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Success Rate of Individual Pollinizers for the Pear Cultivars "Ingeborg" and "Celina" in a Nordic Climate

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Abstract: Norwegian pear production is low due to climatic limitations, a lack of well-adapted cultivars and suitable pollinizers. However, nowadays it is increasing as a result of newly introduced and bred pear cultivars. Since cross pollination is necessary for high yields and good fruit quality, the aim of this investigation was to find the most suitable pollinizers for the pear cultivars "Ingeborg" ("Conference" × "Bonne Louise") and "Celina" ("Colorée de Juillet" × "Williams"). Self-pollination of "Ingeborg" and "Celina", together with "Conference", "Belle Lucrative", "Anna", "Clara Frijs", "Herzogin Elsa", "Kristina" and "Fritjof" as potential pollinizers, were studied in this experiment during the 2017 and 2018 seasons in Norway. The success rate of each pollinizer was tested under field conditions, while the monitoring of pollen tube growth was done using the fluorescence microscopy method. All reproductive parameters (pollen germination, number of pollen tubes in the upper part of the style, pollen tube number in the locule of the ovary, number of fertilized ovules, initial fruit set, and final fruit set) in all crossing combinations were higher in 2018 due to much warmer weather. Based on the flowering overlap and success rate of each individual pollinizer and fruit set, the cultivars "Anna" and "Clara Frijs" can be suggested as pollinizers for the cultivar "Ingeborg", while "Fritjof", "Anna", "Kristina" and "Herzogin Elsa" for the cultivar "Celina". An even distribution of two compatible pollinizers having overlapping flowering times with the main commercial pear cultivar is a general recommendation for commercial pear production.

Keywords: *Prunus communis* L.; cultivars; pollination; pollen tubes growth; fruit set

1. Introduction

Pear (*Pyrus communis* L.) cultivation has a long tradition in Norway. In 2018, the total acreage was only 62 ha with an average yield of 9 tons per ha [1]. Pear production has declined in recent decades, mainly due to climatic limitations, a lack of well-adapted cultivars and suitable pollinizers. Unfavorable environmental conditions for pear pollination during the Nordic spring can have a very negative effect on yield quantity in the pear orchards [2,3]. The "Ingeborg" pear ("Conference" × "Bonne Louise") was developed at Balsgård-SLU (Swedish University of Agricultural Sciences) and is currently the most important commercial pear variety grown in Norway in terms of yield tonnage. The cultivation of "Ingeborg" is mainly located in the Hardanger district, western Norway. Although "Ingeborg" possesses good pomological traits and is suited for cultivation under Nordic conditions, fruit set and subsequent yields of this cultivar tend to vary between orchards and can be significantly lower than for other pear varieties grown in Norway [3,4].

Nowadays, there is increasing interest in pear production due to introducing of new pear varieties resulting from the Norwegian breeding program started in 1984 [5]. The cultivar "Celina" QTee® ("Colorée de Juillet" × "Williams") is the most promising one and it was released in 2010. In Norwegian orchards, this cultivar flowers medium to late and produces attractive fruits with red blush and good fruit quality, storability and shelf life. In Norwegian climatic conditions it ripens in the beginning of September. The process of commercialization and planting has been started throughout European countries, especially Belgium, Switzerland and Spain and other temperate regions of the world, such as South Africa [6]. Significant acreage is planted in Norway too [7]. Insufficient cultivar adaptation to changes in environmental conditions, as well as the lack of suitable pollinizers affects the success of the cultivation of these pear cultivars in specific climatic conditions in Norway [8]. The cultivar "Celina" has been included in the assortment of some European countries and has shown better adaptability to local climatic conditions and gives large yields in combination with appropriate pollinizers [5].

Since pear is an allogamous species, for obtaining higher yields and profitable production, it is necessary to have adequate pollinizers. The pollen donors must have annual flowering and should produce viable pollen at the most fertile stage for the recipient in order to have effective pollination and fertilization of the flowers [9,10]. The effectiveness of pollination depends on several factors: pollen viability [11], stigmatic receptivity [12], ovule longevity, the effective pollination period [13], the considerable reduction in female fertility of some triploid selections [14] and the diversity, abundance and efficiency of pollinators in relation to orchard design [15]. In addition to these factors, in vitro pollen germination and the efficiency of the pollen tube growth in the style and ovary tubes/progamic phase of fertilization can indicate the best pollinizers in tested combinations of pollination [16]. In addition, the pollen germination and pollen tube growth in the pistil are highly genotype-dependent in pears [17,18].

Although some European pear (*Pyrus communis* L.) cultivars, like "Abugo" and "Ceremeño", were discovered to be self-compatible [19], most of them are completely self-incompatible or partially self-compatible [20]. In pear and other species of the *Rosaceae* family, there is a gametophyte system of incompatibility (GSI) that is controlled by a single polymorphic locus (S-locus) [21]. Pollen tube growth is inhibited in the style when the S-allele of the pollen grain matches one of the S-alleles of the style. In these crosses, pollen tube growth stops somewhere along the style length and practically no pollen tubes can be observed at the base [22,23]. Self-incompatibility is one of the most efficient mechanisms to promote out-crossing in plants but could be a problem for fruit production [24]. So far, 19 S-RNase alleles have been cloned and sequenced for European pear, which can be used to characterize more than 130 cultivars [25].

Climatic factors, such as temperature and humidity, greatly affect the factors listed above [26]. Both the male and female organs of the flower are especially sensitive to temperature fluctuations both during their development, before and during flowering and in the post-pollination stage [27]. This phenomenon is bounded with gradual changes in the distribution of cultivars, by favoring those better adapted in reproductive behavior to certain air temperatures [28]. Therefore, planting one or more cultivars in an orchard is needed in order to provide satisfactory yields. Although some cultivars have a certain level of parthenocarpic fruit set, cross pollination is always favored [29].

In this study, we assessed the efficiency of pollen tube growth (from the pistil up to the ovule) of different pollinizers and fruit set of the pear cultivars "Ingeborg" and "Celina" under specific ecological factors for the Nordic climate. The aim of this investigation was to find the most suitable pollinizers for these two cultivars to be planted within the same orchard in order to provide high and stable yields of good fruit quality.

2. Material and Methods

2.1. Plant Material

The pear cultivars "Ingeborg" and "Celina" were used as pollen recipients. As pollen donors/pollinizers, the cultivars "Conference", "Belle Lucrative", "Anna", "Clara Frijs", "Herzogin Elsa", "Kristina" and "Fritjof" were used, in addition to the self-pollination combination of "Ingeborg" and "Celina". The studies were conducted in an experimental pear orchard at Njøs Fruit and Berry Center Leikanger, Western Norway during 2017 and 2018.

The orchard was established in 2012, with all cultivars grafted on the rootstock "Quince Adams". The tree spacing was 4 × 1 m and trees were trained as slender spindle trees. Trees used for the experiment were selected for uniformity of flowering and represented the average bloom intensity and tree size of the orchard. Orchard floor management consisted of grass in the inter-rows and a 1-m wide vegetation-free strip in the intra-row space. The trees were irrigated by drip irrigation when water deficits occurred.

2.2. Climate Conditions Phenology

The typical climate for this part of western Norway is an average annual air temperature of 6.6 °C and an annual rainfall of 994 mm. Unfavorable environmental conditions, cold temperatures and rain during spring can have a negative effect on pear pollination in some years. Daily temperatures (mean, max and min) and precipitation (mm) during the flowering period in both experimental years are presented in Figure 1.

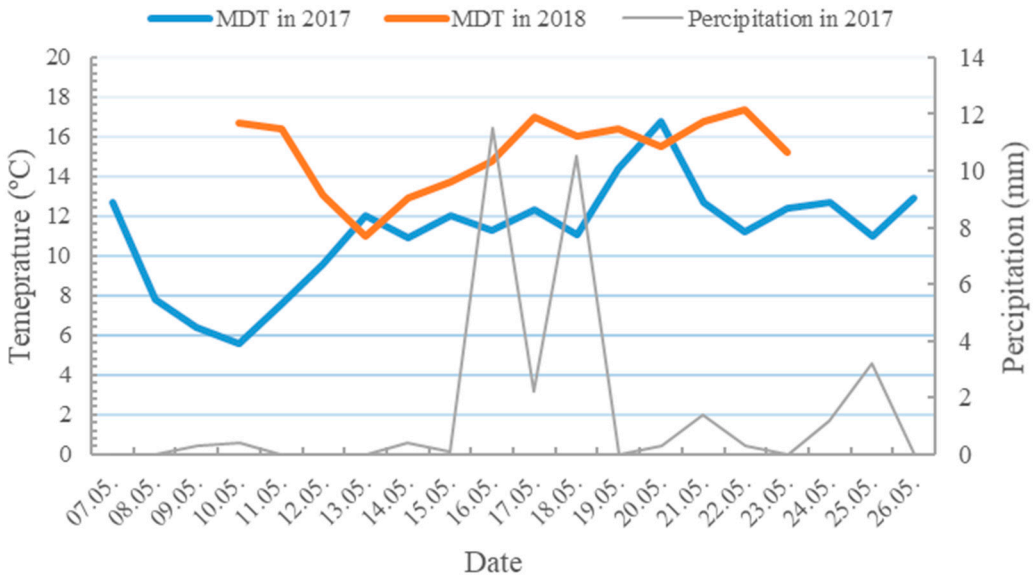

Figure 1. Two-year average of daily temperature and precipitation during the flowering of all studied pear cultivars. MDT—mean daily temperature.

