

EFFECTIVE POLLINATION PERIOD IN 'OBLAČINSKA' SOUR CHERRY CLONES

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Fotirić Akšić M., V. Rakonjac, D. Nikolić, S. Čolić, D. Milatović, V. Ličina, D. Rahović (2014): *Effective pollination period in 'Oblačinska' sour cherry clones*. - Genetika, Vol 46, No. 3, 671- 680.

To obtain high yields there should be high flower density and fruit set in sour cherry (*Prunus cerasus* L.) production. Furthermore, in order to ensure successful fertilization, there should be satisfactory stigma receptivity, rapid pollen tube growth along the style, as well as adequate ovule longevity. This manuscript presents the study of the effective pollination period (EPP) of four 'Oblačinska' sour cherry clones (II/2, III/9, XI/3 and XIII/1) that differs in pollen germination, fruit set and yields. In order to estimate EPP, pollination was conducted in six different stages of flower development: balloon stage, 2 d before anthesis (-2), at anthesis (0), and 2, 4, 6 and 8 d after anthesis (DAA). The initial (IFS) and final fruit set (FFS) were recorded under the field conditions. Alongside with this, the rate of pollen tubes growth in the style was observed with fluorescent microscopy. The experimental design was completely randomized, a two-factorial analysis of variance was carried out and individual testing was performed using LSD test ($p \leq 0.05$; $p \leq 0.01$). The experiment was set in triplicates. Regarding FFS, clones II/2 and III/9 showed the best results ($p \leq 0.01$) in 4 and 6 DAA. The number of pollen tubes in the style of the pistil decreased with subsequent terms of pollination, while its number in the ovule increased up to sixth day after pollination, followed by a decline. Clones II/2 and III/9 showed EPP which lasted from 6 to 8 d, while EPP found in clone XI/3, lasted only 2 d. It is concluded that only clone having long EPP should be used as parents for creating new sour cherry cultivars.

Key words: *Prunus cerasus*, stigma receptivity, fluorescent microscopy, pollen tube growth, fruit set

INTRODUCTION

The 'Oblačinska' sour cherry (*Prunus cerasus* L.) is an autochthonous and heterogeneous cultivar, considered to be self-compatible; besides, it is also highly resistant to leaf spot and to bitter rot (GRZYB and ROZPARA, 2004). Long-term cultivation in diverse agro-ecologic conditions and the use of both vegetative and sexual propagation have influenced the 'Oblačinska' sour

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cherry to become a mixture of numerous genotypes (RAKONJAC *et al.*, 2010). The first selection from a diverse gene pool was conducted by local farmers with the aim to obtain certain desirable traits. NIKOLIĆ *et al.* (2005a, b) and RADE *et al.* (2008) noticed that despite the variability in vigor and other pomological traits, differences in fruit set and yields are significant.

For sour cherry, pollination and flower fertilization are considered the most important factors affecting both fruit set and fruit yield. Some biological features of sour cherries, such as low flower fertility potential of cultivars, short period of pollination and low pollen quality, cause the low effectiveness of pollination level and may be the reason for poor fruit set (SZPADZIK *et al.*, 2008). From a practical point of view, in order to obtain the expected yields it is necessary to determine the time lag from pollination to fertilization.

Frequent fruit set failure in apple led WILLIAMS (1965) to develop the concept of the effective pollination period (EPP). The EPP is defined as the number of days during which pollination is effective in producing a fruit and is determined by the longevity of the ovules minus the time lag between pollination and fertilisation. Therefore, it links female fertility and pollination and is an expression of the likelihood that the flowers set fruit. Theoretically, each normally-developed flower is able to set a fruit if pollinated with the appropriate pollen just after anthesis (BURGOS *et al.*, 2004). Delays in stigma maturation or a short receptivity period may limit the EPP (SANZOL *et al.*, 2003). The rate of pollen tube growth in *Prunus* pistils depends of pistilar genotype, pollen genotype, pollen competition and environmental factors (HEDHLY *et al.*, 2004; HEDHLY *et al.*, 2005), while ovule viability on cultivar and temperatures during anthesis (CERVOIC and MIĆIĆ, 2000). The duration of the EPP in fruits is highly variable, depending on species, cultivar, and environmental conditions, and varied from 2 days in apricot (SANZOL and HERERRO, 2001) and *Citrus unshiu* (MESEJO *et al.*, 2007) to 12 days in some olive cultivars (CUEVAS *et al.*, 2009). The EPP has been proved to have great impact on post-fertilization fruit drop, and it plays a significant role in year-to-year cropping variability.

