

# Dynamics of water and carbohydrate content in branches of peach tree during the winter in South Region of Brazil

Leticia Neutzling Rickes<sup>1</sup>, Fabiano Simões<sup>2</sup>, Robson Ryu Yamamoto<sup>3</sup>  
Luciano do Amarante<sup>1</sup>, Flavio Gilberto Herter<sup>1</sup>

<sup>1</sup> Universidade Federal de Pelotas, Avenida Eliseu Maciel, Campus Universitário, s/nº, Capão do Leão, RS. CEP 96010-900. E-mail: leticiarickes@hotmail.com, lucianoamarante@yahoo.com.br, flavioherter@gmail.com

<sup>2</sup> Universidade Estadual do Rio Grande do Sul, Avenida Antônio Ribeiro Branco, 1060, Parque dos Rodeios, Vacaria, RS. CEP 95200-000. E-mail: fabiano-simoes@uergs.edu.br

<sup>3</sup> Universidade Federal de São Carlos, Rodovia Lauri Simões de Barros, SP-189, km 12 – Aracaçú, Buri, SP. CEP 18290-000. E-mail: yamamoto@ufscar.br

ISSN 2448-0479

**Abstract** - The objective of this work was to determine the water and carbohydrate content in peach tree branches during the winter in South region, Brazil. Fortnightly collections of branches of peach trees 'Jubileu' and 'Eldorado' were done every 15 days between the months June and August of 2010. These branches were divided into three portions: basal, median, and apical, for determination of the water content, these were divided into two tissues: bark and wood. For carbohydrate content, in the portions of the branches, the contents of total soluble sugars, sucrose and starch were analyzed by the anthrone method. The water content in the bark tissue showed no alterations in the two cultivars over the period investigated. It was found that the dynamics of the water (tissue wood) and carbohydrate presented a great increase mainly in the apical portion of the branch close to sprouting. It follows that the water content and carbohydrate dynamics may be considered physiological markers for resumption of growth for temperate climate fruit trees.

**Keywords** - Branch. Bark. Wood. Temperate climate fruit trees. Total soluble sugars.

**Resumo** - O objetivo deste trabalho foi determinar o conteúdo de água e de carboidratos em ramos de pessegueiro durante o inverno, na região Sul do Brasil. Foram realizadas coletas quinzenais de ramos do ano de pessegueiros 'Jubileu' e 'Eldorado', entre os meses

de junho e agosto/2010. Os ramos foram divididos em três seções: basal, mediana e apical, sendo que para determinação do conteúdo de água, estes foram divididos em dois tecidos: casca e lenho. Para o teor de carboidratos, foram analisados nas seções dos ramos o conteúdo de açúcares solúveis totais, sacarose e amido pelo método antrona. O conteúdo de água no tecido da casca não mostrou alterações nas duas cultivares ao longo do período analisado. Verificou-se que a dinâmica do conteúdo de água (tecido lenho) e de carboidratos apresentou um grande aumento principalmente na seção apical do ramo próximo à brotação. Concluem-se que a dinâmica do conteúdo de água e carboidratos podem ser considerados marcadores fisiológicos para a retomada do crescimento para frutíferas de clima temperado.

**Palavras-chave** - Ramo. Casca. Lenho. Frutíferas de clima temperado. Açúcares solúveis totais.

**Recebido em:** 02 de dezembro de 2015

**Aprovado em:** 15 de março de 2016

## 1 INTRODUCTION

The field-cultivated plants will always be targets of different environmental conditions, affecting both their growth and metabolism and bringing about reductions in the crop yield (WANG *et al.*, 2007). The south region of Brazil enables the growing of

temperate climate fruit species, but the climate is characterized for being subtropical and presenting great irregularities of environmental factors, within a same season and over the different years.

