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Relationships between apple tree rootstock, crop-load, plant nutritional status and yield

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Impact of the choice of rootstock and crop-load on apple tree nutritional status and yield relationships between biochemical parameters were analysed. Apple cultivar 'Ligol' was grafted onto semi-dwarfing rootstock M.26; dwarfing rootstocks M.9, P 67 and B.396; and super-dwarfing rootstock P 22. Crop-load was adjusted to 60, 105 or 150 inflorescences per tree. Flower buds were removed at the pink bud stage. Super-dwarfing P 22 rootstock under intensive crop-load (150 inflorescences per tree) resulted in N deficiency in the leaves, which caused an accumulation of leaf sugars. Leaf elements were influenced by rootstock and crop-load. With the dwarfing rootstocks P 67 and B.396, a significantly larger leaf area and a decrease in photosynthetic pigment and leaf carbohydrate content were observed. Apple tree yield was directly correlated with the crop- load and the number of fruits, independent of the rootstock onto which they were grafted. Average fruit weight, independent of rootstock, was inversely related to cropload. Generally, yield, fruit weight were crop-load, while metabolite changes were rootstock dependent. Mineral sufficiency was dependent on rootstock and crop-load. Super-dwarfing P 22 rootstock resulted in the smallest yield and fruits.

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1. Introduction

Different genetic and technological tools, such as grafting to rootstocks and crop-load manipulation, can allow increases in yield, tolerance to abiotic stress or increased vigour. Seedling rootstocks and the fruiting clones grafted onto them within the same botanical genus or species have been investigated since the 19th Century. Recently, molecular aspects of rootstock-scion interactions, explaining connections between gene expression and the protein functions underpinning changes in physiology, have been examined [\(Harada,](#page--1-0) [2010\).](#page--1-0) Few studies have focussed on the underlying vegetative and reproductive responses to different rootstocks and crop-loads by examining physiological and biochemical mechanisms [\(Wünsche](#page--1-0) et [al.,](#page--1-0) [2005\).](#page--1-0) Understanding the relationships between the key physiological (growth, yield, fruit quality etc.), and physical (graft union repair, water usage and transport, hormones, nutrition) aspects mediated by the rootstock is important for the growth of high quality and commercially attractive fruit production ([Koepke](#page--1-0) [and](#page--1-0) [Dhingra,](#page--1-0) [2013\).](#page--1-0) The effects of rootstocks

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[http://dx.doi.org/10.1016/j.scienta.2016.08.027](dx.doi.org/10.1016/j.scienta.2016.08.027) 0304-4238/© 2016 Elsevier B.V. All rights reserved. and crop-loads are some of the most important factors influencing yield and fruit quality in orchard management. [Meland](#page--1-0) [\(2009\)](#page--1-0) found the highest fruit weight, soluble solids contents and return bloom with the lowest crop-load in 'Elstar' apple trees. Increasing crop load resulted in a decrease in leaf area, dry mass per unit leaf area, and an increase in chlorophyll content in peach ([Nii,](#page--1-0) [1997\)](#page--1-0) but no difference in the ratio of chlorophyll a to b in pistachio trees [\(Vemmos,](#page--1-0) [1994\)](#page--1-0) was observed. Moreover, crop-load, defined as number of fruits per light intercepting green area, is known to affect carbohydrate production and partitioning in apple. An increase in crop load conditioned an increased in chlorophyll concentration, and a decrease in leaf starch content [\(Wünsche](#page--1-0) et [al.,](#page--1-0) [2005\);](#page--1-0) whereas soluble non-structural carbohydrates in leaves are affected by crop-load to a lesser extent [\(Klages](#page--1-0) et [al.,](#page--1-0) [2001\).](#page--1-0) Relationships between sugar accumulation and leaf characteristics appear at the fruit development stage, when fruits become major sink organs and carbohydrates are typically transported from leaves to fruits ([Nii,](#page--1-0) [1997\).](#page--1-0) The apple tree is unique in metabolism and carbohydrate accumulation, because the primary products of photosynthesis are sorbitol, sucrose and starch ([Teo](#page--1-0) et [al.,](#page--1-0) [2006\).](#page--1-0) Moreover, almost all of the sorbitol and half of the sucrose is converted to fructose, so most of the total carbon flux goes through fructose ([Li](#page--1-0) et [al.,](#page--1-0) [2012\).](#page--1-0) Sucrose and hexoses are storage compounds, being the transport compounds for export from source

