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Field Evaluation of Recycled Water for Avocado Irrigation

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Field Evaluation of Recycled Water for Avocado Irrigation

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CHAPTER 1

INTRODUCTION

INTRODUCTION

The advent of efficient systems for wastewater treatment offers a valuable opportunity for improving quantity and reliability of water supplies for irrigation of high-value crops. In turn, wastewater treatment plants can sell a product that can help offset operational costs or even contribute to profitability. To this end, it is important to determine the level of water treatment that will meet the needs of agricultural users. Farmers using this water also want to understand how to optimize their irrigation practices to facilitate the use of water with higher salinity to avoid yield losses and deterioration of soil quality. In 2013, a project addressing these issues was initiated with financial support from the San Diego County Water Authority, the Mission Resource Conservation District and the City of Escondido Water Utilities through a grant provided to the Escondido Growers for Agricultural Preservation (EGAP).

In this document, we report the results of a field experiment that compared the performance of young avocado trees that were irrigated either with potable water relatively low in salinity or with tertiary-treated wastewater (commonly referred to as "purple pipe water"). This wastewater contained both elevated salt concentrations as total dissolved salts (TDS) and higher concentrations of potentially toxic elements, including chloride, sodium, and boron. The recycled water also contained a relatively high concentration of nitrate-nitrogen that may negatively affect fruit yields. Furthermore, recycled water can alter pH of the soil, and alter solubility of macro- and micronutrients (AP Figure 2). Trees irrigated with recycled water were deficient in a number of micronutrients, most notably iron and zinc. This is most likely due to combined effects of elevated pH and excess nitrogen, both factors that have been shown to reduce iron uptake in avocado (Bar and Kafaki, 1992).

Ruehle (1940) has also demonstrated that high pH also induces zinc deficiency in avocado. A chart of plant nutrient availability at a range of soil pH values is shown in AP Figure 2. Significant deviation from optimal plant nutrition disrupts normal plant physiology and is likely to elicit a reduction in fruit yield or quality. Although not considered deficient, leaf magnesium concentrations were also lower in trees irrigated with recycled water. Iron and magnesium are involved in chlorophyll synthesis (Heldt and Heldt, 2005). Lower levels of magnesium and iron in leaf tissue were correlated with reduced chlorophyll content, and we can reasonably assume that this reduced photosynthetic rates. Trees irrigated with recycled water could not reach their full potential in terms of growth and yield, due to osmotic stress and nutrient imbalances.

Fruit quality was assessed for visual appearance, and data for fruit quality for consumption was obtained via a taste survey. Fruits from trees irrigated with recycled water had a higher rate and greater severity of fruit rot than fruit from trees irrigated with potable water. However, trees irrigated with recycled water actually earned the highest marks for flavor and aftertaste. This conclusion is somewhat skewed since rotten fruits were not served to taste survey participants. Poor fruit quality is often due to a low calcium-to-nitrogen ratio in the fruit. Fruit nutrient concentrations were not assessed, so leaf nutrient concentrations were used as an estimate of fruit nutrient status. Poor fruit quality in the form of fruit rot and a slimy coat was indeed associated with low leaf Ca:N ratios.

Presently, there are general guidelines for the suitability of different water supplies for irrigation of avocado, but the extent to which different water chemistries affect plant growth is not yet well understood. Current recommendations for the avocado industry are to irrigate with water having less than 600 mg/L of total dissolved salts (equivalent to ~1 dS/m electrical conductivity EC) and having chloride concentrations less than 80-100 mg/L.

The soil type further influences availability of different mineral ions for plant uptake. Soils having a clay texture are generally considered to be more problematic with respect to salinity management. Clay particles are negatively charged and have high surface area relative to sand or silt particles. This results in ample sites for ion adsorption; that is, chemical adherence to surface of clay particles. Clay soils have smaller pore size, leading to reduced infiltration and percolation rates. For these reasons, soils high in clay are difficult to leach and can accumulate ions when irrigated with recycled water, particularly boron.

Boron is an essential micronutrient needed for fruit set, but plants have a narrow range between deficiency and toxicity (Coetzer et. al, 1993). For most agricultural crops, boron concentrations in the irrigation water should not exceed 0.5 mg/L, and amounts above 1 mg/L can lead to accumulation of soil boron levels that are toxic to most crop plants. Avocado has a relatively low threshold for boron toxicity; the recommended upper limit for boron concentrations in irrigation water used for avocado production is 0.3 mg/L. Avocado growers should be cautious when switching to a new water supply with higher boron levels, even if it falls below the 0.3 mg/L threshold. Growers should also monitor boron concentrations via leaf and soil analyses on an annual basis. Avocado growers should also be careful to avoid over-irrigating, as this will introduce more boron than necessary to the soil. Unfortunately, once the critical point of excess boron has been reached, reducing the boron content of the soil may be slow due to the strong adsorption tendencies and it is very difficult to reverse damage to trees. Thus prevention is the best practice. (Joy, 2013)

Still another adjustment that must be made when using recycled water is to account for the additional nitrogen typically present in recycled wastewater. High levels of nitrogen can encourage vegetative growth at the expense of fruit production, and thus can result in reduced yields. This problem can be corrected by including the additional nitrogen from recycled water in fertilizer calculator programs, and is essentially a free fertilizer when the supply concentrations do not exceed plant demand.

The ability to use water supplies with elevated salinity and potentially toxic ions depends on good irrigation practices optimized to prevent salt accumulation in the root zone. This requires the use of a leaching fraction, which is a defined quantity of water to flush salts from the root zone. The precise volume of water needed to leach salts further depends on the soil texture class and the subsoil profile where water can become perched and salts precipitate into hardpans.

The site that was selected for this study has predominantly sandy loam soil with good drainage, and thus represented good conditions as compared to orchard locations on clay soils that are much more susceptible to salinization. However, clay content of the subsoil varied considerably across the site, and as described later, was correlated with salt and chloride retention. The trees used for the experiment were clonal trees with Hass avocado scions grafted on DUSA (Merensky 2) rootstocks. These are the most salt-tolerant of commercially used rootstocks, as compared to non-clonal seedling Mexican rootstocks that comprise approximately 80% of commercial plantings in California (Whiley, 2002). The trees were planted in late July 2013. All trees received high-quality potable water for the first two months to assure that they were well established before beginning the salinization treatments on the plots that were irrigated with recycled water. The study used 90 trees, half (45) receiving potable water for the entire time, and the other half receiving recycled water after the trees had become established. The two irrigation blocks were further segregated into groups of 15 trees each receiving different volumes of water, which was implemented by use of pressure-compensating micro-sprinklers delivering 9, 12, or 14 GPH (gallons per hour). The 14 GPH irrigation rate provided a leaching fraction of approximately 20%; 12 GPH met evapotranspiration demand without leaching, and 9 GPH resulted in a water deficit and no leaching. Irrigation with recycled water began in September 2013, after which tree growth parameters were recorded for two years. Leaf samples were collected annually in the Fall for tissue analyses to determine the concentrations of nutrient elements in the foliage and the concentrations of the potentially toxic elements chloride, sodium, and boron. We further monitored the soil water and salinity status continuously, and measured tree health via a number of parameters. Fruit production data were obtained in December 2014 and 2015. Fruit were harvested and analyzed for quality in March 2016.

Overall, the levels of salts and potentially toxic ions provided in the recycled water were very high as compared to those recommended for the industry. Average total salinity ranged from 1.4 – 1.7 dS/m with an average of 193 ppm chloride. This compares to maximum recommended values of 1 dS/m for irrigation water salinity, and 80-100 ppm for chloride. By comparison, salt concentrations of the potable water used for the control treatments were 0.89 dS/m and 80 ppm chloride. To monitor soil moisture and salt accumulation in the soils receiving the different irrigation treatments, the root zones of three trees per treatment were fitted with probes at 20 cm depth, approximately 0.5 meters from the trunk to measure the volumetric water content, soil water potential (plant available water) and electrical conductivity of the soil pore water over time. Soil data was collected over the entire 2 years at 30-minute intervals using data loggers. Soil cores were collected annually to record the distribution of salts in the soil profile and to determine the efficacy of the leaching that was obtained using the different water supplies and application levels. Data from soil cores collected on 1/1/16 are discussed in this report.

Results showed that, as expected, salts accumulated in the foliage of the trees receiving lower volumes of water and the trees that were irrigated with recycled water. However, due to variations in the clay content of the B horizon of the soil profile, we also observed high variability and increased salt accumulation in the pore water of trees irrigated at the highest water volume using 14 GPH emitters. This was attributed to possible perching of water on top of clay layers that precluded effective leaching. Application of large volumes of water also translates to increased amounts of salts that are introduced into the soil and that must thereafter be leached. The amount of water available as a leaching fraction also depends on the amount of water that is removed by the trees. Trees irrigated with potable water grew significantly larger than their counterparts irrigated with recycled water; thus these trees had higher water demand, and left less water available for leaching/percolation below the root-zone. The use of the soil water monitoring equipment allowed us to document and investigate these relationships in some detail, and provides guidance on the increased leaching requirement for trees receiving recycled water.

