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RESEARCH ARTICLE

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Pollen tube growth and fruit set in quince (*Cydonia oblonga* Mill.)

Aleksandar Radovic (Radovic, A)[1], Radosav Cerovic (Cerovic, R)[2], Dragan Milatovic (Milatovic, D)[1], and Dragan Nikolic (Nikolic, D)[1]

[1] *University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Republic of Serbia.*
[2] *University of Belgrade, Innovation Center of Faculty of Technology and Metallurgy, Karnegijeva 4, 11120 Belgrade, Republic of Serbia.*

Abstract

Aim of study: To determine the self-compatibility level of eight quince cultivars.

Area of study: The region of Belgrade (Central Serbia).

Material and methods: Pollen tube growth *in vivo* and fruit set in two pollination variants (self- and open-pollination) were studied in eight quince cultivars. The quantitative parameters of pollen tube growth (average number of pollen tubes in the upper and middle third of the style, base of the style and in the ovary; the dynamics of pollen tube growth through these parts of the pistil) was determined using the fluorescence microscopy.

Main results: The parameters of pollen tube growth and fruit set were primarily dependent on the genotype and variants of pollination. All studied parameters were significantly higher in the open-pollination variant compared with the self-pollination in all cultivars. In the self-pollination variant, 'Leskovacka' and 'Vranjska' had the highest number of pollen tubes that penetrated the ovary (2.10 and 0.54 in average, respectively), as well as the largest percentage of pistils with the penetration of pollen tubes in the nucellus of ovules six days after pollination (40.09% and 14.74%). Also, they had the highest percentage of initial fruit set (17.01% and 28.52%) and final fruit set (9.32% and 9.86%). Based on this, 'Leskovacka' and 'Vranjska' can be classified as self-compatible cultivars, while the others are self-incompatible.

Research highlights: The majority of quince cultivars were self-incompatible. When establishing new orchards with these cultivars, care should be taken about the choice of pollenisers in order to achieve high yields.

Additional keywords: *Cydonia oblonga* Mill.; pistil; self-pollination; open pollination; pollen tube growth *in vivo*; fluorescence microscopy.

Abbreviations used: ISI (self-incompatibility index).

Authors' contributions: Design of the study, analysis and interpretation of data: AR, RC & DN. Performing the study: AR & DM. Writing the paper: AR. Critical reading and improving the paper: RC, DN & DM. All authors read and approved the final manuscript.

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Correspondence should be addressed to Aleksandar Radovic: radovicaleksandar@yahoo.com

Introduction

Quince (*Cydonia oblonga* Mill.) belongs to the *Rosaceae* family and the *Maloideae* (*Pomoidae*) subfamily. The *Maloideae* subfamily has about 1,000 species and 30 genera, including the genus *Cydonia*, which has only one species, *Cydonia oblonga* Mill., with a larger number of varieties (Bell & Leitão, 2011). Quince is very interesting for growing, not only in gardens, but in commercial orchards as well. It is characterized by high-quality and aromatic fruits that are suitable for different purposes, especially for processing into juice, compote, jam, jelly, and more recently a brandy that is highly appreciated due to its particular aroma (Patel *et al*., 2011; Rop *et al*., 2011). Comparing to other fruit species, the production of quince is low in Serbia. It amounts to 12,556 t, 2015-2017 average (http://faostat.fao.org). In the assortment local cultivars are largely dominated. The leading cultivar is

'Leskovacka', while 'Vranjska' is used as its polleniser. 'Leskovacka' is characterized by high quality of fruits, but relatively low yield. Contrary, 'Vranjska' is characterized by higher yields, but lower fruit quality (Radović *et al*., 2015, 2016a). Therefore, work is needed to introduce more cultivars into the production, which will achieve high yields and have quality fruits suitable for different purposes, especially for processing (Radović *et al*., 2015).

For successful introduction of new quince cultivars in commercial orchards, adequate knowledge of the processes related to their reproductive biology, in addition to their technological and pomological characteristics, is required. For achieving good fruit set and high yields, it is necessary to conduct successful pollination and fertilization where many biophysical, biochemical and physiological processes must take place at the right time (De Graaf *et al*., 2001). These processes depend on several factors: the success of pollen transfer; pollen quality; stigma receptivity; pollen germination and pollen tube growth; the appearance of incompatibility and ovule longevity (Stösser *et al*., 1996; Guerrero-Prieto *et al*., 2009).

Sexual incompatibility represents a dynamic barrier in the fertilization process. In species of the *Rosaceae* family, there is a gametophyte system of incompatibility, in which the fertilization outcome is determined by the haploid *S*-genotype of pollen (Hegedűs *et al*., 2012). This type of incompatibility is controlled by two genes of *S*-locus, with one of them controlling the style component (*S*-RNases) (Bošković *et al*., 2006; Sonneveld *et al*., 2006) and the other the pollen component (*S* specific F-box protein) (Sonneveld *et al*., 2005). There are self-compatible mutants in some fruit species, *e.g.* in Japanese pear (Sassa *et al*., 1993). There is no natural self-compatible mutant *S*-haplotype in apples, while pears and Japanese pears are characterised by non-functional alleles and stylar-part mutants. Although these gene components are identified in the style and pollen, their specific interaction is still not elucidated. It is believed that other loci have a decisive influence on the self-incompatibility system, although the isolation of such a modifier locus from tree fruit species has not yet been achieved (Hegedűs *et al*., 2012). So far, sexual incompatibility research based on molecular markers has not been conducted on quince.

Self-incompatible cultivars often produce a lower yield, as fruit set depends on the abundance of pollen transfer from other trees (Milatović *et al*., 2013). From the aspect of production practice and breeding, self-fertile cultivars are of the highest value, because when growing partially self-fertile and self-incompatible cultivars it is necessary to provide adequate pollenisers (Nikolić & Milatović, 2010). In addition to the appearance of incompatibility, low pollen germination and slow growth of the pollen tube may affect the poor fruit set in some combinations of pollination (Sharafi *et al*., 2010). The level of self-compatibility can be determined on the base of fruit set in field conditions after self-pollination (But & Klimenko, 2001; Ruiz & Egea, 2008). However, this method for the determination of self-compatibility is not always reliable, because its results are heavily dependent on environmental conditions (Milatović & Nikolić, 2007; Sanzol & Herrero, 2007). Therefore, a more reliable method for determining the level of self-compatibility is to monitor the growth of pollen tubes through the style and ovary using fluorescent microscopy (Martin, 1959). Besides the genotype, environmental conditions also have a significant influence on all reproductive processes in fruit trees (Nyéki & Szabó, 1996; Hedhly *et al*., 2005).

The aim of this study was to examine the pollen tube growth in the pistils and fruit set in eight quince cultivars in the self-pollination and open-pollination variants. The purpose was also to define the level of self-compatibility and the selection of self-compatible cultivars.

