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Community Response to False Hellebore (*Veratrum californicum* Durand) Harvest 18 Years after Treatment

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A thesis submitted to the faculty of
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In partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

Community Response to False Hellebore (*Veratrum californicum* Durand) Harvest 18 Years after Treatment

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Discoveries revolving around false hellebore (*Veratrum californicum* Durand) have caused a paradigm shift in treatment from eradication to harvest and preservation. Test plots set in place 18 years ago to analyze the effectiveness of eradication treatments (tilling, herbicide, mow, and remow) give us a better idea of how false hellebore communities might respond to disturbances caused by harvest. We focused mainly on the tilling, mow, and remow treatments because of similarities to harvest techniques.

We found that mow and remow treatments have little effect on the population of false hellebore in the wild. Tilling treatments were effective in reducing the population dramatically; however some recovery in numbers has taken place. Tilled plots showed a significant decrease in mid seral plant populations, and a significant increase in early seral populations. Tilled treatments were also opened up to intermediately desirable and undesirable plants. Mow and remow treatments reacted similarly to each other, with mow treatments showing decreases in mid seral species and with both treatments showing neither an increase nor a decrease in the other seral stages. Mow and remow plots showed a decrease in desirable plants, but an increase in intermediately desirable plants and no increase in undesirable plants.

Greenhouse experiments were unsuccessful due to phenological disruptions caused by removal from the native habitat as well as climate and temperature differences.

Keywords: Veratrum californicum, false hellebore, rhizome, propagation, harvest, treatment

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Chapter 1

Introduction

False hellebore (*Veratrum californicum* Durand [Liliaceae]) is a native perennial plant known to cause birth defects in lambs after maternal consumption (Binns et al. 1963). To facilitate safe grazing, various studies have been implemented to determine the effectiveness of different treatments in the removal of false hellebore from ecosystems (Williams and Kreps 1970, Williams and Cronin 1981, Williams 1991, Anderson and Thompson 1993, Cosgriff et al. 2004). Spread of false hellebore through a plant community is thought to be facilitated by overgrazing (Loft et al. 1987, Anderson and Thompson 1993). False hellebore is known to grow in “meadows and stream banks in grass-forb, aspen, mixed conifer, and spruce-fir communities at 1830 to 3115 m…” (Welsch et al. 2003). False hellebore is thought to take around 10 years to reach maturity, and may survive up to 100 years (Taylor 1956). Recent discoveries have found cyclopamine, a secondary compound produced by this plant, to be effective in cancer treatment (Taipale et al. 2000, Chen et al. 2002, Olive et al. 2009). Cyclopamine, which is found in greatest concentration in the rhizome of false hellebore, is known to disrupt the sonic hedgehog signaling pathway and thus inhibit cell division (Incardona 1998). These recent discoveries regarding the usefulness of compounds found in false hellebore rhizomes have caused a shift from viewing false hellebore as a plant that must be controlled and eradicated, to a view that false hellebore must be protected and preserved for pharmaceutical use.

Earlier research into the uses of plant secondary compounds led to interest in propagation of false hellebore and other similar species in a greenhouse setting (Taylor 1956). Seed germination and rooting from vegetative cuttings have been shown to be labor-intensive and unsuccessful. Taylor (1956) stated that the best method to obtain the valuable secondary compounds in false hellebore is to harvest it from the wild. Because knowledge of false hellebore and its physiological and ecological traits have centered mostly on control or eradication of the plant, little research has been done specifically to understand how this plant and the alpine community in which it thrives might react to harvest. By analyzing plots set up nearly 20 years ago to test the effectiveness of tilling, herbicide, mowing, and seeding treatments in the eradication of false hellebore, we have gained insight into recovery time and plant community response to major disturbances. We gathered plant community data to determine occurrences of desirable, intermediately desirable, and undesirable plants, as well as data to determine occurrence of plants in early, mid, and late seral stages in each treatment area. Early seral species are those plants that recover most quickly from disturbance and are among the first plants to repopulate the area. Late seral species are plants that rely on the occurrence of earlier seral species in the area in order to thrive. A high recovery rate within a plant community is directly related to a high occurrence of late seral species in the area. Study sites revisited 18 years after initial treatments are particularly insightful because of similarities in false hellebore rhizome harvest to tilling treatments and of vegetative harvest to mow and remow treatments.

Our hypotheses associated with this study are 1) that false hellebore populations would recover from all treatments over time, 2) that false hellebore recovery would be slowest in the tilled treatments, 3) that mow and remow treatments would have the highest recovery of false hellebore and the lowest occurrence of undesirable plants, and 4) that seeding would increase desirable plant species in the community across the treatments.

Materials and Methods

Our study site is located in "…Spring Canyon of the Price Ranger District (Manti-Lasal National Forest), Sanpete County, Utah (N 390 36' W 1110 17'). The area is a mountain wet meadow at 2,880 m, running along the base of a steep slope and subjected to deep snow drifts annually. The soil is classified as a silty clay loam, 36 to 76 cm deep (Ellison 1954). Soil pH is approximately 6.3 and soil moisture varies from 40 to 15% between spring and late fall (Ellison 1954). Annual precipitation varies between 60-70 cm, most of which occurs between November and May. The area is grazed by sheep from 1 July to 1 October, but historical grazing pressures were much higher. Dense patches of false hellebore occupy a high percentage of this drainage" (Cosgriff et al. 2004).

