Photosystem II thermostability of apple tree leaves: effect of rootstock, crown shape and leaf topology

Peter Ferus¹, Marián Brestič¹, Katarína Olšovská¹, Anna Kubová²
¹Department of Plant Physiology, Slovak Agricultural University in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
²Experimental Orchard, Slovak Agricultural University in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, e-mail: Marián.Brestic@uniag.sk

Apple tree leaves usually experience extremely high summer temperatures, which might cause disturbances to their photosynthesis and negatively influence fruit loading and quality. In this respect, in apple trees of cv. ‘Idared’ we evaluated the effect of rootstock (very dwarfing M.9 and vigorous MM.104), crown shape (modified Slender spindle and modified Schlüsser palmette) and leaf topology (leaves from the top of annual shoots, from the middle of the annual shoots and from short sprouts on the tree trunk) on the photosystem II (PS II) thermostability at the end of summer in 2006. For this purpose we analysed chlorophyll a fluorescence induction curves after exposure of leaf samples to 30 minutes of 42 °C. Neither rootstock types nor crown shapes caused any changes in the leaf PS II thermostability; however, significant differences in these characteristics were found in relation to leaf position in the apple tree crown. In comparison to leaves from annual shoots, which exhibited only moderate thermotolerance, a considerable increase was observed in leaves from short sprouts on the tree trunk. Measured high capacity of PS II thermostolerance is discussed in respect to plant polarity principles.

Key words: photosystem II thermostolerance, rootstock, crown shape, leaf topology, apple tree (Malus domestica Borkh.).

Introduction. Leaves are the main photosynthesizing plant organs providing assimilates for plant growth and fruit development. Leaves of apple tree (Malus domestica Borkh.) are developed from the leaf buds or combined floral-leaf buds. Passing exogenous dormancy, they expand to annual shoots with numerous leaf insertions. Intensive growth of annual shoots is timed particularly during spring and autumn. In summer their growth is usually decreased or almost stopped.

During vegetation period, developing leaves in the tree crown experience different endogenous and exogenous conditions determining their morphological, anatomical and biochemical characteristics, and hence their photosynthetic activity (Pearcy et al., 2005). In southern Slovakia, summer air temperatures usually reach 40 °C, which can have detrimental effects on photosynthesis (Salvucci and Crafs-Brandner, 2004) and, in a consequence, on fruit loading and quality. The final negative effect depends on leaf protective capacity on one side, and stress intensity and duration on the other side (Larcher, 2003).
Supraoptimal air temperature usually limits leaf photosynthesis through stomatal limitation of CO$_2$ uptake (Rahman, 2005; Cui et al., 2006), Rubisco deactivation (Law and Crafs-Brandner, 1999), thylakoid membrane injury (Liu and Huang, 2000), and photochemical PSII efficiency decline as a result of antenna detachment or impairment of electron transport chain components (Srivastava et al., 1997; Strasser, 2004). In the past two decades, a number of mechanisms alleviating these negative constraints was identified, including thermal dissipation (Bukhov et al., 1998); switch from the non-cyclic to cyclic electron transport (Bukhov et al., 1999); enhanced photorespiration (Sharkey, 2005); electron flux to oxygen (Mehler reaction) associated with up-regulated antioxidant system (Sairam et al., 2000) and chlororespiration (Wang et al., 2006); changes in the membrane composition in favour to saturated fatty acids (Larkindale and Huang, 2004) and accumulation of chemical (Kuznetsov et al., 1999) and molecular chaperones (Iba, 2002; Schroda, 2004).

Apple trees are composed of scion engrafted into a rootstock, which markedly regulates their growth (Webster, 1995). Tree crowns are formed into particular shapes in order to intercept more light and optimise fruit growth and ripening process. Both rootstock and crown shape influence hormonal composition of the scion (Webster, 1995; Tworkoski et al., 2006), and therefore can have significant effect on many physiological processes in leaves as well. Moreover, light conditions regulate leaves expansion and evoke different acclimation syndromes (Kull, 2002).

Very little is known about how these circumstances modify photosynthetic characteristics of apple tree leaves and what effect they have on photosynthetic processes in combination with elevated air temperature. In this work we focused on how rootstock differing in growth intensity, different crown shapes and leaf topology influence photosystem II (PS II) thermostability of leaves exposed to conditions of summer heat.

Object, methods and conditions. Plant material and cultivation. Apple trees (Malus domestica Borkh.) of cv. ‘Idared’, were cultivated in Experimental orchard of Slovak Agricultural University in Nitra, Slovakia, which is located on Nitra river alluvium (loamy-clayed soil substrate). The scion cultivar was engrafted into rootstock MM.104 (vigorous) and formed to modified Slender spindle or modified Schlusser palmette (20 years old trees), while apple trees engrafted into rootstock M.9 (very dwarfing) were formed to Slender spindle (8-years-old trees). Rows were oriented in north-southern direction, spaced 5 metres from each other, with in-row plant distance of 5 metres (older trees) and 1 meter (younger trees). Trees were approximately 3 (older trees) and 2 metres high (younger trees), and their crown diameter/width was 2.5 m (older trees, modified Slender spindle), 1.25 m (older trees, modified Schlusser palmette) and 1 m (younger trees, Slender spindle), respectively.

