

CONTRIBUTIONS TO THE ONTOGENETIC STUDY ON THE SPECIES *Silphium perfoliatum* L. UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

The ontogenetic peculiarities of the species *Silphium perfoliatum* L. cv. 'Vital', known as a high-potential melliferous, fodder and energy species, the periods and age stages that the plants go through under the climatic conditions of the Republic of Moldova have been described. The research was carried out in the experimental sectors of the "Alexandru Ciubotaru" National Botanical Garden (Institute), during four growing seasons. In the ontogenetic cycle, 4 ontogenetic periods (latent, pregenerative, generative, postgenerative) and 6 age stages (seed, seedling, juvenile, immature, virginal, senile) were described. The end of the ontogenesis in *S. perfoliatum*, in the Botanical Garden, has not been recorded yet. The duration of the active growth period varies between 197 and 234 days depending on the weather conditions recorded in the Republic of Moldova and the age of the plants. *S. perfoliatum* plants are characterized by long flowering stage, being able to provide honeybees and other pollinating insects with pollen and nectar at the end of summer - beginning of autumn.

Key words: *Silphium perfoliatum*, ontogenetic periods, age stages, phenological stages.

INTRODUCTION

Silphium perfoliatum L. is a species in the family Asteraceae Bercht. & J. Presl, tribe Heliantheae, genus *Silphium*; it is native to North America. The species of this genus are diploid, with seven pairs of chromosomes, and genome size 2.5 times larger than in sunflower ($2C = 16.6-16.9$ pg) (Bai et al., 2012), which allowed the researchers to demonstrate the polymorphism of the species. In some older sources (Hegi, 1906; Poletico & Mishenkova, 1967) cup plant has been described using several synonyms: *S. connatum* L.; *S. scabrum* Moench.; *S. conjunctum* Willag.; *S. integrifolium* Michx.; *S. laeve* Hook.; *S. speciosum* Rybd. Currently, according to The Plant List, the accepted name of this species is *Silphium perfoliatum* L.

It is a mycorrhizal species (Dhillion & Friese 1992), with strong roots, which grow deep into the soil (Wynia, 2009), is drought tolerant (Ouyang et al. 2007) and makes good use of wet and eroded soils, contaminated with heavy metals, but does not tolerate well marshy soils (Țiței & Roșca, 2021). Recently, cup plant was included in the list of eligible species for areas of ecological interest, being recognized by the

European Union for the benefits brought to the environment and the soil (European Commission, 2018). In Europe, *S. perfoliatum* was introduced in the 18th century, being initially used as an ornamental plant in gardens and parks in Germany, France, Switzerland and the Great Britain (Vavilov & Condratiev, 1975; Uteus, 1991). Currently, it is known as a non-traditional perennial, herbaceous forage crop that produces large amounts of fresh mass with rich biochemical content. It is resistant to unfavourable conditions (-30...+30°C) and, in spring, comes out of dormancy when the soil temperature reaches values of 3-5°C. Cup plant is a mesophile and prefers loamy-clayey and deeply loosened clayey soils (Țiței & Roșca, 2021). In "Al. Ciubotaru" (National) Botanical Garden (NBGI), the species *S. perfoliatum* was introduced in the second half of the 20th century; from the initial plants, biological material was later selected for breeding. Researchers from NBGI, as a result of many experiences and analyses, created a new, indigenous cultivar of *S. perfoliatum* - 'Vigor', registered in the Catalogue of Plant Varieties of the Republic of Moldova in 2012 and patented at the State Agency for Intellectual Property

(AGEPI) in 2016. The new plants obtained are characterized by a greater amount of green mass, increased resistance to adverse conditions and high protein content. The basic qualities of cup plant are high productivity and viability of about 15 years on the same plot. As a fodder crop, it is eaten by a wide range of animals (cattle, sheep, pigs, goats, rabbits etc.), the green mass being used as fodder and for the preparation of vitamin-fortified flour. The productivity of plants is 100 (120) t/ha (Abramov, 1992; Țiței & Roșca, 2021). *S. perfoliatum* is also known as a high-potential honey plant (150-450-560 kg honey/ha) (Koltowski, 2005), the nectar productivity can vary, between 205.2 and 611.6 kg/ha, being closely related to the quality of the soil and the amount of mineral fertilizers applied (Savin & Gudimova, 2019). In addition to nectar, cup plant is also a source of bee bread (flower pollen used by bees as food for the larvae in the hive), besides, cup plant honey does not crystallize for a long time, being a source of food for bees during the winter (Abramov 1992). In the flowering stage, the flowers are visited by a large spectrum of pollinating and honey-producing insects (Cîrlig, 2022); as a result, seed productivity also increases.

