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The effects of pollinizers on pollen tube growth and fruit set of European plum (*Prunus domestica* L.) in a Nordic climate

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Abstract:

Norwegian plum production is characterized by climatic limitations, different flowering time, deficiently of wholly-adapted cultivars and appropriate pollen donors for cultivars that can be grown in this region. This study evaluated the progamic phase of fertilization and fruit set in four European plum cultivars (´Mallard´, ´Edda´, ´Jubileum´, and ´Reeves´) after crossing with different pollinizers over two years (2018/2019). Reproductive parameters, *in vitro* pollen germination, number of pollen tubes in the upper part of the style and locule of the ovary, number of pistils with ovule penetrated by pollen tube, fruit set in all crossing combinations, and fruit set in open pollination of pollen recipient cultivars showed different adaptability of both recipient and donor cultivars to the specific ecological conditions prevailing in Western Norway. The pollinizers ´Victoria´, ´Opal´ and ´Č. Lepotica´ proved to be a very good pollinizers for cultivar ´Jubileum´, while pollinizers ´R. C. Souffriau´ and ´Valor´ for the cultivar ´Reeves´. Cultivars ´Opal´, ´R. E. Prolific´ and ´Mallard´ are excellent pollinizers for ´Edda´ in conditions of higher temperatures during flowering period and post-flowering period. Cultivar ´Č. Lepotica´

proved to be the best pollinizer for ´Edda´ in conditions when the temperatures were lower. Cultivars ´Opal´ and ´R. E. Prolific´ can be considered as good pollinizers for ´Mallard´.

Key words: Cultivars, Flowering, Pollination, Pollen germination, Climate

1. Introduction

The European plum (*Prunus domestica* L.) is a very adaptable fruit species that can be grown in different climatic conditions, from Mediterranean region and north to the Siberian zone. *P. domestica* production has a very long tradition in Norway, which starts in the medieval period of Western Europe (Sekse, 2007). In the structure of Norwegian fruit production, plum ranks second, with harvesting areas of 424.3 ha and 1524.9 tons (FAOStat, 2020). Main plum fruit production areas are located along the western coast in the fjord areas, with Hardanger district as leading production area in Norway. Plum production growing areas are situated between 58° N and 62° N and is under the influence of the Gulf Stream bringing heat from the tropics and heats atmospheric boundary layer (Hjeltnes, 1994). Thus, plum growing at these latitudes is restricted by climatic factors, and short and relatively cool growing season is limiting the choice of plum cultivars that can be grown (Redalen, 2002). Two main self-fertile plum cultivars ´Opal´ and ´Victoria´ are grown over the last decades (Sekse, 2007). Lately, many other cultivars such as ´Edda´, ´Mallard´, ´Reeves´, and ´Jubileum´ are being introduced in commercial plum orchards (Vangdal et al., 2007a). The consumers expect a large and tasty fruits, which are keeping a good quality (external and internal) for fresh consumption after storage (Vangdal et al., 2007b). Breeding and introducing new plum cultivars need to anticipate the achievement of high and stable yield of good fruit quality. Previous experience has demonstrated that cultivars ´Edda´, ´Mallard´ and ´Reeves´ showed self-sterility (Hjeltnes and Røen, 2019). Unlike the cultivars listed above, ´Jubileum´ showed partial self-fertility (Hartman and Neumuller, 2009).

There are many internal factors of reproductive biology (genetics and development) of male and female parts of flowers which can affect crop yield. The male sterility is induced (indirect male sterility) and is due to the mutation, causing defects in final phases of pollen development in some plum cultivars (Schwalm et al., 1995; Botu et al., 2002). The European plum exhibits gametophytic self-incompatibility (GSI), a reproductive barrier that is defined as the ability of the pistil to discard genetically related pollen thus preventing inbreeding, while favoring allogamy. The mechanism of GSI is genetically controlled by two *S*-locus genes, one controlling the style (*S*-RNase), and the other governing the pollen (*S*-specific F-box protein) (Sutherland et al., 2008; 2009). To achieve high stable yields, partially self-fertile and self-sterile cultivars require compatible pollinizers which overlap with the main cultivar during flowering period.

Besides genetically controlled mechanisms, the yield stability of plum cultivars depend on many factors which are necessary for the successful reproductive process, such as: presence of pollinators (Jacobs et al., 2009), flower attraction (Guffa et al., 2017; Fotirić Akšić et al., 2019) pollen viability (Koskela et al., 2010; Hjeltnes and Røen, 2019), stigmatic receptivity (Bayer and Stösser, 2002); pollen tube growth (Stösser, 2002; Nikolić and Milatović, 2010; Glišić et al., 2017; Đorđević et al., 2019a); embryo sac development (Ruiz et al., 2010; Jia et al., 2008; Đorđević et al., 2019b), ovule longevity (Stösser and Anvari 1990; Cerović et al., 2000); effective pollination period (EPP) (Williams 1970; Sanzol and Herrero, 2001) and fruit set (Botu et al., 2002; Nikolić et al., 2012; Đorđević et al., 2016). In addition to these factors, the application of different chemical thinning agents can optimize fruit set, yield, fruit size, weight and quality and return bloom in some plum cultivars (Meland et al., 2014; Meland and Kaiser, 2016).

Also, climate factors, such as temperature and humidity, have a major impact on both pre- and post-fertilization processes (Hedhly et al., 2009). The climate change is projected to increase stress in crops, especially in regions already vulnerable to climate variability and drought. Appearance of extremely high or extremely low temperatures differently affects male and female reproductive structures during flowering.

The plum growing has a short growing season in western Norway (Sekse, 2007). No matter that soil fertility, pruning, pest control, irrigation and all other cultural practices are fulfilled, in some season's yields can be unsatisfactory. One of the reasons can be a fact that plum flowering periods in this region is characterized by rapid changes of temperatures, frequent rainfalls, low sunlight and winds. In addition, temperatures during blooming can be too low for the bees (and other pollinators such as *Bombus* sp. and *Osmia* sp.) to be active as pollinators (Sekse, 2007). For this reason, creating a plum cultivar suitable for Norwegian growing conditions is one of the most important long-term breeding goals in this country (Hjeltnes, 1994). Besides, the solution should be sought in gradual changes in the distribution of old and new cultivars, by favoring those better adapted plum cultivars in reproductive behavior to these climatic changes.

In this study, we assessed the number of pollen tubes in the upper part of style and locule of ovary and dynamics of pollen tube growth (from the style down to the ovule) and fruit set in four plum cultivars ´Mallard´, ´Edda´, ´Jubileum´, and ´Reeves´ pollinated with different pollinizers, under specific ecological factors of a western Nordic climate. The aim of this study was to evaluate the progamic phase during fertilization and assess suitable pollinizers for these four cultivars. By completing this knowledge, it will be possible to recommend which cultivar should be planted within the same plum orchard for providing high and stable yields of good fruit quality under specific climate conditions in western Norway.

2. Material and Methods

2.1. Plant material

The plum cultivars ´Mallard´ (old English plum seedling), ´Edda´ (´Czar´ × ´Pêche´), ´Jubileum´ (´Giant´ × ´Yakima´), and ´Reeves´ (´Prune Peche´ x open pollinated, Canadian plum seedling) were used as mother plants (pollen recipients). Cultivars ´Rivers Early Prolific´ (´R. E. Prolific´), ´Mallard´, ´Edda´, ´Victoria´, ´Opal´, ´Jubileum´, ´Reine Claude Souffriau´ (´R. C. Souffriau´), ´Čačanska Lepotica´ (´Č. Lepotica´), and ´Valor´ were used as pollen donors. During 2018 and 2019, the study was done in Leikanger (61° 10' 43.2" N, 6° 51' 34.3" E) at the Njøs Fruit and Berry Center, western Norway.

The planting of the orchards was in 2012, where St. Julien A was used as a rootstock. The tree row orientation was East-West; trees trained as slender spindle trees and limited 2.5 m height. Grass strips were in the inter-rows, while one-meter-width wowed plastic was in intra-rows. The irrigation system was established, so whenever water deficits occurred, drip irrigation was used.

2.2. Air temperature and precipitation

The fjord areas of western Norway have a marinate climate, with comparatively cool summers and mild winters. Western winds that are usually coming from the Atlantic Ocean are bringing clouds, rain, and wind throughout the year. Thus, the average climatic elements for the last few years in this part of the Norway, have recorded average annual air temperature of 6.6 °C, with precipitation of 994 mm. Unlike this long-term average, annual temperature in both years of study (2018 and 2019) was same, 8.1 °C. In 2018, the temperature varied from the lowest (-11.9 °C) on March 1 to the highest (31.6 °C) on July 28. The following year lowest temperature was -6.6 °C (on February 6) and the highest 32.5 °C (on July 28). There were more rainy days in 2019 (133) than in 2018 (115).