Material and methods

Site and plant material

The studies were carried out in the collection orchard of quince on the 'Radmilovac' Experimental Station of the Faculty of Agriculture of the University of Belgrade. It is located near Belgrade, at latitude 44º 45', longitude 20º 35', and an altitude of 130 m. The climate is temperate continental, with an average annual temperature of 11.7ºC, an average temperature over the growing season (April–October) of 17.5ºC and a total annual rainfall rate of 693.9 mm. The orchard was planted in the spring of 1999, with spacing of planting 4.5 × 3 m. The rootstock is 'quince MA', and the training system is modified central leader. The studies were conducted in the period from 2010 to 2012. The following eight quince cultivars were used for the analyses: 'Asenica', 'Hemus', 'Leskovacka', 'Morava', 'Pazardzijska', 'Portugal', 'Triumph' and 'Vranjska'. These cultivars had previously showed good agronomical and pomological traits in environmental conditions of Serbia (Radović *et al*., 2015; 2016a).

Air temperature during the flowering

During the flowering period, the air temperature in the orchard was monitored, and average mean daily temperature was calculated for each cultivar, from the beginning of full flowering until the sixth day after it. The air temperature was measured at a height of 1.5 m by an automatic MeteosCompact weather station (Pessl Instruments

GmbH, Austria) placed in the immediate vicinity of the experimental orchard.

Average mean daily temperatures from the beginning of full flowering until the sixth day after it by years were: 20.9, 14.0, 19.3ºC ('Leskovacka'); 17.4, 17.0, 14.5ºC ('Vranjska'); 18.2, 16.5, 17.0ºC ('Morava'); 18.9, 15.2, 17.0ºC ('Pazardzijska'); 18.2, 16.5, 14.5ºC ('Hemus'); 17.4, 17.0, 14.5ºC ('Asenica'); 17.4, 17.0, 14.5ºC ('Portugal') and 18.2, 15.9, 15.3ºC ('Triumph') (Fig. 1). Average mean daily temperatures during flowering in all cultivars were highest in 2010, while in most cultivars they were lowest in 2012.

Pollen germination *in vitro*

For examination of pollen germination, branches with flower buds in the 'balloon' phase were taken and carried to the laboratory. In order to collect pollen from the flower buds, anthers were isolated in Petri dishes. They were stored at room temperature (20±2ºC) for 24-48 h to dry and to release the pollen. Then, the pollen was sown with fine brushes in Petri dishes (9 cm diameter) on the previously prepared nutrient medium consisting of 15% sucrose and 0.7% agar-agar. After incubation of 24 h at 20±2ºC, the Petri dishes with the sowed pollen were observed under a Leica DM LS light microscope (Leica Microsystems, Wetzlar, Germany) for counting of germinated pollen grains. The experiment was done in three repetitions, each including at least 300 pollen grains. Pollen grains with tubes exceeding their radius were considered as germinating (Galleta, 1983).

Pollination and pistil fixation

The pollination tests were performed under field conditions in two variants (self- and open-pollination). In the self-pollination variant, during the late 'balloon' stage, emasculation of flowers was performed. In each cultivar, three branches were chosen, containing about 450 flowers in total. At the beginning of full flowering (two days after the emasculation), hand pollination of the emasculated flowers of each cultivar with its own pollen was carried out. Isolation of flowers with a paper bag after hand pollination was not done, because emasculated flowers are not visited by insects. Besides, temperatures inside the bags are often higher than those in the open air that can cause poor fertilization (Vuletin Selak *et al*., 2011). On the same day that the self-pollination was carried out, three branches for testing the open-pollination were selected (450 flowers per cultivar), where the flowers were left to develop freely. In the three successive periods (2, 4 and 6 days after pollination) the fixation of the pistils was performed, in the formaldehyde-acetic acid-alcohol (FAA) fixative consisting of 70% ethanol, glacial acetic acid and formaldehyde in relation to 90:5:5 parts by volume. The fixed material was stored in a refrigerator at +4ºC until staining.

Staining and microscopic observation of pistils

For the study of pollen tube growth in the pistils, the staining was performed with 0.1% aniline blue dissolved in 0.1 N K_3PO_4 (Preil, 1970). To prepare pistils for microscopic examination, the styles were separated from the ovary. The styles were squashed, while the ovary was cut with a razor blade to detect a penetration of pollen tubes in the ovules (Cerović & Ružić, 1992). The examination of pistils was carried out using a fluorescent microscope Leica DM LS (Leica Microsystems, Wetzlar, Germany) with the use of filters A (wavelength 340-380 nm) and I3 (wavelength 450-490 nm).

The pollen tubes were counted in the style (upper third, middle third and base) and in the ovary, and the results are presented as the average number of the three fixation periods. The dynamics of pollen tube growth from the stigma up to their penetration in the nucellus of the ovule is presented as percentages of pistils in which the pollen tubes penetrated to their particular parts. From each cultivar, for both types of pollination, 30 pistils were analyzed.

Fruit set

The initial and final fruit set were determined in two variants of pollination (self- and open-pollination). Three weeks after the full bloom, the percentage of initial fruit set was determined. Percentage of final fruit set was determined just before the harvest. For both methods of pollination, 300 flowers of each cultivar were analyzed. The classification of the cultivars for the self-compatibility level was done using self-incompatibility index (ISI). It is calculated as a ratio: final fruit set in self-pollination/final fruit set in open-pollination. According to this index, cultivars can be divided into three groups: ISI > 0.3 = self-compatible, ISI 0.29 to 0.1 = partially self-incompatible, and ISI 0.1 to 0.0 = completely self-incompatible (Koubouris *et al*., 2014).

Statistical analysis

The data were statistically analyzed using a two-factor analysis of variance (ANOVA). For the results expressed in percentages, the arcsin square-root data transformation was performed. The significance of differences between the mean values was determined

