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MICROSPOROGENESIS AND DEVELOPMENT OF POLLEN GRAIN IN HYBRIDS OF SEA BUCKTHORN (*Hippophaë rhamnoides* L.) OF VARIOUS ECOGEOGRAPHIC ORIGIN

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Summary

***Hippophaë rhamnoides* L. is of interest as feed and medicinal plant. Its fruits contain the C, B_1, B_2, E, P vitamins, polyunsaturated fatty acids, carotene, other biologically active compounds. The temperature conditions during winter-spring period is main limiting factor in sea-buckthorn introduction, and the male generative sphere is the most sensitive to unfavorable conditions. The authors investigated a microsporogenesis in *Hippophaë rhamnoides* L. hybrids of various ecologo-geographic origin of Nizhny Novgorod State Agricultural Academy breeding. The stage of male gametophyte development was determined on squash temporary preparation. As result of analysis the two groups of hybrids were revealed, which differ on the time of beginning and a course of the definite stages of microsporogenesis. The first group involves the hybrids with prevalence of morphophysiological determinants of Katunskii ecotype (Debyut, 5/87, 1/89 and 1/91), the second one — with prevalence of morphophysiological determinants of Baltic ecotype (Gerakl, 31/89, 1/90 and 5/93). In hybrids of the first group the beginning of microsporogenesis and the formation of microspore tetrads occur at the low sum of positive temperatures (respectively, 48.5-49.5 and 114.8-118.5 °C) than in hybrids from second group (62.0-71.1 and 135.6-147.0 °C). However, the maturation of pollen and blossoming (anthesis) of male plants in studying ecotypes pass practically at the same time, at the sum of positive temperatures from 171.8 to 216.6 °C and from 276.3 to 343.9 °C, respectively.**

Keywords: sea buckthorn, hybrids, microsporogenesis, sum of positive temperatures.

In ontogeny plant organisms are exposed to various biotic and abiotic factors, one of which often becomes the limiting factor so significant that the other can be neglected. Knowing the influence of external factors on reproductive process of plants is essential for their introduction and breeding.

Sea buckthorn (*Hippophaë rhamnoides* L.) is a relatively recently introduced berry crop with high nutritional and medicinal value. Its fruits contain vitamins C, B1, B2, E, P, polyunsaturated fatty acids, carotene, and other biologically active compounds. *H. rhamnoides* is used as a source of tannin and dyes, and as a plant of soil erosion control. Its natural populations grow on large areas in the territory of Russia and the former Soviet Union. Within the distribution area, *H. rhamnoides* has a number of relatively isolated and distinct ecotypes, and smaller intraspecific taxa - climatypes (1-3), among which Baltic and Katun ecotypes are the most important for breeding dioecious individuals. Climatic (4-8) and edaphic conditions of growing sea buckthorn (9, 10) have been studied in detail. The major limiting factor in introduction of sea buckthorn is temperature conditions of winter-spring period.

Winter hardiness of *H. rhamnoides* in Nizhny Novgorod Oblast was studied by M.A. Korovina (11), V.V. Selekhov (12), and M.P. Smertin (13), who found that adverse conditions of winter-spring period are most critical for male reproductive organs of *H. rhamnoides*. However, the effect of temperature conditions on early stages of reproductive processes in male plants of sea buckthorn haven't been yet described in the available scientific literature.

It is known that development of anthers in *H. rhamnoides* includes two stages that occur during two growing seasons. The first stage (from archesporial cell to formation of sporogenic tissue and anther wall) occurs from late July to October, the second (microsporogenesis and formation of pollen grains) – in spring (14-16).

The purpose of this work – to establish the relationship between microsporogenesis in *Hippophaë rhamnoides* staminate hybrids of different eco-geographical origin and temperature conditions in spring.

Technique. Investigations were carried out in 2005-2007 on the base of Nizhniy Novgorod State Agricultural Academy (NGSKhA). The objects of investigation were anthers and male gametophytes derived from 8 promising breeding hybrids *H. rhamnoides* established in NGSKhA; these plants of different eco-geographical origin showed a prevalence of morphophysiological characters of Katun ecotype (Debut, 5/87, 1/89, 1/91) and Baltic ecotype (Gerakl, 31/89, 1/90, 5/93), for which they were divided into two groups, respectively, I and II. Total number of studied staminate plants – 40.

Samples for cytological analysis were collected from upper brunches of trees (no less than 15 inflorescences from each hybrid) after the transition of average decade temperatures over 0 °C. Annually it was performed 15-16 samplings depending on the year of research. The development of male gametophyte was assessed in each tier of inflorescence (top, middle, and bottom) separately. Developmental stages of male gametophyte were determined in squashed preparations using a standard technique with aceto-carmine staining (17, 18).

