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Chilling Requirements to Break Dormancy of *Veratrum californicum*

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***Abstract. Veratrum californicum*, a native of the western United States, has long been used in herbal medicine and now also has potential pharmaceutical uses. As a result of a projected increasing demand for *V. californicum* biomass for pharmaceutical purposes, the development of a chilling protocol for enhanced cultivation efficiency is needed. To study the effects of chilling on the growth of *V. californicum*, field-collected rhizomes with attached bulbs and roots were potted, stored at 10 °C for 2 weeks, and subsequently chilled at 5 °C for 30 to 180 days before transfer to a greenhouse or growth room. Twenty plants were transferred to the greenhouse every 30 days to observe growth. Ten plants were harvested at shoot emergence and the remaining 10 when leaves were fully expanded. In addition, 10 plants were transferred from 5 °C to a growth room every 30 days where net photosynthetic rates were measured. Longer chilling duration correlated with a reduction in days to shoot emergence and leaf expansion. The net photosynthetic rates of *V. californicum* plants chilled for 120, 150, or 180 days were higher than those of plants chilled for only 30, 60, or 90 days. Plants exposed to longer chilling durations were taller and had larger, more numerous leaves. Interestingly, *V. californicum* shoot emergence was also observed in the dark at 5 °C after the bulbs had been stored for 210 days. Growth of the root systems of plants was also observed during chilling. In conclusion, chilling was necessary at 5 °C for a minimum of 120 days to force early emergence and vigorous growth of *V. californicum*.**

Veratrum californicum Durand (corn lily) is an herbaceous perennial monocot native to wet meadows across much of western North America [USDA NRCS (Natural Resources Conservation Service), 2011]. *Veratrum californicum* has long been used in herbal medicine and now also has potential pharmaceutical applications. In recent years, the *V. californicum*-derived phytochemical cyclopamine and its derivatives have been explored as promising therapeutic agents for the treatment of tumors arising from activation of the Hedgehog signaling pathway (Berman et al., 2002; Chen et al., 2002; James et al., 2004; Taipale and Beachy, 2001; Tremblay et al., 2009). To meet the projected pharmaceutical demand for this alkaloid, a dependable cultivation system will be required. To that end, we are developing cultivation protocols for the greenhouse production of *V. californicum*.

Extensive field surveys were conducted to select planting stock with high concentrations of cyclopamine and related alkaloids. An ecotype of primary interest was identified in numerous high-elevation bogs and meadows in Utah and Idaho where plants have had the highest yields of alkaloids over several successive years of observation (unpublished data). In these higher (above 2400 m) regions with native *V. californicum* populations, bulbs undergo prolonged (seven- to eight-month) periods of winter dormancy under a deep snow pack.

Average daily soil-temperature data collected between 2003 and 2010 at three depths (5, 20, and 51 cm) from several independent USDA weather station locations in Utah proximal to natural *V. californicum* populations of interest show that soil temperatures typically drop below 10 °C by 1 Oct. and do not return above 10 °C until after 1 May. Field observations report a rapid burst of vegetative growth after the recession of the snow line. Taylor (1956) reported that a period of exposure to cold temperatures is necessary to permit successful vegetative growth of *V. californicum* and that this prolonged exposure to cold is essential for successful cultivation. Dormancy is defined as a lack of growth because specific conditions have not been met (e.g., a period of low temperature). We anticipate that a successful production protocol for *V. californicum* collected from natural populations in temperate regions, sites above 2400 m in elevation, will include defined chilling treatments.

In this study we report the influence of varied periods of chilling on shoot emergence and growth using mature, field-collected *V. californicum* plants. Our goal was to force *V. californicum* to emerge before a hypothetical natural period of snow cover as a means of shortening the production cycle. Thus, we determined the minimum length of cold treatment required to break dormancy and whether shortened dormancy affected shoot emergence and vigor. Growth of the shoot systems was also observed in both greenhouse and artificially illuminated growth rooms.

Materials and Methods

Chilling. A few hundred plants of *Veratrum californicum*, rhizomes with attached bulbs and roots, were selected for uniformly large size from a wild population of thousands dug in a meadow ≈1000 m^2 (mechanically harvested for the purpose of pharmacological research) in Boulger Canyon, UT (lat. 39°36′ N, long. 111°13′ W, elevation 2671 m). This site was chosen because the planting stock from which the plants were collected had high concentrations of cyclopamine and related alkaloids (unpublished data). The next day, 14 Sept. 2010, plants were shipped overnight to Clemson, SC, and on arrival, the rhizomes, each with one bulb, were sorted into two size groups based on bulb circumference [12.2 ± 1.0 cm (mean ± SD) and 10.2 ± 1.4 cm for large and small bulb sizes, respectively] and potted into 7.6-L (large) or 3.8-L (small) plastic containers filled with Fafard 3B mix [45% Canadian sphagnum peatmoss, 25% processed pine bark, 15% perlite, 15% vermiculite, starter nutrients (40 to 230 $mg \cdot L^{-1}$ nitrogen; 5 to 30 $mg \cdot L^{-1}$ phosphorus; 40 to 200 $mg \cdot L^{-1}$ potassium, calcium, and sulfur; 25 to 80 $mg \cdot L^{-1}$ magnesium), wetting agent, dolomitic limestone; Conrad Fafard, Inc., Anderson, SC]. Each plant was drenched with 330 ppm Subdue® [25.1% Metalaxyl: *N*-(2, 6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester, 74.9% inert ingredients; Syngenta Crop Protection, Inc., Greensboro, NC] to prevent root rot.

Veratrum californicum plants were first stored in the dark at 10 °C and 70% relative humidity for 2 weeks (pretreatment) and then chilling treatments were initiated at 5 °C and 65% relative humidity for 30, 60, 90, 120, 150, and 180 d in a controlled environment room (Model# 120-208; Climate Technologies, Inc., Laytonsville, MD) in the Clemson University Biosystems Research Complex. Bulbs and rhizomes not included in these experiments were handled in the same manner as experimental units and were retained under experimental conditions for 220 d. All bulbs and rhizomes were watered at 2-week intervals and substrate volumetric water content was maintained above 44.2% ± 5.8% as measured

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