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REPRODUCTIVE FEATURES OF THREE INVASIVE ALIEN SPECIES OF *ERIGERON* (ASTERACEAE) IN BULGARIA

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(*Submitted by Academician V. Golemansky on September 25, 2012*)

Abstract

Embryological studies have been carried out on some established populations of three invasive alien species of the genus *Erigeron* in Bulgaria: *E. bonariensis*, *E. canadensis* and *E. sumatrensis*. The mode of reproduction and features of the reproductive system, especially these which facilitate the successful distribution and invasiveness of the species were established: a high amount of viable pollen; multicellular antipodal complex; antipodal and endosperm haustoria; simultaneous formation of mature embryos in the florets of the capitulum. All observed embryological features together with the enormous number of flower heads per individual and long flowering period, self-pollination, self-compatibility, autonomous production of achenes and their high dispersal capacity undoubtedly increase the invasive potential of the studied *Erigeron* species.

Key words: embryology, *Conyza*, *Erigeron*, invasive species, male and female gametophyte, reproduction

Introduction. *Asteraceae* is one of the largest families of Angiospermae and it comprises the highest number of invasive species [1]. In the Bulgarian flora, the genus *Erigeron* is represented with four naturalized alien species which are all invasive: *E. annuus* (L.) Pers., *E. bonariensis* L., *E. canadensis* L. and *E.*

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sumatrensis Retz. The latter three species have been treated until recently within the genus *Conyza* Less. in most Bulgarian botanical sources, e.g. DELIPAVLOV [2].

Reproduction is of fundamental importance for the establishment of populations of invasive species, since its type and specific characteristics have an effect on the successful naturalization and invasiveness [3]. According to GREEN [4], the reproductive system should be included as a key element in the protocols for risk assessment of the invasiveness of the most invasive species. Despite the fact that the reproduction mode and potential are connected with the abundance and distribution of the invasive species, the information on the specific reproductive features that influence invasiveness is rather limited [3, 5, 6]. The three invasive *Erigeron* species, object of the present work, are poorly studied regarding their mode of reproduction (amphimixis and/or apomixis) and the specific features of the embryological processes and structures that influence the reproductive capacity, level of invasiveness and adaptability of the populations.

The aims of the present work were:

- to establish the mode of reproduction and the specific features of the reproductive processes and structures in the three invasive species of *Erigeron*;
- to estimate the reproductive capacity after testing of the pollen viability as the studied species are self-compatible (autogamy).

Material and methods. A comparative embryological study on naturalized populations of three invasive species of the genus *Erigeron* in Bulgaria has been carried out as follows:

***Erigeron bonariensis* L.** (syn. *Conyza bonariensis* (L.) Cronquist) – native to tropical America. In Bulgaria it is distributed in the warmer parts of the country: the Black Sea Coast, the Valley of Struma River, the Thracian Lowland and the Tundzha Hilly Country floristic regions [7]. The material for the study was collected from two localities in the Thracian Lowland: the town of Svilengrad, the right bank of Maritsa River, 41.77537°N, 26.18864°E, 08.09.2010, coll. V. Vladimirov; the town of Lyubimets, the left bank of Maritsa River, 41.86752°N, 26.08421°E, coll. V. Vladimirov.

***Erigeron canadensis* L.** (syn. *Conyza canadensis* (L.) Cronquist) – native to North America. In Bulgaria it is widely distributed in all floristic regions [7]. The material for the study was collected from the Tundzha Hilly Country, the right bank of Tundzha River near the Galina Hotel between the villages of Srem and Knyazhevo, 42.06391°N, 26.50576°E, 09.09.2010, coll. V. Vladimirov.

