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# Field woody indicators for determination of *Plum pox virus* in the Czech Republic

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**ABSTRACT:** Suitability and reliability of PPV woody indicators *Prunus tomentosa* Thunb., *Prunus domestica* L. cv. Čačanská rodná and *Prunus cerasifera* Ehrh. GF 31 were verified under the field conditions of the Czech Republic on the 38 cultivars and hybrids of stone-fruits of the genus *Prunus*. *P. tomentosa* was found to be less suitable PPV indicator for its worse affinity to apricots. Despite the leaf symptoms in the indicator *P. domestica* cv. Čačanská rodná develop somewhat later compared with *P. tomentosa* its affinity to prune and apricot cultivars and hybrids is very good. The indicator *P. cerasifera* Ehrh. GF 31 myrobalan reacts more quickly but intensity of symptoms is sometimes lower. We recommend the myrobalan GF 31 as the main woody indicator of PPV under field conditions. The second suitable one is the plum cv. Čačanská rodná. Combination of these two indicators is recommendable.

**Keywords:** *Plum pox virus*; woody indicators; field conditions; *Prunus tomentosa*; *P. domestica* cv. Čačanská rodná; *P. cerasifera* GF 31

In connection with identification and reliable determination of *Plum pox virus* (PPV), family *Potyviriidae*, in the past search for suitable woody indicators was carried out. First plum of Yugoslav provenance, *Prunus domestica* L. cv. Požegača which exhibited severe symptoms in leaves and fruits was used as the indicator (JOSIFOVIĆ 1956). Internationally it was admitted as woody indicator later (POSNETTE et al. 1961). At that time *Prunus tomentosa* was recommended as better indicator by JORDOVIĆ (1961) and in Hungary peach cv. Elberta started to be used (NÉMETH 1963). In the middle of seventies (POSNETTE et al. 1976) peach seedling GF 305 was included into the list of internationally approved PPV woody indicators and in the case of *Prunus tomentosa* two its clones – IR 473/1 and IR 474/1 which appeared to be suitable for greenhouse indexing were recommended. NÉMETH and KÖLBER (1981) exploited for this purpose myrobalan hybride GF 31 which developed distinctive rusty brown necroses in the bark of annual shoots as the symptom of infection.

Obligation to realize the complex programme of stone-fruit certification i.e. selection of superelite trees for technical isolators, keeping of technical and space isolators and production of certified plant material initiated verification of usefulness and reliability of the internationally approved PPV woody indicators under natural conditions of the Czech Republic. It will be necessary to chose one or two of them which will be the best for local certification system.

## MATERIAL AND METHODS

### Investigated stone-fruit species and cultivars

At the locality Hrušky, district Břeclav eight years old apricot trees cv. Vegama infected with PPV were selected in the orchard. Occurrence of PPV was proved by ELISA and by visual evaluation of leaf symptoms (diffuse spots and ringspots). Branches of infected apricot trees cv. Vegama were grafted with selected stone-fruit species and cultivars as follows:

1. *Prunus domestica* L.: cv. Brompton
2. Interspecies crossings of the genus *Prunus*: Ferdor, Fereley, Marianna
3. *Prunus cerasifera* Ehrh.: Myrobalan, MY-BO-1, MY-KL-A
4. *Prunus armeniaca* L.: apricot cvs. Goldrich, Harlayne, Harval, Henderson, Lovaňská, Marii de Cenad, Stark Early Orange (SEO), Sundrop, Vegama, 18 hybrids, 3 autochthonous seedlings originating from South Moravia
5. *Prunus brigantica* Vill.

### Woody indicators and their characteristics

1. Myrobalan GF 31 – a hybrid of *P. cerasifera* Ehrh. and *P. salicina* Lindl.
2. *Prunus tomentosa* Thunb. – an ornamental species
3. *Prunus domestica* L. cv. Čačanská rodná – resulted from crossing of *P. domestica* cultivars Stanley and Požegača

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For field experiments aimed at suitability of PPV three internationally recommended woody indicators were used. *P. domestica* cv. Požegača was substituted for cv. Čačanská rodná which appeared to be more suitable for growing under environmental conditions of the Czech Republic and peach-tree GF 305 was eliminated for its lower cold hardiness and replaced by myrobalan GF 31. *P. tomentosa* was already used in the years 1994–1996 for to verify the presence of PPV in resistant and immune cultivars of apricot (POLÁK et al. 1997), but certain problems with the affinity appeared.

Species, cultivars and hybrids of stone fruits grafted on apricot trees cv. Vegama were next year budded with three different woody indicators: *Prunus tomentosa* Thunb., *Prunus domestica* L. cv. Čačanská rodná and *Prunus cerasifera* Ehrh., myrobalan GF 31.

#### Evaluation of infected species and cultivars of the genus *Prunus*

Infected species and cultivars of the genus *Prunus* were observed in the years 1997–1999. Leaf symptoms of PPV were evaluated in the period from mid-May till the end of August. In following years symptoms were watched also in fruits in the course of their ripening. Presence of PPV in leaves was checked by DAS-ELISA.

#### Evaluation of infected woody indicators

Symptoms of PPV developed in leaves and bark of woody indicators budded onto *Prunus* species and cultivars were rated from mid-May till the end of August. Occurrence of PPV in infected indicators was verified by DAS-ELISA.

#### Immunoenzymatic determination of PPV

For immunoenzymatic determination of the virus both in indicators and in *Prunus* species and cultivars standard procedure of DAS-ELISA (CLARK, ADAMS 1977) was used. Antibodies against PPV-Vegama isolate (PPV-M), IgG and conjugate with alkaline phosphatase were prepared in the virological laboratory of the Research Institute of Crop Production, Prague-Ruzyně.

## RESULTS AND DISCUSSION

It was found that the woody indicator *Prunus tomentosa* Thunb. had good affinity with prune cultivars and hybrids but worse with apricot cultivars. Rating of symptoms in the year of budding was possible by the end of June or in July but reliable evaluation of symptoms was in May or June in the second year after budding. Leaves of the indicator revealed chlorotic to yellow mosaic (Fig. 1) or oak leaf mosaic. By the end of June and at the beginning of July necroses along the midrib and leaf blade deformations developed.

The woody indicator Čačanská rodná had very good affinity both with prune and apricot cultivars and hybrids. Rating of symptoms in the year of budding was possible

in June. Intensity of symptoms was higher at the end of June or in July and remain distinctive even in August. In the second year after budding the evaluation was possible in May but the symptoms were milder than in the later period. In leaves of the indicator diffuse light green spots or rings developed (Fig. 2). Sometimes vein necrosis or leaf deformation occurred.



Fig. 1. Chlorotic and yellow mosaic in leaves of indicator *Prunus tomentosa* infected with PPV



Fig. 2. Chlorotic diffuse spots and rings in leaves of indicator Čačanská rodná infected with PPV

The woody indicator myrobalan GF 31 had good affinity with prune and apricot cultivars and hybrids. Rating of symptoms in the first year after budding was possible from mid-June, in the next year the most intensive symptoms occur from mid-May till the end of June. Leaves of the indicator revealed clearing of veins and diffuse light green spots and rings (Fig. 3). In the lower parts of annual shoots splits of bark and necroses occurred.

Presence of PPV in woody indicators as well as in individual species and cultivars grafted onto infected cv. Vegama was checked and verified by DAS-ELISA. Leaf symptoms were closely watched from mid-May till the end of August, symptoms in fruits in July and August, when they ripe. In-

Table 1. The comparison of properties of field woody indicators of PPV

Field woody indicator	Affinity with			PPV symptoms in leaves	Another PPV symptoms
	plum	myrobalan	apricot		
<i>Prunus tomentosa</i>	good	very good	weak	chlorotic and yellow mosaic, vein necrosis	—
Plum Čačanská rodná	very good	very good	good	large diffuse chlorotic spots and rings	—
Myrobalan GF 31	very good	very good	good	vein clearing, diffuse yellow-green spots and rings	bark necroses in one-year shoots occasionally

tensity of symptoms in susceptible genotypes was milder compared with those developed in the woody indicators.

The comparison of properties of three woody indicators of PPV is presented in Table 1. On the basis of comparative rating of 38 plum and apricot cultivars as well as further *Prunus* species infected with PPV using three woody indicators we can conclude that less suitable indicator is *Prunus tomentosa* Thunb. for its worse affinity with apricots. On the other hand the indicator Čačanská rodná has very good affinity with various species and cultivars and reacts by typical PPV symptoms which unfor-

tunately sometimes develop later than in *P. tomentosa*. The indicator GF 31 reacts more quickly than Čačanská rodná but sometimes the intensity of symptoms is lower.

Therefore we can recommend myrobalan GF 31 as the main woody indicator of PPV under field conditions of the Czech Republic. This indicator was checked and found to be reliable even under glasshouse conditions and can be fully recommended for the certification system of stone fruits in the Czech Republic. As the second suitable indicator we recommend the plum cv. Čačanská rodná. Combination of these two indicators is recommendable.

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Fig. 3. Vein clearing and diffuse yellow-green spots in leaves of indicator GF 31 infected with PPV

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## Polní dřevinné indikátory pro stanovení viru šarky švestky v České republice

**ABSTRAKT:** Vhodnost a spolehlivost dřevinných indikátorů viru šarky švestky (PPV), *Prunus tomentosa* Thunb., *Prunus domestica* L. cv. Čačanská rodná a *Prunus cerasifera* Ehrh. GF 31, byla ověřována v polních podmínkách České republiky na 38 odrůdách a hybridních peckovin rodu *Prunus*. Nejméně vhodným indikátorem PPV byl *P. tomentosa* pro horší afinitu s meruňkami. I když se příznaky PPV na listech indikátoru *P. domestica* cv. Čačanská rodná vyvíjejí o něco později než na *P. tomentosa*, má dobrou afinitu ke kultivarům a hybridům slivoní a meruněk. Indikátor *P. cerasifera* Ehrh. GF 31 myrobalán reaguje rychleji než *P. domestica* cv. Čačanská rodná, avšak intenzita příznaků je někdy nižší. Jako hlavní dřevinný indikátor PPV pro polní podmínky doporučujeme myrobalán GF 31. Druhým vhodným indikátorem je švestka domácí cv. Čačanská rodná. Je možné doporučit kombinaci obou dřevinných indikátorů.

**Klíčová slova:** virus šarky švestky; dřevinné indikátory; polní podmínky; *Prunus tomentosa*; *P. domestica* cv. Čačanská rodná; *P. cerasifera* GF 31

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# Evaluation of differences in growth vigour of apricot genotypes from 6 to 11 years after planting

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**ABSTRACT:** Growth vigour of 12 apricot genotypes grafted on apricot seedling rootstock (*Prunus armeniaca* L.) was evaluated on the base of girth measurements of stem in the period from 6 to 11 years after planting. There were significant differences in growth vigour of genotypes under study. The control cultivar Velkopavlovická LE-12/2 belonged to the cultivars with an above-average growth vigour. During the whole evaluation period, the rank of growth vigour of genotypes did not change. This was proved by highly significant coefficients of correlation existing between individual pairs of years 1994–1999 ( $r = 0.84++$  to  $r = 0.98++$ ). In individual years, the variability of growth vigour was relatively low and ranged from 13.48% to 15.98%. Three genotypes with a low intensity of growth were found, viz. VS-023/187, California and 3/2 A. In all years, their growth vigour was significantly and/or highly significantly lower than that of the control cultivar Velkopavlovická LE-12/2. Percentual differences ranged from -9.8% to -36.5%. Genotypes with lower growth intensity can be used in further breeding or as components inhibiting the growth in indirect vegetative propagation.

**Keywords:** apricot; growth vigour; genotypes; differences; correlations

Depending on their growth vigour, apricot genotypes with low, medium and high growth vigour can grow to the heights of 4–10 m (SMYKOV 1989). In pomological publications, the growth vigour is evaluated as weak, medium and strong (KUTINA et al. 1991). In spite of the fact that e.g. girth is an easily measurable parameter, concrete values are usually not given. The girth reduced by 10% reduces the crown diameter by approximately 0.5 m (VACHŮN 1971).

Differences in growth vigour should be respected when deciding about spacing of trees. As far as the intensification of production is concerned, there are tendencies to increase the numbers of trees per hectare in a similar way as for instance in apple plantations. This requires to look for genotypes and combinations cultivar – rootstock and/or cultivar – stem forming cultivar – rootstock with a lower intensity of growth (NITRANSKÝ 1996; VACHŮN 1971).

To obtain genotypes with a lower growth vigour is one of the most important objectives of stone-fruit trees selection because it enables not only to use a denser spacing but also to reach an earlier onset of high yields per hectare. In apricots, this was not the main objective of selection but it was also not fully neglected (VACHŮN et al. 1981). Significant differences were observed not only among cultivars but also among clones; as compared with the control cultivar Velkopavlovická, these differences made as much as 30% (VACHŮN 1995, 1996; VACHŮN et al. 1981). Genotypes showing a reduced growth vigour were stud-

ied also in Italy (QUARTA et al. 1996) and in some other countries.

Growth vigour represents above all a genetic trait but it can be influenced by the site, pruning and health conditions of propagated clone and/or rootstock. An ill rootstock (infected by virus or by phytoplasma) reduces the growth vigour by 10–15%, increases consumption of nutrients by as much as 50% and decreases the yields of grafted cultivars by 10–15% (DCSBA et al. 1991).

The aim of the research work was the evaluation of differences in growth vigour of apricot genotypes and to find genotypes with a lower intensity of growth for using them in further breeding work or as components inhibiting the growth in indirect vegetative propagation.

## MATERIAL AND METHODS

The experimental plantation was established in spring 1989. Apricot seedlings (*Prunus armeniaca* L.) were used as rootstocks. This study evaluates the growth vigour of twelve genotypes (from the 6<sup>th</sup> to the 11<sup>th</sup> year after planting) and the data obtained cover completely a period of six years. The total number of trees was high enough to enable a reliable statistical analysis. Experimental plantation was established in blocks. Each genotype had five replications with individually evaluated trees. As control, the cultivar Velkopavlovická LE-12/2 was used.

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Experimental set of trees involved not only older Czech and foreign cultivars but also some new breedings and clones. Genotypes with initial letters LE and MVA-3 L.Ž. originated from the Faculty of Horticulture (Mendel University of Agriculture and Forestry) in Lednice na Moravě, genotypes with initial letters VS were from the Plant Breeding Station Veselé (Slovakia), genotype Orange Red (NJA 32, Bhart) was from USA and P 2981 originated from France. Remaining genotypes (without data about the place of origin) were obtained within the framework of exchange of genetic resources. Numbers and letters behind the name of the genotype indicate clones.

Statistical analysis of data about growth vigour was carried out by analysing data from each year separately us-

ing intervals of confidence. In this way it was possible to test the significance of differences between the evaluated genotype and the control cultivar Velkopavlovická LE-12/2. As significantly or highly significantly different from the control only those genotypes were considered in which significant and/or highly significant differences were recorded in all six years.

## RESULTS AND DISCUSSION

Within the whole six-year period, the growth vigour of control cultivar Velkopavlovická LE-12/2 was higher than the average of the whole set of genotypes under study (Table 1). During this period, the average girths of stem of individual genotypes increased from 75 to 208 mm, i.e. by 28.3–68.2%. In 1999, the girth of the most intensively growing genotype MVA-3 L.Ž. was higher by 16.3% than the control. On the other hand, that of the weakest genotype VS-023/187 was lower by as much as 36.5% (Table 2). Confidence intervals for the significance levels of 95% and 99% were calculated separately for each year of the experimental period. As an example, confidence intervals calculated for the significance level of 99% in years 1994 and 1999 are presented in Figs. 3 and 4. In all experimental years, a significantly lower growth vigour was observed only in the genotype VS-023/187. The growth vigour of genotypes California and 3/2 A was highly significantly but in some years only significantly lower than that of the controls. A survey of differences observed in all experimental years is presented in Table 3.

