

## Article

# The Effect of Different Temperatures on the Viability and Senescence of Plum Ovules (*Prunus domestica* L.)

Milena Đorđević <sup>1</sup>, Radosav Cerović <sup>2</sup>, Mekjell Meland <sup>3,\*</sup> and Milica Fotirić Akšić <sup>4,\*</sup><sup>1</sup> Fruit Research Institute, Kralja Petra I/9, 32000 Čačak, Serbia; mdjordjevic@institut-cacak.org<sup>2</sup> Innovation Centre of the Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11120 Belgrade, Serbia; radosav.cerovic@gmail.com<sup>3</sup> NIBIO Ullensvang, Norwegian Institute of Bioeconomy Research, Ullensvangvegen 1005, 5781 Lofthus, Norway<sup>4</sup> Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia

\* Correspondence: mekjell.meland@nibio.no (M.M.); fotiric@agrif.bg.ac.rs (M.F.A.)

**Abstract:** This paper reports on a study investigating the viability and senescence of plum ovules when exposed to different constant temperatures over two years. The research was conducted on the primary and secondary ovules of four plum cultivars: ‘Mallard’, ‘Edda’, ‘Jubileum’, and ‘Reeves’. The results show that the first indication of ovule viability loss was callose accumulation, which was detected using the fluorescent dye aniline blue. All cultivars had viable ovules, in different percentages, at 8 °C on the twelfth day after anthesis. However, at higher temperatures, distinct patterns emerged, indicating the adaptability of each cultivar at certain temperatures. The first indication of callose accumulation became visible at the chalazal pole. After anthesis, the ovule’s ability to remain viable gradually reduced, followed by callose deposition throughout the ovary. The cultivars ‘Edda’ and ‘Reeves’, from 6 days after anthesis onward, in both years, showed the highest percentage of nonviable ovules. In contrast, the ‘Jubileum’ cultivar demonstrated the highest percentage of viable ovules. The loss of viability of secondary ovules followed a similar pattern to that of the primary ovules in all cultivars. This research provides valuable insights into embryological processes, which can help in the following breeding programs, and to cultivate plum cultivars in Western Norway’s climate conditions.

**Keywords:** stone fruit; female sporophyte; callose; degenerative processes



**Citation:** Đorđević, M.; Cerović, R.; Meland, M.; Akšić, M.F. The Effect of Different Temperatures on the Viability and Senescence of Plum Ovules (*Prunus domestica* L.). *Plants* **2024**, *13*, 1359. <https://doi.org/10.3390/plants13101359>

Academic Editors: Jose A. Egea, David Ruiz and Raquel Sánchez-Pérez

Received: 12 April 2024

Revised: 2 May 2024

Accepted: 12 May 2024

Published: 14 May 2024



**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

European plum (*Prunus domestica* L.) is mainly cultivated in the temperate regions of the Northern Hemisphere, but it can also grow well in cooler climates [1]. This fruit species is highly genetically diverse [2] and is used for various purposes, such as table consumption, drying into prunes, distilling, and processing multiple food products. European plum is a rich source of sugars, bioactive, antioxidant, and phenolic compounds, which can provide many health benefits to humans [3,4]. Furthermore, it is an essential fruit crop globally, with China being the largest yearly producer [5].

In Norway, plum production is mainly located at a latitude around 60° north in the fjord districts of the southwestern part and around lakes in the eastern part [1]. Short growing seasons and low summer and winter temperatures are some problems affecting plum growing. At the same time, some benefits include the long days, high global irradiation, delayed flowering, and a low risk of spring frost [6]. Plum-growing at this latitude is possible thanks to a warm gulf stream from the Atlantic Ocean [7]. The main cultivars are ‘Opal’ and ‘Victoria’, while ‘Jubileum’ and ‘Reeves’ have significant production areas [8]. All of the fruit produced is mainly used for fresh consumption.

The female sporophyte of plums consists of a monocarpellary pistil that contains two ovules. These ovules are attached to the placenta through a vascular bundle that runs

through the funiculus and ends at the chalaza [9]. To produce a good fruit set, it is necessary to fertilize one of the ovules. Certain *Prunus* species have been found to have low fruit sets and EPP (effective pollination period, i.e., the period for which a flower retains its ability to give a fruit) due to unfavorable environmental conditions. The temperature of the air is a critical factor in the growth and development of fruit. Temperature fluctuations throughout the seasons can affect various biological processes. Studies have shown that changes in temperature patterns have impacted the growth of wild plants, fruit trees, and crops across 21 European countries [10]. In particular, high temperatures in the days or weeks after a full bloom (anthesis) can significantly affect the fruit set [11,12]. According to the Climate Model (CMIP6), Europe is expected to experience an increase in air temperature of 2 °C to 6 °C by the end of this century [13]. However, some experts argue that the timing and frequency of extreme temperatures, rather than an increase in average temperatures, have a more significant impact on fruit production [14]. These temperature changes represent challenges not only to plant productivity, but also to their geographic distribution. Heat stress at each stage of the reproductive process affects fruit trees and ultimately impacts fruit sets [15].

Various species of *Prunus* have been reported to be affected by high temperatures that can affect male gametophyte development, leading to reduced pollen viability, germinability, and pollen tube growth, both in vitro and in vivo [14,16–20]. The sporophytic anther wall, particularly the tapetum or the outer layers, can degenerate prematurely or in an untimely fashion due to the effects of high temperatures [21,22]. This degeneration can result in pollen sterility and anther dehiscence [23,24]. Earlier research disproved that female sporophytes and gametophytes were less sensitive to high temperatures than pollen [25]. However, several studies have suggested that temperature can impact the structures of female sporophytic and gametophytic tissue, leading to a decrease in stigmatic receptivity [26], shorter style length [11], changes in carbohydrate reserves [27], and accelerated ovule aging [28,29].

Callose, a cell wall component, is known for its high impermeability, rapid synthesis, and easy degradation [30]. Its production is triggered by wound healing, pathogen infection, and physiological strains [31]. As embryological data indicate, callose is temporarily deposited in a cell wall during normal reproductive processes [32]. However, extended callose deposition in ovules leads to their reduced viability [33,34].

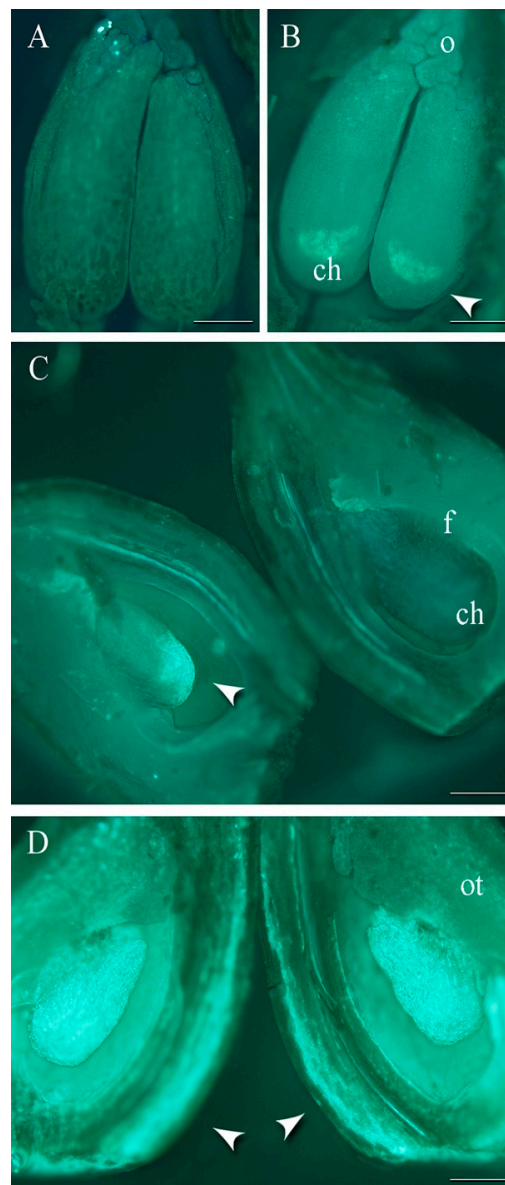
The objective of this study was to establish the temperature threshold necessary to forecast the length of ovule viability at steady temperatures of 8 °C, 12 °C, and 16 °C for up to 12 days after anthesis in four cultivars of plum: ‘Mallard’, ‘Edda’, ‘Jubileum’, and ‘Reeves’, cultivated in Western Norway. These temperatures are commonly experienced during the plum blossoming period in a specific western Nordic climate. The study’s findings will aid in developing successful plum cultivation strategies by considering the impact of temperature on ovule viability, and also support future breeding programs.

