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RESEARCH ARTICLE

Germination potential of sweet cherry seeds from commercial cultivars in Chile and implications for PNRSV dissemination risk

Marlene Gebauer[1*], Yohaily Rodríguez-Alvarez[1], Marco Muñoz[2], Claudia Vergara[2], Claudio Moore[2], and Paulina Escudero[3]

[1]Pontificia Universidad Católica de Chile, Facultad de Agronomía y Sistemas Naturales, Santiago, Chile.
[2]Servicio Agrícola y Ganadero/Ministerio de Agricultura Chile, Santiago, Chile.
[3]Frutas de Chile, Santiago, Chile.
[*]Corresponding author (mgebauer@uc.cl).
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ABSTRACT

Chile is one of the main exporters of cherries to China, with 207 504 t fresh fruit exported in the last four seasons. To determine if seeds of fresh sweet cherries (*Prunus avium* (L.) L.) intended for human consumption could be a dissemination route for *Prunus necrotic ringspot virus* (PNRSV), authorities from both countries agreed to evaluate the associated phytosanitary risk. An experimental study was conducted using the six most widely exported commercial cultivars to assess seed germination potential under conditions simulating a post-consumption scenario. The experimental design aimed to reproduce the sequence of conditions that seeds may experience in China, including export storage, and subsequent exposure after fruit consumption or disposal of whole or partially consumed fruit. These conditions were simulated using one control and six treatments, combining cold-chain storage at 0 °C with winter and spring temperature regimes representative of major Chinese cherry-importing provinces (Shanxi, Shanghai, and Shandong). No germination was observed under these conditions in any cultivar. In contrast, in the control treatment, where pits were stratified at 4 °C for 3 mo prior to transfer to greenhouse conditions, germination ranged from 1.7% to 20.3%. This result is primarily attributed to the lack of sufficient cold stratification required to overcome seed dormancy. Thus, our results suggest that the likelihood of Chilean cherries acting as a source of PNRSV dissemination via seeds under the evaluated conditions is very low.

Key words: Commercial sweet cherry cultivars, phytosanitary risk, *Prunus avium*, seed germination, seed viability.

INTRODUCTION

As a high-value fruit product, sustainable production of sweet cherry (*Prunus avium* (L.) L.) is important for the world market (Xu et al., 2023). In the Southern Hemisphere, Chile is the largest exporter of sweet cherries, and its long-distance markets include China (Ayala et al., 2019).

In the 2020-2021 season, the General Administration of Customs China (GACC) detected the presence of *Prunus necrotic ringspot virus* (PNRSV) in shipments of fresh cherries from Chile. The PNRSV is an Ilarvirus that affects plants of the Rosaceae family, with a worldwide distribution reported in at least 56 countries across five continents (EPPO, 2024), and is spread by pollen, seeds, as well as through contaminated tools and grafting (Kinoti et al., 2017; Farzadfar and Pourrahim, 2019; Rodriguez and Cieniewicz, 2022; EPPO, 2024; Tayal et al., 2024).

To determine the risk of virus dissemination through fruit, the Servicio Agrícola y Ganadero (SAG) agreed with GACC, and the support of the Chilean private sector, represented by Frutas de Chile, to carry out an investigation to assess whether cherry tree seeds are capable of germinating and becoming viable plants, for which the Pontificia Universidad Católica de Chile (PUC) was in charge. The authorities of both countries defined the parameters to be evaluated.

Seed germination begins with water absorption by the seed and ends with the elongation of the embryonic axis and the emergence of the radicle. For germination to initiate, the seed must be viable, not dormant, and free of physiological, physical, or chemical barriers. In addition, it requires suitable environmental conditions including adequate water availability, temperature, oxygen supply, and light, all of which influence the germination process (Graeber and Linkies, 2022; Šerá and Hnilička, 2023; Wang et al., 2024).