There was a highly significant relationship between the rank of growth vigour of individual genotypes in individual pairs of years. This was demonstrated by highly significant correlation coefficients from 0.84++ to 0.98++ (Fig. 1). Values of variability coefficients ranged from 13.48% to 15.98% (Fig. 2). Variability in the growth vi-

Table 1. Evaluation of stem girth (in mm) in apricot genotypes within the period 1994–1999. Plantation established in the spring of 1989

No.	Genotype	1994	1995	1996	1997	1998	1999
1	ORANGE RED	325	360	393	408	443	455
2	VP-LE-111	335	375	395	415	435	450
3	CALIFORNIA	273	290	328	352	393	398
4	VS-74-105-714	283	310	330	378	403	423
5	VP-LE-126	293	311	344	375	398	415
6	<b>VP-LE-12/2*</b>	<b>328</b>	<b>350</b>	<b>380</b>	<b>406</b>	<b>425</b>	<b>441</b>
7	ROXANA	310	340	370	388	403	415
8	LE-1132	260	283	305	338	360	380
9	P 2981	270	297	324	368	402	420
10	VS-023/187	195	200	225	248	270	280
11	3/2A	265	275	303	313	330	340
12	MVA-3 L.Ž.	305	373	395	440	475	513
Average		287	314	341	369	395	411

\*Control genotype VP LE-12/2 (Velkopavlovická LE-12/2)

Table 2. Comparison of stem girth of apricot genotypes expressed in mm and in percentages at the beginning (in 1994) and at the end of the evaluation (in 1999) and percentage difference in the growth vigour to the control

No.	Genotype	Stem girth (mm) in the year		Increment within the period 1994–1999 (%)	Difference to control (%) in 1999
		1994	1999		
1	ORANGE RED	325	455	40.0	103.2
2	VP-LE-111	335	450	34.3	102.0
3	CALIFORNIA	273	398	45.8	90.2
4	VS-74-105-714	283	423	49.7	95.9
5	VP-LE-126	293	415	41.9	94.1
6	<b>VP-LE-12/2*</b>	<b>328</b>	<b>441</b>	<b>34.7</b>	<b>100.0</b>
7	ROXANA	310	415	33.9	94.1
8	LE-1132	260	380	46.1	86.2
9	P 2981	270	420	55.6	95.2
10	VS-023/187	195	280	43.6	63.5
11	3/2A	265	340	28.3	77.1
12	MVA-3 L.Ž.	305	513	68.2	116.3

\*Control genotype VP LE-12/2 (Velkopavlovická LE-12/2)

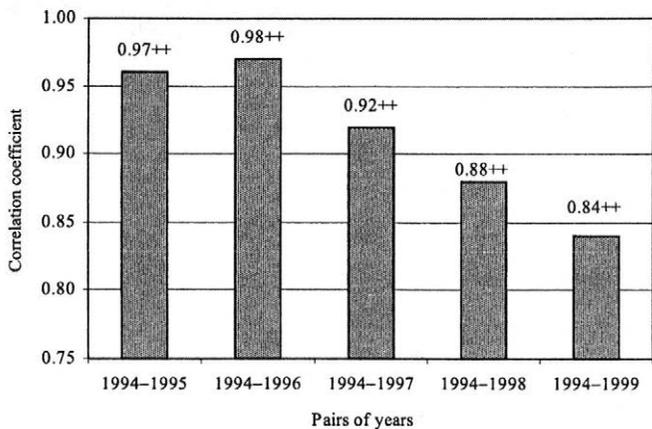


Fig. 1. Coefficients of growth vigour correlation existing between apricot genotypes evaluated on the base of girth of stem measured within the period of 1994-1999

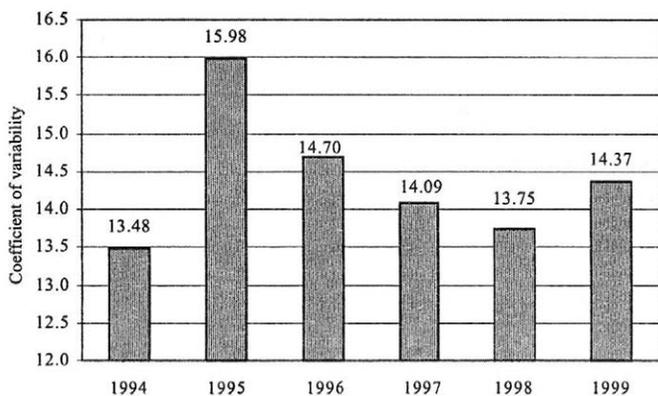


Fig. 2. Coefficients of variability in growth vigour of apricot genotypes in individual years of the period 1994-1999 (evaluated on the base of girth value of stem in mm)

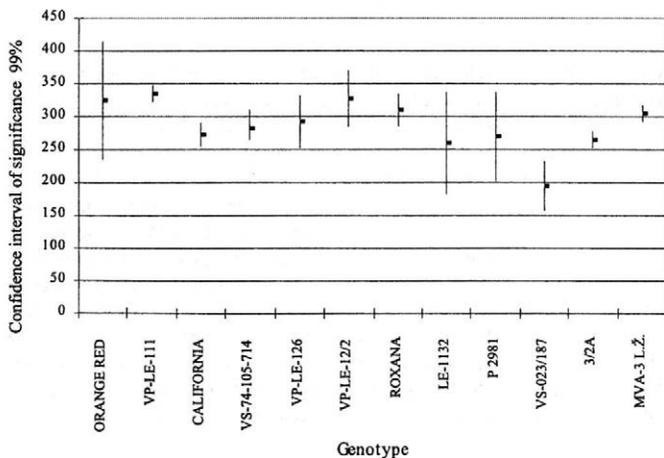


Fig. 3. Confidence intervals for girth of stem in apricot genotypes in the year 1994

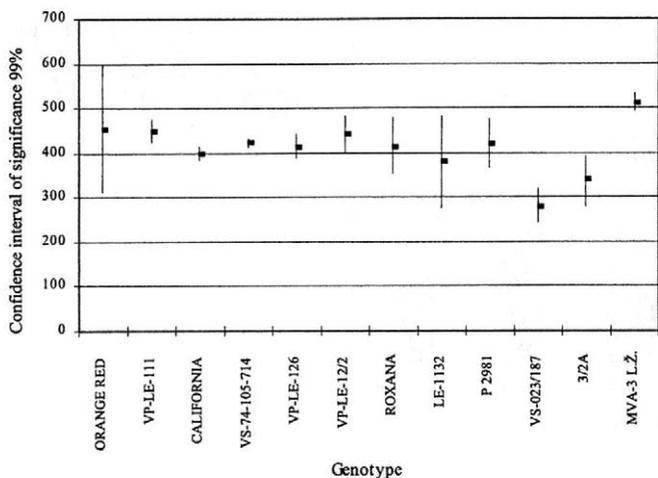


Fig. 4. Confidence intervals for girth of stem in the year 1999

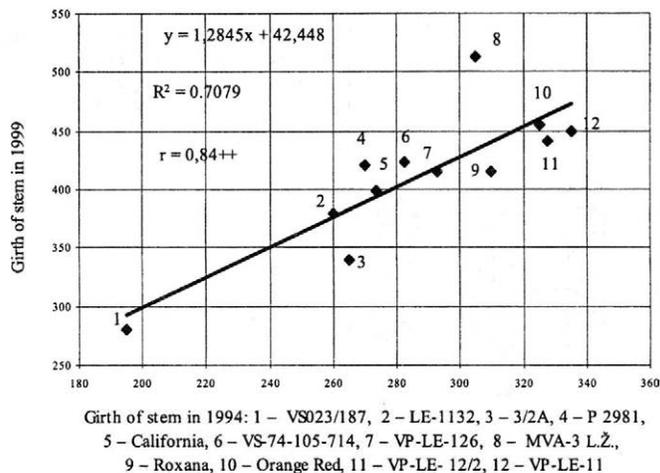


Fig. 5. Correlation between girth values of stem (mm) in 1994 and 1999

gour of grafted cultivars could be increased not only by the genotype but also by the generatively propagated rootstock *Prunus armeniaca* L. Although in individual years the variability of girth values was not high, it could be expected that it would be reduced if such selected apricot rootstocks (e.g. M-LE-1, M-VA-2 and others) would be used. Unfortunately, these rootstocks were not available for these experiments.

A generally lower danger of the occurrence of virus diseases is one of the most important advantages of using generatively propagated rootstocks. By pollen and seeds only some viruses are disseminated, e.g. *Prunus necrotic ring spot virus* (PNRSV). Seed material used for the production of rootstocks was obtained from parent trees non-

selected for the occurrence of PNRSV so that it was not possible to eliminate a potential effect of this and/or some other of until now uncontrolled factors.

The evaluation of our set of genotypes indicates that it was possible to find cultivars with a significantly lower intensity of growth. Provided that they meet the requirements of fruit production and quality, such genotypes could be important for programmes of increasing the numbers of trees per hectare because, for example, a reduction of growth vigour by 30% enables to reduce the spacing by at least 1.5 m even when using vigorous rootstocks. This property can be used also in programmes of selection of suitable stem-forming varieties.

Table 3. Significance of differences in growth vigour of apricot genotypes evaluated on the base of stem circumference (girth) in relation to the control variety Velkopavlovická LE-12/2 within the period 1994–1999

Genotype	Significance of differences					
	1994	1995	1996	1997	1998	1999
ORANGE RED	0	0	0	0	0	0
VP-LE-111	0	0	0	0	0	0
CALIFORNIA	+	+	++	++	++	+
VS-74-105-714	0	0	0	0	0	0
VP-LE-126	0	0	0	0	0	0
VP-LE-12/2*	0	0	0	0	0	0
ROXANA	0	0	0	0	0	0
LE-1132	0	0	0	0	0	0
P 2981	0	0	0	0	0	0
VS-023/187	++	++	++	++	++	++
3/2A	++	++	+	+	++	++
MVA-3 L.Ž.	0	0	0	0	0	0

\*Control variety Velkopavlovická LE-12/2 (VP LE-12/2)

Note: ++ – stem circumference (girth) highly significantly lower than in control

+ – stem circumference (girth) significantly lower than in control

0 – non-significant difference

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## Hodnocení rozdílů ve vzrůstnosti genotypů meruněk od 6. do 11. roku po výsadbě

**ABSTRAKT:** V šestém až jedenáctém roce po výsadbě byla hodnocena vzrůstnost 12 genotypů meruněk na podnoži meruňkový semenáč měřením obvodu kmene (tab. 1). Z vyhodnocení průkaznosti rozdílů v jednotlivých letech a sumárním vyhodnocením těchto rozdílů za šest let vyplývá, že vzrůstnost hodnocených genotypů byla významně rozdílná. Kontrolní odrůda Velkopavlovická LE-12/2 patřila mezi odrůdy s nadprůměrnou vzrůstností. Pořadí vzrůstnosti genotypů se za celé období hodnocení nezměnilo. Prokázaly to vysoce průkazné koeficienty korelace mezi jednotlivými dvojicemi let 1994–1999 s  $r = 0,84$  až  $r = 0,98$ . Variabilita vzrůstnosti v jednotlivých letech byla relativně nízká. Hodnota koeficientu variability se pohybovala v rozmezí 13,48–15,98. Byly zjištěny tři významně slaběji rostoucí genotypy: VS-023/187, California a 3/2A. Jejich vzrůstnost byla ve všech letech průkazně nebo vysoce průkazně nižší než vzrůstnost kontrolní odrůdy Velkopavlovická LE-12/2. Jako příklad jsou uvedeny konfidenční intervaly pro rok 1994 a 1999. V procentuálním vyjádření to bylo u uvedených odrůd od –9,8 % do –36,5 % ve srovnání s kontrolou. Slabě rostoucí genotypy mohou být využity pro další šlechtění nebo jako růst oslabující kmenotvorné odrůdy při nepřímém vegetativním rozmnožování.

**Klíčová slova:** meruňka; vzrůstnost; genotypy; rozdílů; vztahy

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# Possibilities of herbicide applications in the yarrow cultivation (*Achillea collina* Becker)

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**ABSTRACT:** Possibilities of herbicide applications to yarrow cultivation (*Achillea collina* Becker) were tested in the years 1997–1999. The influence of pre-emergence application of linuron, propyzamide, trifluralin, alachlor, combined applications of prometryne + metachlor and trifluralin + linuron on weeds occurrence and on yarrow seeds emergence were studied. Prometryne and linuron were also applied at the post-emergence stage. From the herbicides mentioned above, combined applications of prometryne + metachlor and trifluralin + linuron can be after registration recommended for weed control in early development stages of yarrow, in further development stages of plants the use of prometryne or linuron is possible. None of the other herbicides studied had a sufficient effect on annual weeds. Phytotoxicity on yarrow was not noticed in any of pursued variants.

**Keywords:** *Achillea collina* Becker; herbicides; linuron; propyzamide; trifluralin; alachlor; prometryne; metachlor

*Achillea* L. (yarrow), a wide spread herbaceous perennial from the family *Asteraceae*, is a rich source of bioactive chemical components (essential oils, flavonoids, tannins, sesquiterpene lactones, etc.). Infusa of its flowering tops and inflorescence have been used medicinally since ancient times because of their antiinflammatory, antiphlogistic and spasmolytic activities (KASTNER et al. 1993; VETTER et al. 1996).

The species, mostly defined as a complex, is divided into several subspecies with diploid to octaploid forms. *Achillea collina* Becker differs from other subspecies grown in the Czech Republic by its high content of essential oils and chamazulene. Pharmacopoeia bohémica XCVII requests a minimal content of essential oils (2 ml in kilogram of drug) but does not mention the differences among *Achillea* subspecies, consequently contemporary demand for yarrow is still provided by picking herbs up from nature. To ensure sufficient quantity and quality of herbs and to avoid devastation of nature, the introduction of *Achillea collina* into field growing seems to be necessary.

A lot of authors (e.g. BAIER, BOHR 1997; DOVJAK et al. 1988; HORNOK 1992) have reported on yarrow growing specially the influence of nutrition on yield of herbs and oils were studied. Information about application of herbicide in cultivation of *Achillea* is very rarely published in literature (PANK, MARLOW 1980; PANK et al. 1983) or they only relate to ornamental yarrows (STAATS et al. 1998). To control weeds in *Achillea*, a rotary cultivation is usually used (HORNOK 1992; RUMÍNSKA 1983) or Synfloran (trifluralin) and Roundup (glyphosate) are applied to prepare fields before sowing (ORAVEC 1986). BAIER and BOHR (1997) have even reported that no herbicides are

currently suitable for use in *Achillea*. On the other side, a lot of scientists have been working on finding a herbicide against yarrow as a weed (BOURDOT, HURRELL 1984; BOURDOT, BUTLER 1985).

Because of the long germination of *Achillea* seeds (about 5 weeks after sowing), the young plants are not able to compete with other weeds and just at this time the use of herbicides is necessary. Later, when a leave rosette is developed, plants are vital enough to grow even without herbicides. As for residues content, STŘELEČ (1999) has reported that after pre-emergence application of linuron and prometryne there is time enough for residues elimination from soil before harvest. REIFENSTEIN and PANK (1975) have also confirmed that no content of residua was analysed in *Achillea* drugs after application of prometryne.

In this study, the testing of six selected herbicides and their mixtures for chemical control of weeds in cultivation of *Achillea collina* are reported.

## MATERIAL AND METHODS

According to the Metodická příručka pro ochranu rostlin (1997), three registered herbicides suitable for growing medicinal plants were evaluated in 1997: Afalon 50 WP (linuron), Kerb 50 W (propyzamide) and Synfloran 48 EC (trifluralin). To examine the effect of herbicides, seeds of *Achillea collina* Alba were sown on April 14<sup>th</sup>, 1997 on 10m<sup>2</sup> experimental plots (sowing rate 2.5 g on 10 m<sup>2</sup>, row distance 0.4 m). Herbicides were applied to the soil surface at recommended rates in the case of Synfloran 48 EC before sowing and in the others after sowing the seeds.

Experiments were set up in a completely randomised block structure with four replicates on experimental fields, Faculty of Horticulture of Mendel University of Agriculture and Forestry, Lednice na Moravě, Czech Republic (174 m above sea level, average temperature 9°C, loamy soil).

In 1998, seeds were sown on April 20, on 5 m<sup>2</sup> plots. The herbicides used were Kerb 50 W (propyzamide), Synfloran 48 EC (trifluralin), Lasso 50 EC (alachlor), combined application of Gesagard 80 (prometryne) + Dual 500 EC (metachlor), Synfloran 48 EC + Afalon 50 WP (linuron) in pre-emergent application, Afalon 50 WP and Gesagard 80 in post-emergence treatment. In the second case, herbicides were applied on young plants in 6–10 leaf stage, which were sown on October 2, 1997. All herbicides and using rates are mentioned in Tables 1 and 2.

In 1999, the same herbicides and methods were again used like in 1998 year; sowing of seeds was done on April 26.

Based on the Metodiky pro hodnocení biologické účinnosti herbicidů EPPO No. 99 (1987), the first evaluation of plots was done before herbicide applications, the second evaluation was done in the week following germination of *Achillea* seeds, the other two evaluations were done 3, respective 6 weeks after germination. As for post-emergence treatment, the first evaluation of plots was done on the same day as herbicide application, the three others followed in 2 week intervals after treatment. Weed counts and phytotoxicity evaluation was taken from three randomly chosen subplots (0.25 m<sup>2</sup>) on each plot. The plots were cultivated after the last counting.

In 1997, flowering stems were hand-harvested at the stage of full flowering, air-dried and analysed for content of essential oil. In 1998, the harvest was not done because of damage of young plants by strong and wrong regulated irrigation on a neighbourly field.

