Effect of life cycle on the production of mullein (*Verbascum phlomoides* L.)

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Summary: Aim of the present investigations was the optimalization of the production of the annual cultivar 'Napfény' of *Verbascum phlomoides* L. Quantitative data on morphology (growth, leaf and flower size, branching) yield and content of active materials (mucilages, flavonoids) were studied at six sowing times.

We established, that sowing time may be one basic factor in the production of the annual variety. The major yield was obtained by sowing either late autumn (end of October) or early spring (middle March). At these plots the fresh mass of the flowers was 257–288 g/plant, the drug mass 28–29 g/plant, by 45–70% more than that of the mean of other treatments.

It was established, that under optimal cultivation conditions the annual form of mullein may reach higher individual yields than the plants of the indigenous wild growing population.

Key words: *Verbascum phlomoides* L., cultivation, life cycle, morphology, production, mucilages, flavonoids

Introduction

Mullein (*Verbascum phlomoides* L.) is a biennial species belonging to the family of *Scrophulariaceae*. Under Hungarian climate it develops a leaf rosette in the first year of vegetation and after overwintering it starts to flower in June of the next year (*Boros*, 1974). It is indigenous in Asia Minor, North Africa, North America. According to experimental data on one of the related species *Verbascum thapsus* L. the life cycle depends –among others– on the latitude. At the Northern regions (South-Canada) this plant developed flowering shoots only in the third year, while in South-Texas it flowered already in the first one (*Reinartz*, 1984). Mullein is indigenous at the most part of Europe, in Hungary it grows on meadows, sunny, dry slopes, weed associations, road sides, thin oak forests. It is a pioneer species, occupying rural areas very soon (*Bencze*, 2000).

Drug of mullein, *Verbasci flos* (dried petals without calyces, but with the stamina grown on them) is an official one in several European Pharmacopoeia. Some other species such as *V. densiflorum* and *V. thapsus* belonging to the subsectio *Thapsus* of the genus give medicinal drugs, too (*Boros*, 1974). In Pharmacopoeia Hungarica (VII), *V. phlomoides* and *V. densiflorum* are the officially registrated medicinal plants. Recently, also the leaves (*Verbasci folium*) containing mainly saponins, are requested at the market (*Bencze*, 2000).

Main active components of the flowers are mucilages (polysaccharides) accumulating at about 3 per cent or even up to 8 per cent (*Petri*, 1991), flavonoids and saponins (*Bencze*, 2000). The drug contains flavonoids at about 3.8% such as hesperidin and rutin (*Petri*, 1991). *Vogl* (1909) was the first isolating hesperidin, one of the compounds giving the colour of the petals. Afterwards *Hein* (1959) proved the presence of flavonglycosids hesperidin and verbascoside. *Tóth* (1976) described luteolin and apigenin in the drug of *V. phlomoides*. The drug contains saponins at 0.04 per cent (e.g. verbascogene). Crocetine, a carotinoid dyeing agent plays a role in development of the yellow colour of the petals, too. Further, the drug contains saccharids (10 per cent), bitter materials, and a few essential oil (*Petri*, 1991).

In the flower of *V. densiflorum* flavonoids at 1.5–4 per cent have been proved, such as rutin, hesperidin, apigenin-luteolin-7-O-glycosids, kaempferol (*Wagner & Bladt*, 1996).

The drug *Verbasci flos* had been applied in the folk medicine against catarrh, as expectorant and diaphoretic agent. In the up-to-date phytoterapy it is used similarly, as raw of antitussive and expectorant tea-mixtures together with marshmallow, anis or liquorice. Cosmetics, shampoo for blond hair is made from it (*Bencze*, 2000).

Today, the demand for the drug can hardly be assured by collection of wild growing populations. Copping the flowers needs extremely much time and labour. Harvesting should be made during flowering by hand, continuously, between 9 and 11 a.m. daily (*Boros*, 1974). *Szklanowszka & Denisow* (1999) observed, that opening of the flowers started between 0.30 and 4.00 in the night, the majority of flowers were open at around 5 o'clock a.m. and no more flowers opened after 9–10 a.m.