## 2. Results

Like other species of the *Prunus* genus, the plum has two ovules in the ovary that remain the same size throughout the flowering period. One of these ovules is considered the primary ovule and remains in the ovary’s locule to continue growing, while the other is the secondary ovule.

### 2.1. Viability of Primary Ovules

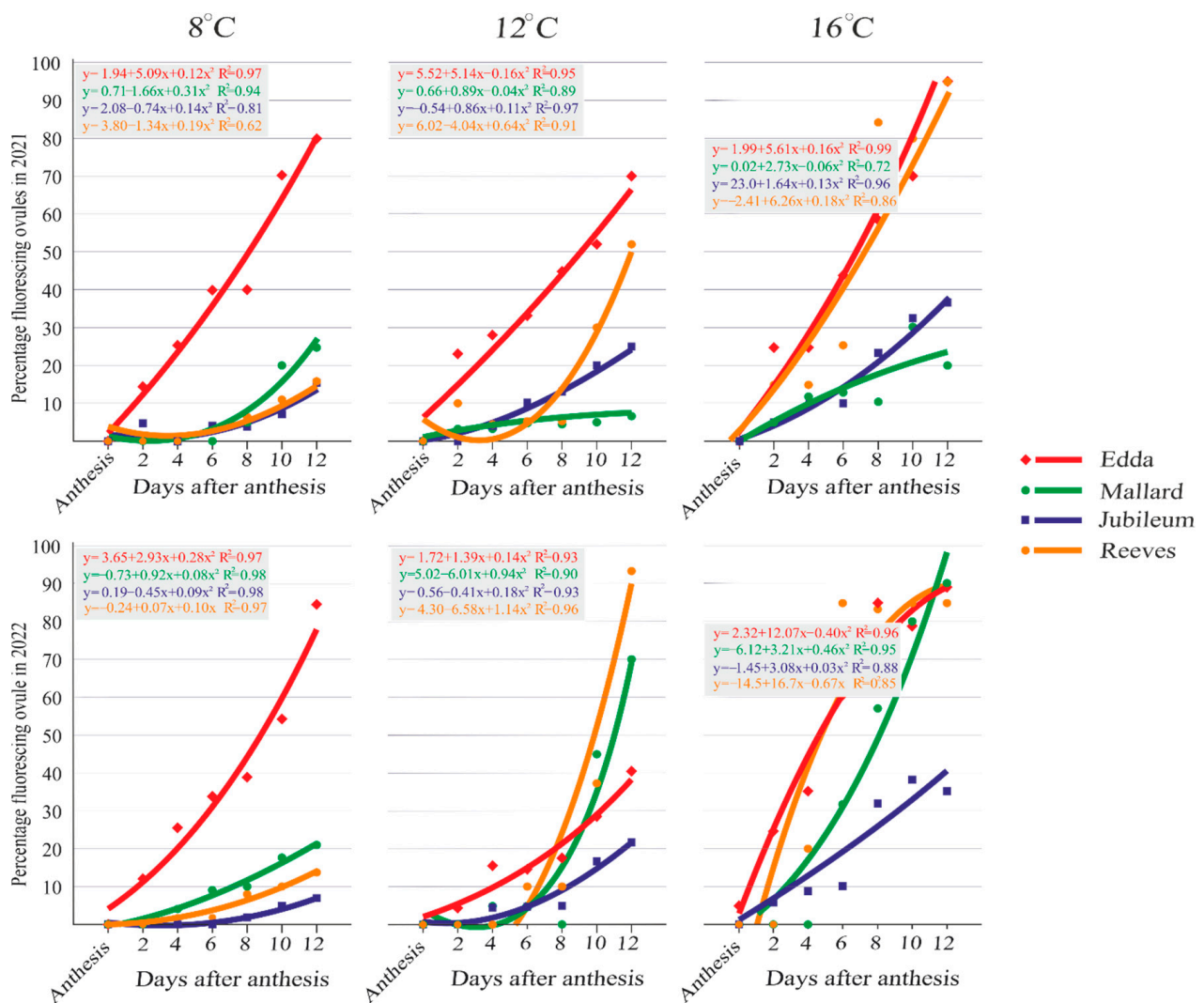
In both years, all tested cultivars showed no evidence of callose accumulation in the ovary at anthesis (Figure 1A, Appendix A Figures A1 and A2). The cultivar ‘Edda’ had the lowest percentage of viable ovules in the days following pollination at 8 °C in both years. Only 20% of ovules were viable in the first year and 15% in the second year (Figure 1B and Appendix A Figures A1(A) and A2(A)). During both years, the cultivar ‘Mallard’ had more than 80% viable ovules at 10 DAA, while ‘Jubileum’ and ‘Reeves’ showed the same results at 12 DAA (Figure 1C and Appendix A Figures A1(B–D) and A2(B–D)).



**Figure 1.** Stained ovule. (A) Ovaries at the anthesis. Callose fluorescence in the ovule and ovary tissue: (B) ‘Edda’ on the 2 DAA at 8 °C; (C) ‘Jubileum’ on the 12 DAA at 8 °C; (D) ‘Reeves’ on the 4 DAA at 16 °C. o—obturator, ch—chalaza, f—funiculus, ot—ovary tissue. Arrow—points to the accumulation of callose in the tissue of ovule and ovary; bars = 1 mm.

The percentages of viable ovules of ‘Edda’ decreased at 12 °C in both years. At 12 DAA, the percentage of viable ovules was 30%, whereas in the second year, that percentage was over 50% on the same day. During the first year, at 12 °C, more than 60% of ovules were viable in ‘Reeves’, while in ‘Mallard’ and ‘Jubileum’, that percentage was around 80% on the same day. In the second year, on the same day, the percentage of viable ovules was lower, ranging from 42 to 55%, except for ‘Jubileum’, where over 80% of the ovules were viable (Appendix A Figures A1(E–H) and A2(E–H)).

The cultivars ‘Edda’ and ‘Reeves’ showed reduced ovule viability in both years when exposed to 16 °C (Figure 1D, Appendix A Figures A1(I,L) and A2(I,L)). In cultivars ‘Jubileum’ in both years and ‘Mallard’ in the first year, a high percentage of ovule viability was observed at 16 °C (Appendix A Figures A1(J,K) and A2(J,K)). Figure 2 depicts the regression line, which shows an upward trend in the percentage of ovules with fluorescence in the tested cultivars for several days after anthesis. Ovule degeneration was significantly associated with cultivars, temperatures, and the days after anthesis.



**Figure 2.** Regression analysis of fluorescing percentage in the primary ovules during the studied period in the tested plum cultivars. Each plot symbol in the regression line indicates the cumulative value of all stages of ovule degeneration observed in the fixation term of the cultivars shown in the legend. Equations and  $R^2$  values are located above or below the corresponding line.

