BREAKING THE GLASS CEILING OF 'HASS' AVOCADO YIELDS: PROVIDING THE PERIODIC DEMAND FOR NUTRIENTS

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ABSTRACT
The main objective of this study was to assess the seasonal nutrients requirement of 'Hass' avocado trees grown in lysimeters, especially during flowering and the early period of fruit development that may affect later on the fruitlet abscission and determine crop yield. The experimental design included three fertigation treatments applying a fixed nutrient solution at three different starting dates of fertigation: (a) T1 - continuous fertigation, including macro nutrients (N-P-K) and micronutrients application, all over the year; (b) T2 – no fertilization (only irrigation) until 15 March, and fertigation as T1 since then; (c) T3 - no fertilization (only irrigation) until 15 May, and fertigation as T1 since then. Absence of fertilization during the winter period induced leaf-chlorosis while healthy, dense and plenteously green leaves characterized the fertilized trees (T1). The beneficial effect of early fertigation on fruit yield was statistically significant, mostly because of higher fruit number. Leaf analyses are commonly used in the avocado industry as a guide for fertilization yet; fruits rather than leaves are the main products of avocado orchards. Consequently, fruit rather than leaf analyses should determine fertilization management. Based on fruit growth data and nutrient concentration in the fruit, the N, P and K quantities removed by 'Hass' avocado fruit yield of 30 tons ha^{-1} were 120, 25 and 240 kg ha^{-1}. Taking into account common efficiency consideration (nutrient quantities removed by fruit yield divided by quantities added), the annual quantities of N, P and K required for attaining high quality avocado yield are 250-300, 80-120 and 500-600 kg ha^{-1}, respectively. Thus, fertilization rate together with nutrient combination should be modified in order to insure optimal fruit development.

Keywords: fertigation scheduling; fruit analyses; Lysimeter; *Persea Americana*; Zn nutrition

INTRODUCTION
'Hass' avocado (*Persea Americana Mill.*) trees are vigorous and the potential photosynthetic capacity of mature trees can support commercial crop yields higher than 30 t ha^{-1} (Wolstenholme, 1986). However, alternate bearing and fruit abscission generally limits the long-term average crop yield to less than 10 t ha^{-1} (Garner and Lovatt, 2008). High 'Hass' avocado yield equivalent to approximately 40 to ha^{-1} was obtained in a lysimeter irrigation experiment few years ago (Silber et al., 2012). The experimental irrigation treatments in that study have practically not affected the vegetative growth, flowering or fruit-set processes, but have induced significant differences on fruitlet abscissions and accordingly, on fruit yield. Fruitlet abscission was the outcome of a multifaceted process starting few months before it occurred, rather than a sudden and abrupt event as one could imagine from the visible aspects of this phenomenon. The causes for fruitlet abscission were attributed to malfunction of embryo or seed induced by water and\or nutrient deficiency during the early period of fruit development (Silber et al., 2012).

The major and most important role of plant leaves is to convert light energy into chemical energy throughout the photosynthesis process by transforming carbon dioxide and water into carbohydrates, the driving forces for all plant-growing. Reproductive organs in avocado are strong sinks for carbohydrates (Whiley and Wolstenholme, 1990; Wolstenholme, 1986), and nutrients (Silber et al., 2013) from early stages of bloom throughout embryo seed formation and fruit ripening. In case of nutrient deficiency in the reproductive organs, nutrients are mobilized from the leaves towards the new developing reproductive organs in order to ensure satisfactory development. In cases of severe nutrient deficiency, the leaves become chlorotic and may abscise by a mechanism of programmed cell death. Thus, beyond their major role in carbohydrate production, leaves may also serve as an "operational reservoir" for nutrients to meet the peak demand in essential plant processes.

