



Leaf carbohydrates in 'Ponkan' mandarin fruit quality under chemical thinning

Rodrigo Amato Moreira^{1*}, José Darlan Ramos¹, Maria do Céu Monteiro da Cruz², Lílían de Araújo Pantoja³ and Alexandre Soares dos Santos³

¹Departamento de Agricultura, Universidade Federal de Lavras, Cx. Postal 3037, 37200-00, Lavras, Minas Gerais, Brazil. ²Departamento de Agricultura da Universidade Federal dos Vales Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil. ³Laboratório de Bioprocessos e Biotransformação, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil. *Author for correspondence. E-mail: amatomoreira@yahoo.com.br

ABSTRACT. This study was performed to verify the influence of the levels of leaf carbohydrates on the fruit quality of 'Ponkan' mandarin trees subjected to chemical thinning for three years using different Ethephon concentrations. Five concentrations of Ethephon were tested: 0, 200, 400, 600 and 800 mg L⁻¹, which were applied during the stage of fruit development when fruits were from 25 to 30 mm in transverse diameter. The experimental design was in randomized blocks, with four blocks and four plants per plot. Interactions were observed between the concentrations of Ethephon and the year of assessment for the percentage of thinning, transverse diameter, longitudinal diameter, mass, soluble solids, acidity and the ratio of fruits. For the contents of carbohydrates, there was an interaction between the concentrations of Ethephon, the years of assessment and the leaf sampling times (flowering and harvest). Maintenance of high levels of leaf carbohydrates resulted in improvement of the quality of 'Ponkan' mandarin fruits. Chemical thinning conducted with Ethephon concentration of 600 mg L⁻¹ maintained carbohydrate levels suitable to improve the quality of 'Ponkan' mandarin fruits.

Keywords: *Citrus reticulata* Blanco, ethylene, growth regulators.

Carboidratos foliares na qualidade de frutos de tangerineira 'Ponkan' sob raleio químico

RESUMO. O trabalho foi realizado com o objetivo de avaliar a influência dos teores de carboidratos na qualidade de frutos de plantas de tangerineira 'Ponkan' submetidas ao raleio químico por três anos com diferentes concentrações de Ethephon. Foram testadas cinco concentrações de Ethephon: 0; 200; 400; 600 e 800 mg L⁻¹, aplicadas quando os frutos estavam no estágio de desenvolvimento de 25 a 30 mm de diâmetro transversal. O delineamento experimental foi em blocos casualizados, com quatro blocos e quatro plantas por parcela. Foram observadas interações entre as concentrações de Ethephon e os anos de avaliação para o percentual de raleio, diâmetro transversal, diâmetro longitudinal, massa, sólidos solúveis, acidez titulável e *ratio* dos frutos. Para os teores de carboidratos foliares ocorreu interação entre concentrações de Ethephon, anos de avaliação e época de amostragem das folhas (floração e colheita). A manutenção dos teores de carboidratos foliares elevados proporcionou a melhoria na qualidade dos frutos de tangerineira 'Ponkan'. O raleio realizado com a concentração de 600 mg L⁻¹ de Ethephon manteve os teores de carboidratos adequados para melhorar a qualidade de tangerinas 'Ponkan'.

Palavras-chave: *Citrus reticulata* Blanco, etileno, reguladores de crescimento.

Introduction

The 'Ponkan' mandarin (*Citrus reticulata* Blanco) stands out as one of the most popular and appreciated mandarins for fresh consumption as well as for industrial processing (RAMOS et al., 2009). For fresh fruit to be accepted in the market by the consumer, in addition to the size of the fruit, five characteristics are noted: color, juice content, soluble solids, acidity and soluble solids/ acidity ratio (JACKSON, 1991).

Production of an excessive amount of fruit per plant is a recurring feature of mandarin trees, which affects the size and quality of fruits, making marketing

them difficult (CRUZ; MOREIRA, 2012; MOREIRA et al., 2011). In years of high production, there is depletion of reserves, especially of carbohydrates accumulated in the roots and leaves of plants (GOLDSCHMIDT; KOCH, 1996).