Results of this study showed that recycled water may be used for avocado irrigation, but that irrigation management becomes critical to prevent detrimental effects of soil salinization on tree growth. Additional treatment is also essential to maintain high-quality California avocados. With respect to biomass production (canopy size), trees watered with recycled water at the 14 GPH application rate were comparable to those watered with potable water at the 12 GPH rate. Thus approximately 15-20% more water may need to be applied when using recycled water. The chloride levels for trees receiving either potable water or recycled water treatments were all above the level (0.25%) that is predicted to decrease yields. In 2014, chloride levels in leaf tissues were 0.15-0.25% greater for trees watered with recycled water as compared to those irrigated with potable water at the same application rate. The higher leaf chloride content in trees irrigated with recycled water was not proportional to differences in the chloride content of the two water supplies. This can be attributed to the lower activity of chloride in water containing high levels of other soluble ions; i.e. high water salinity may partially offset and reduce the uptake of chloride. Leaf chloride levels in trees irrigated with potable water were also higher than expected, and this appears to be related to differences in the clay content in the soil under individual trees. This further demonstrates the need to lower chloride concentration in the wastewater, especially since not all orchards in Escondido may be as sandy as the present study site. Ideally, chloride levels in irrigation water having an EC < 1 dS/m would not exceed 80 ppm. More data is still required to determine the reduction in yield potential and uptake of chloride in water having different EC salinities to derive an economic optimization. A full economic optimization will also require information on the cost of the recycled water at different treatment levels. Since yield potential is lowered by increased chloride, and more water needs to be applied, recycled water must be competitively priced to offset yield reductions and the cost of the extra water for soil leaching.

Additional considerations with the use of recycled water will include the need for careful management of the nitrogen budget to prevent over-fertilization of the trees. Excess nitrogen can have as dire an effect on fruit yields as elevated chloride. On clay soils where there is concern for possible boron accumulation, growers should include a boron test in their annual soil and leaf testing to monitor the extent to which boron toxicity may become a problem. Altogether, the results demonstrate that recycled water can potentially be used for avocado production if treated to the recommended guidelines specified in this report. The experiment further illustrated the importance of monitoring soil water to assure good tree growth and to adjust the irrigation regime for effective soil leaching. Open questions include possible effects of recycled water on fruit quality, photosynthetic rates, root distribution, and pathogens and pests. Additional data also are needed to obtain yield information over several growing seasons to assess the long-term effect of salinity on yield potential. If possible, the experiment should be continued to obtain these data for an economic optimization and fair pricing of this valuable water supply. The detailed report follows.

Scientific background: salinity effects on soils and plant growth

Salinity affects plant growth through two mechanisms. The first involves decreased water availability due to the high osmotic potential of the soil pore water as salts accumulate in the root zone. Water enters plant roots via a process involving osmosis, in which water moves into the root cells in response to plant uptake of mineral ions. The availability of water is measured as the sum of forces that include the matrix potential (adsorption to soil particle surfaces) and the osmotic potential. When the water potential of the soil pore water becomes more negative than the water potential established by the presence of mineral ion salts in the root tissues, water can no longer be taken up by the plant. For avocado, this occurs at a salt concentration in the soil corresponding to ~ 4 decisiemens per meter (dS/m) when measured as electrical conductivity (EC) for a saturated paste extract. The relationship between irrigation water EC and the soil pore water EC has been well studied and suitability of different irrigation waters for plant species with a range of salinity tolerances has been established by the USDA Salinity Lab. (See AP Figure 1, Appendix). Previous work by Oster and Arpaia (2002) has shown that avocado yields for trees on Mexican rootstocks are detrimentally affected when trees are irrigated with water having greater than 0.8 dS/m.

The second mechanism by which salinity affects plant growth is via specific ion toxicities (Ayers and Westcott, 1984). While the relationship between irrigation water chloride and leaf chloride levels are still a subject of debate, a five-year study of six hundred avocado trees carried out by Dr. Crowley and funded by the California Avocado Commission has shown that yields decrease in a linear fashion as leaf chloride concentrations increase above 0.25% Cl. At leaf chloride concentrations of approximately 1%, fruit yield potential is close to zero, and avocado trees show severe leaf burn symptoms. Well before the appearance of leaf burn, root growth is impaired, leading to less efficient water and nutrient uptake, and leaf chlorophyll concentrations are decreased, leading to reduced photosynthesis and biomass production. With optimization of all nutrient elements in the tree, particularly calcium, there can be some offset of the harmful effects of chloride. However, production function models based on artificial neural network analysis of nutrient-yield patterns for avocado predict 80% losses for even the best trees when leaf chloride levels reach 1% (Crowley, personal communication).

Other indirect effects of saline water are incurred in clay soils where sodium ions cause dispersion of clay particles, sealing of the soil, and impaired drainage (Ayers and Westcott, 1984). Divalent cations (Ca^{2+} and Mg^{2+}) are able to bind 2 clay particles together. Sodium, a monovalent cation, can only bind to one clay particle, and it also attracts water molecules, which form a hydration sphere (Ayers and Westcott, 1984). At the micro-scale, this pushes clay particles apart; at the macro-scale, this reduces soil aggregate stability and creates a cement-like crust. This crust results in slower infiltration of water, increased water runoff, soil erosion, and poor soil aeration, which can result in simultaneous drought and hypoxia for the plant roots. In many plant species, hypoxia results in disruption of root cell homeostasis and the release of chloride from the vacuoles of the root cells into the xylem, allowing chloride to move to the plant leaf tissues. Avocado is especially sensitive to hypoxia (Haas, 1935). Thus poor soil aeration in conjunction with salinity can result in flash burns to the tree canopy caused by rapid accumulation of chloride in the leaf tissues in a period of days following a hypoxia event. The effects of sodium on soil aggregate dispersion are mitigated by calcium and magnesium, such that sodium effects are determined as a function of the concentrations of all 3 ions, also known as the sodium adsorption ratio (SAR). The current proposed target SAR for this recycled water is 2.7; avocado trees prefer irrigation water with SAR values of less than 2.

Recommendations to remove excess salts that accumulate in the root zone typically call for 10% excess water to be applied beyond that which is required to bring the top 20 cm of the soil to field capacity. Field capacity is defined as the water content that remains in the soil after excess water has drained and is usually reached 1-3 days following irrigation or a significant rainstorm. The water-holding capacity of the soil depends on the soil texture and soil pore space, which is typically about 50% of the soil volume. The water-filled pore space can range from 10% of the total porosity for a sandy soil to greater than 40% for a clay soil, leaving only 10% air-filled pore space to supply oxygen to the roots. Research from Raul Ferrera's group in Chile indicates soil having less than ~18% air-filled pore space can result in hypoxia. In the field experiment in this study, the orchard was planted on a sandy soil, with a loamy sand surface horizon on top of a sandy clay layer in the B horizon below 20 cm depth. Thus the site had potential for clay dispersion and perching of the water, even though the surface soil was initially well-drained. The accumulation of salts can be monitored using an electrical conductivity probe to measure salt concentrations with increasing depth by collection of soil cores. Alternatively, the salt accumulation can be measured with an *in situ* salinity probe. In the present experiment, we continuously monitored salinity in the root zone with a Decagon 5TE probe and also measured the distribution of salts in the soil profile annually.

Boron is a concern not only for avocado production, but also for any agricultural land where irrigation water contains high concentrations of this element. Although boron is essential for plant growth, it has a narrow range between deficiency and toxicity. Measuring boron in the soil is complicated by the fact that its concentration in irrigation water is not indicative of the boron concentration in the soil and depends on the soil mineralogy. Unlike other ions in irrigation water such as chloride and sulfate, boron cannot easily be leached and moves slowly through the root zone where it is held on anion exchange sites on clay particles. As the soil profile occupied by plant roots fills with high levels of boron, it then becomes increasingly toxic and very difficult to remediate. The rate of boron accumulation in soils is most closely correlated with soil clay content: the greater the percentage of clay in the soil, the more rapid the rate of boron accumulation.

Finally, the nitrogen and phosphorus contents of recycled water should be considered in the fertilization program. The current recommendation for optimal leaf nitrogen content is at 2.2-2.4% leaf N. Excess nitrogen is associated with decreased fruit yield, excessive vegetative growth, and poor-quality fruit that does not do well during post-harvest storage and ripening. Many online fertilization calculators will incorporate the additional nutrients present in the irrigation water. (http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=9361). Calculations are based on the acre-feet of irrigation water supplied and the nitrate concentration of the water in ppm, such that every 10 ppm NO_3^- in the irrigation water is equal to 6.2 lb of nitrogen per acre-foot of water applied. If a typical amount of 4 acre-feet is applied, this provides a substantial portion of the crop requirement. Determining proper fertilization requirements that complement the nutrients provided with recycled water is thus an important part of the nitrogen management.

