2004

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Recommended Citation

Sanders, Suzanne, "Tests for causes of rarity in goldenseal (Hydrastis canadensis L.)" (2004). *Graduate Theses, Dissertations, and Problem Reports*. 2091.
https://researchrepository.wvu.edu/etd/2091

Tests for causes of rarity in goldenseal (*Hydrastis canadensis* L.)

Suzanne Sanders

Dissertation submitted to the
Eberly College of Arts and Sciences
at West Virginia University
in partial fulfillment of the requirements
For the degree of

Doctor of Philosophy
in
Biology

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Morgantown, West Virginia
2004

Keywords: breeding systems, GIS, *Hydrastis*, microsite, rarity, reciprocal transplant, restoration

ABSTRACT

Tests for causes of rarity in goldenseal (Hydrastis canadensis L.)

Suzanne Sanders

Hydrastis canadensis L. (goldenseal) is becoming more uncommon within the eastern deciduous forest. I performed a series of studies that tested hypotheses about population decline and causes of rarity. First, I assessed the time-trend of a natural *H. canadensis* population in an Indiana nature preserve which had been censused 26 years prior. We found a negative population trajectory between the two time periods. The second goal of this dissertation was to determine the breeding system of *H. canadensis*. The breeding system type appears unlikely to be a major factor limiting the distribution or abundance of *H. canadensis*. My third objective was to assess the response of *H. canadensis* populations to harvest. I found variation in patch regrowth which suggests timing of harvest may be important. My studies in chapter 5 focused on abiotic microsite factors. I experimentally assessed the role of temperature, humidity, and light gradients in the distribution of *H. canadensis*. The relatively weak association between environmental variation and plant performance across the forested cove reinforces other studies suggesting that *H. canadensis* has a relatively broad ecological niche, and its rarity is unlikely due to availability of suitable abiotic habitat conditions. My fifth goal was to understand ecologically relevant aspects of *H. canadensis* genetics. I determined if *H. canadensis* contained ecologically important genetic variation (*i.e.* ecotypic differentiation) at the population level. I conducted a classical reciprocal transplant experiment with four natural populations to test the hypothesis that ramets planted in their home site would have greater survival and performance than alien *H. canadensis* ramets planted into the same site. This research is the basis of Chapter 6. My final goal was to determine whether *H. canadensis* range is limited due to high habitat specificity and limited availability of suitable habitat. This study found no evidence of a lack of suitable habitat for *H. canadensis*. Overall, these studies serve to rule out several possible causes of rarity of *H. canadensis*, although this research does suggest some role of understory light availability in species decline. This idea, along with other hypotheses, is discussed further in the general conclusions.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. James McGraw for all of the time and effort that he has invested in this work. I am also grateful to my committee members, Jonathan Cumming, William Grafton, Andrew Liebhold, Richard Thomas, and Timothy Warner. They have all been more than willing to assist me whenever asked.

I am grateful to numerous individuals who have assisted me locating plant populations. For this, I am grateful to Jan Garrett, Russ Richardson, Sam Norris, Ashton Berdine, Brian Streets, Charlie Baer, and Juergen Wildman. I am also indebted to all of the individuals, corporations, and agencies that allowed me access to land for field research. I am especially grateful to all of the private landowners who allowed me access to their property for field surveys. This work would not be possible without their help.

I owe many thanks to all members of my field crews: Gera Jochum, Nathan Kota, Sara Lightner, Emily Mooney, Mary Olive, Sarah Wennerberg, and Bridget Wright. Finally, I wish to thank all of the other graduate students in the Department of Biology who have helped my over these past five years.

This research was funded by grants 99HQAG0045 and 1434-HQ-97-RU-01563, both from the U. S. Department of the Interior and the U. S. Geological Survey. This work was also supported by the West Virginia University Swiger Doctoral Fellowship.

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CHAPTER 1

General Introduction

Hydrastis canadensis L. (goldenseal, yellow root) is a herbaceous perennial herb native to the central range of the eastern deciduous forest. Although historical (prior to 2000) reports documenting abundance are not known, there is a general consensus among botanists, ecologists, and land managers that this species is experiencing population decline. Indeed, it was this concern that led to its listing on Appendix II of the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) in 1997. Further, observational studies of Canadian *H. canadensis* populations have shown population loss in southern Ontario (Sinclair and Catling, 2000).

Hydrastis canadensis is native to the eastern deciduous forest of North America. The range of *H. canadensis* extends from southern Ontario south to Tennessee and the surrounding states, and from Missouri, east to the eastern edge of the Appalachian Mountains (Small and Catling, 1999). Within this range, *H. canadensis* grows in mesic forests, forming dense, clonal patches comprised of between a few to several thousand ramets (Sinclair and Catling, 2000). Nonreproductive ramets are comprised of a single leaf while reproductive ramets have a second (rarely third) leaf. The pedicel arises from the base of the second (or third) leaf and supports a single flower.

The cause of *H. canadensis* population decline is unclear. One of the earliest reports of pressures on *H. canadensis* is from Bowers (1892) who noted that the clearing of land for human settlement has reduced much of the habitat of this species. The author elaborated further by noting that *H. canadensis* required deep shade, nearby decaying logs, and a thick layer of leaf mold. Although more recent authors (Sinclair and Catling, 2000) also support this description of *H. canadensis* habitat, noting the requirement of mature forests and moist soils, numerous populations have been observed on sunny south-facing aspects, as well as near ridgetops in areas that are never flooded (McGraw et al., 2003). These contradictions in habitat requirements suggest that one of two alternatives is occurring. Either *H. canadensis* has very specific habitat requirements that are not yet completely understood or, alternatively, habitat may not be limiting distribution and abundance. If this is the case, a large amount of unoccupied habitat would be expected to be suitable for *H. canadensis* growth and reproduction. Clearly, highly specific habitat requirements could promote population decline if suitable habitat is becoming rarer. Ideal habitat may be related to large mesoscale features, such as aspect or elevation, or it may be a reflection of microsite availability.

Spatial patterns of understory plant distribution are often related to abiotic microsite conditions. Suitable microsites of widely distributed species include those associated with distance from the forest edge (Gehlhausen et al., 2000), those associated with a particular suite of overstory species (McCarthy and Bailey 1994), and those resulting from mesoscale topography (Pornon et al., 1997). Abiotic variables affected by these factors include light, temperature, and humidity (Matlack, 1993). Recent anthropogenic changes that impact these microsite factors could limit the number and extent of suitable microsites available for *H. canadensis*.

In addition to habitat requirements, another factor that may contribute to *H. canadensis* decline is the effect of harvest. *Hydrastis canadensis* is believed by many people to have curative abilities. Due to this belief, the rhizome is harvested from natural populations either for personal consumption or for sale on the herbal market (Charron and Gagnon, 1991). The consequence of harvest on *H. canadensis* population growth is unknown. Like American ginseng (*Panax quinquefolius* L.), it is the belowground rhizome that is valued, and is what is removed during harvest. Unlike ginseng, however, *H. canadensis* reproduces vegetatively *via* prolific rhizome growth. When *H. canadensis* is harvested, the large storage rhizomes at the base of aerial stems are removed and are broken off from the lateral roots, which remain in the soil. Regrowth of new aerial ramets can arise from buds on the lateral roots (Van der Voort et al., 2003). This may allow populations to withstand harvest pressure, providing the time between harvests is sufficient.

Hydrastis canadensis population decline may also be occurring in response to environmental change, if the populations are not able to adapt. Ecological genetic variation in plants allows populations to respond to environmental change (Antonovics et al., 1971; Davison and Reiling, 1995; Benkman, 1995). This is important for adapting to sudden differences in habitat upon dispersal (Gomulkiewicz and Holt, 1995) and to *in situ* directional changes in the environment (Rice and Emery, 2003). Intraspecific genetic variation was demonstrated in common garden experiments by Turesson (1925) and later in reciprocal transplant studies by Clausen et al. (1940). These early studies led to the recognition of "ecotypes" or locally adapted populations that have higher fitnesses in their local microsite conditions than conspecific plants found in contrasting environments. Failure of populations to adapt may threaten their persistence by limiting response to long term environmental change and population establishment after dispersal (Antonovics, 1976).

A reduction in the total number of populations, whether in response to harvest, environmental change, or *via* other mechanisms, can further promote decline as a result of mate limitation. Breeding systems are most likely to limit seed set if a species obligately or preferentially outcrosses and/or available mates are spatially separated across the landscape (Demauro, 1993; Weekley and Race, 2001). Mate limitation resulting from obligate outcrossing has been associated with rarity in numerous studies (Evans et al., 2003; Messmore and Knox, 1997). In clonal plant species, only one or a few genets may be present within a given patch. Thus, for clonal species with an obligate outcrossing requirement, viable seed set may necessitate pollen flow between patches (Wilcock, 2002). Therefore, if *H. canadensis* is an obligate outcrossing species, seed production and viability may be hindered due to mate limitation.

The goals of the current research were six-fold, and were based on documenting decline, and understanding rarity, of *H. canadensis*. First, I assessed the time-trend of a natural *H. canadensis* population in an Indiana nature preserve. Fortunately, the population was mapped in detail and partially censused 26 years prior to my census. The objective was to determine the net trajectory of population change between the two time periods. Specifically, I wanted to determine (1) if there were changes in the overall spatial extent of *H. canadensis* within the preserve, and (2) if there were net changes in the abundance of *H. canadensis* within predetermined quadrats between censuses. This project is the basis of Chapter 2.

The second goal was to determine the breeding system of *H. canadensis*. This would allow me to determine the importance of the degree of patch isolation on fruit set. I performed crosses to test for apomixis, passive autogamy, active autogamy, and outcrossing, both within and between populations. The results of this research are reported in Chapter 3.

My third objective of this dissertation was to assess the response of *H. canadensis* populations to harvest. I focused on regrowth of three experimentally harvested patches as well as that of an illicitly harvested natural patch near Morgantown, West Virginia (Van der Voort et al., 2003). First, I wanted to determine if trends in regrowth were consistent across multiple harvested patches. I compared all four patches and asked whether the effect of the length of time since harvest on ramet leaf area, a measure of recovery rate, varied between patches. I also compared ramet leaf area of the three experimentally harvested patches at three times: immediately prior to harvest, and at one and two years post-harvest. I asked whether ramet leaf area varied between the time periods. If so, I also wanted to know whether pre-harvest size structure could be attained by two years following harvest. This study is detailed in Chapter 4.

My fourth goal was to understand ecologically relevant aspects of *H. canadensis* genetics. Within this effort, my first objective was to determine if *H. canadensis* contained ecologically important genetic variation (*i.e.* ecotypic differentiation) at the population level. I conducted a classical reciprocal transplant experiment with four natural populations to test the hypothesis that ramets planted in their home site would have greater survival and performance than alien *H. canadensis* ramets planted into the same site. Because population introduction may become a necessary strategy for augmenting abundance and promoting persistence, I wanted to test if survival and establishment of introduced *H. canadensis* populations were affected by the genetic composition of the transplanted individuals. My second objective associated with *H. canadensis* ecological genetics was, therefore, to determine if there were differences in performance between populations established from one natural source and those established from multiple natural sources. I conducted a three year population establishment study to test the hypothesis that introduced populations comprised of mixtures perform better than those comprised of monocultures. I also tested whether plants from different source populations differed in their establishment potential in a new site. This research is described in Chapter 5.

Because descriptive studies of *H. canadensis* site microsite conditions can only lead to hypotheses about actual controls over distribution, I experimentally assessed the role of temperature, humidity, and light gradients in the distribution of *H. canadensis*. Specifically, I wanted to determine if these environmental variables could explain the success of *H. canadensis* phytometers placed in different positions within a forested 'cove'. I planted transects of *H. canadensis* at equal distances away from a stream so that they spanned gradients of temperature, humidity, and light. The design was such that one of these transects was adjacent to a natural patch which was postulated to be in suitable habitat. My first objective was to determine if there were differences in phytometer performance between aspects and with distance from the stream. My second objective was to quantify environmental differences between each transect location and the transect at the suitable site and to relate these differences to plant performance. This research is the basis of Chapter 6.

My final goal was to determine whether *H. canadensis* range is limited due to high habitat specificity and limited availability of suitable habitat. I used geographic information systems to develop predictive models both by regression and multivariate techniques. These models were developed for a 23,000 km^2 area of north-central West Virginia. I then performed 50 field surveys within this area to validate the models. This work is presented in Chapter 7.

Overall, these studies will provide insight into the population decline of *H. canadensis* by attempting to answer key questions about its biology. Obviously, the potential factors influencing decline are numerous. It is my goal to address as many of these as possible, and to provide answers to many of my questions. I hope that this research provides a solid background of information on which to build and direct future research.

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CHAPTER 2:

Distribution, Abundance, and Population Dynamics of Goldenseal (*Hydrastis canadensis* L.) in an Indiana Nature Preserve, USA

Abstract

The expanding market for herbal remedies has stimulated increased harvest of goldenseal *(Hydrastis canadensis* L.) from the wild. I examined net population change of *H. canadensis* over 26 years in Bryan Nature Preserve, central Indiana, USA. Individuals were mapped and censused in 0.1 ha quadrats over the entire preserve, repeating a similar procedure carried out by others in 1974. The net trajectory of abundance was negative: *H. canadensis* was present in 46 quadrats in 1974, but had become extinct in 31 of those quadrats and decreased in 15; three quadrats contained new patches. Only 10 of the 99 patches present in 1974 were relocated. Fifteen new patches were found in 2000. The total number of quadrats with *H. canadensis* declined between the two censuses. The number of quadrats in which the abundance trajectory was positive between the two time periods was significantly less than the number showing a negative trajectory. Patches in edge quadrats had higher survival than those in the interior. Extinction probability was not dependent on population size. There is no known cause of the decline in *H. canadensis* in the preserve, although one possibility is the damage from a severe wind and ice storm that occurred in February 1991. Harvest history is unknown, although recent harvest was not evident. Periodic recensusing of this preserve is needed to understand the causes of *H. canadensis* decline.

Introduction

Goldenseal *(Hydrastis canadensis* L.) is a perennial herb of the eastern deciduous forest of North America. While this speciesis not considered threatened or endangered throughout most of its range, it is uncommonly observed, even in apparently suitable habitat. *Hydrastis canadensis* is harvested from the wild and sold on the herbal market where the dried root commanded US $66.00 kg -1 from 1998 to 1999 (Bailey 1999). Demand and price provide incentive to harvest the plant as an income supplement, particularly in areas where unemployment is high. *Hydrastis canadensis* is currently listed on Appendix II of the CITES treaty (Convention on International Trade in Endangered Species of Flora and Fauna). Therefore, the species must be monitored, and the federal government must certify that its harvest remains "non-detrimental," to permit international trade. Little is known about the biology of *H. canadensi*s, particularly with regard to rarity. In general, species abundance is positively correlated with size of geographic range (Brown 1984, Gaston 1994), so that most rare species have small ranges. *Hydrastis canadensis* is in a relatively uncommon class of rarity (Rabinowitz 1981): it has a wide geographic distribution, relatively narrow niche, and genets are sparsely distributed at a local scale (S. M. Sanders and J. B. McGraw, pers. obs.). In particular, it is not known whether the rarity of the species is due to external factors such as harvest, changes in land use, and loss of seed dispersers, or to inherent constraints such as breeding system limitations, lack of genetic variability, or specific habitat requirements. The purpose of this study was to assess the time-trend of a natural goldenseal population in an Indiana, USA, nature preserve. The area was mapped and partially censused 26 years prior to our census. Our objective was to determine the net trajectory of population change between the two time periods. Specifically, I wanted to determine (1) if there were changes in the overall spatial extent of *H. canadensis* within the preserve, and (2) if there were net changes in the abundance of *H. canadensis* within predetermined quadrats between censuses.

Natural history of *H. canadensis*

Hydrastis canadensis inhabits moist rich woods of the eastern deciduous forest from New York and southern New England west to southern Wisconsin and south to Tennessee. This range includes southern Ontario in the north and Missouri and northern Arkansas in the west. *Hydrastis canadensis* often grows in and above the flood zone of low- to mid-order streams, and on periodically spring-flooded plateaus (Sinclair and Catling 2000a). The general lore about *H. canadensis* among herb harvesters is that this species is found almost exclusively on north- and north-west-facing slopes, where cooler and moister conditions occur. Field surveys conducted in West Virginia and four surrounding states (S. M. Sanders and J. B. McGraw, unpubl. data), however, found no aspect preference by *H. canadensi*s. Commonly associated species include blue cohosh *(Caulophyllum thalictroides* [L.] Michx.), black cohosh *(Cimicifuga racemosa* [L.] Nutt.), and mayapple *(Podophyllum peltatum* L.). I have also observed several instances where mature *H. canadensis* patches are in immediate proximity to thriving twinleaf *(Jeffersonia diphylla* [L.] Pers.) patches (nomenclature follows Gleason and Cronquist 1991). *Hydrastis canadensis* primarily reproduces clonally via lateral rootlets; reproduction by seed appears to be of secondary importance (Van der Voort et al., West Virginia University, Morgantown, unpubl. data). Nonreproductive plants develop one palmately lobed leaf, and reproductive plants produce a second smaller leaf (rarely a third) above the primary leaf. The pedicel arises at the base of the uppermost leaf. The flower is apetalous with deciduous sepals that fall soon after opening. Numerous stamens surround a gynoecium with 2–20 carpels. At maturity, the fruit is a red berry resembling a raspberry *(Rubus* L. sp.). The clonal growth of *H. canadensis* promotes dense patches. It is not known whether reproduction in patches occurs entirely vegetatively, whereby entire patches are all of one genotype, or whether multiple genotypes occur within a patch. Due to this uncertainty, all aboveground stems are referred to as ramets, leaving the number of genotypes unknown.

Methods

Field sampling

Bryan Nature Preserve is an 11-ha old-growth oak-hickory forest located 18 km east of Lafayette, Indiana. Dominant overstory species include white oak *(Quercus alba* L.), red oak *(Q. rubra* L.), and shagbark hickory *(Carya ovata* [Miller] K. Koch.). The topography of the preserve is nearly level with scattered, poorly drained depressions. Soil in these depressional areas is a Ragsdale silty clay loam (Typic Argiaquoll), which remains saturated throughout the spring and much of the summer. Soil on the better drained uplands is a Fincastle silt loam (Aeric Epiaqualf) (Johnson et al. 1974). The preserve is surrounded on all sides by agricultural fields. *Hydrastis canadensis* occurrences were mapped in 1973 and 1974, and randomly selected patches were censused in both years (Davis 1976, Eichenberger and Parker 1976). A grid system used in the original mapping subdivided the preserve into 0.1-ha (31.6-m x 31.6-m) quadrats. This design created 132 quadrats, of which 42 formed the perimeter (edge quadrats) and 90 were located in the interior (interior quadrats). I reestablished the grid in June 2000 using electronic distance measuring devices (Sonin Pro, Sonin, Inc., Scarsdale, N.Y.) and handheld compasses. Each quadrat was thoroughly searched by an experienced four-person survey crew. When a patch (or isolated plant) was found, I recorded its location as the distance and angle from the nearest quadrat marker. Patches were defined as groups of ramets, none of which were greater than 0.46 m apart, consistent with Davis's (1976) definition of "clump." I recorded the total number of ramets in each patch, and noted the number of those that were reproductive, both with and without fruit. Direct comparisons of ramet counts between 1974 and 2000 were not possible for most patches and quadrats because ramets in only 25 of the 99 patches located in 1974 were counted. These 25 patches were randomly chosen in 1974 and included 8 patches in edge quadrats and 17 in interior quadrats. I averaged the number of ramets in 1974 edge-quadrat patches and in 1974 interior-quadrat patches. The average value of ramet abundance was then multiplied by the number of patches in each quadrat in 1974, and the product was used as the expected number of ramets in 2000. Using this procedure, I estimated numbers of ramets in 1974 (based on those that were counted) and compared this with the observed number in 2000. Thereafter, I categorized the changes in *H. canadensis* abundance in each quadrat as “unchanged,” “increased,” “decreased,” “extinct,” or “new.” Within a quadrat, abundance “decreased” if the observed number of ramets was at least 20% less than the expected number. Likewise, abundance “increased” if the observed number of ramets was at least 20% greater than the expected number. I chose changes of 20% because I felt an increase of this level suggests patches that are growing strongly, while decreases of greater than 20% might be cause for concern. Abundance changes of less than 20% were labeled “unchanged.” For example, patches in edge quadrats averaged 60 ramets (+ 23.2 SE) in 1974. Since quadrat 5 had three patches in 1974, I would expect 180 ramets present in quadrat 5 in 2000. Interior quadrats averaged 129 ramets (+ 40.0 SE) per patch. Since quadrat 40 had 1 patch in 1974, I would expect 129 ramets present in 2000. Since quadrats 5 and 40 contained only 76 and 11 ramets in 2000, respectively, ramet abundance in both of these quadrats was scored as “decreased.”

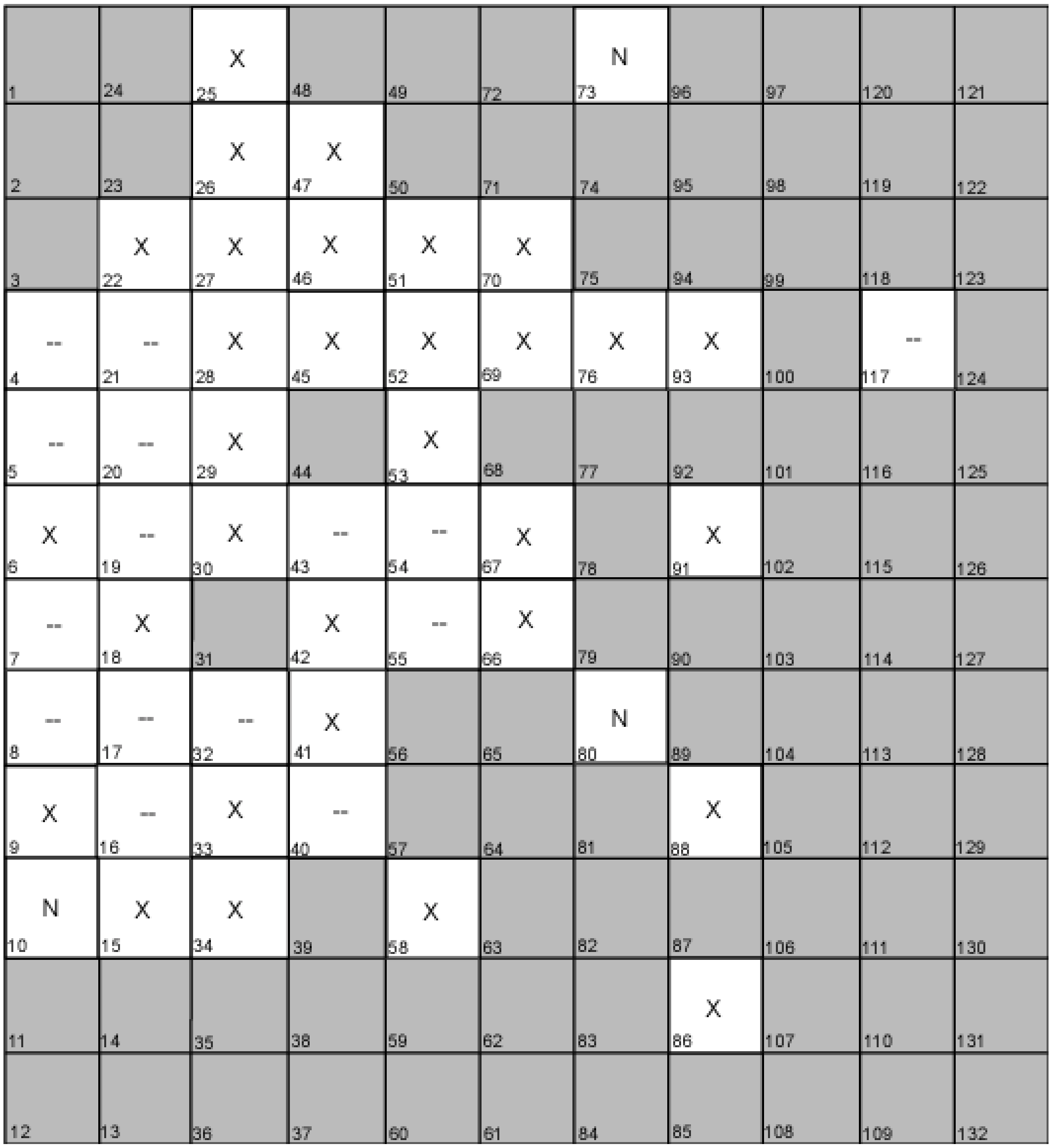
Data analysis

I used a G-test (Sokal and Rohlf 1995) to compare the number of quadrats containing *H. canadensis* in 1974 to the number containing ramets in 2000. I tested the null hypothesis that the overall spatial extent of *H. canadensis* within the preserve remained the same across years. Second, I used a G-test to compare the observed number of quadrats showing positive abundance trajectories (“increased” or new patch) and negative abundance trajectories (“decreased” or “extinct”) with the expected number. I anticipated that there would be some quadrats where abundance would remain unchanged. In quadrats with changing abundance, our null hypothesis was that there would be an equal quantity of quadrats with increasing numbers and decreasing numbers of ramets. Two statistical tests of survival were also used. Because small preserves tend to have greater edge vs. interior habitat, I tested whether habitat would affect *H. canadensis* patch survival. A G-test was used to test the null hypothesis that patches located in border quadrats had the same survival rates as interior patches. I then examined the relationship between survival probability and patch size. It is often assumed that smaller patches will have a greater extinction probability than larger patches. I tested this hypothesis with a logistic regression of extinction occurrence on 1974 ramet number for a patch (n = 25 patches censused in 1974). Finally, I used a G-test to determine if the relative number of reproductive stems differed between the two censuses. This tested the null hypothesis that the percentage of reproductive stems remained un-changed. I determined the percentages of reproductive and nonreproductive stems in censused patches in 1974 and based the expected numbers in 2000 on these percentages.