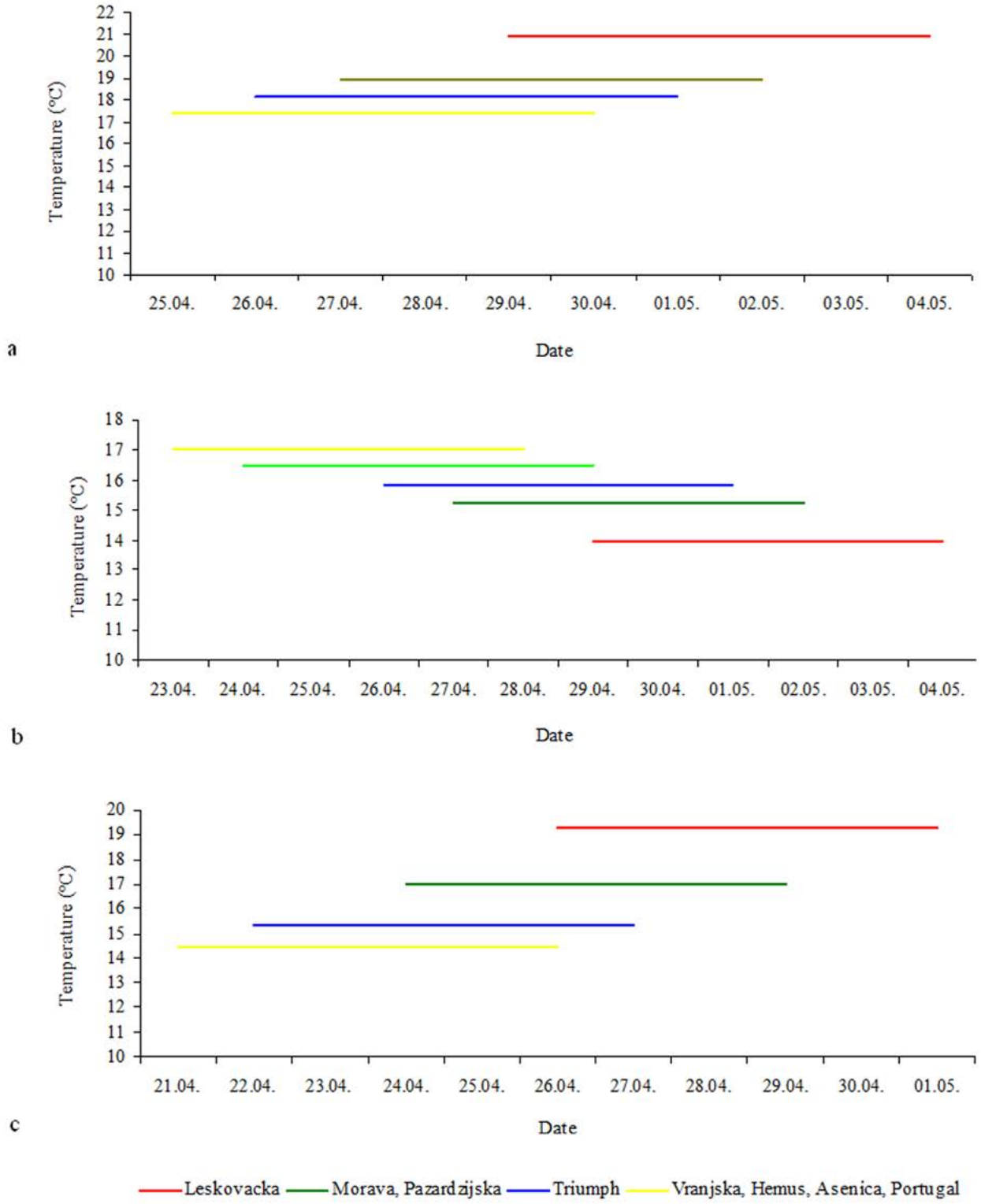

Figure 1. Average mean daily temperatures from the beginning of full flowering until the sixth day after it: a) 2010, b) 2011, and c) 2012.

using the Tukey's test for significance level $p \leq 0.05$. Correlations among the parameters were determined by correlation-regression analysis and Pearson's correlation coefficients. The analysis has been conducted in two pollination variants (self- and open-pollination). Data analysis was performed using the statistical software package Statistica, Version 8 (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results

Pollen germination *in vitro*

In vitro pollen germination was very high in the investigated cultivars (Fig. 2). It was significantly different among the quince cultivars. It averaged from 64.62% in 'Leskovacka' to 86.43% in 'Asenica'. 'Leskovacka' had a significantly lower pollen germination compared to the other cultivars. In 2012, there was a lower pollen germination compared to the other two years of research.

Pollen tube growth efficiency

After pollen grains reach the stigma, they start to germinate and lengthen in the pollen tubes (Fig. 3a). Pollen tubes penetrate the style through the so-called 'transmitting tissue' to its base and enter the locule of the ovary (Figs. 3b,c,d,e). Then, they pass through the obturator, penetrate the ovule micropyle and eventually penetrate the nucellus (Fig. 3f).

Pollen tube growth efficiency in the style and ovary was examined using two parameters. The first one was the number of pollen tubes in certain regions of style (upper third, middle third and base) and the locule of the ovary. The second parameter was the dynamics of pollen tube growth through the upper third, middle third and base of the style, ovary locule, and finally the nucellus of the ovule.

The number of pollen tubes in the various sections of the pistil differed between genotypes and years and there was also a genotype by year interaction (Table 1). In a variant of self-pollination the number of pollen tubes in the upper third of the style ranged from 17.48 ('Morava') to 23.67 ('Hemus'), in the middle third of the style from 0.40 ('Pazardzijska') to 10.60 ('Leskovacka'), and in the base of the style from 0.03 ('Pazardzijska and 'Triumph') to 7.22 ('Leskovacka'). The declining number of pollen tubes continued into the ovary. Excluding the 'Leskovacka' (2.10) and 'Vranjska' (0.54) cultivars, the number of pollen tubes in other cultivars was negligible (less than 0.15). In contrast, the number of pollen tubes following open pollination was higher in all parts of the style, as well as the ovary. It amounted from 27.55 to 35.44 in the upper third of the style, then from 6.75 to 17.85 in the middle third of the style, from 2.97 to 12.02 in the base of the style and from 0.85 to 2.66 in the ovary. Overall, the number of pollen tubes heavily declined in all of the examined cultivars from the upper third of the style to the ovary. This decrease was particularly evident in the variant of self-pollination. In the upper third of the style incompatible pollen tubes were found. These pollen tubes have typical swellings at the tips due to higher accumulation of callose, but there were also a number of short and thickened pollen tubes (Figs. 3g,h). Sometimes these incompatible pollen tubes with a presence of callose could also be detected, although less frequently, in the variant of open pollination.

The dynamics of pollen tube growth in the style and ovary of the pistil differed between genotypes, years and the pollination method (Fig. 4). In the variant of self-pollination, on the second day after the pollination a penetration of pollen tubes in the locule of the ovary was observed in the 'Leskovacka' cultivar during all three years of study, in the 'Portugal' cultivar in 2011 and 2012 and in the 'Vranjska' cultivar in 2010. The penetration of

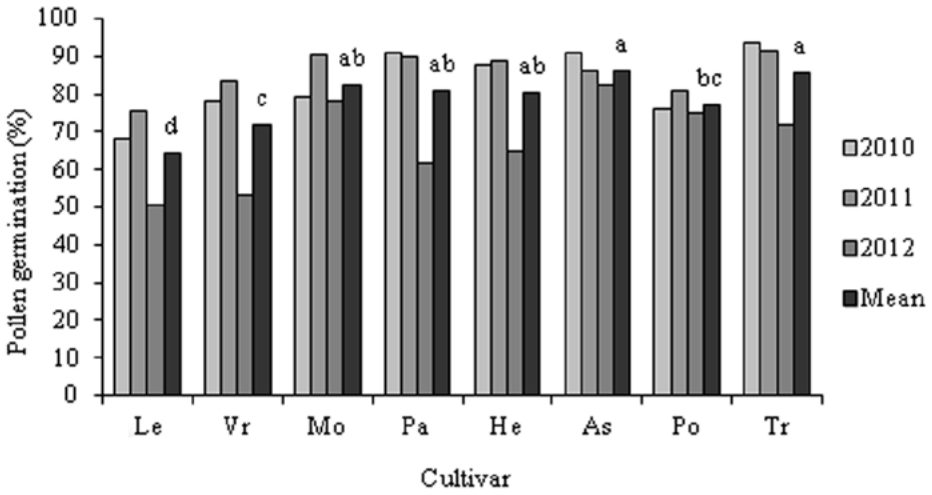

Figure 2. Pollen germination *in vitro* of quince cultivars. Le, Leskovacka; Vr, Vranjska; Mo, Morava; Pa, Pazardzijska; He, Hemus; As, Asenica; Po, Portugal; Tr, Triumph. Different letters above the histogram bars indicate significant differences between groups ($p \leq 0.05$, Tukey's test).

pollen tubes in the locule of the ovary in this period was most frequent in the 'Leskovacka' cultivar in 2011 (in 14.29% of the pistils). For other cultivars, the penetration of pollen tubes in the locule of the ovary was determined on the fourth day after pollination ('Morava' and 'Hemus' in 2012, 'Pazardzijska' in 2011 and 'Asenica' in 2010 and 2012). However, for the 'Triumph' cultivar, pollen tube penetration into the locule was determined only on the sixth day after pollination (in 2011).

