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MORPHOLOGICAL AND ANATOMICAL COMPARISON OF MERICARPS FROM DIFFERENT TYPES OF UMBELS OF *HERACLEUM SOSNOWSKYI*

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Abstract

Jurkonienė S., Žalnierius T., Gavelienė V., Švegždienė D., Šiliauskas L., Skridlaitė G., 2016: Morphological and anatomical comparison of mericarps from different types of umbels of *Heracleum sosnowskyi* [Sosnovskio barščio skirtingų tipų žiedynų merikarpių morfologinės ir anatominės sandaros palyginamoji analizė]. – Bot. Lith., 22(2): 161–168.

Sosnowsky's hogweed (*Heracleum sosnowskyi* Manden.) mericarps were collected from satellite and stem branch umbels for comparative anatomical investigation. Located near Vilnius city, the habitat of *Heracleum sosnowskyi*, formerly a natural forest edge has recently been densely occupied by plants of this species. SEM micrographs of abaxial and adaxial surfaces of mericarps obtained from satellite and stem branch umbels of *H. sosnowskyi* were similar, but morphometrical analysis revealed statistically significant differences in mericarps collected from satellite umbels, which were longer and wider than mericarps from stem branch umbels. The data on longitudinal sections of *H. sosnowskyi* mericarps clearly showed that embryos of satellite umbels were at later torpedo stage compared to embryos of stem branch umbels, which were at earlier heart stage. These data represent unequal development of the embryos in mericarps from different types of umbels. Such different development can be treated as an adaptation of the invasive plant to occupy the current habitat and survive in the seed bank by allowing the embryo to complete development within a seed and germinate when new conditions permit.

Keywords: embryo of Sosnowsky's hogweed, invasive, mericarp, schizocarp, seed morphology and anatomy.

INTRODUCTION

Sosnowsky's hogweed (*Heracleum sosnowskyi* Manden.) is an extremely harmful invasive species that can outcompete local flora, rapidly invade not only open areas, but also spaces along water basins, roads and forests (Laivinš & Gavrilova, 2003; Kabuce, 2006; Gudzinskas, 1998). *H. sosnowskyi* as well as relative *H. mantegazzianum* contain the toxic photoallergic compound furanocoumarin and essential oils (Tkachenko, 2006; Jakubowicz et al., 2012). Even the smell is enough to induce allergenic symptoms in sensitive people. This plant is dangerous for humans, because the plant sap causes photosensitivity and burns (Jakubowicz et al., 2012; Baležentienė et al., 2013). Reproduction for all plants is very important event in their lifetime (Crawley, 1997), especially for monocarpic plants such as *H. sosnowskyi*, which bear fruits only once in their life and after setting of mericarps they die (Devlin, 1969; Jakubowicz et al., 2012). According to Gudžinskas & Rašomavičius (2005), this species in Lithuania propagates only by seeds. Fruits of *H. sosnowskyi* are broadly winged schizocarps. They consist of two mericarps connected in pairs by a carpophore. When mature, the shizocarps split to separate mericarps in turn (Grigas, 1986). Sosnowsky's hogweed can produce about 5000–8000 fruits (schizocarps) (Tkatschenko, 1989;

Gudzinskas, 1998), while other researchers indicate higher seed production – about 15000–16000 one seed containing mericarps (Jakubowicz et al., 2012; Baležentienė et al., 2013) per plant, most germinate in early spring of the following year (Moravcová et al., 2006; Baležentienė et al., 2013). It is known that even at the beginning of seed-setting, the broken off inflorescence of this species can mature seeds (Nielsen et al., 2005). Dispersal and spread of *H. sosnowskyi* have been described in many studies. It is very problematic to eradicate or to stop the distribution of species as the seeds remain viable for many years and the roots are difficult to remove (Laiviņš & Gavrilova, 2003; Nielsen et al., 2005, 2007; Kabuce, 2006; Musikhin & Sigaev, 2006; EPPO, 2015). Due to the reproductive peculiarities of *H. sosnowskyi*, especially fruit abundance, we can highlight a differentiation in fruit ripening time. To all numerous studies on ecological, biological and other different aspects of *H. sosnowskyi*, carpological investigations are lacking or minimal. The descriptions of pericarps and seed-coat patterns by using scanning microscope (SEM) of four *Heracleum* taxa occurring in Poland (*H. sosnowskyi* among these) have been well presented by polish researchers (Klimko et al., 2013), but knowledge about the anatomy of mericarp and especially embryo is still lacking. To date, the specialists testing new methods for the extermination of weeds are in great need of carpological studies. As it was mentioned above, *H. sosnowskyi* as well as other hapaxanthic plants usually die after flowering (Satsiperova, 1984; Gudžinskas & Rašomavičius, 2005; Jakubowicz et al., 2012), and populations would become extinct if there were no possibility for recovery from seeds. Therefore, investigations on seed characteristics are of high importance to prevent further expansion of the invasive plant. The aim of this research was to compare the mericarps of *H. sosnowskyi* from different types of umbels, and find out if there were any differences in their morphology and structure at different stages of maturity depending on umbel type.