Results

Major changes in *H. canadensis* distribution and abundance occurred in the 26 years between mappings. Of the 99 patches present in 1974, only 10 were relocated. Fifteen new patches were found that were not located in 1974. *Hydrastis canadensis* was present in 46 quadrats in 1974. By 2000, *H. canadensis* had become extinct in 31 of those quadrats (Figure 2.1) and decreased in 15 others. In no quadrat did *H. canadensis* abundance remain unchanged or increase, although 3 quadrats did contain new patches that were not located in 1974. The number of quadrats where *H. canadensis* was present decreased significantly between the two censuses (G-test, df = 1, $P < 0.001$). The trajectory of abundance for *H. canadensis* was negative in 46 quadrats and positive in only 3. Since 49 quadrats contained *H. canadensis* in either 1974 or 2000, and abundance did not remain unchanged in any quadrat, I would expect a positive and a negative abundance trajectory each in 24.5 quadrats. The observed number of positive and negative trajectories of *H. canadensis* abundance within quadrats differed from the expected values (G-test, df = 1, $P < 0.001$), demonstrating a greater than expected number of quadrats with declining ramet numbers. Of the 99 original patches, 14 were in edge quadrats (14%) and 85 were in central quadrats (86%). Of the 10 patches relocated in 2000, 4 were located in edge quadrats (40%). By 2000, there was a significant increase in the ratio of patches found in edge quadrats to those in interior quadrats vis-à-vis the ratio found 26 years earlier (Figure 2.2; G-test, df = 1, $P < 0.05$). Logistic regression of extinction occurrence on patch size, performed on the 25 patches censused in 1974 showed that the probability of extinction did not depend on patch size ($r 2 = 0.06$, $P = 0.3$). The relative number of reproductive stems of *H. canadensis* decreased significantly

Figure 2.1. Bryan Nature Preserve with quadrats showing *H. canadensis* changes. Declines reflect losses of greater than 20% between the expected value and the observed value.

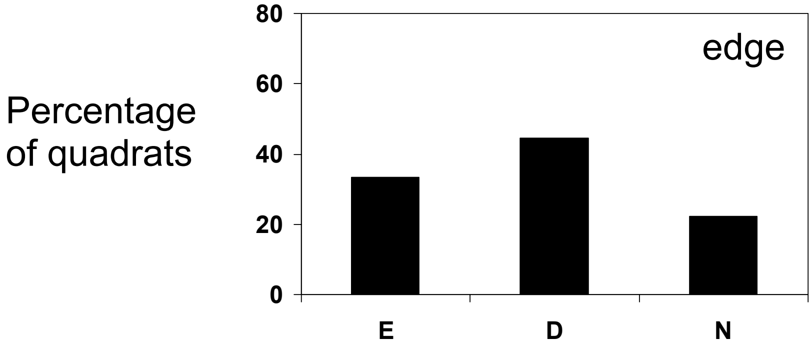


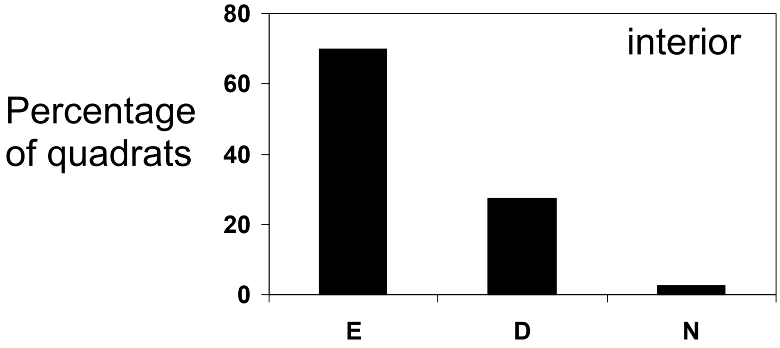
1	24	X 25	48	49	72	N 73	96	97	120	121
2	23	X 26	X 47	50	71	74	95	98	119	122
3	X 22	X 27	X 46	X 51	X 70	75	94	99	118	123
-- 4	-- 21	X 28	X 45	X 52	X 69	X 76	X 93	100	-- 117	124
-- 5	-- 20	X 29	44	X 53	68	77	92	101	116	125
X 6	-- 19	X 30	-- 43	-- 54	X 67	78	X 91	102	115	126
-- 7	X 18	31	X 42	-- 55	X 66	79	90	103	114	127
-- 8	-- 17	-- 32	X 41	56	65	N 80	89	104	113	128
X 9	-- 16	X 33	-- 40	57	64	81	X 88	105	112	129
N 10	X 15	X 34	39	X 58	63	82	87	106	111	130
11	14	35	38	59	62	83	X 86	107	110	131
12	13	36	37	60	61	84	85	108	109	132

Legend

X = Extinct
-- = Decline
N = New
▨ = No goldenseal present in either census

Figure 2.2. Fate of *H. canadensis* patches within edge and interior quadrats in the preserve. E = went extinct, D = declined, N = new population appeared.


Percentage of quadrats
80
60
40
20
0
edge
E
D
N


Percentage of quadrats
80
60
40
20
0
interior
E
D
N

between the two time periods (G-test, df = 1, $P > 0.001$). In 1974, 23% of stems were reproductive and only one patch lacked reproductive stems. In 2000, 11% of the stems were reproductive and 14 of the 25 patches lacked reproductive stems.

Discussion

The dramatic decline in *H. canadensis* over 26 years at Bryan Nature Preserve is surprising, given that this is a long-lived clonal plant. Because I did not directly observe patches in the interim period, it is difficult to ascertain causes of this decline. Although harvest is not permitted in this preserve, illegal poaching is one possible cause of decline. Harvesters remove the underground rhizome, discarding the above-ground portion of the plant. These rhizomes produce numerous lateral rootlets, which are broken off from the rhizome when it is harvested. The lateral rootlets are capable of sprouting new above-ground stems. Elsewhere, harvested *H. canadensis* patches were shown to contain numerous, densely clustered, small ramets, few of which were reproductive (Van der Voort et al., West Virginia University, Morgantown, unpubl. data). The *H. canadensis* ramets observed at Bryan Nature Preserve in 2000 varied in size, and included numerous two-leaved, reproductive stems. Furthermore, the hiking trail through the preserve passed directly adjacent to population # 171, which had 50 ramets and was clearly visible from the trail. Any recent illegal poaching probably would have targeted this patch. It is not known how long harvested *H. canadensis* patches require to return to the original, pre-harvest size structure. Van der Voort et al. (2003) examined recovery of an illegally harvested patch in West Virginia. Although the original size structure was not known, they found that leaf size increased each year for the three years that recovery was monitored. This suggests that, if poaching did occur at Bryan Nature Preserve, it was sometime after the 1974 census until possibly as late as the mid 1990s. Forest disturbance was clearly evident at Bryan Nature Preserve. A severe wind and ice storm occurred during February 1991 (Tom Swinford, Indiana Department of Natural Resources, Indianapolis, pers. com.), downing numerous large trees, especially in the western interior of the preserve. This area is where *H. canadensis* was the densest in 1974. In 2000, saplings were growing in the gaps created by the event, although there was still considerably more light reaching the floor in these disturbed areas compared with nondisturbed areas. Many of these gaps supported dense patches of mayapple, while a few also included *Urtica* L. sp. and *Impatiens* L. sp. The high rates of extinction indicate that *H. canadensis* may not respond well to the environmental changes brought about by overstory loss. These changes could include higher light levels and lower mid-season water potentials (Maschinski et al. 1997), as well as greater competition from gap-invading species. One hypothesis to explain the higher survival rates of edge versus interior patches is that plants in edge patches may be either acclimated or adapted to higher light levels and corresponding lower soil water potential associated with this habitat. Nonstochastic events also may be affecting *H. canadensis* patches. The site appears to be in a state of transition from an oak-hickory forest toward a sugar maple *(Acer saccharum* Marshall) dominated stand. A long-term study of the Tipton Till Plain of central Indiana showed that mid-seral species, including oaks *(Quercus* L. spp.) are gradually being replaced by shagbark hickory *(Carya ovat*a) and sugar maple (Parker et al. 1985). Shifts in canopy composition may influence understory light levels (Küppers 1989, Brown and Parker 1994). Changes in the light level associated with the canopy transition may have reduced the number of suitable sites for development of *H. canadensis* patches. The reduction in flowering stems between the two census periods must be interpreted with caution. The year prior to our census, 1999, was an abnormally hot and dry year in central Indiana. Between 17 July and 31 July 1999, daily high and low temperatures in the region averaged 3.3 o and 4.4 °C above normal, respectively (Palecki and Changnon 2001). High temperatures were coupled with elevated dew points, reducing precipitation. Since the flower bud of *H. canadensis* is formed as early as July of the prior year (Tobe and Keating 1985), the environmental conditions of 1999 may have stressed plants and reduced flower preformation, leading to a reduction of flowering stems in 2000. Seedling establishment may be a constraint on *H. canadensis* population establishment and spread in this preserve. *Hydrastis canadensis* seedlings are distinguishable from older plants, and were not observed during the mapping and census of 2000. Germination rates of *H. canadensis* seed under horticultural conditions are low and variable (Davis and McCoy 2000), and there may be a physiological inhibitor preventing germination. It is unclear whether seed dispersal may limit distribution of *H. canadensi*s. Sinclair et al. (2000) observed red-winged blackbirds *(Agelaius phoeniceus* L.) in a goldenseal population, including one bird that removed a berry and flew to the canopy. It is not known to what extent similar activity occurs at other populations. Another possible constraint on *H. canadensis* spread is altered disturbance regimes at the preserve. Fires historically occurred in this area, and increased *H. canadensis* growth has been observed after controlled burns in the understory of mature forest (Dan Drees, Missouri Department of Natural Resources, Jefferson City, pers. com.). The growth associated with these burns appeared to be primarily via clonal spread and not by increased seedling establishment. Increased nutrient release associated with burns could facilitate growth of *H. canadensi*s. Our findings of decline of *H. canadensis* contrast with those of Sinclair and Catling (2000a, b). They censused the 26 known natural *H. canadensis* patches in Canada, and conducted surveys to find additional patches. Only 3 of the 26 patches could not be relocated and were assumed extirpated. Two areas with previously known patches could not be surveyed. Of the 21 remaining patches, 7 had 100–300 ramets, 2 had 400–500 ramets, 3 had 600–800 ramets, 1 had slightly more than

1000 ramets, and 1 had greater than 4000 ramets. These patches had not been previously censused, and it appeared that ramet size was still increasing in some patches. Although attempts to locate new patches were unsuccessful, the authors speculate that there has been little, if any, decline in *H. canadensis* in Canada since 1991. There is no Canadian equivalent to the United States' Endangered Species Act, thus *H. canadensis* is not a legally protected species in Canada and harvest from the wild is not prohibited. The authors also noted that many of the Canadian patches occurred in areas disturbed by logging, flooding, footpaths, and drainage. Interestingly, many of these patches were located in forest fragments, and habitat size was negatively correlated with number of *H. canadensis* stems. In our study, I found lower extinction rates and higher founding rates on patches in edge versus those in interior habitats. This observation suggests that *H. canadensis* might grow best with intermediate light levels, or when exposed to relatively high light levels for part of the day, as would occur at a forest edge. Metapopulation dynamics occur on spatial scales larger than that of the local population. These dynamics frequently apply to species whose dispersal and colonization are limited, as appears to be the case with *H. canadensi*s. Metapopulations exhibit continued colonization and extinction of local populations, with a number of suitable habitats remaining unoccupied at any given time. It is not clear whether these limitations are due to high habitat specificity or limitations of seed production and dispersal, although both are likely to be factors. Metapopulation theory suggests that there is a threshold number of populations, below which extinction will be greater than colonization and the species will eventually be lost (Hanski 1991).

Smaller metapopulations will have fewer local populations and a greater susceptibility to stochastic events. Our mapping and census showed that the probability of local patch extinction did not depend on patch size, suggesting that environmental stochasticity is an important factor in the metapopulation dynamics of *H. canadensis* at our study site. It is unclear whether *H. canadensis* patches at Bryan Nature Preserve will be able to rebound and attain previous levels of abundance. Periodic re-censusing of this preserve, possibly every 5 to 10 years, would give me much-needed insight on the population dynamics of this species.

Acknowledgements

I thank Tom Swinford of the Indiana State Department of Natural Resources for providing climatological data as well as insight on the ecology of the preserve. I thank George Parker of the Purdue University Forestry Department for information about past research conducted at Bryan Nature Preserve. I are also grateful to the anonymous reviewers of this paper for their valuable comments regarding its content.

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CHAPTER 3:

Does Breeding System Contribute to Rarity of Goldenseal (*Hydrastis canadensis*)?

Abstract

Goldenseal (*Hydrastis canadensis* L.) is an herbaceous perennial species that is becoming more rare within its range. *Hydrastis canadensis* populations are highly isolated and pollen flow between these populations may be restricted. I examined the breeding system of *H. canadensis* to determine if it may be limiting seed set due to increasing isolation of individuals and populations from one another. I tested fruit set in treatments designed to detect the presence of apomixis, passive autogamy, active autogamy, short distance outcrossing, and long distance outcrossing. No fruit set occurred in flowers that were emasculated and bagged, suggesting the species is incapable of apomixis. However, low rates of fruit set were found in all other treatments, suggesting a mixed mating system in which both selfing and outcrossing may occur. Pollen transfer between highly isolated populations can result in fruit set, as can within-population pollen transfer. The breeding system type appears unlikely to be a major factor limiting the distribution or abundance of *H. canadensis*, however, overall low rates of fruit set may be important demographically.

Introduction

Goldenseal, *Hydrastis canadensis* L., is a long-lived herbaceous perennial plant of the eastern deciduous forest. Recent investigations have shown that this species is in decline; previously documented populations have either become reduced in size or extirpated (Sinclair and Catling, 2000a; Sanders and McGraw, 2002; Mulligan and Gorchov, 2003). Suggested causes of this decline include harvest (Robbins, 2000; Mulligan and Gorchov, 2003), stochastic events (Sanders and McGraw, 2002), succession (Sanders and McGraw, 2002), and changes in disturbance modes and patterns (Sinclair and Catling, 2000b). Species that have experienced some level of decline may be further susceptible to other stresses, leading to a feedback loop. One such feedback loop may exist between species abundance and breeding system characteristics.

Breeding systems are most likely to limit seed set if a species obligately or preferentially outcrosses and/or available mates are spatially separated across the landscape (Demauro, 1993; Weekley and Race, 2001). Mate limitation resulting from obligate outcrossing has been associated with rarity in numerous studies (Evans *et al*., 2003; Messmore and Knox, 1997). In clonal plant species, only one or a few genets may be present within a given patch. Thus, for clonal species with an obligate outcrossing requirement, viable seed set may necessitate pollen flow between patches (Wilcock, 2002).

Hydrastis canadensis is a clonal species that may be adversely affected by breeding system characteristics. This species forms dense patches of a few to greater than 1,000 ramets. These patches are frequently sparsely distributed across the landscape, such that many patches are isolated from others by great distances (McGraw *et al*., 2003). While the degree of genetic variation within patches is unknown, the extensive clonal growth pattern suggests that only one or a few genotypes may be present within a given patch. Thus, if *H. canadensis* is an obligate outcrossing species, seed production and viability may be hindered due to mate limitation.

Preliminary studies conducted at two Ontario, Canada sites indicate *H. canadensis* may be self-compatible (Sinclair *et al*., 2000), however, the authors used only three plants to infer this. While this suggests that autogamy occurs, the degree of passive vs. active autogamy, and the relative contribution of selfing and outcrossing to reproductive success remains unknown. The objective of the present study was to determine the breeding system of *H. canadensis*. I performed crosses to test for apomixis, passive autogamy, active autogamy, and outcrossing, both within and between populations.

Methods

Plant material from three natural *H. canadensis* sources in north central West Virginia was used: Cheat Canyon, Morgantown, and Jane Lew. These populations were relatively large and separated by 20 - 120 km. At each site, stems were selected for study during August, 2001.

Stems selected for study were all reproductive in 2001 and an assumption was made that stems reproductive in one year were likely to be reproductive the following year. I based the number of rhizomes collected on the total number of stems at each site that were reproductive in 2001; because I did not want to deplete the patches of reproductive ramets, I selected approximately half of those that were flowering in 2001. The total number of rhizomes removed at each site was 130 from Cheat Canyon, 80 from Jane Lew, and 60 from Morgantown. A numbered aluminum nail was placed in the ground immediately uphill of these stems. A map was drawn so that general locations of stems, and thus rhizomes, were known.

Rhizomes were relocated prior to emergence on March 30, April 1, and April 4, 2002, with the map and a metal detector. Rhizomes were removed, labeled, and replanted in plastic pots (6 cm diam., 18 cm deep) in the West Virginia University Plant Science Greenhouse. Shoots emerged during the second week of April, whereupon, shade cloth was placed over the pots.

To determine breeding system type, five treatments were implemented: (1) emasculation followed by bagging (test for apomixis), (2) bagging with no hand transfer of pollen (test for passive autogamy), (3) bagging with hand transfer of pollen within flowers (test for active autogamy), (4) emasculation plus within-source pollen transfer followed by bagging (test for short distance outcrossing), and (5) emasculation plus between source pollen transfer followed by bagging (test for long-distance outcrossing). Crosses were made so that there were at least 20 replicates in each treatment (Table 3.1). Since each reproductive ramet produces only one flower, there were thus, at least 20 flowers per treatment. Because not all ramets flowered, the total number of ramets in the greenhouse treatments (141) was less than the number collected (270). Crosses were made so that the total number of flowers in each treatment would be approximately equal. However, because a primary interest was to determine if *H. canadensis* has the ability to self-fertilize, I allotted more ramets in the test for active autogamy. Crosses were initiated on April 12, 2002 and all treatments were completed by April 19, 2002.

All emasculation was performed by clipping anthers off filaments with scissors prior to anthesis. Pollen was transferred by removing at least 3 stamens with tweezers and brushing the anthers over the desired stigma. Flowers were selected as a pollen source when at least one anther had a tinge of brown, indicating dehiscence had begun. Crosses between a pollen source and the designated treated flower were then made on consecutive days until all remaining anthers on the source flower were clearly no longer viable. In most instances, crosses between a given pollen source and a designated treated flower lasted over a three day period. Flowers were bagged using a fine mesh cut into disks of approximately 10 cm

Table 3.1. Fruit set percentage for each treatment. The number of flowers represented from each site is shown indented and in parentheses.

Treatment	Number of flowers in treatment	Number of flowers setting fruit	Fruit set percentage
Emasculation	25	0	0
Cheat Canyon	(3)	(0)	(0)
Jane Lew	(8)	(0)	(0)
Morgantown	(14)	(0)	(0)
Passive	25	6	24.0
Cheat Canyon	(8)	(2)	(25.0)
Jane Lew	(7)	(0)	(0)
Morgantown	(10)	(4)	(40.0)
Active	45	8	17.8
Cheat Canyon	(14)	(6)	(42.9)
Jane Lew	(21)	(1)	(4.8)
Morgantown	(10)	(1)	(10)
Within source	22	3	13.6
Cheat Canyon	(7)	(1)	(14.3)
Jane Lew	(7)	(0)	(0)
Morgantown	(8)	(2)	(25.0)
Between sources	24	3	12.5
Cheat Canyon	(6)	(2)	(33.3)
Jane Lew	(4)	(1)	(25.0)
Morgantown	(14)	(0)	(0)
Field sites	831	316	38.0
Cheat Canyon	(106)	(21)	(19.8)
Jane Lew	(544)	(165)	(30.3)
Morgantown	(181)	(130)	(71.8)

diameter and threaded around the perimeter with embroidery floss. The floss was tied around the pedicel, tightly enough that it would not easily come off, but not to tight as to be constricting. Ramets were considered to have set seed when at least one pistil of the flower developed into a fruit. Seeds were collected in July as they matured and tested for viability using the tetrazolium test (Baskin and Baskin, 1998). *Hydrastis canadensis* seeds exhibit morphophysiological dormancy. After fruit maturity on the parent plant, seeds require a period of time during which the embryo enlarges. During this time, *H. canadensis* seed requires exposure to a warm period followed by a cold period (Baskin and Baskin, 1998). Additionally, seed germination percentage is low and more than one year is often required to observe maximum germination rates (Davis and McCoy, 2000). Because of this, I believe the tetrazolium test was a more sensitive indicator of crossing success than seed germination.

Differences among treatments were tested using contingency analysis (SAS JMP, V.5.0; SAS, Inc., 2002). The independent factor was treatment and the response variable was fruit set. Apomixis was determined by whether or not fruit set occurred in emasculated, bagged ramets (treatment 1). To test for self fertilization, I observed whether fruit set occurred in treatments 2 (passive autogamy) and 3 (active autogamy). The difference in fruit set between treatments 2 and 3 was used to determine whether pollinators could potentially increase fruit set. To test for ability to outcross, I observed whether fruit set occurred in treatments 4 (within source pollen transfer) or 5 (between source pollen transfer). To test if there is a preferred outcross mating distance, I compared treatments 4 and 5. Finally, I wanted to determine if *H. canadensis* exhibits a preference for selfing vs. outcrossing. I contrasted fruit set in the two pooled selfing treatments (2 and 3), with fruit set in the two pooled outcrossing treatments (4 and 5). For all analyses, significance was determined using a log likelihood (G) test (Sokal and Rohlf, 1995).

In addition to the greenhouse study, I also noted fruit set in the field. In late June, 2002, in each of the three populations from which the greenhouse study rhizomes were collected, I counted the total number of reproductive (two- and three-leaved) ramets and noted how many of these set fruit. I calculated the overall fruit set percentage in the field, which allowed me to determine if fruit set in the field was comparable to that in the greenhouse. I also calculated the individual fruit set percentages at each of the three populations where collections were made. This allowed me to test for natural variation in fruit set among populations using a log likelihood test. For all statistical comparisons, $\alpha = 0.05$ was the threshold level at which tests were considered significant.

Results

Overall, there was significant variation in fruit set among treatments in the greenhouse (contingency analysis, $P < 0.0001$). Fruit set occurred in all treatments except treatment 1 (the test for apomixis, Table 3.1) in which none of 25 ramets set fruit. Given the fruit set rate in the field (37.8%), the probability of obtaining zero of 25 ramets setting fruit is < 0.0001. In the test for passive autogamy (treatment 2), 6 of 25 ramets (24.0%) set fruit. In the test for active autogamy (treatment 3), 8 of 45 ramets (17.8%) set fruit (Table 3.1). Fruit set of treatment 2 did not differ from that of treatment 3 ($P = 0.5342$). In the tests for ability to outcross, 3 of 22 within source crosses (13.6%, treatment 4) set fruit and 3 of 24 between source crosses (12.5%, treatment 5) set fruit. These two treatments did not differ ($P = 0.9090$). Fruit set of the two selfing treatments (2 and 3) did not differ from that of the two outcrossing treatments (4 and 5) ($P = 0.3319$), showing that *H. canadensis* does not exhibit a preference for selfing vs. outcrossing. All seeds produced in the greenhouse study were shown to be viable using the tetrazolium test.

Fruit set percentage in the three field populations ranged from 19.8 – 71.8% (Table 3.1) and differed between treatments ($P = < 0.0001$).

Discussion

An overall goal of the current research was to assess the role of the breeding system of *H. canadensis* as a causal agent of its rarity. The rarity of *H. canadensis* is atypical in that this species occupies a broad geographical range, but within this range, local population size is small and niche breadth appears narrow (McGraw *et al.*, 2003). In ecology, there is a general association between species abundance and range size, whereby species with broad geographical ranges tend to be both locally abundant and evenly distributed within that range while species with small geographical ranges tend to be very habitat specific, thereby having both limited distribution and abundance (Rabinowitz, 1981; Johnson, 1998). *Hydrastis canadensis* does not follow this relationship between range size and species abundance. The range of *H. canadensis* extends eastward from Missouri and northern Arkansas to the Appalachian Mountains, as far south as Tennessee and parts of the neighboring states and north to New York state and southern Ontario, Canada (Small and Catling, 1999). Within this range, *H. canadensis* grows clonally, typically in dense patches, although dispersed patches and isolated individuals are occasionally observed. It is not clear why *H. canadensis* is restricted to the areas where these patches are located, however, because *H. canadensis* possesses a mixed breeding system, it would appear that the distances separating populations are not adversely affecting the ability to set fruit. Within a population, fruit set can occur via pollen transfer either between two ramets (which, due to the clonal nature, may or may not represent two distinct genotypes) or within a ramet. Additionally, fruit set via pollen transfer between populations is possible.

Despite the mixed breeding system, there were marked differences in fruit set in the field between the three source populations. At two of these populations, Cheat Canyon and Jane Lew, fruit set in the field was comparable to that observed in the greenhouse, averaging 19.8 and 30.3%, respectively. At the Morgantown population, however, fruit set was 71.8%. One factor that may influence site dependent reproductive success is light availability (Kato and Hiura, 1999). The Morgantown site was on a south facing slope with a canopy somewhat more open than that of the other two sites. The greater light availability here could have facilitated photosynthesis, in turn, promoting carbon gain and fruit set. Increased reproductive success associated with light has been reported in other forest understory species. Niesenbaum (1993) found that light availability, but not pollen limitation, influenced fruit set of *Lindera benzoin* (L.) Blume, an understory shrub often associated with *H. canadensis*. Devlin (1988) reported reduced mean seed number of *Lobelia cardinalis* plants subjected to 10% of available light compared with those receiving 27% of available light.

In addition to light, another factor that may influence reproductive success of a population is the degree of genetic variation present (Ehlers, 1999; Schmidt and Jensen, 2000). Unfortunately, the degree of genetic variation present in *H. canadensis* patches is currently unknown. Due to the extensive clonal nature of this species, however, it is unlikely that there are a large number of genotypes present within any given *H. canadensis* patch.

Collectively, factors contributing to low fruit set at some sites may have important demographic consequences for this species. There are some indications that *H. canadensis* has high habitat specificity (McGraw *et al.*, 2003). Additionally, recruitment by seed appears to be relatively uncommon (Sanders and McGraw, 2002). Species with specific habitat requirements, such as *H. canadensis*, may require greater seed production to increase the likelihood of deposition into suitable habitat, where germination may occur and new patches may establish.