In autumn, phosphoric and potassium fertilization combined with cultivation was applied. During the vegetation period, trees were fertilized by nitrogen and for prevention treated against powdery mildew (Podosphaera leucotricha), apple scab (Venturia inaequalis) and herbivores. Weeds were regulated mechanically.

In the fruit growing and ripening growth stage (beginning of September), young
5th leaves of east-oriented annual shoots, leaves of middle part of the same shoots and leaves of south-oriented short sprouts on the tree trunks were collected in the mornings of three consecutive sunny days, transferred to laboratory, acclimated to darkness for 30 minutes and subjected to photosystem II (PS II) thermostability test.

Photosystem II thermostability test. Samples of leaf halves were enclosed in the test tubes and submerged into water bath of 42 °C for 30 minutes in the darkness. Before and after exposure of samples to high temperature, chlorophyll a fluorescence induction kinetics of the samples (12 leaves per treatment, 5 replicates per a leaf) were determined by Handy-PEA, Hansatech Instruments Ltd., UK. Their normalizations enabled to determine relative variable fluorescence at J and I step (V_J and V_I), ratio of fluorescence at K and J step of the induction curves (F_K/F_J) and maximal photochemical efficiency of PS II (F_V/F_M). After that, the JIP test (Biolyzer 4HP v. 3.06 software, Ronald Rodriguez) was applied to the fluorescence induction curves, which revealed further characteristics of the PS II bioenergetic state (Strasser et al., 2000; Strasser et al., 2004), such as maximal quantum yield of primary photochemistry (Φ_Po), exciton transfer efficiency to electron transport chain (Ψ_o), electron transport yield (Φ_Eo) and thermal dissipation yield (Φ_Do).

Determination of photosynthetic pigments concentration. For this purpose, second halves of leaf samples were utilized. Segments of the samples were homogenised in the presence of sea sand, MgCO_3 and 100 % acetone. After acetone vaporisation the homogenates were transferred into 80 % acetone and after centrifugation for 2 minutes at 2 500 rpm light absorption of the solutions was read at 470, 647 and 663 nm using spectrophotometer Jenway, UK. The pigment concentrations per leaf area unit were calculated according to Lichtenthaler (1987).

During the summer months, maximal daily air temperatures and daily rainfall in the experimental orchard was recorded by automatic agrometeorological station.

Statistical data analysis (ANOVA) was accomplished using application Statgraphics Plus v. 4.0.

Results. Photosynthetic apparatus of apple trees before high temperature treatment. Chlorophyll a fluorescence induction curves of leaves from modified Slender spindle apple trees engrafted into vigorous rootstock MM.104 (Fig. 1) showed very similar course at the beginning (O step). At J step they started to differ significantly with much more steeper fluorescence rise to I step in the leaves of south-oriented short sprouts on the trunk (inner-crown leaves) followed by slighter rise to P step. Fluorescence transients from J to P step in the 5th and middle leaves of east-oriented annual shoots were almost parallel. However, the latter gained significantly lower values.
Fig. 1. Chlorophyll a fluorescence induction curves (OJIPs) in control (full symbols) and high temperature (30 min of 42 °C in the darkness) treated (empty symbols) leaves from apple trees of cultivar ‘Idared’ formed to modified Slender spindle and engrafted into vigorous rootstock MM.104: 5th leaves of east-oriented annual shoots (circles), leaves of the middle part of the same shoots (squares) and leaves of the south-oriented short sprouts on the tree trunks (triangles).

Parameters derived from fluorescence induction curves, such as basal fluorescence ($F_0$) and relative variable fluorescence at the J and I step of the induction curves ($V_j$ and $V_i$) (Table 1) also confirm this tendency. On the other hand, maximal photochemical efficiency of PS II ($F_v/F_m$) did not show statistically significant difference between the leaves. Fluorescence at K versus J step of the induction curves ($F_k/F_j$) was slightly enhanced in the inner-crown leaves. JIP test applied to the fluorescence induction curves revealed significantly higher exciton transfer efficiency to electron transport chain ($\Psi_o$) and electron transport yield ($\Phi_{Eo}$) in the inner-crown leaves, but they had no effect on maximal quantum yield of primary photochemistry ($\Phi_{Po}$) (Fig. 2). Thermal dissipation yield ($\Phi_{Do}$) also showed balanced values in each leaf position.
Table 1. Parameters derived from chlorophyll $a$ fluorescence induction curves measured in 5th leaves of east-oriented annual shoots, leaves of middle part of the same shoots and leaves of south-oriented short sprouts on the apple tree trunks (inner-crown), of cultivar ‘Idared’ before the high temperature treatment (30 min at 42 °C in the darkness): $F_o$ – basal fluorescence, $V_j$ and $V_i$ – relative variable fluorescence at J and I step of the fluorescence induction curve, $F_k/F_j$ – ratio of chlorophyll $a$ fluorescence at K versus J step of the induction curve, and $F_v/F_m$ – maximal photochemical efficiency of PS II. Letters indicate statistically significant difference at P = 0.01.