## 2.2. Callose Detection in Primary Ovules

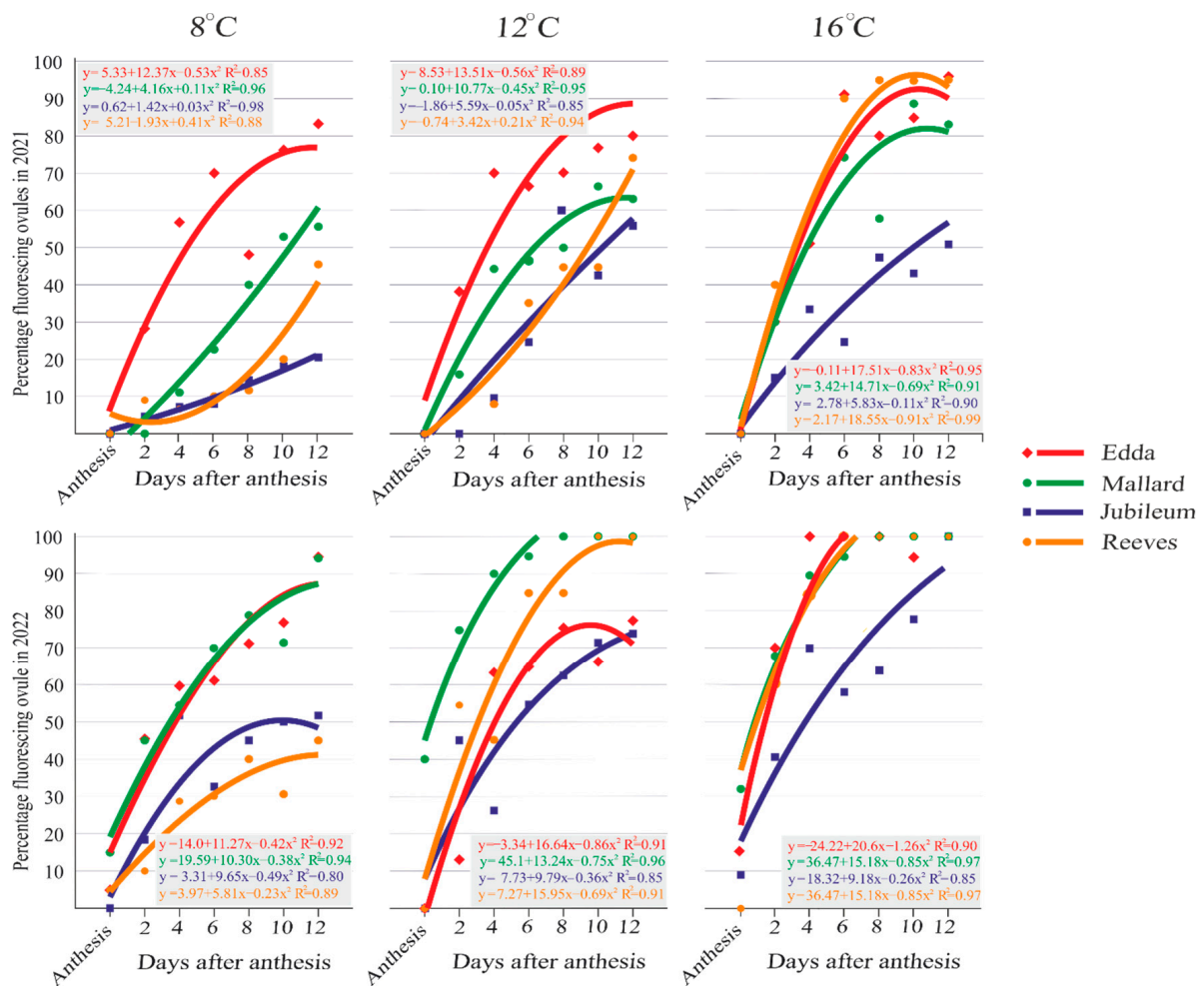
Generally, the percentage of primary ovules with signs of fluorescence increased in the days following anthesis. Up to 4 DAA, most fluorescent ovules were at stages 1 or 2 in all cultivars at all tested temperatures. At a constant temperature of 8 °C, the callose at the chalazal part (stage 1) was observed earlier in the ovary of the cultivar ‘Edda’ at 2 DAA in both years, and in the other part of the ovary, it was detectable in the following days. All stages of fluorescence during ovule senescence with variation within cultivars and temperatures could be found at 6 DAA, while stages 5 and mostly 6 could be found at 12 DAA. The gradual progression of ovule fluorescence was most apparent in the cultivar ‘Edda’ at 8 °C in both years (2021 and 2022:  $R^2 = 0.97$ ). This cultivar’s ovule fluorescence at stage 6 varied from 9.99% at 6 DAA to 56.61% at 12 DAA in 2021, and from 27.55% at 8 DAA to 47.38% at 12 DAA in 2022. The highest percentage of ovule fluorescence at stage 6 at 12 °C was observed in ‘Edda’ in the first year (from 9.32% at 6 DAA to 32% at 12 DAA), while this stage in the second year at 12 DAA was found in 47.6% of ‘Reeves’ ovules. At stage 6, ovule fluorescence was observed at 16 °C in cultivar ‘Edda’, the highest percentage in both years. In 2021, it ranged from 39.96% at 6 DAA to 95% at 12 DAA. In 2022, it ranged from 10% at 2 DAA to 55.5% at 12 DAA. During both years at 16 °C, the ‘Jubileum’ cultivar

had ovule fluorescence lower than 39% at stage 6. The values of the multiple correlation coefficients (R), ranging from 0.62 to 0.99, show that these stages of ovule degeneration were highly correlated with the fixation term, in both years, for all studied cultivars.

### 2.3. Secondary Ovules—Viability and Callose Detection

All cultivars showed no fluorescence at the anthesis of the secondary ovules (Appendix A Figures A3 and A4). The secondary ovules followed the events in the primary ovules. In the days following anthesis in the first year in the cultivar ‘Edda’, a drop in ovules without fluorescence was evident at 8 °C at 12 DAA (16%), whereas the cultivars ‘Mallard’, ‘Reeves’, and ‘Jubileum’ showed a high percentage of ovules without fluorescence (44%, 53%, and 93%, resp.). The percentages of viable ovules in the ‘Jubileum’ and ‘Reeves’ were around 50% at 8 °C on the 12 DAA in the second year. In both years, at 12 °C and 16 °C, a significantly lower number of ovules without signs of fluorescence were observed (Appendix A Figures A3(E–L) and A4(A–L)).

The occurrence of stage 1 of ovule degeneration was determined at 2 DAA, whereas on the other days, all other stages of fluorescent ovules were present at different percentages. The percentages of ovules with fluorescence in the cultivars tested on the days following anthesis manifested a type of parabola. The correlation coefficient index was between 0.80 and 0.99, which indicates that the analyzed factors are highly intercorrelated (Figure 3).



**Figure 3.** Regression analysis of fluorescing percentage in the secondary ovules during the period analyzed in the tested plum cultivars. Each plot symbol in the regression line indicates the cumulative value of all stages of ovule degeneration observed in the fixation term of the cultivars shown in the legend. Equations and R<sup>2</sup> values are located above or below the corresponding line.

The fastest growth trend in the number of fluorescent ovules in the first year at the days after anthesis at 8 °C was found in the cultivar 'Edda', while at 12 °C and 16 °C it was matched by 'Reeves'. In the second year at 8 °C, the fastest trend was seen in 'Edda', while at 12 °C it was seen in 'Mallard' and 'Reeves'. At 16 °C, almost all the analyzed cultivars showed the same growth trend for ovule degeneration with strong fluorescence, except that it was slightly weaker in the cultivar 'Jubileum'.

### 3. Discussion

We are currently investigating how temperature impacts the viability of plum ovules. Given that air temperatures are expected to increase due to global warming, we use various constant temperatures from anthesis up to 12 DAA. Temperature changes can significantly affect biological processes in fruits, which may result in poor fruit sets. High air temperature has been shown to affect various reproductive aspects, such as stigma receptivity, pollen vitality, pollen tube growth, and ovule longevity [35–39], although many parts are still unknown.

