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Current and Predicted Future Winter Warm Spells Would Affect Douglas Fir (*Pseudotsuga menziesii* (Mirb.), Franco) Seeds in the Early Stage of Germination More Than in the Late Stage of Germination

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Abstract: Most tree species in the temperate climatic zone (including Douglas fir) disperse seeds in autumn. Some of them must be exposed to cold (0–10 °C) and moist conditions (cold stratification) to overcome dormancy and trigger germination. In the Northern Hemisphere, winter warm spells occur more frequently and last longer than in recent decades from eastern Canada to Europe. Our main research objective was to investigate the influence of current (1 or 3 days at day/night temperatures: 15 °C/10 °C) and future predicted (5 days at day/night temperatures: 25 °C/15 °C) winter warm spells on dormancy breaking and germination traits (germination energy—GE; germination capacity—GC; final germination capacity—FGC) of Douglas fir seeds from four old-growth stands in northern Poland. For this purpose, we interrupted cold stratification of seeds at different time points, i.e., after 3 weeks; 6 weeks; 9 or 3 weeks and 6 weeks; 3 and 9 weeks; 6 and 9 weeks; 3 and 6 weeks and 9 weeks. We found that for GE and GC, all main effects (populations—P; days of warm spell—D; stratification duration—W) and interactions were significant (except interaction P×D). FGC was significantly affected by the effects P and D and interactions of D × W and P × D × W. In addition, we found that the predicted warm spells negatively affected the early germination stage (GE and GC) of Douglas fir, but both current and future predicted winter conditions will not negatively affect the late germination stage (FGC).

Keywords: seed dormancy; climate change; winter warm spells; germination; Douglas fir

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1. Introduction

Climate change, especially rising temperatures and decrease in snow cover during winter, could have serious consequences for seed biology and ecology [1]. The result of these consequences could be manifested in changes in population dynamics by increased seedling mortality and/or a complete lack of plant regeneration from seeds. These responses would be particularly devastating for species that are sensitive to climate conditions during flowering, seed and fruit set and for species with dormant seeds that require a cold period to break dormancy [1,2]. In the Northern Hemisphere, winter warm spells last longer and occur more frequently than in the last five decades from eastern Canada [3] to Europe [4,5]. In central Europe, research showed that over the study period (1966/1967–2015/2016) the air temperature increased in winter seasons, which translated into an increased number of warm days [6]. An average of three to five warm spells was recorded per 10 years. The most numerous warm spells occurred during three winter seasons, i.e., 1989/1990, 2006/2007 and 2015/2016. Moreover, the temperatures during the entire winter periods have increased, while the thickness of snow covers has decreased during the last decades [7]. Vegetation in the temperate, boreal and arctic zones will be impacted by these changes taking place in winter [8]. Most species in the temperate climatic zone disperse seeds in autumn. Some of them must be exposed to cold (0–10 °C) and moist conditions (cold stratification) to overcome dormancy and trigger germination. Hence, germination occurs during late winter or early spring, thereby allowing seedlings to grow in a period before a hot and dry summer [9]. The consequences of winter warm spells in connection with the forecasted increasing temperatures during the dormancy break and germination periods have not been widely described in the literature, especially for trees [1,10]. Faster and enhanced germination of seeds from exotic (non-native) species in comparison to the native ones indicates that seeds of the former feature possess weak dormancy and could react more strongly to winter warm spells [10–13].

Douglas fir (*Pseudotsuga menziesii* (Mirb.), Franco) was introduced to Europe from North America at the beginning of the 19th century [14]. The species is the second most cultivated non-native conifer tree in Europe (0.8 million hectares) after the Sitka spruce (1.2 million hectares). In Europe, including Poland, Douglas fir is the most frequently cultivated species due to its rapid growth and high production of wood mass [15].

The occurrence of tree species of foreign origins in forest ecosystems is a contentious issue because of habitat alternations and their negative impact on forest biodiversity. In central Europe, it is well evidenced in alien deciduous trees, e.g., *Ailanthus altissimus* (Mill.) Swingle, *Robinia pseudoacacia* L., *Quercus rubra* L. and *Prunus serotina* Ehrh. [16–18].

