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The diversity of polyprenol pattern in leaves of fruit trees belonging to *Rosaceae* and *Cornaceae*[o]

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The polyprenol pattern in leaves of fruit trees belonging to the *Rosaceae* (genera: *Prunus, Malus*) and *Cornaceae* (genus: *Cornus*) families is presented. The content of polyprenyl acetates varied within plant species between 10–50 mg per gram of dry weight. In genus *Prunus*, *Cornus* and in representatives of species *Malus domestica*, a mixture of polyprenols composed of 18, 19, 20, 21 isoprene units was found. In six species of genus *Prunus* (sour-cherry): *P. serrulata-spontanea, P. yedoensis, P. fruticosa. P. kurilensis, P. subhirtella* and *P. incisa* the presence of a second polyprenol family, i.e. the group of prenologues consisting of prenol -35, -36, -37, etc. up to -42 was detected.

Plant polyprenols represent a large diversity of chain length and variations in polyprenol pattern and content. The occurrence of polyprenols composed of 11 or 12 isoprene units in plants was first reported by Wellburn & Hemming (1966) over 30 years ago. Further examination of various systematic groups of gymnosperm plants demonstrated the multiplicity in polyprenol families in which the dominant alcohols are built of 17 or 18 and 23 or 24 isoprene units (Ibata *et al.*, 1984, Świeżewska & Chojnacki, 1988). The studies on polyprenols in leaves of *Rosaceae* have demonstrated also the complexity of polyprenol families in some genera, e.g. *Potentilla* and *Rosa*, as multiple families were characteristic of almost all species studied (Świeżewska *et al.*, 1992).

It was previously demonstrated on a limited number of species of genera *Malus* and *Pyrus* that the dominant prenologues are prenol-20 - 21 and -22 while in genus *Prunus* prenols are composed of 19, 20, 21 units (Świeżewska & Chojnacki, 1996). It was also found that in one species of genus *Prunus* (*P. incisa*) an additional polyprenol family was present, consist-

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Abbreviations: TLC, thin-layer chromatography; HPLC, high-pressure liquid chromatography.

ing of prenologues composed of about 40 isoprene units.

The aim of this study was to examine a number of plants of *Rosaceae* and *Cornaceae* fruit trees in order to find whether these exceptionally long chain polyprenols are more common. For this purpose various rare old varieties of *Malus, Prunus* and *Cornus* from old local collections were examined.

MATERIALS AND METHODS

Plant material. Leaves of fruit trees belonging to the *Rosaceae* and *Cornaceae* families were collected from plants grown in the open air in old orchards in the Bolestraszyce region (including Bolestraszyce Arboretum), in Kórnik Arboretum (Institute of Dendrology, Polish Academy of Sciences) (September 1996 and 1997) and in the Botanical Garden, Polish Academy of Sciences in Powsin near Warsaw (September 1997). The leaves were dried at room temperature and stored in paper envelopes for 2–3 weeks.

Chemicals. Kiesel Gel TLC plates and RP-18 plates with concentrating zone and Kiesel Gel (200–400 mesh) for column chromatography were from Merck (Darmstadt, Germany). Organic solvents used for HPLC were from J.T. Baker B.V. (Deventer, Holland) and from Merck. Standard substances: single prenologues, a mixture of polyprenyl acetates, and a mixture of polyprenol palmitates were from the Collection of Polyprenols, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw. Acylation of polyprenols was performed according to Keller & Adair (1977).

Extraction and quantitative estimation of polyprenols in plant leaves. A sample of dry leaves (0.5 g) was homogenized in a homogenizer with metal blades (Ultra Turrax, Germany) at the top speed in 4 ml of acetone/hexane (1:1, v/v). The homogenate was stored in the dark for 24 or 48 h at room temperature. The dark-green extract (10 μl) was used for thin-layer chromatography.

Thin-layer chromatography was performed on Kiesel Gel plates in solvent A (hexane/toluene, 1:1, v/v). On the RP-18 plates polyprenols were run in solvent B (acetone/hexane, 9:1, v/v). The chromatographic run was performed three times in the same direction in solvent B. Spots of polyprenols were detected with iodine vapours. Semiquantitative estimation of polyprenols was performed by comparing the size and intensity of the spots with that of a known amount of standard substances on Kiesel Gel plates in solvent A.

