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BRIEF COMMUNICATION

Variability and bimodal distribution of size in microspores of *Aesculus hippocastanum*

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Abstract

Size variability of uninucleate microspores was studied in horse chestnut (*Aesculus hippocastanum* L.). Microspores were isolated from buds of different size (3, 4, and 5 mm) taken from lower, middle and upper segments of inflorescences. All analyzed buds showed bimodal distribution of microspore size which confirmed the presence of pollen dimorphism.

Additional key words: horse chestnut, microspore diameter, pollen dimorphism.

Pollen dimorphism has been detected in anthers of numerous herbaceous and some woody species. This phenomenon has also been observed in horse chestnut (Radojević 1989, 1991) and red chestnut anthers (Marinković and Radojević 1992), showing differences in pollen size, shape, viability, staining intensity, fluorescence and embryogenic potential (Radojević *et al.* 2000). Light, scanning and transmission electron microscopy of 13 species of the genus *Aesculus* confirmed considerable differences in pollen size, width of colpi and position of pore, shape of colpus ends, sculpturing of colpal membrane, sculpturing of mesocolpia and, in the case of striate sculpture, ratio of stria and perforation sizes (Pozhidaev 1995).

Aesculus hippocastanum L. flowers are positioned on a 20 - 30 cm long inflorescence. Those located in the basal part of the panicle are female and fertile (segment A). The flowers in the middle are bisexual (segment B) and those on the top of the panicle are male (segment C; Heywood 1978). The female flowers have both pistils and stamens but the stamens fade prematurely and the anthers do not open. The male flowers have underdeveloped pistils and never form fruits. The term bisexual flowers (segment B) refers to flowers with normally developed and functional pistils and stamens

Anthers were isolated from flower buds of different size (3, 4, and 5 mm) developed on A, B and C inflorescence segments. The content of anthers was squeezed out and stained with 1 % orcein solution, prepared in 45 % acetic acid. Hundred microspores were analyzed from each bud. Orcein-treated microspores were observed with *Leica, DMRB* microscope (Wetzlar, Germany), and analyzed by *UTHSCSA Image Tool version 3.0* (San Antonio, USA) software program. A camera was connected to the microscope. The results were analyzed according to *Fig.P BIOSOFT version 6.0* (Ferguson, USA) software package, using completely randomized design.

Least significant difference (LSD) test showed that flower buds of 3 mm in length contained significantly smaller microspores than 4 and 5 mm long flower buds in all of the segments (Table 1). In addition, average microspore size varied depending of the segment. In corresponding bud size, the smallest microspores were in the segment A, and biggest in segment C (Table 1).

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Variability in horse chestnut pollen size was confirmed by frequency distribution of different microspore diameter classes in segments A, B and C (Fig. 1). Two characteristic peaks for all flower buds (size

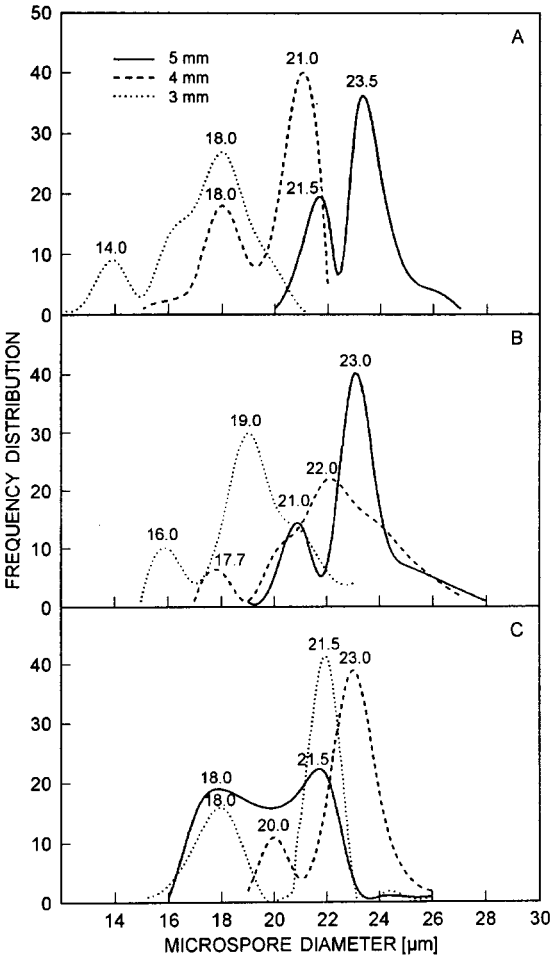

Fig. 1. Bimodal distribution of microspore size (in 3, 4 and 5 mm buds) from segments A (*A*), B (*B*) and C (*C*) of *Aesculus hippocastanum* L. inflorescences.

Table 1. Average diameters of microspores originating from A, B and C segments of horse chestnut inflorescences. Means ± SE, $n = 100$. In each column, the values with different letters are significantly different at $P = 0.05$ according to protected LSD test.

Bud length [mm]	Microspore diameter [µm] A	B	C
3	16.90 ± 0.18c	18.98 ± 0.16b	19.29 ± 0.16b
4	20.46 ± 0.20b	22.14 ± 0.17a	22.31 ± 0.16a
5	22.34 ± 0.17a	22.88 ± 0.14a	23.43 ± 0.20a

3, 4 and 5 mm) indicated the presence of different size groups of horse chestnut microspores. We also observed that the two peaks, lower and higher, had different values for microspores isolated from flower buds of the same length, but originated from different segments. Similarly, Nägeli (1998) and Nägeli *et al.* (1999) had identified two distinct peaks in the distribution frequency of microspore size in maize.

Diameter of microspore in all analyzed buds (3, 4 and 5 mm in length) that were isolated from A, B and C segments showed bimodal distribution (Fig. 1). Thus in all buds were microspores with shorter and with longer diameter. These results are in correlation with already published papers about pollen dimorphism in *A. hippocastanum* (Radojević 1989, 1991) and in *Aesculus carnea* (Marinković and Radojević 1992). It was suggested that uninuclear microspores can be grouped into two types: large, densely staining (with acetocarmine) non androgenic, and small, lightly staining, androgenic (Radojević 1989, 1991). However, more recently androgenesis was successfully induced from microspores of all *A. hippocastanum* segments (Radojević *et al.* 2000). This can be explained by the presence of small, *i.e.* embryogenic microspores in all flowers (A, B and C segments) which was confirmed in the present work.

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