In July of 2009, we followed the same patterns of site analysis used on these populations 18 years ago to classify plant community response to the various treatments. "A baseline inventory of false hellebore stem density, nested frequency, and species richness in the patches along the drainage allowed for 3 uniform patches to be selected as blocks for the study design. In June of 1991, a block measuring 30 by 60 m was measured and divided into 10 plots (6 by 30 m) from each patch. The study was arranged as a Generalized Randomized Complete Block Design (Steele and Torrie 1980). Five treatments were randomly assigned twice within each block, giving 2 replications per block. This allowed for testing treatment response differences between patches within the meadow" (Cosgriff et al. 2004). The study sites were enclosed by a livestock fence to ensure that grazing would not impact the study sites. After the 1999 sampling, these livestock fences were no longer maintained, so complete grazing exclusion could no longer be assured in the 2009 samples. Fences never excluded wildlife from entering the study area.

"Each block was split, half of which was seeded in October of 1991 with a mixture of perennial grasses and forbs using a John Deere Flex-Planter. Treatment response data were collected from 10, 0.25 m^2 quadrats located along a central transect line. Exact placement of quadrats from year to year was not possible due to disruption of initial markers by sheep grazing and small mammal activity, therefore, quadrats were located independently along a central transect line for each year of data collection. Data collection included a count of the number of stems of false hellebore and assignment of a nested frequency score (BLM 1996) for each species found within each quadrat. These counts and scores were recorded and averaged by year, treatment, seeding, replication, and block. Species which occurred consistently (in at least 5% of the 0.25 m^2 quadrats) were included in the data analysis" (Cosgriff et al. 2004). In 2009, only one analysis was done for in the herbicide treatments in block three because initial markers were no longer distinguishable and no physical difference was evident between the two treatments.

Data were gathered as to the occurrence of plants in early, mid, and late seral species in each of the treatments each year using cumulative nested frequency scores. Cumulative nested frequency scores were determined by averaging nested frequency scores measured in ¼ m^2 quadrats across year, treatment, replication, seeding, and block. Seral groupings (table 1) were based upon research by Ellison (1954). Plants were also classified according to desirability (table 2), and measurements were taken in each treatment each year. Plant desirability was determined by forage potential (Anderson and Thompson 1993), and is pertinent because of current use of the test area by the U.S. Forest Service as multiple-use land with livestock grazing as a major use. Cumulative nested frequency scores were used to determine occurrence of desirable, intermediately desirable, and undesirable species, and were collected in the same way as for seral grouping. Stem density of false hellebore was also measured in each treatment each year, to better understand population recovery.

Data were analyzed using a procedural mixed model in SAS. To analyze the seral stage recovery and desirability class within the plots, replications were set as random variables, and seeding, treatment, and year were set as fixed variables. Replications and blocks were set as random variables because of the similarity in species occurrence in these areas. Analysis of variance was used in conjunction with Tukey's test to determined differences in mean species occurrence between treatments and years. Significant effects for mean separation were defined at $p < .05$.

Results

False Hellebore Recovery

Using stem density measurements collected over 18 years, we found that year by treatment variables were significant (table 3). Though an increase of 3-6 stems/m^2 was found in treatments from 1993 to 1999, the highest increase from 1999 to 2009 was of about 4 stems/m^2 in the tilled treatments (figure 1). Untreated areas had the highest stem densities in 2009, averaging about 30 stems/m^2.

Seral Stage Recovery

Seral stages of monitored plants give us an idea as to the recovery of the ecosystem over time (tables 4, 5, and 6). In all test plots except for the tilled and untreated plots, there was a significant decrease in late seral plants over time (figure 2). Nested frequency scores in mow, remow, and herbicide test plots decreased anywhere from .5 to 3.

Mid-seral species decreased in mow, herbicide and till treatments (figure 3). Mid seral species decreased only slightly in mow and herbicide treatments, but more noticeably in till treatments (from a nested frequency score of 3.8 in 1993 to a score of 1.75 in 2009).

Mow and remow treatments showed no increase in early-seral species, while herbicide and till treatments showed significant increases, with the nested frequency score increasing from 6.3 in 1993 to 11.2 in 2009 till treatments, and from 5.6 in 1993 to 9.8 in 2009 herbicide treatments (figure 4).

Desirability Class Recovery

Desirable species decreased significantly in both mow and remow treatments from cumulative nested frequency scores of 5.2 to 2.7 in mow treatments and from 4.7 to 3.3 in remow treatments (figure 5, tables 7, 8, and 9). Herbicide and till treatments had no significant decreases in desirable species occurrence.

Intermediately desirable species increased in all treatments across time. The most notable gains in intermediately desirable species were found in remow treatments with cumulative nested frequency scores increasing from 4.4 in 1993 to 9.5 in 2009 (figure 6).

Undesirable species increased significantly over time in till treatments with cumulative nested frequency scores of 2.4 in 1993 to 4.3 in 2009 (figure 7). Seeding had no significant impact on reduction of undesirable species in the area.

Discussion

False Hellebore Recovery

Should false hellebore populations be recovering from disturbance (control treatments), stem density counts would increase through time. Increase of false hellebore populations in tilled treatments and stable to increasing stem density in herbicide treatments support our hypothesis that false hellebore populations may recover over time. However, this recovery is much slower than may be expected for members of an alpine tall forb community. The development of new rhizomes and subsequent stems of false hellebore in areas with high levels of disturbance may take decades. Mow treatments may have already reached the maximum false hellebore densities, so no increase in stem density was noted. Decreased plant vigor was noted, however in the first years after initial mow treatments. Mow and remow treatments are most similar to the untreated plots, having never been reduced significantly in stem densities and having recovered vigor as indicated by stem heights within the first decade.