Alkaline hydrolysis was performed according to Stone *et al.* (1967) and the unsaponifiable fraction was analyzed by thin-layer chromatography on Kiesel Gel plates in solvent C (toluene/ethyl acetate, 95:5, v/v) and on the RP-18 plates in solvent B.

High-pressure liquid chromatography (HPLC) was performed on a Hypersil ODS 3 μm reversed-phase column (Knauer, Germany) with the Waters Ass. (U.S.A.) dual pump apparatus, gradient programmer, UV detector (210 nm) and an integrator. The elution system used for chromatography was isopropanol/methanol/water (8:12:1, by vol.) in pump A. Hexane/isopropanol (7:3, v/v) in pump B was added from 0% to 60% according to the gradient type "6" or from 0% to 69% according to type "5". The solvent flow rate was 1.5 ml per minute and the analysis was performed for 60, 80 or 90 min.

RESULTS

The results of quantitative estimation of polyprenols in various species of genus *Prunus* are shown in Table 1. In all the species the leaves of plants contained 5–50 mg of polyprenols per gram of dry weight (for details see Materials and Methods). Thin-layer chromatography on RP-18 showed that in

some of the studied species of *Prunus* two groups of polyprenols were present, the first with dominating prenol-18 to -20, and the second with longer chain polyprenols: prenol-34, -35, -36 to -42 (not shown). On the TLC (Kiesel Gel plates, solvent A) record we observed 4–5 spots, but only one of them, corresponding to prenyl-19 acetate, was quite intensive. In *P. serrulata-spontanea, P. yedoensis, P. fruticosa, P. kurilensis, P. incisa* and *P. subhirtella* a second spot moving slightly ahead of prenyl-19 acetate was observed. It corresponded to the additional group of longer chain polyprenyl esters (-34, -35, to -42). In all samples of *P. avium* growing also in Bolestraszyce Arboretum this second spot had much lower intensity. According to chromatographic identification in all these species the polyprenols occurred as acetic acid esters.

The extracts of all plants were subjected to alkaline hydrolysis. In unsaponifiable fraction of *P. serrula* and *P. maximowiczii, P. tomentosa, P. pumila-depressa, P. sachaliensis, P. japonica, P. avium* only one, distinct spot at R_F 0.5 (solvent C) was noticed and this result corresponds with the absence of the second spot of polyprenyl esters in the unhydrolyzed fraction. In *P. kurilensis* and *P. fruticosa* the presence of the second spot confirms our earlier observations made on TLC records of non hydrolyzed extracts. The HPLC records performed after alkaline hydrolysis of *P. kurilen-*

Table 1. Polyprenols in leaves of fruit trees belonging to genus *Prunus*.

Content and type of polyprenols were estimated as described in Materials and Methods.

Prunus species	*Locus	Polyprenols	
		Content (mg/g dry weight)	Chain length (number of isoprene units)
1. *P. serrulata-spontanea*	K	**10–20** **5–10**	18,**19**,20 34,35,36,...41
2. *P. yedoensis*	K	**10–25** **1–5**	18,**19**,20 34,35,36,...42
3. *P. fruticosa*	K	**10–20** **1–5**	18,**19**,20 34,35,36,...41
4. *P. kurilensis*	K	**20–40** **5–10**	18,**19**,20 34,35,36,...41
5. *P. incisa*	K	**10–20** **1–5**	18,**19**,20 34,35,36,...41
6. *P. serrula*	K	**10–20**	18,19,20
7. *P. subhirtella*	K	**10–20** **5–10**	18,**19**,20 34,35,36,...41
8. *P. tomentosa*	K	**10–20**	18,**19**,20
9. *P. maximowiczii*	K	**20–50**	18,**19**,20
10. *P. pumila depressa*	K	**10–20**	18,**19**,20
11. *P. sachaliensis*	K	**10–25**	18,**19**,20
12. *P. japonica*	K	**10–15**	18,**19**,20
13. *P. avium*	A	**5–15**	18,**19**,20
14. *P. avium*	B	**5–15**	18,**19**,20
15. *Prunus* sp.	BA	**10–20**	18,**19**,20
16. *Prunus* sp.	BA	**10–20**	18,**19**,20
17. *Prunus* sp.	BA	**10–20**	18,19,20

*The leaves were taken from plants grown in: A, Aksmanice (Bolestraszyce region); B, Berdehowice (Bolestraszyce region); K,Kórnik Arboretum; BA, Bolestraszyce Arboretum.

sis (Fig. 1A) confirm the occurrence of two almost separated groups of polyprenols. In *P. maximowiczii* (Fig. 1B) similarly as in *P. tomentosa, P. pumila-depressa, P. sachaliensis, P. japonica* and *P. avium* the presence of longer chain polyprenols was not detectable by HPLC.