Adverse environmental effects, especially low temperatures and high depositions can often occur during period March-May. During the study the total amount of annual precipitation was similar in both years with 1024 mm in 2018 and 1033 mm in 2019. During March, April and May, which preceded processes of swelling buds, flowering and leafing, and in which flowering, fertilization and fruit set took place, the amount of precipitation was quite different. During this period in 2018, the total amount of rainfall was 82.5 mm, while in 2019 in was 171 mm. The average temperature for same period was 7.1 °C in 2018 and 7.3 °C in 2019.

For the purpose of this study thermal conditions (including minimal, maximal and average air temperatures) together with the daily amount of rainfalls (mm) were recorded during the blossoming time of plum cultivars.

2.3. Flowering, pollen germination in vitro, pollination procedure and fruit set

For the determination of the flowering phenophases in the tested plum cultivars, a BBCH scale was used (Meier, 2001). Pollen gathering and study of *in vitro* germination was done according to Cerović et al. (2020a). For the purpose of this study uniform, healthy and well-formed trees were used. On each tree, long fruiting branches with numerous flower buds were marked. For every crossing combination in each plum cultivar, 250-300 flowers were labeled and emasculated. Pollination procedure was performed as previously described in Cerović et al. (2020a). For the purpose of this study the following crossing combinations were done for two seasons:

1. ´Mallard´ × ´R. E. Prolific´,

8. ʿJubileum´ × ´Victoria´,

2. ´Mallard´ × ´Edda´,
3. ´Mallard´ × ´Opal´;

4. ´Edda´ × ´Mallard´,
5. ´Edda´ × ´R. E. Prolific´,
6. ´Edda´ × ´Opal´,
7. ´Edda´ × ´Č. Lepotica´;

9. ´Jubileum´ × ´Opal´,
10. ´Jubileum´ × ´Jubileum´,
11. ´Jubileum´ × ´Č. Lepotica´;

12. ´Reeves´ × ´Victoria´,
13. ´Reeves´ × ´Opal´,
14. ´Reeves´ × ´R. C. Souffriau´,
15. ´Reeves´ × ´Valor´.

In order to determine which crossing combination of ´Mallard´, ´Edda´, ´Jubileum´, and ´Reeves´ gave the best results, initial and final fruit set was determined. Both fruit sets were calculated based on the number of flowers and number of fruits counted a month after full bloom (initial fruit set) and prior harvest (final fruit set).

2.4. Pollen tube growth in vivo

Gathered pistils (three repetitions × 10 pistils) from every combination of crossing were picked third, sixth, ninth and twelfth days after pollination (DAP) and placed in the mixed 90:5:5 FPA solution made of 70% ethanol propionic acid and formaldehyde, respectively. According to the Preil (1970) and Kho and Baër (1971) method, immersed pistils into FPA solution were stored at + 4 °C before processing and staining with aniline blue. In order to make native preparations, each pistil was halved by removing the ovary from the style. Ovary was also cut with a razor blade along suture, in order to observe the penetration of the pollen tubes into the micropyle and nucellus. In each combination of pollination pollen, and in each fixation day (third, sixth, ninth and twelfth DAP) tubes were counted and its dynamic of growth from stigma toward nucellus was followed. The dynamic of pollen tube growth through the upper, medium and basal part of the style, locule, micropyle and nucellus was given as the percentage of pistils where pollen tubes can be observed. A method of fluorescent microscopy [microscope Leica DM LS made by Leica Microsystems, (Wetzlar, Germany) with filters A (wavelength 340–380 nm) and I3 (wavelength 450–490 nm)] was used to study pollen tube growth *in vivo*.

2.5. Statistical analysis

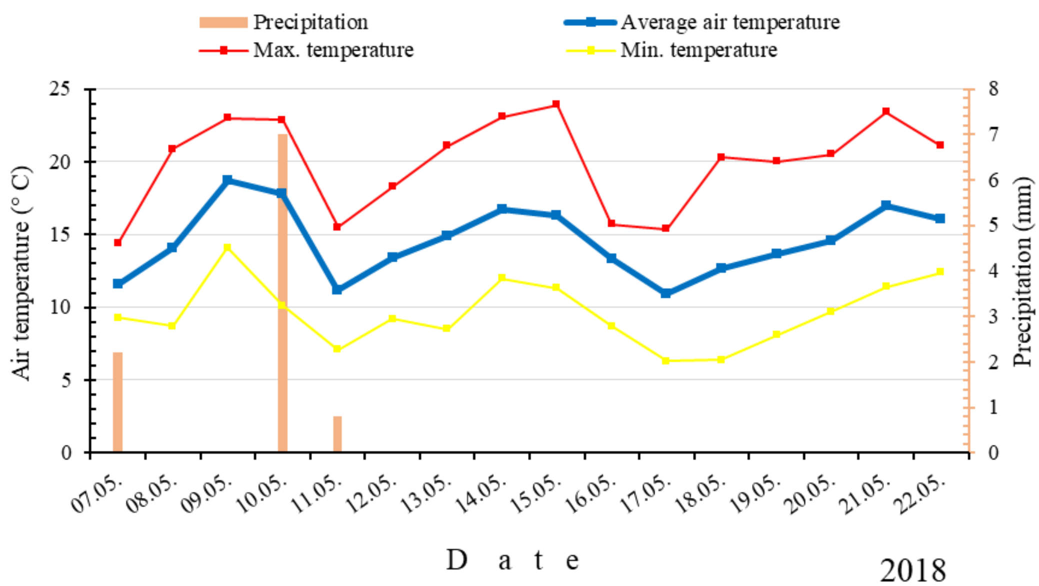
Pollen germination values were transformed to arcsin square root values before statistical analysis. All obtained data were statistically processed using two-way Fisher's ANOVA, prior to the least significant difference (LSD) mean separation. Differences at the level of $p < 0.05$ were considered to be significant. Statistically significant differences are shown in the figures. Pearson correlation coefficient ($p \leq 0.05$) was used to assess the relationship between *in vitro* pollen germination, pollen tube number in the first third of the style, number of pollen tubes in the ovary's locule and final fruit set. This treatment was carried out using Software package STATISTICA for Windows 6.0 (StatSoft Inc., Tulsa, Okla, USA).

3. Results

3.1. Climate - air temperature and precipitation

The weather these two years was completely different. The average temperatures during the growing season (April–September) were 13.6 °C in 2018 in contrast to 12.9 °C in 2019. However, in April and May 2018 the average temperatures were 6.3 °C and 14.8 °C versus 9.1 ° C and 9.9 °C in 2019, respectively. This relatively warm beginning of spring in 2019 promoted very early flowering taking place end of April which is almost two weeks earlier than regular plum flowering in this Nordic environment. Average temperature in May 2018 was 4.9 °C higher than the year after giving good conditions for fruit set and early fruitlets developments.

The mean average daily air temperature during flowering period (from the beginning of flower opening of the earliest cultivar until the petal fall of the latest cultivar) was 14.6 °C (Fig.1) in 2018. In the same year, the mean average of maximum temperature was 20.3 °C, while the minimal mean average was 9.9 °C. In the flowering period of 2019, mean average daily temperature was 13.3 °C, mean average maximum temperature was 17.9 °C, while mean average minimal was 9.7 °C. The mean average daily temperature during the 10-day after flowering period in 2018 was 19.2 °C (with absolute maximum of 29.4 °C) while in the following year it was only 5.9 °C (absolute maximum of 11.5 °C, and minimum of 0.3 °C).



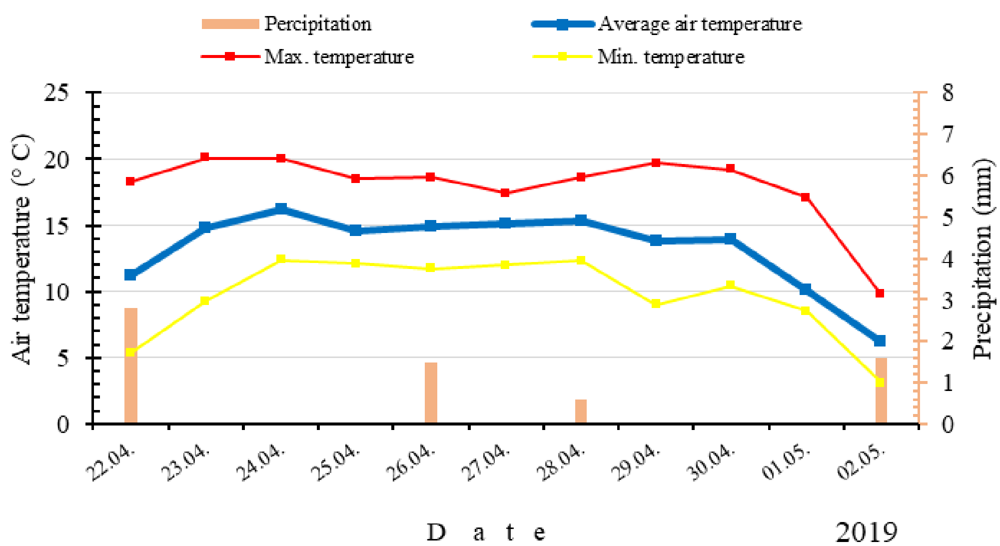


Fig. 1. Daily air temperatures (average, max., and min.) and precipitation during the flowering of plum cultivars in 2018 and 2019.