<table>
<thead>
<tr>
<th>Leaf Lapas</th>
<th>$F_o$</th>
<th>$V_j$</th>
<th>$V_i$</th>
<th>$F_k/F_j$</th>
<th>$F_v/F_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fifth leaf, 434 ± 38 $a$</td>
<td>0.487 ± 0.040 $b$</td>
<td>0.742 ± 0.021 $a$</td>
<td>0.628 ± 0.024 $ab$</td>
<td>0.808 ± 0.019 $a$</td>
<td></td>
</tr>
<tr>
<td>Middle leaf, 401 ± 34 $a$</td>
<td>0.490 ± 0.061 $b$</td>
<td>0.736 ± 0.022 $a$</td>
<td>0.618 ± 0.022 $a$</td>
<td>0.805 ± 0.026 $a$</td>
<td></td>
</tr>
<tr>
<td>Inner-crown leaf, 426 ± 25 $a$</td>
<td>0.414 ± 0.043 $a$</td>
<td>0.830 ± 0.016 $b$</td>
<td>0.642 ± 0.011 $b$</td>
<td>0.826 ± 0.008 $a$</td>
<td></td>
</tr>
</tbody>
</table>

M.9, Slender spindle

| Fifth leaf, 449 ± 33 $b$ | 0.512 ± 0.038 $b$ | 0.738 ± 0.019 $a$ | 0.630 ± 0.028 $a$ | 0.789 ± 0.021 $a$ |          |
| Middle leaf, 365 ± 43 $a$ | 0.451 ± 0.062 $ab$ | 0.729 ± 0.017 $a$ | 0.615 ± 0.025 $a$ | 0.805 ± 0.029 $a$ |          |
| Inner-crown leaf, 384 ± 19 $a$ | 0.413 ± 0.052 $a$ | 0.792 ± 0.024 $b$ | 0.619 ± 0.010 $a$ | 0.833 ± 0.008 $b$ |          |

MM.104, modified Slender spindle

| Fifth leaf, 459 ± 31 $a$ | 0.479 ± 0.031 $a$ | 0.743 ± 0.015 $a$ | 0.642 ± 0.025 $ab$ | 0.796 ± 0.019 $a$ |          |
| Middle leaf, 396 ± 38 $a$ | 0.452 ± 0.066 $a$ | 0.722 ± 0.030 $a$ | 0.630 ± 0.026 $a$ | 0.801 ± 0.028 $a$ |          |
| Inner-crown leaf, 468 ± 31 $b$ | 0.462 ± 0.035 $a$ | 0.839 ± 0.021 $b$ | 0.669 ± 0.024 $b$ | 0.810 ± 0.017 $a$ |          |
Fig. 2. Parameters derived from chlorophyll $a$ fluorescence induction curves measured in the 5th leaves of east-oriented annual shoots, leaves of middle part of the same shoots and leaves of south-oriented short sprouts on the tree trunks (inner-crown) from apple trees, of cultivar ‘Idared’, formed to modified Slender spindle and engrafted into vigorous rootstock MM.104, before and after the high temperature treatment (30 min of 42 °C in the darkness): $\Phi_{Po}$ – maximal quantum yield of primary photochemistry, $\Psi_o$ – exciton transfer efficiency to electron transport chain, $\Phi_{Eo}$ – electron transport yield, $\Phi_{Do}$ – thermal dissipation yield.

Letters indicate statistically significant difference at $P = 0.01$: italic – before, and normal – after the high temperature treatment.
In Slender spindle apple trees engrafted into very dwarfing rootstock M.9, the middle leaves on annual shoots and the inner-crown leaves displayed lower fluorescence values at J step of the fluorescence induction curves, followed by generally slower fluorescence increase to P step in comparison to leaves of apple trees engrafted into rootstock MM.104 (Fig. 3).

Fig. 3. Chlorophyll a fluorescence induction curves (OJIPs) in control (full symbols) and high temperature (30 min of 42 °C in the darkness) treated (empty symbols) leaves from apple trees of cultivar ‘Idared’, formed to Slender spindle and engrafted into very dwarfing rootstock M.9: 5th leaves of east-oriented annual shoots (circles), leaves of the middle part of the same shoot (squares) and leaves of the south-oriented short sprouts on the tree trunks (triangles).

