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Modeling the Effects of Temperature and Gibberellic Acid Concentration on Red Huckleberry Seed Germination

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Abstract. **Low seed germination percentages have been reported for red huckleberry (*Vaccinium parvifolium* Smith). Attempts to improve germination percentages and the speed of germination for red huckleberry are described. Red huckleberry seeds from two collection sites were given gibberellic acid potassium salt (GA-K) treatments (0, 500, 1000, and 1500 mg·L^{-1}) and were germinated under three temperature regimens [constant 22 °C, 22 °C day/5 °C night (22/5 °C), and 20 °C day/13 °C night (20/13 °C) with a 12-h photoperiod]. A logistic regression model was used to assess the effects of temperature regimens and GA-K treatments on the maximum cumulative germination percentages, rates of increase, and germination lag times. For seeds untreated with GA-K, the 20/13 °C temperature regime resulted in germination percentages ranging from 30% to 61% and lag times (i.e., time to reach one-half of the maximum cumulative germination percentage) of 29 to 35 d for the two accessions. In comparison, the 22/5 °C temperature regime produced germination percentages of 12% and 38% and lag times of 38 to 64 d. The 22 °C constant temperature produced germination percentages ≤1%. Maximum germination percentages of up to 75% were obtained with 1500 mg/L GA-K. Rates of germination were generally unaffected by GA-K treatments, and germination lag times were reduced by an average of 10 d when compared with without GA-K. Improved germination percentages and reduced lag times for red huckleberry seeds were obtained by using a 20/13 °C temperature regime and 1000 to 1500 mg·L^{-1} GA-K.**

Red huckleberry is an indigenous *Vaccinium* sp. from southeastern Alaska to central California along the Pacific coast and Cascade Mountain ranges, with small disjunct populations reported in southeastern British Columbia (Vander Kloet, 1988). The berries are tart, rather than sweet, and are harvested commercially from the wild for processing. Bearing red leaves in autumn and red berries that remain on the plants into late autumn or early winter, red huckleberry has potential for managed commercial fruit production and as an edible ornamental plant. Adaptability to a range of growing sites, vigorous growth, upright habit, and the potential for mechanical harvesting are important traits of this species for breeding programs and commercial production (Barney, 2003).

Little research has been published regarding sexual propagation practices for red huckleberries. Various temperature regimens can affect *Vaccinium* sp. germination. Although Stark and Baker (1992) reported 70% germination of *V. membranaceum* at a constant 20 °C, some *Vaccinium* sp. need temperature fluctuations to germinate (Baskin et al., 2000; Minore et al., 1979; Stushnoff and Hough, 1968; Vander Kloet, 1983). Relatively low germination percentages and a slow germination process are a characteristic of red huckleberry seeds (Vander Kloet, 1983). Vander Kloet (1983) reported up to 47% germination of freshly extracted red huckleberry seeds under a temperature regime of 22 °C day/5 °C night with a 14-h photoperiod (time after harvest of berries not reported). The study also included a 28 °C day/13 °C night temperature treatment with a 14-h photoperiod resulting in 21% germination, but detailed information about the process of germination (i.e., how seeds germinate over time) was not given.

Gibberellic acid (GA) is used to help break seed dormancy of many angiosperms (Taiz and Zeiger, 2002). For instance, at 4 weeks after treatment with 900 ppm GA_3, Dweikat and Lyrene (1989) found 50% of *V. corymbosum* seeds had germinated, whereas only 4% of nontreated seeds had germinated. Higher concentrations of GA_3 failed to significantly increase germination. In contrast, GA treatments failed to influence *V. ashei* seed germination (Ballington et al., 1976). Similarly, Austin and Cundiff (1978) reported that neither GA_{4+7} nor GA_{4+7} plus benzyladenine stimulated *V. ashei* seed germination, although GA_{4+7}-treated seedlings reached transplant size 2 to 4 weeks earlier than did control seedlings or seedlings from GA_3-treated seeds. Additionally, Maznaya and Lyanguzova (1999) found that imbibing nonstratified seeds of *V. uliginosum* for 48 h in 100 to 500 mg/L GA_3 solutions increased the germination percentages from 3% to between 65% and 80%.

Devlin and Karczmarczyk (1975, 1977) determined that without germination enhancement treatments, *V. macrocarpon* seeds are photoblastic (i.e., require light to germinate) and suggested an interaction between the need for light and GA treatments. Devlin and Karczmarczyk (1975) determined that *V. macrocarpon* seed germination was enhanced in the dark when seeds were soaked with GA after they were treated with concentrated sulfuric acid. The seedcoat is a barrier for the uptake of exogenously applied GA and dark germination was enhanced by GA treatments after seed scarification with concentrated sulfuric acid. In fact, treatment with 1000 ppm GA enhanced seed germination in the light and dark (Devlin and Karczmarczyk, 1977). Smagula et al., (1980) also determined that early germination of *V. angustifolium* seeds kept in the dark was stimulated by GA at 500, 1000, 2000, or 4000 ppm. Unlike *V. ashei*, *V. angustifolium* seed germination increased with increased GA concentrations in the light.

Therefore, the objectives in conducting this research were to develop a regression model of the germination process for red huckleberry seeds and to use the regression model to assess the effects of temperature and GA concentration on cumulative germination percentage, rate, and lag time of red huckleberry seeds.

