

Failure of black cohosh (*Actaea racemosa* L.) rhizome transplants: potential causes and forest farming implications

Christine J. Small · James L. Chamberlain ·
Christopher M. Nuckols

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Abstract Black cohosh (*Actaea racemosa* L.) rhizomes are harvested extensively from eastern North American forests and sold worldwide for treatment of menopausal symptoms. While forest farming is encouraged to reduce wild-harvest impacts, little information is available to aid landowners in successfully cultivating black cohosh. This study examined survival and multi-year growth of 200 black cohosh rhizomes collected from an Appalachian deciduous forest and transplanted to a similar forest type. By the second year after transplant, fewer than 40 % of rhizomes produced above-ground growth and mean rhizome biomass declined by more than 50 %. Shoot biomass was the greatest predictor of below-ground biomass; however, leaf area provided a reasonable, non-destructive means of estimating this commercially-important biomass. Our results suggest that pre-transplant rhizome condition is particularly important to transplant success. Low biomass, lack of roots, and fungal infection all were associated with reduced plant survival. Based on this and associated studies, we recommend careful site selection for propagation, including circumneutral or lime-amended soils and

light to moderate shading. Well-drained soil appears particularly important to discourage fungal infection. Understanding conditions for successful transplanting of black cohosh rhizomes can improve forest farming and contribute to sustainable management of this and other non-timber forest products.

Keywords Deciduous forest herbs · Forest farming · Fungal root rot · Medicinal plant cultivation · Non-timber forest products

Introduction

In eastern North American forests, non-timber products are of considerable economic and cultural value, yet many are extracted with little management for long-term population viability or natural resource conservation (Ticktin 2004; Vaughan et al. 2013). Most wild-harvested medicinal plants are slow-growing perennial herbs, with reproduction and population expansion largely from clonal growth and slow recovery after disturbance (Gilliam 2007; Small et al. 2011; Whigham 2004). This is particularly problematic for species commercially valued for roots and below-ground storage organs (e.g., rhizomes), as harvesting destroys the plant or greatly reduces clonal reproduction. Resulting declines in understory forest herbs such as American ginseng (*Panax quinquefolius* L.), ramps (*Allium tricoccum* Aiton), and goldenseal

C. J. Small (✉) · C. M. Nuckols
Department of Biology, Radford University, Radford,
VA 24142, USA
e-mail: cjsmall@radford.edu

J. L. Chamberlain
USDA Forest Service Southern Research Station,
Blacksburg, VA 24060, USA

(*Hydrastis canadensis* L.) have been associated with excessive wild harvests (McGraw 2001; Nantel et al. 1996; Robbins 2000; Sinclair et al. 2005).

Black cohosh (*Actaea racemosa* L.) is an eastern forest perennial used in the non-prescription treatment of menopausal symptoms (Predny et al. 2006). This species has been listed as a top 10 selling herbal supplement each year since 2002, with estimated 2003 sales in excess of \$15.7 million (Blumenthal 2003). The American Herbal Products Association (AHPA 2012) estimated that from 1997 through 2005 more than 1 million kilograms of black cohosh rhizomes were harvested from eastern US forests—more than any other medicinal plant tracked by the Association. During that same period, AHPA reported just 25,000 kg of black cohosh rhizomes (~2 % of commercial sales) from cultivated sources. Like many medicinal herbs, black cohosh is harvested primarily for its roots and rhizomes, and field experiments suggest that recovery occurs slowly or not at all after harvests (Small et al. 2011). Increased demand and harvest pressure has led The United Plant Savers (2014) to list black cohosh as an “at-risk species” and NatureServe (2012) to predict 10–30 % declines across its natural range over the next decade, unless sources of cultivated plant material are established.

