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Which stem parts of Slender speedwell (*Veronica filiformis*) are the most successful in plant regeneration?

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Abstract: Regeneration of Slender speedwell (*Veronica filiformis*) from small parts of stem without using stimulative agents was the focus of the investigation. Four different short fragments of shoot (main terminal, secondary terminal, nodal segment and internodal segment) were cultivated under greenhouse and natural conditions. All tested vegetative segments induced roots, rooted in a soil substrate and in a semi-natural lawn, survived winter, and flowered. Multiplication of clonal plants was confirmed for both the main and secondary terminal segments. These terminal segments had the best response in number of growing individuals, flowering stems, and weight of dried biomass. The manipulative experiment revealed that clonal success of speedwell is connected with possibilities to hive off of all tested above-ground segments. Establishment of plantlets from the segment in natural condition was not successful when accompanied by certain grass species.

Key words: clonal; invasive species; regeneration; shoot; stem; terminal; vegetative reproduction

Introduction

Slender speedwell (*Veronica filiformis* Sm.) belongs to the alien flora of Europe and North America, which originated in the Caucasus. Its common name is Creeping speedwell, too. It is a subtle, stoloniferous perennial of plagiotropous stature and the lack of terminal inflorescence, belongs to family Plantaginaceae (formerly Scrophulariaceae), genus *Veronica*, subgenus *Pocilla* (Albach et al. 2004), which is usually noticeable only in full bloom. From March to May it creates striking bright blue blossoms. Although this perennial species has flowers annually both in Europe and in North America, it does not produce viable seeds (Thaler 1953 sec. Harris & Lovell 1980; Neal & Senesac 1993). It propagates vegetatively and fills in unoccupied space in the lawn, its preferred places (Soják & Šourek 1959; Jehlík 1961).

Slender speedwell grows vegetatively very fast on bare soil or in a grass sward and may double its diameter during the vegetation period (Harris & Lovell 1980). It is expected to have high invasive potential (Weber & Gut 2004). The aim of this investigation was to find out which stem part is most useful for vegetative propagation of this species; which part of the plant is useful for the plant regeneration in a lawn.

Material and methods

Plant material
The Slender speedwell plant material was obtained from Velké Skaliny village (N 48°44′48.6, E 14°34′43.4) in South Bohemia. These maternal plants were taken from a mowing lawn in a peripheral part of the village and then introduced into the city grown grass turf on the property of the Biology Centre AS CR in České Budějovice town in South Bohemia. These pre-cultivated plants were cut and thus four different types of stem segments were prepared: A = the main terminal segment (i.e. the top of the main stem with one node, two leaves and a bud), B = the secondary terminal segment (i.e. the top of the secondary branch of the stem with one node, two leaves and a bud), C = the nodal segment (i.e. a middle part of the stem with one node and two leaves), and D = the internodal segment (i.e. a middle part of the stem with one internode, two nodes and four leaves) (Fig. 1).

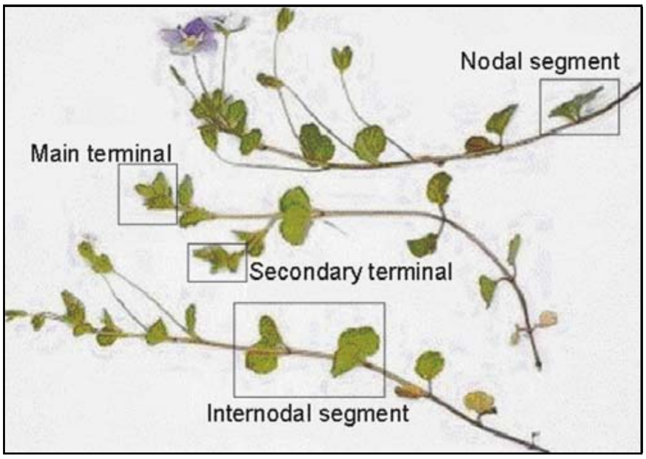

Fig. 1. Four tested segments from stem of Slender speedwell (*V. filiformis*).

