Occurrence of 23-epi-26-deoxyacteain and Cimiracemoside A in Various Black Cohosh Tissues Throughout the Growing Season

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Abstract. Black cohosh [Actaea racemosa L.; Cimicifuga racemosa (L.) Nutt.] is a perennial herb native to North America that is commonly used for the treatment of menopausal symptoms. The plant is almost exclusively harvested from the wild and is being threatened by overharvesting in some regions. As demand for this plant continues to increase, the potential for profitable cultivation of this species is becoming realistic. Little is known about the effect of various cultivation practices, soils, environments, and harvest times on the multitudes of phytochemicals that occur in black cohosh. Furthermore, although the rhizome is the organ that is traditionally consumed, other tissues also contain various quantities of important phytochemicals, but this has not been well documented. The objectives of this study, therefore, were to ascertain any environmental effects on the production of two representative phytochemicals (23-epi-26-deoxyacteain and cimiracemoside A) and to elucidate any season-long patterns or variations in the production of these compounds within five black cohosh tissues (leaf, rachis, rhizome, root, and inflorescence). All black cohosh tissues contained 23-epi-26-deoxyacteain with substantially more, as a percentage of dry weight, detected in inflorescence (28,582 to 41,354 mg kg−1) and leaf (8250 to 16,799 mg kg−1) compared with rhizome (2688 to 4094 mg kg−1), and all tissues experienced a linear season-long decrease in occurrence of this compound. Cimiracemoside A was not detected in leaf tissues. The highest levels were found in rhizome (677 to 1138 mg kg−1) and root (598 to 1281 mg kg−1), which likewise experienced a significant season-long decrease in this compound, whereas levels in the rachis (0 to 462 mg kg−1) increased over time. In general, environmental factors did not affect production of either compound. Varying seasonal patterns in phytochemical production, combined with differences in phytochemical content among plant tissues, point to the potential for more targeted horticultural production of these and other medicinal compounds within black cohosh.
excellent horticultural potential for this species. Although some guidelines for the cultivation of black cohosh have been published (Cech, 2002; Persons and Davis, 2005), little research has been conducted to determine ideal cultivation methods for this species, which, like many woodland perennials, will not thrive if conditions are not suitable. For example, plants cultivated in heavy soil in wet years have proven to be highly susceptible to a variety of fungal diseases (Abad et al., 2005; Thomas et al., 2006). Also, potential influences of cultivation methodologies on phytochemical content have not been thoroughly explored. In one black cohosh study, McCoy et al. (2007) found no differences in triterpene glycoside production, as a percentage of dry weight, in plants cultivated within three different environments (managed agricultural shadehouse, forest, or disturbed forest edge). In other species, however, it has been shown that environmental conditions can have a significant impact on phytochemical content (e.g., Hornok, 1986; Mudge et al., 2004).

Another question is when should plants be harvested to maximize phytochemical yield; the concentration of a.i. in a medicinal plant, as a percentage of dry weight, may be relatively constant throughout the growing season (e.g., Rushing et al., 2004) or may display significant temporal variation (e.g., Sen and Datta, 1986). Although black cohosh is traditionally harvested in the fall, Popp et al. (2002) reported that triterpene glycosides and isoflavonoid acid increased in mid-May and June in black cohosh cultivated in Germany. Additionally, there is the question of which plant tissues might yield maximum quantities of desirable phytochemicals. The use of renewable aboveground tissues such as leaves in place of roots or rhizomes that are at risk of unsustainable wild collection has been explored or suggested for some threatened species such as ginseng (Popovich and Kitts, 2004; Xie et al., 2004). To date, there has been little inquiry into chemical content of plant tissues other than those traditionally used for black cohosh or many other important root crops. Our objective for the present study, therefore, was to quantify any seasonal patterns or variations in the content of two representative bioactive triterpenoids (23-epi-26-deoxyactein and cimicarimoside A) within black cohosh leaf, rachis, rhizome, root, and inflorescence tissues across multiple environments.

Materials and Methods

The study was conducted at two geographically different sites in Missouri that are 304 km apart: the University of Missouri–Columbia’s Southwest Research Center near Mt. Vernon in southwest Missouri (lat. 37°4’N, long. 93°53’W, alt. 378 m) and the Shaw Nature Reserve of Missouri Botanical Garden at Gray Summit in east-central Missouri (lat. 38°28’N, long. 90°49’W, alt. 174 to 188 m). Both of these sites are at the northern edge of the natural range of black cohosh in Missouri (Steyermark, 1977).

