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SOME AGROBIOLOGICAL PECULIARITIES AND QUALITY INDICES OF BIOMASS OF *Macleaya cordata* 'MIHAELA'

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Abstract

Plume poppy – Macleaya cordata (Willd.) R.Br., Papaveraceae family is a perennial herbaceous species with medicinal, melliferous, ornamental and energy biomass utility. The harvested stem biomass from Macleaya cordata can be used as feedstock for the production of solid fuel – briquettes and pellets. The objective of this research was to evaluate some agrobiological peculiarities and quality indices of biomass of Macleaya cordata 'Mihaela' grown in experimental field of the NBGI MSU Chişinău, Republic of Moldova. At the end of the flowering period, Macleaya cordata shoots reach 303-320 cm in height, with 35.8-38.2% leaf share. The content of alkaloids in Macleaya cordata leaves was: 7.36-8.12 mg/g sanguinarine, 5.91-6.82 mg/g chelerythrine and 0.659-0.757 mg/g fagaridine. It has been determined that the stem dry matter productivity of Macleaya cordata harvested in August was 1.19-1.40 kg/m^2, but – of plants harvested in November – 1.67-1.79 kg/m^2. The comparative analysis of cell wall components revealed that Macleaya cordata substrate contained 406-503g/kg cellulose, 212-243 g/kg hemicellulose and 91-104 g/kg acid detergent lignin, the estimated theoretical ethanol yield from cell wall carbohydrates averaged 448.7-541.8 L/t in Macleaya cordata substrates.

Key words: *agrobiological peculiarities, alkaloids, biomass, gross calorific value, Macleaya cordata 'Mihaela' theoretical ethanol potential.*

INTRODUCTION

The introduction and adaptation of new plant species would help meet the actual demands for food, forage, fibers, fuel, pharmaceuticals, chemicals and other important raw materials, and also could stimulate economic growth.

M*acleaya cordata* (Willd.) R. Br. is a species in the family Papaveraceae, which includes 44 genera and 825 species, most of them are herbaceous plants, distributed mostly in the temperate regions of the northern hemisphere.

This botanical family includes many cultivated oilseed crops, others are very important as medicinal and ornamental species.

The genus *Macleaya* consists of 2 species: M*acleaya cordata* (Willd.) R. Br. and *Macleaya macrocarpa* (Maxhn.) Fedde, besides, there is the hybrid *Macleaya cordata x Macleaya macrocarpa*, also mentioned in the specialized literature as *Macleaya x kevensis* Turrill. (Abizov, 2004; Bykova et al., 2023).

Macleaya cordata (Willd.) R. Br. = *syn. Bocconia cordata Willd.* syn. *Bocconia cordata* Willdenow, *Bocconia japonica* J.N. Haage & E. Schmidt; *Marzaria cordata* (Willd.) Raf., *Marzaria cordata* (Willd.) Raf., fam. Papaveraceae, is a perennial species with laticifers, native to South-East Asia, namely, to China and Japan. It is an herbaceous plant with cylindrical stem, erect, glaucous, slightly branched in the upper part, growing 230-356 cm tall, green, covered with a gray waxy layer, at the base – strongly lignified, bluish-orange in color.

The leaves are alternate, with a 1-12 cm long petiole, cordate, with 5-7 lobes, those growing at the base of the shoot are 25-35 cm long and 13-20 cm wide, those at the top are much smaller, 12-15 cm long and 6-9 cm wide, the upper part of the leaf blade is glabrous, brownish-green to gray-green, and the underside is pubescent, gray or gray-yellow.

