GENETIC VARIATION IN *HYDRASTIS CANADENSIS* POPULATIONS IN WESTERN NORTH CAROLINA

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ABSTRACT

GENETIC VARIATION IN *HYDRASTIS CANADENSIS* POPULATIONS IN WESTERN NORTH CAROLINA

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Goldenseal (*Hydrastis canadensis* L.) is a herbaceous perennial that is broadly distributed in patches in eastern deciduous forests. This plant is a valuable medicinal herb, and overharvest has been a cause of population decline along with loss of habitat. Because goldenseal reproduction mostly occurs clonally through rhizome growth and patches are usually small, dense, and highly isolated, genetic diversity within a patch is thought to be relatively low, but little is actually known about goldenseal genetics. Genetic variation is important for species in changing environments because a diversity of alleles provides a possibility for genetic adaptation. This project measured the genetic variation in six natural populations of goldenseal in western North Carolina using an allozyme analysis on leaf material collected from the field to measure molecular genetic diversity and a common garden experiment using rhizomes transplanted from the field to measure phenotypic genetic diversity. Half the rhizomes for the common garden experiment were grown in a greenhouse and the other half under a lath house. Rhizomes were cut in half, producing genetic clones, and one half was given a high fertilizer treatment and the other half low fertilizer. Phenological traits, measured at the end of the growing season, showed more phenotypic variation between than within populations for

emergence and dieback dates, percent reproduction, and biomass. Fertilizer was a significant factor in differences in biomass and dieback timing, but growth area was not a significant source of variance for any trait. Additionally, no significant interaction between genetics (population) and the environment (fertilizer level) was found (i.e. no GxE), which indicated a lack of local adaptation and suggested goldenseal may be a genetic generalist. A separate field study revealed significant differences among populations in rhizome content of medicinal alkaloids. Results from the allozyme analysis on the same populations as used in the common garden indicated more genetic variation within than between populations, so these populations are not diverging genetically. However, even within populations diversity was very low. The molecular diversity present could be due to sexual reproduction. To encourage sexual reproduction and the maintenance of genetic diversity, it may be beneficial to add substrate disturbance and canopy gaps to populations with the greatest conservation needs. Additionally, because fertilizer increased biomass, which was associated with higher reproduction, adding fertilizer at time of disturbance could increase population growth through increased clonal spread and sexual reproduction.

Keywords: rare plant, medicinal herb, genetic diversity, allozymes, common garden

CHAPTER 1: INTRODUCTION

Habitat fragmentation can decrease population sizes and leave species vulnerable to a loss of genetic diversity (Young et al. 1996, Honnay et al. 2005, Kramer and Havens 2009). Loss and alteration of habitat can be especially detrimental for plants that rely heavily on clonal growth and have low population growth rates (Lei 2010). *Hydrastis canadensis* is a medicinal woodland herb native to eastern deciduous forests experiencing habitat loss and reduced population sizes and is rare in much of its range (Van Fleet 1914, Sinclair et al. 2005, Sanders and McGraw 2005). Overcollection for the herbal market, beginning as early as the mid 1800's, has put this species at risk of extinction (Bowers 1891, Van Fleet 1914).

Knowledge of a species' genetic diversity is important when assessing the stability of small isolated populations. Low genetic diversity can result in a population's inability to adapt to variability in the environment (Ouborg et al. 2006), which is especially important if a species is a genetic specialist; a species genetically adapted to specific environmental conditions (van Tienderen 1991). Little is known about the genetics of goldenseal, but due to isolation of populations, slow growth rates, and clonal reproduction, genetic diversity is thought to be low (Sanders 2004). Assessing patterns of genetic variation can inform us of genetic distributions that should be protected or restored, and can inform land managers of appropriate conservation plans (Kramer and Havens 2009).

The purpose of this experiment was to describe goldenseal genetic variation in natural populations in western North Carolina and to determine the relative contribution of genetics and the growing environment on phenotype. The questions were twofold: (1) do natural populations have more genetic variation among or within populations, and (2) do genetics and the growing environment differ in affect on phenotypic responses in emergence, reproduction, biomass and dieback?

An allozyme analysis was chosen to test for molecular genetic variation in this study because an organism's genetic variability can be quantified using this technique (Soltis and Soltis 1989). The protein products of various alleles are called allozymes and can be visualized using protein gel electrophoresis. Allozyme analysis is a relatively simple, fast, and cost-effective way to determine the amount of homozygosity versus heterozygosity in a population, and can inform us if sufficient gene flow (represented by high heterozygosity) between populations is occurring to allow for genetic stability (Soltis and Soltis 1989).

The relative effects of genetics and growing environment were measured using a common garden experiment (Salmore and Hunter 2001). Goldenseal production is not recommended in a greenhouse, but is successful in cleared forest sites or lath houses (Persons and Davis 2005). Because the focus of this study was genetics, and not commercial growing conditions, I chose to grow half the plants in a greenhouse to see if it would be successful, and the other half of the plants under a lath house in case the greenhouse plants did not survive. Also, this allowed for a statistical comparison of growth areas. Although growing goldenseal in a greenhouse may not be preferable to commercial growers, it is beneficial for scientists to know if goldenseal can grow

successfully in a greenhouse to allow for experimental analyses in a controlled environment with this plant. Also, information gained in the greenhouse can be applied to more traditional farming practices to maximize traits of interest such as alkaloid production and size (Laidig et al. 2008).

Combining molecular analysis with a quantitative genetic study provided a chance to quantify levels and patterns of genetic diversity in natural populations of goldenseal, along with degree of plasticity in response to differing environmental conditions (Kramer and Havens 2009). These data are required for effective conservation planning including population restoration either through seed establishment or re-introduction of plants (Kramer and Havens 2009).

In this experiment, data are from the first year of a two-year study. Goldenseal in the common garden experiment will be grown for a second year and will have phenotypic data collected again. These data will be compared to the first year data and will provide a more accurate measurement of phenotypic response in goldenseal. In addition, after the second growing season for the common garden experiment, final rhizome weights will be collected to determine if there was a response in rhizome growth to differing environments. Rhizomes will also be analyzed for alkaloid content to determine if alkaloid content is a plastic or adapted trait in differing environmental conditions. This information could be beneficial to commercial growers trying to maximize medicinal content of their goldenseal.

CHAPTER 2: BACKGROUND

Population Structure and Dynamics of Woodland Herbaceous Perennials

Fragmentation of suitable habitat is a leading cause of extinction for species worldwide (Honnay et al. 2005). One consequence of habitat fragmentation is reduction in population sizes. When populations are small and isolation increases, the probably of extinction increases as well (Honnay et al. 2005). If habitats become unsuitable due to degradation, plants must adapt genetically, migrate through dispersal, or adjust through phenotypic plasticity. However, migration can be difficult for if habitat is too fragmented (Kramer and Havens 2009).

As pollination exchange between plant populations becomes limited due to fragmentation, populations can diverge from each other genetically and become locally adapted, especially if there are selective pressures (Young et al. 1996, Honnay et al. 2005, Honnay and Jacquemyn 2006, Jacquemyn et al. 2012). This can be a detriment to long-term survival of a species in fluctuating environments (van Tienderen 1991, Callaway et al. 2003, Gianoli 2004). However, woodland perennials have evolved reproductive strategies to adapt to heterogeneous environmental conditions such as long generation times and clonal growth (Piquot et al. 1998, Honnay et al. 2005).

Woodland perennials are usually long-lived and use some amount of clonal growth to perpetuate, and seed dispersal is often across short distances (Piquot et al. 1998, Honnay et al. 2005). Benefits of clonal reproduction include the ability to share resources between parent and offspring, less energetic output, and greater chance of

survival (Lei 2010). However, vegetative growth results in offspring that are genetically identical to the parent so no new allele combinations are introduced leaving the population vulnerable to change or biological invasion (Lei 2010). Sexual reproduction provides populations with dispersal and colonization capabilities, and recombination can produce new genotypes, allowing for ability to adapt to environmental change. However, it takes more energy to produce flowers and fruit, especially when many seeds are produced but only a few germinate and survive (Cruse-Sanders and Hamrick 2004, Goertzen and Boyd 2007, Lei 2010).

Some plants can reproduce both clonally and sexually and can alter strategies depending on environmental conditions (Piquot et al. 1998, Gardner and Mangel 1999, van Kleunen et al. 2001). For example, in *Ranunculus reptans* L., clonal reproduction is used more often in open suitable habitats, and sexual reproduction occurs when environments become less favorable and adaptation or dispersal is needed (van Kleunen et al. 2001, Lei 2010). Other species such as *Paris quadrifolia* L., respond oppositely in heterogeneous environments; allocating more energy to clonal growth in unfavorable environments (Jacquemyn et al. 2006). Still other species including *Sparganium erectum* L. respond differently at different landscape scales with sexual reproduction as a response at the meta-population level and clonal spread favorable at the population level (Piquot et al. 1998).

Genetics

Genetic variation can play a critical role in the survival of a species because genetic diversity provides species with adaptive capabilities in a changing environment

(Wright 1931, Ouborg et al. 2006). Habitat fragmentation causes populations to diverge due to increased genetic drift, inbreeding, local extinction, and decreased gene flow (Wright 1931, Young et al. 1996). As habitats are fragmented or altered, population sizes often go down. Small populations are at risk of extinction because they are more likely to lose random alleles through genetic drift that may be advantageous to survival. When alleles are lost from a population, genotype proportions shift from heterozygous to homozygous. This in turn can lower fitness and adaptability (Wright 1931, Ouborg et al. 2006). It is beneficial to determine the amount of genetic variation in a species with small populations to better understand and more effectively manage for potential genetic risks within the species.

Isolation has differing affects on population structure and genetic diversity for woodland perennials including clonal and non-clonal species. For example, the clonal species *Convallaria majalis* L. has little to no within population variation (most were a single genotype) and high population structuring (F_{ST}=0.052) (Vandepitte et al. 2010), and *Panax quinquefolius* L., a non-clonal species, also has low observed heterozygosity (H_o=0.076) (Cruse-Sanders and Hamrick 2004). Conversely, high diversity has been measured in the clonal species *Clematis socialis* Kral (H_o=0.302) (Goertzen and Boyd 2007) and also in the non-clonal *Euphorbia telephioides* Chapm. (H_o=0.310) (Trapnell and Hamrick 2012).

Quantitative genetics is a good way to assess population genetic diversity because quantitative traits are adaptive and acted upon by selection. However, to really understand the genetics of a species, quantitative and molecular variation should be studied together. Determining the amount of molecular variation in a species can inform

us of within- versus among-population genetic divergence that should be protected or maintained in conservation plans. These data can also illuminate possible genetic drift or disruptions in gene flow (Kramer and Havens 2009), allowing land managers to develop strategies to remediate these problems. Only a subset of plants can be studied genetically due to time and budget constraints, so focus should be on rare, economically important, or keystone species (Aravanopoulos 2011). *Hydrastis canadensis* L. is an ideal candidate for genetic analysis because it is both rare and economically important.

Goldenseal, *Hydrastis canadensis* L.

Goldenseal (*Hydrastis canadensis* L.) is a herbaceous perennial and is broadly distributed in eastern US; extending south to Mississippi, west to Oklahoma and north to Canada. However, this species is sparsely distributed within its geographic range (Bowers 1891, Van Fleet 1914, Sanders and McGraw 2005). Goldenseal is usually found in moist deciduous forests with rich, well drained soils (Bowers 1891, Van Fleet 1914). Goldenseal can reproduce sexually through seed, or asexually from rhizomes and has a patchy growth habit characterized by dense spread (Bowers 1891, Van Fleet 1914). Shortly following emergence in mid April to early May, plants produce a white flower which ripens into a red berry late June to August (Bowers 1891, Van Fleet 1914, Eichenberger and Parker 1976, Persons and Davis 2005).

