

Embryology of Two Invasive Alien Species of *Solidago* L. (Asteraceae) in the Bulgarian Flora

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Abstract: An embryological study of two invasive alien species in Bulgaria – *Solidago canadensis* and *Solidago gigantea* (Asteraceae) was carried out. Both taxa are native to North America. The features of the reproductive structures and processes were examined in order to estimate which traits may facilitate the invasiveness of these species. We found that seeds in the Bulgarian localities of both species are formed exclusively by sexual means. The successful sexual reproduction is ensured by the high amount of viable pollen (ca. 75%), almost stable embryological structures, normally running processes in the male and female generative spheres, multicellular antipodal complex and antipodal haustoria (in *S. gigantea*), as well as the simultaneous formation of mature embryos in the florets of the capitulum. In addition, both species are long-lived herbaceous perennials and reproduce asexually through underground rhizomes. During the study, some degenerations were found, mainly in the male and female generative spheres of *S. gigantea*, e.g. degenerated mature embryo sacs and embryos in the florets of all studied capitula. These may influence to some extent the effectiveness of the sexual reproduction of this species. On the contrary, it was observed that in *S. canadensis*, the sexual reproduction is possibly more effective than that in *S. gigantea*. Obviously, the long-distance dispersal of both species is achieved by seeds, but once established in a new location, the individuals reproduce also asexually and form large polycormons.

Key words: *Solidago canadensis*, *Solidago gigantea*, male and female gametophyte, reproductive system

Introduction

Reproductive traits are of crucial importance for the establishment and spread of introduced species, both directly and through interactions with other life-history traits and extrinsic factors (GIBSON et al. 2011). Thus, reproduction can affect the success of naturalisation and invasion (PYŠEK & RICHARDSON 2007, BARRETT 2011, BURNS et al. 2013). Research on reproductive biology of species can help understand the naturalisation and invasion processes and control the invasive aliens. However, the information on the reproductive traits of invasive plants, especially those facilitating invasiveness, is still limited (PYŠEK & RICHARDSON 2007, YAN et al. 2016).

The aim of the present work was to conduct an embryological study of two invasive alien species in the Bulgarian flora – *Solidago canadensis* L. and *S. gigantea* Aiton, in order to describe the embryological structures and processes in the naturalised populations and reveal those that may facilitate the invasive success of the species. Both taxa belong to Asteraceae, the largest plant family and also the richest in alien and invasive alien species in Bulgaria and worldwide.

Solidago canadensis is native to North America (HEGI 1979, KUZMANOV 2012). Alien distribution history of introduction and geographical spread of

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the species represents one of the earliest ornamental introductions from North America to Europe (WEBER 2000). In its native range, *S. canadensis* can occur in any crop, but is not a serious weed in annual crops, since it can be controlled by tilling. However, it invades poorly managed pastures and can be a considerable weed in forest nurseries, in perennial gardens and crops (WERNER et al. 1980). In Europe, *S. canadensis* is considered an environmental weed (WEBER 2003) because it is a serious weed of forest edges, dry meadows, wetland edges, railwaysides, roadsides and wasteland. Large areas infested by the species are often the result of the inappropriate land-use management. The dominance of the weed may be enhanced by allelopathy (SUN et al. 2006, WANG et al. 2006). The distribution of *S. canadensis* in Bulgaria is still limited – the species is confined only to the Sofia Region (VLADIMIROV 2003), Balkan Range (Western) (VLADIMIROV & PETROVA 2010) and Northeast Bulgaria floristic regions (PETROVA & VENKOVA 2015).

Solidago gigantea is native to North America as well. The species was transported to Europe in the middle of the 17th century as an ornamental plant and the first reports of its naturalisation date back from the middle of the 19th century. It is highly invasive in Central Europe, where it can become dominant in various habitats such as forest edges, riverbanks and wetlands (WALTER 1987, SCHULDES & KÜBLER 1990). In Bulgaria, *S. gigantea* was also introduced as an ornamental plant and the oldest herbarium specimens are from 1902 (PETROVA et al. 2013). It is more widespread than *S. canadensis* (PETROVA et al. 2013), occurring in semi-natural and natural habitats – near roads and railways, rivers banks, landfills, meadows and pastures, forest edges. *Solidago gigantea* forms dense independent groups (polycormons), which can reach considerable size, in at least 12 floristic regions (out of 20 for the whole country). It competes with native species for space, changes the chemical composition of the soil in the top 10 cm layer, and thus, affects negatively the local distribution and establishment of the native plants (PETROVA et al. 2013).