Table 2 presents the results of semiquantitative estimation of polyprenols in two species of the genus *Cornus* from Bolestraszyce Arboretum. The group of *Cornus mas* shows some differences in the content of polyprenols per dry weight (20–50 mg/g). In all the plants studied the dominating component was prenol-19 (evaluation by RP-18 chromatography, not shown). This single band observed on TLC (Kiesel Gel plates, solvent C) of hydrolyzed lipid extracts corresponds the one group of polyprenols found on HPLC records (Fig. 1C). Lipid composition of *C. japonica* was different but the dominating spot observed at the front of the plate (TLC of non hydrolyzed fraction) has not been identified. It is evident from HPLC estimation that this plant contained only one family of polyprenols with dominating polyprenol-18 (Fig. 1D).

The results of quantitative estimation of the prenol content and evaluation of the chain length in genus *Malus* are shown in Table 3. The leaves of plants contained 10–50 mg of polyprenols per gram of dry weight. In all studied species prenol-19, -20, -21 was the main component. On TLC (Kiesel Gel, solvent A) of crude extract we observed a dominating single spot at R_F 0.5, corresponding to prenyl-19 acetate standard. Additional 2–3 spots (differing in intensity) were also noticed. As shown by TLC of unsaponified lipid fraction (not shown) in selected samples: *M. domestica* var. Sztetyna Zielona, var. Grochówka, var. Reneta Baumana, not identified the presence of the group of longer chain polyprenols was never detectable in the experimental conditions used (the leaves were collected during two vegetative seasons). The absence of the second spot in unsaponifiable fraction of all samples was confirmed by HPLC records (Fig. 1E – a representative record).

Additionally, about 20 "berry apple trees", which were remainders of the many formerly popular decorative cultivars, were examined and the polyprenol pattern observed was typical of genus *Malus*.

DISCUSSION

Our studies were undertaken to describe the type of the polyprenol pattern and especially the presence of multiple families of prenologues in a large number of species (in the case of genera *Prunus* and *Cornus*) and in various varieties (in the case of *Malus domestica*). In our previous paper we reported that the spectrum of polyprenols consisted of one group with prenol-19 or prenol-20 being the dominating prenologues in the species *Malus* and *Pyrus*, while among the species of genus *Prunus* we found the presence of two groups of polyprenols, i.e. a group of prenols with dominating prenol-19 and a group of longer chain prenologues, prenol-36, -37, etc. up to -42 in one case (*Prunus incisa*) (Świeżewska & Chojnacki, 1996).

The results of the present studies confirmed the presence of the second polyprenol family, i.e. the group of prenologues consisting of prenol-34, -35, -36, etc. up to -42, in other species of *Prunus*: *P. serrulata-spontanea, P. yedoensis, P. fruticosa, P. kurilensis, P. subhirtella*. The leaves of these species exhibited a pattern of polyprenols similar to that reported previously for *P. incisa*. One third of the studied species of genus *Prunus* exhibited this multiple pattern of polyprenols. All of them represent sour-cherry plants. In the remaining two thirds, i.e. 9 species of sour-cherry and one sweet-cherry, only one group of polyprenols was detected (prenol-18, -19, -20). This may point to the taxonomic complexity of the genus *Prunus*. The differences of the polyprenol pattern observed in these studies in various