Goldenseal is valued for its medicinal qualities attributed to the many alkaloids produced in the rhizomes and roots: mainly hydrastine and berberine (Govindan and Govindan 2000). These alkaloids each occur in concentrations of around 2% (Weber et al. 2003), but they can range from 2%-10% (Persons and Davis 2005). Because

goldenseal is both rare and sought after for its value as a medicinal herb, it would be beneficial for growers to determine which environmental or genetic factors maximize traits of interest, for example alkaloid content in this case, so that, at least for some markets, fewer individuals would be needed by the medicinal plant industry (Laidig et al. 2008).

Despite the broad geographic range, populations of *Hydrastis canadensis* have become more rare and smaller in size (Sanders and McGraw 2005). Goldenseal is naturally rare because seed production and germination in the wild are uncommon (Bowers 1891); most population growth occurs within patches through vegetative propagation instead of seed dispersal (Van Fleet 1914). Also, after flowering, goldenseal will more often return to a sterile state instead of flowering a consecutive year (Christensen and Gorchov 2010).

Reasons for the increased rarity include over-harvesting for the herbal market and habitat loss (Sinclair and Catling 2003). As a result of its rarity, the small, dense patches are highly isolated. Because most reproduction occurs clonally through rhizome growth, genetic diversity within a patch is thought to be relatively low (Sanders 2004). However, little is known about the actual genetic variation in goldenseal. If genetic diversity is low, goldenseal could be especially vulnerable to population declines from continued overharvesting and habitat alteration. When overharvest occurs, populations are slow to recover. Following a simulated harvest of goldenseal in West Virginia that left 4 remaining plants, 819 plants regenerated after 4 years, showing goldenseal can repopulate a patch from mainly rhizome fragments (Van der Voort et al. 2003). However, the age structure was affected; the majority of the populations remained small and un-

reproductive, and it did not really recover during the length of the study (Van der Voort et al. 2003).

Goldenseal population sizes and abundance are decreasing due to habitat loss. In the central Appalachian region, McGraw et al. (2003) conducted a population study on presence and abundance of goldenseal and were unable to detect specific habitat requirements due to low occurrence of the plant in the study locations even in the core of its range. In Ohio, almost half of documented herbarium populations from 1845 – 1998 had become extinct by 2002, and 13% of these historical populations had experienced habitat loss (Mulligan and Gorchov 2004). In Ontario, the population growth rate was found to be basically stationary, which is comparable to other woodland herbs (Sinclair et al. 2005). However, in Ohio, population growth was found to be decreasing for two of the three populations studied. Here, vegetative growth contributed more to population growth than seed (Christensen and Gorchov 2010).

It is believed that goldenseal evolved in an environment with frequent disturbances such as flooding and fire at the northern edge of its range (Sinclair and Catling 2003, Sanders 2004, and Sinclair et al. 2005). These disturbances modified forest canopies and created variable growing conditions on the forest floor that have been related to increased growth and seed production in goldenseal (Sinclair and Catling 2003, Sanders 2004, and Sinclair et al. 2005). Goldenseal has also shown phenotypic variation in response to disturbance and nutrients in the wild (Sinclair and Catling 2004), and Sanders and McGraw (2005) suggested goldenseal's variable responses reflect varying plasticity. However, it is unknown if these phenotypic responses are adaptive or plastic under different growing conditions.

Loss of natural disturbance from goldenseal habitats may be a factor in population decreases in the north. In Ontario, simulated disturbance effects of flooding and animal impacts were tested on field populations of goldenseal in attempts to approximate former conditions of disturbance in forests (Sinclair and Catling 2003). The first season after the artificial disturbance, goldenseal plants increased in size, flower numbers, and fruit set (Sinclair and Catling 2003). After the second year these same results held, and it was noted that canopy gaps were not needed to provide a disturbance effect (Sinclair and Catling 2004). Because disturbance can provide more light and nutrients to the habitat, Sinclair et al. (2005) recommend adding disturbance back into goldenseal habitats as a conservation strategy and to encourage population growth.

Increased knowledge about the relative effects of genetics and environmental conditions can help improve conservation and reintroduction of goldenseal populations (Kramer and Havens 2009). Extensive habitat loss and risk of plant extinction means humans may need to intervene on behalf of species either through habitat restoration, species re-introduction and/or population augmentation, and artificial gene flow (Kramer and Havens 2009).

CHAPTER 3: MANUSCRIPT

Introduction

Goldenseal (*Hydrastis canadensis* L.) is a herbaceous perennial that is broadly, though sparsely, distributed throughout eastern deciduous forests (Bowers 1891, Van Fleet 1914). The historical range of goldenseal extended from the Mississippi River to the Allegheny and Appalachian Mountains, and into southern Ontario, Canada (Bowers 1891). The current range in the US comprises 27 states (USDA PLANTS, Figure 1), with the core of its range in Ohio, Indiana, West Virginia, and Kentucky (Van Fleet 1914).

Typical habitats for goldenseal are sloped, relatively open areas near streams, or in moist but well drained soils with a thick layer of leaf litter (Bowers 1891, Van Fleet 1914). Plants known to co-occur with goldenseal include white trillium (*Trillium grandiflorum* (Michx.) Salisb., violets (*Viola* L. ssp.), Jack-in-the-pulpit (*Arisaema triphyllum* (L.) Schott), wild ginger (*Asarum canadense* L.) (Harrelson and Matlack 2006), black cohosh (*Actaea racemosa* (L.) Nutt.), and bloodroot (*Sanguinaria canadensis* L.) (Harrelson and Matlack 2006, Albrecht and McCarthy 2006). Goldenseal reproduces sexually, but more commonly asexually from bright yellow rhizomes, resulting in patchy populations and distributions (Bowers 1891, Van Fleet 1914, Davis 2007).

Goldenseal plants emerge in mid April to early May, and white, apetalous flowers bloom shortly after in late April to early May. Pollination is most commonly by

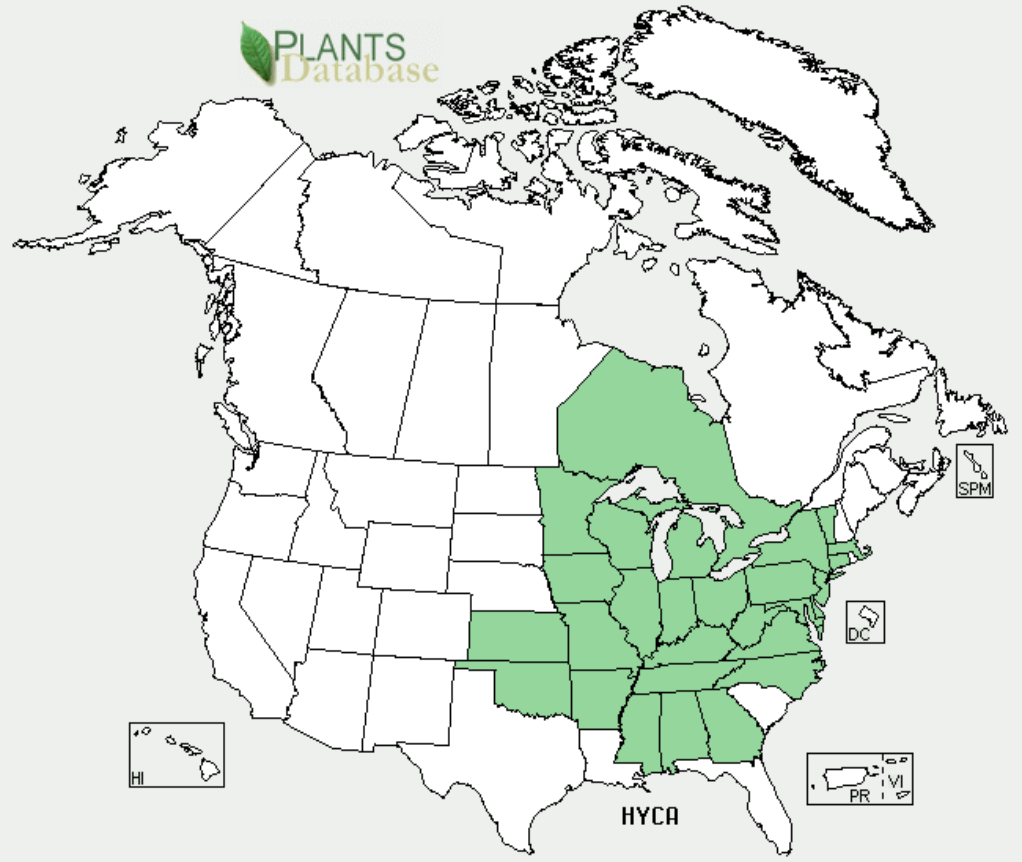

Figure 1. Current range of *Hydrastis canadensis.* USDA PLANTS Database (http://plants.usda.gov/java/)

small bees (*Dialictus* sp. and *Evylaeus* sp.), but flies (*Eupiodes* sp.) and larger bees (*Andrena* sp., *Augochlora pura pura* (Say), and *Bombus* sp.) have been observed as well (Sinclair 2002). Goldenseal fruit is a red berry, resembling a raspberry, that ripens late June to August (Bowers 1891, Van Fleet 1914, Eichenberger and Parker 1976), and each berry produces 10 – 30 black seeds (Van Fleet 1914). Goldenseal has palmately veined leaves with five to seven lobes. Young, non-reproductive plants have a single leaf while plants older than a few years have 2 – 3 leaves alternately arranged on the stem. Stems range from 10 – 30 cm in height and leaves are 15 – 30 cm in wide (Bowers 1891, Persons and Davis 2005).

Goldenseal is collected for its medicinal qualities, which have been known for hundreds of years (Persons and Davis 2005). Native Americans and settlers used goldenseal for ailments of the mouth, eyes, stomach, and liver (Bowers 1891, Van Fleet 1914). Currently, goldenseal is used as a topical antiseptic and to boost the immune system (Davis 2007). The medicinal qualities of goldenseal are often attributed to two of the alkaloids produced in the roots: hydrastine and berberine (Weber et al. 2003, Persons and Davis 2005).

Goldenseal became a popular product on the herbal market in the mid 1800's, and this demand put a major strain on wild populations. The overharvesting was so great that by the late 1800's and early 1900's, goldenseal was thought to be too rare to stay on the market and was considered at risk of extinction (Bowers 1891, Van Fleet 1914). Overharvest has led to a reduction in wild populations (i.e., Davis 2007, Gagnon 1999) and changed the age structure of populations; skewing classes toward younger, non-reproductive plants (Van der Voort et al. 2003). In 1997, *Hydrastis canadensis* was listed

on Appendix II of CITES, Convention on International Trade of Endangered Species of Flora and Fauna, in order to monitor and regulate commercial trade of the plant (Persons and Davis 2005, Sinclair et al. 2005).

In addition to over-harvest, endangerment of goldenseal is due to habitat loss from development (Sinclair and Catling 2003, McGraw et al. 2003, Persons and Davis 2005). As a result of these factors, patch size and density are decreasing (Sanders and McGraw 2005). Habitat loss has affected goldenseal distribution even in the core of its range. In Ohio, almost half of documented herbarium populations from 1845 – 1998 had become extinct by 2002, and 13% of the locations had experienced habitat loss (Mulligan and Gorchov 2004). Habitat fragmentation and subsequent increased patch isolation can have negative genetic and demographic effects on woodland perennials through decreased pollination and migration, and habitat changes (Honnay et al. 2005).