2.3. Flowering and Fruit Set

The phenophase of flowering of each individual cultivar was recorded according to the "Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie" (BBCH) scale [30]. The beginning of flowering (BBCH stage 61) was reported when approximately 10% of flowers were open, full bloom (BBCH stage 65) was noted when 50% of flowers were open, while end of flowering was documented when the majority of petals were fallen (BBCH stage 67). The overlapping of the flowering periods for "Ingeborg" and "Celina" and the other pollinizers are presented in Figure 2.

In all crossing combinations, together with open pollination in both cultivars, the percentage initial fruit set was assessed approximately one month after full bloom (before the "June drop"), while final

fruit set was determined just prior to the harvest. Both initial and final fruit sets were calculated as follows: Initial fruit set percentage/final fruit set = (number of developing fruitlets/total number of flowers) × 100. Since "Ingeborg" is a triploid cultivar, the number of parthenocarpic fruits was not counted in the fruit set.

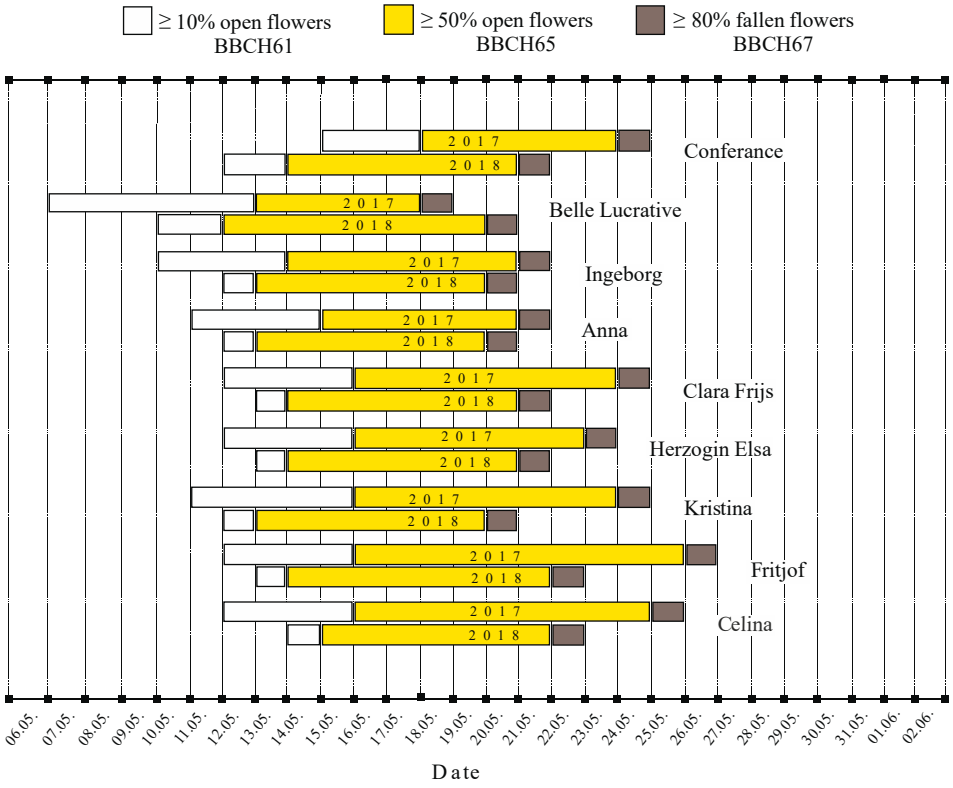

Figure 2. Flowering phenophases of all studied pear cultivars in 2017 and 2018.

2.4. Pollen Germination In Vitro

The anthers at the late balloon stage (BBCH code 60) of each cultivar were collected and left to dehisce for 24–48 h at room temperature (−22 °C). For the estimation of in vitro pollen germination in all tested pollinizers, pollen was placed in Petri dishes with artificial medium (1% agar and 14% sucrose), which were kept at 20 °C for 24 h. The number of germinated pollen grains was determined for the total of three microscopic observation fields under a Leica DM LS microscope. Pollen grains with pollen tubes exceeding their radius were considered as germinating.

2.5. Pollination Treatments In Vivo

At the balloon stage, branches with flowers of the cultivars "Ingeborg" and "Celina" were chosen and tagged. Open flowers were eliminated and the rest were emasculated. Approximately 250–300 flowers were prepared (emasculated and tagged) for each crossing combination. For hand pollination, "balloon" flowers were collected, and anthers were extracted in the Petri dishes that were kept open at an ambient temperature in order to dry and start shedding pollen grains. After pollen was released, dishes were kept closed and refrigerated at +4 °C. Pollination was performed 24 to 72 h after emasculation, in the early hours, when mother trees were in full bloom (flowers on surrounding branches were wide open and anthers started to change color) and when stigma secretion was evident. Before pollination, closed dishes were shaken in all directions to cause vibration throughout the whole area, to cause anther breakage and pollen grains release. The hand pollination of emasculated flowers was done first by dipping a finger into the Petri dish with pollen and then by touching the exposed stigma two times. Pollination was considered successfully done when yellowness was observed on

the stigma. For the purpose of this study, the following combinations were done: self-pollination in "Ingeborg" and "Celina" and cross-pollination of these two cultivars with the following cultivars: "Conference", "Anna", "Clara Frijs", "Herzogin Elsa" and "Kristina". The cultivar "Belle Lucrative" was also included as a pollinizer for "Ingeborg".

2.6. Pollen Tube Growth In Vivo

A total of 30 pistils (three repetitions × 10 pistils) of each crossing combination were collected and fixed 3, 6, 9, and 12 days after pollination (DPA) in FPA (70% ethanol, propionic acid and formaldehyde, 90:5:5 percentages by volume). Fixed materials were kept at +4 °C until staining with aniline blue according to the Preil [31] and Kho and Baër [32] method. To prepare pistils for microscopic examination, the styles were separated from the ovary. The styles were squashed, while the ovary was cut across with a razor blade to detect the penetration of pollen tubes in the parts of the ovary. Pollen tubes were determined in the upper third of styles and the ovary for each crossing combination after 3, 6 and 9 days after pollination (DAP). For the pistils fixed 12 DAP, ovules were removed from the ovary locule in order to observe the penetration of the pollen tube into the ovules [33,34]. This study was done under the fluorescence microscopes Leica DM LS (Leica Microsystems, Wetzlar, Germany) and Olympus BX61 (Tokyo, Japan).

2.7. Statistical Analysis

The data obtained for the pollen germination test, the number of pollen tubes in the upper part of the style and ovary and fruit set were statistically analyzed using Fisher's model of two-factor analysis of variance (ANOVA). The significances of the individual differences for the investigated factors (cultivar, year and interaction of cultivar × year) were determined using the Least Significant Difference (LSD) – test, with 0.05 = 95% confidence. Correlations among the parameters were determined by correlation analysis and Pearson's correlation coefficients. Statistical analyses were conducted using STATISTICA for Windows 6.0 (StatSoft Inc., Tulsa, Okla).

3. Results

3.1. Air Temperature, Rain Precipitation and Time of Flowering

The temperature registered during the month of May, when all the investigated pear cultivars flowered, was on average 4 °C higher in 2018 (15 °C compared to that of 2017 (11 °C)). Warm and dry weather during the flowering period is completely unusual for this location in Norway. The mean daily temperature during the flowering period (from the beginning of flower opening in the earliest cultivar until the petal fall of the latest cultivar studied herein) was 11.2 °C in 2017 (Figure 1), while the following year's mean daily temperature was 4.0 °C higher (15.2 °C). The average temperature during the 7-d post-bloom period in 2017 was 12.5 °C (with a maximum daily temperature of 21.7 °C), whereas in 2018, it was 16.3 °C (with a maximum daily temperature of 25.6 °C). The precipitation was much higher in May of 2017 (52.6 mm) compared to 2018 (15.0 mm), but most of it was concentrated in 3 to 4 days, thus not disrupting the pollination of pear flowers, which on average lasted more than ten days in 2017. During the period of flowering in 2017, the mean daily precipitation was 1.6 mm. Unlike in the previous year, in 2018, there was no rain during the period of flowering.