Both species are included in the List of ‘Worst invasive alien species threatening biodiversity in Europe’ (LARSSON et al. 2007) and in the EPPO list of invasive alien plants (https://www.eppo.int/INVASIVE_PLANTS/ias_lists.htm#IAPList, accessed March 2017).

Materials and Methods

The plant material was collected from the following naturalised localities of the two species in Bulgaria: for *S. canadensis* – Sofia floristic region, grasslands

in the area of the Botanical garden of the Bulgarian Academy of Sciences, ca. 670 m, N 42.64466, E 23.29890, 10.08.2009, leg. V. Vladimirov; for *S. gigantea* – Valley of River Mesta floristic region, by the road from Bansko to Gotse Delchev, ca. 5 km before Gospodintsi Village, ca. 590 m, N 41.68916, E 23.71222, 01.08.2009, leg. V. Vladimirov.

The flower buds and open flowers were collected in the field and fixed immediately in a mixture of FAA (formalin : glacial acetic acid : 70% ethanol in correlation 5:5:90 parts). Then, the fixed plant material was treated according to the classical paraffin methods (SUNDARA 2000), involving the following major steps: embedding in paraffin, cutting into 6-12 µm sections with a rotary microtome, staining of the sections with Heidenhain’s haematoxylin, and embedding in Entellan in order to get permanent slides. The differential staining allowed us to reveal the features of the embryological structures and processes in the male and female generative spheres during the ontogenesis, as well as to evaluate the quality of mature pollen grains.

The pollen viability (potential fertility) was evaluated by counting 1000 mature pollen grains from each of the two studied populations in a visual field at a magnification 100×. The pollen grains were classified into two groups according to the intensity of differential staining: 1. Viable pollen grains – potentially fertile and effective in the pollination process (stained pollen grains with a clear reticulated sculpture and clearly distinguished vegetative and generative cells or sperms); 2. Non-viable pollen grains – sterile and inefficient (colourless or darkly stained degenerating pollen grains, in which the vegetative and generative cell or sperms are not clearly distinguished, not discernible and also empty, transparent, shrunken, without a clear sculpture).

The embryological structures and processes in the male and female generative spheres, as well as the pollen fertility were studied using a Light Microscope Olympus CX2. The microphotographs were made using the ‘Infinity Lite’ Digital Camera 1.4 Mpx.

Results and Discussion

Male generative sphere – anthers and development of the male gametophyte

The anthers are tetrasporangiate. The anther wall develops according to the Dicotyledonous-type (DAVIS 1966) and is four-layered, consisting of epidermis, endothecium, a middle layer and tapetum. A clear differentiation of these layers occurs during the meiosis in microspore mother cells (MMCs) in the anther locules; the differentiation is earlier in *S.*

