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**PRODUCTION BIOLOGICAL EVALUATION OF *VERBASCUM PHLOMOIDES*
L. AND *SALVIA SCLAREA* L. BIOTYPES OF DIFFERENT LIFE-CYCLE**

THESIS OF PHD DISSERTATION

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The background and the aims of the research

Cultivation of medicinal plants is increasing compared to the traditional collection from wild habitats. In Hungary, two-third of the drugs (about 25-35.000 tonnes per year) are produced in cultivation. In line with the increasing requirements for quality control and environmental protection in recent years, there has been a growing tendency in the introduction of species. Besides intensification of the cultivation technology, the profitability of production is ensured by suitable, controlled biological basic material with high yielding potential. Bearing this in mind, in several countries great efforts have been made to breed for such new varieties.

Production can be enhanced by increasing the rate of valuable plant raw used as drugs, as well as by increasing the regeneration ability and individual biomass production (ZÁMBORINÉ, 2003). There are several examples known from both the domestic and international practice of medicinal plant production proving the success of attempts in which annual (Th) types growing reproductive organs already in the first year were obtained from biennial (TH) and perennial (H) species. Such varieties and selected populations can be encountered in several plant families. Among the species belonging to the *Apiaceae* family, attempts have been made to obtain annual caraway (*Carum carvi*) in order to increase yield, maximise land use, and enhance carvone-production in all major producing countries, where the major part of production by now is reached with annual varieties (PANK and QUILTZSCH, 1996; TOXOPEUS and LUBBERTS, 1998; TRAUTWEIN, 2007). Obtaining productive genotypes with shorter lifecycle by introducing wild species into cultivation has also proved to be a task of high importance, for which the orange mullein (*Verbascum phlomoides*) with anticatarrhal properties is a good example. The profitability of clary sage (*Salvia sclarea*), which - due to its essential oil content - provides valuable material for the fragrance industry, is to be enhanced by selecting the ecotypes flowering from the first year (LAWRENCE, 1994).

However, the shorter juvenile stage and the faster development in the above-mentioned species raise difficulties, parts of which can not be foreseen, and which question the required increase itself in biomass and active ingredient production. Therefore, according to the experiences, biotypes shortening their rosette stage often produce lower yields or lower active substance content (ZÁMBORINÉ and TÉTÉNYI, 1990; SVÁBNÉ and NÉMETH, 2000). Despite the fact that such facts may doubt the success of works intended to produce and spread annual ecotypes, the afore-mentioned phenomenon has not hitherto been examined scientifically. Therefore, in the laboratory and field trials our aims were to clarify the questions as follow:

- to what extent do the annual and biennial types differ from each other regarding their physiological (germination, frost tolerance, flowering rate, vernalisation requirements), phenological (growth rate, flowering dynamics), production (yield) and phytochemical (active ingredient content) characteristics and how do these properties range;
- how do ecological factors (growing site, year) influence the production of the annual and biennial types;
- is it possible to influence the effects of ecological factors by technological practices (sowing time) optimally?

The aim of my research was to find answers to the afore-mentioned theoretical and practical questions, by using *V. phlomoides* and *S. sclarea* –two medicinal plant species belonging to different plant families and grown in Hungary– as model plants.

Material and methods

The experiments were carried out at the experimental station of the Department of Medicinal and Aromatic Plants of the Corvinus University of Budapest in Soroksár and at Teichmann Station of the Agricultural Research Centre of the University of Debrecen, in Kisvárda between 2002 and 2005.

The seeds of annual and biennial *V. phlomoides* and *S. sclarea* (Table 1) were sown in open field plots in both cultivation sites. Seeds were obtained from the selected populations of the previous year, and also from collection. Pre-sowing seed treatment (freezing) was only performed in the biennial *V. phlomoides* in order to enhance germination. For this reason, seeds were kept in plastic bags in the deep freezer for about two weeks.