On the other hand, only a few species were naturalized in Europe, e.g., *Picea sitchensis* (Bong.) Carrierre, *Pinus contorta* Douglas ex Loudon, *Pinus strobus* L. and *P. menziesii* [19–21]. However, the invasive potential of these species in Europe is also low and they rarely spread in forests [22,23]. *P. menziesii* is phylogenetically close to the native European conifers and, similarly, shapes the abiotic environment and biotic communities [24]. Nevertheless, contemporary trends in forest management and protection of natural resources encourage such species to be treated as undesirable elements. As such, it is recommended to prohibit their introduction to tree stands or to eliminate them where they are already established [25]. Nevertheless, the observed decline of tree stands of native gymnosperms, such as *Abies alba* Mill., *Picea abies* (L.) H. Karst. and *Pinus sylvestris* L., indicates the need to search for solutions aimed at mitigating the effects of climate change. This need has become more pressing since the results of research on predictive modelling of the future ranges of native gymnosperm trees indicate the loss of the majority of tree stand areas of these species in southern and central Europe [26,27].

The aim of the research was also to investigate the influence of the current short-term warm spells in winter on the breaking of dormancy and the germination of Douglas fir seeds from four old-growth stands in northern Poland. This study also determined the impact of future thermal conditions on the dormancy and germination of Douglas fir seeds in terms of forecasted climate change. To our knowledge, our study is the first to determine the impact of winter conditions on the dormancy break and germination of seeds of this species.

Specific research questions were: (1) Does the increase in temperature and duration of winter warm spells inhibit or promote germination in different populations of Douglas fir in the east-west transect? (2) Does the germination characteristics of seeds that have been exposed to current and future winter warm spells during the cold stratification period differ from seeds without a break in cold stratification? (3) During which period of cold stratification are the seeds most sensitive to winter warmth?

2. Material and Methods

2.1. Plant Material

Seeds originated from four 140-year-old Douglas fir stands located in northern Poland were used (Jamy—JAM, 53°27′46″ N, 19°21′56″ E; Lębork—LEB, 54°31′25″ N, 17°48′27″ E; Czaplinek—CZA, 53°42′4″ N, 16°25′50″ E; and Chojna—CHO, 52°56′33″ N, 14°18′25″ E), Figure 1. The seeds used to establish Douglas fir in Poland were probably imported from British Columbia (Canada) or the state of Washington (USA) by the German scientist Adam Schwappach in 19th century. All of the analyzed populations are well adapted to environmental conditions they grow in. Unfortunately, regenerations (seedling establishment) in these stands are very poor. We chose this population based on the climatic gradient (east-west, see Climatic criteria) and due to their role in forest reproductive material management. All of them are selected or managed seed stands [28]. Seeds from each population were harvested from 50 trees/population in autumn 2019. Since many seeds may be infested by the Douglas Fir Seed Chalcid (*Megastigmus spermotrophus* Wachtl), the seeds were sorted in an air screen separator and additionally X-rayed. This resulted in a high degree of purity (close to 100%).

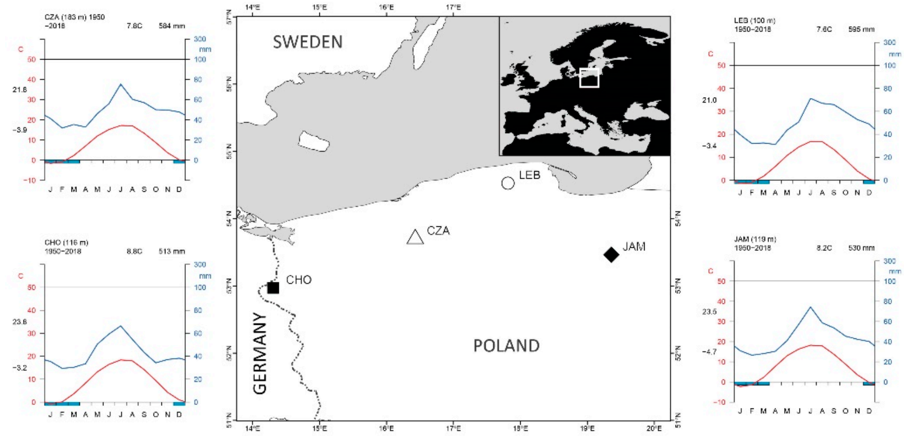

Figure 1. Locations of Douglas fir stands and climatic diagrams. Plots of the precipitation and temperature monthly averages, annual averages of both elements (in the upper part) and monthly averages of the daily maximum and minimum temperatures of the warmest and coldest months, respectively (at the left margin); blue rectangle under the 0 °C axis—frost likelihood; JAM—Jamy, LEB—Lębork, CZA—Czaplinek and CHO—Chojna.