In our research, the lowest constant temperature used was 8 °C. During both years of the investigation, the observed viability of primary ovules in the tested cultivars remained the same at this temperature. Among the tested cultivars, 'Jubileum' and 'Reeves' showed a high percentage of ovule viability on 12 DAA, while the cultivar 'Edda' showed a high rate of ovule viability on 8 DAA. This finding is consistent with Đorđević's research [40], which suggests that the European plum has long ovule viability in natural conditions, lasting up to 10 days from the moment of full flowering.

According to the study, when the temperature was increased by 4 °C and 8 °C in the tested cultivars, the period during which the primary ovule remained viable was reduced, resulting in a shorter time to 8 DAA and 10 DAA. Among these cultivars, the fastest loss of ovule viability was observed in 'Edda' at these temperatures, except in the second year at 12 °C, where a relatively high percentage of viable ovules was observed up to 12 DAA. These results are consistent with previous research by Cerović and Ružić [16] and Cerović et al. [29], which indicates that cherries and plums lose ovule viability as temperatures increase. A study of five European plums found that high ovule viability (80–100%) was observed when pistils were exposed to 5 °C, 10 °C, and 15 °C during the first ten days after full flowering. However, all tested plum cultivars showed a drastic drop in ovule viability at a constant temperature of 20 °C. Previous research suggests that pollen tubes do not enter ovules with intense fluorescence, as they are considered non-viable or senescent [41]. However, in the majority of cases for sour cherry, under the controlled conditions, and in a few cases under field conditions for sweet cherry, when primary ovules were fluorescent, a clear penetration of pollen tubes was observed [16,39]. The lower percentage of fluorescent primary ovules in open pollination compared to cross-pollinated cultivars may be due to the absence of flower emasculation [14,42].

The exact factors that cause the degeneration of one of the two ovules, a natural process in the flowers of *Prunus* species, are unclear. In Japanese plum flowers, the primary and secondary ovules are the same size when opening, but each embryo sac has different developmental stages [43]. The sizes of both degenerated and vital ovules may be influenced by adverse environmental conditions before flowering, according to Guerra et al. [42]. In some European plums, the secondary ovule atrophies as soon as full bloom begins [29]. Previous studies have shown that the first sign of secondary ovule degeneration is the appearance of callose at the chalazal pole of the ovules [34,35,42]. Our research has observed the first stages of fluorescence in secondary ovules at all constant temperatures in both years on the 2 DAA. In cherries, most ovules become senescent at 5 DAA at 5 °C, while at 20 °C, this period is shorter [36].

Several studies have demonstrated the crucial role played by callose in various processes of plant development and the plant's response to different biotic and abiotic stresses [31,44–46]. The occurrence of callose at the chalazal pole in tested plum cultivars is a result of increased ethylene concentration, according to Gonkiewicz and Nosal [46].

Whether ethylene stimulates callose production or inhibits endosperm development, leading to callose synthesis, is uncertain. According to Pimienta and Polito [33] and Rodrigo and Herrero [34], the deposition of callose in the ovule blocks the flow of assimilates and depletes starch. However, it is still unclear whether the synthesis and deposition of callose in the chalazal pole is responsible for the degeneration of the ovule. Recent findings in unpollinated flowers of *Arabidopsis* indicate that ovule integuments degenerate before the gametophytes, and that the later degeneration of gametophyte cells occurs via passive starvation, a consequence of integument degeneration [47]. These findings are consistent with previous research conducted on the senescence of ovules in *Prunus* species [16,35].

The annual variation in ovule senescence suggests that genetic factors, adverse environmental conditions, and the nutritional stage of the flower influence it [43]. In our research, the cultivar specificity affected the expression, i.e., its sensitivity to certain constant temperatures. The tested cultivar's genetic backgrounds could be one reason for this behavior. All cultivars were from cooler geographic regions: 'Mallard' from England, 'Edda' from Norway, 'Jubileum' from Sweden, and 'Reeves' from Canada. The cultivars 'Jubileum', 'Mallard', and 'Reeves' have shown more resistance to temperature stress. The highest percentage of viable ovules was observed with the 'Jubileum' cultivar at all temperatures. This cultivar exhibited the highest percentage of viable and fertilized embryo sacs and fruit sets, whereas the lowest was observed in 'Reeves', as Cerović et al. [48] reported. Previous research by Fotirić Akšić et al. [1] indicated that 'Jubileum' has a longer effective pollination period of five days, in contrast to 'Edda', 'Mallard', and 'Reeves', where it lasts only three days. The authors suggested that temperature differences during the flowering and post-pollination periods could contribute to the fruit-set discrepancy.

## 4. Materials and Methods

### 4.1. Plant Material

This research was conducted during 2021 and 2022 with plum branches from the orchard located in Ullensvang, Hardanger region, western Norway (15 m a.s.l., 60.318655 N, 6.652948 E, with a mean annual temperature of 7.6 °C and 1705 mm precipitation/year, and 12.3 °C and 638 mm for the growing season). For the study of the effects of different constant temperatures on ovule longevity and senescence, branches from the 8-year-old plum trees of the cultivars 'Mallard', 'Edda', 'Jubileum', and 'Reeves' were used. All trees were grafted on the 'St. Julien A' rootstock. Branches taken from the periphery of the canopy of the selected trees mostly contained flowers in the same phenological phase (BBCH59; Meier [49]) (flowers in the earlier or later phenophase on branches were removed).

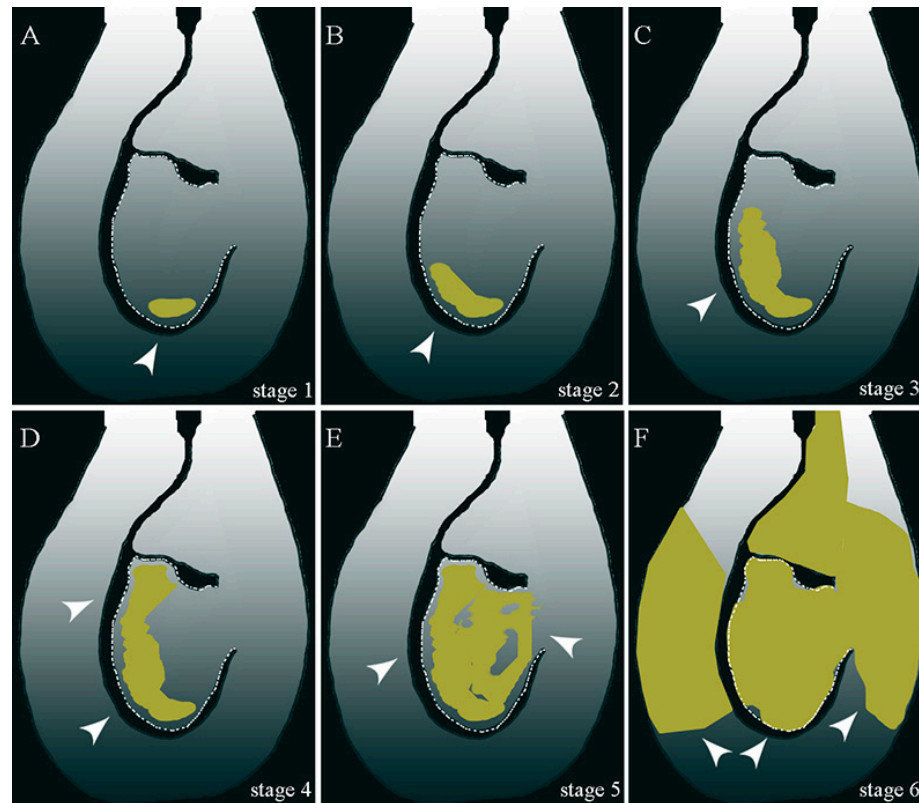
### 4.2. Experimental Designs, Staining, and Sample Preparation

In the lab, cut branches were placed in jars filled with water. When the branches reached the complete flowering phase (BBCH61), they were transferred to chambers with three different constant temperature regimes (8 °C, 12 °C, and 16 °C) and distinct light cycle regimes. For each cultivar, 50 pistils (flowers) were collected every other day, starting from the day of full flowering (day 0) and continuing until the 12th day. The pistils were fixed using FPA fixative (70% ethanol:propionic acid:formaldehyde, 90:5:5, v/v) and refrigerated at +4 °C. The water in the glasses containing the branches was replaced daily until the fixation process was completed. The viability of ovules was assessed using the fluorescent microscopic method described by Anvari and Stösser [50]. The primary and secondary ovules were observed under UV light (blue filter) using an Olympus BX61 fluorescence microscope and DP70 camera. A total of 1440 ovules were analyzed, including 720 primary and 720 secondary ovules.