These carbohydrate reserves are used in the development of flowers and fruits (CRUZ et al. 2007; MONERRI et al., 2011; RUIZ et al., 2001), and according to Bustan and Goldschmidt (1998), the daily production of carbohydrates in the leaves is not sufficient during the period of anthesis.

Thus, management practices are necessary to ensure high 'Ponkan' mandarin yields during

productive years by maintaining the carbohydrate reserves in plants, promoting flowering the next year and developing fruits of higher quality. Among these management practices, chemical thinning that reduces competition between sinks, improving the characteristics of remaining fruits by increasing the supply of metabolites, has been highlighted (GARCIA-LUIS et al., 2002).

Among the growth regulators used to promote thinning, Ethephon, which releases ethylene when in contact with plant tissue, thus promoting abscission, has been considered to be more efficient compared to NAA (DOMINGUES et al., 2001); 3,5,6-TPA; 2,4-DP; Phenothiol and Ethylclozate (SERCILOTO et al., 2003).

The results obtained by thinning fruit in relation to the physical and chemical characteristics of fruits are variable and have been reported only for single years in which thinning was applied. Cruz et al. (2009) showed that application of Ethephon to 'Ponkan' mandarin improved the quality of fruit in terms of all examined characteristics. Ramos et al. (2009) employed chemical thinning of 'Ponkan' trees and obtained an increase in fruit size and in the ratio and a decrease in acidity. In contrast, Rufini and Ramos (2002) investigated the effects of manual thinning alone and observed an increase in the size of fruit, but no change in the chemical properties of 'Ponkan' mandarin.

Although the literature shows that application of chemical thinning in a single year promotes improvement of the quality of 'Ponkan' mandarin (CRUZ et al, 2009, 2011; RAMOS, et al., 2009), it is necessary to evaluate the effect of regular application of this practice in subsequent years, as reducing the number of remaining fruits in just one year can affect the reserves of carbohydrates and promote excessive production of fruit of irregular sizes in the following year.

Thus, this study was performed to verify the influence of the levels of leaf carbohydrates on the fruit quality of 'Ponkan' mandarin trees subjected to chemical thinning for three years using different Ethephon concentrations.

Material and methods

The study was conducted in an unirrigated mandarin 'Ponkan' orchard in the city of Perdões, which is situated in the southern region of Minas Gerais, Brazil, at 21° 05' 27" (S) and 45° 05' 27" (W) from October 2008 to June 2011, including three years of agricultural assessment: 2008/2009, 2009/2010 and 2010/2011.

According to the Köppen classification, the type climate in the region is Cwb, with hot, humid

summers and dry, cold winters. The variations in the maximum temperature, minimum temperature, average temperature, precipitation and relative humidity were recorded during the evaluation period (Figure 1).

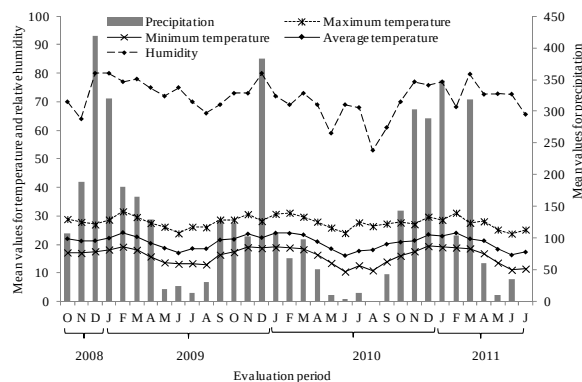


Figure 1. Monthly values of precipitation (mm), maximum temperature (°C), average temperature (°C), minimum temperature (°C) and relative humidity (%) during the evaluation period.

The examined mandarin 'Ponkan' trees (*Citrus reticulata* Blanco) were twelve years old. The trees were cultivated with a spacing 6 x 3 m, grafted on 'Rangpur' lime (*Citrus limonia* Osbeck).

Prior to application of the treatments, the plants were selected on the basis of their productive potential, such that all plants subjected to chemical thinning presented significant amounts of fruits. In the second and third years of thinning, the same chemical concentrations were applied to the plants.

Five concentrations of Ethephon were tested: 0, 200, 400, 600 and 800 mg L⁻¹, which were applied after the period of physiological fruit dropping in Jan. 2009, Jan. 2010 and Jan. 2011 when the fruits were in stage of development where they presented a transverse diameter of 25 to 30 mm. The experimental design was in randomized blocks, with four blocks and four plants per plot.

