Effect of growth regulators on apple tree cv. ‘Jonagold King’ photosynthesis and yield parameters

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The primary biochemical and physiological effect of plant growth regulators determines secondary effects in fruit trees, which might differently affect photosynthesis apparatus and influence fruit loading. Experiments were carried out at the Lithuanian Institute of Horticulture in 2005–2008. Investigations included cv. ‘Jonagold King’ apple trees sprayed with growth regulators: Regalis (prohexadione-calcium), Cerone 480 SL (brand ethephon plant regulator) and Paturyl 10 SL (10 % benzyladenine). This study examined the effect of different action plant growth regulators on photosynthetic pigment system in apple tree leaves and variation of non-structural carbohydrates (fructose, glucose, maltose) concentrations in treated apple tree shoot bark and yield parameters.

Regalis and Paturyl 10 SL application increased and Cerone 480 SL application decreased accumulation of chlorophylls in fruit tree leaves. Storage of researched carbohydrates was found to be more active in apple trees treated with Regalis and Paturyl 10 SL. Fruit trees sprayed with Cerone 480 SL accumulated the lowest general quantity of sugars during winter and spring in the shoot bark tissues. Paturyl 10 SL and Cerone 480 SL application decreased fruit yield. The highest fruit weight was found in apple trees treated with Paturyl 10 SL.

Key words: carbohydrate, fruit weight, growth regulators, photosynthetic pigments and yield.

Introduction. There are a number of applications of plant hormones in agriculture, horticulture, and biotechnology. The development and use of exogenous plant growth regulators as growth retardants is based on knowledge that endogenous plant hormones play significant role in shoot growth. Plant growth regulators modify growth and development in various ways. Plant growth regulators can be well integrated into orchard production systems. Gibberellin biosynthesis inhibitors have received the most attention because of their key role in cell elongation (Luckwill, 1970; Rademcher, 2000). Prohexadione-Ca (Regalis) is an inhibitor of late stages of gibberellin biosynthesis. This compound is under actual development for use as a growth retardant in different crops. Trials with prohexadione-Ca to control vegetative growth of apple, pear and plum trees were demonstrated by other authors (Buban et al., 2003, Basak and Rademacher, 2000). Synthetic auxins and chemicals releasing ethylene gas are successfully applied
for fruit tree growth regulation (Miller, 1988). A precondition of precocious bearing in young trees is the development of a canopy structure, which has good cropping potential and this can be achieved by using benzyladenine (Paturyl). Fruit thinning with benzyladenine in mature trees can result in larger fruit size and increased return bloom the following year. Ethephon (Cerone) releases ethylene, which usually has inhibitory effect on plants. Ethylene promotes abscission of leaves and fruits, inhibits shoot elongation and favors caliper development (Buban, 2000; Jacyna and Puchała, 2004). Ethephon has been widely used chemical for apple thinning purpose for many years (Wertheim, 2000).

However, the temperature dependence of the thinning response remains a problem to be resolved. The efficiency of plant growth regulators is determined by the physiological age of trees, by the environmental conditions at application and by the application methods used. The primary biochemical and physiological effect of growth regulators can determine secondary effects.

The content of chlorophylls and carotenoids, the main pigments of leaf, provides valuable information about plant physiological status. Chlorophylls are essential pigments for the conversion of light energy to stored chemical energy. The amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content; thus, chlorophyll content can directly determine photosynthetic potential and primary production (Curran et al., 1990; Filella et al., 1995). Many researchers state that the amount and variety of carbohydrates found in plants differ in various plant organs and conditions all throughout the growing season. Although storage polysaccharides have significance in plant physiology, mono and disaccharides play a central role in metabolism, soluble sugars help regulate many developmental and physiological processes in plants (Smeekens, 2000; Gibson, 2000). Carbohydrates reserves allow completing phenological developments without the benefit of current photosynthesis. In apple trees, leaf and early fruit development in the spring depend on carbohydrate reserves within the tree (Mcqueen et al., 2004). Thus the storage of adequate supplies of carbohydrate is critical for both fruit yield and quality. Knowledge of the status of carbohydrate reserves is important for experiments involving manipulation of the carbon balance of fruit trees (Tustin et al., 1997).

The aim of this study was to investigate the influence of several chemicals, whose action is based on different hormones metabolism regulation, on photosynthesis and yield parameters of apple cv. ‘Jonagold King’.

