

Cultivar and Maternal Plant Environment Influence Cold Stratification Requirements and Germination Rates of *Vitis* Species

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Abstract. Open-pollinated seeds from grapevines in Parlier and Davis (in California) and Geneva (in New York) were collected in 2016, 2017, and 2018. Seeds were subjected to a series of cold stratification treatments of varying lengths and germinated in incubators to compare germination rates. Two *V. vinifera* cultivars (Chardonnay and Cabernet Sauvignon) and three other cultivars (*V. labrusca* hybrids) with a similar genetic background were compared across three locations to test for maternal environmental effects on germination rates under different cold stratification durations. Two interspecific hybrids (‘Salamander’ and ‘Sovereign Rose’) and three genotypes each from two species, *V. riparia* and *V. cinerea*, were evaluated to compare germination rate variability at different cold stratification durations among and within species and hybrids. Large variability in germination rates was evident among and within grape species, with some accessions requiring little to no cold stratification, and others requiring 10 to 12 weeks. These differences could be useful for breeding grapevines with high or low dormancy requirements. The maternal plant environment impacted the seed weight and total seed germination across years and locations.

Dormancy, the state in which a tissue or organism is in a temporary state of minimal metabolic activity, can occur as a result of external factors (eco-dormancy) or internal cues (endo-dormancy) in plants. In seeds of some perennial plants, endo-dormancy is used to regulate

the timing of germination and may also moderate the spatial distribution of a species (Schutz and Milberg 1997; Van Klinken et al. 2008). Endo-dormancy strength is mediated by both environmental cues experienced by the mother plant (e.g., environmental conditions during seed development) and genetics (Penfield and MacGregor 2017). The genetic mechanisms and environmental factors affecting seed dormancy have been extensively studied in annual model organisms such as *Arabidopsis*; less is known about the effects of those same factors on seed dormancy in perennial species (Graeber et al. 2012; Leida et al. 2012).

Maternal parent genetics and age of the plant can influence seed endo-dormancy and seedling fitness (Chettoor et al. 2016; Ellner 1986; Miller et al. 2012; Rees 1996; Singh et al. 2017). Paternal effects have a role, but they are less impactful than maternal effects because the tissues surrounding the embryo of the seed are maternally derived, with the exception of some hybrid species (Andersson

et al. 2008; Pieskurewicz et al. 2016). In seeds, the maternal environment has been shown to have an important role in seed dormancy affecting germination and seedling vigor (Auge et al. 2017; Bischoff and Muller-Scharer 2010; Cendan et al. 2013; Chen et al. 2014; Fenner 1991; Grass and Burris 1995; Kolodziejek and Patykowski 2015; Nguyen et al. 2021; Penfield and MacGregor 2017; Roach and Wulff 1987; Sawhney et al. 1984; Seglias et al. 2018). The severity of these effects vary among locations and years (Andersson and Milberg 1998; Hoyle et al. 2014). For some perennial species, lower dormancy is associated with high temperatures, short days, drought, and high nitrogen levels experienced by the maternal parent during seed development (Fenner 1991). These types of environmental conditions can result in physical changes, such as a reduced seedcoat thickness, or epigenetic changes (Iwaski et al. 2019; Penfield and MacGregor 2017).

When comparing dormancy requirements and germination rates among different environments, many studies have relied on populations of perennial or annual plants (Andersson and Milberg 1998; Hoyle et al. 2014; Seglias et al. 2018). For annual plants, environmental conditions can select favorable heritable changes that can be passed to subsequent generations (Piskurewicz et al. 2016). Because each plant has only one opportunity to reproduce, these changes are necessary for the population to survive. For perennials, each plant typically has multiple opportunities to reproduce. This multiyear fertile life span allows more attempts for an individual to create successful progeny. Perennial plants with short seed dispersal ranges were found to preferentially use dormancy to stagger seed germination to increase the likelihood of successful germination and development and minimize competition with the mother plant (Penfield and MacGregor 2017).

Grape, *Vitis* sp., is a perennial vine that includes more than 80 different species distributed worldwide. European grape (*Vitis vinifera*), used for raisin and table grape production, is the most important fruit crop in the world; it is valued at \$4.7 billion (USD) in the United States and \$189 billion globally in 2020 (Statista.com). European grape is clonally propagated and cultivars that are decades to thousands of years old are grown across the world in climates from New York to South Africa. In northern climates, grape vines are subjected to short growing seasons with long days and cool nights; however, in southern climates, the same grapevine cultivars may be exposed to long growing seasons and intense prolonged heat. These divergent environments could have large effects on the rate of germination and seed viability within a cultivar. Low temperatures during anthesis or bloom have been shown to improve germination; however, low temperatures during fruit ripening can reduce germination in some species (Chen et al. 2014). For grape, it is expected that seed dormancy is predominantly controlled by maternal genetics, but the role of maternal environment on cold stratification requirements for germination has not been

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explored. Even viticulture practices can affect seed development, with one study showing that seed weight could be reduced using minimal pruning systems (Hardie and Aggenbach 1996). Clonally propagated perennial agricultural crops, like grape, provide a unique opportunity to study the effects of the environment and maternal parent on the rate of germination (e.g., loss of endo-dormancy) in a perennial woody species. Improving germination rates and reducing cold stratification requirements can be useful for breeding within different grape classes and may be predictive of bud dormancy requirements, an important consideration for grape cultivar grown in northern regions (Vahdati et al. 2012; Wang et al. 2016).

Grape seed is primarily animal-dispersed, and endo-dormancy is well-documented, with most *V. vinifera* cultivars requiring a 2- to 3-month stratification period at 4°C for uniform germination (Wang et al. 2009). Studies have evaluated stratification requirements (time and temperature) for different *Vitis* species, as well as the effects of water content, phenolic compounds, and treatment with gibberellic acid and hydrogen peroxide. Reports of the stratification (cold treatment) times needed to break dormancy have varied from 8 to 12 weeks (Chohan and Dhillon 1976; Flemion 1937; Kang et al. 1968; Orru et al. 2012; Scott and Ink 1950), depending on the temperature and species evaluated. Treatment with hydrogen peroxide and gibberellic acid was found to improve the percentage of normal germination of *Vitis vinifera* after only 21 d of cold stratification (Ellis et al. 1983). An inconsistent, but trending, improvement in germination was also observed in muscadine grapes (*Vitis rotundifolia*) treated with hydrogen peroxide and/or gibberellic acid (Conner 2008). Other studies have determined that oscillating temperatures can break seed endo-dormancy more rapidly than a constant temperature, and that species variability exists (Wang et al. 2009, 2011). However, evaluations of the genetically induced and environment-induced variations in stratification time needed to reduce endo-dormancy within *Vitis vinifera* and other related *Vitis* sp. have not been performed.

The objectives of this study were to evaluate the variability in seed germination rates for *Vitis* sp. after exposure to varying cold stratification durations and assess changes in germination rates based on maternal parent location (e.g., environment). These data will provide useful information to improve breeding strategies for selecting grapevines without dormancy requirements and are the first steps in separating genomic from environmental effects surrounding seed dormancy and germination in grape.

Materials and Methods

Seed material and germination experiments.

Mature grape clusters from multiple cultivars were harvested from the US Department of Agriculture Agricultural Research Service of San Joaquin Valley Agricultural Sciences

Center (Parlier, CA), National Clonal Germplasm Repository at University of California Davis Wolfskill Experimental Orchard (Winters, CA, referred to as Davis for clarity) and the United States Department of Agriculture Agricultural Research Service Plant Genetics Resources Unit (hybrid genotypes) or Ravines Vineyard (*V. vinifera* cultivars) (Geneva, NY) (Table 1). For maternal environment comparisons, 'Chardonnay' and 'Cabernet Sauvignon' representatives from Geneva, Davis, and Parlier were selected. Vines grown at the Davis repository received minimal care including a single systemic pesticide for phylloxera and other insect management at the beginning of the season, sulfur sprays for powdery mildew management, and cane-pruning to reduce crop load. Vines received no fertilizer. At the Parlier location, vines were treated with synthetic fungicides as needed to manage powdery mildew (approximately every 10 d). No fertilizer was applied, and the crop load was not managed. In Geneva, 'Iona' received no nutrients or crop load management. Vines received synthetic fungicides as needed for disease management. Commercially grown vines ('Cabernet Sauvignon' and 'Chardonnay') collected in Geneva received standard grower practices for fertilizer, crop load, and disease management. The *V. labrusca* × *V. vinifera* hybrid cultivars were not replicated across locations, and cultivars with a similar genetic background were used instead. Cultivar Isabel mulata (Davis) is believed to be a sport of Isabella, whereas Iona (Geneva) is an open-pollinated seedling of a *V. labrusca* × *V. vinifera* hybrid with features and a flavor profile similar to that of Isabella. 'Isabella'+ indicates 'Isabella'-like genotypes and includes 'Isabella', 'Iona', and 'Isabel mulata'. Grapes were harvested for seed collection from September to October each year in all locations.

