

Optimal Nutrient Concentration Ranges of ‘Hass’ Avocado Cauliflower Stage Inflorescences—*Potential Diagnostic Tool to Optimize Tree Nutrient Status and Increase Yield*

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Additional index words. fertilization, tissue nutrient analysis, nutrient ratio, *Persea americana*

Abstract. Optimizing ‘Hass’ avocado (*Persea americana* Mill.) tree nutrient status is essential for maximizing productivity. Leaf nutrient analysis is used to guide avocado fertilization to maintain tree nutrition. The goal of this research was to identify a ‘Hass’ avocado tissue with nutrient concentrations predictive of yields greater than 40 kg of fruit per tree. This threshold was specified to assist the California avocado industry to increase yields to $\approx 11,200$ kg·ha⁻¹. Nutrient concentrations of cauliflower stage inflorescences (CSI) collected in March proved better predictors of yield than inflorescences collected at full bloom (FBI) in April, fruit pedicels (FP) collected at five different stages of avocado tree phenology from the end of fruit set in June through April the following spring when mature fruit enter a second period of exponential growth, or 6-month-old spring flush leaves (LF) from nonbearing vegetative shoots collected in September (California avocado industry standard). For CSI tissue, concentrations of seven nutrients, nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sulfur (S), zinc (Zn), and copper (Cu) were predictive of trees producing greater than 40 kg of fruit annually. Conditional quantile sampling and frequency analysis were used to identify optimum nutrient concentration ranges (ONCR) for each nutrient. Optimum ratios between nutrient concentrations and yields greater than 40 kg per tree were also derived. The high nutrient concentrations characterizing CSI tissue suggest current fertilization practices (timing or amounts) might be causing nutrient imbalances at this stage of avocado tree phenology that are limiting productivity, a possibility that warrants further investigation. Because CSI samples can be collected 4–6 weeks before full bloom, nutritional problems can be addressed before they affect flower retention and fruit set to increase current crop yield, fruit size, and quality. Thus, CSI nutrient analysis warrants further research as a potential supplemental or alternative tool for diagnosing ‘Hass’ avocado tree nutrient status and increasing yield.

The global avocado industry is dominated by the ‘Hass’ cultivar despite production problems of low fruit size, small fruit size, and alternate bearing (Garner et al., 2011). In particular, the California ‘Hass’ avocado industry suffers from low yields, with a mean yield for the last 20 years of only 6847 kg·ha⁻¹ (California Avocado Commission, 2017). With the increasing costs of producing avocados (water, fertilizer, labor, land, etc.), growers need to increase yield per acre to increase net income and to sustain this commodity-based industry. Optimizing tree nutrient status is an essential and cost-effective strategy for improving yield (Lovatt, 2013).

Nutrient analysis of plant tissues is a popular tool used to assess the nutrient status of horticultural crops to design fertilization programs to achieve high yields (Snyder, 1998). Tissue analysis complements soil analysis by reflecting the amounts of nutrients present in the soil that were actually taken up by the root system and transported to the canopy. When used with soil analysis, tissue analysis is a useful tool for confirming soil nutrient deficiencies and excesses. Furthermore, diminished nutrient levels in tissue samples in contrast to soil analysis indicating good availability of the respective nutrients in the soil solution indicate the need to analyze soil characteristics, such as pH, cation exchange, and salinity, which compromise root uptake of nutrients. When soil characteristics are optimal for plant nutrient uptake, such results suggest the need to investigate other potential factors limiting nutrient absorption by the roots (e.g., low or high temperatures, insufficient or excessive water, root damage caused by disease, insect and nematode pests, or mechanical injury).

Worldwide, growers in many avocado-producing areas rely on nutrient analysis of 6-month-old leaves from nonbearing terminal shoots from the current year’s spring vegetative shoot flush collected between August and September (Northern Hemisphere). Avocado ONCRs based on leaves collected at this time identify tissue nutrient concentrations above and below which yields might be anticipated to decline to a commercially unacceptable level and are used to guide replacement fertilization to assure adequate nutrient supply for next year’s crop (Embleton and Jones, 1972; Embleton et al., 1959). Thus, careful management is required to prevent under-fertilization of high yield trees following a low crop year, which could reduce potential yield, or over-fertilization of low yield trees the year following a heavy crop, which could promote vegetative growth over reproductive development or increase the potential for nutrient contamination of orchard runoff water and groundwater. Leaf standards for N, iron (Fe), and Zn were derived from field experiments testing the effects of fertilization rates on yield (Arpaia et al., 1996; Crowley, 1992; Crowley and Smith, 1996; Embleton and Jones, 1972; Embleton et al., 1959; Lovatt, 2001; Salvo and Lovatt, 2016; Yates et al., 1993). Other nutrient standards were borrowed

Table 1. Latitude, longitude, and elevation (m above sea level) and soil characteristics (percent sand, silt, clay and organic matter, soil depth in cm, and pH) of the six commercial orchards used in the research.

Orchard site	Latitude	Longitude	Elevation	Name of soil type	Sand	Silt	Clay	Organic matter	Soil depth	pH
San Luis Obispo	35°8'N	120°31'W	86	Diablo and Cibo clays	26.1	29.2	47.5	2.50	99	7.3
Santa Barbara	34°27'N	119°44'W	176	Lodo-Sespe complex	35.4	33.6	31.0	2.50	28	7.6
Santa Paula	34°19'N	119°7'W	100	Sorrento silty clay loam	18.1	50.9	31.0	2.44	>200	7.3
Santa Paula foothills	34°20'N	119°8'W	106	Sorrento loam	42.0	21.0	37.0	3.00	>200	7.5
Irvine	33°43'N	117°44'W	119	Bosanko-Balcom complex	35.4	33.6	44.6	1.50	79	8.2
Pauma Valley	33°18'N	116°58'W	478	Cieneba-Fallbrook rocky sandy loam	68.5	19.0	12.5	0.75	25	5.8

from *Citrus sinensis* and are not related to any avocado yield or fruit quality parameter. In the past decades, numerous experiments have documented that avocado leaf analysis is not sensitive enough to detect changes in tree N, P, or K status or differences in N, P, or K fertilization rates that caused changes in the yield (Arpaia et al., 1996; Embleton and Jones, 1964, 1972; Embleton et al., 1959; Lovatt, 2001; Lovatt and Witney, 2001; Salvo and Lovatt, 2016; Yates et al., 1993).

For many commercial tree crops, nutrient concentrations of tissues including flowers, entire inflorescences, leaf petioles, pedicels (stems of individual flowers and fruit), and young developing fruit have proven to be valuable sources of supplemental information or alternatives to leaf analysis for diagnosing tree nutrient status (Castillo-Gonzalez et al., 2000; Khelil et al., 2010; Martinez et al., 2003; Nyomora et al., 1997; Razeto and Castro, 2007; Razeto and Salgado, 2004; Sanz and Carrera, 1994). The greater sensitivity of inflorescence and fruit pedicel tissues than leaves for quantifying changes in tree nutrient status relative to yield has been demonstrated for avocado trees in Chile (Razeto and Castro, 2007; Razeto and Salgado, 2004). Thus, in the preliminary research presented herein, the overall goal was to determine whether nutrient concentrations of inflorescence or fruit pedicel tissues collected at different stages of development, which represented different stages in the phenology of the 'Hass' avocado tree, had potential utility for diagnosing nutritional problems related to the yield of 'Hass' avocado trees in commercial orchards in California. The specific objective was to identify ONCRs that maximize the probability of obtaining a yield greater than 40 kg/tree to assist the California avocado

industry in meeting its goal to increase average production to $\approx 11,200$ kg·ha⁻¹.