Patch isolation can result in population extinction (Honnay et al. 2005) or genetic divergence through increased genetic drift, inbreeding, or decreased gene flow (Wright 1931, Young et al. 1996). Even without these genetic consequences of patch isolation, genetic diversity within patches is expected to be low due to the naturally small, dense populations that spread primarily asexually (Sanders 2004). This naturally low genetic diversity can leave species especially vulnerable to negative effects of population declines from continued overharvesting and habitat loss. Although little is known about genetic variation in goldenseal, one study using RAPDs reported more variation between populations (66.67%) than within (23.58%) (Kelly 2009). American ginseng (*Panax quinquefolius* L.), a woodland medicinal herb, shares a similar habitat and range as well as over-harvesting and habitat loss pressures with goldenseal (Van der Voort et al. 2003,

Cruse-Sanders and Hamrick 2004). Ginseng has been studied extensively and unprotected harvested populations have been found to have excess homozygosity, less genetic variation, and more genetic structure between populations compared to populations that were protected (Cruse-Sanders and Hamrick 2004). Goldenseal could be vulnerable to a similar loss of genetic diversity.

Loss of natural disturbance can make existing habitat less suitable (Sinclair and Catling 2003, Sanders 2004, Sinclair et al. 2005). It is believed some goldenseal habitats used to have more frequent disturbance such as flooding and fire than are present today (Sinclair and Catling 2003, Sanders 2004, Sinclair et al. 2005). These disturbances modified forest canopies and created variable growing conditions on the forest floor that have been related to increased growth and seed production in goldenseal (Sinclair and Catling 2003, Sanders 2004, Sinclair et al. 2005). Goldenseal has also shown variable phenotypic responses to environmental changes (Sinclair and Catling 2004, Davis 2007). Sanders and McGraw (2005) suggested theses variable responses reflect varying plasticity, but it is unknown how much of these phenotypic responses to environmental variation have a genetic basis.

Determining patterns of genetic variation in goldenseal can inform us of genetic distributions that should be protected or maintained in conservation plans, can illuminate possible negative genetic processes (drift or disruptions in gene flow) that should be addressed, and can measure degree of plasticity in response to differing environmental conditions (Kramer and Havens 2009). This information is required for effective conservation plans that might include population restoration through supplementation.

The purpose of this experiment was to describe genetic variation among and within goldenseal populations in western North Carolina and to determine the relative contribution of genetics and the growing environment on phenotype. The questions are twofold: (1) do natural populations have more genetic variation among or within populations, and (2) do genetics and the growing environment differ in affect on phenotypic responses in emergence, reproduction, biomass and dieback?

Methods

Overview

Phenotypic variation in goldenseal was quantified using a common garden experiment with rhizomes collected from wild populations. Plants were grown under different growing conditions to evaluate relative effects of genetics versus the environment on phenotype. Variation among populations in alkaloid content was measured using the same wild populations collected for the common garden experiment, and these wild populations were also used for an allozyme analysis to quantify genetic variation in order to compare within population variation to between population variation. The common garden used a lath house and greenhouse to provide different environments to compare phenology and growth under controlled conditions. Rhizomes were divided to create genetic clones to determine the relative contributions of genetics (the clone) and environment (fertilizer treatment) on phenotype. The different components of this study will provide a more complete description of the genetic diversity of goldenseal by quantifying neutral genetic diversity (with allozymes) and phenotypic plasticity (with a common garden experiment).

Common Garden Experiment

The common garden experiment was conducted at the North Carolina State University (NCSU) Mountain Horticultural Crops Research Station in Mills River, North Carolina. Goldenseal rhizomes were collected from six wild populations in western North Carolina. Collection permits were obtained from the United States Forest Service and the North Carolina Plant Conservation Program. Population locations were provided by the United States Forest Service. Because these are protected populations, coordinates to populations are not disclosed. Populations "Balsam Sales" and "Balsam Lot" were located on private property in Jackson County, and "Hench Knob" populations were also collected from Jackson County, but located in the Nantahala National Forest. The "Moore Knob" population was in the Nantahala National Forest in Macon County, and the "Big Ivy" population was in Buncombe County in the Pisgah National Forest (Table 1). Elevations of the populations ranged from 842 – 1069 meters (Table 1). All collection sites were steep, moist, mixed hardwood coves (typical goldenseal habitat) except Big Ivy, which was in an open field in full sun (atypical for goldenseal) (Persons and Davis 2005). The number of patches sampled per population and the rhizomes collected per patch are listed in Table 1. Variation in the number of rhizomes collected per patch reflects variation in patch size where no more than an estimated 5% of a patch was collected to minimize disturbance to the patch. In addition, rhizomes were selected as far apart from each other in a patch so they would represent different individuals (genets).

A total of 235 rhizomes, the complete underground portion of an individual plant, were collected from the wild populations when plants were dormant (December 2010 and

Table 1. *Hydrastis canadensis* collection summary and experimental design in the greenhouse (GH) and lath house (LH). Patches that were included in the alkaloid analysis = *, allozyme analysis = **, and both = ***. Individuals = # clones that produced plants.

Population	Patch #	# Rhizomes Collected	# Individuals[1]	
			# in GH	# in LH
Balsam Sales (842 m)	1	5	4	4
Jackson County	2	5	3	3
Private Property	3***	14	14	10
	4***	14	10	12
	5**	8	10	6
	6	8	7	3
	7	10	10	4
Moore Knob (853 m)	1*	6	4	6
Macon County	2***	6	8	2
Nantahala National Forest	3	11	10	9
	4**	7	8	5
	5	5	2	5
Balsam Lot (927 m)	1	12	11	9
Jackson County	2	8	8	6
Private Property	3	8	8	8
	4**	12	10	9
	5**	10	4	6
Hench Knob A (953 m)	1	7	2	2
Jackson County	2***	10	3	2
Nantahala National Forest	3***	8	3	1
	4	6	3	2
	5	10	8	**4**
	6**	8	1	**3**
Hench Knob B (1048 m)	1***	7	0	0
Jackson County	2	7	1	0
Nantahala National Forest				
Big Ivy (1069 m)	1**	10	8	9
Buncombe County	2**	9	9	7
Pisgah National Forest	3	4	2	2

[1]Each individual was represented by two clones so the number of plants grown is twice the number of individuals . Where the number of individuals is $\leq$4 for the greenhouse or lath house, the patch is not represented in all blocks. Where the number of individuals is $\geq$5 for the greenhouse or lath house, the patch is represented in some blocks by more than one individual. Numbers in bold do have individuals represented in all blocks.

January 2011). All rhizomes were stored moist in a cooler overnight after collection and planted in bulk the following day in a peat-vermiculite mix in 19 liter pots with patches planted in separate pots. All rhizomes were stored in an overwintering structure at the NCSU greenhouse facility that provided shelter from the elements, and the rhizomes were monitored weekly and watered as needed. In February 2011, rhizomes were removed from the overwintering pots and washed, weighed, cut in half, and potted individually in fresh peat-vermiculite potting mix in 20 cm diameter pots. The rhizomes were cut in half to create genetic clones to analyze genetic by environmental interactions. The shape and size of rhizomes was highly irregular, so decisions on where to cut rhizomes were based on making growth potential of both clones as equal as possible. For example, when only one bud was visible on a rhizome, the clone lacking the bud was allowed more fibrous roots. Rhizomes were weighed to control for variation in above-ground traits that might be due to different initial rhizome biomass and ranged from 0.07 – 11.72 g. All pots were kept under a lath structure until emergence was considered complete (June 6). The lath house, an open outdoor wooden structure, provided an environment of approximately 80% shade with a slatted roof and polypropylene shade cloth. Plants were then divided into seven blocks; four blocks were placed inside the greenhouse and three remained under the lath house. Unequal collection sample sizes and variable emergence success meant plants could not be divided equally between growth areas and blocks (Table 1) creating an incomplete block experimental design. When assigning clones to blocks, the criteria used in order of importance were 1) clones of the same plant were kept together in a block, 2) within the greenhouse or lath house,

blocks had equal numbers of plants in each, and 3) patches were dispersed between all blocks as evenly as possible.

The greenhouse (1,296 square feet of useable space) was temperature controlled, with temperatures never exceeding 100^{o}F, with daytime temperatures averaging 80^{o}F and nighttime temperatures averaging 73^{o}F through the growing season. Here, plants were grown under a polypropylene shade cloth that provided 80% shade. Rhizome halves were randomly assigned to high or low fertility treatments. The macronutrient fertilizer used was Southern Agricultural Insecticides Inc., 20-20-20 Powerpak Water Soluble Fertilizer, administered as a drench, and micronutrients (Southern Agricultural Insecticides Inc., Essential Minor Elements, granular), were administered as top dress. Fertilizer concentrations were based on nutrient recommendations of Ingestad (1972), thus 50% of the fertilizer product's recommended rate was used for the high fertility treatment and 25% of the recommended rate was used for the low fertility treatment. Clones in the high fertilizer treatment group received 3.96 ml/l macronutrients and 1.25 ml/clone micronutrients. Clones in the low fertilizer treatment group received 1.98 ml/l macronutrients and 0.625 ml/clone micronutrients. Fertilization took place once a week in June 2011, which was after clones were put into blocks and before growth data were measured.

Phenology (emergence and dieback dates) and reproduction (flowering and seed production dates) were measured beginning March 21 and ending October 11, 2011. Emergence was defined as the first day an erect stem with an open leaf was observed and dieback date was recorded when more than half the leaf began to die. For reproductive

traits, flowering was recorded as the date a fully open florescence occurred, and seed production was recorded as the date when the whole berry had turned red.

Above-ground variables of height and leaf size were measured on August 8 on all plants that had not already died back. Stem height was measured as the length in centimeters from the soil to the base of the leaf, and leaf width was measured from the base of the leaf to the apex. Rhizomes will be sampled at the end of the second growing season for weight and for percent alkaloid content. These data are beyond the scope of the current project.

Allozyme Variation

Allozyme analyses were used to quantify variation within and among populations and patches, and to describe genetic structure (Soltis and Soltis 1989). A sample of goldenseal leaf tissue was collected in May 2011 and sent to the USDA Forest Service National Forest Genetics Electrophoresis Laboratory (NFGEL, Institute of Forest Genetics, Placerville, California) who determined the buffer systems and stains for best visualizing allozymes of this species. All subsequent collection and laboratory protocols for my allozyme analyses followed NFGEL's Standard Operating Procedures. Fresh leaf samples from the same six natural populations used for the common garden experiment were collected June 7 – 23, 2011. Two patches per population and twelve leaves per patch were collected from Moore Knob, Hench Knob B and Big Ivy, and three patches per population and twelve leaves per patch were collected from populations Balsam Sales, Balsam Lot, and Hench Knob A. Collected leaf tissue was stored overnight in a refrigerator in plastic bags with moist paper towels and processed in buffer the following

day. Extracts were stored in a freezer until gel electrophoresis could be preformed. One buffer system (morpholine-citrate pH 6.1 or MC6) and four stains (diaphorase or DIA, malate dehydrogenase or MDH, 6-phosphogluconate or 6PGD, and isocitrate dehydrogenase or IDH) were used to visualize 8 loci: DIA3, MDH1, MDH2, MDH3, 6PGD1, 6PGD2, 6PGD3, and IDH2. The visualized loci bands were scored by allele number and whether the individual was homozygous or heterozygous.

Alkaloid Content

Rhizomes from the lath house and greenhouse could not be destructively sampled for alkaloids because the common garden experiment was continuing beyond the current project. Therefore, the alkaloids berberine and hydrastine were measured using rhizomes collected from the wild from four of the six natural populations included in the common garden experiment (Table 1). Rhizome samples were collected at the same time leaf material was collected for the allozyme analysis. Ten rhizome samples were collected from two patches each at Hench Knob A, Balsam Sales, and Moore Knob, and ten rhizomes from one patch were collected from Hench Knob B. A subset of patches was sampled to minimize disturbance from populations that had already been sampled for the common garden experiment. Alkaloid content was quantified by Dr. Jason Clement in the Chemistry and Physics Department at Western Carolina University using the Soxhlet extraction method with extracts analyzed by HPLC (Weber et al. 2003).

