UNIVERSITY OF PÉCS

Biological Doctoral School

Floral attractivity of *Cydonia oblonga* Mill. cultivars

PhD Thesis

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PÉCS, 2013

INTRODUCTION

Quince (*Cydonia oblonga* Mill.) is grown in the temperate zone of the whole northern hemisphere. Approximately 30-50 cultivated quince varieties are known. From 1995 the propagation of six cultivars was permitted in Hungary. Since quince is grown chiefly in home gardens, the investigation of local Hungarian varieties can be essential from the aspect of enriching the cultivar assortment.

Cross pollination is crucially important for quince. The fertility relations of this fruit tree remain a matter of controversy in the literature. Although three groups, self-fertile, partly self-fertile and self-sterile cultivars are usually distinguished, the fruit set of quince is very low with natural autogamy so it requires cross-pollination to achieve sufficient yield. On the other hand, for an adequate fruit quality more than 70 ovules need to be fertilized in a quince flower, so it needs more pollen for cross-pollination than other Maloideae fruit crop plants. Furthermore, flower density of quince is low as compared with other fruit species so it needs at least 20-25 per cent fruit set to reach an adequate yield.

Flowers offer **pollen and nectar** as reward for honey bees and bumble bees, which usually serve as pollinators for quince. Accordingly, the study of nectar and pollen, which ensure the insect attraction of flowers, is very important.

The main aim of the present investigation was to contribute to a better understanding of the floral attraction and pollination biology of quince. Our results can contribute to widening the cultivar assortment of quince and to increasing yield safety.

OBJECTIVES

My investigations attempted to answer the following questions:

- Which flower morphological features are characteristic to the investigated quince cultivars?
- What are the typical structural features of the nectary and stigma?
- Is there any relationship between the histological structure of the nectary and the quantity, concentration or sugar composition of the nectar?
- How many flower biological types can be distinguished on the basis of the dynamics of nectar production and anther dehiscence, as well as the activity of floral organs?
- Do quince cultivars differ in their pollen viability?
- How is insect attraction of flowers influenced by the volume, sugar concentration and composition of the nectar and flower biological types?
- What is the apicultural significance of the investigated floral traits?
- Do the above features differ from each other in various years of the period 2004-2006?

MATERIALS AND METHODS

Location and time of experiments

Field experiments and sample collection were carried out in the **quince gene bank** of the Research and Extension Centre for Fruitgrowing, Újfehértó. Samples were processed in laboratories of Újfehértó and of University of Pécs, Department of Botany, from 2005 at the Department of Systematic and Ecological Botany and Department of Plant Physiology.

For the majority of the investigations samples were collected in three years (**2004-2006**). Endogenous rhythm of nectar secretion was also observed in this three-year-long period, but only in two years in each cultivar. Histology of the nectary and nectar sugar composition were studied only in 2004 and 2005.

Microclimatic relations

In Újfehértó, there is a complex computer controlled microclimatic apparatus. It recorded the main meteorological data every hour during the whole flowering period. Besides these data we also measured the local air **temperature and humidity** with a digital hygro- and thermometer during the investigations.

Plant material

28 quince cultivars were studied between 2004 and 2006, including six **commercially grown** varieties (cvs. '*Angers*, '*Bereczki*', '*Pear-shaped Dunabogdány*', '*Champion*', '*Constantinople*' and '*Mezőtúri*'), the **collected** cv. '*Aromate*' and 21 **local Hungarian cultivars**. The investigated local Hungarian cultivars were the following:

Apple-shaped wild	*Késői*	*Örsi*
Pear-shaped Bólyi	*Kúti*	*Perbál I.*
Bori	*Mezőkövesdi*	*Szentlőrinci*
Cserszegi	*Apple-shaped*	*Szobi*
Apple-shaped	*Pear-shaped*	*Tinnye 220*
Fehérvári I.	*Olasz 3*	*U.V.13.*
Horváth Antal	*Óriás*	*Váli*

Investigation of flower morphology

Flowers were investigated with a hand magnifying glass (6x). Measurements were taken in 30-70 flowers per cultivar each year. The following

characters were recorded: a) number of stamens, b) colour of petals, c) colour, shape and size of anthers, d) colour, shape and size of stigma, e) relative position of anthers and stigmata, f) colour of nectary.