Since the EPP is limited by stigma receptivity, pollen tube kinetics and ovule longevity, there must be a good synchronization among them. However, genetic factors may unbalance this process and, therefore, decrease fruit setting. The most favorable condition for fructification is when ovules are at intermediate stages of development (embryo sacs with four to eight nuclei) at anthesis (ALBURQUERQUE *et al.*, 2002). Besides, external factors such as temperature and chemical treatments, flower quality is responsible for modification of EPP (SANZOL and HERERRO, 2001).

EPP has been studied in different fruit tree species, such as apple (GUERRERO-PRIETO *et al.*, 2009), pear (SANZOL *et al.*, 2003), japanese quince (KAUFMANE and RUMPUNEN, 2002), peach (REZAIIE *et al.*, 2011), sweet cherry (HEDHLY *et al.*, 2003), almond (ORTEGA *et al.*, 2004), olive (ORLANDI, 2005; CUEVAS *et al.*, 2009), rabbiteye blueberry (BREVIS *et al.*, 2006) and mandarin and orange (MESEJO *et al.*, 2007).

So far, EPP and their relation with fruit productivity in 'Oblačinska' sour cherry are unknown. Therefore, the objective of this study was to determine the duration of EPP in four 'Oblačinska' sour cherry clones (with different fruiting potential) under orchard conditions and in laboratory using fluorescent microscopy.

MATERIALS AND METHODS

This study was carried out at the Experimental Station Radmilovac, University of Belgrade, Faculty of Agriculture. The experiment started by forming a collection of 'Oblačinska' sour cherry accessions. The orchard was planted in 1993, planting distance was 4 x 2 m and the

trees were trained as spindle bush, under non-irrigated and standard cultural practices. After five years of diverse accessions investigation, we selected four genotypes using values of pollen germination, fruit set and yields. Genotypes II/2 and III/9 were distinguished by its *in vitro* high pollen germination, high fruit set and high yields; while genotypes XI/3 and XIII/1 showed lowest values of pollen germination, fruit set and fruit yield.

Shoots with flower buds at the balloon stage were collected, transported to the laboratory and placed in jars with water and kept at room temperature (20 ± 2 °C). Emasculation of flowers was done immediately. To follow the flower development one branch in balloon stage was separated. Pistils were hand-pollinated 24 h after emasculation with pollen mixture of Oblačinska' sour cherry clones. *In vitro* germinability of pollen was tested on a germination medium containing agar (0.3 %) and sucrose (14 %). All pollen tested showed values of germination over 80 % viable (data not shown). Hand cross-pollination was conducted at 6 stages of flower development: 2 d before opening (-2), during anthesis (0), 2 d, 4 d, 6d and 8d after anthesis. For each date of pollination (-2, 0, 2, 4, 6, 8), fixation of pistils was done 5 d (120 h) after pollination in a 5:5:90 (v/v/v) mix of 40 % (v/v) formaldehyde, glacial acetic acid, and 70 % (v/v) ethanol (FAA). Fixed material was kept at + 4 °C until staining to allow compatible pollen tubes to reach the ovary and ovule (MILATOVIĆ and NIKOLIĆ, 2007).

Staining pistils with anilin-blue and their preparation for microscopic examination was done as described by MILATOVIĆ and NIKOLIĆ (2007). Examination of pistils was carried out by fluorescence microscopy using a Leica DM LS microscope (Leica Microsystems, Wetzlar, Germany), equipped with an I3 blue filter (wavelength 450 – 490 nm). Throughout the process, we analyzed at least 20 pistils from each cultivar, but the only pistils analyzed were the ones with > 20 pollen grains on the stigma.

The dynamics of pollen tubes growth was analyzed and showed as the average number of pollen tubes in: 1) the upper third of the style, 2) at the bottom of the style, and in the 3) ovary.

