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Influence of Scion/Rootstock Interaction on Seasonal Changes of Phenols

By

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With 7 figures

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Summary

USENIK V. & ŠTAMPAR F. 2002. Influence of scion/rootstock interaction on seasonal changes of phenols. – *Phyton* (Horn, Austria) 42 (2): 279–289, with 7 figures. – English with German summary.

The influence of the rootstock/scion interaction on phenol changes in scion tissues during growing season was studied in relation with dwarfing effect. The experiment comprised 4-year-old sweet cherry trees (*Prunus avium*) of 'Lapins' grafted on rootstocks of different genetic origin: 'F 12/1', 'Gisela 5' and 'Weiroot 158'. Samples for HPLC analyses were collected six times in 2000. The semi-dwarfing rootstocks Gisela 5 and Weiroot 158 caused higher concentrations of analysed phenolic substances in the phloem of annual shoots and leaves during the growing season than did the invigorating rootstock 'F 12/1'. Our results show that concentrations of phenols in the scion tissues are related with rootstock used and tree vigour.

Zusammenfassung

USENIK V. & ŠTAMPAR F. 2002. Der Einfluss von Propfreis und Unterlage auf die jahreszeitlichen Änderungen in den Phenolen. – *Phyton* (Horn, Austria) 42 (2): 279–289, 7 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Im Hinblick auf Schwachwüchsigkeit wurden die Wechselbeziehungen von Unterlage und Propfreis auf die Änderungen in den Phenolen der Gewebe des Propflings während der Wachstumsperiode untersucht. Die Untersuchungen erfolgten an

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vierjährigen Kirschbäumen (*Prunus avium*) der Sorte „Lapins“, welche auf verschiedene Unterlagen gepropft waren: „F 12/1“, „Gisela 5“ und „Weiroot 158“. Die Proben für die HPLC-Analysen wurden im Jahr 2000 sechsmal aufgesammelt. Die schwachwüchsige Unterlage „Gisela 5“ und „Weiroot 158“ führten im Phloem der Jahrestriebe und in den Blättern während der Wachstumsperiode zu höheren Konzentrationen an phenolischen Verbindungen als es bei der starkwüchsigen Unterlage „F 12/1“ der Fall war. Unsere Ergebnisse belegen, dass die Konzentration an phenolischen Substanzen in den Geweben der Propfreiser in enger Beziehung zur Unterlage und zur Wüchsigkeit stehen.

Introduction

Dwarfing and invigorating effects produced by grafting scions onto different rootstocks are well known (JONES 1986). Although dwarfing rootstocks have been used in apple culture for more than two thousand years, little is known how dwarfing rootstocks effect scion vigour, flower induction and cropping (WEBSTER 1998, KAMBOJ & al. 1997, SOUMELIDOU & al. 1994). One possible hypothesis is that dwarfing rootstocks affect the production and basipetal translocation of plant hormones (SOUMELIDOU & al. 1994). The graft unions of dwarfing rootstocks appear to deplete the solute of the xylem sap (JONES 1986). LOCKARD & SCHNEIDER 1981 proposed that dwarfing rootstocks affect the scion growth by controlling the auxin passed through the rootstock's bark. The bark of the more dwarfing apple rootstocks causes a higher rate of auxin destruction (GUR & SAMISH, 1968). The amount of degradation depends on the amount of IAA oxidase, peroxidase and phenols in the phloem. The function of IAA oxidase regulators can be assigned to polyphenols. They play a role of internal physiologic regulators and chemical transmitters in plants (SEIGLER 1998). Phenols affect the synthesis, level and polarity of IAA transport and cell wall permeability (LOCKARD & SCHNEIDER 1981, STENLID 1976, TREUTTER & FEUCHT 1988).