Materials and Methods

Plant material. 'Hass' avocado trees in six different commercially producing orchards (ranging in age from 7 to 20 years, with different but known rootstocks, and planting densities of 148–400 trees/ha) were used in this research. The orchards were located across the major northern and southern avocado-growing areas of California, from 33°80'N to 33°18'N and 120°31'W to 116°58'W at elevations from 86 to 478 m above sea level, respectively, representing a range in soil types and microclimates (Table 1). All trees were in good health with no visible signs of nutrient deficiencies, salinity damage, or pest problems. The orchards were managed according to each grower's standard cultural practices. Tissues (40 organs/tree) collected and analyzed included the following. 1) Inflorescences (whole panicles) were collected at the cauliflower stage of development (CSI), when 50% of the trees in each orchard had 50% of the tree at Stage 8, based on the floral development scale of Salazar-García et al. (1998) (March) (Fig. 1A). 2) Inflorescences (whole panicles) were also collected at full bloom (FBI), when 50% of the trees in each orchard had 50% of the tree at Stage 11 (Salazar-García et al., 1998) (April). 3) Pedicels from young fruit of average size were collected during the period that included June drop, exponential fruit growth, and mature fruit drop (end of June–beginning of July) (FP1). 4) Pedicels from fruit of average size were collected at the same time as leaves (September) (FP2). 5) Pedicels from fruit of average size were collected at the end of fall vegetative shoot growth (November) (FP3). 6) Pedicels from mature fruit of average size were collected when the trees reached the cauliflower stage of inflorescence development (as described previously) the following year (March) (FP4) and 7) pedicels from mature fruit of average size were also collected when the trees reached full bloom (as described previously) the following year (April) (FP5). 8) LF (40/tree) were collected from 6-month-old non-bearing terminal vegetative shoots from the spring flush at a height of 1.4 m above the ground from the four quadrants (NE, SE, SW, and NW) of each data tree, according to the industry standard (Embleton et al., 1959) (September).

Tissue nutrient analysis. All tissue samples were collected from the same 16 individual

trees (replications) located diagonally across each orchard for two consecutive crop years. Samples were immediately placed in brown paper bags, stored on ice, and taken to the University of California, Riverside. Upon arrival, tissues were washed thoroughly with dish soap, rinsed three times with distilled deionized water, oven-dried at 60 °C for 72 h, and ground in a Wiley mill to pass through a 40-mesh (420 μ) screen (Embleton et al., 1959). The ground samples were sent to the UC-Division of Agriculture and Natural Resources Analytical Laboratory (Davis, CA). Total N was determined after combustion at 1050 °C by thermal conductivity (Leco Corp., St. Joseph, MI) (AOAC, 2006). The concentrations of P, K, calcium (Ca), Mg, S, Zn, manganese (Mn), Fe, boron (B), and Cu were determined after nitric acid–hydrogen peroxide microwave digestion by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Meyer and Keliher, 1992).

Yield assessment. Individual trees were harvested to determine total kilograms of fruit per tree in March to July, according to each grower's standard management practice (11–15 months after full bloom). For all harvests, the percentage of dry matter content of the fruit was greater than the industry required 20.8% (Dixon, 2013). Total yield was determined as kg/tree by removing and weighing all fruit produced by a tree. In addition, at harvest, a randomly selected sample of 100 to 150 fruit/tree, representing 30% to 100% of the total number of fruit on a tree for each year of the experiment was collected for each data tree and the fresh weight of each fruit in the sample was determined as grams per fruit. These data were used to calculate pack-out, i.e., the kg of fruit of each packing carton size per tree. The following packing carton fruit sizes (grams per fruit) were used: size 84 (99 to 134 g), size 70 (135 to 177 g), size 60 (178 to 212 g), size 48 (213 to 269 g), size 40 (270 to 325 g), size 36 (326 to 354 g), and size 32 (355 to 397 g). Packing carton fruit sizes are based on the number of fruit in an 11.34-kg box within a tolerance of ± 0.23 kg.

The alternate bearing index (ABI) was calculated for each data tree using the following equation: $ABI = (\text{year 1 yield} - \text{year 2 yield}) / (\text{year 1 yield} + \text{year 2 yield})$ in which yield is total kg of fruit per tree and the difference in yield between years 1 and 2 is expressed as an absolute value. An ABI of zero means no alternate bearing, whereas an ABI of one is complete alternate bearing (Pearce and Dobersek-Urbanc, 1967).

Soil, air temperature, and rainfall data. Orchard soil characteristics were obtained

Received for publication 25 Aug. 2017. Accepted for publication 3 Oct. 2017.

This research was supported in part by the Citrus Research Center and Agricultural Experiment Station of the University of California-Riverside, National Institute of Food and Agriculture of the US Department of Agriculture, California Department of Food and Agriculture Fertilizer Research and Education Program, and the California Avocado Commission.

We thank the following grower collaborators, who facilitated the collection of the 'Hass' avocado tissue samples and harvest data: John Grether, Gus Gunderson, Brycen Ikeda, Sam MacIntyre, Jess Ruiz, Jaime Serrato, and the late Dick Whitley and Len Francis.

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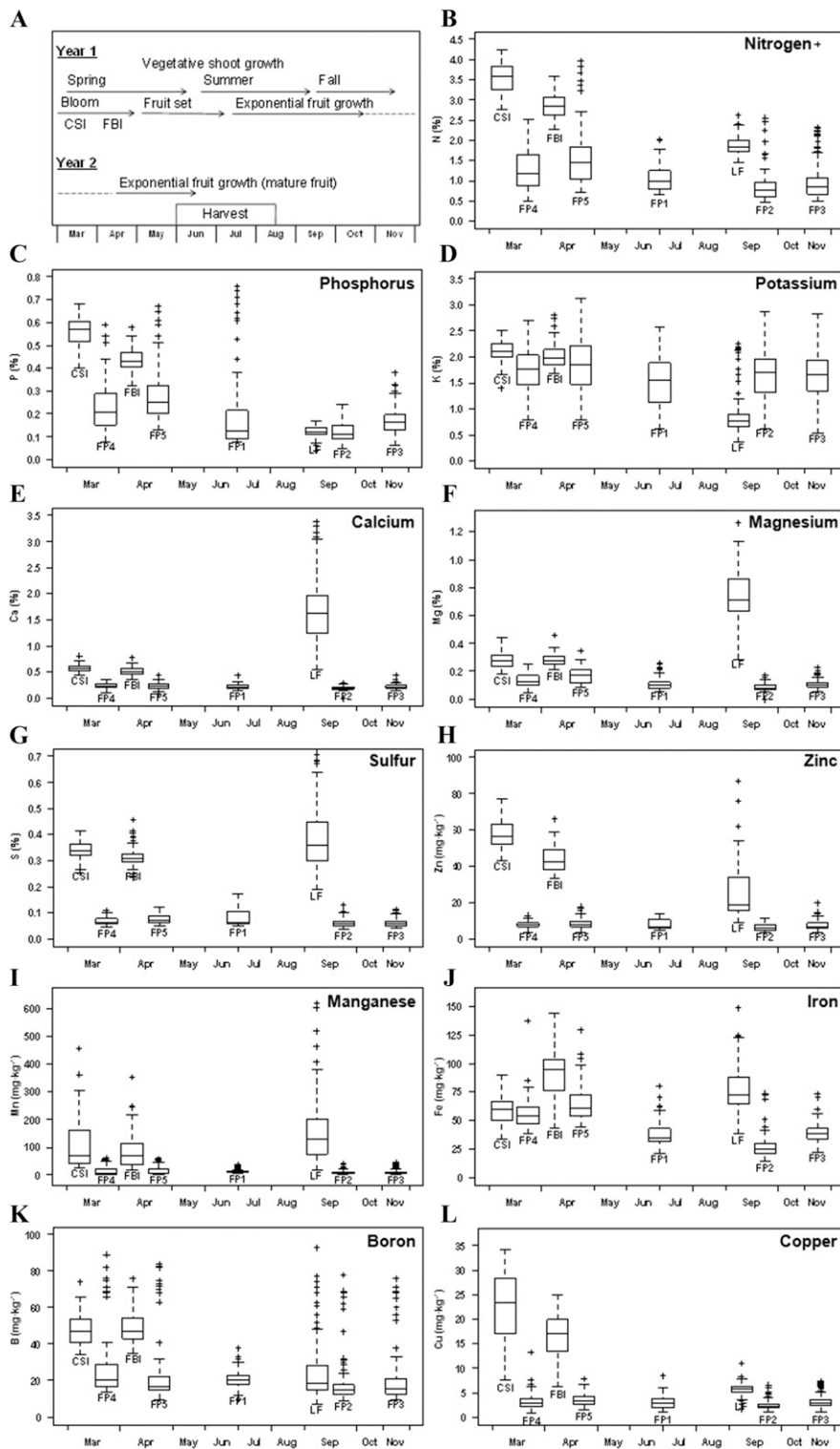