The flowers are small, 6-7 mm, greyish yellow, with cream or orange shades, grouped in erect airy panicles, up to 40 cm long, located at the top of the stems. The flowers are hermaphrodite, actinomorphic, with 2 deciduous sepals and 4 petals. *M. cordata* blooms in July-August and produces fruits in September. The fruit is a flat obovate capsule, up to 8 mm long and up to 4 mm wide, glabrous, attenuate at the base and rounded or obtuse at the apex, with 4-6 ovoid seeds, 1.5-2.0 mm long. The weight of 1000 seeds is 0.6-0.8 g. The root system is a taproot, the plant has rhizomes and numerous dark orange adventitious roots. The rhizome of the plant is vertical, located at a depth of 10-13 cm, short, with numerous buds. The lateral roots are branching and grow mostly horizontally, reaching down to 50 cm deep in the soil. Most of the roots are found in the arable soil layer. One-year-old roots and rhizomes are flexible, dark orange with numerous adventitious roots. Starting in the autumn of the first year of life, adventitious buds are formed on the lateral roots, located in groups, from which root shoots emerge. The younger the rhizome, the smaller and denser the buds are on it. *Macleaya* reproduces by seeds, seedlings and pieces of rhizomes. It is a thermophilic species, so, young shoots can be affected by spring frosts of -3...-4°C. In spring, the plants come out of dormancy at an average temperature of 10°C. Under a snow layer, the plants can withstand temperatures of -20…-25°C. In autumn, when temperatures below 0°C are recorded, the leaves fall off completely. It is a mesophilic plant and makes very good use of the water accumulated in the soil during the dormancy period and from the rains that fall during the growing season. *Macleaya cordata* plants hardly tolerate high temperatures (over +30°C), particularly in the bud formation and flowering periods. Generally, the plants are characterized by low seed productivity, associated with low pollen viability and severe fruit shedding during ripening. Therefore, vegetative propagation of the crop is mostly used for industrial cultivation. It is not demanding to soil, thanks to its deep root system and has high capacity of solubilization and absorption of nutrients (Ţîţei & Roşca, 2021). According to the specialized literature, thanks to this fact, it is able to grow well even on lands with low fertility, with pH = 6.5-8.5, it can be cultivated on degraded soils, fertilized with organic fertilizers and sewage sludge, the plants react to the application of growth stimulators by increasing productivity and the concentration of alkaloids (Sidelnikov, 2014; Lin et al., 2018; Yakhtanigova et al., 2022; Bykova et al., 2023).

Macleaya cordata contains many biologically active compounds. Scientific studies have identified 147 alkaloids, most of these compounds are isoquinoline alkaloids, including sanguinarine, chelerythrine, protopine and allocryptopine (Lin et al., 2018). It is widely used in traditional Chinese medicine for the treatment of injuries, arthritis, rheumatic arthralgia and trichomonas vaginalis. In North America and Europe, *Macleaya cordata* is also considered as a traditional medicinal plant used as a remedy for insect bites and ringworm infection. Extracts from *Macleaya cordata* and their components have many biological properties, such as anti-microbial, anti-fungal, pesticidal and anticancer properties (Satou et al., 2002; Abizov, 2004; Frolova, 2005; Stiborová et al., 2008; Li, 2012; Wang et al., 2012; Baek et al., 2013; Liu et al., 2013; Li et al., 2015; Lin et al., 2018; Liu et al., 2023).

Current studies have shown that *Macleaya cordata* stimulates the growth of animals and poultry, as a feed additive, it can replace antibiotics and is an all-natural feed additive (Köroğlu & Kocabağli, 2019; Buyarov et al., 2020; Toprak, 2020; Manaa et al., 2022; Wang et al., 2022; Chen et al., 2023; Krzykawski et al., 2023; Ling et al., 2023).

The objective of this research was to evaluate some agrobiological peculiarities and quality indices of biomass of *Macleaya cordata* 'Mihaela'

MATERIALS AND METHODS

The local cultivar 'Mihaela' of *Macleaya cordata*, commonly known as plume poppy, created at the "Alexandru Ciubotaru" National Botanical Garden Institute (NBGI) of Moldova State University (MSU) and grown in the experimental collection of the, Chisinau, Republic of Moldova, served as research subject.

The experiments with *Macleaya cordata* started on the experimental field, in late autumn, by planting the rhizomes at a depth of 7-10 cm. The biological peculiarities were studied in the

4th and 5th growing season. The leaf/stem ratio was determined of the end flowering period by separating the leaves and inflorescences from the stem, weighing them apart and establishing the ratios for these quantities (leaves/stems). The dry matter content was detected by drying samples to constant weight at 105°C. For phytochemical analyses, the plant leaves samples were protected from the impact of sunlight and dried in an air oven at 25-30°C. Then, they were milled in a beater mill equipped with a sieve with a mesh size of 1 mm and some assessments of the bioactive compounds, such as sanguinarine, chelerythrine and fagaridine, were made according to standard procedures reported by Casian et al. (2017; 2019).
As energy biomass, the *Macleaya cordata* stems were collected of the end the flowering period, in August, and also at the end of the growing season, when temperatures below 0°C were recorded, in November, apple tree pruning residues were used as control variant. The harvested stems were chopped and disintegrated in a knife mill with a sieve with the mesh size of 1 mm. To perform the analyses, the biomass samples were dried in an oven at 85°C. After that, the total carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) amounts were determined by dry combustion in a Vario Macro CHNS analyzer. The content of ash was determined at 550°C in a muffle furnace HT40AL according to SM EN ISO 18122; the automatic calorimeter LAGET MS10A with accessories was used to determine the calorific value, according to SM EN ISO 18125. The content of cell walls was evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200. Theoretical Ethanol Potential (TEP) was estimated according to methodology, based on the conversion of hexose and pentose sugars into ethanol (Goff et al., 2010).

