SHORT COMMUNICATION

Cultivation of *Cypripedium calceolus* L. *ex vitro* seedlings in outdoor conditions

J. Obdržálek

Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Průhonice, Czech Republic

ABSTRACT: The paper presents first positive results of the experiment with three-year outdoor cultivation of *Cypripedium calceolus* L. *ex vitro* seedlings in the Czech Republic. They were propagated *in vitro* from mature seeds of Carpathian provenance in a Prague private laboratory. In April 2006 after three months cool refrigerating at 4°C the seedlings were prepared for planting. The rhizomes with 4 to 12 roots and visible dormant buds were used. They were planted in two types of substrates: mixture AN on the basis of liadrain (burned clay pebbles) and mixture BN on the basis of granodiorite. Both mixtures were amended with perlite, pumice, sand, zeolite and dolomite lime powder. The mineral substrates proved to be stable and convenient for transfer and cultivation of *ex vitro* seedlings. Additional treatment with lignohumate in other two variants of the experiment did not improve the effect. The seedlings were grown outdoors on a shaded bed till the retracting leaves. They overwintered in a cold glasshouse with temperature close to zero from late November to March. The substrates did not visually influence the phase of sprouting, the phase of growth and retracting of the plants. At the end of the third growing season the yield of 4-year-old seedlings with two to four leaves ranged from 83% to 98% in four variants. In November 2008 seedlings were taken up from the mixes and were evaluated as bare root plants. The number of the living plants with visible new buds and the quality of root system were recorded and evaluated. The average length of roots in mixture A on the basis of liadrain and B on the basis of granodiorite was 14.5 cm and 12.1 cm, respectively. The rhizomes were planted into new mixtures immediately. These seedlings will be able to grow up to the blooming size during two or three seasons. Seven year-old potted seedlings of *C. calceolus* will be planted into gene resource area of the Silva Tarouca Research Institute for Landscape and Ornamental Gardening at Průhonice.

Keywords: *Cypripedium calceolus*; *ex vitro* seedlings; cultivation substrates; wintering; sprouting; buds; roots; yield

Cypripedium calceolus L. is the most striking terrestrial orchid in the Czech Republic. It belongs to the group of critically endangered plants of the Czech flora protected by the Act No. 114/1992, amendment II, § 2. *Cypripedium calceolus* is the only species of hardy orchids included in the European list of protected and endangered plant species according to the EU Council Regulation 338/1997 (Cites), Annex A (I). All other hardy species of the *Orchidaceae* family are listed in CITES Annex B (II). According the Act of the Czech Republic No. 100/2004 growers of *Cypripedium calceolus* have the duty of registration and the dealing of plants of unproved origin is prohibited.

Distribution and habitat

Cypripedium calceolus can be found in the mild humid till cold boreal zone of Eurasian forest region in the west and the north of Europe from England and western Scandinavia to the south over Pyrenees to Spain, across the Alps to the Balkans, in Central Europe, in the Carpathians, to the east

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from Ukraine to the Baltic countries, across Ural, Siberian taiga areas, mountains around the Baikal Lake, to Far East, northeast mountain regions of China and Korea (Cribb 1997; Averyanov 2000).

In the Czech Republic, two phytogeographical regions are recognized where *Cypripedium calceolus* naturally occurs – Pannonic Termophyticum and Bohemia-Moravian Mezophyticum, as described by Skalicky (1988) in Moravec (1994).

C. calceolus grows in partial shade of open woodland among shrubs, conifers and deciduous trees. In warmer areas of Central Europe it grows in light mixed forests with pine *(Pinus sylvestris*), hornbeam (*Carpinus betulus*) and open oak woodland with *Quercus petraea* and *Q. pubescens* (PQ – *Pino-quercetum*, Qp – *Quercetum pubescentis*). In the mountains of Central Europe it grows in submontane and montane zone of beech (*Fagus sylvatica*) woods often among hazels (*Corylus avellana*), i.e. (Fch – *Fagetum sylvaticae,* F – *Eu-Fagion,* FC – *Cephalantero-Fagion*). At higher elevation of Carpathians it grows on grassy stony slopes, usually on limestone in mix mountain forests with spruce (*Picea abies*) and beech (*Fagus sylvatica*) and rarely reaches up to the alpine zone of dwarf pine (*Pinus mugo*).

In Bohemia and Moravia it grows on mineral lime loam rich soils of loess type, rendzin, terra fusca, over limestone and argillite with pH 6–7.3 at the altitude of 280–500 m, in Carpathians up to 1,200 m and in the Alps up to 2,500 m (Cribb 1997; Sekerka et al. 2006).

