Accumulation of nutrients during the development of 'Méndez' avocado fruit
Acumulación de nutrimentos durante el desarrollo del fruto de aguacate 'Méndez'

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SUMMARY

Balanced and timely nutrition is important to ensure the yield and quality of avocado (*Persea americana* Mill.) fruit. This research was carried out in the cv. Méndez with the objectives of a) to describe the dynamics of fruit growth and the process of dry matter accumulation in mesocarp, b) to quantify the concentration and accumulation of nutrients in the mesocarp (pulp) during fruit development. In a commercial orchard with site-specific fertilization, 20 trees were selected and 30 summer flowering inflorescences per production cycle (2014-15, 2015-16 and 2016-17) were marked in each one. Five fruits per tree were randomly collected in five stages of development, from olive size to harvest, and the dry matter of the mesocarp and the concentrations of N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn and B in this tissue were quantified. The fruit required 300 days from anthesis to harvest. It showed accelerated growth in the first three stages of development (S-Olive, S-I and S-II) and lower growth in the last stage (S-III). Dry matter accumulation was constant during fruit development, although the largest accumulation occurred in S-III. In general, the concentration of nutrients in the mesocarp decreased with fruit development and its accumulation increased. The greatest fruit growth occurred in the first 185 post-anthesis days, when the mesocarp accumulated less than one-third of the total nutrients required until harvest. The results indicate the need to review how nutrients are supplied to the tree, to make sure they are adequately provided during fruit development.

Index words: *fruit development, mineral nutrition, phenology,* Persea americana *Mill*.

RESUMEN

Una nutrición equilibrada y oportuna es importante para garantizar el rendimiento y la calidad del fruto del aguacate (*Persea americana* Mill.). Esta investigación se realizó en el cv. Méndez con los objetivos de a) describir la dinámica del crecimiento del fruto y el proceso de acumulación de materia seca en el mesocarpio (pulpa), b) cuantificar la concentración y acumulación de nutrientes en el mesocarpio durante el desarrollo del fruto. En un huerto comercial con manejo de fertilización de sitio específico, Fueron seleccionados 20 árboles y en cada uno se marcaron 30 inflorescencias de los ciclos productivos 2014-15, 2015-16 y 2016-17. Se recolectaron al azar cinco frutos por árbol en cinco etapas de desarrollo, desde el tamaño aceituna hasta cosecha, se cuantificaron la materia seca del mesocarpio y las concentraciones de N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn y B. El fruto requirió 300 días desde antesis hasta cosecha. Presentó un crecimiento acelerado en las primeras tres etapas de desarrollo (S-Aceituna, S-I y S-II) y un menor crecimiento en la última etapa (S-III). La acumulación de materia seca fue constante durante el desarrollo del fruto, aunque la mayor acumulación ocurrió en S-III. En general, la concentración de nutrientes en el mesocarpio disminuyó con el desarrollo del fruto y aumentó su acumulación. El mayor crecimiento del fruto ocurrió en los primeros 185 días posteriores a antesis, cuando el mesocarpio acumuló menos de un

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tercio de los nutrientes totales necesarios hasta cosecha. Los resultados indican la necesidad de revisar cómo se suministran los nutrientes al árbol, para asegurarse que se proporcionen adecuadamente durante el desarrollo del fruto.

Palabras clave: *desarrollo del fruto, nutrición mineral, fenología,* Persea americana *Mill.*

INTRODUCTION

The environmental conditions in which avocados are grown in Mexico influence their phenology and physiology (Salazar-García *et al*., 2016a and b; Álvarez-Bravo *et al.*, 2017; Salazar-García *et al*., 2018). Site-specific fertilization is an approach that seeks to provide the necessary nutritional requirements for the production goal that the environment allows (Salazar-García, 2002; Montgomery-Taboada *et al*., 2017). Studies on vegetables and ornamentals have shown the importance of knowing the dynamics of nutrient extraction to fractionate nutrient supply according to demand (nutrient accumulation curve) and crop development (Sosa *et al.,* 2012; Honorato *et al.,* 1993; Castellanos, 1987[1]; Ortega-Blu *et al.,* 2006; Granjeiro and Filho, 2005). The dynamics of nutrient accumulation by the fruit have been documented in perennial crops, such as apple (Casero *et al*., 2017), coffee (Sadeghian *et al.,* 2012), guava (Araujo *et al*., 1997) and papaya (Fallas *et al*., 2014). These studies show a constant incorporation of nutrients during fruit development, although there are differences between nutrients; also, it has been determined that the stages of fruit development prior to harvest are those of greatest demand for some nutrients.