Table 1. A list of tested pre-emergence herbicides

Date of herbicide application	Commercial name	Active substance	Dose (ha)	Average number of weeds in different evaluation terms <sup>1</sup>		
				2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
14. 4. 1997	Afalon 50 WP	linuron (47.5%)	1.5 kg	24	54.5	70.5
	Kerb 50W	propyzamide (50%)	2.5 kg	37.75	59.75	101.5
	Synfloran 48 EC	trifluralin (480 g/l)	1.5 l	30	42.25	53
	Control			<b>32.5</b>	<b>57.25</b>	<b>121.5</b>
20. 4. 1998	Kerb 50W	propyzamide (50%)	2.5 kg	1.25	4	3.75
	Synfloran 48 EC	trifluralin (480 g/l)	1.5 l	3.75	3	0
	Lasso 50 EC	alachlor (480 g/l)	4 l	0.75	4	5.25
	Synfloran 48 EC + Afalon 50 WP	trifluralin (480 g/l) + linuron (47.5%)	1.5 l + 2 kg	1.25	0.75	0
	Gesagard 80 + Dual 500 EC	prometryne (80%) + metachlor (500 g/l)	1 kg + 3 l	0.75	1.5	1
	Control			<b>2</b>	<b>3.5</b>	<b>5.25</b>
26. 4. 1999	Gesagard 80 + Dual 500 EC	prometryne (80%) + metachlor (500 g/l)	1 kg + 3 l	0	0	0
	Lasso 50 EC	alachlor (480 g/l)	4 l	0	1.25	0
	Control			<b>0.25</b>	<b>0.25</b>	<b>0.25</b>

<sup>1</sup>1<sup>st</sup> evaluation – no weeds

Table 2. A list of tested post-emergence herbicides

Date of herbicide application	Commercial name	Active substance	Dose (ha)	Average number of weeds in different evaluation terms			
				1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
20. 4. 1998	Gesagard 80	prometryne (80%)	1 kg	2.75	3	1.25	0
	Afalon 50 WP	linuron (47.5%)	1 kg	7.5	5	2.25	2
	Control			<b>7.75</b>	<b>8.25</b>	<b>9.25</b>	<b>12</b>
26. 4. 1999	Gesagard 80	prometryne (80%)	1 kg	0.25	0.5	0	0.75
	Afalon 50 WP	linuron (47.5%)	1 kg	0	0	0	0.5
	Control			<b>0.5</b>	<b>0.75</b>	<b>2</b>	<b>2.75</b>

Analysis of variance was carried out for all obtained results; significant differences among variants were proved by Tukay testing.

## RESULTS AND DISCUSSION

In 1997, the influence of pre-emergence herbicides applied on plant germination was studied. About 6 weeks after sowing, the germination of seeds started in all observed plots, no difference and no phytotoxicity was found in treated plots and control. These data confirm the observation of STAATS et al. (1993), who tested the influence of herbicide on germination of weed seeds (among them *Achillea*) in pot experiment.

A few weeks later at the stage of elongated growth and at the beginning of flowering, plants treated by trifluralin differed visually but not significantly in the higher num-

ber of flowering stems from the control and from other variants as well (Table 3).

Due to unbalanced development of plants and gradual germination of *Achillea* seeds during the whole season, the yield of harvested plants could not be found out. However, content of essential oil was analysed and it was confirmed that herbicides tested had no influence on oil content (Table 4).

The following annual weeds mainly appeared on experimental plots: *Amaranthus retroflexus* L., *Chenopodium album* L., *Galinsoga parviflora* Cav., *Capsella bursa-pastoris* Med., *Sonchus oleraceus* L.), later also *Echinochloa crus-gali* and *Portulaca oleracea* L.

There was observed a difference among particular variants in weed control. The visually lowest number of weeds was found on plots treated by trifluralin. In spite of the fact, that all control plots clearly showed the highest number of weeds, the difference among treated and control plots

Table 3. Criteria of the identity of variances in number of flowering stems, in the 4<sup>th</sup> evaluation term in 1997 (*F*-test)

Year	Average number of flowering stems in different variants				<i>F</i> -test		
	linuron	propryzamide	trifluralin	control	<i>F</i> -criterion tabulated		<i>F</i> -criterion calculated
					$\alpha = 0.05$	$\alpha = 0.01$	
1997	21.25	24	30.25	12	3.86	6.99	1.77
1998 <sup>1</sup>	-	-	-	-	-	-	-
1999 <sup>2</sup>	-	-	-	-	-	-	-

<sup>1</sup>in 1998 the young plants were damaged by strong irrigation

<sup>2</sup>in 1999 the plants did not germinate

Table 4. Criteria of the identity of variances in essential oil content (*F*-test)

Year	Average essential oil content in different variants				<i>F</i> -test		
	(%)				<i>F</i> -criterion tabulated		<i>F</i> -criterion calculated
	linuron	propryzamide	trifluralin	control	$\alpha = 0.05$	$\alpha = 0.01$	
1997	0.38	0.36	0.33	0.33	3.86	6.99	0.94
1998 <sup>1</sup>	-	-	-	-	-	-	-
1999 <sup>2</sup>	-	-	-	-	-	-	-

<sup>1</sup>in 1998 the young plants were damaged by strong irrigation

<sup>2</sup>in 1999 the plants did not germinate

Table 5. Criteria of the identity of variances in pre-emergence herbicide – treated plots and in control ones (*F*-test)

Year	<i>F</i> -test					
	<i>F</i> -criterion tabulated		<i>F</i> -criterion calculated			
	$\alpha = 0.05$	$\alpha = 0.01$	2 <sup>nd</sup> evaluation	3 <sup>rd</sup> evaluation	4 <sup>th</sup> evaluation	
1997	3.86	6.99	2.71	2.13	10.59*	
1998	2.91	4.57	1.05	0.29	1.28	
1999	5.14	10.92	3.01	3.01	0	

\* significant difference

was not proved, except in one case, as statistically significant. The only significant difference was found between control and plots treated by trifluralin in the last evaluation of trial (it means the 4<sup>th</sup> one) ( $\alpha = 0.05$ ; Table 5).

Against expectation, none of the tested herbicides controlled the occurrence of *Portulaca oleracea* L. very well, the effect of linuron on *Galinsoga parviflora* Cav. was also very low.

In 1998 in the cases of individual herbicide application, the germination of seeds was observed one week later than in control; in the cases of combined application of herbicides, the very irregular germination was observed even

2 weeks later than in control. During plants development these differences have been equalised. Influence of used herbicides was visual in all observed variants. The most effective weed control was provided by treatment of combined herbicides prometryne + metachlor and trifluralin + linuron, that controlled wider range of weeds than individual herbicides (alachlor, propyzamide, trifluralin) (Table 1). The results mentioned gave again no statistical significant difference (Table 5). It is supposed that it could be caused by perennial *Cirsium arvense* Scop. influencing the occurrence of annual weeds on those experimental plots.

Table 6. Significance of differences in pre-emergence herbicide – treated plots and in control in the 4<sup>th</sup> evaluation, 1997

	Linuron	Propyzamide	Trifluralin
Control	51.5	20.0	68.5*
Trifluralin	17.0	48.5	
Propyzamide	31.5		

\* significant difference (tabulated minimal significant difference D(0.05) = 64.4; D(0.01) = 114.85)

The post-emergence application of herbicides resulted only in *Achillea* leaves getting yellow during 7 days after treatment. Newly developed leaves had again the same colour (dark green) like untreated control. The plant development and beginning of flowering was slightly inhibited as well. Difference of yield could not be proved by damage of young plants by irrigation. As for weeds, the small necrotic spots, dry and convoluted margins appeared on the leaves. Most of annual weeds dried, the recommended dose of prometryne and linuron was strong enough. Both of these herbicides significantly differed from the control almost at all evaluation terms (Table 7), but no significant difference was found between them (Tables 8 and 9). Treated perennial weeds only stopped their growth and 2 weeks later emerged again.

In 1999, irregular and very bad germination of seeds was observed in all plots, both in tested and in control ones. Also occurrence of weeds was very low (Tables 1 and 2). As within 10 weeks no changes in germination were revealed this experiment was finished without harvest. It is supposed that the germination problems were

Table 8. Significance of differences in post-emergence herbicide – treated plots and in control

a) 1<sup>st</sup> evaluation, 1998

	Linuron	Prometryne
Control	0.25	5*
Prometryne	4.75	

\* significant difference (tabulated minimal significant difference D(0.05) = 4.98; D(0.01) = 7.37)

b) 3<sup>rd</sup> evaluation, 1998

	Linuron	Prometryne
Control	7*	8*
Prometryne	1	

\* significant difference (tabulated minimal significant difference D(0.05) = 6.63; D(0.01) = 9.67)

c) 4<sup>th</sup> evaluation, 1998

	Linuron	Prometryne
Control	10*	12**
Prometryne	2	

\* significant difference (tabulated minimal significant difference D(0.05) = 7.12)

\*\* highly significant difference (tabulated minimal significant difference D(0.01) = 10.39)

Table 9. Significance of differences in post-emergence herbicide – treated plots and in control, 1<sup>st</sup> evaluation, 1999

	Linuron	Prometryne
Control	2*	2*
Prometryne	0	

\* significant difference (tabulated minimal significant difference D(0.05) = 1.45; D(0.01) = 2.11)

caused by warm and dry weather in spring or according MITICH (1990) likely by inhibiting effect of *Achillea* plants that were grown on this field for the third year.

Table 7. Criteria of the identity of variances in post-emergence herbicide – treated plots and in control ones (*F*-test)

Year	<i>F</i> -test					
	<i>F</i> -criterion tabulated		<i>F</i> -criterion calculated			
	$\alpha = 0.05$	$\alpha = 0.01$	1 <sup>st</sup> evaluation	2 <sup>nd</sup> evaluation	3 <sup>rd</sup> evaluation	4 <sup>th</sup> evaluation
1998	5.14	10.92	5.86*	3.79	8.14*	15.33**
1999	5.14	10.92	1.80	1.24	11.99**	3.71

\* significant difference

\*\* highly significant difference

## CONCLUSION

To find optimal agrotechnology for cultivation of *Achillea*, an experimental trial for testing pre-emergence and post-emergence herbicides was set up in 1997–1999. It was found that using of herbicides to control weed is necessary especially at the beginning of plant development to compete with faster germinated weeds.

Based on results obtained, combined application of pre-emergence herbicides Gesagard 80 (prometryne) + Dual 500 EC (metachlor) and Synfloran 48 EC (trifluralin) + Afalon 50 WP (linuron) were recommended for weed control of yarrow cultivation in the fields without perennial weeds. If necessary, 2 other solutions are possible: the use of post-emergence application of Afalon 50 WP (linuron) or Gesagard 80 (prometryne) to control weeds till closing stand or the rotary cultivation in later stages of plant development. From the second year of cultivation and after early spring hoeing, yarrow is able to eliminate the occurrence of weeds itself.

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## Možnosti použití herbicidů při pěstování řebříčku chlumního (*Achillea collina* Becker)

**ABSTRAKT:** V letech 1997–1999 byly zkoušeny možnosti použití herbicidů při pěstování řebříčku chlumního (*Achillea collina* Becker) v kultuře. Byl sledován vliv preemergentní aplikace linuronu, propyzamidu, trifluralinu, alachloru, dále kombinované aplikace prometrynu + metachloru a trifluralinu + linuronu na výskyt plevelných druhů a na vzházení osiva řebříčku. Aplikace prometrynu a linuronu proběhla i postemergentně. Z uvedených herbicidů lze ke kontrole výskytu plevelů v raných stádiích vývoje řebříčku doporučit po registraci kombinované aplikace prometrynu + metachloru a trifluralinu + linuronu, v pozdějších stádiích vývoje rostlin potom prometryn nebo linuron. Ostatní použité herbicidy nevykazovaly dostatečný účinek na jednoleté plevely, fytotoxické účinky však nebyly pozorovány u žádných sledovaných varianty.

**Klíčová slova:** *Achillea collina* Becker; herbicidy; linuron; propyzamid; trifluralin; alachlor; prometryn; metachlor

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# Genome induced mutation in *Callistephus chinensis* Nees – I. Effect of colchicine application on the early plant development

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**ABSTRACT:** Colchicine was chosen for polyploidy induction in 2 cultivars of *Callistephus chinensis* Nees Gerda and Princess Armida. The first type of application was done by swelly seeds soaking in solution of colchicine (concentrations of colchicine were 0.01, 0.03, 0.05, 0.1, 0.3% and operating times were 6, 12 and 18 hours). The second method was dropping colchicine solution on the apex between cotyledons leaves (concentrations of colchicine were 0.1, 0.3, 0.5, 1.0, 1.5% and operating times were 2, 3 and 5 days). After the seed treatment, a considerable influence of colchicine on the emergence rate was noticed. Especially higher concentration and longer operating time of colchicine solution expressively suppressed this rate. Seedling morphology was also notably changed, namely at a higher concentration of colchicine solution and at longer operating time. Seedlings hypocotyledons were considerably truncated and plumped. In variants with higher colchicine concentration and longer operating time, roots regenerated very badly and this caused high dieback of seedlings.

**Keywords:** *Callistephus chinensis* Nees; colchicine; seedlings morphology; emergence rate and survival rate after colchicine treatment; polyploidy

The aim of *Callistephus chinensis* polyploid breeding was higher tolerance to *Fusarium oxysporum* f. spp. *calistephi* (Blach) Snyder a Hausen (next called FOC).

The early plant development was expressively affected by colchicine.

Applications of colchicine were performed on the swelly seeds and on the apex of seedlings between cotyledons leaves after their opening. These two methods of somatically polyploidisation considerably affected next cultivation, vitality and efficiency of polyploidisation.

The roots regeneration is a big problem after colchicine treatment on swelly seeds (UHLÍK 1981; LAPTEV 1988). A substrate of perfect quality must be used for plant cultivation. A common problem is root rot of colchicine treated seedlings.

Colchicine application on the apex has not any incidence on roots and is consequently better for plant vitality but the efficiency of polyploidisation is lower (LAPTEV 1988).

The mitotical polyploidy was induced in these experiments, when only meristemata cells were affected by colchicine during cell division. Relatively high number of chimerical, mixoploid plants occurred in the generation treated in this way (ACKERMAN, DERMEN 1972; SCHIFINO, FERNANDES 1987; LAPTEV 1988). Also the morphological and vitality imbalance is higher in the first generation after colchicine treatment ( $C_0$  generation).

## MATERIAL AND METHODS

For polyploidy induction two cultivars of *Callistephus chinensis* were chosen on the base of their tolerance level to FOC. Cultivar Gerda is high tolerant to FOC, cv. Princess Armida is semitolerant (KOVÁČOVÁ 1995; VEGRICHTOVÁ 1998).

The DMSO (dimethylsulfoxide, 3%) was added to colchicine solution, for better penetration into plant tissue.

The first method of polyploidy induction was soaking of swelly achenes in a solution of colchicine (concentrations of colchicine were 0.01, 0.03, 0.05, 0.1, 0.3% and operating times were 6, 12 and 18 hours). Each variant of colchicine application included  $3 \times 220$  seeds. The seeds were kept for 24 hours on wet filter paper before colchicine application. Flush of treated seeds succeeds colchicine application for taking out the rests of colchicine from tissue. Next the seeds were placed again on wet filter paper for roots regeneration. Due to a rot appearance the seeds were sown into peat substrate in greenhouse (day 22°C, night 18°C). The seedlings were treated with fungicides Rovral 0.25%, Previcur 0.25%, Fundazol 0.1%.

The second method was dropping colchicine solution between cotyledons leaves after their opening (concentrations of colchicine were 0.1, 0.3, 0.5, 1.0, 1.5% and operating times were 2, 3 and 5 days), application was performed every day in the morning, between 7 and

8 o'clock in laboratory (22°C). 3 × 100 seedlings were treated in each variant. After colchicine treatment the seedlings were covered by polyethylene folie and dewed by distilled water several times a day.

### CHARACTERISTICS OBSERVED

#### Emergence rate

The emergence rate was evaluated in variants with swelly seeds soaking in colchicine solution only. The percentage of emerged seedlings from numbers of sown seeds was calculated.

#### Seedlings morphology

Colchicine considerably affected morphology of *Callistephus chinensis* Nees young plants. It was affected especially by higher concentration and longer operating time of colchicine solution action. The number of deformed seedlings, length and width of hypocotyledons were monitored. Twenty randomly chosen seedlings were measured in each variant of colchicine application.

#### Survival rate

The survival rate is given as a comparison between emerged seedlings number after colchicine treatment and

survived plants, which were planted in the field (to 1. 6. 1999).

This part is not directly related to influence of colchicine on young plants but it is related to vitality of colchicine treated plants of *Callistephus chinensis* Nees.