In the flowering period mean daily precipitation was 0.7 mm in 2018 and 0.6 mm in the following. The light showers occurred twice in 2018 and four times in 2019.

3.2. Flowering Time

In 2018, all plum cultivars (pollen recipients and pollinizers) flowered in May (Fig. 2). Cultivars ´Mallard´, ´R. E. Prolific´, ´Edda´, ´Victoria´, ´Opal´, and ´Jubileum´ were in the group of “early” cultivars while ´Reeves´, ´R.C. Souffriau´, ´Č. Lepotica´ and ´Valor´ were “late”. Cultivars ´Mallard´ and ´R. E. Prolific´ started flowering the earliest (7 May). The cultivars ´Reeves´, ´R. C. Souffriau´ and ´Č. Lepotica´ started flowering on May 11, while only cultivar ´Valor´ flowered on May 12. A period from full flowering (BBCH stage 65) till petal fall (BBCH stage 67) for “early” cultivars lasted 6 days (9–15 May), while for “late” it was longer, from 7 days (´Reeves´, 13-20 May) and to 9 days (´R. C. Souffriau´, ´Č. Lepotica´, and ´Valor´, 13–22 May). During this year, a period from 10% open flowers of the earliest cultivar until the 80% of petal fall in the latest cultivar lasted 16 days.

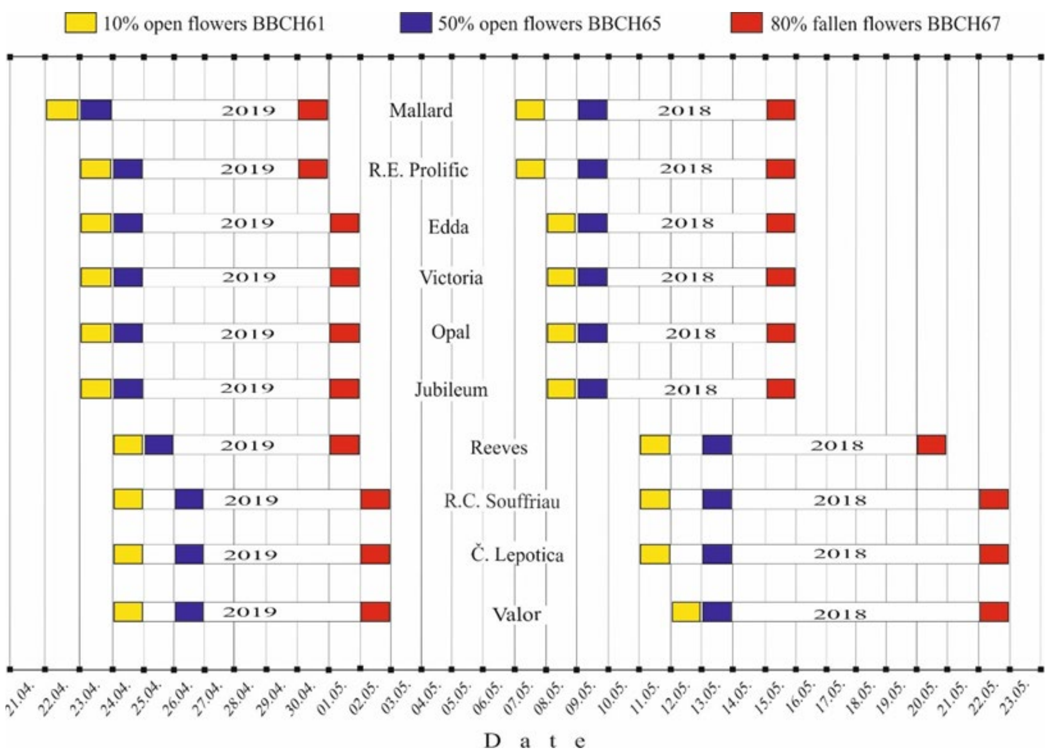


Fig. 2. Flowering phenophases of plum cultivars in 2018 and 2019.

Unlike in the previous year in 2019, all examined plum cultivars flowered in April, 15 to 18 days earlier than in 2018. The earliest beginning of flowering was recorded in ´Mallard´ (22

April), one day before beginning of flowering of ´R. E. Prolific´, ´Edda´, ´Victoria´, ´Opal´ and ´Jubileum´ and two days before the beginning of flowering of ´Reeves´, ´R. C. Souffriau´, ´Č. Lepotica´, and ´Valor´ (24 April). In this year, for all cultivars, a period from full flowering (BBCH stage 65) till petal fall (BBCH stage 67) lasted only 6–7 days. However, the total interval of flowering, from the beginning of flowering of the earliest plum cultivar until the end of flowering of the latest, was 11 days. Although time of flowering was weather conditions depended, the sequence of flowering of the different cultivars was same in both experimental years.

3.3. Pollen germination in vitro

Testing pollen germination *in vitro* is needed to assess pollen viability of the pollinizers. Statistical analysis of pollen germination *in vitro* indicated the existence of significant differences between cultivars, years and their interactions (Fig. 3). In all tested pollinizers, average pollen germination *in vitro* was in average higher in 2018 (69.6%) in comparison to 2019 (48.2%). The highest average percentage of pollen germination *in vitro* was recorded in ´R. C. Souffriau´ (93.1%) in 2018 and the lowest in ´R. E. Prolific´ (13.8%) in 2019. Besides ´R. C. Souffriau´, pollinizers ´R. E. Prolific´, ´Mallard´ and ´Victoria´ had average percentage of pollen germination *in vitro* over 90% in 2018, while such a high germination rate in 2019 had only cultivar ´Valor´ (90.8%). However, the cultivars ´Victoria´, ´Opal´, ´Jubileum´ and ´R. C. Souffriau´ had relatively high germination percentage in 2019 as well.

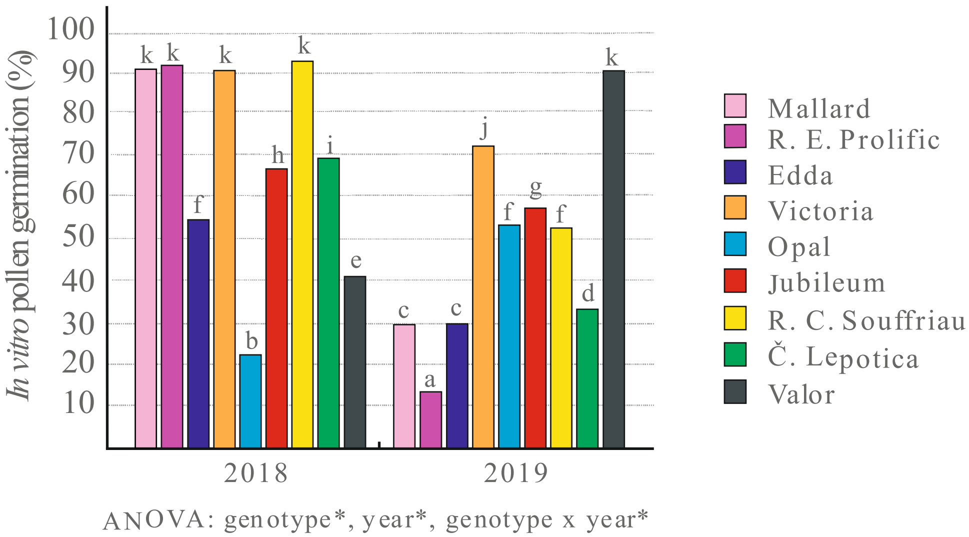


Fig. 3. *In vitro* pollen germination (%) of nine plum cultivars in 2018 and 2019. (Different letters above the bars denote a significant difference between cultivars according to the LSD test, $p < 0.05$).

3.4. Pollen tube growth in the pistil

The pollen tube growth in the pistil commenced with germination of pollen grains on the funnel-like surface of the stigma, i.e., specialized receptive part of a style necessary for capturing pollen grains (Fig. 4 A). The pollen tubes penetrated in the "bound" form intercellular through the "conducting tissue" of the solid type of the style, progressing further through tissue, over obturator to the locule of the ovary. During the growth process through the different part of style (upper, medium and basal) the number of pollen tubes is greatly reduced (Fig. 4 B). Their further progress toward micropyle or nucellus was clearly observed (Fig. 4 C, D, E and F). Sometimes, pollen tubes in the locule of the ovary showed occurrence of uncommon pollen tube growth (Fig. 4 G).