In these leaves $F_o$ reached markedly lower values than in the 5th leaves of annual shoots (Table 1). The 5th leaves also dominated in $V_j$, while $F_v/F_j$ did not change with leaf position. $F_v/F_m$ was the highest in the inner-crown leaves. Significant differences among the JIP test parameters were only obtained in the 5th leaves of annual shoots and inner-crown leaves (Fig. 4). The inner-crown leaves reached higher $\Phi_{Po}^\prime$, $\Psi$, $\Phi_{Eo}^\prime$, and lower $\Phi_{Do}$ than the 5th ones.
Fig. 4. Parameters derived from chlorophyll a fluorescence induction curves measured in the 5th leaves of east-oriented annual shoots, leaves of middle part of the same shoots and leaves of south-oriented short sprouts on the tree trunks (inner-crown) from apple trees of cultivar ‘Idared’, formed to Slender spindle and engrafted into very dwarfing rootstock M.9, before and after the high temperature treatment (30 min of 42 °C in the darkness):

- $\Phi_{Po}$ – maximal quantum yield of primary photochemistry,
- $\Psi_o$ – exciton transfer efficiency to electron transport chain,
- $\Phi_{Eo}$ – electron transport yield,
- $\Phi_{Do}$ – thermal dissipation yield.

Letters indicate statistically significant difference at $P = 0.01$:
- italic – before, and normal – after the high temperature treatment.

Fig. 4 pav. Išvestiniai parametrai iš chlorofilo a fluorescencijos indukcijos kreivių, matuotų modifikuotos laibosios verpstės formos ‘Idared’ veislės obelų su M.9 poskiepiu penktuose lapuose nuo į rytus orientuoto metūgio, lapuose nuo vidurinės šakų dalies ir lapuose nuo į pietus orientuotų vaisinių šakučių. Analizės atliktos prieš ir po temperatūros poveikio (42 °C 30 min tamsoje).

- $\Phi_{Po}$ – maksimali pirminės fotochemijos kvantų išeiga,
- $\Psi_o$ – eksitono perdavimo į elektronų transporto grandinę efektyvumas,
- $\Phi_{Eo}$ – elektronų transporto išeiga,
- $\Phi_{Do}$ – šilumos sklaidos išeiga. Raidės žymi statistiškai patikimus skirtumus, kai $P = 0.01$, pasvirasis šifras – prieš, normalus – po temperatūros poveikio.
Except of markedly lower fluorescence values at O and J step of the fluorescence induction curves in the middle leaves of annual shoots, leaves form Schlösser palmette apple trees engrafted into vigorous rootstock MM.104 (Fig. 5) exhibited very similar fluorescence transients to those from modified Slender spindle apple trees engrafted into the same rootstock.

![Chlorophyll fluorescence induction curves (OJIPs) in control (full symbols) and high temperature (30 min of 42 °C in the darkness) treated (empty symbols) leaves from apple trees of cultivar ‘Idared’, formed to modified Schlösser palmette and engrafted into vigorous rootstock MM.104: 5th leaves of east-oriented annual shoots (circles), leaves of the middle part of the same shoot (squares) and leaves of the south-oriented short sprouts on the tree trunks (triangles).](image)

Consequently, F_o was reduced in the middle leaves of annual shoots, and V_j (Table 1) with JIP test parameters Y_o and F_Eo revealed no difference between leaf positions (Fig. 6).

Photosynthetic pigments concentration and chl./car. ratio exhibited no significant difference between leaf positions in the modified Slender spindle apple trees engrafted into rootstock MM.104 (Table 2). However, chlorophyll a/b ratio of inner-crown leaves was the lowest among all leaf positions.

Despite of significant reduction of chlorophyll a concentration in the inner-crown leaves of the Slender spindle apple trees engrafted into rootstock M.9 (this rootstock – crown shape combination exhibited the highest chlorophyll a concentrations), leaf position did not influence the chlorophyll a/b ratio. Chlorophyll b and carotenoids concentrations and chl./car. ratio were also very similar.

Among rootstock – crown shape combinations, markedly lower chlorophyll a, b and carotenoids concentrations accompanied by decrease of chlorophyll a/b ratio...
in the inner-crown leaves were only observed in modified Schlösser palmette apple trees on the rootstock MM.104. Besides, 5th and the middle leaves on annual shoots of these apple trees dominated in carotenoid concentration.

**Fig. 6.** Parameters derived from chlorophyll \( a \) fluorescence induction curves measured in the 5th leaves of east-oriented annual shoots, leaves of middle part of the same shoots and leaves of south-oriented short sprouts on the tree trunks (inner-crown) from apple trees, of cultivar ‘Idared’, formed to modified Schlösser palmette and engrafted into vigorous rootstock MM.104, before and after the high temperature treatment (30 min of 42 °C in the darkness):

- \( \Phi _{Po} \) – maximal quantum yield of primary photochemistry,
- \( \Psi _{o} \) – exciton transfer efficiency to electron transport chain,
- \( \Phi _{Eo} \) – electron transport yield, \( \Phi _{Do} \) – thermal dissipation yield.