At Mt. Vernon, black cohosh rhizomes were transplanted into five proximate locations within a 0.5-ha area on varying slopes, aspects, and soils in a natural, undisturbed dry-mesic oak-hickory (Quercus-Carya) forest. Indigenous populations of black cohosh were not found in the vicinity of these plantings; however, the species is occasionally found in the region. The soils at this site consisted of a Waben-Cedarberg cherty silt loam complex (Hughes, 1982). The Waben cherty silt loam (loamy-skeletal, siliceous, mesic Uplandalfs) is a deep, nearly level to gently sloping, well-drained soil on narrow terraces and footslopes with moderately rapid water permeability and medium surface runoff, whereas the Cedarberg cherty silt loam (loamy-skeletal, mixed, mesic Cumulic Hapludolls) is a nearly level, somewhat excessively drained soil on narrow flood plains with moderately rapid permeability and slow surface runoff. Both have a low water-holding capacity (Hughes, 1982). Soil fertility tests indicated pH 5.0, organic matter 3.6%, cation exchange capacity 10.4 meq/100 g, low levels of P and K, and adequate levels of Ca and Mg based on recommendations for vegetable production.

At Gray Summit, rhizomes were transplanted into two locations 300 m apart on east to southeast-facing 5% slopes within a mature, mesic, oak-hickory forest. Native populations of black cohosh were likewise not found in the vicinity of this planting; however, a population of a related species, Actaea pachyphylla Ell., which prefers a similar habitat to black cohosh, occurred within 0.5 km of the study site. The soil was a Haymond silt loam (coarse-silty, mixed, nonacid, mesic Typic Udifluvents), which is a deep to very deep, gently sloping to steep, well-drained, silty soil on uplands (Held, 1989). A soil test indicated pH 4.8, organic matter 2.1%, cation exchange capacity 12.3 meq/100 g, very low P levels, low Ca levels, and adequate levels of K and Mg.

The black cohosh plant material for these experiments was acquired from Elixir Farm Botanicals, Brixey, MO, who originally obtained propagules from wild plants occurring in the surrounding Ozark forests. These materials were vouched and confirmed to be Actaea racemosa L. [A. Thomas 35, 36, 37 (MO)]. More than 350 large, robust, mature black cohosh rhizomes were inspected, sorted, and then randomly assigned to the two sites. The 175 rhizomes per site were randomized before transplantation on 7–8 Nov. 2002 at Mt. Vernon and 9 Oct. 2002 at Gray Summit. Spacing was arbitrary and widespread (minimum 1 m apart) in an attempt to simulate somewhat naturally occurring populations of the species. The plants were not irrigated or fertilized, and no pesticides were used.

