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ARTICLE

Morphological investigations on anthers and pollen grains of some quince cultivars

Helga Nagy-Déri

Plant Ecology Research Group of Hungarian Academy of Sciences, Institute of Botany and Ecophysiology, Faculty of Environmental and Agricultural Sciences, Szent István University, Gödöllõ, Hungary

ABSTRACT In the present study we investigated some pollen morphological characters and anther size of *Cydonia oblonga*. Anthers of cultivars with apple- vs. pear-shaped fruits were also compared. In 2005 the anthers of cultivars with oblate vs. suboblate pollen showed significant differences. Anthers of the oblate group were notably shorter and smaller in size, than those of the suboblate group. Equatorial area of pollen in the former group was also smaller. In 2006 P/E ratio of pollen also correlated with length, width and size of anthers. However, shape and size of pollen grains did not show any relationship in the latter year. Equatorial area of pollen grains was not connected with anther size in 2005-2006. Fruit shape correlated with pollen shape only in 2005. P/E ratio of pollen in cultivars with apple-shaped fruits was lower than in cultivars with pear-shaped fruits. We could not demonstrate any relationships between anther morphology and fruit shape of the investigated quince cultivars.
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KEY WORDS

anther
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The 3-whorled androecium of *Cydonia oblonga* L. consists of 15-20 stamens with purplish filaments and light yellow anthers (Halmágyi and Keresztesi 1975). Size of anthers is influenced by flower size (Delph et al. 1996). The level of pollen production is directly related to anther size and inversely related to pollen grain size (Lopez et al. 1999). In apple (*Malus domestica* Borkh.), which belongs to the same subfamily as quince, Kocsis-Molnár et al. (1994) reported that the mean width of anthers was 1.71 mm and their length was 2.46 mm in 1989, while in 1990 they measured 1.98 mm and 2.66 mm, respectively. Benedek et al. (1989) found that the size of apple anthers was 2.20x1.53 mm and their relative surface was 3.37 mm^2.

Quince has yellow pollen grains (Mohácsy and Porpáczy 1958). Shape and size of pollen often change with variable air temperature and humidity and other environmental factors (Thakur and Thakur 1970). Pollen size of apple increases with higher levels of ploidy (Fogle 1977; Orosz-Kovács 2001). Pollen size also depends on pollination strategies of plants (Harder 1998). According to Baker and Baker (1979) pollen grains collected by honeybees are smaller than those which are transferred by butterflies and birds.

Structure of stigma, length and thickness of style can also be related to pollen size (Cruden and Lyon 1985; Kirk 1993).

In subfamily Maloideae (Rosaceae) pollen shape is highly diverse. Typical pollen grains are oblate or spheroidal, with a triangular or circular equatorial outline (Halbritter and Schneider 2000). Quince possesses spheroidal pollen grains (P E, P/E=0.88-1.14), which are round in equatorial view (Erdtman 1966, Halbritter and Schneider 2000). According to the classification of Erdtman (1966), the trizonocolporate pollen grains of quince were considered as large (50-100 µm) by Erdtman (1966) and Sótonyi et al. (2000), contrasting the view of Halbritter and Schneider (2000), who classified quince pollen in the medium (26-50 µm) category. According to Sótonyi et al. (2000), length of *C. oblonga* pollen changed between 50.4-54.4 µm, its width was in the range 23.6-24.1 µm, and the average length and width ratio was 2.19. In comparison, the mean length of pollen grains in apple was 44 µm, mean width 23.1 µm and mean shape index was 1.9 (Sótonyi et al. 2000).

Quince cultivars are divided into two groups on the basis of their fruit shape. They have apple-shaped [*C. oblonga* var. *maliformis* (Mill.) Schneid.] or pear-shaped [*C. oblonga* var. *pyriformis* (Dierb.) F. Zinn.] fruits (Nyéki 1990). Besides the above two varieties, cultivars were also bred from *C. oblonga* var. *lusitanica* (Mill.) Schneid. for fruit growing. This variety possesses pear-shaped fruits (Mohácsy and Porpáczy 1958). However, intermediate fruit types also occur which have fruit shape between apple and pear (Nyéki 1990). The same trees can bear apple-shaped or pear-shaped fruits year by year in turns. It is connected partially with the number of fully developed seeds. Fruits, which contain less seed, are more elongated. Shape and size of fruits can also be influenced by fruit density and flowering time (Soltész 1998, Nyéki 2004).