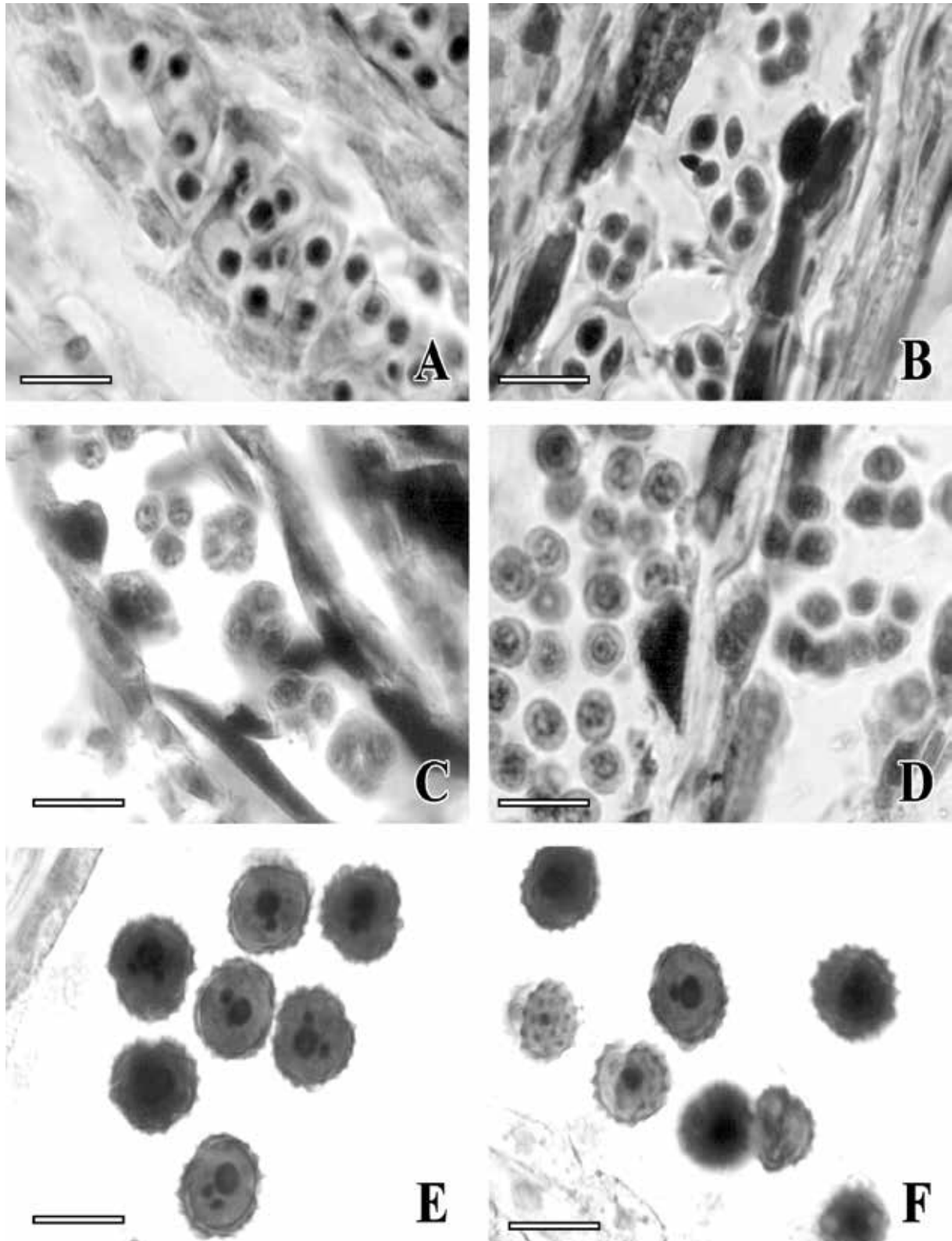


Fig. 1. Anther and development of the male gametophyte: **A** – An anther wall and multilayered sporogenous tissue in *S. canadensis*; **B** – Tetrahedral and isobilateral microspore tetrads in the anthers of *S. canadensis*; **C** – Tetrahedral and isobilateral microspore tetrads in the anthers of *S. gigantea*; **D** – Microspore tetrads and disorganisation of the tetrads with formation of one-celled young pollen in *S. gigantea*; **E** – Viable three-celled pollen in *S. canadensis*; **F** – Viable and sterile mature pollen in *Solidago gigantea*. Scale bar = 20 μ m