### 4.3. The Stages of Ovule Fluorescence during Its Senescence

At anthesis, due to the lack of fluorescence, ovules were indicated as fully viable (0 stage). In the days following anthesis, we categorized the ovary into six stages based on the varying intensity of aniline blue staining in different regions. The ovary's first stage

showed fluorescence in the chalaza pole (Figure 4). The fluorescence spread laterally in the second stage, mainly to the side opposite the funiculus. During stage 3, fluorescence spread towards the micropylar end. The micropylar end started to fluoresce in stage 4, and during stage 5, weak fluorescence could be observed in the whole ovule. In stage 6, strong fluorescence was visible in the entire ovule as well as the tissue of the ovary. To determine the percentages of ovules with and without fluorescence, a study was conducted on 30 flowers, considering the cultivars, temperatures, and fixation periods.



**Figure 4.** Scheme of ovule fluorescence/callose accumulation stages (yellow-golden area). The arrow points to fluorescence/callose accumulation in the ovule and ovary tissues.

#### 4.4. Statistical Data

Stats Blue online statistical software was used to obtain regression analyses and the correlation coefficient ( $R^2$ ) between ovules displaying fluorescence and the days after anthesis.

#### 5. Conclusions

Depending on their genotype, it was concluded that different plum cultivars have varying reactions to constant temperatures during the first 12 days after full flowering. The tested plums had the highest percentage and longest ovule viability at the lowest constant temperature, reflecting their origin. On the other hand, the results obtained on ovule viability at higher temperatures may be related to the weaker/better adaptability of the genotypes. Since temperatures tend to rise during the flowering period, this reproductive parameter could be used to predict the reactions of these cultivars, taking into account the specific climatic conditions of western Norway. Besides supporting plum production in cooler climatic conditions by planting orchards with cultivars having high potential fertilization success and fruit sets, the results of this study can influence future breeding programs to consider using cultivars with longer longevity of ovules.



**Author Contributions:** Conceptualization and design of the experiment: R.C., M.F.A. and M.M. Performance of the experiment in the field: M.M. Performance of the experiment in the laboratory: M.M. Analysis and interpretation of the data: M.Đ. and R.C. Writing—original draft preparation: M.Đ. Writing—review and editing: R.C., M.F.A. and M.M. Visualization, M.M. Supervision, M.F.A. Project administration, M.M. Funding acquisition, M.M. All authors have read and agreed to the published version of the manuscript.

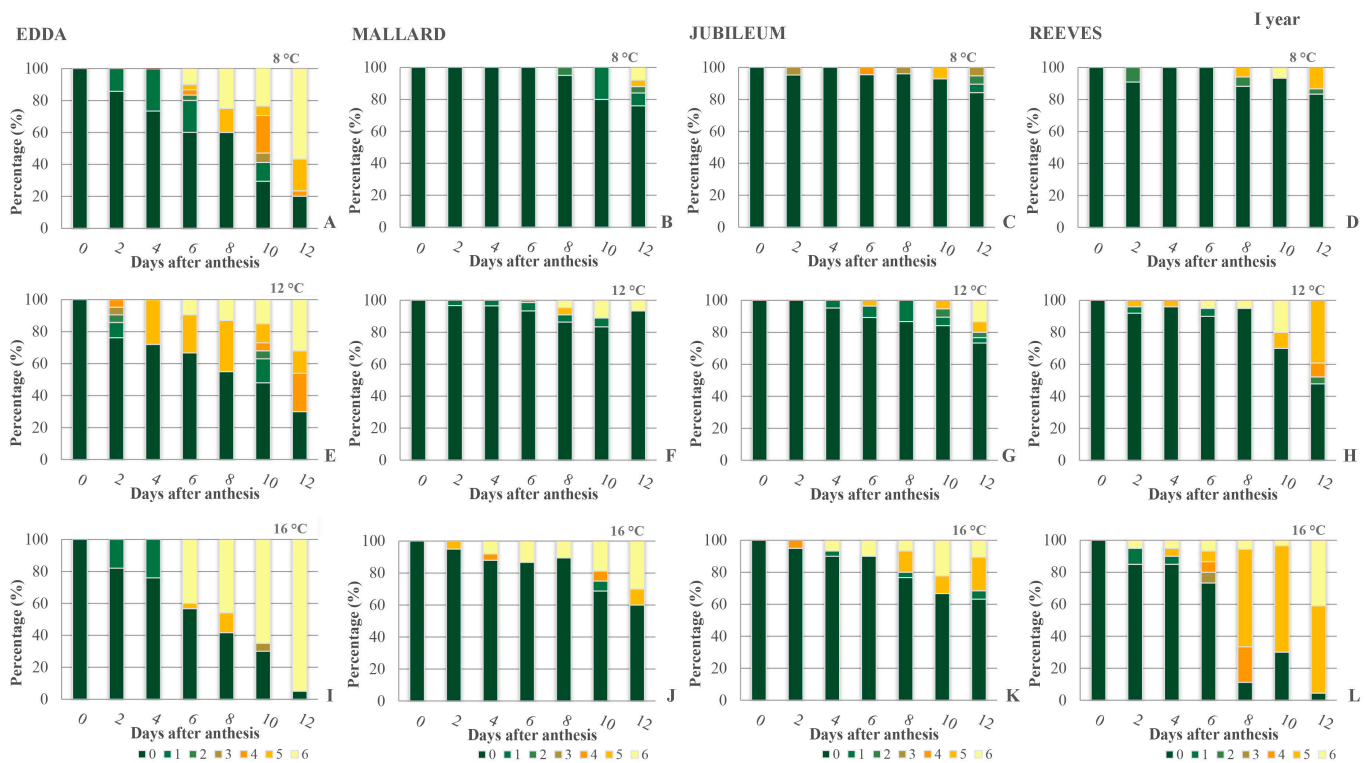
**Funding:** This research was conducted with the support of The Research Council of Norway (project No. 309248).