In recent decades, cultivation of commercially-important native plants has been encouraged to supplement wild harvests, particularly for species in which there is concern for population declines and the sustainability of current or future wild harvesting practices (Burkhart and Jacobson 2009; Chamberlain et al. 2009). Forest cultivation offers a number of potential benefits. “Wild-simulated” forest farming, in particular, offers landowners potential revenue streams with minimal site preparation or costs, and produces harvested material similar in appearance and value to that of wild harvest (Burkhart and Jacobson 2009; Chamberlain et al. 2009). Given the economic value, high demand, and intense wild harvest pressure on black cohosh, it appears to be an ideal candidate for commercial propagation. Previous studies suggest that black cohosh can be cultivated under suitable site conditions (Chamberlain et al. 2009; McCoy et al. 2007). However, while commercial cultivation of some particularly valuable medicinal root crops has been well-studied (e.g., ginseng and goldenseal), relatively little information or support is available to aid forest landowners in profitably cultivating black

cohosh and many other economically important forest herbs (Chamberlain et al. 2009).

Few studies have examined growing conditions and other factors associated with low productivity or failure of black cohosh rhizome transplants (McCoy et al. 2007; Naud et al. 2010; Thomas et al. 2006). This study examined survival and multi-year growth of transplanted black cohosh rhizomes relative to pre-transplant rhizome size and vigor. We used our results to consider rhizome and growing conditions necessary for successful forest cultivation and likely causes of transplant failure. A second objective of our study was to examine relationships between above-ground plant growth parameters and below-ground biomass, to help in gauging harvest timing to optimize commercially important rhizome yields.

Methods

Field methods

In July 2010, more than 200 black cohosh rhizomes were collected from robust natural populations, as part of an experimental harvest study. (Harvest experiments detailed in Small et al. 2011.) Collection sites were located in closed-canopy Appalachian mixed oak forests (Stephenson et al. 1993) in the George Washington-Jefferson National Forest, near the border of Grayson and Wythe Counties, Virginia (36.760164°/–81.216017°). Slopes were north-facing and moderately steep, at approximately 1,180 m elevation. Soils were dominated by McCamy fine sandy loams (Typic Hapludult) formed from weathered residuum of phyllite and metasandstone (Soil Survey Staff 2013). These are well-drained, with typical A horizon depth of 13 cm, approximately 2.25 % organic matter, and strongly acid reaction (pH 4.0). Minimum depth to bedrock is 66 cm. Harvested rhizomes were transferred to eight 30 gallon plastic tubs and buried approximately 5 cm deep in a 1:1 mixture of potting soil and field soil collected from the harvest site. At least five 3 cm holes were cut in the bottom of each tub for drainage. Tubs were stored outside, under partial tree canopy, from late July to early October 2010.

In early October 2010, rhizomes were rinsed and air dried, and pre-transplant fresh-weight determined. Those with evidence of mold or deterioration on more than 1/4 of the rhizome were eliminated from the study. Weighed rhizomes were immediately transplanted to a

mixed oak forest in Floyd County, Virginia (36.911756°/–80.488090°). Elevation at the transplant site was ~850 m, with gentle slopes (<10 %). Soils were similar to those of the harvest site, dominated by Sylco-Sylvatus silt loams (Typic Dystrudept/Lithic Dystrudept) derived from weathered residuum of phyllite and metasandstone (Soil Survey Staff 2013). These are well- to somewhat excessively drained, with typical A horizon depth of 5–10 cm, approximately 1.25 % organic matter, and extremely to strongly acid reaction (pH 3.5–5.0). Minimum depth to bedrock is 40–69 cm. The tree canopy at the transplant site was well-developed (~85 % canopy cover) and dominated by *Acer rubrum* L., *Quercus prinus* L., *Quercus alba* L., and *Fagus grandifolia* Ehrh.