The cultivar "Ingeborg" had a 12-day flowering interval and the onset of full bloom (≥50% open flowers) was 14 May, two days earlier than in the cultivar "Celina" in 2017 (16 May) (Figure 2). Both cultivars, "Ingeborg" and "Celina", had long overlaps with all the pollinizers studied herein, except with "Conference". This pollinizer started flowering five days later than "Ingeborg" and three days later than "Celina". In this year, "Ingeborg" overlapped with "Conference" for only three days. In 2018, "Ingeborg" had an 8-day flowering interval with the onset of full bloom on 14 May, one day earlier than in the cultivar "Celina" (15 May). The pollinizers "Belle Lucrative" (8 days), "Anna" (7 days) and "Kristina" (7 days) mostly overlapped during the flowering period of "Ingeborg". In "Celina",

the situation was different, only the cultivar "Fritjof" had the same overlap during the flowering time (7 days). In this second year, the cultivar "Conference" overlapped with both mother cultivars.

3.2. Pollen Germination In Vitro

The testing of pollen germination in vitro is one of the main indicators of pollen functional viability. Statistical analyses of the data of pollen germination in vitro revealed the existence of significant differences between cultivars, experimental years and their interactions (Figure 3). In all pollinizers except "Belle Lucrative", the germination of pollen in vitro was higher in 2018 than in the previous year. On average, in all the cultivars studied, the highest percentage of pollen germination was recorded in "Fritjof" (77.7%) and the lowest in "Belle Lucrative" (7.3%). "Conference" showed the largest differences in pollen germination in vitro by year (2.6% in 2017, and 88% in 2018).

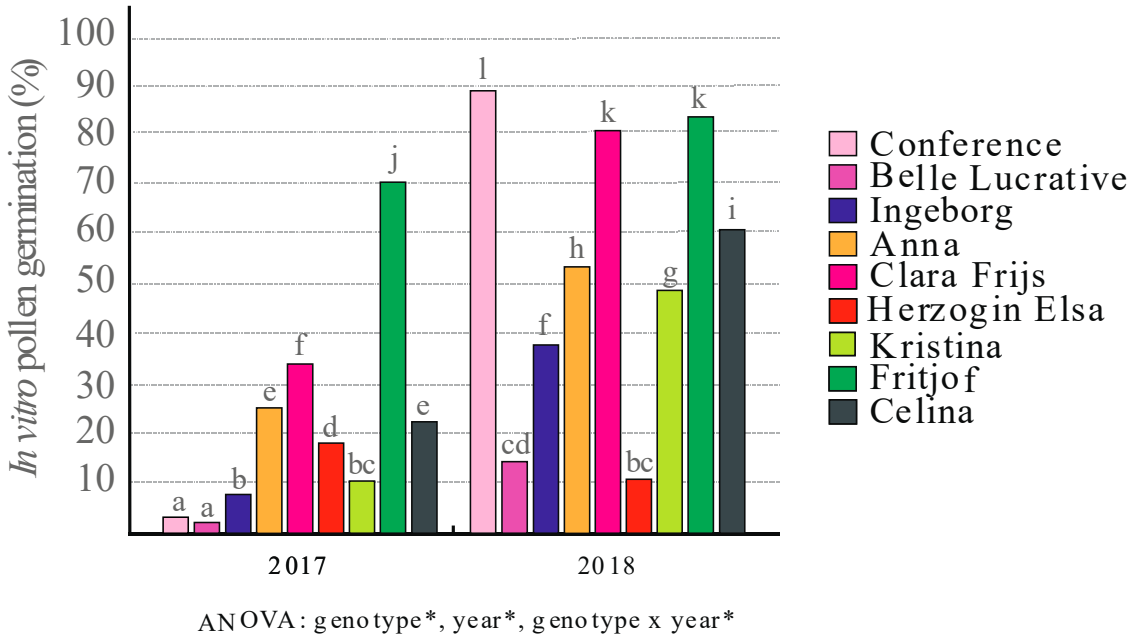

Figure 3. In vitro pollen germination (%) of nine pear cultivars in 2017 and 2018. (Different letters above the bars denote a significant difference between clones according to the LSD test, $p < 0.05$).

3.3. Pollen Tube Growth in the Pistil

Pollen tube growth in all combinations of pollination begins with the germination of pollen grains on the surface of the stigma (Figure 4a). After the penetration of the style cells, pollen tubes progressed through the "transmitting tissue", which extends along the whole length of the style (Figure 4b,c). The number of pollen tubes decreased gradually from the stigma surface to the base of the style (Figure 4d). In the ovary, pollen tubes continued their growth through the locule and penetrated the ovules (Figure 4e,f).

The upper part of the styles and ovary represent main regions for assessing the efficiency of pollen tube growth. The number of pollen tubes in these parts of the pistil differed between crossing combinations, years of study and their interaction (Figure 5).

The average number of pollen tubes in the upper part of the style and the ovary was higher in 2018 compared to 2017, except in "Ingeborg" × "Herzogin Elsa". These differences were mostly pronounced in some crossing combinations (up to five times higher). The highest average number of pollen tubes in the upper part of the style was recorded in "Celina" × "Fritjof" (9.4) and "Ingeborg" × "Herzogin Elsa" (6.0) in 2017. In the following year, the self-pollination of "Celina" (52.5) and "Ingeborg" × "Clara Frijs" (43.8) had the highest average number of pollen tubes in the upper style of the pistil. The average number of pollen tubes in the locule of the ovary showed a similar tendency as in the previous traits.

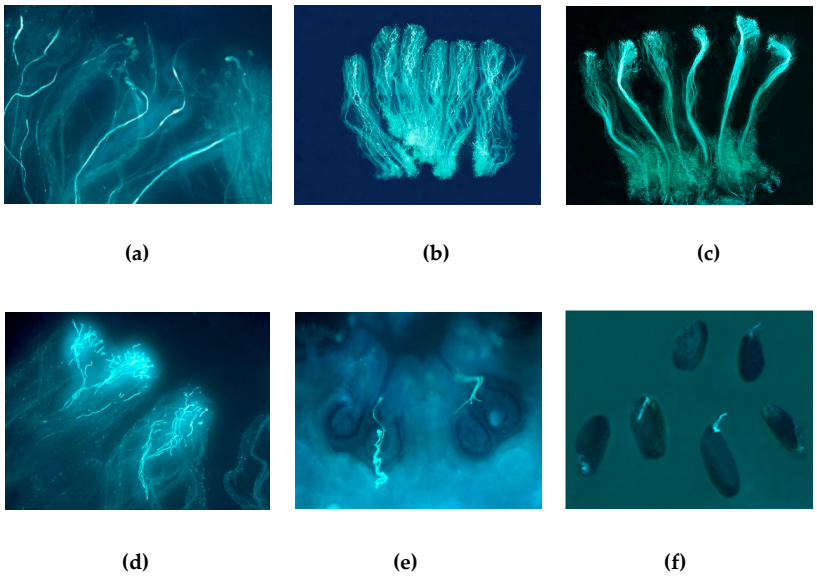

Figure 4. Pollen germination in the upper part of the style with the stigma—"Celina" × "Kristina", 3 DAP (**a**); Pollen tube growth through separate styles—"Celina" × "Fritjof", 6 DAP (**b**); Pollen tubes entering the basal part of the styles—"Ingeborg" × "Anna", 9 DAP (**c**); Incompatible pollen tubes in the upper part of the style—"Ingeborg" × "Conference"—6 DAP (**d**); Penetrated pollen tubes into the locules of the ovary—"Ingeborg" × "Clara Frijs", 9 DAP (**e**); Fertilized ovules—"Celina" × "Kristina", 12 DAP (**f**).

The higher number of pollen tubes in the upper part of the style and locules of the ovary occurred in all crossing combinations of "Celina" in relation to "Ingeborg". In "Celina", the highest average number of pollen tubes in the ovary was detected in the crossing combinations "Celina" × "Kristina" (2.3) in 2017 and "Celina" × "Conference" (6.9) in 2018. The highest average number of pollen tubes that was recorded in the ovary of "Ingeborg" was observed in the combinations with the pollinizers "Fritjof" (3.8) in 2017 and with "Anna" (4.2) in 2018.

