

EFFECT OF DIFFERENT DWARFING METHODS ON CALCIUM CONTENT IN DIFFERENT APPLE TREE ORGANS

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Abstract. The paper presents the results of chemical analyses of different organs of 'Jonica' apple trees. The trees were dwarfed with: rootstocks (M.9 and P60), and additionally, with bark grafting (in normal and inverted position), microinterstocks (M.9, P22, M.27) and retardant (Paclobutrazol). Microinterstock is an additional way of dwarfing, where a ring of bark taken from dwarfing rootstock is grafted on the tree's trunk. In the years 1996–1999 fruit, leaf, wood, bark and root samples of each treatment were collected. After washing, samples were dried, grounded and subjected to mineralization and analysed for calcium by atomic absorption spectrophotometry. The highest concentration of Ca was noted in the bark of investigated trees (1.53% d.w.). Relatively high concentration of Ca was noted in leaves and roots (0.98% d.w. and 0.48% d.w. respectively). The lowest content was noted in wood and fruits (0.09% d.w. and 0.02% d.w.). Analysed rootstocks did not affect significantly the leaves calcium content. On the contrary rootstocks influenced fruit Ca amounts; the lower Ca content was noted in fruits from trees grafted on P60 rootstock. Bark graftage or microinterstocks did not influenced or lowered Ca fruit content. The only exception was microinterstock P22 which significantly increased fruit Ca amounts. No correlation between leaf and fruit Ca content was found. Bark grafting and microinterstocks decreased root Ca content. This effect was especially evident in the first year after grafting.

Key words: *Malus domestica*, mineral content, rootstocks, bark grafting, microinterstocks

INTRODUCTION

The rootstocks have a significant effect on the growth and yields of trees. They also show great differences with respect to the uptake of mineral constituents from soil. Interstocks or microinterstocks show a varied capability of conducting them. Owing to the genetically conditioned capability of the uptake and transmission they can change

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the balance in the content of elements in a fruit tree and hence prevent disturbances caused by the deficiency of certain mineral constituents. Therefore the basic problem of using rootstocks or dwarfing interstems is to assess what interactions occur between them, the cultivar and the environment. Some authors [Hrotko 2004] postulate such a comprehensive holistic approach.

In the present work the main issue was calcium – an element, which of old has been investigated by physiologists [Buchloh 1974, Poovaiach 1993, Zocchi and Mignani 1995]. Most papers concerning this element deal with the accumulation of calcium in leaves [Tukey et al. 1962, Lockard 1976] or in fruit [Tomala and Dilley 1990, Słowińska and Tomala 2001]. However, only a few publications deal with the mineral composition of other organs of the apple tree [Hrvina et al. 1989, Duric et al. 1997], which also take part in the mineral economy of trees. The present work was an attempt to fill this gap.

MATERIAL AND METHODS

The experiment was established in spring 1995 in the Experimental Station of the Horticultural Faculty in Garlica Murowana near Krakow. The soil of the plot where the fruit trees were planted was in the valuation class II b. It is of the brown soil type developed from loess and represents a species determined as silt loam. Soil properties are given in tables 1 and 2.

Table 1. Granulometric soil content of experimental orchard
Tabela 1. Skład granulometryczny gleby sadu doświadczalnego

| Fraction diameter, mm Średnica frakcji, mm | 1.0–0.1 | 0.1–0.05 | 0.05–0.02 | 0.02–0.006 | 0.006–0.002 | <0.002 |
|---|---------|----------|-----------|------------|-------------|--------|
| % share of fraction % frakcji | 2 | 10 | 48 | 22 | 5 | 13 |

Table 2. Acidity, macro- and microelement content in soil before orchard plantation (mean values)
Tabela 2. Odczyn oraz zawartość makro- i mikroelementów w glebie przed założeniem sadu (wartości średnie)

| Depth of sampling Głębokość pobierania próby | pH _{KCl} | Available mineral content, mg · 100 g ⁻¹ Zawartość przyswajalnych składników, mg · 100 g ⁻¹ | | | | Fe % | Zn ppm | Cu ppm |
|--|-------------------|--|-------|--------|------|---------|-----------|-----------|
| | | P | K | Mg | Ca | | | |
| 0–20 cm | 5.3 | 4.91* | 10.9* | 6.56** | 36.5 | 1.82 | 101 | 23.3 |
| 20–40 cm | 4.9 | 4.08 | 8.97 | 9.15 | 39.8 | 1.73 | 127 | 26.4 |