CASE STUDIES

Case Study 1: Witman Ranch

A few avocado orchards are currently irrigated with treated municipal wastewater in Southern California. One such site, Witman Ranch, is located in Ramona, California and is irrigated with treated wastewater from the Ramona Wastewater Treatment Plant. Ramona's drinking water source is at an elevation of 500' and delivered by the San Diego County Water Authority. Ramona sits at an elevation of approximately 1,500' above sea level. To get potable water to the Ramona residents, water must be pumped up this 1,000' elevation differential, and the cost is passed onto customers. For agricultural users, potable water in Ramona costs $3,000/acre-foot; this is not financially feasible for avocado irrigation. The on-site well only yields 10 gallons per minute, which does not provide enough water for irrigating 65 acres. Thus the only option available for avocado irrigation at this particular site is the use of recycled water. The treatment plant is located at approximately 1,300' and the avocado orchard is a located up a steep hill, one mile away at an elevation of 1,800'. Ramona is not able to dispose of its wastewater into any water bodies, and thus must be able to reuse it locally.

Twenty years ago, Witman Ranch entered an agreement with the Ramona Wastewater Treatment Plant to buy treated wastewater at a cost of $35/acre-foot plus $200/acre-foot for the cost of pumping, for a total cost of $235/acre-foot. Initially, Witman Ranch received 500 acre-feet per year, and the volume has been slowly decreased to 220 acre-feet per year, as the demand for recycled water increases in the community. Lake Sutherland was the original water source for Ramona's potable water, however the community now uses water supplied by San Diego County Water Authority, which tends to be more saline, thus the recycled water from this water source is also more saline. Witman Ranch irrigates its mature trees at a rate of ½ gallon per hour for 18-24 hours, every 3-5 days, depending on weather (hot, dry, and/or windy days increase evaporative demand and require more frequent or deeper irrigation). The terrain is not as steep as many avocado orchards in Southern California. Much of the orchard is situated on soil with a hardpan at a depth of 12-18" below the soil surface.

The orchard was originally planted in 1986. When Witman Ranch first acquired the property in 1996, there were 150 acres of trees: 90 acres of avocados and 60 acres of grapefruit. The avocado trees are Hass fruit grafted onto Zutano rootstock. Due to reduction in water allocation, the grapefruit trees had to be removed and avocado acreage has been reduced to 65 acres. This provides enough water to remaining trees in order to ensure they are adequately irrigated. The fallowed land is currently bare, although a riparian zone has been planted with palms to curb water runoff and provide an additional source of income.

Prior to the installation of the reverse osmosis (RO) system, the average yield for years 2005-2009 was 7,643 lb/acre. After the installation of the RO system, the average yield has increased to 9,379 lb/acre for years 2010 – 2015. The trees strongly exhibit alternate bearing, which is a common phenomenon among avocado orchards in warmer climates. In 2015 for example, avocado yields were 3,000 lb/acre, and in 2016, avocado yields are an estimated 18,000 lb/acre. The manager attributes the increase in average yields to the recent installation of the reverse osmosis system at the Ramona Wastewater Treatment Plant in 2010, and average yields are still on an upward trend after accounting for alternate bearing; yields from 2015/2016 are higher than just after the installation of the RO system. With time, the soil is moving towards a new equilibrium more conducive for tree health and higher yields due to higher quality water. Prior to the installation of the RO system, water was more saline and contributed to root die-back, split fruit, and iron deficiency. Water analysis and leaf tissue analysis are shown in Tables 1 and 2, courtesy of Witman Ranch and Dellavalle Laboratory, Fresno, CA.

Leaf tissue analyses suggest the trees are in remarkable health, and the trees show negligible signs of salt burn on leaf tissue. However, boron and chloride concentrations in the leaf are potential cause for concern. As stated earlier, boron is an essential plant micronutrient that can quickly exceed toxic thresholds. Avocado is particularly sensitive to boron toxicity due to its low threshold relative to other crops. Furthermore, wastewater typically has higher concentrations of boron due to residential use of laundry and dish detergents containing boron. Caution should be exercised to avoid boron toxicity. Of alarming importance is that once the toxic threshold is reached, little can be done to save the orchard. This is particularly critical for soils with high clay content, shallow hardpans, or poor drainage.

Part of the agreement between the Ramona Wastewater Treatment Plant and Witman Ranch includes an allocation of potable water that amounts to 10% of the orchard's annual water use. This can be used whenever, however, and wherever the manager sees fit. It can be blended in with the recycled water, or used exclusively on one part of the orchard year-round, or used seasonally on a larger part of the orchard, perhaps in areas more prone to salt build-up in the summer, such as south-facing slopes or soils higher in clay.

The Witman Ranch-Ramona partnership demonstrates that avocado irrigation with recycled water can be successful. Furthermore, such programs are mutually beneficial to both avocado growers and wastewater treatment plants when properly coordinated.

Water Quality Parameter	**Normal Range**	**Ramona Wastewater** (12/2014)
Electrical Conductivity (dS/m)	0.60 – 1.30	1.09
Calcium (mEq/L)	5.0 – 10.0	**2.77**
Magnesium (mEq/L)	1.1 – 5.0	1.70
Sodium (mEq/L)	< 4.0	**5.20**
Sodium Adsorption Ratio (SAR)	< 0.1 – 2.0	**3.50**
Adjusted SAR	< 0.1 – 2.0	**6.10**
Chloride (mEq/L)	0.1 – 1.2	**4.20**
Carbonate/Bicarbonate (mEq/L)	0.1 – 2.5	2.10
Sulfate, SO_4^{2-} (mEq/L)	0.1 – 5.0	3.60
Boron (mg/L)	0.01 – 0.4	**0.40**
NO_3-N (mg/L)	0.1 – 5.0	**7.5**
Iron (mg/L)	< 0.20	< 0.10
Manganese (mg/L)	< .20	< 0.02
pH	6.8 – 7.9	**8.4**
Langelier Index	-0.3 – 0.5	**0.7**

Table 1. Ramona Wastewater Water Quality. Values in the right-hand column shown in blue indicate lower-than-optimal values, and values shown in red indicate higher-than-optimal values. Black values indicate within normal range. Courtesy of Dellavalle Laboratories, Fresno, CA.

Tissue Nutrient Analysis	**Normal Range**	**Witman Ranch** (September 2015)
Total Nitrogen (%)	2.4 – 2.7	2.26
Total Phosphorus (%)	0.14 – 0.35	0.17
Total Potassium (%)	1.0 – 2.5	1.35
Total Zinc (mg/kg)	40 – 120	53
Total Manganese (mg/kg)	30 – 500	66
Total Sodium (%)	< 0.08	< 0.01
Total Boron (mg/kg)	40 – 80	**111**
Total Calcium (%)	1.0 – 3.0	2.04
Total Magnesium (%)	0.25 – 0.80	0.71
Total Iron (mg/kg)	50 – 300	85
Total Copper (mg/kg)	5 – 500	**4**
Chloride extract (%)	< 0.40	**0.6**

Table 2. Ramona Leaf Tissue Analysis. Values in the right-hand column shown in blue indicate lower-than-optimal values, and values shown in red indicate higher-than-optimal values. Black values indicate within normal range. Courtesy of Dellavalle Laboratories, Fresno, CA.

Figure 1. Riparian zone planted with palm trees to reduce irrigation runoff. In the background, the trees on the left side of the hill recently died in a wildfire.

Figure 2. 30-year old trees at Witman Ranch doing well under irrigation with recycled water.

Figure 3. View of the healthy avocado orchard near the water reservoir.

Figure 4. Another view of the Witman Ranch avocado orchard near the water reservoir.

All photos of Witman Ranch were taken on April 20, 2016.

Case Study 2: City of Escondido 1996 Avocado Pilot Project

A previous five-year study from 1991-1996 by Dr. Gary Bender investigated the potential use of Escondido recycled water for commercial avocado irrigation. The study was performed on mature trees (15-20 years old) with 4 irrigation treatments: A) Potable water (control), B) 50/50 blend of recycled and potable water, C) 100% recycled water to meet evapotranspiration, and D) 140% recycled water (a 0.4 leaching fraction). While the initial study demonstrated that tertiary reclaimed water is unsuitable for avocados, it is likely that additional water treatment can make this feasible. (Bender, et. *al*. 1996).

The site was located on well-drained granitic soil with steep slopes typical of avocado orchards in the region. Results from the study showed that soil salinity, and particularly chloride, increased to a greater degree in soils irrigated with reclaimed water relative to the soils under potable water irrigation. Winter rains leached the salts from the soil annually, thus avoiding an overall increase in salinity throughout the trial. The potable water treatment (Treatment A) showed the lowest salinity, while the 100% recycled water had the highest increases in salinity (Treatment D). Treatments B (50/50 blend) and D (140% recycled water) showed the same pattern in regards to soil salinity and were higher than Treatment A and lower than Treatment D. Treatment D flushed salts from the upper layers of the soil profile. Reclaimed water lowered the soil pH to a greater extent than soils under potable water irrigation, likely due to low buffering capacity of the soil. The Sodium Adsorption Ratio (SAR) of the water was not problematic owing to the coarse granitic soil. Authors of the study emphasized that sodium may likely be an issue in soils with higher clay content.