At the transplant site, eight 5 × 5 m plots were located randomly, with a minimum distance of 20 m between plots. Plots were established on sites with at least 25 m² free of mature tree boles or large blow downs, to allow establishment of a regular planting grid. In each plot, 25 rhizomes were planted at regular 1 m intervals in a 5 × 5 configuration. Each rhizome was planted to a depth of 5.0 cm and covered with approximately 2.5 cm of field soil and hardwood leaf litter from the plot. No soil amendments or chemicals were added to transplant sites. Above-ground plant height, stem density, and leaf crown (canopy) area were measured for each rhizome in June 2011 and July 2012 (Year 1 and 2 after transplant). Height was measured from the ground surface to the top of the main canopy of leaves, excluding flowering or fruiting peduncles. Leaf area was calculated based on two perpendicular measurements, including the widest point of the leaf canopy. In July 2012, all rhizomes showing above-ground growth were excavated, washed, and weighed for above- and below-ground fresh-weight biomass. Rhizomes were replanted after weighing. Transplant methods followed McCoy et al. (2007) for season, planting depth, lack of pre-treatment, Naud et al. (2010) for season and depth, and Thomas et al. (2006) for depth, as these authors reported high transplant success or recommendations for significant improvement.

Data summary and analysis

Repeated measures analysis of variance tests were used to examine changes in rhizome biomass from 2010 to 2012 and to compare above-ground measures (plant

height, leaf area, stem density) in 2011 and 2012. Only plants surviving through 2012 were included in the analysis. Individual plants or rhizomes were treated as within-subject variables, measurement year as a fixed within-factor effect, and plot as a random between-factor effect. Before analysis, data were log₁₀ transformed to meet normality and homogeneity of variance assumptions. The Geisser-Greenhouse adjustment was used to correct for potential non-circularity in the repeated measures covariance matrix. Corrected *F*-ratios and associated probabilities are reported for all results. Data failing to meet statistical assumptions were compared using the non-parametric Wilcoxon Signed-Rank Z-Test. To control for family-wise error, a Bonferroni correction was used to adjust α for experiment-wise error across tests (Scheiner 1993). GLM ANOVA was used to compare initial (2010) rhizome biomass for surviving and non-surviving plants (plants producing or not producing above-ground in 2012). Survival was used as a fixed treatment effect and plot as a random effect in the ANOVA model).

Chi square tests of independence were used to examine variations in categorical variables associated with initial rhizome conditions (e.g., presence of roots; presence of mold or rot) relative to above-ground emergence and survival. Yates correction was used to account for expected frequencies <5 in more than 20 % of cells. Least squares multiple regression was used to determine the effectiveness of above-ground measures in predicting 2012 below-ground rhizome biomass. Plant height, crown area, and shoot biomass (2012 only) were treated as independent variables in the regression model. Data were checked for linearity and transformed as necessary to meet normality and variance homogeneity assumptions. Independent variables were checked for multicollinearity and significantly intercorrelated variables were removed prior to final analysis. Stepwise selection was used to determine the subset of independent variables that best predict rhizome biomass based on changes in cumulative *r*² and root mean square error. All statistical analyses were performed using NCSS statistical software (Hintz 2007).

Results

In the first growing season after transplant, 71 % of rhizomes showed above-ground growth (Table 1). By 2012, only 39 % produced above-ground shoots, and

Table 1 Rhizome survival, fresh-weight, and measures of above-ground emergence (height and leaf crown area) of black cohosh rhizome transplants from 2010 to 2012

Year	# rhizomes or plants	Rhizome biomass (g)	Shoot height (cm)	Stem density (#)	Leaf area (cm ²)
<i>Pre-transplant (2010)</i>					
All rhizomes	200	45.6 ± 4.6	–	–	–
Failed by 2012	122	37.1 ± 3.8	–	–	–
Surviving to 2012	78	59.0 ± 10.1	–	–	–
<i>Year 1 (2011)</i>					
All rhizomes	141	–	15.3 ± 0.7	1.34 ± 0.09	111.4 ± 11.1
Failed by 2012	63	–	12.9 ± 0.9	1.39 ± 0.16	77.7 ± 10.7
Surviving to 2012	78	–	17.8 ± 1.0	1.30 ± 0.08	146.4 ± 18.8
<i>Year 2 (2012)</i>					
All rhizomes	78	22.1 ± 3.3	14.1 ± 0.7	1.51 ± 0.11	328.3 ± 45.7