In sweet cherry seed propagation is mainly used in breeding programs to generate new hybrids (Sivakumar et al., 2024; Maldonado et al., 2025). Germination of this species requires overcoming deep seed dormancy, typically achieved through a period of cold and moist stratification. Seeds commonly undergo cold stratification at low temperatures (around 4 °C) for approximately 12 wk, though the duration may vary among different *Prunus* species (Kim et al., 2024). In addition to stratification, the application of growth regulators such as gibberellic acid (GA_3) has been shown to effectively enhance germination rates (Çetinbaş and Koyuncu, 2006; Sivakumar et al., 2024). Warm stratification followed by cold stratification also promotes dormancy break and improves germination performance (Kim et al., 2024).

In this research, the objective was to determine whether seeds of six sweet cherry cultivars can germinate and form plants under conditions that simulate the export of the fruit to China and thus assess the risk that PNRSV can establish and spread to hosts from fresh cherries intended for human consumption.

MATERIALS AND METHODS

Plant material

Fruit of six commercial sweet cherry (*Prunus avium* (L.) L.) cultivars (Royal Dawn, Santina, Regina, Bing, Lapins and Sweet Heart), obtained from Chilean growers through the Servicio Agrícola y Ganadero (SAG) and Frutas de Chile (former Asociación de Exportadores de Fruta de Chile A.G.), was used in the study. This fruit was harvested and stored at 0 °C for 30 d, simulating the temperature to which the fruit would be subjected during the export journey to China. The study was conducted during the 2022 and 2023 growing seasons at the Faculty of Agronomy and Natural Systems of Pontificia Universidad Católica de Chile, under controlled temperature, light and humidity conditions, using cold chambers and greenhouse facilities.

Germination test

To establish the different germination treatments, fruits and pits were placed in plastic boxes with lids containing moistened sandy loam soil collected from the 'Los Tachos' farm (Rancagua, Libertador General Bernardo O'Higgins Region, Chile). The soil had a pH of 7.5 and an electrical conductivity of 1.6 dS m^{-1}, representing typical agricultural soil conditions with moderate salinity and relatively low aeration compared to substrates commonly used for seed germination.

The boxes were placed in cold chambers under controlled conditions of darkness and approximately 80% relative humidity. Light exposure was excluded to simulate post-consumption conditions, if fruits would remain buried or covered by snow, thereby limiting light availability.

The temperature regimes used in the different treatments were based on average monthly climate data representative of the provinces of Shanxi, Shanghai, and Shandong. These data were derived from publicly available global climate datasets (e.g., WorldClim, www.worldclim.org) and were selected to represent typical environmental conditions in major cherry-importing regions of China.

In total, six treatments (T1-T6) and one control (T0) were evaluated. The control treatment (T0) consisted of pits placed in moistened perlite and stratified at 4 °C. In all simulated treatments (T1-T6), the substrate was the same sandy loam soil, whereas the variables were the temperature regime associated with each Chinese province and the type of plant material used. Thus, T1-T2 corresponded to Shanxi, T3-T4 to Shanghai, and T5-T6 to Shandong; within each province, one treatment used whole fruits (T1, T3, T5) and the other used pits (T2, T4, T6).

The temperature regimes were designed to mimic the sequence of average monthly temperatures recorded from January to May in Shanxi, Shanghai, and Shandong, which are major consumption hubs for Chilean cherries. Mean monthly air temperatures for each province were applied sequentially during cold-storage and greenhouse phases to reproduce as closely as possible the progression of winter and early spring conditions in those regions (Table 1).

Table 1. Treatments used to evaluate germination of six commercial sweet cherry cultivars (Royal Dawn, Santina, Regina, Bing, Lapins and Sweet Heart), simulating three different monthly average temperatures of the most relevant provinces in China for the export of Chilean cherries (Shanxi, Shanghai and Shandong).