Materials and Methods

Plant materials. Red huckleberry seeds were collected in two locations during Aug. 2003: the Olympic National Forest in Hood Canal Ranger District, Mason County, Washington (accession no. VAPA 023) and near the Mount Saint Helens National Volcanic Monument, Skamania County, Washington (accession no. VAPA 024). Seeds were extracted from fresh ripe fruits using a blender and macerating the berries with a sieve, followed by repeated water washes.

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Seeds were then air dried for 7 d at room temperature (20 °C–22 °C) and put in cold storage at 2 °C to 4 °C for 30 months in sealed plastic bags.

Seeds were removed from cold storage and sieved to remove those <600 µm in diameter. Undersized, broken, and deformed seeds were removed under a magnifying lens. Subsamples (i.e., a replication per treatment per accession) of 50 seeds were put into 1.5-mL microcentrifuge tubes and covered with tap water to imbibe the seed. After 24 h, the water was poured off and a 0.45% (w:v) sodium hypochlorite solution plus three drops of Tween 20 wetting agent per liter of solution was added and left for 20 min to sterilize the seed surface. Seeds were then rinsed three times for 20 min each with distilled deionized water. Surface sterilization was done before GA potassium salt (GA-K) treatments were applied because sterilization after the treatment seemed to inhibit GA-K effect in preliminary trials (O.A. Lopez, unpublished data). After rinsing the seeds, the microcentrifuge tubes were filled with 1 mL of distilled deionized water (control) or GA-K solutions at 500, 1000, and 1500 $mg \cdot L^{-1}$. Before treatment application, GA-K solution pH was adjusted to 7 and was filter sterilized using 0.22-µm syringe filters (Fisherbrand, Pittsburgh, PA) under sterile conditions in a laminar flow hood.

Tubes were then put in a rotary shaker for 48 h after which the treatment solutions were removed by aspiration. Seeds were placed into 15- × 100-mm plastic petri dishes lined with one sheet of sterile filter paper (#4; Whatman, Maidstone, UK) moistened with 3 mL of distilled deionized water and then the petri dishes were then sealed with plastic film. Distilled deionized water was added later, as needed, to keep the filter paper moist. Each petri dish represented one replication or experimental unit. A total of four replications per temperature and GA-K treatment were used per each seed accession.

Temperature and light. Thirty-two petri dishes (four GA-K treatments × two accessions × four replications) were placed in one incubator per temperature regime. Seeds were treated under three different temperature regimens: 22 °C constant temperature, 22 °C day/5 °C night (22/5 °C), and 20 °C day/13 °C night (20/13 °C), all with 12-h photoperiods (model 818; Precision Low Temperature Illuminated Incubators, Winchester, VA). Light in the incubators was provided by Econ-o-watt F34CW/RS/EW 34 W cool-white fluorescent light tubes. The average light intensity in the fluctuating temperature germination chambers was 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and was 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the constant temperature regime chamber.

Data collection. Seeds were counted as germinated when radical emergence was ≥1 mm and they were removed from the petri dishes without replacement. Determination of germination began 7 d after treatment and every 7 d thereafter for 84 d in the 22 °C and 22/5 °C temperature regimens. After 14 d, germination data were collected every 2 to 3 days for 14 d then every 7 days up to 56 d for seeds in the 20/13 °C temperature regime. The difference in observation times was designed to ensure that rapid flushes of germination were accurately recorded.

Statistical procedures. A logistic regression model was used previously to describe the seed germination process for *V. membranaceum* (Shafii and Barney, 2001). This model provides parameter estimates for the cumulative germination percentage and speed of germination (rates of increase and lag times). The specific model used was of the form:

$$y = M\{1 + \exp[-K^* (t - L)]\}^{-1} \quad [1]$$

where y is the cumulative germination percent at time t, M is the asymptote (theoretical maximum of y), K is the rate of increase, and L is the lag time, i.e., time to reach 50% of M.

Nonlinear estimation was carried out separately for each of the eight GA-K-temperature regime treatments within each accession. Cumulative germination percentage results of seeds incubated under 22 °C constant temperature and untreated with GA-K was only reported because of the very little germination obtained. A weighting procedure (i.e., a process that uses the inverse of the variance and makes the variance to be one when it is zero to evenly distribute residual points) was used during the estimation process to account for changes in germination variability across time. Following model fitting, standard regression residual analysis, graphical plots, and parameter significance and correlations were used to assess the adequacy of each model. Comparisons of GA-K treatments and temperature regimens were carried out using single and multiple-df contrasts within a full dummy variable model [i.e., a dummy variable model is used to compare parameter estimates of the same model specification, individually (single df tests) and collectively (multiple df contrasts) among treatments]. The GA-K/temperature treatment comparisons were conducted separately for each accession. All analyses were carried out using SAS (2004; SAS, Cary, NC).