Abbreviations: Car, carotenoids; Chl, chlorophyll; Fru, fructose; Glu, glucose; NBI, nitrogen balance status; Sorb, sorbitol.

leaves to sink tissues, but they may also control photosynthesis [\(Smith](#page--1-0) [and](#page--1-0) [Stitt,](#page--1-0) [2007\).](#page--1-0) The relationships between plant leaf mineral status and carbohydrate metabolism are complex. [Peuke](#page--1-0) [\(2010\)](#page--1-0) described relationships between plant growth reduction or inhibition by mineral deficiency and increases in sugar concentrations in Ricinus communis. Deficiency in N leads to an accumulation of carbohydrates in Arabidopsis leaves ([Remans](#page--1-0) et [al.,](#page--1-0) [2006\).](#page--1-0) N deficient plants accumulated higher contents of sugars that led to reduced photosynthesis, probably due to feedback metabolite regulation [\(Martin](#page--1-0) et [al.,](#page--1-0) [2002\).](#page--1-0) [Hermans](#page--1-0) et [al.](#page--1-0) [\(2006\)](#page--1-0) suggested that starch and disaccharide metabolism related genes are significantly over-represented among the differentially regulated genes in the shoots of N deficient plants. P is the second most limiting mineral nutrient after N. [Lemoine](#page--1-0) et [al.](#page--1-0) [\(2013\)](#page--1-0) described the relationships between the lack of leaf P, photosynthesis and reduced carbon assimilation. Low P resulted in the accumulation of sugar, starch and anthocyanin in Arabidopsis leaves [\(Zakhleniuk](#page--1-0) et [al.,](#page--1-0) [2001\).](#page--1-0) A common phenomenon in Mg deficient plants is leaf carbohy-drate accumulation. [Hermans](#page--1-0) et al. (2005) stated that Mg deficiency affects sucrose loading by decreasing Mg-ATP and consequently H⁺-ATP activity. Generally, it can be stated that sucrose transport from the leaves is a necessary signal for responses to N and P starvation, but not for responses to K or Mg deficiency. Increased leaf sugar content, especially sucrose, is stress related ([Lemoine](#page--1-0) et [al.,](#page--1-0) [2013;](#page--1-0) [Peuke,](#page--1-0) [2010\).](#page--1-0)

Due to their root system, trees on the super-dwarfing rootstock P 22 are more winter hardy than those on the dwarfing rootstock M.9, which is the most commonly used rootstock in western Europe ([Foster](#page--1-0) et [al.,](#page--1-0) [2015\).](#page--1-0) The dwarfing rootstocks P 67 and B.396 are good alternatives to the M.9 rootstock, due to their desirable characteristics of growth vigour, high yield and yield efficiency in areas where high winter hardiness is required [\(Kviklys](#page--1-0) et [al.,](#page--1-0) [2013\).](#page--1-0) As roots absorb water containing dissolved minerals, it can be assumed that the rootstock will influence the ability to provide nutrients for the whole plant. Thus, nutrient sufficiency is also an important factor affecting fruit production. The relationships between different rootstocks and differences in leaf nutrient levels have been documented in peach ([Tsipouridis](#page--1-0) [and](#page--1-0) [Thomidis,](#page--1-0) [2005\),](#page--1-0) pear ([North](#page--1-0) [and](#page--1-0) [Cook,](#page--1-0) [2008\),](#page--1-0) apple [\(Kviklys](#page--1-0) et [al.,](#page--1-0) [2012;](#page--1-0) [Tomala](#page--1-0) et [al.,](#page--1-0) [2008;](#page--1-0) [Wünsche](#page--1-0) et [al.,](#page--1-0) [2005\)](#page--1-0) and other woody plants. [Kviklys](#page--1-0) et [al.](#page--1-0) [\(2012\)](#page--1-0) described the effect of eleven apple rootstocks on tree size (expressed as the tree cross sectional area), cumulative yield and yield efficiency, fruit weight and quality. [Tomala](#page--1-0) et [al.](#page--1-0) [\(2008\)](#page--1-0) showed that fruits from trees on P 60 and B.396 rootstocks contained more soluble solids.

In this study, physiological insights into the influence of rootstock and/or crop-load on the relationships between some photosynthetic indices (chlorophyll, carotenoid content, leaf area), primary photosynthesis metabolites, leaf nutritional status and yield will be presented.