One of the characteristics of the temperate climate fruit species is the need of accumulating cold for the overcoming of dormancy. During that period, the accumulation of cold is associated with the changes in carbon hydrate content among other substances (WANG *et al.*, 2007). In addition to carbohydrates, the water dynamics is of great importance to the tissues of temperate climate fruit plants, mainly during the dormancy period and sprouting induction, acting upon the biochemical processes, like the mobilization of the reserves and of activation of enzymatic processes (CITADIN *et al.*, 2009; MARAFON *et al.*, 2011a, SIMÕES *et al.*, 2014) since, the translocated solutes, chiefly, carbohydrates are dissolved in water. Water displacement has been the focus of several studies in temperate climate fruit plants (CITADIN *et al.*, 2009; YAMAMOTO *et al.*, 2010; MARAFON *et al.*, 2011a; SIMÕES *et al.*, 2014).

The objective of this study was to determine the water and carbohydrate content in peach tree branches during the resting phase under the mild winter conditions region in the state of Rio Grande do Sul.

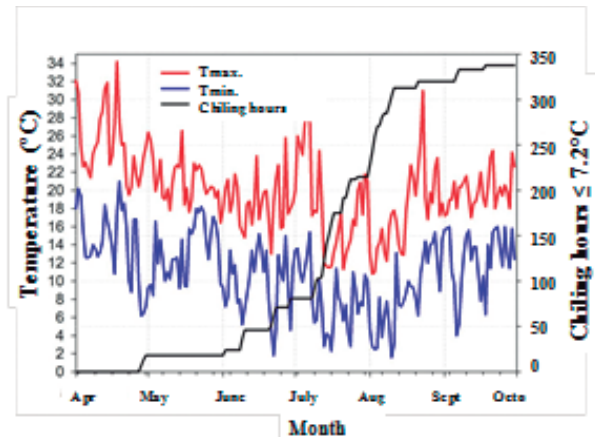
## 2 MATERIAL AND METHODS

### 2.1 Plant Material

The experiment was conducted during the 2010 winter in the Palma Agricultural Center (Centro Agropecuário da Palma) orchard of Faculdade de Agronomia Eliseu Maciel, UFPel, in the municipality of Capão do Leão, with geographic coordinates of latitude 31°52'00" S, longitude 52°21'24" O and altitude of 13 m. The climate is of the Cfa subtype according to the Koeppen classification. Two cultivars of peach tree (*Prunus persica* L.), namely Jubileu, with requirement of 300 hours of cold (HF)  $\leq 7,2^{\circ}\text{C}$  and Eldorado, with requirements of 350 HF  $\leq 7,2^{\circ}\text{C}$  were utilized. The collections were carried out in the months of June (day 18), July (days 1, 16 and 30) and August (day 13). Six plants of each cultivar were sorted, from which four branches one year old, about 30 cm, arranged at the median height in all the quadrants of the plant, were collected. The collections were done always around 10h in the morning.

### 2.2 Meteorological data

The meteorological data were obtained by Estação Agroclimatológica de Pelotas, Terras Baixas Weather Station, situated at about 13 km away from the experiment site. The daily maximum and minimum temperatures as well as the HF  $\leq 7,2^{\circ}\text{C}$  accumulated are represented in Figure 1.



**Figure 1** – Daily maximum (—), minimum (—) temperatures and chilling hours  $\leq 7,2^{\circ}\text{C}$  (—) accumulated, obtained in the Pelotas Weather Station (Capão do Leão) - Terras Baixas Weather Station during the months of April to September of 2010. UFPel, Pelotas, RS- Brazil. 2010.

### 2.3 Determination of water content

The freshly collected branches were packed into closed plastic packages to avoid the dehydration and taken to the Plant Physiology Laboratory (Laboratório de Fisiologia Vegetal) of UFPel for determination of water content (WC). Each branch was segmented into three portions: basal, median and apical, separating the bark and wood. From each position a 1 cm branch sample was taken off.

Immediately after the separation of each tissue of each sample, the fresh biomass (FB) was determined by using an analytical balance of 0.1 mg (model AAA-250/L). Soon after the preparation, the samples were stored into paper packages and placed into oven under forced ventilation at  $65^{\circ}\text{C}$  until constant weight.

The determination was conducted according to the formula:  $\text{WC} = (\text{FB}-\text{DB})/\text{DB}$ , being WC = water content, FB = fresh biomass and DB = dry biomass, expressed in  $\text{g g}^{-1}$  DM.

