

Initial and final fruit set of introduced apple cultivars depending on pollenizer

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Abstract. The aim of the paper was to underline the impact made by the pollenizer on the level of initial and final fruit set in four commercially important apple cultivars, using them as a base for determining and recommending cultivar combinations suitable for planting within a single orchard. The research was conducted over a three-year period, at the Preljinsko Brdo facility of the Fruit Research Institute, Čačak. The examination included the flowering phenophase, pollen germination *in vitro*, as well as the initial and final fruit set in cultivars ‘Gala Must’ (S_2S_5), ‘Red Elstar’ (S_3S_5), ‘Rajka’ (S_5S_7) and ‘Topaz’ (S_2S_5), depending on the pollenizer. The research was conducted in two pollination variants: cross- and open pollination. In the cross-pollination variant, each compatible cultivar was used as the pollenizer and the pollinated cultivar (10 combinations) and together with the open pollination variant, a total of 14 combinations were analysed. The average earliest flowering was recorded in ‘Rajka’ (April 8th), whereas the latest average flowering occurred in ‘Red Elstar’ (April 10th). Regarding the flowering time, all tested cultivars overlapped well during the three-year period of examination. In addition, the assessed cultivars had good *in vitro* pollen germination, ranging from 44.00% (‘Topaz’) to 55.72% (‘Gala Must’). Based on the fruit set, it can be concluded that neither cultivar proved itself as the universal pollenizer for the other cultivars. The highest percentage of the initial (36.95%) and the final (21.38%) fruit set for ‘Gala Must’ was recorded in the open-pollination variant, while the best fructification results for ‘Red Elstar’ occurred in the combination with ‘Topaz’ (41.84% and 23.76%, resp.). The highest values of the initial and final fruit set in ‘Rajka’ (39.26% and 18.81%, resp.) and ‘Topaz’ (38.66% and 26.30%, resp.) were achieved with ‘Gala Must’, i.e. ‘Rajka’ as the pollenizer.

Key words: *Malus × domestica*, cultivar, flowering, pollen germination, fruit set

Introduction

Apple (*Malus × domestica* Borkh.) like other species of Rosaceae family exhibits gametophytic self-incompatibility which is genetically controlled by two S-locus genes, one controlling the style (*S-RNase* gene) and the other governing the pollen (*SFB* gene) (Minamikawa et al., 2010). Depending on the S-genotype, apple cultivars can be fully compatible (when the cul-

tivars differ in both of their S-alleles), semi-compatible (when they differ in one of their two S-alleles), or incompatible (when both cultivars carry the same S-alleles). Therefore cross-compatible apple cultivars are planted together to allow successful pollination and to achieve high and stable yields (Matsumoto, 2014). In addition to genetic compatibility, for cross-pollination to be effective, it is very important that the main and pollenizer cultivars bloom at approximately the same

time, as well as that pollenizer produces the sufficient quantity of viable pollen (Javid *et al.*, 2017). Garratt *et al.* (2014) reported that at least two cross-compatible cultivars that flower simultaneously are recommended for commercial apple production. Fertilization efficiency of apple is under the strong impact of the main cultivar which is reflected in stigma receptivity and ovule longevity (Skendrović-Babojelić *et al.*, 2015). Among environmental conditions, air temperature at the time of pollination has a tremendous impact on different stages of the reproductive process and fertilization success in pome fruits such as stigma receptivity, pollen germination *in vivo*, pollen tube growth rate, ovule longevity (Sanzol *et al.*, 2003; Jahed & Hirst, 2017). Also, Sheffield (2014) pointed out that the seed set (number and size of seeds), for which the impact of pollenizer is of crucial importance, affect fruit size and quality, as well as the marketability of fruit.

According to aforementioned, investigation of the best pollenizers choice is required before introduction of new apple cultivars into commercial orchards of some areas. This work aimed to study the impact made by the pollenizer on the level of initial and final fruit set in four cultivars ‘Gala Must’, ‘Red Elstar’, ‘Rajka’ and ‘Topaz’. According to the previous results, these cultivars can be important for commercial growing in agro-ecological conditions of the Republic of Serbia (Lukić & Marić, 2012; 2015). The results obtained in this work will be useful for determination and recommendation of cultivar combinations suitable for planting within a single orchard.