Both the avocado cultivar and the management practices and conditions where it is cultivated influence the concentration of nutrients in the fruit. This has made it possible to quantify the removal of nutrients by the crop of ‘Hass’ and ‘Méndez’ avocados in different environments (Montgomery-Taboada *et al*., 2017; Salazar-García and Ibarra-Estrada, 2017; Salazar-García *et al*., 2009; Mellado-Vázquez *et al*., 2015; Rosecrance *et al*., 2012; Hofshi and Hofshi, 2003[2]) and indicates that a fertilization program should be designed for each case.

In addition to the diagnosis of foliar nutrient status and the amount of nutrients removed by the crop, precise fertilization management requires considering the assimilation dynamics of the different nutrients during fruit development, in order to schedule fertilization according to the stages of greatest nutrient demand and thereby ensure the quality of the fruit, both at harvest and postharvest (Arpaia, 1994; Salazar-García and Lazcano-Ferrat, 2001; Fernández-Montoya *et al*., 2017). Since the mesocarp is the edible part of the avocado fruit, its quality at consumption maturity usually identifies whether adequate orchard nutrition management was carried out. As the mesocarp reflects the response to fertilization more quickly than the leaves (Salazar-García *et al*., 2008 and 2014), opportune modifications can be made to the fertilization program by monitoring its nutrient status (Salazar-García *et al*., 2014).

Salazar-García *et al*. (2016c) developed an Internet software for the nutritional diagnosis of the mesocarp in five key stages of the development of 'Méndez' avocado fruit in Jalisco, Mexico. However, more information is needed on the physiology of fruit nutrition. The objectives of this research on cv. Méndez were a) to describe the dynamics of fruit growth and the process of dry matter accumulation in mesocarp, b) to quantify the concentration and accumulation of nutrients in the mesocarp during fruit development.

MATERIALS AND METHODS

The research was carried out with fruit set by the summer (August-September) flowering. It started in 2014 and included three production cycles (2014-15, 2015-16 and 2016-17).

Orchard Characteristics

The study was conducted in the “Colorín-1” orchard, owned by the Agro González Company, which covers 3 ha and is located in Atequizayan, municipality of Zapotlán el Grande, in the state of Jalisco (19° 42´ 57.1" N and 103° 31´ 11.9" W), at 1556 m. (CONABIO, 1998). According to Köppen (García-Amaro, 1998) the predominant type of climate is warm subhumid [(A)C(w0)] and [(A)C(w1)] of group C, with an average

[1] Castellanos, J. Z. 1987. Las curvas de acumulación nutrimental en los cultivos hortícolas y su importancia en los programas de fertilización. In: Memorias del 2do Simposio Internacional de Fertirrigación. FIRCO. Querétaro, México.

[2] Hofshi, R. and S. Hofshi. 2003. Total Fruit nutrient removal calculator for Hass Avocado in California. http://www.avocadosource.com/tools/NutRemCalc.htm Accessed November 2018.

annual temperature greater than 18 ºC, temperature of the coldest month less than 18 ºC, temperature of the warmest month greater than 22 ºC. Annual rainfall is 540 mm, in the driest month is less than 40 mm and the winter rainfall ranges from 27 to 55 mm.

The soil type is Haplic Phaeozem with coarse texture (INIFAP and CONABIO, 1995). The orchard was five years old at the beginning of the study, trees were at a 7 × 3.5 m spacing, and it has a micro-sprinkler irrigation system through which nutrients are supplied. The fertilization program varied among years (Table 1) as it was designed to fulfill nutrient orchard requirements for a production target, which may change year after year, as described by Salazar-García (2002) and Salazar-García *et al*. (2009). Fertilizers were applied through the micro-sprinklers with a flow rate of 60 L h^{-1} and were fractionated during the production cycle according to the phenology of cv. Méndez in the region (Salazar-García *et al.,* 2018). Within the orchard, a group of 20 trees was selected, with a height of no more than 6 m, without overcrowding and with a production history of at least 60 kg·$tree^{-1}$.