Climate types and plant formation

Central and Eastern Europe lies in the temperate zone with a cold winter period – type of climate VI(VIII)b, VI(X)b (Walter, Lieth 1967), plant formation 7 (eastilignosa): deciduous and mixed forests, transitive to plant formation 8 (aciculignosa): coniferous woods.

C. calceolus is a hardy species according to its gene type in the boundary of the hardiness zone 3 to 7 with minimum temperature up to –40°C on Siberia and Far East. The climate in Central Europe corresponds to zones 5, 6, 7 with minimum winter temperatures from –12°C to –29°C (Sekerka et al. 2006).

Propagation

C. calceolus has been cultivated longer than any other slipper orchid. Traditionally, an easy way of propagation of *Cypripediums* is to divide clumps with developed buds, if it has a reasonable number. Also, a single growth and short rhizome with reserve buds can be cut from the clumps (Perner 1997). The best term for vegetative propagation is early spring (March/April) before sprouting of buds, and autumn (September/November). The generative method of propagation was used until recently by sowing seeds under mother plants. Using the technology *in vitro* under laboratory conditions enables to propagate *Cypripediums* in high quantities. *C. acaule*, *C. calceolus, C. macranthos, C. kentuckiense, C. parviflorum* var. *parviflorum, C. parviflorum* var. *pubescens, C. reginae* and their vital hybrids propagated *in vitro* are available as young seedlings and mature plants in registered enterprises in the Europe and overseas (Steele 1996; de Jong 2004; Lambe 2005; Malmgren 2005; Tullock 2005). *C. calceolus* is easy to propagate from half-mature seeds. They are hardy and vital even as very small plants, but they have very slow growth from seedling to flowering plant, often 7–8–9 years, which makes commercial propagation difficult (Malmgren 2005).

MATERIAL AND METHODS

Plant material

One-year-old vernalized *ex vitro* seedlings of *C. calceolus* (Carpathian provenance), propagated *in vitro* in a Prague private laboratory from mature seeds were used (Fig. 1). The removed rhizome of seedlings had four to twelve roots with a minimum length of 3 cm and had visible dormant buds (Fig. 2). They were moistened with distilled water and saved for three months in clean plastic bags in a refrigerator at 4°C to reduce dormancy. After the phase of cooling, 132 seedlings were planted in mineral-base substrates. Higher number of plants was not available for the first experiment. (*Cypripedium* seedlings do not produce leaves unless they have had a cold treatment with temperatures of about 4°C at least three months.)

Substrates and growing conditions

Ex vitro seedlings were planted in polystyrene trays, where two substrates and effect of lignohumate treatment were tested. Substrate A consisted of liadrain 30% vol., pumice 20% vol., perlite 30% vol., sand 10% vol., zeolite 10% vol. + dolomite lime powder 20 g/10 l (pH 6.4, conductivity 0.20 mS/cm).

Fig. 1. *Cypripedium calceolus ex vitro* seedlings after cold treatment (April 2006)

Substrate B consisted of granodiorite 30% vol., pumice 20% vol., perlite 30% vol., sand 10% vol., zeolite 10% vol. + dolomite lime powder 20 g/10 l (pH 7.7, conductivity 0.17 mS/cm). Lignohumate solution was applied in the ratio of 12.5 ml/5 l water per m^2 immediately after planting of seedlings. There were four variants: AN (substrate A, without lignohumate treatment), BN (substrate B, without lignohumate treatment), AL (substrate A, with lignohumate treatment), and BL (substrate B, with lignohumate treatment) in four replications. The number of plants was 42 for AN and BN variants, total seedlings 84; and 24 for AL and BL variants, total number of seedlings 48. (The chemical properties of substrates were evaluated according to the European Standards. Electric conductivity and pH value were determined in water leaches (1v:5v). Lignohumate solution as a source of humic acids for seedlings planted into mineral mixtures was used.)

The substrates were treated with biofungicide Supresivit (*Trichoderma harzianum*) by watering in the ratio of 2.5 g/10 l water per 2 m^2 immediately after planting of seedlings.

The plants were grown in trays on a shady bed under green screen textile (50% shading effect). On extremely hot sunny days with temperatures over 28°C the shading was strengthened by using a green textile with 75% shading effect and from the sunny side a polystyrene mat with light permeability 15% was placed. The plants were sprayed and watered only in cool mornings if needed.