Besides the laboratory tests, we tested fertilization in field conditions. Approximately 100 flowers were used for each pollination date. On each genotype, six cardinaly-oriented branches, each with 25-30 flower clusters, were selected and labeled. Open flowers (if any occurred) and immature buds were removed and the flowers at the balloon stage were emasculated. Hand pollinations, using a paintbrush, with the pollen mixture was done in the same terms like in laboratory conditions (-2, 0, 2, 4, 6 and 8 d after anthesis). Each branch was used for one pollination term. We evaluated initial fruit set (IFS) for each date as the percentage of pollinated flowers that became fruits 21 d after pollination and final fruit set (FFS) just before harvesting.

The experimental design was completely randomized and data was analyzed using Statistica (StatSoft, Inc., Tulsa, OK, USA) with a two-factorial analysis of variance. F-test estimated the significance of influence of clones and terms of pollinations, as well as their interaction. Individual testing was performed using LSD test ($p \leq 0.05$; $p \leq 0.01$).

RESULTS AND DISCUSSION

SANZOL and HERRERO (2001) report that the stigma is receptive in the anthesis in numerous fruit species, but its short life span can affect low fruit set in some fruit species. However, the receptive period can vary with species or cultivar (YI *et al.*, 2006). Stigma receptivity in all four 'Oblačinska' sour cherry clones was not delayed, indicating that young flowers supported pollen germination since genotypes set fruits during anthesis (0) or even in the balloon stage (-2), in some

case over 20 %. Similar results were obtained for almond by EGEA and BURGOS (2000), in the cases when acceptable fruit set was achieved from the first day after pollination. Surprisingly, our experiments have shown that pollen grains have germinated even on stigmas that turned brown. Even at 8 DAA, normal germination of pollen grains has occurred on the stigma despite the fact that papillae were totally collapsed. Percentage of IFS that varied from 6.75 to 55.45 % was observed in flowers pollinated at both younger and older flower stages (Figure 1) with statistically significant differences between the clones (Table 1). Similar results were observed for FFS (Figure 1). Clones II/2 and III/9 showed higher values of fruit set (78-89%) than in clones XI/3 and XIII/1. Furthermore, FFS showed moderate to satisfactory values in all pollination dates, and for all clones studied. Also, it was noticed that in all pollination terms, except for the first and last one, the differences between IFS and FFS were similar and were probably the result of the physiological fruit drop.