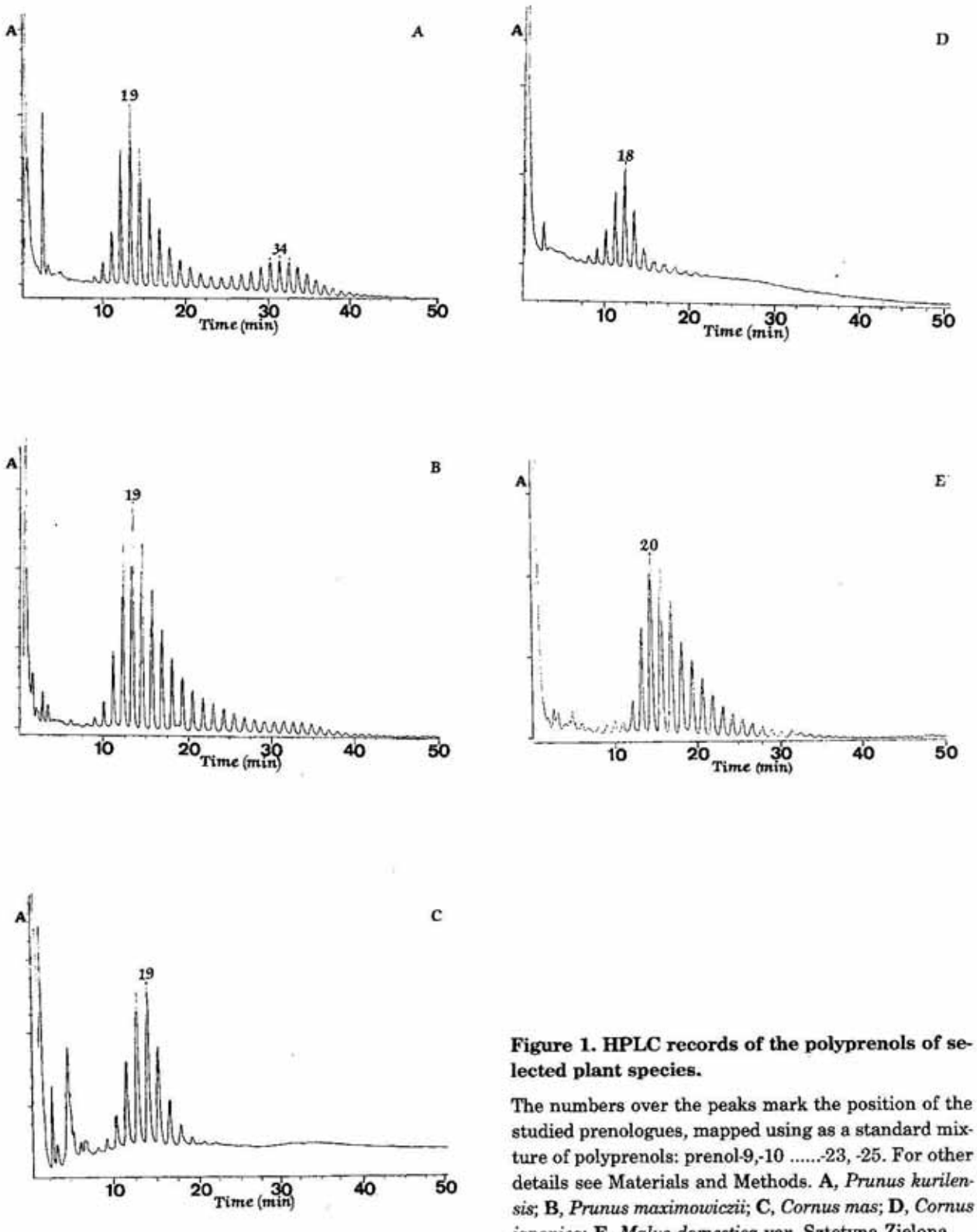

Figure 1. HPLC records of the polyprenols of selected plant species.

The numbers over the peaks mark the position of the studied prenologues, mapped using as a standard mixture of polyprenols: prenol-9,-10 .....-23, -25. For other details see Materials and Methods. **A**, *Prunus kurilensis*; **B**, *Prunus maximowiczii*; **C**, *Cornus mas*; **D**, *Cornus japonica*; **E**, *Malus domestica* var. Sztetyna Zielona.

species of *Prunus* (sour-cherry) can be explained by the fact that, according to the botanical systematics, some representatives of this genus may be evolutionarily rather young, arising from natural crossing or mutations or due to changes accompanying domes-

Table 2. Polyprenols in leaves of fruit trees belonging to genus *Cornus* growing in Bolestraszyce region (B).

Eight individual plants of *C. mas* and one of *C. japonica* were tested. Content and type of polyprenols were estimated as described in Materials and Methods.