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*Corresponding author. E-mail: deri.helga@mkk.szie.hu

Table 1. Length (L), width (W) and size (L*W) of anthers in the investigated quince cultivars in Újfehértó in 2005-2006. Data are shown as mean±SE.

Cultivars	Anther length (μm)		Anther width (μm)		Anther size (L*W) (mm²)	
	2005	2006	2005	2006	2005	2006
'Angers'	3298.46±74.53	3446.62±22.98	1312.37±26.77	1364.61±22.98	4.34 ±0.17	4.70±0.14
'Apple-shaped Dunabogdány'	3259.86±24.78	3392.03±29.50	1368.51±27.68	1337.32±29.50	4.46 ±0.08	4.53±0.09
'Apple-shaped wild quince'	-	3712.52±34.38	-	1424.65±34.38	-	5.31±0.22
'Aromate'	3779.19±30.07	3694.02±28.57	1375.53±38.37	1400.41±28.57	5.20±0.15	5.18±0.12
'Bereczki'	3484.44±62.56	3413.30±13.61	1368.51±15.69	1381.27±13.61	4.76±0.07	4.71±0.07
'Champion'	3768.67±62.38	3670.41±19.61	1477.29±17.70	1449.22±19.61	5.57±1.48	5.32±0.13
'Constantinople'	3445.84±80.49	3454.42±25.79	1350.97±31.06	1337.35±25.79	4.66±1.80	4.63±0.14
'Mezőtúri'	-	3729.11±19.72	-	1419.58±19.72	-	5.30±0.10
'Pear-shaped Bólyi'	3670.41±52.36	-	1410.62±21.44	-	5.18±1.29	-
'Pear-shaped Dunabogdány II'	3670.41±30.14	-	1438.69±10.46	-	5.28±0.04	-
'Pear-shaped Noszvaji'	3576.26±38.74	3639.79±16.64	1441.61±17.52	1419.55±16.64	5.16 ±0.09	5.17±0.08

Table 2. Results of one-way ANOVA test. Differences between quince cultivars possessing oblate vs. suboblate pollen grains in some morphological features of androecium (length (L), width (W) and size (L*W) of anthers, equatorial area of pollen) in 2005. SS – sum of squares, MS – mean squares, F – value of Fisher test, p – significance level. Df=16, critical value of F test=4,5431; * indicates significant difference at $p < 0.05$.

Factors	SS	MS	F	p
Anther length (μm)	1472313.08	376923.71	5.1615*	0.0382
Anther width (μm)	102916.67	20280.95	3.6814	0.0743
Anther size (L*W) (mm2)	7.86E+12	1.99E+12	5.0926*	0.0394
Equatorial area of pollen (μm2)	128987.45	53563.42	11.3626*	0.0039

The present study was based on the hypothesis that a relationship exists between certain pollen morphological characters (shape and size) and anther size. Our additional aim was to investigate and compare the anther morphology of cultivars with apple- vs. pear-shaped fruits.

Materials and Methods

The studied material was taken from the quince genebank of the Research and Extension Centre for Fruitgrowing, Újfehértó, Hungary during 2005-2006. Anther size, pollen shape and size was studied in 11 quince cultivars. 5 of the investigated cultivars had apple-shaped fruits (Ang - 'Angers', A Dun - 'Apple-shaped Dunabogdány', A wildq - 'Apple-shaped wild quince', Const - 'Constantinople', Mezt - 'Mezőtúri'), while 6 cultivars (Aro - 'Aromate', Ber - 'Bereczki', Cham - 'Champion', P Bóly - 'Pear-shaped Bólyi', P Dun - 'Pear-shaped Dunabogdány II', P Nosz - 'Pear-shaped Noszvaji') possessed pear-shaped fruits. Samples were collected from 30-70 pollen shedding flowers per tree, from 2–3 trees per cultivar. 100-500 data were measured per cultivar.