* determined by Egner-Riehm method – mierzone metodą Egnera-Riehma

** determined by the universal method – mierzone metodą uniwersalną

The investigated material was composed of one-year old budded trees of Jonica cultivar on two rootstocks P60 and M.9. In the orchard the soil cultivation system was herbicidal fallow in rows and grass in inter-rows. The apple trees were spaced 1.5 × 1.5 m. The crowns of trees were trimmed in a spindle form. The protection of the trees was

carried out according to the recommendations accepted for commercial plantations. However, no preventive spraying with calcium salts was applied.

The experiment was established in a randomized blocks design, each treatment being represented by four replications – plots of five trees each. The following treatments were used in the experiment:

1. Control I (=Control/M.9) – Jonica apple trees budded on M.9 rootstock.
2. Control II (=Control/P60) – Jonica apple trees budded on P60 rootstock.
3. Grafting with a bark ring in normal position (= Ring 0°) – Jonica trees budded on P60; a bark ring taken from the tree trunk was grafted in the same place with its polarity preserved.
4. Grafting with a bark ring in an inverted position (= Ring 180°) – Jonica apple trees on P60 rootstock; a bark ring taken from a tree was inverted by 180° and grafted in an inverted position.
5. Microinterstock M.9 – Jonica apple trees on P60 rootstock; a bark ring taken from the trunk was replaced with a bark ring from the M.9 rootstock in the normal position.
6. Microinterstock P22 – Jonica apple trees on P60 rootstock; a bark ring taken from the tree trunk was replaced with a bark ring from P22 rootstock in the polar position.
7. Microinterstock M.27 – Jonica apple trees on P60 rootstock; a bark ring taken from the tree trunk was replaced with bark ring from M.27 rootstock.
8. Paclobutrazol – Jonica apple trees on P60 rootstock; Paclobutrazol (Cultar) applied to the root neck in spring. No microinterstock.

In May 1996 in treatments 3–7 bark rings 2 cm in width were grafted. The grafting was carried out on the whole circumference of the trunk at a height of 40 cm. The treatment was conducted only in 1996 while every year in the same period Cultar (20 mg/l) was applied to the root necks of trees in the form of a lanolin paste.

Fruit and leaf samples for a chemical analysis were taken directly from the trees growing in the experimental orchard while the samples of roots, bark and wood were taken from trees, which were purposefully planted on additional reserve plots. Each treatment was represented by eight samples.

Tree roots were sampled during the period of tree rest (late autumn). The collected material was washed in tap water to remove soil, then rinsed in distilled water and dried. At the same time the samples of bark¹ and wood were separately taken from below and above the microinterstocks. Leaf samples were collected in the last 10 days of July. From each plot a sample of 100 leaves with petioles and stipules was taken from a middle part of long shoots. From each plot 25 apples were also sampled, their flesh cut in thin slices and dried at 60°C. Then all the samples were ground in a beater mill.

The prepared air dry samples were subjected to wet mineralization in a mixture of nitric and perchloric acids (at 3:1 ratio) and their composition was assessed using an atomic absorption spectrometer (AA 140, Varian).

The measurements were listed and subjected to analysis of variance. Differences between the means were ascertained with a multiple Duncan Test, using a Statistica 6.0 program. The mean values for the combinations labeled with the same letters do not significantly differ at the significance level $\alpha = 0.05$.

¹The word “bark” was used for all tissues located in the trunk outside of cambium and constituting the secondary bark. It includes phloem, parenchyma of core rays and multi-annual peryderma.

RESULTS

Calcium level found in the different organs of apple trees differed to a great degree (tab. 3). The greatest content was assessed in the bark of trees (1.53% d.w.), a smaller one in leaves (0.98% d.w.) and in roots (0.48% d.w.). The lowest level was found in wood and fruit (0.09% d.w. and 0.02% d.w. respectively).