Unlike in self-pollination, penetration of pollen tubes in the locule was determined on the second day after the open pollination for most cultivars included in the

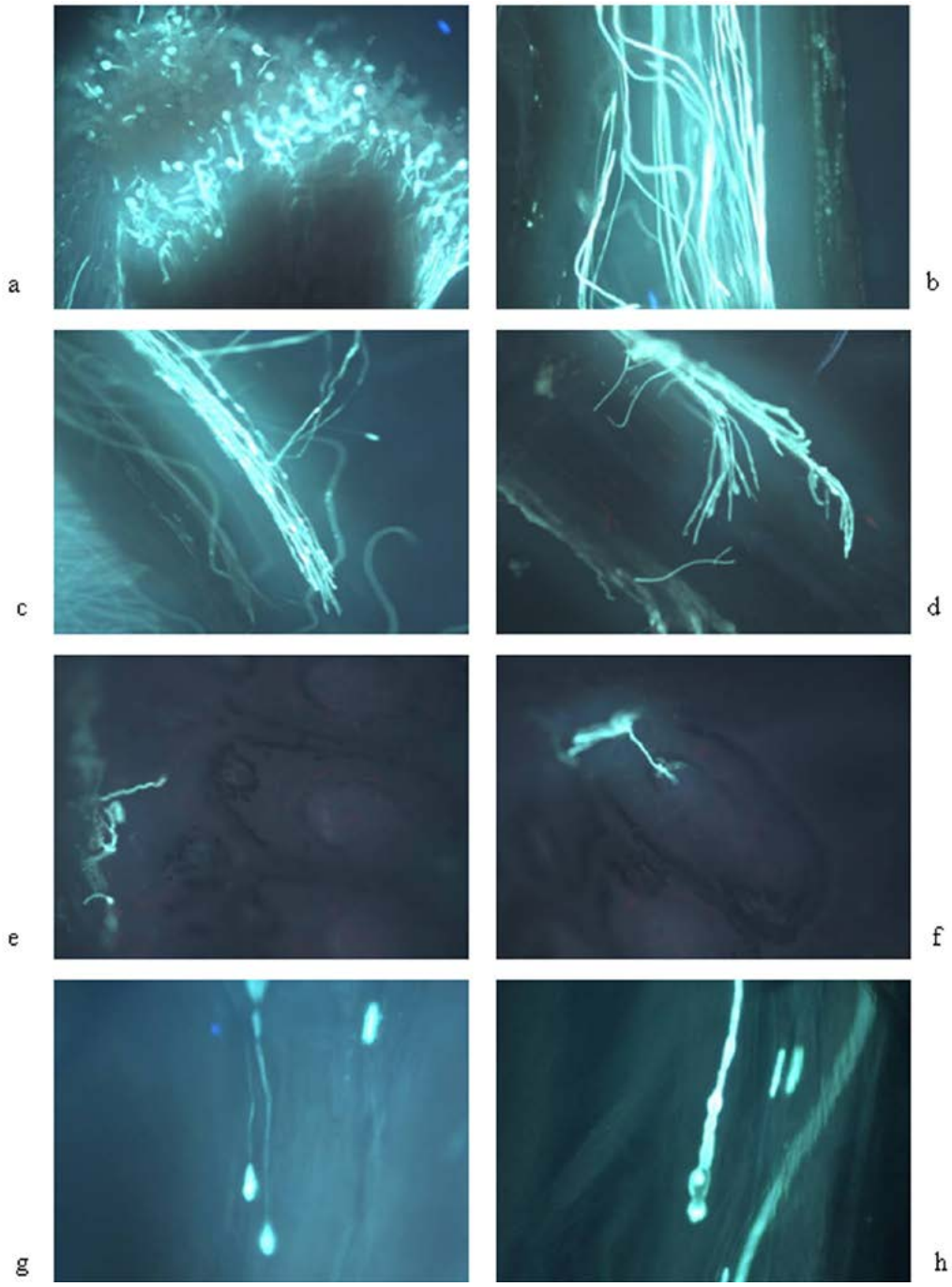

Figure 3. Pollen tube growth in pistils of quince: a) germinated pollen grains on the pistil stigma at cv. 'Morava' in the variant of open pollination, b) pollen tubes in the middle third of the style of 'Vranjska' in self-pollination, c) pollen tubes in the base of the style of 'Vranjska' in self-pollination, d) pollen tubes in the ovary of 'Leskovacka' in self-pollination, e) growth of pollen tube towards the ovule of 'Hemus' in open pollination, f) entry of pollen tube in the top of the nucellus of 'Hemus' in open pollination, g) incompatible pollen tubes with swellings at the tips under the stigma of 'Triumph' in self-pollination, h) incompatible pollen tube thickened along the entire length of the upper third of the style of 'Hemus' in self-pollination.

Table 1. Number of pollen tubes in the pistils of quince cultivars (average, 2010–2012).