Treatments	Substrate	Plant material	China Provinces	Average temperature (°C)				
				Cold storage			Greenhouse	
				Month 1	Month 2	Month 3	Month 4	Month 5
T0 (Control)	Perlite	Pits	-	4.0	4.0	4.0	20 ± 4	20 ± 4
T1	Soil	Fruit	Shanxi	-5.0	0.0	5.5	12.5	18.0
T2		Pits						
T3	Soil	Fruit	Shanghai	5.0	6.0	10.0	15.0	20.0
T4		Pits						
T5	Soil	Fruit	Shandong	0.0	3.5	9.0	16.0	21.5
T6		Pits						

For the establishment of the control treatment, fruits were pulped to obtain the pits, which were subjected to two washes with potable water and a superficial disinfection with a 10% solution of commercial chlorine with agitation for 15 min. The pits were then washed with distilled water and partially dried with absorbent paper. They were placed in hermetically sealed plastic bags containing perlite moistened with a fungicide solution composed of 1 $g \cdot L^{-1}$ benomyl and 0.8 $g \cdot L^{-1}$ captan (Anasac, Santiago, Chile). These pits were subjected to a period of stratification at 4 °C in darkness for 3 mo. During this period, the perlite was moistened weekly by spraying the fungicide solution mentioned above. After 2 mo stratification at 4 °C, pits were checked weekly to evaluate seed germination by radicle emission. At the end of the stratification period, all the material was transferred to seedling trays containing soil and taken to a greenhouse with a temperature range of 20 ± 4 °C and relative humidity of 75%, where germination was evaluated every 15 d until 45 d were completed.

Viability test

After 45 d in the greenhouse, seed viability was evaluated in the non-germinated samples using a tetrazolium chloride test using the methodology described by AOSA/SCST (2010). Seeds from the six sweet cherry cultivars of the different treatments that did not germinate after 45 d in the greenhouse (T0-T6) were evaluated. Fruits and pits were recovered from the trays, washed with abundant water to remove soil debris and de-stalking. The seeds obtained were selected for viability testing, discarding all those in which fungal necrosis, rotting or total dehydration was observed. The seeds were then placed on glass plates with filter paper, covered with the 0.5% tetrazolium solution and incubated in darkness (approximately 12 h) at 30 °C. At the end of the exposure time seeds were washed and kept in distilled water until evaluation, for which the testa was removed to examine the staining.

Seed viability was indicated by intense red staining of the embryonic axis and cotyledons (Salazar et al., 2019). Viability percentage was calculated as the number of viable seeds relative to the total number of non-germinated seed in each treatment and cultivar. Seed viability (%) was calculated as (Number of viable seeds/Total number of non-germinated seeds) × 100.

Experimental design

A completely randomized experimental design was used, with seven treatment scenarios (one control plus six treatments; T0-T6), two types of plant material (whole fruits and pits), and six cultivars (Royal Dawn, Santina, Regina, Bing, Lapins and Sweet Heart), as summarized in Table 1.

Statistical analysis

Due to the complete absence of germination (zero values) in all simulated treatments (T1-T6), no statistical comparisons were performed among these treatments or with the control, and results were interpreted descriptively. Statistical analysis (ANOVA) was applied only to the control treatment (T0), where variation among cultivars was observed. Data were square root transformed prior to analysis, and Tukey's test was used at a significance level of 5%. Analyses were performed using Statgraphics (2023, Statgraphics Technologies, The Plains, Virginia, USA).

RESULTS AND DISCUSSION

When germination of the six sweet cherry cultivars ('Royal Dawn', 'Santina', 'Lapins', 'Bing', 'Regina' and 'Sweet Heart') was evaluated under simulated export conditions corresponding to the temperature regimes of Shanxi, Shanghai and Shandong provinces, no germination was observed after 45 d under greenhouse conditions (Table 2). These treatments were therefore not subjected to statistical analysis and are presented descriptively.

Table 2. Germination evaluation of six commercial sweet cherry cultivars after 45 d under greenhouse conditions. Values represent number and percentage of germinated seeds. No germination was observed under simulated temperature conditions and these treatments were not statistically analyzed. Within the control (T0), means followed by the same letter do not differ significantly (Tukey, $p = 0.05$).