Table 3. Mean calcium content in different organs of apple tree
Tabela 3. Średnia zawartość wapnia w różnych organach jabłoni

| | Ca content, % d. m. Zawartość Ca, % s.m. |
|------------------|---|
| Fruits – Owoce | 0.02 a* |
| Leaves – Liście | 0.98 c |
| Wood – Drewno | 0.09 a |
| Bark – Kora | 1.53 d |
| Roots – Korzenie | 0.48 b |

* Means followed by the same letter do not differ at $\alpha = 0.05$; Duncan's Multiply Range Test – Średnie oznaczone jednakowymi literami nie różnią się przy poziomie istotności $\alpha = 0,05$

Table 4. Calcium content in fruits in years 1997–1999
Tabela 4. Zawartość wapnia w owocach w latach 1997–1999

| Treatment Kombinacja | Ca % d.w.– % s.m. | | | Mean Średnia |
|--|----------------------|-----------|----------|-----------------|
| | 1997 | 1998 | 1999 | |
| Control/M.9 – Kontrola/M.9 | 0.021 b* | 0.022 abc | 0.034 d | 0.026 bc |
| Control/P60 – Kontrola/P 60 | 0.019 ab | 0.019 a | 0.029 c | 0.023 a |
| Ring of bark 0° – Pierścień 0° | 0.014 a | 0.021 ab | 0.031 cd | 0.022 a |
| Ring of bark 180° – Pierścień 180° | 0.015 a | 0.033 d | 0.025 b | 0.024 ab |
| Microinterstock M.9 – Mikrowstawka M.9 | 0.019 ab | 0.021 ab | 0.034 d | 0.025 ab |
| Microinterstock P22 – Mikrowstawka P 22 | 0.024 b | 0.026 c | 0.032 cd | 0.028 c |
| Microinterstock M.27 – Mikrowstawka M.27 | 0.021 b | 0.021 ab | 0.029 c | 0.024 ab |
| Paclbutrazol | 0.018 a | 0.025 bc | 0.020 a | 0.021 a |
| Mean – Średnia | 0.019 a | 0.023 b | 0.029 c | |

* Means followed by the same letter do not differ at $\alpha = 0.05$; Duncan's Multiply Range Test – Średnie oznaczone jednakowymi literami nie różnią się przy poziomie istotności $\alpha = 0,05$

Analyses of fruit showed that the rootstocks affected the accumulation of calcium (tab. 4). The level of Ca was lower in fruit from trees on P.60 in comparison with trees on M.9. In comparison with the control the content of calcium in trees on P60 increased only in the case of a microinterstock from P22, hence it was similar to that found in trees on M.9 rootstock. The remaining treatments did not influence this trait. In the successive years of the experiment the content of calcium in fruit increased.

The differentiation in calcium content was recorded in the years of the investigation; in 1996 and 1998 the values were higher in comparison with the year 1997 (tab. 5). However, no differences between the control plots (M.9 and P60) were ascertained in the different years. Neither did the means for the whole period of the experiment show differences between the control trees on P60 rootstock and the remaining treatments. A tendency to a limited accumulation observed in leaves was due to the microinterstock

M.9 and to the bark ring inversion while an increase was caused by the microinterstock P22 and the paclobutrazol treatment.

Table 5. Calcium content in leaves in years 1996–1998
Tabela 5. Zawartość wapnia w liściach w latach 1996–1998

| Treatment Kombinacja | Ca % d.w.– % s.m. | | | Mean Średnia |
|--|----------------------|---------|---------|-----------------|
| | 1996 | 1997 | 1998 | |
| Control/M.9 – Kontrola/M.9 | 0.99 a | 0.75 b | 1.07 a | 0.93 a |
| Control/P60 – Kontrola/P 60 | 1.08 ab | 0.80 b | 1.08 a | 0.98 abc |
| Ring of bark 0° – Pierścień 0° | 1.17 b | 0.61 a | 1.16 ab | 0.98 abc |
| Ring of bark 180° – Pierścień 180° | 0.95 a | 0.78 b | 1.09 a | 0.94 a |
| Microinterstock M.9 – Mikrowstawka M.9 | 1.07 ab | 0.73 ab | 1.06 a | 0.95 ab |
| Microinterstock P22 – Mikrowstawka P 22 | 1.22 b | 0.72 ab | 1.15 a | 1.03 c |
| Microinterstock M.27 – Mikrowstawka M.27 | 1.12 b | 0.75 b | 1.14 a | 1.01 bc |
| Paclobutrazol | 1.09 ab | 0.75 b | 1.27 b | 1.04 c |
| Mean – Średnia | 1.09 b | 0.73 a | 1.13 b | |