		Self-pollination				Open-pollination			
		Stu	**Stm**	**Bs**	**Ovr**	**Stu**	**Stm**	**Bs**	**Ovr**
Cultivar									
'Leskovacka'		19.71 cd	10.60 a	7.22 a	2.10 a	29.81 bc	17.85 a	12.02 a	2.66 a
'Vranjska'		22.28 abc	10.16 a	4.33 b	0.54 b	30.13 bc	14.57 a	6.01 bc	1.09 b
'Morava'		17.48 d	3.21 bc	0.87 cd	0.06 c	32.61 ab	11.75 b	6.59 bc	1.34 b
'Pazardzijska'		20.34 bc	0.40 e	0.03 e	0.03 c	29.70 bc	6.75 c	2.97 d	1.07 b
'Hemus'		23.67 a	2.59 cd	0.19 de	0.10 c	34.66 a	11.18 b	4.42 cd	1.17 b
'Asenica'		22.94 ab	2.32 cd	0.28 de	0.05 c	35.44 a	12.00 b	5.07 cd	0.85 b
'Portugal'		21.54 abc	4.25 b	1.19 c	0.14 c	27.55 c	13.66 b	7.86 b	1.04 b
'Triumph'		21.80 abc	1.24 de	0.03 e	0.01 c	31.68 ab	12.47 b	6.05 bc	1.23 b
Year									
2010		17.88 b	3.84 b	0.78 c	0.18 b	34.19 a	12.44 b	5.45 b	0.70 c
2011		22.49 a	3.49 b	1.63 b	0.46 a	27.79 c	10.54 c	6.89 a	1.90 a
2012		23.28 a	5.71 a	2.89 a	0.50 a	32.37 b	14.60 a	6.78 a	1.32 b
Cultivar × Year									
'Leskovacka'	2010	14.09	6.44	2.42	1.12	30.13	13.59	5.61	1.56
	2011	20.15	10.30	8.43	2.74	29.84	22.55	17.70	3.76
	2012	24.89	15.06	10.81	2.42	29.47	17.41	12.76	2.65
'Vranjska'	2010	17.95	7.97	1.90	0.17	32.19	13.72	4.74	0.38
	2011	24.68	7.87	2.49	0.41	27.38	14.44	7.35	1.69
	2012	24.20	14.65	8.60	1.06	30.81	15.54	5.92	1.19
'Morava'	2010	12.84	2.24	0.23	0.02	35.27	11.17	6.50	0.90
	2011	17.78	1.52	0.45	0.03	28.25	7.33	5.06	2.02
	2012	21.81	5.86	1.92	0.13	34.33	16.76	8.23	1.11
'Pazardzijska'	2010	15.07	0.61	0.00	0.00	30.01	5.29	2.36	0.41
	2011	24.35	0.17	0.08	0.08	27.79	4.62	2.76	1.79
	2012	21.62	0.42	0.00	0.00	31.32	10.35	3.79	1.03
'Hemus'	2010	21.75	2.01	0.04	0.00	39.70	11.22	4.02	0.97
	2011	23.12	1.19	0.31	0.23	28.79	7.76	5.44	1.58
	2012	26.14	4.59	0.24	0.06	35.48	14.54	3.79	0.97
'Asenica'	2010	20.06	4.62	0.37	0.09	40.44	15.30	6.36	0.21
	2011	24.11	0.91	0.17	0.00	28.74	6.22	4.05	0.93
	2012	24.64	1.44	0.31	0.07	37.14	14.47	4.79	1.40
'Portugal'	2010	18.92	5.57	1.28	0.01	28.42	15.83	8.64	0.39
	2011	24.82	4.31	1.05	0.14	23.91	10.35	6.34	0.99
	2012	20.88	2.88	1.24	0.26	30.34	14.80	8.59	1.75
'Triumph'	2010	22.40	1.23	0.00	0.00	37.35	13.43	5.39	0.76
	2011	20.94	1.68	0.10	0.02	27.65	11.02	6.39	2.47
	2012	22.06	0.80	0.00	0.00	30.05	12.96	6.37	0.47
ANOVA									
Cultivar		**	**	**	**	**	**	**	**
Year		**	**	**	**	**	**	*	**
Cultivar × Year		**	**	**	**	**	**	**	ns

Stu: upper third of the style; Stm: middle third of the style; Bs: base of the style; Ovr: ovary. Mean values followed by different lower-case letters in columns represent significant differences at $p \leq 0.05$ according to Tukey's test. **,* indicate the level of significance at $p \leq 0.01$, and $p \leq 0.05$, respectively. ^{ns}: absence of significance (F test).

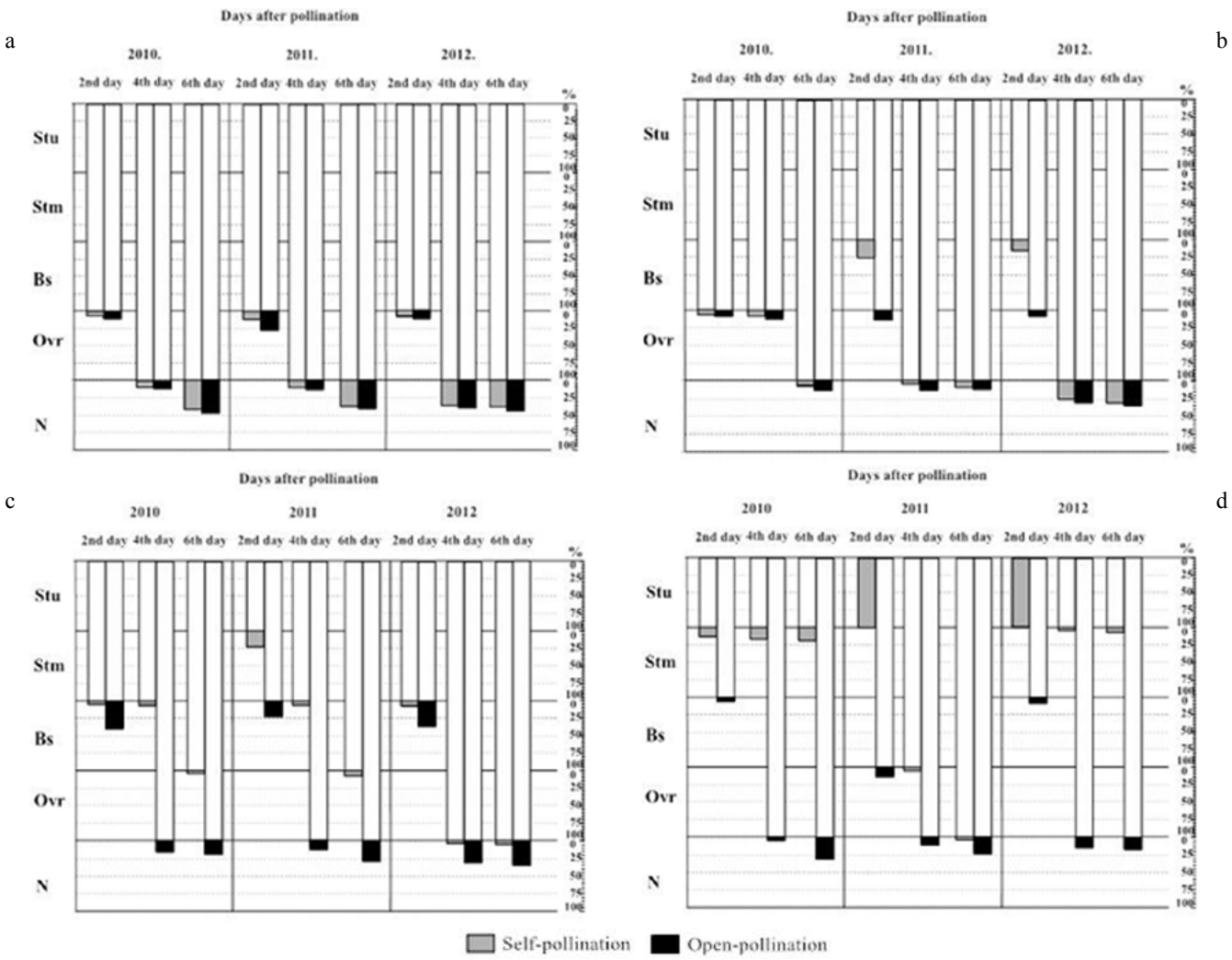

Figure 4. Dynamics of pollen tubes growth in the pistils of quince cultivars: a) 'Leskovacka', b) 'Vranjska', c) 'Morava', d) 'Pazardzijska', e) 'Hemus', f) 'Asenica', g) 'Portugal' and h) 'Triumph'. Stu, upper third of the style; Stm, middle third of the style; Bs, base of the style; Ovr, ovary; N, nucellus.

research, with the highest values in the 'Leskovacka' cultivar (28.57% in 2011). On the fourth day after pollination in all cultivars, the penetration of pollen tubes into the locule of the ovary was determined.

