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Floral anatomy and flower visitors of three persimmon (*Diospyros kaki* L.) varieties cultivated in Central Europe

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ABSTRACT

We report flower and pollination biological traits of three persimmon (*Diospyros kaki* L.) varieties cultivated under suboptimal conditions in the temperate climate of Central Europe. In order to observe flower visiting insects and floral morphology, and to determine the nectar producing capacity of persimmon flowers, field studies were conducted in 2018 and 2019. The anatomical studies were performed with light microscopy. Quantitative floral traits were analysed with two-sample t-test. The main flower visitors were honeybees (*Apis mellifera* L.) and bumblebees (*Bombus* sp.), which can act as pollinators, while searching for nectar. The studied persimmon varieties belong to the gynoecious type, the solitary pistillate flowers consisting of four-membered calyx and corolla, reduced androecium and a pistil with superior ovary and 3 to 5 stigmata. The size of the calyx was significantly different in different varieties, but corolla diameter did not differ within the same year of study. The diameter of both the calyx and corolla of the same variety was bigger in 2019 compared to 2018, due to favourable climatic conditions. The sepals are covered by cuticle, stomata are typically mesomorphic, and both cover hairs and capitate glandular hairs can be observed. The mesophyll is made up by spongy parenchyma, with closed collateral vascular bundles and substantial proportion of intercellular cavities. The cuticle-covered petals are thicker and consist of more cell layers, but contain smaller proportion of intercellulars, compared to sepals. The ovary contains orthotropous ovules, and stigma papillae are structurally suitable for receiving pollen. The horn-shaped nectary is automorphic, supplied with vascular bundles. Despite the presence of fully developed nectaries, we were not able to extract any nectar from the flowers, which may be due to suboptimal climatic conditions for nectar production.

Keywords : bumblebee, honeybee, nectar, pistillate flower, pollination

The genus *Diospyros* from family Ebenaceae is pantropical, but a few species can grow in temperate regions, as well. The main area of distribution includes Asia, South Europe, parts of Africa and the United States, and Brazil (Rauf *et al*. 2017). The commercially important species are valued for their edible fruit, leaves used in traditional medicine and timber.

Diospyros species are deciduous or evergreen trees or shrubs, with simple, alternate leaves. Flowering habit ranges from dioecious to monoecious. Pistillate flowers are generally solitary, whereas staminate flowers are produced in cymous clusters of 3 to 5 actinomorphic flowers. Most commercial varieties are pistillate constant, while staminate bearing varieties can be important as pollinizers. The berry fruits contain 1 to 16 seeds (George *et al*. 1997, Rauf *et al*. 2017).

Pollination is insect mediated with European honeybee (*Apis mellifera* L.) or other bee species such as *Trigona* spp. (George *et al*. 1993, 1997).

Various species of the genus were indicated as potential sources of honey: *D. virginiana* in the United States (Lieux 1972), *D. abyssinica* in Ethiopia (Belay *et al*. 2015) and *D. whyteana* in South Africa (Mensah *et al.* 2017). However, no data are available regarding the actual nectar producing capacity of persimmon flowers.

Although persimmon (*Diospyros kaki* L.) is typically grown in subtropical regions, it can be successfully cultivated in temperate climate, as well. However, data are lacking regarding the flower and pollination biology of various varieties under these suboptimal climatic conditions. The objectives of our research were to provide data on (1) the flower morphology of three persimmon varieties grown in a Central European plantation; (2) flower visiting insects and their potential pollination biological significance; as well as (3) the nectar producing capacity of flowers. In addition, since no data were available regarding the histology of persimmon flowers, we intended to (4) analyse the anatomy of various floral parts.

MATERIALS AND METHODS

Present study was conducted on three persimmon (*Diospyros kaki*) varieties, 'Rojo Brillante', 'Sharon' and 'Vaniglia', all of which belong to the gynoecious type, bearing only pistillate flowers.

1. **Field studies**—Observations were carried out in a persimmon plantation located in Boda, South Hungary (N 46° 4' 46.72", E 18° 2' 51.99") in the years 2018 and 2019. Flower morphological traits, and the type and frequency of flower visiting insects were recorded on two different

occasions in each year: on 18 and 23 May in 2018, and on 28 May and 6 June in 2019.