Ontogenesis is the sequence of all the developmental stages of an individual from the embryo stage to the death of the individual or to the death of all vegetative descendants (Smelov, 1937), a process that does not take place permanently in the same way, and it can be different under various environmental conditions but also under similar conditions. The main processes of ontogenesis are: growth, development, aging (devitalisation) and rejuvenation (Timciuc et al., 2019). Smirnova et al. (2002), explain that determining the absolute age of plants is sometimes impossible due to the permanent renewal of perennial organs, but the classification of the ontogenetic state of plants is quite real.

The researches initiated, carried out and described in this article are aimed at highlighting the ontogenetic peculiarities of *S. perfoliatum* plants under the climatic conditions of the Republic of Moldova, determining the life periods and age stages characteristic of this species in correlation with the meteorological conditions recorded in different years.

MATERIALS AND METHODS

The plants of the species *Silphium perfoliatum* L., cultivar 'Vigor', served as subjects for the study. The experiments were conducted in the experimental sector of the "Plant Resources" Laboratory of "Alexandru Ciubotaru" (National Botanical Garden. NGBI is found in the South-East part of Chisinau, on an area of 104 ha. NBGI hosts a unique gene pool of plants, its collections count about 8500 taxa of spontaneous, fodder, energy, honey, medicinal, aromatic, spicy and ornamental plants.

The research was carried out in four growing seasons (2019, 2020, 2021, 2022) being studied plants aged 1, 2 and 10 years, in order to establish the full ontogenetic cycle.

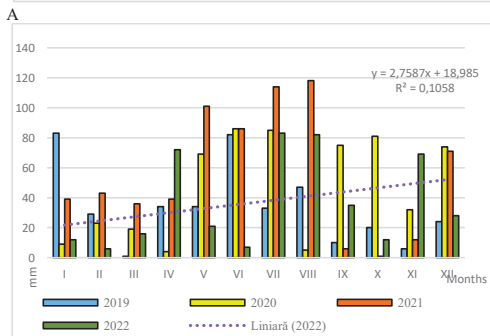
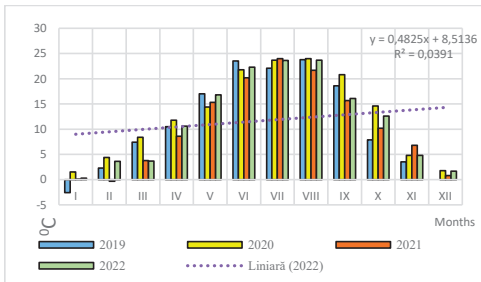
The ontogenetic study was conducted according to the methodology proposed by Rabotnov (1950) who proposed the principle of periodization of ontogenesis, later completed by Uranov (1975) and by Florea (2006). Phenological observations were made and plant growth and development stages were evaluated according to the methodological guidelines used in the Botanical Garden (Metodica fenologhiceskih nabliudnenii v botanicheskikh sadah SSSR 1972). Seed germination was researched according to the indications mentioned in the book "Metodicheskie uczania po semenovedeniu introdutentov" (1980). Special attention was paid to the flowering stage of the plants, being researched as plants with melliferous potential. The planting material was obtained by applying the seedling production methodology used in the cultivation of vegetables (Taracarov, 2003). The experiments were mounted in cell trays in protected environment, followed by the appropriate stages of obtaining healthy seedlings.

The climatic conditions were systematized and characterized with the help of the information provided by the State Hydrometeorological Service, comparatively, in the years during which the research was carried out.

RESULTS AND DISCUSSIONS

The climatic conditions of the Republic of Moldova (Figure 1 A, B) with inhomogeneous temperature regime and amount of precipitation, are favourable for the growth and development

of cup plant and the unfolding of its ontogenetic program. The agro-meteorological conditions recorded during the growing season of 2022, characterized by high temperatures and precipitation deficit (spring with fluctuating temperatures, hot summer) did not affect the growth and development of plants, thus, they managed to complete the full cycle of vegetative and generative phenological stages. Besides, the favourable temperatures in autumn favoured the prolongation of the flowering stage in some specimens, the plants becoming an additional source of food for entomofauna. As compared with 2021, the air temperature was by 1.0-1.5°C higher and the amount of precipitation was lower, and compared with 2020, the atmospheric temperature was by 0.5-1.5°C lower and the amount of precipitation - higher.