### 2.4 Carbohydrate extraction

For extraction of carbohydrates, 300 mg of BMS of each sample (bark+wood) together, were utilized, which were ground in cutting mill

(model CE-430/Mini) till a sample of extremely fine particle size was obtained. The extraction of total soluble sugars and sucrose were carried out using 80% of ethanol repeated for three times. The precipitate retained in that extract was dried in oven at 65°C for 48h for determination of starch. The precipitate was re-suspended in 5 mL of perchloric acid (HClO<sub>4</sub>) at 30% and put into water bath at 80°C for 10 min and centrifuged at 6000 rpm for 10 min. This procedure was repeated once more (DISCHE, 1962).

### 2.5 Determination of total soluble sugars

Aliquots of 200 µL of the extracts were transferred to test tubes followed of the addition of 3 mL of solution of 0.15% anthrone in concentrated H<sub>2</sub>SO<sub>4</sub> (DISCHE, 1962) in ice bath followed of agitation in vortex. Afterwards, the tubes were taken to water bath for 10 min at 100°C. The absorbance readings were conducted in spectrophotometer (Biospectro, model SP-22) at 620 nm and the results were expressed in mg g<sup>-1</sup> MS.

### 2.6 Sucrose determination

In the quantification of sucrose were utilized aliquots of 200 µL of the extract, transferred to each tube, followed of the addition of 200 µL of 15% potassium extract. Next, the tubes were led to water bath at 100°C for 10 min. Afterwards, 3 mL of the reagent anthrone (0.15% p/v in 70% H<sub>2</sub>SO<sub>4</sub>) were added slowly to each tube and again taken to water bath at 40°C for 15 min. The absorbance readings were recorded in spectrophotometer (Biospectro, model SP-22) at 620 nm and the results were expressed in mg g<sup>-1</sup> MS.

### 2.7 Starch determination

The starch quantification was carried out by the anthrone method. Aliquots of 200 µL of the extracts were added to 3 mL of anthrone solution, 0.15% H<sub>2</sub>SO<sub>4</sub> maintaining the test tubes in ice bath. After that stage, the tubes were boiled for 10 minutes at 100°C and then cooled in ice bath. The absorbance readings were recorded in spectrophotometer (Biospectro, model SP-22) at 620 nm and the results were multiplied by the factor of correction 0.9 for conversion into starch contents according to McCready *et al.* (1950) and expressed in mg g<sup>-1</sup> MS.

### 2.8 Experimental design and statistical analysis

The experimental design utilized was completely randomized containing six replications. The factorial arrangement to analyze the variable WC was (2x5), obtained from the combination of the factors tissue (2 part of branches: wood and bark) and collection data (5 collection dates), and variables related to carbohydrates was (3x5) obtained from the combination of the factors (3 parts of branches: basal, median and apical) and collection data (5 collection data) for each cultivar.

The means were submitted to the analysis of variance (p<0.05) and the significant variables were compared by the Tukey test at 5% of probability.

## 3 RESULTS AND DISCUSSION

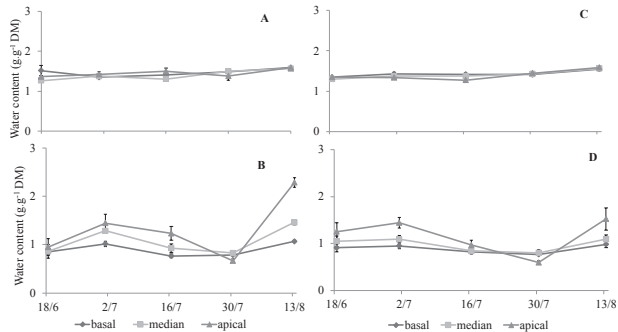
In the year of 2010, according to data made available by Estação Agroclimatológica de Pelotas, the amount of hours of cold ≤ 7.2°C accumulated in the region was approximately 350 HF during the months of April to September. The month of July presented temperatures close to 30°C, the monthly mean of maximum temperature was of 18.4°C followed by the minimum of 8.2°C (Figure 1).