Clusters were returned to the laboratory and seeds were manually extracted, rinsed, and allowed to air-dry. Seeds with a hard brown seedcoat were considered "ripe"; the surface was disinfested with a 10% bleach solution (0.0825% sodium hypochlorite) for 1 min, rinsed with de-ionized water, and allowed to air-dry for 6 weeks to 2 months, depending on the harvest date. For weeks 10 to 12 in 2018, seeds received an extra 2 to 3 months of maturation time because of disruptions caused by the United States federal government shutdown.

Seed weight was calculated based on the total weight of 50 bulked randomly selected seeds. Fifty seeds from each genotype were placed onto moistened blue blotter seed germination paper (Anchor Paper Co., St. Paul, MN) in clear plastic germination boxes that were placed in a cold room (4°C) in the dark for one of the 13 predetermined cold stratification durations (Supplemental Table S1). The approximate cold stratification hours were calculated based on the total number of hours that each box was left in the cold room at a constant 4°C based on the Utah chilling model (Luedeling and Brown 2011; Richardson et al. 1974). Three individual boxes or replicates were used for each genotype and treatment, unless otherwise noted. After the predetermined

amount of cold stratification, germination boxes were moved to a growth chamber [16 h light (28°C) and 8 h dark (20°C)] according to recommendations by the Association of Seed Analysts (Wiersema and Waibel 2013). Water was added to boxes as needed to maintain moisture, and Pageant® Intrinsic® fungicide (BASF, Ludwigshafen, Germany) was applied weekly to minimize fungal growth. Seed germination, defined as radical emergence, for each replicate and genotype was evaluated weekly for 8 weeks. Germinated seeds were removed from each box during evaluation each week and counted. Seeds were evaluated for location effects (Expt. 1), variability among *V. vinifera* and hybrids (Expt. 2), or within-species variability for non-*V. vinifera* (*V. cinerea* and *V. riparia*) (Expt. 3) (Table 1). Each experiment was performed with seed collected in 2 or 3 years from 2016 to 2018.

Data collection and statistical analyses. Cumulative counts of germinated seeds over 8 weeks of incubation (woi) for each cold stratification treatment were used to calculate the total percentage of seed germination (out of 50) and germination rate. The germination rate was calculated using the area under germination progress curve (AUGPC) based on the area under the disease progress curve equation developed by Shaner and Finney (1977) to measure progression in relation to time (Campbell and Madden 1990). The AUGPC was calculated using the function area under the disease progress curve in R statistical free software (R Core Team 2021). Similar to the mean germination time described by Ellis, the AUGPC accounts for the number of seeds and speed of germination across multiple evaluations (Ellis and Roberts 1981). However, in contrast, the AUGPC averages across timepoints and accounts for low germination percentages. For example, a seed lot with low total germination would result in a low AUGPC, whereas the mean germination time would be unaffected by low total germination. A high AUGPC value means a greater number of seeds germinating more quickly, whereas a low AUGPC value means a lower number of seeds germinating over time. Based on previous studies, the seed germination percentage after 8 woi for genotypes with 12 weeks of cold stratification (woc) was used as the baseline for comparison of genotypes within a location (Wang et al. 2009). Two or three runs of data were used for each genotype, depending on seed availability.

During the first experiment, a three-way analysis of variance (ANOVA) was used to assess the effect of cold stratification duration, location, and year on total seed germination and rate of germination (AUGPC) within each of the three genotypes, 'Cabernet Sauvignon', 'Chardonnay', and 'Isabella'+, represented at more than one location (Table 1). The AUGPC values (12 woc only) were used in the two-way ANOVA to determine the effect of year and location on the total germination percentage within each of the three cultivars (Cabernet Sauvignon, Chardonnay, and Isabella+). During the second experiment, a one-way ANOVA with AUGPC

Table 1. Mature grape cultivars and germplasm materials used in germination experiments.

Accession	Species	Location ⁱ	PI identifier	50 seed wt (g) ⁱⁱ			Exp. ⁱⁱⁱ
				2016	2017	2018	
Cabernet Sauvignon	<i>V. vinifera</i>	Parlier	DVIT 8024/1350/677	1.333	1.705	1.527	1
		Davis			1.275	1.566	1,2
		Geneva ^{iv}			1.306	1.325	1
Chardonnay	<i>V. vinifera</i>	Parlier	DVIT 8034/916/950		1.183	1.067	1
		Davis		1.606	1.409	1.185	1,2
		Geneva			1.220	1.265	1
Isabella	<i>V. labrusca</i>	Parlier	PI 588193	1.473	1.200	1.169	1
Iona	<i>V. labrusca</i>	Geneva			1.276	1.615	1
Isabel mulata	<i>V. labrusca</i>	Davis	DVIT 75		3.044	2.718	1,2
Salamander	<i>Interspecific hybrid incd. V. labrusca, V. vinifera</i>	Davis	DVIT 140	2.111	2.213	1.897	2
Sovereign Rose	<i>Interspecific hybrid incd. V. labrusca, V. vinifera</i>	Davis ^{iv}	DVIT 2153		1.645	1.348	2
C208	<i>V. cinerea</i>	Geneva	PI 588208				3
C218	<i>V. cinerea</i>	Geneva	PI 588218				3
C221	<i>V. cinerea</i>	Geneva	PI 588221				3
R275	<i>V. riparia</i>	Geneva	PI 588275				3
R054	<i>V. riparia</i>	Geneva	PI 588054				3
R771	<i>V. riparia</i>	Geneva	PI 588771				3

ⁱ Location of the maternal plant from which seeds were harvested.ⁱⁱ Combined weight of 50 randomly selected seeds.ⁱⁱⁱ Seed from this cultivar was used in location-specific comparisons of germination variability among grape seed collected in Davis, CA, or intraspecies variability among *V. riparia* and *V. cinerea*.^{iv} Indicates which experiments seeds were used for 1) multi-site comparisons 2) location specific comparison of hybrids from Davis and 3) intra and inter species variability among *V. riparia* and *V. cinerea*.

values of five Davis-grown genotypes ('Cabernet Sauvignon', 'Chardonnay', 'Sovereign Rose', 'Isabel mulata', and 'Salamander') was performed to compare the effect of cold stratification time (0–11 woc) on germination rates compared with 12 woc within each cultivar. During the third experiment, the AUGPC values for three genotypes of *V. riparia* and *V. cinerea* were compared within and between species using a two-way ANOVA.

All statistical analyses were conducted using R statistical software (R Core Team 2021). Genotypes that had significant ANOVA results ($P < 0.05$) between runs/years were analyzed separately, whereas genotypes that had no significance difference between runs were combined. Tukey's honestly significant difference test was used to detect significance among treatments and compare individual woc stratification to 12 woc stratification. Differences between means were considered significant at $P < 0.05$. Weather data were collected from nearby stations through the California Irrigation Management Information System (<https://cimis.water.ca.gov/WSNReportCriteria.aspx>) for Parlier and Davis locations and from NEWA (<https://newa.cornell.edu/>) for Geneva (Supplemental Table S2).