Table 1: Seed parameters of tested plant species

	life form	variety/population/line	origin	germinating capacity
<i>Verbascum phlomoides</i>	annual	'Napfény' variety	variety maintenance, Soroksár	>90%
	biennial	seeds collected from wild population	Pestszentimre	60-80%
<i>Salvia sclarea</i>	annual	1 year old strain selected from 'Akali'	selection, Soroksár	>80%
	biennial	'Akali' variety	variety maintenance, Soroksár	>80%

Seeds were sown in drills in all three years of testing and at both cultivation sites, 3 times in the autumn and 3 times in spring. In case of *S. sclarea* in Soroksár in 2002-2003, seeds were only sown in March (Table 2).

Table 2: Times of sowing in situ in the years of the examinations (Soroksár and Kisvárda, 2002-2005)

	Soroksár			Kisvárda		
	2002-2003	2003-2004	2004-2005	2002-2003	2003-2004	2004-2005
1.	04.09.2002.	26.09.2003.	06.09.2004.	05.09.2002.	29.09.2003.	07.09.2004.
2.	02.10.2002.	13.10.2003.	29.09.2004.	03.10.2002.	15.10.2003.	30.09.2004.
3.	30.10.2002.	24.11.2003.	27.10.2004.	06.11.2002.	06.11.2003.	14.10.2004.
4.	19.03.2003.	18.03.2004.	19.03.2005.	26.03.2003.	22.03.2004.	29.03.2005.
5.	03.04.2003.	06.04.2004.	05.04.2005.	10.04.2003.	05.04.2004.	12.04.2005.
6.	16.04.2003.	16.04.2004.	15.04.2005.	-	19.04.2004.	-

The plot size of the plant populations was 10 m² in Soroksár, and 5 m² in Kisvárda, and plots were divided by routes of 0,5 m. Row spacing was 50 cm, and plant density (planting space of

25-30 cm) was adjusted in the second decade of May, by thinning the plants at the stage of 6 true leaves. Thus the optimal number of plants/plot was about 80 in the 10 m² plots. During the vegetation period continuous mechanical weed control was applied. The necessary water supply (depending on the weather conditions) was provided by trickle irrigation in Soroksár, and by sprinkler irrigation in Kisvárda.

In early spring, complex fertiliser (Genezis, N:P:K=15:15:15) was applied to the plots in a dosage of about 48 g/m².

Pest control was necessary in *V. phlomoides* populations, primarily against the larvae of *Nothris verbascella* (Denis & Schiffermüller, 1775) (*Gelechiidae* family), which damaged the rosette leaves in early spring. After the appearance of the larvae, a 0,2-0,3% solution of Unifosz 50EC with dichlorfos active agent was sprayed every 8-10 days, 3 times.

In both sites, a two-variable test was carried out, examining the following characteristics in each sowing time in both life forms:

- **initial growth rate:** assessment at the end of November and the beginning of May, 25 plants/plot, measured parameters: diameter of root collar (mm), length of biggest leaf blade (cm), number of leaves (nr),
- **plant density:** assessed in May, frost tolerance in plots where seeds were sown in autumn: the rate of all (overwintered) plants/plot compared to the adjusted number of plants/plot (about 80 plants/plot) in %; in plots where seeds were sown in spring: the rate of all (emerged) plants/plot compared to the adjusted number of plants/plot (about 80 plants/plot) in %,
- **flowering rate:** evaluation throughout flowering period, rate of flowering plants compared to all plants in plot in %,
- **flowering dynamics:** assessed weekly in vegetation period, rate of flowering plants compared to all plants (except in Kisvárda in the first year),
- **production:** samples from *V. phlomoides* were handpicked without calyces throughout the period of full flowering, every 2-3 days, in the morning hours. Samples were spread out in thin layers on drying frames. Due to the unavoidable flower dropping, the production of plants can only be measured indirectly. 50 of the dried corollae were weighed, from which the average weight (g) of a corolla was calculated. In autumn (September-October), after the deflorescence of the plants, the fruits on the flower stalks were counted (nr/plant, 10 plants/plot), from which the number of flowers produced on a plant during the vegetation period was estimated. Multiplying the average dry weight of corollae by the number of fruits, the drug production of *V. phlomoides* per plant (g/plant) was calculated. *S. sclarea* was harvested about 10 days after full flowering, in the morning hours, cutting the stem

under the first leaf pair below the flower stalk. The weight of fresh flowers (g/plant) was measured in 10 plants/plot. Taking freezing damage and the rate of flowering into account in each plot, the yield per plant was calculated for 1 m², thus obtaining the yield per plot unit (g/m²).