The introduction of dwarfing rootstocks for cherries has enabled the regulation of cherry tree vigour. Some *Prunus* species (*P. canescens*, *P. cerasus*) and *Prunus* hybrids (the 'Weiroot', 'Gisela', 'M x M' series, etc.) have been proved as dwarfing rootstocks (CALLESEN 1998). Exposure of plants or cell cultures to biotic and abiotic stress factors may induce the formation of phenolic metabolites (HELLER & al. 1994). Accumulations of phenolic compounds above the graft union were found in sweet cherry cultivars grafted on rootstocks of different genetic origin (GEBHARDT & FEUCHT 1982, TREUTTER & FEUCHT 1991, DIRR & al. 1994). Stress situations can lead to the accumulation of phenol compounds which have been implicated in different mechanisms with regard to scion-stock relations. Concentrations of phenols in the heterospecific *Prunus* grafts are different from homospecific ones (GEIBEL & FEUCHT 1991, FEUCHT & TREUTTER 1991, TREUTTER & al. 1987, USENIK & ŠTAMPAR 2000b). Phenols are a sign of a plant's altered metabolism, as a consequence of grafting two different genotypes (Mu-

SACCHI & al. 2000). Different concentrations of polyphenols above the graft union prove that the rootstock influence the whole metabolism of a grafted plant (USENIK & ŠTAMPAR 2000a). Phenol composition, influenced by the different developmental stages of the tree, changes seasonally in sweet cherry phloem tissues (SCHWALB & FEUCHT 1999).

The aim of the study is to establish what influence rootstocks for cherries of different genetic origin have on the concentrations of some phenol substances in scion tissues (phloem from annual shoots and leaves) during the growing season. The possibility of involvement of these substances in the dwarfing effect will be discussed.

Material and Methods

The experiment comprised the 4-year-old trees of the 'Lapins' on semi-dwarfing rootstocks 'Gisela 5' ('G 5') (*Prunus cerasus* L. x *Prunus canescens* Bois.), 'Weirroot 158' ('W 158') (*Prunus cerasus* L.) and vigorous rootstock 'F 12'/1 (*Prunus avium* L.). All plants were grown in the same orchard in the Research Centre Bilje near Nova Gorica (south – west Slovenia).

From each stock/scion combination 4 trees were analysed. The experimental design was randomised blocks. The vigour of trees was calculated according to the mean trunk sizes. For polyphenol analyses samples were extracted from leaves and annual shoots. The leaf sampling was performed on 14 April, 17 May, 13 June, 17 July, 17 August and 28 September. Samples from shoots were collected on 14 April, 17 May, 13 June, 17 July and 28 September. The sampling in August was omitted due to the poor vegetative growth. At the sampling a special attention was paid to the selection of equal shoots. When sampling leaves, five leaves per tree were selected; namely, every third or fourth undamaged leaf of the comparable current shoots were chosen. Leaves were frozen in liquid nitrogen and prepared for analyses. Small sections of phloem with cambium were removed from annual shoots and immediately frozen in liquid nitrogen.

Samples were extracted with acetone-water (80:20, v/v) containing Triton X-100 (0.4%) for 4 days (leaf samples) and for 10 days (phloem samples) at 4 °C according to TREUTTER 1988. The HPLC equipment consisted of X-ACT™ degasser, P2000 TSP pump, Chromsep SS (250 x 4.6 mm) Hypersil 5 ODS, reversed phase column, guard column Chromsep Guard SS (10 x 3 mm) reversed phase, autosampler AS 1000, detector WellChrom K-2500 for detection at 280 nm (UV), and OS/2 Warp IBM Operating System (1994). For the leaf samples solvent A was acetic acid-water (1:95, v/v) and solvent B methanol with a gradient range according to TREUTTER 1988. For the phloem samples solvent A was acetic acid-water (1:99, v/v) and solvent B methanol-butanol (5:1, v/v) with a gradient range according to MARTELOCK & al. 1994. Flow rate was 0.5 ml/min. Injection volume was 5 µl.

The individual phenolic compounds (catechin, chlorogenic acid, rutin, p-coumaric acid and eryodictiol 7-glucoside (ERG) were identified by comparison of their UV absorbance spectra with authentic samples (Fluka Chemical). The peak area of the internal standard (6-methoxy-flavon) was used to estimate the relative amount of prunin and dihydrowogonin 7-glucoside (DWG) in the tissue samples (MARTELOCK & al. 1994).

The means were compared using Duncan's Multiple Range Test and LSD tests ($P < 0.05$).