“Surviving rhizomes” includes only rhizomes producing above-ground shoots through 2012

mean rhizome biomass declined to less than half of pre-transplant weight ($F_{1,70,156} = 228.7$, $p < 0.00001$; Table 1). Only a single rhizome increased in biomass from 2010 to 2012. Emerging plants also showed changes in above-ground morphology from 2011 to 2012. In 2012, plants were significantly shorter ($F_{1,61,138} = 14.30$, $p = 0.0069$), with greater stem density ($Z = -2.06$, $p = 0.021$) and more than double the crown area than those in 2011 ($F_{1,61,138} = 43.48$, $p = 0.0003$; Table 1).

Initial rhizome biomass (2010) was a strong predictor of biomass in Year 2 ($r^2 = 0.729$; $F_{1,78} = 204.6$, $p < 0.0001$; Fig. 1). Initial biomass seemed critical to rhizome success, as the 78 rhizomes that survived through Year 2 began with significantly higher initial biomass than non-survivors ($F_{1,7,199} = 7.29$, $p = 0.031$; Table 1). Surviving plants also showed strong relationships between above- and below-ground components. Larger rhizomes were associated with greater above-ground shoot biomass ($r^2 = 0.733$, $F_{1,78} = 208.8$, $p < 0.0001$), plant height ($r^2 = 0.490$, $F_{1,78} = 72.9$, $p < 0.0001$), and leaf crown areas ($r^2 = 0.550$, $F_{1,78} = 93.0$, $p < 0.0001$; Fig. 2). Shoot biomass alone was a strong predictor of rhizome biomass, effectively explaining 73 % of biomass variation (Table 2). Addition of other above-ground measures added no predictive strength to the model. A second multiple regression excluding shoot biomass showed that leaf area can be used to obtain a reasonable (but slightly weaker) below-ground biomass estimate (Table 2). This suggests that marketable below-ground biomass can be estimated effectively using non-destructive measures (i.e., without

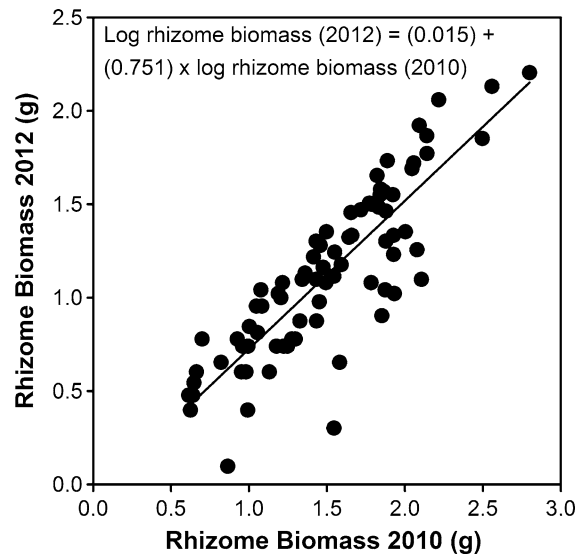


Fig. 1 Linear regression of 2012 black cohosh fresh-weight rhizome biomass to 2012 rhizome biomass for transplanted black cohosh rhizomes. Data log transformed +1 for normality and homogeneity of variance

removing or damaging above-ground plant tissues). Other parameters added little strength to the predictive model.

To better understand factors limiting transplant success, we examined rhizome conditions prior to transplant. Rhizomes lacking roots (~10 % of pre-transplant rhizomes) had significantly lower Year 2 survival than those with roots present (Table 3). Fungal infection (mold) or rot (~5 % of rhizomes) appeared to have little effect on plant survival. While only one-third of rhizomes with mold or rot emerged in 2012,