Scanning Electron Microscopy

For scanning electron microscopy (SEM) fresh materials were prefixed in glutaraldehyde, and postfixed in osmium tetroxide. Dehydration of samples in ascendant ethyl alcohol series was followed by critical point drying in isoamyl acetate and gold coating. Micrographs were taken with ASID-4 SEM adapted to a YEOL 100 C electron microscope in the Central EM Laboratory of the Medical School, University of Pécs.

Histological studies on the nectary

Flower samples were dehydrated in an ascending acetone series, followed by embedding in paraplast. Longitudinal sections (8–10 μm thick) were cut with a rotary microtome. After staining with toluidine blue, samples were mounted in Canada balsam. Histological examinations were done with a 'NIKON H600L Eclipse 80i' research microscope. Digital images were recorded with 'SPOT 4.0.4' computer program. For measurements of histological features we used 'ImageTool 3.0' computer program, and for data processing 'Microsoft® Excel 2002 .

Dynamics of nectar secretion and activity of floral organs

For investigating the **reproductive organs and the endogenous rhythm of nectar secretion**, nectar was extracted from 15-20 labelled flowers with calibrated capillaries every daylight hour, and the number of dehisced anthers and the time of the presence of stigma exudates were also recorded in the same flowers. The dry matter content of the nectar was measured with a hand refractometer.

Study of pollen

Pollen viability was investigated with the isatin staining method, counting at least 500 pollen grains per cultivar. **Proline content of pollen** was determined by spectrophotometric method. Proline was detected with ninhydrin assay in acidic media.

Study of nectar

Nectar volumes (μl), produced by flowers during **24 hours**, were measured with calibrated microcapillaries in 20-30 flowers which have previously been isolated with an isolator-net. The **dry matter content** of the nectar was measured with a hand refractometer. Samples were collected at different blooming stages (beginning of bloom, full bloom and end of bloom) and at different developmental stages. Sugar-value of nectar was calculated following: (nectar μl x refraction %)/100.

Nectar sugar components were determined by thin layer chromatography (TLC), quantitative evaluation was carried out by densitometry.

Statistical analysis

Besides descriptive statistics (average values, standard error), morphological and histological features of flowers were also analysed by Pearson's correlation and analysis of variance (ANOVA). Relationship between two investigated features was characterized by correlation coefficient (r). Strength of linear regression was classified in one of the following categories: weak ($r<0.4$), medium ($r=0.4$-0.7), strong ($r=0.7$-0.9), very strong ($r>0.9$). Calculations were performed using 'Microsoft Excel' and 'Statistica 5.1 for Windows' softwares. Tukey's HSD test was used to test for significant differences between the samples. Means were compared at $p \leq 0.05$ significance level.

RESULTS AND DISCUSSION

Morphology of quince flowers

Morphological characters of quince flowers were in accordance with those reported previously. In the triplostemon **androeceum** various types of anther dehiscence could be observed. Some cultivars had pollen grains with pale yellow colour, which was similar to that of the young anthers. In other cultivars the colour of both the pollen and the stigma surface was bright yellow. **Stigma mimicry** was often characteristic for the latter cultivars.

Quince cultivars, investigated at the beginning of anther dehiscence, were divided into three groups on the basis of their **pistil** morphology:

1. Greenish-yellow pistils with small, undivided stigmas and long, thin styles.

2. Bright yellow pistils with medium-sized, 3-lobed stigmas and long, thin styles. Sometimes purplish red papillae can also be found on their surface.

3. Greenish-yellow pistils with large, 3-lobed stigmas and short, stout styles. Purplish red papillae are typical in this group.