Results

Vigour of trees

The vigour of trees was calculated according to the mean trunk section areas. As it can be seen in Table 1 the grafted trees exhibited similar vigour at the beginning of planting. The influence of the rootstocks on the tree vigour was absent during the second growth period. The 'Lapins' trees which were grafted on the 'G 5' and 'W 158' rootstocks reached 10% less vigour than those grafted on the 'F 12'/1 rootstock in the third year. In the fourth year the vigour of the 'Lapins' trees was by 25.5% and 20.2% weaker on the 'W 158' and 'G 5' W rootstocks, respectively, than it was on the 'F 12'/1.

Phenols in leaves

The concentrations of chlorogenic acid and rutin in the leaves were observed. Fig. 1 shows the concentrations of chlorogenic acid during the growing season in the leaves of 'Lapins' grafted on the rootstocks 'F 12'/1, 'G 5' and 'W 158'. The results show different concentrations of chlorogenic acid in the leaves depending on a rootstock used. The content comparison indicated lower concentrations of the chlorogenic acid in 'F 12'/1 than in 'G 5' and 'W 158', with the exception of April and June when the chlorogenic acid content was higher than in 'G 5'. In April and June the differences between 'F 12'/1, 'G 5' and 'W 158' were not significant. In May, July and September the values were significantly lower in 'F 12'/1 than in 'G 5' and 'W 158', and in August significantly lower than in 'W 158'.

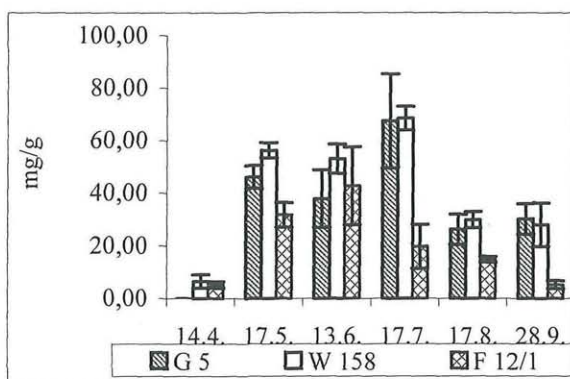


Fig. 1. Concentration (mg/g) of chlorogenic acid in leaves of 'Lapins' on different rootstocks ('F 12'/1, 'G 5' and 'W 158') during the growing season.

Table 1.
Trunk section area (cm²) of 'Lapins' grafted on different rootstocks.

Rootstocks	Trunk section area (cm ²)			
	1997	1998	1999	2000
F 12/1	3.01	4.26	12.58	23.22
Weirroot 158	3.04	3.86	9.65	17.31
Gisela 5	2.85	3.81	10.84	18.54

The concentrations of rutin in leaves were significantly altered during the growing season, and indicative differences among 'F 12'/1, 'G 5' and 'W 158' during individual sampling times were noted as well (Fig. 2). In April the value in 'F 12'/1 was significantly higher than in 'G 5' and higher than in 'W 158'. In May the concentrations significantly increased. The highest concentrations were determined in 'G 5', which also exhibited more rutin than 'F 12'/1 and 'W 158'. In June the concentrations significantly decreased in 'G 5' and increased in 'F 12'/1. In July and August there was a significant decrease in the concentration of rutin in 'F 12'/1 which lasted until September, but in both 'G 5' and 'W 158' the concentrations in July increased, in August they decreased and in September there was an increase in concentrations noted again.

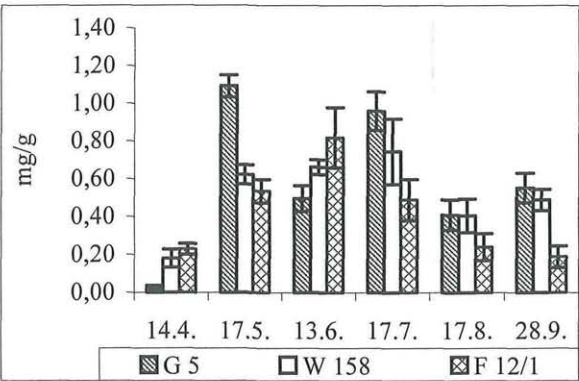


Fig. 2. Concentration (mg/g) of rutin in leaves of 'Lapins' on different rootstocks ('F 12'/1, 'G 5' and 'W 158') during the growing season.