* Means followed by the same letter do not differ at $\alpha = 0.05$; Duncan's Multiply Range Test – Średnie oznaczone jednakowymi literami nie różnią się przy poziomie istotności $\alpha = 0,05$

Table 6. Calcium content in wood of apple tree in years 1996–1998
Tabela 6. Zawartość wapnia w drewnie w latach 1996–1998

| Treatment Kombinacja | Ca % d.w.– % s.m. | | | Mean Średnia |
|--|----------------------|-----------|----------|-----------------|
| | 1996 | 1997 | 1998 | |
| Control/P60 – Kontrola/P60 | 0.093 bc | 0.093 bc | 0.093 c | 0.093 bc |
| Ring of bark 0° ↑ – Pierścień 0° ↑ | 0.096 bc | 0.096 bc | 0.087 bc | 0.093 bc |
| Ring of bark 0° ↓ – Pierścień 0° ↓ | 0.085 ab | 0.076 a | 0.086 b | 0.082 ab |
| Ring of bark 180° ↑ – Pierścień 180° ↑ | 0.099 c | 0.102 c | 0.087 bc | 0.096 c |
| Ring of bark 180° ↓ – Pierścień 180° ↓ | 0.089 b | 0.083 ab | 0.086 b | 0.086 b |
| Microinterstock M.9 ↑ – Mikrowstawka M.9 ↑ | 0.097 c | 0.089 abc | 0.096 c | 0.094 bc |
| Microinterstock M.9 ↓ – Mikrowstawka M.9 ↓ | 0.085 ab | 0.081 ab | 0.079 ab | 0.082 ab |
| Microinterstock P22 ↑ – Mikrowstawka P 22 ↑ | 0.092 bc | 0.100 c | 0.078 ab | 0.089 bc |
| Microinterstock P22 ↓ – Mikrowstawka P 22 ↓ | 0.081 a | 0.079 ab | 0.077 ab | 0.079 ab |
| Microinterstock M.27 ↑ – Mikrowstawka M.27 ↑ | 0.090 b | 0.090 bc | 0.080 ab | 0.087 bc |
| Microinterstock M.27 ↓ – Mikrowstawka M.27 ↓ | 0.078 a | 0.077 ab | 0.070 a | 0.075 a |
| Mean – Średnia | 0.089 b | 0.088 b | 0.083 a | |

* Means followed by the same letter do not differ at $\alpha = 0.05$; Duncan's Multiply Range Test – Średnie oznaczone jednakowymi literami nie różnią się przy poziomie istotności $\alpha = 0,05$

↑ sample taken above the graftage – próbka pobrana nad miejscem szczepienia

↓ sample taken below the graftage – próbka pobrana pod miejscem szczepienia

Analyses of wood (tab. 6) showed a negligible effect of the treatments on the differentiation of the calcium level. However, in almost all the cases a greater concentration of this element was noted above the grafting site.

The site of bark sampling had a stronger effect on the content of calcium in the bark than the kind of treatment (tab. 7). Both in the different years and in the averages from the whole investigation period a greater content of calcium was found below the site of grafting. The bark sampled below the grafting always contained more calcium than the

control treatment. In the bark above the grafting place the content of Ca was smaller or at the outmost the same. In analyses of both the bark and the wood the perfect stability of the mineral composition is striking in the control treatment on P60 rootstock in the successive years of the experiment.