2.2. Climatic Criteria

Warm spells are defined as 3–7 consecutive days of daily mean temperatures >80th or >90th percentile for the normal period [3,29,30]. The climate of the study sites experiences mean annual temperatures from 7.6 to 8.8 °C (LEB and CHO, respectively), while periods with an absolute minimum below 0 °C may occur from December to March in all sites. Mean annual precipitation reached from 513 to 595 mm (CHO and LEB, respectively), taking place mainly in May–July (from 165 to 177 mm; LEB and CHO, respectively), Figure 1. Mean winter months temperature (i.e., from November to March) ranged from 0.6 °C in JAM to 1.8 °C in CHO. In order to determine the climate of study sites, we used E-OBS 0.25° × 0.25° gridded climate data including daily precipitation sums as well as daily mean, minimum temperatures and maximum temperatures for the common observation period between 1950 and 2018 [31]. E-OBS is a gridded climate data set interpolated from climate station data across Europe [32]. After an analysis of the number of warm days in winter (with average temperature > 10 °C) over the last 30 years, we defined warm spells with temperatures of 15/10 °C (day/night) lasting 1 or 3 days as a normal (current) condition (Supplementary Material—Table S3). According to KLIMADA [33], the Polish National Strategy for Adaptation to Climate Change, winter temperatures are expected to rise by 2.5 °C from 2021 to 2050 and by 4.5 °C for 2071–2100, especially in the northeastern part of Poland. With an assumption that the lengths of warm spells along with their temperatures will increase with climate change, we selected 5 days at 25/15 °C for an extreme (future) warm spell (Table S4).

2.3. Experimental Design

In total, 21 temperature-time variants were used for each population. Seeds were divided into separate samples for each experimental condition and stored in paper bags at room temperature. Afterward, seeds were soaked in distilled water for 24 h and all samples were placed in a cold room at 3 °C (cold stratification) for 12 weeks in darkness. Seeds were not moist during stratification. In addition to samples that remained constantly in the cold room for the whole stratification period (12 weeks), which served as a control, other samples were divided additionally for 3 time-temperature conditions. These samples were removed from the cold room and placed for 1 or 3 days at 15/10 °C (day/night) or 5 days at 25/15 °C in two growth chambers (Mytron WB 750 KFL) to simulate the interruption of cold stratification by current (15/10 °C) and future (25/15 °C) winter warm spells. In order to determine in which period of cold stratification the seeds are the most sensitive to winter warm spells, the interruption of the 12 week cold stratification period was conducted after week 3, 6, and/or 9 (Figure 2). Following the interruption, seeds were placed back at 3 °C for the remainder of the 12 week period. After cold stratification, seeds were placed in 9 cm diameter plastic Petri dishes on 5 mm of swimming pool filtered sand (Ø 0.2–0.5 mm) for the germination test. The sand was moistened to saturation with distilled water prior to seed addition and re-moistened as required throughout the duration of the experiment. One hundred seeds were placed in each dish, with three replicates (dishes) per treatment (JAM, CZA and CHO), or fifty seeds per dish, with three replicates (LEB). In total, 22,050 seeds were used (6300—JAM; 6300—CHO; 6300—CZA; 3150—LEB). The reason for the differences in the number of seeds used among populations was due to their accessibility in each population. Dishes were placed singly (i.e., not stacked) on growth chamber shelves.

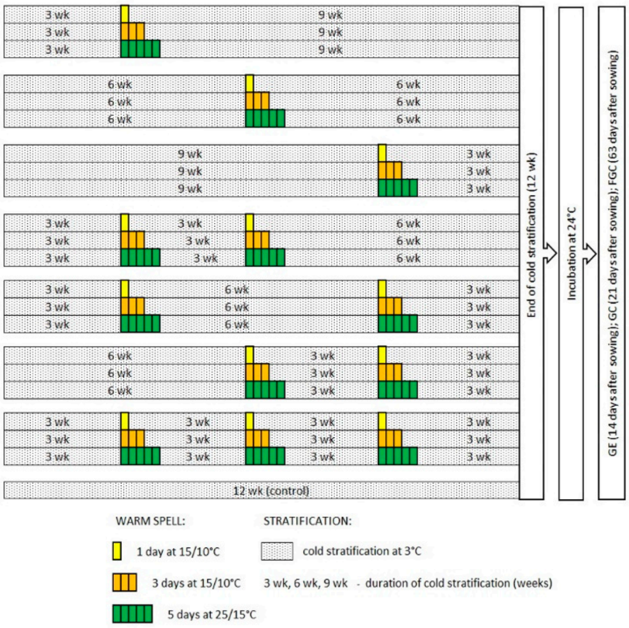

Figure 2. Schematic of treatments to examine the interruption of cold stratification by warm spells differing across time (week 3, 6 and/or 9), in lengths (1, 3 and 5 days) and in temperatures (15/10 °C and 25/15 °C). GE—germination energy; GC—germination capacity; FGC—final germination capacity; wk—duration of cold stratification (weeks). Concept of figure based on Flanigan et al. [10].