**Data Availability Statement:** Data are contained within the article.

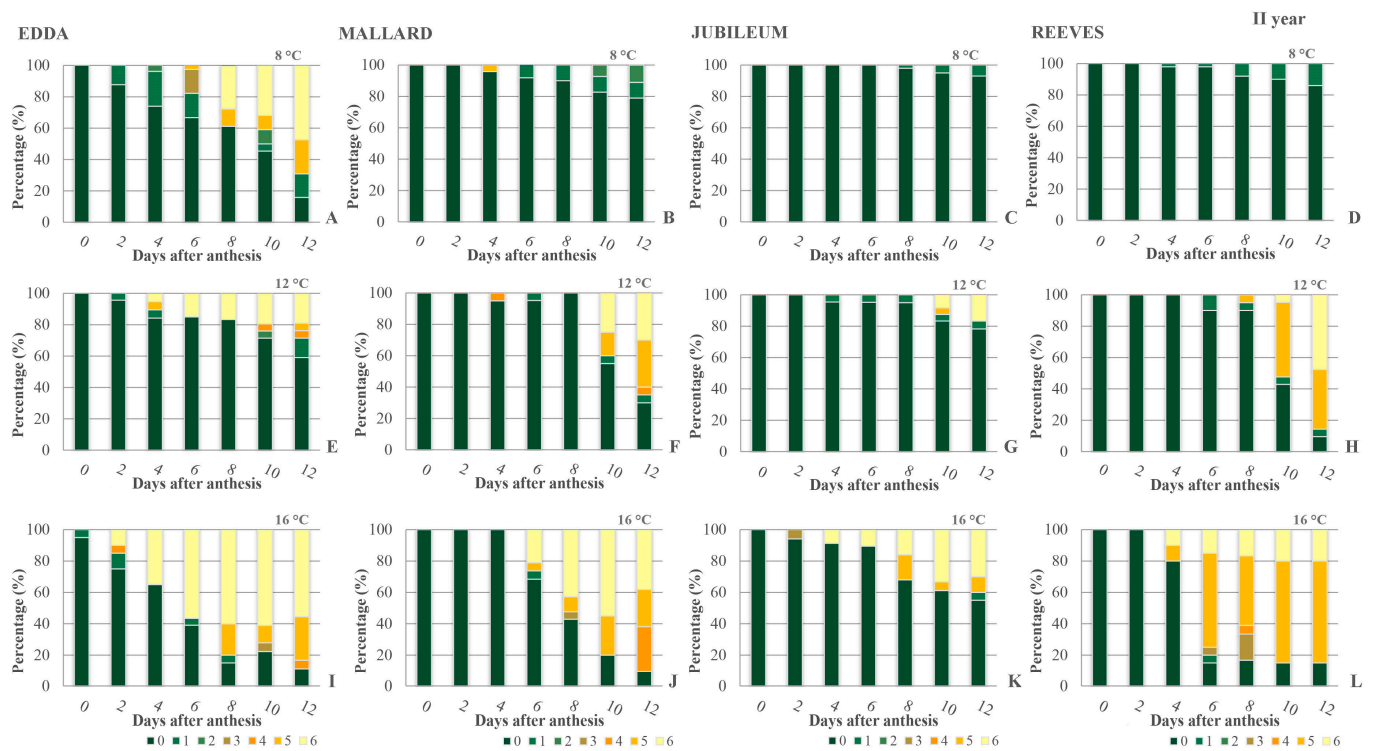
**Acknowledgments:** The authors thank Marianne Hotle, NIBIO Ullensvang, Norwegian Institute of Bioeconomy Research, Lofthus, Norway, for technical support working with the flowering branches in the growth chambers. In addition, the authors would like to thank the Ministry of Science, Technological Development and Innovation of the RS, contracts No. 451-03-66/2024-03/200215, 451-03-65/2024-03/200116.