Table 1. Amount of nutrients applied (kg ha^{-1}) in the Colorín-1 orchard.

Nutrient	2014-15	2015-16	2016-17
N	96.7	249.8	147.4
P_2O_5	37.6	122.9	73.5
K_2O	120.3	250.2	162.9
CaO	5.1	11.8	50.6
Mg	17.7	27.8	24.3
Fe	1.21	1.24	1.76
Mn	1.11	1.24	1.01
Zn	0.92	2.16	2.45
B	3.24	5.15	2.13

Fruit and Mesocarp Sampling

Thirty inflorescences were marked in each of the 20 selected trees. At each sampling date, a variable amount of fruit was collected in each of five trees, according to their stage of development (Álvarez-Bravo and Salazar-García, 2017; Figure 1).

The collected fruit were taken to the laboratory where they were washed with distilled water plus sodium hypochlorite (200 mg L^{-1}) and weighed on a precision scale (Ohaus model P2001, Florham, NJ, USA). Fruit length and diameter were obtained with a digital Vernier caliper (MTC500-196, Mitutoyo Corp., Sakado, Japan). Subsequently, the mesocarp was extracted and cut into thin slices to be dehydrated in a forced-air digital oven (Lab-line Imperial 5, 3488M USA) at 70 °C to constant weight. The dry matter was determined by the difference in weight between the fresh and dry samples and expressed as a percentage. The dry mesocarp was pulverized in a stainless steel micro-mill (IKA MF10 basic) with a No. 40 sieve (1 mm mesh size). Separate samples were prepared for each tree (five per sampling date). Each sample was made up of the mesocarp of the fruit obtained from each tree. The concentration of N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn and B was determined in Fertilab laboratory, which is accredited by the North American Proficiency Testing (NAPT) Program operated by the Soil Science Society of America.

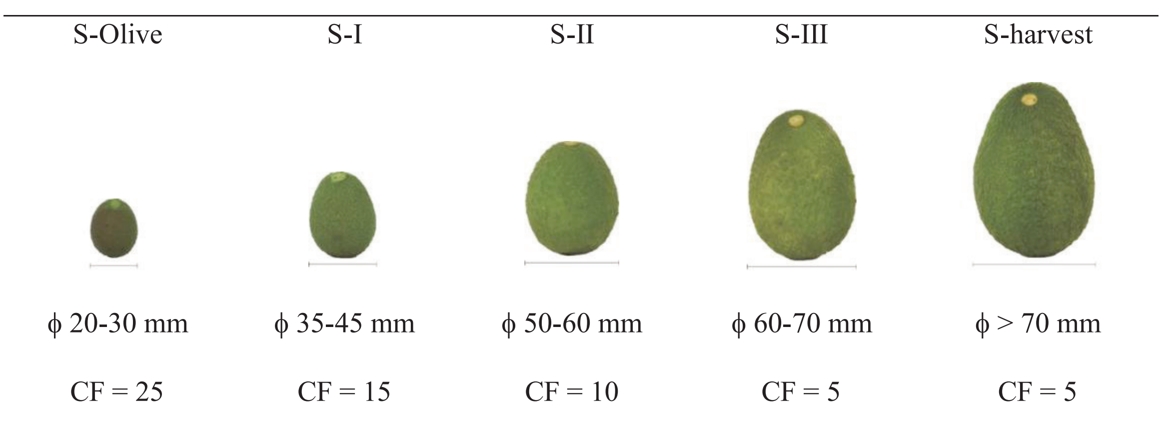

Figure 1. Amount of collected fruit (CF) per tree at each stage (S) of fruit development (mm diameter).

Data Processing

Data on fruit diameter and dry matter were organized in MS Excel 2013. For each stage of fruit development, the data of the three cycles were averaged; subsequently, a graph was prepared for the dynamics of fruit growth and dry matter accumulation. To calculate the concentration of nutrients in the mesocarp, the concentration was averaged for each stage of development (of the three productive cycles). Additionally, with the three-year data set, an analysis of variance and Tukey's multiple comparison test ($P \leq 0.05$) were performed, as well as the linear regression calculation to obtain the polynomial equation and the coefficient of determination for each nutrient. Statistical analysis was done with Minitab 17 statistical software. To calculate the accumulation of nutrients in the mesocarp, the concentration of each nutrient per analyzed sample was converted to grams per 100 grams of dry mesocarp, obtaining an average per nutrient per stage of fruit development. The values per stage were added to obtain 100% accumulation and then the proportion of each nutrient accumulated per stage was calculated. This information was plotted with SigmaPlot 10 software.