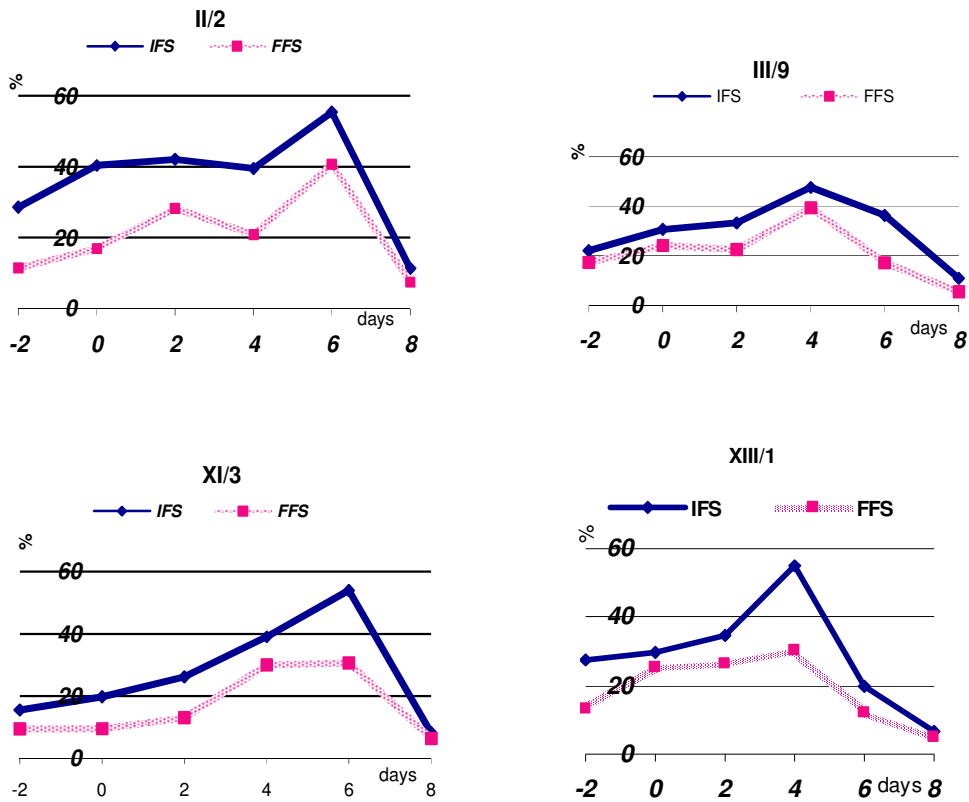


Figure 1. Initial (IFS) and final (FFS) fruit set in four 'Oblačinska' sour cherry clones

The maximum IFS and FFS ($p \leq 0.01$) in clone II/2 (55.45, and 40.77%, respectively) were observed 6 d after anthesis (DAA) (Figure 1). In clone III/9 the maximum FFS (39.49%, $p \leq 0.01$) was attained from pollinations carried out 4 DAA, while this percentage decreased with later pollinations. Genotype XI/3 had the highest IFS and FFS 6 DAA (53.86, and 30.6% respectively), while the peak was not significantly higher than in 4 DAA. XIII/1 achieved the maximum FFS (30.48%) from pollinations carried out 4 DAA but not significantly different from the results obtained in 2 DAA (26.16%). Our results correspond to the findings of CUEVAS *et al.* (2009) who found that in many species, good correlations have been found between the percentages of fruit set following delayed pollination. For all four clones studied herein, fruit set values decreased abruptly and become significantly lower with pollination realized at 8 DAA. As for the maximum of IFS and FFS, the differences between the clones can be explained by the fact that genotypes III/9 and XIII/1 probably have early ovule degradation due to higher temperatures as flowering progressed (data not shown). SANZOL and HERRERO (2001) showed that high temperatures during flowering, shorten ovule longevity affecting EPP. For IFS, there were significant differences between clones and terms of pollination, where its interaction also being significant. Regarding FFS, no differences were found between clones, while the difference in the terms of pollination was significant (Table 1).

Table 1. Degree of freedom (df), mean square (MS) and LSD values from ANOVA for initial fruit set (IFS) and final fruit set (FFS) in four 'Oblačinska' sour cherry clones

Sources of variation	df	IFS			FFS		
		MS	LSD _{0.05}	LSD _{0.01}	MS	LSD _{0.05}	LSD _{0.01}
Clone	3	142.96 [†]	4.09	5.48	48.59	-	-
Pollination term	5	2644.78 ^{††}	5.01	6.7	1515.14 ^{††}	4.51	6.04
Interaction	15	592.02 ^{††}	10.02	13.41	455.86 ^{††}	9.04	12.09
Error	48	36.905			29.988		

[†] $p \leq 0.05$; ^{††} $p \leq 0.01$.

Considering the fact that the fruit set above 30% are related with high yields, we consider the fruit set between 15-25 % gives average yields. The evaluation of the EPP using sequential pollination showed that all examined 'Oblačinska' sour cherry clones showed different patterns. Since clone II/2 achieved 16.94% fruit set in 0 DAA that gradually increased to the maximum fruit set (40.77%) in 6 DAA and decreased rapidly in 8 DAA, we assume that its EPP is 6 days. On the other hand clone III/9 showed satisfactory yields if pollination was carried out in the balloon stage (-2 DAA). The maximum yield was in the 4 DAA (39.49 %), but it was still enough in 6 DAA (17.22 %), thus, its estimated EPP is 8 d. Clones II/2 and III/9, which have long EPP can easily withstand unfavorable environmental conditions during flowering time and therefore enable high yields. Clone XI/3 had no economically acceptable yields up to the 4 DAA. Its maximum of FFS was reached in 6 DAA (30.6 %), so its appraised EPP is only 2 d. Clone XIII/1 in 0 DAA had 26.16 % of fruit set keeping satisfying yields up to the 4 DAA (30.48 %), so its assessed EPP is 4 d.