The plants were sprayed over the full extent of the canopy (internal and external) with approximately two liters of solution. This volume was determined via a blank test in which water was applied.

The commercial product Ethrel® was obtained in the form of a soluble concentrate containing 240 g L⁻¹ 2chloroetil phosphonic acid (Ethephon). It was applied using the adhesive spreader Wilfix® with a backpack sprayer nozzle cone at a pressure of 6 kgf cm⁻², which had the ability to deposit approximately 70 to 100 particles cm⁻² in droplets with diameters ranging from 100 to 200 microns.

During the study period, plants were maintained in accordance with the recommendations for mandarin trees in relation to cultivation, fertilization and the control of pests and diseases.

A total of 48 leaves were collected from productive branches per plot to assess the levels of soluble carbohydrates (mg g^{-1}), reducing carbohydrates (mg g^{-1}), starch (mg g^{-1}) and total carbohydrates (mg g^{-1}) during the flowering period (Oct. 2009, 2010 and 2011) and at harvest time (June 2009, 2010 and 2011).

The leaves were washed in distilled water and dried with forced air at 65°C for 72 hours, until reaching constant mass. The samples were ground and transported to the laboratory for analysis of the levels of carbohydrates in the dry matter.

Extracts for determination of carbohydrates were prepared from 40 mg of the dried leaves following maceration. Preparation of extracts for analysis of soluble sugars was performed using an alcoholic solution ($80\% \text{ v v}^{-1}$) and starch with a perchloric acid solution ($30\% \text{ v v}^{-1}$). Measurements of starch and soluble sugars were carried out using anthrone sulfuric acid according to the methodology proposed by McCready et al. (1950), and while analysis of the levels of reducing sugars followed Miller (1959).

The percentage of thinning was determined on two marked branches per plant by counting the fruits present on the day of application and the fruits remaining 15 days after the application of Ethephon in 2009, 2010 and 2011 when the abscission of fruits was complete.

To evaluate the physical and chemical characteristics, twenty representative fruits per plot were collected from the middle part of the crown in June 2009, 2010 and 2011.

The physical analyses of the fruits included a determination of the transverse diameter (mm), longitudinal diameter (mm), mass (g) and juice yield (%), as determined by the ratio of the volume of juice extracted divided by its mass.

In the chemical analyses of the fruit juice samples, acidity was measured in juice titrated with 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator, with the results expressed in terms of the % of citric acid in the juice. Additionally, the soluble solids in degrees brix ($^{\circ}\text{Bx}$) were determined using a digital refractometer. The ratio was calculated as soluble solids / acidity.

The obtained data were subjected to analysis of variance and polynomial regression including the concentrations of Ethephon and characteristics evaluated. The models were chosen using significance tests of parameters and the regression coefficient, $p < 0.05$.

Results and discussion

Interactions were observed between the concentrations of Ethephon and the year of assessment for the percentage of thinning (PT), transverse diameter (TD), longitudinal diameter (LD), mass (MA), soluble solids (SS), acidity (A) and ratio (RA) of fruits (Table 1).

With respect to the contents of carbohydrates in the leaves, there were interactions detected (Table 2) between the Ethephon concentrations, years of assessment and leaf sampling times (flowering and harvest).

Table 1. Summary of analysis of variance for the percentage of thinning (PT), mass (MA), acidity (A), soluble solids (SS), transverse diameter (TD), longitudinal diameter (LD), and ratio (RA) of mandarin 'Ponkan' fruits under different Ethephon concentrations in three years.

ANOVA	PT	MA	AC	SS	TD	LD	RA
Ethephon concent.	1224.88*	1222.61*	0.01*	1.41*	51.74*	11.61*	5.16*
Blocks	9.86 ^{ns}	4.12 ^{ns}	0.01 ^{ns}	2.34*	2.78 ^{ns}	5.53 ^{ns}	0.78 ^{ns}
Error 1	29.43	197.99	0.00	0.08	6.31	5.48	1.20
Years	704.08*	10001.61*	138.71*	374.09*	231.69*	69.85*	147.59*
Ethephon x Years	234.43*	292.81*	5.52*	2.81*	52.24*	15.04*	6.48*
Error 2	62.29	158.46	0.00	0.08	4.81	3.36	1.30
CV ₁ (%)	48.31	8.15	8.02	2.34	3.43	3.63	6.55
CV ₂ (%)	70.28	7.29	8.30	2.33	2.99	2.84	6.82

*Significant; ^{ns}not significant ($p < 0.05$).