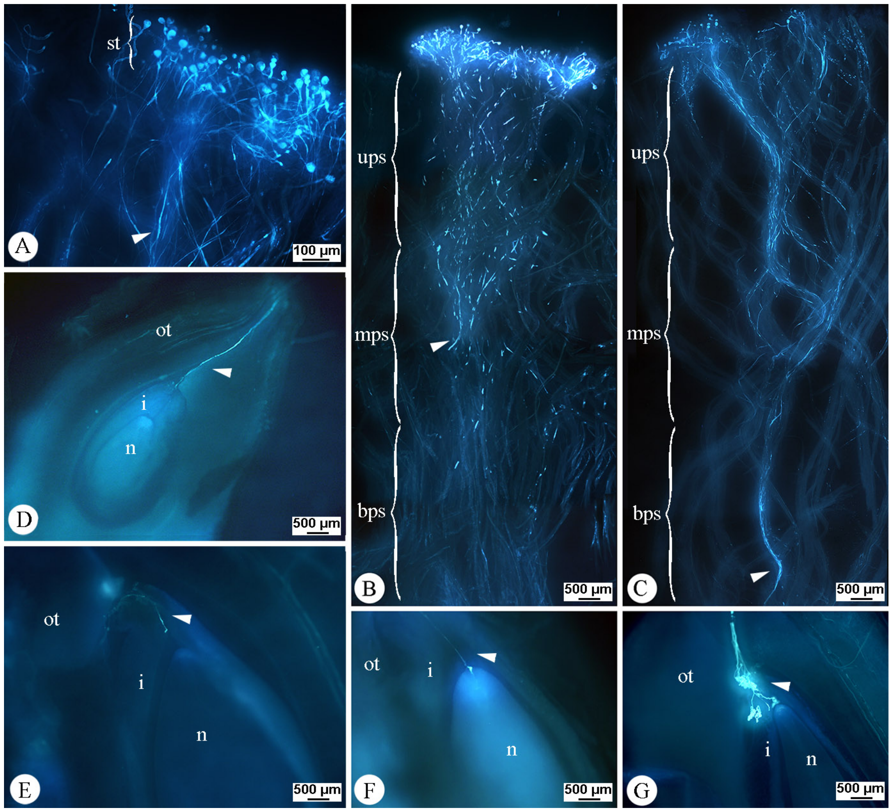


Fig. 4. Growth of the pollen tubes in the pistils − ′Jubileum′ × ′Victoria′, 3 days after pollination (DAP) (**A**); Penetration of the pollen tubes into the middle part of the style − ′Edda′ x ′R. E. Prolific′, 3 DAP (**B**); Pollen tubes in the basal part of the style − ′Mallard′ × ′Opal′, 6 DAP (**C**); Penetration of the pollen tubes into the locule of ovary − ′Edda′ × ′Č. Lepotica′, 6 DAP (**D**); Penetration of the pollen tube into the micropyle − ′Reeves′ × ′Victoria′, 6 DAP (**E**) or in the nucellus − ′Mallard′ × ′Opal′, 9 DAP (**F**); Uncommon pollen tubes growth in the micropyle − ′Jubileum′ × ′Victoria′, 12 DAP (**G**). (st = style; ups, mps, bps = upper, medium and basal part of style; ot = ovary tissue; i = integuments; n = nucellus; arrows = indicate pollen tubes in different region of pistil).

The detailed data about average pollen tube growth in the upper part of style and locule of ovary for each crossing combinations (cultivar/pollen recipient × cultivar/pollinizer) are shown in Fig. 5. Statistical analysis of the data indicated the existence of significant differences between combinations of pollination. The average number of pollen tubes in the upper part of the style (24.5) and in the locule of the ovary (2.9) was higher in 2018 compared to 2019 (19.4 and 1.7, respectively). The highest average number of pollen tubes in the style was recorded in

´Jubileum´ × ´Č. Lepotica´ in both year (42.3 in 2018 and 38.6 in 2019) while the lowest average number was recorded in ´Mallard´ × ´R. E. Prolific´ (14) in 2018 and ´Mallard´ × ´Edda´ (3.5) in 2019. The highest average number of pollen tubes in the locule of the ovary was recorded in ´Juileum´ × ´Victoria´ (5.9) in 2018, and ´Reeves´ × ´Opal´ (3.3) in 2019. In all crossing combinations the lowest average number of pollen tubes in the locule of the ovary was observed in ´Mallard´ x ´R. E. Prolific´, (1.2) in 2018. In the following year cross-pollination of ´Mallard´ × ´R. E. Prolific´, ´Edda´ × ´Mallard´, and ´Jubileum´ × ´Jubileum´ did not have any pollen tubes penetrated the ovary`s locule. Cultivar ´Jubileum´ was the best as a pollen recipient because it had the highest average number of penetrated pollen tubes (for all pollinizers) in the upper style in both year (33.2 in 2018 and 32.1 in 2019). ´Reeves´ as pollen recipient had the highest average number of penetrated pollen (for all pollinizers) in the locule of the ovary (4) in 2018 and (2.7) in 2019.

The dynamics of pollen tube growth (in 3, 6, 9 and 12 DAP) for all combinations of pollination are shown in Fig. 6a and Fig. 6b for both years (2018 and 2019).

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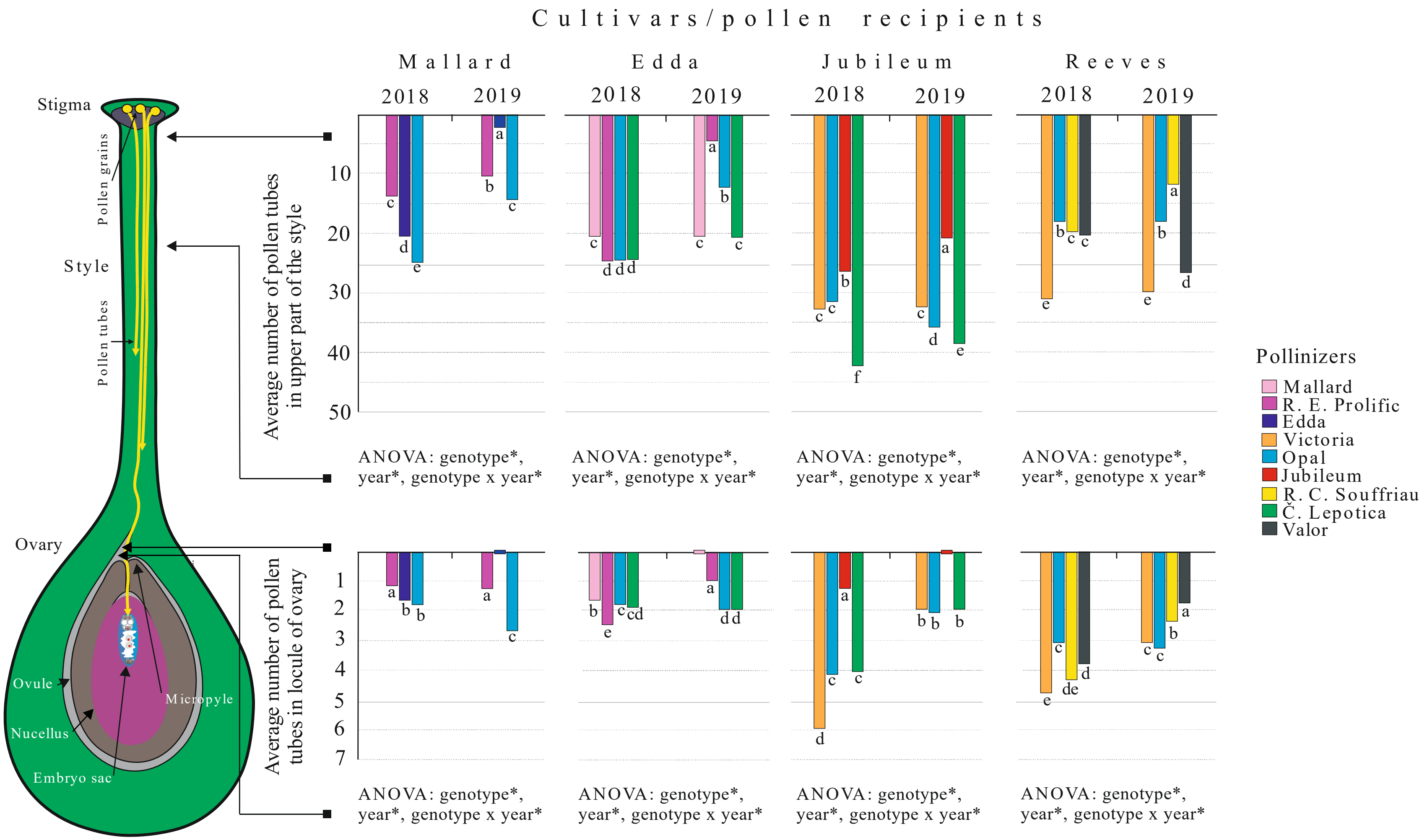

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Fig. 5. The average number of pollen tubes in the upper part of style and ovary of four plum cultivars/pollen recipients pollinated with different pollinizers in 2018 and 2019. (Different letters above the bars denote a significant difference between pollinizers according to the LSD test, $p < 0.05$).