Materials and Methods

Plant material and experiment design. The study was carried out during 2007–2009 at the experimental apple orchard at ‘Prelijnsko Brdo’ facility (43°54’N; 20°24’ E, 350 m a.s.l.), belonging to Fruit Research Institute, Čačak (West Serbia). The orchard was established in the spring 2005 using ‘knip’ nursery trees with five and more lateral shoots of cultivars ‘Gala Must’, ‘Red Elstar’, ‘Rajka’ and ‘Topaz’, grafted on M9 rootstock. The trees were spaced at 4 × 1 m and trained as slender spindles. The standard orchard management (spring and winter pruning, drip irrigation, pest and disease control, anti-hail net) was applied in the trial. The experiment was set up as a completely randomi-

zed block system with five trees in three replications (fifteen trees per cultivar).

Flowering phenophase. Flowering phenophase investigation included monitoring and taking notes of flowering onset (10% of the flowers were open), full flowering (80% of the flowers were open) and end of flowering (90% of petals fallen) (Wertheim, 1996).

Pollen germination in vitro. Flowers in the late balloon stage were taken from the experimental orchard in order to collect pollen samples. Anthers were separated from flowers in the laboratory and stored in paper boxes until they burst and release pollen grains (for 24–48 h at temperature of 20 °C). Pollen of each cultivar was grown 24 h at 20 °C in two Petri dishes on nutrition medium containing 1% agar and 12% sucrose. Three observation fields of each Petri dish with at least 100 pollen grains were monitored using OLIMPUS BX61 microscope, Olympus, Japan (light regime). The germinated pollen grains were counted and the average percentage of germination was calculated.

Pollination procedure and initial and final fruit set. Among the assessed cultivars, three following *S*-genotypes were identified: S_2S_5 (‘Gala Must’ and ‘Topaz’; Marić & Lukić, 2013), S_3S_5 (‘Red Elstar’; Marić S., pers. comm.) and S_5S_7 (‘Rajka’; Marić S., pers. comm.). Therefore, the cultivars ‘Gala Must’ and ‘Topaz’ are cross-incompatible. The research included two pollination variants: cross- and open pollination. In the cross-pollination variant, each compatible cultivar was used as the pollenizer and the pollinated cultivar (10 combinations) and together with the open pollination variant (O.P.), a total of 14 combinations were analysed (‘Gala Must’ × ‘Red Elstar’, ‘Gala Must’ × ‘Rajka’, ‘Gala Must’ O.P., ‘Red Elstar’ × ‘Gala Must’, ‘Red Elstar’ × ‘Rajka’, ‘Red Elstar’ × ‘Topaz’, ‘Red Elstar’ O.P., ‘Rajka’ × ‘Gala Must’, ‘Rajka’ × ‘Red Elstar’, ‘Rajka’ × ‘Topaz’, ‘Rajka’ O.P., ‘Topaz’ × ‘Rajka’, ‘Topaz’ × ‘Red Elstar’, ‘Topaz’ O.P.). To determine fruit set in the variant of cross-pollination, the branches with flowers at late balloon stage were isolated using paper bags. Isolated flowers of each cultivar were hand pollinated at the full blooming using prepared samples of pollen of the compatible pollenizer. The cross was represented by 100 pollinated flowers, so that in each experimental year 200 flowers per cultivars ‘Gala Must’ and ‘Topaz’, as well as 300 flowers per cultivars ‘Red Elstar’ and ‘Rajka’ were hand pollinated. To determine fruit set per cultivar in