Leaves from trees irrigated with recycled water accumulated higher amounts of chloride, while boron did not accumulate in leaves in any of the irrigation treatments. More recent evidence has shown that soil texture, in particular the amount of clay present, is most predictive of orchards that will be plagued with boron toxicity under waters with elevated boron concentrations (Joy, 2013). Contrary to expectations, leaf nitrogen levels in trees irrigated with recycled water were equivalent to trees irrigated with potable water, despite elevated levels of nitrogen in the reclaimed water. There was no apparent benefit from elevated nitrogen concentrations in the reclaimed water. Potassium was the only nutrient elevated in trees irrigated with recycled water.

Yield among the trees irrigated with recycled water were significantly lower than trees irrigated with potable water. Yields for Treatments B and D (50/50 blend and 140% ET recycled water), were both 27% less than yields for Treatment A (Potable water). Yield was reduced by 42% for Treatment C (100% ET recycled water) relative to Treatment A.

This study was performed for 5 years, since a change in water supply may not produce noticeable differences in yields from mature avocado trees for at least 3 years. Authors concluded that in order to manage a successful avocado irrigation program with recycled water, it is necessary to improve water quality prior to its arrival at the treatment facility; this implies the need to reduce pollutants and salts entering the waste stream. Additional technologies and techniques have become available for wastewater treatment, and if these prove cost-effective, this could also help improve water quality for avocado irrigation. A breeding program for salt-tolerant root-stocks would be equally beneficial.

CHAPTER 2

SOIL CONDITIONS

Materials and Methods

The block design, layout of the trees, and layout of the irrigation lines for the field experiment reported here are provided in the Appendix (AP Figure 3). In brief, the trees were arranged in irrigation blocks with each block receiving either potable water or recycled water. Within the blocks, three lines were laid out with emitters providing 9, 12, or 14 gallons per hour. One year-old trees with clonal DUSA rootstocks and Hass scions were planted at 15’ spacing between trees within the rows, and 20’ spacing between rows. The trees were planted in July 2013. The irrigation regime was controlled by an automated system with manual over-rides for irrigation control on each block. Recycled water was delivered by truck and stored in 10,000 gallon tanks until used for irrigation. The irrigation water amounts that were applied are shown in relation to the estimated water requirement for avocado based on evapotranspiration (ET) data from the California Irrigation Management Information System (CIMIS). As shown in Figure 5, the amounts supplied by month closely matched ET, and provided levels both below and above the ET value with an avocado crop coefficient value of 0.72. Cumulative volumes of water applied per tree are listed in the Appendix AP Table 1.

The effects of the irrigation regimes on soil water status were followed using measurements of soil water potential and volumetric water content (VWC) using Decagon soil water potential probes that were placed in the same location with the 5TE probes for measurements of VWC. As shown in Figure 6, the irrigation regime maintained the soil water potential at greater than -100 centibars (cb) for the first year, with soil drying events occurring frequently during the summer. In the second year, the soil was considerably drier and the trees watered with only 9 GPH emitters experienced frequent drought with soil

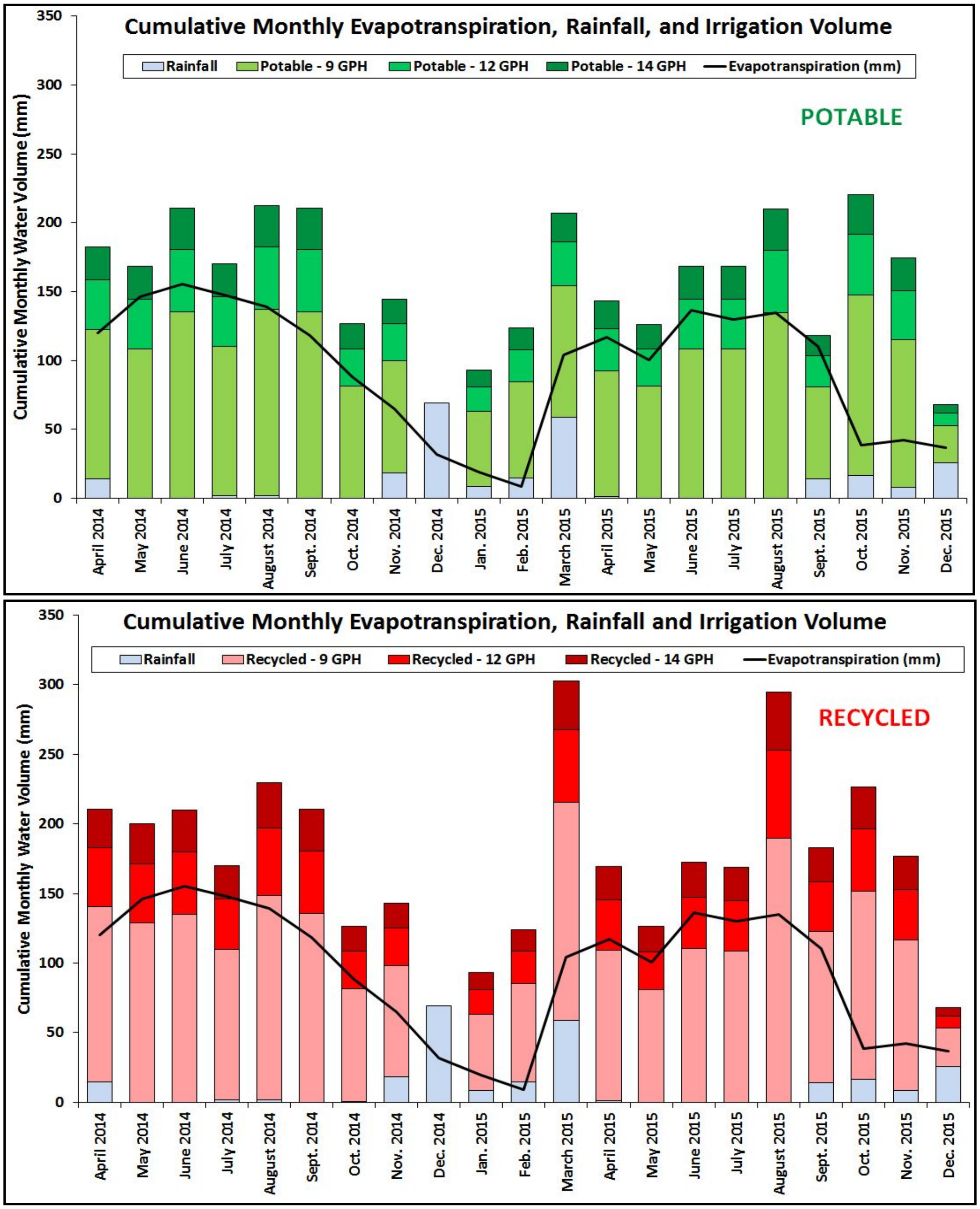
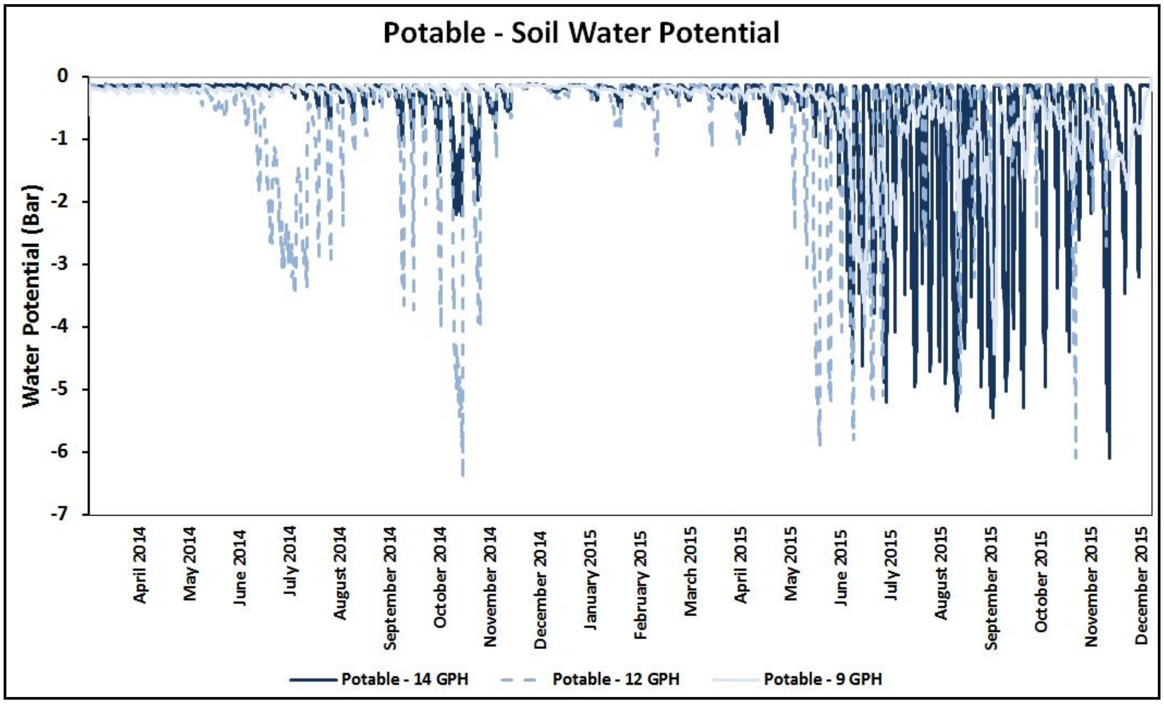