Seral Stage Recovery

Decreases in late seral occurrence could be explained because the area continues to be grazed annually by sheep and palatability is often positively correlated with seral position in the herbaceous layer. The tilled plot might have less of a decrease in late seral species because of a low initial response of these species in 1993 due to the magnitude of disturbance.

Mow and remow treatments do not remove false hellebore domincance and hence do not open the community for establishment of early seral species. This confirms our hypothesis that mowing treatments would have less negative impacts on the plant community over time. Increases seen in early seral species are likely a result of the large disturbances caused by both herbicide and till treatments. Seeded plots contained significantly fewer occurrences of early seral species over time, suggesting that seeding may decrease invasion of early seral species. Treated areas are likely in a mid seral state because of the small changes over time in the occurrences of mid seral species.

Desirability Class Recovery

Decreases in desirable species in mow and remow treatments are likely due to false hellebore reestablishing the competitive dominance of the pre-treatment community. A lack of decrease in desirable species in herbicide and till treatments is likely because the initial treatment destroyed false hellebore dominance and plants that established were able to propagate in a lower competition environment. Increase of intermediately desirable species in all treatments shows that the community had enough time to recover to some degree from all treatments. Increases in undesirable species in till treatments show that this disturbance opened up the area to invasive species (predominantly annuals).

Until recently, false hellebore was considered an invasive plant that decreases grazing value. Decreased grazing intensity over the last 75 years may have arrested the invasion of false hellebore, which is of some concern to those currently interested in compounds found in false hellebore. In tilled treatments, higher instances of false hellebore were found where the plant had propagated from the rhizomes of plants in adjacent plots. Below ground rhizome growth may currently be the only viable way this plant can propagate.

Current false hellebore harvest practices include rhizome harvest. This practice is very similar in ground disturbance incurred by tilling treatments. Tilled treatments showed the highest occurrence of undesirable species, likely due to the magnitude of ecosystem disturbance. Though similar to untreated plots in cumulative nested frequency scores for desirable species, to the casual observer, these tilled treatments seem to include almost a monoculture of tarweed (*Madia glomerata* [Asteraceae]). The removal of shade provided by false hellebore, coupled with major disturbances to soil and plants, leaves the area open for early seral and undesirable plants.

Because of the recent paradigm shift that has changed the treatment of false hellebore from one of eradication and control to one of preservation and protection, limits may need to be put in place to ensure the survival of false hellebore populations. These limits will need to be put in place because many false hellebore populations exist on public land. These areas are multiple-use areas, where an emphasis is put on sustained yield. Management approaches to false hellebore recovery may need to be similar to those relating to timber recovery rather than grazing recovery because of the time needed for full recovery of false hellebore populations.

18 years after initial treatments, the most similar treatment to current harvest practices showed some recovery of false hellebore populations. The time required for a full recovery from tilled treatments, however, may be much longer. If rhizome harvest (similar to tilling) remains the current practice in false hellebore harvest, severe damage could result to the plant community. This damage could be minimized through block or strip harvesting, which would

allow the false hellebore to recover through rhizome propagation. Block or strip harvesting, along with reseeding, could also provide intermediate or desirable species recovery because sufficient shade may be preserved through these types of harvest. The results of this study show the importance of time in false hellebore recovery.

Further Study

Cyclopamine has been found in highest concentration in false hellebore rhizomes, but also exists in lower concentrations in the leaf tissue of the plant. St. Clair et al. (2009) showed that aspen defense compounds increased in leaf tissue two to three times after frost damage.

Because plants can respond to stresses, such as frost or herbivory, by increasing the amount of defense compounds in leaf tissue, false hellebore leaf tissue may contain higher concentrations of cyclopamine after mow or remow treatments. Further tests could confirm this increase in leaf tissue cyclopamine concentrations before and after stress, and analysis could determine whether this concentration increases to such a degree as to make tissue harvest worthwhile. Because communities seem able to recover from both mow and remow treatments without long-term deleterious effects to the ecosystem, further research into this type of vegetative harvest may prove to be very beneficial to the future of false hellebore.

Tables

Table 1

Early-Seral Species
Descurainia pinnata (Walter) Britton
Polygonum douglasii Greene
Chenopodium album L.
Androsace septentrionalis L.
Pseudostellaria jamesiana (Torr.) W.A. Weber & R.L. Hartm.
Madia glomerata Hook
Galium boreale L.
Collomia linearis Nutt.
Mid-Seral Species
Viola purpurea Kellogg
Thalictrum fendleri Engelm. ex A. Gray
Stipa lettermanii (Vasey) Barkworth
Epilobium brachycarpus C. Presl
Hackelia floribunda (Lehm.) I.M. Johnst.
Arabis holboellii Hornem.
Achillea millefolium L.
Liliaceae *spp.*
Late-Seral Species
Poa reflexa Vasey & Scribn. ex Vasey
Elymus trachycaulus (Link) Gould ex Shinners spp. trachycaulus
Delphinium x occidentale (S. Watson) S. Watson (pro sp.) [barbeyi x glaucum]
Osmorhiza occidentalis (Nutt. Ex Torr. % A. Gray) Torr.
Bromus carinatus Hook. & Arn.
Polemonium foliosissimum A. Gray
Cicuta maculata L.
Rudbeckia occidentalis Nutt.
Hymenoxys hoopesii (A. Gray) Bierner
Hydrophyllum capitatum Douglas ex Benth.
Osmorhiza chilensis Hook. & Arn. var. *cupressimontana* (B. Boivin) B. Boivin

Table 1. Species of false hellebore/tall forb alpine communities grouped by seral stage. Groupings based on descriptions by Ellison (1954).