RESULTS AND DISCUSSIONS

As a result of the study of the biological peculiarities of *Macleaya cordata*, we observed that, by the end of April, in the first year of vegetation, from the planted rhizomes, at the soil surface the young plants emerged, which, in the first days of June, developed already erect stems that, by the end of the growing season, grew 160-180 cm tall and 3-5 mm thick at the base, with 8-12 leaves. Some plants also produced inflorescences. In the following years, in spring, when the air temperature exceeded 8-10°C, *Macleaya cordata* 'Mihaela' started the growth and development of the new plants from the dormant buds formed on the rhizomes, and then, the plants went through all stages of ontogenetic development. It was observed that in 4th year of vegetation (2022), the plants came out of dormancy in the middle of April, and in 5th year of vegetation (2023) in the first days of April. It has been established that the number of shoots per plants may vary depending on the climatic conditions and the age of plants. Thus, in the 4th year of vegetation there were 5-6 shoots/plant and in the 5th year of vegetation 8-9 shoots/plant. We found that, in May, the growth rate of the stems was 33-45 mm/day, and in June the growth and development intensified even more, reaching values of 56-64 mm/day. In the second half of June, the *Macleaya cordata* plants formed inflorescences and, at the end of June, the flowering stage started. The flowering stage lasted about 27-34 days.
Some biological peculiarities and the structure of the phytomass harvested from *Macleaya cordata* 'Mihaela' at the end of the flowering period are presented in Table 1.
In the 4th year of vegetation, the mass of a shoot reached 737.1 g of fresh matter or 235.3 g of dry matter, the share of leaves being 38.2%, and in the 5th year of vegetation, the mass of a shoot reached 542.2 g of fresh matter, 161.9 g of dry matter, respectively, the leaves representing 35.8%.
Natural products and phytochemicals have always been of great importance in the treatment of various diseases in humans and animals. The accurate determination of the bioactive compounds in the plant mass is very important for the more comprehensive quality control of the raw materials for the pharmaceutical industry. It is generally known that the leaves have a higher and more diverse content of bioactive compounds as compared to the stems. Among the multi-target natural products, alkaloids have demonstrated a variety of pharmacological properties as anti-inflammatory, anticancer, cardio protective and

neuroprotective, which support their potential in the treatment of chronic multifactorial diseases (Abizov, 2004; Frolova, 2005; Liu et al., 2013; Liu et al., 2022).
The results of the study on the content of some isoquinoline alkaloids in the leaves of *Macleaya cordata* are presented in Table 2. The content of alkaloids in *Macleaya cordata* leaf blade was: 8.12 mg/g sanguinarine, 6.82 mg/g chelerythrine and 0.757 mg/g fagaridine, but in leaf petiole 1.88 mg/g sanguinarine, 1.63 mg/g chelerythrine and 0.108 mg/g fagaridine.

Table 1. Some biological peculiarities and the structure of the harvested phytomass at the end flowering period of *Macleaya cordata* 'Mihaela'

Growing season	Plant height, cm	Stems, g		Leaves, g		Inflorescences, g	
		green mass	dry matter	green mass	dry matter	green mass	dry matter
4th year of vegetation	320	358.3	112.1	281.5	90.0	97.6	33.2
5th year of vegetation	303	273.2	85.2	218.9	58.0	50.1	18.7

Table 2. The alkaloid content in *Macleaya cordata* leaves, 5th year of vegetation

Alkaloids	Leaves	Petioles	Leaves with petioles
Sanguinarine, mg/g	8.12	1.88	7.36
Chelerythrine, mg/g	6.82	1.63	5.91
Fagaridine, mg/g	0.757	0.108	0.659

