ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LVIII 7 Number 2, 2010

Salvia verticillata L. IN THE CZECH REPUBLIC – VARIABILITY OF MORPHOLOGICAL CHARACTERISTICS, SEED QUALITY AND ESSENTIAL OIL CONTENT

K. Dušek, E. Dušková, K. Smékalová

Received: October 29, 2009

Abstract

DUŠEK, K., DUŠKOVÁ, E., SMÉKALOVÁ, K.: *Salvia verticillata L. in the Czech Republic – variability of morphological characteristics, seed quality and essential oil content.* Acta univ. agric. et silvic. Mendel. Brun., 2010, LVIII, No. 2, pp. 61–68

Lilac sage (Whorled sage), as one of the medicinal plants chosen as perspective for the recultivation of flowering meadows in the Czech Republic, was studied for the variability of its morphological characters, seed quality and content of essential oil. Seven particular populations of this genus were studied in the Czech Republic and there were found statistically significant differences in morphological characters (height and width of plants, length and width of leaves and length of inflorescences) but not in the content of the essential oil. Studied populations reached only between 0.028 and 0.072% of essential oil in dry mass and also the quality of seeds was found very low (germination between 0–52%) in the seeds from natural localities but this fact could be influenced by testing method.

Lilac sage, Whorled clary, content of essential oil, germination, variability, biodiversity

Medicinal plants are important components of flowering meadows. They help to create the character of a landscape, make an aesthetic impression and they are also important nutrition components for pasture animals. For the recultivation of natural flowering meadows, it is necessary to use complete mixtures of grass and dicotyledonous plants. Of course there are some specific requests for the species used: they should be relatively easily reproducible (low cost seeds), not too aggressive (oppressing other species), they should have a good germination and/or emergence rate, they should not be toxic for animals and they should be reasonable to maintain (not too exacting in their care). Together with these necessary requirements, a flowering meadow that has been successfully revitalised should preclude a risk of decreasing the natural biodiversity of such meadow phytocenoses. Therefore especially autochthonous (it means material originating on site) plants should be used (ŠRÁMEK *et al.*, 2001) but usually it is difficult to manage or it is very expensive. On the other hand only detail knowledge of variability and biodiversity of plants native in large area can enable its using in allochthonous localities.

Salvia verticillata (Lilac sage, Whorled clary) was chosen by the employers of Czech protected landscape areas as one of the perspective medicinal plants for the recultivation of natural meadows. The evaluation of plants from different population and study of the morphological characters variability, pharmaceutically active substances content and the seed quality was the main goal of the project. The selection of the genotypes perspective usable in the pharmaceutical industry was the accessory goal.

Salvia verticillata L. is a perennial growing in grasslands, on sunny slopes, in fallow areas and railway stations in south and south-east Europe, Caucasus, west Siberia, north Iran, Syria and Asia Minor but it is also naturalized in Central Europe. It has stems up to 80 cm high, erect and often hairy. Leaves are simple or lyrate, petiolate, ovate-triangular. Verticillasters are with 8–30 flowers, calyx is with glandular hairs and corolla has a lilac-blue colour (TUTIN *et al.*, 1972; ŠTĚPÁNKOVÁ, 2000; HANELT *et al.*,

2001). The drug contains especially essential oils, flavonoids and tannins but it is not used in official medicine (it is not mentioned in the Czech pharmacopeia) or in traditional medicine. Nevertheless, a pharmaceutical use of the flowering tops is possible and its medicinal effect is expected to be similar to *Salvia officinalis* L. – to treat the symptoms of gastrointestinal disturbance, in mouthwashes for oral hygiene (ULUBELEN, TOPCU; 1989; BRUNETON, 1995; KRSTIC *et al*., 2007). It is richly melliferous (PURGAR, HULINA; 2007) and it is one of the perspective species for the founding of flowering meadows (BĄBA, 2004).

MATERIALS AND METHODS

Plant populations from 7 natural sites in 4 protected landscape areas (PLA) in the Czech Republic (Tab. I) were chosen for experiments and collecting missions of the plant material were realised in 2004 and 2005. Mother plants (at least 10 plants per locality) were used for the founding of a field nursery in Olomouc in 2004. All plants were organized to 50 m long double-rows with 2 m spacing and 40 cm between the plants. The plants were grown under the same field and climatic conditions in the field nursery and nursed (watering, weeding, harvesting etc.) in the same way.