Results and Discussion

Estimates for maximum cumulative germination (M), rate of increase (K), and time to reach 50% germination of M or lag time (L) are shown in Table 1 and were significant ($P \leq 0.05$) except for the predicted maximum cumulative germination of the control (0 $mg \cdot L^{-1}$ GA-K) seeds for VAPA 023 incubated under the 22/5 °C temperature regime. Residual plots for each model showed no patterns or trends, with the majority of the data points within two standard deviations of the mean. An example of a fitted curve and the associated data for the 1500 $mg \cdot L^{-1}$ GA-K treatment under the 22/5 °C temperature regime is presented in Fig. 1. In this example, L was 31 d to reach 50% of M, which was 75%. The value, 0.18 (K), defines the rate of increase. In general higher values of K lead to faster rates of increase. Other treatment-temperature combinations produced similar fitted curves suitable for treatment comparisons.

Table 1. Parameter estimates of the logistic regression model for seeds of two accessions treated with for four gibberellic acid potassium salt (GA-K) treatments under two temperature regimens.

		22 °C/5 °C			20 °C/13 °C		
Treatments ($mg \cdot L^{-1}$ GA-K)	Parameter[z]	Estimate	SE	$P > t$	Estimate	SE	$P > t$
		VAPA 023					
0	M (%)	11.50	6.40	0.07	29.70	4.00	<0.0001
	K	0.08	0.04	0.04	0.14	0.02	<0.0001
	L (d)	64.10	17.10	0.0002	35.00	2.60	<0.0001
500	M (%)	17.90	1.80	<0.0001	50.90	3.10	<0.0001
	K	0.11	0.02	<0.0001	0.16	0.02	<0.0001
	L (d)	37.80	3.80	<0.0001	28.80	1.30	<0.0001
1000	M (%)	24.10	1.50	<0.0001	39.60	2.40	<0.0001
	K	0.16	0.03	<0.0001	0.20	0.04	<0.0001
	L (d)	32.00	2.20	<0.0001	21.80	1.20	<0.0001
1500	M (%)	37.20	1.90	<0.0001	54.10	2.40	<0.0001
	K	0.17	0.03	<0.0001	0.20	0.03	<0.0001
	L (d)	33.50	1.90	<0.0001	22.90	0.80	<0.0001
		VAPA 024					
0	M (%)	37.80	2.00	<0.0001	61.00	3.00	<0.0001
	K	0.15	0.03	<0.0001	0.16	0.02	<0.0001
	L (d)	37.50	2.00	<0.0001	28.60	1.10	<0.0001
500	M (%)	49.40	1.80	<0.0001	68.20	2.20	<0.0001
	K	0.16	0.03	<0.0001	0.20	0.03	<0.0001
	L (d)	29.20	1.60	<0.0001	19.60	0.70	<0.0001
1000	M (%)	54.60	2.00	<0.0001	74.00	1.80	<0.0001
	K	0.13	0.02	<0.0001	0.26	0.03	<0.0001
	L (d)	32.80	1.60	<0.0001	18.30	0.50	<0.0001
1500	K	0.18	0.02	<0.0001	0.19	0.02	<0.0001
	L (d)	30.60	0.90	<0.0001	20.70	0.60	<0.0001

[z]M = asymptote (theoretical maximum cumulative germination percentage); K = rate of increase; L = lag time (days to reach 50% of M).

Effects of temperature. In the constant 22 °C temperature, maximum germination percentages were 0% and 1% for VAPA 023 and VAPA 024, respectively, and statistical analysis was not completed because of the low germination percentages obtained. Temperature effects on seed germination were assessed through comparison of model parameter estimates within each accession and temperature regime. The overall regression curves (i.e., the 0 mg·L^{-1} GA-K-treated seeds incubated under the 22/5 °C and 20/13 °C temperature regimens) were compared first, followed by subsequent tests on their associated parameter estimates. For both accessions, there were significant differences between the estimated curves for temperature regimens. Further analyses indicated that temperature regime significantly affected M and L for the two accessions tested, whereas seeds in both temperature regimens germinated at similar K (Table 2). The 20/13 °C regime increased M percentages from 12% to 30% and 38% to 61% for seeds from accessions VAPA 023 and VAPA 024, respectively, relative to the 22/5 °C regime. Compared with L in the 22/5 °C regime, L in the 20/13 °C regime were significantly reduced from 64 d to 35 d and 38 d to 29 d for accessions VAPA 023 and VAPA 024, respectively.

Based on these results, 30-month cold-stored red huckleberry seeds require diurnal temperature fluctuation to germinate. As previously mentioned, other authors also have reported the need for temperature fluctuation to promote germination for *Vaccinium* species (Baskin et al., 2000; Minore et al., 1979; Stushnoff and Hough, 1968; Vander Kloet, 1983). The results of this trial support these previous reports because seeds incubated under 22 °C constant temperature failed to germinate or germinated at low germination percentages (1%). In our study, the warmer night temperatures of 13 °C increased cold-stored seed germination and reduced L compared with the 5 °C night temperature. In contrast, Vander Kloet (1983) reported 47% germination for fresh seeds under a 22 °C day/5 °C night and 14-h photoperiod and only 21% germination of fresh seeds in a warmer day and night, 28 °C day/13 °C night and 12-h photoperiod regime. As opposed to what occurred in our study, the colder night temperature regime in the Vander Kloet (1983) study produced lower germination (38%) and longer germination times for dried and cold-stored seeds compared with the 61% germination of cold-stored seed in the warmer night temperature regime. In addition, factors such as drying the fresh seeds and cold storage can affect germination characteristics of *Vaccinium* species. Vander Kloet (1983) obtained greater germination percentages after seeds were dried and cold stored at 2 °C for 6 to 12 months for *V. ovalifolium*, *V. deliciosum*, *V. scoparium*, *V. caespitosum*, and *V. myrtillus*. Opposite results were obtained for *V. parvifolium* and *V. membranaceum*. Shafii and Barney (2001) stated that air drying *V. membranaceum* seeds for 7 d