Cultivation of the biennial wild type does not seem to be economic. *Heeger* (1956) already suggested the selection of an annual variety for drug production. *Auster & Schäfer* (1958) mentioned that it may be successful by early sowing and selection of specimens showing annual behaviour. The

selection proved to be successful after several years. Selected strains of *Csáki* (1982) flowered at 63–75 per cent in the year of sowing. Development of a cultivar has been realized at the beginning of the nineties (*Szépréthy & Zámboriné*, 1995). The annual cultivar 'Napfény' has been registrated in 1997.

Flowering percentage in the first year is stable, around 100%. In case of late sowing or slow germination flowering starts later and the yield may be significantly lower. The annual variety does not need vernalisation, and there is no need for chilling the seeds before sowing. *Szépréthy & Zámboriné* (1995) established, that the biennial type of *V. phlomoides* is superior to the annual type ('Napfény') in plant height, number of shoots and flowers. The yields of dry flower drug showed also a considerable difference: 11 g/plant was measured in case of the biennial plants while it was 2.7–3.8 g/plant in the annual variety. Beside genetic characteristics, other factors, such as pruning (*Lortie & Aarssen*, 1997) or nutrient supply – especially potassium (*Bence*, 2000) – may influence the production.

The aim of the present study was the optimalization of drug production and content of active ingredient of the annual cultivar 'Napfény'. We studied the effect of sowing time as a basic factor in biomass production of mullein. Parallelly, also individuals of an indigenous population had been investigated and sampled as control.

Material and method

The experiment has been carried out in 2002–3003 in Soroksár, Budapest at the research station of the Faculty of Horticultural Sciences. Seeds of the annual variety 'Napfény' were sown at six dates:

1.: 2002. September 4.
2.: 2002. October 2.
3.: 2002. October 30.
4.: 2003. March 19.
5.: 2003. April 3.
6.: 2003. April 6.

Sowing was carried out to a row distance of 50 cm, 0.5–1.0 cm deep. The density within the row was installed to 30–40 cm by thinning the stand in the second half of May, at 4–8 leaves-stage. The plants received drop irrigation. An indigenous population of biennial, wild specimens growing at a distance of 1 km from the experimental plots were compared as control.

For the morphological investigations 25–25 plants were randomly selected both in the cultivated population (plots sown in March) and in the wild one, and measured in the second half of June – beginning August. For calculation of the yield and measuring contents of the active ingredients, the flowers were picked during full flowering, thorough one month every second day. The yield was measured in 10 repetitions, the active ingredients from representative samples in three replications. The content of mucilages was determined by the swelling value according to the PhHg VII., while the content of total flavonoids according to the DAB 10.

Evaluation of the results was carried out by one way ANOVA.

Results and discussion

Morphological variability

The stands of the Hungarian cultivar 'Napfény' sown at the beginning of March and the wild population exhibited considerable differences concerning their morphological characteristics. The height of plants and the diameter of the flowers in the annual variety exceeded those of the wild plants in the natural stand by 25%. The inflorescences were by 50% longer than that of the biennial plants.

According to the results, the cultivar 'Napfény' developed four times more branches and had three times higher flower mass/plant than measured in the natural population. The difference between the two forms of *V. phlomoides* concerning morphological traits was significant at 99% probability level (*Table 1*).

Biomass

The rate of flowering plants of cultivar 'Napfény' proved to be 100% on the plots of each sowing date. The time of the

Table 1 Morphological, productional and chemical characteristics of the annual cultivated plants (sown at middle March) and individuals of the indigenous population