Results

Environment effect on seed germination of three V. vinifera cultivar and variation across years and locations. Germination, total percentage, and AUGPC varied greatly among cultivars, locations, and cold stratification treatments. The effects of location, year, and/or their interactions on seed germination of 'Cabernet', 'Chardonnay', and 'Isabella'+ were significant (ANOVA; $P \leq 0.05$) (Table 2). However, the response to location and year variability was cultivar-dependent, with some cultivars showing greater fluctuation in total

germination and AUGPC than others. Year, cold stratification, and the interaction effect were significant when comparing the AUGPC values for all cultivars (Cabernet, Chardonnay, and Isabella+) after 12 woc (ANOVA; $P \leq 0.05$) (Tables 3 and 4). Location was only significant for cultivars Chardonnay and Isabella+. Temperature, bloom period, and precipitation varied widely among years and locations (Table 5, Supplemental Table S2). Seed weight did not vary among locations for seed collected from 'Chardonnay' or 'Cabernet' vines, but it showed a significant difference among genotypes for Isabella+. Isabella+ seed collected from Parlier ('Isabella') and Geneva ('Iona') locations were not significantly different from each other ($P > 0.05$), whereas seed collected from Davis ('Isabel mulata') were significantly larger ($P \leq 0.01$).

Cabernet Sauvignon. For 'Cabernet Sauvignon', significant differences in AUGPC were detected among years (locations combined), but not locations (years combined) (Table 3). The AUGPCs for seed collected in 2018 and 2016 were higher than that for seed collected in 2017. Pairwise comparisons between locations in the same year showed that

in 2016, Davis and Geneva were significantly different from Parlier, but not from each other in terms of total germination and AUGPC. Seed weights (years combined) did not significantly vary by location for 'Cabernet Sauvignon', although the AUGPC and germination percentage did (Tables 1 and 3, Supplemental Fig S1). Seed collected from both Davis and Geneva had a higher AUGPC at 6 to 12 woc than Parlier at the same cold stratification duration points in 2016 (Fig. 1A). Because of limited seed, data were not collected for 1, 3, and 8 woc for seed collected from Geneva in 2016, and for 1, 3, 5, 7, and 9 woc in 2017. In 2017, seed collected from Geneva had a significantly higher AUGPC compared with that collected from Parlier and Davis at 6 woc, but it showed no significant difference from Parlier or Davis with any of the other shared cold stratification treatments (Fig. 1B). In 2018, the only difference in the AUGPC among locations and cold stratification time was detected from 10 to 12 woc, when seed collected had been allowed more maturation time (Fig. 1C).

Within a location, pairwise comparisons among the cold stratification durations in

Table 2. Two-way ANOVA of the effect of location (L) and year (Y) of study and their interaction on total seed germination after 12 weeks of cold stratification within cultivars. Area under the germination progress curve (AUGPC) values of three replicates of grape cultivars at 12 weeks of cold stratification and 8 weeks of incubation were used in the analyses.

Cultivar	Dependent variable	Factor	df	Mean sq	F value	P value
Chardonnay	AUGPC	L	2	500,198	27.987	<0.001
		Y	2	978,777	54.765	<0.001
		L × Y	3	116,490	6.518	0.005
Cabernet Sauvignon	AUGPC	L	2	33,229	1.135	0.356
		Y	2	418,477	14.297	<0.001
		L × Y	3	1,139,659	38.937	<0.001
Isabella+	AUGPC	L	2	817,987	24.44	<0.001
		Y	2	1,116,236	33.35	<0.001
		L × Y	3	348,503	17.485	<0.001

HSD_{0.05} = Tukey's honestly significant difference test.

Table 3. Pairwise comparisons (ANOVA) between the effects of different locations (P = Parlier, G = Geneva, D = Davis) and years of study and the rate of germination area under the germination progress curve (AUGPC) at week 12 of cold stratification within each *V. vinifera* cultivar. AUGPC values of week 12 of cold stratification and 8 weeks of incubation for three replicates were used in the analyses. The values presented in the table are *P* values.

Contrasts	<i>V. vinifera</i> cultivars		
	Chardonnay	Cabernet Sauvignon	Isabella+
G vs D	<0.001	0.9902	<0.001
P vs D	0.440	0.4247	0.170
P vs G	0.006	0.4256	<0.001
2016 vs 2017	<0.001	0.0200	<0.001
2016 vs 2018	0.640	0.0775	0.007
2017 vs 2018	<0.001	0.0030	0.888

HSD_{0.05} = Tukey's honestly significant difference test.

Davis in 2016 showed significant differences between the first 0 to 5, 7, and 9 woc (Fig. 1A) compared with 12 woc. For seed collected from Davis in 2017, the AUGPC increased gradually with the increased cold stratification duration (Fig. 1B). Starting at 4 woc, the AUGPC was not significantly different from that at 12 woc, although germination was low (mean germination across replicates was 34%) overall. For seed collected in 2018, little to no germination was observed until 10 woc.

In Parlier in 2016 and 2017, germination was low and slowly increased as seeds received more cold stratification hours. However, germination was low even at 12 woc, with mean germinations of 20% and 40% in 2016 and 2017, respectively (Supplemental Fig. 1). Starting at 4 woc, no significance differences in the AUGPC was observed compared with that at 12 woc (Fig. 1A and 1B). However, in 2018, the germination percentage was high (>75%) and AUGPC increased at 10 woc, which coincided with extra seed maturation time.

In Geneva, the AUGPC increased significantly from 5 to 6 woc (2016) or 4 to 6 woc

(2017), and it was consistently high through 12 woc (Fig. 1A and 1B). Similar to Parlier and Davis, germination for seed collected from Geneva in 2018 had an increased AUGPC at 10 woc (Fig. 1C). No significant differences were detected in seed weight across years grouped by location.

Chardonnay. For 'Chardonnay', when years were combined, the pairwise comparison detected significant differences in AUGPC at 12 woc between both California sites (Parlier and Davis) and Geneva, with Geneva having significantly higher germination than either California site (Table 3). However, seed weights (years combined) did not significantly vary by location. When comparing AUGPCs among years (combined locations), seed collected in 2017 had a significantly lower germination rate than seed collected in 2016 or 2018 for 'Chardonnay'. The pairwise comparison between locations in the same year showed that in 2016, the AUGPC was not significantly different after 2 woc (Fig. 1D). In 2017, Geneva was not represented in the comparison because of insufficient seeds available for replicates at 1, 3, 5, 7, 9, and 11 woc. However, at 10 woc, the germination of seed collected from Geneva had significantly

higher germination than that from Davis, but not from Parlier (Fig. 1E). In general, seed collected from Davis and Parlier were not significantly different in terms of seed germination in 2017 (Fig. 1E). In 2018, no significant differences in seed germination were detected between the three locations, except at 9 woc, when seed collected from Geneva had significantly higher seed germination than that from Davis and Parlier (Fig. 1F).

The pairwise comparison among woc in Davis in 2016 showed that cold stratification had no significant effect on seed germination and overall germination was high (~80%) (Fig. 1D, Supplemental Fig. S1). For seed collected from Davis in 2017, germination gradually increased with the cold stratification duration (Fig. 1E). For seed collected from Geneva in 2016, a stable significant increase in the AUGPC was observed at 8 woc compared with that at earlier weeks, which was consistent through 12 woc (Fig. 1D). For seed collected from Geneva in 2017, a significant increase in germination was detected from 10 woc to 12 woc (Fig. 1E). For seed collected from Parlier in 2017, germination increased significantly starting at 6 woc (Fig. 1E). In 2018, in Davis, Parlier, and Geneva, germination increased significantly at 10 woc (Fig. 1F).

For seed collected from 'Chardonnay' grown in Davis, germination in 2016 was significantly different from that in 2017 and 2018 at all cold stratification weeks, except 10 to 12; that in 2016 was not significantly different from that in 2018. Germination rates for seed collected in 2017 and 2018 were not significantly different from each other, except from 10 to 12 woc (Fig. 1D, 1E, 1F). For seed collected from Geneva, the AUGPCs for seed collected in 2016 and 2017 were significantly different from that in 2018 at 6 and 8 woc (Fig. 1D, 1E, 1F). In Parlier, seed germination in 2017 was significantly higher at cold stratification weeks 6, 7, 8, and 9 than in 2018; however, at week 10 and week 11, seed germination in 2018, which had longer maturation times, was higher than that in 2017 (Fig. 1E and 1F).

