituents or standardizing to only one or two constituents is that many medical herbs contain a multitude of active constituents, which may use multiple molecular pathways and reduce side effects. Sometimes, the traditional use of the whole herb or specific structures of the herb (ie. folium, flora, semen, radix) can be more clinically useful than isolated constituents. As in studies with Feverfew, *Tanacetum parthenium* L. (Asteraceae), attempts to use an isolated constituent responsible for its action can lead to poor effectiveness.

Phytochemistry of Medicinal Herbs

The constituents of an herb refer to all the chemical compounds normally found in the plant. This clearly varies by the solvent used for extraction (water, alcohol, oil, glycerin, CO_2, vinegar) and part of the plant used (bark, root, flower, leaves, young shoots, seeds). Since plants are dynamic living organisms, concentrations and even presence of these constituents can also vary by age, time of year, soil minerals, and sun exposure. What may not be obvious for those not versed in plant sciences is that factors such as nearby plants, altitude, microbes, and nearby animals may in some cases be factors as well. When Chinese herbal medicine calls for the plant to be found on a specific mountain, or Cherokee medicine calls for the plant to have companions (other specific plants nearby), or European herbalism calls for the plant to be harvested in a specific manner, these can affect the chemical profile of the herb.

The reason that many medically active constituents are so easily affected by the environment is that the majority of them are what is known as secondary metabolites. Primary metabolites are chemicals found in plants used for structural, growth, and other metabolic purposes. These are necessary for the plant to maintain itself even under ideal conditions. These include sugars and carbohydrates used in structure, transportation and metabolism, lipids used in cell membranes or storage (in seeds), and nucleic acids and proteins uses in information storage and enzymes (Harborne, Baxter 1995). The distinction between primary and secondary metabolites is not always clear, for instance plant growth hormones are usually described as primary metabolites but they also belong to chemical classes usually classified as secondary metabolites (Harborne, Baxter 1995).

Often isolated to only a few families or even species of plants, secondary metabolites are usually the medically active or toxic constituents and are less ubiquitous and more varied than primary metabolites. They are usually not constitutive and have “no direct function in growth and development” (Buchanan *et al.* 2000). They are thought to be of use in plants to protect against herbivory and infection, act as attractants, and to serve in plant to plant interactions, called allelopathy (Buchanan *et al.* 2000). The three major groupings of phytochemicals that have medical benefits are mostly composed of secondary metabolites: nitrogen containing compounds; phenolics; and terpenes (Harborne, Baxter 1995; Buchanan *et al.* 2000).

Nitrogen Containing Compounds

There are over 15,000 know nitrogen-containing compounds synthesized in plants (Harborne, Baxter 1995). This includes the diverse group of over 10,000 alkaloids, the majority of nitrogen-containing compounds found in plants, which require nitrogen, but are not all formed via the same amino acid pathway (Harborne, Baxter 1995; Buchanan *et al.* 2000). Alkaloids are found in 20% of plant species making up 0.1-12% of dry weight of the plant and can be produced by fungal symbionts or the plant itself (Harborne, Baxter 1995; Buchanan *et al.* 2000). Alkaloids can act as herbivory deterrents, nitrogen storage, toxins to vertebrates, and have been shown to increase with initial damage to the plant (Buchanan *et al.* 2000).

Isoquinolines make up the largest group of alkaloids, are derived from Tyrosine and Phenylalanine precursors, and contain many medically active compounds such as morphine and papaverine (Harborne, Baxter 1995). Over 1,200 indole alkaloids have been identified including toxic species and medically interesting species such as reserpine and ergotamine (Harborne, Baxter 1995). Pyrrolizidine alkaloids are a diverse group of secondary compounds, thought to reduce herbivory through deterrent, repellent, or toxic effects on a wide range of generalist herbivores (Joosten, vanVeen 2011). Pyrrolidine and piperidine alkaloids are found mostly in the family Solanaceae and include nicotine (Harborne, Baxter 1995). Quinoline alkaloids are found in the family Rutaceae and a few others and several have shown pharmacological activity. Quinolizidine alkaloids found in the Fabaceae family have antiherbivory effects and potential medical uses (Harborne, Baxter 1995). Steroidal alkaloids are formed from triterpenoid (GDP) synthesis, found in the families Solanaceae, Apocynaceae and two other families, with members of this group having been found to have antihypertensive and other medical qualities (Harborne, Baxter 1995). Tropane alkaloids are found mostly in the families Solanaceae and Erythroxylaceae (Coca family) and to a lesser extent in 8 other families and have both toxic and medicinal compounds (Harborne, Baxter 1995).

Cyanogenic Glycosides and Glucosinolates are synthesized from amino acid precursors, often vary by plant population, and are bitter and/or toxic (Harborne, Baxter 1995). Both of these groups can serve a similar function; when plant tissue is crushed, glycosidase or thioglucosidase (respectively) cleaves the sugar from these compounds, releasing unpleasant aromatic cyanide or sulfur compounds that can be used as feeding deterrent (Buchanan *et al.* 2000). Cyanogenic glycosides are often bitter and toxic (Harborne, Baxter 1995). Glucosinolates compromise over 10,000 known compounds, have an acrid taste and are found mostly in the Brassicaceae family and others of the order Capparales (Harborne, Baxter 1995; Zenk, Juenger 2007).

Many of the other nitrogen-containing compounds are primary metabolites, such as, protein building amino acids, nucleic acids, and proteins used by the plants

(Harborne, Baxter 1995). As deterrents, nonprotein amino acids, a group of 250 compounds, can be toxic in animals and are stored for protection in seeds (Harborne, Baxter 1995, Zenk, Juenger 2007).

Phenolic Compounds

There are over 10,000 known phenolic compounds found in plants, with nearly half of these being flavonoids (Harborne, Baxter 1995; Buchanan *et al.* 2000). Almost all phytochemicals classified as phenolics are derived from the Shikimate pathway via aromatic amino acids (Buchanan *et al.* 2000; Ribereau-Gayon 1972).

Flavonoid synthesis diverge from phenylpropanoids using chalcone synthase to form its precursors (Buchanan *et al.* 2000). Flavonoids have limited distribution throughout the plant kingdom and have been used medicinally due to their antioxidants, antiinflammatory, antimicrobial, free radical scavenging and metal chelating properties (Harborne, Baxter 1995; Perez *et al*. 2014). Flavones and flavonols are sometimes used as pigments and feeding attractants (Harborne, Baxter 1995). They include many medicinally active and antiinflammatory compounds such as: kaempferol, quercetin, myricetin, apigen and luteolin (Harborne, Baxter 1995). Almost exclusive to Fabaceae, isoflavonoids have over 600 recognized compounds, many of which are defensive compounds and beneficial to human health (Broeckling *et al*. 2005).

Phenylpropanoids are initially formed via the same pathway as flavonoids until they diverge at the enzyme, phenylalanine ammonia-lyase (PAL). PAL has been shown to be induced by UV light exposure, which is consistent with the functions of these compounds including: free radical scavenging, vascularization, pigmentation, phytoalexins, UV protectant, and signaling molecules (Broeckling *et al*. 2005; Hamberger, Bak 2013). Phenylpropanoids also contribute to flavor and aroma profiles, are insect deterrents, and have antimicrobial and antibiotic along with of medicinal uses (Harborne, Baxter 1995).

Phenolic acids can be used as primary metabolites in cell wall structures (lignin) or can be used as secondary metabolites such as THC, which is neuroactive in mammals or urushiol, which causes contact dermatitis (Harborne, Baxter 1995). Over 700 coumarins are known with members showing allergenic, insecticidal, blood thinning, antibacterial, brachycardic, and antitumor activities (Harborne, Baxter 1995).

Over 200 lignan compounds have been identified with most members found in the wood where they provide insecticidal properties (Harborne, Baxter 1995). Several extractable lignans have shown medical properties, such as, antiviral, antitumor, and antihepatotoxic properties. Stilbenoids can be fungal resistant and are often found in woody materials or glycosylated (Harborne, Baxter 1995). Tannins have astringent properties, which can be unpalatable but have beneficial properties in wound and burn healing (Harborne, Baxter 1995).

Terpenoid Compounds

Terpenes are the largest class of secondary metabolites with over 20,000 known compounds and have a more diverse range of uses in plants including: membranes components, defense compounds, phytohormones, and signaling molecules (Buchanan *et al.* 2000; Hamberger, Bak 2013). Terpenes are formed by geranylgeranyl diphosphate pathway using five carbon isoprene units. Terpenes are hydrophobic compounds forming important constituents of essential oils (Harborne, Baxter 1995; Buchanan *et al.* 2000). Most terpenoids are secondary metabolites, but the primary metabolites – gibberellins, sterols, and carotenoids – are used as phytohormones (gibberellins and abscisic acid), membrane components, and accessory photosynthetic pigments respectively (Buchanan *et al.* 2000; Zerbe *et al.* 2013).

There are over 600 identified monoterpenes (2 isoprene unit compounds) from plants with most being found in the essential oil and contributing to the aroma (Harborne, Baxter 1995). Many of these, like thymol, also have antiseptic and antiinflammatory properties (Harborne, Baxter 1995). Iridoids are a bitter tasting subgroup of monoterpenes, which are usually found in a glycosylated form acting as a feeding deterrent (Harborne, Baxter 1995). Iridoids also have medicinal uses as tonic bitters, are antimicrobial and anti-inflammatory, but some members are toxic (Harborne, Baxter 1995).

Over 10,000 different diterpenes (4 isoprene compounds) have been identified in plants; most are secondary metabolites but it also includes the primary metabolites gibberellins, a class of plant hormone, which consists of over 71 compounds (Harborne, Baxter 1995; Zerbe *et al.* 2013). These are normally hydrophobic and can be aromatic, so they are rarely glycosylated, although stevioside triglucoside is a noted exception (Harborne, Baxter 1995). Although many diterpenes are toxic, several are of great use to the pharmaceutical industry such as: taxol, a chemotherapeutic agent from Taxus sp.; forskolin, a vasodilator from Coleus forskohlii (Lamiaceae), and marrubiin, an analgesic and antidiabetic drug candidate from Marrubium sp. (Lamiaceae); to name just a few (Zerbe *et al.* 2013). The annual market value of diterpenes alone discovered from plants is in the billions of dollars from pharmaceutical, fragrance, herbal, and other industries (Zerbe *et al.* 2013).

Triterpenes derivatives include cardenolides, bufadienolides, and saponins. Cardenolides and bufadienolides, including digoxin, are known for their cardiac effects on vertebrates (Harborne, Baxter 1995; Buchanan *et al.* 2000). They are found in the families Apocynaceae, Asclepiadaceae, Moraceae, and Scrophulariaceae (Harborne, Baxter 1995).

Triterpene saponins are been found in over 100 families and have been shown to have defensive properties: antiherbivory, anti-nutritional, allelopathic effects, and are toxic to cold blooded animals, insects and mollusks (Harborne, Baxter 1995; Broeckling *et al*. 2005). They have pharmacological impact through their antimicrobial, antiinflammatory, hemolytic, anticholesterolemic, and cytotoxic activities (Harborne, Baxter 1995; Broeckling *et al*. 2005).

Steroidal saponins are found in the families Agavaceae, Dioscoreaceae, Scrophulariaceae, and Liliaceae (Harborne, Baxter 1995). Steroidal saponins have detergent properties, low human toxicity, can be used to stun fish, and include diosgenin, the precursor for synthetic progesterone (Harborne, Baxter 1995).

There are thousands of known sesquiterpenes, many of which are aromatic (Harborne, Baxter 1995). This class contains several biologically active secondary metabolites and the primary metabolite, abscisic acid, which acts to control plant growth (Harborne, Baxter 1995). Tetraterpenes, which are commonly referred to as carotenoids, are lipid soluble and used as accessory pigments in photosynthesis and as antioxidants and vitamin A precursors in animals (Harborne, Baxter 1995; Buchanan *et al.* 2000).

Synthesis Pathways

In order to understand how the environment can affect these chemicals, it is important to review their biosynthetic pathways. The Shikimate pathway (Figure 1) is the most well understood, perhaps because it is ubiquitous throughout the kingdom and is the origin of most phenolic compounds (Ribereau-Gayon 1972; Taiz, Zeiger 2002; Tohge *et al.* 2013). It produces the aromatic amino acids tryptophan, phenylalanine, and tyrosine, which are the initial components for phenol synthesis (Ribereau-Gayon 1972). In higher vascular plants phenylalanine is used predominantly as the precursor for phenolic compounds (Harborne, Baxter 1995). This pathway also provides precursors for chlorogenic acid, alkaloids, glucosinolates, auxin, tannins, suberin, tocopherols, and betalains (Tohge *et al.* 2013).

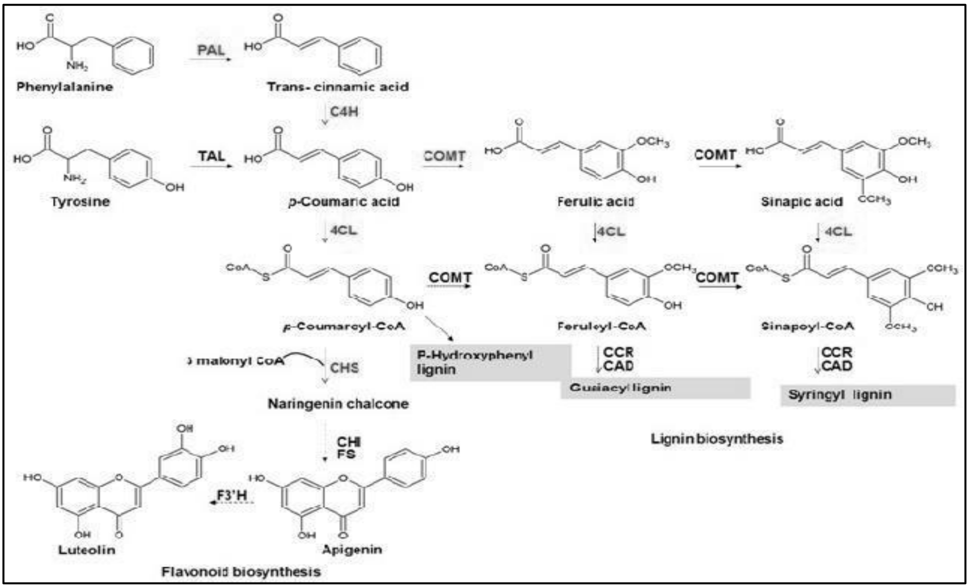

Figure 1. Shikimate pathway, showing the branching between Flavonoid and Phenylpropanoid biosynthesis

From phenylalanine, the metabolism can directed towards the synthesis of phenylpropanoids (coumarins, lignans) or towards flavonoids (Ribereau-Gayon 1972). The phenylpropanoid pathway is first catalyzed by Phenylalanine ammonia-lyase (PAL), which produces cinnamic acid (Ribereau-Gayon 1972; Taiz, Zeiger 2002; Docimo *et al.* 2013). Low nutrient levels, low light levels, and fungal infections have all been shown to increase the activity of Phenylalanine ammonia lyase (PAL), thus increasing synthesis of phenolic compounds based on cinnamic acid (Ribereau-Gayon 1972; Taiz, Zeiger 2002; Docimo *et al.* 2013). Whereas the enzymes in the Shikimate pathway are fairly conserved, the genes for this pathway vary across taxa and there can be variation between different tissues in the same plant, allowing for a great specificity of elicitors and products (Taiz, Zeiger 2002; Tohge *et al.* 2013). Chalcone synthase (CHS) catalyzes the first specific step towards flavonoids, and this pathway has been well elucidated (Docimo *et al.* 2013).

Phenylalanine ammonia-lyase (PAL), Cinnamic acid 4-hydroxylase (C4H) and 4-Coumarate: CoA ligase (4CL) catalyzes the first three steps of the general phenylpropanoid pathway whereas chalcone synthase (CHS) catalyzes the first specific step towards flavonoids (Docimo et al. 2013).

Because alkaloids do not have a uniform classification and are such a diverse group of compounds, only sharing in common that most contain a Nitrogen in a heterocyclic ring and that most compounds are basic, they are synthesized from a variety of pathways. Many may originate from amino acids, including those from the Shikimate pathway, while others originate from terpene synthesis.

Some of the basics of terpene synthesis are well understood, while all the enzymes responsible for individual compounds have yet to be identified (Broeckling *et al*. 2005). This pathway combines isoprene, a 5-carbon branched molecules, to form terpenes and is alternately known as the Isoprenyl pyrophosphate (IPP), the Geranyl pyrophosphate (GPP), or the Geranyl diphosphate (GDP) pathway (Taiz, Zeiger 2002).

Since many secondary metabolites are unique to one or two families or species, much attention is directed at the evolution of the biosynthetic pathways for these molecules. One group of enzymes (P450s) has numerous isotypes and is thought to be a key in specialized phytochemicals since they have been found to be involved in the synthesis of several separate types of secondary metabolites: cyanogenic glucosides, glucosinolates, terpenes (mono-triterpenes), and phenylpropanoids (Hamberger, Bak 2013). In *S. miltiorrhiza* multiple P450s are thought to be involved in catalyzing the unknown steps in phenolic acid and tanshinone synthesis (Luo *et al.* 2014).

Genetic and transcription data available to researchers allows for comparisons of metabolic pathways through transcription profiles to compare transcription sequences across species and families to look for potential links in evolutionary pathways. In one study the transcript profile of curcumin, *Curcuma longa* L. rhizome, was compared with those of other important terpenes: taxol, vinblastine, artemisinin, and acridone alkaloids (Annadurai *et al.* 2013). The authors were able to show that 25% of each of the different terpenes biosynthesis transcripts overlapped with menthol, and that taxol shared 8.11% in common with other terpenes (Annadurai *et al.* 2013).

Evolution, Physiology and Native Plants

Plants lack mobility and have evolved strategies to deal with stressors such as light, poor nutrition, microbes, and herbivores (general and specific). Most of the pathways used to react to these stressors may overlap with each other and growth and development pathways. For instance, light signaling pathways have been shown to overlap with pathways associated with wounding, pathogen attack, ozone exposure, and oxidative stress involving the use of reactive oxygen species, salicylic acid, jasmonate, and ethylene (Nawkar *et al.* 2013).

Some defenses of plants can be intrinsic or structural, such as pigments that absorb excess light, lignification, epidermal thickness, hairs, and thorns, while others are chemical, such as antioxidants, antimicrobials, and deterrents to herbivores (Pankoke, Müller 2013). Secondary metabolites are variable to reduce resource use, and the mechanisms for their evolution are theorized to be highly adaptable (Sønderby *et al.* 2010). Secondary metabolite concentrations differ between tissues and with metabolic levels of the tissue. Mature leaves, which act as a carbon store and are much less metabolically active than growing leaves, have shown less induced resistance to herbivory than growing leaves (Pankoke, Müller 2013; Tallamy, Raupp, 1991).

The two most widely discussed and researched signals to recognize potential stressors and pathogens in plants are jasmonate and salicylic acid (and their methylated forms). Jasmonate has been shown to act as a downstream signaling molecule in NO and H2O2 mediated stress induced by fungi (Ren, Dai, 2012). Salicylic acid occurs constitutively at low concentrations and is also involved in stomatal closure, transpiration, photosynthesis, nutrient uptake, chlorophyll and protein synthesis (Perez *et al*. 2014). Methyl salicylate has been shown to be transmitted through the air to different parts of the plant and nearby plants and has been shown to directly affect many insect species (Taiz, Zeiger 2002; Pickett *et al.* 2007).

There are four basic types of plants in most environments: native, naturalized, invasive, and cultivated. Cultivated species or subspecies may simply be clones or offspring of wild plants or show little resemblance to their wild ancestors. When a plant is introduced to a new environment, it can either: survive only with assistance; become naturalized; or become invasive. Invasive species are those that spread broadly within their newly occupied regions, while naturalized species survive at a moderate level (Bezemer *et al.* 2014).

Factors Shown to Alter Constituents

Abiotic factors, such as moisture, sunlight, soil nutrients, and toxins can alter the constituent profile of a plant. Usually there is a well-established set of ideal growing conditions for a given species or variety. This ideal is usually based on increasing the rate of growth for the commercially useful part of the plant.

Nitrogen deficiency conditions show increased assimilation of ammonium into the GS/GOGAT system and increased PAL activity, stimulating the recycling of nitrogen containing phenolics and antioxidants (Kováčik, Klejdus 2014). In nitrogen-deficient *Nicotiana tabacum* (Solanaceae) there was an observable increase in phenolic acids and in *Matricaria chamomilla* (Asteraceae) an increased lignification, elevations in chlorogenic acid, umbelliferone, flavones, and growth, but decreased flavonols (Kováčik, Klejdus 2014).

Light can be a potent stimulus for induction of secondary metabolites. PAL activity increases with reductions in light, while P450s can be activated by light (esp 450nm). UV-B radiation can be severely damaging to plant growth and development and can induce defenses including: increased salicylic acid (SA) and increased responsiveness to jasmonate (Nawkar *et al.* 2013). A study of UV-B induction of glycosyl flavonoids orientin, isoorientin, vitexin, and isovitexin in *Passiflora quadrangularis* (Passifloraceae) showed an increase of all four flavonoids, with a 40 times greater increase in isovitexin by UV-B exposure than by induction with methyl jasmonate (Antognoni *et al.* 2007). Overall the flavonoid production antioxidant activity increased from 28-76% in UV-B treated callus versus untreated (Antognoni *et al.* 2007).

Another study found UV-B added to herbivory increased glucosinolates in *Brassica oleracea,* broccoli (Mewis *et al.* 2012). Yet another study showed SA and using transgenic comparisons based on the model organism *Arabidopsis metacaspase* (Brassicaceae) was able to show that salicylic acid and jasmonate's involvement in the UV signaling pathways (UVR8-COP1-HY5) increases reactive oxygen species and sunscreen pigments (secondary metabolites) in response to UV levels (Nawkar et al. 2013).

Biotic Factors

Biotic factors such as pollinators, herbivores, soil microbes, infections, and nearby plants can also alter constituents. Many plants have specialized pollinators, which have evolved with them. Some may also be capable of being fertilized by foreign pollinators when introduced to a new environment, but this is not always the case. The pollinator must notice the plant through scent or visual cues; it must be capable of extracting the pollen and depositing it on the next plant and it must do this during the flowering stage, which can change in a new climate. *Forsythia suspense* (Oleaceae) fructus is an example of a useful herbal medicine, which is limited by its inability to produce seed capsules. As a medical herb used for its activities as a broad spectrum antibiotic, an antifungal, an antipyretic, antinausea, diuretic and hepatoprotective properties, it is grown in Shanxi, Henan, and Shandong in China, where the fruit production is greatest (Foster, Yue 1992). While forsythia grows well (to the point of being classified sometimes as invasive in northeastern America, possibly due to the lack of insect pests), it does not produce fruit (Foster, Yue 1992).

Insects also affect plants as herbivores, but the effects can differ due to the presence of specific oral enzymes and the different patterns of damage during their feeding. For example, in *V.vinifera folium* culture, saliva *Manduca sexta* larva (Lepidoptera), was able to induce seven times the production of 3-O-glucosyl-resveratrol in 24 hours (Cai *et al*. 2012). A study with *Plantago lanceolata* L. (Plantaginaceae) showed clipping reduced sugars; only with feeding by generalist, *Grammia incorrupta* (Lepidoptera) was leaf iridoid content increased (Pankoke, Müller 2013). In contrast, for *N. tabacum* exposed to tobacco hornworm, *M. sexta*, cutting of equivalent amounts of leaf tissue, or cutting of identical patterns of leaf tissue, it was found that all treatments significantly increased alkaloids concentrations but both cutting treatments were significantly more effective than feeding, suggesting the specialist saliva may be reducing the response (Tallamy, Raupp, 1991).

Microbes, bacteria and fungi that bind to roots can also affect constituents. Joosten and van Veen found that soil microorganisms had a significant effect on pyrrolizidine alkaloid content in both the roots and shoots of *Jacobaea vulgaris* (Asteraceae). (Joosten, vanVeen 2011). Since microbial infections can stimulate defense pathways and predated herbivores as a stimulus in plant evolution, they theorize that pyrrolizidine alkaloids might have evolved initially as a pathogen defense (Joosten, vanVeen 2011).

A study found that infection with *Xylella fastidiosa*, pathogenic bacteria responsible for Pierce's disease, resulted in induction of phenolic compounds in *V. vinifera* 'Thompson Seedless' (Wallis, Jianchi 2012). The full effect was reached by 2 months, but after 6 months, levels had dropped below control, likely due to loss of defensive capabilities after resources had declined, photosynthesis had declined (Wallis, Jianchi 2012).

Similarly, induction of phenolic compounds occurs with *Colletotrichum lupini* spores applied to *Lupinus angustifolius* L. (Fabaceae) (Wojakowska *et al.* 2013). Metabolites (20-hydroxygenistein and phytoalexins: wighteone and luteone) increased within 24 hours with a maximum concentration at 7 days, after that genistein and the previous compounds were reached levels 50 times greater than in controls (Wojakowska *et al.* 2013).

Yeast has shown increased *M. truncatula cell* cultures isoflavonoid production benzophenanthridine alkaloids induction in *Eschscholzia californica* Cham. (Papaveraceae) suspension cell cultures (Broeckling *et al*. 2005; Cho *et al.* 2008). Derckel *et al.* found that the less virulent strain of a pathogenic gray mold, *Botrytis cinerea*, led to an increase in secondary metabolites, chitinase, b-1,3-glucanases and defensive proteins, while the more virulent strain showed no increase in secondary metabolites and delayed weaker induction of chitinases and b- 1,3-glucanases in infected *V.vinifera* tissues (Derckel *et al.* 1999). They reported similar findings with several agricultural plants: french bean (*Phaseolus vulgaris)*, apple (*Malus domestica)*, strawberry (*Fragaria* × *ananassa)*, carrot (*Daucus carota* subsp. *Sativus)*, and potato (*S. tuberosum)* (Derckel *et al.* 1999).

Many microbes help to form the complex interaction of plants with their environment, helping to fix nitrogen in the soil, and in some cases aiding in production of defensive chemicals either directly or through inducing the plants' defenses. For some medicinal plants, bacteria and fungi have been shown to be necessary for the production of active constituents, such as microbial endophytes used in TCM (Schmidt *et al.* 2014). A co-culture of the fungus, *F. mairei,* with *Taxus chinensis* L. (Taxaceae) showed a 38-fold increase in taxol over the plant culture alone (Soliman *et al.* 2013). Similarly taxol-producing fungi, *Paraconiothyrium* SSM001, does not function in the absence of *Taxus spp.* tissue, but with the addition of the wood and bark material has been shown to yield a 10-30 fold increase in taxol (Soliman *et al.* 2013).

Studies have shown rhizobacteria can affect aroma profiles in strawberries and grapes (Schmidt *et al.* 2014). Biosynthesis of all major classes of secondary compounds (alkaloids, phenolics, and terpenes) have been shown to be stimulated by Arbuscular mycorrhizal fungi (Schmidt *et al.* 2014). Strains of rhizobacteria have been shown to secrete salicylic acid in beans, *Phaseolus spp.,* to increase resistance to the mold *B. cinerea* (Derckel *et al.* 1999).

Signaling Molecules

Nearby plants can affect each other through both damage signals such as methyl jasmonate and allelopathic compounds, where the plant secrete chemicals to inhibit competition. Secondary metabolites found to be active in allelopathy come from all major groups: phenolics (flavonoids), terpenes, and alkaloids (Macías *et al.* 2007). An example of negative allelopathic effects (plants using phytochemical against other plants) comes from the invasive *Centaurea maculos*a Lam. (Asteraceae) which secretes catechin, adversely affecting the growth of nearby natives in North America (Bezemer *et al.* 2014). The antimalarial compound, artemisinin from *Artemisia annua* L. (Asteraceae) has been shown to inhibit seedling growth in a number of plants (Bharati *et al.* 2012).

Methyl jasmonate has been shown to induce defense genes and secondary metabolites inducing GDP synthesis and taxadiene synthase enhancing taxol production from *T. canadensis* and taxane from *T. chinensis* var. *mairei* (Sun *et al.* 2013). *M. truncatula* cell cultures showed an increase in triterpene saponins and the primary metabolites (b-Ala, GABA, and succinic acid) levels following elicitation with methyl jasmonate (Broeckling *et al*. 2005). The authors were uncertain as to the function of the primary metabolites, but GABA is highly neurotoxic in many insects.

Salvia miltiorrhiza radix (Lamiaceae), Danshen, is used in TCM to treat cardiovascular, cerebrovascular, and menstrual disorders and methyl jasmonate has been shown to stimulate both genes responsible for and the synthesis of tanshinones (terpenes) and phenolic acids (Luo *et al.* 2014). Similarly, *Atractylodes lancea* (Asteraceae) incubated with the fungus endophytic *Gilmaniella sp*. AL12 used in TCM for its antimicrobial volatile oil, were shown to have increased oil production and increased sesquiterpene components when exposed to jasmonate (Ren, Dai, 2012). Methyl jasmonate also increased benzophenanthridine alkaloids in *E. californica suspension* cell cultures (Cho *et al.* 2008).

The other major defensive signaling molecule in plants is salicylic acid, which in its methylated form, methyl salicylate, is aromatic. It has been shown to control aphids on cereal crops in fields (Pickett *et al.* 2007). Its production is increased with UV-B exposure (Nawkar *et al.* 2013). Salicylic acid has been shown to double taxol production in fungi and increase it in *Taxus spp.* cultures (Soliman *et al.* 2013). It has been shown to increase growth (height, branching, and number of leaves) in several members of Lamiaceae: *Mentha piperita*, *Ocimum basilicum* and *O. majorana* (Perez *et al*. 2014).

Under normal conditions, *M. piperita folium* contains 19-23% phenolics per dry weight, rutinoside making up much of the flavor and medicinal properties of the plant, with 12% as flavonoids composed of rosmarinic acid, hesperidin, eriocitrin, luteolin, and 7-O-rutinoside (Perez *et al*. 2014). Salicylic acid has been shown to increase growth in *M. piperit*a at 2mM concentration, but only increase phenolic compounds at 0.5mM and 1mM concentrations, and to produce phenolics (sinapic acid, rutin and naringin) not seen in the controls (Perez *et al*. 2014). Similarly in *Zingiber officinale* (Zingiberaceae) *folium*, salicylic acid increased total phenolics by 20% and presented phenolics (ferulic and vanillic acid) not found in controls (Perez *et al*. 2014).