Phenols in shoots

Catechin, p-coumaric acid, ERG, prunin and DWG were analysed. There was an increase observed in catechin concentrations in annual shoots, but in May a decrease in the concentrations was noted (Fig. 3). In April in the 'Lapins' shoots there was significantly less catechin in 'F 12'/1

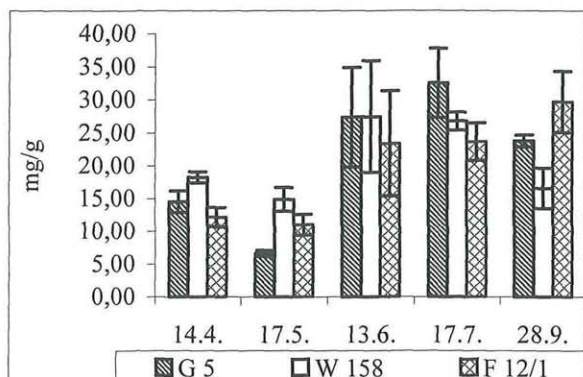


Fig. 3. Concentration (mg/g) of catechin in the phloem of the shoots of 'Lapins' on different rootstocks ('F 12'/1, 'G 5' and 'W 158') during the growing season.

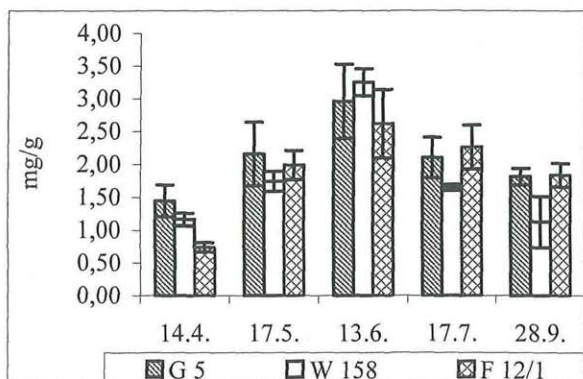


Fig. 4. Concentration (mg/g) of p-coumaric acid in the phloem of the shoots of 'Lapins' on different rootstocks ('F 12'/1, 'G 5' and 'W 158') during the growing season.

than in 'W 158'. All three rootstocks exhibited less catechin in May than in April followed by a great increase in June. In June an increased concentration was significant in 'G 5'. In 'G 5' and 'W 158' the concentrations of catechin decreased between July and September, however, in 'F 12'/1 an increase was observed.

In the shoots of the 'Lapins' cherry tree the concentrations of p-coumaric acid increased between April and June when the highest concentrations were achieved, and decreased thereafter. In April the values in 'F 12'/1 were lower than in 'W 158' and significantly lower than in 'G 5'. In May there was less p-coumaric acid in 'F 12'/1 than in 'G 5' and more than in 'W 158'. The concentrations were the lowest in June, and the highest in August and September in 'F 12'/1 in comparison with 'G 5' and 'W 158' (Fig. 4).

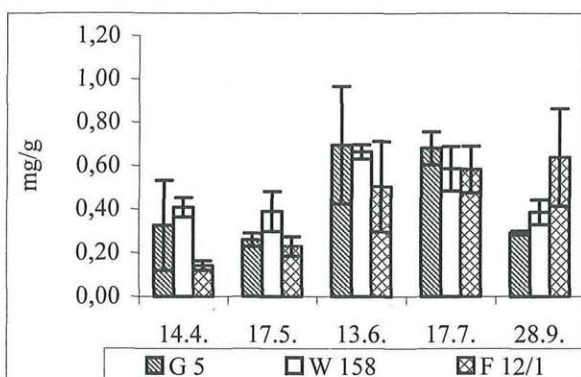


Fig. 5. Concentration (mg/g) of ERG in the phloem of the shoots of 'Lapins' on different rootstocks ('F 12/1', 'G 5' and 'W 158') during the growing season.