In order to examine the effects of life form, sowing time and growing site, the content of active ingredients was measured in the laboratory of the Department of Medicinal and Aromatic Plants at the Corvinus University of Budapest. In both species this was carried out by using representative samples collected from each plot, in three repetitions, according to the specifications of the VII. Hungarian Pharmacopoeia (PhHg. VII., 1986) being in force at the time of the experiments.

The **mucilage content** of the *V. phlomoides* drug was determined by measuring viscosity (ml). (The drug samples of the biennial population sown in March 2003 and those of the annual plants sown in April were damaged by *Plodia interpunctella* to such an extent that measuring could not be performed.)

In case of *S. sclarea*, fresh flowers were prepared (cut) in Soroksár for the laboratory examinations and on the same day the essential oil was distilled from the fresh samples. The extraction of essential oil from the fresh flowers collected in Kiszárda was not possible, and as a consequence the dry samples were examined. Oil extraction was obtained by Clevenger steam distillation. The **essential oil content** was calculated regarding 100g dry weight content (ml/100g).

The main **essential oil components**, such as linalool, linalyl acetate and sclareol, of *S. sclarea* were determined (% , v/v) with capillary gas chromatograph (6890N GC Agilent Technologies) equipped with flame ionisation detector (FID). The parameters were the following: HP-1 capillary column of 25 m X 0,2 mm, film layer of 0,33µm, injector (split: 50:1). Temperature programming: 50°C (0,5 min), 250°C (4°C/min), 250°C (15 min). Nitrogen was the carrier gas, and for flame ionisation a hydrogen generator and an air compressor were used. 1µl of essential oil was injected and analysed in a programme of 75 min, where the components were determined by standards, using peak detection method.

Results and conclusions

Verbascum phlomoides

On the basis of the examinations carried out in **Soroksár** for three consecutive years, it has been concluded that the annual 'Narfény' variety and the biennial ecotype differ in most characteristics tested. Regarding their physiology, only their frost tolerance is similar (in leaf rosette stage with 2-4 true leaves), whereas their germination characteristics (which influence population density) and flowering rates are different. With respect to their phenological characteristics, they showed difference in both their growth rate and flowering dynamics. It has been observed that the inflorescence of the annual 'Narfény' precedes that of the biennial ecotype by 8-10 days, even if germination times are the same. Also in production (drug yield per plant and per unit area) and phytochemical (mucilage content) characteristics (BODOR ET AL., 2006a), stable, heritable differences have been found between the two biotypes. These results reflect that the two ecotypes represent different *convarietas* of the species. Therefore it is suggested that the cultivated stable annual type should be named as *Verbascum phlomoides convarietas annua* 'Narfény'.

In the open field trials it has been verified that in the annual 'Narfény' variety, sowing time influences the growth-rate, flowering rate in plots sown in spring, flowering dynamics and drug yield per unit area. With regard to the active substance content, the drug samples originating from the populations of the annual variety reached the specified amounts. According to the results, it can be stated that the optimal sowing time for the variety is the end of October, late November (pre-winter sowing) or mid-March. Sown at these times, the population density of the annual variety is optimal, and due to its maximum (100%) flowering rate it produces the highest yield of drugs (202-263 g/m²), the quality of which complies with the specifications (viscosity: 8-11,8 ml).