***Erigeron sumatrensis* Retz.** (syn. *Conyza sumatrensis* (Retz.) E. Walker) – native to South America. In Bulgaria it is distributed in the Black Sea Coast, Northeastern Bulgaria, the Danubian Plain, the Forebalkan (Western), the Balkan Range (Eastern), Sofia Region, West Frontier Mts, the Valley of Struma River, Belasitsa Mt., the Valley of Mesta River, Pirin Mt. (Northern), Rila Mt., the Thracian Lowland, the Tundzha Hilly Country floristic regions [7, 8].

The material was collected from the Forebalkan (Western), Montana town, an abandoned garden in the centre, 43.41111°N, 23.22667°E, 07.08.2009, coll. V. Vladimirov.

The material for the study (flower buds and flowers at different development stages) was fixed in a mixture of FAA (formalin : glacial acetic acid : 70% ethanol in correlation 5:5:90 parts) and treated according to the classical paraffin methods [9]. The paraffin sections, 8–18 μm thick, were stained with Heidenhain's and Delafield's stains, and afterwards embedded in Enthelan. To estimate the pollen viability, 1000 mature pollen grains at the permanent slides for each of the studied species were counted. These with clearly distinguished generative and vegetative cells were considered as viable, while deformed, colourless and dark stained – as nonviable.

The observations were made with LM "Olympus" CX21 and microphotographs were taken with "Infinity Lite" Digital Camera 1.4 Mpx.

Results and discussion. The main embryological features of the three studied *Erigeron* species are reported below with particular comments on the features that influence the invasiveness.

Anther and development of the male gametophyte. The anthers are tetrasporangiate in the three studied species, which is congruent with earlier studies in *E. bonariensis* and *Conyza stricta* Willd. [10] and *E. canadensis* [11]. Because of underdevelopment of two of their locules in *E. bonariensis*, bisporangiate anthers were found. Four-layered anther wall develops after Dicotyledonous type and consists of epidermis, endothecium, a middle layer and tapetum. The same characteristic of the anther development is reported for the Asteraceae family [12], and in particular for *Solidago canadensis* and *Conyza stricta* [13]. The middle layer is ephemeral and degenerates during the meiosis in the microspore mother cells (MMCs). Endothecium cells develop fibrous thickenings of their walls after one-celled pollen stage. Initially 1-, 2-nuclear cells of the one-rowed glandular tapetum become multinuclear as a result of consecutive mitotic divisions, which has been reported in *E. bonariensis* and *Conyza stricta* too [10]. After the formation of one-celled pollen, the glandular tapetum transforms into false periplasmodium in *E. canadensis* and *E. sumatrensis* or real periplasmodium in *E. bonariensis*. The periplasmodial tapetum was reported for representatives of the family Asteraceae [12, 14], including *E. bonariensis* and *Conyza sctricta* [10]. The sporogenous tissue is one-, two-layered and its cells directly function as microspore mother cells (MMCs) (Fig. 1A). After simultaneous microsporogenesis, predominantly tetrahedral microspore tetrads and rarely isobilateral ones form in the anthers (Fig. 1B). The tricolporate mature pollen grains with echinate exine are three-celled (Fig. 1C). In the three *Erigeron* species, the estimated pollen viability was more than 80% (87.5% in *E. canadensis*, 86.3% in *E. sumatrensis* and an average of 83.2% for the two studied populations of *E. bonariensis*). In *E. canadensis* and *E. sumatrensis*, morphologically-uniform mature pollen forms