2. **Flower morphology**—On each day of the study 20 flowers were selected randomly from at least 5 different trees in each variety. Variety specific floral morphological characters were recorded; and the diameter of the calyx and the corolla were measured to the nearest millimetre with a ruler. Quantitative flower morphological traits were analysed with two-sample t-test.
3. **Flower anatomy**—From each cultivar 20 flowers were collected randomly and preserved in ethanol : glycerol : distilled water (1:1:1) until further processing. Samples were dehydrated in ascending ethanol series, and embedded in artificial resin Technovit 7100. Longitudinal sections of the gynoecium and cross sections of the sepals and petals were cut with a rotary microtome (Anglia Scientific), stained with toluidine blue and mounted in NeoMount (Merck). Microphotos were taken with a Motic 102M light microscope equipped with digital camera. The thickness of the sepal and the petal, as well as the size of the nectary were measured with Motic Images Plus 2.0 software.
4. **Nectar measurements**—We attempted to gather nectar from persimmon flowers with two different methods. In 2018 microcapillaries bearing microliter marks (Duran) were used, but no nectar could be obtained with this method. In 2019 we used the washing method of Morrant *et al.* (2009), developed specifically for sampling low volume nectars, with slight modifications. The nectar of seven flowers was sampled from each variety; flowers were cut from the plant, then placed in a 30-mL vial and 2 mL distilled water was added using a micropipette. The sealed vials were agitated manually for 1 min, then placed in cooling bags until laboratory analysis. The percentage of total sugar was measured with an ATAGO N-50E hand refractometer.

RESULTS AND DISCUSSION

Flower visitors —In both years of the study, and in each variety, persimmon flowers were visited by honeybees (*Apis mellifera*, Apidae) and bumblebees (*Bombus* sp., Apidae). In a few instances, we recorded the presence of butterflies (Fig. 1), hoverflies (*Epistrophe balteata*, Syrphidae) and ants (Formicidae). We observed bumblebees in the largest number, followed by honeybees on each variety in each year. Previous studies also mentioned honeybees among the most important pollinators of persimmon flowers (George *et al*. 1993, 1997), but our study was the first to record the above mentioned other flower visiting insects, which may also act as potential pollinators. It has to be noted that ants were observed only around the flowers of variety 'Sharon', and mostly outside the calyx, so they cannot be counted on as pollen vectors.

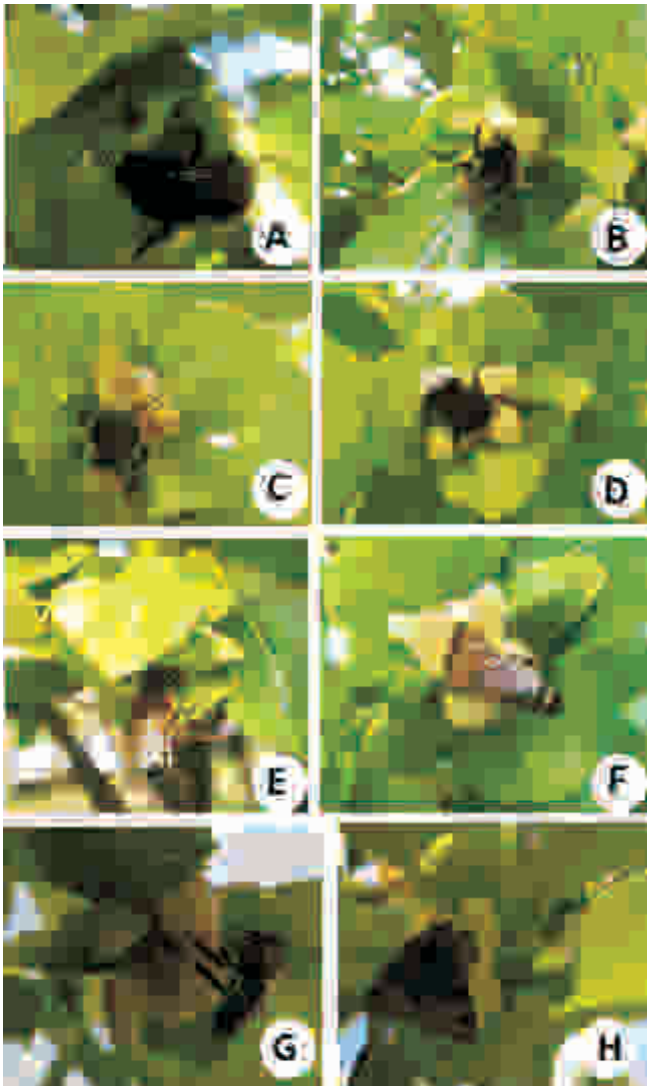

Fig. 1– Flower visiting insects of persimmon flowers. Bumblebees on the flowers of variety 'Rojo Brillante' in (A) 2018 and (B) 2019; variety 'Sharon' in (C) 2018 and (D) 2019. Honeybees on the flowers of variety 'Rojo Brillante' in (E) 2018 and (F) 2019; variety 'Vaniglia' in (G) 2019. (H) Butterfly on the flower of variety 'Sharon' in 2018.