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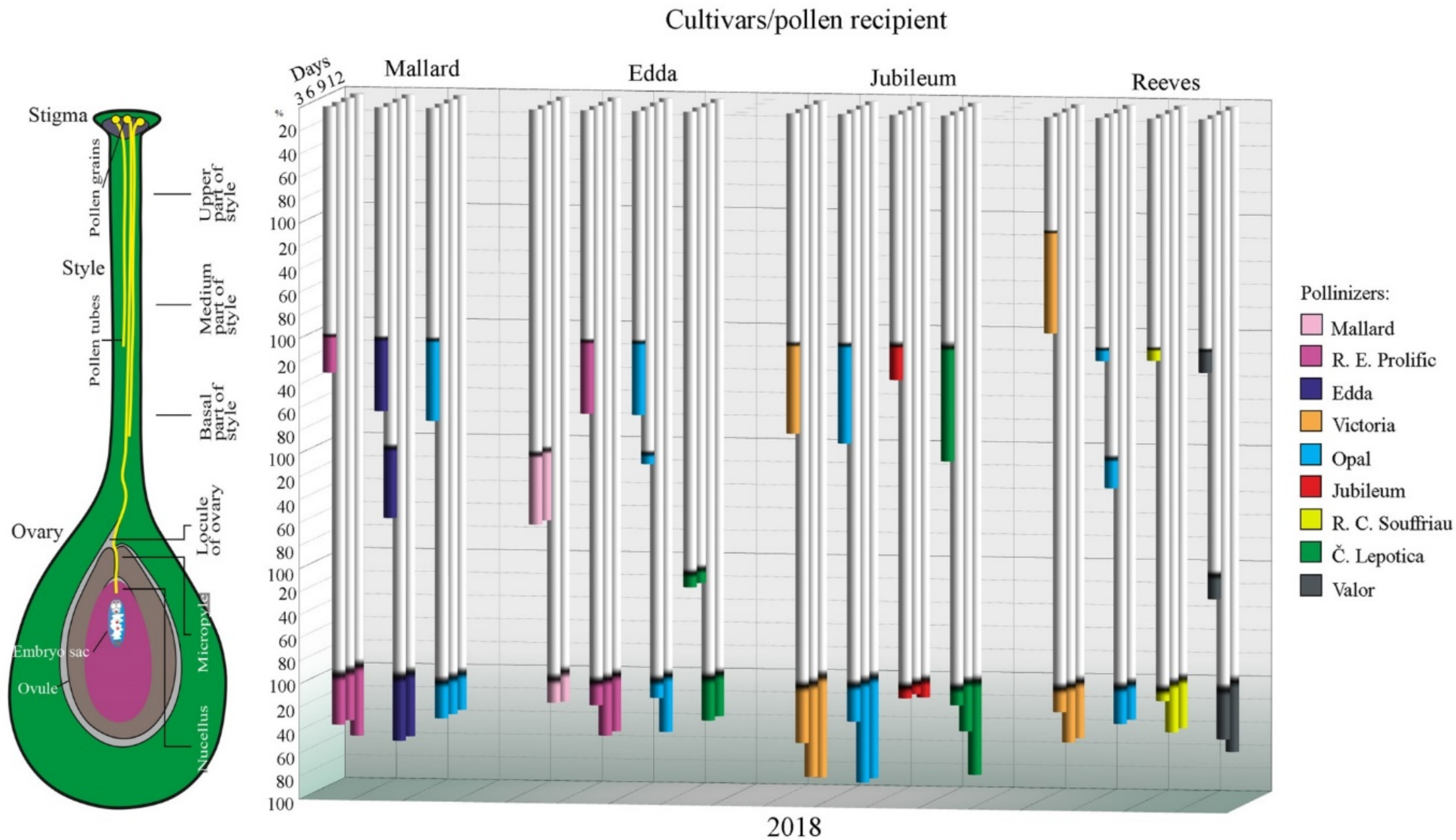

Fig. 6a. The dynamics of pollen tube growth through certain pistil parts (3, 6, 9 and 12 DAP) of cultivars/pollen recipients cross-pollinated with different pollinizers in 2018 and 2019.

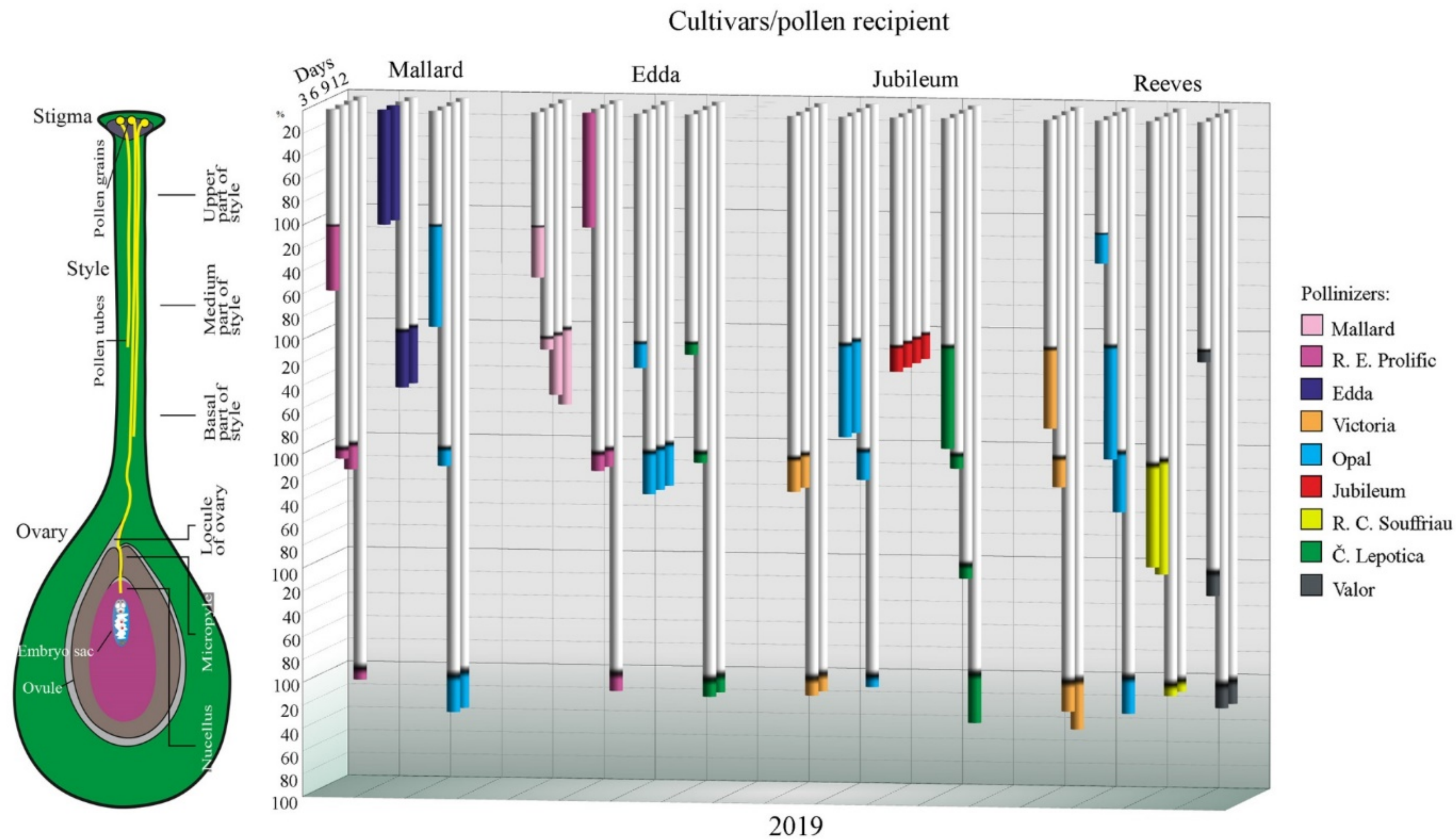

Fig. 6b. The dynamics of pollen tube growth through certain pistil parts (3, 6, 9 and 12 DAP) of cultivars/pollen recipients cross-pollinated with different pollinizers in 2018 and 2019.

In 2018, the fastest growth of pollen tubes through the pistil was recorded in ´Edda´ × ´Č. Lepotica´. The pollen tubes from this cross-combination penetrated the micropyle in 12.5% styles 3 DAP (Fig. 7 A, D). In this year the pistils with the tubes that reached the nucellus were already recorded 6 DAP. The highest average number of pistils where pollen tube penetrated the nucellus were recorded in ´Jubileum´ × ´Victoria´ (50%) 6 DAP (Fig. 7 B, E). The highest numbers of these pistils (87.5 %) were reached in ´Jubileum´ × ´Victoria´ and ´Jubileum´ × ´Opal´ 12 DAP. Cultivar ´Jubileum´, as a pollen recipient showed the highest percentage of pistils with pollen tube penetrated the nucellus, except in self-pollination (´Jubileum´ × ´Jubileum´) only 16.7%. In ´Mallard´, ´Edda´ and ´Reeves´ as mother plants, the percentage of pistils with pollen tube that penetrated the nucellus was lower and not exceeded 60%. The lowest average number of pistils (25%) with pollen tubes that penetrated the nucellus, was recorded in ´Edda´ × ´Mallard´, following ´Mallard´ × ´Opal´ and ´Reeves´ × ´Opal´ (33.3%, in both).