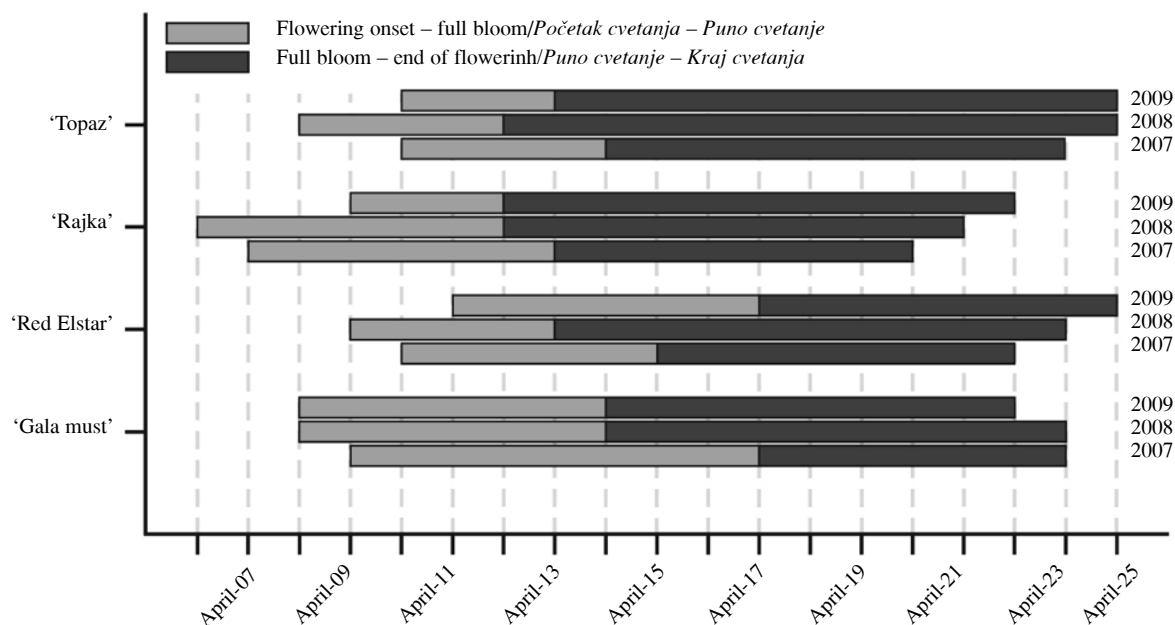
the open pollination variant, the branches with a total of 100 flowers at full blooming were chosen and marked (without bagging). The initial and final fruit set were recorded five weeks after the pollination and before harvest, respectively. Initial and final fruit set are expressed as a fruit number per 100 pollinated (cross-pollination variant) or marked (open pollination variant) flowers.

Statistical data analysis. The data were statistically analysed using the analysis of variance model (ANOVA). The significance of differences among mean values was determined using LSD test at $P < 0.05$.

Results and Discussion

Flowering phenophase. The blooming time of apple cultivars is conditioned by genotype, growing conditions (altitude, exposition, temperature, precipitation, etc.) and system of cultivation (Soltész, 2003). The flowering phenophase of assessed apple cultivars in Čačak's conditions are shown in Graph 1.

The studied cultivars were characterized by quite uniform flowering phenophases during the period of investigation. The average earliest flowering onset (April 8th), full flowering (April 13th) and end of flowering (April 23rd) were recorded in 'Rajka'. The latest average flowering onset (April 10th) occurred in 'Red Elstar', the latest average full flowering (April 16th) in 'Gala Must' and 'Red Elstar', while the latest average end of flowering (April 25th) was observed in 'Topaz'. Based on flowering time observed in this work, 'Rajka' can be classified as medium-early flowering cultivar, whereas other studied cultivars belong to the medium-late flowering apples (Soltész, 2003). An important criterion for successful pollination is flowering overlap among compatible cultivars (Stern *et al.*, 2004). Pejkić (1998) stated that this requirement is fulfilled if compatible pollenizers belong to the same or neighbouring groups according to their flowering time. Results of our study showed that all assessed cultivars overlapped well in flowering time during the three-year period of examination and were prone to pollination among themselves.