Flower Morphology—Since all three varieties in this study belong to the gynoecious (pistillate) type, we could observe practically no male or hermaphrodite flowers. The pistillate flowers are solitary, located in leaf axils. The calyx, with a diameter of about 40 mm (Table 1), consists of four green sepals. In variety 'Sharon' the sepals are wavier than in the other two varieties. The size of the corolla is approximately half of that of the calyx, with a diameter of about 20 mm; consisting of four creamy yellow petals, the tip of which is curling back, in variety 'Vaniglia' to a lesser degree. The androecium is lacking or very much reduced, only short filaments can be observed in some flowers. The gynoecium is made up by a superior ovary, the number of stigmata varies between 3 and 5 (Fig. 2).

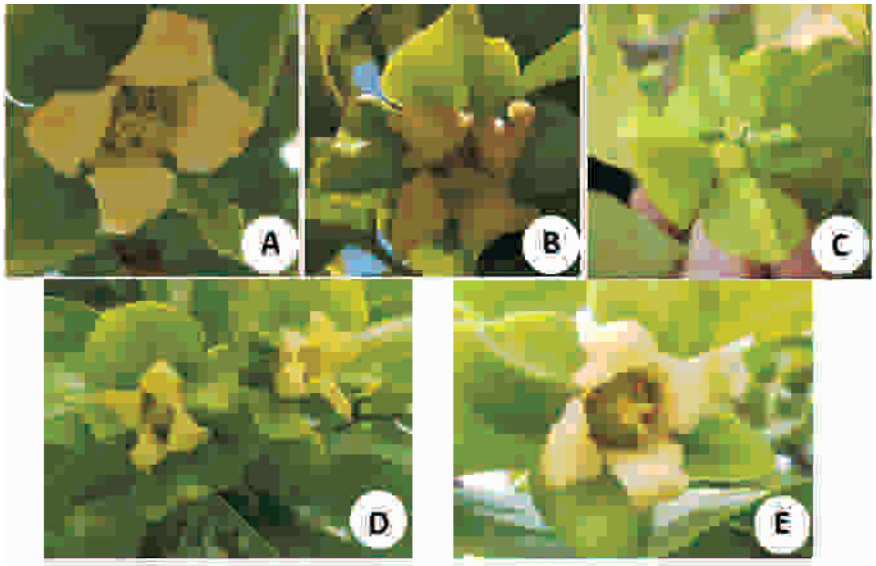

Fig. 2 - Four-membered flowers of persimmon variety (A) 'Rojo Brillante' in 2018, (B) and (C) 'Vaniglia' in 2018, (D) 'Sharon' in 2019 and (E) 'Fuyu' in 2019. (C) Both corolla and androecium are missing. (E) Androecium is reduced to filaments.

Table 1 — Diameter of calyx and corolla in three persimmon varieties in two different years. Data are presented as mean ± standard deviation. Different letters within columns indicate significant differences at P<0.01, according to the results of two-sample t-test (N=20).

	Calyx diameter (mm)		Corolla diameter (mm)	
Variety	2018	2019	2018	2019
Rojo Brillante	42.26 ± 2.79a	46.52 ± 2.34a	20.84 ± 2.12a	24.19 ± 1.86a
Sharon	39.25 ± 3.37b	46.04 ± 3.34ab	19.50 ± 1.54a	23.54 ± 1.88a
Vaniglia	38.25 ± 1.71b	43.41 ± 3.4 b	22.60 ± 1.70b	24.59 ± 1.72a

In both years of the study, the diameter of the calyx was significantly different in different varieties within the same year. In each year, the variety 'Vaniglia' was characterised by the smallest calyx diameter. Corolla diameter values were found to be similar in all three varieties within the same year, the only significant difference $P < 0.01$ was measured between the corolla size of 'Vaniglia' compared to both 'Rojo Brillante' and 'Sharon' in 2018 (Table 1). When comparing different years, we found that both calyx and corolla diameters of the same variety were bigger in 2019 than in 2018 at $P<0.001$ (except corolla size in 'Vaniglia', where $P<0.01$). The reason for this increase in flower size might be due to more favourable weather conditions in the period directly preceding bloom and at full bloom. Due to an uncommon, prolonged rainy period in the May of 2019, the blooming period started two weeks later (first half of June) than in 2018 (second half of May). Therefore, higher temperatures were measured in the period of full bloom, which, together with abundant precipitation in the bud forming period, proved to be beneficial in terms of flower size.