RESULTS AND DISCUSSION

Fruit Growth

The 'Méndez' avocado fruit showed growth of the simple sigmoid type. This coincides with the description made by Bower and Cutting (1988) for the cv. Hass. The time required from anthesis to fruit harvest was 303 calendar days (10 months). Salazar-García *et al*. (2018) reported a similar duration for summer flowering 'Méndez' fruit in the same region of the present study. In the case of the 'Hass' fruit of the main flowering (winter), in similar climatic and elevational conditions in Michoacán, this process was 1 or 2 months shorter (Rocha-Arroyo *et al*., 2011), which coincides with the 8 months that 'Hass' takes in the warm climate of the state of Nayarit (Cossio-Vargas *et al*., 2008). Another study in Colombia (Bernal-Estrada *et al*., 2017) showed that 'Hass' required 8 to 9 months from anthesis to harvest in a low-elevation orchard (1340 m). In contrast, in temperate climates 'Hass' fruit require up to 12 months to complete their development (Reyes-Alemán *et al*., 2017).

It is recognized that environmental conditions modulate the phenology of cv. Hass; however, the fact that 'Méndez' is a spontaneous mutation of 'Hass' results in important phenological differences (Illsley-Granich *et al*., 2011), although the time required for the main flowering fruit (summer) to reach maturity is similar to 'Hass'.

In the present study, the 'Méndez' fruit showed accelerated growth from fruit set until 150 days after anthesis and a final stage (from 220 days after anthesis and until harvest) of fruit development, slightly slowing its growth between 150 and 220 days after anthesis. About 70.8% of initial fruit growth occurred 185 days after anthesis (until the beginning of S-III), which represents 60.4% of the time required to reach S-harvest. Like 'Méndez', 'Hass' has its highest fruit growth rate in the first months after anthesis (Bower and Cutting, 1988; Cossio-Vargas *et al*., 2008; Rocha-Arroyo *et al*., 2011; Bernal-Estrada *et al*., 2017; Reyes-Alemán *et al*., 2017). The stage with the least 'Méndez' fruit growth occurred between S-III and S-harvest with less than 10 mm in diameter (12.8% of total fruit growth) and 39.6% of the time required from anthesis to S-harvest, which corresponds to 120 days (Figure 2A).

Accumulation of Dry Matter in the Mesocarp

The official Mexican standard norm (NMX-FF-016-SCFI-2006) establishes 21.5% as the minimum dry matter content in the mesocarp to consider that the 'Hass' fruit has reached physiological maturity. However, for safety purposes, it is commercially harvested with higher dry matter content. In the present study the 'Mendez' fruit were harvested with an average of 23.3% dry matter, which is slightly higher than the optimum harvest maturity suggested for 'Méndez' (22.7%) (Herrera-González *et al*., 2017). On the other hand, in Colombia, Carvalho *et al*. (2015) mention that 'Hass' should be harvested with a minimum content of 24% dry matter for good postharvest behavior.

In this research, the accumulation of dry matter in the 'Méndez' mesocarp was constant during its development (Figure 2A). This behavior is typical for avocados produced in tropical environments and is different from that presented by 'Hass' in the dry Mediterranean climate of California (Rosecrance *et al*., 2012), where it presented double sigmoid growth (the accumulation of dry matter slowed during the winter).