Fig. 1. 'Hass' avocado tree phenology (A) and nutrient concentrations of cauliflower stage inflorescences (CSI) collected in March, full bloom inflorescences (FBI) collected in April, fruit pedicels (FP) collected at the end of June-beginning of July (FP1), in September (FP2), in November (FP3), the following year in March (FP4) and April (FP5), and 6-month-old leaves collected in September from nonfruiting terminals (LF) for (B) nitrogen (N), (C) phosphorus (P), (D) potassium (K), (E) calcium (Ca), (F) magnesium (Mg), (G) sulfur (S), (H) zinc (Zn), (I) manganese (Mn), (J) iron (Fe), (K) boron (B), and (L) copper (Cu). For each box plot, the horizontal line within the box is the median nutrient concentration, the bars indicate 25th and 75th percentiles, and the dashes indicate the most extreme data points not considered outliers, with outliers plotted individually as plus signs (+).

from the United States Department of Agriculture Natural Resources Conservation Service Web Soil Survey (2013) and used to

determine whether specific soil properties were determinants of yield among the orchards. To determine the influence of climate

on annual yield, monthly average maximum and minimum air temperatures and rainfall for the two crop years of the research were downloaded from the California Irrigation Management Information System website (California Department of Water Resources, 2009) for the closest station to each orchard. Average monthly maximum and minimum temperatures and rainfall for overlapping sequential 3-month periods (e.g., January–March, February–April, March–May, etc.) encompassing key stages of 'Hass' avocado tree phenology (floral development, flowering, fruit set, June drop, exponential fruit growth, phase transition, and vegetative shoot flushes) were computed and compared across orchards and crop years. These data were used to determine whether differences in these aspects of climate occurring during important periods in 'Hass' avocado tree phenology were related to differences in annual yield among orchards.

Statistical analyses. The data set (>8500 data points) was analyzed using a knowledge discovery approach (Benjamini and Leshno, 2010; Raveh, 2013), which consisted of step-by-step implementation of different statistical methods. First, the yield data were evaluated for uniform distribution, and the ABI for each tree in the data set for the two crop years of research was calculated to determine the severity of alternate bearing within each orchard and among orchards. Second, the strength of relationships between orchard soil or climate factors and yield were analyzed by estimating Pearson product-moment correlation coefficients (r). Third, the statistical distribution of the concentrations of each nutrient for each tissue was evaluated using box plots and one-way analysis of variance (ANOVA). Fourth, frontier of production (envelope) analysis, a nonlinear approach based on quantile sampling (Cade et al., 1999; Webb, 1972), was applied to determine nutrient concentrations that maximized the probability of obtaining yields greater than 40 kg/tree (the goal of the California avocado industry). Conditional quantile sampling and frequency analysis (Cade et al., 1999; Raveh, 2013) were further used to identify ONCRs associated exclusively with yields greater than 40 kg/tree. In addition, R^2 -coefficient and corresponding P value were used to identify significant ratios between nutrient-pairs and yields greater than 40 kg/tree.

Results

Yield. Yield for all trees in the data set ranged from 0 to 336 kg/tree, with 35% of the trees in the data set yielding less than 10 kg/tree, 33% yielding between 10 and 40 kg/tree, and 32% yielding more than 40 kg/tree (data not presented). The mean yield for all trees in the data set was 31.4 kg/tree (8534 kg·ha⁻¹, based on the typical planting density used in reporting yield data to the California Avocado Commission). Trees producing more than 100 kg/tree (27,180 kg·ha⁻¹) comprised 10% of the data set.

As total yield increased, the yield of commercially valuable large (CVL) fruit (packing carton sizes 60 + 48 + 40; 178–325 g/fruit) and small fruit (SF) (packing carton sizes 84 + 70; 99–177 g/fruit) increased ($r = 0.83$; $P < 0.0001$ and $r = 0.81$; $P < 0.0001$, respectively); however, yield of CVL fruit consistently comprised the majority (54% to 62%) of the crop (Fig. 2). The results are consistent with earlier results documenting that yield of CVL fruit increased with total yield through 190 kg/tree for ‘Hass’ avocado trees in California (Lovatt et al., 2015). At higher yields, the proportion of SF increased more dramatically than that of CVL fruit, but yield of CVL fruit remained greater than SF even at 300 kg/tree. Thus, in this research, only total yield was analyzed.

Total yield was positively related to the percent sand in the orchard soil ($r = 0.50$; $P < 0.0001$) (data not presented), but was not related to any other soil characteristic evaluated (Table 1). Soil EC (measured in a 1:1 soil:water paste), an indicator of soil salinity, was equal to or less than $1.0 \text{ dS}\cdot\text{m}^{-1}$ for all orchards used in this research (United States Department of Agriculture Natural Resources Conservation Service Web Soil Survey, 2013), consistent with no visual signs of salinity damage on trees in any orchard.

There were significant differences in the average maximum and minimum temperatures during important stages of ‘Hass’ avocado tree phenology among orchards and between years (data not presented). Despite these significant differences, there was no significant relationship between the average maximum or minimum temperature related to the change in temperature for each sequential 3-month period and total yield (data not presented). Sequentially deleting and adding monthly average temperature data to the 3-month period being evaluated did not identify any month with a strong effect on yield in any orchard or year. This is likely due to two factors: 1) no major climatic events (excessively low or high temperatures, excessive wind or rain) occurred in any orchard during the two crop years of the research; and 2) the number of fruit per tree within a single orchard was not uniform. Average total

monthly rainfall was not significantly different across orchards or between years and not related to total yield (data not presented). Thus, for the years included in this research, yield was not dictated by orchard microclimate (temperature or rainfall).