Flower Anatomy—The 350-500 µm thick sepals are covered by cuticle, typically on both sides. In some cases, capitate glandular hairs can be observed. The adaxial surface of the sepals in variety 'Vaniglia' is densely covered with uniserial cover hairs. Stoma guard cells are located generally in the same level as the epidermal cells (mesomorphic type). However, we observed few hygromorphic stomata, as well, in variety 'Sharon', where the guard cells were raised above the level of epidermis. While the presence of a thick cuticle and cover hairs can indicate adaptation to dry environments, the meso- or even hgygromorphic position of stoma guard cells refers to sufficient water supply. On the other hand, both cover hairs and glandular hairs may have a protective role in case of frost, which commonly occurs in temperate climate when the flowers are in the bud stage. The mesophyll is made up by spongy parenchyma, with several intercellular cavities and closed collateral vascular bundles.

The petals are thicker (400 to 900 µm thick) and consist of more cell layers compared to sepals. Also petals can be covered by cuticle and in some cases by cover hairs, as well. The mesophyll contains smaller proportion of intercellulars, compared to the sepal. Closed collateral vascular bundles represent the vascular tissues.

Regarding the pistil, the ovary contains orthotropous ovules, and the elongated stigma papillae are structurally suitable for receiving pollen. The horn-shaped nectary is automorphic, its size ranges from 15,000 to 25,000 μm^2 in the medial longitudinal section of the flower. Nectary tissues are

well distinguishable from those of the receptacle. The nectary is supplied with vascular bundles (Fig. 3).

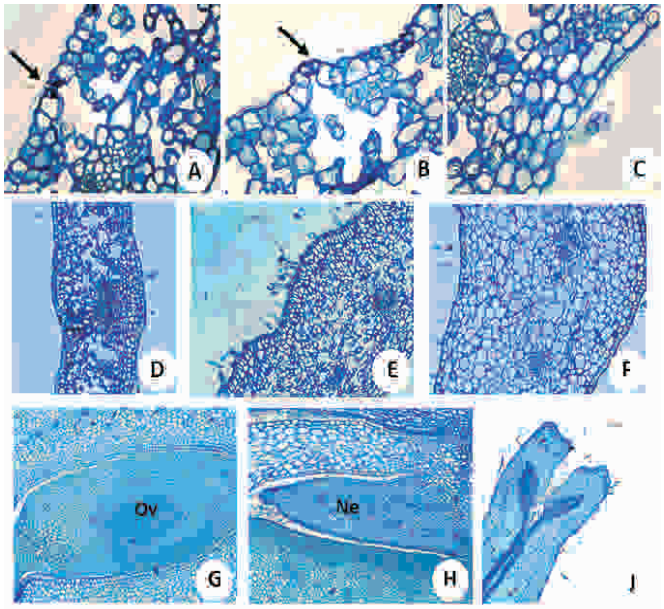

Fig. 3 – (A)–(C) Cross section of sepal in variety 'Sharon', (D) 'Rojo Brillante' and (E) 'Vaniglia'. (A) Mesomorphic and (B) hygromorphic stoma (indicated by arrow) in sepal epidermis. Trichomes: (C) and (D) Capitate glandular hair and (E) non-glandular hairs on sepal. (F) Cross section of petal in variety 'Rojo Brillante'. (G) Ovule (Ov) and (H) nectary (Ne) in variety 'Vaniglia'. (J) Stigma lobes of variety 'Sharon'.

Nectar production—As shown above, the nectaries are structurally capable of producing nectar. However, we were not able to extract any nectar from the flowers of any persimmon varieties in the two years of the study, even though it was attempted with a variety of methods. This may be due to suboptimal climatic conditions for nectar production. It is possible that the trees are not able to produce measurable amounts of nectar under temperate climate, or the microclimatic conditions were not favourable in the two years of the study. On the other hand, the flowers attracted a large number of flower visiting insects. In the lack of viable anthers and consequently lack of pollen, it is possible that persimmon flowers provided sufficient amount of nectar to attract these insects, but these nectar volumes were too low to be measured by us.

Our study on persimmon varieties cultivated outside their native region revealed that flower morphology is consistent with previous descriptions of *Diospyros kaki*, and the size of the perianth may vary both with the variety and climatic conditions. The main pollinators belong to various bee species, but in addition, other flower visiting insects, such as hoverflies and butterflies were recorded for the first time. The appendages of the sepal, i.e. the cover hairs and the glandular hairs may protect the flowers in case of frost or dry periods. The anatomical studies revealed functional nectaries in each variety studied, but no measurable nectar was found in any of the flowers, which may be due to suboptimal climatic conditions for nectar production.

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