

CONTRIBUTION TO THE KNOWLEDGE OF THE
INVASIVE ALIEN SPECIES OF *HERACLEUM* (APIACEAE)
IN BULGARIA

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Abstract

Heracleum mantegazzianum and *H. sosnowskyi* (Apiaceae) have been recently published as naturalised alien species in the Bulgarian flora. The present article summarises the data about the distribution of the species in the country and provides new data about the habitat preferences of the taxa, chromosome number, genome size and reproductive potential. Diploid chromosome number of $2n = 2x = 22$ has been established in both species from Bulgarian accessions. The estimated genome size is $1C = 1.81$ pg for *H. mantegazzianum* and $1C = 1.75$ pg for *H. sosnowskyi*. Studies of the flower/young fruit number and soil seed bank of *H. sosnowskyi* suggests that the species has a great invasive potential.

Key words: alien plants, chromosome number, genome size, Giant hogweed

Introduction. Two alien species of *Heracleum* L. (Apiaceae) have been published recently for Bulgaria – *H. mantegazzianum* Sommier & Levier and *H. sosnowskyi* Manden. [1,2]. *Heracleum mantegazzianum* is native in the Western Caucasus whereas *H. sosnowskyi* originates from the Central and Eastern Caucasus, Western, Central, Eastern, South-west Transcaucasia, and North-east Turkey

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[3]. Both species had been introduced to many European countries where they became naturalised and somewhere invasive. Due to their high negative impact on the native biodiversity and threats to human health, they were included in the List of invasive alien species of Union concern [4, 5]. The list is related to the implementation of Regulation (EU) No. 1143/2014 of the European Parliament and of the Council of 22 October 2014 [6].

The aim of the article is to summarise the currently known distribution data and to provide some new data about the alien *Heracleum* species in the Bulgarian flora such as chromosome number, genome size, reproductive potential, etc.

Material and methods. Field studies were carried out in the period 2020–2022. Plant material (live plants, seeds) was collected for further studies. A few plants from each species have been cultivated in the Greenhouse of the Institute of Biodiversity and Ecosystem Research. Data about the habitats are based on authors' personal observations in the field.

Chromosome counting was done in root meristem. Potted live plants were used for the study. Root tips were collected and pre-treated with colchicine solution (0.01%) for ca. 90 min, then fixed in acetic alcohol (1:3) for at least 2 h at room temperature, hydrolysed in 1N HCl for 25 min at 60 °C, treated with 1:1 solution of concentrated HCL in diethyl ether for 5–8 min at 60 °C, and stained in Gomori's haematoxylin [7] for 30 min at 60 °C. The root tips were finally squashed in 45% acetic acid and observed with a light microscope.

Nuclear DNA content was estimated by CyFlow SL Green flow cytometer (PARTEC, Germany), equipped with a green (532 nm) solid-state laser. Fresh leaves from *Heracleum* plants were used for the study. *Pisum sativum* 'Kleine Rheinländerin' (1C = 4.38 pg, [8]) was applied as an internal standard. The plant material was treated with the extraction and staining kit 'CyStain® PI Absolute P' (SYSMEX) following the protocol prescribed by the manufacturer. Altogether 5000 PI-stained particles were measured per run, with 5 runs per preparation. Runs with a CV above 5% for the studied species or the standard were discarded.

Soil seed bank was studied in a stand of *H. sosnowskyi* near Borino village in the Western Rhodope Mts. A specially constructed device was used for collecting soil samples. The device was made from a water tube with Φ 160 mm which allowed taking samples from the top 5 cm soil substrate with an area of $S = 0.0191 \text{ m}^2$. Altogether, 30 samples were collected from evenly spaced locations in 3 rows (5 m distance between the rows, 2 m distance between the sampling locations in each row). Soil samples were put in plastic bags, labelled and stored in a refrigerator for a couple of days until required. Seed counting was performed in a laboratory, with the soil spread out and sieved, and the seeds and seedlings carefully counted in each sample.

Results and discussion. Distribution in Bulgaria. Since the first reports of *H. mantegazzianum* and *H. sosnowskyi* as new alien species in the Bulgarian flora, a few more localities have been discovered. The currently known

distribution areas of both species in Bulgaria are presented below.

Heracleum mantegazzianum: Sofia Region (Sofia city, Lyulin residential area [1]), the population is small, of a few dozens of specimens and subjected to regular mowing; Vitosha Region (Vitosha Mt., near Balkaniti Mountain Hut [9]), the population is small, of a few dozens of specimens and subjected to eradication measures by staff of the Vitosha Nature Park Directorate.

The species has been recorded in the following EUNIS habitats: **G4.6**: Mixed *Abies* - *Picea* - *Fagus* woodland; **X23**: Large non-domestic gardens.

Heracleum sosnowskyi: Danubian Plain (Koilovtsi village [10]), the population comprised a few hundred specimens; Sofia Region (Mirkovo village [11]), the population comprised perhaps about a thousand specimens, and Rhodope Mts (*Western* – Borino village, the population comprised a few thousand specimens; *Central* – Buynovo gorge, only a few plants observed) [2].

The species has been recorded in the following EUNIS habitats: **C3.6**: Unvegetated or sparsely vegetated shores with soft or mobile sediments; **E2.3**: Mountain hay meadows; **E2.7**: Unmanaged mesic grassland; **G1.1**: Riparian and gallery woodland, with dominant *Alnus*, *Betula*, *Populus* or *Salix*; **X25**: Domestic gardens of villages and urban peripheries.