Retracted seedlings overwintered from the end of November to March in a cold glasshouse with

Fig. 2. *Cypripedium calceolus* seedlings before planting (April 2006)

temperature above zero on the bed under contact white textile Pegatex (mass 45 g/m^2).

In March the seedlings were placed again on the shady outdoor bed. During the vegetation period the seedlings were fertilized with Kristalon blue (N 19, P_2O_5 20, K_2O 6, MgO 3), diluted liquid feeding in the dose of 10 ml of 15% concentrate per 10 l water/2 m^2 four times in 14 days intervals.

Prevention from fungi pathogens of genera *Pythium* was made with systemic fungicides Previcur® SL (active agent: Propamocarb) in the ratio of 0.15% – watering once in full summer.

Snails and slugs invasion were regularly controlled by slug pellets of metaldehyde.

Evaluation of the experiment

In May 2006 the number of sprouted seedlings of *C. calceolus* planted in four variants of mineral substrates was evaluated for the first time. After the first wintering under outdoor conditions (in April 2007) the percentage of sprouted 3-year-old seedlings was evaluated again. In November 2008 the seedlings were planted in new substrates. The numbers of living 4-year-old seedlings after three growing seasons before transplanting were recorded. The length of roots (in cm) and quality of plants were evaluated as well. The length of growing season (in weeks) since the sprouting of the seedling buds till retracting was recorded.

The results were evaluated statistically. The minimal conclusive difference was determined by the Duncan's multiple range test at the significance level of $\alpha = 0.05$ ($P = 95\%$).

RESULTS AND DISCUSSION

On April 20, 2006 *ex vitro* vernalized *Cypripedium calceolus* seedling rhizomes with four to twelve roots and visible dormant buds were planted in mineral base substrates. The plant buds started stretching within May 7 and May 20.

Plants retracted their two leaves in the middle of October after a temporary decrease of the morning temperatures below 0 (–2°C on October 17, 2006). The vegetation period of *C. calceolus* grown in outdoor conditions lasted 22 to 24 weeks in the first year.

In mild winter from December to March 7, 2006 the temperature in the glasshouse during the day ranged from 4°C to 18°C, during the night from 0°C to 12°C.

After first overwintering in the cold glasshouse the seedlings were grown in outdoor conditions on a shaded bed. The plants sprouted up between April 17 and 23. The grown up seedlings with two and three leaves are shown on Figs. 3 and 4.

In November 2008 after third growing season 4-year-old seedlings were taken up from the mixes and ware evaluated as bare root plants (Figs. 5 and 6).

The substrates did not visually influence the phase of sprouting, the phase of growth and retracting of the plants in the first two vegetative seasons. In the third growing season the stronger seedlings' shoots grew up with three to four leaves.

The significantly different percentage of the living seedlings was not observed when the variants of substrates were evaluated by one-way ANOVA. Survey of the yield of 4-year-old seedlings was 98% for plants grown in mixture AN (on the basis of liadrain with addition of pumice, perlite, sand, zeolite and dolomite lime powder) and 93% for seedlings grown in mixture BN (on the basis of granodiorite with addition of pumice, perlite, sand, zeolite and dolomite lime powder). Additional treatment with lignohumate in other two variants of the experiment did not improve the effect. Yield of seedlings grown in mixture AL as 92%, in mixture BL it was 83% (Table 1). When the sprouted green seedlings were evaluated by factors (of substrate and of treatment) by two-way ANOVA positive, significantly different results were obtained for the plants in untreated substrates N in the first and second growing season only (see Table 2).

Significant differences of both substrates were visible in the length of roots. The seedlings grown in mixture AN on the basis of liadrain had significantly stronger and longer roots from 7 cm to 29 cm (on average 14.5 cm) that those in mixtures BN and BL (Table 1, Fig. 5). The length of roots recorded in mixture B was from 6 cm to 20 cm (on average 12.1 cm). In the mixture with granodiorite the roots of the seedlings were shorter, in higher number, and several of them were also stronger.

The new roots of the seedlings grown in mineral substrates were whitish or pale yellowish with active tips and did not show any damage. The older roots of some seedlings mainly from mixtures B and BL were greyish with black tips (Figs. 3 and 5). The seedling rhizomes were with visible single buds. Six strong seedlings with two buds were observed in mixture A and 5 two-bud seedlings of the same quality in mixture B.