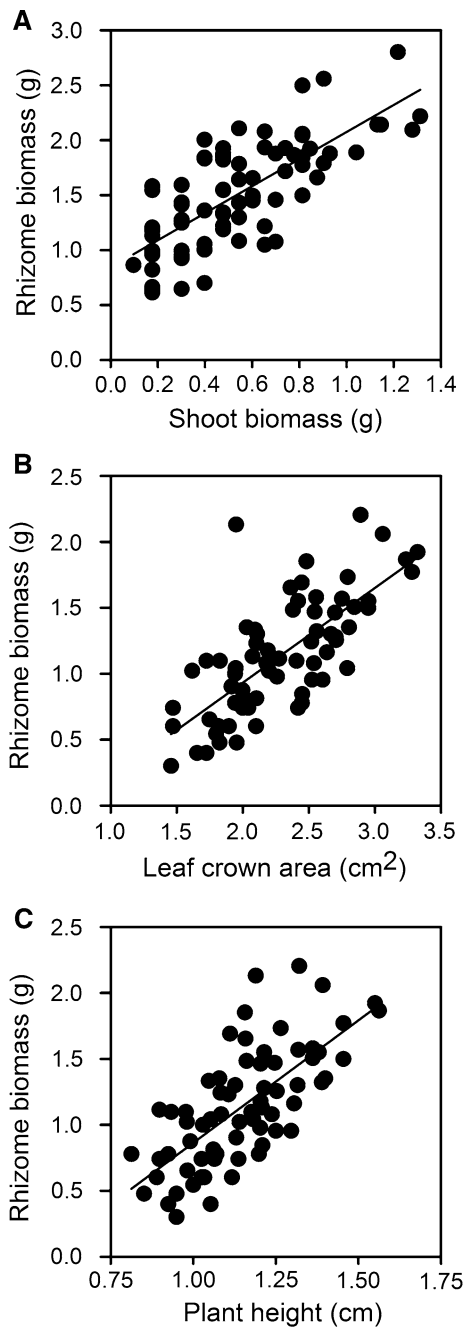


Fig. 2 Linear regression of 2012 rhizome biomass (fresh-weight) to 2012 above-ground growth parameters. All data log transformed +1 for normality and homogeneity of variance: **a** Rhizome biomass = (0.416) + (1.318) × shoot biomass (fresh-weight); **b** Rhizome biomass = (−0.538) + (0.731) × leaf crown area; **c** Rhizome biomass = (−1.033) + (1.882) × plant height

emergence was similar for apparently healthy rhizomes. Approximately 12 % of pre-transplant rhizomes had mold or rot and lacked roots. Survival was lower than for

Table 2 Predictors of black cohosh below-ground biomass, based on multiple regression and stepwise variable selection procedure

Step	Independent variable	r ² cumulative	F	p
<i>All independent variables included in model</i>				
1	Shoot biomass	0.733	208.8	<0.0001
2	Leaf area	0.734		
3	Plant height	0.734		
<i>Shoot biomass excluded from model</i>				
1	Leaf area	0.550	93.0	<0.0001
2	Plant height	0.559		

Data from 2012 plant measurements only. Exclusion of shoot biomass provides a non-destructive means of estimating below-ground biomass

Table 3 Chi square matrixes for 2012 surviving and non-surviving black cohosh rhizome transplants relative to initial rhizome condition variables

Variable (2010)	Black cohosh rhizomes (2012)		Chi square (p value)
	% surviving (n = 78)	% non-surviving (n = 122)	
<i>Roots on rhizomes</i>			
Root present (181)	41.4	58.6	4.75 (0.029)
Roots absent (19)	15.8	84.2	
<i>Rot/mold on rhizomes</i>			
Mold/rot absent (190)	39.5	60.5	0.36 (0.550)
Mold/rot present (10)	30.0	70.0	
<i>Roots + mold/rot</i>			
Healthy rhizomes (roots present; mold or rot absent, n = 177)	41.2	58.8	3.26 (0.071)
Unhealthy rhizomes (roots absent; mold/rot present, n = 23)	21.7	78.3	

DF = 1 for all Chi square tests

“healthy” rhizomes, but this appeared less important than the presence of roots (Table 3).