Graph 1. Flowering phenophase of introduced apple cultivars (2007–2009)

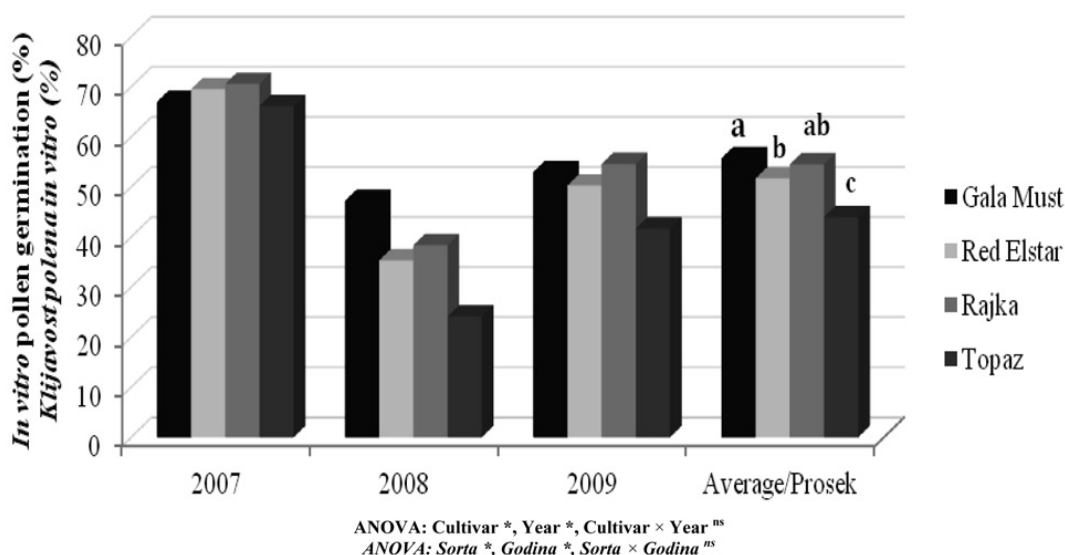
Graf. 1. Fenofaza cvetanja introdukovanih sorti jabuke (2007–2009. godine)

Pollen germination in vitro. Pollen viability and its germination capability are essential factors affecting the fruit set (Imani *et al.*, 2011) and they depend on genotype and environmental conditions, particularly air temperature (Javid & Rather, 2019). This study revealed differences among the assessed cultivars, as well as the experimental years, while a genotype by year interaction did not influence pollen germination *in vitro* (Graph 2).

On average, the highest values of pollen germination *in vitro* occurred in ‘Gala Must’ (55.72%) and ‘Rajka’ (54.53%), while the lowest value was noticed in ‘Topaz’ (44.00%). All assessed cultivars were characterized by the highest value of pollen germination *in vitro* in the first, and the lowest in the second year of study. Wertheim (1996) reported that a cultivar may be a satisfactory pollenizer if its pollen germination value is higher than 25%. Accordingly, all cultivars included in our examination can be considered as good pollenizers. The values of pollen germination *in vitro* obtained in our study are lower in relation to the values that other authors referred to as diploid apple cultivars (more than 90%) (Milutinović *et al.*, 1996; Petrisor *et al.*, 2012).

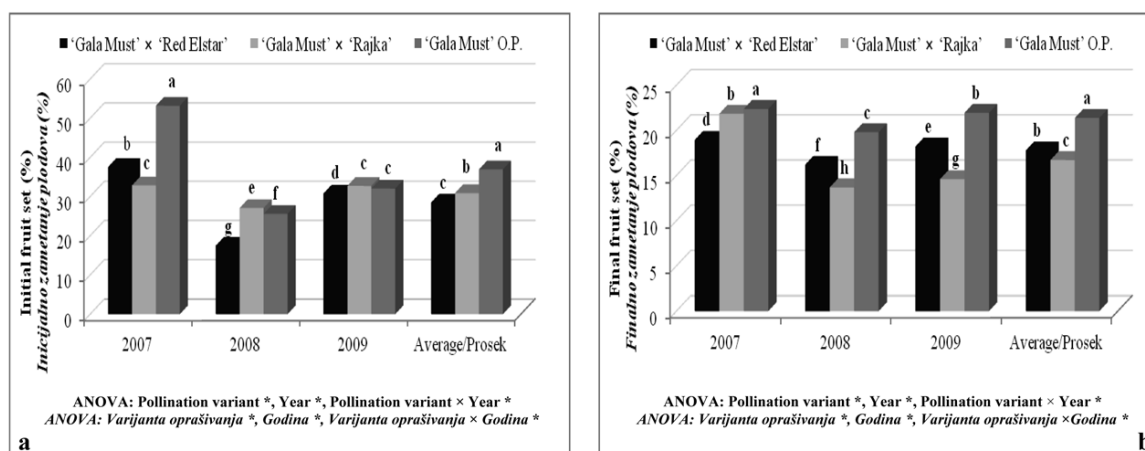
Fruit set. The initial and final fruit set of ‘Gala Must’ were influenced by the pollination variants and year, as well as by the interaction of these factors (Graph 3a–b). The highest average percentage of the initial (36.95%) and the final (21.38%) fruit set for ‘Gala Must’ was recorded in the open-pollination variant. Significantly lower average values of aforementioned parameters were obtained in crosses with ‘Red Elstar’ (28.48% and 17.78%, resp.) and ‘Rajka’ (30.91% and 16.72%, resp.). Also, the lowest average values were recorded in 2008 and the highest in 2007, but this temporal trend was not typical for both pollination variants.