**Object, methods and conditions.** Investigations of different growth regulators effect on apple tree non-structural carbohydrate variation (TNC) variation, concentrations and photosynthetic pigments were carried out at the Lithuanian Institute of Horticulture. Investigations included cv. ‘Jonagold King’ on M.9 rootstock. The orchard was planted in the spring of 1999. Planting distances were 3 × 1 m. Trees were trained as slender spindles. The trial consisted of four replications with 3 trees in each. Treatments were: untreated control; sprayed with Regalis after flowering, two times each 30 days (1.25 %); sprayed with Paturyl 10 SL after flowering, two times each 7 days (0.2 %); sprayed with Cerone after flowering, tree times each 10 days (1 time – 0.025 %, 2 and 3 times – 0.015 %).

Carbohydrate samples were prepared by grinding ~1 g of fresh weight (FW) material and extracted with 4 mL hot bidistilled water. After 24 h extract was filtered
through cellulose and membrane (pore diameter 0.2 µm) filters. Chromatographic analysis was carried-out using Shimadzu 10A HPLC system with refraction index detector (Shimadzu, Japan) and Adsorbosil NH2- column (150 mm × 4.6 mm; Alltech, USA). Mobile phase: 75 % acetonitrile. Flow rate: 1 ml min⁻¹. Data analysis was performed using MS Excel software.

The measurements of chlorophyll were performed from 5 plants of each apple tree variety. The fourth fully developed leaf from the east side of the tree was taken for chlorophyll extraction in 100 % acetone and evaluation by the spectrophotometric method according to Weittstein (Гавриленко, 2003). The concentrations of pigments were detected by Spectrophotometer – Genesys 6 (ThermoSpectronic, USA). Statistical calculations were performed by ANOVA for MS Excel vers. 3.43 (Duncan’s Multiple Range t-test procedure (P ≤ 0.05). The error bars presented in figures are the standard deviations of tree analytical measurements.

In 2004 and 2007, during flowering and after it, occurred -3–-2.5 °C frost and the weather was cool. Such cross conditions negatively affected yield parameters. In 2005 the daily temperature was high and suitable for growth regulators application. In 2006 during flowering and after it the weather was cold, but there were no frosts, and such condition didn’t affect yields. Still they could increase growth regulators efficiency.

**Results.** Application of different growth regulators significantly affected photosynthetic apparatus of apple tree cv. ‘Jonagold King’. Used implements, strongly activated photosynthetic pigments accumulation in 2005. However, growth regulators decreased photosynthesis efficiency. The chlorophyll a/b ratio in analyzed fruit trees leaves didn’t differ from the treated ones (Table 1).

In 2006 chlorophyll accumulation in all investigated apple tree leaves was comparatively low. The highest content of photosynthetic pigments in 2006–2007 was detected in apple trees treated with Regalis. However, chlorophyll accumulation in apple tree leaves sprayed with Paturyl and Cerone 480 SL decreased. Due to relatively high amount of chlorophyll b in treated tree leaves chlorophyll a/b ratio was lower or the same as in not treated ones (Table 1).

**Table 1. Photosynthesis pigment variation in leaves of apple trees cv. ‘Jonagold King’ treated with different growth regulators**

<table>
<thead>
<tr>
<th>Growth regulators</th>
<th>Chlorophyll a/b ratio</th>
<th>Chlorophyll a/b content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not treated</td>
<td>3.2 ab</td>
<td>2.8 b</td>
</tr>
<tr>
<td>Regalis</td>
<td>3.0 a</td>
<td>2.8 b</td>
</tr>
<tr>
<td>Paturyl 10 SL</td>
<td>3.2 ab</td>
<td>2.7 a</td>
</tr>
<tr>
<td>Cerone</td>
<td>3.0 a</td>
<td>2.7 a</td>
</tr>
</tbody>
</table>

* Values followed by the same letters within the columns are not statistically different at P ≤ 0.05.
* Tomis pačiomis raidėmis stulpeliuose pažymėti skaičiai iš esmės nesiskiria (P ≤ 0.05).
The variation of investigated non-structural carbohydrates (TNC) (fructose, glucose and maltose) concentrations were determined by different action growth regulators and plant development stages (Fig. 1, Fig. 2, Fig. 3).

**Fig. 1.** Carbohydrate distribution in bark tissue of apple trees cv. ‘Jonagold King’ treated with different growth regulators in August

In August, when apple tree buds were in vegetative stage, higher contents of soluble carbohydrates in treated apple tree bark tissues were found only in 2005. During later studies clear differences among the investigated fruit tree sugars accumulation was not determined (Fig. 1).