Table 7. Calcium content in bark of apple trees in years 1996–1998

Tabela 7. Zawartość wapnia w korze w latach 1996–1998

| Treatment Kombinacja | Ca % d.w.– % s.m. | | | Mean Średnia |
|--|----------------------|----------|---------|-----------------|
| | 1996 | 1997 | 1998 | |
| Control/P60 – Kontrola/P60 | 1.26 b | 1.26 cd | 1.26 a | 1.26 b |
| Ring of bark 0° ↑ – Pierścień 0° ↑ | 1.15 b | 0.95 a | 1.71 b | 1.27 b |
| Ring of bark 0° ↓ – Pierścień 0° ↓ | 1.81 c | 1.62 d | 2.36 c | 1.93 d |
| Ring of bark 180° ↑ – Pierścień 180° ↑ | 0.92 a | 0.86 a | 1.34 a | 1.04 a |
| Ring of bark 180° ↓ – Pierścień 180° ↓ | 1.59 c | 1.23 c | 2.25 c | 1.69 c |
| Microinterstock M.9 ↑ – Mikrowstawka M.9 ↑ | 1.10 ab | 1.00 ab | 1.53 ab | 1.21 ab |
| Microinterstock M.9 ↓ – Mikrowstawka M.9 ↓ | 1.68 c | 1.50 d | 2.22 c | 1.80 c |
| Microinterstock P22 ↑ – Mikrowstawka P 22 ↑ | 0.97 ab | 1.26 abc | 1.58 ab | 1.27 b |
| Microinterstock P22 ↓ – Mikrowstawka P 22 ↓ | 1.72 c | 1.89 d | 2.44 c | 2.02 d |
| Microinterstock M.27 ↑ – Mikrowstawka M.27 ↑ | 0.93 a | 1.09 ab | 1.36 a | 1.13 ab |
| Microinterstock M.27 ↓ – Mikrowstawka M.27 ↓ | 1.57 c | 1.89 d | 2.15 c | 1.87 cd |
| Mean – Średnia | 1.34 a | 1.32 a | 1.84 b | |

* Means followed by the same letter do not differ at $\alpha = 0.05$; Duncan's Multiply Range Test – Średnie oznaczone jednakowymi literami nie różnią się przy poziomie istotności $\alpha = 0,05$

↑ sample taken above the graftage – próbka pobrana nad miejscem szczeplenia

↓ sample taken below the graftage – próbka pobrana pod miejscem szczeplenia

Table 8. Calcium content in roots of apple trees in years 1996–1998

Tabela 8. Zawartość wapnia w korzeniach w latach 1996–1998

| Kombinacja Treatment | Ca % d.w.– % s.m. | | | Mean Średnia |
|--|----------------------|--------|----------|-----------------|
| | 1996 | 1997 | 1998 | |
| Control/P60 – Kontrola/P60 | 0.51 c | 0.47 a | 0.54 bc | 0.51 c |
| Ring of bark 0° – Pierścień 0° | 0.49 b | 0.43 a | 0.55 c | 0.49 bc |
| Ring of bark 180° – Pierścień 180° | 0.49 b | 0.43 a | 0.57 c | 0.50 bc |
| Microinterstock M.9 – Mikrowstawka M.9 | 0.46 a | 0.39 a | 0.51 abc | 0.45 a |
| Microinterstock P22 – Mikrowstawka P22 | 0.48 ab | 0.46 a | 0.50 ab | 0.48 abc |
| Microinterstock M.27 – Mikrowstawka M.27 | 0.46 a | 0.43 a | 0.47 a | 0.45 a |
| Mean for years – Średnia dla lat | 0.48 b | 0.44 a | 0.52 b | |

* Means followed by the same letter do not differ at $\alpha = 0.05$; Duncan's Multiply Range Test – Średnie oznaczone jednakowymi literami nie różnią się przy poziomie istotności $\alpha = 0,05$

In the first year of the investigation all the interstocks limited the content of calcium in the root system; in the second year no differences were recorded and in the third a decrease in Ca accumulation was only found in the case of M.27 microinterstock (tab. 8). Analyses of data from three years showed that in comparison with the control, in roots of trees with microinterstocks M.9 and M.27 the level calcium was lower. A differentiation also appeared in the years of the experiment; in 1997 the roots contained less calcium than in 1996 and 1998.

DISCUSSION

The distribution of mineral constituents in the plant is irregular [Parups et al. 1958, Mason and Whitfield 1960, Ford 1966, Martin et al. 1970, Baghdadi and Sadowski 1998]. This was confirmed in the present study and was obviously due to the different functions of the particular fruit tree organs.