Isabella+. When comparing AUGPCs among years (combined locations) and locations (years combined), significant differences were detected. Seed collected from Geneva had a significantly lower AUGPC than seed collected from Davis or Parlier. 'Isabella'+ seed collected in 2016 had a significantly lower AUGPC and total germination percentage than seed collected in 2017 and 2018. Pairwise comparisons between locations in the same year showed no significant differences in 2016 or 2018 between locations (Fig. 1H and 1I). A significant increase in seed germination was detected between seed collected in Geneva ('Iona') and seed collected in Parlier ('Isabella') or Davis ('Isabel mulata') in 2017 at 4 and 6 woc (Fig. 1G). However, overall germination percentages varied widely among all three cultivars at 12 woc, depending on the year (2016: 3% to 6%; 2017: 43% to 58%; 2018: 0% to 66%). 'Isabella'+ had a significantly higher seed weight for seed collected from Davis

Table 4. Three-way ANOVA of cold stratification (C), location (L), and year (Y) effects and their interactions on the rate of seed germination within each *V. vinifera* cultivar based on area under the germination progress curve (AUGPC) values for each cold stratification treatment (0 to 12 weeks) after 8 weeks of incubation.

Cultivar	Dependent variable	Factor	df	Mean sq	F value	P value
Cabernet Sauvignon	AUGPC	C	12	2,969,988	93.764	<0.001
		L	2	1,113,996	35.169	<0.001
		Y	2	1,156,253	36.503	<0.001
		C × L	24	71,198	2.248	0.0026
		C × Y	24	355,596	11.226	<0.001
		L × Y	4	1,537,283	48.533	<0.001
		C × L × Y	37	140,821	4.446	<0.001
Chardonnay	AUGPC	C	12	4,669,163	94.275	<0.001
		L	2	3,372,837	68.101	<0.001
		Y	2	11,743,595	237.115	<0.001
		C × L	24	253,509	5.119	<0.001
		C × Y	22	763,987	15.426	<0.001
		L × Y	3	978,873	19.764	<0.001
		C × L × Y	26	134,212	2.71	<0.001
Isabella+	AUGPC	C	12	858,624	43.079	<0.001
		L	2	1,265,257	63.48	<0.001
		Y	2	8,405,877	421.736	<0.001
		C × L	23	89,567	4.494	<0.001
		C × Y	24	377,603	18.945	<0.001
		L × Y	3	348,503	17.485	<0.001
		C × L × Y	16	56,654	2.842	<0.001

Table 5. Average weather conditions experienced by grapevines from 2016 to 2018 for Parlier, Geneva, and Davis. Average temperature and precipitation from each region's respective bloom time to harvest.

Location	Yr	Temperature (°C)	Avg daily temp (°C)	Precipitation (mm)	Total rainfall (mm)
Parlier	2016	17.4–27.4	23.3	0–20.9	54.1
	2017	16.1–28.1	23.6	0–26.9	37.8
	2018	17.0–29.0	22.5	0–15.4	15.5
Davis	2016	19.4–23.2	21.6	0.2–22	35.4
	2017	19.4–24.2	22.6	0–19.1	19.6
	2018	18.4–24.0	21.3	0.3–5.8	8.5
Geneva	2016	18.3–23.1	20.8	16.5–56.4	193.1
	2017	17.3–21.1	19.2	52.8–188.5	573.8
	2018	18.4–22.8	20.5	63.5–162.6	480.4

(‘Isabel mulata’) than that from Parlier (‘Isabella’) and Geneva (‘Iona’) ($P = 0.006$).

The pairwise comparison among cold stratification weeks of seed collected from Davis in 2017 showed a significant increase in germination at 6 woc and continued to increase significantly compared with 0 woc (Fig. 1H). For seed collected from Davis in 2018, no significant increase from 0 to 9 woc was observed. No data were collected after 9 woc because of insufficient seed quantities (Fig. 1I). Comparisons of germination rates for seed collected from Geneva in 2016, 2017, and 2018 could not be made for most cold stratification time points because of few shared treatments (Fig. 1G, 1H, 1I). For seed collected from Parlier in 2016, no effect of cold stratification on germination was detected (Fig. 1G). In 2017, germination gradually increased with the cold stratification period, and a significant increase in germination was observed at 6 woc, which continued to increase significantly after 8 woc

(Fig. 1H). In 2018, germination only significantly increased after 10 woc in seed from Parlier (Fig. 1I).

Effect of the cold stratification duration on seed germination of V. vinifera and hybrids collected from Davis. The percentage of seeds germinated from 0 to 12 woc was the highest for genotype Salamander compared with the other Davis-grown genotypes. The seed germination percentage of ‘Salamander’ was highest at 10 woc (>85%). ‘Isabel mulata’ had the second highest germination percentage (60%) at 10 woc. Chardonnay had the third highest total seed germination, followed by cultivars Cabernet Sauvignon and Sovereign Rose (>52%, 46%, and 33%, respectively) at 12 woc (Fig. 2). The AUGPC values from 0 to 12 woc were used for pairwise comparisons within genotypes. Significant differences among runs for genotypes ‘Salamander’, ‘Sovereign Rose’, ‘Chardonnay’, and ‘Isabel mulata’ were not detected, but significant differences between

runs of data for Cabernet Sauvignon were detected (ANOVA; $P < 0.05$). The AUGPC for the four genotypes that had no run effect was averaged, and data of ‘Cabernet Sauvignon’ were analyzed separately in the pairwise comparison between woc to determine cold stratification hours needed to reach the same level of seed germination as 12 woc with 8 woi (Fig. 3). For ‘Salamander’, the AUGPC and germination percentage at 2 woc were not significantly different from those at 12 woc; however, a trending increase in the AUGPC was observed (Fig. 3). For ‘Sovereign Rose’, the AUGPC was significantly different between all woc in relation to 12 woc until 9 woc (Fig. 3). For ‘Cabernet Sauvignon’, total germination was significantly different for seeds chilled between 0 and 2 woc and 0 to 7 woc in relation to 12 woc for run 1 and 2, respectively (Table 2, Fig. 3). For ‘Chardonnay’, the cold stratification duration was not significantly different from 12 woc after 10 woc (Fig. 3). Finally, ‘Isabel mulata’, had no significant difference in the germination percentage and AUGPC compared with 12 woc after 6 woc (Figs. 2 and 3).

Cold stratification effect and variability in V. riparia versus V. cinerea. The cold stratification effect within accessions of *V. riparia* and *V. cinerea* showed that there were significant differences in the seed germination rate (AUGPC) and percentage among stratification weeks ($P \leq 0.05$) (Table 6, Fig. 4). A pairwise comparison of 0 to 11 woc for *V. riparia* and *V. cinerea* with germination at 12 woc among genotypes of the same species showed species variability similar to that observed within and among *V. vinifera* genotypes and hybrids (Table 7). At 9 or 10 woc,