Table 2

Desirable Species
Elymus trachycaulus (Link) Gould ex Shinners spp. Trachycaulus
Bromus carinatus Hook. & Arn.
Thalictrum fendleri Engelm. ex A. Gray
Poa reflexa Vasey & Scribn. ex Vasey
Intermediate Species
Polemonium foliosissimum A. Gray
Stipa lettermanii (Vasey) Barkworth
Galium boreale L.
Viola purpurea Kellogg
Rudbeckia occidentalis Nutt.
Hackelia floribunda (Lehm.) I.M. Johnst.
Less Desirable Species
Hymenoxys hoopesii (A. Gray) Bierner
Madia glomerata Hook
Descurainia pinnata (Walter) Britton

Table 2. Species of false hellebore/tall forb alpine communities grouped by desirability class. Groupings based on descriptions by Anderson and Thompson (1993).

Table 3

Effect	Num DF	Den DF	F Value	Pr > F
Year	2	145	6.97	0.0013
Treatment	4	145	222.07	<.0001
Year*Treatment	8	145	2.23	0.0285
Seeded	1	145	4.9	0.0284
Year*Seeded	2	145	1.27	0.2847
Treatment*Seeded	4	145	1.47	0.2156
Year*Treatment*Seeded	8	145	0.62	0.7561

Table 3. ANOVA table for false hellebore Stem Density. Year*Treatment interactions were found to be significant (figure 1).

Table 4

Effect	Num DF	Den DF	F Value	Pr > F
Year	2	106	8.68	0.0003
Treatment	4	47	7.31	0.0001
Seeded	1	47	0.86	0.3589
Year*Treatment	8	106	2.27	0.028
Year*Seeded	2	106	0.05	0.9535
Treatment*Seeded	4	47	1.61	0.1876

Table 4. ANOVA table for desirable species of the false hellebore/tall forb alpine community. Year*Treatment interactions were found to be significant (figure 5).

Table 5

Effect	Num DF	Den DF	F Value	Pr > F
Year	2	106	32.38	<.0001
Treatment	4	47	28.45	<.0001
Seeded	1	47	2.43	0.126
Year*Treatment	8	106	2.56	0.0138
Year*Seeded	2	106	0.9	0.4081
Treatment*Seeded	4	47	0.54	0.7036

Table 5. ANOVA table for intermediately desirable species of the false hellebore/tall forb alpine community. Year*Treatment interactions were found to be significant (figure 6).

Table 6

Effect	Num DF	Den DF	F Value	Pr > F
Year	2	106	2.41	0.0947
Treatment	4	47	4.37	0.0044
Seeded	1	47	1.22	0.2755
Year*Treatment	8	106	6.01	<.0001
Year*Seeded	2	106	1.1	0.3358
Treatment*Seeded	4	47	0.76	0.5578

Table 6. ANOVA table for undesirable species of the false hellebore/tall forb alpine community. Year*Treatment interactions were found to be significant (figure 7).

Table 7

Effect	Num DF	Den DF	F Value	Pr > F
Year	2	106	35.08	<.0001
Treatment	4	47	38.06	<.0001
Seeded	1	47	9.64	0.0032
Year*Treatment	8	106	4.39	0.0001
Year*Seeded	2	106	3.64	0.0295
Treatment*Seeded	4	47	1.32	0.2761

Table 7. ANOVA table for early seral species of the false hellebore/tall forb alpine community. Year*Treatment interactions were found to be significant (figure 4).

Table 8

Effect	Num DF	Den DF	F Value	Pr > F
Year	2	106	6.63	0.0019
Treatment	4	47	30.18	<.0001
Seeded	1	47	1.55	0.2195
Year*Treatment	8	106	3.22	0.0026
Year*Seeded	2	106	1.93	0.1499
Treatment*Seeded	4	47	0.16	0.9597

Table 8. ANOVA table for mid seral species of the false hellebore/tall forb alpine community. Year*Treatment interactions were found to be significant (figure 3).

Table 9

Effect	Num DF	Den DF	F Value	Pr > F
Year	2	106	8.16	0.0005
Treatment	4	47	11.24	<.0001
Seeded	1	47	1.54	0.2203
Year*Treatment	8	106	3.55	0.0011
Year*Seeded	2	106	0.6	0.5494
Treatment*Seeded	4	47	2.35	0.0681

Table 9. ANOVA table for late seral species of the false hellebore/tall forb alpine community. Year*Treatment interactions were found to be significant (figure 2).