Healthy and dense foliage is certainly required to achieve high yields, and leaf nutritional status is commonly used for fertilization management decisions in the avocado industry (Lahav and Aycicegi-Lowengart, 2003). The leaf nutrients thresholds were developed empirically in various studies that demonstrated the relationships between leaf mineral concentration and fertilization (mainly N) regime (Koen and Plessis, 1992; Lovatt, 1995, 2001; Salvo and Lovatt, 2016). Yet, the nutrients fertilization thresholds may vary with many factors such as: (i) leaf age (Castillo-Gonzalez et al., 2000; Lahav, 1995; Lahav and Kadman, 1980, Lahav et al., 1990; Salazar-Garćıa et al., 2015); (ii) rootstock (Young and Koo, 1977); and (iii) physiological and phenological status (Salazar-Garćıa et al. 2015). Salazar-Garćıa et al (2015) proposed a mathematical model to determine the correct time for leaf sampling recently. So far, no relationships were found between yield and fruit size of 'Hass' avocado and Leaf-N concentration (Lovatt, 2001; Lovatt and Witney, 2001). Lahav (1995) concluded that the nutrient demand of avocado trees is low and crop yield did not respond to N-P-K fertilization. It is important to note that all the above studies were based on field experiments focusing mainly on N fertilization, without P addition to the fertilization system (except in Koen and Plessis, 1992). Furthermore, solid fertilizers rather than fertigation (simultaneous addition of water and soluble fertilizers through the irrigation system) were applied, which introduces variations in nutrient availability throughout the season. An alternative approach of monitoring fruit peduncle (Razeto and Salgado, 2004) and fruit pulp was reported (Razeto and Palacios, 2007, Razeto and Castro, 2007) to represent nutrient status compared with leaf analysis. Recently, Campisi-Pinto et al (2017) reported that analyses of nutrient concentration in the cauliflower stage inflorescence may serve as a good predictor for yield. In summary, previous studies indicated that leaf analysis is certainly useful; however, it is insufficient for optimal management of avocado orchards. Avocado trees may survive 30-50 years and therefore, such long-term tree fertility imposes that the effective annual quantities of nutrients added should correspond to the annual quantities removed by the fruits. Otherwise, nutrient deficiency may be the limiting factor for achieving high yields. Lahav and Kadman (1980) and Lahav (1995) reported that 10 tons of 'Hass' avocado fruits removes 11, 2 and 20 kg of N, P and K, respectively, and that fertilization with 55 kg of $(NH4)_2SO_4$ and 33 kg of KCl are sufficient to compensate for the removed N and K, respectively. Higher values of N, P and K quantities removed by 'Hass' avocado fruit of, 22-26, 4-5 and 30-40 kg ha^{-1}, respectively (based on 10 t ha^{-1} yield) have been reported by Salazar-Garćıa and Lazcano-Ferrat (2001) and Rosecrance et al. (2012).

The current fertilization recommendation for avocado orchard in Israel include application of 50 kg ha^{-1} of N at the beginning of March and later on 200-250 kg ha^{-1} of N and K from April to October (Noy, 2006). Commonly, P is not applied during the growing season and only added in October-November in form of phosphoric acid (20-30 kg P $ha^{-1)}$ as a practical tool for cleaning the irrigation system and preventing emitter clogging.

Developing a proper seasonal fertigation protocol accounting for the actual demand for nutrients is therefore a major challenge. To address this challenge, we have investigated the response of 'Hass' avocado trees grown in lysimeters to different fertigation regimes. Although the root volume and water stress sensitivity of a lysimeter-grown tree differ from those of a field-grown tree, this setup provides a major advantage as it enables direct measurement of plant water and nutrients uptake at high temporal resolution during successive growth stages.
The main objective of this research was to assess the seasonal nutrients 'Hass' avocado, especially, during flowering and the early stage of fruit development which may affect later on the fruitlet abscission and determine crop yield.