Results. It is known that cell division in flower buds of sea buckthorn starts after the transition of average daily temperatures over 0 °C (19). According to V.R. Kondorskaya (14) and O.B. Proskurina (20) who studied this issue in Leningradskaya Oblast, and I.A. Kasimovskaya (21) – in Tambovskaya Oblast, this occurs in the period of April 20-30. The information about any correlations between early development of male gametophyte and the sum of positive temperatures wasn't found in the literature, although such dependence is known for leafing, inception and development fruits.

Determining developmental stages of the male gametophyte in sea buckthorn is complicated by asynchronous microsporogenesis in different parts of inflorescence: in the bottom buds it starts earlier, which is consistent with the literature data (14, 22). In different parts of one inflorescence were observed the anthers at different stages of development. The most significant differences were found in early stages of microsporogenesis. However, dispersion of pollen in a whole inflorescence was synchronous.

Like other flowering plants (23), in *H. rhamnoides* developmental stages of male gametophyte are manifested in size and color of anthers. Differences between the studied groups of hybrids were more pronounced during the active division in sporogenic tissue: they all had similar structure of anthers and development of male gametophyte, but they differed in time of starting particular stages of microsporogenesis (Fig. 1, 2).

In 2005, in the sample of April 11 it was observed only a distinct tapetum and sporogenous tissue. Individual flower buds showed a slight increase in tapetum cells and the beginning of cytokinesis. The first meiotic division in the lower buds occurred on April 13 (Fig. 1, 2), while the upper buds at that time contained only microspocytes.