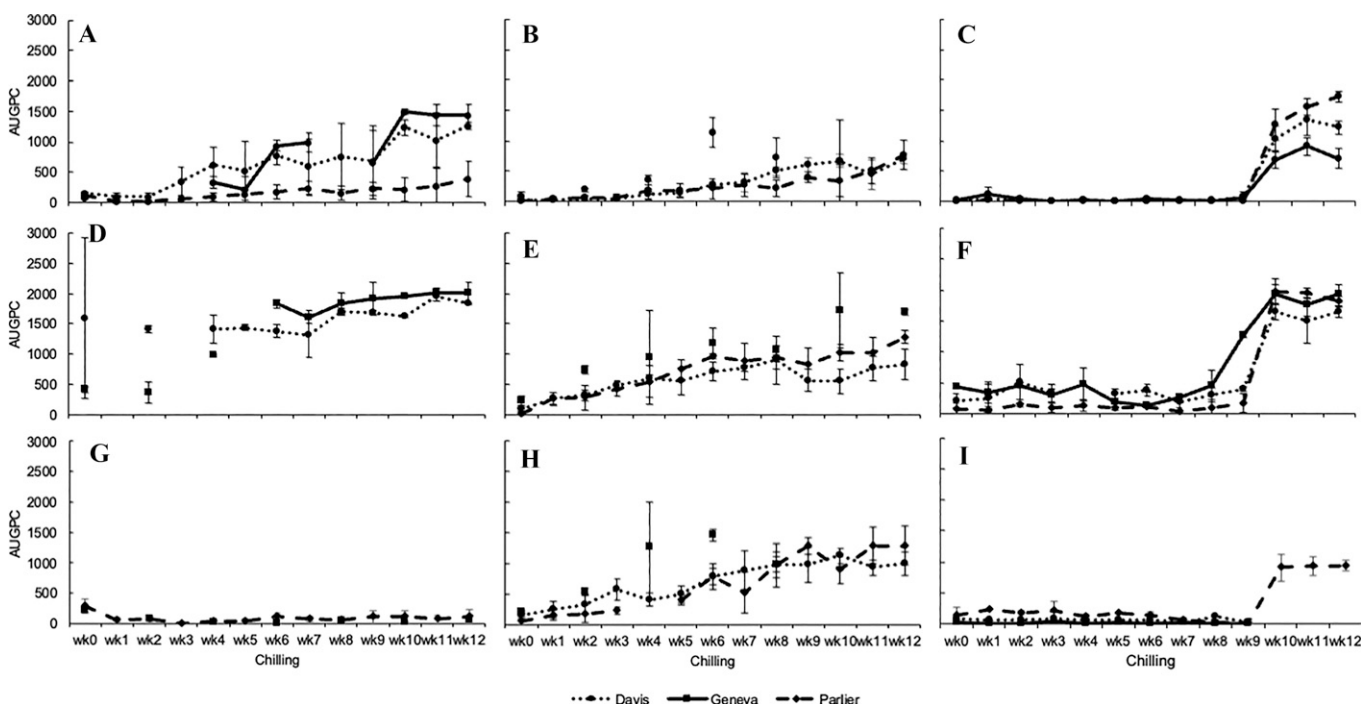


Fig. 1. Germination progression curve as a line with error bars indicating the SD of the mean area under the germination progress curve (AUGPC). (A) ‘Cabernet Sauvignon’ seed germination experiment at three locations in 2016, (B) 2017, and (C) 2018. (D) ‘Chardonnay’ seed germination experiment at three locations in 2016, (E) 2017, and (F) 2018. (G) ‘Isabella’ + seed germination experiment at three locations in 2016, (H) 2017, and (I) 2018.

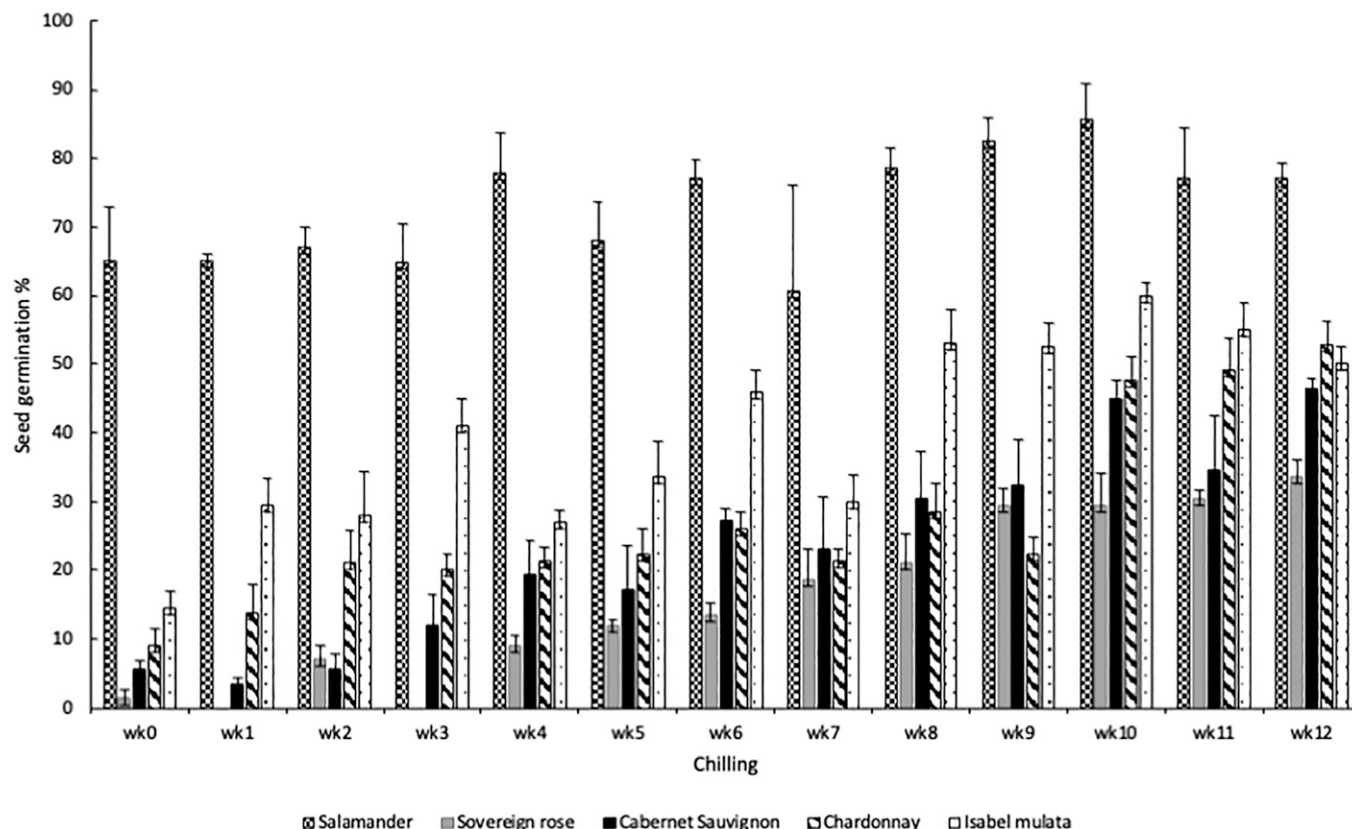


Fig. 2. Total seed germination percentages of Davis-grown cultivars subjected to chilling from 0 to 12 weeks. Seeds were counted as a cumulative after 8 weeks of incubation for three replicates. The seed germination percentage was calculated as the number of germinated seeds to the total number of tested seeds (50). Data are an average of two runs.

seed germination of the three accessions of *V. cinerea* were consistently not significantly different from germination at 12 woc (Table 7). For *V. riparia*, germination at 6 or 7 woc was

not significantly different from 12 woc (Table 7). A pairwise comparison of the germination percentage across accessions of *V. riparia* and *V. cinerea* showed that across

0 to 9 woc (Table 8, Fig. 4), there were significant differences in the germination percentage. However, at 10 to 12 woc, there were no significant differences in the percentage of