Figures

Figure 1

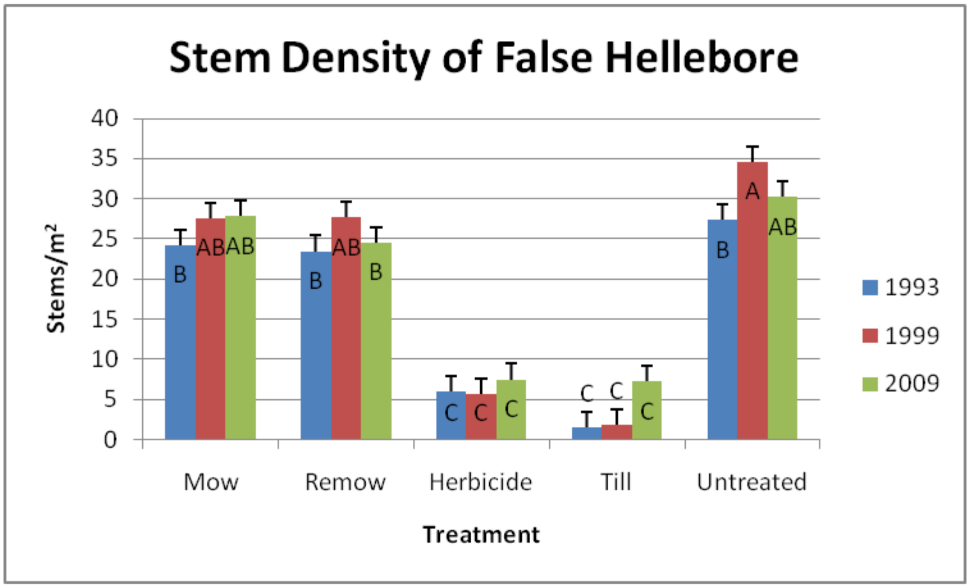

Figure 1. Stem density of false hellebore at $p < .05$. Till treatments showed the highest increase from 1993 to 2009 of about 5.7 stems/m^2. Standard error for Herbicide 2009 = 2.047. Standard error for all other treatments = 1.939.

Figure 2

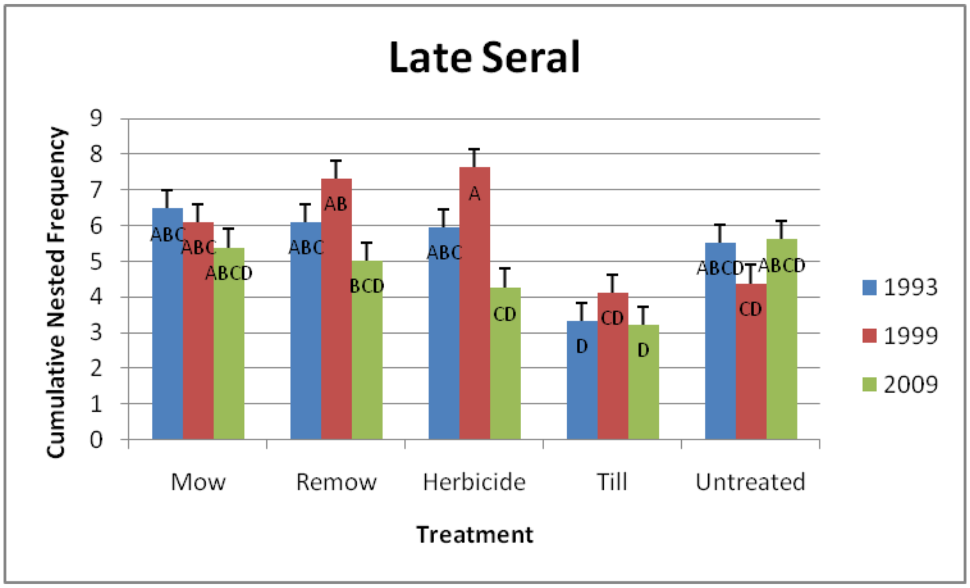

Figure 2. Cumulative nested frequency scores of late seral species of the false hellebore/tall forb alpine community found in each treatment across years. Seral groupings are based on descriptions by Ellison (1954). A decrease was found in all test plots except for tilled and untreated plots. Decreases are likely due to grazing of late seral species. Standard error for Herbicide 2009 = .5598. Standard error for all other treatments = .5132.

Figure 3

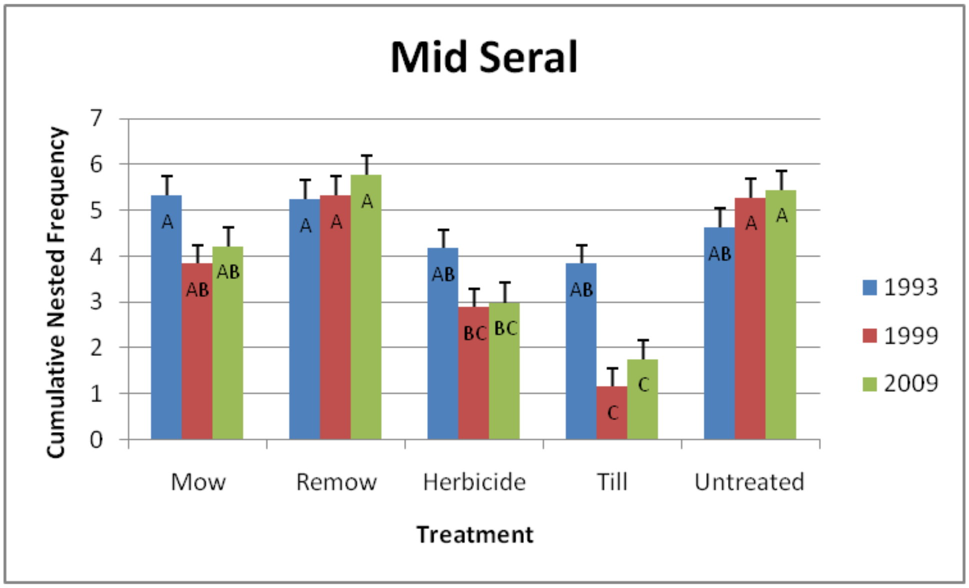

Figure 3. Cumulative nested frequency scores of mid seral species of the false hellebore/tall forb alpine community found in each treatment across years. Seral groupings are based on descriptions by Ellison (1954). Mid seral species decreased significantly in tilled treatments. Standard error for Herbicide 2009 = .4542. Standard error for all other treatments = .4146.