B
Figure 1. Main meteorological indices in the 2019-2022 research periods: A - average monthly temperature; B - the monthly amount of atmospheric precipitation

The variations in temperature and precipitation did not significantly influence the annual development of plants, only some changes occurred in the seasonal growth rate and duration of the growth season. Regardless of the weather conditions recorded in Republic of Moldova, *S. perfoliatum* plants, starting from the 2nd-3rd years of development, went through

the ontogenetic cycle, producing viable seeds by the end of the growing season.

The research of ontogenetic features is an important step in studying the growth and development of plants, their dependence on climatic conditions and the role of plants in their respective biocenosis. The vital form of *S. perfoliatum*, under the climatic conditions of the Republic of Moldova, is perennial, polycarpic herbaceous plant. Some authors describe the ontogenetic cycle of cup plant as a series of development cycles of monocarpic shoots that grow and die annually. The lifespan of each annual shoot is about 8-10 months (Vavilov & Condratiev, 1975).

The research on the biological cycle of *S. perfoliatum* plants, under the climatic conditions of the Republic of Moldova, allowed determining the life periods and age stages characteristic of the species. Four periods (latent, pregenerative, generative (g), postgenerative) and 4 stages (seeds - sm, seedling (plantlet) - pl; juvenile - j; immature - im; virginal - v; senile - s) were described. The biological cycle of cup plant is divided into the active growth period and the dormancy period. The duration of the active growth period is 197-234 days. *S. perfoliatum* plants are characterized by: high rate of growth and development, intensive growth process, high productivity of seeds.

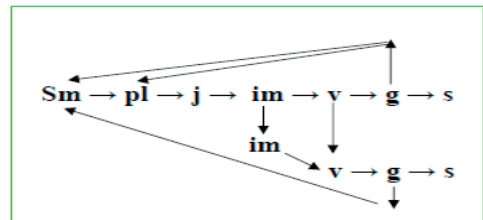


Figure 2. The scheme of the ontogenetic program of *S. perfoliatum* plants under the conditions of the Republic of Moldova

The research carried out demonstrated that the *S. perfoliatum* individual plants from the territory of the "Alexandru Ciubotaru" National Botanical Garden (Institute), depending on the pace of development, can adopt several variants of the ontogenetic program (Figure 2), and the climatic conditions are favourable for the fulfilment of the biological potential of the species.

I. The latent period includes the time from the fertilization of the ovule to the germination of the seeds.

The seed stage. Cup plant produces seeds every growing season. The seed material sown under laboratory conditions, in cell trays, at a depth of 0.8-1.0 cm in soil, being provided favourable conditions for development, began to germinate (Figure 3) at the optimal temperature of 12-15°C. The weight of 1000 achenes is 22-24 g, and the seed productivity reaches 290-450 kg/ha (Țiței & Roșca, 2021).



Figure 3. The stages of seed germination in *S. perfoliatum*

Once the seeds germinate, a new ontogenetic cycle of plant development is initiated.

II. Pregenerative period. It includes the seedling, juvenile, immature and virginal age stages. The growth and development of plants in these stages are closely connected to the weather conditions, thus, unfavourable conditions can make these stages last longer.

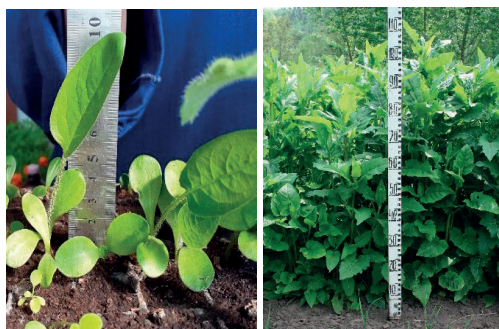


Figure 4. *S. perfoliatum*: A - seedling stage; B - virginal stage

The seedling stage. *S. perfoliatum* plants at this stage were researched under indoor conditions, namely, planting material obtained in cell trays, at a temperature of 18-20°C. According to the position of the cotyledons in the germination

process, cup plant is characterized by epigeal germination. Initially, radicles with absorbent hairs develop. Then, after 5-6 days from the time of incorporation, the thickened, white-greenish hypocotyl emerges at the soil surface. In the next 2 days, the cotyledons come out entirely at the soil surface, being 1.5-1.8 cm long, pale green or yellowish. It takes 14-18 days from the time the seeds are incorporated into the soil until the appearance of the first true leaf (Figure 4A). The plants are in the state of seedling with cotyledons and a true leaf for about 12-18 days. After the 35-39th day, the second true leaf develops.