MATERIALS AND METHODS

General information and site characteristics

The study was conducted between 2014 and 2016 at the Acre Experimental Station, located in the Western Galilee, in Israel (32°57′ N; 35°05′ E; 10 m ASL). The climate is Mediterranean, with mild, wet winters followed by dry hot summers. The rainfall season in the region is between October and May, and annual precipitation at Acre totaled 317, 687 and 280 mm in 2013-4, 2014-5 and 2015-6, respectively. The response of 'Hass' avocado trees grafted on 'Degania 117', a West–Indian avocado rootstock, to different fertigation treatments was studied. The trees were planted in 2006 and grown in 1000-L plastic containers. The containers were covered by a plastic sheet to prevent evaporation. A volume of 50-L of coarse tuff (volcanic material) was placed at the bottom of the container to insure proper drainage and the rest of the volume was filled with perlite of 2-mm grain size. Perlite was chosen as the growth medium because of its high drainage qualities, especially, low air–entry suction. The density and pot capacity of the perlite were 72 g l^{-1} and 0.55 l^3 l^{-3}, respectively. The drainage from the containers was collected through PVC pipes and conducted outside the experimental site. Trees spacing during 2014 and 2015 was 4x5 m (500 trees ha^{-1}) while in 2016 it was changed to 4x6 m (417 trees ha^{-1}). Additional information is detailed elsewhere (Silber et al, 2018).

Experimental design

The experimental design comprised three fertigation treatments with three dates of starting fertigation application at fixed water-nutrient concentration allocated to eight randomized trees. The treatments in 2014 and 2015 were: T_1 - continuous fertigation, including macro nutrient N-P-K and micronutrients application, all over the year; T_2 – no fertilization (only irrigation) until 15 March, and fertigation as T_1 since then; T_3 - no fertilization (only irrigation) until 15 May, and fertigation as T_1 since then; The different treatments started on November 2013, after harvesting the 2013 yield. In 2016, the treatments changed and aimed to examine the commercial assumption that application of P during the growing season is not necessary. As a result, the treatments were: (a) T_1 - continuous fertigation, including macro nutrient N-P-K and micronutrients application, all over the year; (b) T_2 – continuous fertigation, including macro nutrient N-K and micronutrients application until 15 March, and fertigation as T1 since then; (c) T_3 - continuous fertigation, including macro nutrient N-K and micronutrients application until 15 May, and fertigation as T1 since then.
The irrigation system consisted of a loop of 13 2.3 L h^{-1} pressure-compensated drippers (Netafim Inc., Israel) installed around the trunk in each lysimeter. A pulsed irrigation (10-20 min every 30 min) was applied using an irrigation controller. All the treatments received the same daily amount of water. The irrigation amount exceeded the tree evapotranspiration to allow a leaching fraction (ratio of drainage and irrigation amounts) above 0.4, so that the EC

of the draining solution could be kept below 1.5 dS m^{-1} to prevent salt stress. All the horticultural practices were performed uniformly according to the recommendations of the Israeli Extension Service.
The harvest of 2014 yield was on 20 August 2014. The early harvest was chosen for insuring almost similar growth conditions between 2014 and 2015 seasons and to minimize possible effects of fruit load on the floral induction following 2015 spring resulted from alternate bearing process. This early harvest was in accord with Ziv et al (2014) conclusions that picking fruits before November reduce alternate bearing effects. The harvest of 2015 and 2016 seasons was on 15 November 2015 and 7 November 2016, respectively, as fruit dry weight was above 20.5 %. All the fruits from all the trees were collected and the fruits were sorted by weight.

Fertigation solutions and plant analyzes
Samples of the fertigation solutions were collected weekly, the pH, electrical conductivity (EC), and major nutrient concentrations were measured. The pH and EC of the fertigation solution were 7.0 ± 0.3 and 1.0 ± 0.1 dS m^{-1}, respectively. The N, P and K concentrations in the irrigation solution were 40, 10 and 50 mg L^{-1}, respectively. The nutrient solutions were prepared from commercial fertilizers ($(NH_4)_2SO_4$, NH_4NO_3, KNO_3, KCl and H_3PO_4), and tap water containing (mg L^{-1}): 100-120 Ca, 30-40 Mg, 20-30 Na, and 60-70 Cl^-. Micronutrient concentrations (mg L^{-1}) applied was 0.3 Zn, 0.6 Mn, 1.0 Fe, 0.04 Cu, 0.4 B, and 0.03 Mo, all EDTA-based. Productive_organs (florescence, fruitlet and fruit) from five replicates of each treatment were sampled every two weeks, washed with distilled water, dried in a ventilated oven at 60°C, and stored pending chemical analysis. The sampled fruits were separated for seed and mesocarp (pulp) chemical analyses. The dry tissue was ground to pass a 20-mesh sieve, and 100-mg samples were wet ashed with H_2SO_4-H_2O_2 and analyzed for Na, K, organic-N, and P. Ashing in $HClO_4$-HNO_3 was used for Ca, Mg, and micronutrients analyses. Element concentrations were determined as follows: NH_4-N, NO_3-N, and P with an injector Lachat Autoanalyzer (Lachat Instruments, Milwaukee, WI, USA); K and Na with a flame photometer (M410, Sherwood Sci. Ltd, Cambridge, England); Ca, Mg, Fe, Zn and Mn with an Analyst 800 atomic absorption spectrophotometer (Perkin Elmer, Shelton, CT, USA).