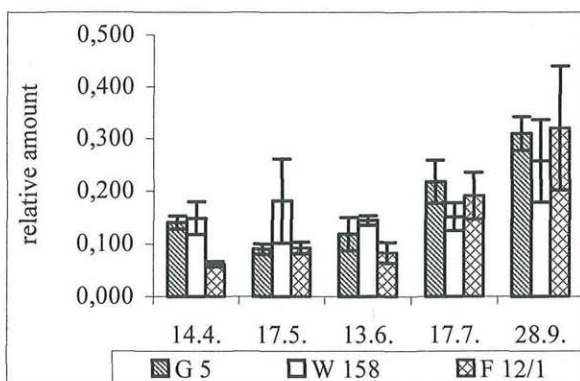


Fig. 6. Relative amount of prunin in the phloem of the shoots of 'Lapins' on different rootstocks ('F 12/1', 'G 5' and 'W 158') during the growing season.

The concentrations of ERG altered throughout the growing season (Fig. 5) as well as did the not significant differences among the rootstocks. The values of ERG were significantly increasing in 'F 12/1' since April until September. In April, May and June the concentrations in 'F 12/1' were lower than in 'G 5' and 'W 158', in July they were the same as in 'W 158', and in September higher than in 'G 5' and 'W 158'. The highest concentration of ERG was noted in June.

An increase of the relative amount of prunin during the growing season (with slight decrease in June) was established with all rootstocks (Fig. 6). The average amount of prunin in 'F 12/1' was in September significantly higher than in April, May and June; in 'G 5' in July it was significantly higher than in May and June and significantly the highest in September. Values of prunin in the shoots of 'Lapins' on the rootstock

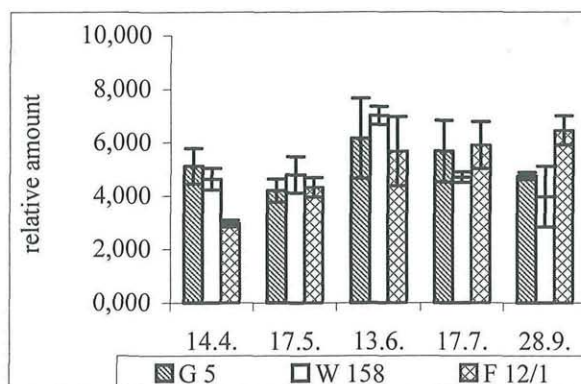


Fig. 7. Relative amount of DWG in the phloem of the shoots of 'Lapins' on different rootstocks ('F 12'/1, 'G 5' and 'W 158') during the growing season.

'F 12'/1 were in April significantly lower than in 'G 5' and 'W 158'. In May they were lower than in 'W 158', in June lower than in 'G 5' and 'W 158', in July lower than in 'G 5' and in September slightly higher than in 'G 5'.

In the rootstock 'F 12'/1 a significant increase in the relative amount of DWG during the growing season was evident while the highest amounts in 'G 5' and 'W 158' were in July. Values of DWG in 'F 12'/1 were in April significantly lower than in 'G 5' and 'W 158' and in September significantly higher than in 'W 158'. The concentrations of DWG were in 'F 12'/1 in May lower than in 'W 158', in June lower than in 'G 5' and 'W 158' and in July slightly higher than in 'G 5' (Fig. 7).

Discussion

The rootstocks 'G 5' and 'W 158' (heterospecific combinations) caused higher contents of phenolic substances in the scion tissues during the growing season than did the rootstock 'F 12'/1 (homospecific combination). Various experiments have proved that in the phloem above (scion), below (stock) and at the graft union higher concentrations of phenolic substances are synthesized in the heterospecific combinations than in the homospecific combinations (GEIBEL & FEUCHT 1991, TREUTTER & al. 1987, USENIK & ŠTAMPAR 2000a & 2000b). The influence of the rootstocks on the content of phenolic substances in scion tissues has not yet been analysed. Results of the research confirm the assumption that the rootstock alters the metabolism of the scion. The concentrations of certain polyphenols increase during stressed situations (ERREA & al. 1994, DIRR & al. 1994, YURI & al. 1990) and are a sign of a plant's altered metabolism, as in the grafting of two different genotypes (MUSACCHI & al. 2000).

The differences in concentrations of phenolic substances during the growing season can be attributed to various seasonal changes in a plant.