Synthesis of hexoses in the investigated fruit trees went significantly up in November. In 2005 the lowest amount of carbohydrates was found in fruit tree shoot bark tissues sprayed with Regalis. By the way, storage of disaccharides in 2006–2007 was found to be more active in apple trees treated with Regalis and Paturyl 10 SL. The lowest general quantity of sugars was found in the bark tissues of fruit trees sprayed with Cerone 480 SL (Fig. 2).

The results of investigations carried out in March showed more active accumulation of maltose in the bark tissues of the fruit trees. Synthesis of glucose and fructose in the fruit trees sprayed with different growth regulators was different. The quantity of non-structural carbohydrate increased in apple trees treated with Regalis and Paturyl 10 SL. It declined or became stable in orchards treated with Cerone (Fig. 3).
Fig. 2. Carbohydrate distribution in bark tissue of apple trees cv. ‘Jonagold King’ treated with different growth regulators in November (1 – 2005/2006, 2 – 2006/2007, 3 – 2007/2008)

Fig. 3. Carbohydrate distribution in bark tissue of apple trees cv. ‘Jonagold King’ treated with different growth regulators in Mach (1 – 2005/2006, 2 – 2006/2007, 3 – 2007/2008)
During investigation years the yield of apple trees treated with Regalis was similar to not treated fruit trees. Paturyl 10 SL and Cerone 480 SL application significantly decreased fruit yield (Table 2).

**Table 2.** Yield of apple trees cv. ‘Jonagold King’ treated with different growth regulators (t/ha)

<table>
<thead>
<tr>
<th>Growth regulators Augimą ribojantys preparatai</th>
<th>Year Metai</th>
<th>2004</th>
<th>2006</th>
<th>2007</th>
<th>average vidurkis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not treated</td>
<td></td>
<td>28.0</td>
<td>43.2</td>
<td>23.0</td>
<td>31.4</td>
</tr>
<tr>
<td>Regalis</td>
<td></td>
<td>26.0</td>
<td>42.8</td>
<td>20.6</td>
<td>29.8</td>
</tr>
<tr>
<td>Paturyl 10 SL</td>
<td></td>
<td>15.6</td>
<td>31.6</td>
<td>12.4</td>
<td>19.9</td>
</tr>
<tr>
<td>Cerone</td>
<td></td>
<td>20.0</td>
<td>30.0</td>
<td>10.9</td>
<td>20.3</td>
</tr>
<tr>
<td>( R_{0.5} )</td>
<td></td>
<td>7.06</td>
<td>9.51</td>
<td>11.21</td>
<td>8.98</td>
</tr>
</tbody>
</table>

**Table 3.** Average fruit weight of apple trees cv. ‘Jonagold King’ treated with different growth regulators (g)

<table>
<thead>
<tr>
<th>Growth regulators Augimą ribojantys preparatai</th>
<th>Year Metai</th>
<th>2004</th>
<th>2006</th>
<th>2007</th>
<th>average vidurkis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not treated</td>
<td></td>
<td>134</td>
<td>169</td>
<td>151</td>
<td>151</td>
</tr>
<tr>
<td>Regalis</td>
<td></td>
<td>130</td>
<td>163</td>
<td>153</td>
<td>149</td>
</tr>
<tr>
<td>Paturyl 10 SL</td>
<td></td>
<td>157</td>
<td>198</td>
<td>237</td>
<td>197</td>
</tr>
<tr>
<td>Cerone</td>
<td></td>
<td>134</td>
<td>171</td>
<td>181</td>
<td>162</td>
</tr>
<tr>
<td>( R_{0.5} )</td>
<td></td>
<td>17.4</td>
<td>23.5</td>
<td>42.3</td>
<td>30.1</td>
</tr>
</tbody>
</table>

Growth regulators Regalis and Cerone 480 SL didn’t significantly affect fruit weight. Paturyl 10 SL application during investigation increased fruit weight (Table 3).

**Discussion.** These investigations present how the growth regulators of different action determine accumulation and distribution of nonstructural carbohydrates among plant tissues and photosynthetic pigment synthesis in their leaves, and how these changes affect plant yield and quality.