These variations in temperature affect very important physiological processes of the plant, for example, changes in cellular structure, enzymatic changes (MARAFON *et al.*, 2011a), carbohydrates flow (YAMAMOTO *et al.*, 2010; MARAFON *et al.*, 2011a; SIMOES, 2011), water content (MARAFON *et al.*, 2011b; YAMAMOTO *et al.*, 2010; SIMOES, 2011), among others.

The cultivars Jubileu and Eldorado presented similar behavior relative to the WC in the bark, considering the different portions on the branch along the winter period (Figure 2A e 2C), having been similar to the one observed by Citadin *et al.* (2009) on walnut tree (*Juglans regia*), where rehydration was less visible at the basal and median section of the branch, both for the bark and for the wood. The meteorological data may have influenced in the decrease of the WC verified at the third collection (16/July) and fourth collection (30/July) at the apical position of the wood in both cultivars (Figures 2B and 2D).

In the wood, the differences were more expressive, stressing a great increase of the WC at the apical portion in both cultivars studied at the fifth collection (13/August), this period being close to sprouting.

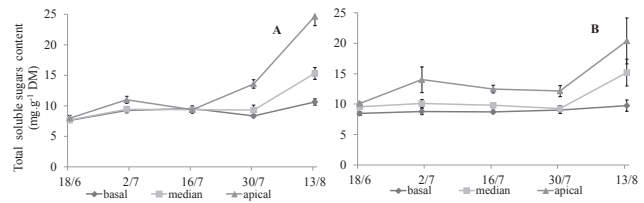
An opposite situation was found by Simões *et al.* (2014) in a study with pear trees 'Housui' in two different regions of Brazil, São Joaquim/SC and Vacaria/RS, where the WC in the wood tissue presented no differences over the period evaluated (May to September).



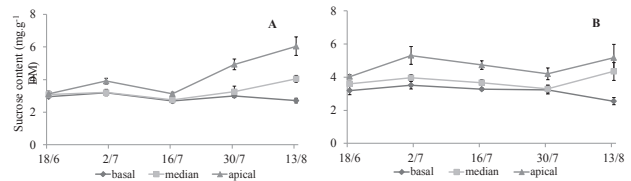
**Figure 2** - Water content ( $\text{g g}^{-1}$  DM) in peach tree cultivars Jubileu (A and B) and Eldorado (C and D), in the bark (A and C) and wood (B and D) during dormancy. UFPEL, Pelotas, Brazil, 2010. Means compared by Tukey test at 5% probability,  $\pm$  SE (n=6).

In both cultivars, greater WC at the apical portion of the wood was found in the second collection (02/July), followed of the fifth collection (13/August), the same behavior can be found for the dynamics of total soluble sugars, sucrose and starch for cultivar Eldorado (Figures 3, 4 and 5 respectively). That dynamics from the latest collection on represents the increase of the xylem sap, to which water migration is ascribed, together with soluble sugars in the phase of induction to sprouting, which confirms the hypothesis of this study that both the WC and the carbohydrate contents are physiological indicators of the growth resumption. This growth of the WC and of carbohydrates found from the fourth collection (30/July) at the apical portion of the branches in the cultivars of the present study, are ascribed to the hydration status of them and has a strong relation with increased metabolic activity and processes of dormancy overcoming, consequently with the sprouting capacity (MARAFON *et al.*, 2011a)

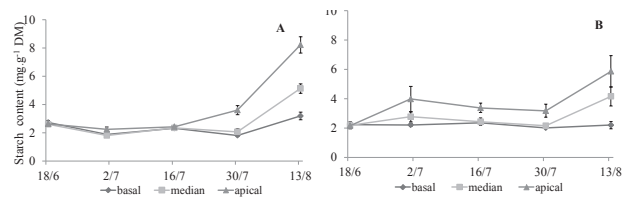
In general, the soluble total sugar content at the apical portion was higher than the other portions in both cultivars, with the exception of the first (18/June) and third collections (16/July) for cultivar Jubileu (Figure 3A), where the three portions investigated presented quite similar soluble total sugar contents. The behavior dynamics was observed for sucrose (Figure 4A) and starch (Figure 5A).



**Figure 3** - Total soluble sugar content ( $\text{mg g}^{-1}$  DM) in peach trees cultivar Jubileu (A) and Eldorado (B) at the basal, median and apical portions during dormancy. UFPEL, Pelotas, Brazil, 2010. Means compared by Tukey test at 5% probability,  $\pm$  SE (n=6).