2003	mean		SD		CV%		p–value
	cultivated	wild	cultivated	wild	cultivated	wild	
length of the lowest leaf (cm)	29.61	28.35	5.11	8.05	17.25	28.40	0.511884
plant height at flowering (cm)	127.09	95.55	16.51	21.89	12.99	22.91	0.000001 **
length of flowers (cm)	84.68	40.78	19.85	15.91	23.44	39.02	0.000000 **
number of branches (pcs)	6.92	1.64	2.06	2.51	29.77	153.33	0.000000 **
diameter of flowers (cm)	4.76	3.56	0.44	0.38	9.34	10.79	0.000000 **
fresh mass of flowering shoots (g/plant)	153.07	48.12	92.04	31.18	60.13	64.80	0.000002 **
dry mass of flowering shoots (g/plant)	50.82	13.20	31.43	8.58	61.86	65.00	0.000001 **
fresh flower yield (g/plant)	288.30	177.55	156.67	0.76	54.34	0.43	0.126725
dry flower yield (g/plant)	29.19	25.54	16.03	22.61	54.91	88.51	0.681834
swelling value (content of mucilages, ml)	8.00	9.16	0.00	0.76	0.00	8.33	0.057235 +
content of total flavonoids (g/100g)	0.1158	0.1068	0.0026	0.0048	2.25	4.50	0.045859 *

**: $p < 0.01$ *: $p < 0.05$ +: $p < 0.1$

Table 2 Flowering time of the cultivar 'Napfény' in the sowing time experiment

sowing time	start of flowering	full flowering	end of flowering
2002. 09. 04.	06. 04.	06. 27.–07. 02.	07. 25.
2002. 10. 02.	06. 11.	07. 02.–07. 15.	08. 05.
2002. 10. 30.	06. 27.	07. 09.–07. 20.	08. 05.
2003. 03. 19.	06. 27.	07. 09.–07. 20.	08. 05.
2003. 04. 03.	06. 27.	07. 15.–07. 25.	08. 27.
2003. 04. 16.	07. 02.	07. 25.–08. 01.	09. 13.

flowering was in connection with the sowing time, flowering started in order of sowing dates after each other. However, the full flowering period of the last autumn sowing (30. 10. 2002.) and the first spring sowing (19. 03. 2003.) could be registered at the same time (*Table 2*).

The mass of the flowers in these populations was significantly higher than that of the other plots. At these plots the fresh mass of the flowers was 257–288 g/plant, the drug mass 28–29 g/plant, by 45–70% more than that of the mean of other treatments. These results are by 40% higher than the fresh flower mass of the wild individuals (177.6g/plant) and by 12% more than the dried mass (25.54 g/plant) of those plants (*Figure 1*).

Production of active ingredients

The swelling value indicating the content of mucilages showed 8.0 ml in case of 'Napfény' while it was 9.2 ml in case of the biennial population. Our data are the first one about content of mucilages in case of the annual cultivar.

The contents of total flavonoids were 0.1158 g/100g in annual and 0.1068 g/100g in biennial plants (*Table 1*). The difference was proved at a level of 95% significance. However, comparing our results with the literature references, the measured data proved to be relatively low. Minimum value in the PhHg,VII (1986) defines 10 ml for the swelling value and at least 1% for the content of total flavonoids in *Verbasci flos*.

Conclusions

The investigations showed, that in cultivation, the flowering and yield of the annual variety 'Napfény' is highly dependent on the sowing time. The mayor biomass production and drug yield could be reached by sowing in late autumn or early spring. The similar behaviour of these two plots may be traced back to the fact, that they both germinated in early spring and developed almost parallel.

We established, that under these circumstances the annual variety may reach even higher individual yields than the wild type plants in the indigenous population. However, further economic calculations may be advisable for exact determination of profit ratio of cultivation and wild collection.

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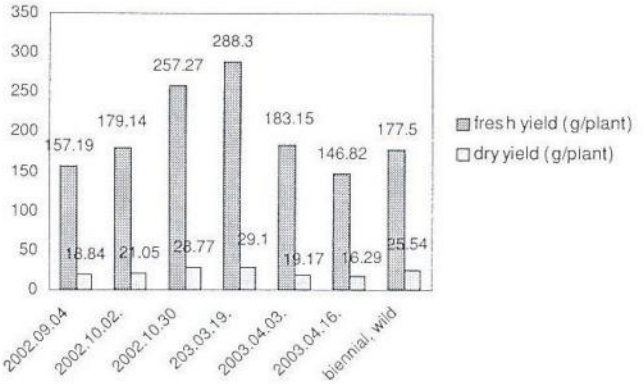

Figure 1 Flower yield of the annual variety sown at different times and that of the biennial wild type individuals

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