Table 2. Summary of analysis of variance for soluble carbohydrates (SCA), reducing carbohydrates (RCA) and starch (STA) in leaves of 'Ponkan' mandarin at the time of leaf sampling in three years under different Ethephon concentrations.

ANOVA	SCA	RCA	STA
Ethephon concentrations	5752.01*	208.72*	951.25*
Blocks	98.99*	8.49*	13.84 ^{ns}
Etheph. conc. x Samp. time	503.48*	37.16*	114.79*
Samp. time	12381.38*	483.70*	1872.69*
Error 1	12.32	1.67	9.20
Years	14305.39*	978.98*	560.82*
Etheph. conc. x Samp. time x Years	908.69*	11.04*	117.31*
Etheph.conc. x Years	359.59*	84.71*	175.50*
Samp. time x Years	5588.84*	416.19*	327.44*
Error 2	3.19	0.53	9.76
CV ₁ (%)	5.17	13.00	9.58
CV ₂ (%)	2.63	7.33	9.87

*Significant; ^{ns}not significant ($p < 0.05$).

The results show that the plants were under similar conditions when they were selected on the basis of their potential production because it was found that during flowering (2008/2009), the plants in different treatments were similar in terms of their soluble carbohydrates, reducing carbohydrates and starch (Figures 2A, 3A and 4A).

At harvest time in the first year after thinning, an increase in the accumulation of soluble and reducing carbohydrates was observed (Figures 2B and 3B). With

respect to the starch content (Figure 4), no difference was observed between treatments, which may be due to the conversion of carbohydrate reserves (starch) into soluble carbohydrates to meet the high demands related to the establishment of fruits.

However, in the second year (2009/2010), there was a linear increase in soluble carbohydrates, reducing carbohydrates and starch as a function of the tested Ethephon concentrations at flowering and harvest time (Figures 2, 3 and 4).

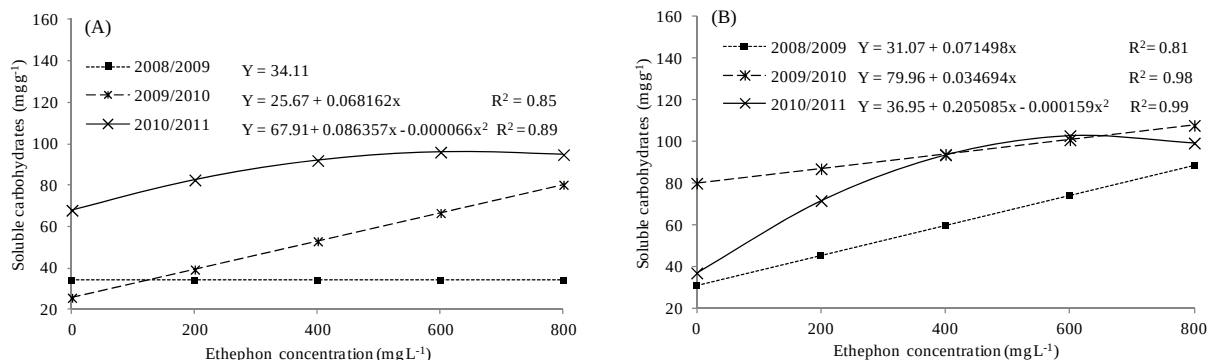


Figure 2. Average soluble carbohydrate contents in leaves of 'Ponkan' mandarin (A) at flowering and (B) at harvest depending on the Ethephon concentrations applied during the three years of evaluation.