It is possible to compare both colchicine application (swelly seeds soaking and dropping colchicine solution on the apex). The differences between number of emerged seedlings and number of plants in the field were relatively high, especially by swelly seeds soaking method of colchicine application.

### RESULTS AND DISCUSSION

#### EMERGENCE RATE

A considerably suppression of emergence rate was observed already after using low concentration of a colchicine solution and after short time of incidence.

The operating time of colchicine solution had higher influence on the emergence rate. Longer operating time of colchicine action and lower colchicine concentration suppressed emergence rate more than higher concentration and shorter operating time (Fig. 1).

The colchicine treated seeds emerged very slowly. The emergence was by one week later than that in the control plants.

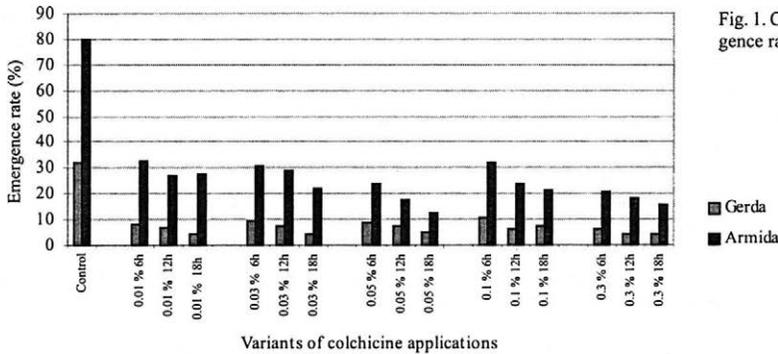


Fig. 1. Colchicine influence on emergence rate of *Callistephus chinensis*

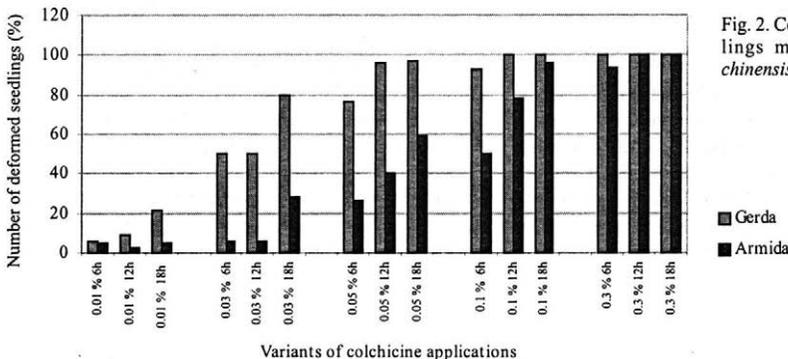


Fig. 2. Colchicine influence on seedlings morphology *Callistephus chinensis*

Speeds of emergence were very different. The control plants emerged uniformly 7–10 days after sowing while the variants with higher colchicine concentration and longer operating time of incidence emerged up to 21 days after.

The emerged seedlings were transplanted into plug-trays into peat substrate. Then they were cultivated in the greenhouse (day 18°C, night 15°C).

#### The problems of roots regeneration

In variants with higher colchicine concentration and longer operating time, the roots regenerated very badly. Especially bad root regeneration was observed in deformed seedlings. These ones were often 2–3 weeks in stage of hypocotyledons with cotyledons leaves without roots. They were very often infected by fungal diseases despite of the regular fungicide protection.

The dieback of plants was relatively lower when the colchicine application was performed on the apex and the roots were not affected by colchicine solution.

According UHLIK (1981) colchicine application on seeds caused big problems with root regeneration. Only few plants survived up to the maturity. It is necessary to avoid root rot. In this view it is better to apply colchicine solution on the plant apex.

LAPTEV (1988) mentioned high negatively colchicine influence on roots especially after application on seeds. The root regeneration on a wet filter paper is suitable.

The roots regeneration of *Callistephus chinensis* was very slow. To prevent a possible appearance of root rot the seeds were not kept on wet filter paper, but immediately sown into peat substrate in a greenhouse (22–24°C) and protected by fungicides.

#### SEEDLINGS MORPHOLOGY

Seedlings morphology was notably changed. The reason of deformed seedlings appearance was probably due to a colchicine influence on cell cytoskeleton and an attendance of polyploid cells already in hypocotyledons.

A higher concentration of colchicine solution (especially in cv. Gerda) provoked high numbers of deformed seedlings within relatively short time of colchicine action (Fig. 2). The hypocotyledons of deformed seedlings were considerably truncated and plumped, the cotyledons leaves were plumped and much smaller (Figs. 3, 4 and 9).

Deformed seedlings were belated in their development. True leaves grew up much later than those of the control plants.

BRINDZA (1998) reported that a change of seedlings morphology (plump of root apex and hypocotyledons) was the first effect of polyploidisation, but it was possible, that not every change of morphology was due to polyploidisation.

MACKEVIĆ (1962) met a similar influence of colchicine solution on young plant morphology of *Populus* sp. He

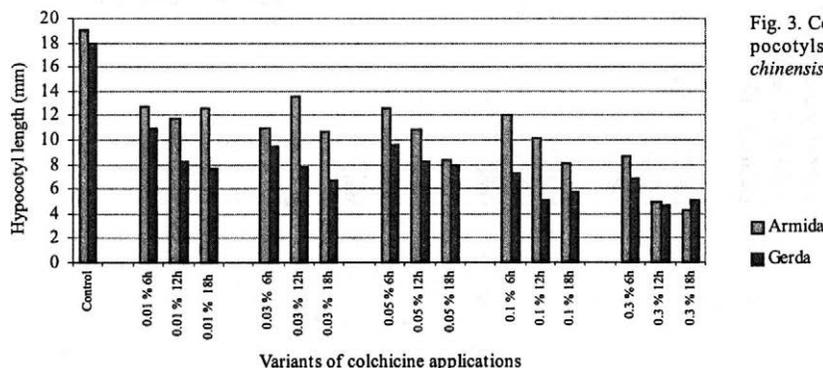


Fig. 3. Colchicine influence on hypocotyls length of *Callistephus chinensis*

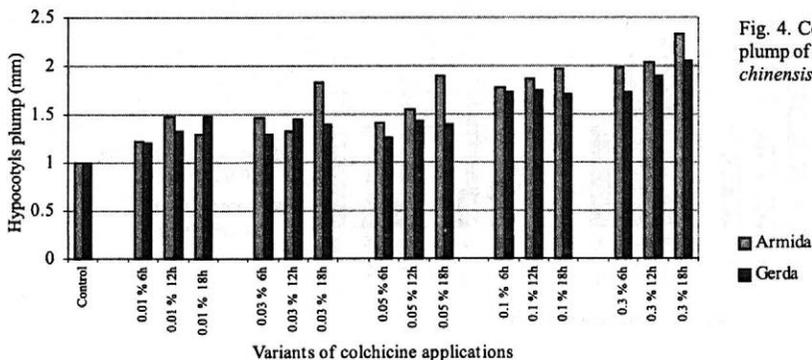


Fig. 4. Colchicine influence on the plump of hypocotyls of *Callistephus chinensis*

says that colchicine concentrations (0.007–0.03%) caused similar morphological deviation (truncated and plumped hypocotyl, slow root regeneration).

MOHINDER and KHOSHOO (1977) found deformed seedlings in the first generation of *Amaranthus* sp. They compared germination and development within  $C_1$  generation and diploid plants of *Amaranthus* sp. They mentioned that tetraploid seedlings had a faster development in the first phases than diploid plants. However, 10 days after their emergence the development of diploid seedlings was always more rapid.

### SURVIVAL RATE

Large differences were found between the two methods of a colchicine application. The soaking of swelly seeds brings considerably suppress of an emergence rate and a high seedling dieback (Figs. 5 and 6). After application of the colchicine solution on the apex between cotyledons leaves, the damage of roots was not so bold and the survival rate was relatively high (Figs. 7 and 8). It is connected with colchicine influence on roots (UHLIK 1981; LAPTEV 1988; MACKEVIČ 1962).

The highest dieback was found in the 5 days variants of a colchicine application on the apex. The influence of a colchicine concentration was here rather peripheral.

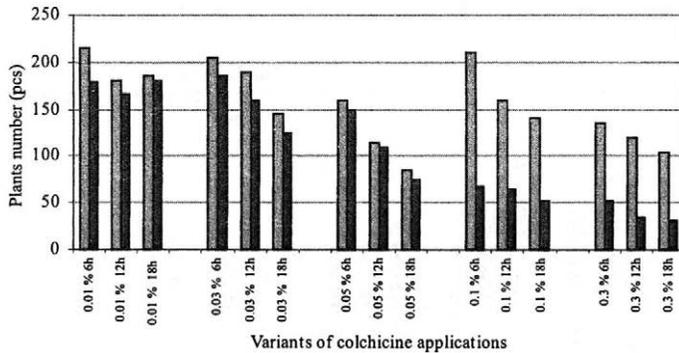


Fig. 5. Survival rate after swelly seed soaking in colchicine solution (Princess Armida)

■ emerged seedlings from 660 seeds (pcs)  
 ■ number in the field 1.6.1999 (pcs)

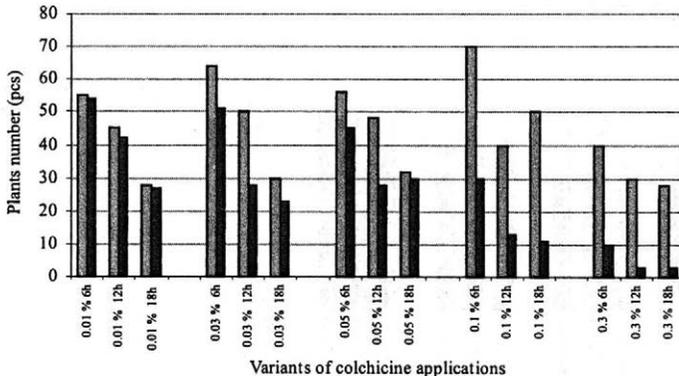


Fig. 6. Survival rate after swelly seed soaking in colchicine solution (Gerda)

■ emerged seedlings from 660 seeds (pcs)  
 ■ number in the field 1.6.1999 (pcs)

ZHAO GANG et al. (1995) indicated that application of a colchicine solution (0.2%) and DMSO (1.5%) on the apex of *Fagopyrum vulgare* seedlings twice a day for 6 days gave a relatively high survival rate (without giving concrete value). They also mentioned a lower growth, a change of leaf morphology, larger size of some flowers and seeds in generation  $C_0$ .

Jakešová (pers. commun.) mentioned different sensitivity among various families *Trifolium* sp.

Colchicine toxic influences were also indicated by JÄHNE and LÖRZ (1995), COHEN and YAO (1996), SONG et al. (1997).

Similar results of colchicine application were obtained in *in vitro* cultivation, too. KAHANE et al. (1997) found in a tissue culture of *Allium cepa* L. that survival rate depended on plant clone and especially on colchicine concentration. The survival rate significantly falled with higher colchicine concentration.

A cytotoxic influence of a colchicine solution in *in vitro* culture was also mentioned by VAN DUREN et al. (1996), STADLER et al. (1989) and HAN et al. (1999), ENDO et al. (1997).

Different sensitivity to colchicine was found in *Callistephus chinensis*. The cultivar Gerda was more sensitive. It was expressed by lower emergence rate, higher number of deformed seedlings and higher seedlings mortality.

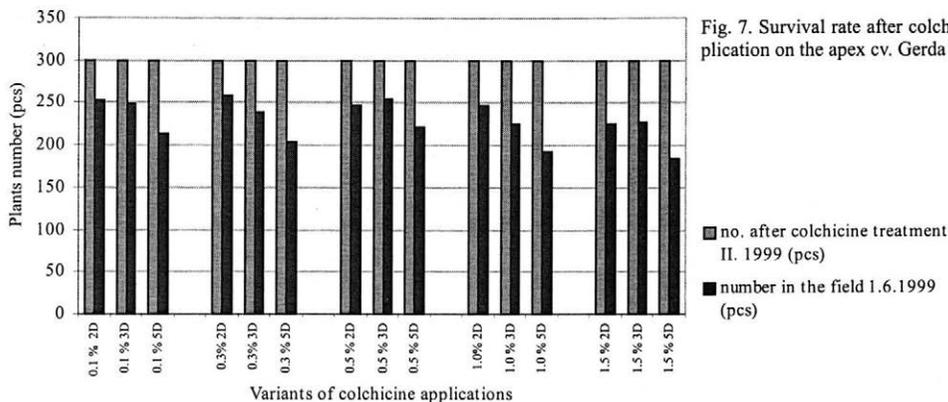


Fig. 7. Survival rate after colchicine application on the apex cv. Gerda

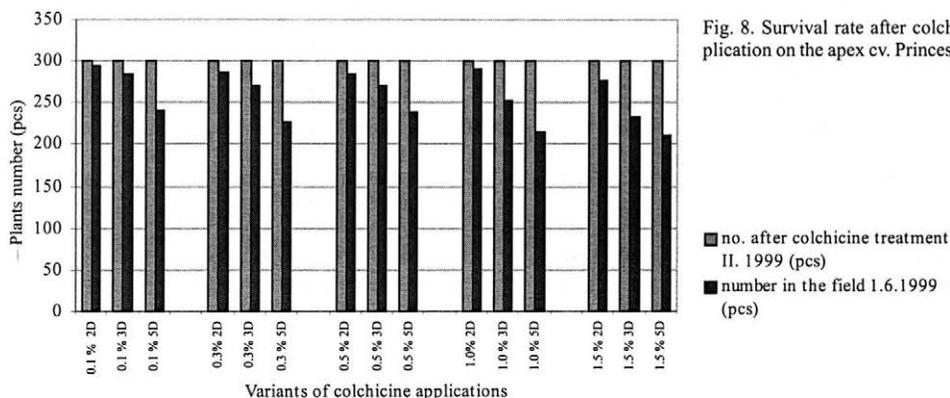


Fig. 8. Survival rate after colchicine application on the apex cv. Princess Armida



Fig. 9. The seedlings of *Callistephus chinensis* Nees (cv. Princess Armida) three weeks after colchicine treatment (swelly seeds soaking in 0.1% colchicine and 12 hours of incidence). The plant without colchicine treatment is on the left

## CONCLUSION

The results of a colchicine application on the seeds and the apex between cotyledons leaves of *Callistephus chinensis* corresponded with results of other authors and with generally known influence of colchicine on plant tissue.

The toxic effect of colchicine on roots was confirmed. It relatively provoked lower emergence rate and dieback of young plants.

When the root system was not affected, plants better tolerated colchicine influence. The problem of a bad root regeneration was connected with the change of young plant morphology, especially with truncation and plumped hypocotyledons. A lot of deformed seedlings died.

Two cultivars were used in the experiments. It is evident that sensitivity to a colchicine influence is different and could depend on cultivars.

The cultivar Gerda was more sensitive to a colchicine influence than Princess Armida. It was proved by higher numbers of deformed seedlings and their high mortality.

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## Indukovaná mutagenéza genomu *Callistephus chinensis* Nees – I. Vliv kolchicinu v raných fázích vývoje rostlin

**ABSTRAKT:** Indukovaná mutagenéza genomu (polyploidizace) u kultivarů Princess Armida a Gerda *Callistephus chinensis* Nees byla provedena máčením nabobtnalých nažek do roztoku kolchicinu (koncentrace 0,01, 0,03, 0,05, 0,1 a 0,3 %, doba působení 6, 12 a 18 hodin) a nanášením kolchicinu na vegetační vrchol mezi děložní lístky aster krátce po jejich rozevření (koncentrace 0,1, 0,3, 0,5, 1,0 a 1,5 % a doba působení 2, 3 a 5 dnů). V raných fázích vývoje kolchicin výrazně ovlivnil vzcházivost (vyššími koncentracemi a delší dobou působení byla výrazně potlačena), vitalitu a morfologii mladých rostlin, a to zejména u varianty máčení nažek. Docházelo ke značnému ztlouštění a zkrácení hypokotylu semenáčků a ke špatné regeneraci kořínků, což následně způsobilo vysoký úhyn mladých rostlin.

**Klíčová slova:** *Callistephus chinensis* Nees; kolchicin; morfologie semenáčků; vzcházivost; míra přežití; polyploidie

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# Genome induced mutation in *Callistephus chinensis* Nees – II. Results of colchicine application – efficiency of polyploidisation

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**ABSTRACT:** Considerable changes morphology of leaf blade can be considered as an additional mark of polyploid plants. It manifested itself by a changed value of leaf index. Leaf index (length and width leaf blade ratio) of diploid plants was closely around 1.5, in the case of polyploid plants it was around 1.05–1.1. Leaves were more rounded and thicker with more entire leaf blade. The primary selection of probably polyploid plants was performed on the base of these morphological changes. Plants chosen in the primary selection were analysed by flow-cytometry. From obtained results, the number of polyploidy (tetraploidy and mixoploidy) plants was determined in population  $C_0$  of *Callistephus chinensis* Nees. The efficiency of polyploidisation is based on the number and on the survival rate of polyploid plants.