Comparisons of Growing Methods Effect on Constituents

Few comparisons of overall growing conditions were found. One showed hydroponically grown *C. officinalis* would increase inflorescence size with supplementation of phosphorus, but it neglected to look into the constituent profile of the inflorescences or compare them to other plants (Stewart, Lovett-Doust, 2003). Another study compared conventional (synthetic urea CO(NH2)2 followed by NH4NO3) versus organic (organic urea) fertilizers in cultivation of *Olea europapea* L. (Oleaceae), olive trees, finding no difference in yield of fruits and only a slight increased bitterness in organic fruits (Rosati, 2014). However the NMR spectrometry showed increased polyphenols in the organic fruits and significant variations in many primary compounds (nucleotides, some amino acids, fatty acids, and glucose) between the two groups (Rosati, 2014).

One of the few studies comparing growing methods and medical constituent quantities looked at bloodroot, *S. canadensis*, which is predominantly wildcrafted, although some is now produced via cultivation (Graf *et al*. 2007). The researchers found that the some of the most medically interesting compounds, the benzophenanthridine alkaloids, sanguinarine and chelerythrine, were consistently higher but more variable in the roots of wildcrafted plants, while the root mass was larger in cultivated rhizomes (Graf *et al*. 2007).

Difficulty with germination is one of the largest obstacles to cultivating many native medical herbs. A study of germination of *Collinsonia canadensis* L.

(Lamiaceae) and *Dioscorea villosa* L. (Dioscoreaceae), which are often wildcrafted or grown from rootstock due to the difficulty with seed germination, indicated that both species require a period of cold temperatures prior to cool temperatures to germinate, similar to the winter period after the seed dispersal (Albrecht, McCarthy 2016). Twelve weeks of cold treatment provided good results for both species; however, they found that neither species will overcome dormancy with a 6-month dry storage treatment and it would take any seeds planted until the following spring to germinate (Albrecht, McCarthy 2016).

In Sardinia the endemic population of *Helichrysum italicum* ssp. *italicum* (Asteraceae), which is valued for anti-inflammatory, antioxidant, and antimicrobial activity (against Staphylococcus aureus and Candida albicans) was examined. Its effects are thought to be due to secondary metabolites – flavonoids, sesquiterpene lactones and essential oils – which depended on both the site of collection and the stage of plant growth (Melito *et al*. 2013). Of the 50 populations examined, analysis of their genes showed two distinct clades varied by elevation, with cluster A at lowland sites, while cluster B was at mid to high altitudes (Melito *et al*. 2013). In each cluster the essential oil contents did not vary significantly, but between the populations it varied significantly based on population. The authors expressed regret that they did not compare growing conditions with secondary metabolites, so it is unclear whether the changes were genetic drift or environmental (Melito *et al*. 2013).

Tims' thesis on *H. canadensis* chemical ecology also looked at microecologies of subpopulations. He found elevation was inversely related to quantity of alkaloids present, toxicity of alkaloids, and combined herbivore and pathogen pressures (Tims, 2006). He found wild populations demonstrate increased alkaloid content with increased rhizome size and increased seed number and theorized that this may be an adaptation to protect seed, which are disturbed by ants that collect them for food (Tims, 2006). Increased growth (increased aerial mass and density of population) was noted in disturbed areas, but fertilization did not increase these parameters (Tims, 2006). He also found that proliferation of rhizome size and leaf biomass were greatest under 70% shade, providing valuable information for propagation (Tims, 2006).

Summary and Conclusions

Because of the complexity of environmental plant interactions and their effects on secondary metabolites, there is much opportunity for future research. Understanding of the complex ecological dynamics of secondary metabolites can create opportunities for conservation and more potent herbal medicines, which may provide research and job opportunities. Those who use plant-based medicines in their practice should be aware of threatened and endangered species included in the materia medica. They may be interested in becoming more educated or involved in cultivation or wildcrafting of herbs. Some may even be interested in investigating opportunities in small-scale alleycropping for their own herbal needs.

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“*Sanguinaria canadensis* L., Bloodroot, highlighting historical and potential uses”

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Abstract

This monograph reviews the identification, distribution, and growing conditions of *Sanguinaria canadensis* with a detailed discussion of the plant's chemical profile and concentrations of important alkaloids and other constituents and their studied effects. I also review the traditional uses by early Americans and the medical community from the 19th century through today including its importance in dental care. An examination of the publication criticizing its use as an escharotic treatment for skin cancers, shows that it focused on the danger of self-treatment, and did not make claims as to damage of healthy skin. Currently bloodroot is used in naturopathic medicine to treat cervical lesions, for polyps, and for infections, but many practitioners avoid using it due to safety concerns not supported by literature or clinical evidence. A growing body of literature in the last decade and a half shows its effectiveness against cancer in vitro and animal studies.

Keywords: bloodroot, monograph, *Sanguinaria canadensis,* escharotic, Sanguinarine, melanoma

Classification

Sanguinaria canadensis L., bloodroot, is the only member of its genus. Its family, Papaveraceae, contains 19 genera and 129 accepted taxa (Moore *et al.* 2017). Several of the common names – bloodroot, puccoon-root (fr. pocan- bright red), redroot, red puccoon, and red Indian paint – and the genus name, Sanguinaria, describe the characteristic red root and/or latex (Harris, 2003).

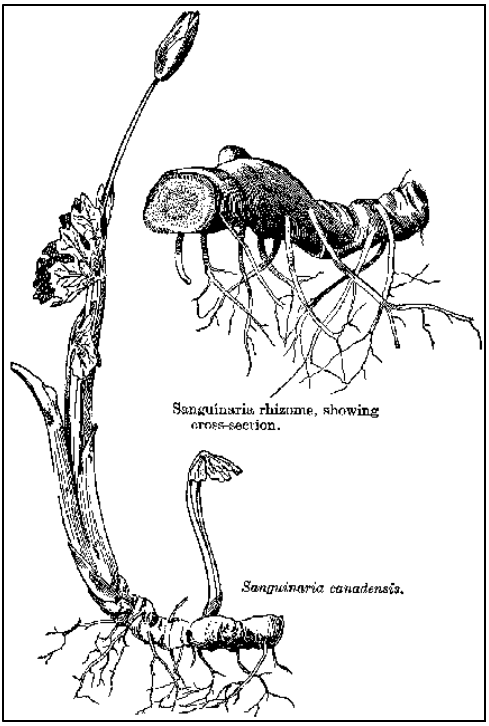

Fig 1. Sanguinaria flower emerging from leaf and rhizome, showing cross-section (Harding 1936)

Botanical description

S. canadensis is a small perennial herb, reaching 6-9 inches in height, whose scape is enveloped by a single leaf until it emerges and blooms in early spring (April-early May) (Harris 2003; Rhoades and Block 2000; Sievers 1930). This solitary radial white flower, which is described as rarely pink by Rhoades and Block has 8-16 spatulate petals and caducous sepals (Millspaugh 1974; Rhoades and Block 2000; van Wyk and Wink 2004). The flower is perfect with rows of 12 stamens whose anthers are covered with golden yellow pollen (Millspaugh 1974; Rhoades and Block 2000). The 2 grooved stigma and short style lead to the hypogynous, 2-placenta, 1-celled ovary (Millspaugh 1974; Rhoades and Block 2000).

The single caudal greenish-blue leaf is cordate, shallowly dissected into 3-9 lobes, sometimes dentate, and expands to 4-7 by 6-12 inches following flowering (Millspaugh 1974; Rhoades and Block 2000). The reticulate venation can appear slightly reddish on the caudal side of the leaf, which tends to have a white glabrous appearance (Millspaugh 1974; Sievers 1930). The reddish-brown seeds are held in a 1 inch 2-valved capsule maturing through June (Millspaugh 1974). The horizontal reddish-brown rhizome can be divided and reach 2-4 inches with a diameter of ½ - ¾ inch (Millspaugh 1974; Wren 1975).

Growing Conditions

S. canadensis prefers rich acidic soils, especially with heavy leaf litter, partial shade to sun, and is hardy to zone 3 (Braly 2007; Kowalchik and Hylton 1987). Although it is found growing along roads or paths and streams, it prefers well-drained forest openings and is classified as an uplands species except in the Midwest and Northcentral and Northeast, where it is classified as a facultative upland species (Rhoades & Block 2000; Moore *et al* 2017).

The flower relies on bumblebees, honeybees, and syphid flies for cross-pollination, resorting to self-pollination if this doesn't occur (Braly 2007; Harris 2003). The seeds form in the pod in the 4-5 weeks following flowering, and when ripe are ejected up to 10 feet (Braly 2007; Glick 2004). Ants then collect the seeds eating the covering and storing them underground (Braly 2007; Glick 2004; Harris, 2003). Due to this, it is often found growing in neat linear patterns.

Cultivation

The recommended method of seed collection requires securing fine netting over the capsules before they open to catch the seeds (Glick 2004). Propagation is more commonly by rhizome division in early autumn with plantings 6-8 inches apart (Kowalchik & Hylton 1987). Early spring propagation is recommended by some, but may damage young tissues reducing flowering and seed production for the year.

S. canadensis is sometimes grown in gardens for its beautiful but short lived early spring flower and lovely foliage. The ornamental *S. canadensis* var *plena* (Multiplex) with extra petals is a showier version, but doesn't produce seeds, so it can only be grown from rootstock.

Distribution/geography

S. canadensis is found in acidic soils of the eastern forests: near openings in the canopy, streams, and roadsides. It is native to Canada and the Eastern United States north of Florida (Figure 2) with a current range from Nova Scotia to Florida in the East to Manitoba and Nebraska in the West (Harris, 2003; Sievers 1930; Moore *et al* 2017). It is common in New England, Long Island, Pennsylvania, and New Jersey (Kowalchik and Hylton 1987). In 2010 the USDA listed it as exploitably vulnerable in New York and of special concern in Rhode Island, as it is still currently listed.

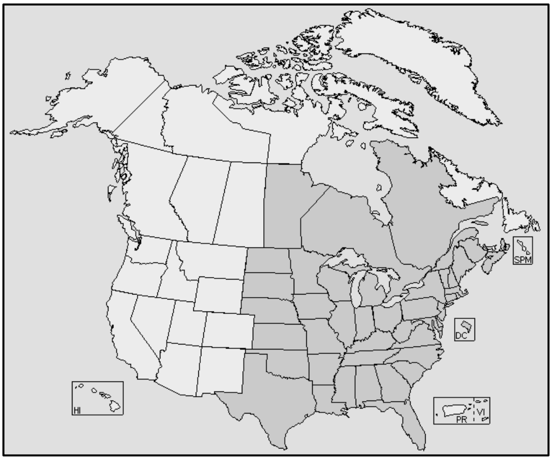

Fig 2. *Sanguinaria canadensis* distribution in North America from the USDA plant database (Moore *et al.* 2017)

Parts used

The dried root or dried root sap have been most commonly used medically. The constituents of the radix differ from the herba. Particularly, the medically active constituent, Sanguinaria, which is the focus of the majority of research is mostly found in the root. The root is collected in autumn and should be stored from moisture (Sievers 1930). The collection time is likely to be more important in wildcrafting, since Graf *et al* (2007) found higher levels of sanguinarine in wildcrafted plants than cultivated plants, but found levels varied more for the wildcrafted plants. The dried rhizome should be reddish brown with a heavy odor and bitter acrid taste (Wren 1975).

Constituents

Herba constituents

The *S. canadensis* herba has been found to have allocryptopine, β-homochelidonine, chelerythrine, dihydrosanguilutine, malic-acid, oxysanguinarine, porphyroxin, sanguirubine, pseudochelerythrine/sanguinarine (low levels), resin, and starch (Foster & Duke 2010).

Allocryptopine and β-homochelidonine are steroisomer isoquinoline alkaloids, which can act as oxytonic agents and are found in several genera of Papaveraceae (Foster & Duke 2010; Harborne & Baxter 1993). Allocryptopine has also been shown to act as an aldose-reductase-inhibitor (IC_{50}=27.9uM in rats), analgesic, antiarrhythmic, anti-fibrillary, and soporific (Foster & Duke 2010).

β-homochelidonine has also been reported as acting as a central nervous system (CNS) paralytic (Foster & Duke 2010).

Chelerythrine exhibits several antimicrobial and antiparasitic actions as an antiseptic, antiviral, gram(+)bactericidal, gram(-)bactericidal, fungicidal (1-5 mg/mL), candidacidal, molluscicidal, and nematicidal (50-100 ug/mL) (Foster & Duke 2010). It has shown promising actions against cancer: immune stimulant, antitumor, antimitotic, cytotoxic, DNA-intercalculator, 12-lipoxygenase-inhibitor, and topoisomerase-I-inhibitor (Foster & Duke 2010). Chelerythrine has also been reported to have antiinflammatory activities (10mg/kg in rats) including 5-lipoxygenase inhibitor effects. It has also been shown to act as an acetylcholinesterase inhibitor, paralytic, irritant, antiaggregant, antitussive, and both hypertensive (3-5 mg/kg IV) and hypotensive effects (Foster & Duke 2010). It was shown to inhibit *Helicobacter pylori* in vitro, but was less active than sanguinarine (Mahady *et al.* 2003).

Malic acid is a fairly ubiquitous compound reported to have a number of antimicrobial actions: antiseptic, antibacterial, mycobactericidal, bruchiphobe, and antitubercular (Foster & Duke 2010). It is also reported to have antitumor, antiatherosclerotic, sialogogic, antiseborrheic, hematopoietic, and antifibromyalgia (300mg 3x/day) (Foster & Duke 2000).

Radix constituents

For *S. canadensis* the dried root or root sap are the portion of the plant most commonly used medically. In the root the total isoquinoline-related alkaloids (common to Papaveraceae) concentration is 18,000-70,000 ppm including: α and β-allocryptopine, berberine, chelirubine, coptisine, sanguidimerine, protopine, sanguinarine, chelilutine, sanguidimerine, sanguirubine, oxysanguinaridine, sanguidaridine, and sanguilutine (Foster & Duke 2000). α-allocryptopine is reported to have antiarrhythmic, cardioactive, and oxytocic activities (Foster & Duke 2000). Its stereoisomer, β-allocryptopine, found in the tribe Chelidonieae in *Bocconia fructescens* L. and *S. canadensis*, and is reported to have anti-vagal activities with highest (Foster & Duke 2000).

Berberine is a yellow pigment found in the bark and leaves of several families with an LD_{50} for humans of 27.5 mg/kg, which can cause hypotension, dyspnea, and cardiac damage (Harborne and Baxter 1993). It has been shown to have effectiveness as an antimicrobial, fungicidal, antimalarial, antipyretic, anthelmintic, bitter, and cytotoxic (Harborne & Baxter 1993).

Chelirubine/Bocconine has been shown to be antibacterial, molluscicidal, nematicidal, and also used as a local anesthetic (Foster & Duke 2000; Harborne & Baxter 1993). Coptisine has been shown to have antiinflammatory, antitumor, and myocontractant properties (Foster & Duke 2000). Sanguidimerine is reported to have antitumor properties (Foster & Duke 2000).

Protopine is found throughout Papaveraceae and has been found to be antiseptic (at 1,000 ppm) and gram(+) bactericidal (Foster & Duke 2000; Harborne & Baxter 1993). It can act as a smooth muscle relaxant, antispasmodic, calcium-antagonist, anticholinergic, and sedative (Foster & Duke 2000; Harborne & Baxter 1993). It can induce bradycardia and act as an antiarrhythmic, but it has also reported to have convulsant effects (Foster & Duke 2000; Lewis & Elvin-Lewis 1977). It has an amphoteric effect on blood pressure, acting as a hypertensive at dosages <1 g/kg and a hypotensive at dosages >1g/kg (Foster & Duke 2000). Other reported effects include: abortifacient (70mg/kg), antispermatogenic, uterotonic, antitussive, aldose-reductase-inhibitor (50uM), amphicholeretic, analgesic, antiaggregant (100-1,000 uM), and as an antiinflammatory (100 mg/kg) (Foster & Duke 2010). It was shown to inhibit *Helicobacter pylori* in vitro, but was less active than sanguinarine (Mahady *et al.* 2003).

Sanguinarine/pseudochelerythrine is found in both tribes Chelidonieae in *S. canadensis* and *Chelidonium majus* and tribe Papavereae in *Argemone mexicana* with concentrations of 6,000-60,000 ppm in *S. canadensis* roots (Foster & Duke 2000; Harborne & Baxter 1993; Adhami *et al.* 2003). Sanguinarine is antiseptic, antiviral, gram(+)bactericidal and gram(-)bactericidal (LD_{50}=292 IV), antitrypanosomal, trichomonacide, fungicide, candidacidal, nematocide, molluscicide (50-100 ug/mL) (van Wyk and Wink 2004; Foster & Duke 2000; Harborne and Baxter 1993; Adhami *et al.* 2003). It was shown to inhibit *Helicobacter pylori* in vitro (Mahady *et al.* 2003).

Sanguinarine also has shown many effects useful against cancer: antitumor, antimitotic, cytotoxic, DNA intercalating agent, and 12-lipoxygenase-inhibitor (vanWyk & Wink 2004; Kim *et al* 2008; De Stefano *et al* 2008; Adhami *et al* 2003). It has shown selective antiproliferative and antiapoptotic effects on carcinomas with milder effects on normal keratinocytes via induction of endogenous apoptosis (Adhami *et al*. 2003). It also showed both antiproliferative and antiangiogenic effects in mice with induced human melanomas (De Stefano *et al*. 2008).

Sanguinarine has also been reported to have antiinflammatory activities including 5-lipoxygenase inhibitor effects, antiedemic, antioxidant (IC_{50}=10 uM), and antisuperoxidogenic (IC_{50}=16 nM) (van Wyk & Wink 2004; Foster & Duke 2000; Harborne and Baxter 1993). Sanguinarine has been found to inhibit the activities of: acetylcholinesterase, cholinesterase, diamine oxidase, and aminotransferase (Foster & Duke 2000; Harborne & Baxter 1993; Adhami *et al* 2003). It is reported as having motor and CNS effects including: CNS-depressant, sympatholytic, emetic, expectorant, sialogogue, gastrocontractant, respiratory stimulant, and vasomotor-stimulant activities (Foster & Duke 2000; Kowalchik & Hylton 1987).

Sanguinarine reduces gingivitis, periodontal disease, and plaque at 2ug/mL (Foster & Duke 2000; Harborne & Baxter 1993; Adhami *et al* 2003). In the cardiovascular system it can: be an inotropic stimulant (0.3-0.65 uM) inhibiting Na+,K+-ATPase; cardiotonic; diuretic; and both a hypertensive and hypotensive (Seifen *et al.* 1979; Foster & Duke 2000; Harborne and Baxter 1993; Kowalchik & Hylton 1987). It has also been shown to cause irritation, increase ocular pressure as a glaucomagenic, and be hepatotoxic at 10mg/kg in rats (Foster & Duke 2000; Kowalchik and Hylton 1987).

Pharmacology

The properties attributed to *S. canadensis* root extracts in traditional and modern use can be explained by the actions of its constituents. The anti-gingival, antiplaque, and antibiotic properties of *S. canadensis* are also found in its constituents – berberine, chelirubine, protopine, and sanguinarine – while antifungal properties are found in berberine and sanguinarine (Foster & Duke 2000; Harborne & Baxter 1993).

Anti-inflammatory properties are found in the constituents berberine, coptisine, protopine, and sanguinarine (Foster & Duke 2000; Harborne & Baxter 1993). Antioxidant properties are found in the constituents berberine and sanguinarine (Foster & Duke 2000; Harborne and Baxter 1993). Spasmolytic properties are found in both protopine and sanguinarine (Foster & Duke 2000). Stomachic properties are found in berberine and sanguinarine, and sanguinarine, chelerythrine and protopine were shown to inhibit *Helicobacter pylori* in (Foster & Duke 2000; Mahady *et al* 2003).

Cardiotonic properties are found in the constituents: α-allocryptopine (antiarrhythmic), coptisine (myocontractant), protopine (antiarrhythmic, amphitensive, myorelaxant, bradycardic), and sanguinarine (inotropic, myocontractant, cardiotonic, and amphitensive) (Foster & Duke 2000; Harborne & Baxter 1993).

Antitumor and escharotic properties are found in: berberine, coptisine, sanguidimerine, and sanguinarine (antitumor, antimitotic, cytotoxic, and intercalculating agent) (DeStefano *et al.* 2008; Foster & Duke 2000; Harborne & Baxter 1993). Research is showing that in addition to its other antitumor actions, sanguinarine anti-angiogenic, reducing vascularization of tumors (DeStefano *et al* 2008).

Sanguinarine has shown to induce apoptosis in human breast cancer cells (MDA-231), especially with the addition of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which are hypothesized to overcome resistance to breast cancer cells containing overexpression of Akt or Bcl-2 (Kim *et al* 2008). Investigations have shown topoisomerase-I and antiangiogenic effects of sanguinarine (with doses of 5mg/kg/day, 5 days/week in rats) against human invasive malignant melanoma tumors (K1375-M2 and B16 M 4A5 lines) (DeStefano *et al* 2008). In a malignant melanoma study, it was noted that 3 of 11 mice were alive at 60 days after the rest had passed and were still alive 77 days later after the conclusion of the study (DeStefano *et al* 2008). Immortalized keratinocytes (HaCAT from hyperproliferative carcinoma) have shown dose-dependent apoptosis with stimulation of Bax and depression of Bcl-2 (Adhami *et al* 2003).

Traditional Actions/Indications

S. canadensis was listed in the United States Pharmacopoeia from 1820-1910 and in the National Formulary from 1925-1965. Traditionally, it has been used as an emetic, expectorant, tonic, spasmolytic, stomachic, snuff for nasal polyps, and escharotic (Millspaugh 1974; Harris 2003; van Wyk & Wink 2004; Wren 1975). Eclectic physicians materia medica included Sanguinarin (the resin) and extracts in several formulas: Pilulae Taroxaci Compositae, Pulvis Ipecacuahae Compositus, and Pulvis Lobeliae Compositus (Millspaugh 1974). The Domestic Physician and Family Assistant in 1836 suggest at the time use centered on emetic, diuretic, emmenagogue (stimulating mense), sialagogue (stimulating saliva and gastric juices), and laxative (Gardner & Aylworth, 1836).

People of the First Nations used *S. canadensis* for similar uses to the traditional medical uses, since many of the doctors and laypeople copied from their knowledge and techniques. Notably *S. canadensis* was used with *Hydrastis canadensis* as an escharotic to remove skin lesions (McDaniel & Goldman 2002). Fell's report of Lake Superior tribes applying the sap to their skin for this use, reputably initiated interest in its use as an escharotic (Lewis & Elvin-Lewis 1977; Kowalchik & Hylton 1987). Using *S. canadensis* as a cosmetic, insect repellant, and rheumatism treatment do not appear to have been copied by settlers (Harris 2003; Lewis & Elvin-Lewis 1977; Kowalchik & Hylton 1987).

Traditional cancer treatments using *S. canadensis* include: dermatologist using Mohs techniques of topical application with zinc and stibnite prior to excision, Fell's breast cancer treatments, and Hoxy's topical red paste (Trost & Bailin 2011; Lewis & Elvin-Lewis 1977; McDaniel & Goldman 2002).

Contraindications and Cautions

Oral toxic doses will cause nausea and vomiting, intense thirst and burning sensations of the mucus membrane, which can be followed with: vertigo, faintness, insensibility, and inhibition of the cardiac, central, and peripheral nervous systems (Millspaugh 1974). Despite warnings of death from cardiac paralysis written in the 1800's, several more current sources claim that neither livestock nor humans are recorded as being poisoned by this herb in recent history, and it is currently being used as a feed additive to enhance animal growth (Millspaugh 1974; Harris 2003; Lewis & Elvin-Lewis 1977; Kowalchik & Hylton 1987). The unpalatable taste may be a reason.

In 2003, the FDA did an extensive literature review on the safety of *S. canadensis* finding in most cases a no-observed-adverse-effect-level (NOAEL) level of 30-50 mg/kg per day (Shuren 2003). Studies found animal mortality at doses of >100 mg/kg per day (4,500 mg for a 100lbs), but found toxicity and mortality to significantly decrease when administered with food (Shuren 2003). Oral extracts are contraindicated during pregnancy and breastfeeding, like most plants with isoquinoline alkaloids, emetics, and emmenagogues.

Topically, it stains the skin and can act as an irritant. However it has longstanding use on the skin as both an escharotic and a cosmetic (First Nations use), but one paper casually called to question its tissue selectivity (McDaniel & Goldman 2002). Recent studies on sanguinarine have demonstrated tissue selectivity in apoptosis induction (DeStefano *et al* 2008; Adhami *et al* 2003). Any treatment of cancer without a doctor's supervision is extremely dangerous because it runs the risk of not fully eliminating the cancerous cells, leading to relapse. This is the contraindication reported by McDaniel & Goldman (2002) in cases of self-treatment of skin cancer.

Dental products containing *S. canadensis* (notably Viadent) have been linked to leukoplakia with chronic use (Damm *et al* 1999; Allen *et al* 2001). The investigational methods used in the first study have been disputed by Munro *et al.* (1999); there is yet to be an explanation of the mechanism of action, and several studies have shown no adverse effects up to 6 months (Shuren 2003). In 2003 the U.S. Food and Drug Administration (FDA), after reviewing the literature, still allowed for use of extracts of 0.03-0.75% in dental products, but caution should be exercised for use longer than 6 months until further investigations determine the exact relationship of this linkage, and the safe period of use (Shuren 2003).

Methods: Recent Research

A pubmed search of the last 10 years shows 58 results for "Sanguinaria canadensis"; 10 could not be used because they either referred to an unrelated species or there wasn't even an abstract available, 3 dealt with ecology, and 4 dealt with the chemistry of sanguinarine. This left 42 articles, of which more than half were either articles about dermatological self-treatment (13) and investigations into potential cancer uses (12). Of the 12 articles covering anticancer properties: 10 researched the component sanguinarine, 1 researched the components sanguinarine and chelerythrine, and only 1 investigated the use as a plant extract.

There were likely 4 more articles about dermatological self-treatment judging from the journal and article titles, bringing the total number of these articles to 17. Most of these focused on 1 or 2 anecdotal cases seen by the author(s), but 3 reviewed the literature. The most common concerns in these articles were scarring and/or cancer not being fully treated. Most of these focused on products, such as black salve, which contain other components such as zinc and other herbs. In addition to dermatology case studies, 2 papers presented cases of experimental internal *S. canadensis* treatments that failed. One was the case of 2 dogs injected with an extract into their tumors, and the second was a case of several doctors treating human subject for an infraorbital tumor with no oversight.

The next largest categories was chronic disease and inflammation with 5 articles: a review of *S. canadensis* use in chronic disease, 3 articles on an antiinflammatory and pathways affected by *S. canadensis* extracts, and 1 article on its potential for osteoarthritis. There were 2 monographs reviewing *S. canadensis* and its uses, and 2 articles reviewing its use in dental hygiene products. The homeopathic use of *S. canadensis* for menopause was the focus of 3 articles with a paper showing anti-proliferative effect on breast cancer cells at 625 and 1,250 µg/ml by the homeopathic Klimaktoplan®, which includes *S. canadensis* as one of its 4 main ingredients (Ahn *et al* 2013). There was also 1 paper investigating anthelmintic effects of different plant extracts, and one investigating its potential use against *Staph. aureus.*

Current Use

S. canadensis has been used as an anti-gingival, antiplaque, analgesic, expectorant, and stomachic (van Wyk & Wink 2004; Kuftinec *et al* 1990). It has been used in over-the-counter products including toothpaste, mouthwash, and cough and cold remedies (Adhami *et al* 2003). *S. canadensis* was used in toothpastes and mouthwashes as a dentifrice having antiinflammatory effects on gingiva and bactericidal effects (Adhami *et al* 2003). Due to concerns with linkages to cases of leukoplakia, most companies have removed it from their formula, but in 2009, Brazilian research found that with a chewing gum containing a tincture of *S. canadensis,* not only was there significant reduction in dental plaque but the alkaloids were retained in the bacterial biofilm for at least 3 hours (Moretti *et al.* 2009).

S. canadensis is popular in homeopathic, non-hormonal menopause supplements. One investigation into one of these products, Klimaktoplan®, showed the additional benefit of anti-proliferative effect on breast cancer cells (Ahn *et al* 2013). In cough and cold remedies its expectorant properties clear mucus and its antimicrobial effects can also be of use in these formulas (Kowalchik &

Hylton 1987, Adhami *et al.* 2003). Currently, extracts are also reported as having antioxidant, antitumor, antibacterial, antifungal, stimulant, tonic, cardiotonic, and anti-inflammatory properties. It is also reported as being used orally for deficient capillary circulation, nasal polyps, rheumatism, warts, fever, and as a general tonic (Millspaugh 1974; Harris 2003; van Wyk & Wink 2004; Wren 1975).

Most controversially, *S. canadensis* is used against cancer and other skin and mucosal lesions. Traditionally it was used by dermatologists, other doctors, and laypeople. Dermatology has begun to condemn the use of *S. canadensis* due to cases of self-treatment leading to severe adverse reactions including scarring, residual tumor formation, and metastatic lesion (McDaniel & Goldman, 2002). These escharotic salves usually contain zinc, which is caustic and may contain other caustic herbs or chemicals. For example, Black salve from Best on Earth Products contains: *S. canadensis*, zinc chloride, distilled water, *Larrea tridentata*, *Trifolium pratense*, *Arctium lappa*, and *Annona muricata*. These are available via the internet and lay people for the removal of skin growths, and the treatment of skin cancers. In Naturopathic medicine, *S. canadensis* and zinc are used to treat early cervical dysplasia. The solution is removed and followed by *Calendula officinalis*. The treatment requires several sessions and histological follow up to confirm the dysplasia has been eliminated.

Discussion

S. canadensis has historically been a medically and economically important herb in America (Sievers 1930), and its “chief” constituent, sanguinarine, is under serious research for both antineoplastic and anti-inflammatory uses. It is currently used in herbal and Naturopathic medicine, but has decreased in popularity in Naturopathic circles due to its reputation for being caustic (potentially due to the preponderance of dermatological papers).

The cases of people self-prescribing “black salve” and the few cases of medical professionals not properly monitoring the treatment and recovery or not suggesting other treatments when warranted does seem to present a serious potential threat to this herb's reputation in natural medicine. While cancer researchers have found evidence to support the efficacy of the constituent sanguinarine and its potential to treat cancer, medical monitoring evaluation is needed to make sure a treatment is successful.