While I do not yet fully understand the rarity of *H. canadensis*, the results of this study can be directly applied in the management of this species. Because fruit set can occur autogamously, protection of small, isolated populations may be as important as the protection of larger metapopulations. Likewise, this research advances our knowledge of *H. canadensis* conservation. If its rarity continues to increase, population establishment in protected areas may be a viable method of species protection. Where source material is limited, population establishment via a single source may be acceptable.

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CHAPTER 4:

Harvest Recovery of Goldenseal, *Hydrastis canadensis* L.

Abstract.

Goldenseal, *Hydrastis canadensis* L., is a herbaceous understory plant of the eastern deciduous forest that is harvested from natural populations due to its medicinal value. The objectives of the current study were to determine if regrowth from harvest varies between patches, and also to relate regrowth to pre-harvest leaf and patch size. I used data from three experimentally harvested natural patches and data on recovery of an illegally harvested patch that had been published earlier. I found variation in patch regrowth which suggests timing of harvest may be important; rhizome removal mid-summer resulted in slower recovery of leaf size relative to the patch that was harvested at the end of the growing season. I also found variation in stem count between patches in response to harvest, which may be due to the pre-harvest size of ramets. The patch with larger ramets pre-harvest showed compensatory growth in the year immediately following harvest while the patches comprised of smaller ramets did not. For all three experimentally harvested patches, ramet leaf area decreased immediately following harvest but, in two of the three populations studied, increased thereafter, while maintaining the size structure in the third. Collectively, our results indicate that response to and recovery from harvest varies between patches and that individual ramet leaf size may be a better predictor of patch recovery than stem count. Because regrowth may be affected by harvest timing, an established harvest season may alleviate some harvest pressure on *H. canadensis*.

Introduction

Harvest of native understory species in the eastern deciduous forest is a time-honored tradition engrained in both the cultural and economic fabric of some regions. Harvested items, and their derivatives, are used locally as food sources, food additives, and medicines, and are sold on the herbal market by harvesters for supplemental income (Bailey, 1999). Harvested understory species include the herbaceous plants, ginseng, *Panax quinquefolius* L., black cohosh, *Actaea racemosa* L. (Nutt.), and ramps, *Allium tricoccum* Ait. (Rock *et al.*, 2004) as well as various mushroom and moss (Peck and McCune, 1998) species. Increases in demand from the herbal market, as well as increasing human population size, have put additional pressures on these species. One species for which harvest pressure may be detrimental is goldenseal, *Hydrastis canadensis* L. (Ranunculaceae).

Hydrastis canadensis is a herbaceous understory species, native to the eastern deciduous forest of North America. This species exhibits highly clonal growth via an extensive rhizome network. This growth habit results in dense patches of *H. canadensis* ramets. Total number of ramets in these patches typically ranges from between a few to several hundred, although populations larger than 1,000 ramets have been reported (Sinclair and Catling, 2000a; Sinclair and Catling, 2000b; Mulligan and Gorchov, 2003). Recent evidence suggests that *H. canadensis* is experiencing population decline, both by the existence of fewer patches (Sinclair and Catling, 2000b; Sanders and McGraw, 2002) and by fewer ramets per patch (Sanders and McGraw, 2002), although stem count in some patches may be increasing (Sinclair and Catling, 2002). Currently, it is unclear what role harvest plays in *H. canadensis* patch dynamics.

Observations of a recently poached patch suggest that harvest may initially stimulate the production of above-ground stems. This can occur because lateral roots are broken and left in the soil as the large storage rhizome is removed from the ground. These lateral roots contain adventitious buds, capable of developing into new aerial shoots (Bowers, 1891). This was demonstrated by Van der Voort *et al.* (2003) who followed the recovery of an illicitly harvested patch near Morgantown, West Virginia. The pre-harvest stem count, size, and stage structures of this patch were unknown, however four ramets were observed during summer, 1995 immediately following harvest. The authors established an 8 m × 8 m permanent grid to monitor regrowth over the next 4 growing seasons (1996-1999). The general trends they reported were an increase in leaf size over the four years monitored, but a decrease in stem number. Unfortunately, this study provides me with recovery information from only one patch, of which pre-harvest size was not known. It is also not known how generalizable these results are across variable environments.

The two objectives of the current study focused on regrowth of three experimentally harvested patches and a comparison with the illicitly harvested natural patch near Morgantown, West Virginia (Van der Voort *et al.*, 2003). First, I wanted to determine if trends in regrowth were consistent across multiple harvested patches. I compared all four patches and asked whether the effect of the length of time since harvest on ramet leaf area, a measure of recovery rate, varied between patches. The null hypothesis was that leaf area recovery would not differ among patches. Our second objective was to relate regrowth following harvest to pre-harvest leaf size. Transition matrix modeling has been used to project that harvest of 10% of stems per year will lead to extinction of the population (Christensen and Gorchov, 2002). However, selective harvesting does not simulate actual harvests where the rhizomes of all ramets in the patch are typically removed at one time, but not spread over years. For our second objective, I compared ramet leaf area of the three experimentally harvested patches at three times: immediately prior to harvest, and at one and two years post-harvest. I asked whether ramet leaf area varied between the time periods. If so, I also wanted to know whether pre-harvest size structure could be attained by two years following harvest. I hypothesized that ramet leaf area would indeed vary between the three time periods, decreasing in the year following harvest, and increasing thereafter. Based on the previous study of one population (Van der Voort *et al.*, 2003), I predicted that the original size structure would not be attained over the two year course of this study.

Methods

Three patches were selected to be experimentally harvested; these sites are referred to in this paper as Cheat Canyon 1, Cheat Canyon 2, and Kingwood. Because I was interested in quantifying recovery within densely clumped patches, and because a population may contain one or multiple patches, I refer to a harvested unit as a "patch" throughout this paper. These patches were selected because, based on pre-harvest leaf size, they appeared to be older, established populations and showed no evidence of recent harvest. The Kingwood site is located on land that had been selectively logged two years prior to harvest while the Cheat Canyon sites are located in older second growth forests dominated by red oak (*Acer rubrum* L.) and tulip poplar (*Liriodendron tulipifera* L.).

The experimental harvests were carried out on July 24 (Cheat Canyon 1 and 2) and July 25, 2001 (Kingwood). Prior to digging, stems were counted and the number that were reproductive was noted. Ramets were dug using a hand trowel with an effort made to remove all rhizomes attached to the visible, above-ground stems. The harvest was conducted in a manner designed to simulate actual harvest; an effort was made to remove all rhizomes, although extra effort was not extended to dig and sift further in the soil for more possible rhizomes, allowing the harvest to be conducted in a timely manner. All plant material was returned to the laboratory where stem height and leaf length were measured on all single-leaved ramets. On two-leaved ramets, the length of each leaf was measured and the area of the two leaves was summed to obtain the total leaf area of the ramet. The rhizomes were cleaned and used for other studies.

Estimates of leaf area were obtained using leaves of 100 additional ramets not included in these studies and regressing the natural log of leaf area on the natural log of leaf length. From the allometric relationship determined by this regression (leaf area = C (leaf length)^a, where $C = e^{0.6068}$ and $a = 1.82848$ ($r^2 = 0.9582$)), I determined leaf area of all harvested ramets. Measurements of regrowth of the experimentally harvested patches were taken during the last week of June, 2002 and 2003. I counted stem number and measured stem height and leaf length of all ramets. I also measured length of the second leaf on reproductive ramets. For the Morgantown patch, I obtained the original data on leaf length from the authors (van der Voort et al., 2003). For this patch, the authors randomly selected 100 leaves for measurement each year. Using this original data, I calculated leaf area for these 100 ramets as described above.

To determine if trends in regrowth were consistent across multiple harvested patches (our first objective) I compared the three experimental patches and the illicitly harvested patch and asked whether the effect of the length of time post-harvest on ramet leaf area varied between patches. I used two-way analysis of variance to test the main effects of time post-harvest (one year or two years) and patch and to determine if there was a differential response among the four populations. The patch effect and the patch × year interaction term were treated as random effects while time post-harvest was treated as a fixed effect in the model. For the Morgantown patch, I used the data from the 100 measured leaves. Because I also wanted to compare the three experimental populations (with common harvest methods and timing), I performed the same analysis described above, omitting the Morgantown patch.

Because changes in leaf area might be offset by inverse changes in the total number of ramets present, I felt that measures of total patch leaf area may be a better measure for assessing year to year harvest recovery. For the three experimentally harvested patches, total patch leaf area was calculated by adding the leaf area of all individual ramets. For the Morgantown patch, the authors randomly measured 100 leaves. Because this patch contained 943 leaves 1 year post-harvest (921 single-leaved ramets and 11 two-leaved reproductive ramets) and 860 leaves two years post-harvest (820 + 20), I calculated the total summed leaf area of the 100 measured ramets each year and multiplied these values by 9.43 and 8.60, to obtain total patch leaf area 1 year and 2 years post-harvest, respectively (this assumes the 100 measured ramets were a random sample of all ramets in the patch). I used one-way analysis of variance to test the effect of time since harvest on total patch leaf area and patch stem count across all four populations. I did not test the patch effect since the total patch leaf area and patch stem count will vary between patches due to inherently different sizes of the patches. Tests for differential responses of patches to the length of time since harvest are not possible due to lack of replication, although summarized data of yearly patch leaf area and stem count are presented and addressed for explanatory purposes.

To address the second objective, relating pre-harvest leaf size to regrowth, I used two-way analysis of variance to test for differences in ramet leaf area of the experimentally harvested patches at three time periods: pre-harvest, and one and two years post-harvest. The main effects were time period relative to harvest (treated as a fixed effect) and patch (treated as a random effect). I examined the significance of the period × patch effect to determine if there was patch dependent recovery of ramet leaf area after harvest. A significant differential response was further explored by one-way analyses of variance to test the effect of the time period relative to harvest on ramet leaf area for each of the three patches individually. Means were separated using the Tukey – Kramer HSD test (Sokal and Rohlf, 1995).

I also used one-way analysis of variance to test the time period effect on the dependent variables of patch stem count and patch leaf area.

Results

Our first objective was to determine if trends in regrowth were consistent across multiple harvested populations. Across all four harvested patches, post-harvest ramet leaf area differed between years (F = 11.72, P = 0.0365, Table 4.1a) and was greater 2 years post-harvest (27.5 ± 3.0 cm^2) than 1 year post-harvest (17.0 ± 2.6 cm^2). Harvest recovery depended on the patch (F = 10.07, P < 0.0001, Table 4.1a); the increase in ramet leaf area of the Morgantown patch between one and two years post-harvest was greater than that of the three experimentally harvested patches (Figure 4.1). When I excluded the Morgantown patch from the analysis to account for possible differences in harvest practices, I found that ramet leaf area was, again, affected by the time since harvest (F = 26.92, P = 0.0140, Table 1b), although it was not patch-dependent (F = 2.05, P = 0.1285, Table 1b).

On a whole-patch basis, recovery from harvest was not evident beyond the initial flush of regrowth in year 1 after harvest. Neither whole-patch stem count (F = 0.22, P = 0.6528, Table 4.2) nor whole-patch leaf area (F = 0.12, P = 0.7401, Table 4.2) differed between the two time periods post-harvest.

The second objective was to relate pre-harvest leaf size of the three experimentally harvested patches to regrowth following one and two years. Changes in ramet leaf area during the three time periods was patch-dependent (F = 73.22, P < 0.0001, Table 4.3). At all three sites, leaf area was less 1 year following harvest relative to that preharvest (Figure 4.1). By 2 years post-harvest, leaf area of both the Kingwood and Cheat Canyon 2 sites had

TABLE 4.1.—a.) Analysis of variance of ramet leaf area of four patches: the three experimentally harvested patches and the poached patch. Time post-harvest is one year and two years.

Effects	df	F	P > F
Time post-harvest	1	11.72	0.0365
Patch	3	11.01	0.0397
Time post-harvest × patch	3	10.07	<0.0001

TABLE 4.1. ─b.) Analysis of variance of ramet leaf area of the three experimentally harvested patches. Time post-harvest is one year and two years.

Effects	df	F	P > F
Time post-harvest	1	26.92	0.0140
Patch	2	10.69	0.0856
Time post-harvest × patch	2	2.05	0.1285

Figure 4.1. Differential response of ramet size to harvest in four populations of *H. canadensis*.

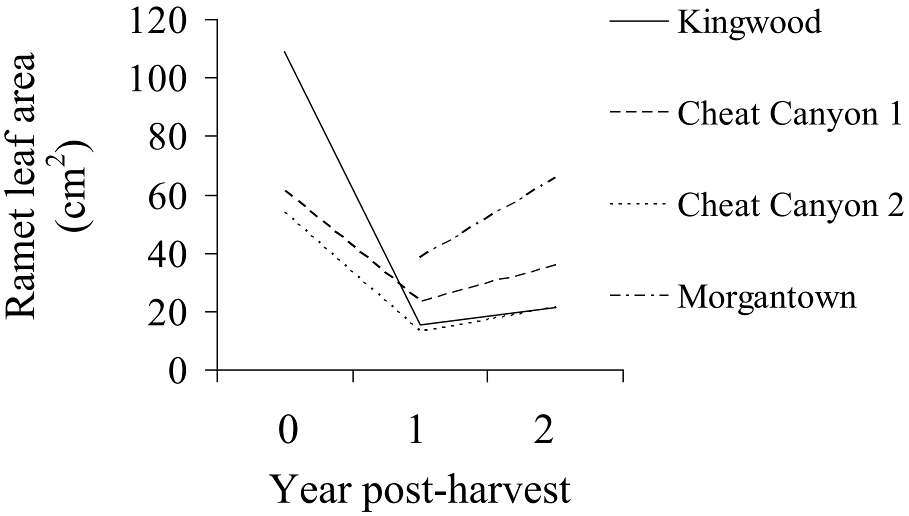

Ramet leaf area (cm²)
120
100
80
60
40
20
0
0
1
2
Year post-harvest
Kingwood
Cheat Canyon 1
Cheat Canyon 2
Morgantown

TABLE 4.2. ─Whole-patch stem count and leaf area for all patches included in this study.

Patch	Patch stem count			Patch leaf area (m^2)		
	pre-harvest	1 yr post-harvest	2 yr post-harvest	pre-harvest	1 yr post-harvest	2 yr post-harvest
Kingwood	163	657	474	2.313	1.014	1.009
Cheat Canyon 1	285	107	67	1.861	0.248	0.241
Cheat Canyon 2	610	392	247	3.264	0.448	0.531
Morgantown	NA	932	840	NA	3.640	5.691

TABLE 4.3. —Analysis of variance table of ramet leaf area of the three experimentally harvested patches. Time relative to harvest is pre-, 1 year post- and 2 years post-harvest.

Effects	df	F	P > F
Time relative to harvest	2	9.56	0.0296
Patch	2	1.12	0.4113
Time relative to harvest × patch	4	73.22	<0.0001

increased relative to that of the previous year, although at neither site did it equal that pre-harvest. At Cheat Canyon 1, leaf area did not increase between 1 and 2 years post-harvest.

On a whole-patch basis, stem count did not differ between the three time periods ($F = 0.21$, $P = 0.8141$, Table 4.2), however changes in stem count between the pre-harvest and 1 year post-harvest time periods varied considerably between the three patches. Stem count of the Kingwood patch increased over 300% between the two time periods (Table 4.2) while stem count of Cheat Canyon 1 and Cheat Canyon 2 patches decreased 63% and 36%, respectively. Total patch leaf area was affected by the time relative to harvest ($F = 13.14$, $P = 0.0064$) and was greater pre-harvest (2.479 m^2) than both one year (0.581 m^2) and two years (0.593 m^2) post harvest (Figure 4.2).

Figure 4.2. Whole-patch leaf area of the three experimentally harvested patches pre-harvest (year 0), and 1 and 2 years post-harvest.

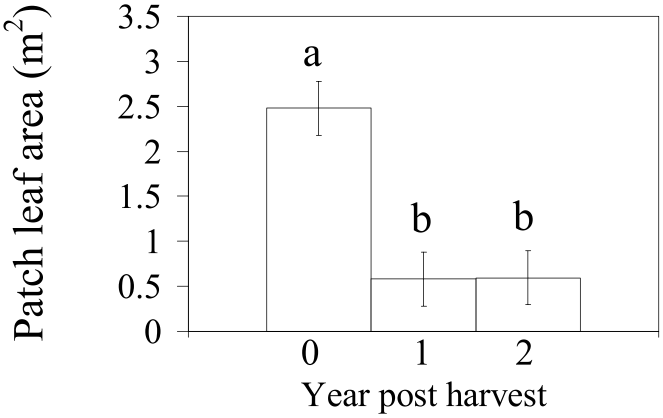
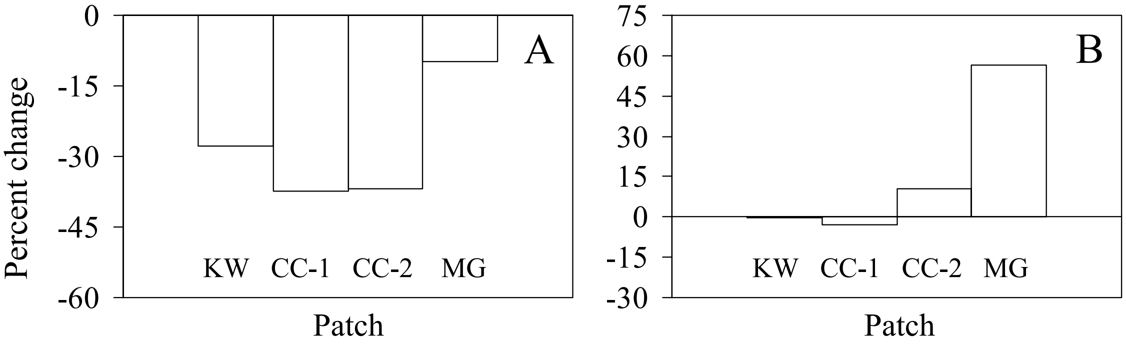

Patch leaf area (m^2)
3.5
3
2.5
2
1.5
1
0.5
0
a
b
b
0
1
2
Year post harvest

Figure 4.3. Percentage change in stem number (A) and patch leaf area (B) between one and two years post-harvest for each of the four patches. KG = Kingwood, CC-1 = Cheat Canyon 1, CC-2 = Cheat Canyon 2, and MG = Morgantown.


A
B
Percent change
0
-15
-30
-45
-60
75
60
45
30
15
0
-15
-30
KW
CC-1
CC-2
MG
KW
CC-1
CC-2
MG
Patch
Patch

Discussion

Our null hypothesis that leaf size would increase in a uniform manner across multiple harvested populations was not supported. Leaf size of the illicitly harvested Morgantown patch increased more between one and two years post-harvest than did the other patches. This could have been due to differences in harvest technique, although I attempted to experimentally harvest our patches in a manner simulating actual harvest. Variation in digging and collecting methods by harvesters could influence how thoroughly a patch is harvested, as could the degree of isolation of the patch and the consequences of a harvester being discovered. Because the Morgantown patch was poached from private land in late August and the experimental harvests were conducted in late July, timing of the harvest may also account for our results.

Seasonal patterns of carbohydrate flux in perennial species generally show a decline in storage reserves during the period of greatest growth (Chapin *et al.*, 1990; Jonsdottir and Watson 1997; Price *et al.*, 2002) followed by a reallocation of resources prior to senescence. In mayapple, *Podophyllum peltatum* L., simulated herbivory immediately following leaf expansion resulted in a reduction of the length of new rhizome segments, measured at the end of the growing season, compared with non-herbivorized plants. In contrast, simulated herbivory during the middle of the growing season did not result in shorter rhizomes (Whigham and Chapa, 1999). In central West Virginia, *H. canadensis* emerges in mid-April and anthesis occurs shortly thereafter; the leaves are fully expanded by early May, and the fruit ripens in mid-July. Aboveground stems are still present in September, although senescence is clearly evident by this time.

The seasonal patterns of carbohydrate flux and rhizome and root development in *H. canadensis* are unknown. *Hydrastis canadensis* rhizomes and lateral roots may be a strong carbohydrate and nutrient sink during the month of August. This could account for the differential regrowth response between the experimentally harvested patches and the poached patch. Because regrowth may be affected by harvest timing, an established harvest season, such as that devised for ginseng, may alleviate some harvest pressure on *H. canadensis*.

Our second objective related size structure of the three experimentally harvested patches following two years of regrowth to size structure prior to harvest. I hypothesized that ramet leaf area would vary between the three time periods, decreasing in the year following harvest, and increasing thereafter. This hypothesis was supported at the Kingwood and Cheat Canyon 2 sites, but not at Cheat Canyon 1 where leaf area did not differ between the two years post-harvest.

Although there was no difference in stem count among years for the three experimentally harvested patches, there was considerable variation between patches in pre-harvest stem count relative to that 1 year post-harvest (Figure 4.3). Stem count of the Kingwood patch tripled while stem counts of Cheat Canyon sites were reduced roughly by half. The variation in stem count during regrowth may be due to different degrees of control of the shoot apex over the adventitious root buds, possibly due to nutrient partitioning. The apical meristem can be a strong metabolic sink, depriving nutrients from other stems or buds (McIntyre, 1977; Cline, 1991; Cline, 1994). Larger stems are likely to be stronger sinks than smaller stems and, consequently, exert stronger suppression of the adventitious buds. This could explain the response of the Kingwood site, where 25% of the ramets pre-harvest were reproductive, a high percentage not commonly observed in natural *H. canadensis* patches. At Cheat Canyon 1 and Cheat Canyon 2, only 5.6% and 0.3% of ramets, respectively, were reproductive. Suppression of adventitious buds at the Kingwood site may have been far greater than at the Cheat Canyon sites. Any decreases in stem counts between 1 and 2 years post-harvest could be due to reestablishment of one or a few strong nutrient sinks by the new shoot apices in the remaining clones (Cline, 1997).

A second possible explanation for the overcompensatory response at the Kingwood site may be related to previous browsing history of the patch. Plants exposed to a history of repeated, low levels of browsing can have greater restraint in bud activation (Tuomi *et al.*, 1994), an insurance against complete destruction by herbivores. In contrast, high levels of browse (or harvest in our case) often result in a compensatory response of the plant whereby latent meristems and buds are activated (Paige and Whitham, 1987). A history of deer browse at the Kingwood site may have promoted activation of a large number of dormant buds and latent meristems, resulting in overcompensatory growth upon harvest.

Our results have shown that the stem count of a patch following harvest varies between patches and does not clearly relate to the preharvest stem number. Therefore, stem counts may not be the most effective measure of patch size (*i.e.* total belowground resources available to the plant) and harvest recovery. In contrast, I found that ramet leaf area appears to be directly, negatively affected immediately following harvest but, in two of the three populations studied, increased thereafter, while maintaining the size structure in the third. Because of the variation in stem count between patches following harvest, measures of changes in leaf size over time may be an effective method to monitor long-term recovery of *H. canadensis* patches following harvest.

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CHAPTER 5:

Response of *Hydrastis canadensis* L. (Ranunculaceae) to microclimate gradients across a mesophytic forest cove

Abstract. Spatial patterns of understory plant distribution can reflect availability of suitable abiotic microsites. *Hydrastis canadensis* is a native, herbaceous perennial whose distribution may be constrained by microsite availability. I planted 5 transects each on south- and north-facing cove hillsides with clonally derived rhizomes of *H. canadensis*. Transects were spaced 20 m, 40 m, 60 m, 80 m, and 100 m from a third-order stream. Because the transect 20 m from the stream on the south-facing hillside was adjacent to a natural *H. canadensis* patch, this transect was postulated to represent suitable habitat. I tested aspect and distance from stream effects on phytometer growth measures (survival, leaf area, rhizome relative growth rate and leaf area change). I also monitored temperature, humidity, and light, then quantified environmental differences in these measures between each transect location and the transect in suitable habitat. Plant growth measures were then regressed on these differences to test hypotheses about factor effects. Neither survival nor relative growth rate depended on aspect or distance from the stream, although leaf area was greater on the north-facing aspect in both years and increased with proximity to the stream in 2003. Rhizome relative growth rate did not depend on any of the environmental distance measures, although leaf area change depended on cumulative light, increasing as the environmental distance from the suitable site increased. The relatively weak association between environmental variation across the forested cove reinforces other studies suggesting that *H. canadensis* has a relatively broad ecological niche, and its rarity is unlikely due to availability of suitable habitat.

Key words: Distribution, Humidity, *Hydrastis*, Light, Microsite, Rare plant, Temperature

Introduction

Spatial patterns of understory plant distribution are often related to abiotic microsite conditions. Suitable microsites of widely distributed species include those associated with distance from the forest edge (Gehlhausen et al. 2000), those associated with a particular suite of overstory species (McCarthy and Bailey 1994), and those resulting from mesoscale topography (Pornon et al. 1997). Abiotic variables affected by these factors include light, temperature, and humidity (Matlack 1993).

Anthropogenic forces can exert profound effects on abiotic factors. These changes have been implicated in the creation of suitable niche space for non-native species (Meekins and McCarthy 2001). For native plant species that are restricted to key abiotic microhabitats, anthropogenic change, such as alteration of disturbance regimes, may be contributing to population decline by reducing the availability of suitable microsites (Bratton et al. 1994).

Hydrastis canadensis is a native understory species of the eastern deciduous forest that is experiencing population decline (Sinclair and Catling 2000a; Sanders and McGraw 2002; Mulligan and Gorchov 2003). This species grows clonally, forming dense patches comprised of a few to several thousand ramets (Sinclair and Catling 2000a). Nonreproductive ramets are comprised of a single leaf while reproductive ramets have a second (rarely third) leaf, with a single flower on a pedicel arising from the base of the second leaf. Although this species is harvested from natural patches due to herbal market demand for the rhizome, it is not clear that this is contributing to population decline (Van der Voort et al. 2003; McGraw et al. 2003; Sanders and McGraw submitted (a)); as the dense storage rhizome at the base of each aerial stem is removed, lateral roots are broken off of the rhizome and remain in the soil. Regrowth can occur from adventitious buds on these roots.