**Keywords:** china aster; polyploidisation; colchicine; leaf index; flow-cytometry; selection of polyploid plants; efficiency of polyploidisation

China aster (*Callistephus chinensis* Nees) is an important annual bedding plant suitable used also as cut flowers. It originates from China and Japan and diploid plants have  $2n = 18$  (COCKSHULL 1985). A considerable problem in china asters cultivation is *Fusarium oxysporum* f. sp. *callistephi* (Blach) Snyder et Hausen (next called FOC), which provokes rapid mortality of plants.

Inducing polyploidy experiments were performed in 1999 in *Callistephus chinensis*. The aim of this work was to enlarge genic variability and to use the polyploid forms of china asters in breeding for resistance to FOC.

Higher tolerance of polyploid plants to virus and fungal diseases was mentioned by BARANOV (1962). This method may increase china aster tolerance to FOC and exploitation of new genic material could enlarge the assortment of *Callistephus chinensis* resistant against FOC.

One of the first reports on tetraploidy form of *Callistephus chinensis* was published by MATVEJEVA (1962). The tetraploid forms differed by larger inflorescence (up to 15 cm), the stem plump was wider. These plants probably did not reach larger expansion because other data are missing.

Colchicine belongs to the group of so called cytoskeleton inhibitors. Its effect reposes in very specific bond to tubulin protein, which leads to temporary failure of dividing spindle (NEČAS 1991). The result is that chromosomes do not go to cell poles during cell division and cells with double chromosomes number appear (NEČÁSEK 1993; NEČÁSEK, CETL 1979; GOTTSCHALK, WOLF 1983; ONDŘEJ 1985).

Colchicine is effective during cell division only (KOVÁČIK 1983). This is the reason why it is the most

effective to apply it on swelly seeds, germinating seeds or seedlings (on meristems generally) (LAPTEV 1988; REIMANN-PHILIPP 1969).

After colchicine treatment a fairly flush of seeds in fluid water for colchicine elimination from tissue is necessary (Jakešová 1998, pers. commun.).

Optimal conditions for colchicine induced polyploidy must be chosen by experiments for every plant species (LAPTEV 1988).

Possibilities to induce polyploidy in *Callistephus chinensis* were not tested sufficiently, it was necessary to prepare the methods by experiments.

Usually used colchicine concentrations generally providing sufficient efficiency of polyploidisation LAPTEV (1988), MATVEJEVA (1962), RANDŽABLI and RUD (1972) were selected for experiments.

Flow cytometry (FCM) allows rapid analysis of relative DNA content of nuclei isolated from leaf tissue. DNA content in  $G_1$  phase of the cell cycle reflects the level of cell ploidy. It is currently used for quick determination of ploidy and the plants have not to be restrained (DOLEŽEL 1998).

## MATERIAL AND METHODS

The ways of colchicine applications on *Callistephus chinensis* Nees are mentioned in HANZELKA and KOBZA (2001).

The *Callistephus chinensis* population counted about 13,000 individuals after colchicine treatment (next called generation  $C_0$ ). Selection of probably polyploid plants (primary selection) was performed by observation of

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clear-cup change of leaf blade morphology. These changes appeared in relatively high number of plants in C<sub>0</sub>.

The secondary selection was done by FCM analysis, which efficiently identified the number of polyploid (tetraploid and mixoploid) plants in generation C<sub>0</sub>.

The coefficient of primary selection effectivity was evaluated as the ratio of plant number selected by secondary and primary selection.

#### VALUE OF LEAF INDEXES

The leaf index (length and width leaf blade ratio) was evaluated in 30 randomly chosen plants of each cultivar in particular variants of colchicine application and separately in diploid and polyploid plants.

Leaf indexes were evaluated only in variants with colchicine application on the swelly seed. These were not evaluated for the colchicine application on the apex between cotyledon leaves because the changes of leaf morphology were very similar as in the first method.

#### FLOW-CYTOMETRICAL ANALYSIS OF PROBABLY POLYPLOID PLANTS (SELECTED BY PRIMARY SELECTION)

A small part of leaf blade was used for FCM analysis. The part of leaf was minced in hypotonic isolation solution (solution A) for cell nuclei extrication. A DNA-specific fluorochrom (DAPI – 4',6-diamidino-2-phenylindole) was added as an inking agent (solution B) (PORTUGAL, WARING 1988; DOLEŽEL 1998).

FCM analysis were performed in Plant Breeding Station Hladké Životice using PARTEC Flow-cytometer (Germany).

##### Composition of solution A (Hladké Životice)

Substance	Concentration	Solution 1,000 ml
Citric acid	0.1 M	21 g
Tween 20 (soaking agent)	0.5%	5 ml
Deionised water		complete

##### Composition of solution B (Hladké Životice)

Substance	Concentration	Solution 1,000 ml
Na <sub>2</sub> HPO <sub>4</sub> · 12H <sub>2</sub> O	0.4 M	143.25 g
DAPI (store solution)	4 µg/ml	50 ml
Deionised water		complete

The result of FCM analysis is a distribution histogram of fluorescence intensity in population of isolation nuclei (DOLEŽEL et al. 1997) (Fig. 4).

#### INTERPRETATION OF INDUCED POLYPLOID RESULTS

The primary selection of probably polyploidy plants in generation C<sub>0</sub> was evaluated according to the so called *coefficient of the primary selection*.

Effectivity of the primary selection =

$$\frac{\text{polyploid plants number (after flow-cytometric analysis)}}{\text{number of plants chosen from primary selection}} \cdot 100(\%)$$

The minimum coefficient is 0 (no polyploid plants were found, effectivity = 0%). The maximum value is 1 (every plant chosen by primary selection was polyploid, effectivity = 100%).

Polyploidisation was generally evaluated as *efficiency of polyploidisation (E)*

Efficiency of polyploidisation is a relative value, which is based on the number of obtained polyploid plants and also on the survival rate of colchicine treated plants. This characteristic comes out from similar work on *Malus × domestica* (BOUVIER et al. 1994).

Interpretation of polyploidisation efficiency based only by the number of obtained polyploid plants in each variant of colchicine application would be considerably misrepresenting.

The efficiency of polyploidisation was calculated as follows:

$$E = \% \text{ polyploid plants} \times \text{survival rate}$$

This efficiency has a maximum of 100, that is, in case that all plants double their chromosome number and survive (BOUVIER et al. 1994).

## RESULTS AND DISCUSSION

### PRIMARY SELECTION AND LEAF INDEXES

The first FCM analysis showed, that a change of leaf blade morphology is considerably correlated with a change of ploidy level in *Callistephus chinensis* (change of ploidy level does not appear in plants without change of leaf blade). Leaves were more rounded and thicker with more entire leaf blade (Fig. 5).

In the case of diploid plants the leaf index was about 1.5 and in the case of polyploid plants it was about 1.05–1.1 (Fig. 1).

MATVEJEVA (1962) noticed the possibility to use change of leaf index value to identify the polyploid plants. The leaf index value was changed in a number of young plants of several plant species in many experiments not further specified. Leaf blades of polyploid plants were usually shorter and wider.

#### Coefficient of primary selection

	Armida	Gerda
Colchicine application on the apex	0.52	0.681
Soaking of swelly seeds into colchicine	0.498	0.502

Number of obtained polyploidy plants from primary selection (%) is given as coefficient of the primary selection × 100

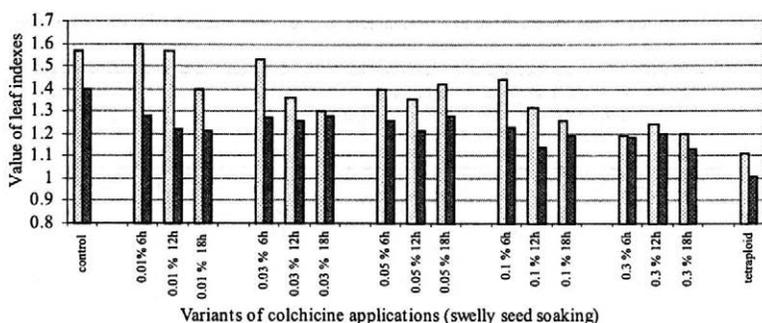


Fig. 1. Leaves indexes values

## SECONDARY SELECTION AND EFFICIENCY OF POLYPLOIDISATION (*E*) (model calculation)

$$E = \text{number of polyploidy plant (\%)} \times \text{survival rate}$$

Variants of colchicine application	Number of evaluated plants	Number of polyploids (%)	Survival rate in % (from 528 plants) **	<i>E</i>
0.1%/6 h	68	7.4	12.9	0.95
0.1%/12 h	65	18.5	12.3	2.28
0.1%/18 h	52	42.3	9.8	4.15

\*\* variants of swelly seed soaking, based on  $3 \times 220$  seeds, cv. Princess Armida, emergence rate 80% (it is 528 plants in control, presupposition 100% survival rate in control plants)

The polyploid plant number was evaluated by flow-cytometric analysis (Flow-cytometer Partec, Münster, Germany). Polyploid plants include both tetraploid and mixoploid plants together (part of tetraploid and part of diploid tissue were found in mixoploid plants).

Table 1. Survey of emerged plants after swelly seed soaking in colchicine solution, survival rate to 1. 6. 1999, number of primary selected plants and final polyploids number

Colchicine application	Emerged plants (from 660 seeds)		Number of plants in the field (1. 6. 1999)		Primary selected plants (pcs)		Final polyploids number (pcs)	
	Armida	Gerda	Armida	Gerda	Armida	Gerda	Armida	Gerda
0.01% 6 h	215	55	179	54	2	6	0	0
0.01% 12 h	180	45	166	42	4	3	0	0
0.01% 18 h	185	28	180	27	3	4	0	0
0.03% 6 h	205	64	185	51	1	7	0	3
0.03% 12 h	190	50	160	28	3	9	1	3
0.03% 18 h	145	30	125	23	4	10	3	4
0.05% 6 h	160	56	150	45	2	9	1	1
0.05% 12 h	115	48	110	28	3	10	2	9
0.05% 18 h	85	32	75	30	6	26	3	16
0.1% 6 h	210	70	68	30	6	20	5	17
0.1% 12 h	160	40	65	13	17	80	12	8
0.1% 18 h	140	50	52	11	31	11	22	8
0.3% 6 h	135	40	52	10	41	10	33	5
0.3% 12 h	120	30	34	3	29	3	21	3
0.3% 18 h	105	28	32	3	32	3	30	2

Table 2. Survey of emerged plants after colchicine application on the apex, survival rate to 1. 6. 1999, number of primary selected plants and final polyploids number

Colchicine application	Number of colchicine treated seedlings (pcs)		Number of plants in the field (1. 6. 1999)		Primary selected plants (pcs)		Finally polyploids number (pcs)	
	Armida	Gerda	Armida	Gerda	Armida	Gerda	Armida	Gerda
0.1% 2D	300	300	294	253	2	5	0	3
0.1% 3D	300	300	285	249	3	3	1	2
0.1% 5D	300	300	241	213	11	13	6	8
0.3% 2D	300	300	286	258	1	2	1	2
0.3% 3D	300	300	271	239	9	9	6	6
0.3% 5D	300	300	227	205	20	10	12	7
0.5% 2D	300	300	285	247	5	7	1	3
0.5% 3D	300	300	270	254	13	14	6	11
0.5% 5D	300	300	239	221	18	15	13	13
1.0% 2D	300	300	290	247	3	7	1	4
1.0% 3D	300	300	252	225	12	15	9	8
1.0% 5D	300	300	215	192	22	17	13	15
1.5% 2D	300	300	277	225	7	16	4	7
1.5% 3D	300	300	233	227	26	12	14	7
1.5% 5D	300	300	210	184	24	22	12	19

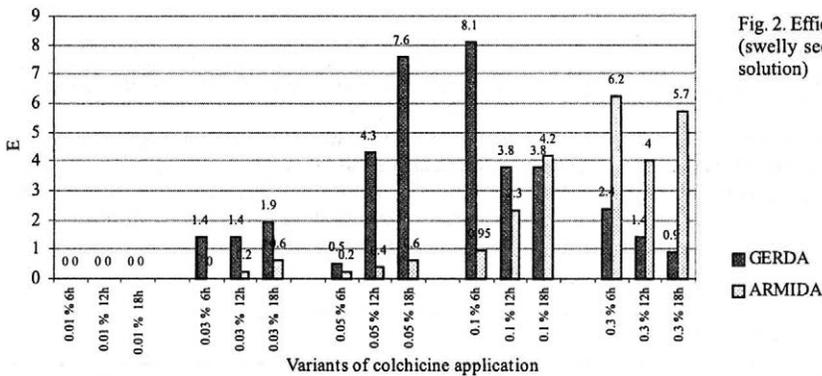


Fig. 2. Efficiency of polyploidisation (swelly seed soaking in colchicine solution)

CAGLAR and ABAK (1997) mentioned on about the submersion of cucumber seedlings into colchicine solution (0.5 and 1.0%, incidence 2 and 4 hours). The chromosomes number was counted in root spike. The most successful variant was 0.5% colchicine concentration and 4 hours of incidence.

LAPTEV (1988) found out that after colchicine treatment it is suitable to place seeds on wet filter paper for better regeneration of involved roots (it is possible to use distilled water or a nutritive solution). This way was unsuitable in the work with *Callistephus chinensis* because root rot in colchicine treated seeds appeared. The colchicine treated seeds were sown 1 day after this treatment into peat substrate and treated with fungicide (Previcur, Rovral, Fundazol).

Application of colchicine solution on the apex between cotyledons leaves gave these results. The most successful

variants were those with 5-days applications of colchicine solution in cv. Princess Armida. Colchicine concentrations were rather second-rate in this way of colchicine application. A relatively high number of polyploid plants was obtained in 3-days colchicine applications and 1.5% colchicine concentration.

In the case of cv. Gerda, 5-days colchicine applications combined with 1.5, 1.0, 0.5% colchicine concentrations were the most successful variants (Fig. 3).

The following range of colchicine concentrations are usually mentioned for plant apex 0.3–1.0% (LAPTEV 1988), 0.5–2.0% (KOVÁČIK 1983).

A colchicine drop was applied on the apex between cotyledons leaves after their opening once a day in the morning. RANDŽABLI and RUD (1972) mentioned that it is better to fixate the colchicine drop with the help of a cotton-wool piece. This way of fixation was not used in our

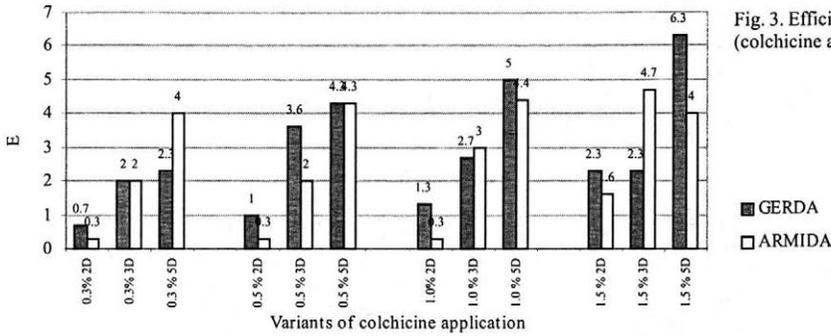
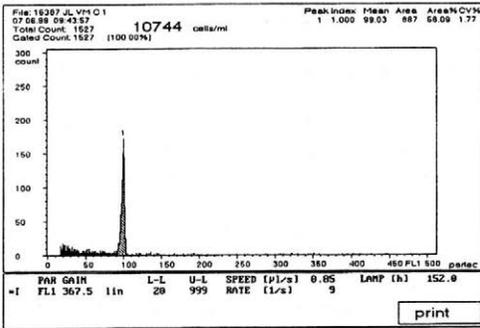
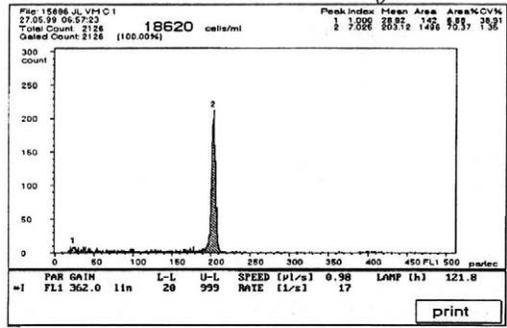


Fig. 3. Efficiency of polyloidisation (colchicine application on the apex)

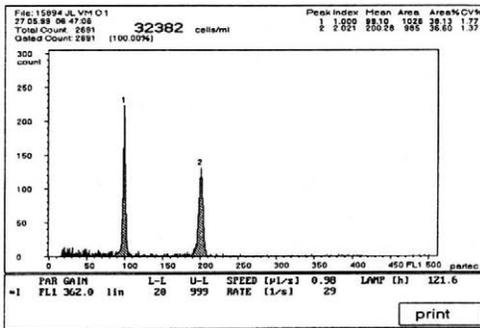
1. Diploid (control) plant



2. Tetraploid plant



3. Tetraploid plant (2) with the control (1)



4. Mixoploid plant

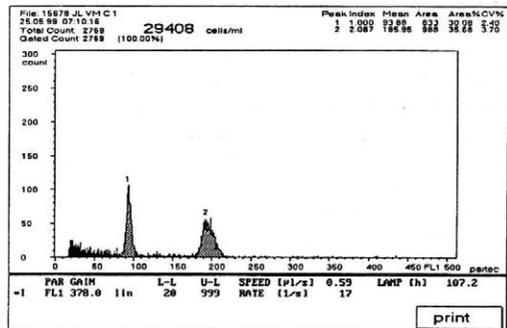


Fig. 4. Histograms of flow-cytometrical analysis of probably polyloid plants (cv. Gerda swelly seed soaking 0.1% colchicine concentration, 6 hours of its incidence)

experiments, because the cotton-wool quickly drain and fall down from apices. This is why colchicine was next applied without this fixation.