At the end of the 12 week cold stratification, the control and all treatments were again transferred to incubators (Mytron Bio-Und Solartechnik GmbH, Gaussring, Germany, model WB750 KFL) with 16/8 light/dark photoperiod and set at a constant temperature of 24 °C and humidity of 70% with illumination from cool white fluorescent light (40 µmol m^{-2} s^{-1}). In the early stage of the germination test, germinated seeds were scored every day and then in 5 day intervals. During each observation, all germinated seeds were removed from the Petri dishes. We defined germination as seeds with visible, normally developed and at least 2 mm length radicle. At the end of all germination tests, the viability of non-germinated seeds was determined using the pinch test: the seeds were pinched with forceps under a dissecting microscope and white and firm embryos were considered viable, while brown and soft embryos were non-viable. Evaluation of all germination traits was determined only for viable seeds.

We evaluated differences in three germination traits:

1. Germination energy (GE)—percentage of germinated seeds at the beginning of the germination test (after 2 weeks of incubation);
2. Germination capacity (GC)—percentage of seeds that would normally germinate under optimal conditions for the species (after 3 weeks of incubation);
3. Final germination capacity (FGC)—maximum (final) germination after 9 weeks of incubation.

2.4. Statistical Analysis

For each trait, a generalized linear model (GzLM) was used:

$$g\left(G_{ijk}\right) = P_i + D_j + P_i \times D_j + W_k + P_i \times W_k + D_j \times W_k + P_i \times D_j \times W_k \qquad (1)$$

where $g(G_{ijk})$ is the log link function and G_{ijk} is the expected mean of the response variable (i.e., GE, GC or FGC) for the i-th population in the j-th day of winter warm spell and k-th stratification duration; P_i is the main effect of the i-th population; D_j is the main effect of the j-th day of winter warm spell; W_k is the main effect of the k-th variant of weeks of stratification durationl $P_i \times D_j$, $P_i \times W_k$, $D_j \times W_k$ are the second-order interaction effects; and $P_i \times D_j \times W_k$ is the third-order interaction effect among the considered model effects. The significance of the model effects was tested by the Wald χ^2 test for a type 3 analysis. Wald's test is equivalent or more powerful than the log likelihood LR test [34]. For significant model effects, pairwise comparisons were made between least-square means with Tukey's post-hoc test.

We used a generalized model with one factor at three levels to examine the effects of current and future conditions and compared them to the control for each study trait (Model 2). Since the control was the same for each $D_j \times W_k$ combination (Figure 2), we applied Model 2 separately for each level of warm spells' duration (W_k). We performed the model analysis and pairwise comparisons analogously to Model 1 (Equation (1)).

Inter-population variability of the examined traits was analyzed based on population (P_i), day (D_j), week (W_k) and $D_j \times W_k$ effects, while intra-population variability was evaluated using third-order interaction ($P_i \times D_j \times W_k$). The interactions $P_i \times W_k$ and $P_i \times D_j$ were analyzed for inter-population as well as intra-population variability (see Supplementary Materials). Calculations were performed using the GENMOD procedure of SAS/STAT® v. 14.3 [35].

3. Results

3.1. Effect of Population, Duration of Winter Spells and Time of Interruption of Cold Stratification on Germination Traits of Douglas Fir Seeds

We found significant influence for all analyzed model effects (except P × D interaction) on GE, GC (except P × D interaction) and FGC (except for the effect of week of stratification (W) and the effects of P × D and P × W interactions). For the sake of better understanding and to make the manuscript more readable, we decided to move all analysis results regarding the significance of all model effects and their interactions to the Supplementary Materials section. In the case of GE and GC, significant differences were found for P × W (Tables S1–S3), D × W (Tables S1–S3) and P × D × W interactions (Figures S1–S3). However, in the case of FGC we only found significant differences for D × W (Tables S1–S3) and P × D × W (Figures S1–S3).

Populations significantly affected the germination of Douglas fir seeds in the case of GE (Wald χ^2 = 11.1, $p < 0.001$), GC (Wald χ^2 = 171.1, $p < 0.001$) and FGC (Wald χ^2 = 194.3, $p < 0.001$). Seeds from LEB and CZA had a significantly lower GE in comparison to seeds from JAM and CHO. Mean GC differed significantly among all populations. The highest average FGC was observed in seeds from JAM and CHO, which were significantly different from population LEB and CZA (Table 1).

Table 1. Germination energy (GE), germination capacity (GC) and final germination capacity (FGC) of Douglas fir seeds. Population (P): JAM—Jamy, LEB—Lębork, CZA—Czaplinek and CHO—Chojna; 1, 3 and 5—number of day(s) of winter warm spell (D); 3, 6 . . . 3/6/9—the week(s) when the warm spell occurred during cold stratification either once (3, 6 and 9) or repeatedly (3/6, 3/9, 6/9 and 3/6/9)—W. Means with the same uppercase letter within a column are not significantly different at $p \leq 0.05$.