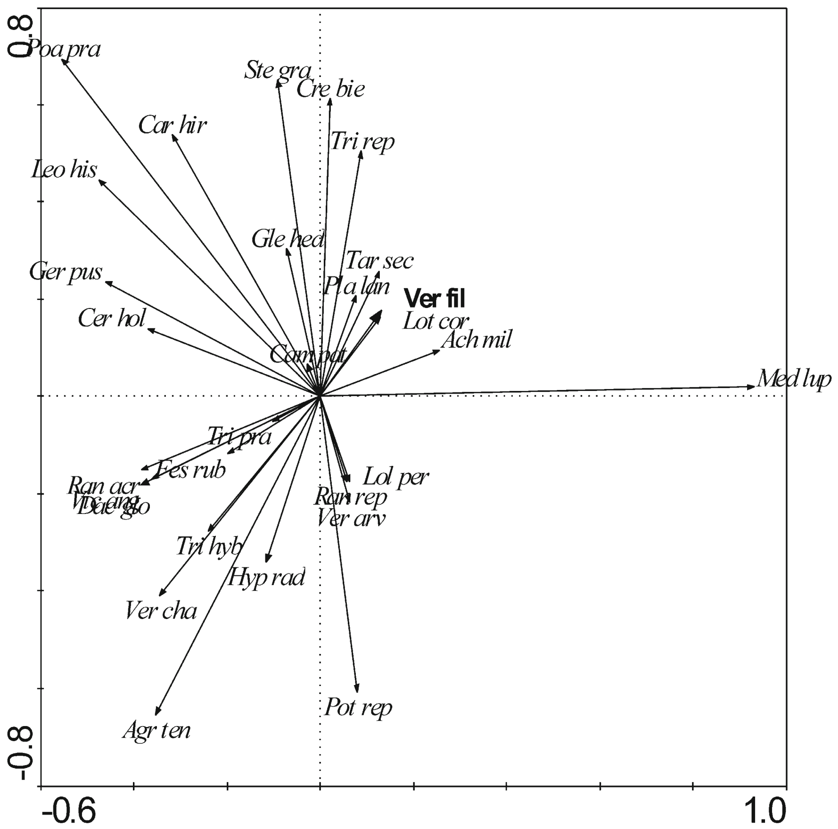

Fig. 2. Partial cluster analysis (PCA) graph showing correlation between regeneration of vegetative parts of Slender speedwell (Ver fil) and dominant plant species in the experimental lawn. More details in text. Dominant and co-dominant species in the plant cover: *Ach mil Achillea millefolium, Agr ten Agrostis tenuis, Cam pat Campanula patula, Car hir Carex hirta, Cer hol Cerastium holosteoides, Cre bie Crepis biennis, Dac glo Dactylis glomerata, Fes rub Festuca rubra, Ger pus Geranium pusillum, Gle hed Glechoma hederacea, Hyp rad Hypochoeris radicata, Leo his Leontodon hispidus, Lol per Lolium perenne, Lot cor Lotus corniculatus, Med lup Medicago lupulina, Pla lan Plantago lanceolata, Poa repens, Ste gra Stellaria graminea, Tar sec Taraxacum sect. Ruderalia, Tri hyb Trifolium hybridum, Tri pra Trifolium pratense, Tri rep Trifolium repens, Ver arv Veronica arvensis, Ver cha Veronica chamaedrys, Vic ang Vicia angustifolia.*