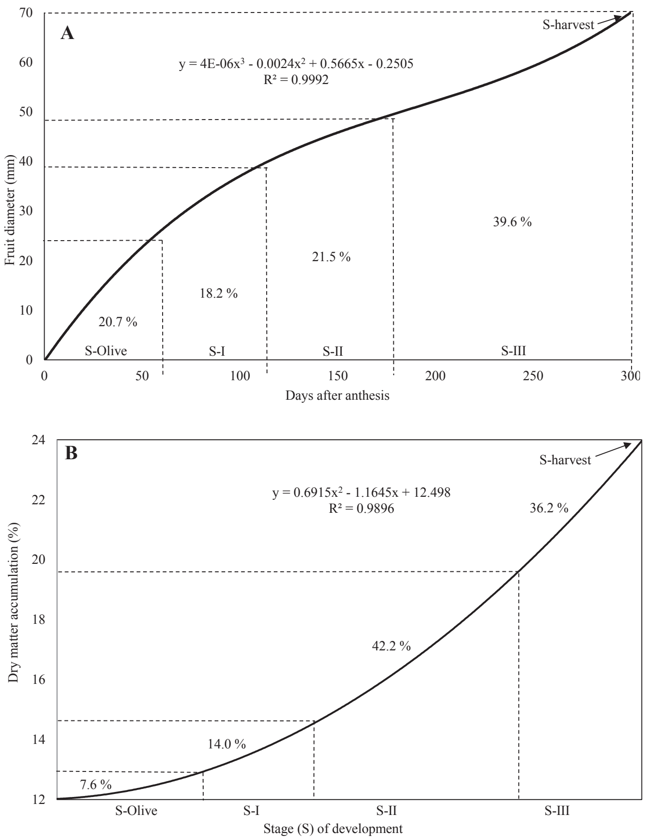

Figure 2. Increase in diameter (A) and mesocarp dry matter accumulation (B) of ‘Méndez’ avocado fruit.

In the cv. Méndez, the stages with the lowest dry matter accumulation rate were S-olive and S-I with 7.6 and 14.0%, respectively. S-II elapsed in 90 days and the highest accumulation of dry matter in the mesocarp (42.2%) was observed. The remaining 36.5% accumulated in 30 days, corresponding to S-III (Figure 2B).

Concentration and Accumulation of Nutrients in Mesocarp

The concentration of most nutrients (N, P, Ca, Mg, Fe, Cu, Mn and Zn) in the mesocarp decreased with fruit development, with an average rate of change between stages of -10 to -30%. In the case of K and S, the last two stages of fruit development showed the highest concentrations, while in B they were S-II and S-III. The ‘Méndez’ fruit in S-harvest showed higher concentrations of N, K, Zn and B than ‘Hass’ fruit while there was similarity in the concentrations of P, Ca, Mg and S (Salazar-García *et al*., 2011; Tamayo *et al*., 2018). The equation that best explained the temporal behavior of the mesocarp concentration for N, P, K, Ca, S and B was the third-degree one (R^2 = 0.99), except S which showed the lowest indicator (R^2 = 0.71). The rest of the nutrients (Mg, Fe, Cu, Mn and Zn) had a second-order trajectory with an average R^2 = 0.94 (Table 2).

Table 2. Polynomic equations of concentration and accumulation of nutrient elements in the mesocarp of ‘Méndez’ avocado fruit.

Nutrient	Equation for concentration	R^2	Equation for accumulation	R^2
N	$y = 0.0093x^3 - 0.0789x^2 - 0.0128x + 2.123$	0.9980	$y = 6.4047x^2 - 14.777x + 12.758$	0.9983
P	$y = 0.0024x^3 - 0.0135x^2 - 0.0093x + 0.3028$	0.9948	$y = 1.3983x^3 - 5.2595x^2 + 12.324x - 5.2471$	0.9988
K	$y = -0.0352x^3 + 0.2814x^2 - 0.5541x + 1.7935$	0.9943	$y = 0.8162x^3 + 0.0483x^2 - 1.1758x + 2.4493$	0.9996
Ca	$y = 0.0137x^3 - 0.1162x^2 + 0.2222x + 0.1714$	0.9964	$y = 3.9644x^2 - 1.045x + 4.8215$	0.9958
Mg	$y = 0.0022x^2 - 0.0288x + 0.211$	0.9693	$y = 6.6535x^2 - 16.328x + 14$	0.9973
S	$y = 0.0005x^3 - 0.0014x^2 - 0.0007x + 0.1505$	0.7110	$y = 1.5851x^3 - 6.3701x^2 + 13.499x - 6.8338$	0.9978
Fe	$y = 0.7429x^2 - 7.5211x + 45.824$	0.8797	$y = 1.3226x^3 - 5.0603x^2 + 13.456x - 6.4727$	0.9984
Cu	$y = 0.4326x^2 - 3.9092x + 20.161$	0.9090	$y = 1.385x^3 - 5.5701x^2 + 14.589x - 7.195$	0.9983
Mn	$y = 1.0879x^2 - 8.8523x + 21.904$	0.9984	$y = 6.0008x^2 - 14.104x + 18.179$	0.9910
Zn	$y = 0.6051x^2 - 4.8893x + 29.833$	0.9543	$y = 1.24x^3 - 4.0112x^2 + 9.7762x - 3.9577$	0.9986
B	$y = -4.7892x^3 + 40.09x^2 - 88.081x + 105.12$	0.9666	$y = 7.6976x^2 - 21.964x + 16.823$	0.9995