Data Analyses

Data from the common garden experiment were analyzed in R (R Development Core Team 2011) using a linear mixed model. A nested Analysis of Deviance was used

to analyze the nesting of clone within plant within patch within population (Underwood 1997) to accommodate the unbalanced design. When significant differences ($p<0.05$) were found, a Tukey's Contrast was used to determine pair-wise differences.

Timing and percent of emergence, flower and seed production were analyzed at the population and patch level using starting rhizome weight as a covariate. Because of poor emergence (3.5%) in the common garden, population Hench Knob B was excluded from further analyses. The main factors for dieback date, sum of stem height, and sum of leaf length were starting rhizome mass, population, fertilizer, block, and growth area (greenhouse or lath house). Sums of stem and leaf sizes were used to analyze size responses as a measure of biomass produced. Rhizome weights and biomass values were log transformed before analyses. Because block was partially confounded with growth area, these factors were analyzed in separate models. Genetic variation was analyzed at the population and patch level.

Data from the allozyme analyses were used to calculate observed (H_o) and expected heterozygosity (H_e), percent polymorphism, and total and average number of alleles per loci. An Analysis of Molecular Variance (AMOVA) and F-statistics were used to determine the distribution of genetic variation among populations (Conner and Hartl 2004) and the results were used to determine if allelic distribution among populations represented adaptive selective pressures, thus causing population divergence (Cruse-Sanders and Hamrick 2004). Allozyme data were analyzed using the computer program GenAlEx (Peakall and Smouse 2006).

Results

Variation Among Populations

Emergence of goldenseal occurred from March 21 to June 13 (Figure 2). Big Ivy was the earliest population to emerge and Hench Knob was last, with an overall average of April 17 (Table 2). Starting rhizome weight was not a significant factor in emergence timing at the population ($p=0.319$) or patch within population level ($p=0.156$) (Table 4). Similar to Hench Knob B, Hench Knob A also had poor emergence. It emerged significantly later than the other populations ($p<0.01$, Figure 3) and also had the fewest rhizomes (35%) that produced plants. The other populations had 78 – 84% of their rhizomes produce plants. Due to poor emergence by Hench Knob A rhizomes, the overall emergence was only 70% and differences among populations were significant ($p<0.001$, Figure 4). Although starting rhizome weight was not a significant factor in when rhizomes produced plants, it was a significant factor in the percent of emergence at the population level ($p<0.001$) and patch-within-population level ($p<0.001$, Table 3).

The percent of emerged plants that flowered ranged from 3% - 46% (Balsam Sales and Big Ivy respectively), averaged 18.1%, and varied significantly ($p<0.001$, Figure 4) among populations. Starting rhizome weight did not significantly affect flower timing at the population ($p=0.797$), or patch-within-population level ($p=0.917$, Table 3), or percent flowering at the patch-within-population level ($p=0.156$). However, starting rhizome weight did affect flowering percent at the population level ($p<0.001$) (Table 3). The Balsam Sales and Hench Knob A populations did not produce any seed while 76.5% of the plants from Big Ivy that flowered produced seed. The percent of flowering

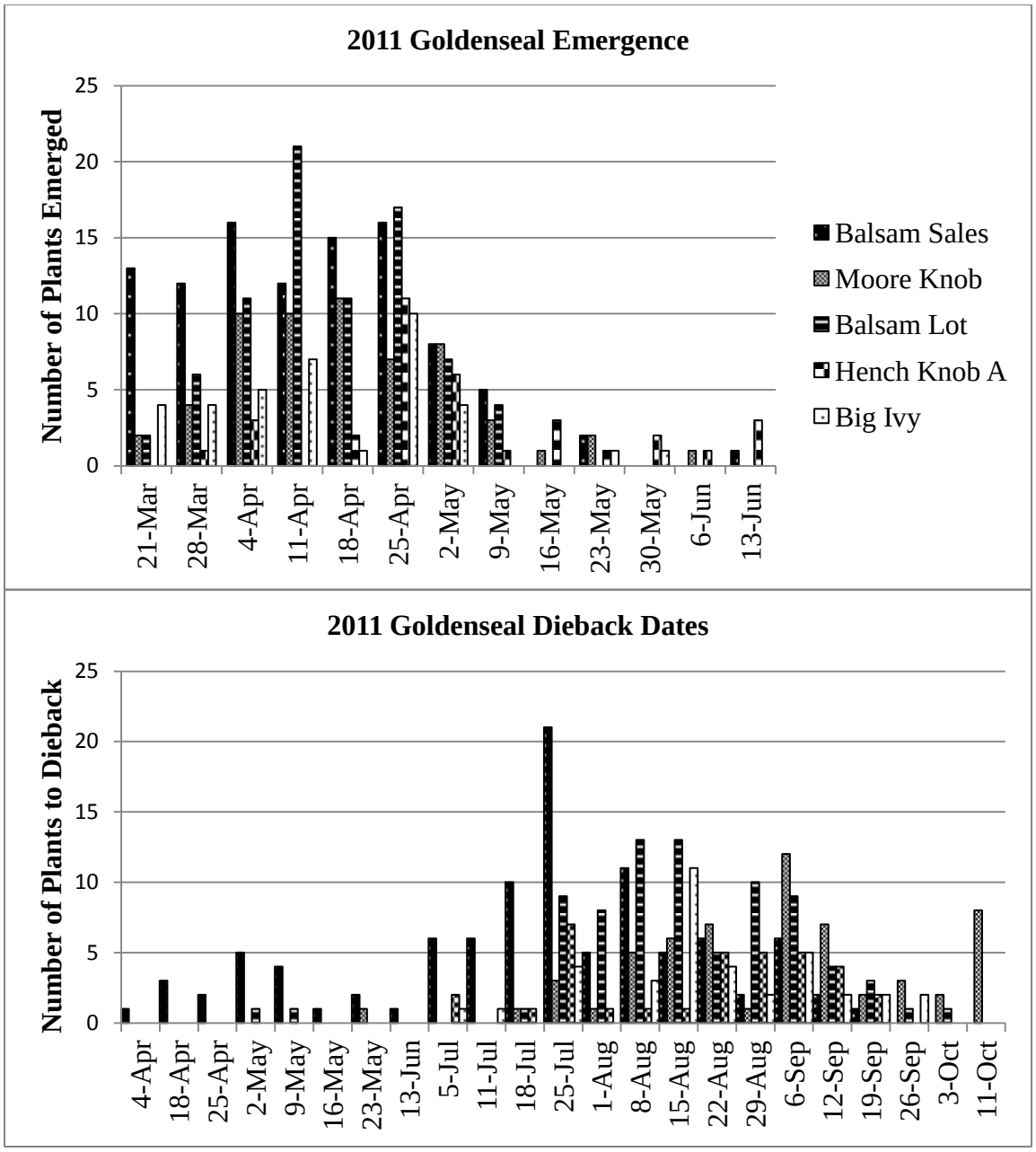

Figure 2. Patterns of emergence and dieback among goldenseal populations from western NC growing in a common garden experiment

Table 2. Variation among *Hydrastis canadensis* populations from western NC growing in a common garden experiment

Trait	Population Range	Average	p-value[1]
Phenology (Date)			
Emergence	April 13 - May 3	April 17	<0.01
Dieback	July 16 - September	August 9	<0.001
Reproduction (Date)			
Flowering	April 9 - April 13	April 11	ns
Seed Production	June 11 - June 20	June 14	ns
Biomass (cm)			
Sum of Stem Height	7.2 - 16.7	11.5	<0.001
Sum of Leaf Length	6.3 - 11.6	8.9	<0.05
Alkaloid Content (%)			
Berberine	2.27 – 3.20	2.66	<0.001
Hydrastine	1.81 – 2.82	2.22	<0.001

[1]From nested Analysis of Deviance

Table 3. Variation among patches within *Hydrastis canadensis* populations from western NC growing in a common garden experiment

Patches within Populations												
Population	**Balsam Sales**		**Moore Knob**		**Balsam Lot**		**Hench Knob A**		**Hench Knob B**	**Big Ivy**		
Patch Variation	Range	Ave	Range	Ave	Range	Ave	Range	Ave	Ave	Range	Ave	P-value
Phenology (Date)												
Emergence	Apr 6 - Apr 18	Apr 13	Apr 10 - Apr 25	Apr 18	Apr 8 - Apr 23	Apr 15	Apr 25 - May 21	May 3	May 9	Apr 7 - Apr 19	Apr 14	ns
Dieback	Jun 10 - Jul 31	Jul 16	Aug 13 - Sep 21	Sep 1	Aug 9 - Aug 20	Aug 15	Jul 25 - Aug 27	Aug 18	Aug 15	Aug 11 - Aug 21	Aug 20	ns
Reproduction (Date)												
Flowering	Apr 11 - Apr 18	Apr 13	Apr 7 - Apr 15	Apr 11	Apr 7 - Apr 15	Apr 11	Apr 11 - Apr 14	Apr 13	None	Mar 31 - Apr 15	Apr 9	ns
Seed Production	None	None	Jun 13 - Jun 17	Jun 15	Jun 20	Jun 20	None	None	None	May 23 - Jun 13	Jun 11	ns
Biomass (cm)												
Sum of Stem Height	7.1 - 11.1	9.4	13.7 - 20.2	16.7	9.8 - 11.5	10.5	3.9 - 9.9	7.2	2.2	13.2 - 15.2	14.1	ns
Sum of Leaf Length	2.6 - 9.0	7.8	8.6 - 13.8	11.6	7.5 - 8.7	8.3	3.5 - 7.9	6.3	3.2	8.3 - 10.2	9.3	ns
Alkaloid Content (%)												
Berberine	1.56 - 3.00	2.28	2.51 - 3.89	3.2	NA	NA	2.36 - 2.98	2.67	2.49	NA	NA	<0.001
Hydrastine	1.61 - 2.12	1.87	2.38 - 2.42	2.4	NA	NA	1.72 - 1.89	1.81	2.82	NA	NA	ns