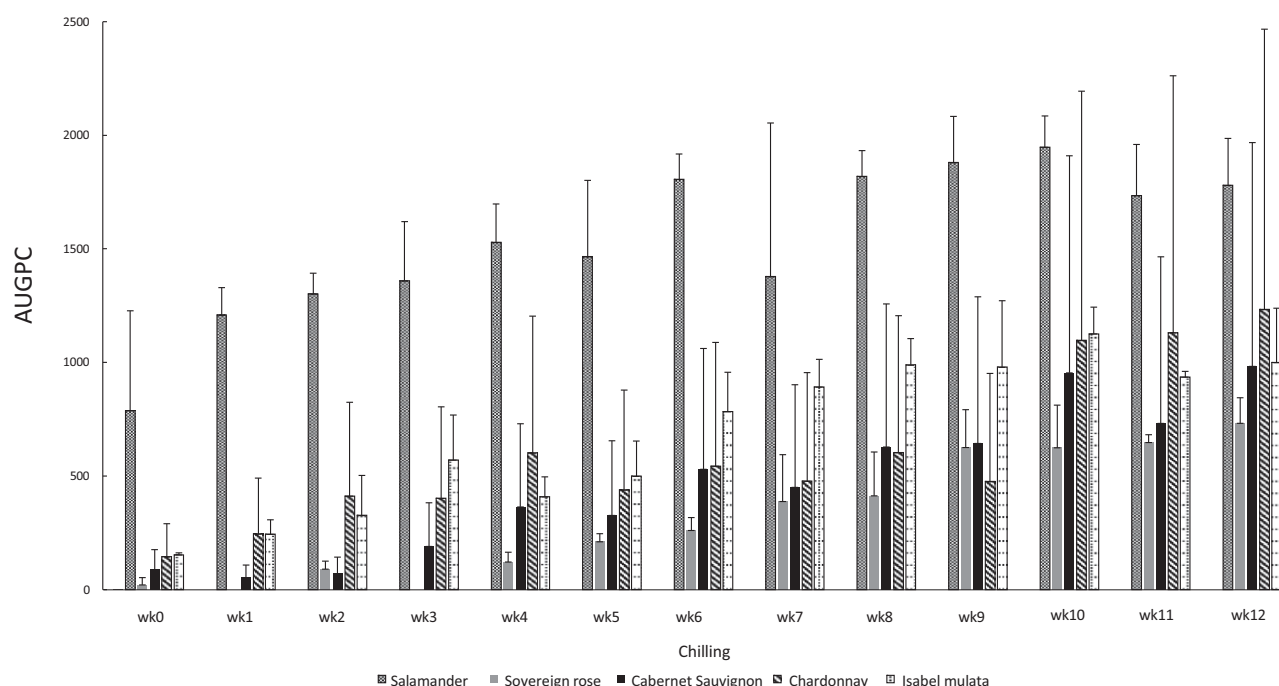


Fig. 3. Averaged area under the germination progress curve (AUGPC) values of two runs of seed germination of Davis-grown cultivars that were subjected to chilling from 0 to 12 weeks. Data are an average of two runs.

Table 6. Two-way ANOVA of cold stratification effect (area under the germination progress curve at 12 woc) within *Vitis cinerea* (C) and *V. riparia* (R) grape accessions from Geneva, NY.

Accessions	df	Sum	Mean	F value	P value
C208	12	23404253	1,950,354	33.99	<0.00
C218	12	23138942	1,928,245	78.41	<0.001
C221	12	21572777	1,797,731	38.34	<0.001
R054	12	29191300	2,432,608	103.2	<0.001
R275	12	18805670	1,567,139	73.78	<0.001
R771	12	10244476	853,706	12.45	<0.001

germination with the exception of *V. riparia* accession R711, which had significantly lower germination than the others (Table 8).

Discussion

Seed dormancy is the suspension of germination until suitable growing conditions are met or internal germination inhibitors have degraded (Hartmann et al. 1997; Hilhorst 1995; Li and Foley 1997). Requirements for temperature, a main factor that regulates seed dormancy and germination, can vary widely among species (Baskin and Baskin 1998; Baskin and Baskin 2004; Fenner and Thompson 2005; Penfield and MacGregor 2017). For *Vitis*, cold stratification for 12 weeks at 3 to 5 °C is considered effective for seed dormancy release of *Vitis vinifera*, although individual seeds may germinate with fewer weeks of cold stratification (Ellis et al. 1983; Singh 1961; Wang et al. 2011). Because 12 woc is considered sufficient for most grape cultivars, we used this as our baseline for maximal

germination for each seed lot. In our study, we observed large variations in time to germination and total germination based on cold stratification hours ranging from 0 to 12 weeks both among and within *Vitis* species, indicating that large variability exists among germplasm and could be targeted for selection. ‘Salamander’, an American hybrid from Missouri with *V. labrusca*, *V. champinii*, *V. vinifera*, and other unknown American species in its background, consistently required the least amount of cold stratification hours (0 weeks) for more than 40% germination to reliably occur compared with other accessions evaluated. In comparison, ‘Isabel mulata’, a *V. labrusca* × *V. vinifera* hybrid from the same location, did not reach more than 40% germination until 3 woc. Whereas ‘Cabernet Sauvignon’, a pure *V. vinifera* cultivar, required at least 10 woc to achieve more than 40% germination. This large variability in germination among cultivars, even after 12 woc, could be because of cultivar-specific or cultivar × environment interaction differences in

seed viability and maturation. Seed maturation or ripening can also have a role in dormancy release, although the importance and variability of this factor in grape have yet to be explored (Ali et al. 2022; Eckpong 2009; Holdsworth et al. 2008; Thomas et al. 1973). Grape seeds used in this study were allowed to air-dry for up to 2 months, which is unlikely to be sufficient time for seed maturation to occur. This was likely the cause of the increased/more consistent germination speeds and percentages for seed collected in 2018 that received an additional 2 to 3 months of maturation time. The low germination rates observed for some cultivars, particularly those from the same location, could be caused by differences in seed maturation requirements or mother vine resiliency to environmental stressors such as heat and drought. Additional work is needed to determine the time and variability for seed maturation among and within *Vitis* species.

Vivipary, germination of the seed within the berry while on the vine, is not common for grape; however, ‘Salamander’ has been observed to exhibit this phenotype, suggesting that dormancy or a seed maturation period is not required. This could be, in part, why germination rates and total germination were so much higher for ‘Salamander’ than for other *Vitis* species evaluated. In this study, differences in seed weight and germination were evident within and among locations and cultivars. For location, germination differences were cultivar-specific, indicating that environmental effects can have a role in germination

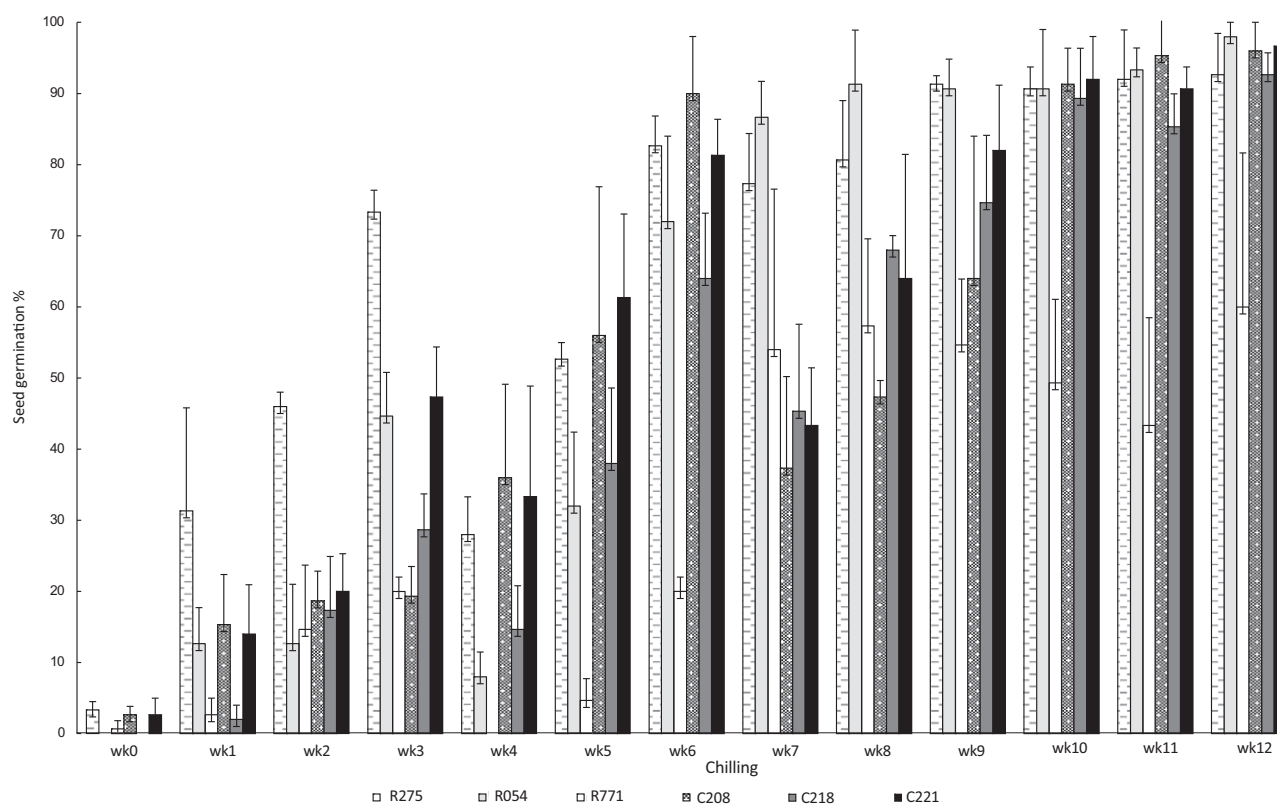


Fig. 4. Germination percentage from 0 to 12 weeks of chilling for accessions of *V. riparia* (R275, R054, R711) and *V. cinerea* (C208, C218, C221) seed collected from Geneva, NY, after 8 weeks of incubation. Data are an average of two runs. Error bars indicate SD.