The impact of sowing time on growth-rate, flowering dynamics and drug yield per unit area has also been detected in the biennial population. According to our experiences, in case of spring sowing seed germination is highly unstable (despite pre-treatment by freezing), thus it should definitely be avoided. From the viewpoint of profitability, however, autumn sowing can be favourable, as population density and flowering rate in the plots were found to be appropriate, the plants behaving like overwintering annuals. Therefore it is suggested that to describe the life form of *V. phlomoides*, the dual denomination of TH (biennial)-Th (overwintering annual) be used. According to our data, its optimal sowing times are September, early October or late November (pre-winter), after which it reaches high yields of drugs (128-228 g/m²) and quality (mucilage content: 9,4-13,3 ml) specified in the Hungarian Pharmacopoeia.

It has been proved that the vegetation year significantly influences the majority of the characteristics tested. However, there is difference between the ecotypes also in this respect: the yield of the selected annual variety is less sensitive to the effect of year (in case of the aforementioned optimal sowing times its drug production exceeded 200g/m² every year), but in its mucilage content it is less stable compared to the biennial ecotype.

The results obtained in **Kisvárda** verify that the two biotypes differ from each other in several characteristics. Differences were observed in the germination characteristics, in population density and drug yield, the latter two being influenced by the first. The annual 'Narfény' starts inflorescence earlier than the biennial type, in this growing site too.

According to the data we established that in Kisvárda sowing time influences mainly germination and phenological characteristics of the annual variety. On the basis of its production it can be stated, that in Kisvárda, apart from September, it can be successfully grown at all sowing times, with maximum (100%) population density and flowering rate, and with a more or less steady yield in drugs. These results coincide with those in Soroksár, according to which the variety is a stable annual plant. In this growing site it was proved that overwintering is not sure in all phenological phases. Moreover, the growing site was found to have an effect on the mucilage content; under the circumstances in Kisvárda, the quality of drug highly depends on the year, in most cases not reaching the specifications of the Pharmacopoeia.

In case of the biennial population, the sowing time in Kisvárda influenced its frost tolerance and flowering dynamics of the populations sown in autumn. According to our experiences, in case of spring sowing germination is highly unstable (similarly to Soroksár), and, consequently, spring sowing should be avoided. Therefore the optimal sowing time is from mid-October until November. Sown during this period, the biennial biotype in the following year produces high yield of drug (206-416 g/m²), the quality of which, however, depends on the year (mucilage content: 7-11,8 ml). The trials carried out in Kisvárda verify the observations on the life form of the biennial population in Soroksár, according to which in case of autumn sowing it behaves like an overwintering annual (Th).

It has been found that the growing site significantly influenced the majority of the characteristics tested. Out of the physiological properties, only the germination characteristics were the same in the two growing sites, and out of the phenological ones, they were similar in flowering dynamics. However, the effect of the growing site was obvious in the fact that vegetative growing in almost all plots in Kisvárda started 2-3 weeks earlier than in Soroksár. By and large, in Kisvárda yields were higher and more stable than in Soroksár. However, the active substance content of the drug samples in the first was lower than in the latter.

Based on our results, it is proved that the species reacts differently to the effects of year in the different production sites. Regarding the yield and quality of its drug, *V. phlomooides* has been found to be highly sensitive to the effects of year in the northern part of the country. In the middle of the country (in Soroksár), however, -presumably due to the more extreme weather conditions -, the year effect was lower.

It has been concluded that in several characteristics the two ecotypes reacted to the different -predominantly climatic- conditions in different ways, and, as a result, their different tolerance level to environmental factors is verified.

Salvia sclarea

Based on the trials carried out in **Soroksár** for three consecutive years, it has been concluded that the annual biotype and the biennial 'Akali' variety differ in most characteristics tested. Regarding their physiology, only their germination rate is similar, whereas their frost tolerance and flowering rate are different. With respect to their phenological characteristics (growth rate, flowering dynamics) they showed similarity, while they differed in their production and phytochemical characteristics. The clary sage population selected for first-year flowering represents a new *chemovarietas* of the species, with a chemically stable difference from the base species.