Also the seed samples were collected in original localities in the periods 10.–23. 10. 2004 and 30. 9.–27. 10. 2005 but with the view of keeping the original population undamaged and guarantee the natural plant recovery, only small seed samples could be collected. Collected seeds were dried in the chamber with controlled air circulation, crumbled and cleaned by hand and until the germination tests it were kept in room temperature condition.

Variability of morphological characters was evaluated according to the Minimal set of descriptors (internal document of CRI) and plant height and width, length and width of leaves and length of inflorescences were measured in the field nursery in 2006 and 2007. Each character was evaluated in all surviving individual plants separately and leaves characters were measured in 10 leaves per plant.

The seed quality was evaluated by germination tests in Jacobsen's germinator. The germination condition (filter paper damped by distil water, 25 °C, 8 h light / 16 h dark) based on method for *Salvia officinalis* (ČSN 46 0610). No any disinfection of seeds was realised and due to limited amount of available seeds only 1 × 50 seeds were tested for each sample. Laboratory germination tests were started 4. 2. 2005 and 12. 5. 2006 and maintained for 22 (2005), respectively 26 (2006) days. During the germination experiments so called maximal germination was determined – it means the „maximal germination" capacity reached by individual sample in every experimental year (BLÁHA, POKOVÁ; 2009).

The content of essential oil was analysed in a mixed sample from each group of plants and each group was analysed separately. All the plant samples from the field nursery were prepared in the same way: the stems, in full flowering stage, were harvested by hand, dried in a temperature lower than 35 °C (drying house with controlled air circulation) and ground by a laboratory homogenizer. Each sample representing the locality was a mixture of all the plants of the group. The content of essential oil was then measured gravimetrically by hydrodistillation (Composite authors 1987). Two repetitions of measurement were used in each sample.

Statistical evaluation of the obtained data was done by ANOVA, one-factor analysis of variance with level of importance $\alpha = 0.05$, resp. 0.01.

RESULTS

Variability of morphological characters in *Salvia verticillata* plants grown in the field nursery in Olomouc is presented in Tab. II. Only 22 plants survived to 2007 from 70 plants which were introduced from

I: *Origin of evaluated plants*

Protected landscape area	Locality
BK – Bílé Karpaty Mts	Suchovské mlýny, Březová, Brumov-Bylnice
ČS – České středohoří Mts	Radobýl
JH – Jizerské hory Mts	Rydvaltice
MK – Moravský kras	Březina lom, Macošská stráň

II: *Variability of morphological characters*

Characteristics	Evaluation in 2006 and 2007	
No. of plants	22	
	Min–Max	Average ± Standard deviation
Height of plants (cm)	31–114	83.6 ± 17.2
Width of plants (cm)	40–186	122.7 ± 32.9
Leaf length (cm)	8–33.5	19.6 ± 4.3
Leaf width (cm)	4–18	9.2 ± 2.0
Length of inflorescences (cm)	16–81	41.9 ± 12.4

original localities. Field cultivation deleted differences between the natural conditions of the original sites, but nevertheless, statistically highly significant differences in both evaluated years were found between samples from individual localities (Tab. III–V) as well as between samples from particular protected landscape areas (Tab. VI–VIII).

III: *Statistical differences in height and width of plants between plants from original localities*

PLANTS – height 2006/2007 //width 2006/2007		(2)	(3)	(4)	(5)	(6)	(7) BK Brumov-Bylnice
ČS Radobýl	(1)	n	n	n	n	n	n
JH Rydvaltice	(2)	n	-/+//+/+	-/++//-/++	-/-//-/+	-/-//-/-	-/+//-/-
MK Macošská stráň	(3)	n		-/-//-/-	+/-//++/-	++/+//++/-	+/++//++/-
MK Březina lom	(4)	n			+/+//+/++	++/++//++/++	+/++//++/++
BK Suchovské mlýny	(5)	n				-/+//+/-	-/+//++/-
BK Březová	(6)	n					-/-//-/-

n = not evaluated; - = no statistical differences; + = statistically significant differences (α = 0.05); ++ = statistically high significant differences (α = 0.01)

IV: *Statistical differences in leaf length and width between plants from original localities*