The distribution of *H. canadensis* may be constrained by specific microsite requirements. Due to its rarity, it has been difficult to draw firm conclusions about the factors influencing distribution and abundance of *H. canadensis* with traditional survey methodologies (McGraw et al. 2003). However, this may be due largely to the sparse distribution of the species. Observational studies have suggested that *H. canadensis* is often located just above floodplains (McGraw et al. 2003), and that it responds through vegetative proliferation to light near edges (Sinclair and Catling 2000b; Sanders and McGraw 2002). Rapid loss of turgor upon severing may indicate rapid water loss (James B. McGraw, personal observation). Collectively, these observations suggest a possible role for humidity, temperature, and light in the distribution. These environmental factors vary in systematic ways across "coves" in the highly dissected Allegheny Plateau, and thereby influence *H. canadensis*' potential distribution.

Descriptive studies can only lead to hypotheses about actual controls over distribution. Therefore, the objectives of the current study were to experimentally assess the role of temperature, humidity, and light gradients in the distribution of *H. canadensis*. Specifically, I wanted to determine if these environmental variables could explain the success of *H. canadensis* phytometers placed in different positions within a forested "cove". I planted transects of *H. canadensis* at 20 m intervals away from a stream on both north- and south-facing aspects in order to span gradients of temperature, humidity, and light. The design was such that one of these transects was adjacent to a healthy, mid-size natural patch which was assumed to be in suitable habitat. Our first objective was to determine if there were differences in phytometer performance between aspects and with distance from the stream. Because of greater irradiance associated with south-facing slopes, and because of known positive responses to paths, edges, and water, I hypothesized that phytometer performance would be greater on the south-facing slope and closer to the stream. Our second objective was to quantify environmental differences between each transect location and the transect at the suitable site and to relate these differences to plant performance. I hypothesized that if the natural population was in a highly suitable site with respect to a critical environmental factor, transects that differed strongly from the 'suitable' site would have reduced phytometer performance.

Methods

Environmental and phytometer growth variation

This study was conducted in a nature preserve approximately 10 km northeast of Morgantown, West Virginia. The site was a wooded cove bisected by a stream flowing from west to east so that the cove hillsides were north-facing and south-facing. A natural *H. canadensis* patch comprised of 700 ramets was located 22 m uphill from the stream on the south-facing slope.

This study incorporated the use of phytometers, an idea initially popularized by Clements in the early 20th century (Clements and Goldsmith 1924; Clements et al. 1929) and later by Antonovics and Primack (1982). With the phytometer method, performance of transplants of a given species is used as an integrated measure of environmental quality.

Phytometer source material for this study was obtained from a single large *H. canadensis* patch in north-central West Virginia. These rhizomes were removed on July 24, 2001 as part of a simulated harvest study. They were cleaned with water, scrubbed lightly to remove soil, air dried, and weighed. The rhizomes were planted at the field site in transects located at distances of 20 m, 40 m, 60 m, 80 m, and 100 m away from the stream on both the north and south-facing hillsides, for a total ten transects (Figure 5.1a). Each transect paralleled the slope of the contour and was comprised of 9 blocks spaced at distances of 20 cm (Figure 5.1b). Four *H. canadensis* rhizomes were spaced 5 cm from the center of each block, so that each transect spanned 1.7 m and was comprised of a total of 36 rhizomes.

Figure 5.1a. Cross section of the cove facing upstream (west), showing north and south facing hillsides, bisected by the stream. Transect locations are noted by flags.

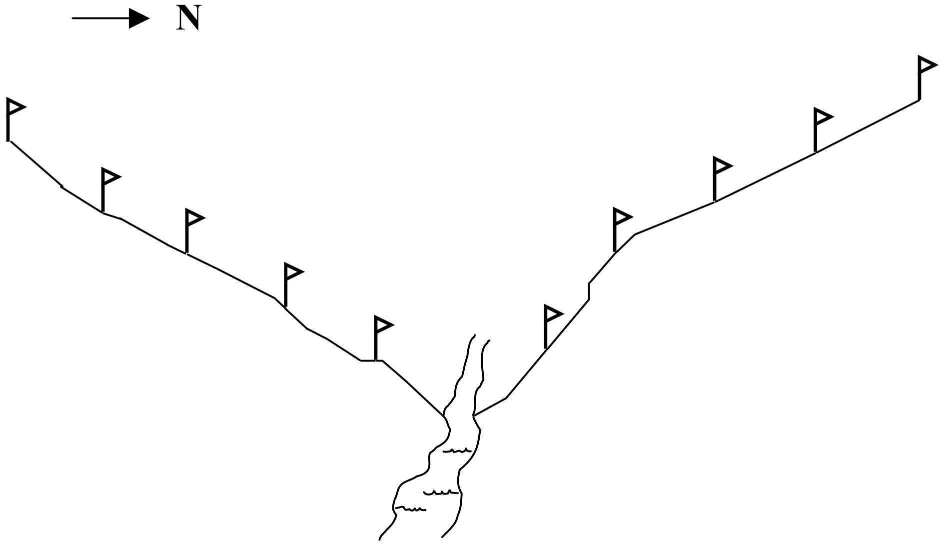
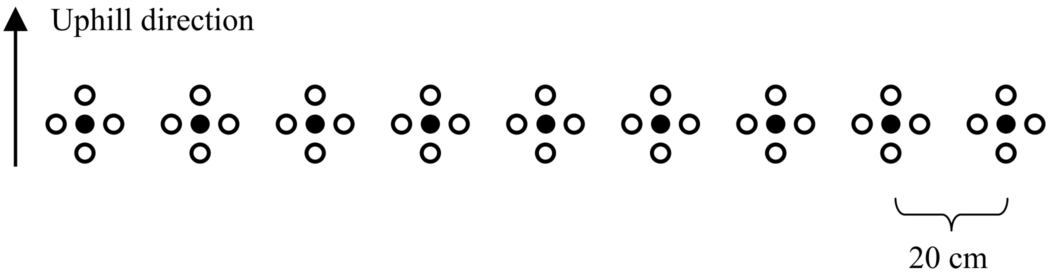

N

Figure 5.1b. Schematic diagram of one transect. Solid circles represent the block centers at which the diazo light sensors were placed. Hollow circles represent *H. canadensis* plants.


Uphill direction
20 cm

To monitor temperature and humidity, data loggers (Hobo Pro relative humidity/temperature logger; Onset Computer Corporation, Bourne, MA, USA) were placed near the center of each transect and programmed to record instantaneous temperature, absolute humidity, and relative humidity at 30 minute intervals. These were put in place in the final week of May 2002 and left until the rhizomes were removed.

To quantify light, I used the light sensitive diazo paper method described by Friend (1961). At the center of each block, a wooden stake was placed in the ground so that the top of the stake was level with the four surrounding *H. canadensis* leaves. A sealed Petri dish containing a 12-layer diazo booklet was placed on each of the stakes in the early evening of July 29, 2003. These were removed 24 h later and developed using standard household ammonia (Austin's Ammonia, 2.5 – 4.0% by wt.; James Austin Co., Mars, PA, USA). A standard curve was also made to relate layer exposure to cumulative light totals (Friend 1961).

Measurements to determine plant growth were made during the final week of June in 2002 and 2003. Stem height and leaf length were recorded. Estimates of leaf area were obtained using leaves of 100 additional ramets not included in this study and regressing the natural log of leaf area on the natural log of leaf length. From the allometric relationship determined by this regression (leaf area = 1.8347 (leaf length)$^{1.8284}$; $r^2 = 0.9582$), I determined leaf area of all measured ramets. No plants were reproductive either year of this study although in a few instances, two nonreproductive aerial stems arose from one rhizome. When this occurred, the leaf area was summed for the plant.

Because I wanted to use initial rhizome dry mass in the statistical analyses, I removed 100 additional rhizomes from the field and washed, air dried, and weighed these as described above. These were then oven dried at 65 °C for 72 h and reweighed. Because the dry mass:fresh mass ratio was not related to initial rhizome fresh mass ($r^2 = 0.0297$), the average value of the ratio was used to estimate an initial dry mass of rhizomes based on their initial fresh mass. Rhizomes were removed from the field on August 8, 2003 and were brought to the laboratory where the stems were removed. They were cleaned and oven dried at 65 °C for 72 h.

Our first objective was to determine if there were differences in phytometer performance as a function of aspect and distance from the stream. I used log-likelihood analysis, with rhizome initial dry mass as a covariate, and tested whether phytometer survival in 2002 and 2003 depended on aspect, distance, or their interaction. Both aspect and distance were considered fixed treatment effects. Aspect was nominal (north-facing, south-facing) while distance was treated as a continuous variable. I examined cumulative survival to June 2002, and August 2003.

To test for differences in leaf area, I used 2-way ANCOVA, again with rhizome initial dry mass as a covariate. I were interested in changes in rhizome mass over the time course of the study since this may be the ultimate measure of growth in a long-lived perennial plant. Rhizome relative growth rate (RGR_R) was calculated by:

$$RGR_R\ (\mathrm{g\ g^{-1}\ y^{-1}}) = \frac{\ln\ (\text{final dry mass 2003}) - \ln\ (\text{estimated dry mass 2001})}{2\ \mathrm{y}}$$

Because RGR_R values may be offset by large increases in leaf area, I calculated the leaf area change as well (Bazzaz and Harper 1977; McGraw and Garbutt 1990):

$$\text{Leaf area change } (\text{cm}^2\ \text{cm}^{-2}\ \text{y}^{-1}) = \ln(\text{leaf area } 2003) - \ln(\text{leaf area } 2002)$$

I used 2-way ANCOVA to test for aspect and distance effects, and their interaction, on both RGR_R and leaf area change. No covariate was used in these analyses as the dependent variable was already relativized to initial size.

To determine how cumulative light varied with aspect and distance to the stream, I again used ANCOVA. As with the analyses on plant growth, both effects were treated as fixed and distance was continuous.

Environmental distance

Our second objective was to quantify environmental differences between each transect location and the transect location at the known suitable site (20 m south-facing; hereafter called 20S) and to relate these differences to plant performance. For each transect, I calculated an environmental distance for five variables from that transect to 20S. These variables were: mean daily temperature, mean daily relative humidity, minimum daily absolute humidity, minimum daily relative humidity, and cumulative light. Environmental distances for temperature and humidity were calculated by

$$D_j = \sqrt{\sum_{i=6/1/02}^{9/30/02} (E_{i,j} - E_{i,20S})^2}$$

where D_j is the environmental distance of transect j from 20S, and $E_{i,j}$ is the mean or minimum daily value on day i for transect j.

Thus, each transect (with the exception of 20S) had one temperature distance value and three humidity distance values. I chose the time interval of June 1 – September 30 because this time frame spans the period of maximum canopy closure. For one data logger, that at 20N, the humidity sensor did not function properly so humidity distances were not calculated for this transect.

To quantify environmental distance with respect to light, the means of the cumulative light values of the nine diazo booklets were calculated at each transect and the absolute value of the difference between the mean of each transect and that at the 20m south-facing transect was considered the cumulative light distance.

To relate environmental distance from the suitable site to plant performance, block means of two plant performance measures (RGR_R and leaf area change) were regressed on each measure of environmental distance. The hypothesis (H_0) for our second objective was that environmental differences had no effect on phytometer performance. Alternatively (H_1), phytometer performance would be expected to decrease with environmental distance for factors that were important for the distribution of *H. canadensis*.

Results

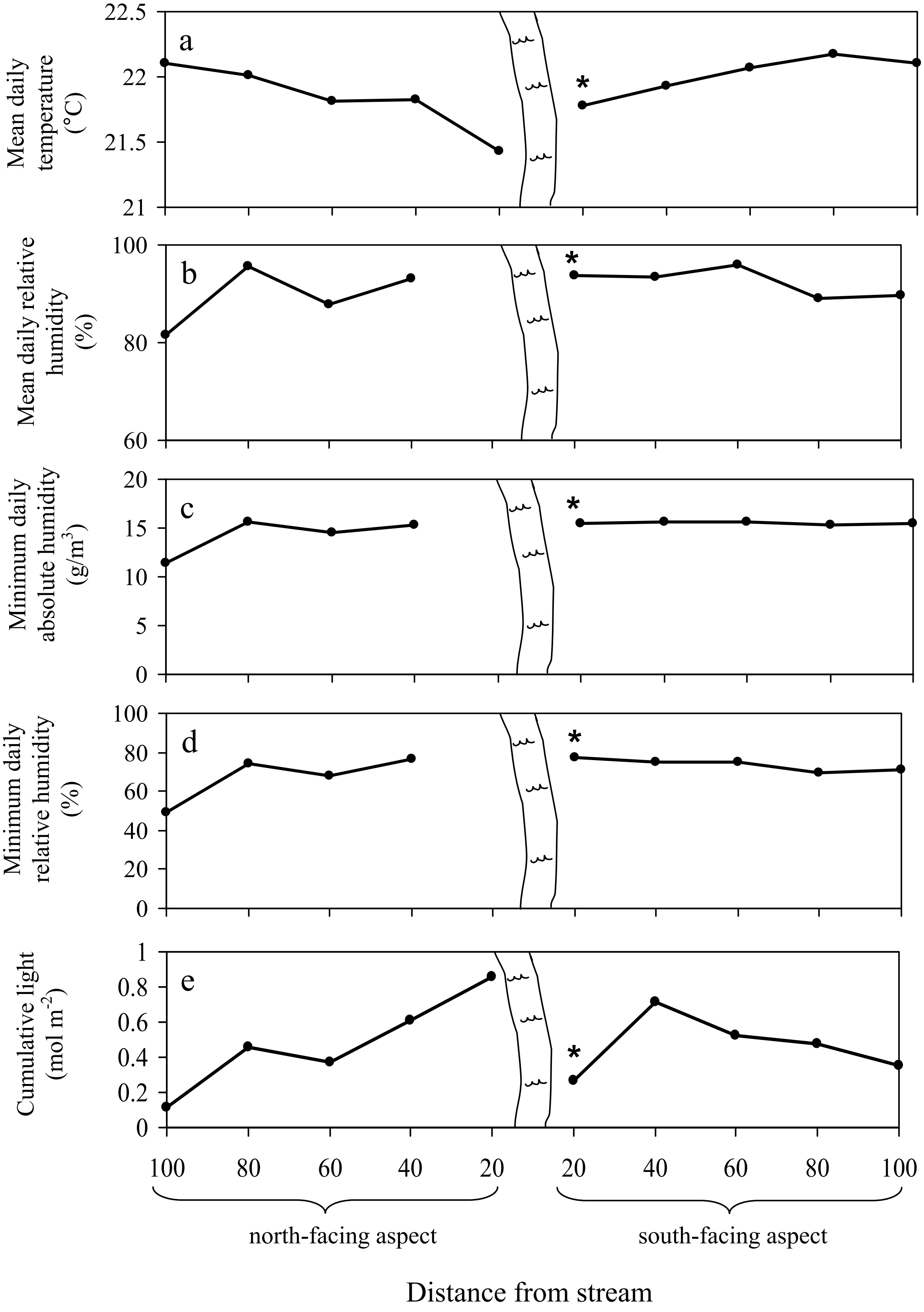
Overall, measures of temperature and light varied between transects more so than did the humidity measures (Figure 5.2). On both aspects, temperature increased with distance from the stream (Figure 5.2a). In contrast, all three humidity measures were relatively constant between transects on both aspects, with the exception of the 100N transect, where humidity was lower (Figures 5.2b, 5.2c, 5.2d).

Our first objective was to determine if there were differences in phytometer performance between aspects and with distance from the stream. There was a differential effect of aspect on cumulative light over the distances tested in this study (aspect × distance interaction, $P < 0.0001$). At all distances except 20 m south-facing, cumulative light was greater on the south-facing slope than at the corresponding distances on the north-facing slope (Figure 5.2e). Omitting 20 m values (both north and south-facing), showed that light differed for the two aspects ($P < 0.0001$) and was greater on the south-facing aspect. Light also varied with distance from the stream and was greater closer to the stream ($P < 0.0001$).

In 2002, overall survival of phytometers was 92.2%. By 2003, cumulative survival was 84.2%, and depended on initial rhizome size; larger rhizomes had lower survival ($P = 0.0350$). Phytometer survival did not depend on either main effect of aspect or distance from the stream, nor did the effect of aspect on survival depend on distance (aspect × distance interaction).

Leaf area per plant differed between aspects in both 2002 ($P = 0.0023$) and 2003 ($P = 0.0237$); in both years, plants on the north-facing aspect had larger leaves than those

Figure 5.2. Means of the daily values for each environmental distance variable during the month of July, 2002. The humidity sensor at 20N did not function properly so values are not available at that transect. The 20S transect, where the natural *H. canadensis* patch is located, is denoted by an asterisk (*).


Mean daily temperature (°C)
22.5
22
21.5
21
a
*
Mean daily relative humidity (%)
100
80
60
b
*
Minimum daily absolute humidity (g/m³)
20
15
10
5
0
c
*
Minimum daily relative humidity (%)
100
80
60
40
20
0
d
*
Cumulative light (mol m⁻²)
1
0.8
0.6
0.4
0.2
0
e
*
100
80
60
40
20
20
40
60
80
100
north-facing aspect
south-facing aspect
Distance from stream

on the south-facing aspect (Figure 5.3). Leaf area also depended on distance in 2003 when those plants closer to the stream had larger leaves ($P = 0.0070$).

Rhizome relative growth rates of phytometers were low and most (88.7%) had a negative RGR, *i.e.* they shrank in size over the two growing seasons. Rhizome relative growth rate did not depend on aspect ($P = 0.3625$) or distance to the stream ($P = 0.1292$). Leaf area changes tended to be greater than those of RGR_R: 72.0% of phytometers exhibited positive leaf area change values. Leaf area change did not depend on aspect, although there was a trend for leaf area change to increase with proximity to the stream ($P = 0.0634$).

Our second objective was to quantify environmental distances between each transect location and 20S and to relate these distances to plant performance. Rhizome relative growth rate did not depend on any of the five environmental distance measures ($P > 0.05$, Table 1). Leaf area change did not depend on any of the three humidity distance measures, although it did did depend on cumulative light ($P = 0.0051$, Table 1), and increased as the environmental distance increased (contrary to prediction if 20S was an “optimal” light environment). Of the nine transects where environmental distance was compared with 20S, light was greater than 20S at eight of these. Therefore, environmental distances represent greater cumulative light than at 20S in 8 of the 9 comparisons. In addition to a dependence on light, there was a trend for leaf area change to depend on temperature ($P = 0.0663$), but again, the tendency was for growth to increase as temperatures deviated from those observed at 20S.

Figure 5.3. Leaf area means (± 1 SE) showing aspect effect in 2002 and in 2003.

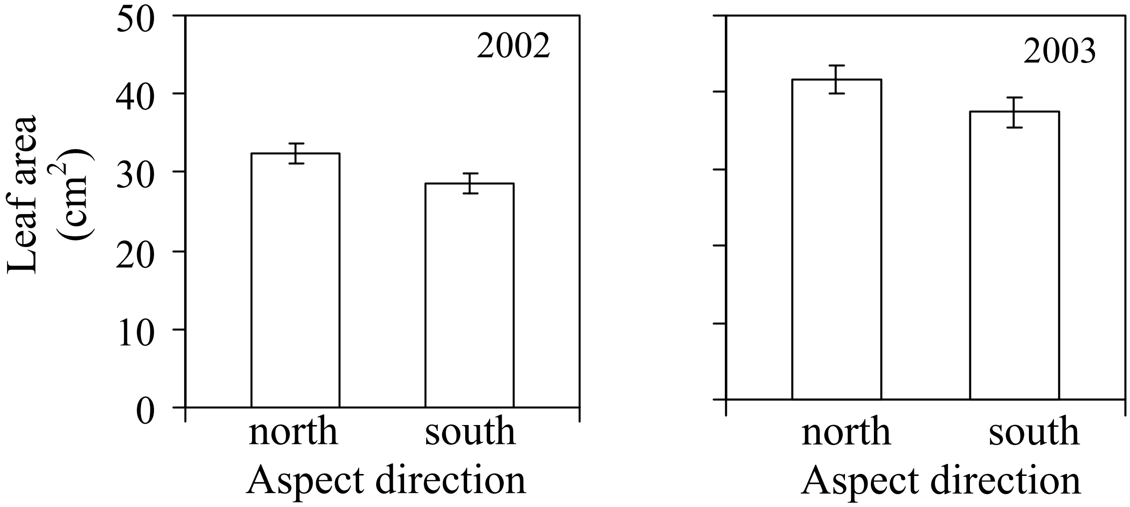

50
40
30
20
10
0
Leaf area (cm²)
2002
north
south
Aspect direction
2003
north
south
Aspect direction

Table 5.1. Slope values for the regression of RGR_R and leaf area change block means on each of the five environmental distance values. The probability values are shown below in parentheses.

Plant performance variables	Environmental distance measures				
	Mean daily temperature	Mean daily relative humidity	Daily low absolute humidity	Daily low relative humidity	Cumulative light
Mean RGR_{R}	0.0017	0.00009	0.0002	0.00004	0.0302
	(0.9222)	(0.8674)	(0.9293)	(0.9167)	(0.8646)
Mean leaf area change	0.0732	-0.0007	-0.0029	-0.0005	1.1185
	(0.0663)	(0.4350)	(0.3370)	(0.3940)	(0.0051)

Discussion

I hypothesized that plant performance would differ between aspects and over distance. Clearly, leaf area depended on both the aspect and the distance to the stream; larger leaved plants were located on the north-facing aspect and, in 2003, closer to the stream. Because I saw a similar aspect and distance effect for cumulative light, larger leaves are likely a response to low light availability. Shifting allocation patterns to obtain limiting resources is a well-documented phenomenon in plant acclimation (Chapin et al. 1987) and increases in leaf area in response to reduced light is a typical pattern seen in numerous studies (Lambers et al. 1998).

The cumulative light recorded at the forest floor may be a reflection not only of mesoscale topography but also of land use history. Until approximately 50 YBP, the south-facing slope from approximately 40 m from the stream and above was pasture. Currently, this part of the study site is dominated by midsuccessional tulip poplar (*Liriodendron tulipifera* L.), shagbark hickory (*Carya ovata* [P. Mill.] K. Koch), and cherry (*Prunus serotina* Ehrh.). Closer to the stream on the south-facing slope, and on most of the north-facing slope is “old growth” forest, with a dominant overstory including large (1 m diameter and larger) red oak (*Quercus rubra* L.) and tulip poplar, with paw paw (*Asimina triloba* [L.] Dunal) also abundant in these areas. On the north-facing slope, the land just above 100 m from the stream was clear cut in 1990. The area near the 100 north-facing transect was dominated by shrubs, mainly spicebush (*Lindera benzoin* [L.] Blume).

Despite the clear pattern of individual plant leaf area in response to mesoscale topography and possibly also land use history, parallel patterns in rhizome relative growth rate were not observed; RGR_R did not depend on either the aspect or distance to the stream. This suggests two possible scenarios. First, *H. canadensis* is capable of acclimating to low light availability by increasing leaf area, although this increase in leaf area does not translate to larger rhizomes. Reduced light would be expected to lower photosynthetic rates per unit leaf area, such that net assimilation is not different from plants having less leaf area but "existing" in higher light environments. Alternatively, there may have been a protracted transplant effect, whereby removal of rhizomes from the original site in August of 2001 resulted in breakage of the fine roots attached to each rhizome. Although a small length of roots remained attached (~1-2 cm), an initial depletion in rhizome reserves may have resulted from increased allocation to new fine roots. Subsequently, allocation to leaf area increased, however consequent rhizome biomass increases had not been observed after two years.

Our second objective related to environmental conditions at the natural *H. canadensis* site. I hypothesized that if the natural population was in a highly suitable site with respect to a particular factor, transects that differed strongly in important microclimate factors from the 'suitable' site would result in reduced phytometer performance. While I found variation in humidity, temperature, and light across the forested cove, there was no evidence that differences in these factors from the inhabited site resulted in lower performance. Although I saw that leaf area change depended on cumulative light environmental distance, it actually increased as environmental distance increased. Likewise, while there was a trend for the dependence of leaf area change on mean daily temperature distance, the performance measure increased with increasing environmental distance. Failure to observe significant regression slopes of RGR_R and leaf area change on most environmental variables suggests that these environmental variables may be poor indicators of phytometer performance and that other variables are influencing *H. canadensis* distribution.

This study examined only three abiotic factors, and did not address effects of soil factors. Lechowicz and Bell (1991) found that three edaphic measures, soil pH, and K^+ and NO^{3-} in the soil solution, can vary at scale of 0.1 m. Clearly, the potential exists for plants in transects spaced 20 meters apart to respond to differences in soil pH or ion concentrations. In addition to abiotic influences, spatial variation in soil biota might also account for our results. Within a given location, small scale spatial structuring of the soil biotic environment occurs and can influence associated plant communities (Wardle 2002; Ettema and Wardle 2002). This can occur either by direct influence on the plant, such as heightened local densities of pathogenic fungi (Packer and Clay 2000) or indirectly by altering competitive interactions between plant species (Brown and Gange 1989; Olff et al. 2000).

Our results showed increased leaf area with decreased irradiance. Because there is a positive response of *H. canadensis* to paths and forest edges (Sinclair and Catling 2000b; Sanders and McGraw 2002), increased leaf area, in response to the low irradiance which typically reaches the forest floor under a closed canopy mid-successional forest, may be unfavorable for *H. canadensis* growth. Although I did not observe an increase in RGR_R at higher irradiance, it may be evident over longer time spans. In contrast to responses to light, I did not find clear relationships between plant growth and temperature or humidity. The relatively weak association between *H. canadensis* performance and environmental variation across the forested cove reinforces other studies suggesting that *H. canadensis* has a relatively broad ecological niche, and its rarity is unlikely due to availability of suitable habitat.