Figure 5. Cumulative monthly evapotranspiration and irrigation. Volume Water regime applied for trees irrigated with different amounts of water using 9, 12, and 14 GPH emitters to provide different levels of leaching. Blue portion of each bar is water from precipitation. Top graph (green bars) represents potable irrigation volumes, bottom graph (red bars) represents recycled irrigation volumes. Emitter rates: light green (light red): 9 GPH; medium green (medium red) 12 GPH; dark green (medium red) 14 GPH.



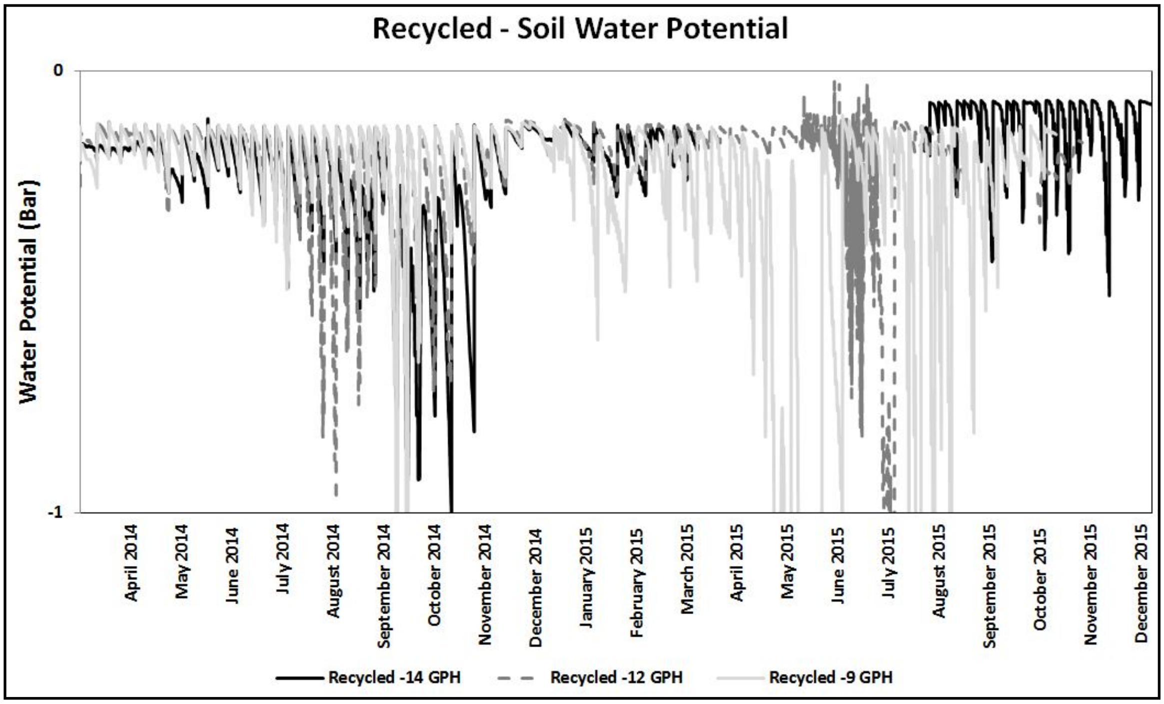


Figure 6. Soil water potential. Plant water availability as determined by measurements of soil water potential for the field experiment examining use of recycled water for avocado irrigation. Note differences in scales for the y axis (water potential) in top and bottom figures.

water potentials approaching -500 cb during the late summer. Altogether, the data demonstrate that the designed irrigation system successfully achieved our goal of establishing different levels of leaching and potential for accumulation of salt via the use of different leaching fractions.

In the field experiment, we hypothesized that trees receiving the lowest quantity of water would experience the greatest problems with salinity and that reductions in plant growth and yield would be more severe in the recycled water treatments. The soils in the Recycled – 9 GPH treatment were expected to accumulate the most salt in the root zone, due to inadequate leaching, whereas trees in the 12 GPH received water in amounts close to ET and thus were leached mainly during precipitation events. Trees receiving 14 GPH of either potable water or recycled water received a 15% leaching fraction. Our second hypothesis was that trees would have reduced growth and higher levels of leaf chloride and nitrogen than trees receiving potable water at the same application rates.

Fertilization

Trees were fertilized with calcium nitrate as needed. In February through October 2014, a total of 5 oz was applied per tree. In February through June 2015, a total of 1.6 lb (25.6 oz) was applied per tree. Leaf nutrient concentration analyses from 1st year of growth showed elevated leaf nitrogen content in trees irrigated with recycled water, so fertilization was halted on these trees in July 2015.

Soil samples

Soil samples were collected as soil cores using a soil auger. Soil cores were extracted to a depth of 24" below the soil surface, at a distance 20" (50 cm) away from the trunk of the tree on the north side. Approximately 40 mL of soil were collected at 6" intervals (0", 6", 12", 18", and 24") below the soil surface. A soil knife was used to extract the soil samples from the intact soil core. Soil samples were double-bagged in plastic Ziploc bags and kept in the shade while the rest of the soil samples were collected. 3 sets of soil samples were collected per irrigation treatment, with the exception of Recycled – 14 GPH irrigation treatment, we were only able to collect 2 soil samples from this treatment on 1/1/16.

Soil samples were analyzed for pH and salinity content using 1:2 soil dilutions. 20 mL of distilled water was added to 50-mL Falcon tubes. Soil was added to the tubes until the total volume for the tubes reached 30 mL. The Falcon tubes were capped, shaken, and allowed to settle for at least 6 hours. 7 mL of water in the tube was removed using a 10 mL pipette and added to a 15 mL beaker. Salinity was measured using an ExStik II Salinity pen (Extech, Nashua NH) and pH was measured using a LaMotte Tracer PockeTester pH/conductivity/TDS/Salinity meter (LaMotte Company, Chestertown, MD). The salinity pen was rinsed with distilled water and blotted dry between sample measurements. Measurements were recorded and multiplied by 4 to provide an estimate of saturated paste extract soil salinity values. Soil clay content was estimated by quantifying the volume of sand, silt and clay after settling in the tube and calculating the percentage of clay as a fraction of the total volume of soil within each tube.

Soil probes collect soil electrical conductivity, temperature, volumetric water content, and soil water potential measurements every 30 minutes. Soil probes were placed

50 cm from the tree trunk on the south side of the tree on 3 trees per treatment. Each tree has a 5TE probe at 20 cm below the soil surface, and one of the three trees also has a 5TE probe at 40 cm below the soil surface, as well as a water potential meter.

RESULTS

Irrigation treatments, leaching, and water potential

The soil moisture profiles from the Decagon soil monitoring equipment illustrate that the target water levels were achieved to provide a distinct contrast between the treatments with regard to the leaching fraction. During the first year, trees irrigated with potable water at 9 GPH were routinely water stressed, with the soil drying to -400 cb. Trees watered at 12 and 14 GPH were mostly maintained at less than -100 cb (Figure 6). This changed during the second year as the trees grew much larger and had increased water requirements. Thus greater water stress was experienced for trees in the potable water treatment, indicating that the soil was not as effectively leached and that the irrigation duration and/or frequency may need to be increased.

Salt accumulation in the soil profile

Soil salinity in the root zone was monitored by *in situ* measurements of salinity measured as electrical conductivity by 5TE Decagon probes. The distribution of salts in the soil was further examined by removal of soil cores and measurement of salinity for 1:2 soil water extracts. Values for 2:1 soil water extracts were multiplied by 4 to convert to approximate values for EC measured for saturated paste extracts. Results from 1/1/16 soil sampling are reported in Figure 7.