This agrees with the findings described by SCHWALB & FEUCHT 1999. Each observed substance has its own curve of seasonal alterations of concentrations. The concentrations of phenols in the leaves differed depending on the rootstocks. The curves of seasonal flow of phenolic substances in annual shoots on various rootstocks were more similar to each other. Why did the concentrations of phenolic substances in the leaves show greater deviations among rootstocks than in the annual shoots? Metabolism in leaves directly responds to various physiological states of a plant but the response of metabolism in shoots is slower and depends on the metabolism in the leaves.

The most interesting phenolic substances in the sense of growth investigations were growth inhibitors and regulators of IAA activity. Phenolic acid, which inhibits growth, also enhances oxidative decarboxylation of IAA (LOCKARD & SCHNEIDER 1981, STENLID 1976, TREUTTER & FEUCHT 1988, SEIGLER 1998). IAA antagonists or auxin-oxidase cofactors are ferulic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid (LOCKARD & SCHNEIDER 1981). The response of different plants and in vitro grown tissues to phenols is not uniform (LAVEE & al. 1994, FEUCHT & TREUTTER 1996, FEUCHT & TREUTTER 1995, YURI & AL. 1990, FEUCHT & al. 1988).

Seasonal observations of phenols show that there were lower concentrations of phenols in those trees which had been grafted on the rootstock 'F 12/1' than in the trees which had been grafted on the semi-dwarfing rootstocks, namely 'G 5' and 'W 158'. However, the statement does not hold true for the rutin concentration in the leaves in June nor for the concentrations of phenols in the phloem in September. During the time of intense vegetative growth (April, May, June) the concentrations of phenols in the annual shoots were by more than 50% lower in those trees which had been grafted on the 'F 12/1' rootstock. Vegetative growth of cherry trees on the rootstock 'F 12/1' was more vigorous than on the 'G 5' and 'W 158'. The differences in phenol concentrations among the rootstocks of similar vigour ('G 5' and 'W 158') were insignificant.

With regard of the contents of polyphenols in the 4-year-old trees in the scion tissues and data of vegetative growth of 'Lapins' it can be assumed that concentrations of phenols in scion are related with both rootstocks and tree vigour. Our data are in agreement with hypothesis of SOUMELIDOU & al. 1994 and results of GUR & SAMISH 1968. Higher concentrations of phenolic substances in the heterospecific combinations which are the consequence of the adjustment of the metabolism of genetic different partners of the graft union and a sign of plant's altered metabolism (MUSACCHI & al. 2000), influence the tree vigour. Further investigation is needed to determine the interaction of polyphenols with tree vigour. It will be of essential importance to investigate the issue in detail.