Table 7. Average area under the germination progress curve (AUGPC) values across 0 to 12 weeks of cold stratification of germination 8 weeks of incubation for three genotypes of *V. riparia* (R275, R054, R771) and *V. cinerea* (C208, C218, C221) compared with 12 weeks of cold stratification for the same genotype using ANOVA.

Accession	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
C208	42 ⁱ	272 ⁱ	426 ⁱ	434 ⁱ	562 ⁱ	1115 ⁱ	2007	838 ⁱ	1001 ⁱ	1439 ⁱ	2037	2385	2233
C218	0 ⁱ	41 ⁱ	393 ⁱ	662 ⁱ	263 ⁱ	720 ⁱ	1433 ⁱ	1037 ⁱ	1593 ⁱ	1744	2126	2022	2182
C221	51 ⁱ	284 ⁱ	446 ⁱ	1049 ⁱ	581 ⁱ	1296 ⁱ	1776	881 ⁱ	1490 ⁱ	1860	2192	2148	2322
R275	64 ⁱ	609 ⁱ	1066 ⁱ	1724 ⁱ	620 ⁱ	1141 ⁱ	1917	1856	1917	2184	2119	2167	2209
R054	0 ⁱ	277 ⁱ	418 ⁱ	1086 ⁱ	154 ⁱ	714 ⁱ	1658 ⁱ	2121	2203	2202	2077	2256	2371
R771	8 ⁱ	37 ⁱ	302 ⁱ	427 ⁱ	0 ⁱ	95 ⁱ	385 ⁱ	1182	1173	1233	1119	940	1328

ⁱ Indicates significant differences between weeks of cold stratification 0 to 11 in relation to week 12 within the same row ($P \leq 0.05$).

HSD_{0.05} = Tukey's honestly significant difference test.

but are superseded by genotype. Seed weight did not significantly vary among locations or years for 'Cabernet Sauvignon' or 'Chardonnay' despite the differences in growing conditions. Isabella+ exhibited differences in seed weight based on location; however, each location represented a related individual and not a clonal cultivar. Isabel mulata, the cultivar grown at the Davis location, is believed to be a sport of Isabella, but it has larger and darker fruit. For some species, seed quality traits such as weight, longevity, and leachate are poor when maternal plants are grown under high temperatures during seed development, but viability is unaffected (Li et al. 2017). Although we did not test leachate or longevity, these factors could be interesting to compare among maternal plants from separate locations in future studies.

For some perennial species, correlations between budbreak and seed dormancy have been found (Vahdati et al. 2012; Wang et al. 2016). It is known that chilling requirements for budbreak have both a genetic and environmental component (Kovaleski and Londo 2019; Mathiason et al. 2009; Min et al. 2017). *Vitis vinifera* L. is a cold-sensitive woody perennial that uses dormancy to avoid freezing injury (Keller 2015). Generally, for perennial plants, bud dormancy is an essential mechanism to survive harsh winter conditions using para-dormancy, endo-dormancy, or ecto-dormancy (Beauvieux et al. 2018; Lang et al. 1987). Endo-dormant buds require exposure to chilling to transit to ecto-dormancy, where they can resume growth under suitable environmental conditions (Fennell 2004; Ferguson et al. 2014). For walnut (*Juglans regia* L.), 6 to 8 weeks of cold were required for seed dormancy break and the best seed germination percentage; a strong correlation was detected between chilling (or cold stratification) requirements for seed and budbreak for

different genotypes of walnut (Vahdati et al. 2012). This was similar for peach (*Prunus persica*), where pathway overlap was demonstrated in bud and seed dormancy breaking (Wang et al. 2016). Numerous studies have shown that grape (cultivated *V. vinifera* and native American species) requires different chilling durations to break bud dormancy (Cragin et al. 2017; Ferguson et al. 2014). Historically, cultivated *V. vinifera* was known as a low-chill species requiring 50 to 400 h at $\leq 7^{\circ}\text{C}$ to complete bud dormancy (Dokoozlian 1999; Magoon and Dix 1943; Weaver and Iwasaki 1977). However, cultivars such as Chardonnay and Cabernet Sauvignon can require up to 750 h and 1250 h at 0 to 7°C to fulfill chilling requirements, respectively (Anzanello et al. 2018; Cragin et al. 2017; Londo and Johnson 2014). This type of cultivar variability was similar for some of the grape cultivars evaluated in this study. 'Chardonnay', which has a lower bud chill requirement, frequently had a higher AUGPC (faster rate of germination) and shorter stratification requirement than 'Cabernet Sauvignon', which has a higher chill requirement for bud dormancy, across locations. Within wild grape species, studies have shown that variability in chilling requirements for bud dormancy can, but does not always, occur (Londo and Johnson 2014; Londo and Kovaleski 2019). Northern wild grape species, such as *V. riparia*, require a short chilling duration to complete endo-dormancy and transit to ecto-dormancy, and they typically achieve budburst within a short period (7 d) under warm conditions. Southern species, including *V. cinerea*, required longer chilling durations and typically have slower budburst rates (Londo and Johnson 2014). *V. riparia* and *V. cinerea* are both used by northern grape breeding programs to incorporate cold tolerance into commercial grape cultivars. For example, *V. riparia* was shown to have

large variability in chilling hours required for budbreak (250–1000 h), whereas *V. cinerea* showed less variability (Londo and Johnson 2014). However, for seed dormancy, both *V. riparia* and *V. cinerea* accessions evaluated showed intraspecific variability in cold stratification requirements. Similar to bud dormancy, *V. riparia* accessions were consistently not significantly different in germination from the maximum at 12 weeks after cold stratification starting at 6 to 7 woc. For *V. cinerea*, germination did not significantly improve after 8 to 9 woc in our study. Although these are, for now, untested associations, it is suggested that bud and seed dormancy may have overlapping pathways similar to other perennial species.

Although breeding cold-hardy grapes is a major focus for northern grape-growing breeding programs, grapes with low chilling requirements are becoming essential for more southern regions as climates change. Cultivars with low cold stratification requirements for germination such as Chardonnay and Salamander could be useful sources for breeding low-chill grapes, whereas cultivars like Cabernet Sauvignon may be useful for breeding higher fruit quality into cold-tolerant grapes. Although further evaluation of the overlap between seed and bud dormancy is needed, using germination assays under little to no chilling may be useful as a phenotypic assay to select individuals with reduced chilling requirements.

Conclusion

In summary, cold stratification requirements for successful germination varied greatly within and among *Vitis* species and hybrids, with some cultivars requiring less than 3 weeks of stratification and others requiring more than 10 weeks to attain

Table 8. Pairwise comparison (ANOVA) of the percentage of germination at each week of cold stratification treatment (0–12) and 8 weeks of incubation across three genotypes of *V. riparia* (R275, R054, R771) and *V. cinerea* (C208, C218, C221). Values in the table are the mean.

Accession	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
C208	2.7 a	15.3 ab	18.7 a	19.3 a	36.0 c	56.0 b	90.0 c	37.3 a	47.3 a	64.0 ab	91.3 b	95.3 b	96.0 b
C218	0.0 a	2.0 a	17.3 a	28.7 a	14.7 abc	38.0 ab	64.0 b	45.3 ab	68.0 abc	74.7 ab	89.3 b	85.3 b	92.7 b
C221	2.7 a	14.0 ab	20.0 a	47.3 b	33.3 bc	61.3 b	81.3 bc	43.3 ab	64.0 abc	82.0 ab	92.0 b	90.7 b	96.7 b
R275	3.3 a	31.3 b	46.0 b	73.3 c	28.0 bc	52.7 b	82.7 bc	77.3 bc	80.7 bc	91.3 b	90.7 b	92.0 b	92.7 b
R54	0.0 a	12.7 ab	12.7 a	44.7 b	8.0 ab	32.0 ab	72.0 bc	86.7 c	91.3 c	90.7 b	90.7 b	93.3 b	98.0 b
R771	0.7 a	2.7 a	14.7 a	20.0 a	0.0 a	4.67 a	20.0 a	54.0 abc	57.3 ab	54.7 a	49.3 a	43.3 a	60.0 a

Letters next to the mean values indicate significant differences between mean values in the same column ($P \leq 0.05$).