Acknowledgments

I am grateful to Allegheny Wood Products for allowing use of the rhizome source material. I are also grateful to Rebecca Kenyon, Sara Lightner, Mary Olive, and Bridgette Wright for field assistance. This research was funded by grants 99HQAG0045 and 1434-HQ-97-RU-01563, both from the U. S. Department of the Interior and the U. S. Geological Survey.

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CHAPTER 6:

Ecological genetics of a threatened plant: variation in plasticity and implications for restoration

Abstract.

Intraspecific genetic variation in plants is frequently associated with adaptation to local environmental differences. Detection of ecotypic differentiation can promote an understanding of a species' distribution and be an important consideration in restoration efforts. I performed a classical reciprocal transplant study using four natural populations of *Hydrastis canadensis* to test for localized adaptation. A second study examined the importance of including multiple sources when introducing new populations for restoration purposes. I established *H. canadensis* populations that were mixtures of three natural sources and also populations that were monocultures of each natural source. In both studies, which were conducted over three years in north-central West Virginia, I measured the dependent variables of survival, plant size, and both rhizome and leaf area relative growth rates. The results of the reciprocal transplant study showed that *H. canadensis* exhibited plasticity in response to site quality variation, but there was no evidence of local genetic adaptation. Additionally, the four *H. canadensis* sources responded differentially to microsites within the transplant site. In the population introduction study, I found that populations established with plant material from single sources performed better than those established with multiple sources. One possible explanation of this result is that opportunistic infection of *H. canadensis* pathogens onto new host genotypes occurred in the mixtures; two other possible explanations are discussed. Collectively, our findings indicate that restoration efforts should involve multiple sources as a bet-hedging strategy to increase the likelihood of suitable source-microsite compatibility. However, within a given restoration site, these sources should be spatially separated, such that numerous populations are introduced, each comprised of only a single source.

Introduction

Ecological genetic variation in plants allows populations to respond to environmental change (Antonovics et al. 1971, Davison and Reiling 1995, Benkman 1995). This is important for adapting to sudden differences in habitat upon dispersal (Gomulkiewicz and Holt 1995) and to *in situ* directional changes in the environment (Rice and Emery 2003). Intraspecific genetic variation was demonstrated in common garden experiments by Turesson (1925) and later in reciprocal transplant studies by Clausen et al. (1940). These early studies led to the recognition of "ecotypes" or locally adapted populations that have higher fitnesses in their local microsite conditions than conspecific plants found in contrasting environments. Failure of populations to adapt may threaten their persistence by limiting response to long term environmental change and population establishment after dispersal (Antonovics 1976).

Reciprocal transplant experiments can be used to test for local genetic adaptation since they allow a partitioning of the total between-population phenotypic variance into components due to environment and components attributable to "genotype" in the broad sense (Heslop-Harrison 1964). Studies of ecotypic differentiation in plant species show that localized adaptation is the norm across a range of species' growth habits and life histories (for reviews see Heslop-Harrison 1964, Langlet 1971, Linhart and Grant 1996). Recent studies using reciprocal transplants have shown locally adapted genotypes in grasses (Rice and Mack 1991, Hufford and Mazer *public communication*), herbaceous dicots (Nagy and Rice 1997, Galloway and Fenster 2000), and shrubs (McGraw and Antonovics 1983, Montalvo and Ellstrand 2000). Although localized adaptation is the rule, exceptions do exist (Aspinwal and Christian 1992, Helenurm 1998, Cheplick and White 2002).

The presence of localized adaptation, as well as its absence, can be used by biologists to understand species' behavior, such as survival after dispersal or disturbance, and to guide conservation efforts (Montalvo and Ellstrand 2000). While reciprocal transplant studies can detect local adaptation and the presence of ecotypes, they do not typically address important questions about compatibility between sources, a focus that has special relevance in the emerging field of population introduction and translocation. Reduced source compatibility can arise either from genetic introgression between sources or via means other than introgression. Where genetic exchange occurs, disruption of co-adapted complexes can result in maladapted offspring genotypes and outbreeding depression (Fenster and Galloway 2000, Wade 2001, Wade 2002). In the absence of introgression, gene swamping may occur via vegetative reproduction if a given genotype (or ecotype) has a fitness advantage at the site of introduction allowing it to compete better. The frequency of this genotype (or ecotype) may then increase more rapidly than that of other genotypes (Saltonstall 2002, Hufford and Mazer 2003). The basis of this fitness advantage may be better adaptation to the local abiotic environment, such as light availability or soil moisture.

Alternatively, a given genotype or ecotype may be more fit in a local environment due to coadaptation with the biotic environment. Localized "ecotypes" of plant pathogens and symbionts are well known, and coadaptation of these with plant populations has been documented (Parker 1985, Chanway et al. 1989, Parker 1995, Thrall et al. 2001, Bever 2003). Because of this tightly knit relationship of plant populations with local biotic and abiotic environments, efforts to introduce or translocate plant populations must focus not only on the potential ability of the species to adapt to the translocation site, but also on the compatibility between introduced sources.

Much of the published work involving plant population introduction has focused on one of two classes of threatened species: 1) those species that are naturally rare, having very high habitat specificity with relatively small amounts of that habitat present (*e.g.*, pitcher's thistle, *Cirsium pitcheri* [Rowland and Maun 2001]), and 2) species that have undergone an extensive reduction in abundance and/or distribution due to habitat loss or other reasons that are less evident (*e.g.*, eelgrass, *Zostera marina* [Williams 2001]). Because species in this second category may be in the initial stages of a population bottleneck, establishment studies examining source compatibility may be especially appropriate.

One species that has experienced declines in both distribution and abundance is *Hydrastis canadensis* (L.). *Hydrastis canadensis* (goldenseal, yellow root) is a herbaceous perennial plant native to the eastern deciduous forest of North America. The range of *H. canadensis* extends from southern Ontario south to Tennessee and the surrounding states, and from Missouri, east to the eastern edge of the Appalachian Mountains (Small and Catling 1999). Within this range, *H. canadensis* grows in mesic forests, forming dense, clonal patches. Although estimates of past abundance (*i.e.*, 100 – 200 YBP) are not available, recent studies indicate a population decline via a reduction in the number of patches present (Sinclair and Catling 2000, Sanders and McGraw 2002, Mulligan and Gorchov *public communication*). It has also been reported that, within the central part of the range, ramet number per patch is declining (Sanders and McGraw 2002), although an increase in stem number was found in many Ontario populations (Sinclair and Catling 2003). Like ginseng (*Panax quinquefolius* L.), this species is harvested from natural populations due to demands by the herbal market (Charron and Gagnon 1991). Unlike ginseng, however, *H. canadensis* is clonal and reproduces vegetatively *via* prolific rhizome growth. When *H. canadensis* is harvested, the large storage rhizomes at the base of aerial stems are removed and are broken off of the lateral creeping rhizomes, which are left in the soil. Regrowth of new aerial ramets can arise from buds on the lateral rhizomes (Van der Voort et al. 2003, Sanders and McGraw *unpublished manuscript*). This may allow populations to withstand harvest pressure, providing the time between harvests is sufficient. *Hydrastis canadensis* patches are spatially separated across the landscape, and are often not present in what is apparently suitable habitat (McGraw et al. 2003). The reasons for this are unclear; obviously harvest pressure is one factor that may account for the patchy distribution, although other possibilities include dispersal limitations and reduced seed germination. A fourth factor that may explain the limited and patchy distribution of *H. canadensis* is a failure to adapt to local habitat, either in response to changes *in situ* or to new environments upon dispersal (Rice and Emery 2003). Lack of ecotypic differentiation may indicate that *H. canadensis* populations lack sufficient ecological genetic variation with respect to potential habitat differences.

I conducted this study to understand ecologically relevant aspects of *H. canadensis* genetics. Our first objective was to determine if *H. canadensis* contains ecologically important genetic variation (i.e. ecotypic differentiation) at the population level. I conducted a classical reciprocal transplant experiment with four natural populations to test the hypothesis that ramets planted in their home site would have greater survival and performance than alien *H. canadensis* ramets planted into the same site. Because population introduction may become a necessary strategy for augmenting abundance and promoting persistence, I wanted to test if survival and establishment of introduced *H. canadensis* populations are affected by the genetic composition of the transplanted individuals. Our second objective was therefore to determine if there were differences in performance between populations established from one natural source and those established from multiple natural sources. I conducted a three year population establishment study to test the hypothesis that introduced populations comprised of mixtures perform better than those comprised of monocultures. I also tested whether plants from different source populations differed in their establishment potential in a new site.

Methods

Reciprocal transplant study

Source material for the reciprocal transplant study was obtained from four locations in West Virginia: Morgantown, Jane Lew, Ellenboro, and Rivesville (precise locations withheld due to conservation concerns). These locations were chosen because they contained older, established populations and covered a large local area. As such, there was a high likelihood that the sampled rhizomes represented multiple genotypes. Additionally, the chosen sites had disparate habitat characteristics (Table 6.1), increasing the likelihood of detecting ecotypic differentiation, if it existed.

Plant material was collected on August 16 and 17, 2000. Since clonal reproduction *via* rhizomes was prolific, all aboveground stems are initially referred to as ramets. At all locations, 120 ramets were collected; these were all non-reproductive (one leaf) at all locations except Jane Lew where half were reproductive (two or three leaves). At all locations, an attempt was made to collect rhizomes spanning the entire spatial extent of the area to maximize the likelihood that distinct genotypes were sampled among the collected rhizomes. All rhizomes were brought to the laboratory where the stems were removed and the rhizomes were cleaned, air dried, and weighed.

Rhizomes from each of the four source populations were planted into each of the four sites. All sites contained three blocks, each of which contained ten replicate rhizomes from each of the four source populations (4 sources × 4 sites × 3 blocks/site × 10 ramets/source-

TABLE 6.1. Habitat characteristics of the four *H. canadensis* source populations in the reciprocal transplant study.

Site	Elevation (m)	Aspect (°)	Soil type	Dominant tree species	Reference
Morgantown	980	210	Gilpin-Culleoka-Upshur silt loam	*Acer saccharum*	Wright et al. 1982
Jane Lew	990	300 – 360	Vandalia silt loam	*Liriodendron tulipifera*, *Acer saccharum*	Pyle 1995
Ellenboro	970	90	Upshur-Gilpin complex	*Quercus alba*, *Carya ovata*	Wright et al., 1986
Rivesville	1,180	300	Culleoka-Westmoreland silt loam	*Quercus rubra*, *Liriodendron tulipifera*	Wright et al. 1982

block = 480 ramets total). Source and site were treated as fixed main effects and block (within site) was treated as a nested random effect. Rhizomes were planted 2 cm deep in random positions within each 1.3 × 1.0 m block at a spacing of 20 cm. Blocks were generally established in microsites with little existing understory vegetation; some small herbaceous plants were removed in a few instances to make room for the transplants, but otherwise, local soil and plants were left undisturbed. An aluminum nail with a unique plant identification number was placed adjacent to each rhizome.

Measurements were taken on all plants during the final week of June in 2001, 2002, and 2003, timed to occur after full leaf expansion but prior to plant senescence. These measurements included noting plant presence, plant height, leaf length, whether the plant was reproductive and whether it fruited. For all plants in the reciprocal transplant study and the population establishment study (see below), estimates of leaf area were obtained using 100 ramets not included in these studies and regressing the natural log of leaf area on the natural log of leaf length. From the allometric relationship determined by this regression (leaf area = 1.8347 (leaf length)$^{1.8284}$; $r^2 = 0.9582$), I estimated leaf area of ramets in both studies. Because I wanted to use initial rhizome dry weight in the statistical analyses, I removed 100 additional rhizomes from the field and washed, air dried, and weighed these as described above. These were then oven dried at 65 °C for 72 hours and reweighed. Because the dry mass:fresh mass ratio was not related to initial rhizome mass ($r^2 = 0.0297$), the average value of the ratio was used to estimate an initial dry weight of rhizomes based on their initial fresh weight.

I used log-likelihood analysis (Sokal and Rolf 1995), to determine effects of source population, site, and their interaction on survival, and reproductive status (flowered/vegetative, fruited/not). For survival, I tested cumulative survival from the start of the study because I was interested in longer term survival rather than year to year differences. Two-way analysis of covariance (with rhizome initial dry weight as covariate) was used to test the model effects (source, site, block (site), and their interaction) on plant leaf area for each year of the study. Because I was also interested in the relative performance of rhizomes planted back into their home site versus, collectively, those planted into alien sites, I created a nominal variable, origin, in which rhizomes were classified as either “home” (25% of the ramets classified) or “away” (75% of the ramets classified). I tested the effect of origin and site on the nominal dependent variables, survival and reproductive status, and on the continuous variable leaf area, for each year in the study. In this model, origin and site were treated as fixed effects, and block (within site) was treated as random. Initial rhizome mass was used as a covariate for all dependent variables.

Rhizomes were removed from the field on August 13 (Jane Lew and Ellenboro) and August 15 (Morgantown and Rivesville), 2003. Stems were removed, rhizomes were rinsed clean, then air dried and rhizome dry weight was determined by oven drying at 65 °C for 72 hours. The rhizome relative growth rate, RGR_R, was then calculated according to Hunt (1982) by:

$$RGR_R\ (\mathrm{g\ g^{-1}\ y^{-1}}) = \frac{\ln\text{(final dry mass 2003)} - \ln\text{(estimated dry mass 2000)}}{3}$$

I also wanted to determine if the rhizome relative growth rate response might be explained by changes in leaf area over time. For example, a small relative growth rate of the rhizomes might be offset by correspondingly high relative increases in leaf area. Therefore, I determined the relative growth rate on a leaf area basis (RGR_{LA}) (Bazzaz and Harper 1977, McGraw and Garbutt 1990) by:

$$RGR_{LA}\ (\mathrm{cm}^{2}\ \mathrm{cm}^{-2}\ \mathrm{y}^{-1}) = \frac{\ln\text{(final leaf area 2003)} - \ln\text{(initial leaf area 2001)}}{2}$$

Two-way nested analysis of variance was used to test for source, site, and interaction effects on RGR_R and RGR_{LA} over the study period. I also tested the effect of origin (home vs. away) on RGR_R and RGR_{LA}. Significant ANOVA and ANCOVA effects were separated using the conservative Tukey - Kramer HSD test (Sokal and Rohlf 1995). For all continuous dependent variables in the reciprocal transplant study, where significant source × block (site) interaction terms resulted, data were separated by site and the source × block interaction was tested individually at each site to examine the form of the differential response. Rhizome initial dry weight was not used as a covariate for RGR_R and RGR_{LA}, since initial dry weight is included in the calculation.

Population introduction study

Source material for the founder populations was obtained from three locations: North Hills, Morgantown, and Jane Lew. As with the reciprocal transplant study, these locations were chosen because they were older, established populations and covered a large local area, increasing the likelihood that the sampled rhizomes would represent multiple genotypes. Plant material was collected from Jane Lew on August 16, 2000; 108 rhizomes were collected, half of which were reproductive and half of which were non-reproductive. Rhizomes from both Morgantown and North Hills were collected on August 17, 2000. At both locations, 108 non-reproductive rhizomes were collected. At all locations, rhizomes were sampled from the entire geographic area of the patch. All plant material was brought to the laboratory where the stems were removed and the rhizomes were cleaned using water and light scrubbing, air dried, and weighed.

The populations were established in a nature preserve located 10 km northeast of Morgantown, West Virginia. This area was chosen primarily because the isolation of this preserve lowered the risk of human disturbance. Additionally, there was one known natural *H. canadensis* population in the preserve, suggesting that this area was suitable habitat for the species. This population was not used as source material because I was attempting to simulate introduction of new populations into a suitable habitat without existing populations.

Eighteen populations were introduced within the preserve, each consisting of 18 randomly selected rhizomes. Nine populations were genetic mixtures containing six rhizomes from each of the three source populations (n = 18 rhizomes / population). The remaining nine populations contained only one source population: three of these were from North Hills, three from Morgantown, and three from Jane Lew, (18 rhizomes / population). In introducing these populations, a small amount of existing vegetation was removed in a few instances, but otherwise soil and surrounding plants were undisturbed. All rhizomes were planted 2 cm deep and marked with an aluminum nail. Populations were arranged in 0.8 m × 0.6 m blocks with 20 cm spacing between rhizomes.

Measurements on all plants were taken during the final week of June in 2001, 2002, and 2003 using the same methodology as in the reciprocal transplant study. To determine if ramets in mixtures performed better than ramets in monoculture, I tested the main effect of composition (mixture or monoculture) and block (within composition) on survival, reproduction, and fruit production using a log-likelihood analysis. Rhizome initial dry weight was used as a covariate. For all analyses, composition effect was considered fixed and block was random. I used a nested one-way analysis of variance to test model effects on leaf area (with rhizome initial dry weight as covariate), rhizome relative growth rate and leaf area relative growth rate.

Because I was also interested in determining if, within each composition treatment, sources differed in their establishment potential, I then tested the effect of source (North Hills, Morgantown, and Jane Lew) on our fitness components of survival, reproduction, fruit production, leaf area, RGR_R, and RGR_{LA}. The blocking within the experimental design precluded a single analysis, therefore two separate analyses were performed. First, testing only populations of mixtures, the model effects were source, block, and the source × block interaction. Source was treated as a fixed effect and the latter two effects were random. Rhizome initial dry weight was a covariate for all dependent variables except RGR_R and RGR_{LA} (here, initial dry weight is included in the calculation). Second, I tested the establishment potential of the sources using only those populations comprised of monocultures. Model effects were source (treated as fixed) and block (within source) (treated as random). Again, rhizome initial dry weight was a covariate for all dependent variables except RGR_R and RGR_{LA}. Significant main effects for all analyses of continuous dependent variables in the population establishment study were separated using the Tukey – Kramer HSD test. All statistical analyses in both the reciprocal transplant and population establishment studies were conducted using SAS JMP (SAS JMP, V.5.0.1.2, SAS, Inc., 2003).

Results

Reciprocal transplant study

Plants from home sites did not have greater survival or reproduction than plants from away sites in any year (no “origin” effect, $P > 0.05$). Likewise, plants from home sites did not have greater leaf area in any year or greater RGR_R, or RGR_{LA} at the end of the three years, than plants from away sites ($P > 0.05$).

Initial size explained some variation in survival after 2 and 3 years ($P < 0.005$, Table 6.2). However, survival was not dependent on source, site, or any interactive effects for any of the time periods examined (1, 2, or 3 years; Table 6.2). These results must be considered in the context of the model. The 48 degrees of freedom were partitioned among the 6 effects, reducing the statistical power to detect differences; therefore, the statistical tests for the reciprocal transplant study tended to be conservative. Although significant effects on survival were not detected, rhizomes from Ellenboro did not survive after 2001 in block 1 at the Morgantown site and block 5 at the Jane Lew site. These two blocks were therefore excluded from analysis of leaf area in 2002 and 2003, and RGR_{LA}. The number of plants that were reproductive and that fruited was very small during each year of the study. The reproductive status of ramets did not depend on source, site, or any interactive effects during any year of the study, although the initial rhizome mass did explain variation in reproductive status in 2001 ($P < 0.0001$). Initial rhizome mass did not explain variation in fruit production in 2001 ($P = 0.5224$); in 2002 only three ramets were reproductive (two fruited) and in 2003 only one ramet was reproductive (and did not fruit) so analyses of variance on fruiting status were not performed for either year.

TABLE 6.2. Log-likelihood values (and probabilities of greater log-likelihood values in parentheses) for survival in reciprocal transplant study.

Source	Survival to 2001	Survival to 2002	Survival to 2003
Rhizome initial dry weight	2.65	9.07	10.52
	(0.1033)	(0.0026)	(0.0012)
Site	0.04	0.19	1.88
	(0.9982)	(0.9796)	(0.5971)
Source	0.03	0.01	0.72
	(0.9988)	(0.9997)	(0.8683)
Block (site)	0.12	5.56	5.69
	(1.0000)	(0.6959)	(0.6817)
Source × site	3.52	9.36	8.07
	(0.9400)	(0.4039)	(0.5266)
Source × block (site)	5.62	9.45	12.75
	(1.0000)	(0.9964)	(0.9700)

Leaf area per plant, a measure of plant size, differed among sources only in 2001 (*P* = 0.0023, Table 6.3) when the Morgantown source had a greater leaf area than that of either Jane Lew or Ellenboro (Fig. 6.1). Source differences disappeared in 2002 and 2003 (P > 0.05, Table 6.3). Leaf area differed among sites only in 2003 (*P* = 0.0236, Table 3) when those plants at Morgantown had a greater leaf area than plants at Jane Lew (Fig. 6.2). Ecotypic differentiation would be indicated by home site advantage within the transplant sites. However, differences among source populations in plant size (leaf area) did not depend on the transplant site during any of the three years of the study.

There was a differential response of leaf area of the four source populations to microsite variation within sites (source × block interaction) in both 2002 (*P* = 0.0032, Table 6.3) and 2003 (*P* = 0.0094, Table 6.3). For example, at the Morgantown site in 2002, the relative leaf area of the Jane Lew or Ellenboro source populations differed between blocks 2 and 3 (Fig. 6.3A), shown by the crossing lines illustrating differential responses to blocks. This general trend was also present at the Morgantown site in 2003 but with a more marked difference in the performance of the Jane Lew source population between blocks 2 and 3 (Fig. 6.4A). In a second example, at the Ellenboro site in both 2002 (Fig. 6.3C), and 2003 (Fig. 6.4C), leaf area of the Morgantown source populations increased in block 9 relative to block 8, although the leaf area of the other sources either decreased between blocks 8 and 9 (2002) or was relatively constant between the two blocks (2003).

Relative growth rate was calculated from rhizome mass, although not all rhizomes could be relocated in August 2003. Blocks in which any one source population was completely absent

FIGURE. 6.1. Least squares means showing source differences in leaf area (2001) in the reciprocal transplant study. Means with different letters are significantly different at $P < 0.05$. MG = Morgantown, JL = Jane Lew, EB = Ellenboro, RV = Rivesville.

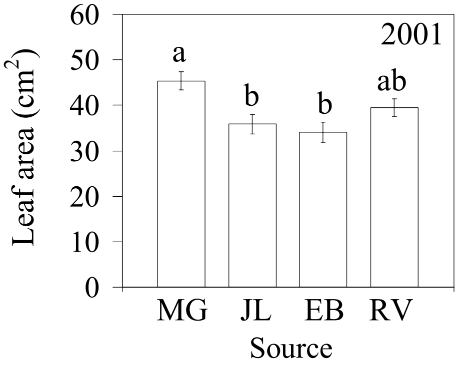

60
50
40
30
20
10
0
Leaf area (cm^2)
2001
a
b
b
ab
MG
JL
EB
RV
Source

TABLE 6.3. *F* ratios for leaf area, RGR_R, and RGR_{LA} for reciprocal transplant study. Probabilities of a greater *F* value are shown in parentheses.

Source	Leaf area 2001	Leaf area 2002	Leaf area 2003	RGR_R	RGR_{LA}
Rhizome initial dry weight	252.42	77.86	27.72	NA	NA
	(<0.0001)	(<0.0001)	(<0.0001)	--	--
Site	0.91	3.72	6.66	1.87	11.05
	(0.4760)	(0.0770)	(0.0236)	(0.2461)	(0.0072)
Source	5.96	1.45	0.62	1.03	0.16
	(0.0023)	(0.2561)	(0.6108)	(0.4055)	(0.9189)
Block (site)	3.94	2.40	1.93	1.73	1.49
	(0.0039)	(0.0682)	(0.1273)	(0.1857)	(0.2315)
Source × site	0.40	0.95	0.29	0.24	0.84
	(0.9253)	(0.5056)	(0.9698)	(0.9562)	(0.5887)
Source × block (site)	0.96	2.24	2.05	2.21	1.16
	(0.5165)	(0.0032)	(0.0094)	(0.0084)	(0.3004)

FIGURE. 6.2. Least squares means showing site differences in leaf area in the reciprocal transplant study in 2003. Means with different lowercase letters are significantly different at $P < 0.05$. MG = Morgantown, JL = Jane Lew, EB = Ellenboro, RV = Rivesville.

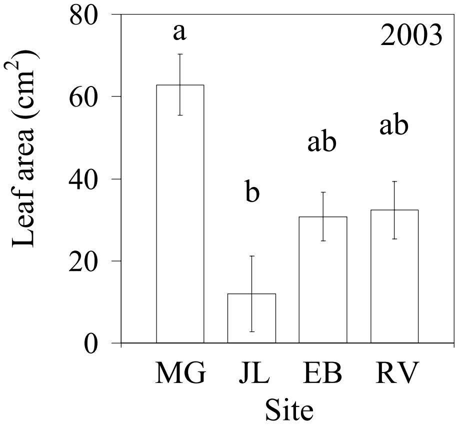

80
60
40
20
0
Leaf area (cm²)
a
b
ab
ab
2003
MG
JL
EB
RV
Site

FIGURE. 6.3. Differential response of leaf area of four source populations to microsite variation within sites in 2002. *P* values indicate source × block interaction. Sites: A = Morgantown, B = Jane Lew, C = Ellenboro, D = Rivesville.