Chromosome number. So far, the chromosome number only for *H. sosnowskyi* from the region near Borino village has been reported – $2n = 2x = 22$ [2]. Diploid chromosome number of $2n = 2x = 22$ has been established also for *H. mantegazzianum* from the both known localities (Sofia city, Fig. 1, and Vitosha Mt, Fig. 2), and this is the first report from Bulgarian accessions. This number confirms earlier counts from elsewhere (e.g. [12]). Both *H. mantegazzianum* and *H. sosnowskyi* are diploid plants and have the same chromosome number which means they cannot be distinguished on this basis.

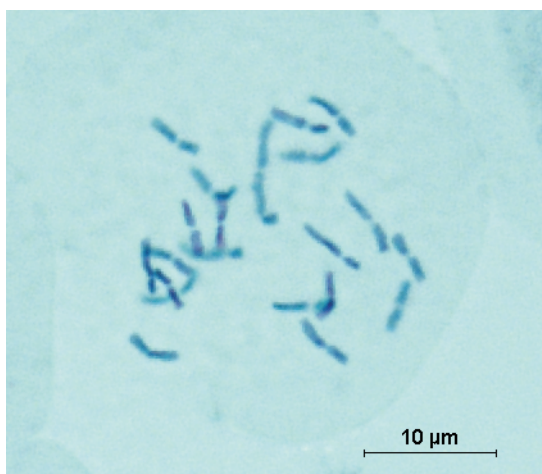


Fig. 1. Metaphase plate of *Heracleum mantegazzianum*, Sofia city, $2n = 22$

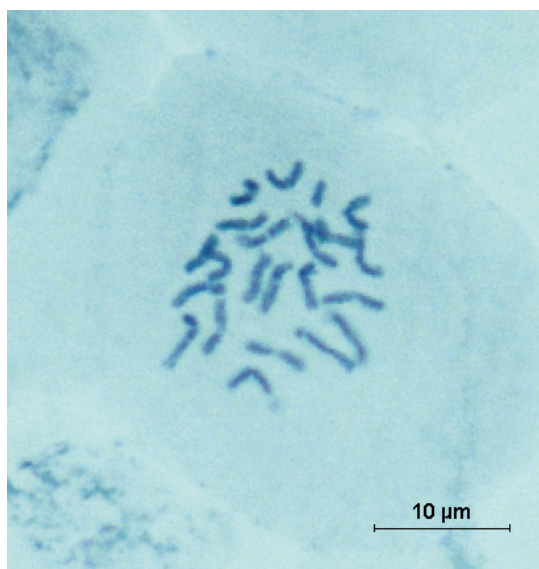


Fig. 2. Metaphase plate of *Heracleum mantegazzianum*, Vitosha Mt., $2n = 22$

Genome size. Search in the Plant DNA C-values Database [13] reveals genome size data were published for only three species of *Heracleum*, including for *H. mantegazzianum*, $1C = 1.78$ pg [14]. ZONNEVELD [15] published data for both species from the Netherlands: *H. mantegazzianum*, $2C = 3.65$ pg, and *H. sosnowskyi*, $2C = 3.58$ pg. Our data are very similar to the already published values. In *H. mantegazzianum*, collected in September 2021 from Sofia Region, Sofia city, Lyulin residential area, $1C = 1.81$ pg or $2C = 3.62$ pg have been estimated. In *H. sosnowskyi*, collected in September 2021 from the Rhodope Mts (Western), Borino village, $1C = 1.75$ pg or $2C = 3.49$ pg have been estimated. These are the first data about the genome size of both species from Bulgarian accessions. Both species have similar values and cannot be distinguished by their genome size, as already noted by Zonneveld [15].

Reproductive potential. Some reproductive traits, such as number of flowers per plant and soil seed bank, have been studied in *H. sosnowskyi*, in the population in the Rhodope Mts (Western), near Borino village. The number of flowers/young fruits in all umbels has been counted in the field in 10 randomly selected live specimens. The results show the number of flowers/young fruits are ca. 5900 on average (average 5916, st. dev. ± 2814) and varied in the range from 2422 to 10224. This data is slightly lower than the published data, for example from the Saint Petersburg area of the Russian Federation – 8836 seeds [16, 17]. However, it is still in more or less the same range bearing in mind that the number of flowers/seeds per plant may vary greatly depending on the soil characteristics and climatic conditions.

Soil seed bank studies revealed that 2978 seeds (± 1914) and 92 seedlings (± 139) on average can be found on 1 m² in a stand of the species. The data varied greatly between the samples in the range from 628 seeds and 0 seedlings to 6702 seeds and 314 seedlings per 1 m².

Conclusions. The study confirmed that both *H. mantegazzianum* and *H. sosnowskyi* are naturalised alien species in the Bulgarian flora. The former species is restricted to only two localities with small populations, whereas the latter is more widespread, and represented by much larger populations, especially in the Western Rhodope Mts, near Borino village. Data on chromosome numbers and genome size from the Bulgarian accession does not differ from that published from elsewhere in Europe. The reproductive traits studied in *H. sosnowskyi* revealed the species forms a large number of seeds and has a great invasion potential.

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