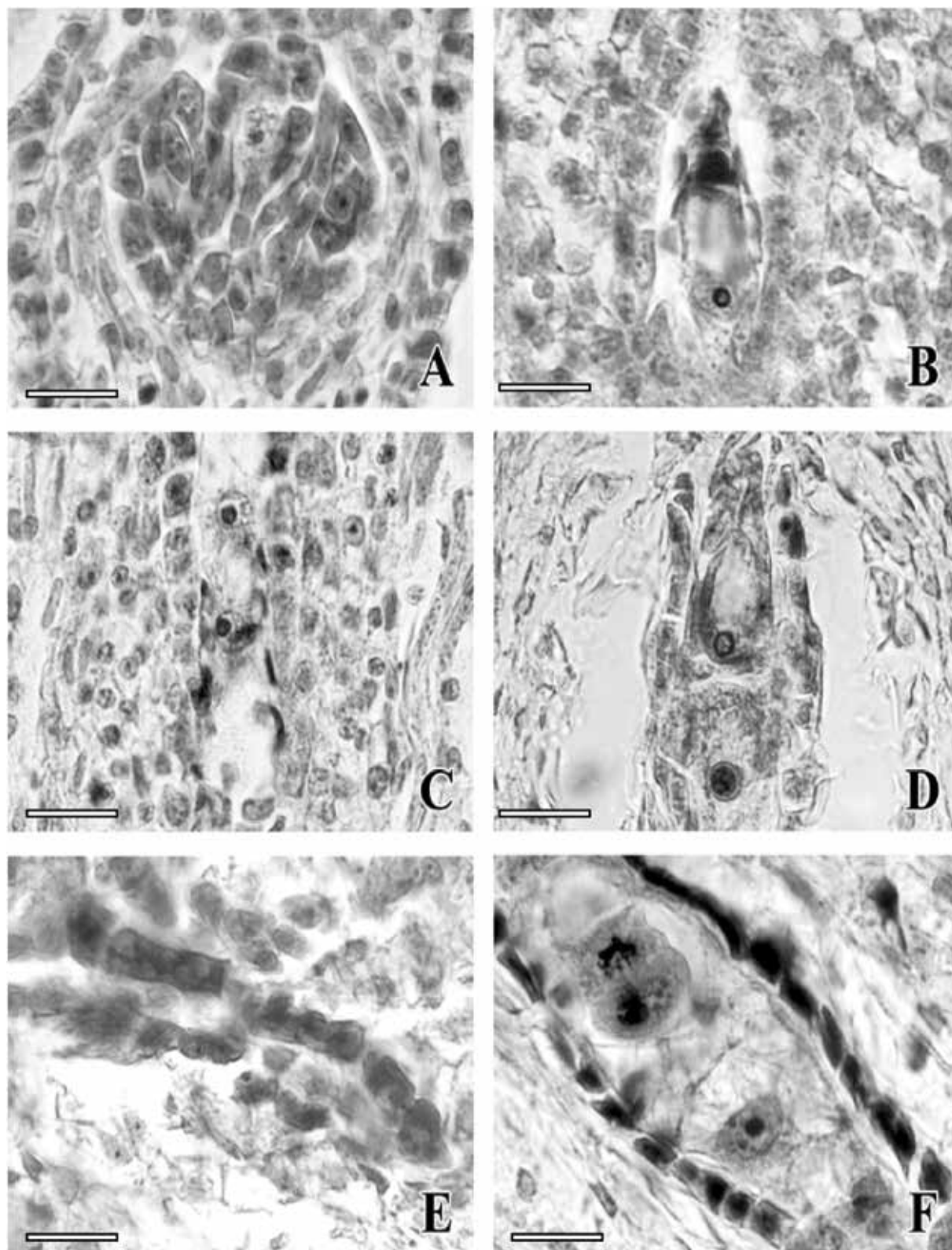


Fig. 2. Ovule and development of the female gametophyte. **A** – A megaspore mother cell in the ovule of *S. gigantea*; **B** – Initiation of the *Polygonum*-type embryo sac (ES) from the chalazal megaspore of linear tetrad in *S. gigantea*; **C** – Removal of ES out of the ES cavity in *S. canadensis*; **D** – An egg cell and central cell in the ES of *S. canadensis*; **E** – A multilayered antipodal complex in the ES of *S. gigantea*; **F** – Initiation of the Asterad-type embryogenesis in ES of *S. gigantea* and nuclear endosperm. Scale bar = 20 μm

