

## Chilling Requirement to Overcome Bud Dormancy in Intermountain West Chokecherry Ecotypes

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### Abstract

Chokecherry (*Prunus virginiana* L.) is a native North American plant that has potential as a regionally important alternative fruit crop. Seedlings from 13 open-pollinated populations were subjected to chilling temperatures (4°C) for 0, 2, 4, 6, 8, 10, 12, 14 and 16 wk, and then held under long day conditions (16 h) at 20 ± 2°C for 4 wk. Bud break and shoot growth were then measured on replicate plants to determine chilling requirement for breaking bud dormancy. No significant bud break occurred when seedlings were chilled for less than 4 wk, whereas plants chilled for 10 wk showed 100% bud break. The amount of chilling required to produce 50% bud break (CB<sub>50</sub>) differed among seedling populations, and ranged from 3.1 to 8.9 wk. The effect of chilling time on terminal shoot elongation also differed among seedling populations. Some populations showed maximum shoot growth after 8 wk of chilling, whereas shoot elongation in other populations continued to increase through 16 wk of chilling. For those populations that reached an obvious maximum growth within 16 wk of chilling, a chilling time to obtain 50% of maximum growth (CG<sub>50</sub>) was calculated. There was no correlation between CB<sub>50</sub> and CG<sub>50</sub>. For the majority of the Intermountain West ecotypes tested, 10 wk of chilling at 4°C maximized both bud break and terminal shoot growth.

Chokecherries (*Prunus virginiana* L.) are native to North America and adapted to a wide range of environmental conditions. The plants survive in USDA cold hardiness zone 2, and with as little as 35 cm annual precipitation (1). Chokecherries grow in a wide range of soil conditions ranging in pH from 5 to 8, and thrive in disturbed sites with little or no top soil. Recent interests in “functional foods” and “nutraceuticals” have drawn increased attention to the potential health benefits of some fruits and vegetables. Assays of oxygen radical absorbance capacity (ORAC) have shown chokecherry to be high in antioxidants (4). The potential health benefits of the fruit, and the adaptability to alkaline soils, cold hardiness and drought tolerance of the plants make chokecherry an interesting alternative crop for the arid Intermountain West region of the U.S (Utah, Idaho, Nevada and Arizona).

A 1995 evaluation of the market potential for chokecherry estimated that annual demand for chokecherries was 8 times greater than supply (5, 9). Currently, companies

marketing chokecherry products rely on hand-harvested fruit from wild stands. Developing methods for commercial fruit production could result in a profitable niche industry. Limitations to the commercialization of chokecherry in the U.S. include the lack of suitable fruiting cultivars and efficient propagation techniques.

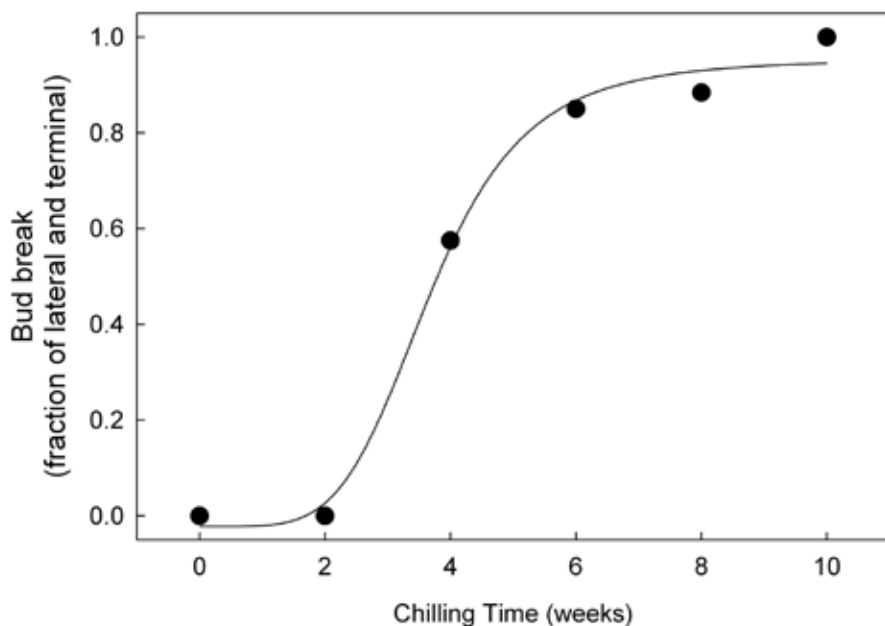
One apparent adaptation of chokecherries to their harsh native environment is a relatively short seedling growth period, where seedlings produce relatively few nodes then set a terminal bud starting a stage of endodormancy. This adaptation, though important for survival in its natural environment, hinders the ability to rapidly propagate and grow chokecherry plants. Determining the minimum requirements for overcoming bud dormancy in these seedlings would be beneficial in commercial chokecherry propagation, and in efforts to develop improved fruiting cultivars. The purpose of the present study was to determine the optimum chilling time to overcome bud dormancy in seedling chokecherry from the Intermountain West.