Letters indicate statistically significant difference at \( P = 0.01 \):
- italic – before, and normal font – after the high temperature treatment.
Table 2. Photosynthetic pigment concentration (mg m\(^{-2}\)) in the 5th leaves on east-oriented annual shoots, leaves of middle part of the same shoots and leaves of south-oriented short sprouts on the tree trunks (inner-crown) from apple trees, of cultivar ‘Idared’, with different rootstock and crown shape. Letters indicate statistically significant difference at P = 0.01.

<table>
<thead>
<tr>
<th>Leaf Lapas</th>
<th>Chlorophyll (a)</th>
<th>Chlorophyll (b)</th>
<th>Carotenoids Karotenoidai</th>
<th>Chlorophyll (a) / Chlorophyll (b)</th>
<th>Chlorophylls / carotenoids Chlorofila / karotenoidai</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM.104, modified Slender spindle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fifth leaf</td>
<td>618.28 ±</td>
<td>225.44 ±</td>
<td>277.75 ±</td>
<td>2.79 ±</td>
<td>3.21 ±</td>
</tr>
<tr>
<td>Penktas lapas</td>
<td>47.96 (a)</td>
<td>36.81 (a)</td>
<td>63.07 (a)</td>
<td>0.35 (b)</td>
<td>0.87 (a)</td>
</tr>
<tr>
<td>Middle leaf</td>
<td>632.21 ±</td>
<td>237.58 ±</td>
<td>322.86 ±</td>
<td>2.67 ±</td>
<td>3.06 ±</td>
</tr>
<tr>
<td>Vidorirnis lapas</td>
<td>87.40 (a)</td>
<td>34.62 (a)</td>
<td>128.17 (a)</td>
<td>0.26 (ab)</td>
<td>1.15 (a)</td>
</tr>
<tr>
<td>Inner-crown leaf</td>
<td>554.05 ±</td>
<td>239.19 ±</td>
<td>306.46 ±</td>
<td>2.35 ±</td>
<td>2.91 ±</td>
</tr>
<tr>
<td>Vaisinių šakučių lapas</td>
<td>76.88 (a)</td>
<td>42.94 (a)</td>
<td>111.98 (a)</td>
<td>0.31 (a)</td>
<td>1.07 (a)</td>
</tr>
<tr>
<td>MM.9, Slender spindle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fifth leaf</td>
<td>720.77 ±</td>
<td>287.90 ±</td>
<td>353.89 ±</td>
<td>2.56 ±</td>
<td>3.03 ±</td>
</tr>
<tr>
<td>Penktas lapas</td>
<td>163.76 (b)</td>
<td>88.44 (a)</td>
<td>110.63 (a)</td>
<td>0.22 (a)</td>
<td>0.82 (a)</td>
</tr>
<tr>
<td>Middle leaf</td>
<td>714.42 ±</td>
<td>276.23 ±</td>
<td>401.58 ±</td>
<td>2.65 ±</td>
<td>2.51 ±</td>
</tr>
<tr>
<td>Vidorirnis lapas</td>
<td>48.32 (b)</td>
<td>46.44 (a)</td>
<td>54.08 (a)</td>
<td>0.47 (a)</td>
<td>0.38 (a)</td>
</tr>
<tr>
<td>Inner-crown leaf</td>
<td>563.97 ±</td>
<td>239.33 ±</td>
<td>326.73 ±</td>
<td>2.37 ±</td>
<td>2.49 ±</td>
</tr>
<tr>
<td>Vaisinių šakučių lapas</td>
<td>60.40 (a)</td>
<td>32.00 (a)</td>
<td>29.12 (a)</td>
<td>0.14 (a)</td>
<td>0.44 (a)</td>
</tr>
<tr>
<td>MM.104, modified Schlösser palmette</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fifth leaf</td>
<td>630.44 ±</td>
<td>256.25 ±</td>
<td>429.65 ±</td>
<td>2.47 ±</td>
<td>2.17 ±</td>
</tr>
<tr>
<td>Penktas lapas</td>
<td>0.96 (b)</td>
<td>30.76 (b)</td>
<td>102.37 (ab)</td>
<td>0.15 (b)</td>
<td>0.57 (a)</td>
</tr>
<tr>
<td>Middle leaf</td>
<td>603.76 ±</td>
<td>242.09 ±</td>
<td>440.26 ±</td>
<td>2.50 (±)</td>
<td>2.14 (±)</td>
</tr>
<tr>
<td>Vidorirnis lapas</td>
<td>9.43 (b)</td>
<td>30.25 (b)</td>
<td>133.51 (b)</td>
<td>0.17 (b)</td>
<td>0.84 (a)</td>
</tr>
<tr>
<td>Inner-crown leaf</td>
<td>411.83 ±</td>
<td>192.25 ±</td>
<td>305.72 ±</td>
<td>2.14 ±</td>
<td>2.02 ±</td>
</tr>
<tr>
<td>Vaisinių šakučių lapas</td>
<td>3.95 (a)</td>
<td>21.58 (a)</td>
<td>52.14 (a)</td>
<td>0.06 (a)</td>
<td>0.37 (a)</td>
</tr>
</tbody>
</table>