MATERIALS AND METHODS

The study plant and collection of material

The fruits of Sosnowsky's hogweed (*Heracleum sosnowskyi*), as in other species of the umbelliferous (Apiaceae) family, are schizocarps (Esau, 1977). When mature, they split up into two one-seed-bearing mericarps (two parts of the schizocarp) (Perglova et al., 2007; Naujalis et al., 2009; Jakubowicz et al., 2012). For our research, the mericarps were collected from satellite and stem branch umbels. The investigated population was located near Vilnius (Lithuania), on formerly natural forest edge that has recently been overgrown with Sosnowsky's hogweed. The area in which the investigated mericarps were collected occupies 0.415 ha and is located between 54°758587' N and 25°260138' E. All mature plants in the experimental plot as well as satellite and stem branch umbels of the experimental plants were chosen randomly (Fig. 1). Mature seeds were collected in late August in 2014–2016. The vegetation period of *H. sosnowskyi* is about 200 days (when the average temperature is higher than 5°C) and it starts in April (Grigas, 1986). The fully ripened fruits and seeds were collected and analysed immediately. They were measured, and seed shape, surface properties, ridges, pits, venation, roughness, thorns, hairiness, gloss and the presence of membranous rim on seeds were described. We collected 1000 seeds from 38 plants. The callipers and a calibrated loupe were used for the measurements.

Fig. 1. Satellite (1) and stem branch (2) umbels of *Heracleum sosnowskyi*

Anatomical investigation of Sosnowsky's hogweed mericarps

All mericarps of each variant were fixed in FAA solution (formalin-acetic acid-ethanol (1:1:20)). After three days fixation stable histological preparations were made: samples of mericarps were dehydrated in a graded alcohol series, embedded in paraffin, cut with a rotary microtome (Leica RM2125) into 5–7 µm thick longitudinal sections, and stained with periodic acid-Schiff´s reagent. These preparations were analysed using a light microscope (Motic B3). Pictures were captured using Moticam 2300.

Preparation of mericarps from different types of umbels was carried out at four stages: a) fruit coat (pericarp) was removed, b) endosperm was removed, c) embryos prepared for morphometrical evaluation, and d) longitudinal sections of embryos were received.

After removing of pericarp, the surface of testa (seed coat) was analysed using SEM.

The embryogenesis stages were identified according to the embryogenesis of *Arabidopsis thaliana* (Goldberg et al., 1994; Park & Harada, 2008; Möller & Weijers, 2009).

Scanning electron microscopy

To prepare the samples for the observation by scanning electron microscopy, the material (mericarps, seeds without fruit coats (pericarps), embryos) was air-dried on filter paper for several seconds until the surface became dry (Barthlott, 1981). Then the samples were used to take backscattered electron (BSE) images. The observations and micrographs were made using FEI Quanta 250 scanning electron microscope.

Statistical analysis

The results reported in the tables are means of the values with standard error (SE). The significant differences between mericarps from different types of umbels were tested using Student's *t*-test. Calculations were done with Statistica 191 for Windows (StatSoft, 2010). The minimum confidence level was set to $p < 0.05$.