A harvest of mature black cohosh plants at both sites began soon after leaf emergence in spring (14 May 2003, 6 May 2004) and continued every 2 weeks throughout the season until leaf senescence (1 Oct. 2003, 23 Sept. 2004). This yielded 11 season-long tissue samplings from two disparate sites over 2 years for laboratory analysis. For each harvest, six random plants per site were dug and then dissected into leaf, rachis (commonly referred to as stem), rhizome, root, and inflorescence (in season). Fresh and dry weights of these separated plant tissues were determined for large numbers of individual plants. To obtain enough dry weight tissue for analysis, the separated tissues from each of the six plants per harvest were combined. Samples were then washed clean of all soil and foreign matter, chopped into small pieces, frozen in a standard (−17 °C) freezer, then later freeze-dried and ground into a fine powder (35 mesh) with a Cyclone mill (Udy Corp., Fort Collins, Colo.). A total of 44 bulked six-plant samples each for leaf, rachis, rhizome, and root, and 10 samples of inflorescence (186 total samples) were prepared and analyzed by high-performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD). Samples were analyzed at the conclusion of each of the two growing seasons, and all HPLC standards were calibrated to the original standards. Our methods were modified from Li et al. (2002) and used a gradient mobile phase recommended by ChromaDex (Santa Ana, Calif.). Ground black cohosh tissue samples (0.5 g) were weighed into 50-ml Falcon Tubes (Fisher Scientific, Pittsburgh, PA), 20 mL methanol: water (80:20) was added, and the tubes placed in a sonicator waterbath at 55 °C for 3 d with occasional shaking. The tubes were centrifuged and an aliquot removed for analysis. The HPLC system consisted of a Hitachi L-7100 (Hitachi, Ltd., Tokyo) pump with gradient flow at 1.0 mL/min; a Hitachi L-7200 autosampler (20-μl injection); and an ELSD Model 301 (ESA, Chelmsford, Mass.) with a Prodigy, 5 μm, C18, 250 × 4.6 mm reversed-phase analytical column fitted with a SecurityGuard C18 (ODS) 4.0 × 3.0 mm guard column (Phenomenex, Torrance, Calif.). The ELSD detector had the evaporation temperature set at 50 °C and the nebulizer temperature set at 35 °C, and the photomultiplier tube set at 850 V. The chromatographic gradient solvent system included H2O, acetonitrile (ACN), and EtOH. The mobile phase was: 0 min, 56% H2O, 21% ACN, 23% EtOH; 35 min, 52% H2O, 14% ACN, 34% EtOH; 36 min, 56% H2O, 21% ACN, 23% EtOH; and 50 min, 56% H2O, 21% ACN, 23% EtOH. Primary standards (500 mg kg−1) of 23-epi-26-deoxyactein and cimicarimoside A (ChromaDex) were prepared in 80% ACN:H2O. Working standards of 250, 125, and 62.5 mg kg−1 were then prepared in 80% ACN:H2O. The retention times were 17.45 min for cimicarimoside A and 29.17 min for 23-epi-26-deoxyactein. Data were recorded and processed by a Hitachi D-7000 data acquisition package with ConcertChrom software on a microcomputer. The presence of 23-epi-26-deoxyactein was further confirmed with additional MS parameters initially derived from He et al. (2000). This system used a Qattro LC mass spectrometer (Micromass, Beverly,
Mass.) with Micromass MassLynx data system (version 3.4) set to positive atmospheric pressure chemical ionization mode with 100 to 1000 amu mass range scanned, 150 °C source temperature, 450 °C desolvation temperature, cone voltage 75 V, and scan time 1 s.

An analysis of variance was conducted using the SAS GLM procedure (SAS Institute, Cary, N.C.) to elucidate differences in the quantities of the two phytochemicals among tissues, between years and sites, and across the growing season. Initial analyses revealed no significant differences in the production of 23-epi-26-deoxyactein and cimiracemoside A between the 2 years and between the two sites (data not shown). Therefore, the data were combined and analyzed as a randomized complete block design using year and site as blocks (n = 4). There were 8 days’ difference in numbered harvests (1 to 11) between the 2 years, but the initial harvest each year was based on physiological stage of plant emergence in spring (primarily affected by weather conditions) rather than a strict calendar date. Therefore, the first through 11th harvests in both years were individually pooled for statistical determination of any season-long trends. To confirm or define any seasonal trends in occurrence of the two phytochemicals, polynomial orthogonal contrasts were performed to test for significant linear, quadratic, or cubic trends across the growing season. Mean separation was determined by Duncan’s multiple range test with differences considered significant at the $P \leq 0.05$ level.

Results and Discussion

The black cohosh transplants emerged and thrived at both locations with little care. In 2006, 4 years after the initial planting, the remaining unharvested plants continued to flourish at both locations, indicating that the sites selected were satisfactory for ordinary growth and survival of black cohosh. Figure 1 provides detail on the season-long occurrence of 23-epi-26-deoxyactein within the five black cohosh tissues during the study. All data are based on dry plant tissue weight. Root and rachis samples generally contained similar amounts of 23-epi-26-deoxyactein with means ranging from 1149 to 1970 mg·kg$^{-1}$ (root) and 598 to 1987 mg·kg$^{-1}$ (rachis). Rhizomes had mean levels ranging from 2688 to 4094 mg·kg$^{-1}$, and leaves much more at 8250 to 16,799 mg·kg$^{-1}$. Inflorescences contained roughly 10 times the amount of 23-epi-26-deoxyactein as a percentage of dry weight compared with rhizomes with mean levels ranging from 28,582 to 41,354 mg·kg$^{-1}$. Statistically significant season-long decreasing linear trends in occurrence of 23-epi-26-deoxyactein were detected in all five plant tissues (Fig. 1).