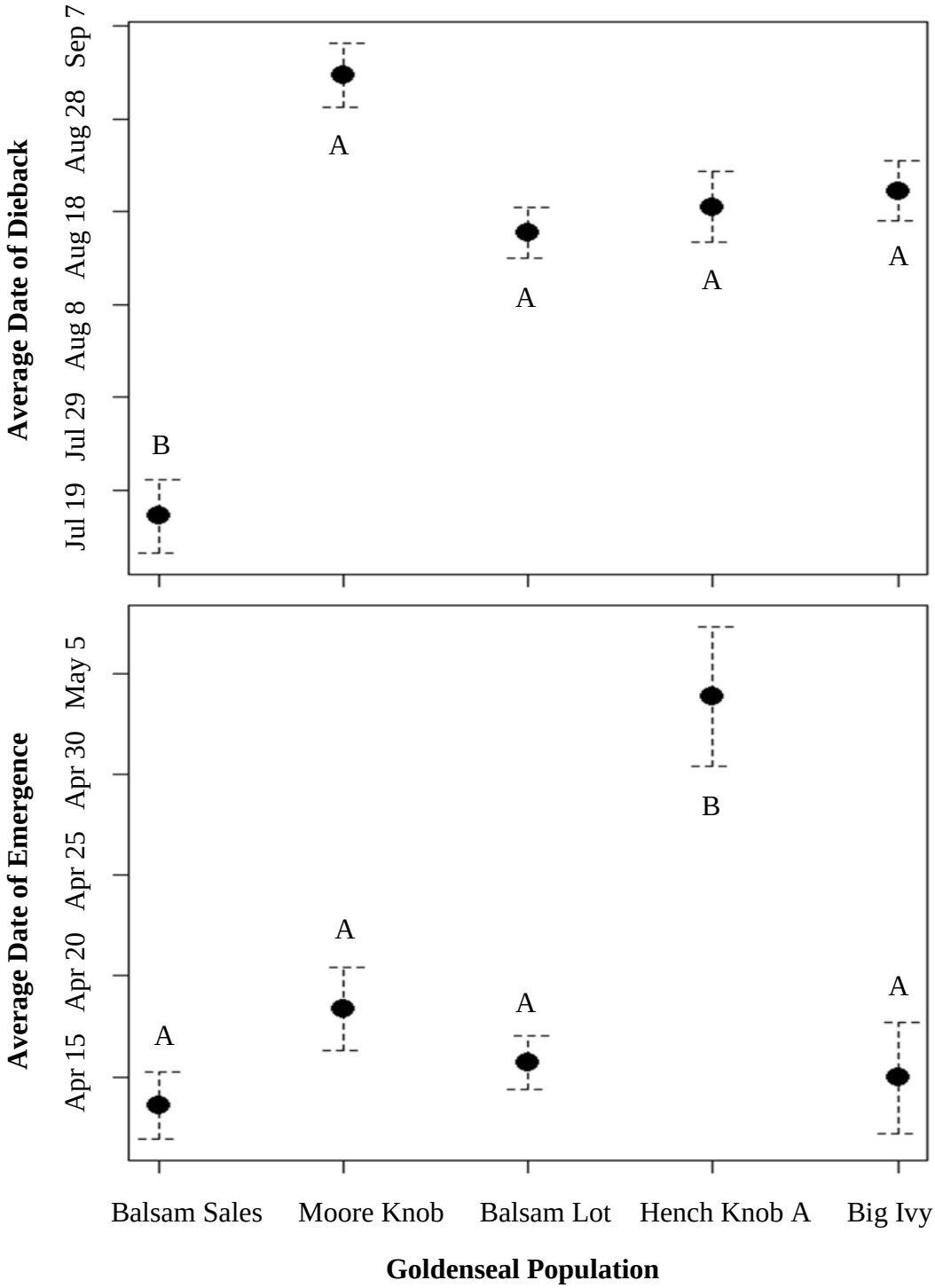

Figure 3. Average emergence and dieback dates (± standard error) for *Hydrastis canadensis* populations from western NC growing in a common garden experiment. Populations are listed in order of lowest to highest elevation. Populations with different letters varied significantly ($p \leq 0.05$) from each other.

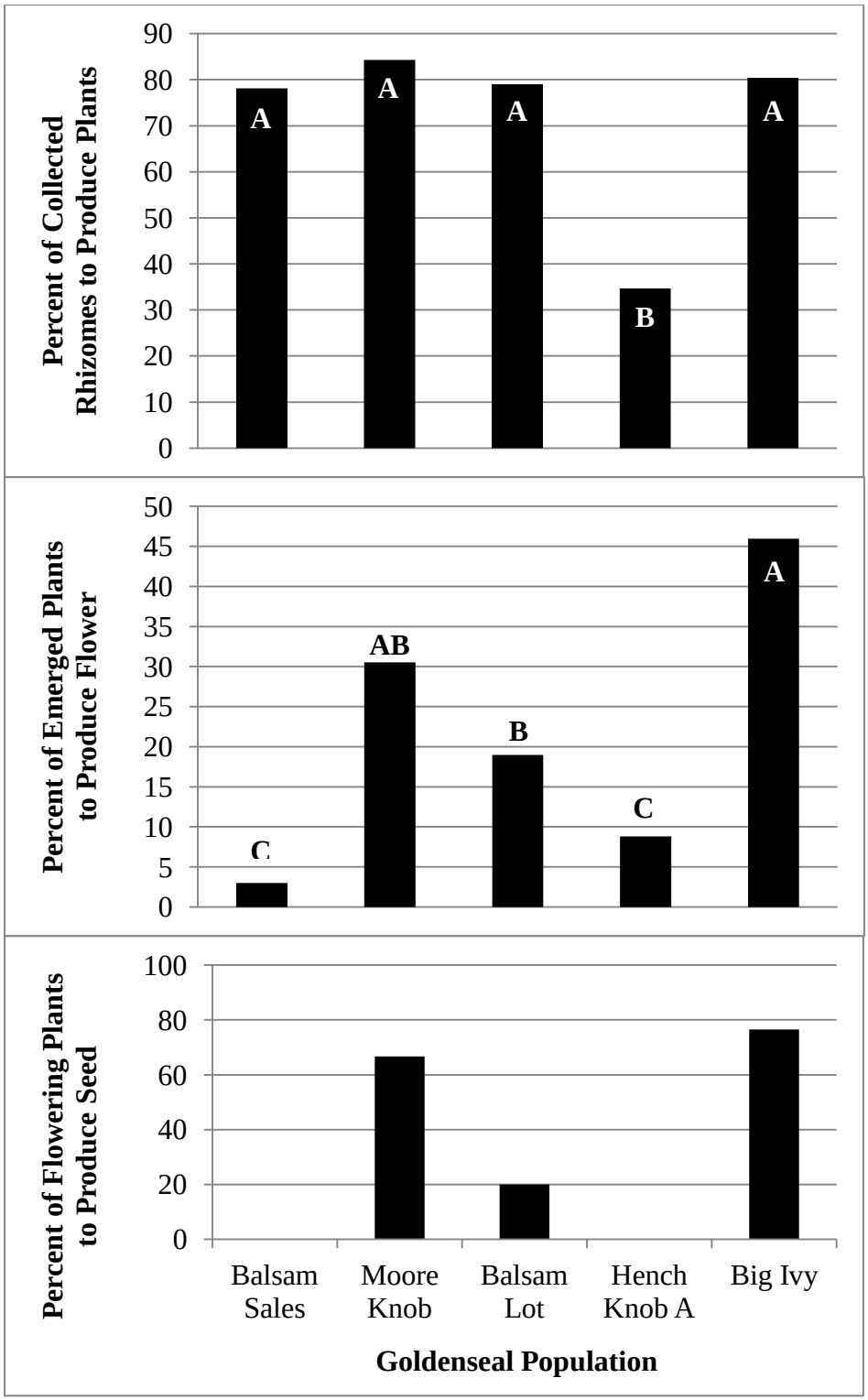

Figure 4. Variation in reproduction among *Hydrastis canadensis* populations from western NC growing in a common garden. Populations are listed in order of lowest to highest elevation. Populations with different letters varied significantly ($p<0.05$) from each other.

Table 4. Sources of variance from nested Analysis of Deviance for timing and percent of reproductive traits in common garden experiment

	Emergence		Flowering		Seed Production	
Source of Variance	df	p-Value	df	p-Value	df	p-Value
Timing						
Rhizome weight	1	ns	1	ns	1	ns
Population	4	<0.01	4	ns	2	ns
Patch	6	<0.05	6	ns	3	ns
Percent						
Rhizome weight	1	<0.001	1	<0.05	1	<0.05
Population	4	<0.001	4	<0.001	4	ns
Patch	6	ns	6	ns	6	ns

plants that produced seed ranged from 0 – 76.47% but variation was not significantly different at the population ($p=0.102$) or patch within population level ($p=0.156$) (Figure 4, Table 3). Variation in starting rhizome weight accounted for the variance in seed production between populations ($p<0.05$) and patches within populations ($p<0.05$), thus differences in seed production were not significant among populations ($p=0.102$) or patches within populations ($p=0.156$) (Table 3).

Dieback dates ranged from April 4 to October 11, which was a greater time span than for emergence (Figure 2). Balsam Sales was the first population whose plants senesced while Moore Knob A was last (Figure 3). Average dieback dates between populations ranged from July 16 – September 1 although most dieback occurred during the last two weeks of August. The overall dieback date was August 9 (Table 2) and varied significantly among populations ($p<0.001$, Figure 3). Although larger rhizome weight was significantly correlated to delayed dieback dates ($p<0.05$, Figure 3 and Figure 6), only a small amount of variation was explained.

Sum of stem height ranged from 7.2 cm at Hench Knob to 16.7 cm at Moore Knob, averaging 11.5 cm (Table 2). Although these differences were influenced by starting rhizome weight (Figure 6), the remaining variance among populations was still significant ($p<0.001$, Table 4). On average, larger rhizomes produced greater sum of stems (Figure 6). Sum of leaf length was the smallest for Hench Knob A plants at 6.3 cm and Moore Knob had the largest leaves at 11.6 cm (Figure 5). The overall average for sum of leaf size was 8.9 cm (Table 2), which was also significantly influenced by starting rhizome weight ($p<0.001$, Table 4). On average, larger rhizome weights increased sum of leaf length (Figure 6).

Table 5. Sources of variance from nested Analysis of Deviance for biomass and dieback from common garden experiment.

	Sum of Stem Height		Sum of Leaf Length		Dieback Date	
Source of Variance	df	p-Value	df	p-Value	df	p-Value
Rhizome Weight	1	<0.001	1	<0.001	1	<0.05
Population	4	<0.001	4	<0.05	4	<0.001
Patch within Population	6	ns	6	ns	6	ns
Fertilizer	1	<0.05	1	<0.05	1	<0.001
Fertilizer:Population	4	ns	4	ns	4	ns
Growth Area	1	ns	1	ns	1	ns

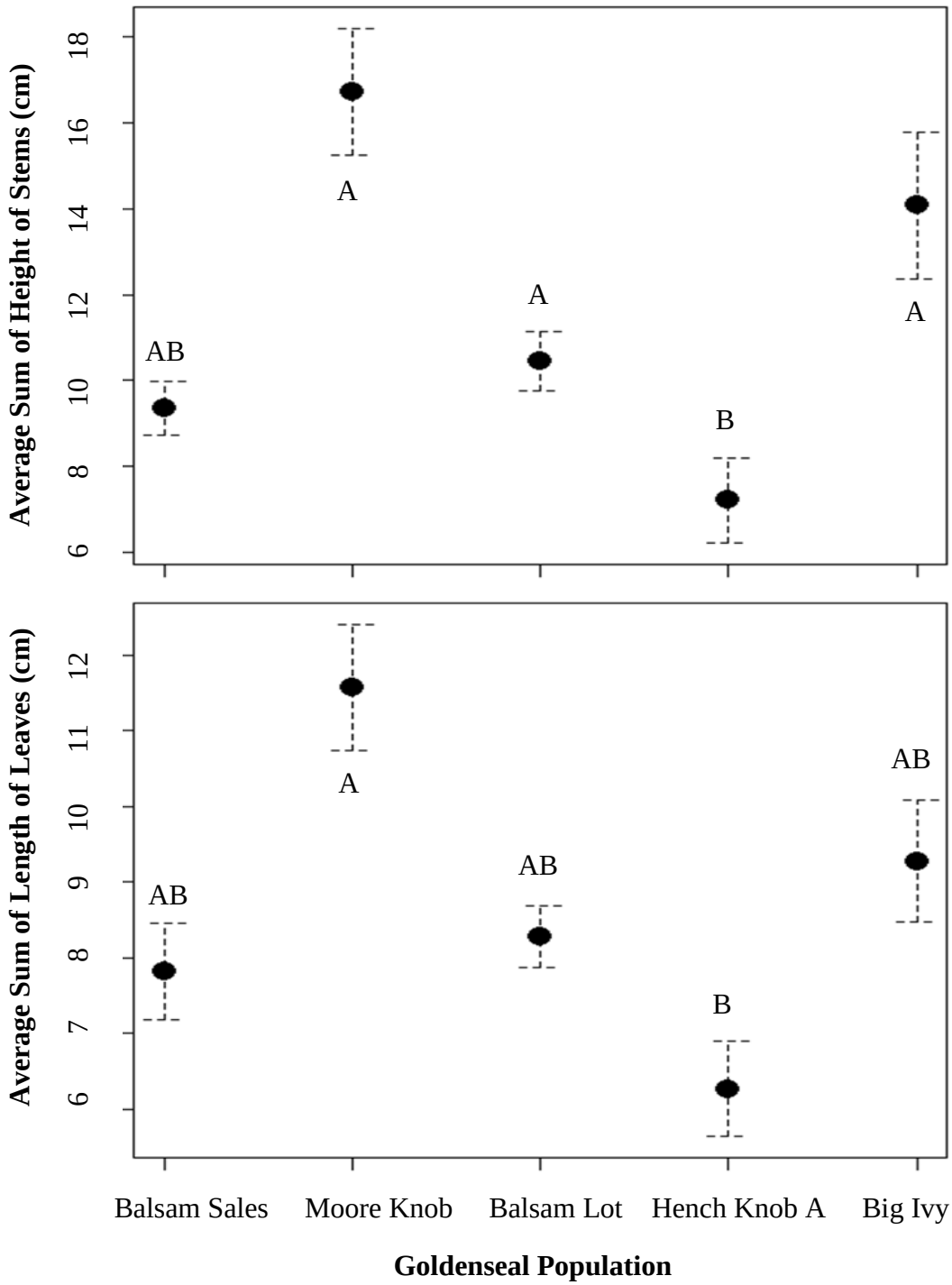

Figure 5. Variation among populations in size (± standard error) for *Hydrastis canadensis* populations growing in a common garden. Populations are listed in order of lowest to highest elevation. Populations with different letters varied significantly ($p \leq 0.05$) from each other.