Treatment for nasal polyp is a widely reported traditional indication, and the current medical treatment of nasal polyps requires surgery (often repeated). Unfortunately, survey of the literature did not reveal studies on polyps, but the use of aerosolized extracts may provide a future treatment. The traditional use as snuff for polyp is not well tolerated by many modern patients, who are not used to this form of drugs.

In dentistry, more research is need to conclude if the leukoplakia cases are a result of *S. canadensis,* and what constitutes safe use in oral hygiene. Although no commercial brands currently use extracts from the plant, some consumers and some in the dental hygiene field are still interested in its use because of its potential effects on biofilms, and the FDA currently lists it as safe for use in these products (Shuren, 2003).

Finally, the growing interest in native plants and shade gardens presents another opportunity for *S. canadensis,* as another lovely flower flower growing well beside *Trillium spp.*

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"Spreading the Ginseng Gospel: Case Study in Ginseng Production and Promotion from Watauga County Cooperative Extension"

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Abstract

Over the last four years, Cooperative Extension has been providing on-farm demonstrations and workshops with forest landowners and intensive ginseng growers in northwestern North Carolina. Wild-simulated ginseng is a viable forest crop option for underutilized woodlands in prime ginseng growing habitat in northwestern NC (and many parts of the Appalachians). Interest in the county's "ginseng program" and programmatic efforts have yielded participation by over 150 landowners who have sown an estimated 3,000 pounds of seed in the last four years. In 2014, Watauga County was home to the first felony conviction of ginseng theft on private property in North Carolina due in part to a coordinated and proactive educational approach directed towards law enforcement and the district attorney's office. Successful elements of Extension's wild-simulated ginseng production program are presented to highlight how other organizations can garner interest from landowners in establishing ginseng on underutilized forest land.

Keywords: ginseng, wild-simulated, Watauga County, Cooperative Extension, workshops, underutilized forestland

Introduction

The wild harvest of ginseng and other forest medicinals has a long history in the High Country region of northwest North Carolina. With its cold winters, ample rainfall, mountainous terrain, and prevalence of rich cove sites, the native forests of this region have the ideal climate and environment for ginseng. Ginseng roots harvested from this part of the southern Appalachians historically have been prized for their shape, quality, and "character" by the export markets.

In 2013, Watauga County Cooperative Extension took on ginseng production and promotion as a programming priority to meet a rekindled demand from private forest landowners who are interested in the plant for home production and to add value to their underutilized forestlands. Over the last four years, several workshops, field days, farm tours, and other Extension programming have led to a significant reestablishment of ginseng in this part of the North Carolina.

Historical Context

Watauga County has been a regional epicenter of the ginseng and herbal trade since the early 1900s, when Grant Wilcox opened Wilcox Drug Company in Boone, North Carolina. According to Wenger (2013), Mr. Wilcox bought and traded ginseng and other forest herbs for decades and by "1976, Butch Wilcox, the third generation proprietor, told a reporter that the business was the largest American buyer of botanicals . . . (buying) about four to six million pounds of botanicals a year, a couple of hundred items from thirty-eight states." In 1982, Wilcox Natural Products was purchased by the Zuellig Group, a Swiss botanicals firm, which closed the historic Boone, NC location in 2000 (Wenger, 2013). In 2015, a historic marker was erected in downtown Boone to recognize the impact of ginseng and the herbal trade to the town and regional economy. Today, several ginseng and herb dealers continue to serve this area, capitalizing on a significant volume of wild ginseng and other herbs that are still wild-harvested each year—most notably, Lowe Fur and Herb in Wilkesboro and Ridge Runner Trading Company in Fleetwood.

Watauga County's Ginseng Extension Program

As the price of wild ginseng increased from 2011 to 2013 (reaching an all-time high of $1,300 per pound in 2013), the Watauga County Extension office began receiving an increased number of calls from private forest landowners seeking information about ginseng. Interest and information requests about ginseng increased even more in 2014 as the History Channel and National Geographic Channel released a series of reality shows which highlighted (and misrepresented, in most cases) the culture and 'underground economy' of the ginseng trade in the mountains of North Carolina and Kentucky: *Appalachian Outlaws*, *Smoky Mountain Money*, and *Filthy Riches*.

Approximately 60% of Watauga County is considered timberland (NC State, 2012). However, timber harvesting is a low priority of landowners in the county. Approximately 50% of land parcels in the county are owned by "non-resident landowners" (Rothrock, et. al, 2003). The county's main economic driver is tourism, and many properties are held for retirement and vacation homes. Additionally, Appalachian State University has a large presence in the county. As a result, property values per acre of land are high and timber harvesting is no longer considered a priority among many landowners who wish to maintain their wooded properties for aesthetic and recreational purposes. However, based on conversations with many private forest landowners via the Extension office, landowners (locals and newcomers) are interested in creating some value in these underutilized forest lands—for conservation purposes, as a hobby, for long-term/retirement investment purposes, or for supplemental income.

In 2013, Watauga County Extension advertised its first formal ginseng workshop. North Carolina A&T State University's Natural Resource Specialist provided some funding for a small quantity of seed and rootlets to distribute to prospective participants, and Dr. Jeanine Davis (Specialty Crops Specialist from NC State) was invited to speak. Despite low expectations for attendance for an evening workshop offered in mid-December, enrollment in the class had to be cut off at 40. Since 2013, over 160 landowners have attended workshops and planting demonstrations that have been offered each subsequent year in the fall. In 2016, 80 landowners participated in four field-based planting demonstrations and evening seminars on ginseng production.

Watauga Cooperative Extension, working with a local non-profit fiscal agent, has received approximately $70,000 in grant funding from the NCDA Specialty Crop Block Grant Program and TVA's Ag & Forestry Fund, to subsidize seed and rootlet purchases for workshop participants, to establish a field demonstration site for ginseng production, to pay for training materials, and for external specialists to visit and tour farms to provide more specialized training for a select group of commercial growers. Additionally, grant funding paid for a marketing study, conducted by ASAP (Appalachian Sustainable Agriculture Project), to analyze the potential for the domestic sale of ginseng in the burgeoning health food, brewery, and other local markets. Since over 90% of all ginseng harvested in the United States is exported to Asia (Davis & Persons, 2014), local growers are interested in the potential for more direct sale of roots to local customers. The ASAP study was published in February, 2017 and is available online:
http://asapconnections.org/wp-content/uploads/Exploration-of-Market-Opportunities-for-Western-North-Carolina-Grown-Ginseng-Root_ASAP.pdf

Watauga Cooperative Extension is also working with a group of ten producers, led by Travis Cornett of High Country Ginseng (http://www.highcountryginseng.com), who are planting ginseng at a higher density per acre rate, but under wild-simulated conditions, to produce high-quality roots. It is estimated that this group of producers has planted approximately 2,800 lbs. of seed on over 40 acres over the last three years. Beyond the potential economic yield of this group's production of ginseng root within the next 7-10 years, it is likely that seed production will be significant within the next two to three years—hopefully opening up an additional source of high-quality forest grown seed to the 'ginseng marketplace'.

While ginseng production on private land has traditionally been a covert and secretive trade, by working with a core group of producers who are willing to share knowledge with each other, we hope that successful cultivation of this crop in a wild-simulated strategy with educational programming and sharing of information can bring growing ginseng 'out of the shadows'. Since 2013, four workshops and farm tours with these commercial producers have been held to discuss disease issues, predator pressure, and alternative fertility and chemical options to optimize production.

Law Enforcement Education

An additional component of the Watauga County ginseng production and promotion program has been focused on education of law enforcement. It is widely known that theft, or 'poaching' as it is more commonly referred, remains one of the biggest threats to ginseng production on private land. Many potential ginseng growers and hobbyists have been discouraged from production after having years of work and entire plantings stolen overnight.

In 2014, Watauga County became the first county in North Carolina to successfully prosecute and receive a felony conviction for ginseng theft on private land (Wood, 2014). Several federal cases have been prosecuted successfully in the Great Smoky Mountains National Park (Taylor, 2016), due in part to Jim Corbin's (NCDA Plant Inspector) ginseng marking efforts in the park boundaries. However, prior to 2014, most ginseng cases on private land in the state have been dismissed or downgraded to misdemeanor trespassing charges. In 2013, the Watauga County Sheriff's Department arrested David Allen Trivette who was caught and confronted by Travis Cornett of High Country Ginseng after leaving his property. Trivette had trespassed onto the property and stolen roots from Mr. Cornett's ginseng plantation on a prior occasion as well. The case languished in the court system for an entire year until Watauga Cooperative Extension set up a meeting with the district attorney's office to highlight the nature of ginseng production in the county and to build awareness of the economic impact and investment that was at risk. Several growers and Ridge Runner Trading Company owner, Tony Hayes, emphasized the importance of setting a precedent with the case. We feel that the case would not have concluded with a felony conviction without informing the prosecuting attorneys about the nature of the burgeoning ginseng industry in the county and the detrimental economic impact of theft of this forest-grown crop.

Additionally, Watauga Cooperative Extension and the Plant Conservation Specialist for the NC Dept. of Agriculture gave a presentation to the Watauga County Sheriff's Department on the ginseng industry in the county in 2015 regarding the threats facing the conservation of the plant and theft risks for producers, and awareness of how ginseng 'thieves' typically operate. Following this meeting, the sheriff's department made two additional arrests of ginseng poachers on private property (Wood, 2015). Interestingly, two members of the sheriff's department also attended Extension's ginseng workshops that year and are planting ginseng themselves—thereby creating a vested interest in the law enforcement community in mitigating the theft threats.

Disease and Predation Issues

Since commercial production of ginseng has not been practiced in the county in the past, disease pressure is relatively light (according to Dr. Mary Hausbeck, Michigan State, based on her recent visit and evaluation). For producers in the county growing ginseng at a higher density, fungal disease risks are there (alternaria blight, phytophthora root rot, pythium, and cylindrocarpon, etc.), but based on the body of existing knowledge and research on ginseng diseases, and a higher prevalence of registered/labeled chemical products that currently exist, so far, producers in the county have effectively managed and mitigated these disease risks. Cooperative Extension and producers continue to explore and diversify the available chemistry and products to mitigate fungal disease risk and monitor resistance.

Predation pressure from deer, turkey, and voles (especially) seems to be a more pressing issue—for small scale landowners and commercial producers alike. While damage and risks from these 'pests' are documented in most ginseng production guides and are common knowledge, there really are not many documented mitigation practices that demonstrate effective control. Plenty of products exist in the marketplace for rodent control, for example, but it is unclear as to which, definitively, are the best for ginseng producers. We are experimenting via trial and error with a variety of products and practices.

Turkey pose a significant risk to newly planted seedbeds, as they rake out and can consume entire beds of seeds in the fall after seeds are typically planted, and in the spring as new seedlings are germinating. Their scratching in search of seed can also unearth seedlings. One landowner in the county recently documented (and Extension confirmed) a seed predation rate of almost 100% in a multiple-bed planting of 1.5 lbs. of seed—after careful searching only six seedlings were found! Turkey have also been verified via trail cameras in recently established seedbeds. One successful mitigation practice discovered by a Watauga grower is the installation of biodegradable landscape matting, which is laid over newly planted seed beds. The filament that holds the matting together discourages turkeys from scratching in beds as their nails get caught. Additionally, the landscape matting prevents the leaf mulch over new beds from getting displaced during wind events that are common in the region.

Deer predation, while not heavily damaging to roots, can reduce seed productivity and impact plant growth as deer consume the leaves and tops of plants as they browse throughout the growing season. As in many areas of the Appalachians, deer populations and pressure is high. Thus far, repellants show some success in discouraging deer predation on larger-scale commercial ginseng plantings in the county. Hunting/shooting offending deer is another method that growers have used to reduce the numbers of deer in plantations. Predation permits are encouraged, if not required, by the North Carolina Fish and Wildlife Service.

Voles have, over the last two years, become the most damaging pest to established ginseng plantings in the county. Chemical control, using anti-coagulant baits (Ramick, in particular), seems to be the most effective method for reducing their predation on roots. As voles do not truly hibernate and feed year-round, winter application of bait is necessary as well. Mild winters in 2016 and 2017, we believe, have led to a proliferation in the vole population in Watauga County. Traps have not been effective. At one farm, over 100 traps were baited and set in high density mature beds that were being ravaged by voles during a two-week period in late spring, 2016, and only yielded three shrews—which perhaps were there to feed on the voles. Based on a literature review, shrews do not seem to favor ginseng for consumption. One grower released ten black rat snakes into debris piles on his farm in an attempt to provide 'biological control' of this pest. However, it's impossible to know if this strategy is effective.

Lessons Learned

With several thousand pounds of ginseng seed now established in the county and continued interest by smaller-scale landowners in getting started, Watauga County Cooperative Extension and its participating ginseng growers have noted the following observations and 'lessons learned' for this burgeoning forest medicinal industry in our county that may be relevant for other efforts to promote the production of wild-simulated (or a variant thereof) ginseng in other rural forested communities where ginseng can be grown:

- There is great interest from private landowners in establishing ginseng in their underutilized forest lands for conservation, diversification, and additional income. Levels of interest vary from individuals wishing to plant a half pound or less of seed per year to some interested in planting over 10 lbs of seed per year (see Appendix 1 workshop evaluation summary).
- While there are resources on how to select a good site, plant, and manage for ginseng disease risks, there are not readily-available sources of information regarding how to reduce predation pressure from animal pests.
- There are very few Extension and other technical resource personnel within agencies and universities who specialize in the production of

ginseng/forest medicinals. The Watauga County Extension office receives more calls each year from landowners from other counties and states looking for information (to speak with a person) about ginseng production. Grant funding was acquired to pay for specialists in the field to come to the county and provide their expertise for commercial-size growers. Social media and online sources provide more opportunity for questions to be answered by peers (and sometimes experts), but there are few local resources for prospective producers. The newly formed Appalachian Beginning Forest Farmers Coalition is attempting to bridge some of these gaps. (http://www.appalachianforestfarmers.org/)

- Getting producers and interested landowners together through Extension or Agency/Organization-facilitated meetings and workshops is a great way to bring this forest 'crop' out of the shadows and allow practitioners to share information. Finding a source of seed and/or rootlets for 'starter-kits' encouraged participation and interest.
- High quality and reliable sources of seed are expensive and difficult to come by. There is a bias, justified or not, against purchasing more-readily-available and less expensive Canadian or Wisconsin cultivated seed. A new source of seed production will hopefully be a by-product of Watauga's programming efforts.
- There is some level of market uncertainty for the larger-scale commercial ginseng growers in the county and certain data points are unavailable/unknown, since there is no published data on this type of production. Estimated yield per acre is unknown due to unknown mortality rates and variability in causes/frequency of mortality (disease, pests, etc.), future price points, and cost of labor – roots will have to be hand harvested, as there is no equipment available to handle the terrain. Producers here are not tilling beds or fertilizing beyond phosphorus and gypsum at the time of planting. Best guest estimates are included below (see Appendix 2: production estimates).
- Ginseng production at an economic level is not for the faint of heart! For a 7-10 year (hopefully) crop, a lot can happen between disease, pest pressure, and the ever-present threat of theft.

Appendix 1: *Watauga County Ginseng Production Course, Evaluation Survey Analysis (2016). 84 total workshop participants. 56 surveys returned (66% survey response rate).*

Attendance

- 76.8% attended **both** the field and classroom sessions.
- 14.3% of the participants only attended the **classroom session**.
- 50% of the participants who only attended the classroom session reported to already know **some** about growing ginseng **before** attending the workshop.

Knowledge about growing ginseng before attending the workshop

- 53.6% of the survey participants reported to know **nothing** about growing ginseng before attending the workshop.
- 35.7% of the survey participants reported knowing **some** about growing ginseng.
- 10.7% of participants reported knowing **a lot** about growing ginseng before attending the workshop; though 5 out of 6 participants report also learning a lot as a result of their attendance, with the remaining one participant reporting to only learn **some.**

Amount of increase in knowledge about ginseng as a result of workshop

- 0 participants report only **a little** increase in their knowledge about ginseng
- 5.4% of the participants report only learning **some** about ginseng.
- 94.6% of survey participants report their knowledge on ginseng increasing **a lot** as a result of their attendance of the workshop.

Workshop value

- 100% of the participants reported the workshop to be worth the money they paid. (Participants were charged $75 for the workshop and received ½ lb of seed).

Consideration of planting more ginseng as a result of attendance of the workshop

- 100% of participants reported that **yes** they would consider establishing more ginseng as a result of the workshops

Amount of ginseng participants would potentially be interested in planting in the future

- 17.9% of participants report they would be interested in planting **1/2 lb or less**
- 39.3% reported they would be interested in planting **1 to 5 lbs** of ginseng

- 17.9% reported a potential interest in planting **5 to 10 lbs** of ginseng in the future.
- 14 of 56 or 25% of participants were potentially interested in planting **10 lbs or more**
- There was no significant correlation between the amount a participant was interested in planting in the future and the rest of their answers to the survey.

Appendix 2. *Watauga County Ginseng Program: Estimated Yields for low & high density, wild simulated planting* (Estimates based on known and historical data related to ginseng production and conservative price per pound estimate. So far, no producer in the county has yet to reach final and comprehensive harvest to provide more accurate figures. Estimates factor in 50% mortality rate of ginseng by final harvest after 9-10 years)

Low density wild simulated planting & yield estimates (no-spray):

43,560 sq. feet per acre. Avg. plantable area = 21,780 sq. feet/acre
1 oz seed/100 sq feet = 218 oz of seed = 13.6 lbs of seed per planted acre
6,000 seed per pound * 13.6 lbs of seed = ~81,600 plants
50% mortality after 7-9 years = 40,800 plants/roots
Takes ~ 300 roots = 1 dry pound; 40,800 roots/300 = 136 dry lbs
136 lbs * $400 per lb = $54,400 gross estimate

High Density "Wild-Simulated" planting & yield estimates (high density/fungicide applied based on weather. Beds untilled and no fertilizer):

6,000 seed per pound * 50 lbs (avg. per acre) = 300,000 plants
50% mortality after 7-9 years = 150,000 plants
~ 300 roots = 1 dry pound
150,000/300 = 500 dry lbs
500 lbs * $400 per lb = $200,000 gross estimate

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“Connecting Appalachian Icons: The importance of conserving plant-animal mutualisms in a changing world.”

(poster presentation)

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Abstract

Alteration of mutualistic interactions can negatively affect population growth and persistence. As a declining species with economic and cultural value, and a life history similar to many other understory herbs, American ginseng has become a focal species for many demographic and conservation-based studies. However, little research has been conducted to understand a critical mutualism for ginseng populations: seed dispersal. Anecdotally, ginseng seeds have been classified as gravity dispersed; but, the production of red, fleshy-fruits suggest a mutualism with animals. Trail cameras were used to identify thrushes, particularly wood thrushes (*Hylocichla mustelina*), and small mammals as potential dispersers. Through feeding studies, thrushes were identified as the primary seed dispersers, while small mammals were identified as predators. Radio transmitters were used to determine potential dispersal distances by wood thrushes and compared with field observations of wood thrush presence/absence and ginseng distribution at 28 populations. Wood thrushes were found to be predictive of ginseng distribution within a site. Dispersal by wood thrushes is likely to be important for the persistence of ginseng as harvest, deer browse, and climate change continue to threaten populations. However, wood thrushes are also a declining species – linking ginseng and wood thrush conservation.

"Antidermatophytic Effect of Black Walnut hull, *Juglans nigra*"

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Abstract

Athlete's foot, ringworm, jock itch and fungal nail infections are all caused by dermatophytes, making dermatophytosis the most common type of fungal infection. More than $500 million is spent worldwide to treat these fungal infections. *Juglans nigra* has a long history of use by herbalists in the treatment of fungal infections. Several studies have been published on the antifungal activity of the related *Juglans regia*, which is used in Europe and Asia, but little research has been published exploring anti-fungal effects of *J. nigra,* which is endemic to eastern North America and preferred by American herbalists. Fresh and dry black walnut hull preparations are used with the fresh often considered more potent than the dry hull. The objective of this project was to investigate the difference in anti-dermatophyte activity of fresh and dry ethanolic, aqueous, and glycerin extracts of black walnut hull.

"RootReport: Measuring the Market for Forest Medicinals"

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Abstract

Few American non-timber forest products (NTFPs) are systematically tracked, meaning that the size and distribution of harvests, value of products and trends in production over time are often unknown. This increases risks for potential growers, harvesters and buyers, and is a barrier to effectively managing wild populations. RootReport (www.rootreport.frec.vt.edu) was created as a Virginia Tech extension program to measure output for medicinal plants other than ginseng being harvested in deciduous forests in the eastern US. A survey was developed and sent to primary buyers of medicinal plants in 15 states, many of whom were also interviewed. The project was designed to that data in a format usable for multiple stakeholders, including participants. An online platform hosts results from previous years, and connects users with other resources, such as materials about growing and stewarding medicinal plants, and other institutions and organizations that support NTFP production. The presentation will show results compiled from three years of data collection and discuss the future of the project.

"Producing wild leek in forest farming under northern climates"

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Abstract

This paper presents the results of experiments we have been running over the past ten years in order to improve wild leek growth under forest farming. Wild leek thrives under low temperatures typical of early spring. Under more northern climates, the plant annual cycle is compressed in time, which reduces bulb growth. Planting wild leek under trees leafing out late (oak, ash or walnut) prolongs wild leek's growing period and improves annual growth. However, seedlings behave differently from mature plants and can continue their growth under shade conditions in the summertime. High natural plant density negatively affects plant growth and appears to expose the plant to pest outbreaks such as spotted snake millipedes. Partial bulb harvest can improve growth of the remaining bulbs by reducing plant density. Leaf harvest can be sustainable if harvest occurs late in the season and the plant is allowed to recover its initial size before being subjected to another leaf harvest. Organic fertilizers improved plant growth whereas gypsum is recommended when planting in soils low in calcium. The presence of litter, although maintaining the soil slightly cooler than in absence of litter, did not influence plant growth, but improved wild leek survival the year following planting. Soil tilling did not improve survival nor plant growth, but could nevertheless be useful in some sites, in addition to facilitate bulb planting. Further testing is needed to optimize fertilization (formula, application rates and frequency), soil tilling and litter, along with pest management studies.

Keywords: agroforestry, *Allium tricoccum*, light response, mineral fertilization, plant density, plant harvest

Introduction

Wild leek or ramp (*Allium tricoccum* L.) is a well-known forest herb in northeastern USA and eastern Canada, due to its culinary properties. There are even some ramp festivals in spring each year. Wild leek mostly thrives in rich hardwood forests: it is present from Tennessee and North Carolina to the southern part of eastern Canada; and from the East Coast up to South and North Dakota (Flora of North America).

As most forest herbs, wild leek exhibits slow growth rate, yet plant populations can be very dense due to clonal propagation (Nault and Gagnon 1993). Nevertheless, there is great concern regarding the capacity of wild leek to tolerate repeated harvests, especially large-scale harvests to support a commercial market (Nantel et al. 1996; Rock et al. 2004). The species is listed as special concern in Maine, Rhode Island and Tennessee, and as endangered in the province of Quebec, where large-scale harvests occurred in the 1970s leading to the extinction or near extinction of many populations (Couillard 1995). In response to this dramatic decline, the provincial government passed a law that strictly forbids selling of wild leek in Quebec, and stipulates that forest owners can only harvest 50 plants per year for their own consumption. Besides commercial harvests, long-term overabundance of white-tailed deer as well as destruction of habitat due to urbanization both threaten populations. Deer consume wild leek to some extent, although it is not one of its preferred plant species (Anderson 1994).

As for other exploited forest herbs, cultivation under forest farming can be an avenue to pursue in an attempt to reduce the pressure on natural populations while fulfilling the demand from consumers. We thus initiated a series of experiments to characterize the conditions that favour the establishment and yield of wild leek planting under forest farming and to quantify the impact of leaf and bulb harvest on plot yields during the following years. The effects of 1) the light environment in the understory, 2) temperature, 3) litter, 4) soil tillage, 5) fertilizers and gypsum, and 6) plant density on plant growth were quantified in plots established from bulbs. We also measured the impact of different intensities of either leaf or bulb harvest on subsequent plant growth. These studies have furthered our knowledge of the biology of the species, which in turn helped us identify the optimal yield conditions.

Material and Methods

Readers are encouraged to refer to the published papers for the details regarding Material and Methods. The effect of the tree canopy has been published by Dion et al. (2017); the effect of light quantity has been published by Dion et al. (2016a); the fertilization trials have been published by Bernatchez et al. (2013); the effect of plant density and of leaf and bulb harvest has been published by Dion et al. (2016b), and the effect of growth temperature has been published by Bernatchez and Lapointe (2012). Hereafter we described the methods for the latest studies not yet published.

Soil Tillage

Plots (1.65 x 0.9 m) were first established in spring 2014 in two sugar maple forests located in the Argenteuil regional county municipality, in the Laurentides region (45° 39′ N; 74° 20′ W). Two treatments, one tilled and a control not tilled were repeated 5 times on each site, for a total of 20 plots. Litter was first removed and soil tilling was performed using a Pulaski to a depth of 15 cm. Organic fertilizers (55–110–82 kg ha^{-1} of N–P_2O_5–K_2O) and gypsum (3000 kg ha^{-1}) were then spread over the plot in June 2014 and May 2015, before litter was put back onto the plots (Bernatchez et al. 2013; see *Amendments* in the Results and Discussion section). Fifty bulbs were planted in each plot in July 2014 at a depth of 5 cm. The following spring, high mortality rates were observed. We moved the few remaining bulbs outside the plots, and planted 50 new bulbs per plot in May 2015. For both plantings, bulbs came from seizure by governmental authorities. Bulbs with a diameter of 10 to 15 mm were selected for the two experiments (Soil Tillage and Litter).

Survival was estimated the following spring based on the number of plants that emerged/number of bulbs planted per plot. Total leaf width was measured at complete leaf unfolding. Bulb diameter was measured in late June after complete leaf senescence. The top of the bulbs were gently dug then the diameter was measured using calipers. For bulbs that divided, we measured each bulb individually, then calculated the diameter of a bulb that would represent the same total surface area as the sum of the individual bulbs (see Dion et al. 2016b for details).

Litter

Plots of the same size as for the Soil Tillage experiment were established in the same two locations. Litter was first removed and soil was tilled before planting. The same doses of fertilizers and gypsum as for the Soil Tillage experiment were incorporated into the plots before planting. Three treatments were compared: no litter, natural litter put back on the plots following tillage, and 3 cm of ramial chipped wood (RCW) spread onto the plots following tillage. The RCW was made of sugar maple branches finely chipped. Each treatment was repeated 5 times for a total of 30 plots. Similarly to the Soil Tillage experiment, a first planting took place in July 2014, and a second planting in spring 2015 with 50 bulbs per plot. Natural litter was removed from the no litter and the RCW plots before the second planting as well as the following spring. Data loggers (iButton, Maxim Integrated, San Jose, CA) were placed at a depth of 5 cm to record soil temperature throughout the year every 2.5h. Mean per day was calculated then averaged over each month. Data presented are for the month of May based on the data recorded in each plot the year planting occurred and the following year. The same plant variables (survival, total leaf width and bulb diameter) as for the Soil Tillage experiment were also monitored.

Results and Discussion

Light environment in the understory

Wild leek senesced later under late canopy closure than under canopies that closed earlier in the season; this longer leaf lifetime translated into larger bulbs over the years (Dion et al. 2017). The composition of the tree canopy thus influences wild leek growth. Tree species that bud burst later, such as *Fraxinus*, *Juglans*, *Quercus*, *Tilia* or *Carya* spp, provide a light environment that can favour wild leek growth, at least at the northern limit of its distribution, as the duration of the high light conditions between complete snow melt and aboveground canopy closure decreases from north to south in hardwood forests (Routhier and Lapointe 2002).

Although mature wild leek plants are exposed to high light conditions in spring, this is not the case for seedlings, which grow under the canopy of mature wild leeks especially in dense patches. We noticed that seedlings and juveniles senesce somewhat later than mature plants. We thus wanted to quantify the response of mature plants to light availability in spring compared to that of seedlings and juveniles. All plants were exposed to 60% of incident light for 30 days simulating conditions in the understory in early spring. They then received either 60, 10 or 4% of ambient light until complete leaf senescence (Dion et al. 2016a). Mature plants and three-year-old juveniles senesced much earlier than two-year-old and seedlings even when subjected to the same light conditions. Within each plant size, wild leeks produced larger bulbs under higher than under lower light conditions. However, while mature plants and three-year-old juveniles kept their leaves longer under higher light conditions in order to produce larger bulbs, seedlings senesced earlier under higher light conditions. Timing of leaf senescence of two-year-old juveniles was not affected by light availability. We concluded that seedlings and two-year-old juveniles behave as summer green plants that maintain their leaves for much longer than mature plants which behave as spring ephemerals (Neufeld and Young 2014). Young wild leeks acclimated their leaves to lower light conditions whereas mature plants exhibited no acclimation and induced leaf senescence once exposed to decreased light availability. Seedlings, which appear source-limited, may require a longer time than mature plants to accumulate the carbon reserves necessary to survive until the next year. Mature plants with their much larger leaf-to-bulb ratio can fill their bulb much faster than seedlings, which may have led to the evolution of the spring ephemeral growth habit.

Temperature

Spring ephemerals need to be able to grow at low temperatures since they sprout early in spring. Yet, most plants adapted to grow at low temperatures still perform better at higher temperatures, more typical of summer time (Lapointe and Lerat 2006). We have already shown that other spring geophytes such as trout lily (*Erythronium americanum*) and spring crocus (*Crocus vernus*) do grow better at 12/8 °C (day/night temperatures) than at 18/14 °C (Lapointe and Lerat 2006; Badri et al. 2007). Trout lily does even better at 8/6 °C than at 12/8 °C (Gandin et al. 2011). We tested the impact of these three growth temperatures on wild leek growth under controlled growth conditions (Bernatchez and Lapointe 2012). Similarly to the other spring geophytes, wild leek final bulb size was greater at 12/8 °C than at 18/14 °C, but bulb size was smaller at 8/6 °C than at 12/8 °C. This study confirmed that spring geophytes are well adapted to grow at low temperatures to the extent that their growth decreases at higher temperatures. We have previously shown that soil more than air temperatures affect growth of geophytes (Badri et al. 2007).