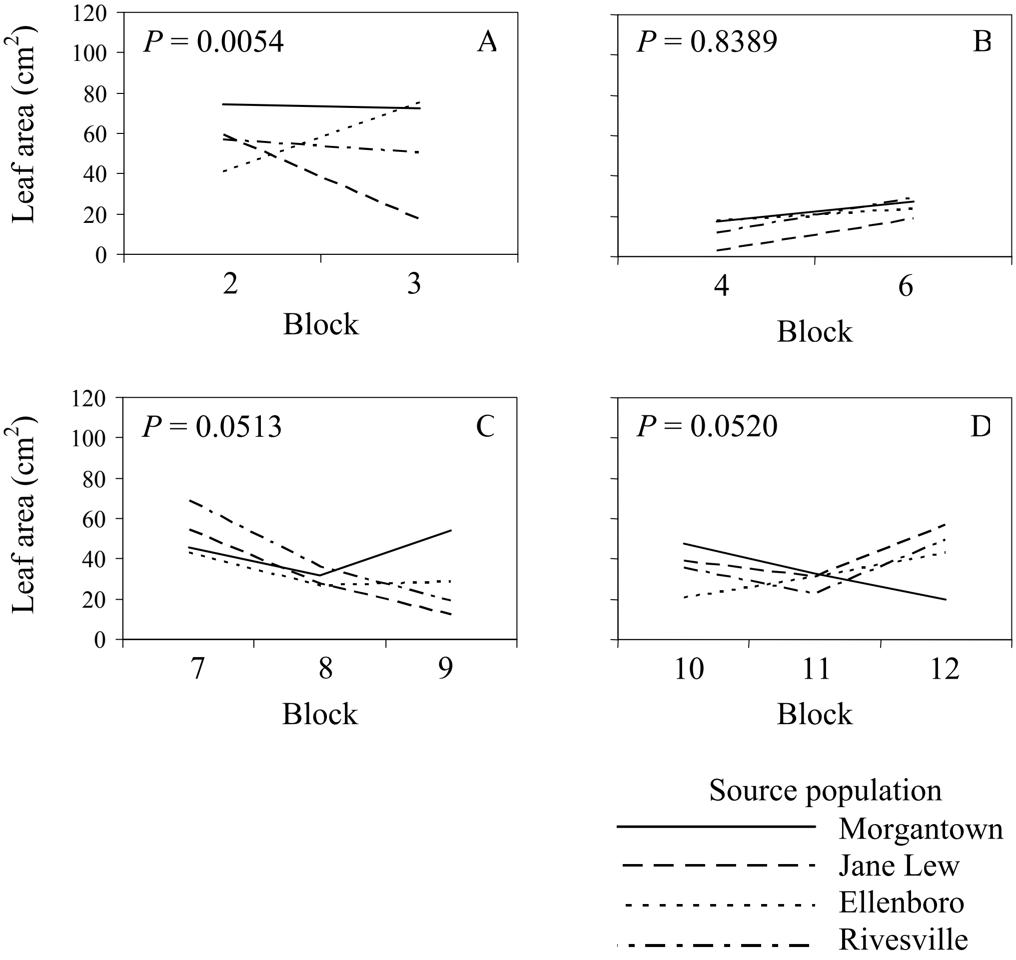
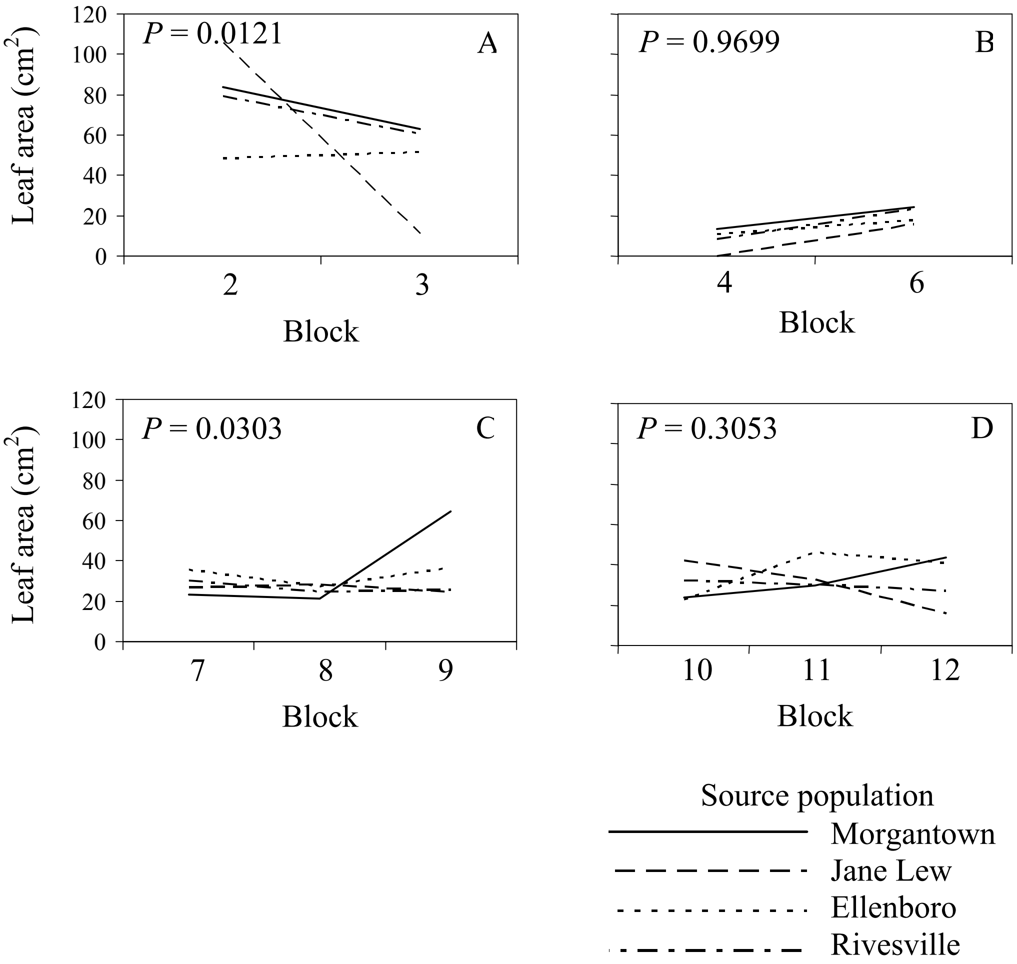

Leaf area (cm²)
120
100
80
60
40
20
0
P = 0.0054
A
2
3
Block
P = 0.8389
B
4
6
Block
P = 0.0513
C
7
8
9
Block
P = 0.0520
D
10
11
12
Block
Source population
Morgantown
Jane Lew
Ellenboro
Rivesville

FIGURE. 6.4. Differential response of leaf area of four source populations to microsite variation within sites in 2003. *P* values indicate source × block interaction. Sites: A = Morgantown, B = Jane Lew, C = Ellenboro, D = Rivesville.

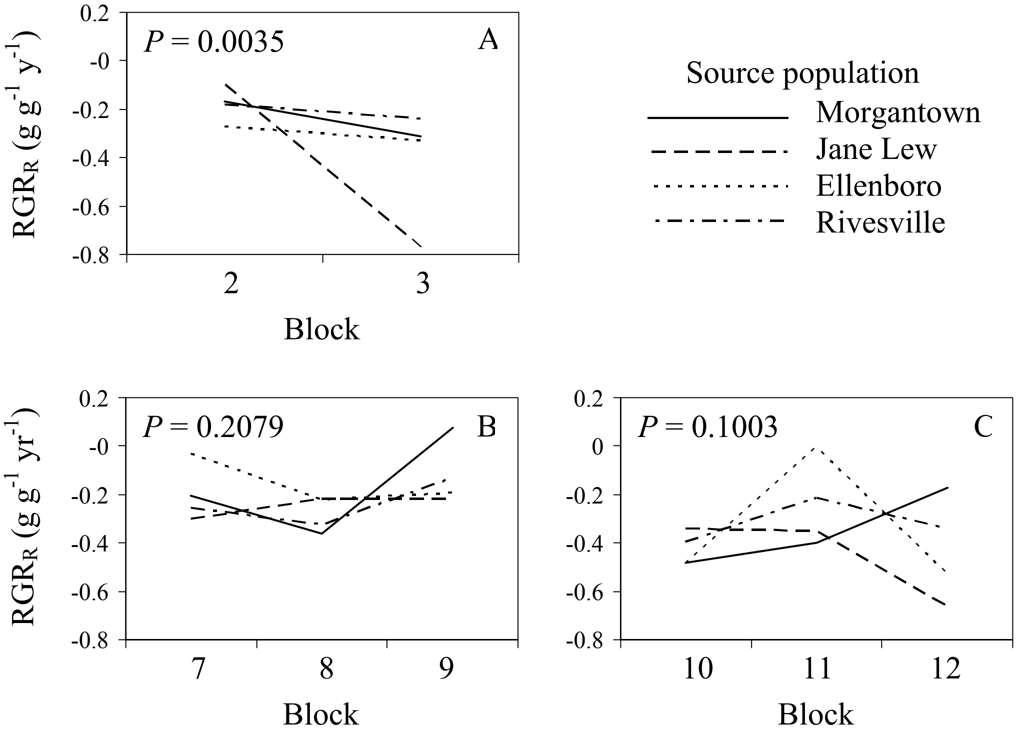

Leaf area (cm^2)
120
100
80
60
40
20
0
P = 0.0121
A
2
3
Block
P = 0.9699
B
4
6
Block
P = 0.0303
C
7
8
9
Block
P = 0.3053
D
10
11
12
Block
Source population
Morgantown
Jane Lew
Ellenboro
Rivesville

in 2003 were deleted from the analysis. Rhizome relative growth rate did not depend on the source populations or the site into which they were transplanted ($P > 0.05$, Table 6.3), however there were differences among source populations in growth response to microsites (*i.e.*, source × block interaction, $P = 0.0084$, Fig. 6.5). The non-parallel lines representing differential microsite response of source populations were most evident between blocks 2 and 3 at Morgantown, 8 and 9 at Ellenboro, and 11 and 12 at Rivesville. Changes in leaf area relative growth rate depended only on transplant site ($P = 0.0072$, Table 6.3); the $\mathrm{RGR}_{\mathrm{LA}}$ was greater at Morgantown than Ellenboro or Rivesville (Fig. 6.6).

Population introduction study

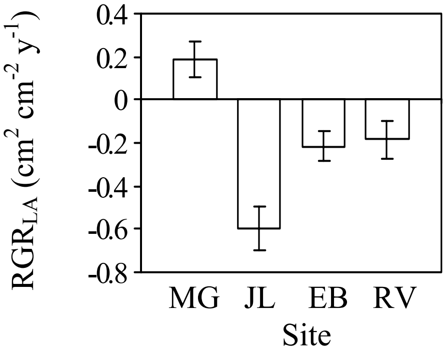
The first goal of the population introduction study was to determine if plants in mixtures performed better than plants in monoculture. Survival did not depend on block composition (mixture vs. monoculture) in 2001 or 2002, although there was a trend by 2003 ($P = 0.0571$, Table 6.4), at which time 61.8% of ramets in mixtures had survived and 70.4% of ramets in monocultures survived. Survival varied among blocks in all three years of the study ($P < 0.05$, Table 6.4). In 2001, the likelihood of ramets either being reproductive or fruiting did not depend on whether the population was a mixture or monoculture (composition effect, $P > 0.05$). In 2002, no ramets were reproductive and in 2003, five ramets were reproductive and four fruited. Therefore, no analyses for composition effect on reproductive/fruiting status were performed either year. Plant size, in terms of leaf area, depended on composition in 2002 ($P = 0.0193$, Table 6.4), an effect that was further

FIGURE 6.5. Differential response of rhizome relative growth rate of three source populations to microsite variation within sites in 2003. *P* values indicate source × block interaction. A = Morgantown, B = Ellenboro, C = Rivesville.



P = 0.0035
A
RGR$_R$ (g g^{-1} y^{-1})
0.2
-0
-0.2
-0.4
-0.6
-0.8
2
3
Block
Source population
Morgantown
Jane Lew
Ellenboro
Rivesville
P = 0.2079
B
RGR$_R$ (g g^{-1} yr^{-1})
0.2
-0
-0.2
-0.4
-0.6
-0.8
7
8
9
Block
P = 0.1003
C
0.2
0
-0.2
-0.4
-0.6
-0.8
10
11
12
Block

FIGURE. 6.6. Least squares means showing site differences on leaf area relative growth rate in reciprocal transplant study in 2003. Means with different lowercase letters are significantly different at $P < 0.05$. MG = Morgantown, JL = Jane Lew, EB = Ellenboro, RV = Rivesville.



RGR$_{LA}$ (cm^2 cm^{-2} y^{-1})
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
MG
JL
EB
RV
Site

TABLE 6.4. Log-likelihood values (survival) and *F* ratios (leaf area, RGR_R and RGR_LA) of individual plants in the population introduction study. Composition effect is plants in either mixtures or monocultures. Probabilities of a greater *F* value or greater log-likelihood value are shown in parentheses.

Source	Survival to 2001	Survival to 2002	Survival to 2003	Leaf area 2001	Leaf area 2002	Leaf area 2003	RGR_R	RGR_{LA}
Rhizome initial dry weight	3.04	1.34	0.173	74.84	19.08	0.35	NA	NA
	(0.0812)	(0.2464)	(0.6744)	(<0.0001)	(<0.0001)	(0.5524)	--	--
Composition	0.04	0.06	3.62	3.30	7.26	14.04	14.26	15.33
	(0.8399)	(0.8019)	(0.0571)	(0.0938)	(0.0193)	(0.0024)	(0.0022)	(0.0017)
Block (composition)	25.06	28.30	26.57	1.54	7.34	1.52	1.88	1.44
	(0.0145)	(0.0050)	(0.0089)	(0.1157)	(<0.0001)	(0.09)	(0.0434)	(0.1581)

amplified in 2003 ($P = 0.0024$, Table 6.4). In 2002, plants in monocultures were 2.3-fold larger than plants in mixtures and in 2003, monoculture plants were 3.1-fold larger than mixture plants (Fig. 6.7). Rhizome relative growth rates depended on composition ($P = 0.0022$, Table 6.4) and were greater in monocultures (Fig. 6.8A). Indeed, rhizomes in monocultures were growing (positive RGR_R), while those in mixtures were declining (negative RGR_R) in size. Leaf area relative growth rate mirrored that of RGR_R; it depended on composition ($P = 0.0017$, Table 6.4, Fig. 6.8B) and was greater in monocultures.

A second goal of the population introduction study was to determine if, within composition type, sources differed in their establishment potential. Within mixtures, rhizome initial size explained some variation in survival in 2001 ($P = 0.0208$, Table 6.5) and showed a trend of explaining survival variation in 2002 ($P = 0.0709$, Table 6.5). Survival was not dependent on source, block, or any interactive effect in any of the three years of the study. Similarly, in 2001, the reproductive and fruiting status did not depend on source, block, or any interactive effect ($P > 0.05$). In 2002, no plants were reproductive and in 2003 only one plant was reproductive; therefore analyses on reproductive variables were not conducted on the 2002 and 2003 data. Leaf area did not differ among sources in any year (no main effect of source), although it varied among the blocks into which transplants were placed in 2002 ($P = 0.0150$, Table 6.5). The difference in mean leaf area among source populations depended on the block into which transplants were placed in 2003 ($P = 0.0007$, Table 6.5), paralleling the differential response to microsites observed in the reciprocal transplant study. Within mixtures, neither the rhizome relative growth rate nor the leaf area relative growth rate depended on any source, block, or their interaction ($P > 0.05$, Table 6.5).

FIGURE. 6.7. Effect of population composition on plant leaf area in populations composed of mixtures and monocultures in 2002 and 2003. LS Means are significantly different at $P < 0.05$.

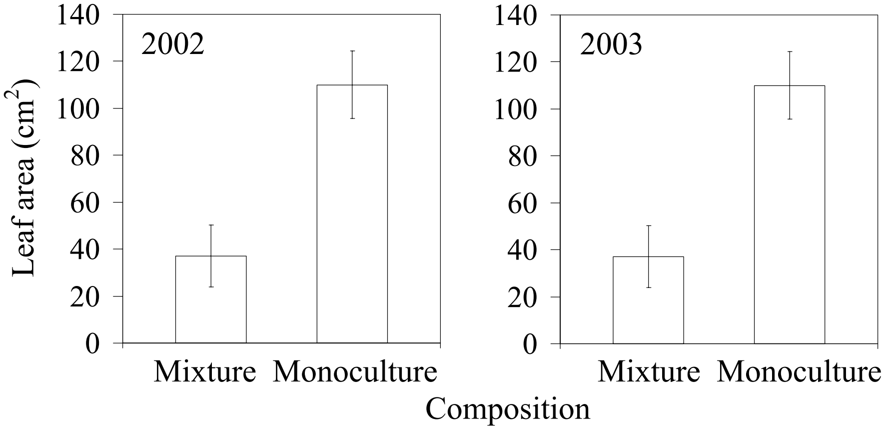

2002
2003
Leaf area (cm^2)
0
20
40
60
80
100
120
140
Mixture
Monoculture
Composition

FIGURE 6.8. Rhizome relative growth rate (A) and leaf area relative growth rate (B) of individual ramets in the population establishment study in plots composed of mixtures and monocultures. LS Means are significantly different at $P < 0.05$.

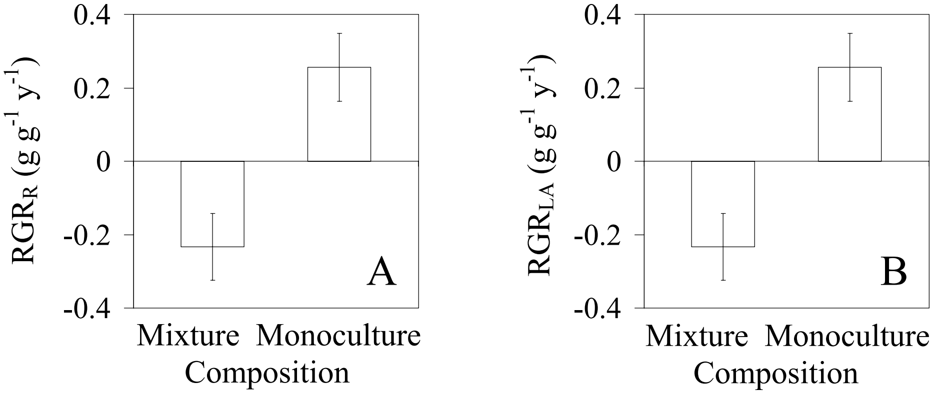

0.4
0.2
0
-0.2
-0.4
RGR$_R$ (g g^{-1} y^{-1})
Mixture
Monoculture
Composition
A
0.4
0.2
0
-0.2
-0.4
RGR$_{LA}$ (g g^{-1} y^{-1})
Mixture
Monoculture
Composition
B

TABLE 6.5. Log-likelihood values (survival) and *F* ratios (leaf area, RGR_R and RGR_{LA}) of mixtures in population introduction study. Probabilities of greater *F* values or greater log-likelihood values are shown in parentheses.

Source	Survival to 2001	Survival to 2002	Survival to 2003	Leaf area 2001	Leaf area 2002	Leaf area 2003	RGR_R	RGR_{LA}
Rhizome initial dry weight	5.34	3.26	1.97	25.98	7.78	1.16	NA	NA
	(0.0208)	(0.0709)	(0.1602)	(<0.0001)	(0.0070)	(0.2852)	--	--
Source	0.02	0.02	0.07	0.21	1.11	1.21	1.60	0.99
	(0.9877)	(0.9892)	(0.9649)	(0.8094)	(0.3443)	(0.3248)	(0.2217)	(0.3906)
Block	5.76	6.07	8.73	0.63	3.76	1.37	2.60	1.73
	(0.5686)	(0.5309)	(0.2725)	(0.7228)	(0.0150)	(0.2864)	(0.0532)	(0.1726)
Source × block	2.58	3.86	9.84	1.29	0.78	3.35	0.61	1.13
	(0.9996)	(0.9963)	(0.7734)	(0.2389)	(0.6819)	(0.0007)	(0.8406)	(0.2680)

I also wanted to determine if, within monocultures, establishment potential differed among sources. Survival of plants in monocultures did not depend on the source of the plant material, although survival did vary among blocks in 2002 ($P = 0.0124$, Table 6.6) and 2003 ($P = 0.0067$, Table 6.6). The likelihood of a plant being reproductive did not depend on either the source or the block in 2001 or 2003 (no plants were reproductive in 2002). In 2001, the likelihood of fruiting did not differ between sources or blocks. Because no plants were reproductive in 2002 and only four were reproductive in 2003 (all fruiting), no analyses on fruit production were conducted for those years. Leaf area of plants in monocultures did not depend on the source of the plants during any year, although it did depend on the microsite into which it was planted (block effect) in 2001 ($P = 0.0494$, Table 6.6) and 2002 ($P < 0.0001$, Table 6.6). Neither RGR_R nor RGR_{LA} differed among sources or blocks in any year (Table 6.6).

TABLE 6.6. Log-likelihood values (survival) and *F* ratios (RGR_R and RGR_LA) of monocultures in population introduction study. Probabilities of a greater F value or greater log-likelihood value are shown in parentheses.

Source	Survival to 2001	Survival to 2002	Survival to 2003	Leaf area 2001	Leaf area 2002	Leaf area 2003	RGR_R	RGR_{LA}
Rhizome initial dry weight	1.03	0.44	0.11	41.05	16.53	0.01	NA	NA
	(0.3097)	(0.5058)	(0.7384)	(<0.0001)	(0.0001)	(0.9154)	--	--
Source	0.02	0.05	4.06	0.52	1.16	2.16	5.11	1.25
	(0.9891)	(0.9770)	(0.1312)	(0.6367)	(0.4212)	(0.2485)	(0.0933)	(0.3915)
Block (source)	7.74	10.88	12.21	2.74	8.65	0.9787	0.70	0.34
	(0.0517)	(0.0124)	(0.0067)	(0.0494)	(<0.0001)	(0.4089)	(0.5543)	(0.7952)

Discussion

The reciprocal transplant study suggests that *H. canadensis* does not exhibit localized adaptation to the four sites in this study, providing no support for our hypothesis. Early 'source' population differences were most likely due to carryover effects from the previous season (Geber et al. 1997, Ohara et al. 2001), since the difference later disappeared. By the end of the study, plant size had responded to site differences, but these responses were not source-specific. The collective results of the source and site effects suggest that Morgantown may be a superior site for *H. canadensis* growth, and goldenseal did respond in a plastic manner to site quality over 3 years.

In a very long-lived perennial plant, plasticity, rather than genetic adaptation, may be more advantageous for long term survival (Grime et al. 1986, Schlichting 1986, Via et al. 1995). Bradshaw (1965) discussed conditions favoring plasticity in relation to plant generation time. He argued that recurrent environmental changes at intervals less than the generation time of the plant, would lead to selection for plasticity, rather than stability of traits. In an extremely long-lived plant such as *H. canadensis*, it seems likely that individuals able to successfully acclimate to *in situ* environmental change will persist and reproduce in the long run.

Studies testing for local adaptation in perennial species typically show that plasticity is common (Scheiner 1993). For example, Sultan and Bazzaz (1993) examined *Polygonum persicaria* genotypes from a moderately moist site and from a site exposed to both seasonal drought and flooding. They found plasticity in root characteristics in response to extremes in soil moisture. Plasticity has also been reported in native perennial species. Like *H. canadensis*, *Polymnia canadensis*, is an understory species of the eastern deciduous forest with a similar range and habitat characteristics. *Polymnia canadensis*, however, is facultative with respect to life history, ranging from winter annual, to biennial, and perennial. Bender et al. (2002) used reciprocal transplant studies to show that variation in life history, as well as growth rate, and age at maturity, occurred in response to environmental conditions and not via genetic adaptation.

The four sites in our study were all in mesic, older second growth forests with apparently suitable habitat. Overstory species at two sites (Ellenboro and Rivesville) included *Quercus alba* and *Q. rubra*, respectively, and differed considerably from the other two sites (Table 1). At the oak-dominated sites, there was a thick layer of leaf litter that may have influenced soil moisture and nutrients. Additionally, older forests with a large percentage of *Quercus* spp. are typically more open, allowing more light into the understory than forests (e.g. Morgantown and Jane Lew) dominated by *Acer saccharum* and *Liriodendron tulipifera* (Brewer 1980). Despite apparent differences between sites in light and moisture, these abiotic influences would be expected to change within a given site via periodic disturbance events such as tree fall and flooding.

Although we did not detect localized adaptation to the sites from which the plant sources were collected, the significant differential response of the four *H. canadensis* sources to blocks within the transplant sites may indicate microsite adaptation. Within a given location, small scale spatial structuring of the soil biotic environment occurs and can influence associated plant communities (Wardle 2002, Ettema and Wardle 2002). This can occur either by direct influence on the plant, such as heightened local densities of pathogenic fungi (Packer and Clay 2000) or indirectly by altering competitive interactions between plant species (Brown and Gange 1989, Olff et al. 2000). Small scale structuring can also occur in response to the abiotic environment. For example, Lechowicz and Bell (1991) found that three edaphic measures, soil pH, and K^+ and NO^{3-} in the soil solution, can vary at scale of 0.1 m. In a plant species with limited dispersal and outcrossing, small scale soil structuring can promote the maintenance locally adapted subpopulations. Thus, the differential response of the four source populations to microsites suggests possible coadaptation with both the local soil biotic community and abiotic components.

The results of the reciprocal transplant study suggest that some differentiation has occurred between sources, specifically with respect to microsite characteristics. The population establishment study was designed to test the significance of source differences when introducing new populations to a new area. Clearly, populations established from mixtures of sources displayed reduced growth compared with populations established with single sources. Because we did not detect differences in source plant performance in populations established with mixtures (Table 5) or in those established from only monocultures (Table 6), these results suggest antagonism between sources in the mixtures.

Reduced competition in clonal plant species as a result of genotype and ecotype recognition has been reported (Mahall and Callaway 1996). Self/nonself recognition by roots via both direct root contact and by soil diffusible substances (Mahall and Callaway 1992) occurs in clonal desert shrubs. Self recognition can promote strategic placement of roots for optimizing nutrient acquisition and reducing competition within a given genotype, even if ramet connections have been broken. While this may sometimes explain greater performance of the plants in monocultures than in mixtures, the relatively small size of the plants in this study, and the spacing between them, makes it unlikely that there was sufficient competition between the plants to elicit this type of response.