Freshly gathered anthers were investigated with stereo microscope (Carl Zeiss, Jena) immediately. Their size was measured with an ocular micrometer. Pollen samples were prepared with the fixation and isatine staining method elaborated by Gulyás and Pálfi (1986). The slides were examined with light microscope (NIKON H600L Eclipse 80i), and micrographs were taken with the software Spot Basic 4.0. For analyzing shape and size of pollen grains software 'UTHSCSA Image Tool' was used.

All statistical analyses of variance (ANOVA) were performed using Statistica 5.1 software. Differences between cultivars that had different pollen shape or fruit shape was analyzed by using a one-way ANOVA (p 0.05). Pearson correlation analysis was also employed to compare pollen size, pollen shape and anther size.

Results and Discussion

Mean length of anthers (L) in various cultivars changed between 3259.86±24.78 μm and 3779.19±30.07 μm in 2005. The smallest values were measured in the flowers of cv. 'Apple-shaped Dunabogdány'. The longest anthers could be found in the flowers of cv. 'Aromate'. In 2006 again, cv. 'Apple-shaped Dunabogdány' possessed the shortest anthers (3392.03±29.50 μm). The longest anthers (3729.11±19.72 μm) could be observed in the case of cv. 'Mezőtúri' (Table 1). Anther length fluctuated 6-30 % per cultivar during the two years.

Mean width of anthers (W) was also very diverse. Its fluctuation rate was 5-35 % in 2005-2006. In 2005 the narrowest anthers (1312.37±26.77 μm) occurred in the flowers of cv.

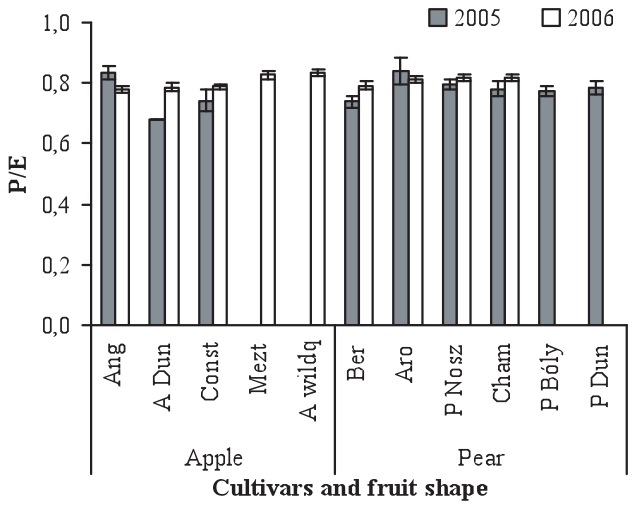

Figure 1. P/E ratio of pollen grains in the investigated quince cultivars in Újfehértó in 2005-2006.

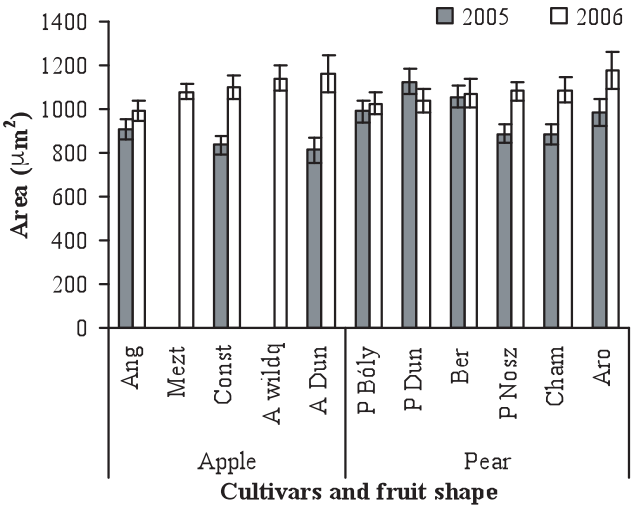

Figure 2. Equatorial area of pollen grains in the investigated quince cultivars in Újfehértó in 2005-2006.