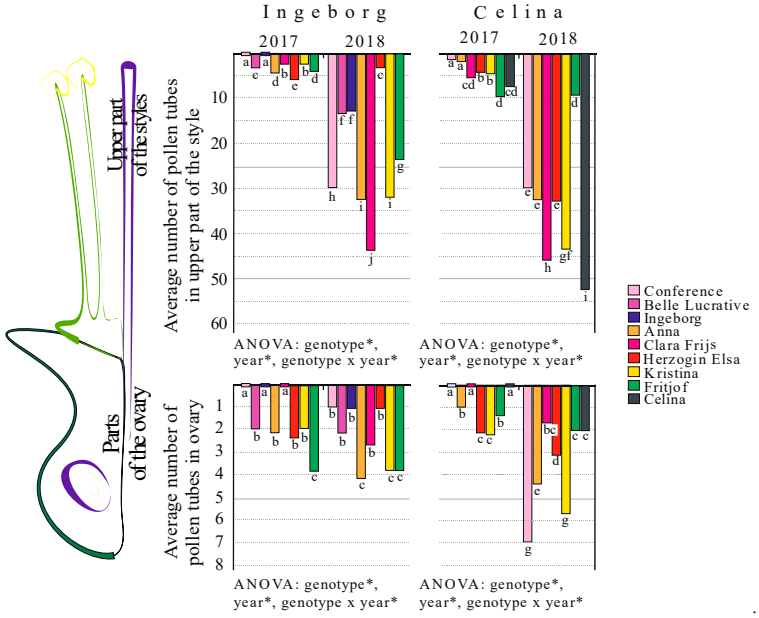

Figure 5. Average number of pollen tubes in the upper part of the style and ovary in the pear cultivars "Ingeborg" and "Celina" in different combinations of pollination in 2017 and 2018. (Different letters below the bars denote a significant difference between clones according to the LSD test, $p < 0.05$).

The dynamics of pollen tube growth in all crossing combinations with "Ingeborg" and "Celina" are shown in Figures 6 and 7. The highest percentages of pistils (100%) with pollen tubes that penetrated the locule of the ovary in "Ingeborg" were recorded in "Ingeborg" × "Clara Frijs" and "Ingeborg" × "Herzogin Elsa" 9 days after pollination (DAP) in 2017. The following year, the highest percentage of pistils (100%) with pollen tubes that penetrated the locule of the ovary were recorded in the combinations of pollination "Ingeborg" × "Clara Frijs", "Ingeborg" × "Kristina", "Ingeborg" × "Fritjof" and "Ingeborg" × "Anna" 6 DAP. The occurrence of incompatibility and pollen tube arrest in the upper third of the style was pronounced in the self-pollination of "Ingeborg" for the whole test period. This combination of pollination had by far the lowest average number of pollen tubes and pollen tube growth rate in the pistil.

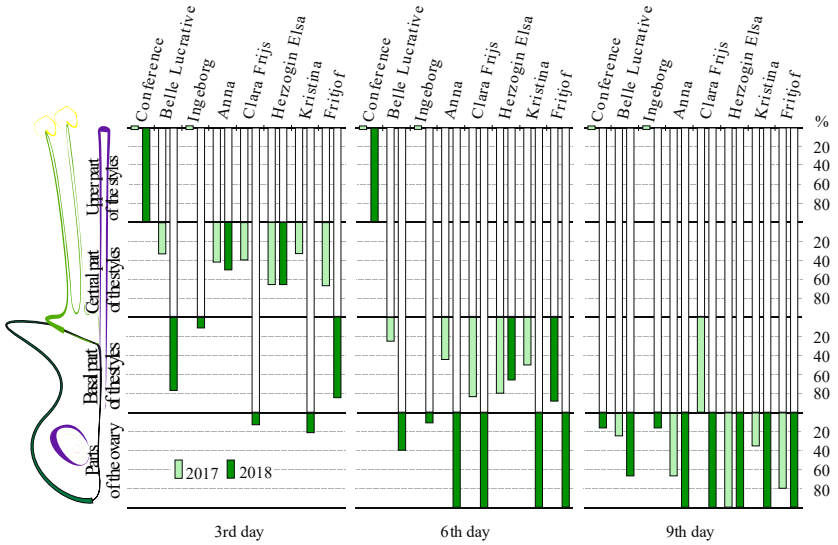

Figure 6. Dynamics of pollen tube growth through certain pistil parts (3, 6 and 9 DAP) of cultivar "Ingeborg" in different combinations of pollination in 2017 and 2018.

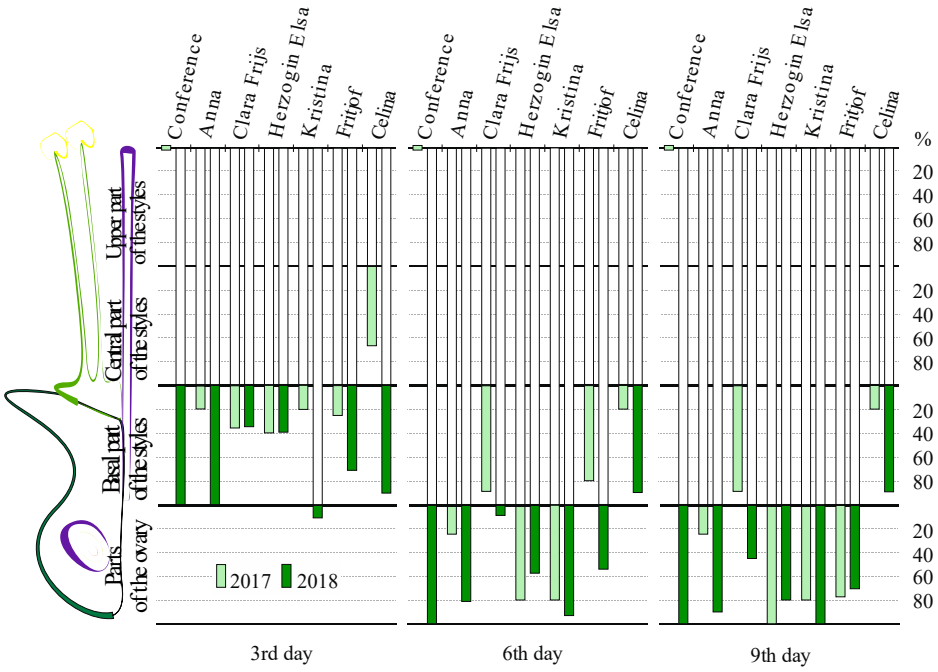

Figure 7. The dynamics of pollen tube growth through certain pistil parts (3, 6 and 9 DAP) of cultivar "Celina" in different combinations of pollination in 2017 and 2018.

In contrast to "Ingeborg", the highest percentage of pistils with pollen tubes that penetrated the locule in 2017 was observed in "Celina", especially in the crossing combinations "Celina" × "Herzogin Elsa" (100%) 9 DAP (Figure 7). In the following year, the best crossing combinations were "Celina" × "Conference" (6 DAP) and "Celina × "Kristina" (9 DAP). There was little evidence of incompatibility in the combinations of pollination. Generally, appearance of incompatibility was evident only in the self-pollination of "Celina" in 2017.

The percentage of pistils which contained different numbers of fertilized ovules can be used for estimating the success of progamic phase fertilization in "Ingeborg" and "Celina" in different combinations of pollination (Figure 8). Twelve DAP, the highest percentage of pistils with fertilized ovules was found in the crossing combinations "Ingeborg" × "Fritjof" (57.1%) and "Ingeborg" × "Anna" (50%) in 2017. The following year, the combinations of pollination "Ingeborg" × "Kristina" (100%) and "Ingeborg" × "Clara Frijs" (79.7%) had the highest percentages of pistils with fertilized ovules.

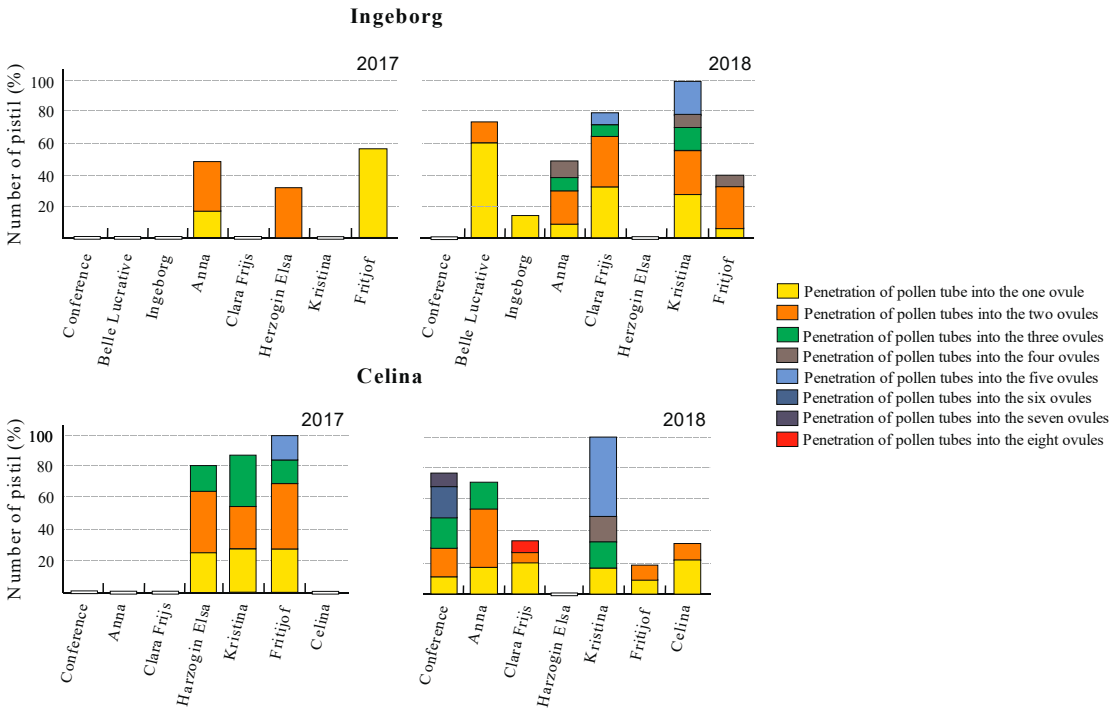

Figure 8. Number of pistils with different numbers of fertilized ovules (12th DAP) of the pear cultivars "Ingeborg" and "Celina" in different combinations of pollination in 2017 and 2018.