In 2019, the dynamics of pollen tubes growth through different structures of pistil showed tendency to have lower values. Pollen tubes that penetrated the nucellus were recorded only 9 DAP, 33.3% in crossing combination ´Mallard´ × ´Opal´. The maximum percentage of pistils (44.4 %) with penetrated pollen tubes into the nucellus were observed in ´Reeves´ × ´Victoria´ 12 DAP (Fig. 7 C, F). Cultivar ´Reeves´ as pollen recipients showed the highest average percentage of pistils with pollen tubes that penetrated the nucellus after control crossings compared to mother cultivars ´Mallard´, ´Edda´ and ´Jubileum´ with their pollinizers. The lowest pistils number (10%) with pollen tubes penetrated the nucellus were detected in ´Mallard´ × ´R. E. Prolific´, ´Jubileum´ × ´Opal´, and ´Reeves´ × ´R. C. Souffriau´ 12 DAP.

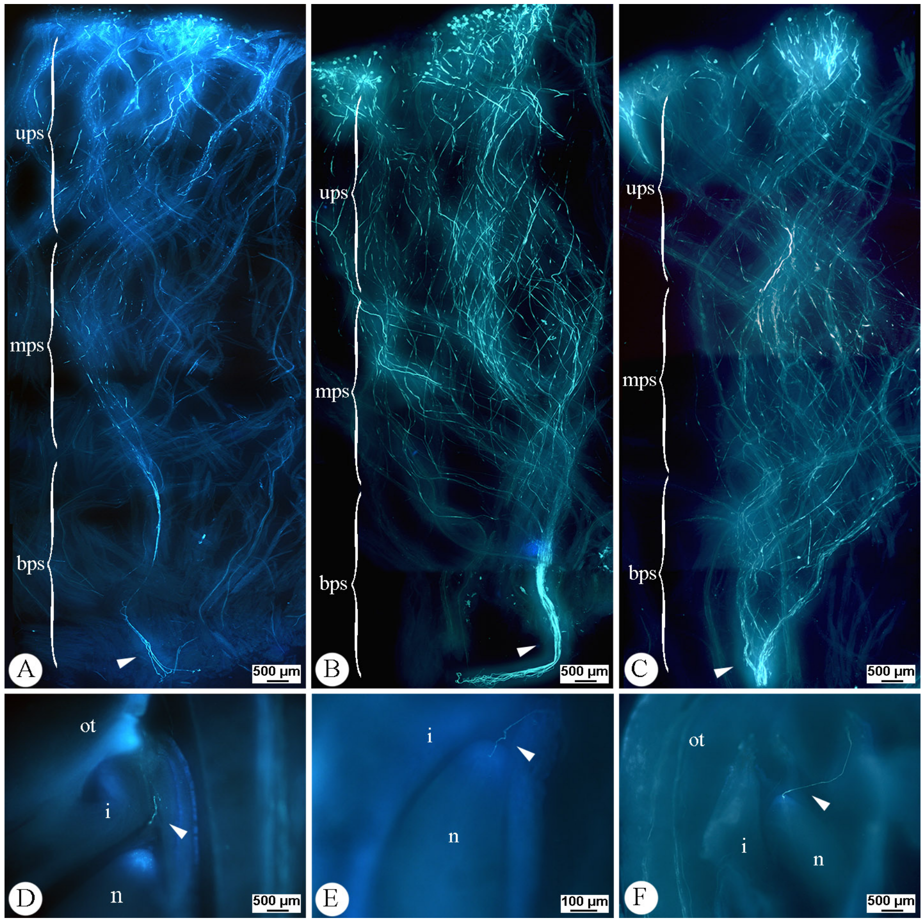

Fig. 7. Pollen tubes growth through different parts of the style and penetration into the micropyle and nucellus: (**A**) (**D**) − ´Edda´ × ´Č. Lepotica´, 3 DAP; (**B**) (**E**) − ´Jubileum´ × ´Victoria´, 6 DAP; (**C**) (**F**) − ´Reeves´ × ´Victoria´, 12 DAP. (st = style; ups, mps, bps = upper, medium and basal part of style; ot = ovary tissue; i = integuments; n = nucellus; arrows = indicate pollen tubes in different region of pistil).

The dynamics of pollen tube growth through different parts of the pistil showed higher number of pistils where pollen tube penetrated the nucellus in all combinations of pollination in 2018 compared to 2019.

3.4. Fruit set

The values of fruit set in each crossing combination (cultivar/pollen recipient × pollinizer) are given in Fig. 8 for both years (2018/2019). All factors studied (cultivar, year of study and their interactions) had significant influence on the fruit set. ´Jubileum´, as mother cultivar, had the highest average fruit set (for all crossing combinations) in both 2018 (32.9%) and in 2019 (35.4%). In contrast to, cultivar/pollen recipient ´Mallard´ had the lowest average fruit set in experimental years, 17.8% in 2018 and 21.2% in 2019. Individually, the highest percentage of fruit set (64.2%) was recorded in combination ´Jubileum´ × ´Victoria´ (64.2%) and ´Edda´ × ´Opal´ (43%) in 2018. In the same year no fruit set was found in combination ´Reeves´ × ´Valor´. Also, pollination combination of ´Mallard´ × ´Edda´ had very low fruit set (3.6%). The following year, crossing combination ´Jubileum´ × ´Opal´ and ´Reeves´ × ´R. C. Souffriau´, had the highest percentage of fruit set (56.8% and 49.3%, respectively). In the same year, the lowest fruit set was recorded in ´Mallard´ × ´Edda´ (0.8%) and selfed ´Jubileum´ (5.1%). Average fruit set for all the tested cultivars in open pollination were more than three folds higher in 2018 (27.3%) compared to 2019 (8.2%). Cultivar ´Jubileum´ had highest fruit set in both years (44.7% in 2018 and 10.9% in 2019). On the other hand, the lower average of fruit set in open pollination was recorded ´Reeves´ (13% in 2018 and 5.4% in 2019).

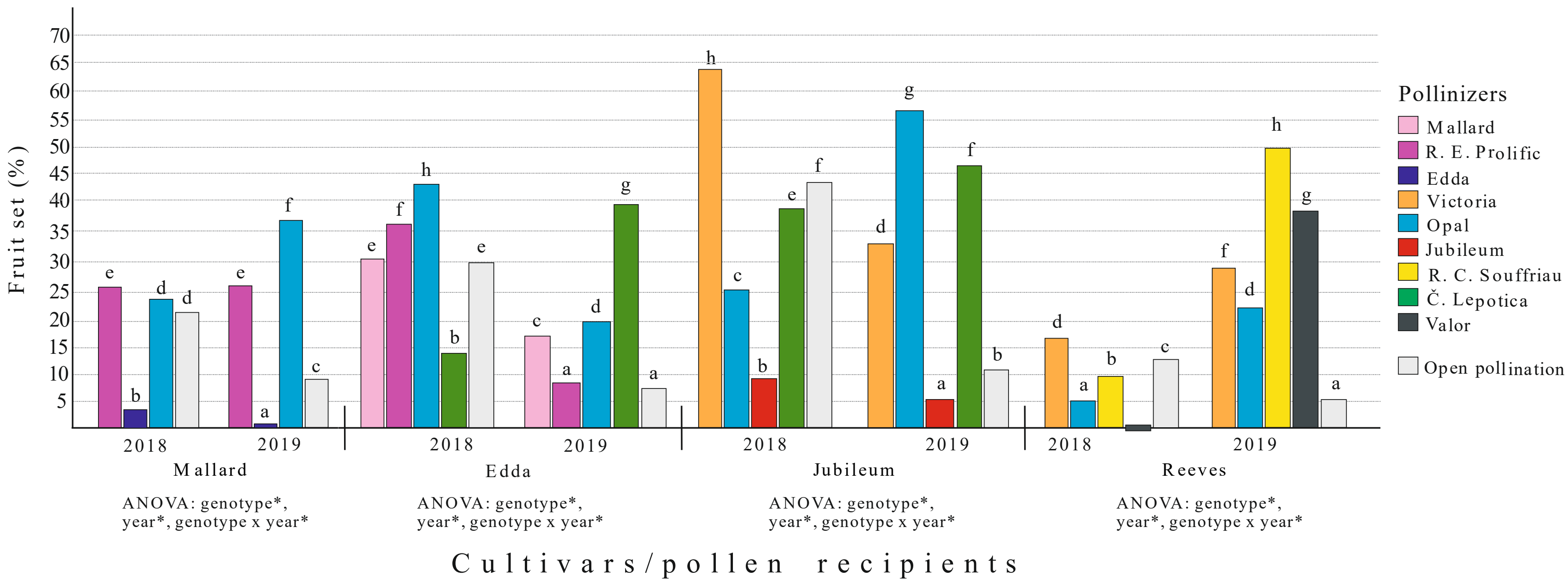

Fig. 8. Fruit set of four plum cultivars in different crossing combinations in 2018 and 2019. (Different letters above the bars denotes a significant difference between different cultivars used as pollinizers for the same cultivar according to the LSD test, $p < 0.05$).