'Angers' and the widest in cv. 'Champion' (1477.29±17.70 µm). In 2006 their width varied between 1337.32±29.50-1449.22±19.61 µm. The smallest values were measured in the flowers of cv. 'Apple-shaped Dunabogdány' and the largest again in cv. 'Champion' (Table 1).

Size of anthers (length * width, L*W) changed between 4.34-5.57 mm^2 in 2005, and 4.53-5.32 mm^2 in 2006. Smallest anthers were measured in cv. 'Angers' and 'Apple-shaped Dunabogdány' in the two years, respectively. Largest anthers could be observed in the flowers of cv. 'Champion' in both years (Table 1).

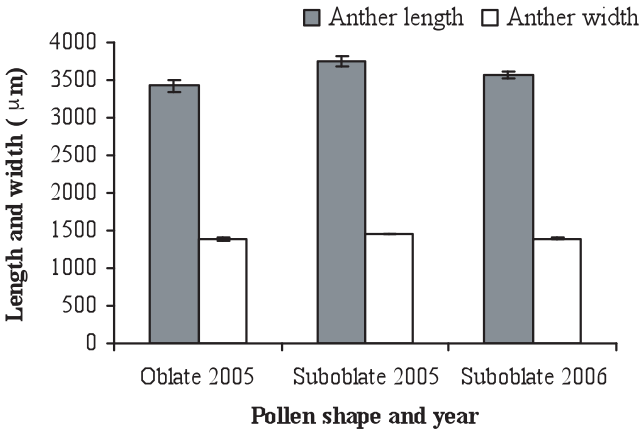

Figure 3. Length and width of anthers in quince cultivars possessing oblate vs. suboblate pollen grains in Újfehértó in 2005-2006.

Shape of the pollen grains was typified with the ratio of the length of their polar (P) and equatorial (E) axis (P/E) and it was ranked by the evaluation system elaborated by Erdtman (1952). According to this ratio, the investigated quince cultivars could be divided into two groups. They possessed oblate (P/E=0.5-0.75) or suboblate (P/E=0.75-0.88) pollen grains. Oblate group could be distinguished only in 2005. In this year P/E ratio of pollen varied between 0.68 and 0.84. The smallest values were reached by cv. 'Apple-shaped Dunabogdány' and the largest ones could be counted in cv. 'Aromate'. Three cultivars, namely 'Apple-shaped Dunabogdány', 'Bereczki' and 'Constantinople', belonged to the oblate category. The other investigated cultivars were characterized by suboblate pollen grains. In 2006 all quince cultivars had suboblate pollen. Their P/E ratio was 0.78-0.83. Pollen grains of cvs. 'Apple-shaped Dunabogdány', 'Bereczki' and 'Constantinople' were typified by the smallest values also in the latter year. The highest P/E ratios were reached by cvs. 'Apple-shaped wild quince' and 'Mezőtúri' (Fig. 1).

In 2005 equatorial area of pollen changed between 813.48±56.94 µm^2 and 1126.46±56.32 µm^2. The smallest pollen grains could be found in the anthers of cv. 'Apple-shaped Dunabogdány', while the largest ones could be detected in cv. 'Pear-shaped Dunabogdány II'. In 2006 area of pollen was larger, than in the previous year. The smallest pollen grains (992.36±49.62 µm^2) were possessed by cv. 'Angers' and the largest ones (1175.73±82.30 µm^2) by cv. 'Aromate'(Fig. 2).

Comparing 2005 and 2006, it can be established that size of anthers was very similar in the two years. Nevertheless, shape and equatorial area of pollen grains was usually smaller in 2005 than in 2006 (Table 1, Figs. 1-2).