The penetration of pollen tubes into the nucellus of the ovules during self-pollination occurred on the fourth day after the pollination in 'Leskovacka' in all three years, 'Vranjska' in 2011 and 2012 and 'Morava' in 2012. The number of pistils, where the pollen tube penetrated into the nucellus of these cultivars, was the greatest on the sixth day after pollination. At this time, the largest number of pistils with the penetration of pollen tubes into the nucellus of the ovules was found in 'Leskovacka' (42.86% of the pistils in 2010) and 'Vranjska' (30.00% of the pistils in 2012). On the sixth day after the pollination, in some cases the penetration of pollen tubes in the nucellus of the ovules was found in some cultivars as well, such as 'Morava' and 'Pazardzijska'. Concerning the other investigated cultivars, penetration of pollen tubes in the nucellus was not observed.

After open pollination, as opposed to self-pollination, pollen tubes penetrated the nucellus of the ovules in most quince cultivars on the fourth day after the pollination. This phenomenon was not observed in 2010 in 'Vranjska', 'Asenica' and 'Portugal'. However, on the sixth day after the pollination, in all of the examined cultivars the penetration of pollen tubes in the nucellus of the ovule was observed in all three years, especially in 'Leskovacka' in 2010 (44.00% of the pistils).

Among the testing parameters affecting pollen tube growth efficiency greater or lesser correlations were found, depending on the pollination variant. A strong positive correlation was found between the number of pollen tubes in the middle third of the style and their number in the style base in both pollination variants (r = 0.95* and 0.90*, respectively), as well as between the number of pollen tubes in the middle third of the style and the ovary in the self-pollination variant (r = 0.81*) (Tables 2, 3). In addition, in both variants of pollination a strong

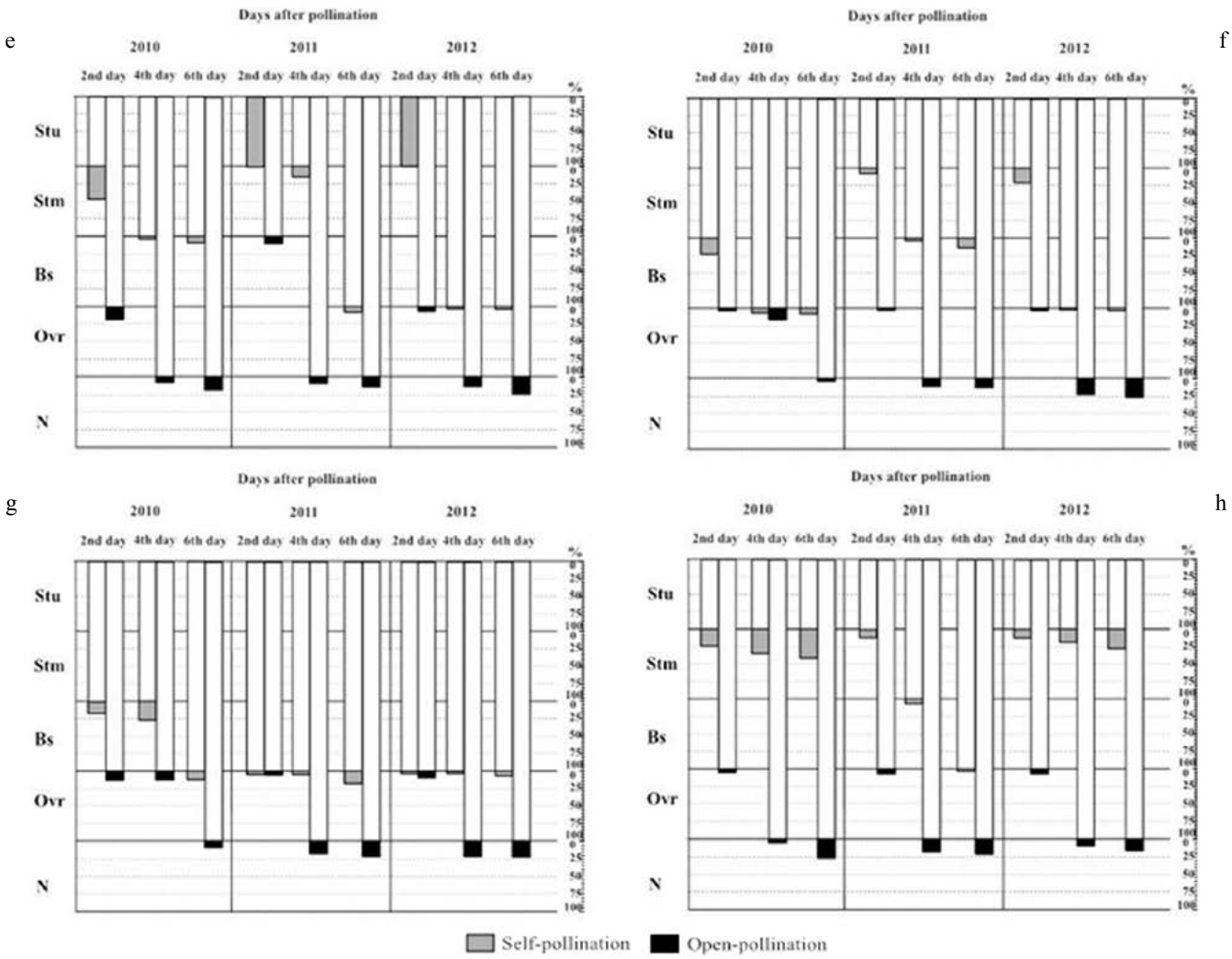

Figure 4. Continued

and positive correlation was established between the number of pollen tubes at the base style and the ovary (r = 0.94* and 0.84*), as well as between the number of pollen tubes at the base of the style and the percentage of pistils with the penetration of pollen tubes in the nucellus (r = 0.97* and 0.74*). Between some reproductive parameters in the open pollination variant there was no correlation, especially when compared with the same parameters in the self-pollination variant. This discrepancy is due to the different times of pollination of flowers under natural conditions in the field in the open-pollination variant.