Figure 4

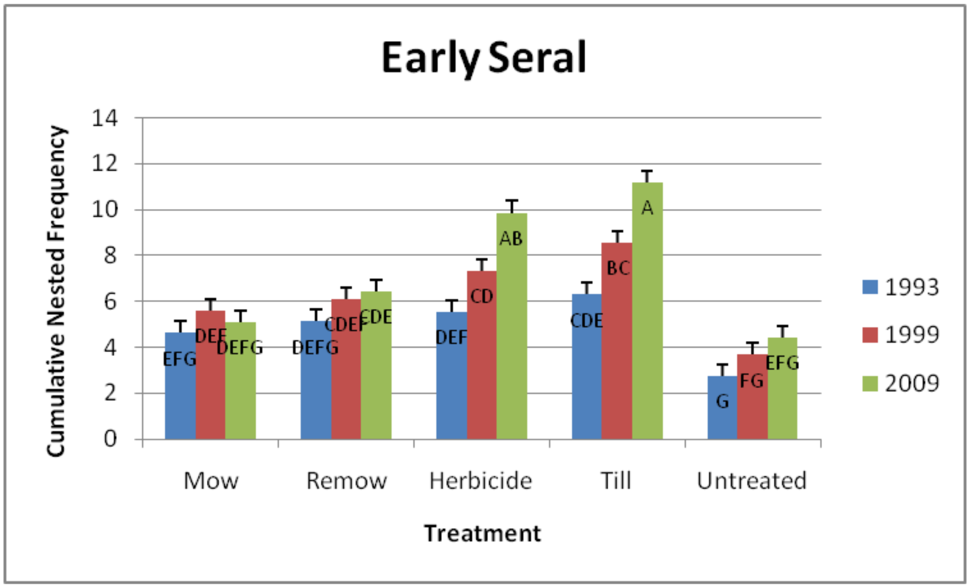

Figure 4. Cumulative nested frequency scores of early seral species of the false hellebore/tall forb alpine community found in each treatment across years. Seral groupings are based on descriptions by Ellison (1954). Herbicide and tilled treatments showed significant increases in early seral species. This is likely a result of the magnitude of disturbance caused by these treatments. Standard error for Herbicide 2009 = .5503. Standard error for all other treatments = .5034.

Figure 5

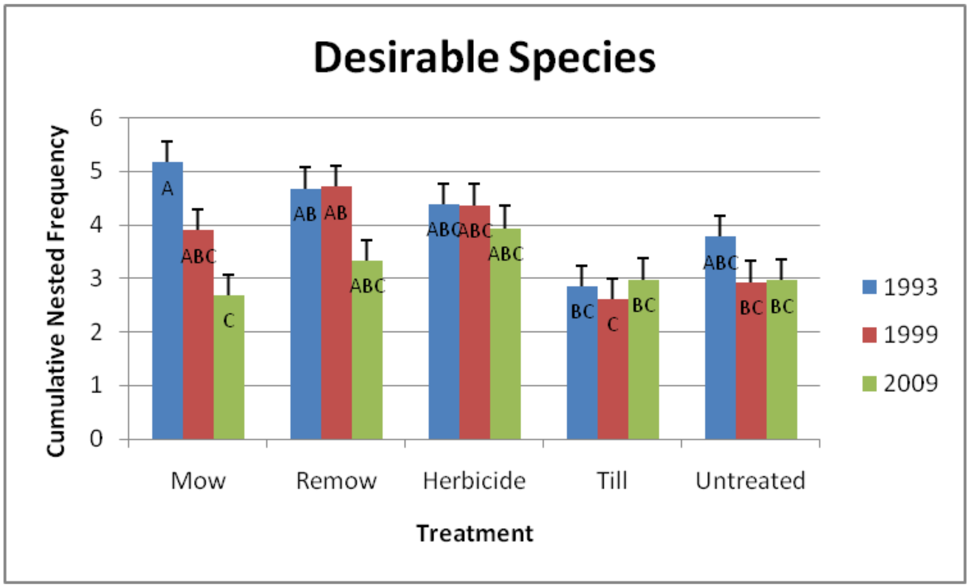

Figure 5. Cumulative nested frequency scores of desirable species of the false hellebore/tall forb alpine community found in each treatment across years. Desirability classes are based on forage potential (Anderson and Thompson 1993). Desirable species decreased in mow and remow treaments. This is likely due to false hellebore reestablishing competive dominance. Standard error for Herbicide 2009 = .4271. Standard error for all other treatments = .3910.