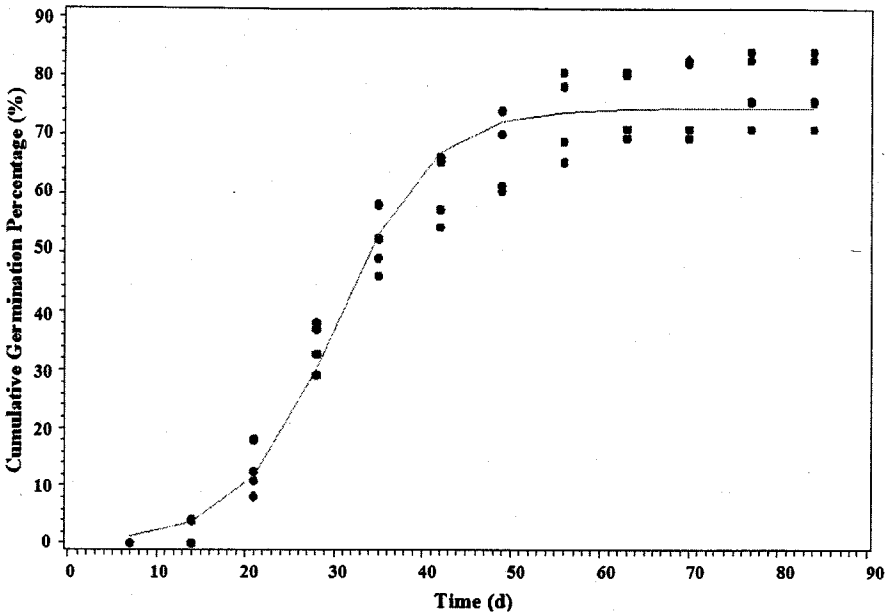

Fig. 1. Estimated logistic regression line with observed data points representing germination of VAPA 024 seeds treated with 1500 mg·L^{-1} gibberellic acid and incubated under 22 °C day/5 °C night temperature regime and a 12-h photoperiod.

Table 2. Contrasts between regression lines of seeds treated with 0 mg·L^{-1} gibberellic acid potassium salt (GA-K) and parameter estimates of the logistic regression model comparing the effects of two temperature regimens (22 °C day/5 °C night and 20 °C day/13 °C night and 12-h photoperiod) on red huckleberry seed germination in two accessions.

Label[z]	DF	*F* value	*P*
VAPA 023			
Lines: 22/5 °C vs. 20/13 °C	3	32.59	<0.0001
M: 22/5 °C vs. 20/13 °C	1	24.73	<0.0001
K: 22/5 °C vs. 20/13 °C	1	0.74	0.3905
L: 22/5 °C vs. 20/13 °C	1	8.12	0.0053
VAPA 024			
Lines: 22/5 °C vs. 20/13 °C	3	61.44	<0.0001
M: 22/5 °C vs. 20/13 °C	1	41.09	<0.0001
K: 22/5 °C vs. 20/13 °C	1	0.15	0.7021
L: 22/5 °C vs. 20/13 °C	1	15.34	0.0002

[z]Accessions VAPA 023 and 024. Lines are represented by the logistic regression model Eq. [1]. Lines = contrasts between all three parameter estimates simultaneously (M, K, and L) where M = the maximum asymptote (theoretical maximum cumulative percentage), K = germination rate, and L = lag time or time to reach 50% of M.

Table 3. Contrasts between regression lines and parameter estimates of the logistic regression model comparing the effects of gibberellic acid potassium salt (GA-K) on red huckleberry seed germination for two accessions (VAPA 023 and 024) and two temperature regimens (22 °C/5 °C and 20 °C/13 °C).

Label[z]	DF	*F* value	*P*
VAPA 023–22/5 °C			
Lines: control vs. GA	3	25.38	<0.0001
M: control vs. GA	1	5.29	0.0225
K: control vs. GA	1	1.89	0.1709
L: control vs. GA	1	3.00	0.0849
VAPA 023–20/13 °C			
Lines: control vs. GA	3	193.25	<0.0001
M: control vs. GA	1	22.66	<0.0001
K: control vs. GA	1	2.66	0.1129
L: control vs. GA	1	15.56	0.0001
VAPA 024–22/5 °C			
Lines: control vs. GA	3	212.57	<0.0001
M: control vs. GA	1	96.71	<0.0001
K: control vs. GA	1	0.14	0.7074
L: control vs. GA	1	9.58	0.0023
VAPA 024–20/13 °C			
Lines: control vs. GA	3	357.48	<0.0001
M: control vs. GA	1	11.79	0.0007
K: control vs. GA	1	7.74	0.0059
L: control vs. GA	1	62.79	<0.0001

[z]Lines are represented by the logistic regression model Eq. [1]. Lines = contrasts between all three parameter estimates simultaneously (M, K, and L) where M = the maximum asymptote (theoretical maximum cumulative percentage), K = rate of increase, and L = lag time or time to reach 50% of M.

reduced M percentage from 73% to 59% compared with fresh seeds although subsequent cold storage at 2 to 4 °C of the dried seeds restored germination rates to those similar to fresh seeds. In our preliminary trials, fresh and dried seeds of *V. parvifolium* germinated at very low cumulative germination percentages (Lopez, 2006), and data were not modeled because of low germination percentages and high variability. Future research is needed on the effect of cold storage times on *V. parvifolium* seed germination.