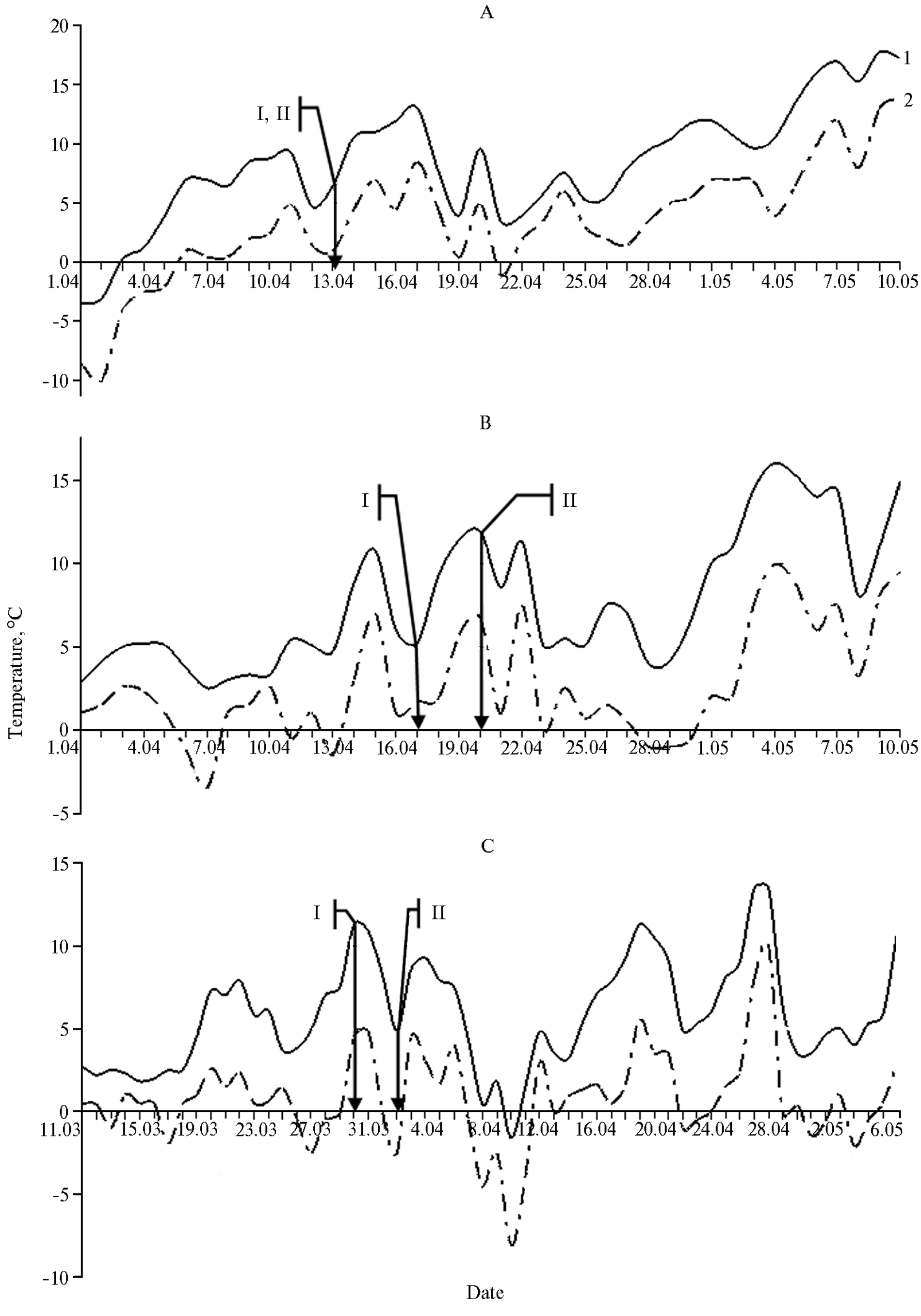

Fig. 1. Average daily (1) **and minimal** (2) **temperatures in spring shown by years of observation:** A — 2005, B — 2006, C — 2007**.** Arrows point on the start of microsporogenesis in hybrids of sea buckthorn (*Hippophaë rhamnoides* L.) of Katun (I) and Baltic (II) ecotypes (experimental station of Nizhniy Novgorod State Agricultural Academy).

In 2005, the hybrids showed no differences in the time of starting the meiosis in anthers as it occurred in a short period from April 13 to 18 (Fig. 2). The authors associate this fact with intense growth of positive temperature (Fig. 1). From April 13 to 18, air temperature didn’t drop below 1 °C, and the sum of positive temperatures accumulated rapidly up to 61.0 °C. Probably, peculiarities of microsporogenesis can be revealed during a more gradual growth of positive temperatures. In 2005, meiosis in anthers started at the sum of positive temperatures 64.0 °C.

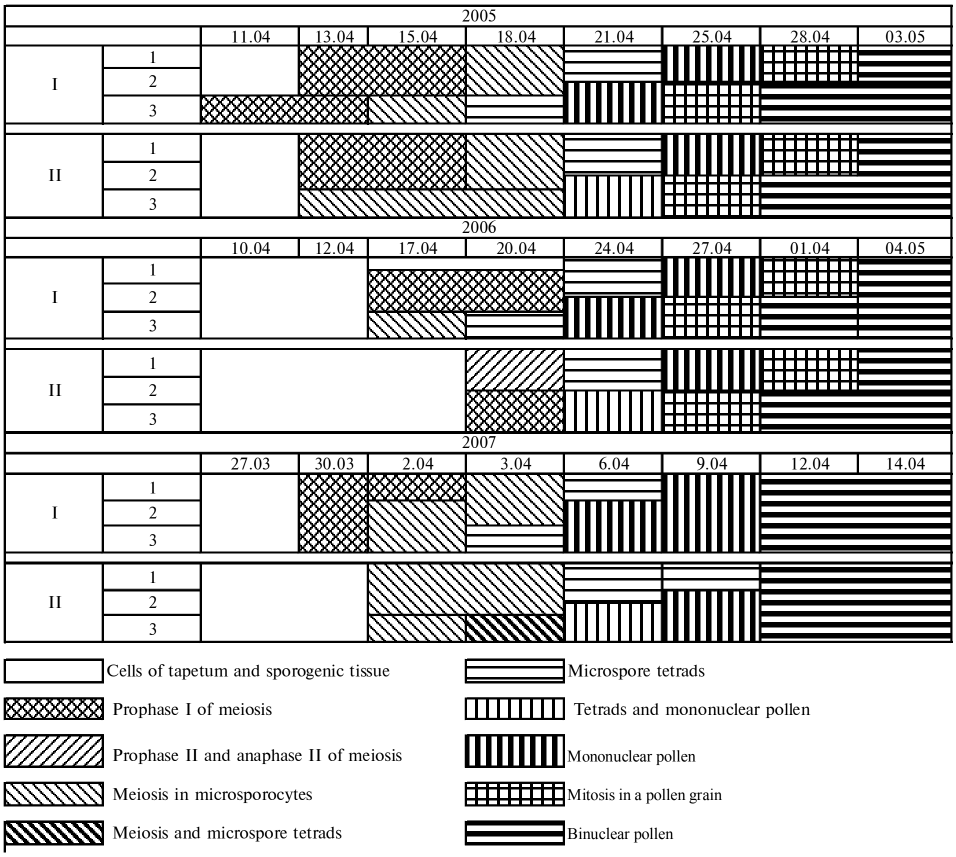

Fig. 2. Microsporogenesis stages in hybrids of sea buckthorn (*Hippophaë rhamnoides* L.) **of Katun (I) and Baltic** (II) **ecotypes, shown by sampling date:** 1- upper, 2 – medium, 3 – lower flower buds in inflorescences (experimental station of Nizhniy Novgorod State Agricultural Academy).