The relative position of stigmas and anthers also changes with the age of the flower. Spatial relations and movements of the reproductive organs are connected with pollinator attraction and pollination mechanisms of the flowers. At the beginning of anther dehiscence filaments usually moved away from the style. Rarely, one or two styles could curve to the freshly dehisced anthers. At the end of pollen shedding receptive pistils often leant out of flowers aspiring to cross-pollination.

Morphology of androeceum

In 2004-2006 the average **number of stamens** varied between 18.12-23.49 in the investigated cultivars. Number of stamens varied significantly with change of weather conditions so it was not characteristic to varieties.

During 2004-2006 the smallest anthers (4.4 mm^2) were measured in cv. '*Szentlőrinci*' and the largest ones (6.7 mm^2) in cv. '*Mezőkövesdi*'. 11 of the studied 24 cultivars had small anthers while the others had medium-sized anthers. The studied features of anthers did not differ significantly in the three years.

According to the classification of Erdtman (1966) and Halbritter and Schneider (2000) quince varieties had medium-sized **pollen** grains. The length of their equatorial axis (E) varied between 31.4-51.7 μm and the length of the polar axis (P) between 25.3-44.1 μm. In 2005 quince cultivars had smaller pollen grains than in 2006. According to the evaluation system based on P/E (Erdtman 1943, 1952), the shape of pollen grains was oblate, suboblate or oblate-spheroidal.

Morphology of androeceum showed relationship with pistil morphology and fertility of cultivars. In 2006 cultivars with large, 3-lobed stigma (third group of pistil morphology) had significantly larger anthers than the cultivars in the other two groups.

Secretory tissues of quince flowers

Stigmatic surface was covered with papillate epidermis and a thin layer of cuticle.

The intrafloral **nectary** of quince was ring-shaped, surrounding styles from the apex of the ovary to the basal part of the stamens. Its shape was partly automorphic. The glandular tissue was protruding out of the basal part of the receptacular tissue, but the apical part of the nectary was epimorphic. The glandular tissue did not always reach the ovary, in which case the nectary was **receptacular**. However, most of the cultivars had **ovario-receptacular** glands

because their nectary reached as far as the ovary. In 2004-2005 the **area** of the nectary changed between 0.92-1.48 mm^2. In 2005 there was a linear correlation between the area of the nectary and the nectar volumes secreted by pollen shedding flowers. Late flowering cultivars had larger nectary, with about 0.2 mm^2, compared to early or mid-season flowering cultivars.

The adaxial epidermis of the nectary was covered by **cuticle** and wax. According to the average of the two seasons, the thinnest cuticle (2.60 μm) was found in cv. '*Örsi*' and the thickest one (3.67 μm) in cv. '*Kúti*'. Guard cells of the **stomata** were usually sunken below the outer anticlinal wall of the epidermis cells, but the inner anticlinal walls were at the level of the epidermal cells. The **area of the epidermal cells** varied between 244.75-366.26 μm^2 and 214.02-413.28 μm^2 in 2004 and 2005, respectively. Based on the quotient of the width (W) and length (L), the **shape of the epidermal cells** was anisodiametric, following MARÓTI's (1966) nomenclature (W/L_{2004}: 2.02-3.46, W/L_{2005}: 2.30-3.91). The stomata of cultivars with rather elongated epidermal cells were more sunken so their nectary could retain more nectar. In quince, anisodiametric epidermal cells could take over the role of nectar chambers, which were not characteristic for any cultivars.

In the majority of varieties the small, isodiametric cells of **glandular tissue** were arranged in columns perpendicular to the surface. The characteristic mosaic structure of the nectary comprised three cell types: glandular cells, nectary parenchyma cells and larger light-stained cells of the receptacle. On the basal part **thickness of glandular tissue** varied between 142.40 μm (cv. '*Váli*') and 239.37 μm (cv. '*Pear-shaped Bólyi*'). In 2004 **area of glandular cells** was 40.31-81.19 μm^2, in 2005 41.37-76.49 μm^2. In 2004 and 2005 the smallest **cytoplasm-nucleus ratios** of the glandular cells were found in '*Pear-shaped Dunabogdányi*' (0.77), and '*Apple-shaped Dunabogdányi*' (0.72), respectively. The highest cytoplasm-

nucleus ratios (1.65 és 1.72) were measured in cvs. '*U.V.13. Martonvásár*' and '*Bereczki*'.