gigantea, in which the consisting cells of epidermis, tapetum and endotecium are bigger than in *S. canadensis*. The epidermis comprises one row of almost rectangular one-nuclear cells, which enlarge tangentially and round up outside during the anthers ontogenesis. After formation of one-nucleate pollen, the endotecium develops fibrous thickenings, which is typical for this layer in Asteraceae. The middle layer is ephemeral and usually degenerates until the second (homotypic) division of the meiosis. The tapetum differentiation begins at the stage of sporogenous tissue in the anthers. Initially, it is cellular with one-nucleate cells. During the anther ontogenesis a rapid multiplication of the nuclei in the tapetum cells occurs as a result of successive mitotic divisions without subsequent cytokinesis. In already strongly elongated in a radial direction tapetum cells (6-8-nucleate, often polyploid, observed more frequently in *S. canadensis*) the nuclei usually are located along the entire length of the cells (Fig. 1C). The tapetum cells remain viable after the formation of MMCs. After this stage, their disorganisation begins and the initially cellular tapetum transforms to a false periplasmodium (Fig. 1D), which is typical for Asteraceae (PODDUBNAYA-ARNOLDI 1982, SOLNTSEVA 1987). The sporogenous tissue is multi-layered (Fig. 1A). Its consisting cells differentiate directly in MMCs after a period of intensive lengthening. At the stage of mature pollen in the anthers, only epidermis and endothecium from the anther wall are preserved, although often they are almost completely destroyed.

The meiosis runs normally, with small deviations that are more diverse in *S. gigantea*. In this species, besides lagging chromosomes, often an unusual location of the division spindles during metaphase and anaphase II was observed. In the anthers of *S. canadensis*, as a result of simultaneous microsporogenesis predominantly tetrahedral microspore tetrads and more rarely isobilateral tetrads are formed (Fig. 1B, C). In *S. gigantea*, various types of microspore tetrads were observed as a result of different disturbances during the meiosis and microsporogenesis, but micronuclei or an inhibition of the meiosis were not found. The mature pollen is three-celled (Fig. 1E), tricolporate, fertile in a high amount (77.8% in *S. canadensis* and 75.6% in *S. gigantea*).

Female generative sphere—ovule and development of the female gametophyte

The pistil is two-carpellate, syncarpous. The ovary is inferior, uniloculate. It has only one ovule with a basal placentation. The style is papillose and

shortly bilobed and the stigma is papillate. The well-developed ovule is anatropous, unitegmic, with poor nucellus. According to existing classifications of the ovule types (VAN TIEGHEM 1898, SCHNARF 1929, KORDYUM 1978, SHAMROV 1999), the ovule of two studied species can be defined as tenuinucellate with poorly developed nucellus, represented by a row of cells of the nucellar epidermis and unicellular archesporium with subepidermal location. Sporadically, in single florets of a small number of capitula two-celled archesporium was observed. SHAMROV (1999), who introduced the criteria for clear determination of crassi- and tenuinucellate ovules, assigned the ovule of Asteraceae to the typical or simpetal variation of the tenuinucellate type (the epidermal layer of the ovule completely covers the developing megasporocyte and the nucellus destroys before fertilisation). According to changes in the integument development, KOLCZYK et al. (2015) defined three types of ovules in Asteraceae, namely '*Taraxacum*', '*Galinsoga*' and '*Ratibida*'. These authors defined the ovule of the genus *Solidago* as '*Taraxacum*' type, in which the nutritive tissue is well-developed, with very swollen cell walls of a spongy structure. Different types of embryo sac development were reported in *Solidago purpurea* (MUKHERJEE & DESAI 1990). In the present study, a reduction in the ovule structure of the studied *Solidago* species was not observed.