Experiment I – greenhouse experiment

The soil substrate was prepared by mixing the Horticultural and Floral Substrate (producer Rašelina Soběslav) and sand in ratio 2:1, without any fertilizers or sterilization. This mixed substrate was sieved in the 3 × 3 mm sieve. The amount of 3 g in 5 L of milled limestone was added to the substrate. Each plastic pot of the size 4 × 4 × 6 cm was filled with 30 mL of the substrate. Rootless segments of plants were individually ranked on the substrate surface and slightly pressed against it. The pots with the same type of segments, sixty of each, were placed on a tray. Each tray contained 1000 mL of water in the start of the experiment. The plant segments were cultivated in a heated greenhouse during three months in the autumn 2007. There was a day/night cultivation, average temperature 20 ℃ by day and 8 ℃ by night. The substrate was watered through trays with the same amount of water. The plants were harvested, counted, above-ground and under-ground masses were dried under 21 ℃ and weighed on the analytical scales (precision 10^{-4} g). The ratio between the weight of the above and underground dry biomass was counted (R/S). One-way ANOVA and Tukey HSD tests were used to evaluate the differences in survival and growth of the used plant segments (Meloun & Militký 1994).

Experiment II – lawn experiment

The four types of plant segments were used for a growing testing in the semi-natural lawn of the investigation area of the Biology Centre AS CR in České Budějovice (syntaxonomically close to aliance of Arrhenatherion Koch 1926, mowing three times a year). In June 2008 and 2009, phytocenological relevés of the squares (estimation of plants cover in percentage) were recorded. Thirty-six vascular plant species were recorded: eight of the rare species and twenty-eight of the other (dominant and co-dominant) species. The list of the twenty-eight lawn species is shown in Fig. 2. Rare species was defined as a species with growth frequency on 1–2 squares and with a cover smaller than one percentage in the square.

Rootless plant segments were slightly pressed to the soil surface in random squares. For each segment, five random 1 × 1 m^2 squares were used, with five segments cultivated on each of them (design see Fig. 3). The experiment started in August 2008 and the individuals of Slender speedwell species were regularly monitored. During the experiment, the lawn was neither mown, nor artificially irrigated. In July 2009, the Slender speedwell plants were harvested. Amount of underground masses was negligible, so only the aboveground masses was dried and weighed.

One-way ANOVA and Tukey HSD tests were used to evaluate the differences in survival and growth of the used plant segments. Multiple regression data analysis (PCA, Partial Cluster Analysis) was used to compare the reached coverage of Slender speedwell with the coverage of the other plant species on 25 squares (Meloun et al. 2005). The

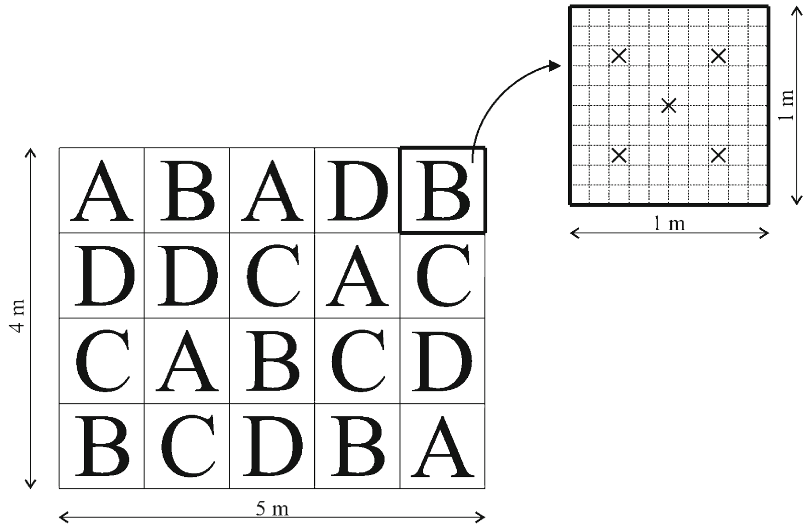

Fig. 3. Design of random squares – lawn experiment. Stem segments of Slender speedwell (*V. filiformis*): A = main terminal, B = secondary terminal, C = nodal segment, D = internodal segment. Mark x shows a cultivation place for one plant segment.