Photosystem II activity in apple tree leaves treated with high temperature. Thirty minutes treatment by temperature of 42 °C in the darkness caused a significant increase of \(F_o, F_o/F_i, \Phi_{po}, \Phi_{p_o}\) and decrease of \(V_i, F_v/F_m, \Phi_{po}\) in all rootstock-crown-shape-leaf position combinations (Table 3). Inner-crown leaves of apple trees on rootstock MM.104 also showed lower \(V_i\) and higher \(\Psi_o\).

In comparison to control measurements, fluorescence induction curves of leaves from modified Slender spindle apple trees engrafted into rootstock MM.104 exhibited higher fluorescence at the O step, reduced fluorescence at the J and particularly L step, followed by markedly lower fluorescence values at the P step (Fig. 1). Parallel to these changes, relations between fluorescence transients of leaves from different positions stayed relatively stable.
Table 3. Evaluation of statistical difference of parameters derived from chlorophyll a fluorescence induction curves between control and high temperature (30 min at 42 °C in the darkness) treated apple tree leaves. $F_o$ – basal fluorescence, $V_i$ and $V_j$ – relative variable fluorescence at J and I step of the fluorescence induction curve, $F_i/F_j$ – ratio of fluorescence at K versus J step of the fluorescence induction curve, $F_v/F_m$ – maximal photochemical efficiency of PS II, $\Phi_{po}$ – maximal quantum yield of primary photochemistry, $\Psi_o$ – exciton transfer efficiency to electron transport chain, $\Phi_{eo}$ – electron transport yield, $\Phi_{do}$ – thermal dissipation yield. Double asterisk indicates on statistically significant difference at $P = 0.01$, asterisks indicates on statistically significant difference at $P = 0.05$ and n. s. – non-significant difference.

From them the lowest $F_o$ and $V_j$ values, and the highest $F_v/F_m$ of inner-crown leaves were derived (Table 4). Leaf positions did not influence values of $V_i$ and $F_i/F_j$. These results were reflected in JIP test parameters: the inner-crown leaves showed the highest $\Psi_o$, $\Phi_{eo}$ and $\Phi_{po}$, but the lowest $\Phi_{do}$ (Fig. 2).
Table 4. Parameters derived from chlorophyll a fluorescence induction curves measured in 5th leaves of east-oriented annual shoots, leaves of middle part of the same shoots and leaves of south-oriented short sprouts on the apple tree trunks (inner-crown), after the high temperature treatment (30 min at 42 °C in the dark): 

<table>
<thead>
<tr>
<th>Leaf</th>
<th>F₀</th>
<th>Vₐ</th>
<th>Vᵢ</th>
<th>Fₖ/Fₐ</th>
<th>Fᵥ/Fₘ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fifth leaf</td>
<td>549 ± 62 b</td>
<td>0.441 ± 0.045 b</td>
<td>0.541 ± 0.065 a</td>
<td>0.817 ± 0.037 a</td>
<td>0.711 ± 0.033 a</td>
</tr>
<tr>
<td>Middle leaf</td>
<td>521 ± 53 ab</td>
<td>0.471 ± 0.078 b</td>
<td>0.539 ± 0.063 a</td>
<td>0.807 ± 0.024 a</td>
<td>0.700 ± 0.039 a</td>
</tr>
<tr>
<td>Inner-crown leaf</td>
<td>475 ± 23 a</td>
<td>0.356 ± 0.031 a</td>
<td>0.593 ± 0.045 a</td>
<td>0.819 ± 0.025 a</td>
<td>0.781 ± 0.016 b</td>
</tr>
</tbody>
</table>

Fluorescence induction curves in the middle leaves of annual shoots and inner-crown leaves from Slender spindle apple trees engrafted into rootstock M.9 did not exhibit lower fluorescence values at the J step than before high temperature treatment (Fig. 3). Except of this difference, fluorescence transients of these as well as the 5th leaves resembled those from modified Slender spindle apple trees engrafted into rootstock MM.104. Also the parameters derived from fluorescence induction curves showed the same tendency as in these apple trees (Table 4, Fig. 4).
treatment indicates the similar characteristics to those measured in the Slender spindle apple trees on the same rootstock (Fig. 5). On the other hand, inner-crown leaves except of the lowest \( F_\alpha \) and \( V_f \), and the highest \( F/F_m \) showed also the highest \( V_i \) (Table 4). However, \( \Phi_{po}, \Psi_o, \Phi_{lo} \) and \( \Phi_{do} \) in these apple trees were also similar to the Slender spindle apple trees (Fig. 6).