No detectable amount of cimiracemoside A was found in any leaf sample, whereas varying amounts were detected in other tissues (Fig. 2). In general, more cimiracemoside A was detected in rachis and root tissues compared with inflorescence and...
rachis. Mean levels of this compound ranged from 677 to 1138 mg kg⁻¹ in rhizome, 598 to 1281 mg kg⁻¹ in root, and 0 to 462 mg kg⁻¹ in rachis. The initial mean inflorescence sampling (1089 mg kg⁻¹) was similar to root and rhizome samples on that date, but levels thereafter quickly declined to below detection limits as the flowering season progressed. Interestingly, although cimiracemoside A was not found in leaf tissues, it was found in significantly increasing levels in rachis tissues across the season, whereas, at the same time, levels in rhizome and root tissues significantly decreased (Fig. 2).

Because no significant differences were detected across the two sites or the 2 years with either compound, the production and accumulation of both 23-epi-26-deoxyactein and cimiracemoside A do not appear to be strongly dependent on environmental circumstances. However, significant season-long differences in phytochemical production, combined with differences in content among various plant tissues, suggest that horticultural refinement in the production and harvest of these and other plant compounds may be possible. Because both compounds in all tissues (except cimiracemoside A in rachis) tended to be present at the highest levels soon after emergence in spring and thereafter declined across the season, we might speculate that the increase in physiological activity associated with the spring awakening and emergence of the plant stimulates the production of high levels of these compounds. Our results, however, do not permit us to satisfactorily speculate as to whether specific plant tissues (as opposed to several or all tissues) produce such compounds nor if such compounds may be translocated throughout the plant for some purpose.

With traditional harvest of cultivated black cohosh rhizome, plants are usually grown for 3 to 5 years before being destructively harvested (Persons and Davis, 2005). If leaves and inflorescences could be used as sources of active compounds, destructive harvest of plants (cultivated or wild) may not be necessary, repetitive annual harvest could be established, and horticultural techniques could possibly be optimized for production of large quantities of inflorescences and leaves. However, the producer must consider the relative amount of a particular phytochemical produced within a specific tissue compared with the total dry matter yield of that and other tissues. Table 1 provides mean fresh and dry weight yields of the various tissues from large cultivated material earlier in the season. If leaves and inflorescences were determined to be useful for the production of 23-epi-26-deoxyactein and cimiracemoside A, along with other phytochemicals that behave similarly in the plant, harvest early in the season would seem to be clearly preferable. Potential harm to these perennial plants incited by removing newly flushed leaves in spring after overwintered carbohydrate reserves have been largely depleted would have to be considered. Additional research would be merited to determine whether secondaryflushes of leaves and inflorescences would be produced and if they would contain similarly high concentrations of phytochemicals. The concept of a repeated harvest of emerging foliage until plants begin to show signs of carbohydrate depletion, as is done with the vegetable asparagus (Asparagus officinalis L.), might also be investigated.

As the horticultural and medicinal knowledge of black cohosh cultivation is gradually elucidated and accumulated, and as specific phytochemicals within black cohosh tissues are individually evaluated clinically for efficacy in treating menopausal and other health-related concerns, the cultivation of black cohosh (as well as other medicinals) will likely and necessarily become more focused toward very specific phytochemical production goals. The results of this study suggest that black cohosh is well suited for this type of horticultural development.

### Literature Cited

Abad, Z.G., J. Phillips, and A.L. Thomas. 2005. Black root and crown rot of black cohosh (Actaea racemosa L.) is associated to Phytophthora and Pythium species. Phytopathology 95:S1.

Blumenthal, M. 1999. Herb market levels after five years of boom: 1999 sales in mainstream market up only 11% in first half of 1999 after 55% increase in 1998. HerbalGram 47:64–65. Blumenthal, M. 2002. Herb sales down in mainstream market, up in natural food stores. HerbalGram 55:60.

Burdette, J.E., J. Liu, S.N. Chen, D.S. Fabricant, C.E. Pierson, E.L. Barker, J.M. Pezzuto, A. Mesecar, R.B. van Bremen, N.R. Farnsworth, and J.L. Bolton. 2003. Black cohosh acts as a mixed competitive ligand and partial agonist of the serotonin receptor. J. Agr. Food Chem. 51:5661–5670.

Cech, R. 2002. Growing at-risk medicinal herbs. Horizon Herbs, LLC, Williams, Ore.