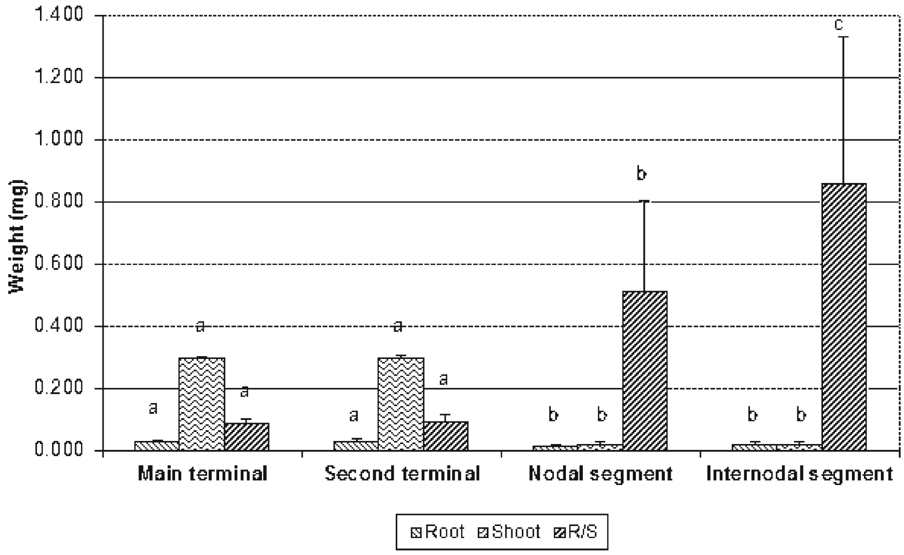

Fig. 4. Dry biomass of Slender speedwell (*V. filiformis*) from the greenhouse experiment. Results of Tukey HSD test are presented, non-significant differences are in the same letters.

twenty-eight of the dominant and co-dominant species growing on the squares were used for this PCA analysis (Fig. 2). Other species of minority representations were not included in the analysis due to their low significance.

At the end of Experiment II, unlike Experiment I, only the aboveground biomass was analysed. The underground biomass, tiny adventitious roots growing in the natural conditions, was not possible to be harvested and consequently analysed. Seeds, low in number, were not germinable.

Results

Experiment I – greenhouse experiment

All four segments were vital and can regenerate. After three months of segment cultivation, the ratio of growing individuals compared to the number of planted segments was as follows: A 90%, B 97%, C 97% and D 93%. All types of plant segments were thus able to root and grow.

Segments A and B showed a significantly higher weight of the above and underground dried biomass than segments C and D (Fig. 4). The weights of biomass from C and D segments were very low. There were significant differences in the biomass ratio R/S among tested groups of segments, but no significant difference was found between A and B segments (Fig. 4). The D segment (i.e. internode with four leaves) was least suitable for regeneration of *V. filiformis* in the greenhouse, when considering the amount of final biomass.

Experiment II – lawn experiment

After four months of growing in natural lawn conditions, the success of total surviving individuals of the tested segments was: A 84%, B 80%, C 60% and D 40%. These percentages did not markedly change over