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### Materials and Methods

Ripe fruit were collected from locations in western Colorado, southeastern Idaho and northern Utah in August, 2006. Each seed lot population was the open-pollinated progeny of a single female parent. Seeds were separated from the fruit and cleaned using a low-speed blender. Cleaned seeds were placed on paper towels and dried at room temperature for 4 days, then stored dry at 3°C. Seeds were germinated in the greenhouse during 2007 after stratification at 3°C for a minimum of 12 wks, as described previously (6). Germinated seedlings were transplanted to 1-L tree pots (6.5 cm wide by 25 cm tall) containing a soil-less potting medium (1:1:1 v:v:v peat:perlite:vermiculite) and grown in the greenhouse at  $20 \pm 2^\circ\text{C}$ . Supplemental lighting (high-pressure sodium) was provided to maintain long day conditions (16 h). Plants received a 20-10-20 fertilizer (Peters Peat Lite Special, Allentown, PA) at  $100 \text{ mg}\cdot\text{L}^{-1}$

actual N at each daily irrigation. By the fall of 2008, all seedlings had set a terminal bud and were typically between 5 and 10 cm in height. Plants of each seed lot were sorted for uniformity and replicate plants were then randomly assigned to one of nine treatments. The number of plants per chilling treatment differed among seed lots due to plant availability, but ranged from 3 to 10 plants per treatment. Plants assigned to eight of the treatment groups were transferred to a walk-in cold room on 18 November 2008 and held at 4°C. Treatment groups were moved from the cold room back to the greenhouse at 2 week intervals (2, 4, 6, 8, 10, 12, 14 and 16 wk). After 4 wk of forcing conditions in the greenhouse, bud break was scored (percent of total buds on the plant) for each plant and terminal shoot growth measured. Plants assigned to the unchilled control group remained in the greenhouse under long day conditions throughout the experiment.



**Fig. 1.** Sigmoid response curve of chilling time to bud break for seedling population 06011. Treatments chilled for 10 or more weeks showed 100% break of both terminal and lateral buds after 4 weeks of forcing at  $20^\circ\text{C}$  under long days.

Curves were fit to percent bud break and terminal growth data using the curve fitting feature of Sigma Plot (SYSTAT Software, San Jose, CA). For those responses showing a sigmoid curve, the response midpoint and standard error were determined using the EC<sub>50</sub> feature.

### Results and Discussion

The effect of chilling time on bud break showed a typical sigmoid response (Fig. 1) for all but one of the 13 seedling populations tested. After 4 wk of forcing at 20°C no bud break occurred in the 0- or 2-wk chilling treatments among any of the seedling populations. However, the 10-wk chilling treatment showed 100% break of both terminal and lateral buds in all seedling populations. The amount of chilling required to produce 50% bud break (CB<sub>50</sub>) differed among seedling populations and ranged from 3.1 to 8.9 wk (Table 1). Only population 06030 did not show a clear sigmoid response due to the unusually high degree of variability among seedlings in the 4-wk and 8-wk chilling treatments (data not shown). This resulted in a

high estimate of CB<sub>50</sub> with a large standard error (Table 1). In the case of this population, total bud break for the 6-wk chilling treatment was 44.9%±0.7.