Contrary to the concentration of nutrients, their accumulation in the mesocarp increased with fruit development (Figures 3 and 4). In S-olive, nutrient accumulation was low (< 9.4% on average for all nutrients); however, Ca and Mn stood out with 18.3 and 16.2% of the total accumulation. From S-I to S-harvest, an average of 91.6% of each nutrient was accumulated, with B being the most accumulated in this period (95.1%), along with Ca (81.7%). S-III was the most active in nutrient accumulation, with an average of 49.1% of each nutrient, although S stood out for accumulating 55.1% of the total in this stage. Ca was one of the most active elements in the early stages of development (S-olive - S-I), showing an accumulation of 18.3%, in contrast to B which in this same period only accumulated 4.9%. What was observed for Ca coincides with the findings reported for ‘Golden Smoothee’ apple, where Ca had a rapid accumulation in the early stages of fruit development (Xucla *et al*., 1999; Casero *et al*., 2017). The period from S-II to S-harvest is highlighted as on average > 73% of each nutrient was accumulated.

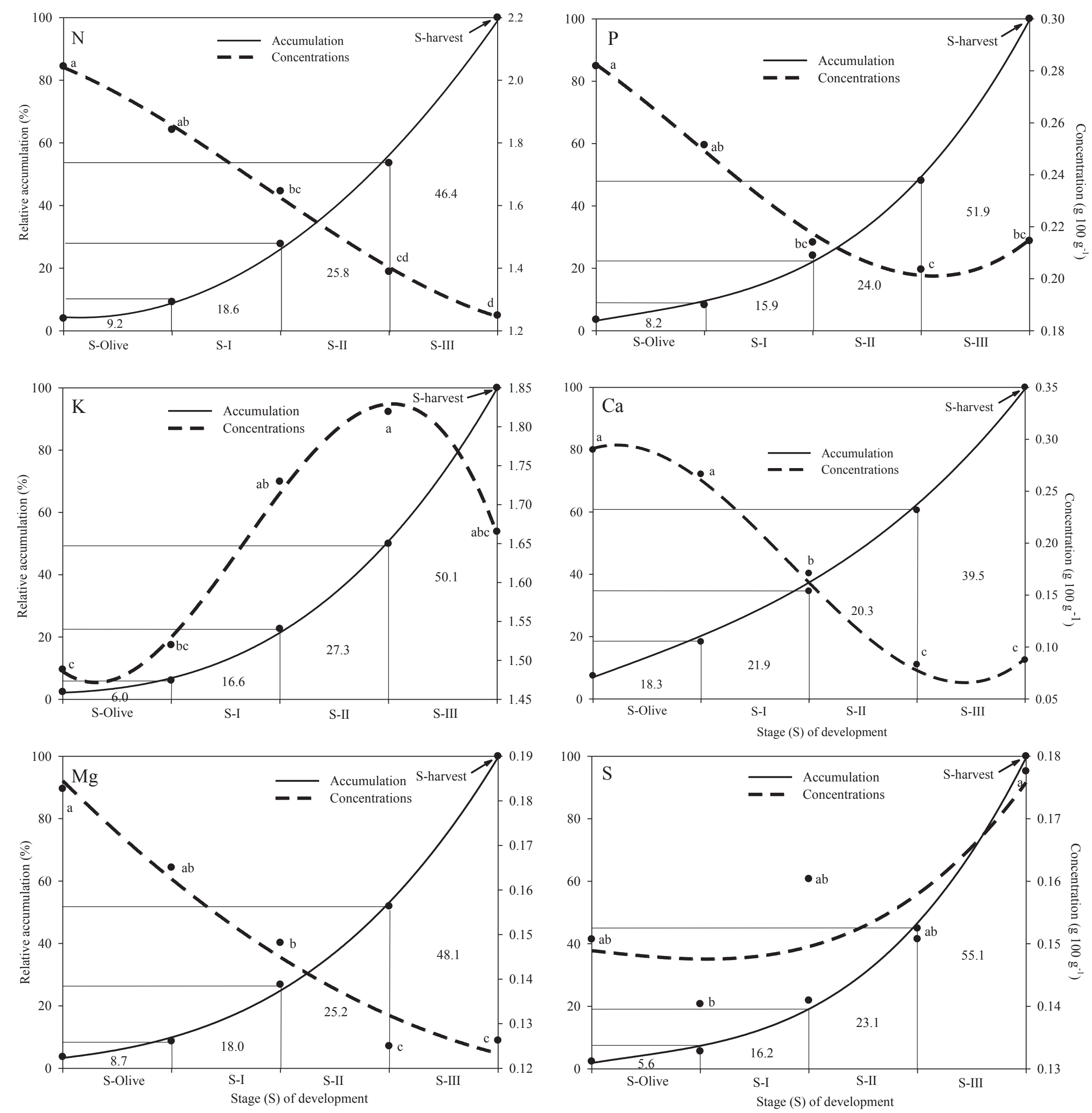

Figure 3. Concentration and relative accumulation of macronutrients in the mesocarp of ‘Méndez’ avocado fruit. Each data point is the average of three production cycles. Means with the same letter are not significantly different (Tukey, $P \leq 0.05$).