According to our results, sowing time affects the growth rate, frost tolerance, flowering rate in the plots sown in spring, flowering dynamics and fresh drug yield per unit area of the selected population. The optimal sowing times for the annual biotype are late November (pre-winter sowing) or mid-March. Sown at these times, its population density and flowering rate are at maximum (100%), and although its fresh drug yield (609-2585 g/m²) and active substance content (essential oil content: 0,26-0,41 ml/100g) highly depend on the year, they are satisfactory. Its further selection is needed. One of its advantages is that its sclareol content exceeds that of the biennial ecotype.

It has been proved that the date of sowing influences the growth rate, frost tolerance, flowering rate, flowering dynamics and fresh drug yield per unit area of 'Akali' variety. It has been known for a long time that the cultivation of the biennial type is reliable and profitable. However, in our experiments it has been found that it can also be grown as overwintering annual (Th). In case of pre-winter sowing (October or the end of November), it reaches outstanding yields (more than 2 kg/m²) already in the next summer, and its essential oil content (more than 0,5%) is similar to that of the samples obtained from the traditional two-year cultivation (BODOR ET AL., 2006b). Economical advantage of growing it as annual overwintering plant, is that it only occupies the land for one year.

The results obtained in **Kisvárd**a verify those of Soroksár. The two biotypes differ from each other in their frost tolerance and flowering rates, but their germination rates is similar. With respect to their phenological characteristics (growth rate, flowering dynamics) they are similar, whereas they differ in their yields and active substance contents, which confirms that the clary sage population selected for first-year flowering represents a new *chemovarietas* of the species.

In our field trials it has been found that sowing time affected the growth rate, frost tolerance, flowering rate and flowering dynamics of both ecotypes, but did not influence the biomass significantly. Although the effect of the sowing time on essential oil content is statistically proved, its tendency in each vegetation cycle is not consequent.

Based on our results, the optimal sowing dates for the annual ecotype is the end of October, beginning of November (pre-winter) and mid-March. Sown at these times, it reaches a population density of 70-100% and maximum flowering rate (100%). Its fresh drug yield (1960-2392 g/m²) and active substance content (essential oil content: 0,19-0,37 ml/100g) are of medium level and depend on the year.

The possible cultivation of 'Akali' variety as overwintering annual (Th) has been verified in Kisvárd

too. In case of pre-winter sowing (mid-October – late November) it produces high yields (1768-2322 g/m²) and favourable essential oil content (0,29-0,54 ml/100g) already in the next summer.

It was established that the effect of the production site significantly influenced the majority of the characteristics tested in the clary sage populations. However, it has been concluded that although in several characteristics the two ecotypes reacted to the different climatic conditions of the sites in different ways, the effect of the site had the same impact on both biotypes.

The annual and the biennial ecotypes were only similar in the two production sites in their germination and growth rate. However, the frost tolerance of the selected population as well as the population density were low in both growing sites. In comparison, the frost tolerance of the biennial type differed in the two sites: plants suffered greater frost damage in Kisvárd

a, where the climate is cooler. The flowering rate and the flowering dynamics of the two ecotypes were different in the two growing sites, and the inflorescence of both ecotypes commenced 1-2 weeks earlier in Kisvárd

a than in Soroksár. Regarding the fresh drug yield per unit area, in most cases both biotypes reached higher yields in Kisvárd

a, which is probably down to the more frequent and abundant rainfalls.

The reactions of each biotype to the effects of year were predominantly the same, with the exception of their characteristics of germination biology (germination rate) and essential oil content, where the influence of the year could not be verified. The flowering rate predominantly influences the success of cultivation, however, it was highly affected by the year at both growing sites.

Comparison of the two model species

Based on our results, both similar and different characteristics and reaction forms have been described between the two species.