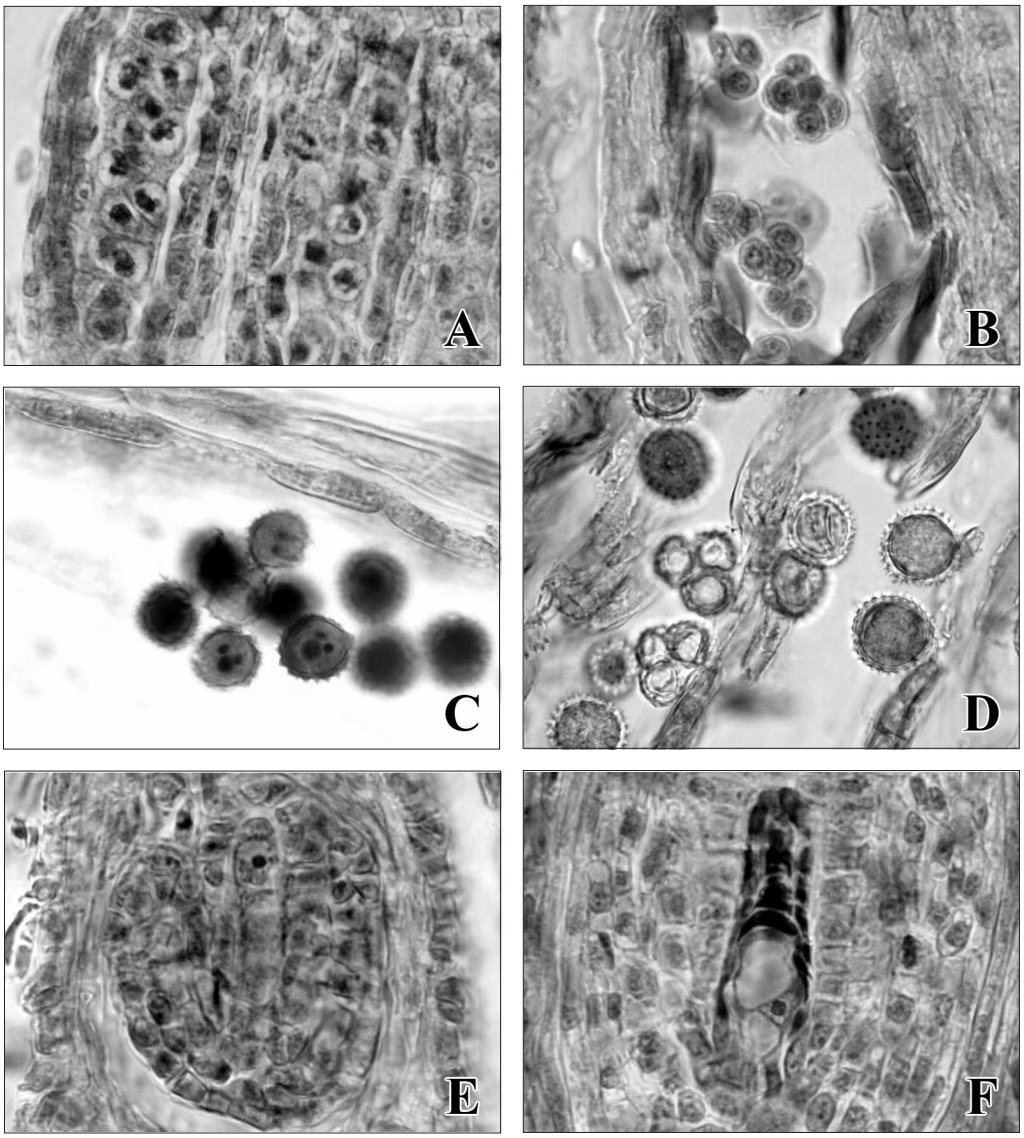

Fig. 1. Development of male and female gametophyte: A) Sporogenous tissue in an anther of *E. sumatrensis*; B) Microspore tetrads in an anther of *E. canadensis*; C) Morphologically uniform, three-celled mature pollen in *E. canadensis*; D) Polymorphous pollen and microspore tetrads in *E. sumatrensis*; E) Megaspore mother cell in an ovule of *E. bonariensis*; F) One-nucleate Polygonum-type ES in *E. canadensis*. ×400