Litter

When wild leek was grown under different tree species, we noticed a positive relationship between litter thickness in spring time and wild leek survival rate (Dion et al. 2017). Soil temperature under a thicker litter was cooler and less variable than under a thinner litter. As tree litter differs in other aspects than litter thickness, we ran a specific experiment to compare the effect of natural litter, a RCW mulch, and no litter on wild leek survival and growth. We included a treatment with RCW mulch as a potential solution on forested sites or tree plantations where natural litter is degraded by early summer, due to the activity of earthworms (Corio et al. 2009). Plants in this experiment were strongly affected by the bulb mite (*Rhizoglyphus robini*) which most likely influenced the conclusions we can draw from this study. We reported an increased survival in plots covered with RCW mulch than in plots with no litter (Fig. 1). Natural litter presented intermediate results. The litter treatment did not affect the total leaf area (data not shown) nor the bulb size (Fig. 1). Soil temperature in spring was lower in plots covered with litter or mulch than in plots without litter (Fig. 2). We could expect that soil temperature differences lead to differential growth of wild leek over time. Furthermore, litter — either natural or as a mulch — could lessen evaporation and maintain a higher soil water content, a condition that favours wild leek growth (Nault and Gagnon 1993; Bernatchez et al. 2013).

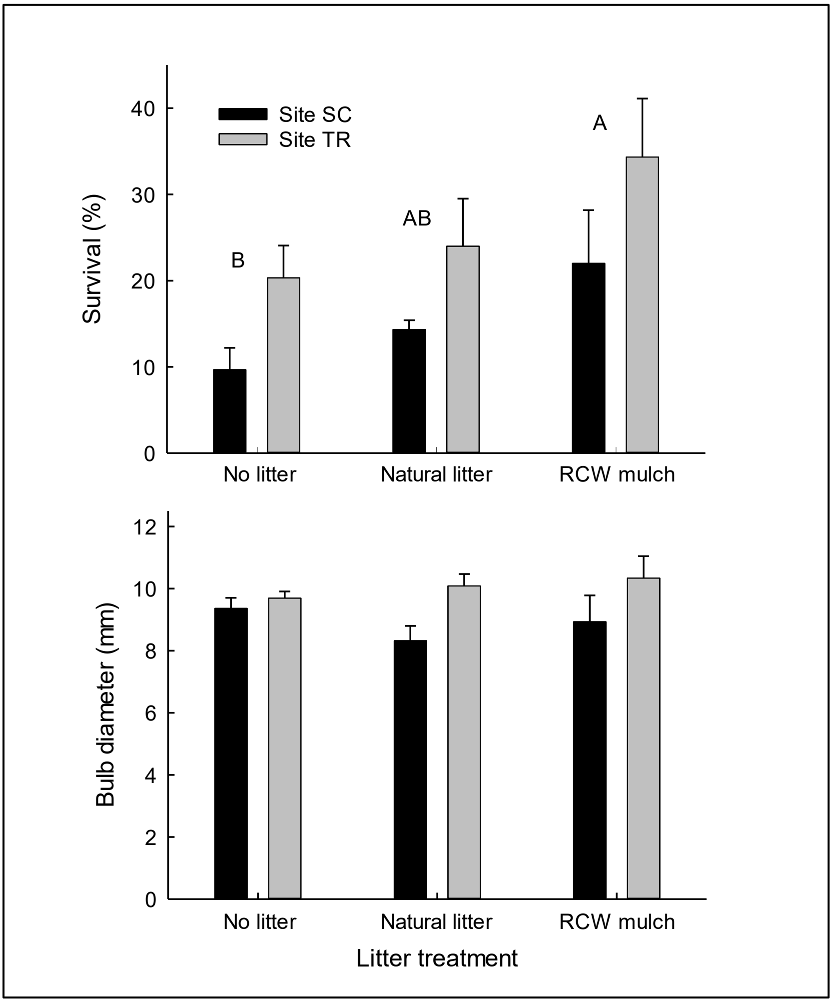

Fig. 1: Effect of the presence and type of litter on wild leek survival and bulb size. Survival was recorded in early spring and bulb diameter was measured in early summer, the year following planting. RCW: ramial chipped wood. Different capital letters indicate differences among litter treatments, both sites confounded

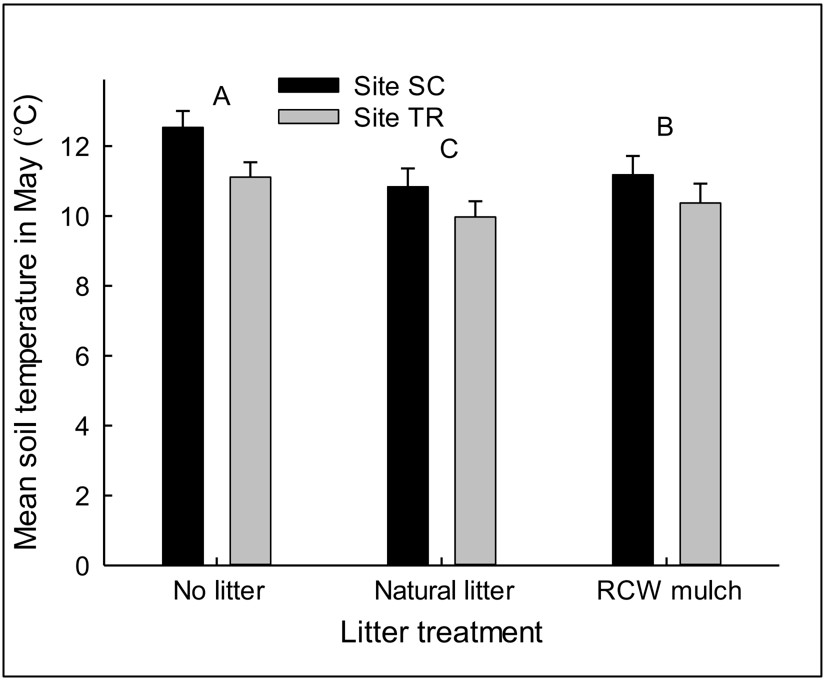

Fig. 2: Effect of the presence and type of litter on mean soil temperature during the month of May. Soil temperature was measured at the same depth as the bulbs, i.e. 5 cm during the year planting occurred and the following year. RCW: ramial chipped wood. Different capital letters indicate differences among litter treatments, both sites confounded

We found very few impacts of litter or mulch on plant mineral nutrition, as estimated from leaf nutrient content (data not shown). Surprisingly, wild leek absorbed more calcium (Ca) in plots without litter (5.6 ± 0.3 mg g^{-1}) than in plots covered with RCW mulch (4.6 ± 0.2 mg g^{-1}), despite the fact that soil Ca availability is strongly correlated with the rate of Ca mineralization from organic matter (Dijkstra 2003). The absence of a litter might increase the rate at which Ca is released from gypsum through higher temperatures and more rapid rainfall penetration in the soil, explaining the higher absorption of Ca by wild leek. Natural litter degraded faster than RCW mulch (pers. obs.) which could explain the slightly higher concentration of Ca in wild leek under natural litter (4.8 ± 0.2 mg g^{-1}) than under mulch.

Soil Tillage

Tillage is prescribed for most herbs grown under forest farming (Persons and Davis 2005), but takes time and effort. We wanted to compare growth of wild leek following direct planting with that of bulbs planted in tilled soil. In the absence of soil tillage, litter still needs to be removed to spread fertilizers and plant the bulbs before putting the litter back onto the ground. We did not see any impact of soil tilling on either plant survival, total leaf area or bulb size (Fig. 3). Soil tillage did not impact plant mineral nutrition the following year (data not shown). Short-term tillage does not always improve nutrient mineralization as demonstrated in other systems (Kingery et al. 1996; Kristenen et al. 2003). Tillage does not seem necessary, although it certainly facilitates bulb planting. However, as results varied between sites (Fig. 3), we need to test the impact of soil tilling in different types of soils to determine whether tilling would benefit wild leek under specific conditions.

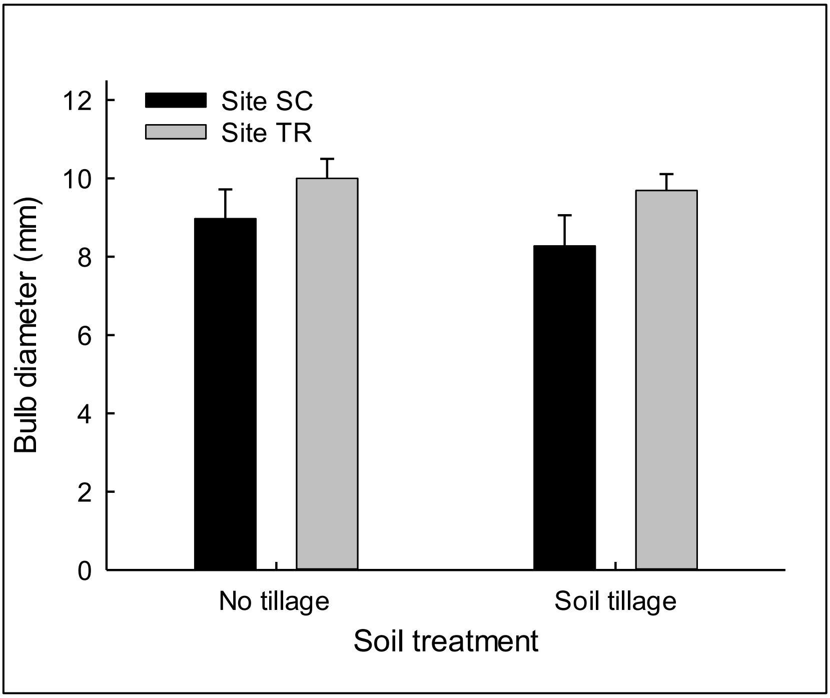

Fig. 3: Effect of soil tillage on wild leek bulb size. Bulb diameter was measured in early summer, the year following planting

Amendments

Wild leek thrives on rich soils with high Ca availability (Rousseau 1974). We thus tested the impact of organic fertilizer and gypsum on its subsequent growth over two years (Bernatchez et al. 2013). Organic fertilizer composition was chosen based on recommendations for cultivated garlic and leek in organic soil and from Nadeau and Olivier (2003) for forest farming of ginseng. We chose Bio-Garden (4–3–6; McInnes Natural Fertilizers, Stanstead, QC, Canada), a granulated slow-release fertilizer made from feather meal, fossil bone meal (natural rock phosphate) and Sul-Po-Mag to which we added more fossil bone meal (0–13–0) to enhance P availability. Two levels of fertilization were tested: 27.5–55–41.3 kg ha^{-1} (N–P_2O_5–K_2O) and 55–110–82.5 kg ha^{-1}. We combined these fertilizer treatments with the addition of gypsum (Uncalcined Gypsum Products, $CaSO_4$, Georgia-Pacific Gypsum Corporation, Atlanta, GA, USA) at a rate of 3000 kg h^{-1}.

Gypsum had limited impact on wild leek (Bernatchez et al. 2013). We reported higher concentration of Ca in the leaves, but no impact on plant growth over two years. The Ca/Mg ratio was affected by the addition of gypsum. The sites were rich in Ca (2486 to 9344 kg ha^{-1}). We ran a second experiment on these same sites where we modulated the amount of Ca (1000 vs 3000 kg ha^{-1} of gypsum) and magnesium (Mg) (addition of chelated Mg to attain 75 kg ha^{-1} vs 41 kg ha^{-1}) to try to establish a better balance between Ca and Mg. Wild leek juveniles exhibited slower growth in plots fertilized with a surplus of Mg compared to plants in control plots (55–110–82.5 kg ha^{-1} of N–P_2O_5–K_2O; 3000 kg ha^{-1} of gypsum), whereas reducing the amount of gypsum did not affect plant growth. The addition of gypsum would need to be adjusted as a function of natural Ca availability in the soil as it can greatly improve wild leek growth in soils low in Ca (Ritchey and Schumann 2005).

Mineral fertilization did improve plant growth (leaf width in year 1/leaf width in year 0) the following year compared to unfertilized plants (Bernatchez et al. 2013). They also produced a larger bulb for a similar leaf size. However, these results were only observed the year following the fertilization. Two years after fertilization, size of fertilized plants no longer differed from that of control plants. Nitrogen (N) was the only nutrient that increased in fertilized compared to unfertilized plants and soil analyses indicate no differences among plots two years later. We thus concluded that either fertilizers would need to be applied each autumn (at the time new roots appear), or that fertilization was mainly useful to recover from transplantation shock. Nitrogen appears to be the most limiting macronutrient, based on the N: P ratios recorded. A second experiment was thus conducted in which fertilizers with either more N (110 vs 55 kg ha-^{1}) or less phosphorus (P) (41 vs 110 kg ha-^{1}) were compared to the initial treatment (55–110–82.5 kg ha^{-1} of N–P_2O_5–K_2O; unpubl. data). We recorded no difference among the three fertilizer treatments in terms of leaf or bulb width. This study indicated that the addition of bone meal (0–13–0) to the Bio-Garden fertilizer is not necessary to insure good growth rate of wild leek.

Plant Density

Wild leek tends to grow in dense patches due to the propagation method by division of the bulb (Nault and Gagnon 1993). Competition among shoots could eventually decrease their growth rate. We tested different planting densities, including densities similar to those recorded in nearby natural populations to quantify the impact of the plant density on plant growth and to determine optimal planting densities (Dion et al. 2016b). Four different densities were tested: 44, 89, 178 and 356 bulbs m^{-2}. Four years after planting, individual plants in the densest plots were smaller than in the three other plot densities. Plant division was also affected by plant density, to the extent that four years after planting we recorded the same number of shoots per plot in the two highest densities whereas in the lowest density there were 50% more bulbs than initially planted. At year 5, mortality started to spread in the plots, starting with the dense plots but eventually affecting all plots in year 6, since they were fairly close to each other. Dying of compact clumps has been reported previously (Nault and Gagnon 1993). The soil was infested with spotted snake millipedes (*Blaniulus guttulatus*). However, further studies are needed to determine if the millipede can be the initial cause of bulb decay or if another pest or pathogen weakens the plant prior to the attack by the millipede. Considering the workload of tilling soil, a density of around 89 bulbs per m^{-2} would be optimal as it represents the best compromise between individual plant growth and division and plant yield per area.

Leaf Harvest

Although wild leek plants are usually harvested as whole plants, harvested leaves can be used fresh or prepared (e.g. pesto) in different culinary dishes (Facemire 2009). From the point of view of the plant, leaf harvest is much less destructive than bulb harvest, but can still affect carbon reserves and therefore bulb size if it occurs too early in the season as shown on onion (Muro et al. 1998). Removing leaves before senescence will also affect plant mineral nutrition status since many of the nutrients located in the leaves are massively translocated to the bulb during leaf senescence (Nault and Gagnon 1988; Rothstein and Zak 2001). We tested the impact of harvesting 50 or 100% of the leaves 15, 20 or 25 days after complete leaf unfolding on subsequent plant growth (Dion et al. 2016b). As expected, plants were less affected when only 50% of the leaves were removed than when all leaves were removed. Plant size, plant division and flowering were affected by leaf harvest. Wild leek plants were also less affected when leaf harvest occurred later in the season. According to these results, we would recommend harvesting half of the leaves 20 to 25 days after leaf unfolding, since plants were able to completely recover within a year or two. However, in a commercial situation, harvesting all leaves would be faster than harvesting half of the leaves on each plant. We thus recommend waiting until leaves of harvested plants have attained their pre-harvest size before subjecting them to another harvest. This might take two to four years depending on the conditions as warm or dry springs do negatively affect wild leek growth (Bernatchez et al. 2013).

Bulb Harvest

As plant density can attain high values in natural populations — 350 to 400 bulbs m^{-2} — we tested the impact of reducing the number of bulbs within a patch on subsequent plant growth (Dion et al. 2016b). All plants were dug out (plots of 100 mature plants) then either all were replanted (control plants to test the impact of digging on subsequent growth), or 20% or 40% of mid-size plants were harvested and the rest replanted. We also followed plots which were not dug out as a second control. Digging strongly affected plant size the following year as reported previously (Vasseur and Gagnon 1994). Digging out all bulbs is however the only efficient method in dense plots to select mid-size bulbs without damaging the large reproductive and small regenerating bulbs which are then replanted in order to favor recruitment from both bulb division and seedling establishment. All the chosen plots were high-density plots but due to large variation in plant density, there was an important overlap in terms of bulb density per plot following bulb harvest which potentially diminished the probability to detect differences among treatments. Two other factors complicate the analysis of such experimental design. Harvesting mid-size plants can influence the mean plant leaf size depending on the proportion of large and small plants in the plot. This can be corrected to some extent by estimating the mean leaf size in control plots after having removed from the data, 20 or 40% of the mid-size plants within these plots. Stochastic events also complicate the analyses. In this study, all plants reduced in size from year 3 to year 4 to the extent that plants were smaller in year 4 than in year 2. Nevertheless, the results suggest that plant growth rate was increased in plots subjected to partial bulb harvest compared to that of control (un-dug) plots. To lessen competition in dense plots would require reducing plant number down to 50 to 100 plants m^{-2} as shown in the Plant Density experiment (Dion et al. 2016b). The grower would need to wait until the plant density has attained high values again (over 200 plants m^{-2}) before harvesting in the same plots; that could take many years due to the slow growth of the plant and to stochastic events.

Conclusions

These studies have allowed us to better define the conditions that improve wild leek growth under forest farming conditions at its northern limit of distribution, along with factors that will require further studies. Wild leek would benefit from being planted under late closing canopy such as under oak, ash or linden. The presence of a thick litter appears to improve growth, although further studies are needed to confirm these preliminary results and determine whether long-term exposure to the litter of some tree species such as that of walnut could be deleterious. Mineral fertilization improves plant growth but since an annual fertilization appears required, economical studies are needed to balance costs and benefits. Furthermore, micronutrients should be monitored as they could be limiting in some soils. Gypsum should be added in soils presenting low Ca level. We did not find any short time benefit to soil tilling, but this factor would need to be tested in different types of soils before drawing conclusions. We recommend a planting density of no more than 100 bulbs m^{-2}, to avoid competition over time as bulbs get bigger and divide, but also to reduce the probability of infestation that can rapidly destroy the whole plot. Indeed, yearly monitoring of bulb mites and millipedes could prevent extensive damage. Leaf harvest is sustainable but should occur at least 20 days after complete leaf unfolding; furthermore, growers should wait until plants have reached pre-harvest size before harvesting the same plants again. Partial harvest of plants in very dense populations could improve subsequent growth of the remaining plants. However, partial harvesting in natural populations should only occur where the species is not endangered and these sites should then be left to recover their initial densities before another partial harvest is allowed.

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"Conservation status of North American forest botanicals: What do we know? Why does it matter?"

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Abstract

Based on the International Union for Conservation of Nature (IUCN) Sampled Red List Index, 20% of plants in the world are threatened with extinction. According to NatureServe rankings, a larger proportion (26%) of the North American flora is threatened. While NatureServe data are more complete for North American plant species, the more limited IUCN global Red List assessment data enable analysis of current knowledge of the conservation status of forest plants in North America and the extent to which they are threatened by biological use, including gathering. The IUCN Plants for People initiative is focused on conservation assessment and action for economically important plants, including non-timber forest products (NTFPs). This initiative invites broad collaboration to improve what we know about the conservation status of forest botanicals and other plant species important to livelihoods, health, and commerce in North America as a basis for conservation and sustainable use.

Keywords: North America, plants, botanicals, non-timber forest products, conservation, sustainable use

What do we know about the conservation status of the world's plant species?

An estimated 391,000 species of vascular plants are currently known to science (Royal Botanic Gardens/RBG Kew 2016). Before 2010, only about 3% (12,873) of identified plant species were included on the global IUCN Red List of Threatened Species (RBG Kew 2012). The majority of those species had been assessed because they are rare endemics or already thought to be threatened. This gave a view of the overall conservation status of plants that was likely biased towards high proportions of threatened species.

Under the United Nations Convention on Biological Diversity (CBD), the Global Strategy for Plant Conservation (GSPC) set a target to achieve "a significant reduction in the current rate of loss of [plant] biodiversity, by 2010" (United Nations Environment Program/UNEP 2002). This target was revised for

2011-2020 to call for “an assessment of the conservation status of all known plant species, as far as possible, to guide conservation action” (UNEP 2011). The limited number of plant global IUCN Red List assessments overall, and the bias towards including assessments of known threatened species in the IUCN Red List have stood in the way of achieving both targets.

In response to these obstacles, IUCN Red List programme and Red List Partners, including RBG Kew, launched the Sampled Red List Index (SRLi) for Plants and began regular conservation status assessments of a significant sample of the world’s flora (RBG Kew 2012). Results of the first SRLi for plants included:

- One in five species is threatened with extinction
- Major threats to plant species worldwide are agriculture – conversion of plant habitat – and biological resource use.

IUCN, working with the plant specialist groups of the Species Survival Commission, and the botanic garden community, led by RBG Kew and Botanic Gardens Conservation International (BGCI), have developed some new plant conservation assessment approaches and tools. These include:

- many comprehensive plant assessments of complete taxonomic groups (e.g., cycads, conifers and gymnosperms, cacti and succulents)
- regional Red List assessments for plants completed or in process (e.g., Europe, South Africa, Brazil, Madagascar)
- annual State of the World’s Plants reports, led by RBG Kew (RBG Kew 2016, 2017)
- ThreatSearch database, created by BGCI (2017).

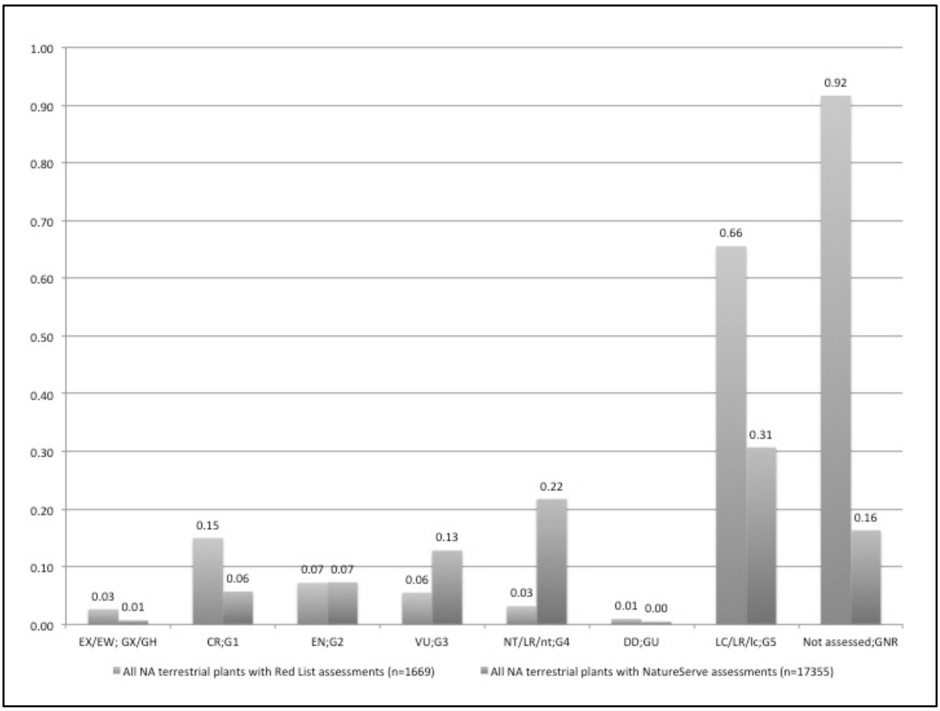
However, economically and socially important plants – plants important to cultures and livelihoods – remain significantly under-represented in IUCN global and regional Red List assessments, including NTFPs and medicinal plants. To address this gap, IUCN created the Plants for People initiative with a focus on medicinal, timber and non-timber trees, palms, and crop wild relatives (IUCN 2017a).

What do we know about the conservation status of North American[1] plant species?

[1] The boundaries of North America are defined slightly differently by IUCN and NatureServe: NatureServe data include just Canada and USA, while IUCN Red List data include the French islands Saint Pierre and Miquelon (just south of Newfoundland) as North American habitat.

There are roughly 20,000 species of vascular plants in the North American flora (Flora of North America 2008). Of these, NatureServe (2017) has ranked 17,355 species (87%). Just 1670 (less than 8%) of North American vascular plant species have published global Red List assessments (IUCN 2017b). Figure 1 compares the results of searches on both platforms of the conservation status of North American plant species. The two systems of threat classification have some differences in definitions and applications of factors (NatureServe) or criteria (IUCN), but are similar enough for this analysis. Annex 1 provides a table comparing definitions of NatureServe ranks (Master et al. 2012) and IUCN criteria (IUCN 2012).

Fig 1. Comparison of the distribution of NatureServe ranks and IUCN Red List categories for North American plant species (n=20,000)



NatureServe Explorer data are far more comprehensive in their coverage of the North American flora than are global Red List assessments, and therefore are likely less biased towards threatened ranks in the overall distribution of conservation status results. However, the IUCN Red List data and searchable attributes more readily provide insight into the conservation status of plants that occur in particular habitats (e.g., temperate forest), threats (e.g., biological resource use), and sub-categories of threat (e.g., gathering terrestrial plants).

The proportion of North American plant species assessed as threatened with extinction by NatureServe (NatureServe ranks G1 – Critically Imperiled, G2 – Imperiled, G3 – Vulnerable) is 26%, approximately equal to the proportion of North American plant species assessed as threatened (28%) by IUCN (IUCN Red List categories CR – Critically Endangered, EN – Endangered, VU – Vulnerable). However, the proportion of North American species assessed by IUCN that met criteria for Critically Endangered is higher than the proportion of species ranked as Critically Imperiled by NatureServe (Figure 1).

What do we know about the conservation status of North American forest botanicals?

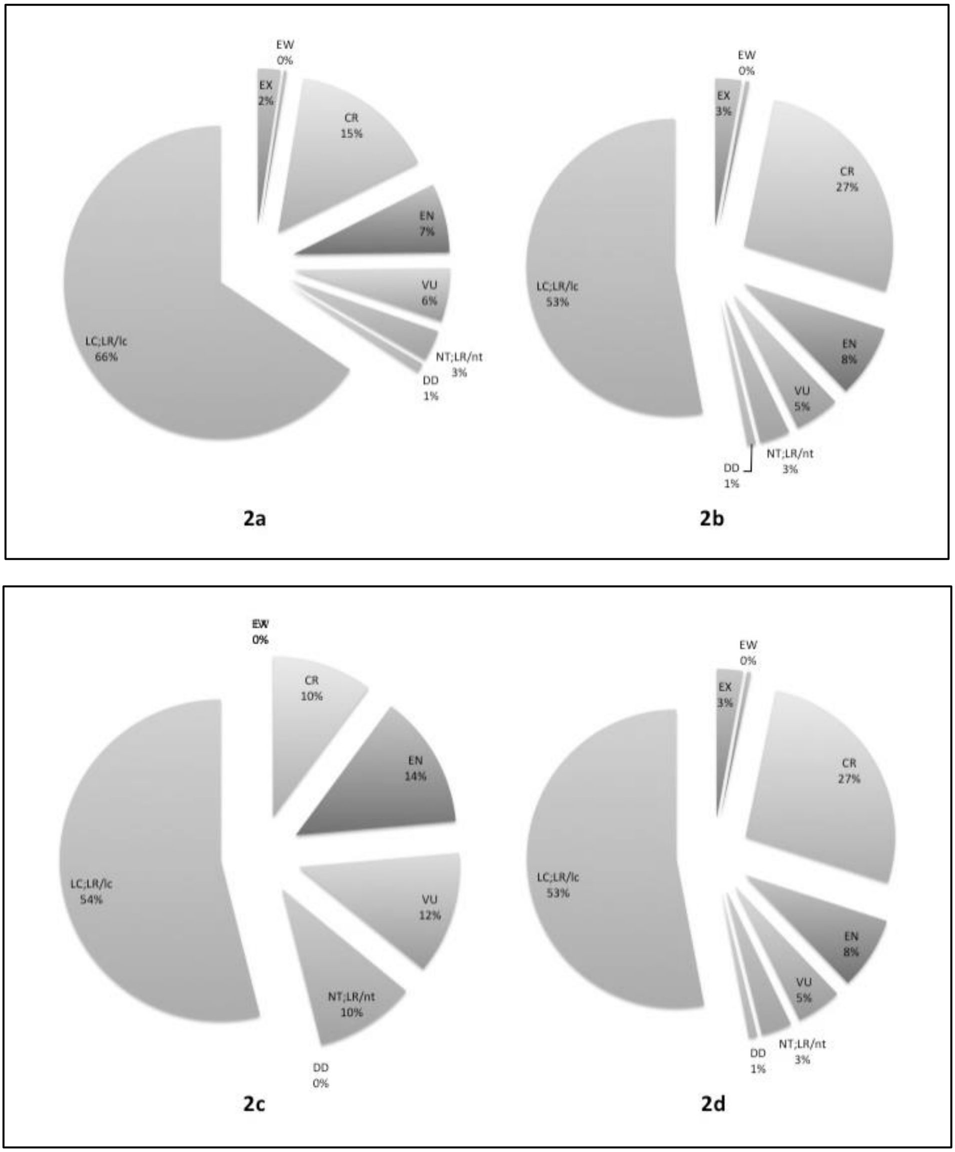
The IUCN Red List data (IUCN 2017b) were searched using the following sets of search criteria:

Search 1: Taxonomy = Plantae; Location = Land regions/North America/Canada + United States; Habitat = Forest
Search 2: Search 1 + Threats = Biological resource use
Search 3: Search 1 + Threats = Biological resources use/gathering terrestrial plants

Results of these searches include the following:

- Of the 1669 North American plant species assessed by IUCN, 670 (40%) occur in forests.
- Forest plants in North America appear to be more threatened than the flora as a whole (CR, EN, VU forest = 40%; NA flora = 28%) (Figure 2a and b).
- Biological resource use is a significant threat to 38% of North American forest species (Figure 2c).
- Gathering terrestrial plants is a type of biological resource use that threatens 49% of North American forest species (Figure 2d).

Fig 2. Comparison of IUCN Red List assessment results for (a) all North American plants with IUCN Red List assessments (n=1,669), (b) all North American plants occurring in forests (n=670), (c) all North American forest plants threatened by biological use (n=89), (d) all North American forest plants with biological uses that are threatened by gathering (n=37)



What can we do with this knowledge?