ZHAO GANG et al. (1995) worked on colchicine application (0.2%, + 1.5% DMSO) on the apex of *Fagopyrum vulgare* seedlings. The application of colchicine solution was performed twice a day (in the morning and in the evening) during six days. They found some differences among cul-

tivar sensitivity to colchicine solution. The rate of obtained polyloid plants reached up to 62.8%. They also found, that DMSO accelerated efficiency of polyloidisation because it increases colchicine penetration in plant tissue. They obtained 30–40% of polyloid plants without DMSO, and 55–65% with DMSO.

Interesting are the possibilities of applying colchicine in *in vitro* cultures of family *Asteraceae*. MIYOSHI and ASA-

KURA (1996) induced diploid shoots of *Gerbera jamesonii* from haploid callus. The colchicine concentration was 0.05% in medium and the incidence time 2, 3 and 6 days. 80.4% of regenerated plants were haploids, 15.2% diploids and 4.3% mixoploids after flow-cytometrical analysis.

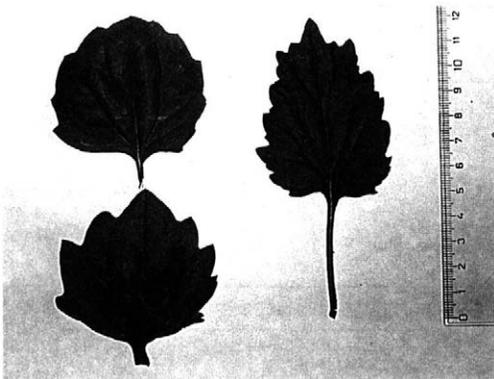


Fig. 5. Change of leaf blade morphology in polyloid form of  $C_0$  generation *Callistephus chinensis*. Leaf from control (diploid) plant is on the right, leaves from tetraploid plant are on the left

ENDO et al. (1997) induced polyploidisation in *in vitro* culture of *Dendranthema × grandiflorum*, colchicine concentrations were 0.05, 0.08, 0.1, 0.2% and incidence time 12, 24 and 48 hours. 96 polyploid plants were selected from 156 plants. The efficiency of each variant was not specified.

TOSCA et al. (1995) observed that in *in vitro* cultures of *Gerbera* sp., the number of polyploids increased with the colchicine concentrations in range from 5.8% to 40%. The colchicine concentrations were 0, 30, 60, 120, 240, 480, 720 and 960  $\mu\text{M}$ . The highest number of dihaploids were obtained with 60  $\mu\text{M}$  colchicine then the rate of dihaploids dropped down. The incidence time was 48 hours.

FUJISHIGE et al. (1988, 1996) mentioned successful polyploidy induction in *in vitro* culture of *Haplopappus gracilis* ( $2n = 4$ ) using Colcemid (0.5 mg/ml) and 3 days of incidence. Most of metaphase cells were found as polyploids 30 days after colchicine treatment (about 85%).

## CONCLUSION

In both cultivars polyploidy was successfully induced by both ways of polyploidy induction. The most successful variants in cv. Princess Armida were 0.3% of colchicine concentration and 6 and 18 hours of incidence on the swelly seeds ( $E = 6.2$  and  $5.7$ ). 5-days variants of colchicine application and 3-days with 1.5% on the apex showed maximum of polyploids ( $E = 4.0$ – $4.7$ ). In the cultivar Gerda the most successful combination was 0.1% colchicine and 6 hours of incidence ( $E = 8.1$ ) and 0.05% and 18 hours ( $E = 7.6$ ). The colchicine application on the apex brings maximum polyploid plants by 5-days of incidence and 1.5 and 1.0% colchicine concentrations.

It is evident that cultivar sensitivity to colchicine is different. The cultivar Gerda was more sensitive than cv. Princess Armida. More plants died, the most successful variants were with lower colchicine concentrations and shorter times of incidence.

Variants with swelly seeds soaking caused higher plant mortality but the number of obtained polyploid plants was higher, too. In variants with colchicine application on the apex (between cotyledons leaves) relatively more plants survived (mortality was minimal) but the number of obtained polyploid plants was relatively lower. These differences between the two ways of colchicine applications were minimised with the relative value *efficiency of polyploidisation*. This value considered the number of obtained polyploid plants (in %) and survival rate in each variant of colchicine application.

## Acknowledgements

The authors thank the management of the Plant Breeding Station Hladké Životice, especially Ing. Hana Jakešová, CSc. a RNDr. Josef Janeček, CSc. for their help and cooperation in polyploid plant identification. Without this help these results would not have been obtained.

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## Indukovaná mutagenéze genomu *Callistephus chinensis* Nees – II. Výsledky aplikace kolchicinu – účinnost polyploidizace

**ABSTRAKT:** Primární selekce pravděpodobně polyploidních rostlin probíhala na základě výrazných morfoloických změn listové čepele, které se projeví zejména změnou velikosti listového indexu (poměr délky a šířky listové čepele). Listový index diploidních rostlin byl blízký hodnotě 1,5. U tetraploidních jedinců se blížil hodnotě 1. Čepel listů byla okrouhlejší, hrubší, s méně členěným okrajem. Rostliny vybrané primární selekcí byly dále analyzovány průtokovou cytometrií a byl stanoven počet polyploidních (tetraploidních a mixoploidních) rostlin generace  $C_0$ . Účinnost polyploidizace byla zvolena jako relativní hodnota zohledňující procentuální podíl získaných polyploidů a míru přežití kolchicinem ošetřených rostlin.

**Klíčová slova:** astra čínská; polyploidizace; kolchicin; listový index; průtoková cytometrie; selekce polyploidních rostlin; účinnost polyploidizace

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*Melissa officinalis* L. – a really medicinal herb

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**ABSTRACT:** This paper summarizes the knowledge of effective compounds of *Melissa officinalis* and principles of their isolation, and research in their effects on microorganisms, viruses and enzymes, which were published in the last decades.

**Keywords:** *Melissa officinalis* L.; essential oils; extracts; content compounds; effects

The efforts to use natural resources of plant and animal origin in the food industry, cosmetics and alternative medicine currently initiate more and more detailed investigations of well-known as well as less known plants to define their activity against negative factors of all types. The well-known *Melissa officinalis* (e.g. HLAVA et al. 1983) has often been described in literature in recent years: not only its positive effects have been confirmed (an official drug is *Melissae herba*, dried leaves collected at the beginning of flowering, which is specified in the pharmacopoeia (sine, 1997) but also new aspects of products made of it have been examined (as analytical and separation methods have developed). This review article covers some of recent papers dealing with newly discovered constituents and parallelly determined activity of *Melissa officinalis* extracts, the variability of which is sometimes surprising.

METHODS OF *MELISSA OFFICINALIS* MATTER PROCESSING

Naturally, fresh matter as well as a drug of *Melissa officinalis* (lemon balm) are processed. Either steam distillation of the material is used or it is extracted with water or water-ethanol mixture at different ratios.

The essential oil (0.2–0.8%) produced by steam distillation contains more than 30 constituents, mainly linalool, citronellal, geranial, geraniol, geranyl acetate, beta-cubeben, alpha-caryophyllene, rose-oxide (SARER, KOKDIL 1991; SHALABY et al. 1995; KREIS, MOSANDL 1994, etc.), that means mostly terpenic compounds.

Besides the above constituents (with respect to the extractant composition), aqueous and hydroalcoholic extracts contain many nonvolatile compounds while important are some flavonoids (MULKENS, KAPETANIDIS 1987) and polyphenolic or tannin-like substances often derived from caffeic acid (KORDANA et al. 1997). It is e.g. melitric acid (AGATA et al. 1993). Another important example is rosmarinic acid, the depside of caffeic acid, which has been identified in lemon balm a long time ago (e.g. HERRMANN 1960).

The name rosmarinic acid is attributed to two compounds in which stereoisomers can be identified. Chemical description of the two compounds:

- 1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester of 3-(3,4-dihydroxyphenyl)propenic acid (Fig. 1a),
- 2-carboxy-1-(3,4-dihydroxyphenyl)ethyl ester of 3-(3,4-dihydroxyphenyl)propenic acid (Fig. 1b),
- R isomer of the former compound (S isomer was detected in *Anthoceros punctatus* only) was isolated from *Melissa officinalis*, some representatives of the genus *Salvia* and *Lavandula*, the latter compound is a constituent of *Perilla frutescens* and *Thymus bashkiriensis*.

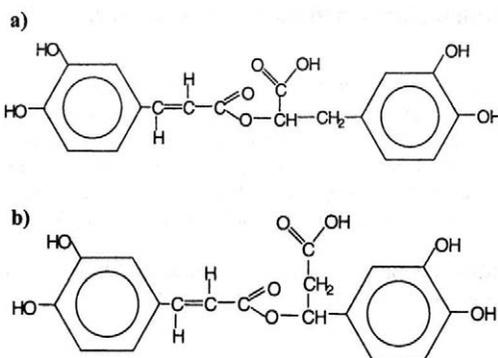


Fig. 1. Rosemary acid

Besides the above compounds, *Melissa officinalis* contains luteolin-7-O-glucoside, isoquercitrin, apigenin-7-O-glucoside (MULKENS, KAPETANIDIS 1987), trienic acid ((9S, 10E, 12Z, 15E)-9-hydroxy-10,12,15-octadecatrienic acid), eugenylglucoside (MULKENS, KAPETANIDIS 1988), rhamnosin (3,5,4'-trihydroxy-7,3'-dimethoxyflavone) (THIEME, KITZE 1973).

The specification of spasmolytic, antimicrobial, antioxidative and virostatic activity of *Melissa officinalis*

(VAVERKOVÁ et al. 1995) is available in recently published papers.

### ACTIVITY AGAINST MULTICELLULAR ORGANISMS

Sedative effects of hydroalcoholic lyophilized extracts of lemon balm were investigated on mice and compared with pentobarbital effect. Higher doses of the extract induced sleep in mice (SOULIMANI et al. 1991). A similar study was published later (SOULIMANI et al. 1993). While the essential oil was not active on the mouse used in tests, the alcoholic extract had significant analgetic effects at doses 800–1,600 mg/kg in a "writhe" test induced by acetic acid. The extract significantly decreased locomotion (maximum response at a dose 25 mg/kg) and significantly induced mouse sleep (doses 3 and 6 mg/kg) and prolonged sleep (doses 6 and 50 mg/kg) induced by sodium pentobarbital. The extract inhibited peristaltic contractions of rat duodenum, but it did not have any effects on contractions induced by carbamoylcholine.

The antifeedant activity of extracts from 21 medicinal herbs against larvae of *Pieris brassicae* (cabbage white) was tested in Switzerland. Two-sample and zero-sample studies demonstrated significant effects of extracts from lemon balm, *Mentha piperita* and some eucalypti (ABIVARDI, BENZ 1984).

The dog tick *Dermacentor reticulatus*, the pictus tick *D. marginatus* and *Hyalomma anatolicum* (a subtropical species) inoculated by a defined amount of tick-borne encephalitis (TBE) were exposed to various essential oils. Extracts from lemon balm, *Pimenta officinalis* (*P. dioica*) and *Artemisia dracuncululus* induced contrary reactions in infected and uninfected ticks. Uninfected ticks were repelled, infected ticks were attracted while the effect on the two sexes was different. An extract from *Populus nigra* had contrary effects on the males and females of *Dermacentor* ticks.

### ANTIMICROBIAL ACTIVITY

Besides essential oils of other plants (*Thymus daensis*, *Artemisia absintium*, *Matricaria chamomilla*, *Allium sativum*) different concentrations of lemon balm essential oil were tested on agar disks (FIROUZI et al. 1998). The essential oil was efficient at a dilution 1:2,560 and its antibacterial activity against a number of microorganisms was the highest of all tested essential oils.

Microatmospheric techniques were used to investigate the antimicrobial activity of lemon balm, *Lavandula officinalis* (*L. angustifolia*) and *Rosmarinus officinalis*. *L. officinalis* essential oil was more efficient against filamentous fungi than other oils. The essential oil from lemon balm showed higher antimicrobial activity (against bacteria and fungi) than other oils (LARRONDO et al. 1995).

The lemon balm essential oil inhibited the growth of *Staphylococcus aureus*, *Streptococcus faecalis* and *Candida albicans* (MIC values 100, 250 and 300 µg/ml), and of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* to a smaller extent MIC values 100,

250 and 300 µg/ml). The constituents with highest antimicrobial activity were citronellol, beta-caryophyllene, thymol, carvacrol and citronellal (KEDZIA et al. 1994).

### EFFECTS ON ENZYMES

Aqueous extracts of lemon balm (without tannins) strongly inhibit protein biosynthesis in cell-free suspensions of rat ribosomes (CHLABICZ et al. 1984). Peptidyl transferase inhibition accounts for about 30% of the inhibitory effects. The principle of these activities was not identified at that time.

Lipoxygenase products, identified as dienic acids, with the capacity to inhibit human blood adenylate cyclase, were detected in dried material of *Glechoma hederacea*. The presence of hydroxylated polyunsaturated fatty acids was investigated in lemon balm (as well as in *G. hederacea*, *Lamium galeobdolon* [*Lamiastrum galeobdolon*], *L. purpureum*, *Monarda didyma* and *Ajuga reptans*). The main active constituent was identified as (9S, 10E, 12Z, 15E)-9-hydroxy-10,12,15-octadecatrienic acid. This acid was localized mainly in cellular esters of leaf lipids (KUHNN et al. 1989).

Besides the extracts from *Lycopus virginicus* and *Lithospermum officinale*, aqueous extract of lemon balm was tested for enzymic inhibition, specifically for enzymic T4-5'-deiodization (AUF'MKOLK et al. 1984). The intensity of extract effect depends on extraction method and plant species. The effect was increased by repeated extraction of lyophilized dry matter of the original extract, the effect is ascribed to secondary metabolites such as rosmarinic acid, ellagic acid and luteolin-7 β-glucoside. Simple diphenolic substances such as cinnamic acid are inactive. A hypothesis that both deiodizations are caused by one enzyme is supported by inhibition of both deiodinases-5' and 5.

Hydro-methanolic extract of *Melisa officinalis* (as well as of *Salvia officinalis* and *Lavandula officinalis*) was tested as an inhibitor of enzyme-dependent and enzyme-independent lipid peroxidation. The active constituents are supposed to be phenolic compounds of the type caffeic acid and its derivatives (HOHMANN et al. 1999).

Rosmarinic acid at a concentration 10<sup>-6</sup> mol/l was found to be an inhibitor of C3-convertase with anti-inflammatory activity (ENGLBERGER et al. 1988).

The above finding was confirmed later (PEAKE et al. 1991) by detailed research on the inhibition of degradation processes caused by convertases.

### ANTIVIRAL ACTIVITY

Hydrous extract of lemon balm has always been used for its antiviral effects as a diaphoretic and stimulant in pharmaceutical drugs to treat skin irritation, in form of emulsion or solution (ZIVANOVIC et al. 1990).

A comparison of the sensitizing effect of lemon balm extract with some antiviral substances (e.g. tromantidine) used for herpes treatment showed that the effect of the former on the skin of pigs was very weak (HAUSEN, SCHULZE 1986).

It was specified by later research (DIMITROVA et al. 1993) that some of the lemon balm extracts showed high anti-

herpes (HSV-1) activity: the herpes was inactivated for 24 hours. The extracts were demonstrated to contain caffeic, rosmarinic and ferulic acids.

A lemon balm cream containing 1% of dried extract was successfully used for herpes treatment. In comparison with placebo, the lemon balm cream shortens the healing time and significantly reduces other herpes symptoms (KOYTCHIEV et al. 1999).