They can be considered as similar in respect that their selected annual types are of stable Th form. Even these biotypes require vernalisation for the induction of flowering, but probably quantitatively less than the base species. The requirements for the induction of flowering of orange mullein and clary sage are probably quite similar. Both species are of qualitative (obligate) vernalisation requirement, with the cold effect taking place even above freezing point, with a maximum at about 15°C. In seedling stage, both biotypes of the two species react differently to the winter cold, the annual form being more sensitive. Also the two types of orange mullein and clary sage differ in their level of active compound accumulation. The phenomenon of life-form transition can be observed in the biennial types of both species, thus winter sowing can be applied, resulting in flowering of the biennial plants as early as in the next summer. The optimal dates of sowing of the two types of the two species are different. Thus, in accordance with the afore-mentioned, the differentiation of the ecotypes of the two species at *convarietas* level is well founded.

It has also been found that the germination characteristics of the two plant species examined are different. The germinating power of clary sage is greater and emergence is more uniform, while the germination of the biennial type of the orange mullein is unsteady. The frost tolerance of *V. phlomoides* is fundamentally better, since it is a native species, while *S. sclarea* is of Mediterranean origin. The *S. sclarea* ecotypes show similar growth dynamics, but the development rates of the two types of *V. phlomoides* are different. Considering production, the difference between the two biotypes of *V. phlomoides* is unstable, whereas it is quite remarkable in the case of *S. sclarea* types. The effect of year are realised for the two *Salvia* types in a similar way, while there are greater differences in the reactions of the two *Verbascum* types. While the climatic effects of the production site influence the two types of clary sage in a predominantly similar way, they affect the annual and biennial types of orange mullein differently.

New scientific results and recommendations for practice

Based on our research conducted between 2002 and 2005, the following new scientific and practically valuable results have been achieved:

- The effects of sowing date and production site on the physiological, phenological, production and phytochemical characteristics of the different life-form types of *Verbascum phlomoides* have been compared in a systematic series of experiments for the first time.
 - It has been proved that there are significant differences between the physiological characteristics of the annual and biennial types of the orange mullein. The germination of 'Napfény' variety was faster and more uniform, while that of the biennial type was extremely unsteady in case of spring sowing. There are no earlier literature data on the germination biology characteristics of the species. Considering the date of flowering it can be stated that 'Napfény' is constantly annual. Autumn sowing facilitates the first-year flowering of the biennial type, but in case of autumn sowing, the induction of flowering is uncertain. Therefore, the orange mullein is a species of qualitative (obligate) vernalisation requirement. The cold effect takes place even above freezing point, its upper limit being around 15°C. The two biotypes differ also in their phenological (growth rate, flowering dynamics) properties: the growth of 'Napfény' variety is faster and its flowering takes place earlier than that of the biennial type. The difference between the production characteristics (drug yield per unit area) of the two genotypes is unstable; it is predominantly influenced by the date of sowing and the year-effect. The difference between the phytochemical properties of the two types is proved and clear: the mucilage content of the drug of the biennial type is higher. According to our results, inheritable and stable differences can be pointed out between the two types, thus their differentiation at *convarietas* level is well founded. Therefore it is suggested that the cultivated stable annual type be defined as *Verbascum phlomoides convarietas annua* 'Napfény'.
 - The results of the field trials have proved that the date of sowing affects the growth rate, flowering dynamics and drug yield of both types of the orange mullein. The optimal sowing times for the annual 'Napfény' variety are in early winter (pre-winter sowing), or in early spring, while the biennial type produces high yields only if sown in the autumn. Since the phenomenon of life form transition in the biennial type has been proved in case of autumn sowing, besides the TH (biennial) definition of *V. phlomoides* the use of Th (overwintering annual) is recommended.

- On the basis of our results it has been concluded that both ecotypes were affected by the production site. Out of the physiological characteristics, only their rate of germination, while among the phenological properties, only their flowering dynamics were identical at the two production sites. The effect of the production site only manifested in that the generative phenological phase commenced 2-3 weeks earlier in Kisvárdá than in Soroksár, in almost every case. Differences in the important production parameters (drug yield, active compound content) were also recorded. Yields in Kisvárdá were greater and more uniform than in Soroksár. However, the active compound content of the drug samples from Kisvárdá was lower than that of the Soroksár samples. It proved to be a site-specific characteristic that the frost tolerance of the annual type was lower in Kisvárdá: in case of September sowing the plants perish from frost.