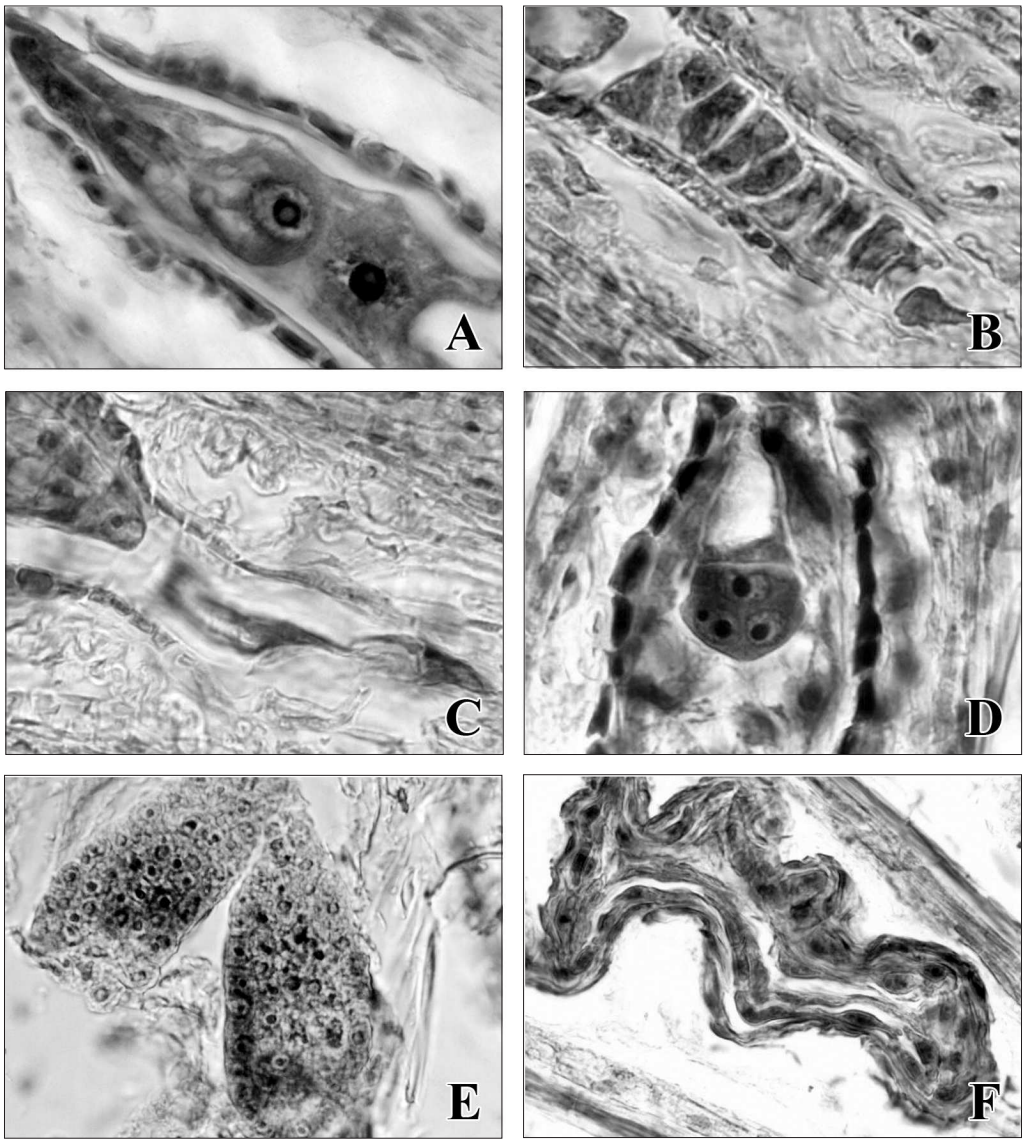

Fig. 2. Development of female gametophyte, embryo- and endospermogenesis: A) Egg apparatus and central cell of mature ES in *E. canadensis*; B) Multicellular antipodal complex in *E. bonariensis*; C) Lowest part of endosperm and antipodal haustorium in *E. canadensis*; D) Asterad-type embryo and free endosperm nuclei in *E. sumatrensis*; E) Polyembryony – legitimate and synergid embryos in *E. sumatrensis*; F) Lowest part of endospermal haustorium in *E. bonariensis* ×400

(Fig. 1C). As a result of some deviations of the meiosis in MMCs and microsporogenesis, presence of polymorphous grains and a higher amount of sterile pollen were established in *E. bonariensis* (Fig. 1D) that had already been observed in the invasive species *Erigeron annuus* [15].