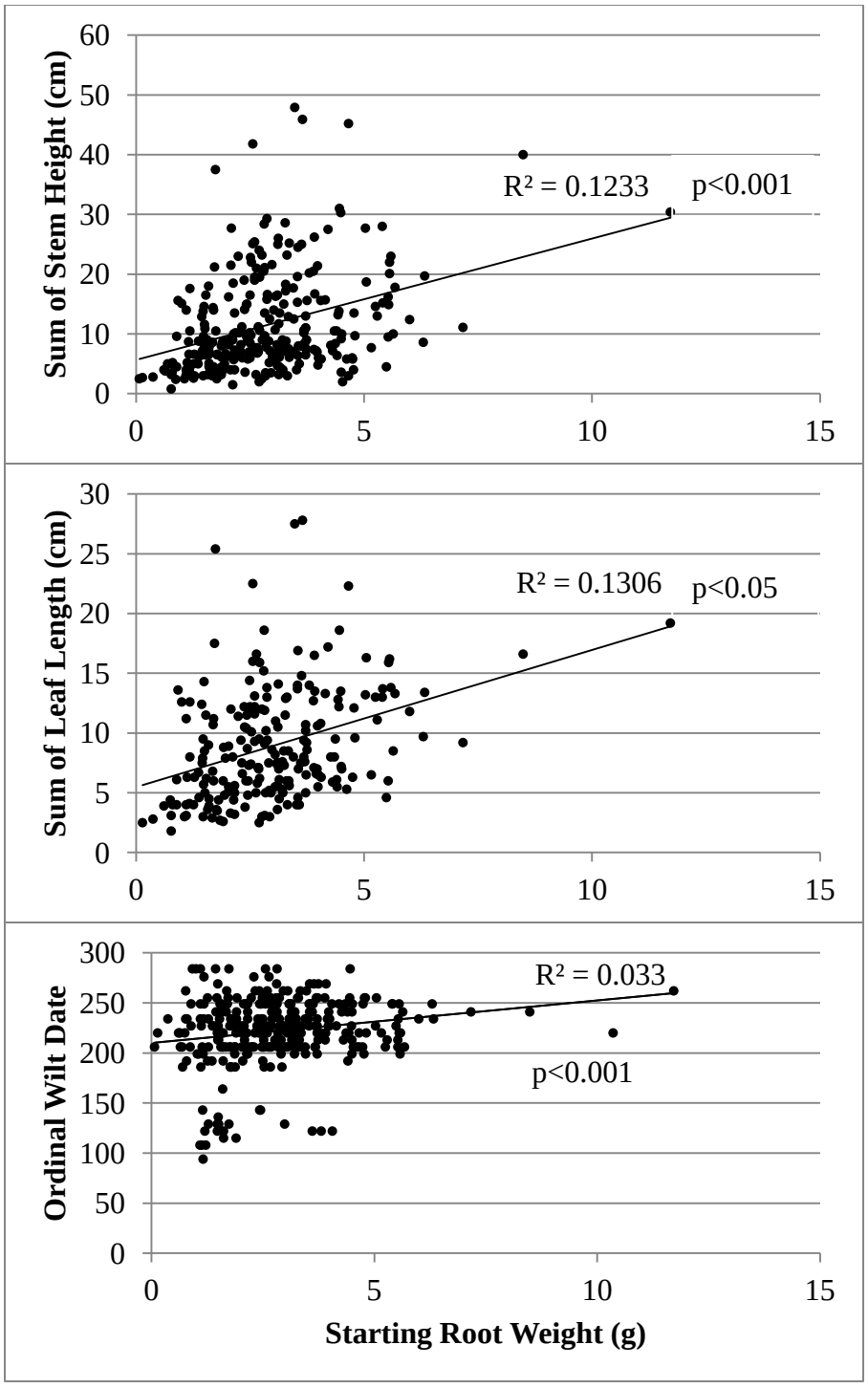

Figure 6. The effects of starting rhizome weight on biomass and dieback date for *Hydrastis canadensis* populations from western NC growing in a common garden

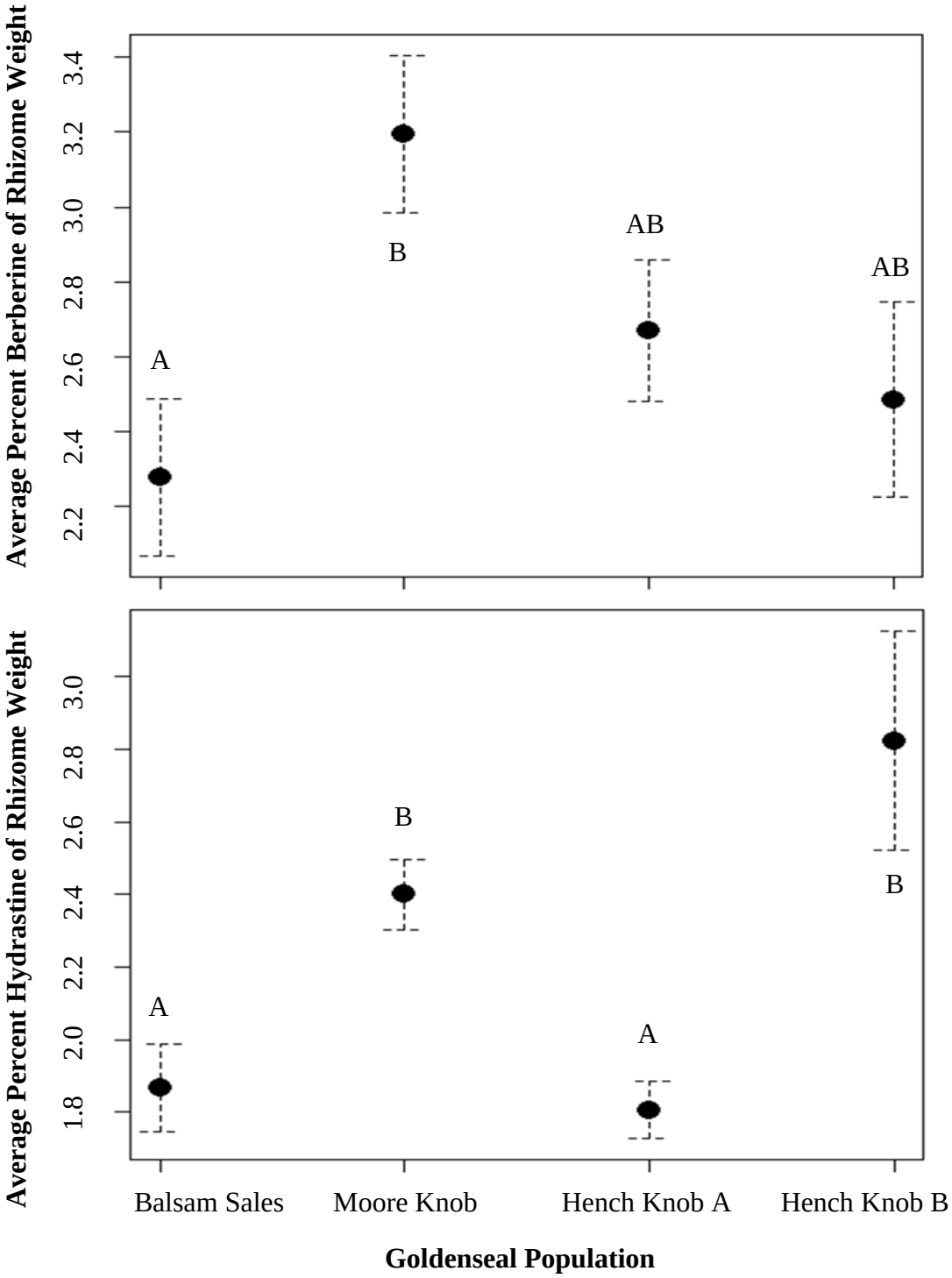

Figure 7. Variation among populations in alkaloid content (± standard error) for *Hydrastis canadensis* populations from western NC growing in a common garden. Populations are listed in order of lowest to highest elevation. Populations with different letters varied significantly ($p \leq 0.05$) from each other.

Average berberine rhizome content was higher than hydrastine rhizome content (Table 2). Moore Knob had the highest berberine rhizome content and the second highest hydrastine content (Figure 7). Significant differences among populations were found for both alkaloids. Balsam Sales had significantly less berberine than Moore Knob (Figure 7, $p<0.05$). The percent hydrastine was found to be significantly greater in Moore Knob and Hench Knob B ($p<0.05$) populations than Balsam Sales and Hench A (Figure 7).

In summary, phenotypic variation among plants grown in the common garden was significant among population for all traits, whereas variation among patches within populations was not significant for any trait. Variance components were close to zero at the patch level and plant-to-plant level for all traits measured, and all variation was due to population differences; therefore, more phenotypic variation was found between populations than within. The populations that grew for the greatest number of days (Moore Knob and Big Ivy) had the largest size, greatest number of plants that flowered, and greatest fruit production. The Moore Knob population also had some of the highest alkaloid contents. Balsam Sales and Hench Knob A grew the fewest days, were the smallest in size, and had the fewest plants that flowered with low to no seed production.

Out of the 8 loci analyzed, 5 were found to be polymorphic: DIA3, MDH1, MDH3, 6PGD3, and IDH2 (Table 6). Because not all samples that were run were able to be scored due to poor band visualization on the gel, the sample sizes (N) were unequal among loci. Big Ivy had three alleles that were unique to that population, but no other populations had unique alleles. One rare allele (occurring <5% of the time) was found at Big Ivy, Balsam Sales, and Hench Knob B. Two rare alleles were found in the Moore Knob population, and three rare alleles occurred at Balsam Lot (Table 6). The average

Table 6. Allele frequencies of polymorphic loci in six *Hydrastis canadensis* populations in western NC. N = number of samples scored for a given locus.