Chen, S.N., W. Li, D.S. Fabricant, B.D. Santariero, A. Mesecar, J.F. Fitzloff, H.H. Fong, and N.R. Farnsworth. 2002a. Isolation, structure elucidation, and absolute configuration of 26-deoxyactein from Cimicifuga racemosa and clarification of nomenclature associated with 27-deoxyactein. J. Nat. Prod. 65:601–605.

Chen, S.N., D.S. Fabricant, Z.Z. Lu, H.H. Fong, and N.R. Farnsworth. 2002b. Cimiracemosides I-P, new 9,19-cyclopanoanetetratripeptide glycosides from Cimicifuga racemosa. J. Nat. Prod. 65:1391–1397.

Chen, S.N., D.S. Fabricant, Z.Z. Lu, H. Zhang, H.H. Fong, and N.R. Farnsworth. 2002c. Cimiracemates A-D, phenylpropanoid esters from the rhizomes of Cimicifuga racemosa. Phytochemistry 61:409–413.

Compton, I.A., A. Culham, and S.L. Jury. 1998. Reclassification of Actaea to include Cimicifuga and Souleia (Ranunculaceae): Phylogeny inferred from morphology, rDNA ITS, and cpDNA trnL-F sequence variation. Taxon 47:593–634.

Düker, E.M., I. Kpanisi, H. Jarry, and W. Wurtke. 1991. Effect of extracts from Cimicifuga race- moso on gonadotropin release in menopausal women. J. Clin. Endocrinol. Metab. 73:1377–1381.
women and ovariecctomized rats. Planta Med. 57:420–424. 
Einbond, L.S., M. Shimizu, D. Xiao, P. Nuntanakorn, J.T. Lim, M. Suzui, C. Seter, T. Pertel, E.J. Kennelly, F. Kronenberg, and I.B. Wein-stein. 2004. Growth inhibitory activity of extracts and purified components of black cohosh on human breast cancer cells. Breast Cancer Res. Treat. 83:221–231. 
He, K., B. Zheng, C.H. Kim, L. Rogers, and Q. Zheng. 2000. Direct analysis and identification of triterpene glycosides by LC/MS in black cohosh, Cimicifuga racemosa, and in several commercially available black cohosh products. Planta Med. 66:635–640. 
Held, R.J. 1989. Soil survey of Franklin County, Missouri. USDA–Soil Conservation Service. U.S. Govt. Printing Office, Washington, D.C. 
Hornok, L. 1986. Effect of environmental factors on growth, yield and on the active principles of some spice plants. Acta Hort. 188:169–176. 
Hughes, H.E. 1982. Soil survey of Greene and Lawrence Counties, Missouri. USDA–Soil Conservation Service. U.S. Govt. Printing Office, Washington, D.C. 
Jarry, H., G. Harnischfeger, and E.M. Düker. 1985. Studies on the endocrine efficacy of the constituents of Cimicifuga racemosa. 2. In vitro binding of constituents to estrogen receptors. Planta Med. 61:221–226. 
Jiang, B., F. Kronenberg, M.J. Balick, and E.J. Kennelly. 2006a. Analysis of formononetin from black cohosh (Actaea racemosa). Phyto- medicine 13:477–486. 
Jiang, B., F. Kronenberg, P. Nuntanakorn, M.H. Qiu, and E.J. Kennelly. 2006b. Evaluation of the botanical authenticity and phytochemical profile of black cohosh products by high-performance liquid chromatography with selected ion monitoring liquid chromatography-mass spectrometry. J. Agr. Food Chem. 54:3242–3253. 
Kennelly, E.J., S. Baggett, P. Nuntanakorn, A.L. Oosaki, S.A. Mori, J. Duke, M. Coleton, and F. Kronenberg. 2002. Analysis of thirteen populations of black cohosh for formononetin. Phytotherapy 9:461–467. 
Lehmann-Willenbrock, E. and H.H. Riedel. 1988. Klinische und endokrinologische Untersuchungen zur Therapie ovarießer Ausfallerschei- nungen nach Hysteretomie unter Belassung der Adnexe. Zentralbl. Gynakol. 110:611–618. 
Li, W., S. Chen, D. Fabricant, C. Angerhofer, H. Fong, N. Farnsworth, and J. Fitzloff. 2002. High-performance liquid chromatographic analysis of black cohosh (Cimicifuga racemosa) constituents with in-line evaporative light scattering and photodiode array detection. Anal. Chim. Acta 471:61–75. 