**Figure 4** - Sucrose content ( $\text{mg g}^{-1}$  DM) in peach trees cultivar Jubileu (A) and Eldorado (B) at the basal, median and apical portions during dormancy. UFPEL, Pelotas, Brazil, 2010. Means compared by Tukey test at 5% probability,  $\pm$  SE (n=6).



**Figure 5** - Starch content ( $\text{mg g}^{-1}$  DM) in peach trees cultivars Jubileu (A) and Eldorado (B) at the basal, median and apical portions during dormancy. UFPEL, Pelotas, Brazil, 2010. Means compared by Tukey test at 5% probability,  $\pm$  SE (n=6).

During winter, starch is the most abundant reserve carbohydrate in woody plants of temperate climate and it is broken down by amylases and converted into smaller molecules such as sucrose for cold protection (YOSHIOKA *et al.*, 1988). The sucrose produced in the reserve tissues is transported by the phloem pathway and hydrolyzed into glucose and fructose for the furnishing of energy and carbon precursors (YOSHIOKA *et al.*, 1988). However, a clear response of this conversion of reserve substances in that work was not found, since a constant dynamics in the three portions till the third collection (16/July) for cultivar Jubileu was verified (Figure 5A), which can also be reinforced as obtained in relation to the total soluble sugar content. That behavior can be associated with the high temperatures during dormancy, which would upset cold accumulation, disturbing the mobilization of these carbohydrates (HONJO *et al.*, 2002).

There was a great increase of these carbohydrates at the apical section from the fourth collection (30/ July) for cultivar Jubileu, but for Eldorado, the same was found only in the fourth collection (13/August). That difference of the dynamics among the branch portions is due to increased capacity of breaking down starch at the apical part of the branch as compared with the base of this same branch, implying into a greater capacity of mobilizing starchy reserves at the apical part of the branch and being able to be related to the acropetal mechanism (CITADIN *et al.*, 2009).

According to Bonhomme *et al.* (2010), close to sprouting, the strong increase of hexoses towards the apical portion seems to be closely linked to the decrease of both sucrose and starch, but that was not found in the present work in both cultivars, where there was a great increase of these carbohydrates at the apical portion. The same dynamics was observed for the sucrose content (Figure 3). The basal and median portions of the branch present a great sucrose-exporting capacity due to fact that an increase of this content takes place at the apical portion of the branch. Sucrose is exported from the parenchyma cells to the xylem vessels through H<sup>+</sup>-sucrose cotransporters, being displaced along with the water towards the buds, where it is absorbed directly or then hydrolyzed in the apoplast, supporting its importation (YOSHIOKA *et al.*, 1988).

The increase of soluble sugars, sucrose and starch towards the apical portion of the branches during the winter period clearly demonstrate the accumulation of nutrients toward the buds for sprouting and this displacement of nutrients would be directly involved with the WC, which in turn is closely linked to sugar mobilization. Although the contents of total soluble sugar and sucrose have increased mainly close to budding, this increase was not accompanied by the reduction in starch, contradicting the results obtained by Bonhomme *et al.* (2005) in peach tree and by Marafon *et al.* (2011b) in pear tree, who found a parallelism between starch hydrolysis and the increase in the content of total soluble sugars in branches during the winter. Maurel *et al.* (2004) in studies with peach tree, found a decreasing concentration of sucrose and sorbitol during the winter and an increase of glucose and fructose a little before sprouting, the same was found in this study with respect to total soluble sugars, where there was a large increase of the same ones close to the sprouting period.

A starch re-synthesis was found, this starch-sugar interconversion, this occurring in the penultimate collection (30/July) when both cultivars presented increases in the content of total soluble sugars and starch simultaneously. In this period, the occurrence of quite high temperatures was verified, there being no need of maintenance of high levels of soluble sugars to protect from possible damages to the sensitive organs caused by cold, causing them to convert the sugars into starch.

#### 4 CONCLUSIONS

The increase of the WC in the wood close to the sprouting period is related to the mobilization of reserves for the resumption of growth after the meeting in cold during endodormancy.