**Conflicts of Interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

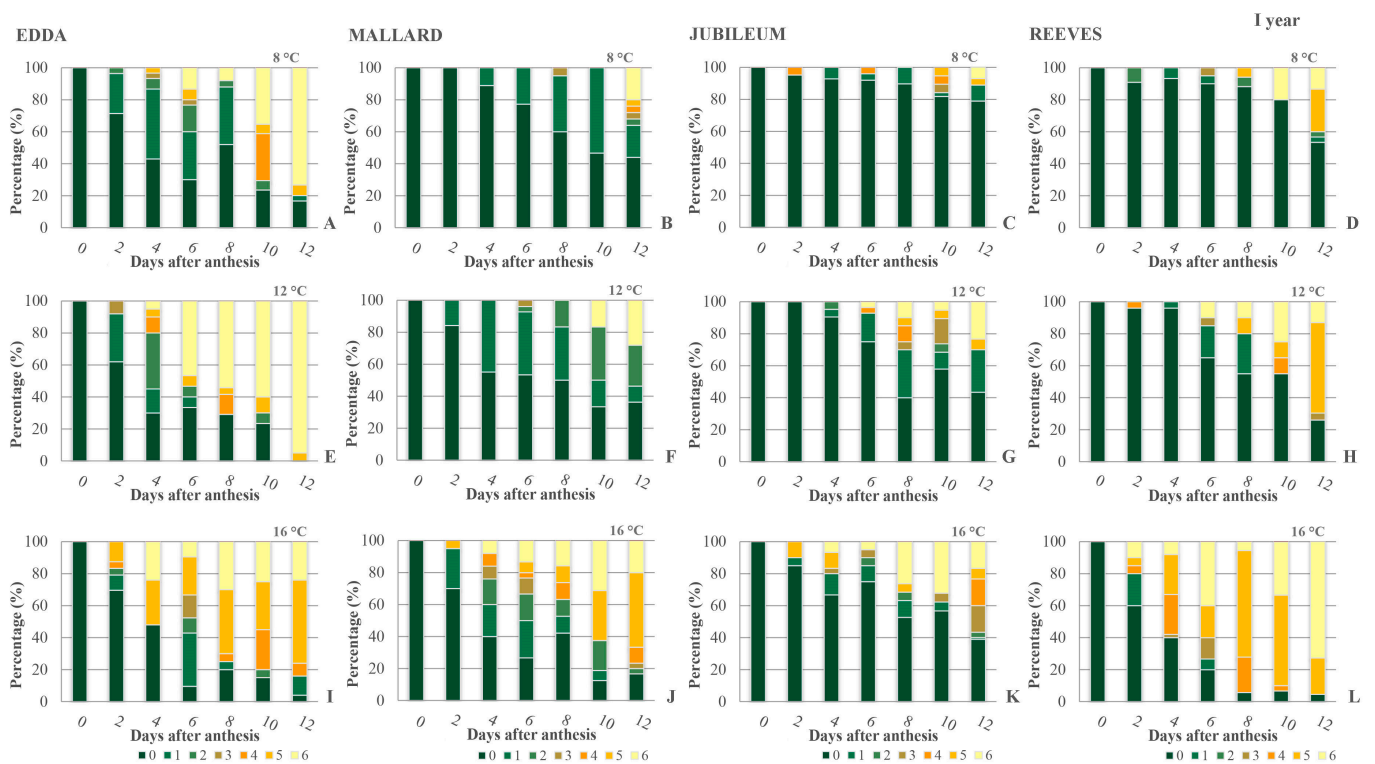
## Appendix A



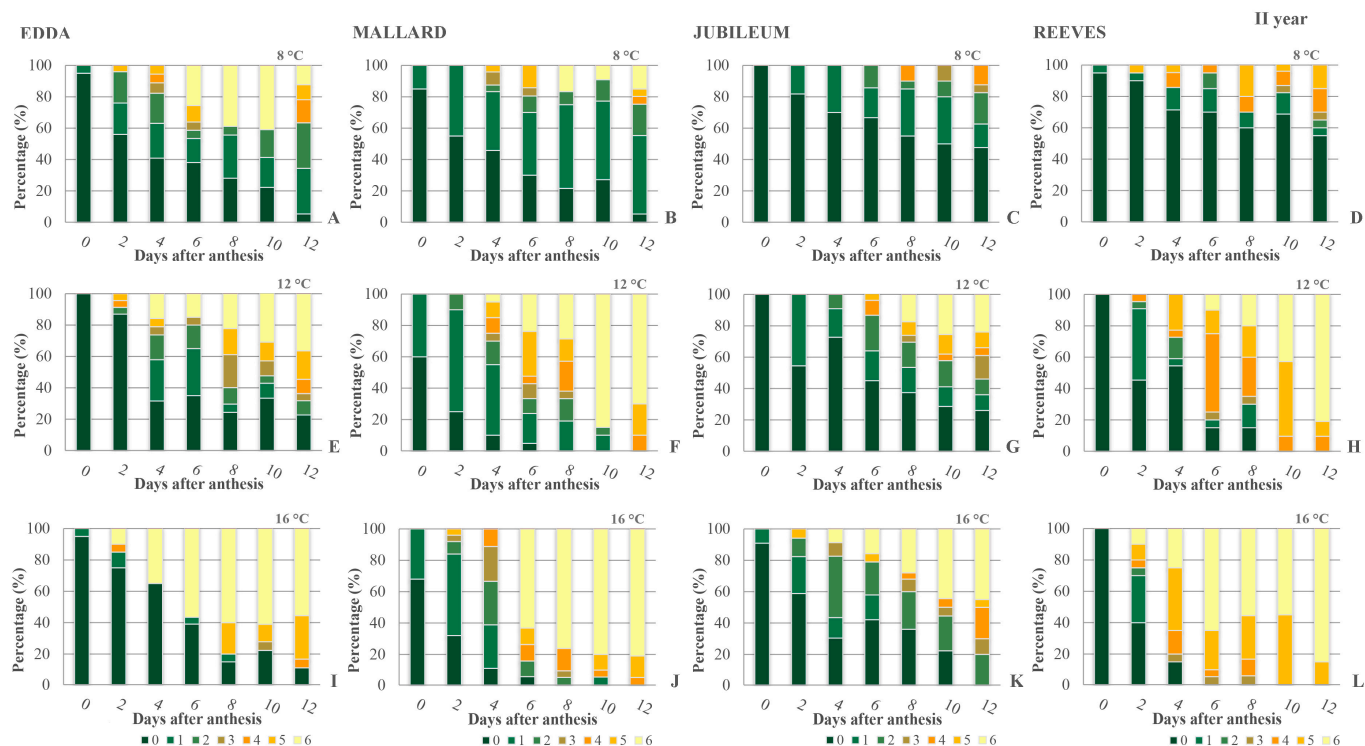
**Figure A1.** Observed stages of degeneration in the primary ovules during their senescences at different constant temperatures in the first year in the tested plum cultivars. (A–D) at 8 °C; (E–H) at 12 °C; (I–L) at 16 °C. Ovule fluorescence stages (0–6) are described in Section 4.3.



**Figure A2.** Observed stages of degeneration in the primary ovule during their senescence at different constant temperatures in the second year in the tested plum cultivars. (A–D) at 8 °C; (E–H) at 12 °C; (I–L) at 16 °C. Ovule fluorescence stages (0–6) are described in Section 4.3.



**Figure A3.** Observed stages of degeneration in the secondary ovule during their senescence at different constant temperatures in the first year in the tested plum cultivars. (A–D) at 8 °C; (E–H) at 12 °C; (I–L) at 16 °C. Ovule fluorescence stages (0–6) are described in Section 4.3.



**Figure A4.** Observed stages of degeneration in the secondary ovule during their senescence at different constant temperatures in the second year in the tested plum cultivars. (A–D) at 8 °C; (E–H) at 12 °C; (I–L) at 16 °C. Ovule fluorescence stages (0–6) are described in Section 4.3.

## References

- Fotirić Akšić, M.; Cerović, R.; Hjeltnes, S.H.; Meland, M. The effective pollination period of European plum (*Prunus domestica* L.) Cultivars in Western Norway. *Horticulturae* **2022**, *8*, 55. [\[CrossRef\]](#)
- Zhebentyayeva, T.; Shankar, V.; Scorza, R.; Callahan, A.; Ravelonandro, M.; Castro, S.; DeJong, T.; Saski, C.A.; Dardick, C. Genetic characterization of worldwide *Prunus domestica* (plum) germplasm using sequence-based genotyping. *Hortic. Res.* **2019**, *6*, 12. [\[CrossRef\]](#) [\[PubMed\]](#)
- Walkowiak-Tomczak, D. Characteristics of plums as a raw material with valuable nutritive and dietary properties—A review. *Polish J. Food Nutr. Sci.* **2008**, *58*, 401–405.
- Fotirić Akšić, M.; Tešić, Ž.; Kalaba, M.; Ćirić, I.; Pezo, L.; Lončar, B.; Gašić, U.; Dojčinović, B.; Tosti, T.; Meland, M. Breakthrough Analysis of Chemical Composition and Applied Chemometrics of European Plum Cultivars Grown in Norway. *Horticulturae* **2023**, *9*, 477. [\[CrossRef\]](#)
- FaoStat. 2021. Available online: <http://www.fao.org/faostat/en/#data/QC> (accessed on 1 April 2024).
- Redalen, G. Plum growing in Norway at 60° N. *Acta Hort.* **2002**, *577*, 385–389. [\[CrossRef\]](#)
- Sekse, L. Plum production in Norway. *Acta Hort.* **2007**, *734*, 23–28. [\[CrossRef\]](#)
- Meland, M. Performance of six European plum cultivars on four plum rootstock growing in a northern climate. *Acta Agric. Scand.-B Soil Plant Sci.* **2010**, *60*, 381–387. [\[CrossRef\]](#)
- Endress, P.K. Angiosperm ovules: Diversity, development, evolution. *Ann. Bot.* **2011**, *107*, 1465–1489. [\[CrossRef\]](#)
- Menzel, A.; Sparks, T.H.; Estrella, N.; Koch, E.; Aasa, A.; Ahas, R.; Alm-Kübler, K.; Bissolli, P.; Braslavská, O.; Briede, A.; et al. European phenological response to climate change matches the warming pattern. *Glob. Chang. Biol.* **2006**, *12*, 1969–1976. [\[CrossRef\]](#)
- Rodrigo, J.; Herrero, M. Effects of pre-blossom temperatures on flower development and fruit set in apricot. *Sci. Hortic.* **2002**, *92*, 125–135. [\[CrossRef\]](#)
- Hedhly, A.; Hormaza, J.; Herrero, M. Warm temperatures at bloom reduce fruit set in sweet cherry. *J. Appl. Bot. Food Qual.* **2007**, *81*, 158–164.
- Carvalho, D.; Cardoso Pereira, S.; Rocha, A. Future surface temperatures over Europe according to CMIP6 climate projections: An analysis with original and bias-corrected data. *Clim. Chang.* **2021**, *167*, 10. [\[CrossRef\]](#)
- Hedhly, A.; Hormaza, J.; Herrero, M. Global warming and sexual plant reproduction. *Trends Plant Sci.* **2009**, *14*, 30–36. [\[CrossRef\]](#) [\[PubMed\]](#)
- Irenaeus, K.S.T.; Mitra, S.K. Understanding the pollen and ovule characters and fruit set of fruit crops in relation to temperatures and genotype—a review. *J. Appl. Bot. Food Qual.* **2014**, *87*, 157–167. [\[CrossRef\]](#)