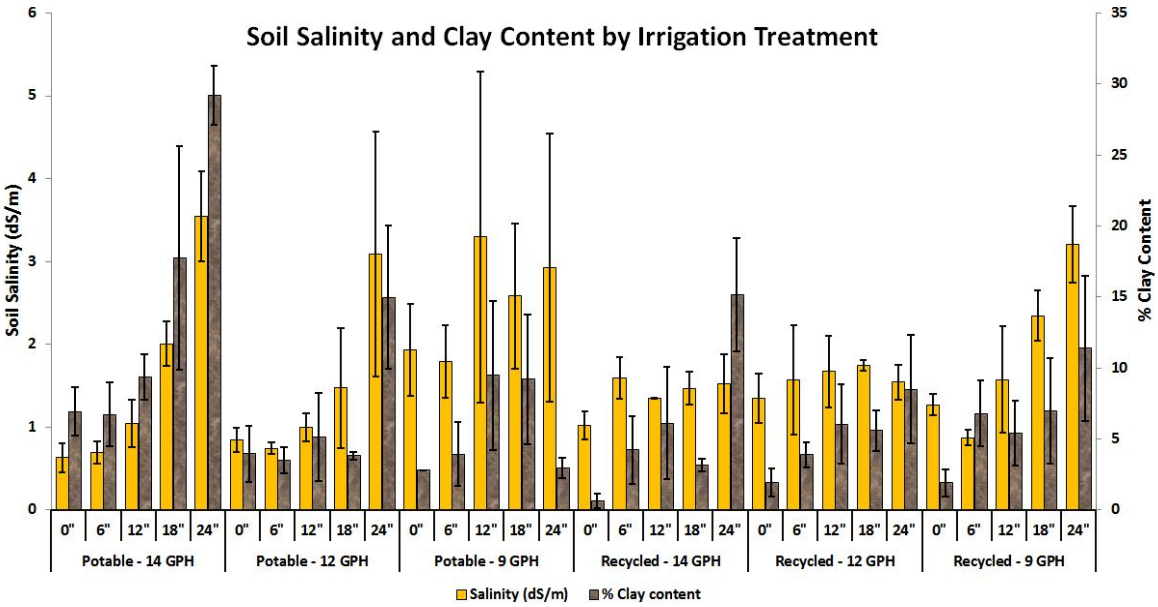


Figure 7. Soil salinity and soil clay content with depth by irrigation treatment (double axis graph). Soil salinity as the saturated paste extract values via 2:1 dilution, represented in dS/m (yellow bars, left axis), and percent clay content (brown bars, right axis). Vertical error bars indicate standard error values.

Soil salinity profiles generated in this snapshot did not follow the expected result that salt accumulation would be higher in the irrigation treatments receiving recycled water, but instead were more closely correlated with the clay content of the soil. The clay content increased with depth in the soil profile and varied across the field. The highest subsoil clay content was found under the trees receiving 14 GPH of potable water. These trees had the highest fruit yields and best growth rates, but also demonstrated symptoms of leaf burn that are typically associated with salinity and chloride toxicity. Trees in this treatment also had the greatest canopy volumes and greater water demand, and received more total salt due to the higher amount of irrigation water applied.

Pore Water Electrical Conductivity

Soil pore water electrical conductivity (EC_p) varies inversely with soil water content. The results are reported here and compared with saturated paste extract salinity values (EC_e), which allow comparison of soils with differences in volumetric water content. EC_p values can temporarily be higher than corresponding EC_e values, as a temporary increase in salinity typically corresponds with a temporary decrease in soil moisture due to intervals between irrigation or rainfall. High salinity readings via EC_p are not necessarily cause for concern as these are not equivalent to recommended EC_e values.

Potable – 9 GPH initially had the lowest pore water salinity values at 20 cm depth (~1-2 dS/m), but started to increase during the late Spring, peaking in Summer 2015 at 6 dS/m. Peak salinity values were highest for Potable – 9 GPH, as might be expected, due to inadequate leaching and thus concentrations of salt in the upper layers of the soil profile. Salinity values for Potable – 9 GPH dropped off starting in Fall 2015, as expected, due to cooler temperatures and leaching from winter rains. At 40 cm depth below the soil surface, soil salinity peaked at 8 dS/m in October 2014, and then decreased.

Potable – 12 GPH consistently had low salinity at 20 cm below soil surface (~1-2 dS/m), with a peak mid-summer at 4 dS/m. The lower salinity can be attributed to lower clay content in this irrigation block, as well an adequate amount water applied to meet evapotranspiration demand. At 40 cm below the soil surface, soil salinity remained relatively constant, at 2-3 dS/m throughout the year.

Potable – 14 GPH consistently had the highest salinity year-round (3-4 dS/m); there was little fluctuation in salinity throughout the year, and winter leaching was delayed. Potable – 14 GPH salinity peaked at 5 dS/m in September 2015, while Potable 9 GPH and

Potable – 12 GPH peaked in June and July 2015. These results are likely due to the higher clay content in the soil, as clay soils tend to retain salts in higher concentrations and for longer periods of time. At 40 cm below the soil surface, soil salinity peaked at 11 dS/m in late Fall and early Winter months. Salinity did not drop off until early Spring 2015, and again started to climb in August 2015. This data confirms a delay in leaching, likely due to the high clay content found in the soil.

Recycled – 9 GPH had an increase in salinity over time that was not leached out by winter rains. Salinity increased at 40 cm during the winter months; this can be attributed to inadequate leaching. Initial salinity was 2.3 dS/m at 20 cm (2 dS/m at 40 cm). Peak salinity was 10.2 dS/m at 20 cm depth, and 5 dS/m at 40 cm depth, suggesting salinity accumulates at upper levels of the soil profile. Average salinity values were 5 dS/m and 3.6 dS/m for 20 and 40 cm depths, respectively.

Recycled – 12 GPH Winter salinity values read around 2.5 dS/m. Salinity showed a slight increase in Summer 2014, reaching 5 dS/m, and peaked in August – October 2015, reaching 8 dS/m.

Recycled – 14 GPH showed the largest seasonal fluctuations in soil salinity at both 20 cm and 40 cm depths. Salinity peaked at 20 cm depth in October 2014 (16 dS/m) and June 2015 (13 dS/m). During winter months, salinity dropped to 3-4 dS/m. The large fluctuation can be attributed to large volumes of saline water applied, which are subsequently leached in the winter months.

Volumetric Water Content

If soil conditions were uniform, it would be expected that Potable – 14 GPH would have the highest volumetric water content (VWC); however, Potable – 12 GPH has the highest volumetric water content at both 20 and 40 cm depths below the soil surface. At 20 cm below the surface, Potable – 12 GPH has a VWC value of 15-18% m^3/m^3; the upper limit of the range occurs in the winter months, while the lower limit of this range occurs in the summer months, as might be expected.

Potable – 9 GPH and Potable – 14 GPH approximated each other, hovering around 14% VWC during winter months with a drop in summer months to 10% for Potable – 9 GPH, and a drop to 12% VWC for Potable – 14 GPH.

At 40 cm below the soil surface, all irrigation treatments showed a trend of decreasing volumetric water content over the course of the experiment; the water content reached a plateau in winter months, and then continued to drop in the following summer months. Volumetric water content was again highest for Potable – 12 GPH (initially 16%, plateau at 14% during winter months, and declined to 12%), and lowest for Potable 9 GPH (initially 13%, plateau at 12% during winter months, and declined to 10%). This pattern can be explained by the high clay content in the Potable – 14 GPH, which retained moisture in the upper layers of the soil profile and prevented percolation to lower soil depths. At 9 GPH, there was not adequate leaching for water to reach the lower depths of the soil profile, as indicated by higher water content at 20 cm (14%). Growing trees will also extract more water as time progresses, thus lowering soil water content, particularly at depth, since less water is available for percolation.

Recycled – 9 GPH water content fluctuated from 10% (summer and fall) to 12% (winter and spring), with field capacity at 19.5% in winter months.

Recycled – 12 GPH had the highest volumetric water content (20% at field capacity during winter months), and the lowest reading 12% just prior to irrigation in the winter months; 18% just after irrigation, and 10% just prior to summer irrigations. Water content at field capacity at 40 cm depth was 24.5%.

Recycled – 14 GPH had the lowest volumetric water content at 20 cm below soil surface (11% in winter and spring months, 9% in summer and fall months). Water content at 40 cm depth was higher, ranging from 12-15%; the lower limit of the range for summer months, and the upper limit of the range during winter months. Water content at field capacity was 19.6% at 40 cm depth.

Table 3. Summary table for soil salinity and water content as measured by Datatrac probes.