Figure 6

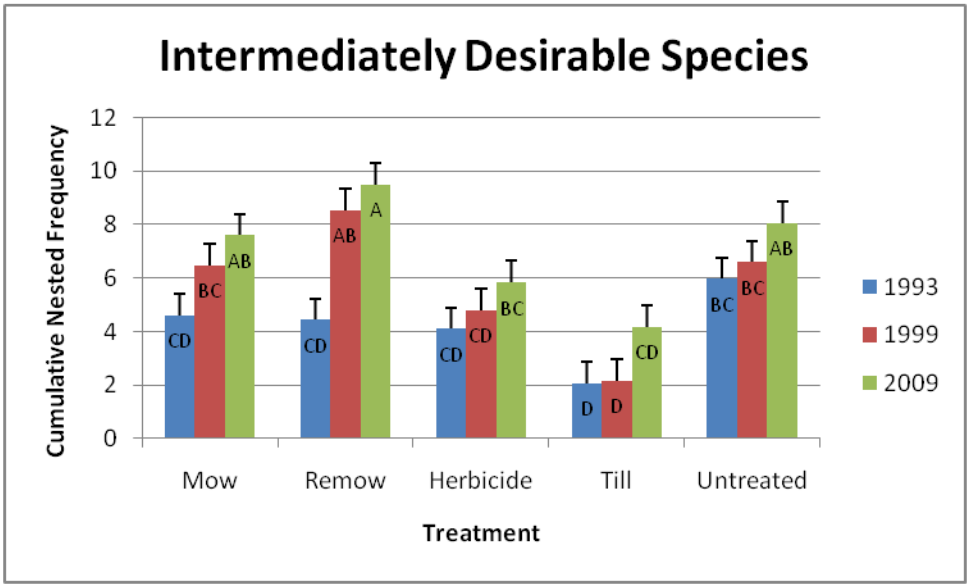

Figure 6. Cumulative nested frequency scores of intermediately desirable species of the false hellebore/tall forb alpine community found in each treatment across years. Desirability classes are based on forage potential (Anderson and Thompson 1993). Increase in intermediately desirable species across all treatments shows that the areas all recovered to some degree from the treatments. Standard error for Herbicide 2009 = .8327. Standard error for all other treatments = .7932.

Figure 7

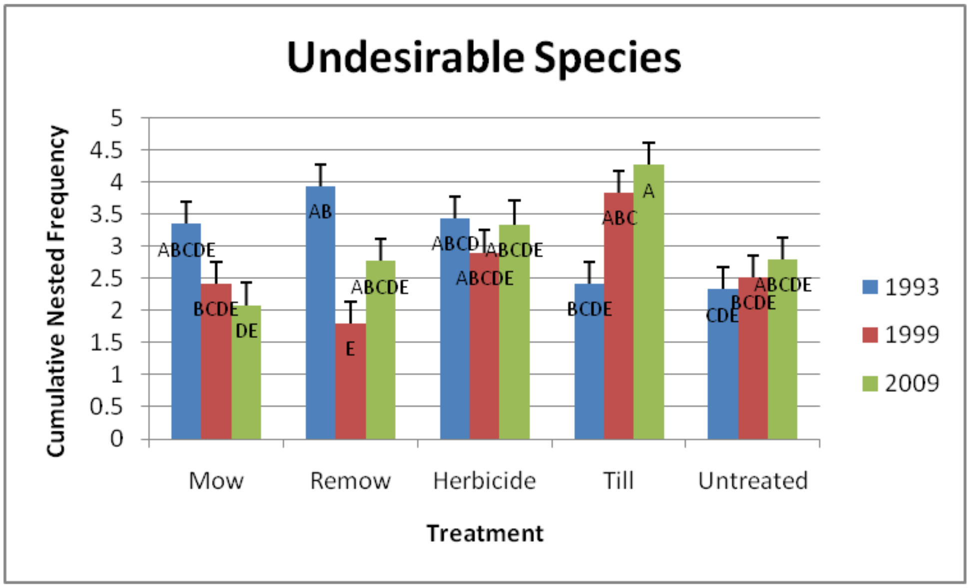

Figure 7. Cumulative nested frequency scores of undesirable species of the false hellebore/tall forb alpine community found in each treatment across years. Desirability classes are based on forage potential (Anderson and Thompson 1993). Undesirable species in tilled treatments increased significantly showing that the disturbance opened up the area to invasive species. Standard error for Herbicide 2009 = .3736. Standard error for all other treatments = .3443.

Chapter 2

Introduction

Research into the uses of plant secondary compounds has led to interest in propagation of false hellebore and other similar species in a greenhouse setting because of the unknown extent of native populations and federal land restrictions. Though in the past greenhouse propagation has been unsuccessful, we tried some new methods to not only determine the feasibility of agronomic growth, but also the effects of harvest phenology and fertilizer treatment on cyclopamine production in the plant.

We harvested false hellebore rhizomes at 5 different phenological stages and transplanted them under greenhouse conditions. We then treated plants with different levels of fertilizer. Our hypotheses were 1) that plants harvested earliest and latest in the season would have the highest recovery rate after transplant, 2) that plants with more fertilization would have higher recovery rates after transplant, and that 3) plants with more fertilization would have higher concentrations of cyclopamine in the plant tissues.

Materials and Methods

Shoots with intact rhizome segments were gathered from three geographically distinct locations to ensure variability in genotype. 15 plants from five defined growth stages were gathered from each site. Stage growth one was defined as plants just emerging as “spears” from the soil. The tissue of the shoot was not veined, and the plants were from 3.5 to 7.5 centimeters tall. Stage growth two was defined as shoots from 20 to 40 centimeters in height. The plant tissue was veined in these plants. Stage growth three was defined as plants approximately one meter tall. These plants have grown significantly, but show no signs of inflorescence. Stage growth four was defined as mature inflorescence. Stage growth five was defined as frost killed shoots. These plant materials were chilled and transported to a greenhouse, where the aboveground growth was trimmed.

In the greenhouse each rhizome section was placed in an eight inch azalea pot. These pots were lined with a single layer of paper towel and filled 1/3 with #3 coarse vermiculite. The false hellebore rhizomes were trimmed to fit in the pot. Each rhizome was measured and treated with wettable 50% captan fungicide on the main cut areas. After cutting, measuring, and treating with captan, the plants were placed in the bottom of the pots. The remainder of each pot was filled with a 1 to 3 mixture of perlite to vermiculite and kept damp to simulate a high water table.