LEAVES – length 2006/2007 //width 2006/2007		(2)	(3)	(4)	(5)	(6)	(7) BK Brumov-Bylnice
ČS Radobýl	(1)	n	n	n	n	n	n
JH Rydvaltice	(2)	n	++/++//++/++	++/++//++/++	++/-//-/++	++/-//-/++	++/++//++/++
MK Macošská stráň	(3)	n		++/++//++/+	++/-//++/-	++/++//++/-	++/-//++/-
MK Březina lom	(4)	n			-/++//++/++	-/++//++/-	-/++//++/++
BK Suchovské mlýny	(5)	n				-/-//-/-	-/-//+/-
BK Březová	(6)	n					-/++//-/+

n = not evaluated; - = no statistical differences; + = statistically significant differences (α = 0.05); ++ = statistically high significant differences (α = 0.01)

V: *Statistical differences in length of inflorescences between plants from original localities*

INFLORESCENCES – length 2006/2007		(2)	(3)	(4)	(5)	(6)	(7) BK Brumov-Bylnice
ČS Radobýl	(1)	n	n	n	n	n	n
JH Rydvaltice	(2)	n	++/++	++/++	++/++	++/-	-/-
MK Macošská stráň	(3)	n		n/++	n/-	n/++	n/++
MK Březina lom	(4)	n			++/++	++/++	++/++
BK Suchovské mlýny	(5)	n				-/++	++/++
BK Březová	(6)	n					++/-

n = not evaluated; - = no statistical differences; + = statistically significant differences (α = 0.05); ++ = statistically high significant differences (α = 0.01)

VI: *Statistical differences in height and width of plants between plants from different protected landscape areas*

PLANTS – height 2006/2007//width 2006/2007	Moravský kras	Bílé Karpaty Mts
Jizerské hory Mts	++/++//++/++	-/-//-/-
Moravský kras		++/++//++/++

- = no statistical differences; + = statistically significant differences (α = 0.05); ++ = statistically high significant differences (α = 0.01)

VII: *Statistical differences in leaf length and width between plants from different protected landscape areas*

LEAVES – length 2006/2007//width 2006/2007	Moravský kras	Bílé Karpaty Mts
Jizerské hory Mts	++/-//++/++	++/+//-/++
Moravský kras		-/+//++/+

- = no statistical differences; + = statistically significant differences (α = 0.05); ++ = statistically high significant differences (α = 0.01)

VIII: *Statistical differences in length of inflorescences between plants from different protected landscape areas*

INFLORESCENCES – length 2006/2007	Moravský kras	Bílé Karpaty Mts
Jizerské hory Mts	++/++	++/-
Moravský kras		++/++

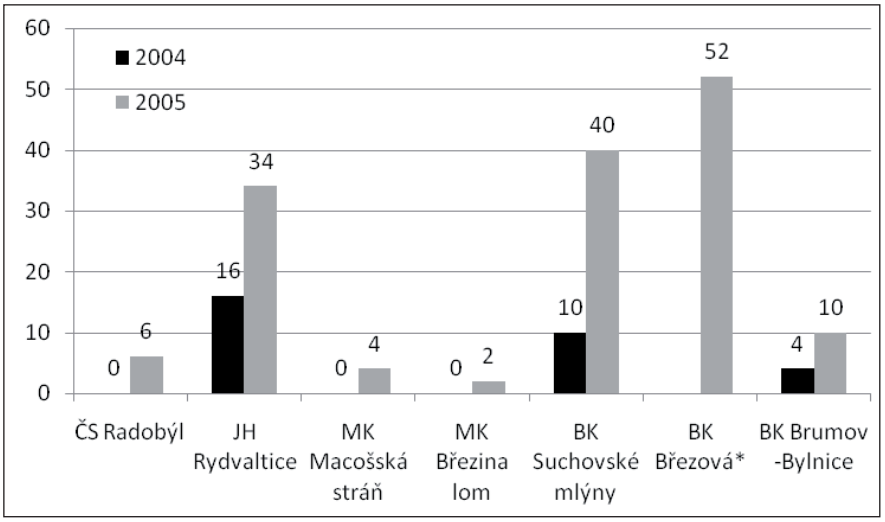
- = no statistical differences; + = statistically significant differences (α = 0.05); ++ = statistically high significant differences (α = 0.01)

Testing of seed quality did not bring satisfactory results. Especially in 2004 the results were very bad – three of six tested seed samples did not germinate at all and other three achieved only 4–16% germinable seeds (Fig. 1). In 2005, when at least one seed had germinated in each sample, the seeds from locality Bílé Karpaty Mts. – Březová achieve the germination 52% which was the best obtained score at all. The most intensive grow of seedlings was found in 5th–7th day of test.