Both types of substrates (A and B) proved to be stable and convenient for transfer and cultivation

Fig. 3. *Cypripedium calceolus* 2-year-old seedlings grown in mineral substrates A (left), B (right) (September 2006)

of seedlings propagated *in vitro.* pH values and EC 1v:5v in water leaches determined on June 27, 2007 showed stability and good physical properties of the mineral compounds used. The values for mixture A were: pH 6.3, conductivity 0.20 mS/cm; for mixture B: pH 7.7, conductivity 0.17 mS/cm.

Extremely hot and dry summer weather was the main cause of difficulty and damages of *Cypripedium* seedlings during growing season. Shading and cooling as precautions towards high temperatures and direct sun as well as good drainage, water and air movement are necessary to prevent wilting and rotting of sensitive young seedlings (Perner 1997).

It seemed that better drainage effect and water and air regime for roots embodied mineral substrate A with liadrain (burned clay pebbles) than substrate B with granodiorite. Compound of granodiorite with higher pH contains more clay parts that may reduce aeration of the mixture in the rainy weather and during dry summer days when the adequate watering has to be very intensive.

The results are consistent with those reported elsewhere. The success of micropropagation and reintroduction programme depends on working on the broadest genetic base. In England, the last perishing population of *C. calceolus* was re-established by planting seedlings from *in vitro* culture into original localities. The seeds from vital natural populations of continental plants were used. Native seedlings were successfully used for the reintroduction programme

Fig. 4. *Cypripedium calceolus* 3-year-old sprouted up seedlings in mix B after overwintering (June 2007)

Fig. 5. *Cypripedium calceolus* 4-year-old seedlings after three growing season in outdoor conditions (September 2008)

(Cribb 1997). Seedlings cultivated for 2–3 years in mineral substrates are available in Belgium (Lambe 2005) and in Sweden (Malmgren 2005).

On the basis of these positive results of the first experiment with outdoor cultivation of *C. calceolus ex vitro* seedlings in the Czech Republic as well as the experience of foreign nursery growers, general requirements and practical recommendations were prepared.

Cold treatment

C. calceolus seedlings from *in vitro* culture do not produce leaves unless they have had a cold treatment (vernalization) with temperatures of about 4°C for at least three months (Perner 1997; de Jong 2004; Malmgren 2005).

– The best term for expedition and planting of *ex vitro* seedlings is autumn (September/November) or early spring (March/April) before the buds sprouting (Steele 1996; Perner 1997; de Jong 2004).
– In the experiment, *C. calceolus ex vitro* seedlings were planted after cold treatment in late April with good result.

Substrate and feeding

The substrates that are used to plant new seedlings must be completely inorganic. Any organic material present in mixes may promote growth of molds and consequently loss of seedlings. The suitable feeding regime would commence when the plants are unfurling leaves (de Jong 2004).

– Suitable growing mixtures contain stabile mineral ingredients as loam pellets, burned clay pebbles (Seramis®), pumice, vulca, perlite, vermiculite and sand (Cribb 1997; de Jong 2004; Lambe 2005; Weinert 2008).

Fig. 6. *Cypripedium calceolus* 4-year-old seedlings – detail of roots from mixture A

Table 1. Survey of yield percentage and root length of *Cypripedium calceolus* 4-year-old seedlings planted in mineral substrates after three growing seasons evaluated by one-way ANOVA and Duncan's multiple range test (2006/2008)

Variant	Number of seedlings	Sprouting (%)		Yield (%)	Root length (cm)	Leaves number
		2006	2007	2008	2008	
AN	42	100[a]	100[a]	98.0[a]	14.5[a]	3.3[a]
BN	42	100[a]	98.0[a]	93.0[a]	12.1[bc]	3.2[a]
AL	24	92.0[a]	92.0[a]	92.0[a]	12.8[ab]	3.1[a]
BL	24	88.0[a]	83.0[a]	83.0[a]	10.1[c]	2.6[a]

Values indicated by the same letter are not significantly different at P = 95%

– The substrates on the basis of Czech compounds as liadrain = ekogrit (burned clay pebbles) and granodiorite with addition of perlite, zeolite and sand proved to be stable and convenient for cultivation of seedlings propagated *in vitro*.
– Using of lignohumate as a source of humic acids for seedlings planted into mineral mixtures is possible.
– Dilute liquid feeding of green seedlings with orchid fertilizers (Dyna-Grow, Wuxal) in half concentrations is beneficial once a month from May to September (Perner 1997; Weinert 2008).
– The liquid feeding with Kristalon blue (N 19, P_2O_5 20, K_2O 6, MgO 3) in the dose of 10 ml of 15% concentrate during third growing season had a positive effect on growth of leaves and root system.
– 1–1.5 g of the slow release fertilizer Osmocote Plus (5–6) can be used. It ensures a constant availability of low levels of nutrients during growing season (de Jong 2004).
– Treatment of planted seedlings with biofungicide Supresivit (*Trichoderma harzianum*) is recommended.