- The effects of sowing date and production site on the physiological, phenological, production and phytochemical characteristics of the annual and biennial life-form of *Salvia sclarea* have been compared also for the first time.
 - It has been proved that there is a difference between the frost tolerance and the first-year flowering rate of the annual and biennial types of clary sage. Among their physiological properties only their germination characteristics are identical. The frost tolerance of the biennial type is definitely better than that of the type selected for annual life form. However, the proportion of plants developing inflorescence in the annual type is higher than that of the biennial type. The clary sage requires vernalisation, but this does not necessarily mean freezing; though a temperature around 15°C is the upper limit of the induction range. The phenological properties of the two types (growth rate, flowering dynamics) can be considered the same, but they differ in their production and phytochemical characteristics. In case of autumn sowings, the fresh yields of the biennial type were higher, and its essential oil contents were significantly higher in all sowing times. The clary sage population selected for first-year flowering represents a new *chemovarietas* of the species.
 - In our field trials it has been proved that the date of sowing affects the growth rate, frost tolerance, flowering rate, flowering dynamics and drug yield per unit area of both ecotypes of the clary sage. There was a difference between the two types in their optimal sowing dates: the highest yield can be obtained by pre-winter or early spring sowing in the annual type, and by autumn (especially pre-winter) sowing in the biennial type. The phenomenon of life form change has been proved in the biennial type in case of autumn sowing. Thus, besides the TH (biennial) definition of *S. sclarea* the use of Th (overwintering annual) is recommended.

- Based on our results, we proved the definitive effect of production site on the plant characteristics. Only the germination characteristics and the growth rate were the same at the two production sites.
- By the scientific description of the life form types of the orange mullein and the clary sage, our results provide a good basis for the improvement of frost tolerance, flowering time, active substance content, as well as for the establishment of new genotypes and candidate varieties by plant breeding.
- For the practice, our data serve as a basis for the improvement of the production potentials of these plant species by agrotechnical methods. In our experiments, the optimal sowing dates of *Verbascum phlomoides* and *Salvia sclarea* have been determined at both production sites for commercial production.
 - The optimal sowing dates for 'Napfény' variety in Soroksár are the end of October and late November (pre-winter sowing), or mid-March. Sown at these times, the vegetation density and the flowering rate of the variety is at its maximum, producing the highest drug yield and the quality of the drug fulfils the requirements of the Pharmacopoeia. With the exception of September, the variety can be successfully grown at any sowing dates in Kisvárda; 100% vegetation density and flowering can be obtained, and its drug yield is fairly uniform. The disadvantage of the production site in Kisvárda is that the quality of the drug is heavily affected by the year, and in most cases it does not reach the required level of active compound content.
 - According to our results, autumn sowing is favourable at both sites, since the vegetation density and the flowering rate proved to be appropriate. Thus, the optimal sowing dates are early October or late November (pre-winter sowing), in order to obtain good quality and ample drug yield.
 - At both production sites, the optimal sowing times of the annual clary sage are late October and late November (pre-winter sowing) or mid-March. Sown at these dates, the vegetation density and the flowering rate of the selected population are at maximum. Although the fresh yield and active compound content are greatly affected by the year, they are satisfactory.
 - The 'Akali' variety can also be grown as an overwintering annual type. In case of pre-winter sowing (mid-October – late November), it produces high yields and essential oil content in the next summer.
- Based on the results of our experiments, it can be stated that the appearance of the different life forms of the two species belonging to distinct families shows species-specific characteristics, and thus, most of the conclusions can not be generalized.