Fruit set

A percentage of initial and final fruit set primarily depended on the pollination variant (Table 4). In the self-pollination variant percentage of initial and final fruit set differed between genotypes, and only 'Vranjska' and 'Leskovacka' had satisfying fruit set (28.52% and 17.01% – initial fruit set and 9.86% and 9.32% – final fruit set). The rest of the cultivars had a significantly lower fruit set (initial fruit set below 10.0% and final fruit set below 2.0%). The year significantly influenced on percentage of initial fruit set, while interaction between genotype and year did not have significant influence on initial and fruit set. In contrast to self-pollination, fruit set percentage was much higher in all cultivars under the open-pollination variant. In this variant, there were not main effects of genotype and year on percentage of initial fruit set, neither genotype × year interaction. Only the influence of year on percentage of final fruit set was significant. In the open-pollination variant the percentage of initial fruit set ranged from 28.83% ('Portugal') to 45.17% ('Asenica'), and the percentage of final fruit set ranged from 16.21% ('Vranjska') to 24.30% ('Triumph'). According to self-incompatibility index (ISI), studied quince cultivars can be divided in two groups: self-compatible ('Vranjska' and 'Leskovacka') and self-incompatible ('Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal', and 'Triumph').

There was a high correlation in both pollination variants between the number of pollen tubes in the ovary and the percentage of pistils with the penetration of pollen

Table 2. Pearson's coefficients of linear correlation between the reproductive parameters (self-pollination variant).

Parameter	STU	STM	BS	OVR	PTN	IFS	FFS
STU							
STM	-0.10						
BS	-0.23	**0.95***					
OVR	-0.25	**0.81***	**0.94***				
PTN	-0.25	**0.85***	**0.97***	**0.99***			
IFS	0.16	**0.87***	**0.77***	0.55	0.65		
FFS	-0.04	**0.97***	**0.93***	**0.79***	**0.85***	**0.94***	

STU: pollen tubes number in the upper third of the style; STM: pollen tubes number in the middle third of the style; BS: pollen tubes number in the base of the style; OVR: pollen tubes number in the ovary; PTN: percentage of pistils with the penetration of pollen tubes in the nucellus sixth day after pollination; IFS: initial fruit set; FFS: final fruit set. *The values are statistically significant at $p \leq 0.05$.

tubes in the nucellus (r = 0.99* and 0.96*, respectively), as well as between initial and final fruit set (r = 0.94* and 0.92*) (Tables 2, 3).

Discussion

Pollen tubes growth efficiency

The efficiency of pollen tube growth in the pistil is generally assessed based on the number of pollen tubes in certain regions of the style and ovary (the upper third, middle third, base of the style, ovary tissue) and dynamics of pollen tube growth through these parts, ending with the penetration in the nucellus of the ovule (Cerović, 1992). Differences in the growth speed of pollen tubes indicate that the choice of polleniser and variant of pollination can significantly influence the efficiency of the pollen tube growth in the style and ovary (Cerović & Mićić, 1996).

In vitro pollen germination and pollen tube growth rate is an important contributor to successful fertilisation and fruit set (Hedhly *et al*., 2005). The pollen germination *in vitro* values for the quince cultivars studied in this research are consistent with the findings of Dalkiliç & Mestav (2011), and within the lower range reported by Sharafi (2011). Pollen germination *in vitro* varied between

Table 3. Pearson's coefficients of linear correlation between the reproductive parameters (open-pollination variant).

Parameter	STU	STM	BS	OVR	PTN	IFS	FFS
STU							
STM	-0.22						
BS	-0.39	**0.90***					
OVR	-0.26	0.66	**0.84***				
PTN	-0.33	0.48	**0.74***	**0.96***			
IFS	0.59	-0.16	-0.14	-0.04	0.01		
FFS	0.59	-0.28	-0.20	-0.11	-0.06	**0.92***	

STU: pollen tubes number in the upper third of the style; STM: pollen tubes number in the middle third of the style; BS: pollen tubes number in the base of the style; OVR: pollen tubes number in the ovary; PTN: percentage of pistils with the penetration of pollen tubes in the nucellus sixth day after pollination; IFS: initial fruit set; FFS: final fruit set. *The values are statistically significant at $p \leq 0.05$.

Table 4. Initial and final fruit set of quince cultivars (average, 2010-2012).

		Self-pollination		Open-pollination		ISI[1]	Diagnostic SC/SI[2]
		Initial fruit set (%)	Final fruit set (%)	Initial fruit set (%)	Final fruit set (%)		
Cultivar							
'Leskovacka'		17.01 ab	9.32 a	36.69	18.68	0.50	SC
'Vranjska'		28.52 a	9.86 a	33.67	16.21	0.61	SC
'Morava'		2.42 c	0.71 b	43.05	23.57	0.03	SI
'Pazardzijska'		2.84 c	0.00 b	36.93	19.58	0.00	SI
'Hemus'		3.69 c	0.46 b	32.89	19.09	0.02	SI
'Asenica'		9.14 bc	1.61 b	45.17	23.03	0.07	SI
'Portugal'		4.09 c	1.01 b	28.83	16.69	0.06	SI
'Triumph'		3.19 c	0.00 b	43.87	24.30	0.00	SI
Year							
2010		8.73 ab	2.82	37.65	19.18 ab	-	-
2011		11.87 a	2.88	39.44	16.70 b	-	-
2012		5.98 b	2.92	35.82	24.54 a	-	-
Cultivar × Year							
'Leskovacka'	2010	16.54	9.84	38.69	17.37	-	-
	2011	15.88	5.75	21.53	12.71	-	-
	2012	18.60	12.37	49.85	25.98	-	-
'Vranjska'	2010	30.30	10.09	35.58	17.10	-	-
	2011	35.60	10.65	42.21	14.67	-	-
	2012	19.65	8.84	23.21	16.87	-	-
'Morava'	2010	2.24	0.67	41.85	20.60	-	-
	2011	1.93	0.40	45.98	20.27	-	-
	2012	3.07	1.05	41.31	29.84	-	-
'Pazardzijska'	2010	2.36	0.00	35.05	18.73	-	-
	2011	6.16	0.00	36.03	12.59	-	-
	2012	0.00	0.00	39.70	27.42	-	-
'Hemus'	2010	3.15	0.00	34.71	17.90	-	-
	2011	6.60	1.39	33.07	19.20	-	-
	2012	1.31	0.00	30.89	20.16	-	-
'Asenica'	2010	8.99	1.92	43.40	21.29	-	-
	2011	16.20	1.78	52.83	17.36	-	-
	2012	2.22	1.11	39.27	30.43	-	-
'Portugal'	2010	4.00	0.00	26.85	16.44	-	-
	2011	5.65	3.04	34.85	16.34	-	-
	2012	2.62	0.00	24.79	17.28	-	-
'Triumph'	2010	2.26	0.00	45.02	24.01	-	-
	2011	6.91	0.00	49.02	20.50	-	-
	2012	0.41	0.00	37.56	28.38	-	-
ANOVA							
Cultivar		**	**	ns	ns	-	-
Year		*	ns	ns	**	-	-
Cultivar × Year		ns	ns	ns	ns	-	-

[1]ISI = Final fruit set self/Final fruit set open-pollination. [2]SC = self-compatibility; SI = self-incompatibility. Mean values followed by different lower-case letters in columns represent significant differences at $p \leq 0.05$ according to Tukey's test. **,* indicate the level of significance at $p \leq 0.01$, and $p \leq 0.05$, respectively. [ns]: absence of significance (F test).

different years. It was the lowest in 2012. It could be possibly influenced by environmental conditions. The most important environmental factor affecting pollen germination is air temperature (Pirlak, 2002). The optimum temperature for pollen germination varies between different fruit species. In apricot and pear, it ranges from 10 to 20°C (Pirlak, 2002; Radović *et al*., 2016b), in sour cherry from 15 to 25°C (Cerović & Ružić, 1992), and in plum from 22 to 23°C (De Ceault & Polito, 2010). Probably the main reason of lower pollen germination in 2012 year in our research was lower temperatures during flowering for most of the studied cultivars. Besides, pollen germination may be also affected by other factors such as soil moisture, and plant nutritional status in the period from the start of flower bud differentiation to flowering onset.