Irrigation Treatment	**Salinity** (dS/m)						**% Volumetric Water Content (VWC)**					
	Minimum		Average		Peak		Minimum		Average		Field Capacity	
Depth	20 cm	40 cm	20 cm	40 cm	20 cm	40 cm	20 cm	40 cm	20 cm	40 cm	0 cm	0 cm
Potable - 14 GPH	1.5	3	4	7	5	11	12	9.5	13	12	ND*	ND*
Potable - 12 GPH	1	2	1.8	2.6	4	3	15	12	16	14	ND*	16
Potable - 9 GPH	1	2	5	3.2	6	8	10	10	14	12	ND*	13
Recycled -14 GPH	3	1.7	8	3.9	16	7	9	12	11	15	16	19.6
Recycled -12 GPH	2.5	1.4	5	ND*	8	4.9	9	10	14	16	20	24.5
Recycled - 9 GPH	2.3	2	5	3.6	10.2	5	10	8	12	12	19.5	17

ND* = No data at time of publication

Soil Sodium Adsorption Ratio

As expected due to higher sodium content in recycled water, the soil Sodium Adsorption Ratio (SAR) was elevated in the recycled water treatments (Figure 8). High SAR values reduce soil water infiltration and soil aeration while encouraging run-off and erosion; the degree of severity increases with the amount of clay present in the soil (Ayers and Westcott, 1984). The maximum recommended soil SAR for avocado is 5 (Bender, 2010). In this study, the soils irrigated with recycled water reached SAR values in exceedance of 6. Thus, sodium content of the water should be reduced.

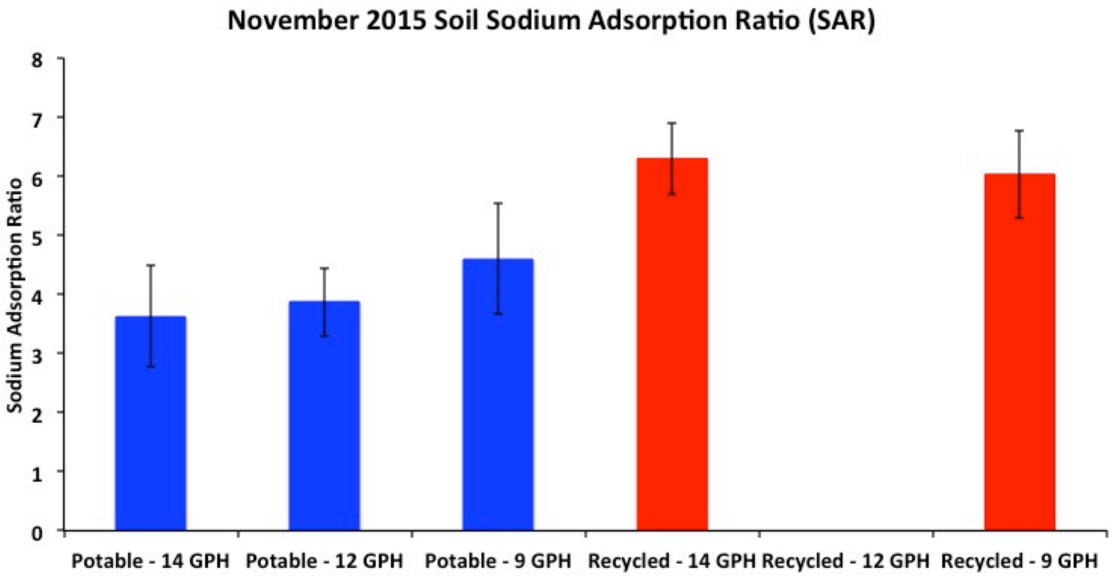


Figure 8. November 2015 Average Soil Sodium Adsorption Ratio (SAR). Data not available for Recycled – 12 GPH. Error bars indicate standard error values.

SAR	POTABLE			RECYCLED		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2015	3.63	3.87	4.60	6.30		6.03

Soil pH

As shown in Figure 9, the average soil pH ranged between 7.0 - 8.0 for all treatments. Soil pH was slightly higher in the recycled water treatments, with the exception of the Potable – 9 GPH treatment. Data should be viewed conservatively, since there were only 3 samples taken per irrigation treatment per year, and there is considerable variation within treatments. The pH of the soil affects mineral solubility and bioavailability for plants. Higher pH limits availability of micronutrients such as iron and zinc (AP Figure 7). Trees irrigated with recycled water in the present study had reduced concentrations of iron in their leaves relative to trees irrigated with potable water (Figure 43).

As mentioned previously, trees at Witman Ranch also exhibited symptoms of iron deficiency. Avocado trees at South Coast Research Station in Irvine, CA were recently switched to irrigation with recycled water, and iron supplementation has since become necessary (Focht, personal communication, 2016). The phenomenon of iron deficiency appears to be pervasive in avocado trees irrigated with recycled water in Southern California and is likely due to higher soil pH values induced by the high mineral content in recycled water. Furthermore, many parent materials in avocado-growing regions are calcareous and thus already naturally have a high pH. Addition of recycled water rich in minerals such as calcium exacerbates any existing problems with high soil pH. The combined experiences and data from existing avocado orchards irrigated with recycled water demonstrates the need to monitor soil pH and leaf nutrient concentrations and make adjustments as needed.

Granular fertilizer was applied as calcium nitrate, and both calcium and nitrate would raise soil pH. This would explain a high pH in trees irrigated with potable water, as they were fertilized with $Ca(N0_3)_2$, while fertilization was stopped on trees irrigated with recycled water because the water already provided needed nutrients. Excessive nutrients are wasteful, pollute groundwater, alter soil chemistry, and can actually harm tree health, reduce yield and fruit quality. Leaf calcium content was reduced in 2015 in trees irrigated with recycled water, potentially due to the elimination of fertilization via calcium nitrate. The average pH of the potable water was 8.0, which was higher than the pH of the recycled water (7.5). This would also narrow the differences in soil pH between potable water and recycled water treatments by elevating the pH in soils irrigated with potable water. Carbonate concentrations tend to be elevated in recycled water (Pettymeyer and Ashano, 1984). Carbonates buffer soil pH and keep it more alkaline. Carbonates also interfere with absorption of micronutrients such as iron. Citrate is a transporter of iron in plants, and in the presence of high soil carbonate concentrations, citrate will preferentially bind to calcium instead of iron or zinc (Morissey and Guerinot, 2009). This can lead to iron and zinc deficiencies, which were observed in trees irrigated with recycled water in the present study.

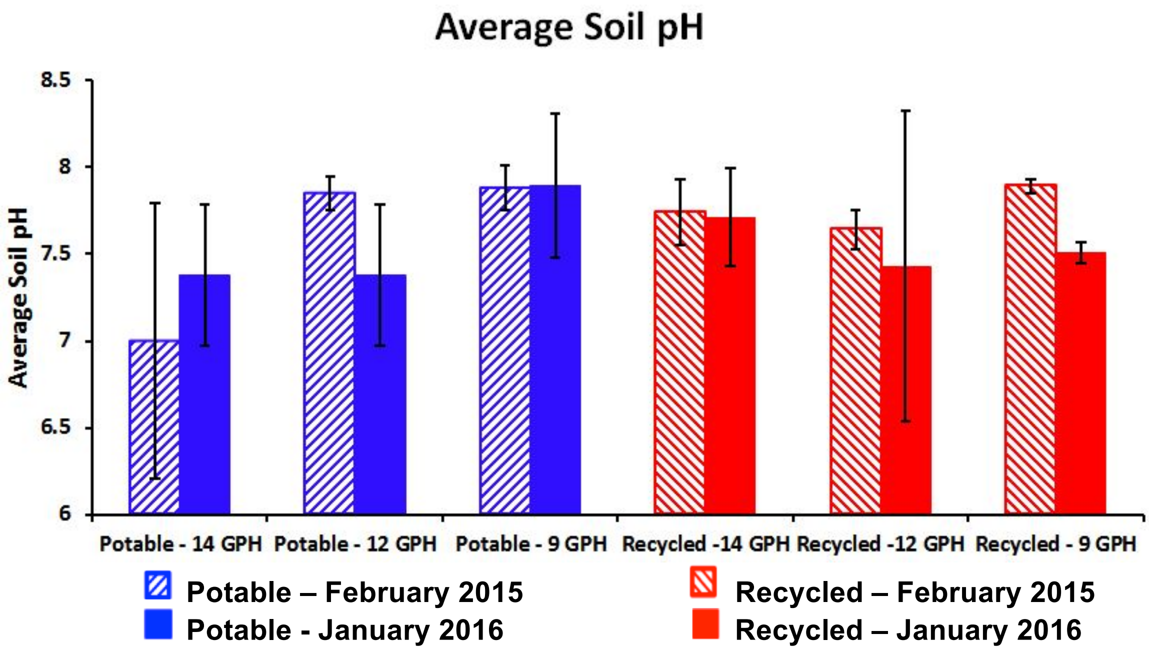


Figure 9. Average Soil pH. Measurements were made on soil taken from 6” below soil surface, 50 cm away from trunk of tree. Note the scale on the y-axis only runs from pH 6 to pH 8.5 for higher resolution. Error bars indicate standard error values. November 2015 soil pH data shown in table below, but not represented in above graph.