In 2006 (Fig. 1, 2), the hybrids of group I (Debut, 5/87, 1/89, 1/91) exhibited earlier start of meiosis (April 17) than group II (Gerakl, 31/89, 1/90, 5/93) (April 20). On hybrids of Katun ecotype (group I) there were individual inflorescences whose flowers of the lowest tier entered the meiosis already on April 12.

Gradual growth of positive temperatures in spring of 2006 along with low positive temperatures in early April and negative night temperatures in the period of April 11-13 contributed to clear differences in time of entering particular stages of microsporogenesis by hybrids of different eco-geographical origin (Fig. 1). The sum of positive temperatures in the period from April 17 to 20 was 37.7 °C. In hybrids of Katun ecotype, meiosis started at the sum of the positive temperature 48.5 °C (Fig. 1, 2), in hybrids of Baltic ecotype – at 62.0 °C.

The autumn-winter period of 2006-2007 with extreme weather conditions (long warm autumn, winter with unstable temperature) caused depletion and death of plants in the hybrids Debut, 1/90, and 5/93. In the spring of 2007, average decadal temperatures quite early exceeded 0 °C. In general, positive temperature increased gradually, but at night there were observed values below 0 °C. Formation of microsporocytes and the first division of meiosis in hybrids of Katun ecotype occurred much earlier (March 30) than in plants of Baltic ecotype (April 2) (Fig. 1, 2). In the first group, meiosis started at the sum of positive temperatures 84.0 °C, while in the second group – at 114.4 °C (Fig. 1). Such significant differences in the sums of positive temperatures probably resulted from a prolonged decline in night temperature (March 17, March 26-29, and April 1-2). Considering the fact that in the period of March 26-30 anther tissues didn't show any active differentiation, the start of meiosis in groups I and II corresponded to the sums of positive temperatures equal to 49.5 and 71.7 °C.

It can be assumed that in cases when pre-meiotic period of anthers coincides with the night temperature decline down to 0 °C and below, the start of meiosis requires a greater amount of positive temperatures.

Formation of tetrads of microspores in hybrids of Katun ecotype started earlier in all years of research (April 18, 2005; April 20, 2006; April 3, 2007) (Fig. 2) than in hybrids of Baltic ecotype (April 21, 2005; April 24, 2006; April 6, 2007). The start of formation of tetrads in group I corresponded to the sum of positive temperatures 114.8-118.5 °C, in group II – at 135.6-147.0 °C.

Formation of mononuclear pollen grains occurred almost simultaneously in all investigated forms regardless of their eco-geographical origin; separation of tetrads to individual microspores and the start of formation of unicellular pollen was extremely rapid (Fig. 2). While in the bottom part of inflorescences it was observed the stage of unicellular pollen grains that had not fully formed wall and cytoplasm, the upper buds contained only tetrads of microspores. In all the years of observation, individual mature bicellular pollen grains were observed earlier in the hybrid Debut than in other hybrids. Mature pollen grains remained in the anthers until dispersion. It was found that mature binuclear pollen was formed at the sum of positive temperatures 171.8- 216.6 °C. Pollen in all plants was completely mature on May 3, 2005; May 4, 2006, and April 12, 2007 (Fig. 2).

Dispersion of pollen began almost simultaneously in all hybrids regardless of eco-geographical origin of their parental forms. The remarkable fact is that dispersion of pollen in cv Gerakl in all years of study started 12-24 hours earlier than in the other hybrids. In 2005-2006, pollen dispersion of sea buckthorn was observed for 2-3 days, in 2007 – 7-8 days, which, in the authors' opinion, was associated with the decline in average daily temperatures and increased air humidity during flowering of male hybrids; this fact was consistent with observations of other authors (12, 24). The release of pollen started at the amount of positive temperatures 276.3 – 343.9 °C. Observational data of the year 2007 are of particular interest because the period from maturation of pollen to its dispersion lasted 20 days.

Thus, in sea buckthorn hybrids with a predominance of morphophysiological characters of Katun ecotype, microsporogenesis and the formation of microspore tetrads occurred at lower amounts of positive temperatures (respectively, 48.5-49.5 °C and 114.8-118.5 °C) than in hybrids of Baltic ecotype (62.0-71.1 °C and 135.6-147.0 °C). Both groups showed no difference in onset of pollen dispersion (flowering). The main factors that affect the start of pollen dispersion – maturity of pollen and weather conditions in the flowering period (temperature and air humidity). The decline in air temperature and growth of air humidity delay dispersion of pollen. It is practically important to establish pollinators with a prolonged period of pollen dispersion and selection of male sea buckthorn hybrids with various onset of anthesis ensuring more efficient fertilization of female flowers and high yields.

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