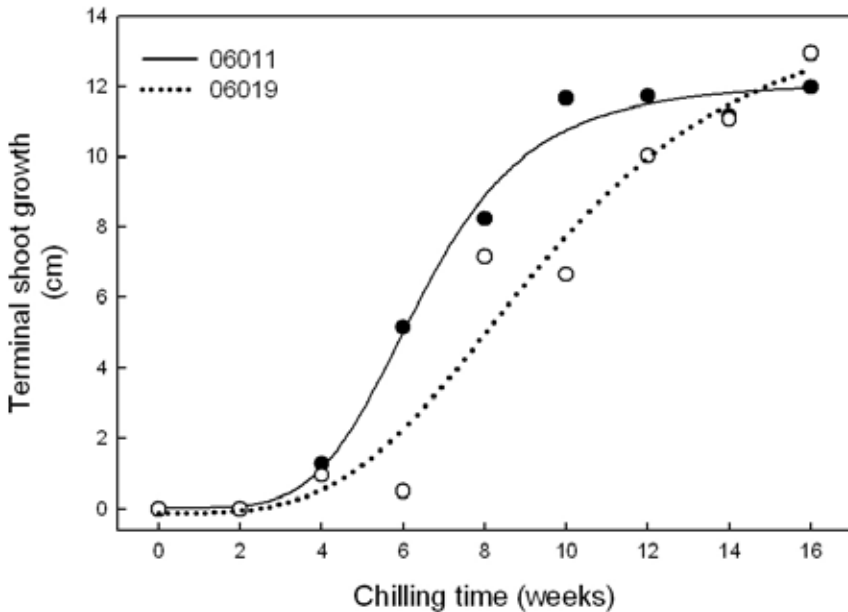
Terminal shoot elongation in response to chilling differed among seedling populations. Nine of the 13 populations studied, including 06011, reached a maximum shoot elongation within 16 wk of chilling (Fig. 2). For these populations, a sigmoid curve was fitted and the chilling time for a growth response midpoint (CG<sub>50</sub>) and growth maximum were calculated (Table 2). However, in the case of populations 06019, 06021, 06025 and 06029, shoot elongation continued to increase with chilling through the 16-wk treatment. For these four populations, a linear regression was calculated for the 4-wk through 16-wk treatments (Table 2). Both of these apparently disparate results agree with those previously reported for peach (*Prunus persica* L. cv. Johnson Elberta) where shoot elongation of cuttings continued to increase with chilling up to 10 wk (2).

There was no significant correlation between chilling requirements for bud break

**Table 1.** Origin and chilling requirement (at 4 °C) for 50% break of terminal and lateral buds (CB<sub>50</sub>) of 13 chokecherry seedling populations (mean ± SE).

Population	Origin		CB <sub>50</sub> ± SE )			(n) <sup>2</sup>
	Latitude (°N)	Elevation (m)				
06022	42.682	1907	3.12	±	0.51	6
06009	42.332	1914	3.34	±	0.29	7
06025	42.328	1864	3.37	±	0.24	4
06007	42.342	1971	3.47	±	0.31	10
06011	42.325	1886	3.68	±	0.23	10
06029	42.311	1892	3.92	±	0.21	8
06020	42.593	2000	4.26	±	0.33	6
06021	42.671	1921	4.32	±	1.22	6
06003	38.413	2477	4.46	±	0.01	4
06019	42.500	2218	4.47	±	2.85	7
06015	42.445	2162	5.08	±	0.31	6
06012	42.325	1886	5.83	±	1.81	3
06030	42.218	1501	8.89	±	10.02	4

<sup>2</sup>n=number of plants sampled for each chilling treatment.



**Fig. 2.** Terminal shoot growth response of two contrasting open-pollinated chokecherry seedling populations. The population 06011 (●) showed a typical sigmoid response, with maximum growth at  $12.1 \pm 0.5$  cm and 50% of maximum growth ( $CG_{50}$ ) at  $6.5 \pm 0.3$  wks at  $4^{\circ}\text{C}$ . Conversely, terminal shoot growth of the seedling population 06019 (○) shows a more linear increase in growth with increased chilling between 4 and 16 weeks.