In 2005 we compared the anthers of the cultivars which had oblate and suboblate pollen and significant differences could be noticed between these groups. Anthers of the oblate

Table 3. Results of one-way ANOVA test. Differences between quince cultivars with apple-shaped vs. pear-shaped fruits in some morphological features of androecium (length (L), width (W) and size (L*W) of anthers, pollen shape (P/E), equatorial area of pollen) in 2005-2006. SS – sum of squares, df – degree of freedom, MS – mean squares, F – value of Fisher test, p – significance level, F crit. – critical value of F test. * indicates significant difference at $p < 0.05$.

Factors and year	SS	df	MS	F	p	F crit.
Anther length (µm) 2005	412530.94	11	58203.28	1.6426	0.2289	4.9646
Anther length (µm) 2006	160718.18	8	7332.22	0.3346	0.5811	5.5914
Anther width (µm) 2005	33753.20	11	2864.19	0.9273	0.3583	4.9646
Anther width (µm) 2006	12770.27	8	2867.40	2.0269	0.1975	5.5914
Anther size (mm2) (L*W) 2005	2.10E+12	11	2.55E+11	1.3803	0.2673	4.9646
Anther size (mm2) (L*W) 2006	8.69E+11	8	9.05E+10	0.8140	0.3969	5.5914
Pollen P/E 2005	1.27E-02	8	9.92E-03	25.0555*	0.0016	5.5914
Pollen P/E 2006	4.66E-03	11	5.74E-05	0.1249	0.7311	4.9646
Equatorial area of pollen (µm2) 2005	86830.28	8	37338.28	5.2810	0.0551	5.5914
Equatorial area of pollen (µm2) 2006	42471.35	10	2420.05	0.5438	0.4796	5.1174

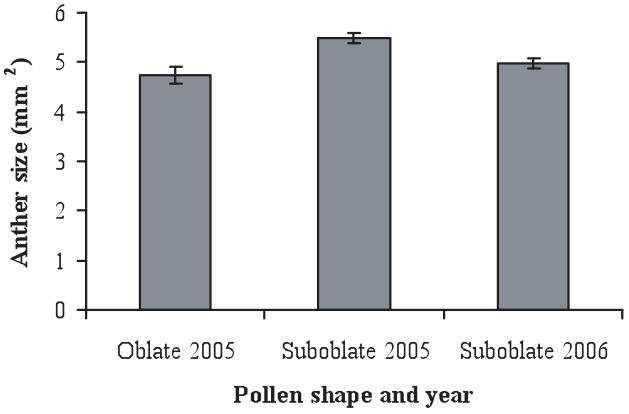

Figure 4. Size of anthers (L*W) in quince cultivars possessing oblate vs. suboblate pollen grains in Újfehértó in 2005-2006.

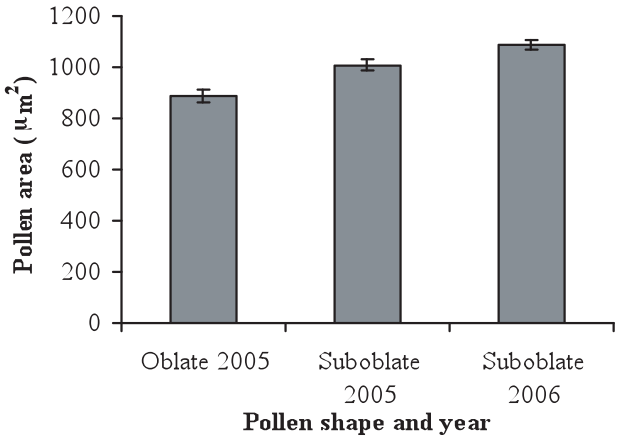

Figure 5. Equatorial area of pollen in quince cultivars possessing oblate vs. suboblate pollen grains in Újfehértó in 2005-2006.