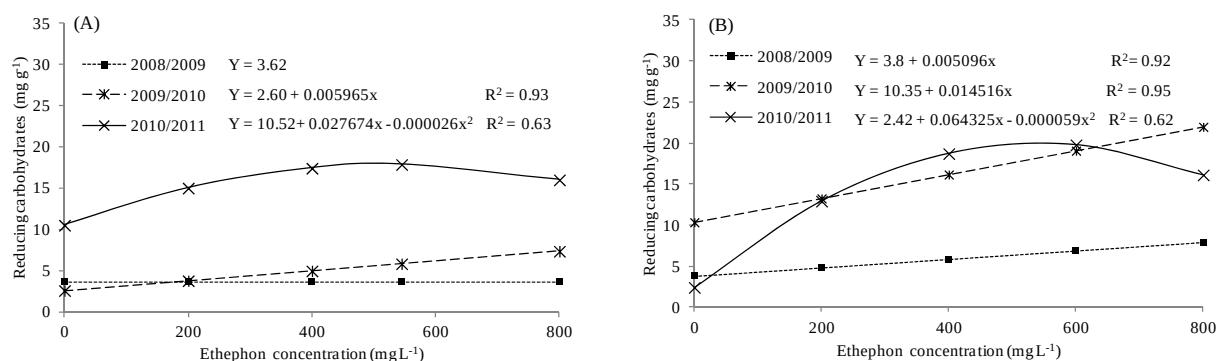


Figure 3. Average reducing carbohydrate contents in leaves of 'Ponkan' mandarin (A) at flowering and (B) at harvest depending on the Ethephon concentrations applied during the three years of evaluation.

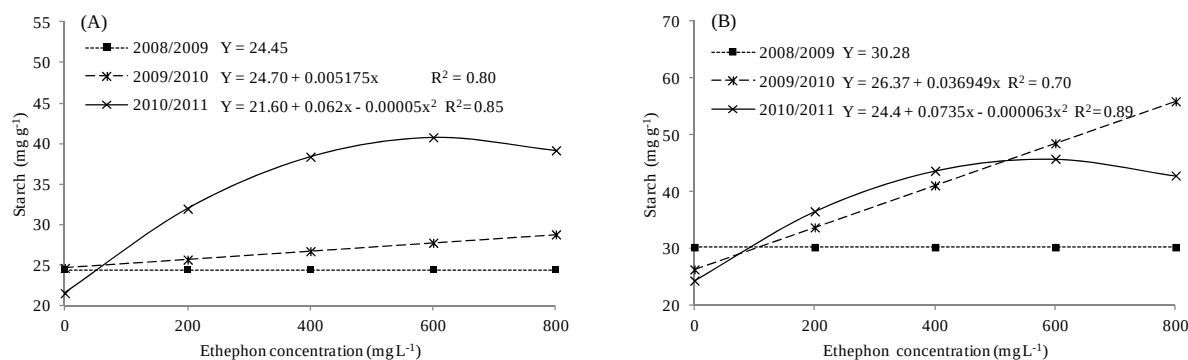


Figure 4. Average of starch contents in leaves of 'Ponkan' mandarin (A) at flowering and (B) at harvest depending on the Ethephon concentrations applied during the three years of evaluation.

In 2010/2011, there was a quadratic increase in soluble carbohydrates at the time of flowering and harvest (Figures 2, 3 and 4). The maximum soluble sugar levels were observed in plants sprayed with Ethephon concentrations of 654 and 644 mg L⁻¹ at flowering and harvest, respectively (Figure 2A and B).

In relation to the measured reducing carbohydrates (glucose and fructose), a pattern similar to that detected for soluble carbohydrates was observed; i.e., the highest levels (19.9 mg g⁻¹) were detected in association with an estimated Ethephon concentration of 532 mg L⁻¹ at flowering, while a content of 17.9 mg g⁻¹ was obtained under an estimated Ethephon concentration of 545 mg L⁻¹ during harvest (Figure 3A and B).

The greatest accumulation of starch content was observed at the time of flowering in the plants treated with an Ethephon concentration of 620 mg L⁻¹ and at harvest time with 583 mg L⁻¹ of Ethephon (Figure 4B).

Regarding the levels of carbohydrates caused by fruit abscission promoted by Ethephon, it was found that there was a linear increase in the percentage of thinning depending on the concentrations of Ethephon applied in the three years evaluated (Figure 5). In plants subjected to a concentration of 800 mg L⁻¹, 28.5, 7.0 and 39.9% thinning was observed in 2008/2009, 2009/2010 and 2010/2011, respectively.