Pollen tube growth in all 'Oblačinska' sour cherry clones started with pollen grains germination on the funnel-like surface of the stigma. After penetrating the stylar tissue during their further

growth, the tubes progressed towards the transmitting tissue which extends, as the central cylinder, along the whole length of the style. The study of pollen tubes in the upper part of the style revealed that stigma receptivity decreases with subsequent terms from flower opening. For the most of the clones studied, the highest number of pollen tubes in the upper part of the style correspond to days -2 and 0, and the lowest number to day 8 (Figure 2). This was probably due to the highest stigma receptivity in early stages of flower development. The number of pollen tubes at the base of the style had a similar pattern but with lower values.

Terms of pollination had the greatest impact to pollen tubes number in different parts of the style, followed by interaction clone x pollination term. Besides, and only for number of pollen tubes in STB, the differences between clones were significant, while for number of pollen tubes in STU and OV no differences were found (Table 2).

Regardless of the fact that the highest number of pollen tubes in the STU and STB were found in the earliest stages of flower development, fruit set in these terms of pollination was the lowest. This could be explained with the fact that maturation of the pistil was not complete, because maturation occurs during the flower life in a basipetal way, starting at the stigma and proceeding along the style down to the ovule. Contrary to this, as flowering progressed the number of pollen tubes in the ovary increased. ALBURQUERQUE *et al.* (2004) demonstrated that, in apricot cultivars with more highly developed embryo sacs, pollen tubes grew faster, and number pollen tubes inside the ovary increased with subsequent pollination. Exception to this rule is the 8 DAA, where probably ovule degeneration started. Nevertheless, the distinction between different terms of pollination regarding number of pollen tubes in all parts of the pistil, for each clone, was not evident neither for IFS, nor for FFS. This means that even a low number of pollen tubes could be enough to ensure fruit set. Similar results were obtained by ORTEGA *et al.* (2004) for almond.

Table 2. Degree of freedom (df), mean square (MS) and LSD values from ANOVA for number of pollen tubes in the upper part of the style (STU), basal part of the style (STB) and in the ovule (OV) in four 'Oblačinska' sour cherry clones.

Sources of variation	df	STU			STB			OV		
		MS	LSD _{0.05}	LSD _{0.01}	MS	LSD _{0.05}	LSD _{0.01}	MS	LSD _{0.05}	LSD _{0.01}
Clone	3	24.57	-	-	14.30 ^{††}	1.35	1.81	0.130	-	-
Pollination term	5	208.02 ^{††}	3.04	4.07	213.17 ^{††}	1.66	2.23	2.821 ^{††}	0.21	0.29
Interaction	15	31.83 ^{††}	6.08	8.14	22.00 ^{††}	3.33	4.45	0.734 ^{††}	0.44	0.58
Error	48	13.579			4.068			0.069		

^{††} p ≤ 0.01.