For all the tested cultivars, a drastic reduction occurred in the number of pollen tubes from the upper third of the style to its lower parts and the ovary in both variants of pollination, especially in the self-pollination variant. The number of pollen tubes penetrating the ovary was several times smaller (depending on the cultivar, and pollination variants) compared to their number in the upper third of the style as determined in the other fruit species (Herrero, 1992; Socias i Company & Alonso, 2004). The exceptions were the 'Leskovacka' and 'Vranjska' cultivars in which there was a greater presence of pollen tubes in the lower regions of the style and the ovary in the variant of self-pollination. For other quince cultivars in this variant of pollination, the occurrence of incompatible pollen tubes was observed. Similar results were obtained with the other fruit species, which showed a difference in the rate of growth of pollen tubes in compatible and incompatible combinations of pollination (Stott, 1972; Kaufmane & Rumpunen, 2002; Alonso & Socias i Company, 2005; Sanzol & Herrero, 2007). In addition to the self-incompatibility, reducing the number of pollen tubes throughout the pistil can be explained by the progressive reduction of the stylar reserves throughout the pistil (Herrero & Hormaza, 1996). It is known that number of pollen tubes has been correlated with the levels of a hormone in the style (Weterings *et al*., 2002; Rodrigo *et al*., 2009). It has been found that after pollination and during pollen tube growth levels of a hormone in the style change, with auxins and gibberellins content increasing while abscisic acid content decreasing (Wu *et al*., 2008). Also it has been shown that genotypic specificities in terms of pollen performance in the style have also existed (Hormaza & Herrero, 1999; Hedhly *et al*., 2004).

Stopping the growth of pollen tubes in certain regions of the pistil is a consequence of the gametophyte incompatibility system (De Nettancourt, 2001; Franklin-Tong & Franklin, 2003). The signs of incompatibility were the most prominent in the upper third of the style, which has been experimentally confirmed in sour cherry (Cerović, 1992), Japanese quince (Kaufmane & Rumpunen, 2002), pear (Sanzol & Herrero, 2007) and plum (Nikolić & Milatović, 2010). The occurrence of different degrees of self-compatibility in quince can be linked to its polyploid origin and high variability. This confirms the results from several authors, who have found high average heterozygosity among quince cultivars (Yamamoto *et al*., 2004; Halasz *et al*., 2009; Azad *et al*., 2013; Yüksel *et al*., 2013). Further research of self-incompatibility in the field of molecular genetics can lead to new insights, which may be useful in commercial quince production.

Environmental factors such as air temperature have a significant influence on the growth of pollen tubes through the style and the ovary during the flowering. In most quince cultivars studied, on the second day after the pollination, faster growth of pollen tubes was determined in 2010 because of higher average daily temperatures during the flowering than in 2011 and 2012. During the first days after pollination, the pollen tubes mostly grow in the style, where its speed growth is usually higher than in the ovary (Kaufmane & Rumpunen, 2002), so they travel a longer distance in a shorter time in the style than in the ovary (Herrero, 1992). It has been confirmed in some studies that there is a correlation between the rate of growth of pollen tubes and the air temperature (Keulemans, 1984). The optimum temperature for growth of pollen tubes through the pistil differs in certain fruit species (Cerović & Ružić, 1992; Pirlak, 2002; De Ceault & Polito, 2010). In addition to this, the optimum temperature for growth of pollen tubes varies between individual cultivars depending on their origin (Hedhly *et al*., 2004). Moreover, low or even high temperatures during the flowering phase may slow down the growth of pollen tubes and lead to poor fruit set (De Ceault & Polito, 2010). Higher temperatures accelerate pollen tube growth rate but also reduce the number of growing pollen tubes along the style and accelerate ovule degeneration. In sweet cherry it was found that even slight increase in temperature during blooming can cause significant decrease in fruit set (Hedhly *et al*., 2007). Bartolini & Viti (2018) reported that in olive weather conditions at fertilization time, particularly high temperatures, may have a key role for reproductive success. These results indicate a differential genotypic response to temperature during the reproductive phase. The temperature during the reproductive phase could act as a selective pressure agent for genotypes better adapted for pollen tube growth in the style (Hedhly *et al*., 2004).

Fruit set

If we analyze the variant of self-pollination, it can be concluded that the number of pollen tubes in the ovary and the percentage of pistils with penetrated pollen tube

into the nucellus are positively correlated with the percentage of initial fruit set, or they are in a strong positive correlation with the percentage of final fruit set. In the variant of self-pollination, only 'Leskovacka' and 'Vranjska' could be distinguished as cultivars with significant fruit set.

The occurrence of self-incompatibility leads to low fruit set and low yields in quince. Dimitrovski & Mitreski (1976) stated that the percentage of final fruit set in self-pollinated quince ranged from 0.44 to 2.25%, while But & Klimenko (2001) reported values from 0.0 to 9.8%. The differences in results can be associated with varying degrees of fertility of certain cultivars. Depending on the cultivar, the percentage of final fruit set in self-pollination in our work ranged from 0.00 to 9.86%. However, some species close to the quince in the *Maloideae* subfamily, such as pear and apple, showed different degrees of parthenocarpy depending on the cultivar. The tested quince cultivars were not prone to formation of parthenocarpic fruits (Unpublished results), so that the emergence of low fruit set in most cultivars is the result of self-incompatibility. Significant differences were reported in fruit set at the same cultivar in different regions. Nyéki *et al*. (2003), citing a larger number of authors who have dealt with the investigation of the 'Bereczki' quince cultivar, state enormously high differences in fruit set in this cultivar in terms of self-pollination, ranging from 0.0% to as much as 19.3%. The resulting differences may be due to the influence of different environmental conditions, as well as different research methodologies.

Based on the obtained results, 'Vranjska' and 'Leskovacka' cultivars can be classified as self-compatible cultivars, while the other cultivars are self-incompatible. However, the obtained results show that the self-compatible cultivars also give much better results in terms of fertilization after open pollination. In addition, results from the open-pollination variant would not indicate the best polliniser for obtaining good yields. However, from a practical point of view, these results can serve as a basis for comparison of the efficiency of pollen tube growth and fruit set in self-pollinated combinations. Therefore, for obtaining high yields in commercial quince orchards, the combination of more cultivars can be recommended.

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