Source of Variance		GE (%. ± SE)	GC (%. ± SE)	FGC (%. ± SE)
P	CHO	25 (0.8) ^{A}	58 (1.1) ^{B}	96 (0.3) ^{A}
	CZA	17 (0.7) ^{B}	43 (1.1) ^{D}	90 (0.5) ^{B}
	LEB	19 (1.0) ^{B}	49 (1.6) ^{C}	90 (0.7) ^{B}
	JAM	28 (0.8) ^{A}	62 (1.1) ^{A}	97 (0.3) ^{A}
D	1	22 (0.7) ^{AB}	55 (1.1) ^{A}	95 (0.4) ^{A}
	3	23 (0.8) ^{A}	52 (1.1) ^{AB}	93 (0.4) ^{B}
	5	20 (0.7) ^{B}	51 (1.1) ^{B}	93 (0.4) ^{B}
	3	29 (1.2) ^{A}	53(1.6) ^{AB}	92 (0.7) ^{A}
	6	24 (1.2) ^{B}	54 (1.7) ^{AB}	92 (0.7) ^{A}
	9	18 (1.1) ^{C}	50 (1.7) ^{B}	94 (0.6) ^{A}
W	3/6	32 (1.3) ^{A}	58 (1.7) ^{A}	93 (0.6) ^{A}
	3/9	21 (1.1) ^{BC}	55 (1.6) ^{AB}	94 (0.6) ^{A}
	6/9	22 (1.1) ^{BC}	52 (1.7) ^{AB}	94 (0.6) ^{A}
	3/6/9	12 (0.9) ^{D}	47 (1.6) ^{B}	94 (0.6) ^{A}

Duration of warm spells also had a significant effect on all analyzed germination traits (GE—Wald χ^2 = 10.6, $p = 0.005$; GC—Wald χ^2 = 8.4, $p = 0.015$; FGC—Wald χ^2 = 12.3, $p = 0.002$). On average, GE was significantly higher after 3 days of warming than after 5 days. In the case of GC, statistical differences were only found between seeds affected by 1 or 5 days of a warm spell. A 1 day warm spell caused a higher increase in mean GC compared to a 5 day spell. A 1 day warm spell caused a significant increase in mean FGC compared to a 3 or 5 day spell (Table 1).

Based on the analyses performed, we have concluded that the time when cold stratification was interrupted significantly affected the germination of Douglas fir seeds. However, those differences were only significant in the case of GE (Wald χ^2 = 190.2, $p < 0.001$) and GC (Wald χ^2 = 25.8, $p < 0.001$). The greatest effect on GE occurred when the warm spell interrupted cold stratification at 3 or 3 weeks and 6 weeks. Interruption of cold stratification after 6, 9, 3 and 9 weeks or 6 and 9 weeks caused significantly lower GE in comparison to the interruption at 3 weeks or 3 and 6 weeks. The lowest GE occurred when cold stratification was interrupted three times, i.e., after 3, 6 and 9 weeks (Table 1). When cold stratification was interrupted after 3 and 6 weeks, GC was significantly higher compared to the seeds for which cold stratification was interrupted after 9 weeks or 3 and 6 weeks and 9 weeks (Table 1). Mean FGC was not affected by the timing of the warm spell interruption.

3.2. *Effect of Current and Future Winter Warm Spells during Cold Stratification on Germination Traits*

Since winter warm spells lasting 1 or 3 days did not differ significantly in the germination traits analyzed (in the case of GE and GC especially), we decided to combine these two treatments. Finally, we compared current winter warm spells with predicted future winter warm spells. As a control, we used seeds without interruption of the cold stratification period (results of Model 2 analysis are available in Supplementary Material—Table S2).

Seeds for which cold stratification was interrupted after 3 weeks by current or future winter warm spells had a significantly higher GE compared to the control (Figure 3A). The same effect was obtained when cold stratification was interrupted after 6 weeks or 3 and 6 weeks. When cold stratification was interrupted after 9 weeks, GE increased in comparison to both future conditions and control. We also observed significantly higher GE in comparison to control when cold stratification was interrupted twice after 6 and 9 weeks. Interruption of cold stratification after 3 and 9 weeks caused significantly higher GE when seeds were subjected to the predicted future winter warm spells. We found no significant differences between current, future and control conditions in the case of the interruption of cold stratification after 3 weeks and 6 and 9 weeks.