On the basal part of the nectary **thickness of parenchyma layer** was 500-900 μm. Parenchyma cells were four times as large as glandular cells. Self-fertile cultivars had smaller glandular parenchyma cells than self-sterile ones. The vascular strands of quince nectary did not enter the secretory tissues.

Floral biological types

Quince cultivars were usually characterized by various flower biological strategies, even the flowers of a single tree in a given year could belong to more than one flower biological group. Besides delayed homogamy beginning with protogyny, several variants of homogamy also occurred in the investigated varieties.

In **delayed homogamous flowers** the **stigma** became receptive in the bursting bud preceding anthesis. In the young open flower some of the stigmatic papillae lost their turgor already, and no stigmatic exudates were detected by the end of anther dehiscence. Nectar production also started in the bursting bud simultaneously with stigmatic activity. The largest yields were produced by the young, opened and pollen shedding flowers. Secretion of nectar continued until the end of anther dehiscence. **Homogamous flowers** of the investigated quince cultivars were classified into three functional groups according to the active period of the pistils and anthers. Their anther dehiscence and stigma secretion commenced at the same time. The stigmas ceased to be active either after all anthers have dehisced (1. and 2. groups) or remained receptive for 2-4 hours after the pollen shedding phase (3. group). In the **1stfunctional group of homogamy** both reproductive organs became active in the bursting bud, and at the same time nectar secretion also started. Highest volumes of nectar with highest sugar concentrations

were measured in the young, opened flowers and in flowers with at least half of the anthers dehisced. Nectar production lasted to the end of pollen shedding. In the **2nd and 3rd functional group of homogamy,** anther dehiscence and stigma secretion commenced in the young opened flowers. Nectar secretion started at the beginning of stigma receptivity. In the 2nd group flowers were the most attractive after half of the anthers have dehisced, by producing the highest volumes of nectar with the highest sugar concentrations. At the end of pollen shedding and stigma receptivity, nectar production also finished. In the **3rd functional group of homogamy** the peaks of nectar secretion were measured after half of the anthers opened and after anther dehiscence has finished. Secretion of nectar lasted until the stigma remained glossy and receptive.

The daily rhythm of the activity of floral organs

Nectar secretion was periodic, production maxima could be observed in every 3.5-4 hour. Stigmatic exudates were present continuously and anther dehiscence was also continuous. The maxima of pollen shedding occurred simultaneously with the peaks of nectar secretion one hour later. In the warmest midday hours the rate of pollen shedding increased.

Pollen viability

According to the results of isatin staining, quince varieties were characterized by low pollen viability, with values ranging between 6.66 % and 47.98 % in the studied cultivars. Significant differences were detected between different years regarding this feature. The most viable pollen was found in cvs. '*Angers*', '*Bori*' and '*Óriás*'.

Proline concentration of pollen was highly variable (100-300 μM). However, percentage of proline was usually above 2 %, thus, quince pollen was of

high quality. Pollen of the early flowering cultivars contained more proline than that of the other groups.

The fertility type of quince varieties also correlated with the results of **isatin test**. The largest proportion of viable pollen grains (about 50 %) was found in self-fertile cultivars, while the lowest proportion of viable grains (20-38 %) was counted in the partly self-fertile group.