Alternate bearing was not a major problem for the two crop years of the research. The ABI for individual trees in each orchard was low. More importantly, each orchard had both low and higher yielding trees in each crop year. In the orchard with the most severe alternate bearing, average ABI was only 0.31. The presence of high and low yielding trees in each orchard annually is likely the reason that yield was not related to rootstock, tree age, or tree size and alternate bearing was not an overriding factor, i.e., there was not a true “on-” and “off-” crop year. Taken together, these results provide evidence that the yield data were not strongly biased by location, including aspects of soil and climate, rootstock, tree age, or tree size. Thus, yield data were not normalized to adjust for any of these factors in any orchard, consistent with the goal of the research to identify a tissue that could be used to diagnose nutritional problems across all avocado orchards independent of these considerations, which is the case with the current use of leaf analysis.

Tissue nutrient concentrations. There were significant variations in the concentrations of the 11 nutrients analyzed among the eight tissue samples collected at various stages of ‘Hass’ avocado tree phenology (Fig. 1). Across orchards and years, CSI tissue concentrations of N, P, Zn, and Cu were greater than those of FBI tissues ($P < 0.0001$) (Fig. 1B, C, H, and L), with CSI K and B concentrations equal to those of FBI (Fig. 1D and K). Both CSI and FBI had concentrations of N, P, K, Zn, B, and Cu that were greater than LF and all FP samples ($P < 0.0001$) (Fig. 1B–D, H, K, and L), and FBI had greater Fe concentrations than all other tissues ($P < 0.0001$) (Fig. 1J). Leaves collected in September had greater concentrations of Ca, Mg, S, and Mn compared with all other tissues ($P < 0.0001$) (Fig. 1E–G and I), whereas LF K concentration was lower than all other tissues ($P < 0.0001$) (Fig. 1D). In

general, nutrient concentrations of FP tended to be low across all collection dates, with all FP samples having lower concentrations of Ca, Mg, S, Zn, Mn, and Cu than all other tissues ($P < 0.0001$) (Fig. 1E–I and L). Thus, despite being collected at the same stage of tree phenology, the concentrations of several nutrients were substantially greater in one tissue type vs. another. For example, S, Zn, and Cu concentrations were each more than 3-fold greater in CSI and FBI tissues than FP4, and FP5 also collected in March and April each year, respectively (Fig. 1G, H, and L). Similarly, Ca, Mg, S, Zn, Mn, and Fe were all significantly (>3-fold) greater in LF than FP2 tissue, which was also collected in September (Fig. 1E–J). Notably, these data also provided evidence suggesting that the accumulation of nutrients in some cases was tissue specific, for example, only LF tissue accumulated high concentrations of Ca and Mg, and only CSI and FBI tissues accumulated high concentrations of Cu (Fig. 1E, F, and L).

Tissue nutrient concentrations associated with yields greater than 40 kg/tree. Frontier of production analysis was used to examine the relationship between tissue nutrient concentrations and yield as kg per tree. Results of this analysis identified seven nutrients (N, P, K, Mg, S, Zn, and Cu) in CSI tissue that had concentrations uniquely associated with yields greater than 40 kg/tree (Fig. 3A–G). For each of the seven nutrients, yields greater than 40 kg/tree were associated with the lower end of the nutrient concentration range, which also included low yields at all but the lowest concentrations. For the seven nutrients in CSI tissue, the high end of the concentration range was always and only associated with lower yields (<40 kg/tree). For CSI, N concentrations greater than 2.7% but less than 3.0% were solely associated with trees producing more than 40 kg/tree (Fig. 3A). Above this range, low yielding trees were also found with high yielding trees. By contrast, N concentrations greater than 3.6% were exclusively associated with trees producing less than 40 kg/tree. Thus, application of this method to the CSI data set identified the concentration range for each nutrient that was associated with yields greater than 40 kg/tree and simultaneously, it identified nutrient concentrations that were never associated with yields above this threshold. The method identified a single nutrient (Fe) in the CSI data set (Fig. 3H), for which the high end of the concentration range (>80 $\text{mg}\cdot\text{kg}^{-1}$) was exclusively associated with yields greater than 40 kg/tree, and also revealed that the number of trees meeting this criterion was too low in the current data set to be statistically reliable. In addition, frontier of production analysis established that for three nutrients (B, Ca, and Mn) in the CSI data set (Fig. 3I–K), there was no concentration related to yields greater than 40 kg/tree that was not also associated with yields less than 40 kg/tree.

Pearson coefficients documented the significant and inverse correlations between

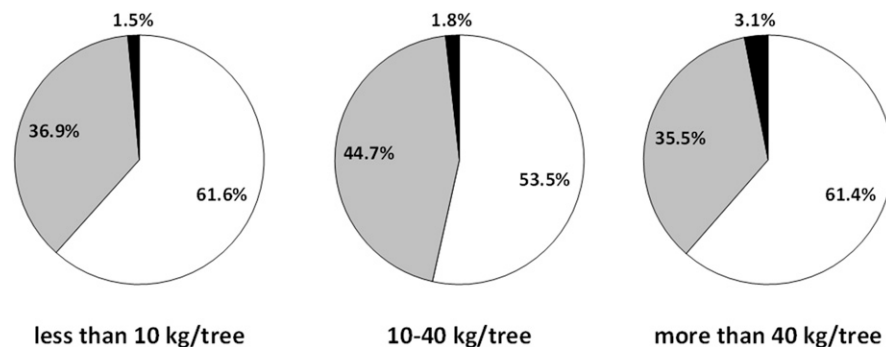


Fig. 2. Distribution of commercially valuable large fruit (packing carton sizes 60 + 48 + 40; 178–325 g/fruit) (represented by white) and less valuable small fruit (packing carton sizes 84 + 70; 99–177 g/fruit) (represented by gray) and very large fruit (packing carton sizes > 40; > 325 g/fruit) (represented by black) as a percent of the total yield of trees producing less than 10 kg per tree, 10–40 kg/tree and more than 40 kg/tree.