RESULTS

Morphology of *Heracleum sosnowskyi* mericarps

All examined fruits of *H. sosnowskyi* both from satellite and from stem branch umbels were elliptic, laterally flattened, shallowly ridged schizocarps composed of two one-seeded not-opening mericarps (the fruit and seed were fused together). They had clearly club-shaped oil ducts. The mericarps were convex in dorsal side, with five thin longitudinal ridges, and four oil canals lying among them (Fig. 2.1). Lateral

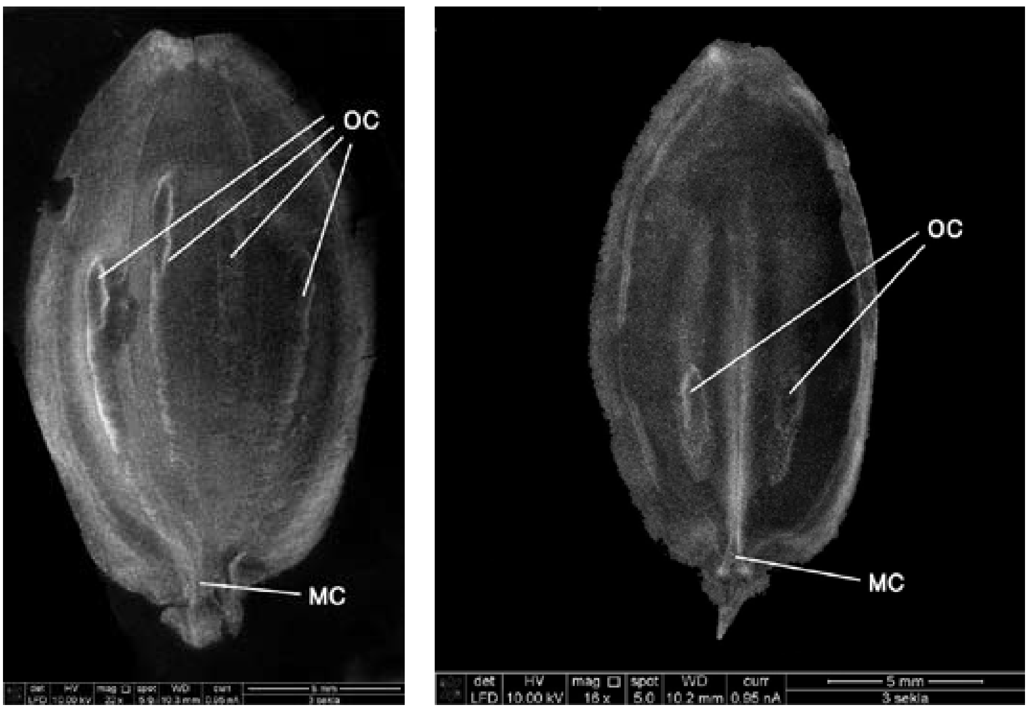

Fig. 2. Scanning micrographs of *Heracleum sosnowskyi* mericarp surfaces gathered from satellite umbels. Abaxial (outside) surface (1) and adaxial (inside) view of mericarp. Abbreviations: OC – oil canals; MC – micropylar canal

ridges were wider, almost winged. Inside of mericarps was slightly concave with three ridges and two short oil canals among them (Fig. 2.2). The oil canals didn't reach the base of both sides of mericarp. All gathered mericarps were smooth, bald, and slightly glossy. SEM micrographs of abaxial and adaxial surfaces of mericarps obtained from satellite and stem branch umbels of *H. sosnowskyi* (Fig. 2.1; 2.2) were similar.

Morphometrical analysis of *H. sosnowskyi* mericarps

The analysis of weight data showed significant differences in average mass per mericarp, which was significantly higher on satellite umbels compared to that maturing on one stage lower stem branch umbels (Student's *t*-test, $p < 0.05$) (Table 1).

Table 1. Average mass of mericarp from satellite and stem branch umbels

Type of umbels	Average mass of mericarp, g
Satellite	0.51 ± 0.25 (n 1000)
Stem branch	0.44 ± 0.22 (n 1000)*

Note: *– differences are statistically significant at $p < 0.5$ (Student's *t*-test).

Morphometrical analysis of *H. sosnowskyi* mericarps from satellite and stem branch umbels revealed that mericarps collected from satellite umbels were significantly longer and wider than mericarps from stem branch umbels (Table 2). The differences are statistically significant (Student's *t*-test, $p < 0.05$). The results of mericarp thickness had the same tendency as of mericarp length and width from satellite and stem branch umbels, but these differences weren't statistically significant at $p \leq 0.5$.