The main effect of pollination variants and year on initial and final fruit set of ‘Red Elstar’ was observed, while interaction of these factors had impact only on final fruit set (Graph 4a–b). On average, the best results of initial and final fruit set for ‘Red Elstar’ occurred with ‘Topaz’ as pollenizer (41.84% and 23.76%, resp.), whereas open pollination variant gave the lowest fructification (28.77% and 15.31%, resp.). Regarding the average initial fruit set, better results were obtained in 2007 and 2009 in comparison to 2008. The best average final fruit set was obtained in 2009 and the lowest in 2008.



The various lowercase letters in respective columns indicate significant differences at $P \leq 0.05$ according to the LSD test/Mala slova u kolonama pokazuju značajne razlike za $P \leq 0,05$ primenom LSD testa

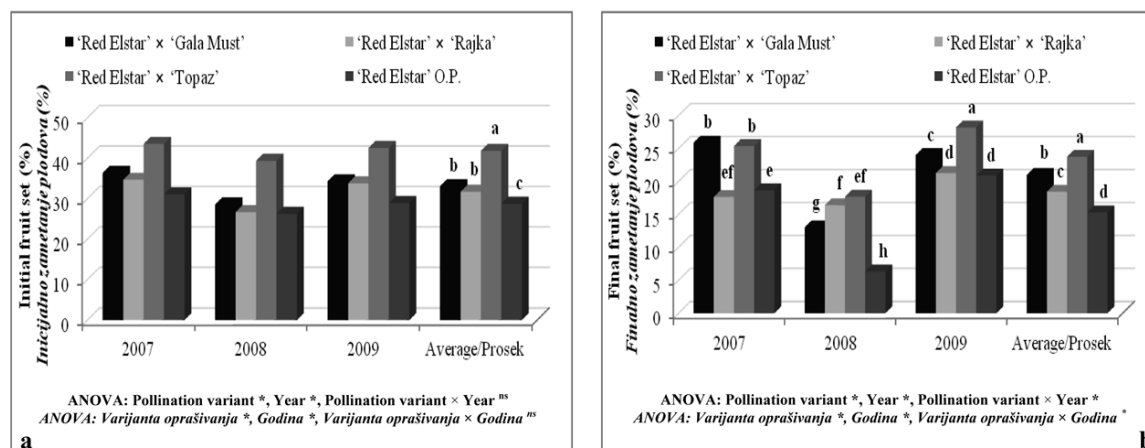
Graph 2. *In vitro* pollen germination (%) of introduced apple cultivars (2007–2009)
Graf. 2. Klijavost polena *in vitro* introdukovanih sorti jabuke (2007–2009. godine)



The various lowercase letters in respective columns indicate significant differences at $P \leq 0.05$ according to the LSD test/*Mala slova u kolonama pokazuju značajne razlike za $P \leq 0,05$ primenom LSD testa*

Graph 3. Initial (a) and final (b) fruit set of 'Gala Must' depending on pollination variant (2007–2009)

Graf. 3. Inicijalno (a) i finalno (b) zamatanje plodova sorte Gala Must u zavisnosti od varijante oprašivanja (2007–2009. godine)