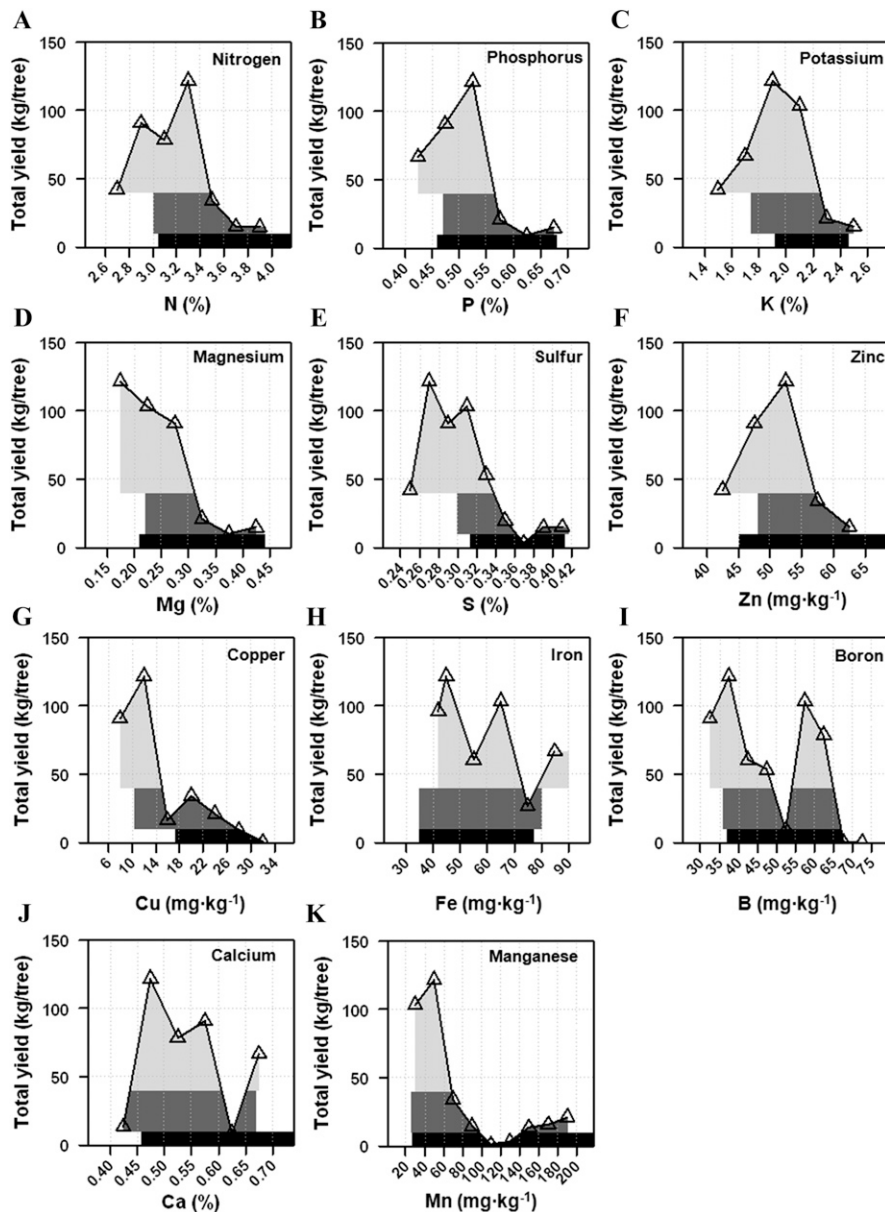


Fig. 3. Geometrical representation of the results of frontier of production analysis of the nutrient concentrations of cauliflower stage inflorescences of the 'Hass' avocado in relation to yield (kg/tree) for nutrient concentrations significantly correlated with yield, (A) nitrogen (N), (B) phosphorus (P), (C) potassium (K), (D) magnesium (Mg), (E) sulfur (S), (F) zinc (Zn), and (G) copper (Cu), and nutrient concentrations not significantly related with yield, (H) iron (Fe), (I) boron (B), (J) calcium (Ca), and (K) manganese (Mn). For each nutrient, the light gray color indicates nutrient concentrations associated with trees producing more than 40 kg/tree, the dark gray color indicates nutrient concentrations associated with trees producing from 10 kg to 40 kg/tree, and the black color indicates nutrient concentrations associated with trees producing less than 10 kg/tree. The segmented black line is the estimated frontier of production, which indicates the maximum yield observed at each nutrient concentration.

yield and concentrations for each of the CSI nutrients (N, P, K, Mg, S, Zn, and Cu, except Fe) identified by frontier of production analysis. The results were consistent with the observation that better yielding trees (>40 kg/tree) were at the low end of the nutrient concentration ranges for these seven nutrients, whereas only low yielding trees were at the higher end of each concentration range. The correlations for the seven nutrients and yield were not strong ($r = -0.52$ for N, -0.50 for P, -0.49 for K, -0.48 for Mg, -0.58 for S, -0.54 for Zn, and -0.76 for Cu), but were

highly significant ($P < 0.0001$) in all cases. No significant correlations were observed in the other tissues, with the exception of N ($r = -0.49$, $P < 0.0001$) and Cu ($r = -0.72$, $P < 0.0001$) concentrations in FBI tissue.

The results of both statistical approaches established that CSI tissue provided a greater number of nutrients that were related to total yield. Thus, the CSI subset was selected for further statistical analysis.

CSI ONCR. To identify CSI ONCRs related only to trees producing greater than 40 kg/tree, the seven nutrients (N, P, K, Mg,

S, Zn, and Cu) were subjected to frequency analysis whereby the yield distribution for trees within uniform subsets of the concentration range for each nutrient was determined (Fig. 4A–G). For example, within our data set, CSI N concentrations between 2.7% and 3.0% were only associated with yields greater than 40 kg/tree (Fig. 4A); there were no data points below 2.7%. As the N concentration incrementally increased beyond 3.0%, the probability of obtaining yields greater than 40 kg/tree decreased in favor of trees yielding less than 40 kg/tree and subsequently in favor of trees producing less than 10 kg per tree. Thus, the CSI ONCR for N is 2.7% to 3.0% (Fig. 4A). The CSI ONCRs for the other six nutrients are P, 0.40% to 0.45% (Fig. 4B); K, 1.4% to 1.7% (Fig. 4C); Mg, 0.15% to 2.0% (Fig. 4D); S, 0.25% to 0.28% (Fig. 4E); Zn, 40 to 44 mg·kg⁻¹ (Fig. 4F); and Cu, 6 to 10 mg·kg⁻¹ (Fig. 4G). The CSI ONCRs are summarized in Table 2.

Important nutrient ratios. To further increase the probability of attaining yields greater than 40 kg/tree, ratios between each of the seven CSI ONCRs and all other nutrients in relation to yield were calculated and statistically analyzed. The results fell into four categories: 1) the ratio that prescribed the necessary concentration of one nutrient relative to another associated with high yields was only significant for high yielding trees ($R^2 > 0.70$; $P \leq 0.05$), with the ratio for low yielding trees nonsignificant (e.g., N:K, N:Ca, N:Mg, N:Fe, Mg:Ca) (Fig. 5A–E); 2) significant ratios existed for both high-yielding and low-yielding trees that were distinctly different and thus clearly specified the corresponding concentration of one nutrient relative to the other that was necessary for high yields (e.g., Cu:P, Cu:Zn) (Fig. 5F and G); 3) significant ratios existed for both high-yielding (>40 kg/tree) and low-yielding (<40 kg/tree) trees, but they were similar and thus, provided no unique information characterizing the nutrient status of high yielding trees (data not presented); and 4) no significant ratio was detected for either high yielding or low yielding trees ($R^2 < 0.70$; $P > 0.05$) (data not presented). Statistical analysis of N:K provided evidence that high yields were attained only when the concentration of K increased in a specific ratio with the increasing concentration of N (Fig. 5A); similar results were obtained for Mg:Ca (Fig. 5E). By contrast, as N concentration increased, high yielding trees were characterized by specific decreases in concentrations of Ca, Mg, and Fe described by the slope of the line in Fig. 5B–D, respectively. The ratios of Cu:P and Cu:Zn indicate that at Cu concentrations within the ONCR (6 and 10 mg·kg⁻¹), low concentrations of P (<0.54%) and Zn (<53 mg·kg⁻¹) sustained high yields (circled dots in Fig. 5F and G).