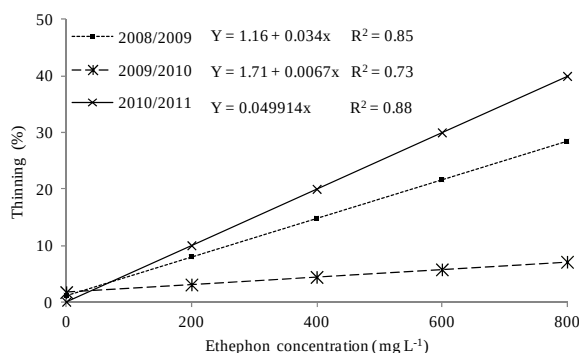


Figure 5. Average thinning (%) of 'Ponkan' mandarin fruits depending on the applied Ethephon concentrations during the three years of evaluation.

The differences between thinning among years can be attributed to the amount of fruit produced by the plants because the action of Ethephon on the thinning intensity is proportional to the amount of fruit on the plant.

Chemical thinning was carried out via application of Ethephon, which increases the level of ethylene released by promoting the abscission of reproductive organs (IGLESIAS et al., 2006) as well as vegetative leaves due to increased cellulase activity in the abscission zone in these organs (GUAN et al.,

1995). This phenomenon was observed with greater intensity in plants subjected to an Ethephon concentration of 800 mg L⁻¹.

Another pattern observed in the present study was that the leaf concentrations of total carbohydrates were increased at the time of harvest compared to flowering (Figure 6). This finding can be explained by fact that the onset of flowering involves high consumption of carbohydrates during the formation of vegetative and flowering shoots, resulting in a reduction in the leaf starch content. After natural abscission, the sink competition for carbohydrates is lower, which can increase the levels of starch in the leaves at harvest time for transportation from the roots (MEHOUACHI et al., 2009) and / or to support increase photosynthetic rates due to the early summer period (PEREIRA et al., 2011).

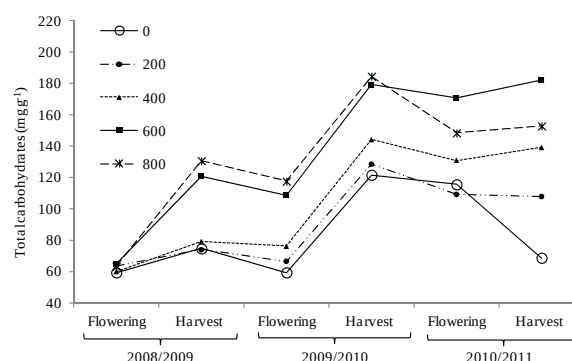


Figure 6. Average total carbohydrate contents in the leaves of 'Ponkan' mandarin trees subjected to different Ethephon concentrations (0, 200, 400, 600 and 800 mg L⁻¹) depending on the time of evaluation.

An influence of the tested concentrations of Ethephon on the levels of total carbohydrates in leaves was observed during the final evaluation, i.e., in the 2010/2011 harvest. The lowest accumulation of total carbohydrates was detected in mandarin trees not subjected to Ethephon treatment, and a pronounced reduction of 2010/2011 flowering was observed in these plants for the harvest this year (Figure 6).

The plants sprayed with a concentration of 800 mg L⁻¹ showed lower levels of total carbohydrates in the third harvest year (Figure 6) compared with the plants treated with 600 mg L⁻¹ Ethephon, probably as a result of the leaf abscission that occurs when applying high doses to promote thinning. In contrast, when lower concentrations (200 and 400 mg L⁻¹) are used, because there is competition for photoassimilates due to the low percentage of thinning (Figure 2), the maintenance of carbohydrates may not be sufficient to improve fruit quality.

The results regarding the levels of leaf carbohydrates showed the influence on the quality of

fruits, as a linear increase was detected in the first year (2008/2009) and a quadratic increase in the second (2009/2010) and third years (2010/2011) with respect to evaluation of the mass and diameter of fruits depending on the Ethephon concentrations applied (Figure 7A, B and C).