Anatomy of embryos of *Heracleum sosnowskyi* mericarps from umbels of different type

It was observed that mericarps of *H. sosnowskyi* contain large amount of endosperm, which completely surrounded a small embryo located at the micropylar end of the seed (Fig. 3). Such naked seeds from satellite and stem branch umbels were compared and location of embryo in both types of mericarps was observed and marked by arrows (Fig. 3.1, 3.2).

Biometrical analysis of *H. sosnowskyi* embryos from mericarps of satellite and stem branch umbels showed that embryos from mericarps of satellite umbels were significantly longer and wider than embryos from mericarps of stem branch umbels: 670.76 ±

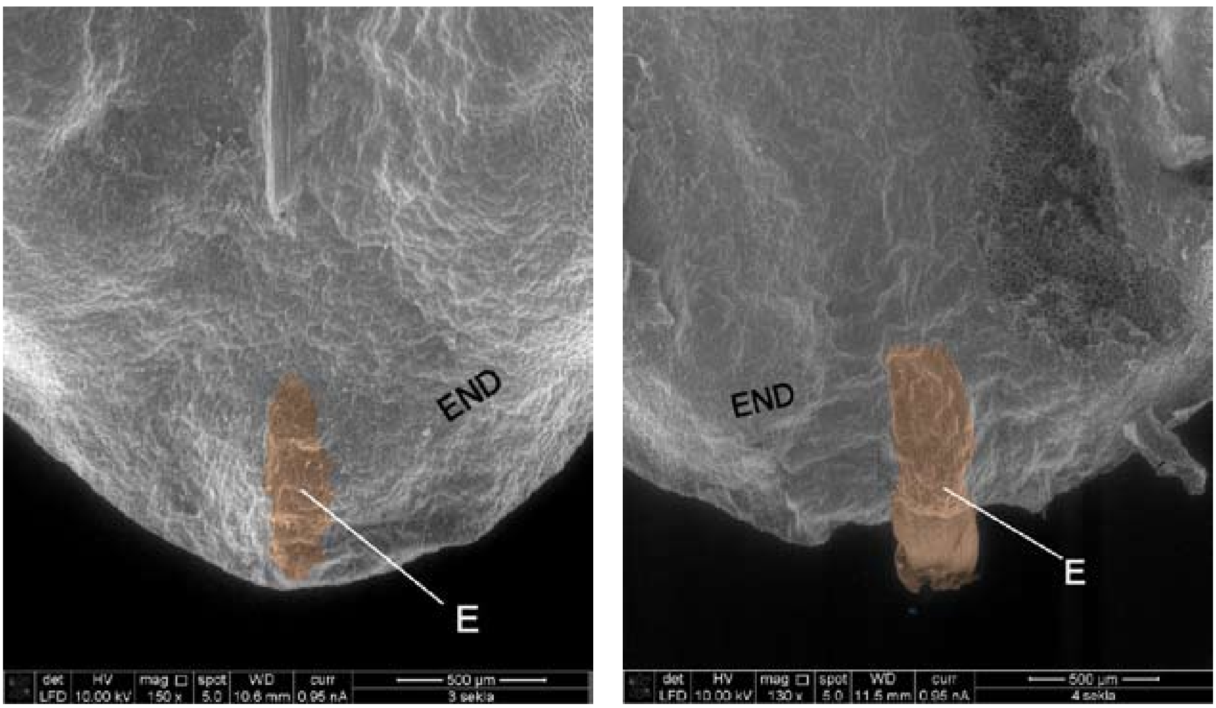

Fig. 3. Micrographs of testa (seed coat) surfaces without fruit coat (pericarp) of mericarps from satellite (1) and stem branch (2) umbels of *H. sosnowskyi*. Arrows mark location of embryos. Abbreviations: E – embryo; END – endosperm

Table 2. Average length, width and thickness of mericarps from umbels of different type

Type of umbels	Length, mm	Width, mm	Thickness, mm
Satellite	10.30 ± 1.25 (n 1000)	6.33 ± 0.78 (n 1000)	0.73 ± 0.06 (n 1000)
Stem branch	9.35 ± 0.86 (n 1000)*	5.83 ± 0.62(n 1000)*	0.68 ± 0.05 (n 1000)

Note: *– differences are statistically significant at $p \leq 0.5$ (Student's *t*-test).