The equations for the solid line describing each of the significant ratios associated with high yields (>40 kg/tree) in Fig. 5A–G are presented in Table 3. These equations can be used to estimate the optimal nutrient concentrations for a specified *nutrient-y* when the

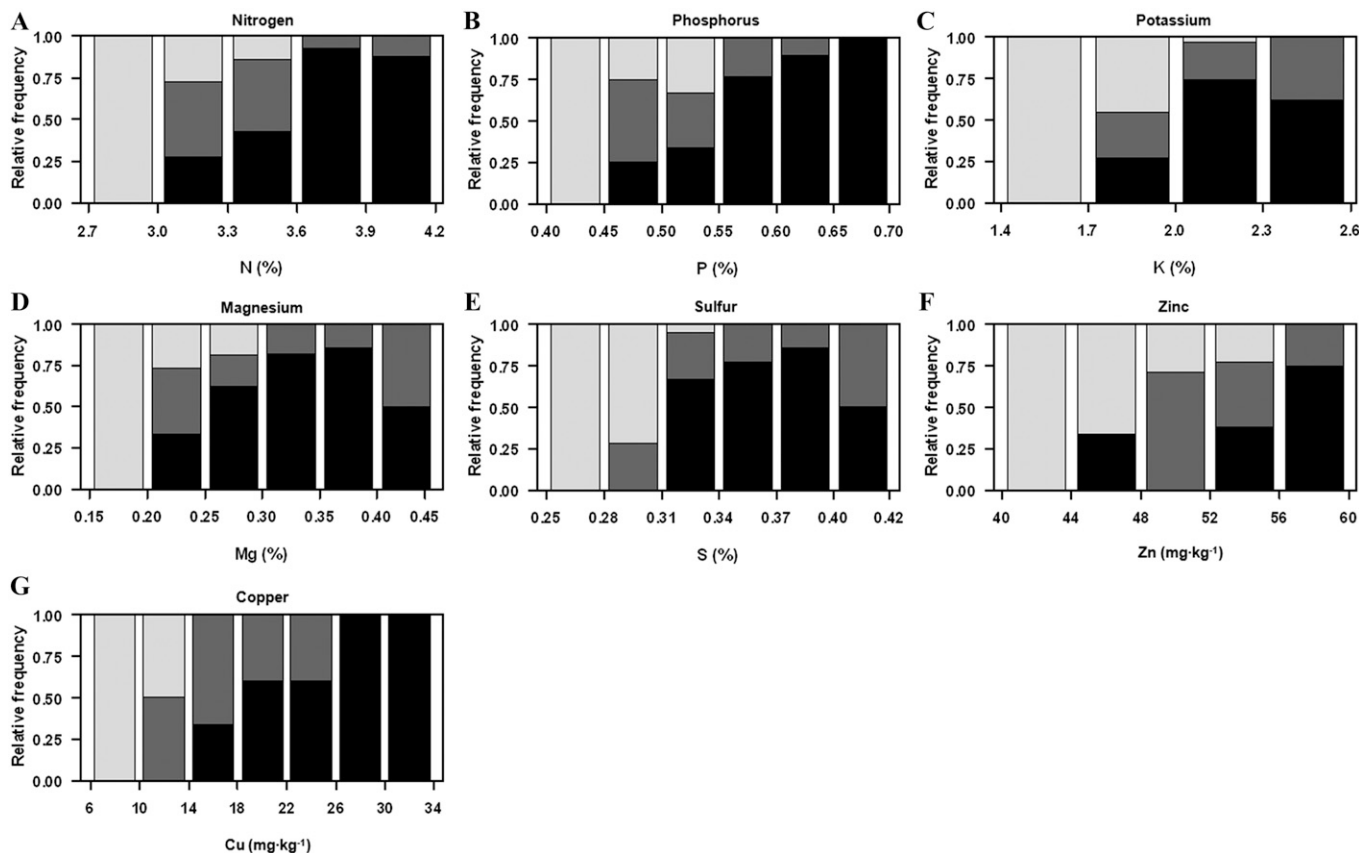


Fig. 4. Frequency of 'Hass' avocado trees based on yield (kg/tree) within nutrient concentration ranges for the cauliflower stage inflorescence: (A) nitrogen (N), (B) phosphorus (P), (C) potassium (K), (D) magnesium (Mg), (E) sulfur (S), (F) zinc (Zn), and (G) copper (Cu). For each concentration category within the span of a bar, the light gray color indicates the proportion of trees with yields greater than 40 kg/tree, the dark gray color indicates the proportion of trees with yields from 10 to 40 kg/tree, and the black color indicates the proportion of trees with yields less than 10 kg/tree.

Table 2. Summary of the optimal nutrient concentration ranges (ONCRs) for the seven nutrients in the cauliflower stage inflorescence (CSI) of the 'Hass' avocado predictive of yields greater than 40 kg/tree.

CSI nutrient	N	P	K	Mg	S	Zn	Cu
	----- (%) -----					---- (mg·kg ⁻¹) ----	
ONCR	2.7–3.0	0.40–0.45	1.4–1.7	0.15–2.00	0.25–0.28	40–44	6–10

concentration of *nutrient-x* is known. For example, when observed N is 2.8%, by using the third equation in Table 3, the corresponding concentration of Mg is estimated to be 0.27%. In addition, by using the estimated value for Mg of 0.27% in the fifth equation in Table 3, the concentration for Ca in this case is estimated to be 0.61%. Note that the equations in Table 3 are valid only for nutrient concentrations that are within the CSI ONCR for *nutrient-x*; nutrient concentrations outside the ONCR are not likely to identify trees that yield more than 40 kg/tree.

Comparison of nutrient concentrations in CSI and LF samples. For CSI and LF tissue, the median, mean, and standard deviation of the concentrations of each nutrient were significantly different ($P \leq 0.001$), indicating that observations for CSI and LF tissues are associated with different underlying distributions (Fig. 6A–E, G, J, and K). No significant linear correlation existed between the concentration of nutrients in CSI and LF tissue, with the exception of Mn ($r = 0.76$;

$P \leq 0.05$). Specifically, Mn concentrations in LF samples are related to Mn concentrations in CSI samples by the following equation, where Mn is mg·kg⁻¹: $Mn_{LF} = 0.95 (Mn_{CSI}) + 76 \text{ mg·kg}^{-1}$; suggesting that Mn uptake and accumulation in leaves continue from March through September in a predictable manner. With the exception of Mn, these results taken together indicate that nutrient concentrations in CSI tissue cannot be used to predict nutrient concentrations in LF tissue, and conclusions based on CSI analysis cannot be extended to LF analysis or vice versa.

Discussion

The results of the present study demonstrated the benefit of using a knowledge discovery approach and stepwise application of statistical methods. Specifically, use of frontier of production analysis successfully identified seven nutrients (N, P, K, Mg, S, Zn, and Cu) in CSI tissue with concentration ranges uniquely related to yields greater than

40 kg/tree. This unique relationship was not observed for Ca, Mn, or B. The results indicated that the nutrient status of trees producing high yields were only at the low end of the concentration ranges for N, P, K, Mg, S, Zn, and Cu, whereas those producing low yields were at the high end of each range. Pearson correlation coefficients confirmed that the concentrations of N, P, K, Mg, S, Zn, and Cu were negatively related to yield, Fe concentrations in CSI tissue were positively (but weakly) related to yield, and Ca, Mn, and B concentrations were not related to yield. Frequency analysis was used to define ONCRs for N, P, K, Mg, S, Zn, and Cu. In addition, significant nutrient ratios were identified for CSI tissue, N:K, N:Ca, N:Mg, N:Fe, Mg:Ca, Cu:P, and Cu:Zn, which expanded the scope of practical information beyond nutrient ONCRs that CSI tissue can provide.