The Medicinal Plant Specialist Group of the Species Survival Commission, IUCN, is proposing a North American project as part of IUCN's global Plants for People initiative (IUCN 2017a). This project is inviting collaboration with North American networks of medicinal plant and NTFP collectors, herbalists, the commercial herbal industry, botanists and citizen scientists, educational and research institutions, government agencies, and non-government agencies – broadly, the people working with plants important to people – to work on shared concerns about the long-term survival of these important plant species in North America. Current efforts focus on five tasks:

Task 1. Red List assessments of priority species of medicinal plants. We hope to draft and publish Red List assessments for all economically important plant species in North America, but we need to start with some clear priorities. These include:

- Medicinal plant species listed in Appendices 1 and 2 of the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) that occur in North America
- North American plant species included in the World Health Organization (WHO) monographs (WHO 1999, 2002, 2007, 2009)
- "At Risk" and "To Watch" species identified by United Plant Savers (UPS 2014)

Task 2. Identification of in situ and ex situ conservation gaps (e.g., occurrence in botanic garden and genetic collections; protection of crop wild relatives; presence in and effectiveness of protected areas).

Task 3. Assessment of vulnerability to climate change.

Task 4. Integrated (in situ and ex situ) conservation strategies emphasizing sustainable wild collection, e.g., application of the FairWild Standard (FairWild Foundation 2010) and various degrees of cultivation, including woods-grown and reintroductions.

Task 5. Communication of key actions needed with those who can and must act to conserve threatened species and ensure that those not currently threatened do not become so.

The goal of the Plants for People initiative is not simply to create a Red List of threatened forest botanicals and other economically important species in North America. By better understanding the conservation status, the threats, and the conservation actions that can prevent the loss of these species, we can act to support the livelihoods, industries, health treatments, and the many additional benefits that people derive from these North American plant species.

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Annex 1: Comparison of IUCN Red List categories and NatureServe ranks

NatureServe Rank and Definition[2]	IUCN Red List Category and Definition[3]
GX – Presumed Extinct (species) Not located despite intensive searches and virtually no likelihood of rediscovery. **Extinct** (ecological communities/systems) Eliminated throughout its range, with no restoration potential due to extinction of dominant or characteristic taxa and/or elimination of the sites and ecological processes on which the type depends.	**EX – Extinct** A taxon is Extinct when there is no reasonable doubt that the last individual has died. A taxon is presumed Extinct when exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), throughout its historic range have failed to record an individual. Surveys should be over a time frame appropriate to the taxon's life cycle and life form.
GH – Possibly Extinct Known from only historical occurrences but still some hope of rediscovery. There is evidence that the species may be extinct or the ecosystem may be	**EW – Extinct in the Wild** A taxon is Extinct in the Wild when it is known only to survive in cultivation, in captivity or as a naturalized

[2] Master et al. (2012)
[3] IUCN (2012)

NatureServe Rank and Definition[2]	IUCN Red List Category and Definition[3]
eliminated throughout its range, but not enough to state this with certainty. Examples of such evidence include (1) that a species has not been documented in approximately 20-40 years despite some searching or some evidence of significant habitat loss or degradation; (2) that a species or ecosystem has been searched for unsuccessfully, but not thoroughly enough to presume that it is extinct or eliminated throughout its range.[4]	population (or populations) well outside the past range. A taxon is presumed Extinct in the Wild when exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), throughout its historic range have failed to record an individual. Surveys should be over a time frame appropriate to the taxon's life cycle and life form.
G1 – Critically Imperiled At very high risk of extinction or elimination due to extreme rarity, very steep declines, or other factors.	**CR – Critically Endangered** A taxon is Critically Endangered when the best available evidence indicates that it meets any of the criteria A to E for Critically Endangered (see Section V), and it is therefore considered to be facing an extremely high risk of extinction in the wild.
G2 – Imperiled At high risk of extinction or elimination due to very restricted range, very few populations or occurrences, steep declines, or other factors.	**EN – Endangered** A taxon is Endangered when the best available evidence indicates that it meets any of the criteria A to E for Endangered (see Section V), and it is therefore considered to be facing a very high risk of extinction in the wild.
G3 – Vulnerable At moderate risk of extinction or elimination due to a restricted range,	**VU – Vulnerable** A taxon is Vulnerable when the best available evidence indicates that it

[4] Possibly Eliminated ecosystems (ecological communities and systems) may include ones presumed eliminated throughout their range, with no or virtually no likelihood of rediscovery, but with the potential for restoration, for example, American chestnut forests.

NatureServe Rank and Definition[2]	IUCN Red List Category and Definition[3]
relatively few populations or occurrences, recent and widespread declines, or other factors.	meets any of the criteria A to E for Vulnerable (see Section V), and it is therefore considered to be facing a high risk of extinction in the wild.
G4 – Apparently Secure Uncommon but not rare; some cause for long- term concern due to declines or other factors.	**NT – Near Threatened** A taxon is Near Threatened when it has been evaluated against the criteria but does not qualify for Critically Endangered, Endangered or Vulnerable now, but is close to qualifying for or is likely to qualify for a threatened category in the near future.
G5 – Secure Common; widespread and abundant.	**LC – Least Concern** A taxon is Least Concern when it has been evaluated against the criteria and does not qualify for Critically Endangered, Endangered, Vulnerable or Near Threatened. Widespread and abundant taxa are included in this category.
G#G# – Range Rank A numeric range rank (e.g., G2G3, G1G3) is used to indicate uncertainty about the exact status of a taxon or ecosystem type. Ranges cannot skip more than two ranks (e.g., GU should be used rather than G1G4).	No equivalent category
GU – Unrankable Currently unrankable due to lack of information or due to substantially conflicting information about status or trends. Note: whenever possible (when the range of uncertainty is	**DD – Data Deficient** A taxon is Data Deficient when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status.

NatureServe Rank and Definition[2]	IUCN Red List Category and Definition[3]
three consecutive ranks or less), a range rank (e.g., G2G3) should be used to delineate the limits (range) of uncertainty.	A taxon in this category may be well studied, and its biology well known, but appropriate data on abundance and/or distribution are lacking. Data Deficient is therefore not a category of threat. Listing of taxa in this category indicates that more information is required and acknowledges the possibility that future research will show that threatened classification is appropriate. It is important to make positive use of whatever data are available. In many cases great care should be exercised in choosing between DD and a threatened status. If the range of a taxon is suspected to be relatively circumscribed, and a considerable period of time has elapsed since the last record of the taxon, threatened status may well be justified.
GNR – Unranked Global rank not yet assessed.	**NE – Not Evaluated** A taxon is Not Evaluated when it has not yet been evaluated against the criteria.
GNA – Not Applicable A conservation status rank is not applicable because the species or ecosystem is not a suitable target for conservation activities.[5]	No equivalent category

[5] A global conservation status rank may be not applicable for several reasons, related to its relevance as a conservation target. In such cases, typically the species is a hybrid without conservation value, of domes c origin, or the ecosystem is non-native, for example, ruderal vegetation, a plantation, agricultural field, or developed vegetation (lawns, gardens etc).

"Taking the Broad View: How Are Wild Ginseng Populations Faring and When Does Conservation Policy Need to Change?"

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Abstract

American ginseng has been harvested from the wild to supply the Asian traditional medicine trade in North America since the early 1700s. However, only since 1975 have federal and state regulations been in place to regulate this trade, and the harvest practices supporting it. Using data from a unique long-term formal census of 30 natural populations in 7 states, my lab examined recent trends from 2004-2014 as an indicator of how wild ginseng populations are faring. Combined with demographic modeling 'experiments' using these census data, my students have also examined alternative projections for the future of ginseng over coming decades, including factors that explain variation in population growth and viability. Over the decade (2004-2014), 25 of 30 ginseng populations declined in size from their initial numbers. The mean decline over this timeframe was 30%. The observed immediate reasons for the decline were (1) harvest; particularly illegal harvest, which was prevalent in about half the populations, and (2) deer browse; also prevalent, and distributed unevenly among populations. Age-specific demographic data clearly showed that the current 5-year age restriction is insufficient to protect wild populations. Additional analyses showed large between population variation in age-specific growth and reproduction, suggesting that an alternative criterion for harvest is needed. Also negatively impacting populations were hot, dry summers that curtailed growth and seed production, as well as intense natural or human-caused tree canopy disruption. In the long-term, landscape level changes to the forest, combined with climate change, are projected to impact ginseng populations negatively in the coming century. While regulations imposed in response to CITES listing may only impact harvest practices, they must be considered in light of the broader array of stresses experienced by natural populations. The precarious state of wild populations even in the absence of harvest pressure suggests that wild harvest practices need to be further adjusted toward the 'stewardship' end of the harvest spectrum. At the same time, forest 'farming' practices using regional ginseng ecotypes that relieve harvest pressure on wild populations need to be encouraged through any regulatory reform that takes place.

Introduction

The most important question concerning wild American ginseng populations is ‘Are populations declining?’ Historical data suggests that certainly individuals in the past were much larger than individuals today. We can see that from historical photographs (Fig. 1). We can also see the same phenomenon when studying herbarium specimens. Herbarium specimens from the 1800s and early 1900s were much larger than herbarium specimens today (McGraw 2001). Other indirect evidence includes anecdotes about fortunes made from the harvest of ginseng in the 1700s and 1800s. This includes large shipments that comprised John Jacob Astor’s fortune and shipments made by Daniel Boone Down the Ohio River. We can also hear anecdotes from harvesters today about how ginseng is getting harder to find.

Fig. 1. Photos showing harvested roots at ginseng dealer warehouses in 1929 and more recently.

One more scientific case study of ginseng harvest also involved herbarium specimens collected at different points in time (Case et al. 2007). This study showed that at least for part of ginseng’s range the rate of collection of specimens by botanists has slowed down relative to rates of collection earlier in the history of herbarium collections.

In Search of Direct Evidence for Population Growth or Decline

Ideally, we would prefer direct evidence for decline in ginseng populations but the kind of sampling required to obtain direct evidence provides unique challenges. One of these challenges is that there are in fact tens of thousands of small ginseng populations arrayed over a wide area of the eastern deciduous forest of the United States. Random sampling of such a large area would involve assigning a random number to each of these small ginseng populations, sorting by the random number, then choosing approximately the top 1000 to visit and follow over time. This statistically robust sampling scheme is impractical in the extreme. However, every practical sampling scheme will end up being either slightly biased or inadequate in some measure. To gauge natural population status, we therefore attempted a more modest goal: to establish a representative subset of populations across a wide range. Henceforth I will call these 'NSF LTREB populations' after the National Science Foundation program that funded the project (McGraw et al. 2017). By 2004, we had established 30 populations located in seven states for our sampling, including three states (WV, KY, VA) that are high harvest states (Fig. 2). These populations occurred across a wide array of elevations (397 - 3504 feet). They also occurred across a wide range of pH levels (3.9 - 6.6). In addition, these populations were found over a wide range of soil types, overstory, land ownership, land use history, and access. In all, we are acquired 457 'population years' of data. Each year, we sampled between 4,300 and 5,200 plants.

Fig. 2. Location of 30 representative NSF LTREB populations used to monitor population change and assemble demographic data sets to assess health of wild populations.

In each population, censusing in year 1 consisted of careful surveys to locate plants. Plants were considered to be in a different population if they were at least 100 m from any other such grouping. This distance was based upon previous studies of pollination and the drop off and seed production as a function of distance from a pollen source (Hackney, 1999). Annual censusing thereafter consisted of visiting populations in spring and fall of each year. The spring census occurred between May 20 and June 20 while the fall census occurred between August 1 and August 20. The spring census was used to measure germination, leaf lengths/widths (and infer leaf area from those using multiple regression), plant height and several other conditions of fully expanded plants. The late summer census was used to assess changes in status over the growing season and to count seeds on every plant in every population (Fig. 3).

Fig. 3. Sara Souther censusing a ginseng population. Flags were used only during the census to mark plants, then were removed to disguise the population. Hand field measurements and notes were entered into the official LTREB data forms later in the lab.

In order to summarize these data, we used a simple general equation for population change:

(1) $N_{t+1} = N_t + \text{births} - \text{deaths} + \text{immigration}$

This was simplified to exclude immigration because this term was assumed to be so small as to be negligible. 'Births' were counted when new seedlings arose by germination, while deaths were designated when plants disappeared from the population for two years or more.

(2) $N_{t+1} = N_t + \text{new seedlings} - \text{deaths}$

For presentation, number changes over time were relativized to an initial population size of 100 for all populations, and the starting year was 2004, the first year for which we had data on all 30 populations. The first year of population change was therefore:

(3) $N_{\text{relative, 2005}} = 100 * N_{2005}/N_{2004}$

Subsequent years were given by the general equation:

(4) $N_{relative, t+1} = N_{relative, t} * N_{t+1}/N_t$

Equation 4 was iterated up to 2014, after which determination of deaths was not possible because not enough years had passed to definitively determine mortality.

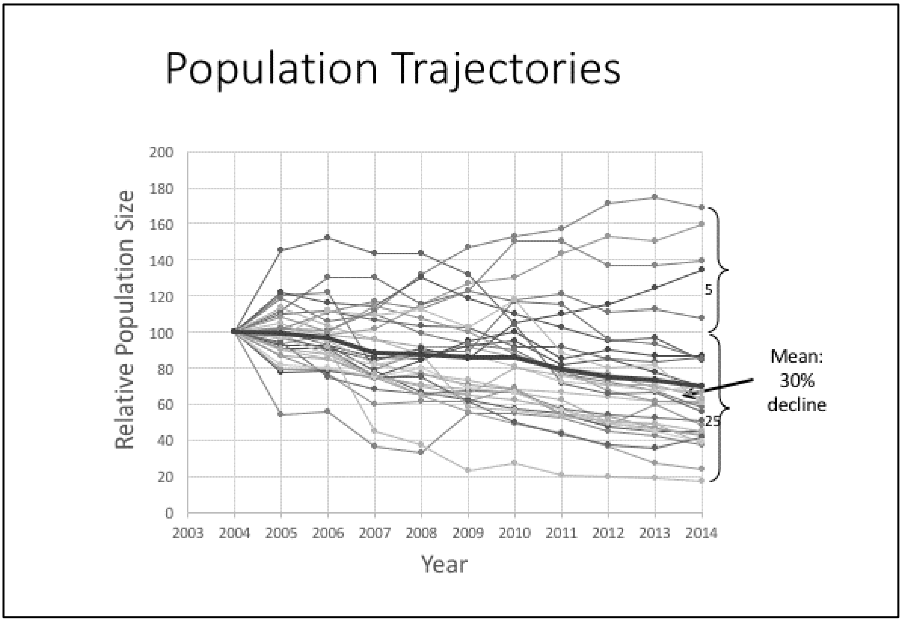

Fig. 4. Relative population change for 30 representative ginseng populations censused from 2004 - 2016.

Figure 4 shows the population trajectories from 2004 through 2014. Significantly more than half of the populations declined over this time frame ($G=25.49$, $p<.0001$). Five populations increased relative to their 2004 values while 25 populations decreased in size. The mean decline over the decade was 30%, while the median decrease was 39%. Some years showed very little decline, e.g., 2007 – 2008 and 2009 – 2010, however most years exhibited a decline in the majority of populations. The fact that five populations actually increased over the decade suggests that ginseng decline is not inevitable, and these populations represent an opportunity to explore the differences between increasing and decreasing populations.

The Role of Harvest in Population Decline

McGraw et al. (2013) suggested that one of the main reasons for population decline was harvest. In one early study of harvest Van der Voort and McGraw (2006) showed that the behavior of harvesters had a significant negative effect on population numbers. Van der Voort et al. (2006) simulated population growth as affected by three harvester behavior types: (a) non-compliant (with existing harvest regulations), (b) compliant, and (c) stewardship harvesters. The most important differences between these are highlighted in Table 1.

Table 1

Harvest Attribute	Non-Compliant	Compliant	Steward
Season	Ignores Harvest Season	Harvests In Season	Delays Harvest Until Berries Ripe
Plant Stage	Harvests 2-leaf, small adults as well as small and large adults	Harvests only small and large adults	Harvests only small and large adults with fruits
Seed Fate	None; seed not mature	Fruit scattered	Fruit planted optimally (2 cm)

These differences in harvester behavior had a significant and important effect on population growth (Van der Voort and McGraw 2006). Relative to unharvested populations, only the stewardship harvest had equivalent population growth (Fig. 5). Non-compliant harvest reduced population growth 15% on an annual basis, while even compliant harvest reduced growth 8%. Populations subject to both non-compliant and compliant harvest would soon go extinct. These results showed that movement of harvest policy toward more compliant behaviors was not sufficient to prevent extinction. Instead, harvest behavior needed to be closer to stewardship in order to be sustainable.

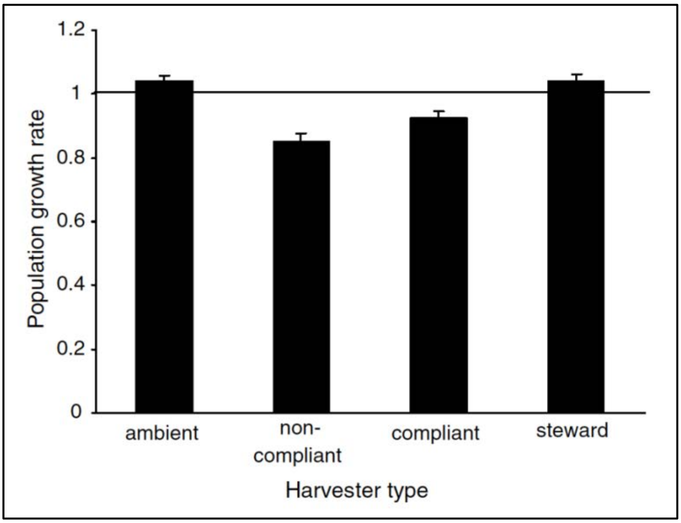

Fig. 5. Effect of harvester behavior on population growth rate in American ginseng. A population growth rate of 1 indicates stable populations, while values below 1 are declining.

A subsequent analysis of actual harvester behavior in harvest events observed in LTREB populations showed that most harvest events were in fact illegal in one of three respects (Fig. 6) (McGraw et al. 2010). Two of these (noncompliance with harvest season, harvest of undersized plants) were shown by Van der Voort and McGraw (2006) to have strong negative effects on population growth rate (Table 1). This rate of noncompliance with existing regulations suggests that solutions to the problem of ginseng conservation need to consider how to regulate in such a way as to improve compliance. New regulations may be well-intended, and biologically sound, but if compliance is lacking, they will be ineffective.

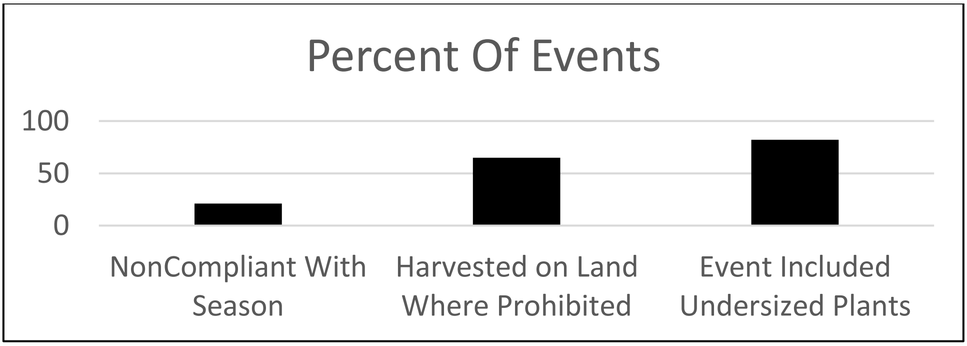

Fig. 6. Percent of harvest events exhibiting illegal behavior.

In the early 2000's, harvest seasons varied widely among states with no apparent rationale based on geography or biology (Fig. 7). However, the finding that across the range seeds were not ripe for dispersal on Aug. 15 (McGraw et al. 2005) led to convergence of harvest season to Sept. 1 (Fig. 7). This policy should, in theory, move harvest more toward a stewardship relationship of harvester to the plant, though this change will be effective only in so far as harvesters comply with the new regulation. For example, a severe harvest was observed June 7, 2017 in Population 29 (of the NSF LTREB populations); one that is sure to have negative consequences as a large portion of the seed-producing plants were taken (Fig. 8.).

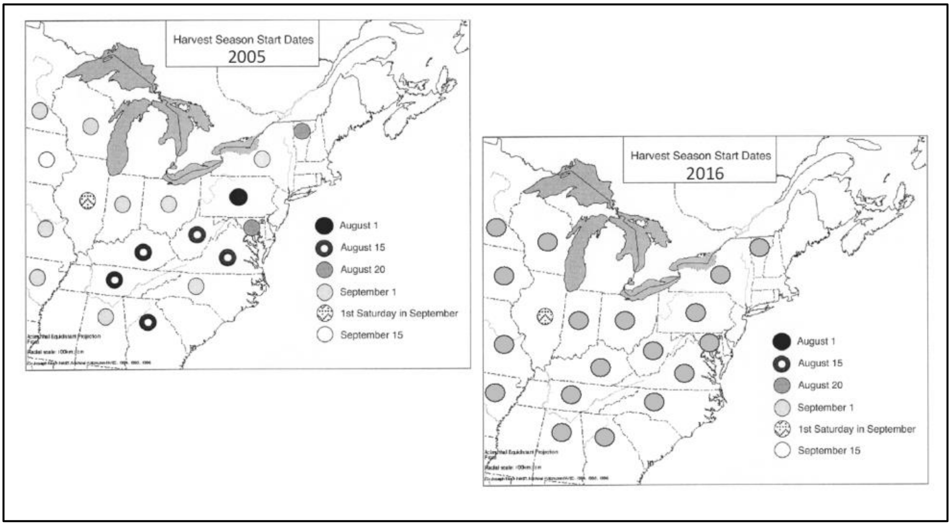

Fig. 7. Change in harvest season onset between 2005 and 2016.

Fig. 8. Tops from harvested roots observed in a remote population of ginseng (Population 29) on June 7, 2017.

The 'Five-Year Rule' and Sustainable Harvest

Many opportunities for revising harvest regulations more extensively could improve prospects for sustainability. One consistent regulation among states involves the so called '5-year rule', whereby US FWS stipulates that plants must be five years of age or older to be exported. Using the LTREB data set, in which individual plants have been followed from 'birth' (germination) allows new analyses of age-specific life histories to evaluate how well the 5-year rule protects populations. Many misconceptions exist regarding the rate at which plants survive, grow, and reproduce with age. Using thousands of new seedlings followed through time, the analysis shows that by age 5, plants may survive at a rate of 90% annually, but on average they only produce 0.6 seeds each (Fig. 9).

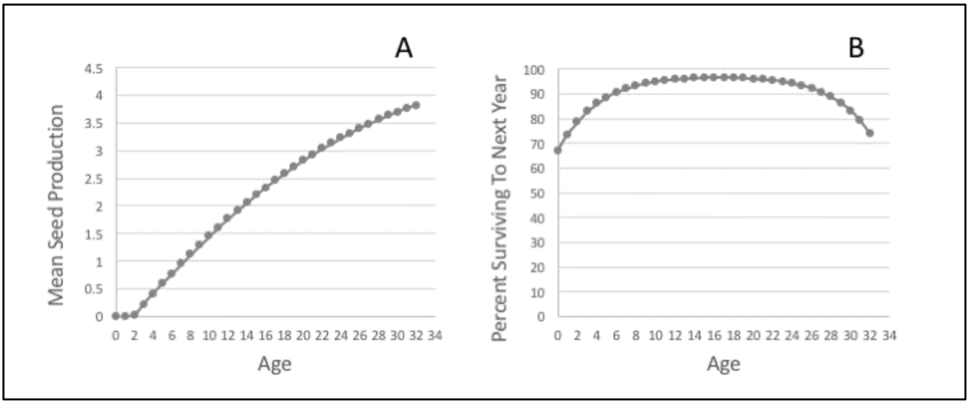

Fig. 9. Age-specific survival and fertility schedules.

Age-specific reproductive and survival schedules do not tell us directly how many seeds would need to be required for a new germinant to replace itself. To estimate that, we can examine the fraction of seedlings remaining alive by age 5 (Fig. 10). In five western LTREB populations (IN and w. KY), only 25% of new germinants remain after 5 years, suggesting that at least four seeds would need to be produced by that age. This number would be 3 seeds in north central populations. However both of these figures do not account for losses of seeds from the seed bank.

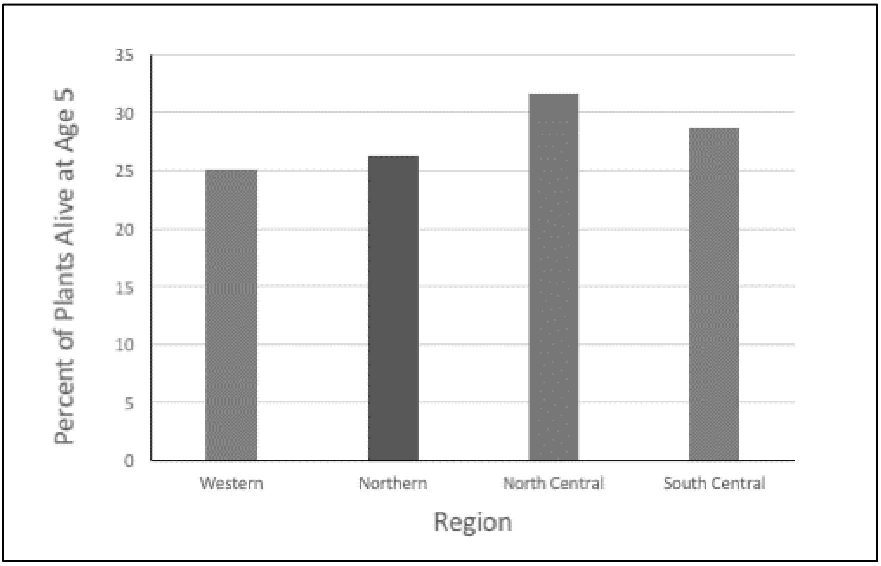

Fig. 10. Survival from germination to age 5 for ginseng plants in four regions sampled by the NSF LTREB populations.

How old do plants have to be before they have literally 'replaced themselves' after germination? This can be determined by performing actual simulations of a cohort of 100 new germinants, then determining how long it takes for this cohort to produce 100 new germinants. One subtlety in making this calculation is the determination of new recruitment. In censusing ginseng populations, we carefully assessed the area for new recruits within 2 meters of each plant. However, this may not include all the new recruits since some dispersal may occur beyond 2 meters. If we make an 'optimistic' projection of our under-censusing of new seedlings, we find that it takes 12 years for a single plant to replace itself. This optimism is based on a fairly limited observation of dispersal beyond 2 m by birds (specifically wood thrushes; Hruska et al. 2014, Elza et al. 2016). Being pessimistic about dispersal by birds, we find that it may take as much as 22 years for a plant to replace itself (Fig. 11). The actual replacement age is probably somewhere between age 12 and 22.

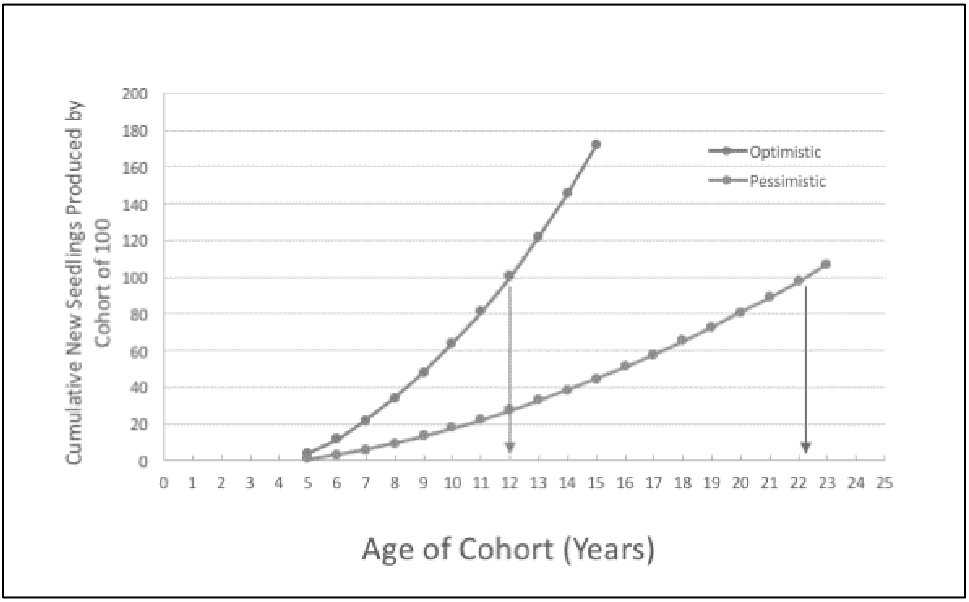

Fig. 11. Cumulative new seedling numbers produced by a cohort of 100 new germinants as a function of age. These cohorts cross the 'replacement threshold' by age 12 (optimistically) and age 22 (pessimistically).

Further convincing evidence that age 5 as a harvest threshold is *not* protecting the population from harvest is the low frequency of flowering (Fig. 12) and the immaturity of plants (Fig. 13) by age 5. Indeed, most states stipulate the plants must have three leaves or more to be harvested, but in all regions, less than 1/3 of plants have reached the 3-leaf stage by age 5.

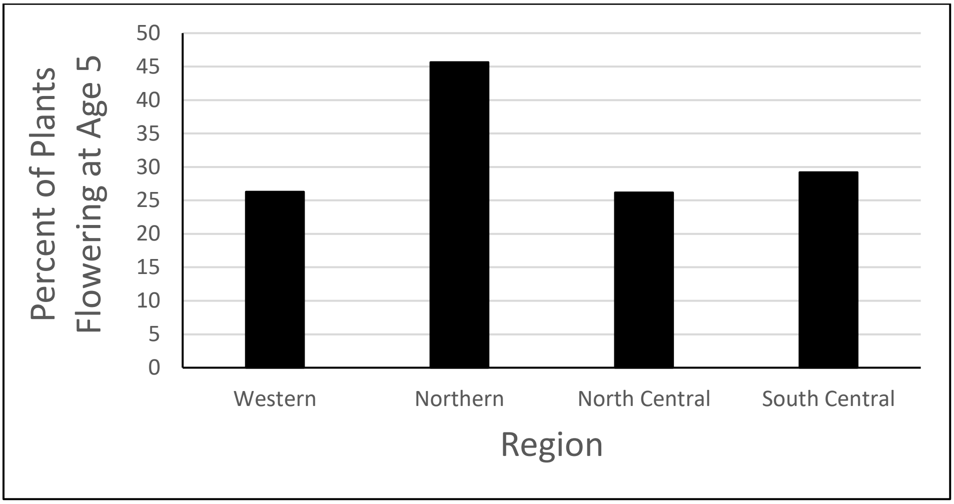

Fig. 12. Flowering rates by age 5 for ginseng plants in four regions.