A second possible explanation for our results involves coadaptation, or at least "co-acclimation," between the plant sources and local pathogens. This could be manifested as preferential attack by a pathogen on specific nearby plant genotypes, or evidenced as acquired resistance of a plant species to local pathogens (Parker 1985, Parker 1989, Burdon 1987, DeNooij and VanDamme 1988, Burdon and Jarosz 1991, Bevan et al. 1993). At the initiation of the population introduction study, rhizomes were removed from their sources and cleaned prior to being planted into the nature preserve. Because these rhizomes were not subjected to sterilization, such as placing in bleach, there is a possibility that pathogenic microbes may have survived on the rhizomes. In introduced populations composed of multiple sources, opportunistic microbial attack of susceptible foreign plant sources may have occurred. Although this may explain the results of the population introduction study, biotic mutualisms may also bear on the reciprocal transplant study, since it also involved random mixing of source material. Source-dependent pathogens could indeed be one of the biotic environmental components that could explain the differential response of sources to microsites. If this mechanism was prevalent, however, we might have expected local genotypes to perform better in the among-site transplant study, and this pattern was not observed.

Relationships with mutualists might also explain the relative performance of the single source introductions compared with those of multiple sources. Greater reproductive output has been demonstrated in plants inoculated with local mutualist isolates compared with non-local isolates (Parker 1995). Root staining of *Hydrastis canadensis* has shown that it is symbiotic with endomycorrhizal fungi (Suzanne Sanders, personal observation). Mycorrhizal colonization of *P. peltatum* has been shown to be age-dependent (Watson et al. 2002), such that colonization of intermediate aged ramets is highest and colonization of younger ramets is non-existent. If mycorrhizal colonization was source-dependent, populations established from multiple sources (i.e. 6 ramets from each source) may not have been sufficiently inoculated with coadapted mycorrhizal fungi to establish as effective a mutualism as in monocultures.

Ecological genetic variation is typically ignored in studies of restoration and in conservation management (Rice and Emery 2003). However, our experimental results show how classical experimental approaches can have important implications for restoration. The differential response of sources both to microsites in the reciprocal transplant study and the population introduction study indicate that multiple sources should preferably be used as a bet-hedging strategy. This would increase the likelihood of compatible source-microsite combinations. However, because populations introduced using rhizomes from multiple sources exhibited reduced performance compared with those of single sources, introductions should be comprised of monocultures within the introduction site. Further, the results of the reciprocal transplant study support the idea that plastic responses to introduction site environment may be more important for long-term survival than overall genetic adaptation. The acceptability of single source population introduction has been corroborated by a recent breeding system study of *H. canadensis* (Sanders *in press*), that showed no differences in fruit set of ramets pollinated within sources from those pollinated between sources. The study also demonstrated the ability of *H. canadensis* to set fruit autogamously, and that fruit set did not differ between selfed and outcrossed ramets. Although the source composition of introduced populations is important, the spatial placement of these populations may be equally vital. The results of the reciprocal transplant study suggest differences in site quality exist, even within a given established population. Future research examining niche requirements, specifically with respect to biotic associations, would enhance our understanding of *H. canadensis* restoration ecology.

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CHAPTER 7:

Habitat Suitability Modelling of *Hydrastis canadensis*, *Actaea racemosa*, and *Panax quinquefolius* with Geographic Information Systems

Introduction

Determining suitable habitat of a species is important for preserve design (Rubino & Hess 2003), species reintroduction (Burnside et al. 2002), and to facilitate locating extant populations (Sperduto & Congalton 1996). Habitat suitability models may be especially useful for species that are experiencing population decline. This is because the current distribution of such species may not reflect the historical distribution of that species such that the current realized niche differs from the ecological niche (Malanson 1997; Ge & Chang 2001). Patterns of rarity, such as narrow niche breadth, are not always clearly evident from on-site, field observations and are in fact, difficult to document with traditional random sampling techniques (McGraw et al. 2003). Thus, habitat suitability models may elucidate habitat requirements and promote an understanding of niche for rare and/or declining species.

Goldenseal (*Hydrastis canadensis* L.), black cohosh (*Actaea racemosa* L. (Nutt.)), and American ginseng (*Panax quinquefolius* L.) are three species for which habitat suitability modeling may promote a better understanding of distribution and niche. The general habitat requirements are similar in that each species occupies "rich" understory areas of the eastern deciduous forest, although within this general context, specific patterns of distribution are unclear. *Hydrastis canadensis* is relatively uncommon, even in apparently suitable habitat, while *A. racemosa* is more abundant and appears to have a wider niche breadth. Like *H. canadensis*, *P. quinquefolius* is often not observed in apparently suitable habitat, although it is more frequently encountered than *H.*

canadensis. Each of these species has long been harvested from natural populations because of alleged medicinal properties, although it is presently unclear whether harvest is influencing the decline and limited distribution of these species. Interest in harvesting quantities of *A. racemosa* is a relatively recent phenomenon, driven in part by the recent search for natural alternatives to hormone replacement therapy that is no longer widely recommended in menopausal women ( ). Habitat suitability modeling, based on current, known locations of these species, may help explain the current distributions of both species, as well as provide estimates of the availability and occupancy of suitable habitat.

Several modeling techniques have been developed in recent years, combining the use of geographic information systems (GIS) and statistical analyses. The choice of modeling approach is often a function of the biology of the species under study. The relative rarity of each species being modeled, but especially of *H. canadensis*, presents a key problem when modeling habitat. Data about a particular species are often restricted to that of areas where the species is present. Examples of this include herbarium records and breeding/migrating bird surveys. It cannot be assumed that areas where a species is absent indicate unsuitable habitat. Because of this problem, use of modeling techniques based on presence data only, may provide viable options for modeling species habitat (Hirzel 2002). Such modeling techniques include the multivariate method, Mahalanobis distance (Boetsch et al. 2003; Farber & Ronen 2003), and operate under the assumption that absence of a species does not necessarily indicate unsuitable habitat, but rather that information about the presence in these areas is not known. If absence data is available, modeling techniques utilizing regression analysis become feasible.

For the present study, I developed models to predict the availability of suitable habitat of *H. canadensis*, *A. racemosa*, and *P. quinquefolius* within north-central West Virginia. The locations of populations for model development were determined by extensive communication with botanists, foresters, and land managers, as well as preliminary surveys conducted during summer, 2001. I also used the data from these preliminary surveys as confirmation of areas where the species were absent. This allowed for the development of models that incorporate absence data. Because habitat modeling of rare species often relies only on presence data, I also developed a habitat suitability models based on presence data only. This would allow me to compare the relative predictions of the two types of models. The models were then validated by 50 field surveys for the three study species within the study area.

The aim of this research was to address two key questions about these species. First, how much habitat for each of these species is available across the landscape? Developing models based on habitat characteristics of current known and unknown locations will provide such an estimate. Second, how do the models based entirely on species presence data compare with those based on confirmed known and unknown locations. I assessed this by looking at the number of errors between the two types of models as well as the overall spatial extent of classified suitable habitat.

Methods

Study Area

Habitat suitability models were developed for a 21,400 km^2 area of north-central West Virginia (Figure 7.1). The western two-thirds of the study area are part of the Appalachian Plateau, typified by rolling hills and with an elevation range of 177 meters to 450 meters. The eastern third of the study area is comprised of several anticlines of the Appalachian Mountains, and is characterized by steeper slopes, mostly of southeast and northwest aspects, and higher elevation, ranging from approximately 450 meters to 1,482 meters.

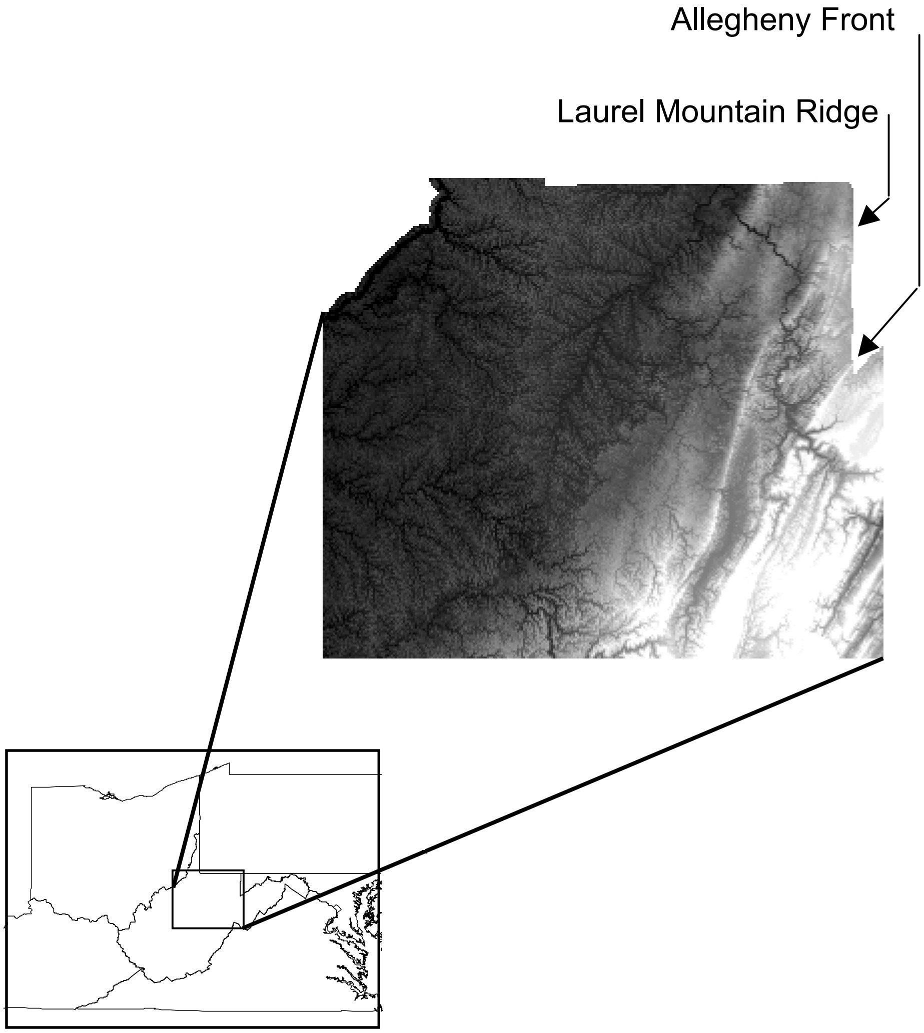
Study Species

Hydrastis canadensis L. is a perennial herbaceous species ranging from New York west to southern Wisconsin and south to Missouri and Tennessee. Non-reproductive ramets consist of a stem supporting a single leaf while reproductive ramets have two leaves with an apetalous flower. This species is highly clonal, reproducing from rhizomes as well as adventitious buds on lateral roots. *Hydrastis canadensis* ramets can grow singly or in large clumps, typically containing from 10 – several hundred ramets.

Actaea racemosa L. (Nutt.) is a perennial species with a range from Massachusetts to Missouri and south to South Carolina, Virginia, and Tennessee.

Although *A. racemosa* is entirely herbaceous, it is large, growing up to 2.5 meters tall and producing numerous

Figure 7.1. *Study location with a detail of topography.*


Allegheny Front
Laurel Mountain Ridge

branches from a central stem. Its large stature makes this species readily observable in its natural habitat. A raceme emerges in May and flowering occurs in June and July within the study area, however, at any given site, only a small percentage (~10%) of ramets has been observed to flower (personal observation). This species is a geophyte, sprouting each year from an underground rhizome. This rhizome, for which the species is harvested, contains alkaloids, glycosides, and salicylates (Small & Catling 1999). *Actaea racemosa* grows as a single plant, or in clumps, ranging from a few individuals to several thousand. Reproduction is largely by seed although little is known about the demography of this species.

Panax quinquefolius L. is a perennial species whose range is similar to *H. canadensis*, although it extends further south, to Georgia, Alabama, and Mississippi (Small & Catling 1999). This species displays stage structured growth, typically of 1 - 4 leaves (colloquially referred to as "prongs"), and each leaf composed of 3 – 5 leaflets (Charron & Gagnon 1991). Reproduction is nearly exclusively by seed although vegetative propagation via rhizome fragmentation has been reported (Lewis 1984; Van der Voort et al. 2003).

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Model Development

The predictive habitat models were developed from 10 data layers, with each layer representing a habitat variable (Table 7.1). Because the three study species grow exclusively in deciduous forests, I used a land cover/land use data layer to eliminate all pixels within the study area that were classified other than “deciduous forest,” or “mixed forest”. The overall spatial extent of the study region is 21,398 km^2, but areas of unsuitable land cover type comprised nearly 5,000 km^2, so that the total amount of land actually classified in the models was 16,595 km^2. All data layers were 30 m resolution and were reprojected as needed to universal transverse Mercator projection and WGS84 datum.

Two approaches to determining suitable habitat were used. First, predictive models were developed using logistic regression and were based on locations where the species were confirmed to be either present or absent (Table 7.2, Figure 7.2). Pixels representing areas where a species was present were assigned a value of 1 and those representing areas where it was absent were assigned zero. I regressed the dependent variable, probability of presence, on X, the pixel values of the independent variables at each location in each data layer. From this, I developed predictive equations:

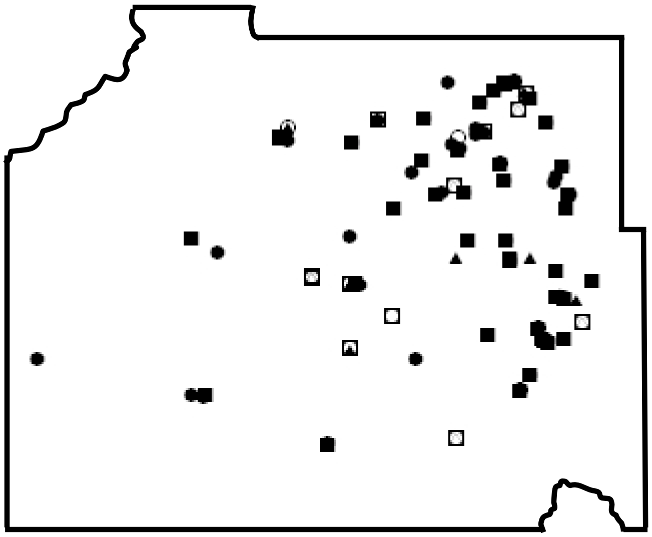
Table 7.1. *GIS data layers used for model development. The 10 continuous layers were used for initial development of the models, after which the land use/land cover layer was used to rule out all pixels classified other than "deciduous" or "mixed forest".*

Name	Description	Value range	Reference
Land use/land cover	assigns pixels into one of 21 classes based on vegetation and use	categorical	National land cover data set
Elevation	height above sea level	177 – 1,482 m	National elevation dataset
Terrain shape index	quantifies mesoscale landforms	-115 – 96	McNab 1989
Relative slope position	position on slope: valley bottoms = 0, ridge tops = 100	0 – 100	Wilds 1996
Planiform curvature	quantifies “steps” and “benches” in the landscape; Negative values depict areas that are upwardly convex while positive values depict upwardly concave values	-9.59 – 5.98	derived from elevation
Profile curvature	depicts “rolls” or areas where drainages cut through hillsides; negative values depict areas that are upwardly convex, such as areas between drainages while positive values depict upwardly concave values, such as the actual drainages	-7.8 – 10.2	derived from elevation
Slope	angle from horizontal	0 – 60.8°	derived from elevation
Aspect	direction of slope	1.0 – 3.0	Beers et al. 1966
Topographic relative moisture index (TRMI)	composite index of slope, aspect, and curvature values; lower values represent dry areas and higher values moist areas	0 - 60	Parker 1982
Solar insolation	represents the length of sunlight based on surrounding topography	39.33 – 210.67	derived from elevation
Distance to streams	horizontal Euclidean distance from all streams on hydrology digital line graphs	0 – 1,460 m	derived from stream vector layer

Table 7.2. *Number of sites used to develop the models where the species was either present or absent.*

Species	Present	Absent
H. canadensis	40	22
A. racemosa	41	14
P. quinquefolius	32	11

Figure 7.2. Hydrastis canadensis *sites are noted by circles,* A. racemosa *by squares, and* P. quinquefolius *by triangles. Solid symbols indicate locations of species presence, while hollow symbols indicate areas of confirmed absence. Not all symbols are clearly evident in the figure due to overlap.*



$$p = 1 / (1 + (\exp (- (b_0 + b_1X_1 + b_2X_2 + b_3X_3 + \ldots)))$$

where p is the probability of species presence in any given cell and ranges from zero to 1, b represents the regression coefficients, and X_i represents the individual cell values for the independent variables which are the GIS data layers. Values at and close to 1 indicate highly suitable habitat and values close to zero indicate low habitat suitability. Because all three study species can be present but unapparent within the understory herbaceous matrix, I limited areas where the species were designated as “absent” to include only those that were systematically surveyed for an earlier study, and in which the species were confirmed absent. For all three species, the absence variable is represented by fewer observations than the presence variable. The conservative nature of our choice to include absence areas should reduce errors in classification whereby areas might be considered relatively poor habitat, when in fact, the opposite is true.

For all three species, GIS layer values at each known and unknown location were determined and the regression coefficients were calculated with the Proc Logistic procedure in the SAS software package (SAS Institute 2001). Because all data layers are not necessarily important predictors of habitat, I also developed regression equations for each species using both forward stepwise logistic regression and backward elimination logistic regression in addition to the full models. For the forward model, the significance levels for entry and removal of variables were 0.3 and 0.35, respectively. For the backward model, 0.2 was used as the significance level for removing variables.

The second approach for modeling habitat used the Mahalanobis distance multivariate statistic. This method is based on location of species presence and does not include values of habitat variables where the species are confirmed absent. This statistic, D^2, is determined by;

$$D^2 = (\boldsymbol{x} - \boldsymbol{y})' \, \mathbf{S}^{-1} \, (\boldsymbol{x} - \boldsymbol{y})$$

where x is a vector of individual values for the independent variable at a known location, y is a vector of the means of that independent variable, and S^{-1} is the inverse of the variance – covariance matrix of all independent variables. Lower D^2 values indicate areas (pixels) that are closer in the multivariate data space to those of the known locations and higher D^2 values, areas more distant. Hence, this modeling metric differs from that of logistic regression, because higher values indicate better habitat.

All data layers were imported into Erdas Imagine image processing software package (Leica Geosystems, 2002). A signature file of presence locations was developed and a Mahalanobis distance classification was performed using the supervised classification routine.

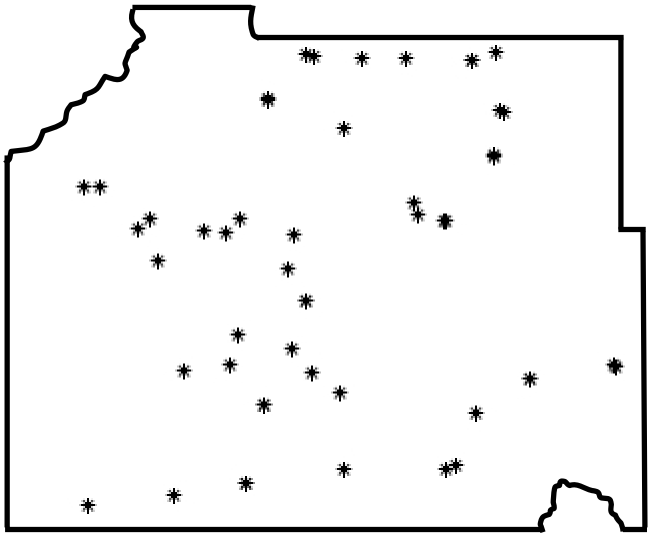
Field Surveys

The models were validated by 50 field surveys conducted during summer, 2002 (Figure 7.3). Survey sites were chosen so that they represented a range of habitat quality. Each survey was a 60 m × 60 m quadrat that was further divided into nine subplots of 20 m × 20 m. A GPS reading was obtained at each of the four corners of the quadrat. A three person field team searched the quadrat for the three study species by walking six 10 meter wide swaths through the area. The number of all *H. canadensis* ramets and *A. racemosa* and *P. quinquefolius* plants were counted and the subplot in which they were located was noted. Each survey required approximately 10 person hours to complete since thorough searching required careful, slow movement across the surveyed area.

Statistical Analysis

The spatial resolution for all GIS data layers was 30 meters, while our surveys were 60 m × 60 m and did not necessarily align with the pixels. As such, there were multiple pixels within each surveyed area. I obtained the mean of all pixels falling within each surveyed area, using the rules for pixel inclusion from Erdas Imagine image processing software (Leica Geosystems, 2002). This mean for each site was then taken to be the predicted habitat suitability value (hereafter referred to at the “prediction value”) for that site.

Figure7.3. *Locations of the 50 model validation surveys.*



I performed Wilcoxon tests to determine if the prediction values of surveyed sites where a species was found differed from the values where the species were not found. This allowed me to determine if the models captured important differences in habitat.

The developed models resulted in pixels classified across a continuous range of prediction values, rather than a categorical classification (*i.e.* suitable or unsuitable). In order to understand whether availability of suitable habitat was limiting to any of these species, I wished to convert the continuous gradient of suitability to a binary classification: suitable vs. unsuitable. I adopted a series of threshold points across the continuous range of prediction values. For the logistic regression models, pixels with values above the thresholds were considered to represent suitable habitat and pixel values below the thresholds were considered unsuitable. Since lower D^2 values in the Mahalanobis distance models indicate habitat more similar to that at the known sites compared to pixels with higher D^2 values, in these models, pixel values at survey sites that were below the threshold points were considered suitable, while those above were deemed unsuitable.

I assessed model accuracies by comparing habitat predictions for each of the surveyed sites with the actual outcome for that survey site. Each survey site was assigned to one of four categories (Liebhold et al. 1998, Table 7.3). Those where a study species was accurately predicted to be either absent or present at a given threshold value were classified as either X_1 (predicted absent and not located) or X_4 (predicted present and found). Errors were classified in a similar manner. Errors of omission, X_2, were those

Table 7.3. *Four accuracy classification categories based on model predictions and survey observations.*

		observed	
		absent	present
predicted	absent	X_1 (correct)	X_2 (error)
	present	X_3 (error)	X_4 (correct)

survey sites where a species was predicted absent at a given threshold, but was found. Finally, errors of commission, X_3, occurred at those sites where a species was predicted present, but it was not located. This allowed me to construct error plots for each species and model combination. At each threshold point, I calculated error 2 and error 3 where:

$$\text{error 2} = X_2/(X_1 + X_2)$$

$$\text{error 3} = X_3/(X_3 + X_4).$$

From these plots, I examined the distribution of error and could determine an optimal predictive value to define habitat as suitable where both errors were minimized.

Results

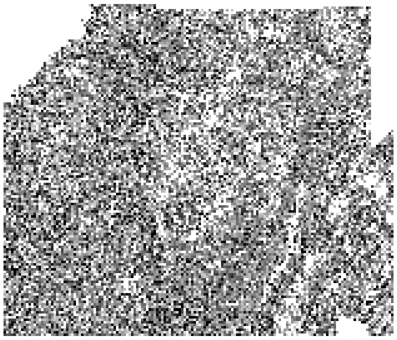
There was a general similarity in all of the models in that they predicted reduced habitat quality within the mountainous regions of the study area compared with the Appalachian Plateau (Figure 7.4). For all models, there is a distinct boundary in habitat quality along Laurel Mountain Ridge (see Figure 7.1), with a secondary boundary in many of the models along the Allegheny Front. For both *H. canadensis* and *A. racemosa*, the three types of logistic regression models tended to be very similar to each other in their predictions for those species. The mean pixel prediction value for the full logistic regression model for *H. canadensis* is 0.62, although the distribution is skewed to the left (Figure 7.5). Both *H. canadensis* partial regression models predicted generally higher habitat quality than the full model, although the general patterns were similar.