After planting and filling, the pots were irrigated in three initial treatments: 1) with water, 2) nitrogen fertilizer at a concentration of 50 mg/L, and 3) fertilizer at a concentration of 200 mg/L nitrogen. These plants were then set on a propagation bench in the greenhouse. The propagation bench is set to receive overhead misting treatments every 3-4 minutes, and also programmed to have bottom heating to 21.1 Celsius. The bottom heat and misting was provided to promote rooting.

After three weeks on the propagation bench, the rhizomes were moved to another bench in partial sunlight, at a temperature ranging 15.5-22.2 Celsius. The plants remained on the bench without watering for 24 hours. The plants were then retreated with water, 50 mg/L nitrogen fertilizer, or 200 mg/L nitrogen fertilizer, as they had been treated before. Subsequently, the plants were then irrigated one to three times a week with water. Shoot regrowth was measured and recorded. Measurements were averaged by plot, treatment, and rep (table 10). New shoot growth was also recorded in a high percentage of all treatments and plots for the first four growth stages (table 11)

We placed the plants in a lath house on January 5, 2010 in order to simulate overwintering for the plants. Arrangements were made with the pharmaceutical company interested in these plants to use their classified bioassay to determine the cyclopamine content of the plants. No measurements were taken, however, because nearly all of the shoots and rhizomes had died by spring.

Results

Two types of growth were found from the transplanted rhizomes. Plants harvested early in the season (during growth stages one and two) had some regrowth of the stem and leaves. Lowest average regrowth of stem and leaves from the transplanted rhizomes was 18 cm, and highest average regrowth of stem and leaves from the transplanted rhizomes was 38.9 cm. New stem growth occurred in plants gathered from each growth stage. Fertilizer seemed to have no significant impact on stem regrowth or new growth. The transplants died before cyclopmamine data could be taken.

Discussion

Large amounts of energy can be stored in plant rhizomes. Regrowth of earlier harvested plants is likely because of this stored energy coupled with growth in a less harsh environment. Previous studies on native populations showed that false hellebore did not recover from mowing treatments in the same year they were implemented (Cosgriff et al. 2004). New growth in the same year is also likely tied to the less harsh environment in which the false hellebore rhizomes were transplanted.

The death of the plants after being moved outdoors is likely linked to temperature, insulation, and remaining energy in the false hellebore rhizome. Native populations of false hellebore are found in elevations ranging "1830 to 3115 m…" (Welsch et al. 2003). These areas are known to get large amounts of winter precipitation in the form of snow. This snow may have an impact on the survival and overwintering of false hellebore plants because of the insulation it provides from the harsh winter temperatures. The plants grown in the lath house were subject to frequent freeze-thaw cycles, common to lower elevation valleys in the area. Due to the lack of snow insulation as well as the deterioration of the potting medium, many of the rhizomes were much less protected than the native populations.

Another factor influencing the survival of the greenhouse populations is the energy expended by the plants before overwintering. The plants harvested in growth stage one, in a less harsh environment than the alpine ecosystem, were able to put on new growth in the same growing season. The energy expended for this growth likely left the plant more susceptible to the outdoor weather conditions.

Conclusion

Our approach to propagate this plant was unsuccessful. It is unknown whether fertilizer or phenological stages at harvest have any impact on the concentration of cyclopamine in false hellebore. Because of the difficulty in propagating false hellebore and the amount of time it takes for false hellebore to recover naturally in the wild, further study is needed to find a sustainable way to propagate false hellebore so that sources of cyclopamine are not lost.

Tables

Table 10

	Untreated					
	Site 1	St. Err.	Site 2	St. Err.	Site 3	St. Err.
Stage 1	18.4	5	38.9	4.3	27.4	5.4
Stage 2	9.9	2.4	9.8	1.8	4.4	1.2
Stage 3	-	-	-	-	-	-
Stage 4	-	-	-	-	-	-
Stage 5	-	-	-	-	-	-
	50 ppm					
	Site 1	St. Err.	Site 2	St. Err.	Site 3	St. Err.
Stage 1	28.3	4.8	30.2	5.4	33.7	3.4
Stage 2	8.6	1.6	20.3	5.1	3.2	0.6
Stage 3	-	-	-	-	-	-
Stage 4	-	-	-	-	-	-
Stage 5	-	-	-	-	-	-
	200 ppm					
	Site 1	St. Err.	Site 2	St. Err.	Site 3	St. Err.
Stage 1	25	9.4	27.7	3	34	4.3
Stage 2	10	2.3	14	2.9	6.2	2.1
Stage 3	-	-	-	-	-	-
Stage 4	-	-	-	-	-	-
Stage 5	-	-	-	-	-	-

Table 3. Average re-growth height of greenhouse plants. Heights were averaged by plot, treatment, and replication. Regrowth occurred in plants harvested earlier in the season, likely because of sufficient energy storage. Regrowth ranged from 18 cm to 38.9 cm.

Table 11

	Untreated			50 ppm			200 ppm		
	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Stage 1	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
Stage 2	80.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	80.00%
Stage 3	80.00%	100.00%	100.00%	80.00%	100.00%	100.00%	60.00%	100.00%	100.00%
Stage 4	60.00%	80.00%	60.00%	80.00%	100.00%	80.00%	100.00%	100.00%	100.00%

Table 4. Percentage of plants from each plot and treatment with new shoot growth.

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