The dynamics of the WC and carbohydrates in peach tree branches close to the budding period presented in the following variation sequence: apical> median> basal.

#### 5 REFERENCES

- BONHOMME, M.; RAGEAU, R.; GENDRAUD, M.; LACOINTE, A. Influences of cold deprivation during dormancy on carbohydrate contents of vegetative and floral primordia and nearby structures of peach buds (*Prunus persica* L. Batch). **Scientia Horticulturae**, Amsterdam, v. 105, n.2, p. 223-240, 2005.
- BONHOMME, M.; PEUCH, M.; AMEGLIO, T.; RAGEAU, R.; GUILLIOT, A.; DECOURTEIX, M.; ALVES, G.; SAKR, S.; LACOINTE, A.; Carbohydrate uptake from xylem vessels and its distribution among stem tissues and buds in walnut (*Juglans regia* L.). **Tree Physiology**, Oxford, v. 30, p. 89-102, 2010.
- DISCHE, Z. General colors reactions In: WHISTLER, R.L.; WOLFRAM, M.L. **Carbohydrate Chemistry**. New York: Academic Press, p. 477-520, 1962.
- HONJO, H.; KOBAYASHI, M.; WATANABE, M.; FUKUI, R. Effect of intermittent periods on bud break and carbohydrate content of Japanese pear (*Pyrus pyrifolia* Nakai) in different endodormancy stages. **Acta Horticulturae**, Amsterdam, v. 587, p. 397-403, 2002.
- MARAFON, A.C.; HERTER, F.G.; HAWERROTH, F.J. Umidade ponderal em tecidos de pereira durante o período de dormência sob condições de inverno ameno. **Pesquisa Agropecuária Brasileira**, Brasília, v. 46, n. 9, p. 1-7, 2011a.
- MARAFON, A.C.; CITADIN, I.; AMARANTE, L.;

HERTER, F.G.; HAWERROTH, F.J. Chilling privation during endodormancy period disturbs carbohydrate mobilization in Japanese pear. **Scientia Agricola**, Piracicaba, v. 68, n. 4, p. 462-468, 2011b.

MAUREL, K.; SAKR, S.; GERBE, F.; GUILLIOT, A.; BONHOMME, M.; RAGEAU, R.; PETEL, G. Sorbitol uptake is regulated by glucose through the hexokinase pathway in vegetative peach tree bud. **Journal of Experimental Botany**, London, v. 55, n.398, p. 879-888, 2004.

McCREADY, R.M.; GUGGOLZ, J.; SILVEIRA, V.; OWENS, H.H. Determination of starch and amylose in vegetables **Analytical Chemistry**, Washington, v. 22, p. 1156-1158, 1950.

SIMÕES, F.; HAWERROTH, F.J.; YAMAMOTO, R.R.; HERTER, F.G. Water Content and carbohydrate dynamics of pear trees during dormancy in Southern Brazil. **Acta Horticulturae**, Wageningen, v. 1058, p. 305-312, 2014.

WANG, H., WANG, E., WANG, G., MAJOURHAT, K. The responses of photosynthetic capacity, chlorophyll fluorescence and chlorophyll content of nectarine (*Prunus persica* var. *nectarina* Maxim.) to greenhouse and field grown conditions **Scientia Horticulturae**, Amsterdam, v. 112, n.1, p. 66-72, 2007.

YAMAMOTO, R.R.; HORIGANE, A.K.; YOSHIDA, M.; SEKOZAWA, Y.; SUGAYA, S.; GEMMA, H. "Floral primordia necrosis" incidence in mixed buds of Japanese pear (*Pyrus pyrifolia* (Burm.) Nakai var. *culta*) 'Housui' grown under mild winter conditions and the possible relation with water dynamics. **Journal of the Japanese Society for Horticultural Science**, Kyoto, v. 79, n. 3, p. 246-257, 2010.

YOSHIOKA, H.; NAGAI, K.; AOBA, K.; FUKUMOTO, M. Seasonal changes of carbohydrates metabolism in apple trees. **Scientia Horticulturae**, Amsterdam, v. 36, n. 3/4, p. 219-227, 1988.