Fig. 4. Embryos received from the seeds of *H. sosnowskyi* satellite (1) and stem branch (2) umbels. Abbreviations: A – axis; C – cotyledons

42 µm and 222.56 ± 12 µm (Fig. 4.1) 495.44 ± 36 µm and 197 ± 11 µm (Fig. 4.2), respectively (Student's *t*-test, $p < 0.05$, values are calculated on the basis of SEM micrographs).

Embryos of satellite umbels differed from those of stem branches in seed anatomy characteristics. A longitudinal section of *H. sosnowskyi* mericarps clearly revealed that embryo of satellite umbel was longer and wider than embryo from stem branch inflorescence. Embryos of satellite inflorescences were found to be at later torpedo stage (Fig. 5.1) compared to stem branch umbel embryos, which were at earlier heart stage (Fig. 5.2).

DISCUSSION

The morphological and anatomical analysis of mericarps from different types of umbels of *H. sosnowskyi* allowed us to make assumption. Mericarp dimensions vary greatly, from largest mericarps observed on satellite umbels of *H. sosnowskyi* to the smallest on stem branch umbels: mericarps of satellite umbels were longer and wider than mericarps from stem branch umbels. These differences in size are in good coincidence with the effects of fruit position on fruit weight in another alien species *Heracleum mantegazzianum* found by Moravcová et al. (2005). According to the literature data, mericarps of the genus *Heracleum* are similar in shape and different in size (Grigas, 1986; Moravcová et al., 2007). Morphologically fruits from both types of umbel were similar; it's interesting to note that Klimko et al. (2013) have stated morphological variation of all fruits of the genus *Heracleum* to be limited to a considerable degree.

Based on anatomical examination of mericarps from different types of umbels, we could hypothesize unequal speed of embryos development in mericarps from different types of umbels. Additionally, the described differences in embryos development of meri-