Calculation and statistics
The single fruit growth rate was estimated based on data from Zilkah and Klein (1987). The N-P-K quantities in the productive organs were calculated based on the seasonal nutrient concentration in the fruitlet and later on in the mesocarp and the seed, assuming a proportion of 80% and 20% of mesocarp and seed, respectively in a single fruit.
Statistical analyses were carried out with JMP(®) 12 software. All data were analyzed for the effects of treatments by means of the general linear model procedure of SAS (SAS Institute, Cary, NC, USA). Differences among means were tested with the standard least squares mode of ANOVA, followed by Tukey HSD pair wise comparison of means. Differences with a probability larger than 95% were considered as significant.

RESULTS
Tree development and yield
Absence of fertilization during the winter period (T2 and T3 treatments) induced leaf-chlorosis while healthy, dense and green leaves characterized the trees fertilized all over the year (T1). The first sign of chlorosis appeared at the beginning of February and its severity significantly increased at the emergence of flower buds (mid-March) and bloom (mid-April). Leaf chlorosis was followed by massive defoliation in the following order: T3>T2>>T1. The

intensity of fruitlet abscission later (May-June) followed the same order. In order to fulfil the main objective of this research i.e., assessing nutrient needs during flowering and the early period of fruit development, the fruits were collected in 2014 on 20 August, earlier than the commercial harvest starting generally around November, to prevent undesirable effect of alternate bearing. In 2015 and 2016, fruit harvest was similar to the common harvest time. In accord with chlorosis and leaf\fruitlet abscission in 2014 and 2015, the beneficial effect of all year fertigation on fruit yield was statistically significant, mostly because of higher fruit number (Silber et al., 2018). The number of fruit harvested and the averaged fruit weight of T1 trees were above the common values in commercial orchards in the region, and consequently, fruit yield was very high. Additional information is detailed elsewhere (Silber et al, 2018).

Seasonal fluctuations of nutrient concentration in productive organs

Figure 1 illustrates the impact of the treatments on N, P and K concentrations in the productive organ: inflorescence (March-mid April, DOY=80-125); fruitlets (mid-April-mid-May, DOY=125-140); Fruit (mid-May to harvest) for 2014. Concentrations of N, P and K in the productive organs (buds, flowers and fruitlets) were significantly higher than in the leaves and were affected by the experimental treatments. Treatment effects on N, P and K concentrations in the productive organs was principally similar to that on leaves, i.e., higher concentration in the all year fertilized trees (T_1), and almost similar concentration as fertilization started later on in T_2 and T_3 trees (Fig. 1). As for leaves, the effect of treatment on nutrient concentration in the productive organs at 2015 was quite similar to that of 2014 and therefore not presented.