Figure 7.4. *Predictive maps of habitat for* H. canadensis*,* A. racemosa*, and* P. quinquefolius*.*

H. canadensis
full logistic regression

H. canadensis
forward logistic regression

H. canadensis
backward logistic
regression



H. canadensis
Mahalanobis distance

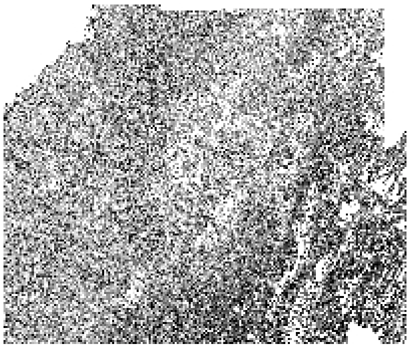
A. racemosa
full logistic regression

A. racemosa
forward logistic regression

A. racemosa
backward logistic
regression

A. racemosa
Mahalanobis distance

P. quinquefolius
full logistic regression

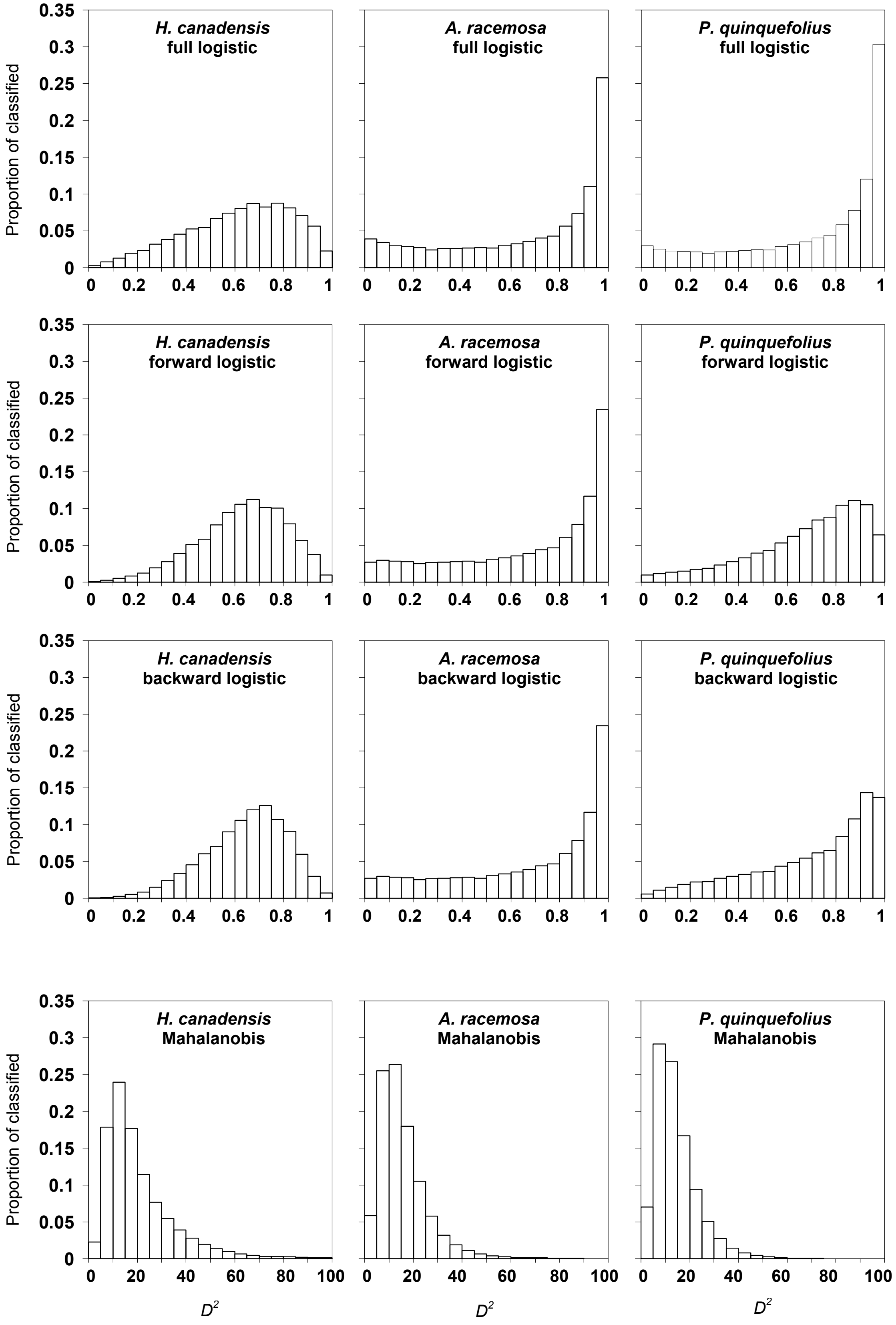


P. quinquefolius
forward logistic regression

P. quinquefolius
backward logistic regression

P. quinquefolius
Mahalanobis distance

Figure 7.5. *Histograms showing the proportion of pixels in suitability classes. The top three rows show this for the logistic regression models, while the lower row shows the frequency distributions for the Mahalanobis distance classifications.*


H. canadensis full logistic
A. racemosa full logistic
P. quinquefolius full logistic
H. canadensis forward logistic
A. racemosa forward logistic
P. quinquefolius forward logistic
H. canadensis backward logistic
A. racemosa backward logistic
P. quinquefolius backward logistic
H. canadensis Mahalanobis
A. racemosa Mahalanobis
P. quinquefolius Mahalanobis
Proportion of classified
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.2
0.4
0.6
0.8
1
20
40
60
80
100
D²

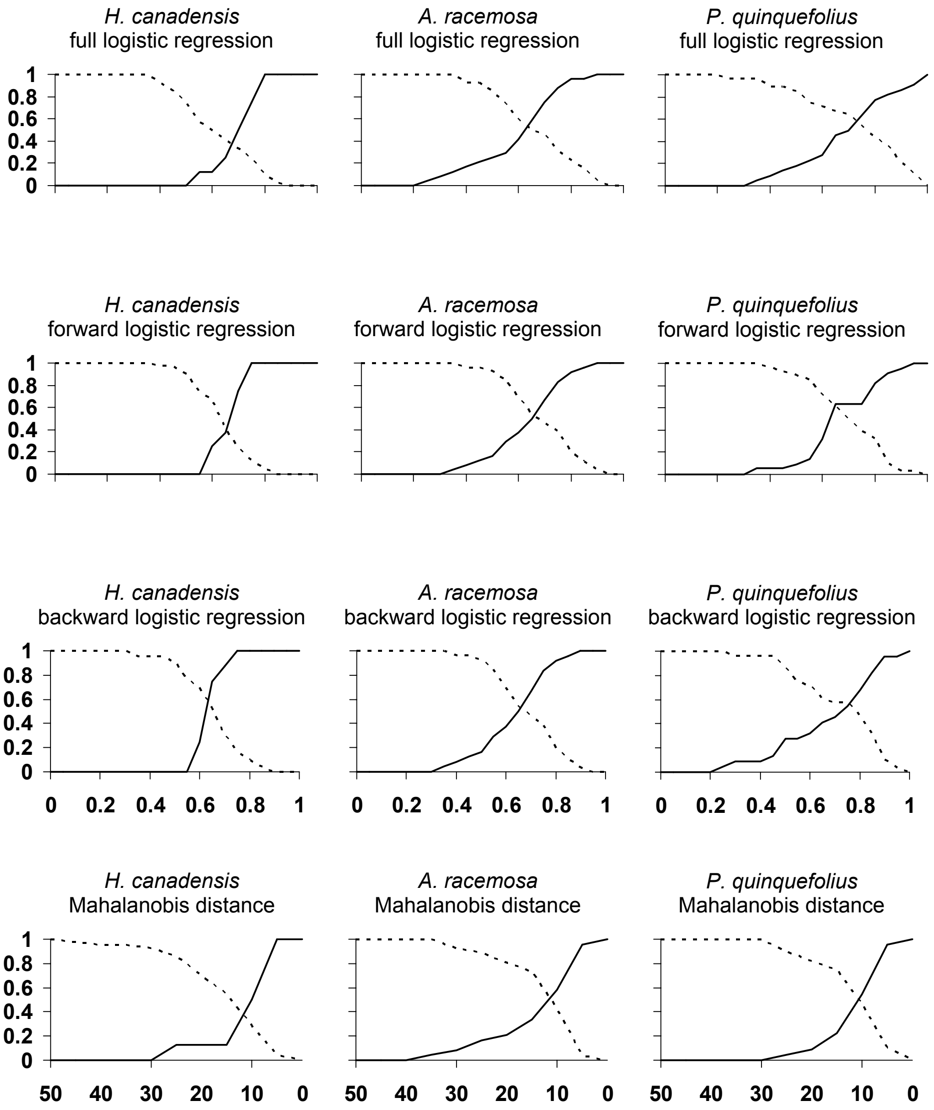
For *A. racemosa*, the stepwise addition and backward elimination regressions resulted in the same partial regression coefficients and hence, the same data layers were included (Table 7.4). Thus, only one map was developed for these two models. The means of pixel prediction values were similar between the two models, 0.674 and 0.681 for the full and stepwise models, respectively. While the *H. canadensis* regression models classified 3.6 – 7.9% of the pixels as having a 0.9 or greater probability, the *A. racemosa* full and partial models placed 37 and 35% of pixels respectively, in this class (Figure 7.5).

In contrast to the models for *H. canadensis* and *A. racemosa*, the three logistic regressions for *P. quinquefolius* resulted in dissimilar habitat predictions. The full model classified 42% of pixels as having a probability of 0.90 or greater and a mean probability of 0.72. The stepwise forward and backward elimination models classified 17% and 28% as a probability of 0.90 or greater (Figure 7.5) with mean probabilities of 0.68 and 0.71, respectively. Four of the ten data layers (elevation, slope, aspect, and stream distance) were included in the full model but not in either of the stepwise models. If the values of the *P. quinquefolius* known locations for any given variable do not differ from the values of that variable as a whole, then that particular variable may not be a good predictor of habitat. This is because there would not be an optimal range of habitat within predictive habitat variable. This could lead to incorrectly large amounts of habitat being classified as suitable. This may indeed be occurring with *P. quinquefolius* for one of the four habitat variables listed above.

Table 7.4. *Data layers included in the stepwise addition and backward elimination logistic regression models.*

Species	Model	Layers
H. canadensis	Stepwise addition	terrain shape index, profile curvature, slope
H. canadensis	Backward elimination	terrain shape index, profile curvature, insolation
A. racemosa	Stepwise addition	terrain shape index, profile curvature, slope, aspect, topographic relative moisture index
A. racemosa	Backward elimination	terrain shape index, profile curvature, slope, aspect, topographic relative moisture index
P. quinquefolius	Stepwise addition	planiform curvature, topographic relative moisture index, insolation
P. quinquefolius	Backward elimination	terrain shape index, relative slope position, profile curvature, topographic relative moisture index, insolation

Figure 7.6. *Error 2 and Error 3 plots for the range of probability and* D^2 *values. Values on the horizontal axis are the prediction values of the surveyed sites, and values on the vertical axis are the proportion in error.*


H. canadensis
full logistic regression
A. racemosa
full logistic regression
P. quinquefolius
full logistic regression
H. canadensis
forward logistic regression
A. racemosa
forward logistic regression
P. quinquefolius
forward logistic regression
H. canadensis
backward logistic regression
A. racemosa
backward logistic regression
P. quinquefolius
backward logistic regression
H. canadensis
Mahalanobis distance
A. racemosa
Mahalanobis distance
P. quinquefolius
Mahalanobis distance
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
50
40
30
20
10
0

The Mahalanobis distance classifications showed overall predictive patterns that were similar to the logistic regression classifications. For *H. canadensis*, only 2.3% of pixels were classified in the highest class where $D^2 < 5$. In contrast, 5.8% of the pixels for *A. racemosa* and 7.0% of the pixels for *P. quinquefolius* were classified as $D^2 < 5$.

Of the 50 surveyed sites, *H. canadensis* was located in eight, *A. racemosa* was found in 24, and *P. quinquefolius* was present at 22 sites (Table 7.5). Wilcoxon test comparisons of prediction values between sites where a species was located and sites where a species was not found were not significant (Table 7.6).

Error plots showed that Error 2 tended to be minimal in the logistic regression models below probabilities of about 0.6 although it differed between the full and partial models. For the full models, there was a sharp threshold in Error 2, such that it was near 0 at probability values of about 0.6, while it was at 1 around probability values of 0.8. In the stepwise logistic regression models, Error 2 tended to increase gradually from probabilities of about 0.3 to 1. In the Mahalanobis distance models, Error 2 showed patterns similar to that of the partial logistic regression models.

Error 3 for all models tended to show a gradual decrease from predictive values of 0.4 to 0.9 in logistic regression and from 30 to 5 in Mahalanobis distance.

Table 7.5. *Number of surveys in which each species was present and absent. For each species, the mean, range, and standard error are presented only for those surveys where the species was present.*

Species	Present	Absent	Mean	Range	s.e.
H. canadensis	8	42	635	1 – 3,054	386
A. racemosa	24	26	317	1 – 2,230	120
P. quinquefolius	22	28	11	1 - 49	2.7

Table 7.6. *Wilcoxan test comparisons of species presence and absence at each of the 50 surveyed sites. The dependent variable is the prediction value: p for logistic regression models and* D^2 *for Mahalanobis distance. Comparisons are calculated based on the number of surveyed sites where each species was present vs. absent:* H. canadensis *(8/42),* A. racemosa *(24/26),* P. quinquefolius *(22/28).*

Species	Model	X^2	p
H. canadensis	Mahalanobis distance	1.55	0.2136
	full logistic regression	1.75	0.1858
	stepwise forward logistic regression	1.12	0.2898
	stepwise backward logistic regression	0.25	0.6151
A. racemosa	Mahalanobis distance	0.18	0.6692
	full logistic regression	1.14	0.2855
	stepwise forward logistic regression	1.40	0.2362
P. quinquefolius	Mahalanobis distance	0.07	0.7844
	full logistic regression	0.64	0.4230
	stepwise forward logistic regression	0.50	0.4878
	stepwise backward logistic regression	1.42	0.2332

Discussion

The habitat model predictions for *H. canadensis* and *A. racemosa* are consistent with other ecological observations. Of the three study species, *A. racemosa* appears to have the broadest niche. This was supported by the models that consistently predicted a relatively high percentage of the study area to be highly suitable for *A. racemosa*. This supports the findings of Hicks (1980) who examined distribution patterns of southern Appalachian herbaceous species. Of 19 "important" understory, herbaceous species, *A. racemosa* had a broad niche breadth, narrower only than *Euonymus* sp., *Laportea canadensis*, *Aster acuminatus*, *Aster divaricatus*, and *Dryopteris noveboracensis*. Like *A. racemosa*, these species all grow over a range of habitat types.

The model predictions for *H. canadensis* are somewhat more conservative than those for *A. racemosa* with regard to the amount of land classified as highly suitable. Nonetheless, over half of the study area was classified by the logistic regression models to have a probability of 0.6 or greater. This is not inconsistent with our observations of *H. canadensis*. Although anecdotal reports allude to strong associations with shade, north facing hillsides, and low slopes, I have not found this to be accurate. The 40 known locations used to develop the model spanned a range of aspects and slope positions. This suggests that distribution of *H. canadensis* is limited not by the availability of suitable habitat, but by some other mechanism.

Predictions for availability of habitat for *P. quinquefolius* varied between the logistic regression models and is likely due to the layers that were included in the stepwise models.

Although the full model predicted a 42.3% of habitat to have a likelihood of *P. quinquefolius* presence of > 90%, the forward addition and backwards elimination models predicted only 16.97% and 28.01% respectively.

Because of the large number of steps involved in developing the habitat models, error will necessarily be incorporated into them. One potential source of error was bias in the locations of known sites used for development of all models. For this study, I made an exhaustive effort to locate as many populations as possible so that they could be included in the models. To accomplish this, I consulted with botanists, foresters, hunters, and land managers. In addition, I examined labels on herbarium specimens for location clues. As a result, there is an evident clustering of known locations toward the eastern half of the study area (Figure 2). This occurs because the individuals I consulted have a greater knowledge of this area and not necessarily because there are more populations in this area. This type of bias is likely not present in the absence sites included in the logistic regression models. These locations were based on randomly located surveys in which the species of interest were not located.

Because these three species are harvested from natural populations, the individuals and populations that do not get harvested represent the realized niche of these species, rather than the ecological niche. While it is possible that these could be identical, the realized niche is likely to be a subset of the ecological niche with the realized niche being those areas where long-term harvest pressure is least. Because our models were developed based on an exhaustive effort to locate all populations within the study area, our models should therefore indicate the realized niche of these three species, rather than the ecological niche. The known sites used to develop the models may have been in suboptimal habitat and are present there because these areas represent refugia where harvesters do not typically search. This could lead to a higher likelihood of predictions for ecologically suboptimal habitat. Rock et al. (1999) modeled *P. quinquefolius* habitat in the Great Smoky Mountains National Park. The authors subsequently located two large populations (approximately 160 plants each) in areas classified as only “moderately suitable”.

The model validation process for a rare species must be interpreted with caution. Although the logistic regression models are developed based on the likelihood that a species will be present in a given area, one must be careful not to make the assumption that habitat with a high probability of presence is expected to be saturated (Capon et al. 1986). For example, where metapopulation dynamics are occurring, only a fraction of suitable habitat is occupied at any given time (Hanski et al. 1996). Habitat validation for a harvested species adds another degree of uncertainty to the validation process. It is difficult to ascertain a clear distinction of whether the habitat is truly unsuitable, or whether the species is not present due to some historical event, *i.e.* harvest. For these collective reasons, Error 3, which occurs when a species is predicted to be present but it is not located, is of less concern to me than Error 2.

Because so much of the area is predicted to be suitable habitat, it might be considered surprising that the study species, and especially *A. racemosa*, were not located in more surveys. It must be noted, however, that there are a number of factors influencing habitat that are not considered in this model, including depth of litter and organic layers, nutrient availability, and pH. Little is known about how these factors influence the three species. One factor that I was unable to incorporate into the model, that has been shown to influence *A. racemosa* distribution is stand age. This is due to the limited dispersal ability of *A. racemosa* (Matlack 1994).

Actaea racemosa is a geophyte, so that vegetative reproduction is limited to resprouting of buds on an underground rhizome. Seed production occurs in only a small percentage of plants, and may be linked to plant age (Small and Catling, 1999). Because the only known dispersal mode is via gravity, dispersal into new and/or distant areas is slow, and leads to the tendency of this species to grow in patches. Matlack (1994) examined migration of herbaceous species from old regrowth forests into contiguous successional stands and found that *A. racemosa* did not migrate (i.e. rate = 0 m yr^{-1}) into the younger stands. He also found differences in frequency of *A. racemosa* in old regrowth stands (9/17 sites) compared with disjunct successional stands (6/26 sites) ($p = 0.05$). The limited vegetative reproduction and relatively long period to seed set suggests a strong, adverse response to harvest. As with *Panax quinquefolius* L., harvest of *A. racemosa* is completely destructive to the plant, and possibly, to entire populations.

Our models showed that availability of suitable habitat is not limiting *H. canadensis* distribution. Two possible mechanisms that may be limiting *H. canadensis* distribution are seed dispersal and seed germination. *Hydrastis canadensis* berries are bright red and clearly visible above the plant, suggesting adaptation for bird dispersal. However, berries are commonly observed to fall to the forest floor upon maturity and remain undigested. A second, related factor possibly limiting *H. canadensis* abundance is poor seed germination. Germination percentage of *H. canadensis* seed is low and variable (Davis and McCoy, 2000) and it is not known what affects germination rate. The combination of these two factors may indicate a reduction of avian dispersal agents.

In addition to seed dispersal and germination limitations, another possible factor contributing to reduction in the number of *H. canadensis* populations may be harvest. Unlike *A. racemosa*, however, harvest of *H. canadensis* rhizomes is not completely destructive to the plant. Van der Voort et al. (2003) monitored an *H. canadensis* patch that had been poached in August, 1995. Although the initial ramet count was not known, four ramets remained following harvest. In 1996, 932 stems were present in the patch. The medium-sized patch was monitored for the following three years, during which time, leaf size tended to increase and there were never fewer than 800 ramets. Regrowth from harvest is possible because harvest of rhizomes causes a breakage of the lateral root. The root, which are left in the ground, have buds that are capable of producing adventitious shoots and hence, new aboveground ramets. Van der Voort *et al*. (2003) found that 12 percent of experimentally planted root material generated aboveground ramets. Because of this, I feel that harvest may not be great enough to produce the observed rarity.

The model predictions for *P. quinquefolius* are less straightforward than for *H. canadensis* or *A. racemosa*. While there are marked differences in the predictions about high quality, they do indicate that suitable habitat for *P. quinquefolius* is not limiting. The impact of harvest on model development may be greater for *P. quinquefolius* than for either *H. canadensis* or *A. racemosa* because harvest pressure is greatest on this species.

This research has shown that, in general, habitat is not limiting for any of the three species, but that the niche for each species may not be fully realized. Although harvest may play a small role in explaining why this occurs, the models presented here suggest that abundance and distribution of these three species in north central West Virginia may be limited by other mechanisms.

Acknowledgments

I am grateful to numerous individuals for assistance with locating populations and conducting field surveys. This work was funded by grant 1434-HQ-97-RU-01563 from the U. S. Department of the Interior and the U. S. Geological Survey.

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General Conclusions

It is becoming increasingly clear that *Hydrastis canadensis* populations are in decline (Sinclair and Catling, 2000). This reality was shown by the present research, and has recently been corroborated by others (Mulligan and Gorchov, 2003). It is essential that the causes of this decline are elucidated in order to prevent further population loss and and advance restoration efforts.

This research tested several hypotheses about causes of rarity, and many of my findings were somewhat unexpected in a species experiencing population decline. In my study to determine the breeding system of *H. canadensis*, I found that this species can produce viable seed *via* self fertilization and both within-patch and between-patch outcrossing. Because patches often contain many flowering ramets, mate limitation is likely not a causal agent of rarity.

The inability of species to adapt to habitats is sometimes cited as a contributing factor to decline (Antonovics et al., 1971; Benkman, 1995; Davison and Reiling, 1995). This has special relavance considering the vast amount of anthropogenic environmental change within the previous century. I found that *H. canadensis* likely exhibited microsite coadaptation with microbial soil biota, and also demonstrated plasticity in response to transplant sites. Collectively, these traits can allow a population to adapt to long term unchanging conditions, but also respond to repeated disturbance and change. It is therefore likely that *H. canadensis* possesses ecologically relevant genetic variation, and a lack therof is not contributing to its increasing rarity.

The availability of suitable habitat can limit a species' distribution, especially if this species is highly specific with regard to habitat requirements. I tested these ideas in two separate studies. In Chapter 5, I tested the response of *H. canadensis* phytometers to temperature, humidity, and light within microsites. My results showed increased leaf area with decreased irradiance. Because there is a positive response of *H. canadensis* to paths and forest edges (Sinclair and Catling, 2000; Sanders and McGraw, 2002), increased leaf area, in response to the low irradiance which typically reaches the forest floor under a closed canopy mid-successional forest, may be unfavorable for *H. canadensis* growth. The degree to which reduced light can inhibit *H. canadensi*s growth and persistance is unknown. A large percentage of the eastern deciduous forest was cleared in the early part of the twentieth century, although much of this land has since been allowed to return to a forested state. As such, many of these forests are currently in the mid-successional stage. Brewer (1980) examined changes in the forest herb community in a maturing *Fagus grandifolia* - *Acer saccharum* forest in Michigan from 1919 – 1977. Of 30 species originally classified as "abundant" at the beginning of the study, only 13 were classified as such by the conclusion. Many of the species that had experienced decline were present only at edges and in ravines by the end of the study period. The increase in dominance of *A. saccharum* and *F. grandifolia* as the forest matured could have reduced light reaching the forest floor. This is because many of the mid-successional shade intolerant trees being replaced by *F. grandifolia* and *A. saccharum*, including *Tilia americana*, *Fraxinus americana*, *Carya cordiformis*, *Liriodendron tulipifera*, *Carya ovata*, and *Quercus rubra*, leaf out later than *A. saccharum* and *F. grandifolia* (Brewer, 1980). The time period between leaf out of *A. saccharum* and that of earlier successional overstory species might be a period of rapid growth for many forest herbs, including *H. canadensis*. As the forest matures, greater shading during critical periods of growth might reduce dominance of certain understory herbs. Indeed, Brewer's findings of spatial patterns of understory herb dynamics are consistant with my findings of *H. canadensis* dynamics at the Bryan Nature Preserve. Both studies reported reductions in abundance of shade tolerant species in the forest interior, but that these species were later primarily located, or even restricted, to forest edges. Longer term studies (*i.e.* 5 – 10 years) of *H. canadensis* performance under mature forests both with and without supplemental shading may elucidate this idea.

In contrast to responses to light, I did not find clear relationships between plant growth and temperature or humidity. The relatively weak association between *H. canadensis* performance and temperature and humidity variation across the forested cove reinforces other studies (see below) suggesting that *H. canadensis* has a relatively broad ecological niche, and its rarity is unlikely due to availability of microsites with suitable temperature and humidity conditions.

Some species' distributions are constrained by habitat topography (Rubina and McCarthy, 2003). In Chapter 7, I explored the relationship between topographic variables at extant *H. canadensis* populations, and the availability of areas of similar topography across north-central West Virginia. I modelled habitat using 3 separate methods, and for each, I found that availability of topographically similar habitat was not limiting.

The final possible causal agent of *H. canadensis*' rarity I explored was that of harvest effects. My research showed two of three harvested patches did exhibit evidence of recovery, by increasing ramet leaf size between one and two years post-harvest. My results also suggest that patch recovery may be affected not only by harvest timing, but also by patch disturbance history. Clearly, a full understanding of responses to harvest will require long-term studies. Additionally, *H. canadensis* response to harvest, both at the species and population level, will depend on harvester behavior. Finally, frequency of harvest will also impact recovery.

I noted earlier that many of my findings were somewhat unexpected in a species experiencing population decline. Indeed, *H. canadensis* is unusual among rare species because it occupies a wide geographical range. In general, species abundance is positively correlated with size of geographic range (Brown, 1984; Gaston, 1994), so that most rare species have small ranges. *Hydrastis canadensis* is in a relatively uncommon class of rarity (Rabinowitz, 1981) because it has a wide geographic distribution, but genets are only sparsely distributed (McGraw et al., 2003). This correlation between abundance and range size is not necessarily inconsistant with the distribution and abundance of *H. canadensis*, at least historically. It is known that the geographic range spans much of the eastern deciduous forest (see Chapter 1). It is also known that this species has experienced population decline within the previous 100 years. It is clearly possible, if not probable, that *H. canadensis* was once a common or even dominant species within the understory. Although my findings implicate changes in understory light availability as a possible causal agent of population decline, there remain other possible agents which were not explored in this research.

Chief among other causal agents of rarity is that germination percentage of *H. canadensis* seeds is low and, when it occurs, variable (Davis and McCoy, 2000). *Hydrastis canadensis* seedlings display only cotyledons during the first season of emergence (Tobe and Keating, 1985) and, as such, can be readily distinguished from older plants. While I am familiar with the appearance of *H. canadensis* seedlings, I have never observed them at field sites, despite intense searching. Further, the bright red, fleshy berry is suggestive of avian dispersal. Although consumption by birds has been reported (Sinclair et al., 2000), I have frequently observed berries on the ground in proximity to reproductive ramets, suggesting that they simply fell after ripening on the plant. Studies testing hypotheses about seed germination, and dispersal, would clarify our understanding of *H. canadensis* rarity.

In addition to germination limitations, changes in disturbance regime may also influence the distribution and abundance of *H. canadensis*. One obvious altered disturbance regime is the reduction in fire within the eastern deciduous. Increased vegetative spread of *H. canadensis* following controlled burns has been observed in Missouri (Dan Drees, Missouri Department of Natural Resources, Jefferson City, personal communication). A second major altered disturbance regime is flooding. Where observed, *Hydrastis canadensis* is often located slightly above the floodplain. High water events that occur on the order of every 10 – 20 years could deposit propagules and scour soil and existing vegetation, creating new microhabitats. Indeed, soil turnover prior to transplantation of rhizomes has been shown to increase transplant size, and flowering and fruiting percentage (Sinclair and Catling, 2003).

The present research provides some answers to critical questions about causes of population decline of *H. canadensis*. It also provides a great deal of insight into the biology of *H. canadensis*, including responses to abiotic and biotic pressures. This insight can, in turn, be used in the direction of future research exploring population decline of *H. canadensis*.

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