The juvenile stage. It is characterized by the more intense development of the first 2-4 true leaves. The leaves are petiolate, pubescent, 8-12 cm long and 3.5-5.0 cm wide. At this stage, plants depend highly on temperature and humidity. At the end of this stage, from the buds in the axils of the cotyledons, other true leaves begin developing. The root system expands, providing the plants with a more stable connection to the soil.

S. perfoliatum can be propagated by seeds directly in open ground as well as by transplanting seedlings obtained indoors. The production of seedlings for transplantation seems to be more expensive and a lengthy process, but some researchers describe this method as more efficient, as a result, the plantation is established earlier, is more uniform, thus making use of the selected land more efficiently and the yield are higher; the explanation would be the low germination capacity of seeds in open ground (Gansberger et al., 2015; Ruf et al., 2019).

Gansberger et al. (2015), Pan et al. (2011), Schoo et al. (2017) recommended the optimal planting density of 40,000 plants/ha, that is, about 4 plants/m².

The immature stage. At this stage, the root system develops more intensively and branches. The aerial part of the plants is a rosette of 7-14 leaves, the growth rate of the foliage is slower, the plants in open ground reach a maximum height of 32-45 cm and remain so until the end of the growing season, in late autumn. In the first year of vegetation, cup plant goes through the ontogenetic cycle until the immature stage of development, which coincides with the phenological stage – leaf development. During

this period it is not recommended to cut the plants for green mass, because the resources of the plants are focused on the formation of the root system and the leaf rosette.

In the second year of life, *S. perfoliatum* plants come out of dormancy early, when the atmospheric temperature reaches 3-5°C. They are characterized by rapid growth, with intensive formation of the leaf rosette and stems in a short time, as well as morphological features similar to the mature plant, which already correspond to **the virginal stage** (Figure 4B). The shape of the leaves is similar to that in the immature stage. The underground part is strongly developed and the plants are prepared for the next important period in their life cycle. In the second year of life, in the spring season, in open field, *S. perfoliatum* plants reach the virginal stage. In 30-35 days from the start of the growing season, the plants reach a height of 18-25 cm and form a rosette of 6-10 leaves. In the next phase, the green mass is formed more rapidly, and the stems grow very fast (h: 250-370 cm). The stems are 4-angled, erect, with hairs. Plants can produce a different number of flower stalks (7-15-23 depending on the age of the plants). The leaves grow larger, are light green, heart-shaped, rough, opposite. The lower leaves are petiolate, but those growing in the middle and apical part of the stem are fused around the stem, forming a cup, which helps the

plant use moisture more efficiently. The leaves growing at the base of the stem (also called "spring" leaves) turn brown when the plants enter the flowering phase. The leaves that are developed during the summer period are more resistant and turn brown only in autumn when temperatures below 0°C are recorded, at the end of the growing season. Mature leaves are 25-35 cm long and 16-22 cm wide (Țiței et al., 2020). The root system - a well-developed tap root - has high capacity for branching and can grow about 3.5 m deep in the soil (Țiței & Roșca, 2021.). Starting from the summer of the second year of life (sometimes from the third year, depending on the recorded weather conditions and the time of planting in the field), the plants go into the generative period.

III. The generative period. In this period, flower stalks and flower buds develop. The assimilation surface reaches maximum size. During a growing season, *S. perfoliatum* plants go through the entire cycle of vegetative and generative phenological stages - bloom, produce fruits and viable seeds. In the generative phase (Figure 5A), the growth of the plant and the formation of the plant mass slows down. The generative phenological stages are staggered. The flowering stage extends over a period of 51-63 days and occurs at the end of June - August or in July - September, depending on the weather conditions (Table 1).

Table 1. The life cycle of the species *S. perfoliatum* throughout a growing season

No.	Life period	Age stage	Length (days)	Phenological stage
I	Latent	Seed (sm)		Morphological dormancy
II	Pregenerative	Seedling (pl)	<30	Germination
		Juvenile (j)	18-75	The development of the first true leaves and of the root system
		Immature (im)		
		Virginal (v)	25-36	Full development of foliage and root system
III	Generative	Generative (g)	115-130	Budding
				Flowering
				Fruit development
				Seed ripening
IV	Postgenerative	Senile (s)	10-15	The end of the growing season
The length of the growing season				197-234 days

In some specimens, the flowering stage lasts even longer, having solitary flowers until the middle of October. During this period, solitary specimens of insects can be noticed on the flowers. An inflorescence consists of 20-30 yellow flowers of 3-5 cm in diameter, each flower produces by 20-30 seeds.