The mechanisms of temperature effect on red huckleberry seeds are yet to be determined, but habitat characteristics could explain the dependence on temperature fluctuation for germination. Seed ecology and germination characteristics are associated with habitat adaptation, climate conditions, and the seed position in the soil that are favorable for seed germination and seedling establishment (Baskin et al., 2000; Berrie, 1984; Thompson et al., 1977). For example, Thompson and Grime (1983) stated that sensitivity to temperature fluctuations evolved in boreal wetland species (e.g., *Chenopodium rubrum*, *Rorippa islandica*, *Gnaphalium uliginosum*, and *Atripex hastate*) as an adaptation to ensure that seeds will germinate when the conditions are more suitable for seedling establishment.

A required warmer daily minimum temperature has been reported in *Vaccinium* species to promote seed germination. Baskin et al., (2000) reported that *Vaccinium myrtillus* and *V. vitis-idaea* seeds germinated when minimum daily temperatures were frequently ≥10 °C in late August and early to mid-September, but no seeds of either species germinated during late May where minimum temperatures ranged from 7 °C to 10 °C.

Based on the results of our study, red huckleberry seed germination is greater in night temperatures of 13 °C compared with germination at 5 °C. In alpine and sub-Alpine ecosystems where red huckleberry is adapted, temperatures naturally fluctuate with high temperatures during the day and low temperatures at night (Vander Kloet, 1988).

Effects of GA-K. GA-K treatments significantly increased M percentages for both accessions and germination temperature regimens compared with germination in the control (Table 3). M for VAPA 023 seed at 22/5 °C increased from 12% to 38% as the GA-K concentrations increased from 0 to 1500 mg·L^{-1} (Fig. 2). Specific comparison of the 1000 and 1500 mg/L GA-K treatments indicates that M increased in seeds treated with more than 1000 mg·L^{-1} GA-K (Table 4). VAPA 023 seeds grown under 20/13 °C showed similar responses except that seeds treated with 1000 mg/L had lower M than those with 500 mg·L^{-1} (Fig. 3). A contrast between these two treatments (500 vs. 1500 mg·L^{-1} GA-K) showed that the M between the mentioned treatments were similar, however (Table 4).

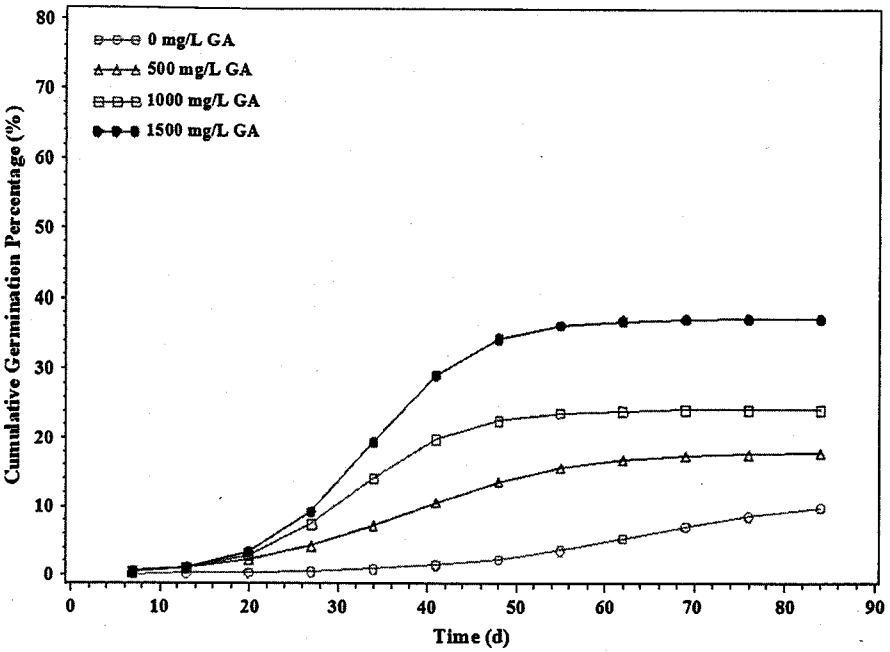

Fig. 2. Estimated logistic regression lines for accession VAPA 023 seeds grown under 22 °C day/5 °C night temperatures with a 12-h photoperiod treated with four gibberellic acid potassium salt (GA-K) treatments.

Table 4. One-df contrasts between parameter estimates of the logistic regression model comparing the effects of gibberellic acid potassium salt (GA-K) doses on red huckleberry seed germination for two accessions (VAPA 023 and 024) and two temperature regimens (22 °C/5 °C and 20 °C/13 °C).