Discussion

Forest cultivation has the potential to reduce wild-harvest pressures on many commercially important forest herbs, including black cohosh (Burkhart and

Jacobson 2009; Chamberlain et al. 2009). To be successful, however, landowners must have sufficient knowledge of propagation techniques and necessary growing conditions (and markets) for profitable cultivation. Black cohosh is cultivated most often from rhizome fragments, as growth from seed requires careful treatment to overcome morphological and physiological dormancies (Albrecht and McCarthy 2011) and may delay growth of rhizomes to harvestable size (rhizomes typically require at least 5–8 years) (Chamberlain et al. 2009). In this study, less than half of the rhizomes emerged in Year 2, and nearly all had declined in biomass. Several factors may have contributed to poor survival and growth, including low pre-transplant biomass, absence of roots, and unfavorable site conditions. However, the most likely cause of failure appears to be fungal infection. These factors, and recommendations for propagation, are discussed below.

Pre-transplant rhizome size and presence of roots

Surviving rhizomes showed strong above- to below-ground relationships. Of the parameters measured, shoot biomass was the greatest predictor of rhizome biomass. Leaf crown area also provided a reasonable estimate of below-ground marketable biomass. This finding is important, as it suggests that above-ground parameters can be used to monitor below-ground biomass increase (or decrease) over time, allowing a non-destructive means of estimating marketable biomass and the timing of harvest. Fischer et al. (2006) and Chamberlain et al. (2013) report similar correlations between rhizome biomass and above-ground growth parameters such as leaf area and height.

While roots contribute little to the below-ground biomass of black cohosh (and therefore little to its economic value), they seem critical to propagation success. Year 2 survival was 60 % higher for rhizomes with roots present at the time of transplant. Surviving plants also began with significantly larger rhizomes. In propagation studies, Fischer et al. (2006) and McCoy et al. (2007) found higher relative growth rates and yields from entire (unsevered) rhizomes with roots and larger, actively growing terminal fragments, as compared to smaller, midsection fragments and those with fewer buds. Like goldenseal, black cohosh produces irregular, knotty rhizomes and numerous adventitious roots (Chamberlain et al. 2013; Van der Voort et al.

2003). Rhizomes are tightly interwoven and break easily, making extraction difficult without damage or loss of roots. Thus, while rhizome and root fragments may aid in population regrowth after harvest, relatively intact rhizomes seem critical to transplant success.

Site conditions

Of all components of forest vegetation, the herbaceous layer is particularly responsive to variations in site conditions (Gilliam 2007; Small and McCarthy 2002). Many widely used medicinal herbs are adapted to moist, deeply shaded conditions typical of deciduous forest understories (Chamberlain et al. 2009; Naud et al. 2010). Forest vegetation studies and habitat prediction models show greatest abundance of medicinal herbs like black cohosh, blue cohosh (*Caulophyllum thalictroides* (L.) Michx.), wild ginger (*Asarum canadense* L.), ginseng, and bloodroot (*Sanguinaria canadensis* L.) in relatively undisturbed or mature cove forests and northeast-facing slopes, particularly those dominated by mesophytic tree species (e.g., *Acer saccharum* Marshall, *Liriodendron tulipifera* L., *Fraxinus americana* L., *Tilia americana* L.). Soils at these sites tend to be rich, with ample moisture and high pH, organic matter, and base cation availability (esp. N, Ca, Mg) (Burkhart 2013; Hutchinson et al. 1999; Small and McCarthy 2002; van Manen et al. 2005). Drier, less fertile sites also inhibit seed germination and seedling survival in many of these species, including black cohosh (Albrecht and McCarthy 2009). Given this, it is not surprising that site conditions should play an important role in the successful cultivation of forest herbs.