Gibberellin inhibition caused by Regalis application results in shoots, with the same numbers of leaves and internodes compressed into a shorter length, formation
Increases in chlorophyll concentrations per unit leaf area, following the application of growth regulator Regalis, were generally found in these experiments (Table 1), what consists with other investigations on prohexadione-calcium (Miller, 2002; Reekie et al., 2005). Glenn and Miller (2005) suggested that the greater leaf photosynthetic activity in apple trees treated with prohexadione-calcium was the result of reduced shading in the canopy. The mechanism for enhancement of photosynthesis promoted by prohexadione-calcium in apple trees may be related to its effects on the concentration of chlorophyll per unit leaf area (Table 1) and carbohydrates content in bark tissues (Fig. 1, Fig. 2, Fig. 3). Environmental shading creates competition for in-season carbohydrates, reducing fruit growth rate and prompting fruit drop. Previous studies by Privé et al. (2004) have shown that prohexadione-calcium can increase light interception to the inner canopies of fully-grown apple trees and thus enhance flower bud formation and possibly yield. In our investigations the yields of apple trees treated with Regalis remained similar to untreated trees as introduced in other authors works too (Medjdoub et al., 2007).

Cytokinins promote shoot development through increased cell division. A reasonable lateral branching for training scaffold limbs and a proper balance between elongated and short shoots which have high flowering potential can be achieved using chemical such as benzyladenine applied in the fruit tree nursery (Buban, 2000). During the expansion of cells in fruit tree leaves sprayed with benzyladenine, the number of chloroplasts per cell increases (Nii and Kuroiwa, 1986). In 2005 and 2007 benzyladenine (Paturyl 10 SL) application maintains the chlorophyll at higher level (Table 1). Increased chlorophyll synthesis was also reported by other authors (Walker et al., 1988). Our data showed that apple tree shoots inhibited by benzyladenine treatment contained higher levels of nonstructural carbohydrates during summer, autumn and spring periods. However, in 2006 chlorophyll content was lower than in not treated fruit trees. Bark tissues also contained lower concentration of nonstructural carbohydrates (Fig. 1, Fig. 2, Fig. 3). This can be enhanced by adverse environmental conditions during application time. Buban and Lakatos (2000) confirmed the temperature dependence of benzyladenine effect. Paturyl 10 SL demonstrated successful fruit thinning effect of benzyladenine in our work, enlarging fruit weight because of enhancement of cell division by benzyladenine (Stover et al., 2001). Effects of benzyladenine include abscission of fruitless as a main effect (Ferree, 1996; Basak and Rademacher, 2000; Buban and Lakatos, 2000) and other effects such as increased size of fruit at harvest (Greene, 1989; Stopar et al., 2003).

Ethephon (Cerone) is absorbed by the plant tissues where it is broken down into naturally occurring compounds: carbondioxid, phosphate and ethylene, which acts as a plant hormone. Chlorophyll accumulation during investigation time went slowly in apple tree leaves treated with Cerone 480 SL (Table 1). Ethylene is known to be involved with both chlorophyll degradation in leaf and with the abscission process (Aharoni et al., 1979). The total carbohydrate concentration after ethephon application in bark tissues in August increased. Exogenously applied ethylene mobilized carbohydrates in woody shoots and established its mechanism of action (Eklund and Little, 1998). However, it was found that glucose and total carbohydrate accumulation decreased in winter and spring (Fig. 1, Fig. 2, Fig. 3). High total carbohydrate concentrations in
shoots during winter storage and spring promoted high flower induction, which resulted in high flower numbers and high yields in the following year (Khan et al., 1998). Ethephon is widely used for apple thinning (Wertheim, 2000). As growth retardant Ethephon is much less useful on bearing trees, because it thins fruit even at rates that have only a small effect on growth. In our study as in other works (Ebert and Bander, 1986; Stopar, 2000; Stopar and Lokar, 2003) Ethephon reduced the final fruit number per tree, while the fruit weight was not enhanced significantly. The main disadvantage of ethephon is that the results obtained after spraying are highly inconsistent and difficult to predict (Wertheim, 2000).

In conclusion, high photosynthesis, carbohydrate concentrations and yield parameters proves proper plant physiological status after prohexadione-calcium application and Regalis suitability as growth regulator. Reduced yield after Paturyl 10 SL application on fruit tree shows its irrelevancy as growth regulator. However, intensive photosynthesis, high carbohydrates accumulation leads to fine fruit quality, and improves its availability as fruit thinner. Low carbohydrate status during winter storage and spring and early post bloom sprays with Cerone, which affect maximum growth control, reduce fruit set and indicates Cerone’s inefficiency as growth regulator.

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Augimą reguliuojančių preparatų įtaka ‘Jonagold King’ veislės obelų fotosintezės ir derliaus rodikliams

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Santrauka


Reikšminiai žodžiai: angliavandeniai, augimą ribojantys preparatai, derlius, fotosintezės pigmentai, vaisiaus svoris.