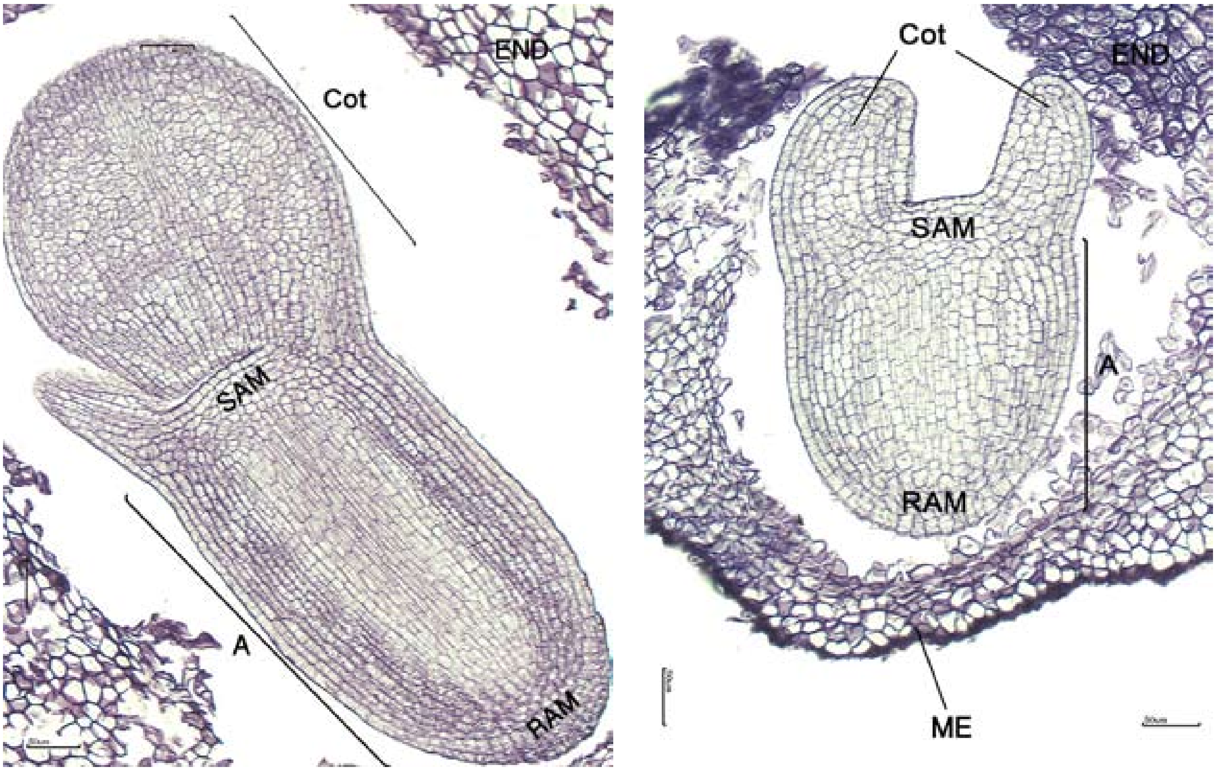

Fig. 5. Longitudinal sections in their middle portion of *Heracleum sosnowskyi* embryos of satellite (1) and stem branch (2) umbels. Abbreviations: A – embryonic axis; Cot – cotyledons; SAM – shoot apical meristem; RAM – root apical meristem; END – endosperm; ME – micropylar endosperm

carps from satellite umbels and from embryos of stem branch umbels lead us assumption about a prolonged ability of *Heracleum sosnowskyi* seeds to germinate for about 5–10 years (Gudzinskas, 1998; Obolevičа, 2009). Failure of seeds to germinate immediately may be a consequence of partial development of the embryos from different umbels. In *H. sosnowskyi*, this question seems to be more complex. Germination will occur only when the embryo development is complete, and this may occur during or before the germination process (Moravcová et al., 2006; Mayer & Poljakoff-Mayber, 2014).

According to Moravcová et al. (2006), morphologically fully developed embryos in mericarps of satellite umbels (Fig. 5.1) mean that such seeds are only physiologically dormant; morphological dormancy must be broken at the time of germination. However, some seeds with an underdeveloped or partially developed embryos on stem branch umbels (Fig. 5.2) keep morphological (or morphophysiological) dormancy (Baskin & Baskin, 2004). Devlin (1969) revealed that immature embryos may be found in *Orchidaceae* as well as in some *Fraxinus* and *Ranunculus* species. Dormancy due to immature embryos can only be broken by allowing the embryo to complete development within a seed in the environment favourable to germination. Such germination can be delayed from a few weeks to a few months (Nikolaeva, 2001; Baskin & Baskin, 2004; Casas et al., 2012).

CONCLUSION

In conclusion, our study on the anatomy of mericarps provided some important new data concerning the embryos of mericarps from satellite inflorescences, which were at later torpedo stage, and the embryos from branch umbels, which were at earlier heart stage. Such different development can be treated as adaptation of the invasive plant to occupy the current habitat and survive in the seed bank by allowing the embryo to complete development within a seed and germinate when new conditions permit.

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SOSNOVSKIO BARŠČIO SKIRTINGŲ TIPŲ ŽIEDYNŲ MERIKARPIŲ MORFOLOGINĖS IR ANATOMINĖS SANDAROS PALYGINAMOJI ANALIZĖ

Sigita Jurkonienė, Tautvydas Žalnierius, Virgilija GAVELIENĖ, Danguolė ŠVEGŽDIENĖ, Laurynas Šiliauskas, Gražina Skridlaitė

Santrauka

Palyginamiesiems anatominiams tyrimams Sosnovskio barščio (*Heracleum sosnowskyi*) merikarpiai buvo surinkti nuo palydovinių ir stiebo šakų žiedynų šios rūšies okupuotoje natūralioje augavietėje – pamiškėje Vilniuje. Palydovinių ir stiebo šakų skėčių merikarpių abaksialinio ir adaksialinio paviršiaus SEM mikrografijos buvo panašios, tačiau merikarpių dydžiai patikimai skyrėsi: palydovinių skėčių merikarpiai buvo ilgesni ir platesni nei stiebo šakų žiedynų. Išilginiai *H. sosnowskyi* merikarpių pjūviai aiškiai parodė, kad palydovinių žiedynų merikarpių embrionai buvo vėlyvesnėje torpedos stadijoje, o stiebo šakų žiedynų embrionai – ankstyvesnėje širdies stadijoje. Šie duomenys parodo nevienodą embrionų vystymąsi skirtingo tipo žiedynuose. Toks skirtingas vystymasis gali būti naudingas invaziniam augalui leidžiant embrionui vėliau baigti vystymąsi sėkloje ir sudygti susidarius tinkamoms sąlygoms.