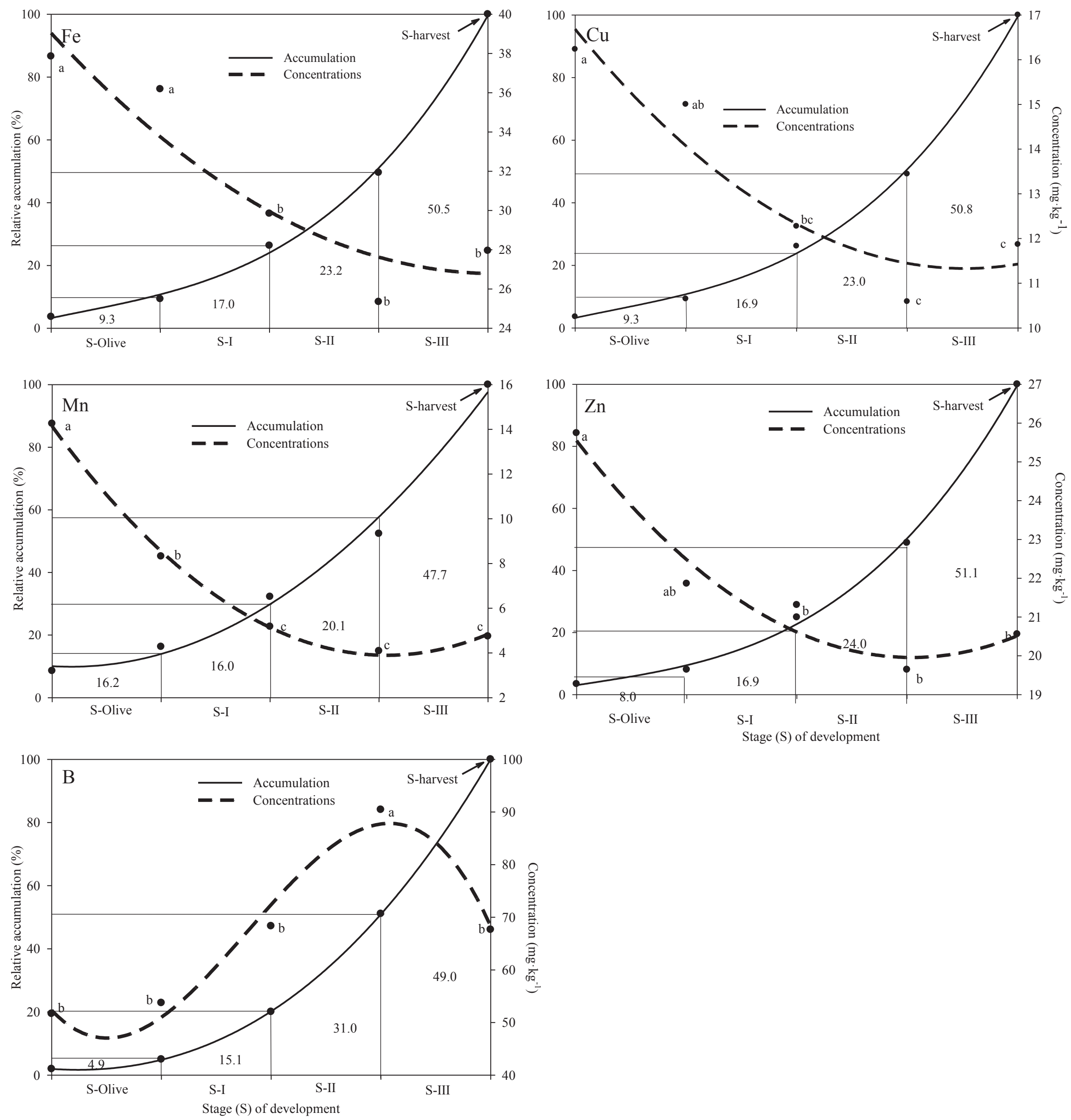

Figure 4. Concentration and relative accumulation of micronutrients in the mesocarp of 'Méndez' avocado fruit. Each data point is the average of three production cycles. Means with the same letter are not significantly different (Tukey, $P \leq 0.05$).

The type of equation generated for the accumulation of N, Ca, Mg, Mn and B was second-order, while for P, K, S, Fe, Cu and Zn it was third order. The average R^2 value for nutrient accumulation was 0.99 (Table 2).

In the present study, the dynamics of nutrient accumulation in the 'Méndez' mesocarp was similar to that described for 'Hass' in California, where an accumulation of 50% of nutrients at 64% fruit development is reported (Rosecrance *et al*., 2012). In the case of 'Méndez', the fruit accumulated on average 50.9% at 60% fruit development, with the exception of K and B as 'Méndez' accumulated 20% more than 'Hass' in the same period.

Mesocarp concentrations of both N and Ca are of relevance for postharvest fruit quality. After four years of differential nitrogen treatments in a 'Hass' avocado orchard in California, a positive relation was found between leaf N to pulp N concentrations (Arpaia *et al.*, 1996). Fruit from trees high in N showed and increased susceptibility to chilling injury following six weeks at 5 °C storage as well as a shorter time to ripen. Similar results were found by Kruger *et al.* (2016) where excess N concentrations in mesocarp of ‘Hass’ avocado fruit, that were stored for one month at 1 °C before being ripened at room temperature, had faster ripening. Excess N in mesocarp also has been associated to incidence of black cold damage and grey mesocarp of avocado during postharvest (Magwaza *et al*., 2008).