The content of essential oil was found only between 0.028 and 0.072% of dry mass (0.063% in average) in our plants. All these results (Tab. IX) are so equal that no statistically significant differences were proved between samples from individual localities in the Czech Republic (significance level α = 0.05: F critical value = 2.70; F value = 0.97) and also no statistically significant differences were found between results in years 2006 and 2007 (significance level α = 0.05: F critical value = 4.30; F value = 1.79) in our experiment.

DISCUSSION

According to the literature, the height of stems should be up to 80 cm (TUTIN *et al.*, 1972); another source shows height as (20–)30–90(–100) cm (ŠTĚPÁNKOVÁ, 2000). Our plants, in field conditions, nevertheless got plant heights between 31–114 cm which makes good the hypothesis that field conditions support the spreading of plants. Leaf length in botanical literature is described as (4–)6–9(–12) cm long leaf blade and (3–)6–10(–15) cm long leaf petiole (ŠTĚPÁNKOVÁ, 2000) and these data are in



1: *Germination of Salvia verticillata seeds (%)*
* seeds from locality BK Březová were not tested in 2004

IX: *Content of essential oils*

Locality	Average (%)*	Standard deviation
BK – Suchovské mlýny	0.050	0.022
BK – Březová	0.072	0.074
BK – Brumov-Bylnice	0.068	0.024
ČS – Radobýl	0.028	0.000
JH – Rydvaltice	0.069	0.027
MK – Březina lom	0.048	0.006
MK – Macošská stráň	0.071	0.063
Total	0.063	0.043

* two repetitions of measurement of mixed sample from each group of plants in the years 2004, 2006 and 2007

accordance with our measurements where leaves were 8–33.5 cm long. Big differences were however found in leaf width. According to ŠTĚPÁNKOVÁ (2000) the leaf blade is (3–)5–7(–9) cm wide but our plants in the field had significantly wider leaves – up to 18 cm. Big differences in the length of inflorescences (16–81 cm), which were always measured as a length of primary inflorescence from first branching to the top, are probably connected with common growing potency of studied plants. Unfortunately, it was not possible to compare this characteristic with literature data.

Our laboratory results of seed germination, which were set between 0–52% depending on locality of origin and harvesting year, are not very satisfactory with a view to using for flowering meadows recultivation. On the other hand there are some possible explanations for such low score. First of all it is necessary to stress the wild character of *Salvia verticillata* species. It was frequently proved that wild forms of cultivated crops as well as wild species in itself have a lower germination capacity compare to crop plants because the genetically controlled dormancy of seeds (HITCHMOUGH *et al*., 2000, HOROVITZ *et al*., 1975, LADIZINSKY, 1998). From this point of view it is not surprising that *Salvia pratensis* as a comparable species (no any information about germination of *Salvia verticillata* was found in literature) show maximal germination only 3% (MAGYAR *et al*., 2004), 18% (BLÁHA, POKOVÁ; 2009) or 37% (STAMPFLI, ZEITER; 1999) even in using of different seed pre-treatments and/or germination conditions. The best germination ability of *Salvia pratensis* – 77% – was gained on non-stratified seeds in darkness and temperature fluctuation 15/22 °C (ZHANG *et al*., 2000). Our results of course could be influenced by only small number of seeds in the samples and better results should be expected also with stratification treatment (3–7 days are seeds placed on wet filter paper in the temperature 6–8 °C and darkness) which is recommended in the newest ISTA handbook (DUCOURNAU *et al*., 2008). The study of *Salvia verticillata* germination ability is planned for following experiments with the plants coming from original localities.

KRSTIC *et al.* (2006) present that the essential oil content ranged from 0.40% to 0.42% of dry mass in *Salvia verticillata* in Serbia. Our plants, however, reached only between 0.028 and 0.072% of dry mass which is extremely low compared to samples from Serbia. Of course this situation is strongly influenced by different climatic and soil conditions in both countries but the differences are too large for such an explanation on its own and we have to reason also about extremely different genotypes. The content of essential oil is quite low compared to other sage species but its composition could be interesting for example for the production of herbal pesticides (PAVELA, NEUGEBAUEROVÁ; 2008).

SUMMARY

Salvia verticillata L. is a surprisingly poorly studied species in the Czech Republic as well as abroad – only a few publications can be found about its variability. Thanks to its positive properties it is recommended as a perspective genus for the recultivation of regional flowering meadows and further study of this genus is advisable.