For "Celina", the highest percentage of pistils with fertilized ovules was recorded when "Fritjof" (100%) and "Kristina" (82.5%) were pollinizers in 2017. In contrast to the first year of study, the percentage of pistils with the highest number of fertilized ovules was recorded in "Celina" × "Kristina" (100%) and "Celina" × "Conference" (80%).

3.4. Fruit Set

In both cultivars and all combinations of pollination, both the initial and final fruit set were higher in 2018 compared to the previous year (Figure 9). Pollinizers, year of study and their interaction, had a significant influence on the fruit set. In 2017, a large difference between initial (34.0%) and final set (4.2%) in all combinations of pollination was found in "Ingeborg". Final fruit set in "Ingeborg" in all combinations of pollination in the second year was several fold higher than in the first year. This cultivar had the highest percentage of final fruit set in the combinations "Ingeborg" × "Kristina" (26.6%), "Ingeborg" × "Fritjof" and "Ingeborg" × "Belle Lucrative" (both 17.2%) in 2018.

In contrast to "Ingeborg", in 2017, the highest percentage of initial fruit set was in the crossing combination "Celina" × "Herzogin Elsa" (37.8%), while the highest final fruit set was obtained in "Celina" × "Herzogin Elsa" (31.9%), "Celina" × "Kristina" (23.6%) and "Celina" × "Fritjof" (21.8%). In 2018, the crossing combinations "Celina" × "Anna" (53.3%) and "Celina" × "Kristina" (47.5%) gave the highest initial fruit set, while "Celina" × "Anna" (47.2%), "Celina" × "Conference" (42%) and "Celina" × "Kristina" (38.8%) recorded the highest percentages of final fruit set. Final fruit set in "Celina" was higher in all crossing combinations in 2018 compared to 2017. This cultivar showed differences in final fruit set in self-pollination in 2018 in relation to 2017. The percentage of final fruit set in the self-pollination of "Celina" in the second year was 21.3% in comparison to 1.3% in the first year.

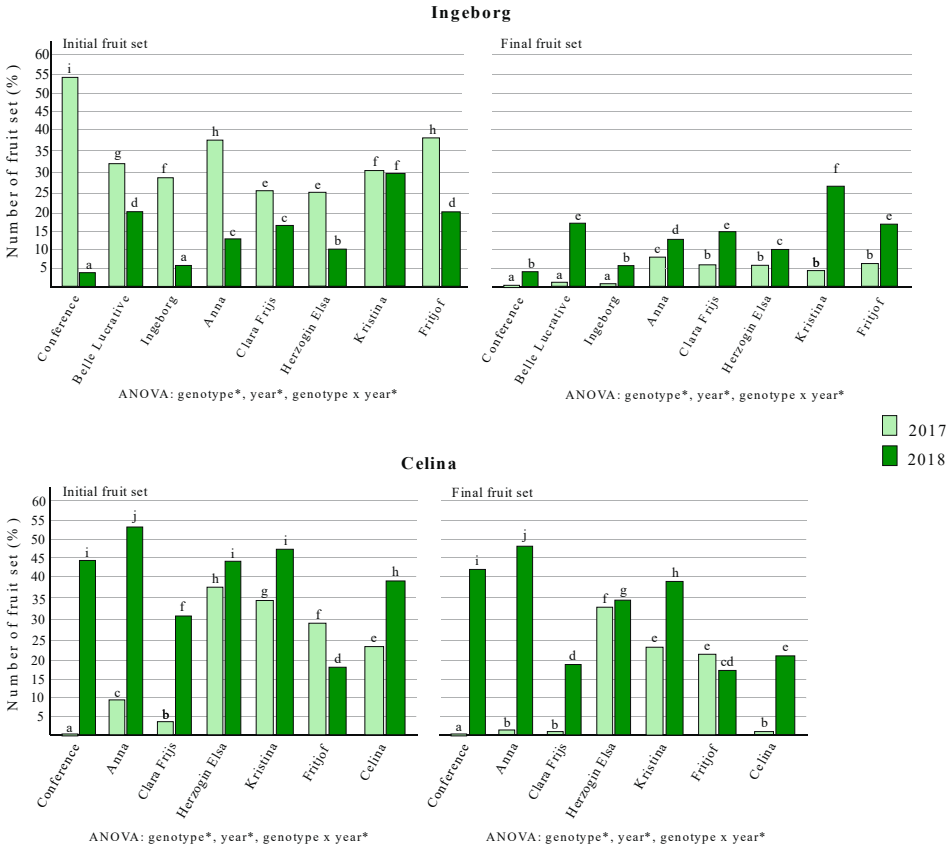

Figure 9. Fruit set of cultivars "Ingeborg" and "Celina" in different combinations of pollination in 2017 and 2018 (different letters above the bars denotes a significant difference between clones according to the LSD test, $p < 0.05$).

3.5. Correlation among Reproductive Parameters

A correlation matrix obtained for both cultivars and crossing combinations showed correlations by the tested reproductive parameters (Table 1). A correlation was found between the average number of pollen tubes in the locule of the ovary, the number of pistils with fertilized ovules and initial and final fruit set (r = 0.51 *, r = 0.58 * and r = 0.61 *, respectively). The number of pistils with fertilized ovules showed a positive correlation with final fruit set (r = 0.51 *). In addition, a negative correlation was determined between pollen germination and final fruit set (r = −0.39 *) and between the number of pollen tubes in the upper part of the style and final fruit set (r = −0.46 *). Finally, a positive correlation existed between initial and final fruit set (r = 0.57 *).

Table 1. Pearson's coefficient of linear correlation between the reproductive parameters.

Parameter	PG	STU	OVR	FOV	IFS	FFS
PG	/					
STU	−0.11 (p = 0.244)	/				
OVR	0.33 (p = 0.062)	0.11 (p = 0.244)	/			
FOV	0.20 (p = 0.121)	−0.07 (p = 0.301)	0.51 * (p = 0.009)	/		
IFS	−0.01 (p = 0.393)	−0.30 (p = 0.088)	0.58 * (p = 0.005)	0.33 (p = 0.062)	/	
FFS	−0. 39 * (p = 0.038)	−0.46 * (p = 0.021)	0.62 * (p = 0.001)	0.51 * (p = 0.009)	0.57 * (p = 0.005)	/

* The values are statistically significant at $p \leq 0.05$. PG: Pollen germination in vitro; STU: Pollen tube number in the upper part of the style; OVR: Pollen tube number in the locule of ovary; FOV: Number of fertilized ovules; IFS: Initial fruit set; FFS: Final fruit set.

4. Discussion

4.1. Pollen Germination In Vitro

Pollen germination in vitro varied from 2.6% ("Conference") to 71.3% ("Fritjof") in the first experimental year and from 11.5% ("Herzogin Elsa") to 88.0% ("Conference") in the second. Those data go are in line with the results obtained by Shafari [35], who studied pear cultivars from the East Azerbaijan Province of Iran and Bhat et al. [36] and Bieniasz et al. [37], who both studied Asian and European pear cultivars. The germination test in vitro showed that almost all pollinizers had higher pollen germination in the second year. In the first year, only three cultivars ("Clara Frijs", "Celina" and "Fritjof") had pollen germination > 30%, while most of the cultivars in the experiment showed much higher average pollen germination (with the exception of the cultivars "Belle Lucrative" and "Herzogin Elsa") in the second year.

Pollen functionality and efficiency of the progamic phase during fertilization are factors that influence the fertilization success and fruit set. Many factors influence pollen germination: time of collection, the season, conditions of pollen storage and others. The obtained results indicated that this trait is under the strong influence of ecological factors, which was previously reported for other fruit species [35,38]. In this study, it was particularly pronounced in the cultivar "Conference", which showed the highest difference in pollen germination in vitro between two years. This variability may be a result of the interaction between exogenous and endogenous factors during the development of the pollen grain and the activation/deactivation of certain enzyme systems present in the pollen grain. The existence of significant and perhaps critical functional differences affects the behavior of pollen growth both in vitro and in vivo [39].