3.5. Correlation among reproductive parameters

A correlation matrix was done for all tested reproductive parameters in all cultivars and crossing combinations during both years of study. According to the Table 1, a positive significant correlation was determined between the number of pollen tubes in the upper part of the style and average number of pollen tubes in the locule of the ovary (r = 0.54*) and with the fruit set (r = 0.63*). Also, a positive significant correlation was found between the average number of pollen tubes in the locule of the ovary and the number of fruit set (r = 0.58*).

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

Parameter	PG	STU	OVR
STU	0.28	\	\
OVR	0.37	0.54*	\
FS	0.14	0.63*	0.58*

*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; FS: Fruit set.

4. Discussion

4.1. Pollen Germination In Vitro

European plum showed different pollen quality (Hartman and Stösser, 1994) which is dependent on the cultivar (Stösser et al., 1996). Wertheim (1996) indicated that a line of 25% pollen germination is threshold between `poor` and `good` pollen germination in the plum. In our study, in 2018, high pollen germination *in vitro* (> 90%) was found in ´Mallard´, ´R. E. Prolific´ and ´Valor´ in the following year only in ´Victoria´. The average *in vitro* germination in all cultivars was higher in 2018 (69.6%) in comparison to 2019 (48.2%). Similar, but slightly lower percentages of pollen germination in ´Opal´, ´Valor´, ´Victoria´ and ´Jubileum´, have been reported by Keulemans (1984), Botu et al. (2002) and Koskela et al. (2010). Hjeltnes and Røen (2019) indicated very low pollen germination in ´Edda´, ´Jubileum´, ´Mallard´, ´Opal´ and ´Victoria´ (< 30%). Also in this experiment, the cultivar ´R. E. Prolific´ showed high variability of pollen germination *in vitro,* 92.1% in 2018 and 13.8% in 2019, which is in accordance with the findings of Hjeltnes and Nornes (2007) who discovered high variability of pollen germination in plum cultivars grown in Norway.

The viability, germination and pollen tube growth in stone fruits vary significantly, depending on a species, cultivars and the applied tests (Hedhly et al. 2004; Sharafi, 2010). High temperatures in the pre-flowering can also influence the number and the development of pollen grains and/or appearance of sterility in the male gametophyte (Kozai et al., 2004). Also, (ir)-regularity of microsporogenesis may influence pollen germination, as reported for sour cherry (Fotirić Akšić et al., 2016). It means that interaction between exogenous and endogenous factors during development of the pollen grain may affect its capacity of germination. The obtained results of pollen germination *in vitro* in this study indicated that this trait is under the strong influence some of above factors, which was previously reported by Hedhly et al. (2008), Pacini and Dolferus (2019).

4.2. Pollen Germination In Vivo

Pollen tube growth in all crossing combinations initiated with the germination of pollen grains at the surface of stigma. Usually, a small portion (< 8%) of the progamic phase accounts for pollen germination, while > 92% is overtaken by the period of pollen tube growth from the stigma to nucellus (Williams, 2012). After penetration in the stylar tissue, pollen tubes continue growth through transmitting tissue along the whole length of the style. The number of pollen tubes decreases gradually from stigma to the locule of the ovary. During the pollen tube growth, evident histological and histochemical changes can be observed, especially in the conductive tissue of the style and tissue of the ovary (Cerović et al., 1999). The pollen quality, stigma receptivity, pollen tube activation and guidance, among other factors, play an important role during progamic phase of the fertilization (Hedhly et al., 2003; Hiscock and Allen, 2008; Zheng et al., 2018). These changes have been accompanied by the emergence of intercellular substances which are responsible for many processes, such as nutrition of the growing pollen tubes (Bayer and Stösser, 2002), pollen tube guidance by different female structure and the appearance of uncommon growth of pollen tubes in the final stages of the progamic phase of the fertilization (Herrero, 2001; Dresselhaus and Franklin-Tong, 2013; Radičević et al., 2018).

No correlation was found between pollen germination *in vitro* and pollen tube number in the upper part of the style. This can be explained by the fact that the analysis of pollen germination is done in the laboratory conditions where Petri dishes are kept at room temperature. On the other side, pollen tube number in the upper part of the style, locule and fruit set was determined in the field conditions, as the average of two years (one with very good climatic conditions – 2018, but the other with very poor temperature regimes – 2019). Since Yoder et al. (2009) determined linear correlations between pollen germination on the stigmatic surface and temperature from 13 to 29 °C, we believe that post blooms temperatures in 2019 (10-days average of 5.9 °C) influenced the absence of such correlations.

The success of progamic phase of fertilization is generally assessed by the number and the dynamic of pollen tubes growth in certain regions of the style and ovary (the upper third, middle third, base of the style, locule ovary tissue, micropyle and nucellus) (Cerović, 1997). In 2018, almost all crossing combinations showed higher average number of pollen tubes in the upper part of style and locule of ovary. Also, in this year, the dynamic of pollen tube growth through certain parts of pistil was much faster in all combinations of pollination. Individually, cultivars/pollen recipient ´Jubileum´ had the highest, while ´Reeves´ was second, regarding the average number of pollen tubes in upper part of style, in locule of the ovary and the percentage of pistils where pollen tubes penetrated nucellus. Next year, in relation to the above parameters, the cultivar/pollen recipient ´Reeves´ had the highest and ´Jubileum´ was second for the same traits. Cultivars ´Victoria´ and ´Opal´ were the most efficient pollinizers for above mentioned cultivars in both years. Other crossing combinations of ´Mallard´ and ´Edda´ had lower values for the number and the dynamic of pollen tubes growth in certain regions of the style and ovary in both years.

As mentioned, this study showed that the average number of pollen tubes in the upper part of style and ovary and percentage of pistils with pollen tubes that penetrated nucellus were under the strong influence of the pollinizers and the year of investigation. Although pollinizer success is temperature dependent, it can be partially modified by the influence of the pollinated cultivar (Radičević et al, 2016). Namely, the environmental conditions, in the first-place air temperature, has directly influence the progamic phase of fertilization (Hedhly et al., 2008). It was found that the temperature, prevailing during the time of pollen tube growth had the greatest effect on progressing pollen tube toward the egg cell in plum (Bayer and Stösser, 2001). This may be related to higher average temperature in 2018 (14.6 °C) and 2019 (13.3 °C). Also, in 2018, the mean average maximal temperature was 20.3 °C while in 2019 it was 17.9 °C. As expected, a positive significant correlation between the number of pollen tubes in the upper part of style and the number pollen tubes in locule of the ovary has been established.

4.3. Fruit Set and Overlapping Flowering

There are many internal and external factors that are influencing the reproductivity which leads to the final fruit set. Primarily, internal factors include synchrony between pollen tube arrival to the ovule and embryo sac maturation, syngamy, and successful early embryo development. In this study, the variation of the percentage of fruit set depended on crossing combinations and experimental years. Environmental conditions, such as temperature and air humidity have also strong effect on the fruit set (Cerović, 1997). This study showed the existence of a strong positive correlation (r = 0.58*) between the number of pollen tubes in the ovary and the percentage of fruit set.

In 2019, cultivar/pollen recipients ´Jubileum´, ´Reeves´ and ´Mallard´ had higher average fruit set (calculated for all pollinizers) (35.4%, 34.7%, and 21.2%, respectively), while ´Edda´ − had highest fruit set (30.4%) in 2018. Individually, ´Jubileum´ × ´Victoria´ had higher fruit set in 2018 (64.2%) and ´Jubileum´ × ´Opal´ in 2019 (56.8%). The cross combination ´Jubileum´ × ´Č. Lepotica´ had high value of fruit set in both 2018 (32.7%) and 2019 (46.4%). The problem in this combination could be the fact that in the warmer year (2018) the full bloom difference between recipient and pollen donor was too large (four days), and could happen that in field conditions wouldn`t be sustainable. Flowering of cultivar/pollen recipient ´Jubileum´ completely overlapped with pollinizers ´Victoria´ and ´Opal´, 8 days in 2018, and 9 days in 2019. Self-pollination of ´Jubileum´ had only 7.4% of fruit set (average of both years). Considering the efficacy of the progamic phase and fruit set, a conclusion can be made that ´Jubileum´ belongs to the group of partially self-fertile plum cultivars, which was already stated by Neumüller (2010) and Koskela et al. (2010). According to the fruit set > 25%, Wertheim (1996) proved that cultivars ´Victoria´, ´Opal´ and ´Č. Lepotica´ are very good pollinizers for ´Jubileum´. Hjeltnes and Nordnes (2007) reported similar values of fruit set in crossing combination ´Jubileum´ × ´Victoria´ obtained earlier in the same climatic conditions.