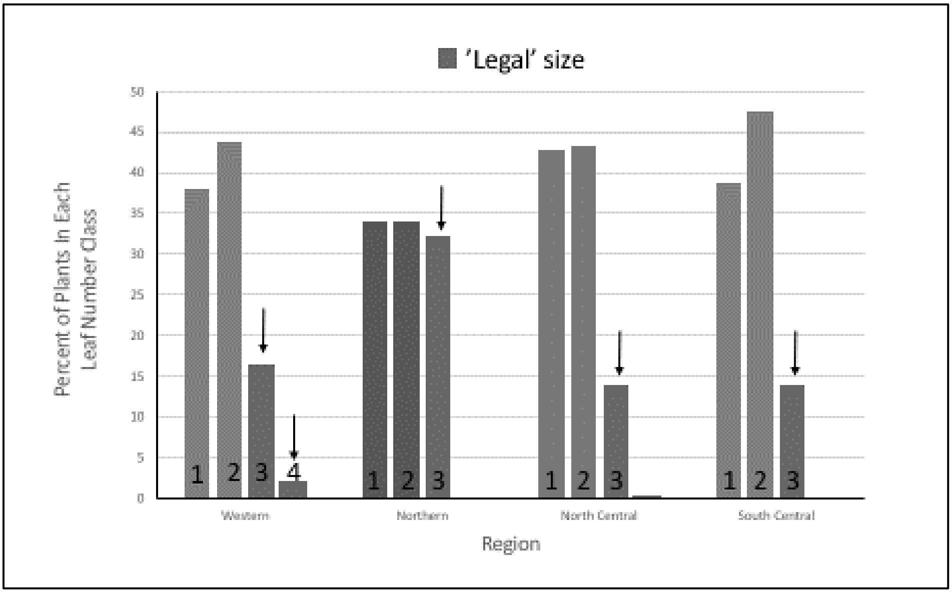

Fig. 13. Stage reached by age 5 ginseng plants in four regions within the 30 LTREB populations.

Alternatives to the Five-Year Rule

As a solution to the inadequacy of the 5-year rule, it would be tempting to choose a new (greater) age limit to protect populations. Indeed one advantage of age as a criterion is that it is verifiable at the points of sale and export. However, the plasticity of plants as a function of age means the choice of any age is likely to be protective in some populations but not in others. This can be seen readily from photographs of plants in different age classes in two populations (Fig. 14). These two populations are comparable in overall size, but one grows in a moderately supportive habitat, while the other is a near-ideal habitat. In Population 27, plants are frequently deer browsed, and have other unknown stresses that cause them to grow slowly. In Population 30, plant progress rapidly through the stage classes and reach 3-leaf reproductive size by age 8 quite often. Harvest of age 5 and above plants in Population 27 would cause a rapid crash because there would be virtually no seed production there, however some plants would produce seeds at age 8 and above in Population 30 (though harvest at age 5 would still be strongly inhibiting).

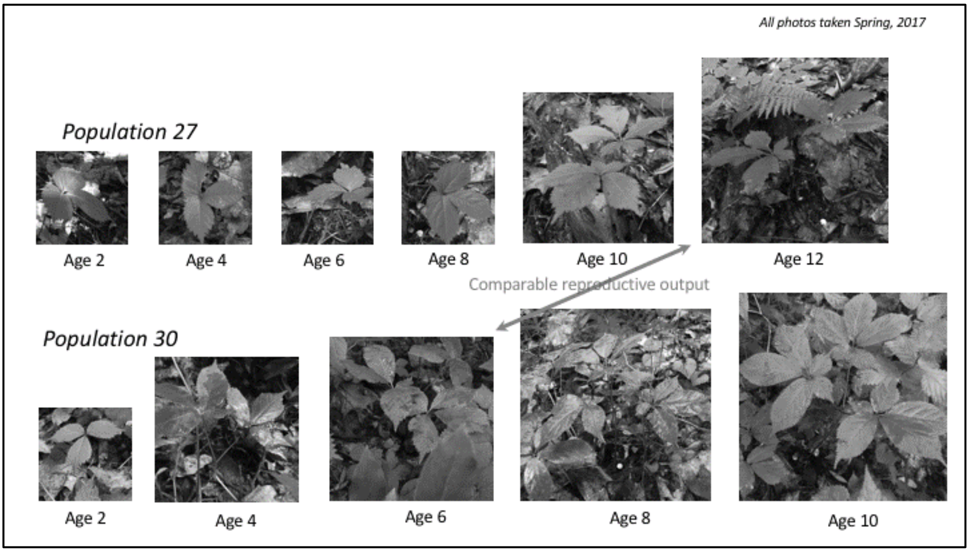

Fig. 14. Photographs showing progression of age and size in two contrasting different populations (Population 27 and 30 the NSF LTREB populations).

By contrast to the age-specific growth patterns in Population 27 and 30, the size-specific patterns are similar (Fig. 15). This suggests that a size-based criterion for harvest could protect these two populations well, while an age-based criterion would leave Population 27 vulnerable.

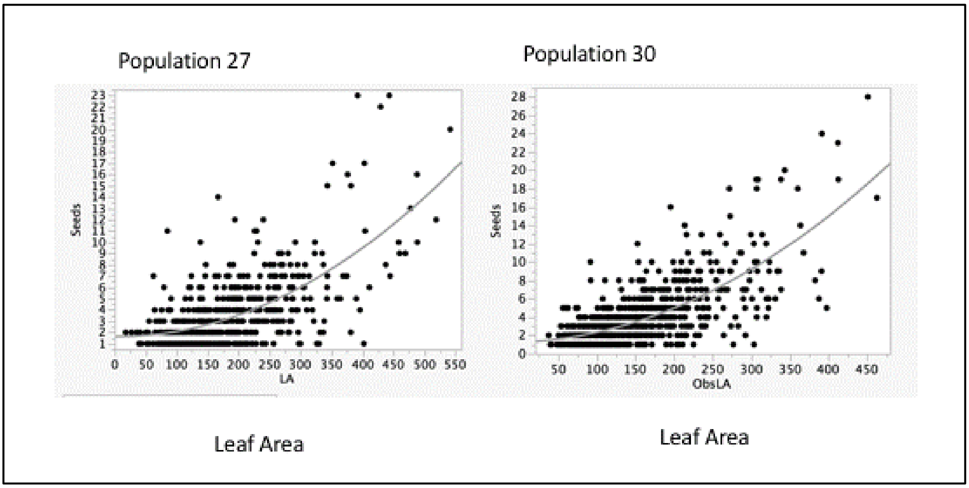

Fig. 15. Similar seed production as a function of leaf area (one measure of size) in two ginseng populations (Population 27 and Population 30) with sharply contrasting age-specific patterns.

To regulate harvest according to leaf area would be unrealistic since harvesters cannot be expected to measure leaf lengths and widths, then use multiple regression to compute leaf area, all while hiking in remote back country! This opens the question, however, of what size-based criterion could be used to good effect, while allowing it to be usable by harvesters, verifiable (preferably at both point of sale and export), and demographically meaningful. It would be tempting to suggest some root size criterion, expect that root harvest is destructive (although planting roots back after excavation is possible, it does cause some losses). Aboveground size dimensions would be possible, but would require tops to be turned in along with roots. If tops have some economic value, this could incentivize this behavior and perhaps also discourage out of season harvest. These are a few of the factors that should be considered in a move to a size-based harvest and export criterion.

The Larger Context

Harvest is one of many stressors acting on ginseng populations to reduce population growth (McGraw et al. 2013; Fig. 16). Two other factors - deer (McGraw and Furedi 2005) and climate change (Souther and McGraw 2011a, b; Souther et al. 2012; Souther and McGraw 2014) have effects of comparable magnitude, and in specific populations other factors can further depress population growth either chronically or episodically (McGraw et al. 2013). If harvest was acting alone, more harvest could occur before population growth would be depressed below 1 to the declining level (Fig. 16), however in the presence of these factors, populations sit precariously close to that tipping point.

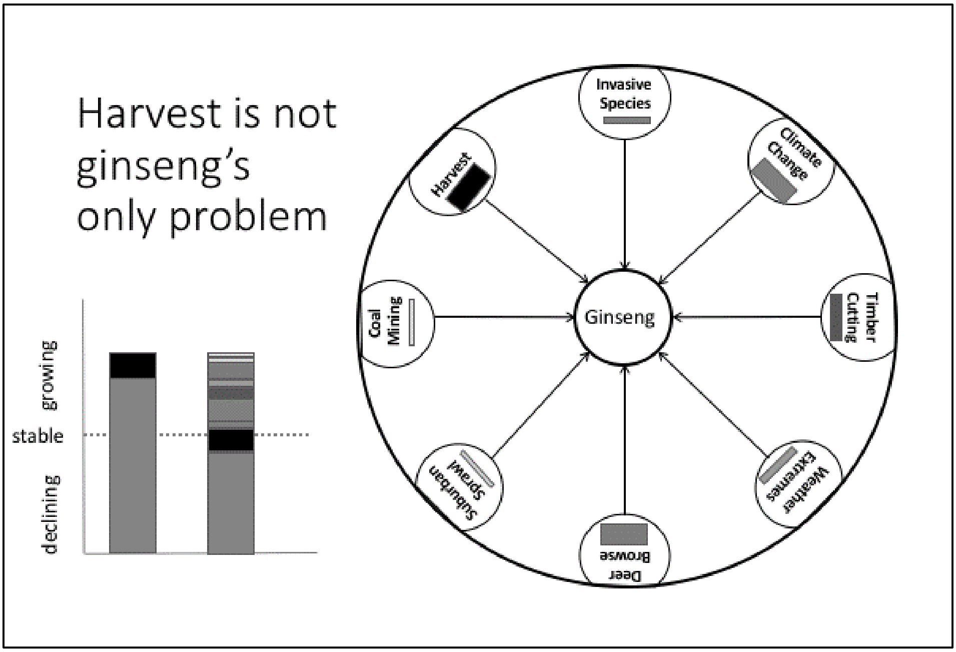

Fig. 16. Harvest is one of many factors reducing population growth of ginseng below its potential.

Summary

The challenge of the 'Ginseng and Forest Botanicals' Conference is to identify specific improvements we can implement in coming years to move toward 'stewardship' harvest from the current state of 'exploitative' harvest of vulnerable populations. This challenge is not easy as it involves multiple stakeholders with competing interests. Nevertheless, meeting this challenge will be necessary if we are to guarantee the persistence of wild ginseng populations for future generations. Shifting our policy goals from the short-term and pragmatic toward goals that are optimistic, idealistic and long-term, can be a driving force for making the necessary changes to the human-ginseng relationship that will preserve that relationship. Purposeful evolution of the human-ginseng relationship toward that of a mutualism, rather than a parasitic or predatory one, may be one measure of our maturity as a species.

Acknowledgments

First and foremost, the author wishes to thank the series of hardworking graduate students who led research teams each summer to do the hard work of data collection that led to the publications cited in this paper. These included: Martha Van der Voort, Mary Ann Furedi, Emily Mooney, Zach Bradford, Kerry Wixted, Sara Souther, Jessica Turner, and Jennifer Chandler. Colleague Anne Lubbers at Centre College was instrumental in censusing Kentucky populations. Emily Thyroff played a key role in curating the data set resulting from this study. Several dozen undergraduate conservation interns also assisted in the hard work of data collection, data entry and data checking. This research was supported by a series of grants from the National Science Foundation (DEB-0212411, DEB-0613611, DEB-0909862, DEB-1118702). Finally, I would like to acknowledge the contributions from my family, who tolerated my summer absences during ginseng census seasons so I could do my part to maintain the consistency of the data collection effort.

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“Population, Distribution, and Threats of American Ginseng (Panax quinquefolius L.) in Indiana and Illinois”

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Abstract

American ginseng, *Panax quinquefolius* L., is one of the most iconic medicinal plants in North America. Although ginseng has been continuously collected over hundreds of years, more than a decade of research shows that it is currently facing increased threats from wild harvest, herbivory, and climate change. These increased threats have recently raised concern about ginseng’s conservation status and sustainable harvest practices. Indiana and Illinois are each one of the top ten states with the greatest legal export. In addition, large-scale illegal wild collection of ginseng root was recently documented from these states. Working collaboratively, botanists from NatureServe, the Indiana Natural Heritage Program, the Illinois Division of Forest Resources, independent companies, and the U.S. Geologic Survey collected current population and genetic data for ginseng in Indiana and Illinois in 2016. A total of 65 sites across 42 counties were surveyed; most sites were impacted by one or more threats, and many populations are considered small. Demographic data revealed that many populations had a higher ratio of juvenile plants to mature plants than expected. The observed pattern suggests harvest pressure, since most well-established ginseng populations have more mature plants than juvenile plants. Results from this study will inform the conservation status of ginseng in these states as well as nationally and globally, and inform future conservation efforts. The forthcoming genotype results will clarify whether the sampled ginseng plants originate from local or non-local seed sources.

Keywords: conservation, threats, Indiana, Illinois, demography, harvest, wild simulated seed, medicinal plants, NatureServe

Introduction

American ginseng (*Panax quinquefolius* L.) is native to forests in the eastern United States. Ginseng is a widespread species that has been continuously harvested for its medicinal use for hundreds of years. Despite having a large range with many occurrences, NatureServe considers American ginseng to be Globally Vulnerable (G3) to extinction, primarily due to numerous threats and a declining trend (2005). The direct and indirect consequences of harvest are of great concern for the conservation of the species. Wild-collected ginseng is harvested throughout its range for export to international markets; however, collection is illegal in Canada. Among U.S. states, Indiana ranks 5th and Illinois ranks 8th in the amount of ginseng harvested for export.

Knowledge of a species' distribution and the health of its populations are fundamental to assessing its extinction risk. Assessing the conservation status of native plants, animals, and ecological communities is central to the NatureServe Network's mission. The NatureServe network, a partnership of 80 natural heritage programs or conservation data centres across North America, conducts field surveys of species' populations which are used to evaluate the extinction risk or conservation status of each species. Conservation status assessments are conducted at subnational (state or provincial), national, and global scales, with a ranking system indicating the relative level of imperilment on a scale from one to five (Faber-Langendoen et al. 2012). These ranks are used by government agencies in the United States and Canada to prioritize species for conservation.

Rarity, threats, and trends are the three factors that underpin conservation ranks. Rarity is generally quantified through population size and number of occurrences, both of which are obtained through field surveys. While ginseng has a broad distribution comprised of many populations, research suggests that ginseng populations have been steadily decreasing. Documented threats to ginseng populations throughout its range include harvest pressure, deer herbivory, and habitat degradation. However, the impact of threats on ginseng has not been comprehensively evaluated throughout its range.

The impact of certain harvesting practices on ginseng populations is a significant conservation concern. Most collectors follow the long-honored tradition of leaving seed in harvested populations, which ensures future harvest. The concern is not over the practice of leaving seed behind, but rather the source and geographic origin of the seed being planted. Rather than leaving seed from the original population during harvest, collectors sometimes leave 'wild-simulated' seed instead. Wild-simulated seed originates from farmed populations typically grown in Wisconsin. Mixing wild-simulated seed with true wild populations can decrease the genetic vigor of the true wild populations. This is because introducing genes that are not adapted to local environmental conditions can reduce a population's ability to adapt to those conditions. If the practice of using wild-simulated seed is occurring on a large scale, it could impact ginseng's long-term viability.

In Indiana and Illinois, the state ranks (Sranks) indicate that ginseng is vulnerable, ranked S3 and S3?, respectively, though the Sranks have not been reviewed in more than 15 years. An S3 indicates that a species is vulnerable due to a restricted range, relatively few populations or occurrences, recent and widespread declines, or other factors making it vulnerable to extirpation (Master et al. 2012). The goals of this project were 1) to collect current population data on *Panax quinquefolius* in Indiana and Illinois to inform the conservation status ranks for these states, and 2) to gather genetic samples that will inform studies on the impacts of wild simulated seed. Genetic samples were collected to leverage funding for the field surveys, but additional funding is needed to process the samples.

Methods

We surveyed 37 sites in Indiana and 28 sites in Illinois from August to October of 2016. Given the time and resource limitations, we focused on sites documented having robust ginseng populations in the past, and that represent wide geographic areas within each state. Site selection was a highly collaborative effort with the Indiana Natural Heritage Program, the Illinois Ginseng Coordinator, and field biologists. We obtained necessary permits and permission to access all sites.

We surveyed populations to determine whether ginseng met the description of S3 as follows: "vulnerable … due to a restricted range, [with] relatively few populations or occurrences, recent and widespread declines, or other factors making it vulnerable to extirpation" (Master et al. 2012). We collected site information including name, ownership, geographic coordinates, habitat characteristics, and associated species. The Indiana Special Plant Survey form was used at each site in both Indiana and Illinois (Appendix 1).

We collected data on ginseng populations such as phenology, number of individuals, population extent, and age class. We documented age classes according to how many palmately compound leaves, or 'prongs' were observed on an individual plant. Plants with a single stem and three leaflets were categorized as seedlings, plants with one to two prongs were categorized as juveniles, and plants with three to four prongs were categorized as adults. Although seedlings were noted at some sites, these data were not consistently collected throughout the study because it was difficult to distinguish ginseng seedlings from other similar looking species.

Threats to ginseng were recorded and described in field forms. Threats were recorded at all sites, even when ginseng was not found. At sites where ginseng was absent, herbivory was recorded when other plant species at the site were disturbed. Wild collection was documented through evidence of freshly dug holes and other human disturbances around plants, (e.g., flagging, litter, paths). In some cases, wild collection was documented by law enforcement officers or landowners with recent first-hand reports of poachers digging ginseng at study sites.

We categorized small populations as those with 20 individuals or fewer, based on a study by Souther and McGraw (2014) that calculated 20 individuals to be the quasi-extinction value within 70 years. Using population viability analyses, the study detected genetic degradation and population decline leading to extinction in populations of 20 or fewer individuals. For this study, using a threshold of 20 individuals to categorize populations as small is conservative considering that many of these populations are predicted to become extirpated within a short timeframe.

Genetic samples were collected using Whatman FTA™ plant saver cards following a protocol designed to capture genetic material from leaves with minimal damage to the plants (Young 2014). FTA cards allow genetic material to be transferred from the leaves and fixed to the cards for long term storage. FTA cards were placed in plastic bags with desiccant and stored in the freezer.

Results

Ginseng was present at 22 of 37 sites in Indiana and 25 of 28 sites in Illinois (Fig. 1). In Indiana, there were 8 populations with 1-20 plants, 10 populations with 21-50 plants and 4 populations with 51-165 plants. In Illinois, there were 14 populations with 1-20 plants, 20 populations with 21-50 plants, and 13 populations with 51-165 plants. There were 15 sites in Indiana, and 3 in Illinois where surveyors failed to find ginseng. The timing of the surveys coincided with the beginning of senescence of ginseng plants, so it is possible, though unlikely, that early senescence led to an overestimate of sites where surveyors failed to find ginseng. Of the inventoried sites with ginseng present, 29% were represented by small populations, as defined by 20 or fewer plants.

Invasive species and herbivory were the most pervasive threats (Fig 2). Invasive species were present at 68% of sites in Indiana and 80% of sites in Illinois. The most frequently encountered invasive species were *Lonicera mackii* (Japanese honeysuckle), *Rosa multiflora* (Multi-flora rose), and *Alliaria petiolata* (Garlic mustard). Herbivory by deer or rabbits was observed at 73% of sites in Indiana and 44% of sites in Illinois. Insect damage was observed on many plants but only appeared to cause superficial damage. Wild collection was documented at 5% of sites in Indiana and at 24% of sites in Illinois (Fig 2).

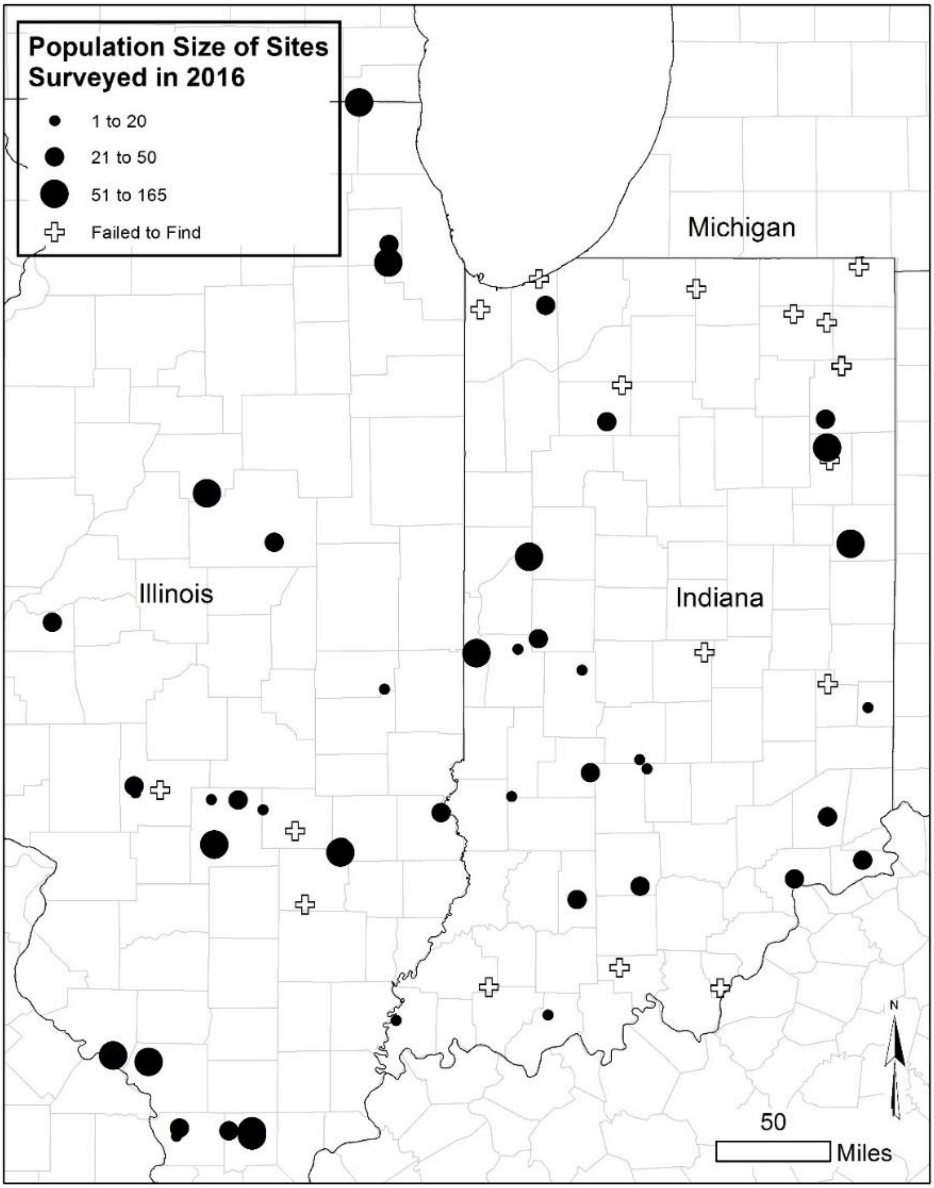
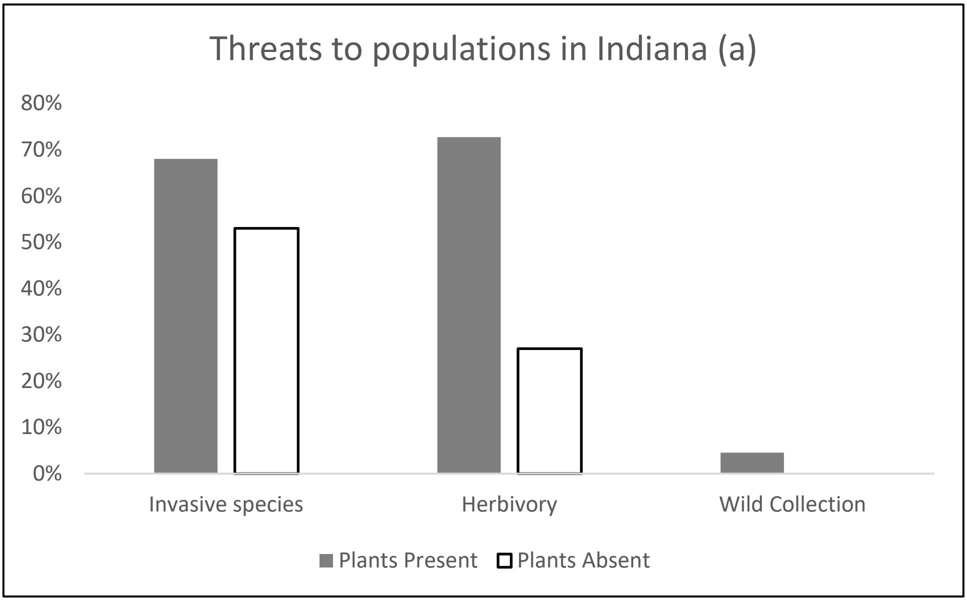


Fig 1. Sites surveyed in Indiana and Illinois in 2016. American ginseng was present at 22 of 37 sites in Indiana and 25 of 28 sites in Illinois. Circle size indicates population size with small circles indicating populations with 20 individuals or fewer, medium circles with 21 to 50 individuals, and large circles indicate populations of greater than 51 individuals. Empty plus symbols indicate sites where surveyors failed to find ginseng (18).



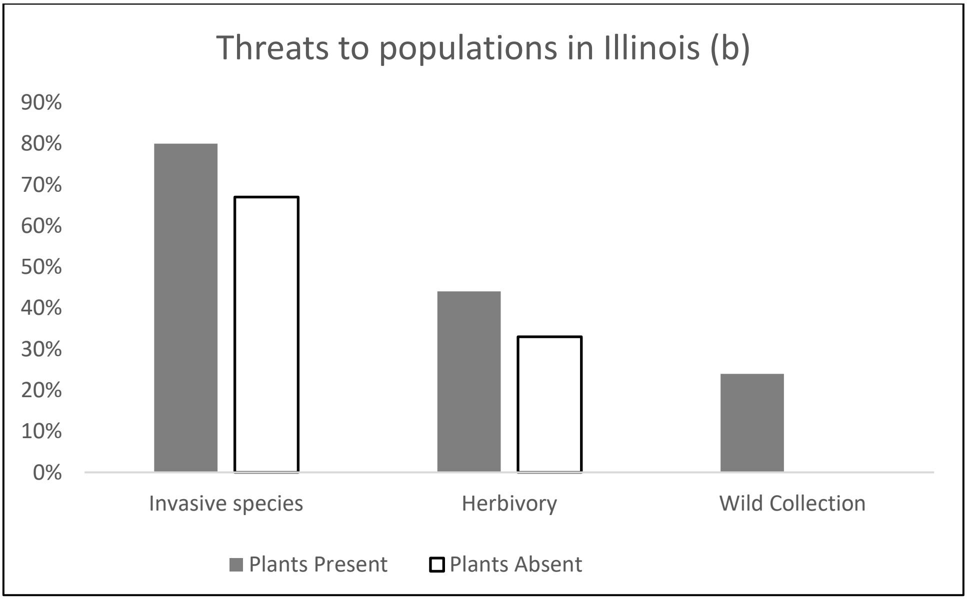


Fig 2. Threats to sites surveyed for ginseng. Invasive species are the most common threat, followed by herbivory. Wild collection was observed at more sites in Indiana (a) than Illinois (b) where ginseng was present.

We analyzed population demography of sites with more than 20 individuals. Of these, we found that 38% of sites in Indiana and 56% of sites in Illinois were comprised of more than 50% juveniles (Figs. 3 and 4). While results from both states indicate high numbers of juveniles within each population, more populations in Illinois had high numbers of juveniles in populations compared to Indiana (Figs. 3 and 4).

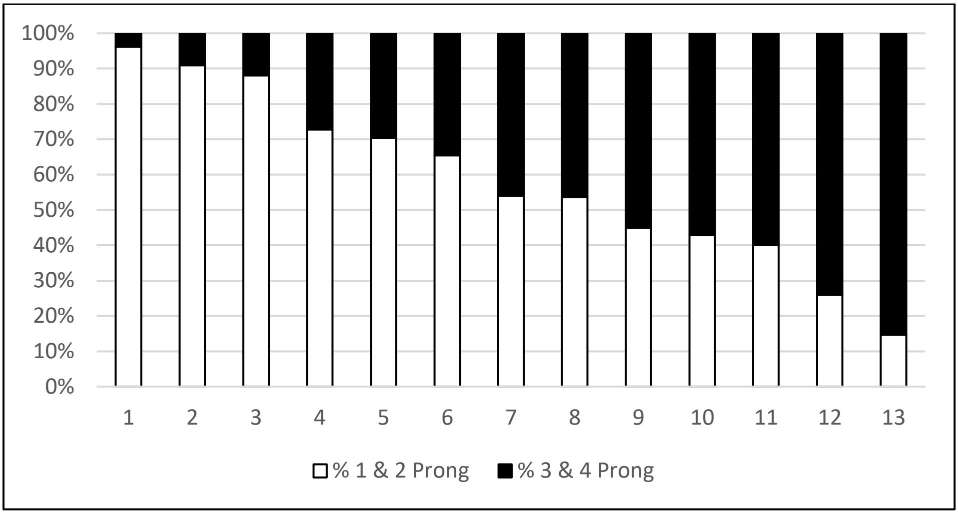


Fig 3. Population demography of 13 sites in Indiana with more than 20 plants. White indicates the percentage of juveniles (1 and 2 pronged plants) and black represents the percentage of mature individuals (3 and 4 pronged plants).