4.2. Pollen Tube Growth in Pistils

The number of pollen tubes decreased gradually, from the stigma, down to the base of the style and into the ovary during the progamic phase of fertilization. This reduction can be associated with the anatomy of the style, ovary and the heterotrophic character of pollen tube growth [40,41]. The pollen–pistil interactions also play an important role in pollen competition and selection during compatible mating [42,43]. The efficiency of the progamic phase of fertilization is under the direct influence of pollen performance, which includes pollen germination, pollen tube growth rate, pollen competition, environmental conditions during flowering and the overlapping of the main cultivars and pollinizers in full bloom. One of the major factors that might significantly affect the progamic phase of fertilization is the temperature [44].

In this study, the average number of pollen tubes in the upper part of style and ovary and the percentage of pistils that contained pollen tubes which penetrated the ovules in "Ingeborg" and "Celina" varied in relation to pollinizers and the year of investigation. Pollen tube growth in the pistil was much faster in 2018 compared to 2017 due to higher temperatures during the flowering stage. The obtained results clearly indicated a specific response of each genotype in relation to temperature variations [26], which is already proved for other pear cultivars [17,45,46]. The temperature during the

reproductive phase could act as a selective pressure agent for genotypes that are better adapted to warm climates [47].

The occurrence of incompatible pollen tubes in the upper part of the style was recorded in the self-pollination of "Ingeborg" and "Celina", and in the crossing combination "Ingeborg" × "Conference" in both experimental years. The combination "Ingeborg" × "Conference" showed typical signs of incompatibility. In compatible combinations, pollen tubes grew fast, parallel with the transmitting cells from the stigma to the style towards the ovary. However, in incompatible combinations, pollen tubes were much slower, made loops, had swollen tips and even stopped growing. The majority of incompatibility signs were observed in the middle part of the transmitting tissue, which was previously proved to be typical for *Rosaceae*, and was described in some pear cultivars by Shaheen et al. [23] and Silva et al. [22]. The cultivar "Ingeborg" is triploid and possesses S-alleles S102, S108 and S121 [2]. Since one of the "Ingeborg" parents is the cultivar "Conference", which has the S108 and S121 alleles [48], the crossing combination "Ingeborg" × "Conference" is incompatible, which was also confirmed by our study.

The transition from self-incompatibility to self-compatibility can happen due to either physiological or genetic changes. The breakdown of the incompatibility barrier may be caused by differences in S-RNase expression, its abundance in the style, the silencing or deletion (partial or complete) of the S-locus or the modification of other major genes [49,50]. This indicates that other significant processes and interactions between the male gametophyte and female sporophyte can occur during pollen tube growth in vivo [47]. In addition, tree or flower age, flower quality and the application of plant hormones, together with environmental conditions, can influence self-incompatibility and reduce it [49]. Seasonal temperatures above the optimum and/or extremes, which can coincide with critical stages of plant development (especially fertilization), can increase selfing in allogamous species [47]. These kinds of changes are always temporary and cannot be transmitted from one generation to the next. In 2017, both cultivars "Ingeborg" and "Celina" had very low fruit set after self-pollination (0.8% and 1.3%, respectively), and both cultivars could be described as self-incompatible. In 2018, the situation was completely different, with numerous pollen tubes growing from the stigma, through the pistil and reaching the ovules. This was much more pronounced in "Celina", which gave 21.3% fruit set, and could be defined as self-compatible. Thus, we believe that extremely high temperatures during full bloom in 2018 resulted in self-incompatibility breakdown in both cultivars, especially "Celina". This is in line with Sanzol and Herrero [34] and Moriya et al. [51], who showed wholly contradictory reports for the same cultivars.

4.3. Fruit Set

The weather conditions, activity of pollinators, compatibility and flowering overlap of the pollinizers represent the main limitation factors in fruit set. The efficiency of the progamic phase of fertilization is an essential prerequisite, among others. This requires successful pollen transfer to the stigma, the growth of pollen tubes through the pistil and the fertilization of the ovules [13]. Almost all parameters of fruit set (percentage of pistils with fertilized ovules, initial and final fruit set) were lower in 2017 for both "Ingeborg" (33.1%, 34.0% and 4.2%, respectively) and "Celina" (57.4%, 20.0% and 11.7%, respectively) compared to 2018 (44.7%, 14.7% and 13.7%, respectively, and 48.0%, 39.6% and 31.5%, respectively). As shown, final fruit set was threefold higher in 2018 compared to 2017 in both cultivars. These results can be explained by the fact that the average daily temperature during full bloom in 2018 was 16.3 °C, which is 4.4 °C higher than the previous year (Figure 1). Hedhly et al. [26] proved a long time ago that temperature can either speed up or slow down the whole or just part of the reproductive process.

It is still unknown what temperatures are optimal for high fertilization, bearing in mind that ovule viability is an important parameter, together with the progamic phase of fertilization [52,53]. The functionality and viability of the embryo sac are major factors, which have a direct influence on the effective pollination period in apples and pears [54]. If pollination is delayed, fertilization depends on the ovules remaining receptive until the pollen tubes reach them [55].

The average initial and final fruit set for "Ingeborg" for both years was 24.3% and 8.9%, respectively, and for "Celina" was 29.8% and 21.6%, respectively, showing that "Celina" had a 2.4-fold higher final fruit set than "Ingeborg". This result was expected, since the cultivar "Ingeborg" is triploid, and those genotypes have a very low fruit set, sometimes even showing no measurable female fertility at all [14]. All crossing combinations where "Ingeborg" was a mother plant (except for "Ingeborg" × "Conference") gave fruits with a commercially accepted size (140–190 g) which were comparable with "Ingeborg" open pollination, but with just two to three seeds/fruit. A similar situation was noticed in "Celina", but the average seed number/fruit ranged from seven ("Celina" × "Herzogin Elsa") up to 14 ("Celina" × "Anna") (data not shown).

Generally, all presented data in this study are in accordance with the studies done by Falk Kühn and Bertelsen [56], who examined the cultivar "Clara Frijs", by Sheiki et al. [57], who worked with pear cultivars native to Iran and by Quinet et al. [58], who studied the cultivar "Conference", but they are higher than the results of Shalan [59] who studied the commercial pear cultivar "Leconte" in Iran and Tatari et al. [60].

4.4. Overlapping in Flowering Time

Based on final fruit set, the cultivar "Ingeborg" showed the longest overlapping period (≥50% open flowers) with the pollinizer "Anna" (6 days), while the shortest with "Conference" (3 days) during the period of full bloom in 2017. In the next year, the overlapping period was the longest with "Anna" (7 days) and "Kristina" (7 days). Based on the evidence that "Anna" overlapped and gave the top fruit set in both years, that cultivar can be recommended as a pollinizer for "Ingeborg", as was previously suggested by Gasi et al. [2]. Although "Fritjof" had the best parameters in terms of pollen germination in vitro and efficiency of the progamic phase of fertilization in combination with "Ingeborg", it cannot be recommended as a pollinizer for "Ingeborg" due to a short flower overlapping period.

In both years of study, all crossing combinations with "Celina" had a higher percentage of final fruit set than "Ingeborg". In 2017, "Celina" shared the longest overlapping period with the pollinizers "Fritjof"—9 days, "Kristina"—8 days, "Clara Frijs"—8 days and "Herzogin Elsa"—7 days. The cultivar "Conference" overlapped with "Celina" to some extent, but started to flower three days later. In 2018, the longest coincidental flowering of "Celina" was shared with "Fritjof" (7 days), followed by a 6-day overlapping with "Conference", "Clara Frijs" and "Herzogin Elsa". According to Sønsteby et al. [61], the late blooming pear cultivars "Celina", "Fritjof" and "Kristina" had a perfect match of blooming periods in the climate conditions of southeast Norway, but they claimed that "Fritjof" and "Anna" were the most suitable pollinizers. Although "Fritjof" overlapped the most, in the condition of high spring temperatures (2018), it showed lower parameters of fertilization and had lower fruit set than "Kristina", "Anna" and "Herzogin Elsa". On the other hand, the controlled crossing combination "Celina" × "Conference" showed high values for fertilization efficiency and also fruit set in the second year. However, insufficient overlapping in flowering with "Celina" in some years (like in 2017) indicates that "Conference" might not be a good pollinizer for this newly bred pear cultivar. On the other hand, "Kristina" was stable in terms of progamic phase efficiency in both experimental years, which differed a lot regarding climatic conditions during flowering time (Figure 1).