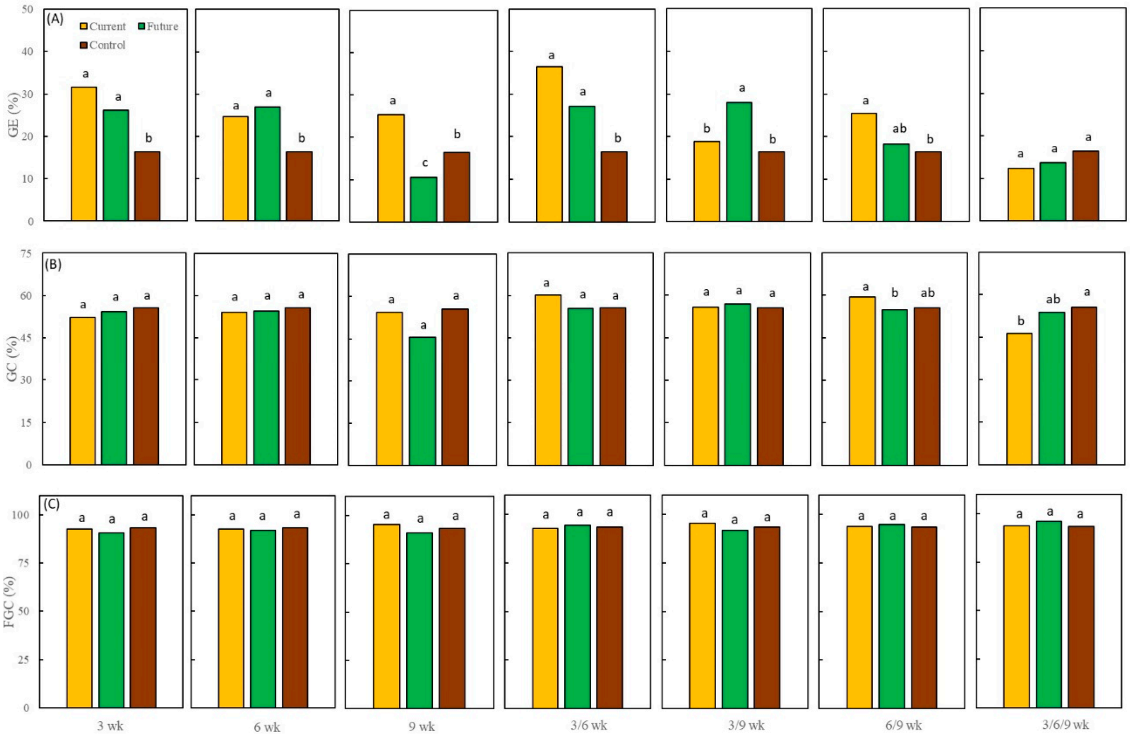

Figure 3. Effect of current (1 or 3 days at 15/10 °C) and future (25/15 °C) warm spells depends on time of interruption of cold stratification on germination energy (**A**), germination capacity (**B**) and final germination capacity (**C**) of Douglas fir seeds compared to the control (12 weeks of cold stratification without interruptions). The numbers 3, 6 . . . 3/6/9—the week(s) when the warm spells occurred during cold stratification either singly (3, 6, 9) or in multiples (3/6, 3/9, 6/9, 3/6/9). Means with the same letter are not significantly different at $p \leq 0.05$.

In the case of GC, we found significant differences between current and future winter warm spells only when cold stratification was interrupted after 6 and 9 weeks or after 3 and 6 and 9 weeks. In the former case, higher GC was found after the occurrence of current winter warm spells than predicted future winter warm spells (Figure 3B). We also found that seed germination after uninterrupted cold stratification (control) did not differ significantly from both current and future conditions. However, in the second case when cold stratification was interrupted three times, we observed that seeds from the control variant germinated significantly better in comparison to seeds affected by current winter warm spells (Figure 3B). We did not find any significant differences in the case of FGC (Figure 3C).

