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Yield and quality of southern sweet-grass leaves as affected by reduction of sunlight intensity

INTRODUCTION

Southern sweet-grass (*Hierochloë australis* (Schrad.) Roem. et Schult.) is a tuft grass growing wild in coniferous and mixed forest in Europe, mostly in Poland (Picture 1-2). Leaves of this plant are collected exclusively from natural sites as a coumarin raw material used for extracts production, utilized in food industry for aromatization of alcoholic beverages (Picture 3). In natural sites the species is seriously endangered because of excessive and uncontrolled harvesting. Introduction into cultivation of southern sweet-grass seems to be the most effective way to protect this plant in the wild.

The aim of investigation was to study the influence of reduction of sunlight intensity on the mass of raw material i.e. leaves and accumulation of coumarin compounds in the first year of plant vegetation.

Picture 3. The usage of southern sweet-grass

Picture 1. Population on natural site

Picture 2. Plants on natural site

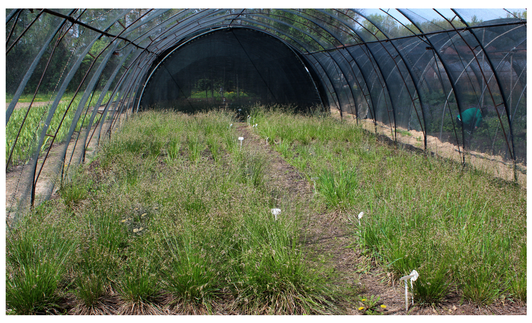
MATERIAL AND METHODS

The object of the investigation was selected population of southern sweet-grass originated from Eastern Poland. The plants were cultivated in full sunlight (control) and under shading nets reducing intensity of sunlight for 30% and 50%. The measurement of solar radiation intensity was carried out using phytophotometer RF-100 (Table 1). During plant vegetation, the relative content of chlorophyll and flavonoids as well as Nitrogen Balance Index (NBI – the ratio of chlorophyll/flavonoids related to nitrogen/carbon allocation) in investigated plants, were measured using Dualex 4 Force apparatus. Plants grown under control conditions and under shading nets were compared in terms of selected morphological traits: the number of leaves, leaf length and width, as well mass of leaves. After harvesting, leaves were dried at 40°C, powdered and subjected to chemical analysis. The content and composition of coumarin compounds was determine using HPLC, on Shimadzu chromatograph equipped with auto sampler SIL-20, photodiode array detector SPD-M10A VP DAD, Phenomenex Kinetex® 2.6 µm, C18, 100 Å, 100 × 4.60 mm column and Class VP 7.3 chromatography software. The TLC analysis of extracts obtained from investigated plants was also carried out. The results were evaluated using one-way ANOVA and Tukey's HSD test at α=0.95 with Statgraphics Plus for Windows v. 4.1 software.

Picture 4. Plants grown under full sunlight (control)

Picture 5. Plants grown under net reducing intensity of sunlight for 30%

Picture 6. Plants grown under net reducing intensity of sunlight for 50%



Picture 7. Experimental cultivation of southern sweet-grass

RESULTS

The highest mass of dried leaves was obtained from plants grown under 30% reduction of natural light. The plants grown in full sunlight were characterized by distinctly higher number of leaves. However, they were considerably shorter than leaves of plants cultivated under shading nets. The leaves of plants grown under 50% reduction of sunlight were characterized by the lowest relative content of flavonoids and the highest content of chlorophyll as well the highest NBI value. High rate of NBI indicates the best growth conditions for plants (Table 2).

The content of main active compound, responsible for aroma of the raw material, i.e. coumarin was considerably higher in leaves from plants grown under 50% light reduction (90.97 mg × 100 g^{-1} dm.) in comparison with full sunlight and 30% light reduction (65.97 and 67.08 mg × 100 g^{-1} dm., respectively). The opposite relation was observe for 3,4-dihydrocoumarin and o-coumaric acid, i.e. the content of these compounds was the highest in raw material from plants grown in full sunlight (Table 3, Fig. 1-5).