Liu, W., Y. Sun, W. Liang, J.F. Fitzloff, and R.B. van Breemen. 2003. Identification of caffeic acid derivatives in Actaea racemosa (Cimici- fuga racemosa, black cohosh) by liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 17:978–982. 
Li, J., J.E. Burdette, H. Xu, C. Gu, R.B. van Breemen, K.P. Bhat, N. Booth, A.I. Constantinou, J.M. Pezzuto, H.H. Fong, N.R. Farns- worth, and J.L. Bolton. 2001. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. J. Agr. Food Chem. 49:2472–2479. 
Lloyd, J.U. and C.G. Lloyd. 1884. Drugs and medicines of North America. Vol. 1. J.U. and C.G. Lloyd, Cincinnati, Ohio. 
McCoy, J., J.M. Davis, N.D. Camper, I. Khan, and A. Bharathi. 2007. Influence of rhizome prop- agule size on yields and triterpene glycoside concentrations of black cohosh. [Actaea racemos- a L. syn Cimicifuga racemosa (L.) Nuttal]. HortScience 42:61–64. 
McKenna, D.J., K. Jones, S. Humphrey, and K. Hughes. 2001. Black cohosh: Efficacy, safety, and use in clinical and preclinical applications. Altern. Ther. Health Med. 7:93–100. 
Moerman, D.E. 1998. Native American ethnobot- any. Timber Press, Portland, Ore. 
Mudge, K.W., W. Lim, J.P. Lardner, and R.L. Beyfuss. 2004. Effects of population and age on ginsenoside content of American ginseng (Panax quinquefolium L.). Acta Hort. 629:161–166. 
Panossian, A., A. Danielyan, G. Mamikonyan, and G. Wikman. 2004. Methods of phytochemical standardisation of rhizoma Cimicifugae race- mosae. Phytochem. Anal. 15:100–108. 
Persons, W.S. and J.M. Davis. 2005. Growing and marketing ginseng, goldenseal, & other wood- land medicinals. Bright Mountain Books, Inc., Fairview, N.C. 
Pethö, Å. 1987. Klimakterische Beschwerden. Umstellung einer Hormonbehandlung auf ein pflanzliches Gynäkologikum möglich? Ärzt. Praxis 38:1551–1553. 
Popovich, D.G. and D.D. Kitts. 2004. Generation of ginsenosides Rg3 and Rh2 from North American ginseng. Phytochemistry 65:337–344. 
Popp, M., R. Schenk, and G. Abel. 2002. Cultiva- tion of Cimicifuga racemosa (L.) Nuttal and quality of CR extract BNO 1055. Maturitas 44(Suppl. 1):S1–S7. 
Rushing, J.W., R.L. Hassell, and R.J. Dufault. 2004. Drying temperatures and stage of develop- ment at harvest influence active principle in feverfew, Tanacetum parthenium L. Acta Hort. 629:167–173. 
Sakurai, N., J.H. Wu, Y. Sashida, Y. Mimaki, T. Nikaido, K. Koike, H. Itokawa, and K.H. Lee. 2004. Anti-AIDS agents. Part 57: Actein, an anti-HIV principle from the rhizome of Cimi- cifuga racemosa (black cohosh), and the anti-HIV activity of related saponins. Bioorg. Med. Chem. Lett. 14:1329–1332. 
Sen, S. and P.P.C. Datta. 1986. Alkaloid quantity of some Apocynaceae leaf drugs in relation to seasons. Acta Hort. 188:177–186. 
Steyermark, J.A. 1977. Flora of Missouri. The Iowa State University Press, Ames, Iowa. 
Struck, D.M., M. Tegtmeier, and G. Harnischfeger. 1997. Flavones in extracts of Cimicifuga race- mosa. Planta Med. 63:289–290. 
Thomas, A.L., R.J. Crawford, Jr., L.J. Havermann, W.L. Applequist, B.E. Schweitzer, S.F. Wood- bury, and J.S. Miller. 2006. Effect of plant- ing depth, planting season, and fungicide treatment on establishment of black cohosh in a poorly drained soil. HortScience 41:374–376. 
Xie, J.T., S.R. Mehendale, A. Wang, A.H. Han, J.A. Wu, J. Osinski, and C.S. Yuan. 2004. American ginseng leaf: Ginsenoside analysis and hypoglycemic activity. Pharmacol. Res. 49:113–117.