Extracts and ethanol extracts from 19 plants were tested for their activity against the virus ECHO9 Hill. The antiviral activity against this virus was detected in lemon balm extract (SKWAREK et al. 1994).

The anti HIV-1 activity of lemon balm extract is very interesting. The lemon balm was highly active in a complex of 46 plants, the extracts of which were tested for this activity. It is peculiar that the antiviral activity was detected in a complex of polar water-soluble constituents while the essential oil did not show any such effects. According to YAMASAKI et al. 1998 the activity consists in HIV-1 inhibition by reverse transcriptase.

#### ANTIHORMONAL ACTIVITY

Ethanol extracts from leaves of *Melissa officinalis*, *Lithospermum officinale*, *Lycopus virginicus* and *Thymus serpyllum* were used to test their activity against TSH (hypophyseal hormone) secretion and TSH-blocking activity in rats (SOURGENS et al. 1982). Besides the lemon balm extract, the antihormonal effects of extracts from *Thymus serpyllum*, *Leonurus cardiaca* and *Rosmarinus officinalis* were confirmed in a subsequent paper (SOURGENS et al. 1986).

While in the original paper (CHLABICZ, GALASINSKI 1986) caffeic acid and another unidentified substance were described as important constituents of lemon balm influencing protein biosynthesis *in vitro*, and potentially having the antitumoral activity, their activity was specified in greater detail ten years later. Lemon balm glycosides, and caffeic acid contained in them, are active against extension factor eEF-2 (GALASINSKI et al. 1996). The authors believe that these inhibitors of protein biosynthesis could be used in oncogenic drugs.

#### ANTIMUTAGENIC ACTIVITY

Hydrous extracts from different samples of foods and plants were tested for antimutagenic activity against 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (Trp-P-1) in *Salmonella typhimurium*. Antimutagenic activity was demonstrated in all tested aromatic plants. The capacity of extract prepared from lemon balm to inactivate Trp-P-1 was high (UEDA et al. 1991).

The knowledge of lemon balm activity was complemented by a study of mutagenic (genotoxic) effects of its extract on *Aspergillus nidulans* (RAMOS RUIZ et al. 1996): these effects were zero.

#### ANTIFUNGAL ACTIVITY

Tests of the activity of essential oils from 28 plants against the fungal species *Microsporum gypseum*, *Trichophyton*

*equinum* and *T. rubrum* demonstrated that the essential oils of *Melissa officinalis*, *Anethum graveolens*, *Artemisia maritima*, *A. nilagarica*, *Cymbopogon flexuosus*, *C. winterianus*, *Cyperus scariosus*, *Santalum album*, *Trachyspermum ammi* and *Vetiveria zizanioides* were toxic to one, two or all tested organisms (DIKSHIT, HUSAIN 1984). Five out of the active essential oils had antifungal effects.

#### OTHER ACTIVITIES

The effects of lemon balm essential oil and extracts from *Lavandula off.* and *Rosmarinus off.* on *Candida albicans* morphology were jointly studied by scanning electron microscopy. Besides against *C. albicans*, the above oils were active against *Spermatophyta*, *Eumycota* and *Deuteromycotina* (LARRONDO, CALVO 1991). The antifungal activity of the plant essential oils in question was not confirmed in this paper.

Low antispasmodic activity was observed in extracts from *Melissa* as well as from *Mentha piperita*, *Rosmarinus officinalis*, *Matricaria chamomilla*, *Foeniculum vulgare*, *Carum carvi* and *Citrus aurantium* (FORSTER et al. 1980).

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## *Melissa officinalis* L. – meduňka (opravdu) lékařská

**ABSTRAKT:** Práce obsahuje literární údaje o účincích meduňky, publikované v rozsahu přibližně posledních 15 let. Ze zpracovaného souhrnu vyplynulo, že účinné jsou jak extrakty, získávané destilací s vodní parou s následnou extrakcí nevodnými rozpouštědly, tak extrakty vodné a vodně alkoholické. Zatímco první z nich obsahují soubor převážně mono-, seskvi- a diterpenických látek, ve vodných extraktech byly nalezeny mnohé flavonoidy a polyfenolické látky včetně derivátů kyseliny kávové – melitrové a rozmarýnové kyseliny, která je depsidem kyseliny kávové. Z anti-aktivit uvedených extraktů byly již vedle antimikrobiálního, antifungálního a antihormonálního působení zaznamenány významné účinky antivirové, účinky na některé enzymy, a dokonce i účinek proti HIV a antimutagenní aktivita. Proto je meduňka, resp. extrakty z ní, mnohostranně pozitivně působícím faktorem a významnou součástí alternativní medicíny.

**Klíčová slova:** *Melissa officinalis* L.; silice; extrakty; obsahové látky; účinky

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## Anthocyanins and carotenoids – major pigments of roses

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**ABSTRACT:** The major pigments of roses could be divided into two groups – carotenoids and anthocyanins. For the yellow and orange coloured roses carotenoids are typical. The dominant compounds found are lycopene, rubixanthin, taraxanthin, lutein and zeaxanthin.  $\beta$ -carotene, its epoxides, auroxanthin, and from  $\alpha$ -carotene derived flavoxanthin and chrysanthemaxanthin were found in all growth stages. Some of the structures were found in a limited number of roses – rosaxanthin and rubixanthin. In some roses (the floribunda rose All Gold) retinol was found. For pink, red or purple coloured roses both anthocyanins and carotenoids are typical. The ratio of carotenoids and anthocyanins determines the shade of rose colour. Among anthocyanins, cyanidin glycosides (cyanin, cyanidin-3-O-glucoside, cyanidin-3-O-malonylglucoside and cyanidin-3-O-malonyllaminaribioside) and peonidin glycosides are present. They are frequently copigmented with galloannin, chlorogenic acid, protocatechuic acid, etc. The main flavonoids are isoquercitrin, quercetin and tiliroside. In flowers and hips of the dog-rose (*Rosa canina* L.) the number of present compounds is higher – from polyphenols there are catechin, epicatechin, galloocatechin, isoquercitrin, kaempferol-3-O-(*p*-coumaroylglucoside), kaempferol-3-O-glucoside and vanillin, and from carotenoids lycopene, rubixanthin, taraxanthin, lutein and zeaxanthin. Ascorbic acid levels in hips of the dog-rose are considerably higher than in those of *Rosa* spp. Ascorbic acid reduces anthocyanins to colourless leucoanthocyanidins and could decrease the stability of anthocyanins. Most represented are tannins (in average 23,500 mg/kg in hips, 180,000 mg/kg in leaves), polyphenols (23,300 mg/kg in hips) and leucoanthocyanidins (15,500 mg/kg in hips).

**Keywords:** roses; dog-rose; carotenoids; anthocyanins; ascorbic acid; stability of pigments; use

Pigments of flowers belong to different chemical classes and structures (GOODWIN 1965). Among them the main classes represent carotenoids, anthocyanins, flavonoids, chalcones and aurones, anthraquinones, etc. The nature of pigments can affect the stability of certain flower pigment and changes in colouration of ornamental plants during their storage and use. In *Rosa* spp. carotenoids, anthocyanins and flavonoids were found to be the dominant pigments.

### CAROTENOIDS IN ROSES

VALADON and MUMMERY (1968a,b) analyzed flowers of ten cultivars of yellow and orange roses, two *Posentilla* spp. and two *Geum* spp. for their carotenoid contents.  $\beta$ -carotene and  $\beta$ -carotene oxide mutatochrome were found in all cultivars. Lutein, the leaf xanthophyll, was found in small amounts in all flowers except in four cultivars of roses. Epoxy carotenoids were obtained in fairly large amounts. Those identified were phytoene, phytofluene,  $\beta$ -,  $\gamma$ -,  $\delta$ -carotene,  $\beta$ -zeacarotene, and lycopene. Rubixanthin, a specific pigment of rose hips, was found in eight of the roses but not elsewhere. In six rose cultivars a

yellow pigment was found. Its trivial name is rosaxanthin. As the main four carotenoids were evaluated  $\beta$ -carotene, auroxanthin, 5,6,5',6'-diepoxide of  $\beta$ -carotene, flavoxanthin and chrysanthemaxanthin (VALADON, MUMMERY 1969). GOODWIN (1965) reported that in the genus *Rosa* the qualitative distribution is similar in many species and rubixanthin is the dominant pigment. Data suggest that roses are yellow or orange depending upon which carotenoids (acyclic and with rings) and the ratio in which they are present (Figs. 1 and 2). VALADON and MUMMERY (1969) found in anthers of fully opened flowers, anthers and styles of ripe hips from floribunda, hybrid tea and dog-rose (*Rosa canina* L.) sixteen carotenoids in flowers and twenty two ones in hips.  $\beta$ -carotene and its epoxides, and from  $\beta$ -carotene derived xanthophyll, auroxanthin and from the  $\alpha$ -carotene derived flavoxanthin and chrysanthemaxanthin were found in all stages studied. With the age the control of carotenoid synthesis was removed and oxidation processes took place, leaving very few members of the  $\alpha$ -carotene series, and giving as main constituents the epoxy carotenoids and their derivatives. Rubixanthin (3-hydroxy- $\gamma$ -carotene) characteristic in rose hips, was abundant in anthers and styles as derived from

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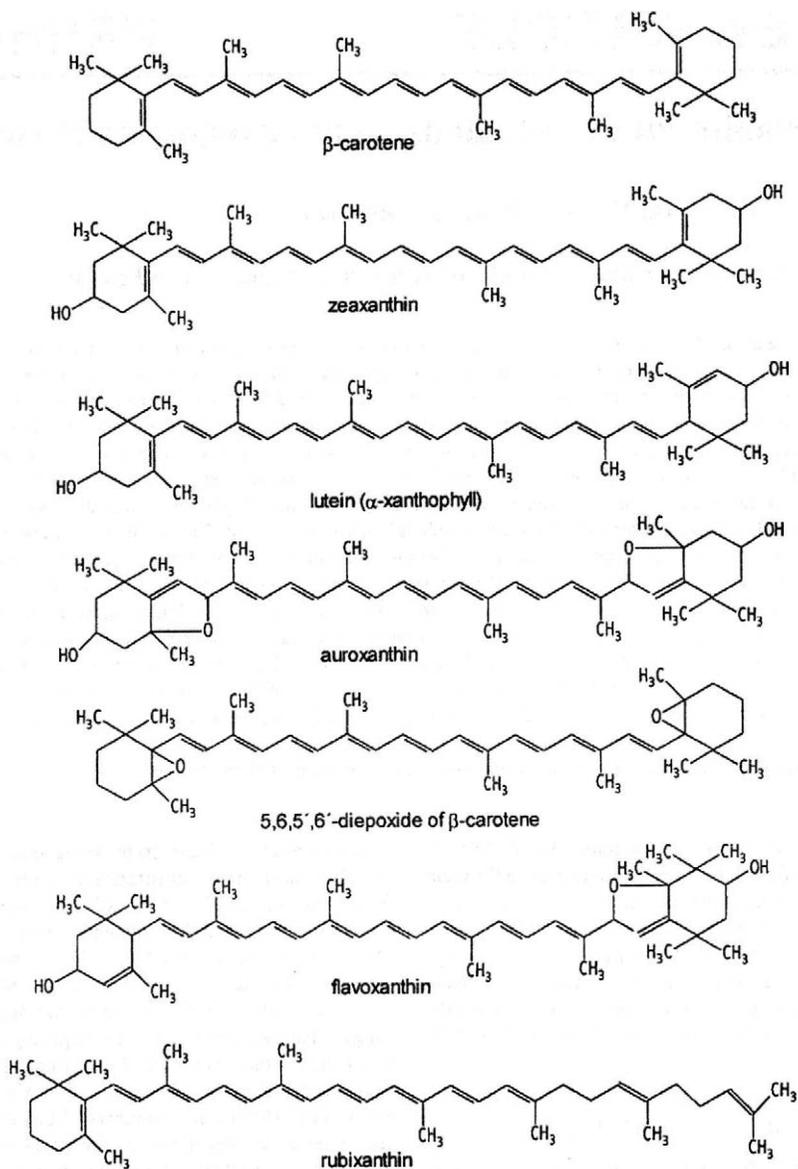


Fig. 1. Major carotenoids of yellow-orange roses with rings

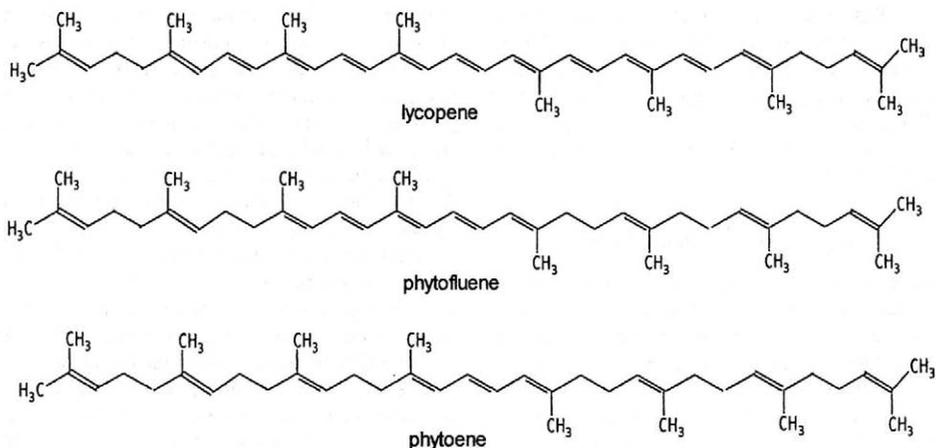


Fig. 2. Dominant acyclic carotenoids of yellow-orange roses

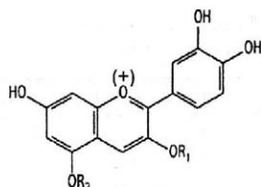
$\gamma$ -carotene. Only the hips of the floribunda rose All Gold contained retinol. It was found that yellow roses contain only carotenoids.

#### ANTHOCYANINS AND THEIR COMPLEXES IN ROSES

In pink flowers of *Rosa rugosa* both anthocyanins and carotenoids were found. Among anthocyanins the pure peonidin is bound in the form of glycosides (GOODWIN 1956). The relative quantity of carotenoids in which they are present, and the noncarotenoid pigments, mainly anthocyanins (Fig. 3) determine the tinge of roses (VALADON, MUMMERY 1968b). In the genus *Rosa* a mixture of glycosides derived from cyanidin and peonidin was found (*Rosa rugosa* and its hybrids), whereas carmine coloured roses contained only cyanidin glycosides (HARBORNE 1963). Copigmentation is a further factor controlling the flower colour in the genus *Rosa*. Lily and purple coloured cultivars (e.g. Reine de Violette) containing cyanin (cyanidin-3,5-O-diglucoside) were investigated. In the cultivars

cyanin was copigmented in greater deal with gallotannin and the spectral shift ranged from 507 to 512 nm (HARBORNE 1963). This copigmentation causes blue shade of certain roses. Copigmentation complexes of cyanidin-3-glucoside, cyanidin-3-malonylglucoside and cyanidin-3-malonyl-laminaribioside with chlorogenic acid and protocatechuic acid were studied intensively (DONNER et al. 1998; DAVIES, MAZZA 1993). The series of twelve structurally related anthocyanins, extracted from the red-purple flowers of *Dendrobium* "Pramot", the pink flowers of *Senecio cruentus*, the orange-red flowers of *Sophronitis coccinea*, the red-purple flowers of *Phalaenopsis* and also red-purple flowers of *Laeliocattleya* cv. Mini Purple and *Bletilla striata* allowed the confirmation of the existence of strong intra molecular copigmentation effects (FIGUEIREDO et al. 1998). Copigmentation of anthocyanins and chlorogenic acid influences the colour of strawberry and chokeberry juices (WILSKA-JESZKA, KORZUCHOWSKA 1996). Anthocyanins having a catechin or pyrogallol group in their structure are widespread in flowers and fruits as glycosylated derivatives of cyanidin, delphinidin and petunidin chromophores. In particular, flowers have developed very efficient strategies in the production of colour which led to the biosynthesis of structurally very complicated bio-organic pigments, e.g. with  $Al^{3+}$  or  $Ga^{3+}$  (ELHABIRI et al. 1998). The important role that anthocyanins could play as antioxidants in living systems may be well explained by their regulation of iron uptake and activity within the most complicated biological media. Values of a complexation constant lie between 85 (5,7-dihydroxy-3,4'-dimethoxyflavylium) to 700 for malvin (GEORGE et al. 1998).