The results of experiment show that from cultivated plants of southern sweet-grass it is possible to obtain high yield of leaves characterized by expected content and composition of coumarin compounds.

Table 1. The solar radiation intensity (µmol photons × m^{-2} s^{-1})

	Cloudless sky	Cloudy sky
Control - full sunlight	2210	660
30% reduction of sunlight	1450	390
50% reduction of sunlight	650	130

Table 2. The relative chlorophyll and flavonoids content in the leaves and nitrogen balance index (NBI)

	Relative chlorophyll content	Relative flavonoids content	Nitrogen Balance Index (NBI)
Control - full sunlight	34.3c	1.39a	25.8c
30% reduction of sunlight	40.0b	1.11b	30.2b
50% reduction of sunlight	44.6a	0.96c	51.3a

Table 3. Selected morphological traits of investigated plants

	Number of leaves (per plant)	Leaf length (cm)	Leaf width (cm)	Dry mass of leaves (g/plant)
Control - full sunlight	132.20a	32.30c	0.99b	18.85c
30% reduction of sunlight	113.00b	38.22b	1.07a	33.75a
50% reduction of sunlight	90.80c	45.36a	0.89c	30.40b

Values marked with the same letters do not differ significantly at α=0.95, Tukey's HSD test

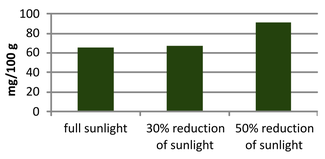


Figure 1. The content of coumarin (mg × 100 g^{-1}dry matter)

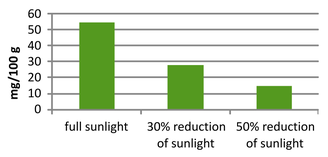


Figure 2. The content of 3,4 dihydrocoumarin (mg × 100 g^{-1}dry matter)

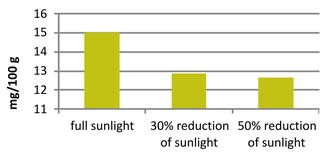


Figure 3. The content of o-coumaric acid (mg × 100 g^{-1}dry matter)

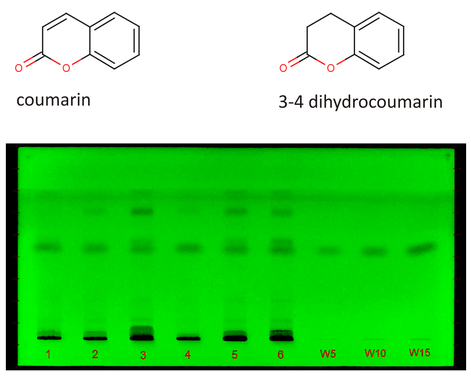


Figure 4. The TLC chromatogram of coumarin separation

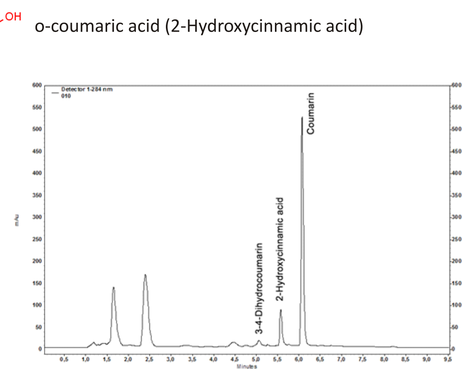


Figure 5. The HPLC chromatogram of coumarin compounds separation

1, 4 - extracts from plants grown in full sunlight (control)
2, 5 - extracts from plants grown under net reducing intensity of sunlight for 30%
3, 6 - extracts from plants grown under net reducing intensity of sunlight for 50%
W5, W10, W15 - coumarin in progressive concentration

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