Publications in the topic of the thesis

Journal publications (peer reviewed):

1. **Bodor Zs.**, Németh É. (2004): Effect of life cycle on the production of mullein (*Verbascum phlomoides* L.). International Journal of Horticultural Science, 10(1): 123-125.
2. **Bodor Zs.**, Németh É., Csalló K. (2006): Produktionspotential ein- und zweijähriger Formen des Muskatellersalbeis (*Salvia sclarea* L.) und Einfluss unterschiedlicher Aussaatzeiten. Zeitschrift für Arznei- und Gewürzpflanzen, 11(1): 40-47.
3. **Bodor Zs.**, Németh É., Csalló K., Rajhárt P. (2006): Az életforma, a vetésidő és a termőhely produktiót befolyásoló hatása a szöszös ökörfarkkóró esetében. Kertgazdaság, 38(4): 79-87.

Conference publications (Hungarian, abstract):

1. **Bodor Zs.**, Zámboriné Németh É. (2003): Életforma hatása a *Salvia sclarea* L. produktiójára. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak. Gyógynövénytudományi Szekció. Budapest, 2003. 11. 06-07. Összefoglalók p. 272.
2. **Bodor Zs.**, Zámboriné Németh É., Csalló K. (2005): A vetésidő és a termőhely hatása a szöszös ökörfarkkóró (*Verbascum phlomoides* L.) egy- és kétéves ökotípusának produktiójára. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak. Gyógynövénytudományi Szekció. Budapest, 2005. okt. 19-21. Összefoglalók p. 122.
3. **Bodor Zs.**, Zámboriné Németh É., Csalló K., Fazekas K. (2007): Vetésidő és termőhely hatása a *Verbascum phlomoides* 'Napfény' fajta produktiójára. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak. Gyógynövénytudományi Szekció. Budapest, 2007. nov. 7-8. Összefoglalók p. 122-123.

International conference publications (full paper):

1. **Bodor Zs.**, Németh É. (2004): Optimalisierung der Production in *Salvia sclarea* L. – Populationen von verschiedener Lebensform. Fachtagung für Arznei- und Gewürzpflanzen. Jena, 07-09. 09. 2004 Tagungsband p. 147-150.
2. Mihalik E., Lehoczki E., **Bodor Zs.**, Z. Németh É. (2005): Photosynthetic and morphological characters of leaves of the annual and biennial *Salvia sclarea* biotypes. 8th Hungarian Congress on Plant Physiology. Szeged, 22-25. 08. 2005 Proceedings p. 161-163.

International conference publications (abstract):

1. **Bodor Zs.**, Németh É., Gimpl A. (2004): Optimalization of the production of *Salvia slarea* L. populations of different life cycle. International Conference on Horticulture Post-graduate (PhD.) Study System and Conditions in Europe. Lednice, 17-19. 11. 2004 Proceedings of Abstracts p. 11.
2. **Bodor Zs.**, Németh É., Kozak A. (2005): Optimierung der Produktion in *Verbascum phlomoides* L. Populationen von verschiedener Lebensform. 15. Bernburger Winterseminar zu Fragen der Arznei- und Gewürzpflanzenproduktion. Bernburg, 22-23. 02. 2005 Kurzfassung der Referate und Poster Teilnehmerliste. p. 33.
3. **Bodor Zs.**, Alberti Á., Zsarnóczai J., Kéry Á., Németh É. (2007): Evaluation of phytochemical markers characterising cultivated and wild mullein flowers (*Verbascum phlomoides* L.). 55th International Congress and Annual Meeting of the Society for Medicinal Plant Research, Graz, 2-6. 09. 2007 Abstracts p. 919.
4. **Bodor Zs.**, Németh É., Csalló K., Gosztola B. (2008): Auswirkung unterschiedlicher Standorte und Aussaatzeiten auf die Produktion der Königskerzensorte 'Napfény'. 18. Bernburger Winterseminar und 5. Fachtagung Arznei- und Gewürzpflanzen, Bernburg, 18-21. 02. 2008 In Press.