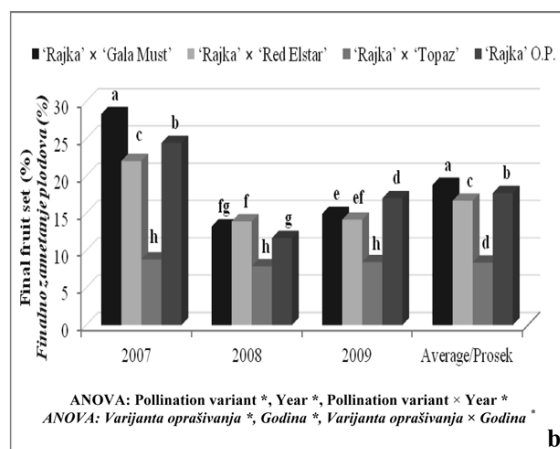
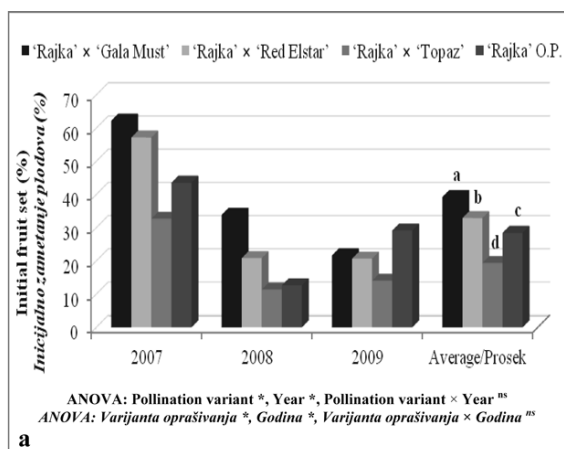
The various lowercase letters in respective columns indicate significant differences at $P \leq 0.05$ according to the LSD test/*Mala slova u kolonama pokazuju značajne razlike za $P \leq 0,05$ primenom LSD testa*

Graph 4. Initial (a) and final (b) fruit set of 'Red Elstar' depending on pollination variant (2007–2009)

Graf. 4. Inicijalno (a) i finalno (b) zamatanje plodova sorte Red Elstar u zavisnosti od varijante oprašivanja (2007–2009. godine)

There were differences among pollination variants with respect to initial and final fruit set of 'Rajka' (Graph 5a–b). The significantly highest values of those parameters were achieved with 'Gala Must' (39.26% and 18.18%, resp.), while the lowest values were typical with 'Topaz' (19.40% and 8.38%, resp.) as pollenizers. Also, Lukić & Marić (2014) reported that the highest fruit weight, dimensions of the fruit and the number of seeds per fruit in 'Rajka' were ob-

tained with 'Gala Must' as the pollenizer. Fructification of 'Rajka' differed among experimental years. The lowest average values of both parameters were recorded in 2008 and the highest in 2007. Mentioned trend was observed in both cross- and open pollination variants regarding the initial fruit set, while the final fruit set was influenced by the interaction of pollination variant and year.



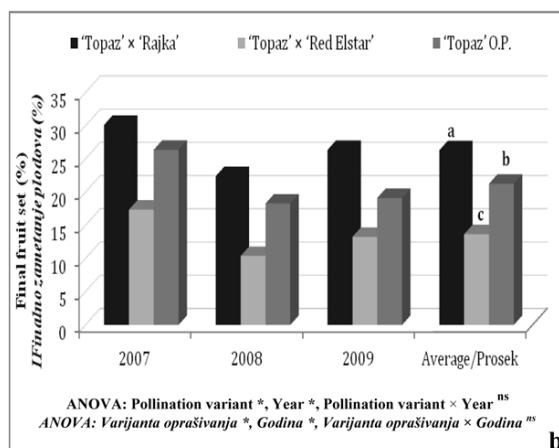
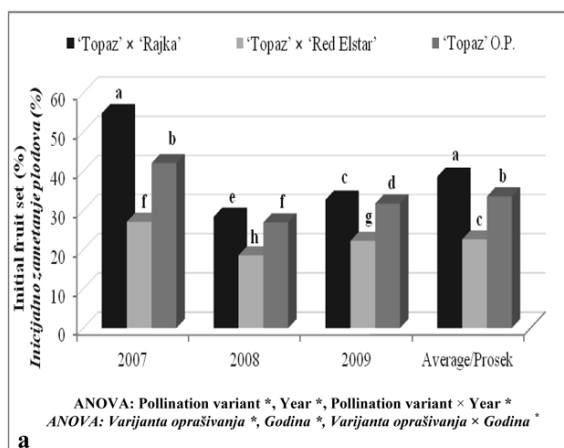
The various lowercase letters in respective columns indicate significant differences at $P \leq 0.05$ according to the LSD test/*Mala slova u kolonama pokazuju značajne razlike za $P \leq 0,05$ primenom LSD testa*