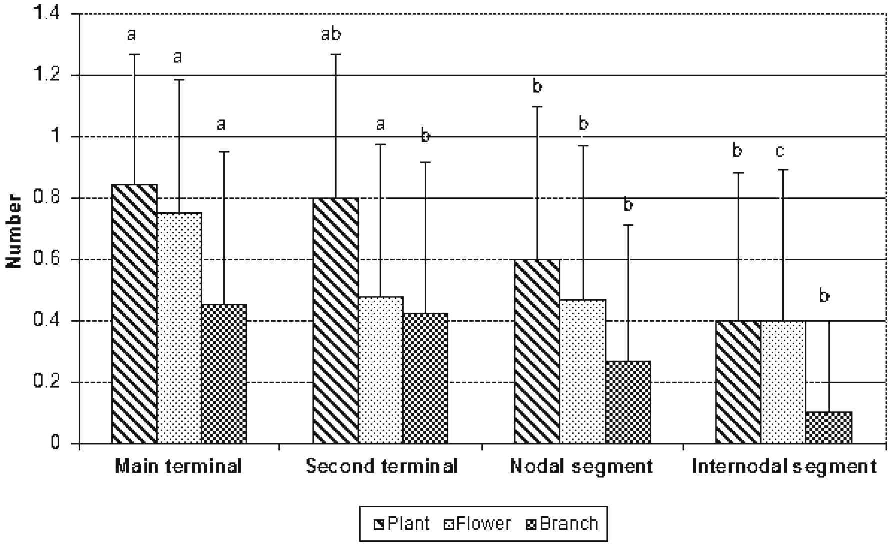

Fig. 5. Mean number of regenerated plants, flowers and branches of Slender speedwell (*V. filiformis*) in relation to cultivation of various segments from the lawn experiment. Results of Tukey HSD test are presented, non-significant differences are in the same letters.

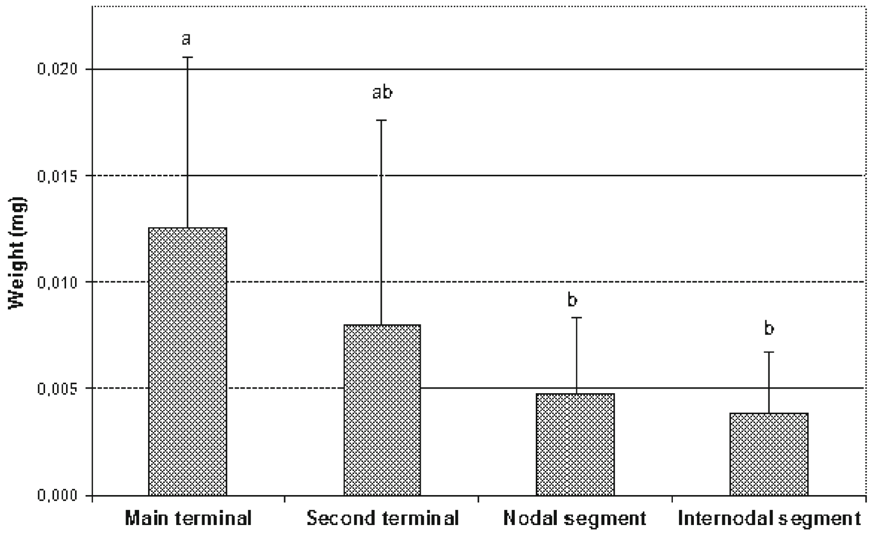

Fig. 6. Above-ground biomass of Slender speedwell (*V. filiformis*) from the lawn experiment. Result of Tukey HSD test is presented, non-significant differences are in the same letters.

the winter. At the end of the experiment, after eleven months of growing, this ratio was: A 76%, B 68%, C 56% and D 36%. There was a significant difference in mean number of individuals per square between A and C, D segments (Fig. 5).

Similar results were shown by the following characteristics: number of blooming flowers (in May), number of new branches shooting from the original stem, and an average weight of the dry mass of the above-ground biomass measured at the end of the experiment (Figs 5–6). The segments of terminals appeared to be the most successful, on the contrary big nodes were the least successful. Although the growth of the node segments (C, D) was significantly smaller in comparison with terminal segments (A, B), they regenerated, survived the winter, managed to grow and had flowers. The higher variability in all measured characteristics from the lawn experiment in comparison with results from the greenhouse experiment was obvious (Figs 4–6).

Multiple regression data analysis showed correlation between occurrence (successful regeneration) of *V. filiformis* and coverage of twenty-eight lawn species (Fig. 2). The plantlets of *V. filiformis* grew better when accompanied by the following meadow species as *Lotus corniculatus, Taraxacum* sect. *Ruderalia, Plantago lanceolata, Trifolium repens* and *Achillea millefolium.* On the other hand, negative correlations were shown between *V. filiformis* and plant species as *Agrostis tenuis, Dactylis glomerata, Festuca rubra, Hypochoeris radicata, Lolium perenne, Ranunculus acris, R. repens, Trifolium hybridum, T. pratense, Veronica arvensis, V. chamaedrys and Vicia angustifolia.*