Several literature sources described the pytochemical composition of *Macleaya* species. According to Abizov (2004) in the flowering period the sum of chelerythrine and sanguinarine alkaloids in *Macleaya cordata* reached 1.18 % in leaves, 0.20% in stem mass and 3.21% in rhizome parts; in *Macleaya macrocarpa* – 1.75% in leaves, 0.29% in stems mass and 4.74% in rhizome parts; in *Macleaya x kevensis* – 1.15% in leaves, 0.22% in stem mass and 3.16% in rhizomes. Frolova (2005) stated that the highest content of isoquinoline, chelerythrine and sanguinarine, in above-ground parts of *Macleaya* plants was recorded in the budding phase – 0.84%, but in the roots and rhizomes 0.96 % in the dormancy period. Kosina et al. (2010) found that *Macleaya cordata* aerial part contained 4.51 mg/g sanguinarine and 2.88 mg/g chelerythrine; seeds contained 0.07 mg/g sanguinarine, 0.02 mg/g chelerythrine, but capsules – 32.08 mg/g sanguinarine, 7.36 mg/g chelerythrine. Pěnčíková et al. (2011) revealed that aerial parts of *Macleaya macrocarpa*, depending on the age of plants, contained 2.43-4.37 mg/g sanguinarine, 2.93-5.75 mg/g chelerythrine, but the underground part 1.68-3.11 mg/g sanguinarine, 2.60-4.98 mg/g chelerythrine. Sidelnikov (2014) revealed that sanguinarine levels in samples of *Macleaya cordata* from Belgorod region and Krasnodar region reached 12.1 and 12.4 mg/g dry matter, and in the Moscow region they were lower, 4.2 mg/g, and chelerythrine levels were 5.9-6.1 mg/g dry matter in Belgorod region and Krasnodar region, and 2.2 mg/g in Moscow region, Russian Federation. Casian et al. (2017) found that *Macleaya microcarpa* leaves, in the budding stage, contained 9.70 mg/g sanguinarine, 8.91 mg/g chelerythrine and 0.377 mg/g fagaridine, but the leaves collected in the flowering stage of the plant contained 8.94 mg/g sanguinarine, 7.06 mg/g chelerythrine and 0.308 mg/g fagaridine. Tuzimski et al. (2022) reported that the content of isoquinoline alkaloids in the aerial part dry matter was 2.41-4.09 mg/g sanguinarine and 3.26-5.36 mg/g chelerythrine, but in root part 1.23-1.78 mg/g sanguinarine and 0.88-1.54 mg/g chelerythrine. Bykova et al. (2023) mentioned that the content of alkaloids in *Macleaya cordata* was 0.162-0.164%, as compared to 0.98-1.04% in *Macleaya x kevensis*. Misiurek et al. (2023) remarked that the content of alkaloids in dry plant material samples of *Macleaya cordata* decreases as follows was: in leaves 0.051 mg/g sanguinarine, 0.046 mg/g chelerythrine; in stems 0.027 mg/g sanguinarine, 0.024 mg/g chelerythrine; in root mass of 0.026 mg/g sanguinarine, 0.019 mg/g chelerythrine.
The use of phytomass and crop residues for energy production includes the production of gaseous, liquid and solid fuels. It has been

determined that stem productivity of *Macleaya cordata* harvested in August was 1.19 kg/m^2 dry matter in 4^{th} year of vegetation and 1.40 kg/m^2 dry matter in the 5^{th} year of vegetation, but plants harvested in November had a productivity of 1.67 kg/m^2 dry matter in the 4^{th} year of vegetation and 1.79 kg/m^2 dry matter in the 5^{th} year of vegetation. The quality indices of energy stem substrates from *Macleaya cordata* 'Mihaela' are shown in Table 3. It has been determined that *Macleaya cordata* stem substrates contained 406-503 g/kg cellulose, 212-243 g/kg hemicellulose and 91-104 g/kg acid detergent lignin, the estimated theoretical ethanol yield from cell wall carbohydrates averaged 448.7- 541.8 L/t.

Table 3. The quality indices of stem energy substrates from *Macleaya cordata* 'Mihaela'

Indices	4^{th} year of vegetation		5^{th} year of vegetation		Apple tree pruning residues
	August	November	August	November	
Minerals, g/kg DM	56	32	49	24	59
Acid detergent fibre, g/kg DM	497	568	528	607	547
Neutral detergent fibre, g/kg DM	709	802	727	850	766
Acid detergent lignin, g/kg DM	91	99	79	104	110
Cellulose, g/kg	406	469	444	503	437
Hemicellulose, g/kg	212	234	283	243	219
Hexose sugars, g/kg	72.73	83.30	80.15	89.87	78.17
Pentose sugars, g/kg	34.87	38.49	46.55	39.97	36.02
Theoretical ethanol potential, L/t	448.7	507.8	528.3	541.8	476.3
Gross calorific value, MJ/kg	18.35	18.98	18.45	19.28	18.90

The concentrations of structural carbohydrates were much higher in *Macleaya cordata* stem substrates collected in the 5^{th} year of vegetation, with positive impact on the ethanol yield in comparison with the control – apple tree pruning residue substrate. The *Macleaya cordata* stem biomass collected in August had high content of minerals and lower content of structural carbohydrates and acid detergent lignin than stem biomass collected in November. The gross calorific value was higher in stem biomass collected in November.

CONCLUSIONS

The *Macleaya cordata* 'Mihaela' leaf biomass is a valuable resource for the pharmaceutical industry. Besides, due to its optimal cellulose and hemicellulose content *Macleaya cordata* stem biomass makes an attractive material for the production of bioethanol and densified solid biofuel.

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