Graph 5. Initial (a) and final (b) fruit set of 'Rajka' depending on pollination variant (2007–2009)

Graf 5. Inicijalno (a) i finalno (b) zametanje plodova sorte Rajka u zavisnosti od varijante oprašivanja (2007–2009. godine)

According to the obtained results showed in Graph 6a–b, the highest average both initial and final fruit set of 'Topaz' were obtained in combination with 'Rajka' (38.66% and 26.30%, resp.). The study of metaxenia effect on pomological traits of 'Topaz' revealed that the highest fruit weight, height and width, as well as number of seeds per fruit were achieved using 'Raj-

ka' as the pollinizer (Lukić *et al.*, 2016). Significantly lower percentages were observed in cross with 'Red Elstar' (22.65% and 13.68%, resp.), as well as in open pollination variant (33.62% and 21.27%, resp.). In the respect of experimental year, the best results were obtained in 2007 and the lowest in 2008. The interaction of main factors had impact on the initial fruit set only.



The various lowercase letters in respective columns indicate significant differences at $P \leq 0.05$ according to the LSD test/*Mala slova u kolonama pokazuju značajne razlike za $P \leq 0,05$ primenom LSD testa*

Graph 6. Initial (a) and final (b) fruit set of 'Topaz' depending on pollination variant (2007–2009)

Graf 6. Inicijalno (a) i finalno (b) zametanje plodova sorte Topaz u zavisnosti od varijante oprašivanja (2007–2009. godine)

The results regarding the initial fruit set obtained in this study are in agreement with results reported by Guerro-Prieto *et al.* (2009). Also, values of initial and final fruit set of studied apple cultivars varied depending on pollenizer which is in agreement with the statements of Arafat *et al.* (1994), Skenderović-Babojelić *et al.* (2015) and Jahed & Hirst (2017). Our study revealed that neither cultivar whose pollen was used for cross-pollination proved itself as the universal pollenizer for the other cultivars. ‘Gala Must’ had the highest average initial and the final fruit set in the open-pollination variant, which can be explained by the fact that semi-compatible pollenizers were used for pollination of this cultivar. Skenderović-Babojelić *et al.* (2015) reported that semi-compatible pollenizers impacted lower fructification. However, this phenomenon was not typical for other studied cultivars, which achieved the highest initial and final fruit set in the cross with semi-compatible pollenizers. This supports the notions that the fruit set besides genetic incompatibility is also conditioned by a series of physiological events, such as pollination, pollen tube growth, ovule longevity and fertilization (Parray *et al.*, 2017), as well as by tree condition (Dafni & Firmage, 2000), climatic conditions (Yoder *et al.*, 2009) and rootstock (El-Shammaa *et al.*, 2011). If we take into account proposed standards for the fruit set of pome fruit species given by Wertheim (1996), we can conclude that all studied cultivars except ‘Red Elstar’ had good fruit set in open pollination variant. The ‘Red Elstar’ was characterized by moderate fruit set. Also, good fruit set was obtained in all combinations of cross-pollination variant, with exception of cross ‘Rajka × ‘Topaz’ which had moderate fruit set.

Conclusion

The results obtained by examining some aspects of the reproductive process of introduced cultivars ‘Gala Must’, ‘Red Elstar’, ‘Rajka’ and ‘Topaz’ – stable overlap of the main cultivars and pollenizers in the full bloom from year to year, pollen quality and fruit set, can contribute to the proper choice of the cultivars for commercial apple orchard. Further studies of their reproductive behaviour, such as pollen tube growth characteristics in the style, in the context of agro-ecological conditions in main fruit growing regions in the Republic of Serbia will give a more detailed insight into the fertilization relationship between these cultivars.