References

- CALLESEN O. 1998. Recent developments in cherry rootstock research. – *Acta Hort.* 468: 219–228.
- DIRR U., FEUCHT W. & TREUTTER D. 1994. Effect of nutrient deficiency on accumulation and leakage of the stress metabolite prunin. – *Acta Hort.* 381: 398–404.
- ERREA S., FELIPE A., TREUTTER D. & FEUCHT W. 1994. Flavanol accumulation in apricot grafts as a response to incompatibility stress. – *Acta Hort.* 381: 498–501.
- FEUCHT W. & TREUTTER D. 1991. Phenol gradients in opposing cells of *Prunus* heterografts. – *Adv. Hort. Sci.* 5: 107–111.
- & — 1995. Catechin effects on growth related processes in cultivated calli of *Prunus avium*. – *Gartenbauwissenschaft* 60: 7–11.
- & — 1996. Effects of abscisic acid and (+)-catechin on growth and leaching properties of callus from four fruit tree species. – *Gartenbauwissenschaft* 61, 4: 174–178.
- , — & SCHMID P. 1988. Inhibition of growth and xylogenesis and promotion of vacuolation in *Prunus* callus by the flavanone prunin. – *Plant Cell Reports* 7: 189–192.
- GEBHARDT K. & FEUCHT W. 1982. Polyphenol changes at the union of *Prunus avium*/*Prunus cerasus* grafts. – *Journal of Horticultural Science* 57: 253–258.
- GEIBEL M. & FEUCHT W. 1991. Flavonoid 5-glucosides from *Prunus cerasus* bark and their characteristic weak glycosidic bonding. – *Phytochemistry* 30: 1519–1521.
- GUR A. & SAMISH R. M. 1968. The role of auxines and auxin destruction in the vigor effect induced by various apple rootstocks. – *Beitr. Biol. Pflanzen* 45: 91–111.
- HELLER W., ERNST D., LANGEBAEDEL C. & SANDERMANN H. 1994. Induction of polyphenol biosynthesis in plants during development and environmental stress. – In: *Polyphenols 94*, Palma de Mallorca (Spain), 23.–27. May, 1994. Paris, INRA, 67–78.
- JONES O. P. 1986. Endogenous growth regulators and rootstock/scion interactions in apple and cherry trees. – *Acta Hort.* 179: 177–184.
- KAMBOJ J. S., BLAKE P. S., QUINLAN J. D., WEBSTER A. D. & BAKER D. A. 1997. Recent advances in studies on the dwarfing mechanism of apple rootstocks. – *Acta Hort.* 451: 75–82.
- LAVEE S., AVIDAN N. & PIERIK R. L. M. 1994. Chlorogenic acid – an independent morphogenesis regulator or a cofactor. – *Acta Hort.* 381: 405–412.
- LOCKARD R. G. & SCHNEIDER G. W. 1981. Stock and scion relationships and the dwarfing mechanism in apple. – *Horticultural Reviews* 3: 315–375.
- MARTERLOCK G., BAUER H. & TREUTTER D. 1994. Characterisation of *Prunus avium* L. varieties with phenolic compounds. – *Fruit Varieties Journal* 48: 81–88.
- MUSACCHI S., PAGLIUCA G., KINDT M., PIRETTI M. V. & SANSAVINI S. 2000. Flavonoids as markers for pear-quince graft incompatibility. – *J. Applied Botany* 74: 206–211.
- SCHWALB P. & FEUCHT W. 1999. Changes in the concentration of phenolic substances in the bark during the annual development of the cherry tree (*Prunus avium* L.). – *Adv. Hort. Sci.* 13: 71–75.
- SEIGLER D. S. 1998. Flavonoids. In: *Plant secondary metabolism*, 151–192. Boston/Dordrecht/London, Kluwer academic publishers, U.K.

- SOUMELIDOU K., MORRIS D. A., BATTEY N. H., BARNETT J. R. & JOHN P. 1994. Auxin transport capacity in relation to the dwarfing effect of apple rootstocks. – J. Hort. Sci. 69: 719–725.
- STENLID G. 1976. Effects of flavonoids on the polar transport of auxins. – Physiol. Plant. 38: 262–266.
- TREUTTER D. 1988. Separation of naturally occurring mixtures of phenolic compounds from various *Prunus* tissues by reversed-phase high-performance liquid chromatography. – J. Chrom. 436: 490–496.
- & FEUCHT W. 1988. Accumulation of the flavonoid prunin in *Prunus avium*/*Prunus cerasus* grafts and its possible involvement in the process of incompatibility. – Acta Hort. 227: 74–78.
- & — 1991. Accumulation of phenolic compounds above the graft union of cherry trees. – Gartenbauwissenschaft 56: 134–137.
- , — & SCHMID S. S. 1987. Ageing-dependent responses of phloem flavonoids of *Prunus avium* graftings: flavanone, flavone- and isoflavone-glucosides. – Sci. Hort. 32: 183–193.
- USENIK V. & ŠTAMPAR F. 2000a. Effect of different rootstocks on polyphenol content in cv. 'Lapins' phloem. – Research Reports, Biotechnical Faculty, 75 (2): 217–222.
- & — 2000b. Influence of various rootstocks for cherries on p-coumaric acid, genistein and prunin content and their involvement in the incompatibility process. – Gartenbauwissenschaft 65: 245–250.
- WEBSTER A. D. 1998. Strategies for controlling the size of sweet cherry trees. – Acta Hort. 468: 229–240.
- YURI A., SCHMITT E., FEUCHT W., & TREUTTER D. 1990. Metabolism of *Prunus* tissues affected by Ca^{2+} – deficiency and addition of prunin. – J. Plant Physiol. 135: 92–97.

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