Treatments	China Provinces	Plant material	Total number of germinated seeds (%)					
			Royal Dawn	Santina	Lapins	Bing	Regina	Sweet Heart
T0 (Control)	-	Pits	5 (1.7)^{bc}	13 (4.3)^{b}	54 (17.7)^{a}	47 (15.7)^{a}	0 (0)^{c}	61 (20.3)^{a}
T1-T2	Shanxi	Fruit-Pits	0	0	0	0	0	0
T3-T4	Shanghai	Fruit-Pits	0	0	0	0	0	0
T5-T6	Shandong	Fruit-Pits	0	0	0	0	0	0

In contrast, in the control treatment (T0), where germination did occur, significant differences were observed among cultivars, with germination ranging from 0% to 20.3% (Table 2). Germination percentages were 1.7% for 'Royal Dawn', 4.3% for 'Santina', 17.7% for 'Lapins', 15.7% for 'Bing', 0% for 'Regina', and 20.3% for 'Sweet Heart'. These differences highlight the importance of adequate stratification conditions for overcoming seed dormancy.

During a previous season, germination of 'Santina', 'Lapins' and 'Regina' was evaluated under more favorable conditions, using seeds stratified at 4 °C in moistened perlite for 3 mo in 'Santina' and 'Lapins' and for 4 mo in 'Regina' reaching 84%, 97% and 96%, respectively.

A high proportion of the evaluated seeds were dead, whereas the remaining seeds showed variable viability, with the highest value reaching 34.7% in 'Sweet Heart' under treatment T6 (pits corresponding to the Shandong province temperature regime) (Table 3). In the control treatment (T0, pits stratified at 4 °C), viability among the six cultivars ranged from 11.2% to 21.3% (Table 3).

Table 3. Viability of six commercial sweet cherry cultivars after being subjected to different temperature treatments simulating conditions of three provinces of China.

Treatments	China Provinces	Total number of viable seeds (%)					
		Royal Dawn	Santina	Lapins	Bing	Regina	Sweet Heart
T0 (Control)	-	33 (11.2)	61 (21.3)	49 (19.8)	40 (15.8)	10 (3.3)	32 (13.4)
T1	Shanxi	0 (0)	0 (0)	0 (0)	0 (0)	7 (2.3)	23 (7.7)
T2		3 (1.0)	1 (0.3)	0 (0)	4 (1.3)	12 (4.0)	69 (23.0)
T3	Shanghai	22 (7.3)	26 (8.7)	5 (1.7)	3 (1.0)	22 (7.3)	88 (29.3)
T4		33 (11.0)	72 (24.0)	17 (5.7)	37 (12.3)	30 (10.0)	43 (14.3)
T5	Shandong	26 (8.7)	25 (8.3)	4 (1.3)	9 (3.0)	0 (0)	63 (21.0)
T6		70 (16.0)	70 (23.3)	38 (12.7)	46 (15.3)	18 (6.0)	104 (34.7)

The fact that no germination was observed in any of the simulated treatments is consistent with the stratification requirements described for this species, which typically needs prolonged cold and moist conditions at around 4 °C for 2 to 4 mo to break deep seed dormancy (Serrano et al., 2009; Kim et al., 2024). Seeds of most *Prunus* species, including *P. avium*, exhibit deep dormancy, an adaptive mechanism that protects seedlings from frost damage during harsh winters by inhibiting germination until an adequate cold period has

been completed, even when moisture, oxygen and temperature are otherwise favorable (Frisby and Seeley, 1993; Javanmard et al., 2014). Seed dormancy can be broken in several ways, and although the best method depends on the species (Heidari et al., 2014), cold stratification has traditionally been used to overcome dormancy in *Prunus* species (Garcia-Gusano et al., 2004), which supports the hypothesis that none of the simulated treatments provided the cold requirements necessary for germination.

A limitation of this study is that the experimental design does not allow the independent evaluation of the effects of temperature, substrate, and seed condition. Therefore, the absence of germination cannot be attributed to a single factor but rather reflects the combined effect of these variables under simulated post-consumption conditions. Consequently, the results should be interpreted as representing a realistic scenario rather than controlled experimental conditions designed to isolate individual factors.

Similarly, low germination rates may also be explained by the presence of the endocarp, which acts as a mechanical barrier to radicle emergence and restricts embryo growth.