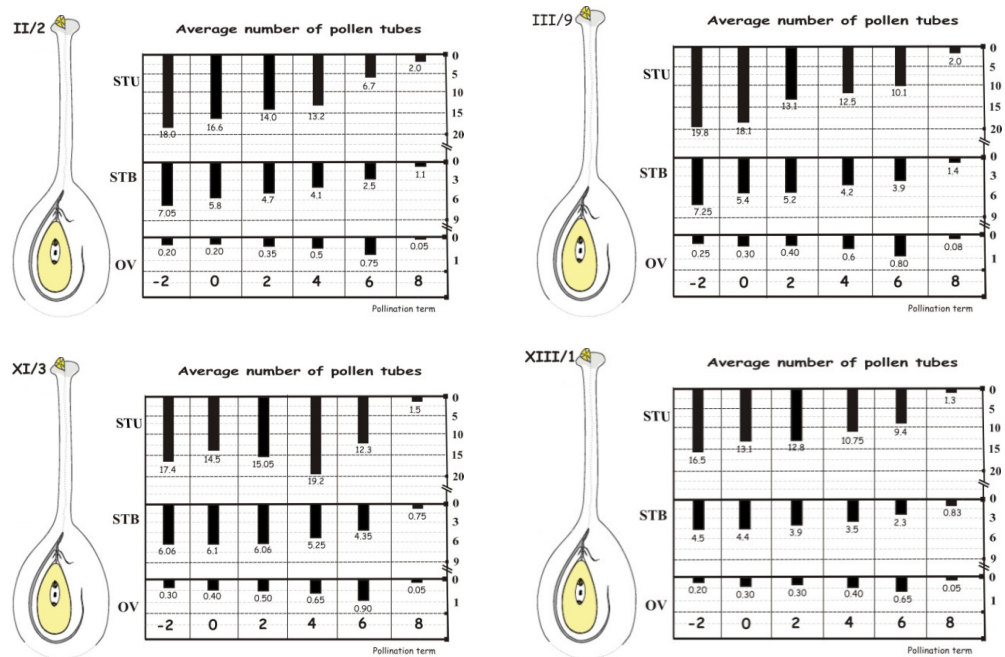


Figure 2. The average number of pollen tubes in different sections of the style [upper part of the style (STU), bottom part of the style (STB), ovule (OV)] at -2, 0, 2, 4, 6 and 8 DAA in four 'Oblačinska' sour cherry clones.

CONCLUSION

Stigma receptivity in 'Oblačinska' sour cherry clones is optimal during anthesis. Stigmas at younger stages of flower development can support pollen germination, but in some clones further maturation of the pistil is required.

Estimated duration of EPP in all studied 'Oblačinska' sour cherry clones was quite different, and it ranged from two days in clone XI/3 up to eight days in clone III/9.

Regarding future breeding work, only clones that have long EPP should be used as parents for creating new sour cherry cultivars, because its ovule longevity can ensure regular and high yields.

ACKNOWLEDGEMENTS

The research is a part of the projects TR 31063 (funded by Ministry of Education, Science and Technological Development of the Republic of Serbia) and FP7 Project AREA 316004.

We would also like to thank Mrs. Zorica Trbuilin, the head teacher and the Chief of the Department for Foreign Language Courses at the Military Academy in Belgrade, for assistance with the text check and correction.

Received August 01th, 2013

Accepted September 05th, 2014

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EFEKTIVNI POLINACIONI PERIOD KLONOVA OBLAČINSKE VIŠNJE

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Izvod

Produkcija cvetova i procenat zamatanja predstavljaju važne osobine u proizvodnji višnje (*Prunus cerasus* L.), jer sa njihovim povećanjem raste i prinos. Pored toga, usled kratke vitalnosti jajne ćelije kod nekih sorti višnje koja sazreva odmah nakon otvaranja cvetova, zadovoljavajuća receptivnost žiga tučka, brzo oprašivanje i brz prodor polenove cevčice niz stubić tučka su jako bitni kako bi se osigurala oplodnja vitalne jajne ćelije. U ovom radu ispitivan je efektivni polinacioni period (EPP) kod četiri klona Oblačinske višnje (II/2, III/9, XI/3 and XIII/1) koji se međusobno razlikuju u stepenu klijavosti polena, zametanju plodova i prinosu. Kako bi se procenio EPP, oprašivanje je izvedeno u šest različitih stadijuma razvoja cveta: faza balona, tj. dva dana pre otvaranja cveta (-2), u vreme cvetanja (0), i 2, 4 i 6 dana nakon otvaranja. Inicijalno (IFS) i finalno (FFS) zamatanje utvrđeno je u poljskim uslovima. Pored toga, broj polenovih cevčica u tučku utvrđeno je metodom fluorescentne mikroskopije. Eksperiment je postavljen po potpuno slučajnom planu sa tri ponavljanja. Primenjena je dvo-faktorijalna analiza varijanse, a individualno testiranje izvršeno je LSD testom ($p \leq 0.05$; $p \leq 0.01$). Uzimajući u obzir FFS, klonovi II/2 i III/9 dali su najbolje rezultate ($p \leq 0.01$) 4 i 6 dana posle otvaranja cvetova. Broj polenovih cevčica u stubiću se smanjivao sa kasnijim oprašivanjem, dok se njihov broj u plodniku tučka povećavao do 6 dana posle otvaranja cvetova, praćen kasnijim naglim padom. EPP kod klonova II/2 i III/9 trajao je od šest do osam dana, dok je kod klona XI/3 trajao samo dva dana. Iz svega ovoga može se zaključiti da klonovi koji imaju duži EPP se mogu koristiti kao roditelji za stvaranje novih sorti višnje.

Primljeno 01. VIII. 2013.

Odobreno 05. IX. 2014.