4. Discussion

In the Northern Hemisphere, climate change that is exacerbated and accelerated by human activities may result in significant changes in the distribution and pattern of seasons in the future. This phenomenon may particularly affect the winter period, which is of great importance for many physiological processes in plants [1]. One such process is cold stratification that overcomes dormancy in seeds. Interrupting cold stratification by increasing the temperature to 16–17 °C together with the desiccation of the seeds results (depending on the species) in the retardation of germination, reduction in germination capacity and the induction of secondary dormancy in ungerminated seeds or to the acceleration of germination [36–38]. In the present study, warming during cold stratification, with temperatures of 15/10 °C lasting 1 or 3 days (current conditions) and with temperatures of 25/15 °C lasting 5 days (predicted future conditions), were tested. For Douglas fir seeds, germination either decreased or increased depending on the time at which scoring was performed. Short-term or long-term warm spells during the initial stratification period tended to have a stimulatory effect on Douglas fir seed germination. A single warming after 3 weeks or a double warming after 3 and 6 weeks of stratification significantly increased seed germination energy. However, for germination capacity, this effect was only significant for seeds for which stratification was interrupted once after nine and three times after 3, 6 and 9 weeks. When the experiment ended on day 63 after the start of incubation, the effect of the stratification period during which winter warming occurred was insignificant. The negative effect of warmings was also observed when the warm spells occurred several times during the cold stratification period (in particular, warmings with a temperature of 25/15 °C that lasted 5 days and occurred three times after 3, 6 and 9 weeks of stratification). Differences also occurred in the response of seeds to the duration of warming depending on seed origin. This effect was more evident in the early stages of germination but no differences were present among populations at the final germination stage (after 63 days from the beginning of incubation).

In a study by Martiník et al. on Douglas fir, germination capacity of non-stratified seeds was only 58%, while seeds were treated as pre-sowing by various stratification periods (from 3 to 16 weeks) reached 92%–96% [39]. In our study, seeds for which the 12 week stratification was not interrupted by warm spells (control) achieved germination of 71% (JAM population), 48% (LEB population), 36% (CZA population) and 67% (CHO population). Thus, for two populations (JAM and CHO) the germination capacity was higher than for unstratified seeds in the study by Martiník et al., but none of our populations had the germination capacity at the level reported by these authors [39]. Such values were reached by seeds in our study after 63 days of incubation (96%—JAM, 88%—LEB, 92%—CZA and 97%—CHO). In contrast, the seed viability traits we analyzed were not affected by the length of the stratification periods.

In the case of Douglas fir, which is not native to Europe, we found a strong and generally positive response to short-term warm spells during the first few weeks after germination started. With increase in the length of the germination (incubation) period, the degree of this effect decreased. After 63 days from the start of incubation, the germination capacity of seeds from each population (except two cases) was no longer dependent on the occurrence of a warm spell, both in terms of its duration and the time during stratification when it occurred. This type of response was consistent with what was documented for non-native species by Flanigan et al. [10]. Those authors analyzed the effects of winter warm spells and warming on germination of selected native and non-native species of woody plants in middle Tennessee (USA). The occurrence of short-term warm spells had the most negative effect on some native plant species, including *Parthenocissus quinquefolia* (L.) Planch. and *Celtis laevigata* Willd. One case of increased seed germination of the native species *Vitis vulpina* L. was also reported. For the non-native species (mainly from

South-East Asia), the authors observed a predominantly neutral seed response to winter warm spells. Only in the case of *Lonicera maackii* (Rupr.) Maxim. was a positive seed response observed after the interruption of the cold stratification period with an increase in germination of about 20% compared to the control. The results regarding Douglas fir may indicate that the populations contain some phenotypic plasticity in relation to the climatic conditions of dormancy and germination. Cold stratification of seeds has a beneficial effect on subsequent germination and displays an increase in the final germination capacity, an increased germination rate and an expansion of the temperature range at which the seeds can germinate [40]. However, we found no clear changes in the germination phenology of Douglas fir seeds due to the interruption of the stratification period by short- and long-term warm spells. Neither current nor predicted future winter warm spells affected the pattern of germination curves compared to seeds for which stratification was uninterrupted (cf. Supplementary Material).

Seeds from all populations analyzed, although differing in the germination energies achieved, began to germinate after the first week of incubation. Germination dynamics also increased relatively uniformly with time in all populations. This can be observed very clearly in the curves showing the course of germination between the third and fifth week of incubation (Figures S1–S3). After this period, there is a characteristic decrease in germination dynamics, but this does not mean that the seeds have stopped germinating. The prolonged germination time also indicates the presence of seed fractions in the population for which primary dormancy is much deeper than that of the others. This may be an intrinsic property of these seeds or, in these cases, may have been induced by the disruption of the stratification period as a kind of secondary dormancy. A study by Taylor et al. [41] showed that there are cold-induced changes in gene expression during stratification of Douglas fir seeds. Whatever the reason, the presence of such seeds significantly increases the chances of avoiding unfavorable weather conditions during the period of germination and growth of a young seedling, thus contributing to the reproductive success of Douglas fir at the individual and population levels.