( $CB_{50}$ ) and for terminal shoot growth ( $CG_{50}$ ). The  $CB_{50}$  of population 06011 was 3.7 wk (among the shortest) and more than 80% bud break occurred after 8 wk of chilling (Fig. 1). Conversely, half-maximal shoot elongation for 06011 occurred after 6.46 wk (among the longest), with an apparent maximum reached by 10 wk. For this same seedling population, Rowley et al. (6) previously reported that the optimum stratification time for seed germination was 16 wk. Whether or not stratification requirements are correlated with bud chilling requirements across populations was not determined, but Frisby et al. (3) reported a strong correlation between bud chilling requirements and seed stratification requirements of peach.

The origin of the study populations ranged from  $38.413^{\circ}\text{N}$  latitude to  $42.682^{\circ}\text{N}$  latitude and from 1501 m to 2477 m in elevation (Table 1), with all but one of the populations

collected in a relatively small geographic area. This represents a very small subset of the natural range of this species. The USDA NRCS PLANTS database (8) lists three varieties of *P. virginiana* with *P. virginiana* var. *melanocarpa* predominant in the Intermountain West with a geographic range from New Mexico to Alaska and from the West Coast to Kansas. Including all three varieties (*melanocarpa*, *demissa* and *virginiana*) the species is distributed throughout the continental U.S. and Canada (8). Among the populations studied here, there was no correlation between chilling requirements and the latitude or elevation of the population origin (analysis not shown). However, such a comparison should be carried out on a wider range of ecotypes. Although not particularly diverse in geographic origin, populations in this study did show wide diversity in a number of characteristics, including adventitious shoot growth

**Table 2.** Effect of chilling time at 4 °C on terminal shoot elongation of 13 chokecherry seedling populations. Nine of the populations showed a typical sigmoid response, reaching an apparent maximum, whereas four populations showed a continued increase in stem elongation with chilling time through 16 weeks of chilling. For populations showing a sigmoid response, chilling time to 50% of maximum growth, or CG<sub>50</sub> was calculated (wk ± SE).

Sigmoid	CG <sub>50</sub>			Maximum growth		
	(wk	±	SE)	(cm	±	SE)
06007	4.94	±	0.2	8.4	±	0.3
06020	5.05	±	0.3	9.4	±	0.5
06030	5.16	±	0.2	9.1	±	0.3
06022	5.23	±	1.3	15.3	±	3.1
06012	5.87	±	0.7	11.0	±	1.2
06003	6.07	±	0.2	10.1	±	0.3
06015	6.33	±	0.4	11.0	±	0.6
06011	6.46	±	0.3	12.1	±	0.5
06009	6.51	±	0.6	13.3	±	1.3
Linear	(slope	±	SE)	R <sup>2</sup>	P value	
06025	0.95	±	0.15	0.906	0.004	
06019	1.07	±	0.14	0.918	0.001	
06021	1.03	±	0.13	0.927	0.001	
06029	1.07	±	0.25	0.786	0.008	

(suckering), powdery mildew susceptibility and growth habit (apical dominance, branching, erect or prostrate).

Among the seedling populations tested, a 10-wk chilling period at 4°C was sufficient for complete bud break, and provided optimum shoot elongation. This information, along with optimum seed stratification requirements (6) provides the opportunity to use a series of chilling and forcing cycles to rapidly propagate and establish chokecherry seedlings. Seedling populations are currently being evaluated for fruiting characteristics, with emphasis on precocity, growth habit, fruit size and quality. Utah’s existing fruit production industry has a significant processing and value-added component (7), and it is anticipated that suitable fruiting cultivars of chokecherry could be rapidly integrated to create a niche industry.

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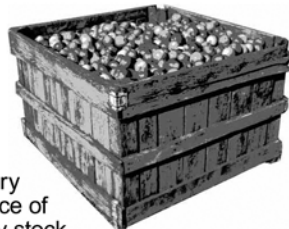
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