Sampled trees did not show visible symptoms of nutrient deficiencies, thus the lower thresholds of CSI ONCRs remain the object of further research. Analysis of an expanded CSI data set might also reveal the ONCR for B related to its well-documented role in avocado flowers for successful pollen germination, pollen tube growth and fertilization, and increased fruit set and yield (Boldingh et al., 2016; Jaganath, 1993; Lovatt, 2001). A CSI data set that includes strongly alternate bearing orchards (ABI = 0.75–1.0) is required

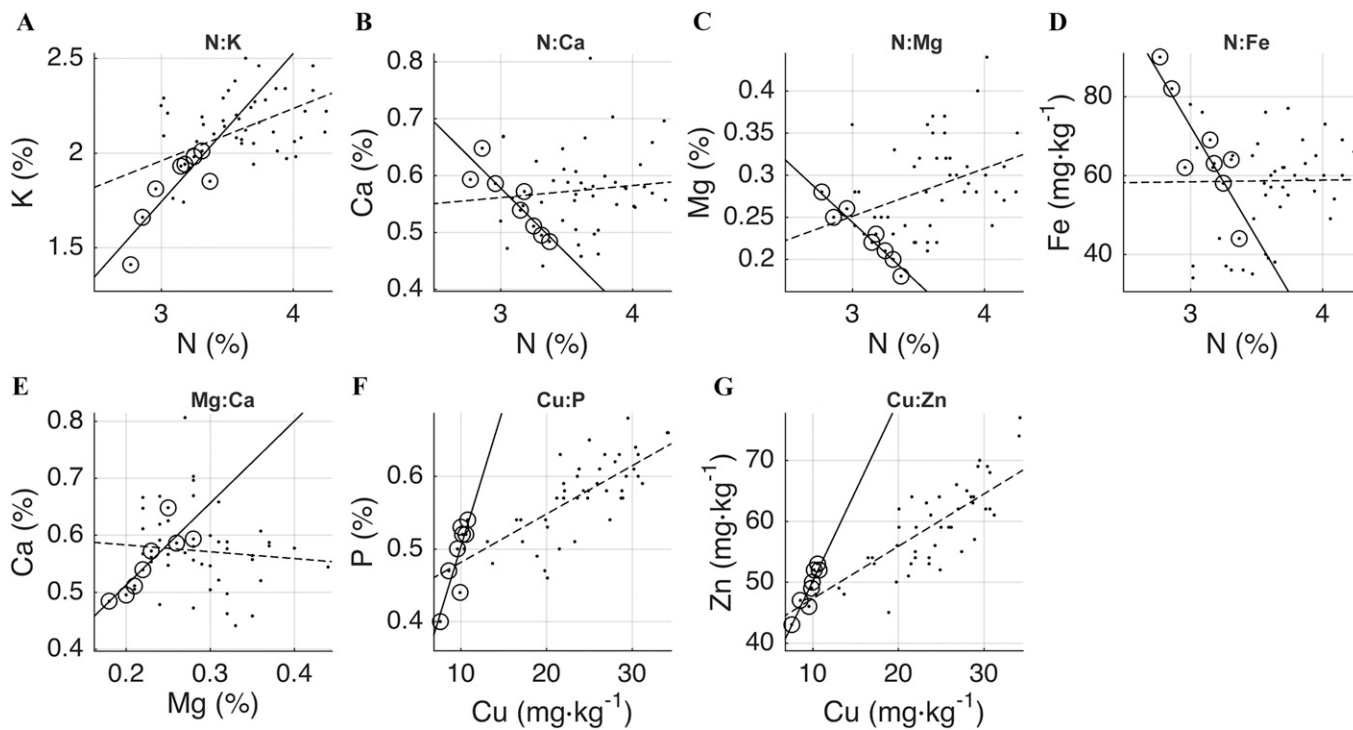


Fig. 5. Ratios between optimal nutrient concentrations (ONCs) (x axis) and other nutrients (y axis) for the cauliflower stage inflorescence in relation to yield of the ‘Hass’ avocado: (A) nitrogen:potassium (N:K); (B) nitrogen:calcium (N:Ca); (C) nitrogen:magnesium (N:Mg); (D) nitrogen:iron (N:Fe); (E) magnesium:calcium (Mg:Ca); (F) copper:phosphorus (Cu:P), and (G) copper:zinc (Cu:Zn). Dots represent trees in all yield categories; circled dots represent trees with yields greater than 40 kg. The dashed and solid lines represent linear interpolations of the corresponding data points, respectively; linear interpolations indicated by a solid line are statistically significant ($R^2 > 0.70$; $P \leq 0.05$).

Table 3. The linear equations for the statistically significant nutrient ratios associated with yields greater than 40 kg/tree presented in Fig. 3, with the 95% confidence intervals for the estimated parameters p_1 and p_2 for each equation.

Nutrient ratio $Y = f(X)$	Equation ² $Y = p_1X + p_2$	95% confidence interval for p_1	95% confidence interval for p_2	R^2	P value
$K = f(N)$	$K (\%) = 0.784 N (\%) - 0.6116$	(0.3034, 1.265)	(-2.108, 0.8846)	0.73	≤ 0.05
$Ca = f(N)$	$Ca (\%) = -0.2302 N (\%) + 1.269$	(-0.3454, -0.115)	(0.9099, 1.627)	0.80	≤ 0.05
$Mg = f(N)$	$Mg (\%) = -0.1458 N (\%) + 0.6818$	(-0.1874, -0.1042)	(0.5522, 0.8113)	0.92	≤ 0.05
$Fe = f(N)$	$Fe (\%) = -54.48 N (\%) + 241.9$	(-88.81, -24.16)	(141.3, 342.6)	0.75	≤ 0.05
$Ca = f(Mg)$	$Ca (\%) = 1.439 Mg (\%) + 0.2242$	(0.54, 2.339)	(0.01663, 0.4319)	0.72	≤ 0.05
$P = f(Cu)$	$P (\%) = 0.0385 Cu (mg \cdot kg^{-1}) - 0.1177$	(0.01336, 0.06371)	(-0.1269, 0.3622)	0.70	≤ 0.05
$Zn = f(Cu)$	$Zn (mg \cdot kg^{-1}) = 2.968 Cu (mg \cdot kg^{-1}) - 20.32$	(1.679, 4.257)	(7.795, 32.85)	0.84	≤ 0.05

²Equations are defined within cauliflower stage optimal nutrient concentration ranges.

to further test the utility of this tissue. The concentration ranges for N, P, K, Ca, and Mg in CSI tissue reported in this study largely correspond to those reported for inflorescences from ‘Colin V-33’ avocado trees in Mexico (yield data were not reported) (Castillo-Gonzalez et al., 2000). The high nutrient concentrations characterizing CSI tissue in both countries raise concern that current fertilization practices (timing or amounts) might be creating nutritional imbalances at this stage of avocado tree phenology that have previously gone undetected and might be affecting yield not only in California and Mexico, but also in other avocado-growing areas, a possibility that warrants further investigation.