Discussion

In the non-native area, Slender speedwell propagates mainly vegetatively (Harris & Lovell 1980; Peniašteková & Zlinská 1995). It grows in meadows, grassy gardens, river banks and edges of roads (Müller & Sukopp 1993; Holm et al. 1979; Jehlík 1998). The results of the two experiments prove that the plant is able to propagate from various stem parts easily. The segments of the main and secondary terminals showed a high probability of surviving and growth (Figs 4–6). In spite of that, even the low values of survival and growth shown by the big nodes (36%) are positively significant for the species regeneration.

Slender speedwell produces only tiny roots, negligible underground biomass (Harris & Lovell 1980), (Fig. 4). When new roots are created, the adventitious roots appear at every node of the stem. These fragile roots mainly fix the plant to the surface. It is the aboveground biomass that is important for water metabolism and surviving the winter season. The plants are sensitive to summer droughts (Jehlík 1998). That was also observed several times when cultivating plants in the lawn at the property of the Biology Centre AS CR in České Budějovice in South Bohemia. The observations showed that the plants are able to use horizontal precipitation (fog and dew).

It is important to realise that the lawn was not mown or disturbed in any other way during the experiment. Mowing, that would, probably be a natural activity under normal conditions, would probably have a positive effect. In the future, the investigation of vegetative propagation of Slender speedwell will focus on its ability to spread under various disturbing conditions.

It was found that if roots start to grow immediately after planting (slight pressing against the soil), the probability of emergence of independent growing individual is high. Clonal propagation by regenerating plant from shoot is common for many plant species (Klimesova & de Bello 2009). Clonal regeneration of Slender speedwell is not based on progressive root propagation. Slender speedwell has probably a similar clonal strategy as *Prunella vulgaris, Lysimachia nummularia* or *Glechoma hederacea.* These species exhibit avoidance of root competition and are not competitively inferior to the species that does not (e.g. *Potentilla reptans, Ranunculus repens*, genus *Fragaria*) (Steinbach & Gottsberger 1994; Leeflang 1999; Vermeulen et al. 2008; Semchenko et al. 2010). It was found that *G. hederacea* invests preferentially in rapid exploitation of unoccupied places, whereas *F. vesca* invests in increasing the competitive ability and local persistence of established ramets (Semchenko et al. 2010). Slender speedwell grew very fast from two-node segments in bare loam soil (Harris & Lovell 1980), but its body is delicate and not so competitive with other species in the lawn. This species must use during its vegetative propagation unoccupied space in lawns or meadows (Soják & Šourek 1959; Jehlík 1961). The surrounding vegetation is probably a limiting factor for its propagation.

Slender speedwell regenerated very well from vegetative segments in the experimental lawn. It was obvious that the species survived and grew markedly better or worse in relation to occurrence some plant species (Fig. 2). An interesting observation was a negative correlation between regeneration of Slender speedwell and grasses such as *A. tenuis, D. glomerata, F. rubra* and *L. perenne* growing in an opposite tendency to regenerating segments of Slender speedwell (Fig. 2). The grass species *Poa pratensis* (the only other species from family Poaceae growing on the experimental lawn) was in a neutral position to Slender speedwell. Two other species of genus *Veronica* were growing in the experimental lawn. Both these species, *V. chamaedrys* and *V. arvensis,* had opposite tendencies in comparison to the tested species, too (Fig. 2). These two examples may be functional correlations between the plant species. These preliminary results will be studied in following experiments.

Investigation of this non-native plant species (Jehlík 1998) is important because of current climate changes (Bernstein et al. 2007; Lapin et al. 2009). The climate changes may increase the migration of organisms. The shift in expansion of naturalized alien species is presumed (e.g. Alford & Backhaus 2005). Taking the presented results and scientific literature into account, it may be assumed that Slender speedwell would be successful not only in golf courses (Neal & Senesac 1993), but it will spread in urban lawns, private gardens, parks and in road edges.

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