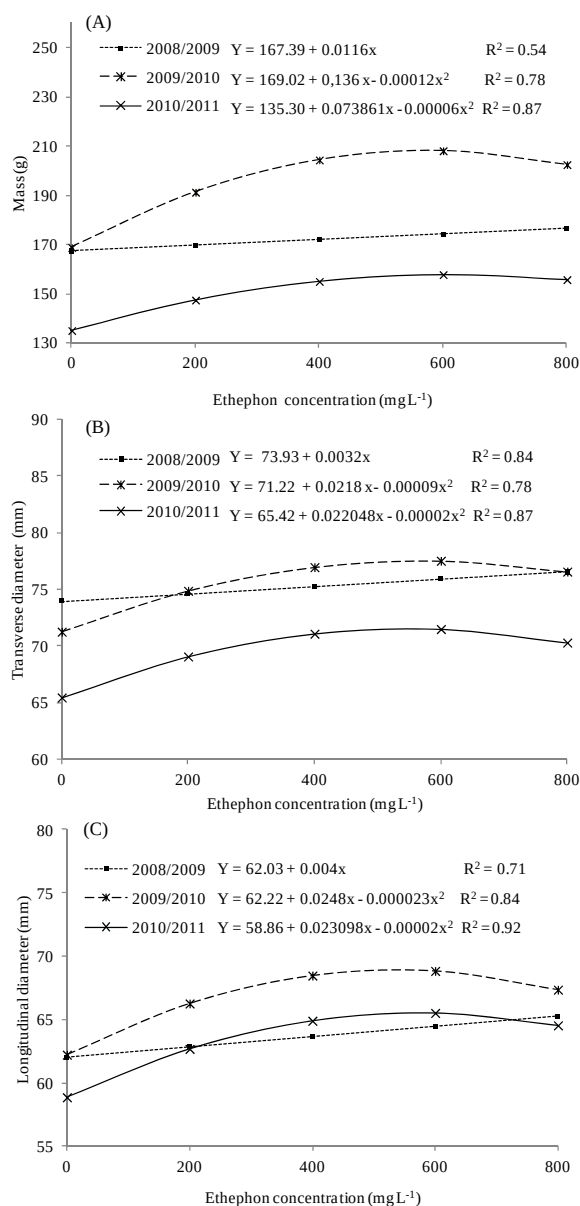


Figure 7. Average (A) mass (g), (B) transverse diameter (mm) and (C) longitudinal diameter (mm) of 'Ponkan' mandarin fruits depending on the applied Ethephon concentrations during the three years of evaluation.

In 2008/2009, it was found that an Ethephon concentration of 800 mg L⁻¹ promoted increases of 5.5% in the mass, 3.5% in the transverse diameter and 5.2% in the longitudinal diameter of fruits compared with those plants that received no Ethephon. In the second and third years of

evaluation, greater increases in the size of fruit were observed under an Ethephon concentration of 600 mg L⁻¹, with increases of 22.4 and 16.8% in mass, 17.6 and 9.3% in transverse diameter and 10.6 and 11.6% in longitudinal diameter being detected, respectively.

Obtaining a larger fruit size was due to the increase of the source/sink ratio as a result of the reduction in the number of fruits per plant and the maintenance of carbohydrates in the leaves, which favor the highest contents of photoassimilates in each fruit (GUARDIOLA; GARCÍA-LUIS, 2000).

Similar results regarding increased fruit size were obtained by Cruz et al. (2009, 2010 and 2011), Ramos et al. (2009), following the application of chemical thinning with Ethephon for one year in 'Ponkan' mandarin trees. However, the continued practice of thinning, according to the results obtained from three years of evaluation, show that further improvement was achieved compared to the results obtained in the first year of the implementation of thinning. This result suggests that chemical thinning should be a regular practice in orchards of 'Ponkan' mandarin whose production is intended for the fresh fruit market and have the characteristic of producing irregularly sized fruits as consequence of the excessive amounts produced per plant (MOREIRA et al., 2012).

In terms of the juice yield, there was no difference associated with the different Ethephon concentrations applied in the three study years, averaging 39% of juice yield. This result may have been obtained because larger fruits produce greater amounts of juice, thus not affecting the yield of juice (juice volume / mass).