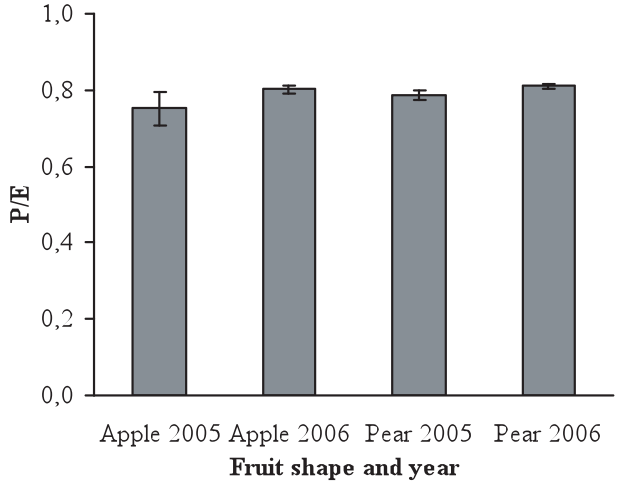

Figure 6. P/E ratio of pollen in quince cultivars having apple-shaped vs. pear-shaped fruits in Újfehértó in 2005-2006.

group were notably shorter and smaller in size, than those of suboblate group (Figs. 3-4). Equatorial area of pollen in the former group was also smaller (Fig. 5, Table 2). None the less all investigated quince cultivars had suboblate pollen in 2006, in which year P/E ratio of pollen also correlated with length (r^2=0.8925), width (r^2=0.7633) and size (r^2=0.9058) of anthers. Cvs. 'Angers', 'Apple-shaped Dunabogdány', 'Bereczki' and 'Constantinople', whose pollen grains could be characterized by lower P/E ratios, had smaller anthers than other investigated cultivars (Fig. 1, Table 1). However, shape and size of pollen grains did not show any relationship (r^2=0.3239) with each other in 2006. Similarly, equatorial area of pollen grains was not connected with anther size either in 2005 (r^2=0.2782) or in 2006 (r^2=0.0005).

Cultivars with apple-shaped vs. pear-shaped fruits did not differ from each other in respect of length, width and size of

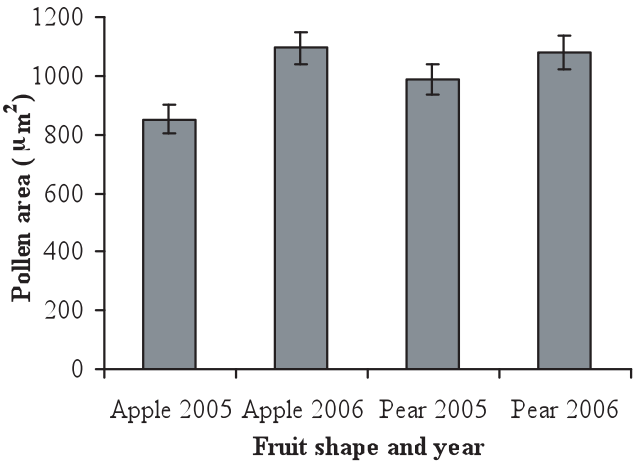

Figure 7. Equatorial area of pollen in quince cultivars having apple-shaped vs. pear-shaped fruits in Újfehértó in 2005-2006.

anthers (Table 3). In contrast, correlation was found between pollen shape (P/E) and fruit shape in 2005 (Fig. 6, Table 3), when P/E ratio of pollen in cultivars having apple-shaped fruits was lower than in cultivars having pear-shaped fruits. Cultivars with apple-shaped fruits usually had oblate pollen grains, whereas cultivars with pear-shaped fruits developed suboblate pollen (Fig. 1). However, in 2006 this relationship could not be observed. Although in 2005 pollen area of apple-shaped cultivars seemed to be smaller than that of pear-shaped ones (Fig. 7), no statistically significant relationship could be demonstrated between pollen size and fruit shape of cultivars in any of the years (Table 3).

In summary, positive correlation was found between anther size and pollen shape, as well as between anther size and equatorial area of pollen. Anthers of apple-shaped vs. pear-shaped quince cultivars also differed from each other. P/E ratio of pollen grains was lower in cultivars with apple-shaped fruits than in the case of pear-shaped cultivars.

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