Label[z]	DF	*F* value	*P*
VAPA 023–22/5 °C			
M: 1000 vs. 1500 mg·L^{-1} GA	1	28.67	<0.0001
K: 1000 vs. 1500 mg·L^{-1} GA	1	0.01	0.9261
L: 1000 vs. 1500 mg·L^{-1} GA	1	0.26	0.6045
VAPA 023–20/13 °C			
M: 500 vs. 1500 mg GA	1	0.66	0.4180
K: 500 vs. 1500 mg·L^{-1} GA	1	2.29	0.1316
L: 500 vs. 1500 mg·L^{-1} GA	1	14.12	0.0002
VAPA 024–22/5 °C			
M: 1000 vs. 1500 mg·L^{-1} GA	1	66.13	<0.0001
K: 1000 vs. 1500 mg·L^{-1} GA	1	3.44	0.0652
L: 1000 vs. 1500 mg·L^{-1} GA	1	1.51	0.2205
VAPA 024–20/13 °C			
M: 1000 vs. 1500 mg·L^{-1} GA	1	0.01	0.9432
K: 1000 vs. 1500 mg·L^{-1} GA	1	3.76	0.0537
L: 1000 vs. 1500 mg·L^{-1} GA	1	9.83	0.0020

[z]Parameter estimates of the logistic regression model Eq. [1] where M = the maximum asymptote (theoretical maximum cumulative percentage), K = rate of increase, and L = lag time or time to reach 50% of M.

As with the VAPA 023 accession, cumulative VAPA 024 seed germination grown under 22/5 °C increased with increasing GA-K concentration. Seeds treated with 500 and 1000 mg·L^{-1} GA-K germinated similarly at 50% and 55%, respectively, whereas seed germination with 1500 mg·L^{-1} GA-K was 75% (Fig. 4; Table 3). All treated seed had M greater than the 38% germination of control seeds. Finally, VAPA 024 seeds grown under 20/13 °C also had a consistent M increase from 61% for the control up to 74% for the 1000 and 1500 mg·L^{-1} GA-K treatments (Fig. 5).

The rate of increase (K) was influenced by GA-K treatments only when VAPA 024 seeds were germinated under 20/13 °C (Table 3). K increased for VAPA 024 seeds after GA-K treatments whereas VAPA 023 rate did not (Table 4). Germination rates for seeds treated with 500 or 1500 mg·L^{-1} GA-K were similar, whereas the germination rate of seeds treated with 1000 mg·L^{-1} was higher compared with that of control seeds (Fig. 5). K for seeds treated with 1000 and 1500 mg·L^{-1} GA-K were similar, and VAPA 024 seeds showed increased speed of germination after GA-K

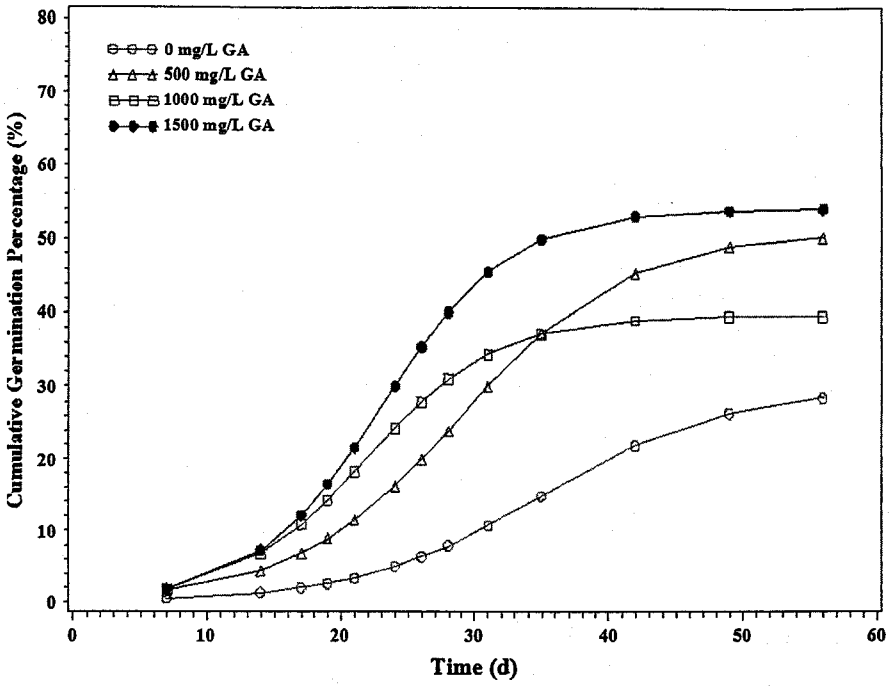

Fig. 3. Estimated logistic regression lines for accession VAPA 023 seeds under 20 °C day/13 °C temperatures and a 12-h photoperiod treated with four gibberellic acid potassium salt (GA-K) treatments.