Nectar production of flowers of quince cultivars

Bursting buds and **young opened flowers** did usually not secrete any nectar. The highest volumes of nectar, 2-4 μl per day, were drained from the **pollen shedding and senescent flowers**. In the pollen shedding flowers, the sugar concentration of nectar changed between 20-30%, in old flowers between 25-40%. Accordingly, the nectar of senescent flowers was attractive for bees due to both high volumes and high sugar concentration. Comparing **blooming stages,** flowers secreted more concentrated nectar at the beginning of bloom than in full bloom. At the end of bloom, pollen shedding flowers were characterized by higher volumes of less concentrated nectar, while senescent flowers produced lower volumes of more concentrated nectar than in full bloom. A correlation was found between the **fertility relations** of quince cultivars and the volume and sugar concentration of their nectar. The lowest volumes of nectar with the lowest sugar concentrations were produced by self-fertile cultivars.

Nectar was characterized by the highest **sugar-values** at the beginning of bloom, and by the lowest sugar-values at the end of bloom The following cultivars can be recommended for apiculture, based on their favourable nectar traits: '*Bori*', '*Pear-shaped Dunabogdány*', '*Horváth Antal*', '*Constantinople*', '*Pear-shaped Noszvaji*', '*Óriás*' and '*Tinnye 220*'.

Main sugar components of the nectar

In 2004 **total sugar content** of quince nectar varied between 160.99-540.61 mg/ml, in 2005 between 146.87-330.22 mg/ml. Floral nectar of quince contained **sucrose** in the largest amount (77.99-364.34 mg/ml), so it was very attractive for honey bees. From the two main monosaccharides, the concentration of **fructose** was higher than that of **glucose**.

According to the evaluation system of Baker and Baker (1983a, 1990), the nectar of 1 and 6 cultivars belonged to the sucrose rich group [**S/(G+F)**=0.50-0.99] in 2004 and 2005, respectively. The nectar of the other investigated cultivars belonged to the sucrose dominant category [S/(G+F)>0.99] in both years.

Considering floral nectar traits which are important from apicultural viewpoint (volume, sugar content and composition), the floral secretory product of *C. oblonga* proved to be less attractive to pollinators with its volume and the most attractive with its sugar composition.

SUMMARY OF RESULTS

The majority of quince (*Cydonia oblonga* Mill.) cultivars requires cross pollination to produce acceptable yield. The main pollen vectors are honeybees, rewarded by nectar and pollen. The main objective of our study was to investigate the features of these two primary attractants, as well as some of the secondary attractants like floral morphological characters. A further aim was to provide an in-depth analysis of the floral and pollination biology of various quince cultivars.

The investigated quince cultivars can be classified into 3 types based on the morphology of their stigma and style. The anthers of cultivars with large stigma and robust style were significantly larger than those in the other two groups. The anthers of self-fertile cultivars were smaller compared to self-sterile and partially self-fertile ones.

The relative position of the stigma and the anthers was changing through various developmental stages of the flower, due to the movements and elongation of the reproductive floral parts.

The nectary of quince is either receptacular or ovario-receptacular. The more protected was the position of the gland, the lower was the monosaccharide content of the nectar. Positive correlation was found between the area of the nectary and the volume of nectar. In the glandular tissue 3 cell types were mixed to show a mosaic-like pattern. The larger size of the glandular cells can correspond to larger sucrose content in the nectar. Compared to self-fertile cultivars, the parenchyma cells in self-sterile cultivars were bigger and were able to store larger quantities of starch, ensuring higher nectar sugar contents and better insect attraction.

In early-blooming cultivars the nectary was smaller, but the proline content of the pollen was higher than in late-blooming cultivars. Proline-content of quince pollen exceeds 2%, however, pollen vitality determined with isatin-staining proved to be rather low.

Quince flowers can be characterised by delayed homogamy or one of the 3 subtypes of homogamy, each type accompanied by a characteristic nectar secretion pattern. Nectar is typically produced with 4 hour intervals, similarly to other pome fruits.

The best nectar producers were the pollen-shedding and the old flowers, providing 1-4 μl nectar with 20.00-40.00% sugar concentration. Sugar values varied to a high degree in different years and blooming phases. For pollinators the most attractive feature of the nectar is its sugar composition, rather than its quantity.

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