Although Douglas fir is a non-native species in Europe, it has been present in European forests for at least 190 years. Moreover, it was already a component of the flora of Europe before the last ice age [42]. With climate change scenarios predicting higher mean annual temperatures and increasing summer drought, the Douglas fir can serve as an alternative tree species even in countries where the timber market for this tree has not yet been developed. However, some studies point to the potential loss of new areas to Douglas fir under an unfavorable (for this species) climate change scenario [43]. For Douglas fir to be introduced in new areas, sufficient seed material is required [44]. It is often the availability and quantity of seeds of certain non-native species that determine the success of a particular taxon in colonizing new areas [45,46]. However, Bradley St Clair and Howe suggested the possibility of genetic maladaptation of coastal Douglas fir seedlings to future climatic conditions [47]. These authors found, based on genealogical models obtained from common garden experiments, that with respect to most traits, including the season of bud development, growth of above-ground and below-ground portions of seedlings and shoot-to-root ratios, the risk of maladaptation of current populations is as high as 90%. Our studies suggest that, with respect to seed germination, repeated warm spells of a few days during winter may negatively affect the reproductive ability of Douglas fir, especially in the early stage. However, we have no evidence on whether this information applies equally to coastal and interior Douglas fir stands.

Considering the analyzed traits in relation to the populations averaged over all treatments, it was found that JAM and CHO stood out in relation to seed viability, while CZA and LEB tended to be in a separate homogeneous group and possessed weaker seed viability parameters. The exception is germination capacity, where all analyzed populations differed significantly. The JAM and CHO populations were the most geographically distant ones, while the LEB population is the northernmost one on the east-west transect analyzed. Liu and El-Kassaby, who investigated the relationship between seed germination timing and temperature-based environmental conditions during seed maturation of three conifer species in natural stands in British Columbia (Canada), concluded that there was a strong and positive correlation between these variables [48]. This pattern persisted in all species and seed lots analyzed within species and confirmed the importance of environmental conditions on seedling traits during seed development and maturation. In addition, a phenotypic plasticity strategy was observed to influence seed germination time. The results also indicated changes in the degree of seed dormancy of conifers in British Columbia north of 54°N, with seeds in this region having deeper dormancy than in other regions. Climate change will also shift the timing of seedling emergence and accelerate their appearance in spring. Our results may also indicate that there is a relationship between temperature at seed maturity and subsequent germination in the Douglas fir, but this relationship requires further studies.

5. Conclusions

Based on our study, it can be concluded that the effect of both current and predicted future winter warm spells on seed dormancy of the Douglas fir is most pronounced in the early stages of germination. Climate change is already altering the magnitude and/or frequency of extreme events, which in turn will have a greater impact on plant fitness than any change in average. Although the fingerprint of anthropogenic warming is well understood in recent phenological records, the impact of extreme events has been largely neglected. Our results suggest that the dormancy of Douglas fir seeds is not as deep as previously believed. On the other hand, the positive effect of warming during the stratification period may indicate that its occurrence is indeed a desirable phenomenon for Douglas fir seed germination. Thus, a positive (or at least unchanged) effect of projected climate change on the potential for native regeneration of the Douglas fir can be expected. However, this should not change the degree of invasiveness of this species in the future. Nevertheless, we need to monitor very closely the changes that are occurring in forest ecosystems. The changes in seed germination and early seedling growth could, in our opinion, be a very useful and sensitive tool for this purpose.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12060796/s1, Table S1: Results of a Wald test according to a type 3 analysis testing the effects of populations (P), days of winter warm spell (D), weeks of cold stratification (W) and their interactions on germination energy (GE), germination capacity (GC) and final germination capacity (FGC), Table S2: Results of a Wald test according to a type 3 analysis testing the effects of current and future winter warm spells during cold stratification on germination energy (GE), germination capacity (GC) and final germination capacity (FGC), Table S3: Germination energy (GE), germination capacity (GC) and final germination capacity (FGC) of Douglas fir seeds from four populations, Table S4: Number of consecutive days with warm spells between 1990–2020 at temperature 10, 13 and 15 °C and their duration in four analyzed sites, Figure S1: Differences between four populations of Douglas fir (CHO, JAM, LEB and CZA) in seeds germination after 2 (GE), 3 (GC) and 9 (FGC) weeks of incubation at 24 °C depend on time when cold stratification was interrupted for 1 day (15/10 °C), Figure S2: Differences between four populations of Douglas fir (CHO, JAM, LEB and CZA) in seeds' germination after 2 (GE), 3 (GC) and 9 (FGC) weeks of incubation at 24 °C depending on the time when cold stratification was interrupted for 3 days (15/10 °C), Figure S3: Differences between four populations of Douglas fir (CHO, JAM, LEB and CZA) in seeds' germination after 2 (GE), 3 (GC) and 9 (FGC) weeks of incubation at 24 °C depending on the time when cold stratification was interrupted for 5 days (25/15 °C).

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