The source of the specific nutrients that accumulated in CSI tissue is unclear. The emergence of the inflorescence from the bud (Stage 7) to CSI (Stage 8) occurs within 2–4 weeks during February to March under low average maximum and minimum air and soil

temperatures (Salazar-García et al., 1998). During this period, stored nutrients may be transported to different degrees from neighboring leaves into the developing CSI. Salazar-García et al. (2007) documented that nutrient recycling in winter from senescing leaves occurred concurrently with floral bud-break. Transport of leaves at that time was $K > Cu > N > P > Fe > S$, with no recycling of Ca, Mg, Mn, Zn, and B. They also documented that summer LF did not recycle Ca and Mg. Differences in the degree of recycling among nutrients might partially explain why some nutrients are in high concentrations in some tissues but not others. In addition, periods of significant uptake of soil available nutrients and accumulation within developing fruit might be anticipated to influence concentrations of these nutrients in other tissues. Comparison of the distributions of nutrient concentrations in inflorescence, fruit pedicel, and LF tissues confirmed that avocado tissues accumulated specific

nutrients to different degrees, for example, CSI had high concentrations of N and Cu and LF had high concentrations of Ca and Mg compared with all other tissue samples, even those collected at the same stage of avocado tree phenology. FP collected on each of the five sampling dates had very low levels of the nutrients that were not recycled from leaves, that is, Ca, Mg, Mn, and Zn (Salazar-García et al., 2007) and did not reflect the major periods of nutrient accumulation by ‘Hass’ avocado fruit in California (Rosecrance et al., 2012). In both California and Mexico, the N concentration of FP was consistently lower than that of inflorescences and leaves. Similar to California, leaves of avocado trees in Mexico had high Ca and Mg concentrations, whereas inflorescences had high P and K concentrations (Castillo-Gonzalez et al., 2000). Comparison of LF and CSI samples by ANOVA confirmed significant differences ($P \leq 0.001$) in the mean concentrations of corresponding nutrients. Consistent with

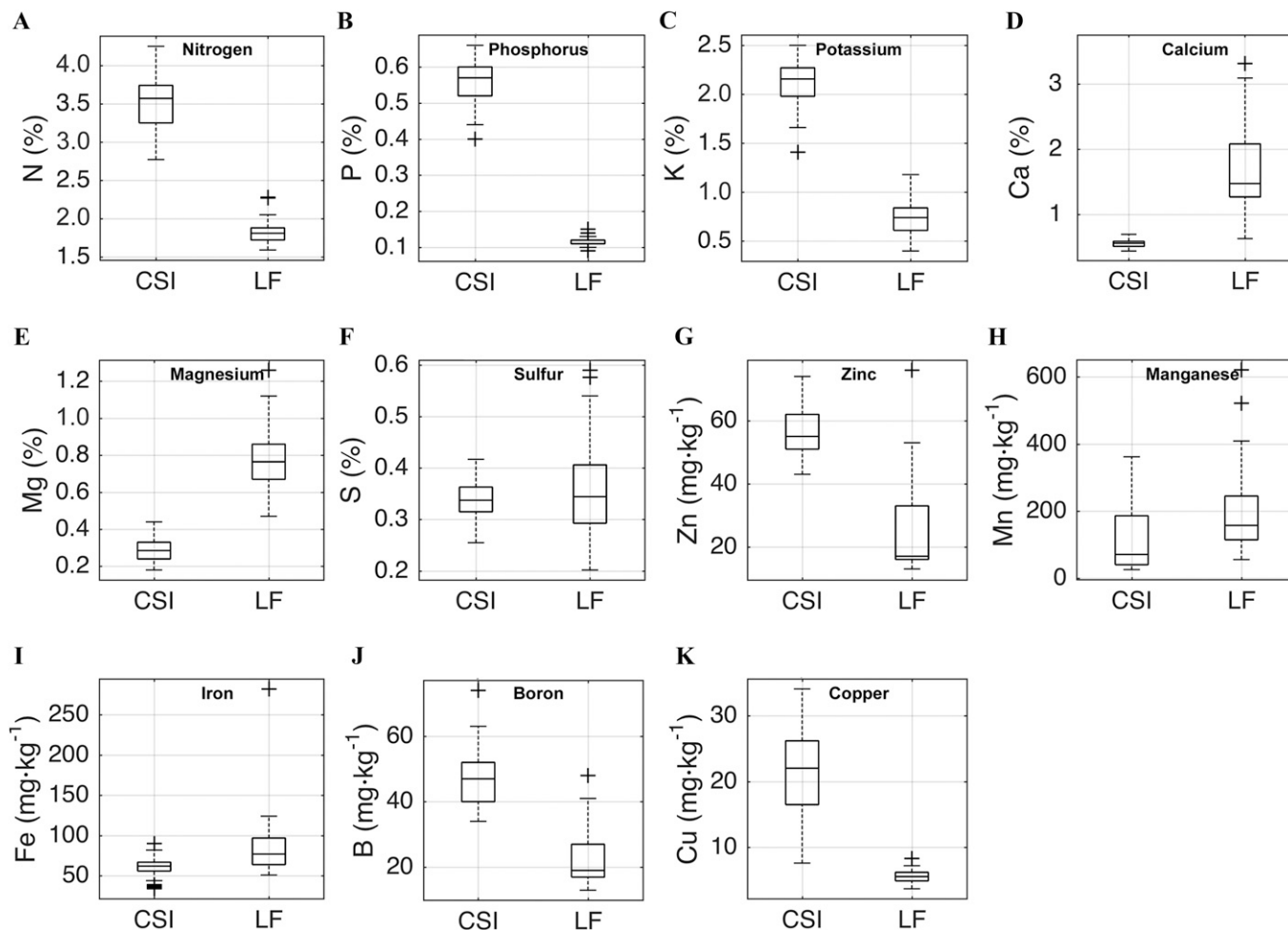


Fig. 6. Analysis of variance of nutrient data arrays for cauliflower stage inflorescences (CSI) and 6-month-old leaves from nonbearing terminal vegetative shoots from the spring flush collected in September (LF) from the same ‘Hass’ avocado trees: (A) nitrogen (N); (B) phosphorus (P); (C) potassium (K); (D) calcium (Ca); (E) magnesium (Mg); (F) sulfur (S); (G) zinc (Zn); (H) manganese (Mn); (I) iron (Fe); (J) boron (B); and (K) copper (Cu). For each box plot the horizontal line within the box is the median nutrient concentration, the bars indicate 25th and 75th percentiles, and the dashes indicate the most extreme data points not considered outliers, with outliers plotted individually as plus signs (+).

the greater rate of export of N, P, K, and Cu out of leaves at spring budbreak, concentrations of these four nutrients were significantly greater in CSI than in LF, with LF having greater concentrations of Ca and Mg, which were not recycled (Salazar-García et al., 2007). It should also be noted that the distribution of CSI nutrient concentrations was not related to the distribution observed by standard LF nutrient analysis; thus, recommendations for LF cannot be extended to CSI and vice versa, with the exception of Mn. As demonstrated by Razeto and Salgado (2004), CSI tissue is characterized by unique physiological and statistical features that render it a promising tissue to supplement the diagnosis of avocado tree nutrient status in relation to yield.

Avocado leaves are 6 months old at the time of collection in August–September and it is notoriously difficult to identify the correct leaf on a terminal spring flush vegetative shoot from one that developed on a terminal shoot in a later flush. Collected this late in the production season, LF analysis is used to guide replacement fertilization in spring, about 7–8 months later. Samples of

CSI tissue are collected in spring (March) and represent a discrete developmental stage of relatively short duration that is easy to identify and collect. As CSI can be collected and analyzed 4–6 weeks before full bloom, sufficient time is provided to correct nutrient imbalances before they affect flower retention and fruit set and negatively affect current crop yield, fruit size, or quality. Taken together, the results of this research provide compelling evidence that further research is warranted for the development of CSI nutrient analysis as a supplemental or alternative tool for diagnosing ‘Hass’ avocado tree nutrient status to increase yield.

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