4.5. Correlation among Reproductive Parameters

In our study, a significant correlation was found between final fruit set and all other reproductive parameters. In seeded plants, like pear, successful fruit set and fruit development depend on both pollination and subsequent fertilization. Since the pollen grain is a key factor in those processes, it must have good functional ability, which requires adequate vitality and satisfactory germination [62]. A correlation between pollen germination in vitro and final fruit set (Table 1) was previously determined in apple by Jahed and Hirst [63] and in sweet cherry by Radičević et al. [64]. According to Zhang et al. [65], low pollen germination influenced the low fruit set, higher nonviable seed ratios and increased numbers of misshapen fruit in pears.

Negative relationships were established between final fruit set and both pollen germination and pollen tube number in the upper part of the style (Table 1). On the contrary, positive correlations were found between fruit set on one side and pollen tube number in the locule of the ovary and the number of fertilized ovules on another. This is due to the fact that the stigma can be a host for hundreds of pollen grains, which germinate and grow toward the ovule. Because of reduced space in the transmitting tissue, an incompatible reaction, non-matching crossing combinations between distant plant species or unfavorable ecological conditions, the number of pollen tubes gradually decreases and the number of pollen tubes that reach the base of the style is much lower. That is why the high number of pollen tubes in the upper part of the style is not a guarantee for fruit set, while the number of pollen tubes in the ovary and the number of fertilized ovules are prerequisites for high yields.

5. Conclusions

For achieving good fruit set and high yields, it is necessary to conduct successful pollination and fertilization, where many processes must take place at the right time. This study evaluated the main reproductive performances of seven promising pollinizers for the pear cultivars "Ingeborg" and "Celina" over two years (2017/2018). The results of this study of pollen germination in vitro and the efficiency of pollen tube growth in the pistil, as well as fruit set, showed that these processes are temperature-dependent. The values of these parameters were higher in the second year of study with a higher average temperature during flowering. Taking into account the overlap in flowering time, the efficacy of the progamic phase of fertilization and fruit set, the cultivars "Anna" and "Clara Frijs" can serve as the best pollinizers for the cultivar "Ingeborg", and the pollinizers "Fritjof", "Anna", "Kristina" and "Herzogin Else" for the cultivar "Celina". These findings are extremely important, because stable and corresponding flowering between the main cultivar and two to three pollinizers within the orchard can secure high and stable yields.

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References

1. FAOStat. 2019. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 17 March 2020).
2. Gasi, F.; Pojskić, N.; Kurtović, M.; Kaiser, C.; Hjeltnes, S.H.; Fotirić Akšić, M.; Meland, M. Pollinizer efficacy of several 'Ingeborg' pear pollinizers in Hardanger, Norway, examined using microsatellite markers. *HortScience* **2017**, *52*, 1722–1727. [CrossRef]
3. Meland, M.; Frøynes, O. Evaluation of new Norwegian pear cultivars in a Nordic climate. In Proceedings of the ISHS 12th Pear Symposium, Leuven, Belgium, 14–18 July 2014; p. 56.
4. Meland, M.; Frøynes, O.; Akšić, M.F.; Maas, F.M. Performance of 'Celina', 'Ingeborg' and 'Kristina' pear cultivars on Quince rootstocks growing in a Nordic Climate. *Acta Hortic.* **2020**, in press.
5. Hjeltnes, S.H.; Vercammen, J.; Gomand, A.; Måge, F.; Røen, D. High potential new Norwegian bred pear cultivars. *Acta Hortic.* **2015**, *1094*, 111–116. [CrossRef]
6. Vilegen-Verschure, A. Celina, the new pear variety. *Eur. Fruitg. Mag.* **2013**, *55*, 14–17.
7. Meland, M.; Frøynes, O.; Kalamujić-Stroil, B.; Lasic, L.; Gasi, F. Pollinizer efficacy of several 'Celina' pollinizers in Norway, examined using microsatellite markers. *Acta Hortic.* **2020**, in press.
8. Meland, M.; Kurtovic, M.; Kalamujic, B.; Pojskic, N.; Lasic, L.; Gasi, F. Microsatellites as a tool for identifying successful pollinators of the pear cultivar 'Ingeborg' in Ullensvang, Norway. *Acta Hortic.* **2018**. [CrossRef]
9. Granger, A.R. Gene flow in cherry orchards. *Theor. Appl. Genet.* **2004**, *108*, 497–500. [CrossRef]

10. Jacquemart, A.L.; Michotte-Van der, A.A.; Raspe, O. Compatibility and pollinator efficiency test on the *Pyrus communis L*-Cv "Conference". *J. Hortic. Sci. Biotechnol.* **2006**, *81*, 827–830. [CrossRef]
11. Ketchie, D.O.; Fairchie, E.D.; Drake, F.R. Viability of different pear pollen and the effect on fruit set of 'Anjou' pear (*Pyrus communis* L.). *Fruit Var. J.* **1996**, *50*, 118–124.
12. Sanzol, J.; Rallo, P.; Herrero, M. Asynchronous development of stigmatic receptivity in the pear (*Pyrus communis*, Rosaceae) flower. *Am. J. Bot.* **2003**, *90*, 78–84. [CrossRef]
13. Sanzol, J.; Herrero, M. The effective pollination period in fruit trees. *Sci. Hortic.* **2001**, *90*, 1–17. [CrossRef]
14. Phillips, W.D.; Ranney, T.G.; Touchell, D.H.; Eaker, T.A. Fertility and reproductive pathways of triploid flowering pears (*Pyrus* sp.). *HortScience* **2016**, *51*, 968–971. [CrossRef]
15. Quinet, M.; Jacquemart, A.L. Cultivar placement affects pollination efficiency and fruit production in European pear (Pyrus communis) orchards. *Eur. J. Agron.* **2017**, *91*, 84–92. [CrossRef]
16. Cerović, R.; Mićić, N. Oprašivanje i oplodnja jabučastih i koštičavih voćaka. *Jugosl. Voćarstvo* **1996**, *30*, 73–98.
17. Vasilikis, M.D.; Porzingis, I.C. Effect of temperature on pollen germination, pollen tube growth, effective pollination period, and fruit set of pear. *HortScience* **1985**, *20*, 733–735.
18. Petropoulou, S.P.; Alston, F.H. Selecting for improved pollination at low temperature in apple. *J. Hortic. Sci. Biotechnol.* **1998**, *73*, 507–512. [CrossRef]
19. Sanzol, J. Pistil-function breakdown in a new S-allele of European pear, S21°, confers self-compatibility. *Plant Cell Rep.* **2009**, *28*, 457–467. [CrossRef]
20. Claessen, H.; Keulemans, W.; Van de Poel, B.; De Storme, N. Finding a Compatible Partner: Self-Incompatibility in European Pear (*Pyrus communis*); Molecular Control, Genetic Determination, and Impact on Fertilization and Fruit Set. *Front. Plant Sci.* **2019**, *10*, 407. [CrossRef]
21. Sassa, H.; Nishio, T.; Kowyama, Y.; Hirano, H.; Koba, T.; Ikehashi, H. Self-incompatibility (S) alleles of the Rosaceae encode members of a distinct class of the T2/S ribonuclease superfamily. *Mol. Gen. Genet.* **1996**, *250*, 547–557.
22. Silva, L.; Sanzol, J.; Herrero, M.; Olivera, C.M. Study of pollen-pistil interactions on crosses between 'Rocha' pear an potential pollinators. *Acta Hortic.* **2008**, *800*. [CrossRef]
23. Shaheen, M.A.; Essa, M.A.; Sayed, R.A.; Abd El-Aziz, Y.S.G. Sexual Compatibility of Le Conte pear cultivar. *J. Hortic. Sci. Orn. Plant* **2011**, *3*, 99–105.
24. Herrera, S.; Rodrigo, J.; Hormaza, J.I.; Lora, J. Identification of Self-Incompatibility Alleles by Specific PCR Analysis and S-RNase Sequencing in Apricot. *Int. J. Mol. Sci.* **2018**, *19*, 3612. [CrossRef] [PubMed]
25. Goldway, M.; Takasaki-Yasuda, T.; Sanzol, J.; Mota, M.; Zisovich, A.H.; Stern, R.A.; Sansavini, S. Renumbering the S-Rnase alleles of European pears (*Pyrus communis* L.) and cloning the S109 RNase allele. *Sci. Hortic.* **2009**, *119*, 417–422. [CrossRef]
26. Hedhly, A.; Hormaza, J.I.; Herrero, M. Global warming and sexual plant reproduction. *Trends Plant Sci.* **2009**, *14*, 30–36. [CrossRef] [PubMed]
27. Hedhly, A. Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environ. Exp. Bot.* **2011**, *74*, 9–16. [CrossRef]
28. Radičević, S.; Cerović, R.; Đorđević, M. Ovule senescence and unusual pollen tube growth in the ovary of sweet cherry as affected by pistilar genotype and temperature. *Span. J. Agric. Res.* **2018**, *16*, 1–12. [CrossRef]
29. Fotirić Akšić, M.; Cerović, R.; Slavković, D.; Hjeltnes, S.H.; Meland, M. Selection of the best pollinizer of 'Celina' pear. *Acta Hortic.* **2018**, *1229*, 365–370. [CrossRef]
30. Meier, U. Growth stages of mono-and dicotyledonous plants. In *Federal Biological Research Centre for Agriculture and Forestry*, 2nd ed.; BBCH Monograph: Berlin & Brunswick, Germany, 2001.
31. Preil, W. Observing of pollen tube in pistil and ovarian tissue by means of fluorescence microscopy. *Zeiss Inf.* **1970**, *75*, 24–25.
32. Kho, Y.O.; Baër, J. Fluorescence microscopy in botanical research. *Zeiss Inf.* **1971**, *76*, 54–57.
33. Sanzol, J.; Herrero, M. Identification of self-incompatibility alleles in pear cultivars (*Pyrus communis* L.). *Euphytica* **2002**, *128*, 325–331. [CrossRef]
34. Sanzol, J.; Herrero, M. Self-incompatibility and self-fruitfulness in pear cv. Agua de Aranjuez. *J. Am. Soc. Hortic. Sci.* **2007**, *132*, 166–171. [CrossRef]
35. Sharafi, Y. Investigation on pollen viability and longevity in *Malus pumila* L.; *Pyrus commonis* L.; and *Cydonia oblonga* L.; In Vitro. *J. Med. Plants Res.* **2011**, *5*, 2232–2236. Available online: https://academicjournals.org/journal/JMPR/article-full-text-pdf/E65178D21212 (accessed on 28 May 2020).