The field nursery was found in Olomouc in 2004 using the plants removed from 7 original localities in four protected landscape areas of the Czech Republic. Selected morphological characters and its variability were studied at these plants in 2006 and 2007. Also the content of essential oils was simultaneously determined in the dry flowering tops and germination of the seeds obtained at the original localities in 2004 and 2005.

The evaluation of morphological characters proved, that the plants in the field nursery are 31–114 cm high and 40–186 cm width, leaves are 8–33.5 cm long and 4–18 cm width and terminal inflorescences 16–81 cm long. Even two years of field cultivation in the identical conditions did not overturn plant natural background and there were found statistically significant differences between plants from different original localities as well as from individual protected landscape areas in all evaluated characters.

Laboratory tests proved the germination of seeds from original localities only 0–52%. These results significantly differ with a view to individual seed origin as well as to seed harvesting year. With regard to capital intensity such a result seems to be not very satisfactory but of course this is just a preliminary result and quality of *Salvia verticillata* seeds will be henceforth studied. The positive influence of previous stratification or other pre-seeding treatment to the seed germination can be expected.

Czech origin populations contain between 0.028–0.072% of essential oil in dry flowering tops. In comparison with other *Salvia* species this content is very low and possible pharmaceutical utilization is possible to recommend only in the view to high efficiency of some essential oil compounds.

SOUHRN

Salvia verticillata L. v České republice – variabilita morfologických znaků, kvality osiva a obsahu silice

Šalvěj přeslenitá (*Salvia verticillata* L.) je jak v české, tak zahraniční odborné literatuře překvapivě málo studovaným druhem – její variabilitě je věnováno pouze velmi málo publikací. Díky svým příznivým vlastnostem je ale doporučována jako vhodný komponent travních směsí pro zakládání a obnovu květnatých luk, a proto je její bližší studium žádoucí.

V roce 2004 byla v Olomouci z rostlin přenesených ze sedmi lokalit ve čtyřech CHKO ČR založena polní školka a v letech 2006 a 2007 byly u těchto rostlin hodnoceny vybrané morfologické znaky a jejich variabilita. Současně byl zjišťován obsah silice v sušené kvetoucí nati a klíčivost semen přenesených z původních lokalit v letech 2004 a 2005.

Při hodnocení morfologických znaků bylo zjištěno, že rostliny v polní školce dosahovaly výšky 31–114 cm a šířky 40–186 cm, u listů byla naměřena délka 8–33,5 cm a šířka 4–18 cm a délka terminálních květenství se pohybovala mezi 16 a 81 cm. I přes dvouleté pěstování v identických podmínkách polní školky byly u všech morfologických znaků zjištěny statisticky průkazné rozdíly mezi rostlinami z jednotlivých původních lokalit i CHKO.

Laboratorní testy prokázaly u semen z původních lokalit klíčivost pouze 0–52 %. Výsledky jednotlivých vzorků se významně liší jak s ohledem na odlišný původ osiva, tak vzhledem k roku sklizně semen. S ohledem na ekonomickou náročnost výroby osiva se takový výsledek jeví jako nepříliš uspokojivý, ale jedná se pouze o předběžný test a kvalita osiva šalvěje přeslenité bude dále studována. Lze předpokládat, že předchozí stratifikace nebo jiný způsob ošetření osiva před výsevem současné výsledky pozitivně ovlivní.

Obsah silice byl u sušené kvetoucí natě šalvěje přeslenité zjištěn v rozmezí 0,028 a 0,072 %. Ve srovnání s ostatními druhy šalvěje je tento obsah velmi nízký a případné farmaceutické využití lze doporučit pouze za předpokladu vysoké účinnosti některých siličnatých složek.

šalvěj přeslenitá, obsah silice, klíčivost, variabilita, biodiverzita

Acknowledgements

The financial support of Research Goal MZe 0002700602 is gratefully acknowledged.

REFERENCES

BĄBA, W., 2004: The species composition and dynamics in well-preserved and restored calcareous xerothermic grasslands (South Poland). Biologia, Bratislava, 59, 4: 447–456.

BLÁHA, L. and POKOVÁ, H., 2009: Test umožňující paralelní rychlé hodnocení klíčivosti druhů s odlišným stupněm dormance. In: Osivo a sadba. IX. odborný vědecký seminář, 10. 2. 2009. p. 153–157.