The JIP test parameters obtained from all the combinations of rootstock – crown shape – leaf position after high temperature treatment showed similar \( \Phi_{po}, \Psi_o, \Phi_{lo} \) and \( \Phi_{do} \) in the 5th and the middle leaves of annual shoots (Fig. 7). However, despite of almost equal \( \Psi_o \) and \( \Phi_{lo} \), significantly higher \( \Phi_{do} \) in the inner-crown leaves from modified Schlüsser palmette apple trees engrafted into rootstock MM.104 led to significantly lower \( \Phi_{po} \) than in the Slender spindle apple trees engrafted into rootstock M.9.

**Fig. 7.** Comparison of parameters derived from chlorophyll \( a \) fluorescence induction curves (\( \Phi_{po} \) – maximal quantum yield of primary photochemistry, \( \Psi_o \) – exciton transfer efficiency to electron transport chain, \( \Phi_{lo} \) – electron transport yield, \( \Phi_{do} \) – thermal dissipation yield) between treatments (rootstocks and crown shapes) in 5th leaves of east-oriented annual shoots, leaves of middle part of the same shoots and leaves of south-oriented short sprouts on the tree trunks (inner-crown) from apple trees, of cultivar ‘Idared’, after the high temperature treatment (30 min at 42 °C in the dark). Letters indicate statistically significant difference at \( P = 0.01 \).
**Discussion.** Leaf photosynthetic characteristics are influenced by internal and external factors and reflect their time-space fluctuations. Despite of homogenous rainfall distribution during summer months (Fig. 8), maximal daily air temperatures often exceeded 35 °C, inducing enhanced thermotolerance in apple trees leaves. How was this acclimatory process influenced by type of rootstock, crown shape and leaf topology within the crown?

![Graph showing the course of maximal daily air temperatures and daily rainfall in summer months in 2006, in the experimental orchard of Slovak Agricultural University (Nitra, Slovakia)](image_url)

**Fig. 8.** Course of maximal daily air temperatures and daily rainfall in summer months in 2006, in the experimental orchard of Slovak Agricultural University (Nitra, Slovakia)

Rootstock mainly influences: (1) the amount and/or ratio of promoting and inhibiting endogenous hormones circulating within tree plant, particularly between the root system and above-ground tree parts; (2) the movement of assimilates (e. g., sugars and amino acids) or mineral elements between the scion and rootstock; and (3) the amount of water taken up and moved through the rootstock to scion (Webster, 1995).

Therefore, we could expect rootstock influence on photosynthetic apparatus as well. Rootstocks with enhanced resistance to *Erwinia amylovora*, provide higher resistance to scion (Jensen et al., 2003). Would it not be possible also in relation to abiotic stressors?

However, no difference in any of the JIP test parameter (Φ_p, Ψ, Φ_o and Φ_d) in leaves with different crown localization, from apple trees engrafted into either vigorous rootstock MM.104 or very dwarfing rootstock M.9 (Fig. 7) implies to the fact that rootstock does not change the PS II thermostability of apple tree leaves. On the contrary, according to Kamboj and Quinlan (1998) and also Kamboj et al. (1999), roots of M.9 accept less auxin and produce less cytokinin and more abscisic acid than MM.104, which is supposed to stress-harden (Wang et al., 2003) the scion in larger extent.
Crown shape optimises light utilization efficiency, but also harmonizes the ratio of fruiting and growing parts of trees. Different branch organisation may also change hormonal composition (auxin-cytokinin ratio) in shoot tips, as mentioned by Tworkoski et al. (2006) and thus also stability of leaf photosynthesis, because cytokinines reduce its susceptibility to heat stress (Liu and Huang, 2002; Gupta et al., 2000). Nevertheless, different crown forms (modified Slender spindle or modified Schlösser palmette) caused no significant change in the PS II thermostability of leaves from any crown position (Fig. 7), suggesting a similar hormonal balance.

Concentrating on PS II reactions to high temperature treatment in leaves from different tree crown positions we detected a pronounced unification in fluorescence induction transients (Fig. 1, 3, 5) and parameters derived from them (Table 4, Fig. 2, 4, 6). The highest electron transport rate to photosystem I (lower disturbances at the acceptor side of PS II reaction centre, different impairment at the donor side are excluded because of balanced \( F_v/F_m \) ratio), enhanced communication between antenna complex and reaction centres of the PS II and the reduced thermal dissipation of excitation energy led to the smallest decrease of photochemical PS II efficiency (Strasser, 2004) and thus the highest PS II thermostability in the inner-crown leaves. Response of the 5th and the middle leaves from the annual shoots was very similar.