36. Bhat, Z.A.; Dhillon, W.S.; Shafi, R.H.S.; Rather, J.A.; Mir, A.H.; Shafi, W.; Rashid, R.; Bhat, J.A.; Rather, T.R.; Wani, T.A. Influence of Storage Temperature on Viability and *In Vitro* Germination Capacity of Pear (*Pyrus* spp.) Pollen. *J. Agric. Sci.* **2012**, *4*, 128. [CrossRef]
37. Bieniasz, M.; Necas, T.; Dziedzic, E.; Ondrasek, I.; Pawłowska, B. Evaluation of Pollen Quality and Self-Fertility in Selected Cultivars of Asian and European Pears. *Not. Bot. Hortic. Agrobo. Cluj-Napoca* **2017**, *45*, 375–382. [CrossRef]
38. Pacini, E.; Dolferus, R. Pollen Developmental Arrest: Maintaining Pollen Fertility in a World with a Changing Climate. *Front. Plant Sci.* **2019**, *10*, 679. [CrossRef]
39. Zheng, R.H.; Su, S.H.; Xiao, H.; Tian, H.Q. Calcium: A Critical Factor in Pollen Germination and Tube Elongation. *Int. J. Mol. Sci.* **2019**, *20*, 420. [CrossRef]
40. Hormaza, J.I.; Herrero, M. Dynamics of pollen tube growth under different competition regimes. *Sex. Plant Reprod.* **1996**, *9*, 153–160. [CrossRef]
41. Herrero, M. Ovary signals for directional pollen tube growth. *Sex. Plant Reprod.* **2001**, *14*, 3–7. [CrossRef]
42. Hormaza, J.I.; Herrero, M. Pollen selection. *Theor. Appl. Genet.* **1992**, *83*, 663–672. [CrossRef]
43. Kumar, A.; McClure, B. Pollen-pistil interactions and the endomembrane system. *J. Exp. Bot.* **2010**, *61*, 2001–2013. [CrossRef]
44. Heslop-Harrison, J. Pollen germination and pollen tube growth. *Int. Rev. Cytol.* **1987**, *107*, 1–78.
45. Marcucci, M.C.; Visser, T. Pollen tube growth in apple and pear styles in relation to self-incompatibility, incongruity and pollen load. *Adv. Hortic. Sci.* **1987**, *1*, 90–94.
46. Rohitha, B.H.; Klinac, D.J. Some observations on the influence oftemperature on the germination of pollen on excised nashi (*Pyrus serotina* Rehder var. culta Rehder) flowers New Zealand. *J. Crop. Hortic. Sci.* **1994**, *22*, 339–342. [CrossRef]
47. Hedhly, A.; Hormaza, J.I.; Hererro, M. Influence of genotype-temperature interaction on pollen performance. *J. Evol. Biol.* **2005**, *18*, 1494–1502. [CrossRef]
48. Sanzol, J. Genomic characterization of self-incompatibility ribonucleases in European pear cultivars and development of PCR detection for 20 alleles. *Tree Gen. Genomes* **2009**, *5*, 393–405. [CrossRef]
49. De Nettancourt, D. *Incompatibility and Incongruity in Wild and Cultivated Plants*; Springer: Berlin, Germany, 2001.
50. Hiratsuka, S.; Zung, S.L. Relationships between fruit set, pollen-tube growth, and S-RNase concentration in the self-incompatible Japanese pear. *Sci. Hortic.* **2002**, *95*, 309–318. [CrossRef]
51. Moriya, Y.; Okada, K.; Yamamoto, K.; Iwanami, H.; Bessho, H.; Takasaki-Yasuda, T. Characterisation of partial self-compatibility in the European pear cultivar, "Grand Champion". *J. Hortic. Sci. Biotechnol.* **2009**, *84*, 77–82. [CrossRef]
52. Stösser, R.; Anvari, S.F. On the senescence of ovules in cherries. *Sci. Hortic.* **1982**, *16*, 29–38.
53. Cerović, R.; Ruzic, Đ.; Mićić, N. Viability of plum ovules at different temperatures. *Ann. Appl. Biol.* **2000**, *137*, 53–59. [CrossRef]
54. Wiliams, R.R. Factors affecting pollination in fruit trees. In *Physiology of Tree Crops*; Luckwill, L.C., Cutting, C.V., Eds.; Academic Press: London, UK; New York, NY, USA, 1970; pp. 193–207.
55. Pratt, C. Apple Flower and Fruit Morphology and Anatomy. *Hortic. Rev.* **1988**, *10*, 273–280. [CrossRef]
56. Falk Kühn, B.; Bertelsen, M. Pollination Experiment with the Pear Cultivar 'Clara Frijs'. *Acta Hortic.* **2004**, *636*, 375–379. [CrossRef]
57. Sheiki, H.; Arzani, A.; Kousheshsaba, M. Determination of self and cross-(in) compatibility of some Asian Pear (*Pyrus serotina* Rehd.) and European Pear (*Pyrus communis* L.) cultivars native to Iran. *Seed Plant Imp. J.* **2016**, *32*, 383–400.
58. Quinet, M.; Buyens, C.; Dobrev, P.I.; Motyka, V.; Jacquemart, A.L. Hormonal Regulation of Early Fruit Development in European Pear (*Pyrus communis* cv. 'Conference'). *Horticulturae* **2019**, *5*, 9. [CrossRef]
59. Shalan, A.M.N. Impact of boric acid spraying date with different concentrations on yield and fruit quality of *Pyrus communis* cv. 'Leconte' pear trees. *J. Plant Prod.* **2013**, *4*, 1479–1491. [CrossRef]
60. Tatari, M.; Ghasemi, A.; Mousavi, A.; Bahrami, H. Study on pollination and selection of the most suitable pollinizers for commercial Pear cultivars (*Pyrus communis* L.) in Iran. *J. Hortic. Res.* **2017**, *25*, 49–57. [CrossRef]
61. Sønsteby, A.; Heide, O.M.; Rivero, R.; Måge, F.; Remberg, S.F. Phenology, flowering and fruit-set performance of six recent pear cultivars of Nordic origin. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2019**, *69*, 578–587. [CrossRef]

62. Fotirić Akšić, M.; Rakonjac, V.; Nikolić, D.; Zec, G. Reproductive biology traits affecting productivity of sour cherry. *Pesqui. Agropecuária Bras.* **2013**, *48*, 33–41. [CrossRef]
63. Jahed, K.R.; Hirst, P.M. Pollen Tube Growth and Fruit Set in Apple. *HortScience* **2017**, *52*, 1054–1059. [CrossRef]
64. Radičević, S.; Cerović, R.; Nikolić, D.; Đorđević, M. The effect of genotype and temperature on pollen tube growth and fertilization in sweet cherry (*Prunus avium* L.). *Euphytica* **2016**, *209*, 121–136. [CrossRef]
65. Zhang, C.; Tateishi, N.; Tanabe, K. Pollen density on the stigma affects endogenous gibberellin metabolism, seed and fruit set, and fruit quality in Pyrus pyrifolia. *J. Exp. Bot.* **2010**, *61*, 4291–4302. [CrossRef]