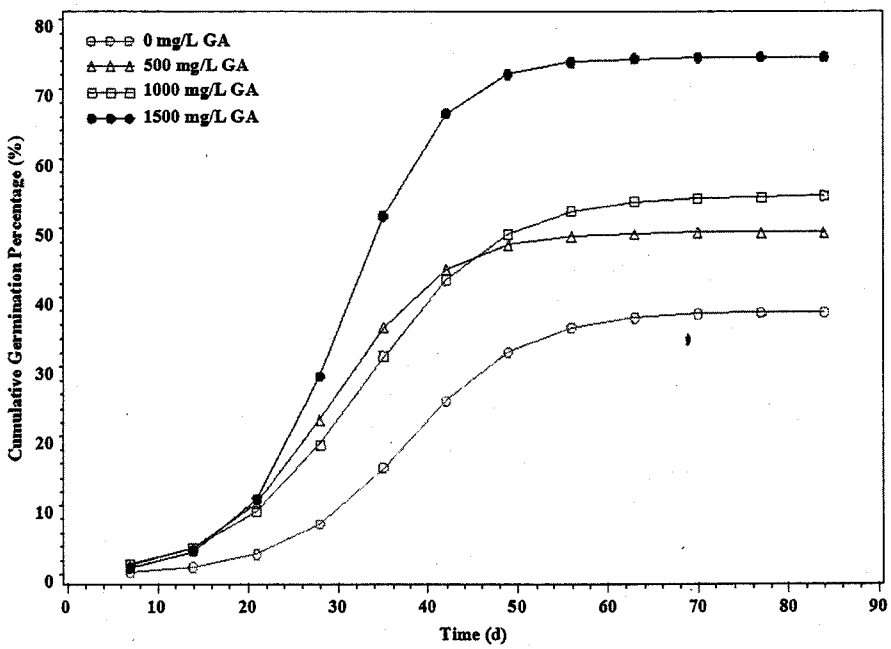

Fig. 4. Estimated logistic regression lines for accession VAPA 024 under 22 °C day/5 °C night temperatures and a 12-h photoperiod treated with four gibberellic acid potassium salt (GA-K) treatments.

treatments whereas VAPA 023 did not (Table 4).

All GA-K treatments significantly reduced L for seed germination compared with the control except when VAPA 023 seeds were grown under 22/5 °C (Tables 1 and 3). However, When comparing the L of 32 d of seeds treated with the 1000 mg·L^{-1} GA-K with seeds treated with 0 mg·L^{-1} GA-K, the L of 64 d was reduced to 32 d, respectively (Fig. 2).

Lag time of VAPA 023 seeds grown under 20/13 °C were reduced from 35 d (control) to 21 or 23 d for 1000 and 1500 mg·L^{-1} treatments, respectively (Fig. 3). In addition, L was shortened as the GA-K dose increased from 500 to 1500 mg·L^{-1} (Table 4). L of VAPA 024 seeds grown under 22/5 °C decreased significantly from 38 d to 29 d for control and 500 mg·L^{-1}, respectively (Fig. 4). In addition, as the GA-K concentrations increased from 1000 to 1500 mg·L^{-1}, seeds germinated at similar L (Table 4). For VAPA 024 seeds grown under 20/13 °C, the 1000 mg·L^{-1} GA-K treatment significantly reduced L from 29 d (control) to 18 d (Fig. 5).

A relatively low germination level of 47% and prolonged germination times of 38 d to first radical emergence have been reported for red huckleberry seeds with no GA (Vander Kloet, 1983). In our study, up to 75% germination and an L as short as 18 d were obtained as a result of endogenously applied GA. These results support reports of improved seed germination with GA treatments in related *Vaccinium* species (Devlin and Karczmarczyk, 1975; 1977; Maznaya and Lyanguzova, 1999; Smagula et al., 1980), and provide a detailed seed germination description resulting from applied GA.

Conclusions

Temperature regimens affected red huckleberry germination characteristics. The 22 °C constant temperature regime with a 12-h photoperiod resulted in little to no germination during the test periods, supporting previous findings that red huckleberry seeds require diurnal temperature fluctuations to germinate. Germination conditions with relatively low night temperatures (22 °C day/5 °C night) and a 12-h photoperiod produced lower germination percentages than a regime with a warmer night temperature (20 °C day/13 °C night and 12-h photoperiod). These findings provide valuable information to growers on germination protocols to apply to produce red huckleberry commercially. The increase in germination speed and germination percentages creates a more valuable scenario for commercial production of red huckleberry plants and increases the possibility of finding potential valuable germplasm for plant breeding purposes.

Although the mechanisms of GA-K effects on germination are yet to be determined for red huckleberry seeds, GA-K had a profound effect on germination of dried, cold-stored red huckleberry seeds. GA treatments generally induced seeds to germinate at higher M percentages with shorter Ltimes compared with control seed germination. The 1000 mg·L^{-1} and 1500 mg·L^{-1} treatments produced the highest germination percentages and rates as well as shortest lag times. For breeding programs and huckleberry production, the use of growth regulators can play an important role in optimizing radical emergence and the potential of selecting valuable genotypes as prospective candidates for commercial production. In addition, with more uniform, rapid, and higher percentages, the use of GA-K can be a valuable tool for growers to grow huckleberry plants commercially by making management and marketing more feasible.