Reduced chlorophyll \( a/b \) ratio in the inner-crown leaves from the older apple trees on rootstock MM.104 (Table 2) points to their shaded character (Kull, 2002). Leaves from modified Schlösser palmette apple trees in comparison to modified Slender spindle were likely more exposed to higher light intensities, therefore a slight movement of photosynthetic pigment characteristics was expected to sunny type. However, this was not observed. Because of massive light transmission into the crown of younger apple trees on rootstock M.9, there was no partition into sun and shade type in leaves.

Higher PS II thermotolerance in the inner-crown leaves is in contrast to their shade character, because shade leaves usually contain less xanthophyll cycle pigments (Demmig-Adams and Adams, 1992), less enzymatic and non-enzymatic radical scavengers (Logan et al., 1998) and have reduced photorespiratory activity (Muraoka et al., 2000), thus their protection capacity is reduced.

Also finding of Niinemets et al. (1999) that increased optimal temperature for maximal photosynthetic electron transport in poplar leaves is correlated with integrated quantum flux density, can not be taken into account because of lower PS II thermostability of sun leaves from annual shoots in comparison to shaded inner-crown leaves, no difference between shaded leaves in apple trees with different crown shape, and difference between leaf positions in apple trees engrafted into rootstock M.9. All these results confirm our suggestion that there is no relation between light acclimation syndrome and PS II thermostolerance in the apple tree crowns. So, is the leaf age responsible for the PS II thermostability differences between leaf positions?

Juvenile elm leaves exhibited lower thermotolerance in respect to electron transfer from PS II antenna complex to reaction centre and electron transport to PS I (mainly limited by oxygen evolving complex (OEC) impairment), compared to expanded ones (Jiang et al., 2006). Balanced \( F_v/F_m \) in every leaf position in apple trees suggests on reaching comparable OEC stability and hence leaf maturity, and further PS II thermostability rise of the inner-crown leaves provides enhanced communication
between antenna complex and reaction centre of PS II and probably higher capacity of protective mechanisms, utilizing electrons from electron transport chain. Secondly, middle leaves of annual shoots, which are older than 5th leaves, did not exhibit enhanced PS II thermostability. Also different period of elevated temperatures, which are required for photosynthetic apparatus acclimation (Verdaguer et al., 2003), can be excluded, because of limited growth of annual shoots during summer.

Therefore, we suppose that the effect of plant polarity and associated changes in hormonal balance could be responsible for the difference in PS II thermostability in these three leaf positions. Larger proximity to root apices and larger distance from shoot apices shift the hormonal composition in short sprouts on the trunk in favour to cytokinins content, which may reduce leaf susceptibility to heat stress (Liu and Huang, 2002; Gupta et al., 2000). Their effect is probably realized through enhanced antioxidant enzymes level (Liu and Huang, 2002) or photorespiration (Tian et al., 2006).

Conclusions. Fruit trees experiencing extremely high summer temperatures usually exhibit disturbances to photosynthetic process. Testing roles of rootstock vigour (vigorous MM.104 and dwarfing M.9), crown shape and branch organization (Slender spindle and Schlüsser palmette) as well as leaf position in apple trees (cv. ‘Idared’) on photosystem II (PS II) thermostability (parameters derived from rapid chlorophyll \(a\) fluorescence kinetics) points to no influence of first two factors. On the other hand, the leaf topology seems to be the most important regulatory feature suggesting on influence of trunk/root apex distance associated with cytokinin concentration.

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**Vaismedžio vainiko formos, poskiepio ir topologijos įtaka obelų lapų II fotosistemos termostabilumui**

**P. Ferus, M. Brestič, K. Olšovská, A. Kubová**

*Santrauka*

Vasara obelys dažnai patiria neigiamą aukštų temperatūrų poveikį, kuris gali lemti fotosintezės sutrikimus ir neigiamai paveikti vaisių derlių ir kokybę. Atsižvelgiant į tai, buvo įvertinta poskiepio (žemaūgis M.9 ir augus MM.104), vainiko formos (modifikuota laiboji verpstė ir modifikuota) ir lapų topologijos (lapai imti nuo metinio ūglio virpūnės, vidurio ir nuo vaisinių šakučių) įtaka 'Idared' veislės obelų II fotosistemos (PS II) termostabilumui 2006 metų vasaros pabaigoje. Tirtos chlorofilo a fluorescencijos indukcijos kreivės, 30 minučių paveikus tiriamus lapus 42 °C temperatūra. Nei poskiepio tipas nei vaismedžio forma nesukėlė lapo PS II termotoleransumo pokyčių, tačiau esminiai skirtumai pastebėti tarp skirtinos lapų pozicijos vaismedžio vainike. Lyginant su lapais nuo metūglio kurie pademonstravo tik nedidelę termotoleranciją, žymus augimas nustatytas lapuose nuo vaisinių šakučių. Išmatuotas PS II termotolerancijos pajėgumas aptartas atsižvelgiant į augalų poliškumo principus.

**Reikšminiai žodžiai:** II fotosistemos termotolerancija, lapo topologija, obelis (*Malus domestica* Borkh.), poskiepis, vainiko forma.