Ovule and development of the female gametophyte. The ovule is anatropous, tenuinucellate and unitegmic. In it only one archesporium cell that directly functions as megaspore mother cell forms hypodermally (Fig. 1E). The meiosis leads to a linear megaspore tetrad in the ovule. In the three species studied, the embryo sac (ES) development follows *Polygonum* (monosporic)-type from the chalazal megaspore (Fig. 1F), already established in *E. bonariensis* [10, 16] and *E. canadensis* [11]. After three mitotic divisions, subsequently 2-, 4- and 8-nucleate ES form. In *E. sumatrensis* the ES moves out of the initial ES cavity at 2-nucleate ES stage, that was established in *Erigeron annuus* too [15].

The mature embryo sac consists of three-celled egg apparatus (an egg cell and two hooked synergids with fibrillar apparatus), two polar nuclei (after their fusion the central cell of the ES forms) (Fig. 2A) and an antipodal complex in the chalaze. After two-nucleate ES stage, an endothelium forms in the ovule. In the three species studied, as a result of multiplication through mitotic divisions, an antipodal complex with different numbers of one-, two-nuclear and vacualized cells forms – from 4 to 6–7 antipodals in *E. canadensis* and *E. sumatrensis* and usually more than 10 in *E. bonariensis* that are often arranged in two rows (Fig. 2B). A multicellular antipodal complex resulting from secondary multiplication is reported for *E. canadensis* [16] and *E. bonariensis* [10, 16]. The antipodals are long-lived and remain vital usually even after the presence of a heart-shaped embryo in the ES cavity. During the study, in *E. canadensis* clearly-expressed antipodal haustorium (Fig. 2C) was often observed after the formation of a globular embryo. The porogamous double fertilization proceeds. In the three species, the legitimate embryo develops according to the Asterad-type (Fig. 2D) that is announced for *E. bonariensis* [10] and *E. canadensis* [11]. In *E. sumatrensis*, besides the legitimate embryo, an additional embryo forms in the same ovule (polyembryony) as a result of synergid apogamety (Fig. 2E). The endospermogenesis begins before the embryogenesis. Initially nuclear endosperm transforms into cellular one after the formation of a multicellular globular embryo. Endospermal haustorium forms in the three species of *Erigeron* but it is more clearly expressed in *E. bonariensis* (Fig. 2F). That structure was also established in *E. canadensis* [11].

Conclusion. As a result of the study, we consider that the following embryological features have an impact on the invasive behaviour of the three *Erigeron* species: a high amount of viable pollen; long-lived multicellular antipodal complex; formation of antipodal and endosperm haustorium; simultaneous formation of mature embryos in almost all florets in one and the same capitulum.

The results show that the generative sphere, especially the female one, is characterized with a high plasticity expressed with highest degree in *E. sumatrensis*.

Besides the above-mentioned features, in *E. sumatrensis* some specific ones, especially connected with the trophism of the female gametophyte and adaptability, were observed: ES moves out of its cavity at two-nucleate stage; polyembryony, as a result of development of an additional embryo from a synergid and clearly-expressed typical endosperm haustorium.

All established features of the reproductive sphere, especially these facilitating the full realization of the reproductive capacity, together with the abundant and long flowering, gynomonoecy, self-pollination, self-compatibility, autonomous rapid production of achenes and their high dispersal capability undoubtedly increase the invasive potential of the three studied *Erigeron* species.

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