The single archesporium cell (Fig. 2A) functions directly as a megaspore mother cell from which a linear megaspore tetrad is formed after meiosis. Usually, after a period of growth and vacuolisation, the chalazal megaspore transforms directly in an embryo sac mother cell – one-nucleate embryo sac (ES) (Fig. 2B), whereas the other three megaspores of the tetrad degenerate. The ES develops according to the basal for the Angiosperms *Polygonum* (monosporic) type. This type of ES formation was also reported for other *Solidago* species as *S. virgaurea* subsp. *alpestris* (MUSIAL 1994). After consecutive mitoses running in the embryo sac mother cell, two-, four- and eight-nucleate ES are formed. In *S. canadensis*, removal of ES out of the ES cavity after two-celled ES-stage was observed (Fig. 2C), which was also found in *Erigeron annuus* and *E. sumatrensis* (YURUKOVA-GRANCHAROVA et al. 2012, 2013). The mature ES comprises a three-celled egg apparatus in its micropylar end (Fig. 2D), two polar nuclei (after their fusion the central cell of the ES is formed) and antipodal complex in the chalaza. The synergids in ES of *S. gigantea* are not typical-hooked as in most representatives of the family Asteraceae. In the ES of the two studied species, the antipodal

complex is most plastic. Usually, the antipodal cells are three. In the ES of *S. canadensis* often two antipodal cells were observed, the upper one usually almost cylindrical in shape, highly vacuolated, two-nucleate, bigger than the lower one (chalazal) and haustorising, while the lower antipodal cell was smaller, one-nuclear, sometimes haustorising too. In both *Solidago* species, a secondary multiplication of antipodals in the ES occurs as a result of successive mitotic divisions followed by cytokinesis. Thus, in the chalazal part of ES an antipodal complex is formed. It is composed not only of three (very often observed in the ES of *S. canadensis*), but also of more than three cells (5-8) in the ES of *S. gigantea*, usually with linear arrangement and penetrating deeply into the chalaza (Fig. 2E). The antipodals remain viable even after the formation of a multicellular embryo, which was observed in the invasive *Erigeron sumatrensis* and *E. bonariensis* too (YURUKOVA-GRANCHAROVA et al. 2012). Long-living antipodals, differentiated as a result of secondary mitotic divisions, either accompanied or not by cytokinesis, were reported in *S. virgaurea* subsp. *alpestris* (MUSIAL 1994). After the stages of one- to two-nucleate ES formation well-differentiated endothelium was observed. This structure is typical of the tenuinucellate ovule (SHAMROV 1999). The legitimate embryo and endosperm are a result of double porogamous fertilisation. Usually, the embryogenesis begins after the endospermogenesis. The embryo develops according to the Asterad-type (Fig. 2F). The endosperm development passes a stage of free nuclei and transforms into cellular endosperm after the multicellular embryo formation. The endosperm in the mature ovules is absorbed by the fast growing embryo and it is completely consumed at the stage of mature embryo. JOHRI & AMBEGAOKAR (1984) reported the presence of an antipodal embryo in *S. canadensis*, but in the present study neither apomixis, nor elements of apomixis were observed.

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Conclusions

The present embryological study revealed that both *S. canadensis* and *S. gigantea* are sexually reproducing in the studied naturalised localities in Bulgaria.

The established high amount of viable pollen (75-77%), almost stable embryological structures and normally running processes in the male and female generative spheres, the multicellular antipodal complex and antipodal haustoria (in *S. gigantea*), and the simultaneous formation of mature embryos in the florets of a capitulum ensure successful sexual reproduction and seed production, thus facilitating the invasion of the species. Moreover, *S. canadensis* and *S. gigantea* are herbaceous perennials, which can reproduce asexually by underground rhizomes, leading to the formation of polycormons of different size.

The deviations registered in *S. gigantea* in the male generative sphere, leading to the formation of a slightly higher percentage of sterile pollen, and the degenerations found in the female generative sphere, including degenerated mature ESs and embryos in some florets of all studied capitula, very likely affect negatively to some extent the effectiveness of the sexual reproduction in this species. On the contrary, the sexual reproduction in *S. canadensis* is more effective. The present study confirms the expectation that in the Bulgarian localities of both species viable seeds can be formed. Thus, the long-distance dispersal of the species is achieved by seeds, whereas, the local spread – by vegetative propagation. Therefore, any measures limiting the sexual reproduction in both species would serve as effective means for the containment of the species.

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