Cultivar/pollen recipient ´Reeves´ showed higher average fruit set in 2019 (34.7%) compared with 2018 (7.9 %). Pollinizers ´R. C. Souffriau´, ´Valor´, and ´Victoria´ with ´Reeves´ had higher fruit set in 2019 (49.3%, 38.1%, and 29%, respectively). In this year all pollinizers had acceptable overlapping period of flowering with ´Reeves´ (8 and 9 days). Hjeltnes and Nordnes (2007) reported similar results for fruit set and overlapping period for pollinizers ´R. C. Souffriau´ and ´Victoria´. Also, Meland et al. (2020) did paternity testing with SSR markers in plum kernels from different crossing combination of plum cultivars in Norway, and proved that ´Opal´ is the best pollinizer for ´Reeves´, while ´Victoria´ gave better results as a pollen donor in a cooler year. On the other hand, the low fruit set in ´Reeves´ in 2018 could be a consequence of specific interaction between female cultivar and the temperature, male cultivar and the temperature, as well as female × male cultivar and its influence on pollen tube growth (Bayer and Stösser, 2001). All possible interactions have a complex effect on the entire reproductive process that can be accelerated or slowed down (Stösser and Anvari, 1990; Hedhly et al., 2008). In 2018 average daily temperature (19.2 °C) during ten days after flowering could affect EPP in ´Reeves´. Although all pollinizers had a high efficacy of the progamic phase when crossing ´Reeves´, the fruit set (average for both years) was the lowest (10.6%). High temperatures during bloom can inhibit pollen germination and tube growth, and adversely affect fruit set in some plum cultivars (DeCeault and Polito, 2010). High temperatures affect loosing viability of ovules, and degeneration of embryo sacs which all together are responsible for fruit set reduction (Stösser et al, 1996; Alburquerque et al., 2002; Đorđević et al., 2019b). Level of ovule maturity at full bloom is closely related to EPP and finally affects fruit set (Ruiz et al., 2010). Also, much higher fruit set of ´Reeves´ in 2019 (when post bloom temperatures were lower) compared to 2018 means that this cultivar is much better adapted to cooler climate. It is assumed that in such temperature conditions all tested pollinizers (´R. C. Souffriau´, ´Opal´, ´Victoria´ and ´Valor´) showed higher fruit set when used as pollinizers of cultivar ´Reeves´.

Cultivar/pollen recipient ´Edda´ had higher fruit set (average of all pollinizers) in 2018 (30.4%) compare to 2019 (21.4%). Cultivars ´Opal´, ´R. E. Prolific´ and ´Mallard´ belong to group of very good pollinizers for the cultivar/pollen recipient ´Edda´ (> 25% fruit set) in 2018. In the following (cooler) year, pollinizer ´Č. Lepotica´ gave the best result with ´Edda´, achieving 39.2% fruit set. On the contrary to that ´R. E. Prolific´, as a pollinizer, gave much better results of fruit set with ´Edda´ in a warmer year (36.1%), while in a cooler it was just

8.5%. Our results indicated the existence of specific response of each genotype to temperature variation, which complies with the Hedhly et al. (2004). The best overlap of flowering of cultivar ´Edda´ had with pollinizers ´Opal´, ´R. E. Prolific´ and ´Mallard´ (8 days) in 2018 and 8−9 days in 2019. In 2018, the cultivars ´Opal´, ´R. E. Prolific´ and ´Mallard´ were the best pollinizers for ´Edda´ when temperatures during flowering period, and immediately after it, were high. According to Meland et al. (2020) cultivar `Opal` is the best pollinizer for ´Edda´ in all kind of weather.

The pollinizers ´R. E. Prolific´ and ´Opal´ when used as pollen donors for ´Mallard´, gave satisfactory average fruit set in both years (26.1% and 30.3%, respectively). Crossing of ´Mallard´ with ´Opal´ gave very high fruit set (36.8%) in 2019, which was previously established as a good combination by Meland (2001) and Meland et al. (2020). In both years, crossing ´Mallard´ × ´Edda´ had the lowest fruit set (3.6% in 2018 and 0.8% in 2019). According to Wertheim (1996) every fruit set in the range of 0-4%, just like in the previous crossing combination, can be classified as a ´poor´ fruit set. Based on this, crossing ´Mallard´ × ´Edda´ can be considered as incompatible combination. This indicates the existence of specific interactions in the ovary during the progamic phase of fertilization between male and female cultivar, but also in the prezygotic and post-zygotic period. On the other hand, the other three pollinizers, ´R. E. Prolific´, ´Edda´, and ´Opal´ had full flowering overlapping period with ´Mallard´ in both years. Based on the fruit set and overlapping of flowering, cultivars ´Opal´ and ´R. E. Prolific´ can be considered as good pollinators for cultivar/pollen recipient ´Mallard´.

In 2018, high percentage of fruit set in open pollination was recorded in ´Mallard´ (21.6%), ´Edda´ (29.6%), and ´Jubileum´ (44.7%). According to Stösser (2002), fruit set in plum is often higher in open then in hand pollination, due to the fact that favorable field temperature conditions during flowering enable often visits of different pollinators to flowers of cultivar/recipient carrying pollen mixtures of different pollinizers. In this way, flowers received different quantity and quality of pollen grains that originated from different cultivars which intensified competition among male gametophytes on the stigma and along the style, and had positive effect on fruit set. The lowest average of fruit set in open pollination was recorded in cultivar ´Reeves´ (13% in 2018 and 5.4% in 2019). These results can be explained by the fact that cultivar ´Reeves´ is susceptible to high temperatures during flowering time which can influence the duration of EPP, making it much shorter, and thus causing poor fruit set (Williams, 1970; Đorđević et al., 2019a).

The situation regarding open pollination in 2019 was completely different. In this year the average fruit set in open pollination was several times lower compared to controlled pollination (8.2% and 28.2%, respectively). The period of full flowering, when pollen was applied in the controlled crossings, was followed with some raining period. Due to the unfavorable weather conditions and impossibility of pollinators to fly around, open pollination happened a bit later. In that case pollen tubes didn't have enough time to reach the ovules, and do the fertilization. A cold period during the 10-day after flowering period (average 5.9 °C) influenced the abortion of such fruits which all together lead to low fruit set in open pollination in contrast to hand pollination which provided enough time for the effective pollen tubes growth, successful fertilization and high fruit set. Previously obtained results in pear under the same climate conditions showed that fertilization success and fruit set were higher in year with higher average temperature during the flowering and after the flowering period (Cerović et al., 2020b).

5. Conclusions

Norwegian plum production is characterized by climatic limitations, different flowering time, insufficiently of well-adapted cultivars and appropriate pollinizers for cultivars that can be grown in this region. This study evaluated the efficiency and dynamic of pollen tube growth (progamic phase of fertilization) and fruit set in four European plum cultivars: ′Mallard′, ′Edda′, ′Jubileum′, and ′Reeves′ after crossing with different pollinizers, over two years (2018-2019). Reproductive parameters (*in vitro* pollen germination, number of pollen tubes in the upper part of the style and locule of the ovary, number of pistils with fertilized ovules, and fruit set) in all crossing combinations and fruit set in open pollination showed different adaptability of both recipient and donor cultivars to the specific ecological conditions prevailing in West Norway. The genotype-dependent response of pollinizers in terms of air temperature during the flowering has not consistently manifested and was partially modified by the influence of the pollinated cultivar. Based on the evaluation of progamic phase of fertilization, fruit set in the controlled crossings and fruit set in open pollination, together with the overlap of the flowering time, pollinizers ′Victoria′ and ′Opal′ proved to be very good pollinizers for cultivar/pollen recipient

´Jubileum´, while pollinizers ´R. C. Souffriau´ and ´Valor´ for the cultivar ´Reeves´. In addition, it was also confirmed that ´Jubileum´ belongs group of partially self-fertile plum cultivars. Cultivars ´Opal´, ´R. E. Prolific´ and ´Mallard´ are excellent pollinizers for ´Edda´ in conditions of higher temperatures during flowering period and post-flowering period. Cultivar ´Č. Lepotica´ proved to be very good pollinizer for ´Edda´, but only when the temperatures were lower, and thus, could be used as a backup. Cultivars ´Opal´ and ´R. E. Prolific´ can be considered as good pollinizers for ´Mallard´. Evenly distribution of two pollinizers having overlapping in flowering times with the main cultivar is a general recommendation for commercial plum production for obtaining high yields of good fruit quality.

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Credit authorship contribution statement

Radosav Cerović: Conceptualization, Methodology and formal analysis, Writing—original draft preparation, Writing—review and editing. **Milica Fotirić Akšić**: Conceptualization, Methodology and formal analysis, Writing—review and editing. **Milena Đorđević:** Methodology and formal analysis, Writing—review and editing. **Mekjell Meland**: Conceptualization, Methodology and formal analysis. Writing—review and editing, Funding acquisition, Project administration. Resources.

Declaration of Competing Interest

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

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