Cornus species	Locus	Polyprenols	
		Content (mg/g dry weight)	Chain length (number of isoprene units)
19. *C. mas*	**B**	**20–40**	18,**19**,20
20. *C. mas*	**B**	**20–40**	18,**19**,20
21. *C. mas*	**B**	**20–30**	18,**19**,20
22. *C. mas*	**B**	**10–20**	18,**19**,20
23. *C. mas*	**B**	**10–25**	18,**19**,20
24. *C. mas*	**B**	**20–40**	18,**19**,20
25. *C. mas*	**B**	**30–50**	18,**19**,20
26. *C. japonica*	**B**	**5–10**	17,**18**,19

tication. On the contrary, the representatives of the old species of *P. avium* (sweet cherry) do not differ much from their wild ancestors known in Tertiary formation. In none of the *Prunus avium* plants tested we were able to find, after alkaline hydrolysis, the second family of longer chain polyprenols composed of 36–42 isoprene units.

In the 8 studied plants of the species *Cornus mas* and *C. japonica* a simple pattern of polyprenols was found. We suggest that this reflects the uniform taxonomic character of this group of plants.

Special attention was given to representatives of *Malus domestica* species. The studied 104 individual plants represented a group of several varieties, both classified and undefined, wild local varieties characteristic of the area of South-East of Poland and adjacent region of Ukraine which was formerly, at the end of the 19th and beginning of the 20th century, an area of rich horticulture. Several studied apple trees were taken from old local orchards, usually about 80 years old. Many of those varieties are unique for this region of Europe and show a considerable biological diversity. In none of them we could detect the presence of more than one polyprenol family. In all of them only this single group of polyprenols, consisting of prenologues composed of 19–22 units, was detected, irrespective of the evident differences in the appearance of the plants studied and the variations in the shape, size and thickness of their leaves. As a rule the content of polyprenols was high in all studied species and the type of the polyprenol pattern was the same, irrespective of the content of polyprenols.

The observed uniformity of the polyprenol pattern in the varieties of *Malus* could be explained by the possibility that these varieties originated from a common ancestor, which underwent qualitative and quantitative evolutionary changes during the domestication process. Similar observations were made by Kazimierczak *et al.* (1997) in their study on the specific pattern of polyprenols in green needles of *Pinus mugo* Turra. The spectrum of polyprenols (prenol-15, -16 and -17) was the same, irrespective of the location of plants and of distinct morphological differences observed between the various selected forms of this species.

The results of our observations and the data on the origin and history of development of the species of fruit trees belonging to *Ro-*

Table 3. Polyprenols in leaves of fruit trees belonging to genus *Malus* growing in Bolestraszyce region.

Content and type of polyprenols were estimated as described in Materials and Methods.

M. domestica variety	Polyprenol	
	Content (mg/g dry weight)	Chain length (number of isoprene units)
„Rarytas Śląski" *N, „Sztetyna Zielona" N, "Boiken" N, "Landsberska" N, "Landsberska" N, "Piękna z Zabergau. N, "Szara Reneta" A. *M. domestica* "n.d"† -N (7 plants), *M. domestica* "n.d." -B (3 plants), *M. domestica* "n.d."- A (6 plants).	**10–20**	**19,20,21**,22
"Ribston" N, "Truskawkowe Wisnera" N, "Sztetyna Zielona" N, "Bukówka" G, "Reneta darc" A, "Glogierówka" A, "Papierówka" A, "Żelaźniak" A, "Bukówka" A. *M. domestica* "n.d." - N (5 plants), *M. domestic* "n.d."-G (6 plants), *M. domestica* "n.d" -B (2 plants), *M. domestica* "n.d" -A (18 plants), *M. domestica* "n.d." -AB (10 plants)	**20–30**	**19,20,21**,22
"Grochówka" N, "Reneta Baumanna" G, "Codle" A, "Grochówka"A, "Książę Albrecht" A, "Żelaźniak" A, "Landsberska" A, *M. domestica* "n.d" - N (3 plants), *M. domestica* "n.d" -G (1 plant), *M. domestica* "n.d" -B (1 plant), *M. domestica* "n.d"- A (8 plants), *M. domestica* "n.d." -AB (11 plants).	**30–60**	**19,20,21**,22

†"n.d" - non defined variety; *Leaves were taken from plants grown in: **N**, New orchards Bolestraszyce No. 40; **G**, Bolestraszyce No. 128 (owner Grażyna Majka); **B**, Berdehowice; **A**, Aksmanice; **AB**, Arboretum Bolestraszyce.

saceae and *Cornaceae* suggest that the type of polyprenol pattern might serve as a reliable chemotaxonomic marker.

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