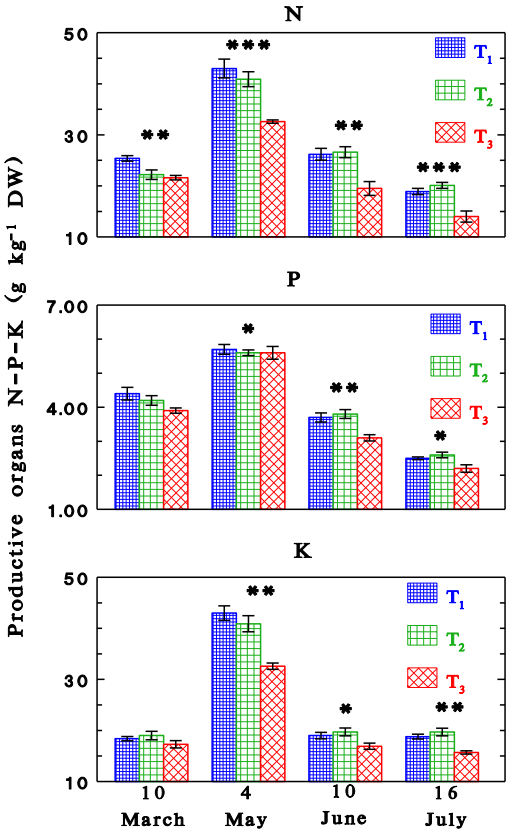

Figure 1. Experimental treatments effects on Fruitlet-N, -P and -K during 2014. Vertical lines designate standard error values. *, ** and *** designates Prob>F of: 0.005>, 0.001> and 0.0001, respectively.

Due to the continuous application of nutrients during the three consecutive years of the experimental period, the data of the T_1 trees were used to delineate the seasonal fluctuations of nutrient concentrations in the bud, flowers, and fruitlet and later on in the mesocarp and the seed (Fig. 2).

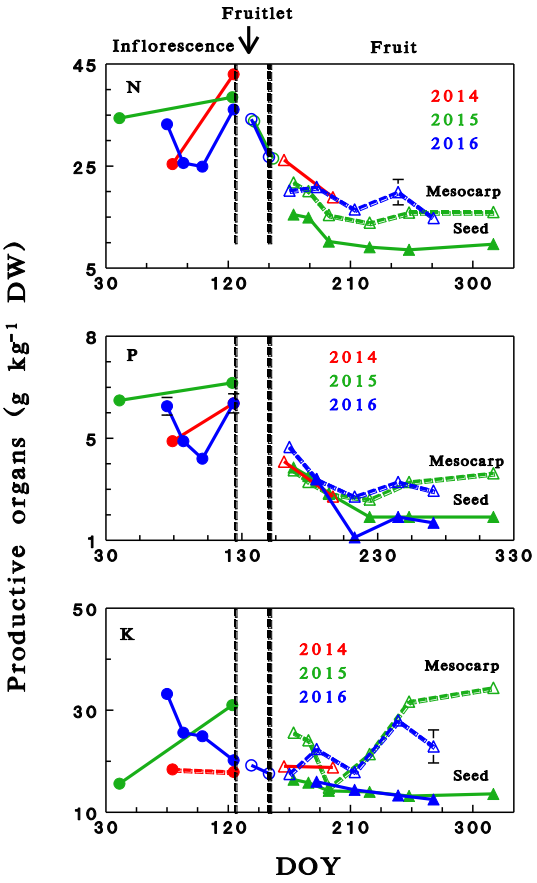

Figure 2. N, P and K concentration in the productive organs of T_1 trees during 2014, 2015 and 2016 experimental years (red, green and blue lines, respectively). Inflorescence: DOY 30 -125 (closed circles); fruitlet: DOY 125-135 (open circle); and fruit-mesocarp (open triangle) and -seed (closed triangle). DOY is day of the year.