2. Materials and methods

2.1. Growth conditions

A field experiment was carried out in an intensive orchard at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in 2014 and 2015. Trees were planted in 2005 year in rows spaced 1.5 m apart with 4 m between the rows and trained as a slender spindle. The commercially important apple cultivar 'Ligol' was grafted on the semi-dwarfing rootstock M.26, the dwarfing rootstocks M.9, P 67 and B.396 and the superdwarfing rootstock P 22. Crop-load was adjusted to 60, 105 and 150 inflorescences per tree. Flower buds were removed before flowering in early May at the pink bud stage. The trail was planted in

eight replicates, single tree represented one replicate. Full randomized design was applied for the experiment. 80 kg/ha of N (40 kg before flowering and 40 kg 2 weeks after flowering) and 80 kg/ha of K fertilizers after harvest were applied annually. The soil in the orchard was Epicalcari-Endohypogleyic Cambisol: heavy clay loam containing 2.8% of humus, 255 mg kg⁻¹ P2O₅, 230 mg kg⁻¹ K₂O, 7077 mg kg⁻¹ Ca, 1873 mg kg⁻¹ Mg, with pH 7.2 (in 1 mol L⁻¹ KCl extract). 80 kg ha^{-1} of N (40 kg before flowering and 40 kg 2 weeks after flowering) and 80 kg ha−¹ of K fertilizers after harvest were applied annually. Pest and disease management was carried out according to the integrated plant protection rules.

2.2. Biometric measurements

Yield (kg per tree) and fruit weight (g) (eight trees represent one treatment) were evaluated in October. To determine the leaf area $\rm (cm²)$, thirty leaves from tree were measured with a leaf area meter (AT Delta-T Device, UK) in July.

2.3. Nitrogen balance index (NBI)

NBI was evaluated in July using no-destructive measurement of leaf chlorophyll and flavonoid content in the epidermis (Dualex® 4, France) of thirty leaves from each tree.

2.4. Determination of photosynthetic pigments

Chlorophylls (Chl) a and b and carotenoids (Car) were measured in the middle of July using the spectrophotometric (Genesys 6, ThermoSpectronic, USA) method of Wetshtein [\(Gavrilenko](#page--1-0) [and](#page--1-0) [Zigalova,](#page--1-0) [2003\)](#page--1-0) in a 100% extract of acetone. 0.2 g of fresh leaves were ground with $CaCO₃$ with a small volumes of acetone, filtered and diluted to a final volume of 50 mL using 100% acetone. The absorbance of the samples was measured at 440.5 and 662 nm for Chl a and b and at 644 nm for Car.

2.5. Carbohydrate content and composition assay

Carbohydrates were analysed in leaves in the middle of July. About 1 g of fresh plant tissue was ground and diluted with 4 mL double distilled water at 70 \degree C. The extraction was carried out for 24 h. The samples were filtered using 0.22 μ m pore diameter cellulose acetate syringe filters. The analyses were performed on a Shimadzu HPLC (Japan) system with a low temperature evaporative light scattering detector (ELSD-LTII), and the oven temperature was maintained at 40 ℃. Separation of fructose, glucose and sucrose was performed on an EC 250/4 NUCLEOSIL Carbohydrate column $(250 \times 4 \text{ mm})$ (Germany), with a mobile phase of acetonitrile: water (79:21, v/v), and a flow rate of 2 mL min^{-1} . Separation of sorbitol was performed on an NUCLEOGEL Sugar 810 Ca column $(300 \times 7.8 \text{ mm})$ (Germany), with a mobile phase of double distilled water, and a flow rate of 0.6 mL min−¹ and the oven temperature was maintained at 85 °C.

2.6. Determination of leaf elements

Macro- (N, P, K, Ca) and micro- (Fe, Mg, Mn) elements were analysed in leaves in the middle of July. Nitrogen content was determined with the Kjeldahl method PN ISO 13878. For other element analysis, 0.4 g (dry weight) of leaves was digested with 6 mL HNO₃ and 2 mL H₂O₂ in a microwave digestion system (Multiwave GO, Antor Paar, Austria) according to [Marin](#page--1-0) et [al.\(2011\).](#page--1-0) The digestion program was as follows: 1) 170 ◦C reached within 5 min, digested for 25 min; 2) 140 \degree C reached within 1 min, digested for 5 min; 3) 120 \degree C reached within 1 min, digested for 2 min; 4) 100 \degree C reached within 1 min, digested for 1 min. The resulting solutions

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