Loci	Allele	Balsam Sales	Moore Knob	Balsam Lot	Hench Knob A	Hench Knob B	Big Ivy
DIA3	**N**	**36**	**24**	**36**	**36**	**24**	**24**
	1	0.500	0.500	0.611	0.250	0.500	0
	2	0.500	0.500	0.389	0.750	0.500	0.813
	3	0	0	0	0	0	0.188
MDH1	**N**	**27**	**15**	**33**	**28**	**19**	**17**
	1	0	0	0	0	0	0.059
	2	0.352	0.967	0.652	0.696	0.263	0.471
	3	0.630	0.033	0.318	0.304	0.737	0.471
	4	0.019	0	0.030	0	0	0
MDH3	**N**	**36**	**21**	**35**	**35**	**20**	**24**
	1	0.125	0.048	0.200	0.214	0.350	0.188
	2	0.083	0.	0.014	0.057	0.025	0
	3	0.792	0.952	0.786	0.729	0.625	0.813
6PGD3	**N**	**30**	**24**	**36**	**36**	**23**	**19**
	1	0	0	0	0	0	0.026
	2	0.900	1.000	0.958	0.861	1.000	0.921
	3	0.100	0	0.042	0.139	0	0.053
IDH2	**N**	**36**	**24**	**33**	**36**	**22**	**24**
	1	0.931	0.938	0.909	1.000	1.000	0.938
	2	0.069	0.063	0.091	0	0	0.063

Table 7. Allele frequencies of polymorphic loci in patches within populations of *Hydrastis canadensis* in western NC. N = number of samples scored for a given loci.

Loci	Allele	Balsam Sales Patches			Moore Knob Patches		Balsam Lot Patches		Hench Knob A Patches			Hench Knob B Patch	Big Ivy Patches	
		3	4	5	2	4	4	5	2	3	6	1	1	2
DIA3	**N**	**12**	**12**	**12**	**12**	**12**	**24**	**12**	**12**	**12**	**12**	**24**	**12**	**12**
	1	0.792	0.667	0.042	1.000	0	0.500	0.833	0.750	0	0	0.500	0	0
	2	0.208	0.333	0.958	0	1.000	0.500	0.167	0.250	1.000	1.000	0.500	0.958	0.667
	3	0	0	0	0	0	0	0	0	0	0	0	0.042	0.333
MDH1	**N**	**10**	**12**	**5**	**12**	**3**	**22**	**11**	**10**	**12**	**6**	**19**	**5**	**12**
	1	0	0	0	0	0	0	0	0	0	0	0	0	0.083
	2	0.950	0	0	0.958	1.000	0.545	0.864	0.850	0.542	0.750	0.263	0	0.667
	3	0.050	1.000	0.900	0.042	0	0.409	0.136	0.150	0.458	0.250	0.737	1.000	0.250
	4	0	0	0.100	0	0	0.045	0	0	0	0	0	0	0
MDH3	**N**	**12**	**12**	**12**	**12**	**9**	**24**	**11**	**11**	**12**	**12**	**20**	**12**	**12**
	1	0.083	0.250	0.042	0.048	0	0.229	0.136	0	0.542	0.083	0.350	0	0.375
	2	0.208	0	0.042	0	0	0.021	0	0.182	0	0	0.025	0	0
	3	0.708	0.750	0.917	0.917	1.000	0.750	0.864	0.818	0.458	0.917	0.625	1.00	0.625
6PGD3	**N**	**8**	**11**	**11**	**12**	**12**	**24**	**12**	**12**	**12**	**12**	**23**	**7**	**12**
	1	0	0	0	0	0	0	0	0	0	0	0	0.026	0
	2	1.000	1.000	0.727	1.000	1.000	0.938	1.000	0.583	1.000	1.000	1.000	0.786	1.000
	3	0	0	0.273	0	0	0.063	0	0.417	0	0	0	0.143	0
IDH2	**N**	**12**	**12**	**12**	**12**	**12**	**23**	**10**	**12**	**12**	**12**	**22**	**12**	**12**
	1	1.000	0.792	1.000	0.875	1.000	0.870	1.000	1.000	1.000	1.000	1.000	1.000	0.875
	2	0	0.208	0	0.125	0	0.130	0	0	0	0	0	0	0.125

number of alleles per locus ranged from 1.8 – 2.4 and averaged 2.133 $\pm$ 0.155, but did not differ significantly (p=0.137) among populations (Table 7). Observed heterozygosity was very low for all populations, but especially for Moore Knob (H_o=0.041). Observed heterozygosity for the other populations ranged 0.182 – 0.277, and Big Ivy had the greatest observed heterozygosity (Table 8). All populations had at least one locus out of Hardy-Weinberg Equilibrium (HWE) except Big Ivy, but no population was out of HWE (Table 8). Results of the AMOVA indicated 11% of genetic variation was distributed between populations and 89% was found within populations (p=0.01). The F_{ST} value was 0.096 which indicated only moderate differentiation among populations.

Variation Among Growing Environments

Averaged across populations, the high fertilizer treatment increased biomass significantly for sum of stem height ($p<0.05$), and sum of leaf length ($p<0.05$), and delayed dieback date ($p<0.001$) (Table 5). Although Figure 8 shows that some populations responded more than others (e.g. Moore Knob vs. Balsam Lot), the population by fertilizer interaction was not significant for sum of stem height (p=0.431), sum of leaf length (p=0.616), or dieback date (p=0.231) (Table 5). Plants grown in the greenhouse had slightly larger sum of stems and leaves when compared to plants grown in the lath house, although the only major response was by Big Ivy (Figure 9), and differences between the greenhouse and lath house were not significant for sum of stem height (p=0.962), sum of leaf length (p=0.295), or dieback timing (p=0.832) (Table 4).

Table 8. Average number (± standard error) of alleles (Na), observed heterozygosity (H_o), expected heterozygosity (H_e), and Hardy-Weinberg Equilibrium (HWE) for *Hydrastis canadensis* populations in western NC

Population	**Na**	**H_o**	**H_e**	**# Loci in HWE**	**Population in HWE?**
Balsam Sales	2.400 ± 0.245	0.230 ± 0.710	0.328 ± 0.076	4 of 5	Yes
Moore Knob	1.800 ± 0.200	0.041 ± 0.019	0.154 ± 0.089	2 of 4	Yes
Balsam Lot	2.400 ± 0.245	0.246 ± 0.103	0.307 ± 0.080	3 of 5	Yes
Hench Knob A	2.000 ± 0.316	0.248 ± 0.095	0.291 ± 0.080	3 of 4	Yes
Hench Knob B	1.800 ± 0.374	0.182 ± 0.136	0.275 ± 0.114	1 of 3	Yes
Big Ivy	2.400 ± 0.245	0.277 ± 0.056	0.286 ± 0.077	5 of 5	Yes

Table 9. Average number (± standard error) of alleles (Na), observed heterozygosity (H_o), expected heterozygosity (H_e), and Hardy-Weinberg Equilibrium (HWE) for *Hydrastis canadensis* patches within populations in western NC.

Population	Na	H_o	H_e	# Loci in HWE	Population in HWE?
Balsam Sales					
Patch 3	1.800 ± 0.374	0.220 ± 0.119	0.175 ± 0.091	3 of 3	Yes
Patch 4	1.600 ± 0.245	0.317 ± 0.135	0.230 ± 0.096	3 of 3	Yes
Patch 5	2.000 ± 0.316	0.163 ± 0.061	0.163 ± 0.066	4 of 4	Yes
Moore Knob					
Patch 2	1.600 ± 0.245	0.067 ± 0.031	0.090 ± 0.043	2 of 3	Yes
Patch 4	1.000	0	0	Mono-morphic	No
Balsam Lot					
Patch 4	2.400 ± 0.245	0.280 ± 0.136	0.352 ± 0.080	3 of 5	Yes
Patch 5	1.600 ± 0.245	0.176 ± 0.073	0.150 ± 0.062	3 of 3	Yes
Hench Knob A					
Patch 2	1.800 ± 0.200	0.333 ± 0.106	0.283 ± 0.081	4 of 4	Yes
Patch 3	1.400 ± 0.245	0.267 ± 0.165	0.199 ± 0.122	2 of 2	Yes
Patch 6	1.400 ± 0.245	0.133 ± 0.097	0.106 ± 0.074	2 of 2	Yes
Hench Knob B					
Patch 1	1.800 ± 0.374	0.182 ± 0.136	0.275 ± 0.114	1 of 3	Yes
Big Ivy					
Patch 1	1.600 ± 0.400	0.102 ± 0.083	0.087 ± 0.069	2 of 2	Yes
Patch 2	2.000 ± 0.316	0.433 ± 0.138	0.324 ± 0.094	3 of 4	Yes

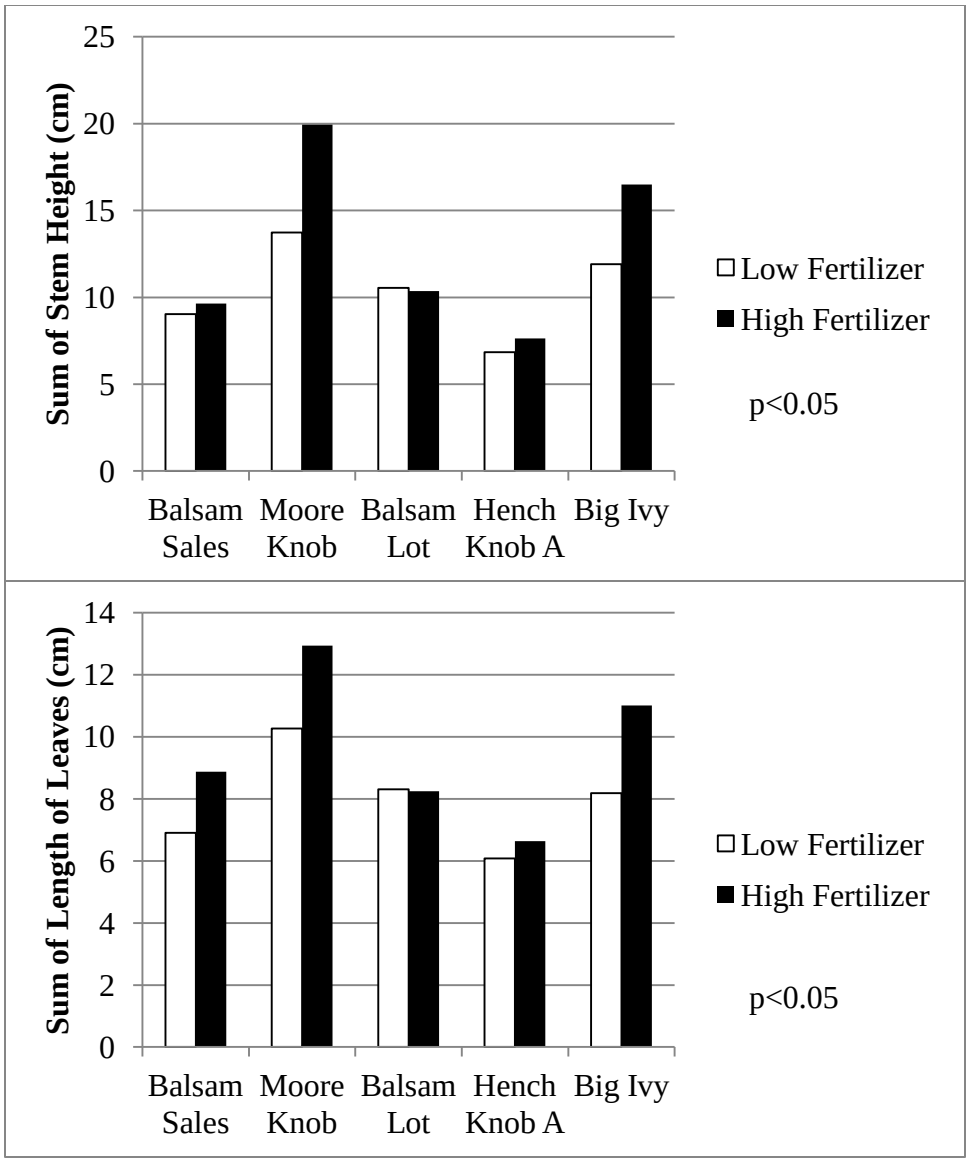

Figure 8. Comparison of fertilizer treatment on size parameters for *Hydrastis canadensis* populations from western NC growing in a common garden