The seasonal variation in nutrient concentration in the productive organs was considerable (Fig. 2). Concentration of N, P and K in the bud, the flower and the fruitlet increased from January, reached a peak at the first week of May (May 5th correspond to DOY 126), and decreased sharply after that. Note that the values of N, P and K concentration on May 5th were double, quadruple and triple respectively, compared with the leaf-concentration at the same date (Silber et al., 2018), which may indicate intense metabolic activity during this period. As this intense metabolic activity ceased, N-P-K mobilization toward the fruitlet slowed down while the fruit continued to grow and consequently, the concentrations decreased.
Concentration of fruit-N in the mesocarp was higher than in the seed (15±2 and 9±2 g kg^{-1} DW, respectively) while that of P and K concentration in the mesocarp increased from the beginning of August (1 August correspond to DOY 214). The increased values of K in the mesocarp could possibly relate to fatty oil production.
Concentrations of Ca and Mg in the productive organs decreased continuously from 4.5±0.5 and 3±0.5 g kg^{-1} DW, respectively in February (buds) to less than 1 and 2 g kg^{-1} DW, respectively, in the mesocarp and the seed at harvest (not presented). Similar pattern as Ca and Mg, Fe and Mn concentration in the productive organs decreased from 40-60 and 20-30 mg kg^{-1} DW, respectively, in February to 20-40 and 2-6 mg kg^{-1} DW, respectively, at harvest.

DISCUSSION

Matching the nutritional demand

Analogous to the irrigation scheduling dilemmas, the magnitude and the timing are the two principal growers' dilemmas regarding the fertilization of agricultural crops. Leaf analyses is commonly used in the avocado industry to address magnitude topic (Koen and Plessis, 1992; Lahav1995; Lahav and Kadman, 1980; Lovatt, 2001, 2013; Salazar-Garćıa et al., 2015; Salvo and Lovatt, 2016), yet, fruits rather than leaves are the main sink in avocado trees. Therefore, fruit analyses should be used as an effective tool for assessing the quantities and timing of nutrient addition through fertilization to avocado orchard. The operative objective of fertilization is to replace/restore the nutrient quantities that were removed by the crop. Based on Fruit-N –P and –K concentration presented in Fig. 2 and assuming fruit growth pattern similar to the one that was described by Zilkah and Klein (1987), N, P and K quantities in a single fruit were calculated (Fig. 3).
The estimated quantities of N, P and K removed by 10 tons per hectare of 'Hass' avocado in the present study were 36, 7 and 71 kg ha^{-1}, respectively. These quantities are significantly higher than the 22-26, 4-5 and 30-40 N, P and K quantities (respectively) reported by Salazar-Garćıa and Lazcano-Ferrat (2001) and Rosecrance et al. (2012). Accordingly, the 2016 crop yield removed 120, 25 and 240 kg ha^{-1} of N, P and K, respectively (Fig. 4). Linear curve characterized N accumulation in the fruits while concave curve characterized P and K accumulation. It is interesting to note that P and K followed the same trend, although the P values are one order of magnitude lower than the K values. Nutrient concentration in the developing productive organs during the winter and early spring was very high (Fig. 2) while soil temperature was far from being optimal for root activity and nutrient uptake (range of 15-18^{o}C, data not presented) and the transpiration rates were very low due short days, low sun angle and low VPD. Thus, the ability to supply the tree with the demand for nutrients was limited in that period. The second dissonance occurred during the late summer and the autumn (August-November) as high P and K quantities accumulated in fruit (1 August correspond to DOY 214, Fig. 4). This high accumulation resulted from the combination of fruit growth (Fig. 3) and increased concentration (Fig. 2) whereas water demand and accordingly irrigation doses decreased. Thus, irrigation- P and more important -K concentration should be modified in order to ensure optimal fruit development.