BRUNETON, J., 1995: Pharmacognosy, Phytochemistry, Medicinal plants. Vol. 2., Intercept Ltd., Hampshire, 540–544.

COMPOSITE AUTHORS, 1987: Československý lékopis 4 (1. sv.) (Pharmacopoeia Bohemoslovenica 4 (Vol. 1)). Avicenum, Prague.

ČSN 46 0610, 1984: Zkoušení osiva. Vydavatelství ÚNM, Praha.

HANELT, P. *et al.* (eds.), 2001: Mansfeld's Encyclopaedia of Agricultural and Horticultural Crops. Vol. 5., 1st Ed., Springer, Berlin, Heidelberg, New York, Barcelona, Hong Kong, London, Milan, Paris, Singapore, Tokyo, 2018–2027.

HITCHMOUGH, J. D., GOUGH, J. and CORR, B., 2000: Germination and dormancy in a wild collected genotype of *Trollius europaeus*. Seed science and technology, 28, 3: 549–558.

HOROVITZ, A., BULLOWA, S. and NEGBI, M., 1975: Germination characters in wild and cultivated *Anemone coronaria* L. Euphytica 24: 213–220.

DUCOURNAU, S., DAVIDSON, S., REMEEUS, P., ZECCHINELLI, R. and IVANOVICS, G., 2008: Working sheet on Salvia. In: Ripka Z. (2008): ISTA Handbook on Flower Seed Testing, 1st Edition, Bassersdorf, Switzerland. ISBN 978-3-906549-45-3.

KRSTIC, L., MALENCIC, D. and ANACKOV, G., 2006: Structural investigations of trichomes and essential oil composition of *Salvia verticillata*. Botanica Helvetica, 116, 2: 159–168.

LADIZINSKY, G., 1998: Plant Evolution under Domestication. Kluwer Academic Publishers, Dordrecht, The Netherlands. ISBN 0-412-822105.

MAGYAR, E. I., OPITZ VON BOBERFELD, W., SZEMÁN, L. and LASER, H., 2004: Keimungsvergalten von Kräutersaatgut unter variierten Umweltbedingungen. Die Bodenkultur, 55, 1: 13–19.

PAVELA, R. and NEUGEBAUEROVÁ, J., 2008: Screening of insecticidal activity of some *Salvia* species on *Spodoptera littoralis* Boisduval larvae. In: RŮŽIČKOVÁ, G. (ed.): Proc. 5th Conf. Medicinal and Aromatic Plants of Southeast European Countries. Mendel University of Agriculture and Forestry, Brno.

PURGAR, D. D., HULINA, N., 2007: The honey plants of Plešivica hills (NW Croatia). Agronomski glasnik, 61, 1: 3–22.

STAMPFLI, A. and ZEITER, M., 1999: Plant species decline due to abandonment of meadows cannot easily be reversed by mowing. A case study from the southern Alps. Journal of Vegetation Science, 10: 151–164.

ŠRÁMEK, P. *et al.* 2001: Zvyšování biodiverzity travních porostů. Zemědělské informace č. 21/2001, ÚZPI, Praha. 34 s. ISBN 80-7271-091-5.

ŠTĚPÁNKOVÁ, J., 2000: *Salvia* L. – šalvěj (*Salvia* L. – sage). In: SLAVÍK, B. (ed.): Květena České republiky 6 (Flora of the Czech Republic 6), Academia, Prague, 694–707.

TUTIN, T.G. *et al.* (eds.), 1972: *Salvia* L. In: Flora Europaea, Vol. 3. Cambridge University Press, Cambridge, New York, Port Chester, Melbourne, Sydney, 188–192.

ULUBELEN, A., TOPCU, G., 1989: Flavonoids and terpenoids from *Salvia verticillata* and *Salvia pinnata*. Journal of Natural Products, 47: 1068.

ZHANG, R., HOFMANN, M. and ISSELSTEIN, J., 2000: The effects of seed stratification and sward disturbance on germination and seedling emergence of grassland species. 44. Jahrestagung der AGGF in Kiel, Wissenschaftlicher Fachverlag Gießen, 2, 157–160.

Address

Ing. Karel Dušek, CSc., Ing. Elena Dušková, Ing. Kateřina Smékalová, Ph.D., Výzkumný ústav rostlinné výroby, v.v.i., Oddělení zelenin a speciálních plodin Olomouc, Šlechtitelů 11, 783 71 Olomouc-Holice, Česká republika, e-mail: Dusek@genobanka.cz