The obtained results show the greatest content of calcium in the bark and the smallest in fruit. This finding is in agreement with the results given by Martin et al. [1970]. The present authors could not have proved a higher accumulation of calcium in leaves caused by P60 rootstocks as numerous authors claimed [Simmons and Swiader 1985, Kruczynska et al. 1990, Jadczyk 1995, Lipecki and Jadczyk 1998, 1999]. In the discussed experiment a tendency to the more efficient uptake and accumulation of calcium in leaves of trees grafted on P60 rootstock in comparison with M.9 can only be indicated. The applied grafting and the paclobutrazol treatment did not significantly affect changes in the concentration of this constituent.

Calcium plays a basic role in the storage performance of apples [Raese and Streif 1990, Autio 1991, Fallahi et al. 1998]. In the present work fruit from trees grafted on M.9 contained more calcium in comparison with those grafted on P60. Słowińska and Tomala [2001] found a contrary tendency.

Arakawa et al. [1997] and Elfving et al. [1991] showed a decrease in calcium content in fruit caused by girdling or grafting with bark rings. The present authors observed a contrary behaviour. The contents were the same or even greater than in the control treatment. The microinterstock P22 had a particularly favourable effect on the content of calcium in fruit. In the whole period of the experiment trees from this treatment showed the greatest concentration of calcium. However, it did not differ from trees on M.9. In the year of the highest yields (1999) the greatest average content of calcium was unexpectedly found in the fruit. Drake et al. [1974] reported a similar finding: he also recorded high contents of calcium in fruit from the year of heavy yielding. In the discussed year particularly favourable conditions of flower pollination occurred and the number of seeds is one of the factors determining the content of calcium in fruit [Tomala and Dilley 1990].

No significant dependence was found between the content of calcium in fruit and in leaves, the correlation coefficient r being only 0.03 ($\alpha = 0.05$, $N = 64$; unpublished data).

CONCLUSIONS

1. Individual organs of a fruit tree are characterized by a pronounced differentiation in calcium content. The highest average content was found in the bark of apple trees, less in leaves, followed by roots. The smallest content of Ca was recorded in wood and fruit.
2. The microinterstock taken from the bark of P22 rootstock favourably affected the content of calcium in fruit and increased its accumulation.
3. No significant dependence was found between the content of calcium in fruit and in leaves.
4. The applied grafting caused decreases in the content of calcium in roots.

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WPLYW RÓŻNYCH METOD SKARLANIA NA ZAWARTOŚĆ WAPNIA W RÓŻNYCH ORGANACH JABŁONI

Streszczenie. Badania prowadzono w latach 1996–1999 na drzewach jabłoni ‘Jonica’. Czynniki skarłającymi drzewa były: podkładki (M.9 i P60), przeszczepione pierścienie kory odmiany (w położeniu polarnym i apolarnym), mikrowstawki (przeszczepione pierścienie kory z podkładki M.9, P22 lub M.27) oraz retardant (Paclobutrazol). Najwyższą średnią zawartość wapnia stwierdzono w korze drzew (1,53% s.m.), mniej w liściach (0,98% s.m.), a następnie w korzeniach (0,48% s.m.). Najmniej Ca zawierało drewno i owoce (odpowiednio 0,09% s.m. i 0,02% s.m.). W przypadku analizy liści nie stwierdzono zróżnicowania zawartości wapnia spowodowanego wpływem podkładki. Owoce pochodzące z drzew na podkładce P60 zawierały mniej Ca niż z drzew rosnących na M.9. Zastosowane wszczyepy nie wpłynęły istotnie lub obniżyły zawartość wapnia. Wyjątkiem jest mikrowstawka P22, która zwiększyła akumulację tego pierwiastka w jabłkach we wszystkich latach badań. Nie stwierdzono istotnej zależności między zawartością wapnia w owocach i liściach; wartość współczynnika korelacji r wynosiła tylko 0,03 ($\alpha = 0,05$, $N = 64$). Przeszczepienie kory i mikrowstawki ograniczyły akumulację wapnia w korzeniach, co było szczególnie widoczne w pierwszym roku badań.

Słowa kluczowe: *Malus domestica*, skład mineralny, podkładki, przeszczepianie kory, mikrowstawki

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