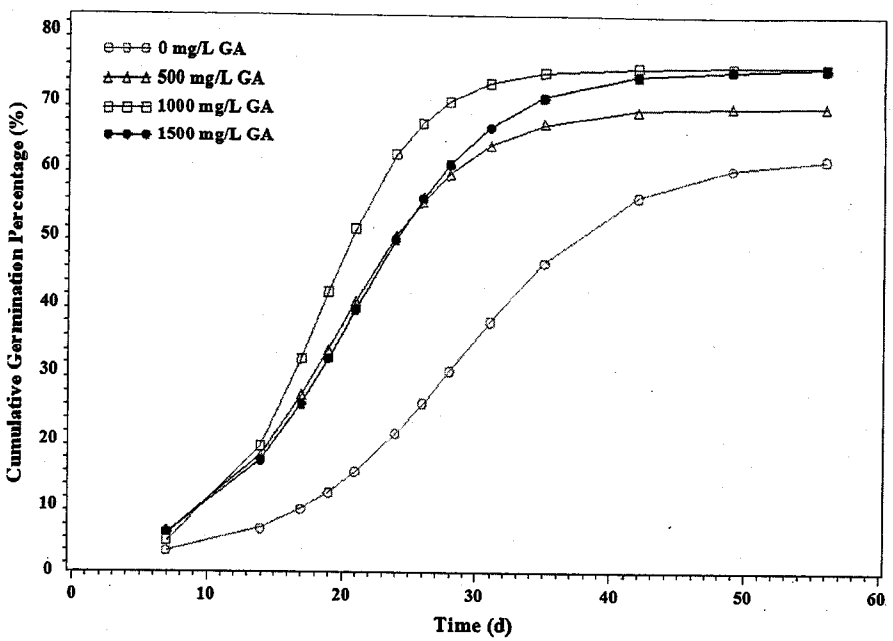

Fig. 5. Estimated logistic regression lines for accession VAPA 024 under 20 °C day/13 °C night temperatures and a 12-h photoperiod treated with four gibberellic acid potassium salt (GA-K) treatments.

Literature Cited

Austin, M.E. and J.S. Cundiff. 1978. Factors affecting rabbiteye blueberry seed germination. J. Amer. Soc. Hort. Sci. 103:530–533.

Ballington, J.R., G.J. Galletta, and D.M. Pharr. 1976. Gibberellin effects on rabbiteye blueberry seed germination. HortScience 11:410–411.

Barney, D.L. 2003. Prospects for domesticating western huckleberries. Small Fruits Rev. 2: 15–29.

Baskin, C.C., P. Milberg, L. Andersson, and J.M. Baskin. 2000. Germination studies of three dwarf shrubs (*Vaccinium*, Ericaceae) of northern hemisphere coniferous forests. Can. J. Bot. 78:1552–1560.

Berrie, A.M.M. 1984. Germination and Dormancy, p. 440–468. In: M.B. Wilkins (ed.). Advanced Plant Physiology. Pitman Press. Marshfield, MA.

Devlin, R.M. and S.J. Karczmarczyk. 1975. Effect of light and gibberellic acid on the germination of "early black" cranberry seeds. Hort. Res. 15:19–22.

Devlin, R.M. and S.J. Karczmarczyk. 1977. Influence of light and growth regulators on cranberry seed dormancy. J. Hort. Sci. 52: 282–288.

Dweikat, I.M. and P.M. Lyrene. 1989. Response of highbush blueberry seed germination to gibberellin A_3 and ^{6}N-benzyadenine. Can. J. Bot. 67:3391–3393.

Lopez, O.A. 2006. Seed and in vitro propagation of *Vaccinium* species native to Western North America. Univ. of Idaho, Moscow, M.S. Thesis.

Maznaya, E.A. and I.V. Lyanguzova. 1999. Biology of seed germination in some species of *Vaccinium* L. (translated title). (In Russian). Rastitel'nye Resursy. 35:53–59.

Minore, D., A.W. Smart, and M.E. Dubrasich. 1979. Huckleberry ecology and management research in the Pacific Northwest. Research Note PNW-236. USDA Forest Service Pacific Northwest Forest and Range Expt. Sta., Portland, OR.

SAS Institute Inc. 2004. SAS Online Doc. 9.1.3. Cary, NC.

Shafii, B. and D.L. Barney. 2001. Drying and cold storage affect germination of black huckleberry seeds. HortScience 36:145–147.

Smagula, J.M., M. Michaud, and P.R. Hepler. 1980. Light and gibberellic acid enhancement of lowbush blueberry seed germination. J. Amer. Soc. Hort. Sci. 105:816–818.

Stark, N. and S. Baker. 1992. The ecology and culture of Montana huckleberries: A guide for growers and researchers. Misc. Pub. 52. Montana Forest and Conservation Expt. Sta.. School of Forestry, Univ. of Montana, Missoula.

Stushnoff, C. and F. Hough. 1968. Response of blueberry seed germination to temperature, light, potassium nitrate and coumarin. Amer. Soc. Hort. Sci. 93:260–266.

Taiz, L. and E. Zeiger. 2002. Plant physiology, 3rd ed. Sinauer Associates Publishers, Sunderland, MA.

Thompson, K. and J.P. Grime. 1983. A comparative study of germination responses to diurnally fluctuating temperatures. J. Appl. Ecol. 20:141–156.

Thompson, K., J.P. Grime, and G. Mason. 1977. Seed germination in response to diurnal fluctuations of temperature. Nature 267:147–149.

Vander Kloet, S.P. 1983. Seed and seedling characters in *Vaccinium* § *Myrtillus*. Le Naturaliste Can. 110:285–292.

Vander Kloet, S.P. 1988. The genus *Vaccinium* in North America. Publication 1828. Res. Branch, Agr. Can., Ottawa, Canada.