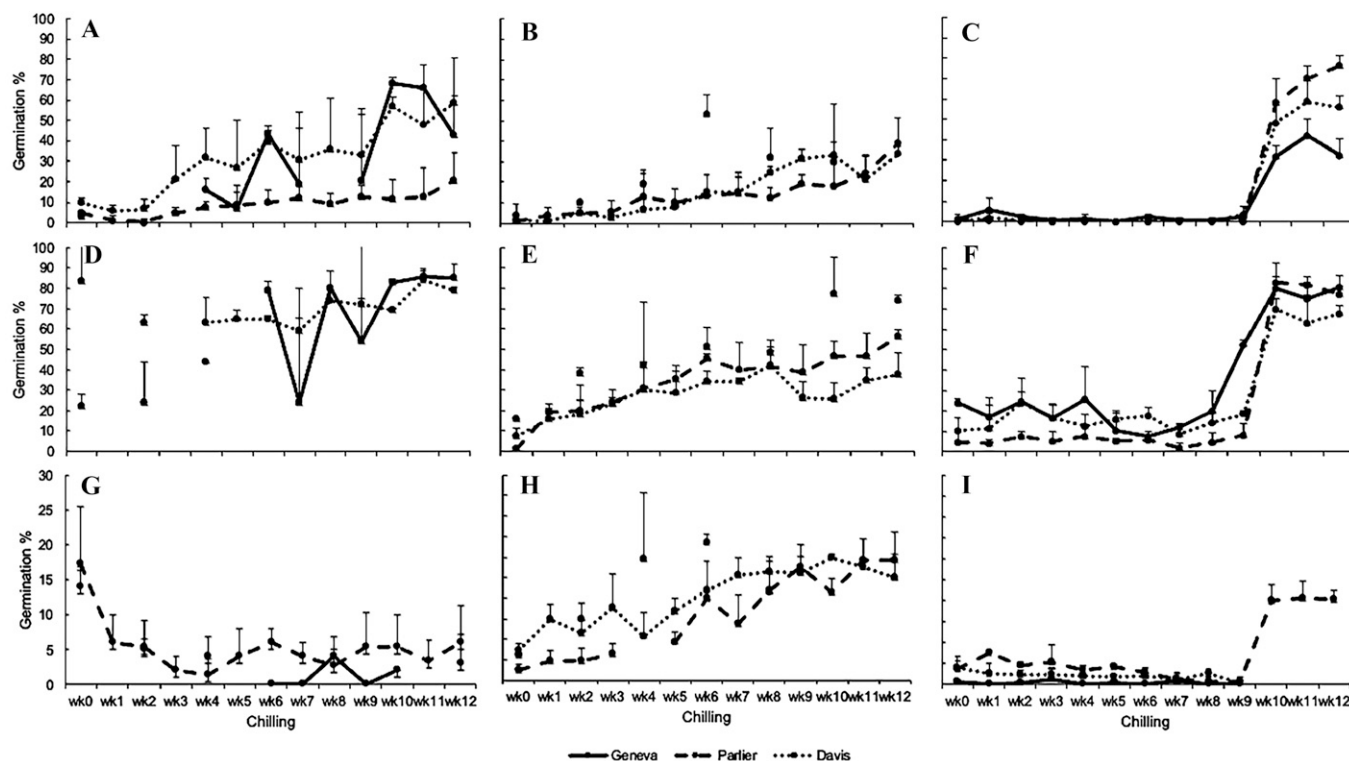
HSD_{0.05} = Tukey's honestly significant difference test.

substantial germination (>40%). *Vitis vinifera* cultivars with low chilling requirements for budbreak often required fewer hours of cold stratification for seed germination to occur than cultivars with high chilling requirements. However, this was dependent on the year the seed was collected and maternal parent environmental conditions during seed development. Variability within and among cultivars was detected and could be useful for breeding low-chill grape cultivars in changing climates.

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Supplemental Fig. S1. Total seed germination percentage based on location by cultivar by year is shown as a line graph with error bars indicating the standard deviation of the mean. (A) ‘Cabernet Sauvignon’ seed germination experiment at three locations in 2016, (B) 2017 and (C) 2018, (D) ‘Chardonnay’ seed germination experiment at three locations in 2016, (E) 2017, and (F) 2018, (G) ‘Isabella’+ seed germination experiment at three locations in 2016, (H) 2017, and (I) 2018. The seed germination percentage was calculated as the number of germinated seeds to the total number of tested seeds (50). Data is an average of three replicates.

Supplemental Table S1. Weeks of chilling and approximate hours of chilling each week.

Treatment	Chilling hours ¹
0	0
1	168
2	336
3	504
4	672
5	840
6	1008
7	1176
8	1344
9	1512
10	1680
11	3360
12	5040

¹ Estimated chilling hours based on the Utah chilling model.

Supplemental Table S2. Average air temperature and total precipitation for three seed collection locations (Parlier, CA, Davis, CA, and Geneva, NY).

Location	Month	2016		2017		2018	
		Avg Air Temp (°C)	Precipitation (mm)	Avg Air Temp (°C)	Precipitation (mm)	Avg Air Temp (°C)	Precipitation (mm)
Davis	January	9.7	130.1	8	278.2	9.8	97.2
	February	12.1	18.2	11.2	194.2	10.5	4.4
	March	13.5	121.5	13.7	70.1	11.7	103.5
	April	16.7	7	15.2	64.7	15	13.2
	May ¹	19.5	22	19.4	0	18.4	5.8
	June	22.7	0.2	23	19.1	22.1	0.3
	July	23.2	0.2	24.2	0.2	24	0.8
	August	21.7	12.2	23.7	0.2	21.7	1.1
	September	20.9	0.8	22.5	0.1	20.5	0.5
	October	16.6	49.2	17.2	3.8	18.3	0.7
	November	12.6	39.2	12.4	31	12.3	74.1
	December	7.2	66.1	8.1	0.7	9.3	50.2
Parlier	January	9.3	87.5	8.7	122.3	10	30.2
	February	11.9	3.2	11.8	58	10	10.3
	March	14.2	57.9	14	32.2	12.7	87.2
	April ¹	17.5	18	16.1	26.9	17	15.4
	May	21.2	14.9	20.9	3.8	20.5	0
	June	25.8	0.1	26	0	24.7	0
	July	27.4	0.2	28.1	0	29	0
	August	26.1	0	27.6	0.2	26	0.1
	September	22.3	0	23	5.2	22.6	0
	October	17.4	20.9	16.4	1.7	17.1	0
	November	12.5	11.7	12.9	9.3	11.6	28.1
	December	7.8	64	7.1	1.2	8.9	11.2
Geneva	January	-3.57	52.832	-0.98	114.3	-4.5	71.12
	February	-0.94	200.66	1.07	80.772	0.39	97.536
	March	3.88	76.2	-0.07	149.098	-0.5	90.932
	April	5.63	75.438	10.78	138.684	4.11	134.112
	May	14.43	107.188	12.92	352.298	17.5	203.2
	June ¹	19.21	41.91	19.1	258.064	18.79	161.29
	July	22.55	65.278	21.07	478.79	22.79	232.918
	August	23.11	143.256	19.5	234.188	22.05	413.004
	September	18.32	132.842	17.32	134.112	18.44	209.804
	October	11.1	448.31	13.47	392.938	9.44	272.796
	November	6.04	108.966	3.77	210.312	1.56	232.156
	December	-1.39	80.01	-2.97	60.706	0.22	186.944

¹ Indicates when flowering time occurred.