Likewise, low germination results are influenced by the presence of the endocarp, which exerts an important barrier to radicle emission, offering not only some resistance to germination, but also functioning as a mechanical barrier to embryo growth. Barros et al. (2012) reported the significant effect of endocarp removal on *Prunus azorica* seed germination, with values of 49% germination using seeds and 15% using seeds with pits, in a relatively short period. Other authors have obtained similar results, where they conclude that the presence of endocarp contributes to the delay of germination (Çetinbaş and Koyuncu, 2006; Esen et al., 2006; Javanmard et al., 2014).

In addition, the substrate used in this study, corresponding to a sandy loam soil with a pH 7.5 and an electrical conductivity of 1.6 dS m^{-1}, can also be considered an impediment to germination. This type of soil may develop a relatively compact structure with lower aeration compared to lighter substrates typically used in nurseries for seed propagation. Reduced oxygen availability, together with moderate salinity, may negatively affect embryo development and radicle emergence, as oxygen is essential for seed respiration and metabolic reactivation during germination (Corbineau, 2022). Commonly, substrates used for seed stratification consist of mixtures of peat and perlite in different proportions, e.g., 2:1 (Krzysztof et al., 2020) or 1:1 (Barros et al., 2012).

The use of mixtures of Sphagnum-type peat moss with perlite enhances substrate aeration and facilitates oxygen availability for root development due to the porous and lightweight characteristics of perlite. In contrast, the soil used in this study lacks these properties, which may further restrict germination. It is important to note that light conditions are unlikely to have limited germination, as cold stratification in darkness is commonly used to overcome dormancy in *Prunus* species. In this study, darkness was intentionally maintained to simulate post-consumption conditions in which fruits may remain buried or covered by snow (Corbineau, 2022).

A common practice reported in numerous studies is the use of pretreatments such as gibberellic acid, potassium nitrate, citric acid and thiourea as germination promoters in species of the genus *Prunus* (Çetinbaş and Koyuncu, 2006; Esen et al., 2006; Barros et al., 2012; Javanmard et al., 2014). These studies show that cherry germination could be increased by using other treatments to break dormancy, such as hormone application, which under natural conditions does not occur.

On the contrary, the results of the control treatment, where the necessary conditions for seed germination are provided (disinfection of pits, stratification period at 4 °C for 3 mo and seedling growth in greenhouse at 20 ± 4 according to Table 1), showed that germination in the different cultivars can occur in a low percentage (1.7%-20.3%), with the exception of the 'Regina' which did not germinate. This low germination is largely due to the impediment generated by the endocarp, which is also not an ideal germination condition.

Seed viability assessed at the end of the germination trial showed that many non-germinated seeds were dead or had low viability, which could be explained by the inadequate stratification conditions imposed during the study. In treatments T1, T3 and T5, which used whole fruits on sandy loam soil and were subjected to the temperature regimes of Shanxi, Shanghai and Shandong provinces, respectively, seed viability differed form that of the corresponding pit treatments (T2, T4 and T6), with a higher proportion of dead seeds in the whole-fruit treatments. These results suggest that the failure to germinate was associated with the combined effect of the temperature regime, the soil substrate and the presence of the stony endocarp surrounding the seeds.

CONCLUSIONS

This study shows that germination of sweet cherry seeds requires specific conditions that were not met under the simulated post-consumption scenarios evaluated. Although the experimental design does not allow the separation of individual effects, the absence of germination across all treatments suggests a low likelihood of seedling establishment under these conditions. Consequently, the probability of *Prunus necrotic ringspot virus* (PNRSV) dissemination through seeds from fresh fruit is likely minimal. However, this conclusion should be interpreted with caution, as the study is based on germination outcomes and does not directly assess viral transmission or infectivity.

Author contribution

Conceptualization: M.G., Y.R-A., M.M., C.V., C.M., P.E. Methodology: M.G., Y.R-A. Validation: M.G., Y.R-A. Formal analysis: M.G., Y.R-A. Investigation: M.G., Y.R-A. Resources: P-E. Data curation: M.G., Y.R-A. Writing-original draft: M.G., Y.R-A. Writing-review & editing: M.G., Y.R-A., M.M., C.V., C.M., P.E. Visualization: M.G. Supervision: M.G. Project administration: M.G. All co-authors reviewed the final version and approved the manuscript before submission.

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