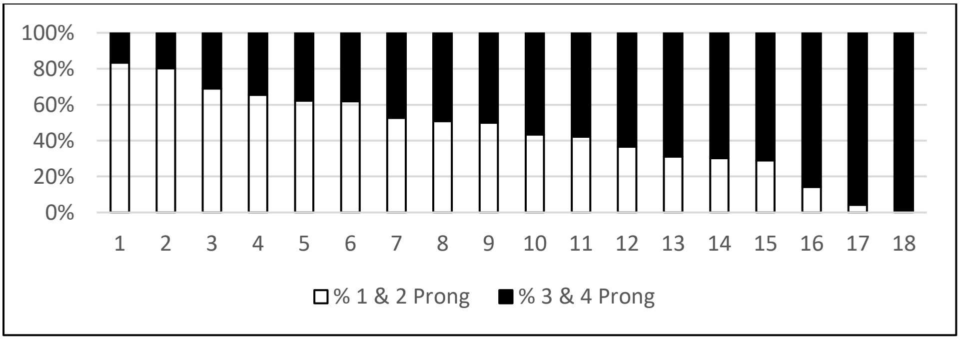


Fig 4. Population demography of 18 sites in Illinois with more than 20 plants. White indicates the percentage of juveniles (1 and 2 pronged plants) and black represents the percentage of mature individuals (3 and 4 pronged plants).

Discussion

The primary objective of this study was to survey ginseng populations in Indiana and Illinois to inform the conservation status of the species in those states. Although limited by a one-time, single visit to each site, we were able to summarize threats, estimate population demography, and identify potentially extirpated sites. Here we relate the results of this study to ginseng conservation throughout its range, focusing on the main factors impacting ginseng population health in Indiana and Illinois. We found that ginseng is significantly threatened by herbivory, invasive species, and potentially unstainable harvest. Nearly 30% of populations in this study were considered so small (20 or fewer individuals) that they are expected to be extirpated within the next 70 years. Many populations were comprised of higher percentages of juveniles than adults suggesting the effects of harvest pressure. Lastly, while this study did not measure trends, it is noteworthy that ginseng was not present at 18 sites where it was previously known.

Threats are a key factor in determining a species' risk of imperilment in both NatureServe's and the IUCN Red List's conservation status assessments (Master et al. 2012, IUCN 2017). In this study, invasive species and herbivory were the predominant threats. Invasive species were pervasive at most field sites in both Indiana (68%) and Illinois (80%). Similarly, Wixted and McGraw (2008) documented invasive species at 63-70% of ginseng populations surveyed across multiple states. *Rosa multiflora* was the most frequent invasive species encountered in this study as well as in the Wixted and McGraw (2008) study. Another commonly encountered invasive was garlic mustard (*Alliaria petiolata*). The presence of garlic mustard has been associated with increased ginseng mortality, perhaps due to allelopathy (Wixted and McGraw 2009). It is highly likely that invasive species are negatively impacting ginseng in Indiana and Illinois by altering landscape condition and degrading habitat.

Herbivory was documented at most sites surveyed in both Indiana and Illinois. This is not surprising as deer-browse is considered a widespread threat to ginseng (McGraw et al. 2013). While herbivory does not immediately kill plants, it does cause decline in population growth over time (Farrington et al. 2009, McGraw and Furedi 2005). Using life history models, Farrington et al. (2009) predicted that deer-browse alone would decrease ginseng's population growth rate by 2.9% over 7 years. In West Virginia, deer-browse resulted in a 2.7% decline in ginseng populations (McGraw and Furedi 2005). Based on the high percentage of deer-browse in Indiana in this study (73% of sites), it is likely that herbivory has contributed to declines in ginseng population growth. While deer-browse was less frequent in Illinois (44% of sites), it is also likely to be contributing to declines in ginseng population growth.

Wild collection or harvest was documented at 24% of Illinois and 5% of Indiana sites where ginseng was present. Wild collection was documented at each site based on the observation of freshly dug holes and reports from conservation officers. However, documenting this threat based on one site visit was challenging. All our field surveys took place in late summer and early fall, coinciding with the start of the ginseng harvest season. Harvest that occurred after our surveys was not documented in this study. Interpreting the wild collection in this study would require more information on the factors impacting collection such as timing of field work in relation to harvest and differing harvesting regulations by state.

Population size and demography

We documented many small populations in both Indiana and Illinois. This result is consistent with previous research indicating that most naturally occurring ginseng populations are less than 150 individuals (McGraw et al. 2013). Small populations are of conservation concern because they are at greater risk of extirpation. Once ginseng populations are reduced to 20 individuals they are considered quasi-extinct because Allee effects and demographic stochasticity lead to population degradation (Souther and McGraw 2014). In their study, Souther and McGraw (2014) also found that population extinction risk was 65% when populations of 140 plants were threatened by climate change and harvest. If we assume that small populations will eventually become extirpated (per Souther and McGraw (2014), nearly 30% of the sites surveyed in the present study will not persist over the next 70 years. Even the largest population in this study (165 individuals) would be considered small in the context of long-term viability, based on ginseng population viability models. For example, one population viability model showed that 172 ginseng plants are needed to maintain a population for the next 100 years, considering stochastic events (McGraw et al. 2013). Another study from West Virginia concluded that 800 individuals are required for a population to remain extant for 100 years under current deer browsing pressure (Furedi and McGraw 2005). Based on the threats documented for Indiana and Illinois, most populations surveyed in this study would not be expected to persist for more than 100 years.

Many populations had a high proportion of juvenile to adult plants, a pattern connected with overharvest. In Indiana, 38% of sites and 56% of sites in Illinois were comprised of more than 50% juveniles. The demographic pattern detected in this study was coined as the “fingerprint” of harvest pressure by Mooney and McGraw (2009), substantiated by other studies. For example, a study by Sanders-Cruse and Hamrick (2004) concluded that harvested ginseng populations in had a higher ratio of juvenile to adult plants than ginseng populations protected from harvest through regulations. The reduction of reproducing adults leads to a decline in population growth and regeneration. If this is repeated over many growing seasons, populations will ultimately decline.

The observed demographic pattern of a high number of juveniles shows harvest-induced evolutionary change, one that mirrors exploited fish stocks

(Mooney and McGraw 2009). Data collected by the U.S. Fish and Wildlife Service for CITES (Convention on International Trade of Endangered Species of Wild Fauna and Flora) show a decrease in the mass of harvested ginseng roots over time (Mooney and McGraw 2009). Harvest induced evolutionary changes in ginseng could result in a reduction in the size of mature plants since diggers often harvest larger plants. We observed the same demographic pattern in Indiana and Illinois, indicating that perhaps harvest-induced evolutionary change leading to smaller mature plants is occurring in Indiana and Illinois.

In this study, the presence of threats, the number of small populations, and a high percentage of populations with more juveniles than adults provide concern over the long-term viability of ginseng in Indiana and Illinois. Based on these results, recalibrating NatureServe's population viability ratings will provide more accurate measures of population health and extirpation risk. Accurate population viability ratings are essential to assesses the conservation status. Even though ginseng has a wide range with many populations, the conservation status may be more imperiled if most populations have poor viability. Results from this study provide anecdotal evidence of a recent declining trend, based on the number of sites where surveyors failed to find ginseng. Assessment of each population's quality under the revised viability criteria, combined with evidence of continuing threats and decline, may result in a change in rank from an S3 to an S2 in both Indiana and Illinois.

Acknowledgements

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Appendix 1: Indiana Special Plant Survey Form

Indiana Special Plant Survey Form

Element Name: ______________________________ EO boundaries mapped: ________

Surveyor (s): ______________________________ Date: ______________

Location: __________ Sec. _____ T _____ R _____ County: ____________________

UTM/GPS coordinates: ____
Show exact location and boundaries of taxon on map. (attach)

Repeat visit: _______ Repeat visit needed: ________ When: __________________

Area name: Ownership info:

Biology:

Phenology	Approx # Indiv	Population Area	Age Class
☐ In leaf	☐ 1-10	☐ 1 yd^2	☐ % Seedlings
☐ In bud	☐ 11-50	☐ 1-5 yd^2	☐ % Immature
☐ In flower	☐ 51-100	☐ 5-10 yd^2	☐ % 1st year
☐ In fruit	☐ 101-1,000	☐ 10-100 yd^2	☐ % Mature
☐ Seed Dispersing	☐ 1,001-10,000	☐ 100 yd^2-2 ac	☐ % Senescent
☐ Dormant	☐ 10,001+	☐ 2 ac +	

Comments on above:

Compared to your last visit to this site:

Approx # Indiv	Population Area	Age Class	Reproduction
☐ more	☐ more	☐ same	Is reproduction occurring? ______
☐ same	☐ same	☐ diff	Type: ☐ sexual, ☐ asexual, ☐ both
☐ less	☐ less		

Threats:

EORANK ____________________ Date ____________________
A = very good example, B = good example, C = average example, D = poor example, X = destroyed

EORANK Comments:

Population Distribution: ☐ solitary, ☐ clumps or dense groups, ☐ small patches or cushions,
☐ small colonies or large carpets, ☐ large, almost pure population stands

Vigor: ☐ 1) very feeble, ☐ 2) feeble, ☐ 3) normal, ☐ 4) exceptionally vigorous

Evidence of symbiotic or parasitic relationships:

Habitat:

Aspect	Slope	Light	Topographic Position	Moisture
☐ N	☐ Flat	☐ Open	☐ Crest	☐ Inundated (Hydric)
☐ E	☐ 0-10'	☐ Filtered	☐ Upper slope	☐ Saturated (Wet-mesic)
☐ S	☐ 10-35'	☐ Shade	☐ Mid-Slope	☐ Moist (Mesic)
☐ W	☐ 35' +		☐ Lower slope	☐ Dry (Xeric)
	☐ Vertical		☐ Bottom	

Elevation: ________ ft. to ________ ft. Surface Relief: ☐ /: ☐ ∪: ☐ ∩: ☐ —: ☐ ~~

Substrate/Soils:	
Associated Natural Community/	
Plant Community: Characteristic Associated species:	

Estimated size of potential Habitat: ____
(as in population area)
NOTE: Collect specimen if a healthy, viable population exists. Collection # ____________

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“Partial root harvest of *Panax quinquefolius* L. (American ginseng): a non-destructive method for harvesting root tissues for ginsenoside analysis”

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Abstract

Panax quinquefolius L. (American ginseng) is an economically important, but increasingly threatened, herbaceous perennial plant native to eastern North America. The roots have long been prized in traditional Asian medicine, and are increasingly being used in producing herbal supplements for other markets. Much research is focused on ginsenosides, medicinally-active compounds found in ginseng roots. Given the conservation concerns regarding wild *P. quinquefolius*, and the use of roots for both commercial and research activities, we began experimenting with a partial-root harvest method in 2014. This approach was designed to extract tissue for ginsenoside analysis without reducing the plant's fitness or resulting in mortality. Partial-root harvest samples were taken from 57 plants in four wild populations from western North Carolina. Of the 57 plants subjected to partial-root harvest in 2014, 51 (89%) reemerged in 2015 and 45 (79%) reemerged in 2016. These resprout rates were similar to paired unharvested plants (86% and 81%, respectively). Fitness (berry production) was not affected by partial-root harvest, however, there were short-term effects on growth. Harvested plants had significantly shorter stems and smaller leaf area than unharvested plants in the first year after harvest. However, there were no significant differences in any plant metric between these two groups of plants by the second year after harvest. Our results suggest that this method could be an effective way for researchers to reduce their impact on wild ginseng populations.

Keywords: American ginseng, non-destructive harvest, *Panax quinquefolius,* phytochemical analysis, sustainability

Introduction

Panax quinquefolius L. (American ginseng) is an economically important, perennial herb endemic to the deciduous forests of eastern North America (Anderson et al. 1993). The roots have long been prized in traditional Asian medicine, and are beginning to be used more in North America and Europe as herbal supplements (Schlag and McIntosh 2006). This use has led to an increased demand for the roots of this species, resulting in overharvesting, increasing rarity, and loss of genetic diversity in much of its native range (Cruse-Sanders and Hamrick 2004). Researchers are interested in determining the composition of root phytochemicals, particularly the triterpenoid saponins known as ginsenosides (Qi et al. 2011), that are major medicinally-active compounds. Previous studies have demonstrated that plants' chemotypes vary between organs (leaves and root) in cultivated (Li et al. 1996) and wild (Searels et al. 2013) plants, and among roots of individual plants (Searels et al. 2013; Schlag and McIntosh 2013). While some variation can be attributed to genetic factors (Schlag and McIntosh 2013), the production of certain ginsenosides (Rb1, Rd, Rc) seems to be environmentally determined (Lim et al. 2005). However, most previous research isolating root ginsenosides for analysis has relied on total root harvest killing the plant.

Given the conservation concerns regarding American ginseng, we began experimenting with a non-destructive, partial-root harvest method in 2014 in an attempt to extract tissue for ginsenoside analysis without increasing plant mortality or reducing plants' vegetative fitness. We predicted that carefully harvesting small amounts (~ 300 mg) of fresh tissue, then immediately replanting the remaining root, would have no negative effects on growth or survival compared to similarly sized unharvested plants. If successful, this partial-root harvest method would allow researchers to assess root phytochemicals with less overall mortality.

Materials and Methods

For this study, we monitored 114 mature (three or four leaf) *P. quinquefolius* plants (Fig. 1) from four protected wild populations in western North Carolina. Of these plants, 57 were partially root harvested in 2014, while the other 57 were unharvested. When roots were exposed, side roots branching from the main root were harvested (Fig. 2). If no side root was present, then part of the side of the main root was harvested with care to not damage the vascular cylinder in the center of the root. Roots were then replanted into the same spot from which they were dug.

Figure 1. A four-leaved (four prong) adult *Panax quinquefolius* plant with inflorescence. (Photo by J. Horton)

Figure 2. *Panax quinquefolius* root showing a side root that was partially harvested. (Photo by J. Horton)

We measured morphological data – reproductive status, number of berries (if present), number of leaves, total number of leaflets, stem height (cm), peduncle length (cm, if present), largest leaf rachis (cm), largest leaflet length (cm), and largest leaflet width (cm) in 2014 before partial-root harvest and again in 2015 and 2016 mid growing season. Leaflet length and width were used in an allometric equation developed by Mooney and McGraw (2009) to calculate leaf area of the largest leaf.

We used Analysis of Variance to compare the number of berries, number of leaves, number of leaflets, stem length, and leaf area between partial-root harvested plants and unharvested plants before harvest and one year and two years after harvest. Survivorship was compared between the partial-root harvested plants and the unharvested plants one and two years after harvest using Chi-squared tests.

Results and Discussion

Before harvest in 2014, there were no differences in morphological metrics between partial-root harvested and unharvested plants (Table 1; Fig. 3), confirming that control and partial-root harvest plants were appropriately paired. There was no significant difference in reemergence between partial-root harvested and unharvested plants either the first year ($\chi^2 = 0.988$, $p = 0.568$, harvested 51/57 – 89% and 49/57 – 86%) or second year ($\chi^2 = 0.999$, $p = 0.815$, harvested 45/57 – 79% and unharvested 46/57 – 81%) after harvest.

Table 1. Statistical results from Analysis of Variance comparing morphological parameters between partial-root harvested and unharvested plants.

	Pre-harvest (n = 114)		1st year after (n = 100)		2nd year after (n = 91)	
Parameter	F	p	F	p	F	p
# of Berries	1.85	0.177	0.00	0.946	0.65	0.421
# of Leaves	0.00	1.000	2.00	0.160	1.08	0.302
# of Leaflets	0.37	0.547	2.74	0.101	2.00	0.161
Stem Length	0.43	0.516	5.08	0.026	0.31	0.579
Leaf Area	0.25	0.616	16.3	< 0.001	1.40	0.239

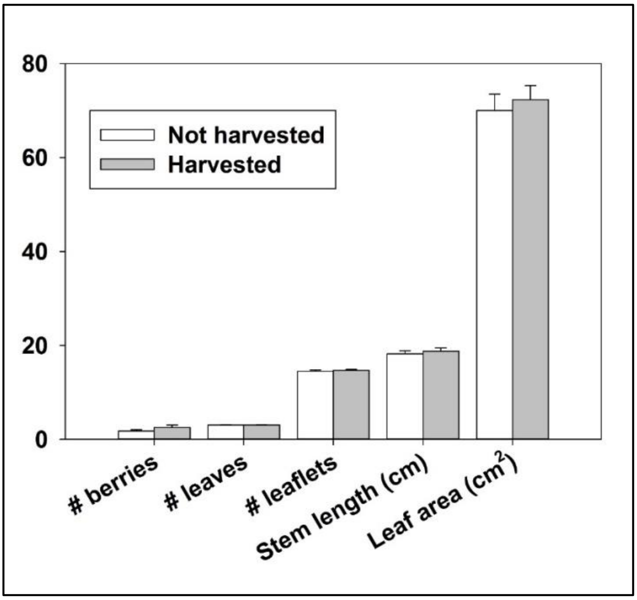

Figure 3. Pre-harvest mean (±1 se) morphological measurements did not vary significantly ($p > 0.05$ for all) between the harvested and unharvested plants.

Fitness (berry production) was not affected by partial-root harvest, however, there were short-term (one year) effects on growth. Mean number of berries decreased in the first year after harvest (2015) for both non- harvested and partial-root harvested plants, and this decrease was not significantly different between the two groups (Table 1; Fig. 4). Possible reasons for this decrease in berry production across both groups and all populations could include inter-annual differences in temperature and/or precipitation, although these factors were not addressed in this study. Neither the number of leaves nor the total number of leaflets per plant differed between partial-root harvested and unharvested plants in either year after harvest (Table 1, Fig. 4 & 5). The differences in number of leaves and leaflets between years is also likely due to environmental conditions and future research should explore the effects of environmental variation of growth. Because ginseng growth is determinant and buds are formed late the previous summer, it is likely environmental conditions in late summer the year before that determine leaf and leaflet number in the current year. Both stem length and leaf area decreased between 2014 and 2015 for partial-root harvested but not for unharvested plants, and these differences were significant (Table 1; Fig. 4). This decrease in stem length and leaf area in partial-root harvested plants could be a response to simulated root herbivory from the partial-root harvest. Root and rhizome herbivory has caused size class reversion and dormancy in subsequent years (Farrington 2006).

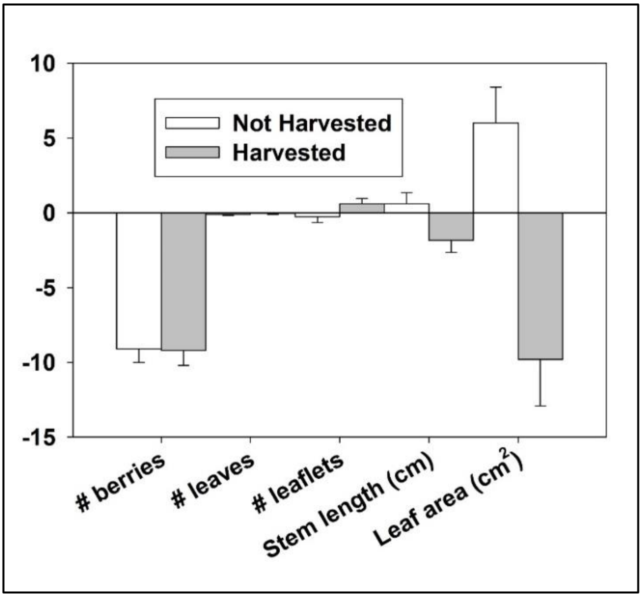

Figure 4. Mean (± 1 se) differences in plant morphological parameters between 2014 and 2015 for both partial-root harvested and unharvested plants.

By the second year after harvest (2016), number of berries, leaves and leaflets and stem length were all slightly lower than they were before harvest (Fig. 5), but were not significantly different between partial-root harvest and unharvested plants. Leaf area of unharvested plants was higher, while area of partial-root harvest plants was lower than pre-harvest values (Fig. 5). These values were highly variable in both groups, however, and were not significantly different between them (Table 1).

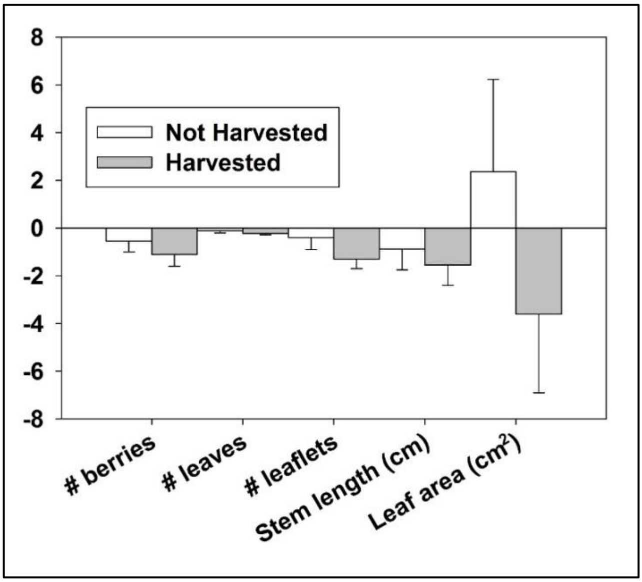

Figure 5. Mean (± 1 se) differences in plant morphological parameters between 2014 and 2016 for both partial-root harvested and unharvested plants.

In summary, partial-root harvest had no significant effect on survivorship one or two years after harvest. There were significant decreases in stem length and leaf area in partial-root harvested plants in the first year after harvest, but these were not present in the second year, suggesting that partial-root harvest has little long-term effect on growth or survivorship over time. Berry production was lower in 2015 relative to 2014, but the decrease occurred in both partial-root harvested and unharvested plants suggesting other factors such as inter-annual variation in weather.

Because the partial-root harvest method had no significant effect on survivorship in either year and had marginal and short-lived effects on morphology, it could prove to be an effective non-destructive method for ginsenoside extraction for research applications. The partial-root harvest method could be used in research projects designed to test the environmental and genetic factors affecting the production of secondary metabolites while preserving plant material in the field. One hindrance to studying the phytochemical makeup of wild American ginseng populations has been the need to destructively harvest root tissue. This method will allow researchers to sample plants from wild populations without causing long-term population declines.

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“Assessing the Status of American Ginseng from Harvest and Monitoring Data”

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Abstract

We summarize trends on the status of wild populations of American ginseng (*Panax quinquefolius*) by drawing inferences from the combined visualization and statistical analysis of four separate sources of data on ginseng populations: 1) Yearly harvests submitted to the U.S. Fish and Wildlife Service by states permitting the export of wild-harvested ginseng for the years 2000-2014. 2) Annual counts of ginseng populations on plots in Arkansas and North Carolina, 2000-2015. 3) Roots per lb. for Georgia dating from the 1984 – present. 4) Locations of ginseng populations throughout the eastern state and federal agency data and herbarium records. We find strong evidence that ginseng harvesting has increased since 2005, which represents a reversal of declines in harvesting from a high point from the mid-1980s to 1990, and that harvesting pressure has: 1) altered the age structure of populations such that mature reproductive plants are a smaller component with obvious implications for growth rates, and 2) led to population extirpations. We also found evidence that harvesting pressure may be leading to selection for reproduction at smaller sizes. Further research into social-ecological conditions seems key to understanding how ginseng populations respond and will persist in the face of heavy harvest pressure.

"Relationships between Genetic and Phytochemical Diversity of American Ginseng from Western North Carolina"

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Abstract

American ginseng (*Panax quinquefolius*) is a threatened and economically valuable woodland herb, distributed throughout forests in eastern North America. Previous research has shown that composition of medicinal compounds, ginsenosides, and genetic profiles vary within and among western North Carolina (WNC) populations. In this study, samples were collected from 3o wild-grown American ginseng plants. Root tissue was non-destructively subsampled for ginsenoside analysis via High Performance Liquid Chromatography, and leaflet samples were collected for analysis of six DNA microsatellite regions to assess genetic diversity. A majority of WNC populations were dominated by the RG (Re/Rg1 < 1) chemotype, while three populations had individuals with I (1 < Re/Rg1 < 2) and RE (Re/Rg1 > 2) chemotypes. Composite genetic distances were not correlated with any ginsenoside measure. Future studies will use commercial seeds and wild transplants into common gardens to determine the relative contributions of genetic and environmental factors to the production of medicinally-active compounds in these plants.

Keywords: American ginseng, chemotype, genotype, ginsenoside, microsatellite

Introduction

American ginseng (*Panax quinquefolius* L., Araliaceae) is a perennial herb inhabiting deciduous hardwood forests from Georgia to Quebec (Anderson et al. 2002) and as far west as Oklahoma (USDA Plants 2017). The plant produces multiple compound leaves and a single umbel; the latter matures to produce berries, which can be dispersed by thrushes (Hruska et al. 2014). The species is threatened, endangered, or of special concern in multiple states throughout its range (USDA Plants 2017). Wild-harvested American ginseng roots had a market value of up to $1,400 per pound for the 2015 season, while the price for dried, cultivated roots have a steady market value of $70 to $80 a pound (Rainey 2015). The high market value for wild-harvested roots has led to overharvesting to the point of near extinction, and in 1972 the species was listed on Appendix II of the Convention on International Trade in Endangered Flora and Fauna (CITES 2017). Population viability analysis of 36 populations predicted that *P. quinquefolius* had a > 99% probability of going extinct in the wild within the next century (McGraw and Furedi 2005). While American ginseng has been commercially cultivated for over two hundred years, wild harvesting has continued as non-cultivated roots earn higher prices on the Asian market due to phenotypic traits (McGraw et al. 2013).

Ginseng's secondary compounds, ginsenosides, are found within leaves and roots, and have been used in western medicine (McGraw et al. 2013). These medicinally active compounds are triterpendoid saponins, and are organized into two classes: 20(S)-protopanaxadiol, also called PPD, and t20(S)-protopanaxatriol, also called PPT. Members of the PPD class, which include Rb1, Rb2, Rc, and Rd, contain a carboxyl group on the C-6 position, while members of the PPT class, which include Re, Rg1, Rg2, and Rh1, do not (Kolodziej et al. 2013). Rb1, Rb2, Rc, Rd, Re, and Rg1 are the forms most commonly found in *P. quinquefolius* (Corbit et al. 2005, Schlag & McIntosh 2013). Many studies have found that Rb1 and Re are the most common ginsenosides found in American ginseng roots (Li et al. 1996, Court et al. 1996). Extracted *P. quinquefolius* ginsenosides have been used to treat immune, endocrine, cardiovascular, and central nervous systems disorders, and may be useful in cancer prevention (Dharmananda 2002, Corbit et al. 2005). In addition, intact American ginseng roots have been wild-harvested since the 1800s for export to the Asian market, where they are used in Traditional Chinese Medicine (TCM) (Carlson 1986).

Ginsenoside species and concentrations vary within plant organs and among individuals, with a plant's unique suite of ginsenosides described as a chemotype. There is greater chemotypic variability in western North Carolina than in other portions of American ginseng's range (Schlag and McIntosh 2013, Searels et al. 2013), and chemotypes can be correlated with genetic variation (Schlag and McIntosh 2013). In addition, a unique chemotype has been found in American ginseng from the southern Appalachians (Searels et al. 2013). The relative contributions of environmental, genetic, and interactive factors to this unique chemotype or to other chemotypic patterns remains poorly characterized, however. The goal of this study was to examine relationships between genetic and chemical factors in wild-collected *P. quinquefolius* plants from western North Carolina.

Methods

Ginsenoside Sample Collection and Preparation

A small portion of root was collected from 30 three-leaved, non-reproductive plants in western North Carolina, leaving most of the root intact. The root drying procedure mimicked commercial procedures, with wet root mass measured and samples placed in a drying oven at ~37 °C for approximately 140 hours. Dry mass was measured, and roots were ground in a Wiley Mill with a 40-mesh screen.

The extraction procedure, adapted from the methanol reflux extraction of Corbit et al. (2005), maximizes ginsenoside yield. For each sample, 100 mg of the powdered plant root was combined with 5 mL of 100% HPLC- grade methanol. Samples were refluxed at ~63 °C for 1 h, then the methanol solution was vacuum filtered through Whatman 41 Ashless filter paper. Another 5 mL of 100% HPLC-grade methanol was added to the remaining root material and allowed to reflux for 1 h. The methanol solution was filtered again through vacuum filtration and added to the previously-extracted liquid. The vacuum flask was rinsed with another 5 mL of 100% HPLC-grade methanol and added to the liquid extraction. Samples were diluted to 20 mL with 100% HPLC-grade methanol and then filtered using a 0.45 µM filter.

Ginsenoside Analysis

Standards were prepared using ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd, obtained from Indofine Chemical Company (Hillsborough, NJ). Ginsenosides in standards and plant extracts were separated by high performance liquid chromatography (HPLC, Thermo-Hypersil Gold, 150 x 3mm, C_{18} column 3 µm particle size, Shimadzu Inc.) using an injection volume of 20 µL with water/acetonitrile gradient elution at a rate of 0.6 mL/min. Gradient shifts were as follows:

0-22 min 95/5
22-40 min 78/22
40-50 min 55/45
50-52 min 45/55
52-58 min 35/65

The column temperature was held at 35 °C, and ultraviolet detection was set at 205 nm. Each ginsenoside was identified by retention time, which remained constant throughout the analyses. The concentration of each ginsenoside was calculated using the peak area and a six-point external standard calibration curve.

Genetic Sample Collection and Extraction

Single leaflet tissue samples were collected from 30 western North Carolina plants and stored at -80 °C until extraction. Then, whole genomic DNA was extracted from leaflets using Qiagen DNeasy Plant Mini Kits (Qiagen, Valencia, CA). DNA concentrations of samples were quantified spectrophotometrically (NanoDrop, Wilmington, DE), with ideal concentrations around 10 ng/µL. High concentrations were diluted with AE Buffer (Qiagen).

Microsatellite Amplification and Analysis

Twelve microsatellite primers specific for *P. quinquefolius* (Young et al. 2012) were ordered from Eurofins MWG Operon (Huntsville, AL), then screened with Polymerase Chain Reaction (PCR). The six most consistently amplifying primer sets for western North Carolina plants (B011, B119, C105, C202, D114, D227) were fluorescently-tagged then used in subsequent PCR amplifications. In each reaction, 7 µL of DNA sample was combined with 1 µL each of the forward and reverse primer (10 µM) and 9 µL of MasterMix (5 PRIME, Gaithersburg, MD). Microsatellite regions were then PCR-amplified (BIO-RAD Thermocycler, Hercules, CA) using the following protocol (Young et al. 2012):
94° C for 2 minutes
   35 cycles of
   94° C for 40 s
   56° C for 40 s
   72° C for 1 min
final extension at 72° C for 10 min

PCR products were visualized via gel electrophoresis (1% agarose gels), and successful products were multiplexed with the LIZ 500 ladder and sent to the DNA Analysis Facility at Yale University for fragment analysis. Peak calls for raw data were made in Geneious 10.2.2 with the Microsatellite 1-4-4 plugin.