16. Cerović, R.; Ružić, Đ. Senescence of the ovules at different temperatures and their effect on the behaviour of pollen tubes in sour cherry. *Sci. Hortic.* **1992**, *51*, 321–327. [[CrossRef](#)]
17. Egea, J.; Burgos, L.; Zoroa, N.; Egea, L. Influence of temperature on the *in vitro* germination of pollen of apricot (*Prunus armeniaca* L.). *J. Hortic. Sci.* **1992**, *67*, 247–250. [[CrossRef](#)]
18. Pirlak, L. The effect of temperature on pollen germination and pollen tube growth of apricot and sweet cherry. *Gartenbauwissenschaft* **2002**, *67*, 61–64.
19. Nava, G.A.; Dalmago, G.A.; Bergamaschi, H.; Paniz, R.; Santos, R.P.; Marodin, G.A.B. Effect of high temperatures in the pre-blooming and blooming periods on ovule formation, pollen grains and yield of ‘Granada’ peach. *Sci. Hortic.* **2009**, *122*, 37–44. [[CrossRef](#)]
20. Sorkheh, K.; Azimkhani, R.; Mehri, N.; Chaleshtori, M.H.; Halasz, J.; Ercisli, S.; Koubouris, G.C. Interactive effects of temperature and genotype on almond (*Prunus dulcis* L.) pollen germination and tube length. *Sci. Hortic.* **2018**, *227*, 162–168. [[CrossRef](#)]
21. Raja, M.M.; Vijayalakshmi, G.; Naik, M.L.; Basha, P.O.; Sergeant, K.; Hausman, J.F.; Khan, P.S.S.V. Pollen development and function under heat stress: From effects to responses. *Acta Physiol. Plant.* **2019**, *41*, 47. [[CrossRef](#)]
22. Sato, S.; Peet, M.M.; Thomas, J.F. Determining critical pre- and post-anthesis periods and physiological processes in *Lycopersicon esculentum* Mill. exposed to moderately elevated temperatures. *J. Exp. Bot.* **2002**, *53*, 1187–1195. [[CrossRef](#)]
23. Hedhly, A. Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environ. Exp. Bot.* **2011**, *74*, 9–16. [[CrossRef](#)]
24. Cecchetti, V.; Celebrin, D.; Napoli, N.; Ghelli, R.; Brunetti, P.; Costantino, P.; Cardarelli, M. An auxin maximum in the middle layer controls stamen development and pollen maturation in *Arabidopsis*. *New Phytol.* **2017**, *213*, 1194–1207. [[CrossRef](#)] [[PubMed](#)]
25. Mascarenhas, J.P.; Crone, D.E. Pollen and the heat shock response. *Sex. Plant Reprod.* **1996**, *9*, 370–374. [[CrossRef](#)]
26. Hedhly, A.; Hormaza, J.I.; Herrero, M. The effect of temperature on stigmatic receptivity in sweet cherry (*Prunus avium* L.). *Plant Cell Environ.* **2003**, *26*, 1673–1680. [[CrossRef](#)]
27. Das, K.; Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2014**, *2*, 53. [[CrossRef](#)]
28. Burgos, L.; Berenguer, T.; Egea, J. Self- and cross-compatibility among apricot cultivars. *Hortscience* **1993**, *28*, 148–150. [[CrossRef](#)]
29. Cerović, R.; Ružić, Đ.; Mičić, N. Viability of plum ovules at different temperatures. *Ann. Appl. Biol.* **2000**, *137*, 53–59. [[CrossRef](#)]
30. Ünal, M.; Vardar, F.; Aytürk, Ö. Chapter 14: Callose in Plant Sexual Reproduction. In *Current Progress in Biological Research*; Silva-Opps, M., Ed.; IntechOpen: Rijeka, Croatia, 2013. [[CrossRef](#)]
31. Chen, X.Y.; Kim, J.Y. Callose synthesis in higher plants. *Plant Signal Behav.* **2009**, *4*, 489–492. [[CrossRef](#)] [[PubMed](#)]
32. Brzezicka, E.; Koziaradzka-Kiszkurno, M. Callose deposition analysis with special emphasis on plasmodesmata ultrastructure during megasporogenesis in *Sedum* (*Crassulaceae*). *Protoplasma* **2024**, *261*, 31–41. [[CrossRef](#)]
33. Pimienta, E.; Polito, V.S. Ovule abortion in ‘Nonpareil’ almond (*Prunus dulcis* [Mill.] D.A. Webb). *Am. J. Bot.* **1983**, *69*, 913–920. Available online: <https://www.jstor.org/stable/2442888> (accessed on 7 April 2024). [[CrossRef](#)]
34. Rodrigo, J.; Herrero, M. Influence of intraovular reserves on ovule fate in apricot (*Prunus armeniaca* L.). *Sex. Plant Reprod.* **1998**, *11*, 86–93. [[CrossRef](#)]
35. Stösser, R.; Anvari, S.F. On the senescence of ovules in cherries. *Sci. Hortic.* **1982**, *16*, 29–38. [[CrossRef](#)]
36. Postweiler, K.; Stösser, R.; Anvari, S.F. The effect of different temperatures on the viability of ovules in cherries. *Sci. Hortic.* **1985**, *25*, 235–239. [[CrossRef](#)]
37. Burgos, L.; Egea, J.; Dicenta, F. Effective pollination period in apricot (*Prunus persica* L.) cultivars. *Ann. Appl. Biol.* **1991**, *119*, 533–539. [[CrossRef](#)]
38. Zhang, L.; Ferguson, F.; Whiting, M. Temperature effects on pistil viability and fruit set in sweet cherry. *Sci. Hortic.* **2018**, *241*, 8–17. [[CrossRef](#)]
39. Radičević, S.; Ognjanov, V.; Marić, S.; Barać, G. The effect of genotype and temperature interaction on pollen performance in the pistils of autochthonous sour cherry cultivar ‘Feketička’. *Zemdirbyste* **2021**, *108*, 271–278. [[CrossRef](#)]
40. Đorđević, M. Cytoembryological Aspects of Fertilisation of Plum Cultivar “Pozna Plava” (*Prunus domestica* L.). Doctoral Dissertation, The Faculty of Agriculture, University of Belgrade, Belgrade, Serbia, 2016. Available online: <https://refri.institut-cacak.org/handle/123456789/87> (accessed on 9 April 2024).
41. Anvari, S.F.; Stösser, R. Fluoreszenzmikroskopische untersuchungen des pollenschlauchwachstums und des zustands der samenanlagen bei sauerkirschen. *Mitteilungen Klosterneubg.* **1978**, *28*, 23–30.
42. Guerra, M.E.; Wunsch, A.; López-Corrales, M.; Rodrigo, J. Flower emasculation as the cause for lack of fruit set in Japanese plum crosses. *J. Am. Soc. Hortic. Sci.* **2010**, *135*, 556–562. [[CrossRef](#)]
43. Ruiz, D.; Campoy, J.A.; Egea, J. Ovule development at anthesis in Japanese plum (*Prunus salicina* Lindl.) cultivars. *Span. J. Agric. Res.* **2010**, *8*, 151–158. [[CrossRef](#)]
44. Verma, D.P.; Hong, Z. Plant callose synthase complexes. *Plant Mol Biol.* **2001**, *47*, 693–701. [[CrossRef](#)]
45. Nishikawa, S.; Zinkl, G.M.; Swanson, R.J.; Maruyama, D.; Preuss, D. Callose ( $\beta$ -1,3 glucan) is essential for *Arabidopsis* pollen wall patterning, but not tube growth. *BMC Plant Biol.* **2005**, *5*, 22. [[CrossRef](#)] [[PubMed](#)]
46. Gonkiewicz, A.; Nosal, K. Anatomical Changes in Plum Fruit *Prunus domestica* L. after Fruit Thinning Using Potassium Salt of Alpha-Naphtaleneacetic Acid. *EJPAU* **2006**, *9*, 50. Available online: <http://www.ejpau.media.pl/volume9/issue4/art-50.html> (accessed on 12 April 2024).

47. Van Durme, M.; Olvera Carrillo, Y.; Pfeiffer, M.; Doll, N.; De Winter, F.; Lin, Z.; Nowack, M. Fertility loss in senescing Arabidopsis ovules is controlled by the maternal sporophyte via a NAC transcription factor triad. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2219868120. [[CrossRef](#)]
48. Cerović, R.; Fotirić Akšić, M.; Đorđević, M.; Meland, M. Viability of embryo sacs and fruit set in different plum (*Prunus domestica* L.) cultivars grown under Norwegian climatic conditions. *Plants* **2022**, *14*, 219. [[CrossRef](#)] [[PubMed](#)]
49. Meier, U. Growth stages of mono- and dicotyledonous plants. In *Federal Biological Research Centre for Agriculture and Forestry*, 2nd ed.; BBCH Monograph; Blackwell Wissenschafts-Verlag: Berlin, Germany, 2001; pp. 1–158.
50. Anvari, S.F.; Stösser, R. Eine neue fluoreszenzmikroskopische Methode zur Beurteilung der Befruchtungsfähigkeit der Samenanlagen bei *Prunus*. *Zeitschrift Pflanzzüchtung* **1978**, *81*, 333–336.

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.