It is accepted that Ca physiology is involved in the manifestation of avocado fruit physiological disorders, such as rapid softening after ripening (Wills and Tirmazi, 1982) and high Ca levels have decreased cold-induced disorders (Chaplin and Scott, 1980), then the factors affecting the preharvest uptake, movement, and deposition of Ca are important (Bower, 1988). According to Bower (1985), the maximum avocado fruit Ca concentrations takes place in the first three months after fruit set. In the present study this timeframe occurs before the beginning of S-III; however, a sharp Ca accumulation in the mesocarp starts at this stage of fruit development making 60% of total mesocarp Ca accumulation at harvest. Our experience with soil applications of Ca to ‘Hass’ avocado during S-III has shown an increase in mesocarp Ca concentrations which prevented Ca deficiency at harvest.

CONCLUSIONS

This research documents for the first time the dynamics of nutrient accumulation in the mesocarp during the development of ‘Méndez’ avocado fruit and sets the opportunity for similar work with other avocado cultivars. It also establishes the difference in the interpretation of results, whether expressed as a concentration or accumulation of nutrients. The greatest fruit growth occurs in the first 185 days post-anthesis and is when the mesocarp accumulates less than one-third of the total nutrients required until harvest. This raises the need to review how nutrients are applied to the tree, in order to ensure their supply in the final stages of fruit development.

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