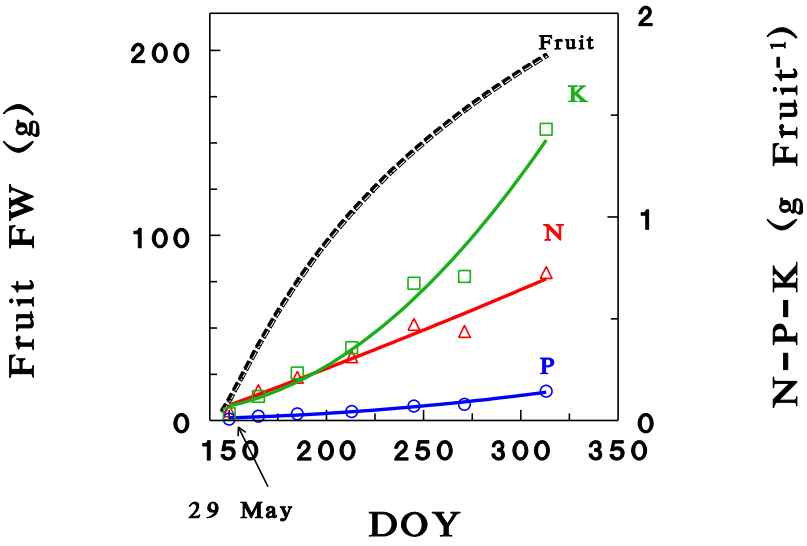

Figure 3. Rate of fruit growth and N, P and K quantities accumulated in a single fruit. Data of fruit growth were taken from Zilkah and Klein, (1987). DOY is day of the year.

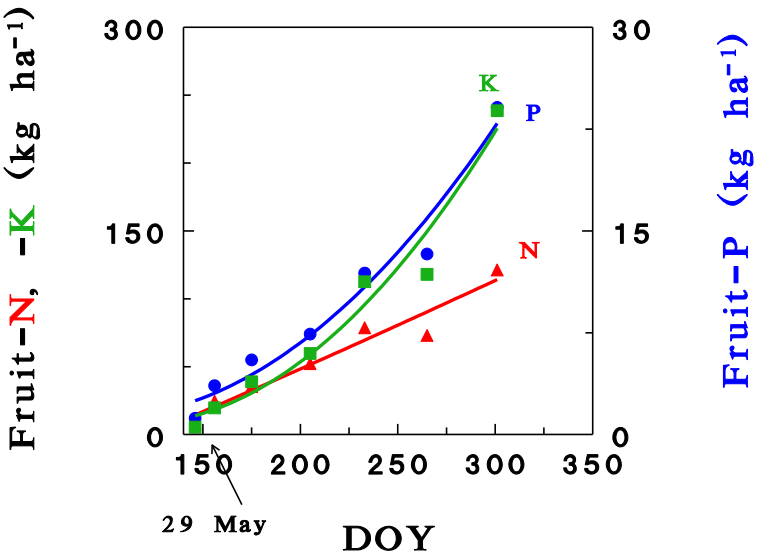

Figure 4. Fruit-N, -P and –K quantities removed by 'Hass' avocado fruit yield of 30 tons ha^{-1}. DOY is day of the year.

The efficiency value (nutrient quantity removed by plant divided by nutrient added through fertilization) are 40-50% for N and K, 20-30% for P (Haneklaus and Schnug 2016; Johnston, 2000), Taking into account these efficiency values, the annual quantities of N, P and K required for attaining 30-ton ha^{-1} of 'Hass' avocado fruit are 250-300, 80-120 and 500-600 kg ha^{-1}, respectively. The annual N quantity is quite similar to the common N fertilization practice in Israel while that of P and K are significantly higher than the common practice of 20-30 and 200-250 kg ha^{-1}, respectively. The common fertilization practices fail to provide the trees with all the amount of P, and K that is removed by the fruit and the timing of N-P-K application during the year introduces nutrients deficiency in certain periods throughout the year, especially in the winter period (November-March), prior to the critical period of flowering. The current study confirms earlier findings on the effect of nutrient deficiency during the period of florescence, fruit-set and early fruit development, that may induce leaf

and later on fruitlet abscission (Silber et al., 2012). In conclusion, the rate of nutrient application should be adjusted to meet the actual demand for nutrients at each phenological stage. Plant methods such as leaf and\or inflorescence analyses should be used for control and surveillance of the fertilization design.

CONCLUSIONS

1. Fruits rather than leaves are the main sink in avocado trees. Therefore, fruit analyses should be used as an effective tool for assessing the quantities and timing of nutrient addition through fertilization to avocado orchard.
2. Plant methods such as leaf and\or inflorescence analyses should be used for control and surveillance of the fertilization design.
3. Continuous fertigation with all the necessary nutrients, including phosphorus and micronutrients, throughout the year is required to ensure high yields.
4. Special effort should be made to determine periodic demand for water and nutrients during the inflorescence and fruit-set processes.

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