Shading and cooling as precautions towards high temperatures and direct sun

Extremely hot and dry summer weather is the main cause of difficulty and damages of *Cypripedium* seedlings during growing period – in warm spells, wilting attracts fungi and bacteria. Good air movement is necessary to prevent rotting.
– Optimal daily temperature in the summer is 17°C to 20°C, maximum 25°C, with the humidity of 70% to 80%. At night the temperature should fall about 10°C. The humidity should not to fall below 40% along the summer period (Perner 1997).
– Shading success against solar shining green textile with 50 or 75% shady effect and polystyrene mats with light permeability 15% should be used.

Table 2. Yield percentage and effect of treatment on the growth of *Cypripedium calceolus* seedlings during three growing seasons evaluated by two-way ANOVA and Duncan's multiple range test (2006/2008)

Factors	Number of seedlings	2006	2007	2008	2008	
					root length (cm)	leaves number
Substrate		0.6655**	0.2794**	0.2290	0.0006	0.1426**
A	42 + 24	95.8[a]	95.8[a]	94.6[a]	13.9[a]	3.2[a]
B	42 + 24	93.8[a]	90.4[a]	88.3[a]	11.4[b]	3.0[a]
Treatment		0.0492	0.0386	0.1353	0.0184	0.0587
N	42 + 42	100.0[a]	98.8[a]	95.4[a]	13.3[a]	3.2[a]
L	24 + 24	89.6[b]	87.5[b]	87.5[a]	11.6[b]	2.9[a]

Values indicated by the same letter are not significantly different at P = 95%, **plants were evaluated using factors (substrate and treatment) as two fields (sets)

– Overhead watering or misting of green seedlings during night or early cold morning were needed only; it is not recommended to water the plants during hot sunny days at temperatures over 24°C.
– Shading and watering regime enabled seedlings to overcome hot days and extreme temperatures (from 30°C to 34°C during July and August 2007 and 2008) without any exceptional damage.

Chemical control

The best precaution towards fungi pathogens is to maintain open mineral substrate as well as good water and air-movement on the beds with plants.
– During hot summer eliminate fungi pathogens of genera *Pythium*, *Phytophtora*, *Rhizoctonia* with systemic fungicides Previcur SL (active agent: Propamocarb) 0.15% – watering, Saprol 1.5 ml/l + Sportak (Prochloraz) 1 ml/l – spraying. *Botritis cinerea* should be controlled with Rovral (Iprodione) 0.1% spraying (Perner 1997; de Jong 2004).
– To prevent aphids attack of the seedlings, spray seedlings with insecticide Pirimor 50 WG (Pirimocarb) 0.15%.
– Protection from slugs/snails is advised and controlled by slug pellets of metaldehyde regularly.

Overwintering

In full dormancy the seedlings required cold constant winter and a little moisture in substrate. Cold semi-dry dormant conditions with temperatures above zero to 4°C are optimal; it should not rise above 7°C and not fall below –2°C for a long time (Perner 1997; de Jong 2004).
– The dormant seedlings safely overwinter in a cold glasshouse on the bed under contact white textile Pegatex (mass 45 g/m).
– Effective protection of seedlings against warm spells initiating bud stretching and growth of shoots during mild winter is necessary (see shading and cooling above).

Actual scientific and professional information on the genus *Cypripedium* is currently completed at www.cypripedium.de (Frosch) (Weinert 2008).

CONCLUSIONS

In this paper we presented the first positive results of the vegetative experiment with three-year outdoor cultivation of *Cypripedium calceolus* L. *ex vitro* seedlings in the Czech Republic.

In April 2006 *Cypripedium calceolus* as one-year-old vernalized seedlings propagated from mature seeds were used in the experiment. The four variants of substrate after planting the seedlings were treated with biofungicide Supresivit (*Trichoderma harzianum*) in the ratio of 2.5 g/10 l water – as prevention to pathogen fungi attack.

Two stabile mixtures for *ex vitro* seedlings proved to be effective:

AN on the basis of liadrain with addition of pumice, perlite, sand, zeolite and dolomite lime powder, pH 6.4. The yield of 4-year-old seedlings was to 98%.