Cup plant flowers are attractive to a wide range of honeybees and pollinators, providing pollen and nectar until autumn, when food sources are scarce. As a result of the entomological monitoring carried out at the National Botanical Garden (Institute) "A. Ciobotaru", 10 species of honey-producing and pollinating insects visiting

cup plant flowers, belonging to 6 families and 4 orders, were identified. The species of the Apidae family (*Apis mellifera*, *Bombus terrestris*, *B. lapidarius*) had the maximum frequency on the flowers (Cîrlig, 2022). The research carried out by Wroblewska (1997) demonstrated the role of insects (especially the honey bee) on the number of seeds produced as a result of pollination. In the inflorescences isolated from insects, only 5-10% of seeds were produced, and in those pollinated by bees, 70-80% of seeds were formed. Cup plant is considered a high-potential honey plant in England, Germany, Russia, Bashkortostan, Bulgaria (Daniel, 1984; Wroblewska, 1997; Hoves, 2017), being able to provide a late-season source of nectar and pollen for insects. An inflorescence of *S. perfoliatum* produces, on average, 122 disk florets, and a disk contains about 14200 pollen grains (Mueller et al., 2020). Mueller et al. (2020) calculated the average productivity of a cup plant flower: 1.75×10^6 pollen grains, 12.5×10^{12} pollen grains/ha 80 kg/ha nectar sugar each season, which would provide the necessary food for 34 honey bee larvae per season and 6 worker honey bee per day.

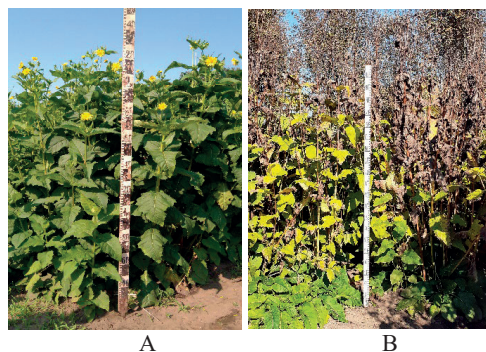


Figure 5. *S. perfoliatum*: A - generative phase, flowering stage; B - senescence stage

The **post-generative period** in higher plants is characterized by the disintegration of the root system and the death of the whole organism. The state of *S. perfoliatum* plants at the end of the growing season corresponds to the **senescence stage** (Figure 5B), the stems turn brown, dehydrate, generative shoots are no longer produced, the area with dead tissues

predominates. However, these physiological processes do not mean the end of ontogenesis of the species. Under the climatic conditions of the Republic of Moldova, *S. perfoliatum* plants have not reached this stage yet.

Once the growing season ends, the plants are of interest for biofuel production. The biomethane production potential is 230 l/kg and the gross calorific value is 17.8 J g^{-1} (Țiței et al., 2020).

CONCLUSIONS

The research carried out at the "Alexandru Ciubotaru" National Botanical Garden (Institute) on *Silphium perfoliatum* L. plants demonstrates the high adaptability of plants to the climatic conditions of the Republic of Moldova. During a growing season, plants go through the ontogenetic cycle of development which includes 4 life periods: latent, pregenerative, generative, postgenerative and six age stages: seed, seedling, immature, juvenile, virginal and senile. In the seedling and immature stages, plant development highly depends on climatic conditions. The temperatures below 0°C in spring can slow down the growth of vegetative organs for a few days. In the first year of life, cup plant goes through the ontogenetic cycle until the immature stage of development, the root system and the leaf rosette develop intensively. The following growing season, the plants go into the virginal stage, during which the plants have a high rate of growth and development of vigorous stems and foliage. The generative period is long and coincides with the generative phenological stages characteristic of this species: budding, flowering, fruit development and seed ripening. The flowering stage lasts about 51-60 days, depending on the recorded weather conditions, becoming a source of food for honeybees and pollinating insects for a long period, and lasts till autumn when very few other species are blooming. The analysis of specialized literature demonstrates the interest of researchers towards *S. perfoliatum* - a multi-purpose crop, characterized by high adaptability and resistance to various climatic conditions, fast growth and high melliferous potential.

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