We found poor survival and growth of black cohosh rhizomes after transplant to a submesic, acidic oak forest. Stunted growth and other changes in above-ground morphology also were suggestive of plant stress. McCoy et al. (2007) compared black cohosh rhizome growth in intact forest, forest edge, and artificial shade environments. Like our study, they found decreases in rhizome biomass (after 3 years) for unsevered rhizomes grown in forests and edges, yet their yields were substantially higher under artificial shade. However, conditions in their artificial shade sites differed markedly, with greater light (78 shade vs. 90–92 % shade), nutrient availability (cation exchange capacity 11.4 vs. 7.7–10.8 Meq/100 g; base

saturation 84 vs. 18–46 %, esp. Ca), and lower acidity (pH 6.9 vs. 5.2) than the “typical woodland” conditions of their forest and edge sites (McCoy et al. 2007). Naud et al. (2010) also report increased biomass of black cohosh transplants in canopy gaps and more fertile, less acidic soils. Their results suggest that pH is more important to black cohosh growth than nutrient or light availability and that lime amendments should significantly improve its performance in acidic soils (Naud et al. 2010; suggested optimum at pH 5–6). It is important to note that soil pH and nutrient levels comparable to our transplant site have been associated with lower black cohosh growth and rhizome yields, but not with the high mortality rates we observed. Three years after transplant, McCoy et al. (2007) and Naud et al. (2010) observed relatively high survival (65–97 %) across all sites. Neither study reported issues with fungal infections.

Fungal infection

In previous black cohosh propagation studies, only fungal root rot has been cited as a cause of mortality as high as in this study. Thomas et al. (2006) suggest that adequate drainage is essential for black cohosh cultivation, as this species is highly susceptible to fungal infection in heavy, poorly drained soils. In their study, just 10 % of rhizome transplants survived in somewhat poorly drained soils or after periods of above average rainfall (Thomas et al. 2006). Mortality was attributed to fungal pathogens, including species of *Phytophthora*, *Pythium*, *Rhizoctonia*, and *Fusarium*. Attempts to reduce disease impacts in later rhizomes transplanted to infected soils included addition of compost to enhance drainage, fungicide treatment, shallower planting depth, and fall (rather than spring) planting. No remediation efforts were successful in reducing disease prevalence or rhizome mortality in poorly drained, infected soil, although survival was slightly higher for shallow- versus deep-planted rhizomes (0.5 vs. 6.5 cm).

We did not anticipate problems with fungal infection in our study. Black cohosh is a hardy landscaping plant, preferring moist, organic-rich soils and partial shade (Pengelly and Bennett 2012; Predny et al. 2006). The high mortality described by Thomas et al. (2006) occurred in soil with standing water or ponding as often as 14 % of the time. Soils at both our collection and transplant sites were well-drained. Temporary

storage tubs experienced natural late season (July–October) temperature and rainfall conditions, under a partial tree canopy. The field/potting soil mix used in these tubs did remain moist, with heavier consistency, but large drainage holes prevented saturation or standing water. Despite this, a small proportion of our rhizomes (<5 %) showed molding and/or rot after 3 months in storage tubs. Rhizomes with evidence of mold or rot were discarded prior to transplant, and our analysis showed no effect of externally obvious fungal infection or rot on rhizome survival. However, it seems reasonable to suspect that some rhizomes may have been diseased or deteriorating with little to no external evidence. Fungal infection may have proliferated during storage in moist soils and contributed to low survival rates. The lower biomass of failing rhizomes also may be suggestive of rhizome rot or early deterioration. If so, this supports the supposition that underlying fungal infection may have been a primary contributor to the high mortality rates in our study.

Conclusions

For forest farming to be profitable and successfully reduce wild harvest pressures, forest landowners need greater information and support for growing and processing medicinal herbs (Chamberlain et al. 2009; Davis 2012). Demand and profitability of organically-grown black cohosh, in particular, has increased in recent years, indicating that wild-simulated forest farming can be a valuable enterprise for landowners, if high yields can be maintained (Davis 2012). Our results, coupled with those of previous propagation studies, suggest that careful site selection is critical for black cohosh cultivation. Circumneutral or lime-amended, organic rich soils and light to moderate shading are recommended to enhance growth and productivity without loss of total active component yields (e.g., McCoy et al. 2007; Naud et al. 2010). In addition, moist but well-drained soils appear to be critically important in reducing the likelihood of devastating fungal infections.

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