BN on the basis of granodiorite with addition of pumice, perlite, sand, zeolite and dolomite lime powder, pH 7.7. The yield of 4-year-old seedlings was to 93%.

Additional treatment with lignohumate in other two variants, BL and AL**,** did not improve the effect. The yield of 4-year-old seedlings ranged from 83% to 92%.

The substrates did not visually influence the phase of sprouting, the phase of growth and retracting of the plants.

Diluting liquid feeding with Kristalon blue (N 19, P 20, K 6, Mg 3) in the dose of 10 ml 15% concentrate per 10 l water/2 m^2 four times was essential during growing season from May to September. The vegetation period of *C. calceolus* grown in open conditions lasted 22 to 24 weeks in the first two years of outdoor cultivation. In November 2008, after third growing season, 4-year-old seedlings were taken up from the mixtures and evaluated as bare root plants. The length of roots in mixture A on the basis of liadrain was on average 14.5 cm, and in mixture B on the basis of granodiorite it was on average 12.1 cm. The rhizomes with visible new buds were planted into new mixtures immediately. These evaluated seedlings will be able to grow up to the blooming size during two or three seasons.

Positive results of the experiments with outdoor cultivation of *C. calceolus ex vitro* seedlings represent the success in the program of reintroduction of endangered species into nature in the Czech Republic. Seven year-old potted seedlings of *C. calceolus* will be planted into gene resource area of the Silva Tarouca Research Institute for Landscape and Ornamental Gardening at Průhonice.

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Pěstování *Cypripedium calceolus* L. *ex vitro* semenáčů ve venkovních podmínkách

ABSTRAKT: V příspěvku jsou uvedeny první pozitivní výsledky s tříletým venkovním pěstováním *ex vitro* semenáčů *Cypripedium calceolus* L. v České republice. Semenáče byly získány výsevem vyzrálých semen karpatské provenience v podmínkách *in vitro* v pražské soukromé laboratoři. Rizomy prodělaly po dobu tří měsíců chlazení – vernalizaci při teplotě +4 °C. V dubnu 2006 byly semenáče se 4 až 12 kořeny a vyvinutými pupeny nasázeny do dvou typů minerálních substrátů – AN směsi na bázi liadrainu a BN směsi na bázi granodioritu. Hlavní komponenty byly shodně doplněny pemzou, perlitem, pískem a zeolitem, pH bylo upraveno mletým dolomitickým vápencem. Minerální substráty vykazovaly stabilitu a osvědčily se pro pěstování *ex vitro* semenáčů ve specifických podmínkách venkovního prostředí. Dodatečné ošetření rostlin v obou substrátech roztokem lignohimatu nevykázalo znatelnější pozitivní efekt. Rostliny po zatažení od konce listopadu do března přezimovaly ve studeném skleníku s teplotou těsně nad nulou. V průběhu dubna postupně vyrašily. Délka vegetace semenáčů v prvních dvou letech trvala 22 až 24 týdnů. Po druhém přezimování na konci vegetačního období se výtěžnost čtyřletých semenáčů se dvěma až čtyřmi listy pohybovala od 83 % do 98 % ve čtyřech variantách. Koncem listopadu 2008 byly semenáče ze směsí vyzvednuty a hodnoceny jako prostokořenné rostliny. Byl zaznamenán počet živých rostlin s dobře vyvinutými pupeny a kvalita kořenového systému. Délka kořenů ve směsi A na bázi liadrainu byla v průměru 14,5 cm; ve směsi B na bázi granodioritu byla v průměru 12,5 cm. Následně byly rizomy nasázeny do nových směsí. Tyto semenáče dorostou do květuschopné velikosti v průběhu dvou až tří sezon. Sedmileté semenáče *C. calceolus* budou vysázeny na výzkumné plochy ve Výzkumném ústavu Silva Taroucy pro krajinu a okrasné zahradnictví, v.v.i. v Průhonicích.

Klíčová slova: střevíčník pantoflíček; *ex vitro* semenáče; pěstební substráty; přezimování; vyrašení; pupeny; kořeny; výtěžnost

Corresponding author:

Ing. Jiří Obdržálek, CSc., Výzkumný ústav Silva Taroucy pro krajinu a okrasné zahradnictví, v.v.i., Průhonice, Květnové náměstí 391, 252 43 Průhonice, Česká republika
tel.: + 420 296 528 308, fax: + 420 267 750 440, e-mail: obdrzalek@vukoz.cz