A linear increase was observed in the soluble solid contents as a function of the applied Ethephon concentrations in the first year, and a quadratic increase was detected in the second and third years (Figure 8A). In 2008/2009, 11.7 °Bx was observed in the juice of the fruits of plants sprayed with a concentration of 800 mg L⁻¹, which amounted to an increase of 3.5% compared with the fruits of control treatment.

There were increases of 1.0% and 7.4% in the soluble solid contents detected under estimated Ethephon concentrations of 373 mg L⁻¹ and 546 mg L⁻¹ for the second and third years, respectively. This smaller increase in the second year can be attributed to the higher values of soluble solids obtained, even in plants in the control treatment (14 °Bx). This difference in soluble solids between years may be related to the precipitation that occurred during the maturation of fruits (Figure 1), as in June 2009 and June 2011, the total precipitation levels were 24.5

and 35.2 mm, respectively, causing dilution of the soluble solids present in fruits, whereas that in June 2010 was only 3.5 mm, causing concentration.

Similar acidity values were observed in the first and third years, while a linear decrease was detected in the second year (Figure 8B). In this year, for the plants subjected to application of 800 mg L⁻¹, 22.1% fruit exhibited less acidity than those of the control plants. This result can be attributed to the decrease in the content of organic acids depending on the use of these acids as a substrate in cellular respiration or for transformation into sugars.

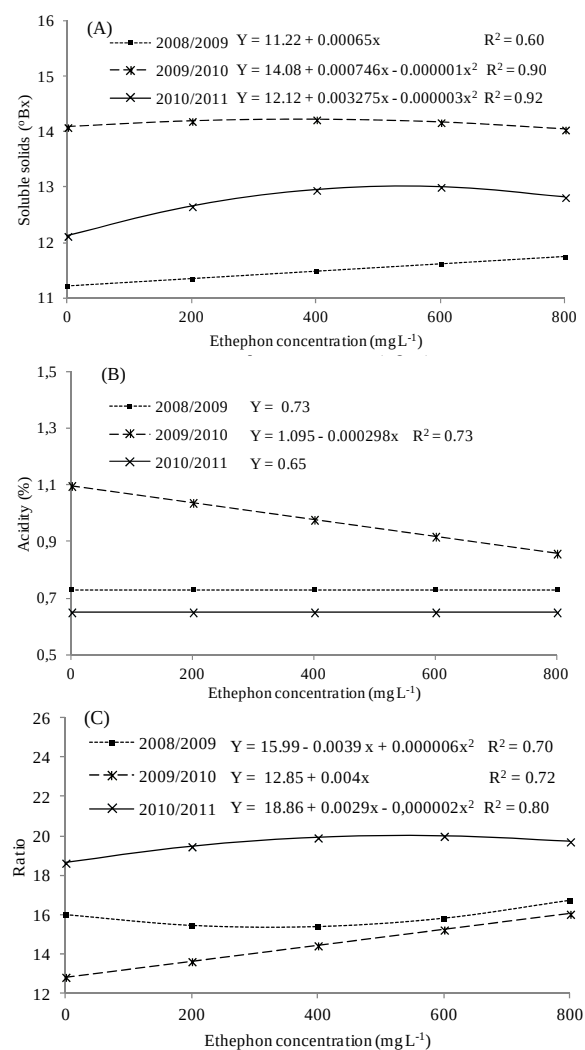


Figure 8. Average (A) soluble solids (°Bx), (B) acidity (mm) and (C) ratio of 'Ponkan' mandarin fruits depending on the applied Ethephon concentrations during the three years of evaluation.

The fruit ratio was observed to increase as a function of the applied Ethephon concentrations in the three years evaluated. Increases of 4.5, 24.9 and 5.5% were observed in plants treated with a concentration of 800 mg L⁻¹ compared with no application in 2008/2009,

2009/2010 and 2010/2011, respectively. This result can be attributed to the increase in soluble solids in the three years of chemical thinning and the reduction of acidity in the second year, as the ratio is calculated from these two variables.

Conclusion

Maintenance of high levels of leaf carbohydrates resulted in improvement of the quality of 'Ponkan' mandarin fruits.

Chemical thinning conducted with Ethephon concentration of 600 mg L⁻¹ maintained carbohydrate levels suitable to improve the quality of 'Ponkan' mandarin fruits.

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