Other types of complexes are interactions between anthocyanins and different flavan-3-ols, as it was studied in young red wines (SANTOS et al. 1998). Formation of complex pigments and its influence on colour were investigated in more stable complex pigments between malvidin-3-O-glucoside and (-)-epicatechin, (+)-catechin, and procyanin-



$R_1 = \text{Glu}, R_2 = \text{Glu}$	cyanin
$R_1 = \text{Glu}, R_2 = \text{H}$	cyanidin-3-O-glucoside
$R_1 = \text{Malonylglu}, R_2 = \text{H}$	cyanidin-3-O-malonylglucoside
$R_1 = \text{Malonylaminaribioside}, R_2 = \text{H}$	cyanidin-3-O-laminaribioside

Fig. 3. Dominant glycosides of cyanidin in red roses

dins (epi-4,8-epi and cat-4,8-cat). Other examples are major pigments in purple lisanthus (*Eustoma grandiflorum*) – delphinidin-3-O- $\beta$ -D-[6-O- $\alpha$ -L-rhamnolactoside]-5-O- $\beta$ -D-[(E and Z)-p-coumaroylglucosides] (MARKHAM et al. 1998). In *Rosa canina* L. tiliroside (7-O-p-coumaroyl-3-O-glucoside of kaempferol) was reported by GOODWIN (1965).

### STABILITY OF ANTHOCYANINS

The interest in the use of anthocyanins as food pigments has increased in recent years (BRIDLE, TIMBERLAKE 1997), however, these colours are affected by a number of factors (FRANCIS 1989). Nearly all reports treating colour and stability of pure anthocyanins under pH influence deal with acid or neutral solutions (BROUILLARD 1988). A comparison between the colour intensity and stability of petanin, i.e. petunidin-3-[6-O-(4-O-E-p-coumaroyl-O- $\alpha$ -rhamnopyranosyl)- $\beta$ -glucopyranoside]-5-O- $\beta$ -glucopyranoside and the anthocyanidin 3-monoglucosides revealed rather big differences (ANDERSEN et al. 1998). After five days at pH 8.1 the visible  $\lambda_{\max}$  absorption of petanin was similar or higher than the corresponding absorption of fresh cyanidin-3-glucoside solutions at any pH. Anthocyanins with two or more OH-groups in ortho-position to each other on the anthocyanidin B-ring showed dramatic hypsochromic shifts ranging from pH 8.0 to 9.0 in comparison with anthocyanins with different number of methoxyl groups, however, with only one OH-group on the B-ring (pelargonidin, cyanidin, peonidin vs. delphinidin, petunidin and malvidin-3-O-glucosides). The pH values of the fluid in pigment-bearing tissues of flowers could be different, e.g. alkaline for cornflowers and acidic for roses. Acylation of cyanidin-3-O-sophoroside-5-O-glucoside with p-coumaric, ferulic, sinapic and malonic acids or their two molecules in red cabbage caused greater stability of these glycosides (BUCKENHÜSKES et al. 1988; LACHMAN et al. 1991).

Many recent works deal with floriculture and landscaping of flowers. Among them the important role play roses (BOSE et al. 1999; PEGRUM 1990; JOYCE et al. 1998). Many of them are dealing with drying of flowers that artificially eliminates some weather hazards and provides opportunity for much smoother marketing of the product (ROBERTS et al. 1998; THAL 1991; EPHRAUMS, IRONSIDE 1992). As reported BASAPPA et al. (1991) each drying method produced different shades of flower colour in the colouration of *H. bracteatum* ranging from canary yellow to cream, orange, copper, crimson red, carmine, amethyst and rose pink. The effects of different drying temperatures were investigated (room, full sun or in the oven at 40 or 50°C). A solar-powered drier was described by SILHOL and DENIS (1994). The drier has to operate at moderate temperatures in order to preserve the colour, flavour and active ingredients of the plant material. ORDUNO-CRUZ and BALTAZER-BERNAL (1995) referred about the effect of different mixtures of sand and borax on the drying of three flower species. Rose, gerbera and carnation flowers were dried for 10, 15 or 20 days in river or sea

sand mixed with borax in various proportions (sand:borax ratios of 1:1, 2:1 or 3:1). Moisture loss was measured and flower quality was assessed in terms of blemishes, texture, general appearance, etc. River sand containing a high proportion of borax and drying time of 15 or 20 days were considered as the best conditions for roses and carnations.

Anthocyanins can be divided into two groups based on the possibility to form intramolecular copigmentation, e.g. *Gymioa bicolor*  $\times$  *Hibiscus* (TSAI et al. 1992). Red anthocyanins from *G. bicolor* possessed better stability toward heating, oxygen and ascorbic acid. The extracts of *Hibiscus sabdariffa* (*Malvaceae*) and its main coloured components were evaluated as a red colour additive. From the colourimetric determination the results revealed existence of a linear relationship between absorbance and concentration of the colour solution at absorption maximum  $\lambda_{\max} = 518$  nm (NOUR et al. 1993). It was due delphinidin-3-O- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranoside (SATO et al. 1991). KOSWIG and HOFSSOMMER (1995) stated that the anthocyanin fingerprints of different products such as blackberry, strawberry, blood orange and blueberry obtained by reversed-phase high-pressure liquid chromatography or a RP-18 column with gradient elution are sufficiently differentiated.

TSAI and ANDI (1996) determined in the roselle (*Hibiscus sabdariffa* L.) with the most intensive red colour that after drying and storage at room temperature (about 25°C) and low temperature (5°C) under light or in the dark the colour remained relatively stable for eight months. The stability of anthocyanins is now very intensively studied (LIN, LI 1992; RASTELLI 1993).

### USE OF ROSES IN FOODS

Being a true member of the apple family, rose hips coloured orange, red or purple during autumn (FRANCIS 1975; HRAZDINA 1981), are edible. They are very rich in vitamin C (Table 1). Stability of anthocyanins is effected negatively by ascorbic acid, because they are reduced to colourless leucoanthocyanidins (LACHMAN et al. 2000). Also rose petals could be sprinkled on salads, used as garnish, or made into wonderful rose-petal jelly or rose-petal wine. *Rosa rugosa alba* is appreciated for its taste.

Table 1. Content of ascorbic acid and polyphenols in *Rosa* spp. and dog-rose (*Rosa canina* L.) hips

Compound	<i>Rosa</i> spp. (mg/kg)	<i>Rosa canina</i> L. (mg/kg)
Ascorbic acid	1,000–12,500 (F)	7,400–25,000 (F)
Catechins	8,000–9,100 (F)	15,000 (R)
Flavonoids	100–3,500 (F)	100–3,500 (F)
Leucoanthocyanidins	13,500–17,500 (F)	*
Polyphenols	20,200–26,400 (F)	*
Pyrogallotannins	*	15,000 (R)
Tannins	*	20,000–27,000 (F), 100,000–260,000 (L)

F – fruits (hips); L – leaves; R – roots; \* – not determined

According to Duke's data (Table 1, DUKE 1992), the dog-rose (*Rosa canina* L.) hips contain on average more ascorbic acid (7,400–25,000 mg/kg) and the same amount of flavonoids (100–3,500 mg/kg) in comparison with *Rosa* spp. (1,000–12,500 mg/kg ascorbic acid, 8,000–9,100 mg/kg catechins). Roses have high polyphenol content in hips (20,200–26,400 mg/kg), *Rosa canina* L. has high content of tannins (20,000–27,000 mg/kg in hips and 100,000–260,000 mg/kg in leaves). Among carotenoids (Table 2), the most common is  $\beta$ -carotene in dog-rose (25–62 mg/kg). A survey of major polyphenols and carotenoids is given in Table 3.

Table 2. Content of carotenoids in *Rosa* spp. and dog-rose (*Rosa canina* L.) hips

Compound	<i>Rosa</i> spp. (mg/kg)	<i>Rosa canina</i> L. (mg/kg)
$\beta$ -carotene	*	25–62 (F)
Carotenoids	100–500 (F)	*

Table 3. Major polyphenols and carotenoids in *Rosa* spp. and dog-rose (*Rosa canina* L.) hips

<i>Rosa</i> spp.	Polyphenols	Compounds
		isoquercitrin (F), quercetin (F), tilioside (F)
<i>Rosa canina</i> L.	polyphenols	catechin (F), epicatechin (F), galloocatechin (F), isoquercitrin (F), kaempferol-3-( <i>p</i> -coumaroyl-glucoside) (F), kaempferol-3-O-glucoside (F), vanillin (F)
<i>Rosa canina</i> L.	carotenoids	lycopene (F), rubixanthin (F), taraxanthin (F), xanthophyll (F), zeaxanthin (F)

## CONCLUSION

In flowers and fruits of roses (*Rosa* spp.) the dominant pigments are carotenoids, anthocyanins and flavonoids. Yellow and orange coloured roses are rich in acyclic and with rings carotenoids – in the anthers of fully opened flowers, anthers and styles of ripe hips from floribunda roses, hybrid tea roses and dog-rose sixteen carotenoids in flowers and twenty two ones in hips were found. The major constituents are lycopene, rubixanthin, taraxanthin, lutein and zeaxanthin.  $\beta$ -carotene, its epoxides, auroxanthin, and from the  $\alpha$ -carotene derived flavoxanthin and chrysanthemaxanthin were found in all stages. Carotenoids are contained in *Rosa* spp. fruits in range 100–500 mg/kg, the most contained  $\beta$ -carotene in the dog-rose in the range 25–62 mg/kg.

In pink flowers of the genus *Rosa* peonidin and its glycosides, glycosides of cyanidin (cyanin, cyanidin-3-O-

malonylglucoside and cyanidin-3-O-malonyllamariboside) were detected. These glycosides are frequently copigmented with gallotannin, chlorogenic acid, and protocatechuic acid, resp.

The dominant flavonoids in *Rosa* spp. are isoquercitrin, quercetin and tilioside, in the dog-rose catechin, epicatechin, galloocatechin, isoquercitrin, kaempferol-3-(*p*-coumaroylglucoside), kaempferol-3-O-glucoside and vanillin were reported. The dog-rose is very rich in catechins, pyrogallotannins as well as tannins, especially in the leaves. It is richer in ascorbic acid in comparison with *Rosa* spp. It confirms and justifies the use of roses, esp. their hips, also as food constituents, esp. in salads, jelly or wine.

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## Anthokyany a karotenoidy – dominantní barviva u růží

**ABSTRAKT:** Hlavní barviva růží mohou být rozdělena do dvou skupin – na karotenoidy a anthokyany. Pro žlutě a oranžově zbarvené růže jsou typické karotenoidy. Jako hlavní látky byly identifikovány lykopen, rubixanthin, taraxaxanthin, lutein a zeaxanthin.  $\beta$ -karoten a jeho epoxidy, auroxanthin a od  $\alpha$ -karotenu odvozený flavoxanthin a chrysanthemaxanthin byly nalezeny ve všech růstových stadiích. Některé struktury byly nalezeny pouze u omezeného počtu růží – rosaxanthin a rubixanthin. V některých růžích (růže floribunda All Gold) byl stanoven retinol. Pro růžově, červeně nebo purpurově zbarvené růže jsou typické jak anthokyany, tak i karotenoidy. Relativní množství karotenoidů a anthokyanů určuje odstín zbarvení růží. Mezi anthokyany jsou zastoupeny peonidin ve formě glykosidů, glykosidy kyanidinu (kyanin), 3-O-glukosid kyanidinu, 3-O-malonylglykosid kyanidinu a 3-O-malonyllaminaribiosid kyanidinu. Jsou často kopigmentovány s gallotaninem, chlorogenovou kyselinou, protokatechovou kyselinou aj. Hlavními flavonoidy jsou isokvercitrin, kvercetin a tilirosid. V květech

a plodech šípku (*Rosa canina* L.) je počet složek vyšší – z polyfenolů je to katechin, epikatechin, gallokatechin, isokvercitrin, 3-O-p-kumaroylglukosid kempferolu, 3-O-glukosid kempferolu a vanillin, z karotenoidů lykopen, rubixanthin, taraxanthin, lutein a zeaxanthin. Šípky jsou mnohem bohatší na askorbovou kyselinu ve srovnání s *Rosa* spp. Askorbová kyselina redukuje anthokyany na bezbarvé leukoanthokyanidiny a může tak snižovat stabilitu anthokyanů. Nejvíce zastoupené jsou taniny (průměrně 23 500 mg/kg v plodech, 180 000 mg/kg v listech), polyfenoly (23 300 mg/kg v plodech) a leukoanthokyanidiny (15 500 mg/kg v plodech). Růže mohou nalézt využití nejen jako dekorativní květiny, ale také jako složky potravin v salátech, jako ozdoba jídel nebo po zpracování ve formě rosolu nebo vína.

**Klíčová slova:** růže; šípková růže; karotenoidy; anthokyany; askorbová kyselina; stabilita barviv; využití

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## Peppers: Vegetable and Spice Capsicums

P.W. BOSLAND, E.J. VOTAVA

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This book is part of series which examines economically important horticultural crops from temperate, subtropical and tropical climatic areas. Emphasis is placed on the scientific principles underlying crop production practises rather than providing empirical recipes for uncritical acceptance. Nowadays, increasing interest can be attributed to peppers (*Capsicum* spp.), which are used not only as vegetables and as flavourings in food products, however also as ornamentals, in cosmetics, pharmaceuticals and some other special uses e.g. to colour flamingos in ZOO.

Book consists of ten chapters, each covering all aspects of production, from background physiology and breeding, to propagation and planting, through husbandry and crop protection, to harvesting, handling and storage. Preface and introduction deal with problems of common name of peppers (or chilli?), showing their history and describe in detail all important attributes of their popularity. Taxonomy, pod types and genetic resources are topics of second chapter. Three *Capsicum* complexes involved domesticate or wild *Capsicum* species (viewed as a primary pool of genetic diversity). Only five species of genus *Capsicum* are domesticated (from totally 25): *C. pubescens* (*C. pubescens* complex), *C. baccatum* (*C. baccatum* complex), *C. annuum*, *C. chinense* and *C. frutescens* (all in *C. annuum* complex). Many species with restricted distribution contain genes for adaptation to unusual environmental conditions as well as disease resistance thus wild *Capsicum* species could be used for breeding for disease resistance.

Chapters 3 and 4 deals with morphology, growth, flowering, pollination and development of fruits of pepper plant, including special chapter only about seeds (seeds formation, production, dormancy and germination). Chapter 5 is focused on pepper genetics, breeding and biotechnology, all of them strictly depends on type of peppers and their final use. A variety of breeding methods can be used to produce new pepper cultivars (mass selection, backcrossing, recurrent selection, mutation breeding). In this chapter are mentioned also hybrid seed production and interspecific hybridization. Biotechnology is being used in pepper to develop new cultivars, map genomes and evaluate genetic resources (isozymes, molecular markers, RFLPs, RAPDs). Genetic engineering in pepper is dependent upon a reliable methods of transformation and tissue culture regeneration. Genetically engineered pepper is not commercially available because of the lack of a transformation system. However, tissue culture of pepper

have standardized protocol, and can include anther culture, protoplast regeneration, embryo rescue and organogenesis.

Chapter 6 in details discussed chemical composition and some special nutritional quality of peppers (as a pungency). Some important facts, as medical used of peppers (e.g. to alleviate pains) and nutrition quality – mainly as important and rich source of vitamin A, C and others are mentioned. The important part of book is chapter 7, which is focused on production. Pepper production occurs in humid tropics, to the dry deserts, to the cool, temperate climates. Pepper can be grown as annual or a perennial crop, outside in the fields or under greenhouses. This adaptability of pepper to growth in wide range of climates, brings wide spectrum of production methods. Authors discussed climate conditions (temperature, moisture or hydroponic cultures in greenhouses, light, CO<sub>2</sub> enhancement), soil type, preparing the land for peppers and compared advantages of some methods as direct seeding or by transplanting of seedlings, use of mulches (for reducing weeds in the field), using of optimal fertilizers, growth regulators and mycorrhizal fungi, integrated pest management.

Next two chapters – 8 and 9 – are concentrated on harvesting and postharvest handling. In first of them are discussed advantages and disadvantages of hand-picking and machine harvesting. In latter there are emphasised eight major areas dealing with postharvest handling of peppers. Peppers can be fresh, canned, brined/pickled, frozen, fermented, dehydrated and extracted for oleoresin.

In chapter 10 is summarized information about main diseases and pests of peppers, with emphasis on fact that every region has its specific diseases and pests. Attention is also paid on abiotic disorders, as air pollution, extreme temperatures, moisture, nutrients, pH, salt problems and wind injury. Book includes also a comprehensive and recent bibliography; index of technical and botanical terms, English and Latin names of plant species, pathogens and pests.

Without any doubt the book provides comprehensive and recent coverage on all aspects of the plant's history, genetics, chemical composition, production, harvesting and related diseases and pests, including large amount of important references. The content of the book will be welcome by university students with horticulture or agriculture courses, as well as is valuable for progressive growers, hobby gardeners, breeders and plant scientists.

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