Statistical Analysis

Statistical analyses were conducted in R 3.1. Composite genotypes were generated with Polysat v. 3.1.3. Euclidean distances among ginsenosides were then calculated in Vegan 2.3-5. Vegan 2.3-5 was used to conduct Mantel tests, with Spearman's rank correlations and 9999 iterations, to discern relationships between composite genotypes and ginsenoside patterns.

Results

Analyses of data for these 30 plants revealed no relationships between genetic and ginsenoside patterns. When individual ginsenosides were used to generate composite distances, they were not related to composite genotypes (Mantel r = 0.02485, P = 0.31); neither was total ginsenoside concentration

(Mantel r = 0.02324, P = 0.33). Geneotype was also not related to chemotype (Re/Rg1 ratio: Mantel r = 0.0088, P = 0.41; Rg1 concentration: Mantel r = 0.0058, P = 0.43; Re concentration: Mantel r = 0.013, P = 0.38).

Discussion

Chemotypic diversity in western North Carolina plants was depressed relative to plants in Maryland (Schlag and McIntosh 2013). This could be due to higher rates of harvesting here, or more consistent environmental conditions among sites that we sampled. This pattern might also be attributed to reduced genetic diversity in western North Carolina populations, although methodological differences between our study and that of Schlag and McIntosh (2013) render direct comparisons impossible.

Preliminary examinations of small numbers of wild-grown American ginseng from western North Carolina showed no relationships between chemical (6 individual ginsenosides) and genetic (composite genotypes with 6 microsatellite loci) properties. Perhaps ginsenoside differences are not correlated with the neutral loci discerned through microsatellite analyses. Alternatively, environmental conditions could exert more control over chemotypic patterns than innate genetic differences.

Future research will require more exhaustive sampling and analysis of these populations as well as additional populations from individuals within western North Carolina. It would also be informative to include a data from commercial seeds grown under field conditions. Finally, as contribution of environmental factors to chemotypic patterns remains uncharacterized, growing different genotypes in common gardens will allow the relative contributions of genetic and environmental factors to be discerned.

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"American ginseng status assessment on four National Forests in the Mid-Atlantic U.S."

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Abstract

In an effort to better understand the distribution, population density, and genetic structure of American ginseng (*Panax quinquefolius*) on mid-Atlantic US National Forests, the USGS, at the request of the USFS, conducted a comprehensive study on four National Forests in 2014-2015. Field surveys, guided by a randomized field survey design and species distribution modeling, were conducted on the Monongahela (WV), Wayne (OH), Pisgah (NC), and Nantahala (NC) National Forests. Data collected in field surveys was used in subsequent statistical population density estimates, population simulation under various harvest and stewardship scenarios, and genetic analysis. We found American ginseng plants generally widely distributed in accordance with predicted habitat, but at low densities in field plots. The highest densities were found on the Nantahala NF, followed by the Pisgah NF, the Monongahela NF, and lowest densities on the Wayne NF. Through population survival modeling we found that probability of extinction decreases with stewardship behaviors, especially re-planting of seeds, but the probability of extinction was never zero. Simulated population viability was highly dependent on initial population size, survivorship scenario, harvest timing, and stewardship type. Results of genetic analysis were highly variable among sample sites, with some sites highly diverse and others consisting largely of selfed progeny. In general, we found that more genetic diversity is held *among* populations within Forests (51%) than within populations (36%), and only a small fraction of genetic diversity is held among the four Forests (13%). These findings suggest that care should be taken to maintain as many individual populations as possible as a large proportion of the existing genetic variation is apparent among populations, and remaining populations, while widely dispersed, currently exist at low densities and are susceptible to harvest pressure.

“An Introduction to Flower Essences: Sustainable Supplements from Forest, Field, and Garden”

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Abstract

Flower essences are a complementary healing approach addressing emotional/feeling states. Originated in England between 1928 and 1935 by Dr. Edward Bach, flower essences are now developed and used in many parts of the world. They are similar to homeopathic remedies in that they derive their treatment not from biochemical properties but from energies that expand through dilutions. Because of the dilution process, a small number of flower blossoms result in a great many 30 ml treatment bottles for individuals, making flower essences a sustainable modality. This paper gives an overview of how flower essences are made. It also presents a chart of six forest botanical flower essences, four of them listed by United Plant Savers as at-risk species. Properties of these essences as derived by their developers are shown in the chart.

Keywords: flower essences, sustainable, forest botanicals, pawpaw

About Flower Essences

Flower essences are a complementary healing approach addressing emotional states. In contrast with psychiatric medications, which can be more dramatic in their immediate impact, flower essences offer gradual and longer-term transformation. They are vibrational rather than biochemical in nature, and they work via resonance with feeling states (Kaminski, 1998). Flower essence remedies have a similarity to homeopathic remedies in that they derive their healing not from biochemical properties but from energies that expand through dilutions.

Flower essence practitioners work to match the feeling state of a client as precisely as possible with a remedy. The practitioner’s study and knowledge of individual flower essences and knowledge of the individual client aid this; it is a correspondence between person and nature. Kinesiology, or muscle testing of the body’s electrical system, may also assist in selecting essences (Wright, M.S. 2011).

Flower essences were first originated and developed by Dr. Edward Bach, an English physician who was a pioneer in the development of vaccines. Between 1928 and 1935 Bach developed 38 different essences, which he called remedies, each of which he classified under seven general kinds of psychological states: fear, uncertainty, insufficient interest in present circumstances, loneliness, over-sensitivity to influences and ideas, despondency and despair, and over-care for the welfare of others (Weeks, N. 1994).

Flower essences are developed in many parts of the world today and include the following. Julian Barnard of Healing Herbs Ltd, located in Walterstone, Hereford (Great Britain), is a specialist producer of Bach flower essences and offers education about flower essences through books and online resources. The Flower Essence Society and its companion organization Flower Essence Services, located in the foothills of the Sierra Nevada in Nevada City, California, develop essences, support research, and provide training and education. FES offers two comprehensive sets of essences that it has developed and researched along with those of Healing Herbs Ltd. The Perelandra essences are developed and made available by the Perelandra Center for Nature Research in Jeffersonton, Virginia. Woodland Essence in Cold Brook, NY develops and produces a line of Woodland flower essences, including a collection of forest floor essences.

Making Flower Essences

Most developers and producers of flower essences describe the details of making their essences; see for example Barnard (2010). The basic steps for sun-infused essences are to 1) gather fresh blossoms in the morning on a sunny day, 2) fill a glass bowl (size depends on size and number of blossoms) with the most pristine water available in the area and float the blossoms on top of the water, and 3) let the bowl sit undisturbed on the earth in the sunlight for several (3-4) hours. Figures 1 and 2 show flowers infusing in early spring sunlight.

Figure 1. Flowers infusing in sunlight: Redbud, Wild Ginger, Violet. (Photograph by Matthew Ziff)

Figure 2. Flowers infusing in sunlight: Pawpaw
(Photograph by Katherine Ziff)

The interaction of water, sunlight, flowers, and earth support the creation of an imprint, or vibration, of the energy pattern of the flower in the water (Kaminski, 1998). The resulting infusion is known as the “mother essence.” It is preserved with brandy in a ratio of about equal parts brandy and essence. This mother essence is diluted to make “stock” bottles by adding a few drops of the mother essence to a one-ounce (30 ml) dropper bottle filled with equal parts water and brandy. While dilution suggestions vary today, the original Bach stock concentrate suggests a 1:400 dilution, or two drops of the mother essence to prepare a 30 ml stock bottle. Thus, 30 ml of a mother essence can be used to produce hundreds of stock bottles, which are made available to stores and to flower essence practitioners. The practitioner will add a few drops from the stock bottle to a 30 ml dropper bottle of equal parts water and brandy, to make a final dilution for a dosage bottle for an individual. A typical usage cycle of a 30 ml dosage bottle is four drops, four times daily for three-four weeks. Thus, from a glass bowl of 500 ml of mother essence, preserved with brandy added 1:1, may be obtained about five million dosage bottles at treatment strength, each bottle enough for one person for a typical usage cycle of three-four weeks (Barnard, 210).

Properties of Flower Essences

Some producers of flower essences maintain a research practice. The Flower Essence Society maintains a broad research program regarding the properties of their essences. The program includes plant study, testing in clinical settings by health practitioners, maintenance of files of practitioner case studies, and support for more narrowly defined experimental designs. Table 1 shows properties identified by the developer(s) of selected flower essences made from forest botanicals.

Table 1. Flower essences from forest botanicals

Plant	Properties	Developed
American Ginseng	Brings vitality, strength, support for depletion. For when releasing fear of expressing true self is needed.	Woodland Essence: Cold Brook, NY http://www.woodlandessence.com/herbal.htm
Black Cohosh	For courage to deal with/heal from abusive situations. Brings release from toxic lifestyles & entanglements. Supports emergence of a bright, strong sense of self.	FES: Flower Essence Services & Flower Essence Society, Nevada City, CA. From their Quintessentials collection. http://www.flowersociety.org/index.html Woodland Essence: Cold Brook, NY
Goldenseal	For those benefitting from energizing and free flow of energy by releasing that which no longer serves, making way for new.	Woodland Essence: Cold Brook, NY
Pawpaw	For aid in the digestion and assimilation of ideas. Encourages scattered plans to come together and take productive form. Offers healing between humans and the natural world.	Briarwood Studios LLC Athens, Ohio
Trillium	Brings a secure sense of personal welfare and well-being; courage and flexibility to flow with changes and cycles of life.	FES: Flower Essence Services & Flower Essence Society, Nevada City, CA. From their Quintessentials collection. Woodland Essence: Cold Brook, NY
Wild Ginger	Brings an invitation to rekindle connection with nature and the forest. Grounding.	Woodland Essence: Cold Brook, NY

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“Alkaloid content in forest grown goldenseal: preliminary results and current directions”

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Abstract

Goldenseal (*Hydrastis canadensis*) is an Appalachian forest herb whose rhizome is used to treat inflammation and digestive disorders. Due to overexploitation concerns and significant demand, goldenseal is a crop option for forest farming. Despite its popularity as an herbal medicine, there is little information on the effects of harvest timing and habitat-related production factors on its medicinal constituents (i.e., Berberine, Hydrastine, and Canadine). The need to satisfy market demand with sustainably harvested, quality assured product requires a better understanding of goldenseal chemistry. Results (using High Performance Liquid Chromatography) in central Pennsylvania suggest that time of harvest can dramatically influence the alkaloid content in the dried root and rhizomes. Alkaloid content was found to peak in July (fruiting stage) and October (senescent stage), while samples between those times fell well below current recommended therapeutic and industry constituent levels (c.f., United States Pharmacopeia). My current research further examines harvest timing effects by expanding the range of the previous study to include (1) aerial and root portions; (2) time of day harvested; (3) full seasonal phenology; and (4) drying temperature. Additionally, I am conducting more exhaustive geographic sampling for associated habitat conditions in Pennsylvania and nearby states. The results of this study will identify production, harvest and post-harvest factors that can influence quality control in forest farmed goldenseal. This, in turn, may help forest farmers garner higher prices and a stronger market edge compared with wild crafted product –contributing to conservation of remaining wild populations by creating a more desirable product.

Keywords: Forest Farming, Goldenseal, Medicinal Plant Chemistry, Isoquinoline Alkaloids, Quality Control

Introduction

Plants have long played a critical role in treating human ailments in cultures around the world. In North America, early European settlers quickly adopted many natural remedies from the Native Americans that were well accustomed to making use of North America’s rich array of natural remedies – many of which grew under the canopy of the vast forested landscape at the time. While herbal popularity faded in the 20th century with the advent of “modern medicine,” it has made a resurgence in recent decades due to public interest in “natural” medicine along with academic interests in new medicinal compounds that can be developed into pharmaceutical drugs.

One medicinal plant that has been particularly popular in North American has been goldenseal (*Hydrastis canadensis*). Historically, Native Americans used goldenseal root for the treatment of numerous maladies including whooping cough, diarrhea, stomachache, tuberculosis, fever, earaches, and general weakness as well as a tonic and wash for inflammation (Moerman, 2003) . Among early European settlers, the most common medicinal uses for goldenseal include an eye wash (hence the common names eye balm and eye root), a bitter tonic, a digestive aid and appetite stimulant, and a treatment for mucus membrane inflammation (Lloyd and Lloyd, 1884). Today, goldenseal is one of the most popular plants in the herbal medicinal market, and is found in many formulations used to treat numerous ailments, and is known to have antibacterial, antimicrobial, anticancer, and immune-stimulant properties (Le et al., 2013). Goldenseal’s medicinal properties are largely attributed to a synergistic action of the alkaloids berberine, hydrastine, and canadine (Avula et al. 2012, Scazzocchio et al., 2001, Weber et al. 2003).

Goldenseal was introduced into cultivation in the United States more than a century ago (Van Fleet 1914) but the adoption of goldenseal as a specialty crop has been hindered by volatile prices and demand, and associated profitability concerns (Burkhart and Jacobson 2009, Person and Davis 2005). It is generally believed that most goldenseal on the market today originates from harvesting from wild populations in the United States (c.f., AHPA 1999, 2003, 2006, 2007) and thus the species is included in Appendix 2 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) due to conservation concerns surrounding continued wild harvests. The timing of harvests, allowing an adequate recovery interval, and attention to site influences are all important components for sustainable harvesting from wild populations (Albrecht and McCarthy 2006, Sanders and McGraw 2005, Sinclair and Catling 2004).

The United State Pharmacopeia alkaloid minimum standards for goldenseal dictate 2.5% for berberine, and 2.0% for hydrastine. However, reports on alkaloid content range from 0.5-6.0% berberine, 1.5-4.0% hydrastine, and 0.5-1.0% canadine, with a total alkaloid range of 2.5-6.0% (Upton, 2001). Studies of other North American medicinal forest plants such as bloodroot, *Sanguinaria canadensis* L. (c.f. Salmore and Hunter 2001), American mayapple, *Podophyllum peltatum* L. (c.f., Zheljazkov et al. 2009) and American ginseng, *Panax quinquefolius* L. (c.f., Lim et al. 2005) have shown that there are often differences in chemistry resulting from when and where plants are harvested, and that these differences can be important qualitative considerations for herb buyers, consumers and herbal practitioners. This study examined alkaloid content – in particular the three major alkaloids berberine, hydrastine and canadine – in wild-harvested goldenseal roots and rhizomes in relation to plant colony and harvest date to evaluate (1) alkaloid variation in wild-harvested roots from colonies co-occurring on a single forested site; and (2) the best post-reproductive phenological stage during which harvest should occur for purposes of maximizing root alkaloid content.

Methods

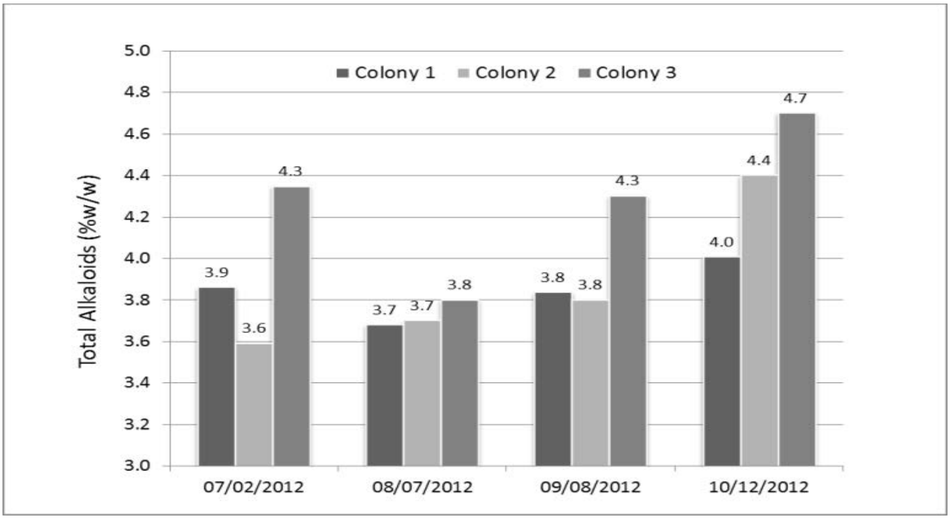
Three spatially distinct goldenseal colonies were sampled in a forested hollow in central Pennsylvania. Colony 1 was situated at the lowermost position in the hollow (elevation 305 m); colony 2 at a middle-upper location; and colony 3 occurred at the upper end on the hollow (elevation 370 m). Each colony was spatially distinct with a distance of approximately 450 m between colonies 1 and 3. Soil conditions varied slightly between colonies, although all soils had a pH between 6 and 7, were low in fertility (by agronomic standards), and had moderately high levels of calcium. Due to conservation concerns, exact locations are withheld from this publication but Global Position System (GPS) coordinates are on-file with the PA DCNR Wild Plant Management Program. Voucher specimens for study populations were deposited in herbaria at the Carnegie Museum of Natural History (Pittsburgh, PA) and the Morris Arboretum of the University of Pennsylvania (Philadelphia, PA).

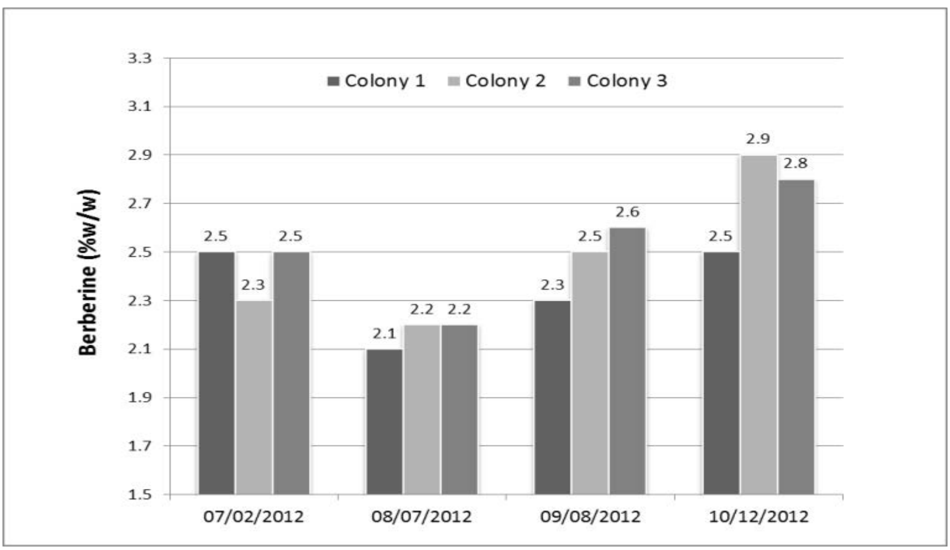
Goldenseal roots and rhizomes were harvested from mature, reproductive stems (i.e., stems bearing 2-3 leaves) on four dates corresponding with the following phenological stages of interest: (July 2) Fruit present and fully mature, foliage green; (August 7) Post fruit bearing, foliage green; (September 8) Post fruit bearing, foliage beginning to yellow; and (October 12) Foliage yellow, plants senescing with ~50% of each colony fully senesced. Root and rhizome material was not differentiated in collecting and processing samples, and samples are hereafter simply referred to as root samples. For each sample date, three root samples were collected from each of the three colonies for a total of nine samples per date and 36 samples total. Roots were washed and dried at 35°C for 24-36 hours until they were dry enough to break cleanly. Root samples were ground to approximately 60 mesh and analyzed using High Performance Liquid Chromatography.

Results and Discussion

There was considerable variation observed both within and between colonies for all three alkaloids (Figure 1). Total 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). Average root alkaloid content was highest in colony 3 on all sample dates (Figure 2), suggesting that local growing conditions and/or genetic predilections could be influential factors in goldenseal alkaloid production. Both of these potential influences have been highlighted in wild chemistry studies of other North American medicinal forest plants (c.f., Lim et al. 2005, Zheljazkov et al. 2009).

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.





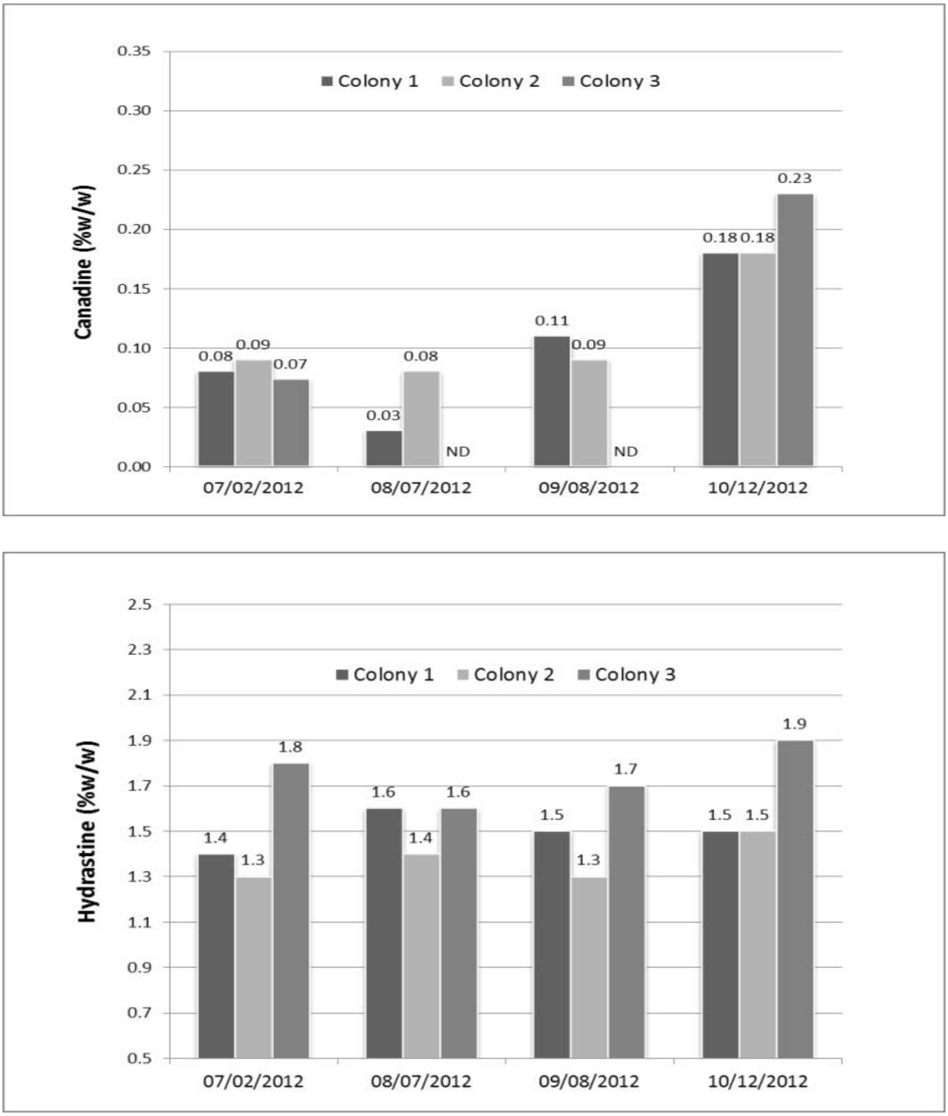
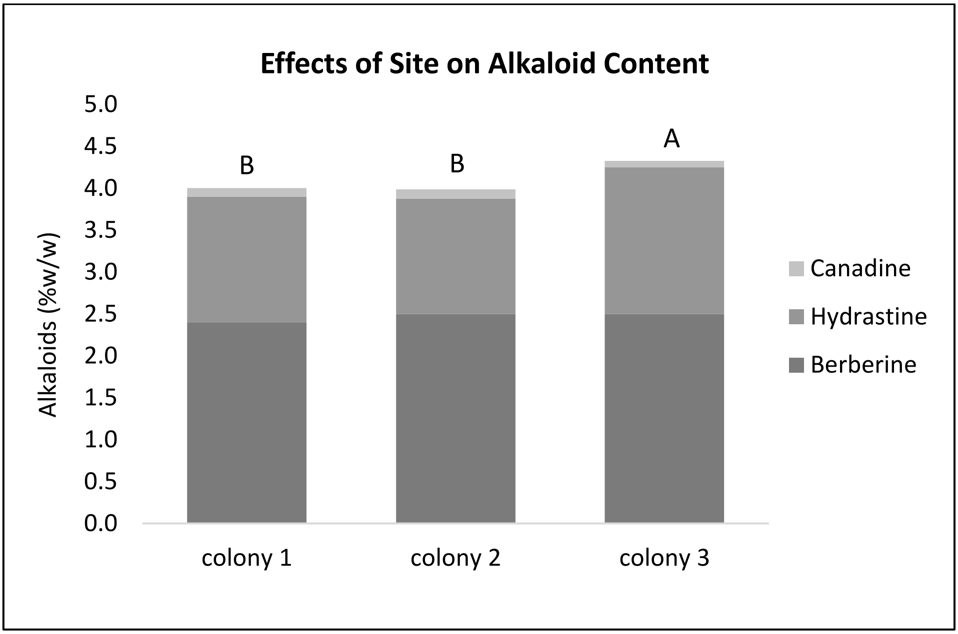


Figure 1: Alkaloid levels in wild-harvested goldenseal rhizomes/roots in relation to colony and harvest date. Clockwise from top-left: total alkaloid content (berberine, hydrastine and canadine), berberine, canadine and hydrastine. Note the different scales on the vertical axes. Harvest dates corresponded 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.

Figure 2: Effect of site on alkaloid content of goldenseal in central Pennsylvania (*Bars with different letters are significantly different based on Bonferroni method and 95% confidence)



Total alkaloid content was highest at plant senescence (Figure 3), which corroborates the long-held belief among wild-crafters that roots and rhizomes should be harvested during the fall months. Total root alkaloid content averaged between 4.0% and 4.7% on the final harvest date (Oct 12) compared with an average of 3.7%-3.8% obtained from samples harvested in August. 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 roots and rhizomes (USP 2013). Out of the 36 wild harvested goldenseal root samples analyzed in this study, slightly more than half (53%, *n* = 19) met this threshold for berberine while only one sample (<1%) met the hydrastine threshold.

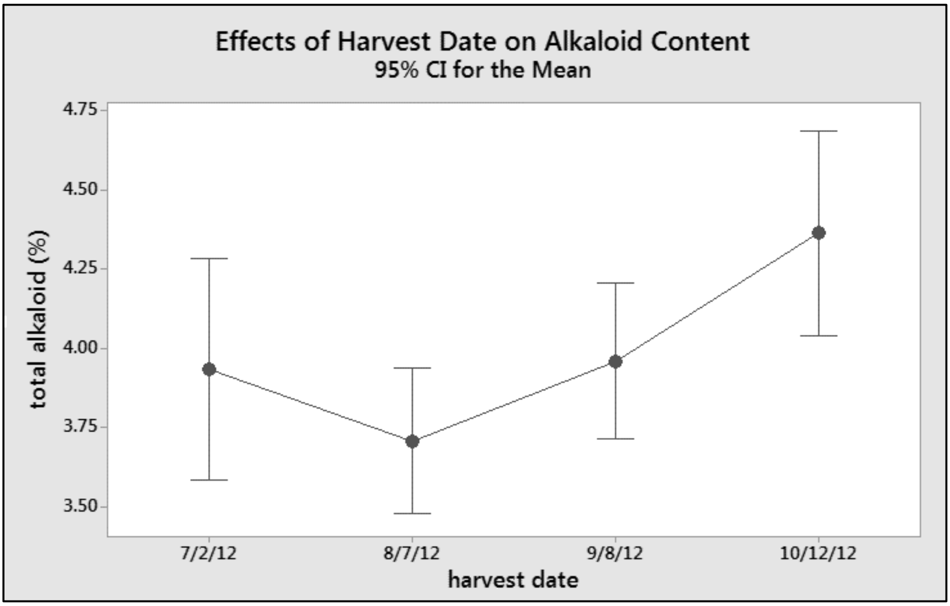


Figure 3: Effect of harvest date on total alkaloid content in goldenseal across three colonies in central Pennsylvania

Conclusion

The harvest of goldenseal from the wild is inherently of concern because it is the underground roots and rhizomes that are harvested, and this can lead to population declines if proper stewardship behaviors are not followed (such as allowing for recovery time between harvests). Over a century ago, there were reported noticeable declines in populations (Lloyd and Lloyd, 1884), and it is common to see statements such as “declining due to excessive collection” in some regional floras today (e.g., Plants of Pennsylvania, 2007). One course of action that has been identified as a viable option for helping sustain goldenseal in the future is to promote forest farming as an alternative for digging the plant from the wild. In order to be successful, it is important to have a more comprehensive understanding of the effects of habitat and harvest timing on alkaloid production in goldenseal.

Results indicate that colonies of goldenseal can vary significantly, and support a fall harvest of goldenseal roots/rhizomes which is in agreement with traditional lore as well as current understandings of sustainable wild-harvest practices (c.f., Albrecht and McCarthy 2006, Sanders and McGraw 2005). Total alkaloid content – and the levels of the individual alkaloids berberine, hydrastine and canadine – were all greatest at plant senescence (early October). However, our inability to distinguish what could be influencing the differences observed between colonies and our finding that alkaloid levels were nearly as high at fruit maturity (early July) as they were at plant senescence (early October) suggests further research is needed to examine additional goldenseal populations and early season alkaloid levels particularly during the period between flowering and fruiting. It may be that alkaloid levels fluctuate during the growing season in relation to key reproductive phenological stages, and that total alkaloid levels – or individual alkaloids – are in equal or greater concentration in roots/rhizomes during this time. While early (pre-fruit maturation) season harvests would negatively impact reproduction in wild populations, early season harvests from cultivated or forest farmed populations would not present such ethical dilemmas.

My current research further examines harvest timing effects by expanding the range of the previous study to include (1) aerial and root portions; (2) time of day harvested; (3) full seasonal phenology; and (4) drying temperature. Additionally, I am conducting more exhaustive geographic sampling for associated habitat conditions in Pennsylvania to illicit more detailed factors that may have an influence on the average alkaloid content in a given colony. The results of this study will identify production, harvest, and post-harvest factors that can influence quality control in forest farmed goldenseal. This, in turn, may help forest farmers garner higher prices and a stronger market edge compared with wild crafted product – contributing to conservation of remaining wild populations by creating a more desirable product.

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