Acknowledgments

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INICIJALNO I FINALNO ZAMETANJE PLODOVA INTRODUKOVANIH SORTI JABUKE U ZAVISNOSTI OD OPRAŠIVAČA

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Rezime

Jabuka je samobesplodna vrsta voćaka čija je redovna i visoka rodnost bazirana na izboru kombinacija kompatibilnih sorti koje imaju sinhronizovano cvetanje. Cilj proučavanja prikazanih u ovom radu bio je da se utvrdi uticaj oprašivača na nivo inicijalnog i finalnog zametanja plodova kod četiri introdukovane, ekonomski značajne sorte jabuke – Gala Must (S_2S_5), Red Elstar (S_3S_5), Rajka (S_5S_7) i Topaz (S_2S_5). Dobijeni rezultati predstavljaju osnovu za utvrđivanje kombinacija sorti koje bi se mogla preporučiti za gajenje u komercijalnim voćnjacima. Proučavanja su trajala tri godine i sprovedena su u eksperimentalnom zasadu jabuke na objektu Preljinsko brdo Instituta za voćarstvo, Čačak.

Istraživanja su obuhvatila proučavanja fenofaze cvetanja i vitalnosti polena, kao i utvrđivanje inicijalnog i finalnog zametanja plodova. Karakteristike fenofaze cvetanja ispitane su putem osmatranja i beleženja datuma početka, punog i kraja cvetanja. Za ispitivanje vitalnosti polena korišćen je test klijavosti polena *in vitro*, koji podrazumeva zasejavanje prethodno pripremljenog polena svake sorte na hranljivu podlogu koja sadrži 12% saharoze i 1% agara, inkubaciju u trajanju od 24 h na temperaturi od 20 °C i utvrđivanje broja klijalih polenovih zrna pod mikroskopom. Inicijalno (pet nedelja po oprašivanju) i finalno (neposredno pred berbu) zametanje plodova proučavanih sorti jabuke utvrđeno je u svim kompatibilnim kombinacijama međusobnog ukrštanja, kao i u varijanti slobodnog oprašivanja, pa je ukupno analizirano 14 različitih

kombinacija. Pomenuti parametri predstavljaju broj zametnutih plodova od 100 oprašenih (unakrsno oprašivanje), odnosno obeleženih (slobodno oprašivanje) cvetova, izražen u procentima. Dobijeni rezultati su statistički obrađeni primenom Fišerovog modela analize varijanse (ANOVA) i LSD testa za prag značajnosti $P < 0,05$.

Rezultati fenoloških proučavanja su pokazali da se sorta Rajka odlikuje srednjeranim, a sve ostale ispitivane sorte srednjekasnim vremenom cvetanja, kao i da se period cvetanja u dovoljnoj meri preklapao u sve tri eksperimentalne godine. Prosečne vrednosti klijavosti polena *in vitro* proučavanih sorti jabuke su varirale u intervalu od 44,00% (Topaz) do 55,72% (Gala Must), stoga se sa aspekta kvaliteta polena sve ispitivane sorte mogu preporučiti kao dobri oprašivači. Dobijene vrednosti inicijalnog i finalnog zametanja plodova su pokazale da je najviši procenat inicijalno (36,95%) i finalno (21,38%) zametnutih plodova sorte Gala Must utvrđen u varijanti slobodnog oprašivanja, dok su najbolji rezultati za sortu Red Elstar utvrđeni u kombinaciji sa sortom Topaz (41,84% i 23,76%, resp.). Najviše vrednosti inicijalnog i finalnog zametanja plodova sorte Rajka (39,26% i 18,81%, resp.) utvrđene su u varijanti oprašivanja sa sortom Gala Must, dok se sorta Rajka pokazala kao najbolji oprašivač za sortu Topaz (38,66% i 26,30%, resp.).

Ključne reči: *Malus × domestica*, sorta, cvetanje, klijavost polena, zametanje plodova