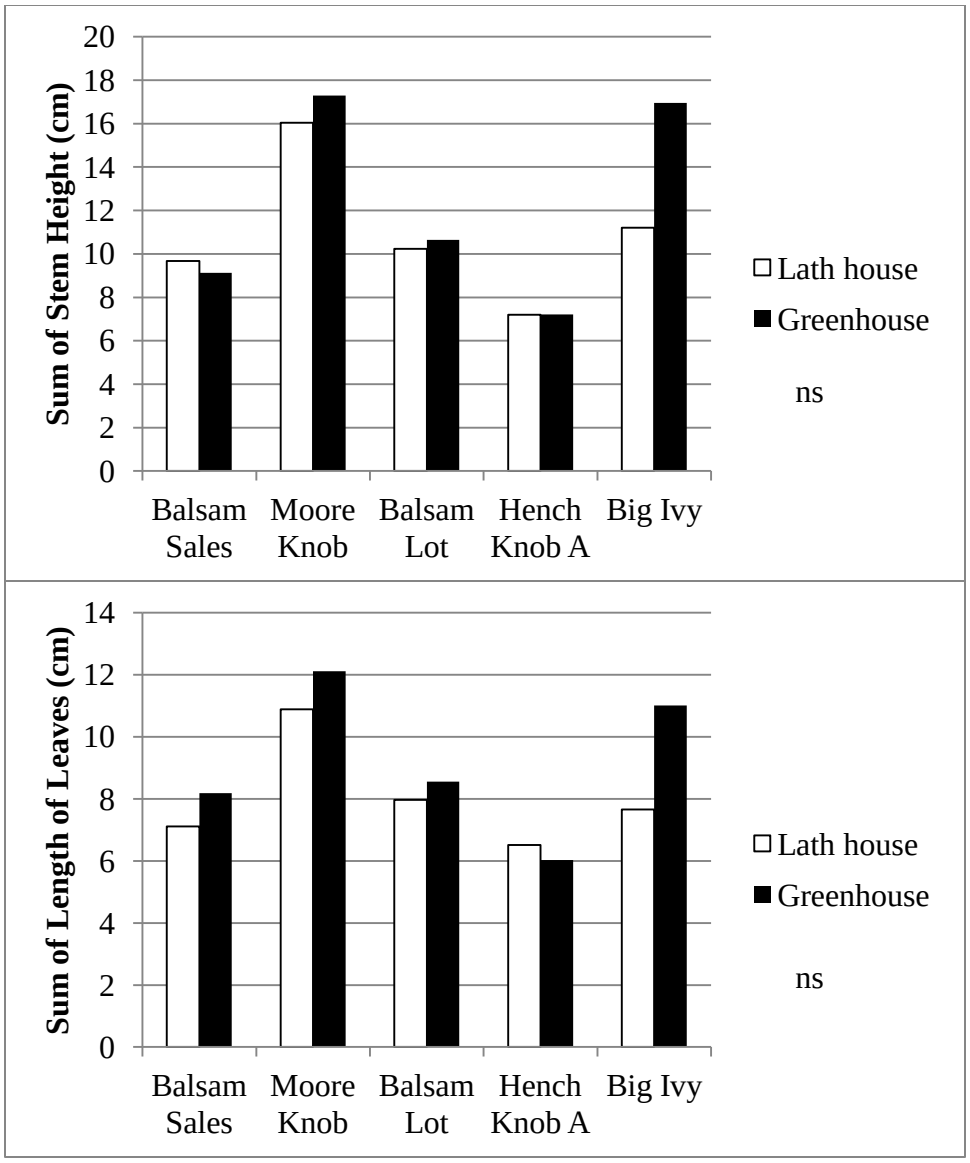

Figure 9. Comparison of greenhouse and lath house on size parameters for *Hydrastis canadensis* populations from western NC growing in a common garden.

Discussion

The purpose of this project was to improve our understanding of genetic variation in *Hydrastis canadensis*. The common garden and alkaloid analyses revealed variation in phenotypic traits among populations for all variables except flowering and seed production. The same trend was seen in a reciprocal transplant study with *Sanguinaria canadensis* L., which showed phenotypic variation and plasticity in response to fertilizer and light in stem and leaf sizes but not reproduction (Marino et al. 1997). A common garden experiment found alkaloid content in *Sanguinaria canadensis* rhizomes to respond plastically to fertilizer and light (Salmore and Hunter 2001), but we will not know if alkaloid content is plastic in goldenseal until the second year data is taken from the common garden.

Sinclair (2002) reported the main pollinator for goldenseal is small bees, and that red winged blackbirds, among others, were dispersers. These observations indicated that pollination and dispersal were not limiting population growth since pollinators were present and seed was removed by dispersers. In my study, there were no observed pollinators in the common garden experiment, but goldenseal has been shown to be capable of self-pollination (Sanders 2004), so pollination does not appear to be a limiting factor for fruit set. Based on the findings of my allozyme study that most genetic variation is within populations, pollination and dispersal may not be limiting genetic diversity either.

Reproductive timing in the common garden experiment did not follow elevational trends, though it was expected based on Hopkins Bioclimatic Law (Pearcy and Ward

1972). The lack in elevational trends could be because the elevational differences between the populations were not great enough (only 744 ft, corresponding to 7 days) to illuminate the pattern. In contrast, *Festuca eskia* Ramond had local adaptation in height and reproduction across an elevational gradient of only 1,000 m (Gonzalo-Turpin and Hazard 2009), and differentiation has been measured in *Ranunculus reptans* L. over 10 m increments (Prati and Schmid 2000). The lack of local adaptation across elevational gradients in goldenseal suggests that it is a genetic generalist (at least across the elevations sampled), because when brought to a novel environment, the plants did not exhibit adaptation to their habitat of origin (Van Tienderen 1991, Kassen 2002).

Plants grown in the greenhouse had larger biomass than plants grown in the lath house, but growth area did not significantly affect any trait. Perhaps the plants were not moved far enough from their origin to elicit a response, or perhaps plants are showing plasticity instead of local adaptation. Plasticity is favorable to specialization in variable environments. If these goldenseal plants are exhibiting plasticity, perhaps they are coming from spatially heterogeneous environments and have evolved to respond to change instead of specializing (Van Tienderen 1991, Callaway et al. 2003). This lack of response to a novel environment also supports the idea that goldenseal is a genetic generalist (Van Tienderen 1991, Kassen 2002). No interactions between genotype and environment were found at the population or patch level. Sanders and McGraw (2005) also found plasticity in phenotypic traits of goldenseal and no signs of local adaptation. However, a lack of GxE detected in this study could also be due to the short range of collection and movement of genotypes. Interactions between genetics and the environment often require greater differences such as changes in hardiness zones, or large

changes in growth environment, and may be limited to a few genotypes anyway (Roth et al. 2007).

Maintenance of genetic variation present in western NC goldenseal, though minimal, could be due to sexual reproduction. In a large scale review comparing phenotypic traits with allozyme diversity, Loveless and Hamrick (1984) showed that long-lived plants exhibiting mixed mating, seasonally synchronous flowers, with small bee pollination and animal ingested dispersal had increased genetic diversity compared to plants with life histories aligned with more typical clonal habits (Loveless and Hamrick 1984). Although goldenseal is mainly clonal, it has the previously mentioned characteristics that may be adding genetic diversity (Sanders 2004, Sinclair 2000).

Since the majority of molecular variation found in my study was within populations, populations are not experiencing the high degree of structuring expected for neutral alleles in populations that grow in isolated patches (Young et al. 1996, Honnay and Jacquemyn 2006, Jacquemyn et al. 2012). The low number of alleles found among these populations meant not much variation was even possible, so variation that was present was found in heterozygosity levels within populations. Although the average number of alleles was very low, observed heterozygosity was consistent with common species (H_o = 0.139) and rare species (H_o= 0.100) (Cole 2003). However, Moore Knob had especially low heterozygosity (H_o = 0.041). *Panax quinquefolius*, often compared to goldenseal due to habitat similarities and medicinal value, had lower expected heterozygosity (H_e=0.076), even in protected populations, than goldenseal (lowest H_e=0.154) (Cruse-Sanders and Hamrick 2004).

Moore Knob was one of the largest populations in this study, whereas Big Ivy was one of the smallest populations based on field observations (data not collected). Yet, Moore Knob had the lowest diversity while Big Ivy was the most diverse. This relationship between diversity and population size is opposite of expectations where small and isolated populations are expected to have lower heterozygosity because of increased genetic drift and inbreeding, and decreased gene flow that often accompanies fragmentation (Wright 1931, Young et al. 1996, Jacquemyn et al. 2006, Jacquemyn et al. 2012). For example, the forest herb *Paris quadrifolia* L. has a patchy distribution like goldenseal and has greater genetic diversity in larger populations than in small populations (Jacquemyn et al. 2006) presumably due to high gene flow and sexual reproduction. Expected heterozygosity in *Panax quinquefolius* is also higher in larger populations of ginseng (Cruse-Sanders and Hamrick 2004). The lack of relationship between population size and genetic diversity observed in my study may be the result of goldenseal reproductive strategy, or it may be due to low sample size or relatively close geographic proximity of populations.

Some species such as goldenseal that can reproduce both clonally and sexually can alter strategies depending on environmental conditions. For some species, clonal reproduction is the best strategy in open suitable habitats, while sexual reproduction is used when habitats are less suitable and adaptation or dispersal is needed (Lei 2010). This has been demonstrated in the clonal plants *Sparganium erectum* L. (Piquot et al. 1998) and *Rannunculus reptans* L. (Prati and Schmid 2000, van Kleunen et al. 2001). The habitat at Big Ivy was the seemingly least typical habitat (based on field observations) out of the populations studied yet this population was the most genetically

diverse. Therefore, this population might be the result of seed dispersal and continued sexual reproduction has occurred to increase chances of adaptation to this novel environment. Evidence for this is seen in the unique alleles found at Big Ivy.

Management Implications

Knowledge of genetic diversity and plasticity is necessary to avoid causing outbreeding or inbreeding depression when combining different genetic sources of plants (Kramer and Havens 2009) such as occurs with reintroduction programs. Since genetic and allelic diversity was found to be relatively low across the populations of goldenseal in this study, it is unlikely that reintroductions into declining populations would lead to genetic loss due to outbreeding depression. It should be noted that Big Ivy had the only unique alleles found in this study, so it would probably make the worst candidate for reintroduction. However, it was the second smallest population in this study, so it may be the best candidate for encouraged reproduction by adding substrate disturbance to the habitat (Sinclair 2002). Although seedling success is usually minimal for clonal species (Cruse-Sanders and Hamrick 2004, Goertzen and Boyd 2007, Lei 2010), even a small amount of added sexual reproduction can counteract the negative effects of genetic drift (Wright 1931, Watkinson and Powell 1993, Young et al. 2006)

Adding additional substrate disturbance into goldenseal habitats is a good way to stimulate population growth rates and should be implemented when resources are available. Sinclair (2002) thinks goldenseal "waits" for a disturbance in the environment before expanding, and is stationary otherwise. He found goldenseal is positively affected by substrate disturbance, and that high light significantly increased biomass, flower

production, fruit production, and seed production, but in all these cases only a small amount of the variability was explained by light. However, the highest amount of variability explained by light was germination and seedling success. He recommends disturbance and fertilizer to encourage population growth and sexual reproduction, especially in high light conditions (Sinclair 2002). In my study, larger sizes were associated with greater reproduction, and size was increased by fertilizer, so adding disturbance and fertilizer to sites could increase sexual reproduction and add genetic variation.

CHAPTER 4: LITERATURE CITED

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