Average Soil pH	POTABLE			RECYCLED		
	14 GPH	12 GPH	9 GPH	14 GPH	12 GPH	9 GPH
February 2015	6.99	7.85	7.88	7.74	7.64	7.89
November 2015*	6.87	7.15	6.92	7.60	No Data	7.02
January 2016	7.38	7.38	7.89	7.71	7.43	7.50

CHAPTER 3

TREE HEALTH

MATERIALS AND METHODS

Monitoring Tree Health

Trees were monitored for the following parameters: tree canopy volume, tree trunk diameter, leaf salt-burn damage, leaf chlorophyll content, general tree health and appearance. Leaf nutrient concentrations and fruit yield quality are discussed in Chapter 6. Statistical differences were calculated via 1-way ANOVA in SPSS, using a p-value of $P < 0.05$.

Calculating Canopy Volume

Trees in this field study most closely resembled the general shape of a cone with an ellipsoid base. Trees were measured using a tape measure. Since trees were wider in one direction at the base, trees were measured at the base in 2 directions perpendicular to each other. The tallest point of the tree was used to measure tree height. Tree canopy volume was thus calculated using the formula for determining the volume of a cone with ellipsoid base: Volume = ½ (base width 1) x (base width 2) x height. In a few cases, the tallest part of the tree was off to the side rather than in the center, so these parts of the tree were "visually moved" to the center of the tree to verify that they tree still resembled a cone in total volume, with volume gradually decreasing towards the top of the tree.

Salt-burn Damage

Trees were visually assessed for salt-burn damage annually in the Fall. Trees were assigned a number 0-5 depending on the severity of salt-burn damage to leaf tissue. No damage was assigned a 0 rating, whereas severe damage covering at least 50% of the leaf tissue on 75% or more of the canopy was assigned a rating of 5. Decimals were used when appropriate, for example a tree showing minimal salt-burn damage on a small number of leaves was given a 0.1 rating, and trees that appeared worse than a 2, but not quite 3, were given a rating of 2.75.

Chlorophyll Content

Leaf chlorophyll content was measured using a Minolta SPAD-502 meter (Konica Minolta, Japan). 5 fully expanded leaves closest to the growing tip of the branch were selected and measured while still attached to the tree. Leaves were measured on all sides of the tree rather than just one side of the tree to account for variations in the tree. To assure that 5 leaves per tree was an adequate number, 25 leaves were measured on 3 trees per treatment to note if the side of the tree significantly affects leaf chlorophyll content; it did not. Leaf chlorophyll concentration is reported in micrograms of chlorophyll per square centimeter of leaf tissue. In October 2014, there was not enough time to measure chlorophyll of all trees, so only 5 trees per treatment were measured (30 trees total for the entire experimental plot). In May 2015 and November 2015 all 90 trees in the plot were measured.

Trunk diameter

Trunk diameter was measured using vernier calipers. Measurements were taken on all trees in October 2014 and March 2016, 5 cm above the rootstock-scion graft where the tree trunk returned to normal diameter.

RESULTS

Tree Growth

Growth of the trees was significantly affected by the quantity and quality of the irrigation water applied. All of the trees receiving potable water grew well, irrespective of the water application rate (Figure 10). In contrast, growth of the trees irrigated with recycled water depended on the water application rate, with trees receiving 14 GPH performing the best, and trees receiving 9 GPH performing the worst. The differences were even more apparent at the end of the second year (Figure 10, Figure 12).

Canopy Volume

Avocado tree growth performance was measured with respect to canopy volume in October 2014 and in March 2015. Most of the trees had a large base with decreasing foliage volume towards the top. Tree canopy volume was thus calculated using the formula for determining the volume of a cone with ellipsoid base:

$$\text{Volume} = \tfrac{1}{2}\,(\text{base width 1}) \times (\text{base width 2}) \times \text{height}$$

As documented in the photographs (Figures 11 and 12) and by physical measurements (Figure 10), the canopy volumes were smallest for the trees in the recycled water treatment with water applied at the 9 GPH rate. At each application rate, trees receiving potable water were larger in size than those receiving the same amounts of water as recycled water. Trees receiving recycled water at 12 and 14 GPH rates were comparable in size to the trees receiving 9 GPH in the potable water treatment. The largest trees were those receiving 12 and 14 GPH of potable water, which were approximately 25% larger than trees receiving recycled water at the same corresponding application rates.

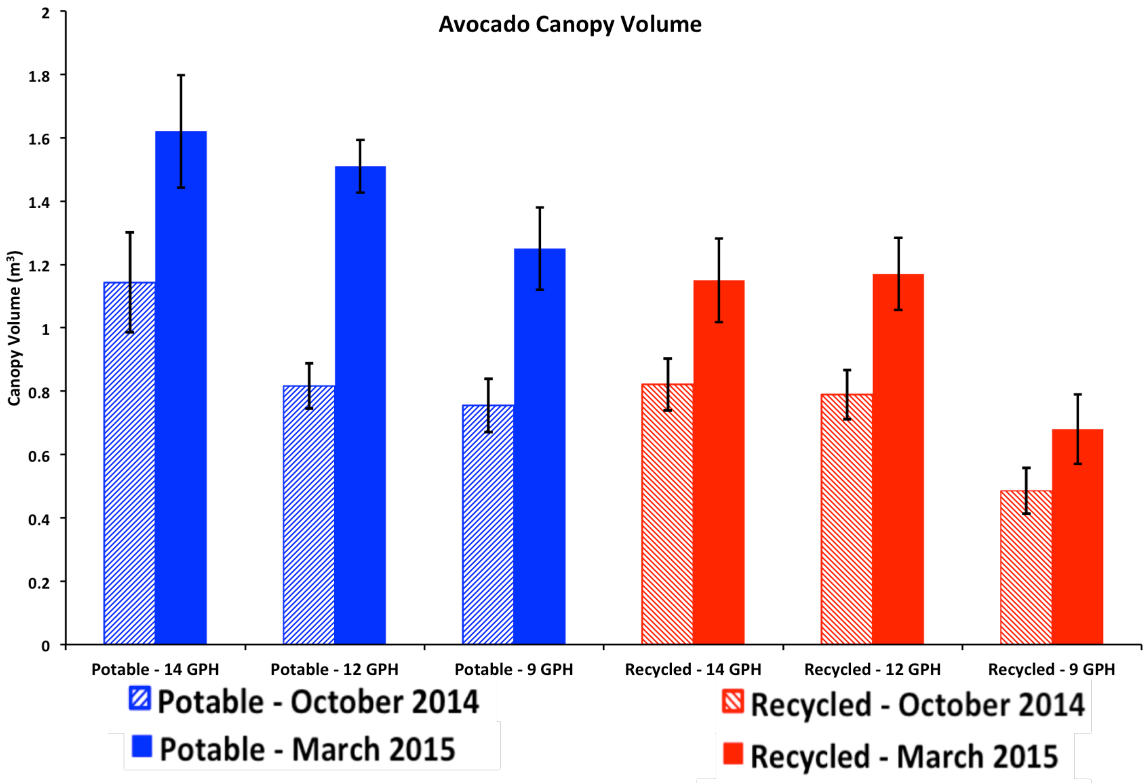


Figure 10. Tree canopy volume. Vertical error bars indicate standard error values. Average values are shown in the table below. $P < 0.05$

Canopy volume (m^3)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
October 2014	1.14	0.82	0.75	0.82	0.79	0.49
March 2015	1.62	1.51	1.25	1.15	1.17	0.68

Canopy volume ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
October 2014	a	ab	ab	ab	ab	b
March 2015	ac	ac	a	a	a	ab

Canopy Volume - October 2014

Potable – 14 GPH **Potable – 12 GPH** **Potable – 9 GPH**

Recycled – 14 GPH **Recycled – 12 GPH** **Recycled – 9 GPH**

Figure 11. Avocado tree canopy volume by irrigation treatment. Photographs were taken of trees that represented average tree canopy volume, with Patricio as a scale reference (Patricio is 5’3”, 160 cm in height). Above photos were taken October 16, 2014.

Trees irrigated with recycled water suffered from osmotic stress due to the presence of higher salt concentrations. Energy that could be invested in fruit production instead had to be expended on extracting water from the soil to overcome the osmotic force in the soil exerted by high concentrations of soluble salts. Smaller trees yielded fewer fruit due to less leaf surface area available for photosynthesis and energy production. Smaller trees were also less able to physically support fruit due to a fewer number of branches. Small tree size was equated with thinner, weaker, and fewer branches, thus small size negatively impacted a tree’s ability to physically support fruit. Trees irrigated with recycled water also had lower leaf chlorophyll content due to a number of environmental stressors, and reduced chlorophyll content contributed to reduced tree growth (Figure 13). Data displayed in Figure 13 is shown in a map in the Appendix in AP Figure 26.

Canopy Volume July 2015

Potable 14 GPH   Potable 12 GPH   Potable 9 GPH

Recycled 14 GPH   Recycled 12 GPH   Recycled 9 GPH

Figure 12. Avocado tree canopy volume by irrigation treatment. Photographs were taken of trees that are representative of the average tree canopy volume, with Jenessa as a scale reference (Jenessa is 5’7”, 170 cm in height). Above photographs were taken July 11, 2015.

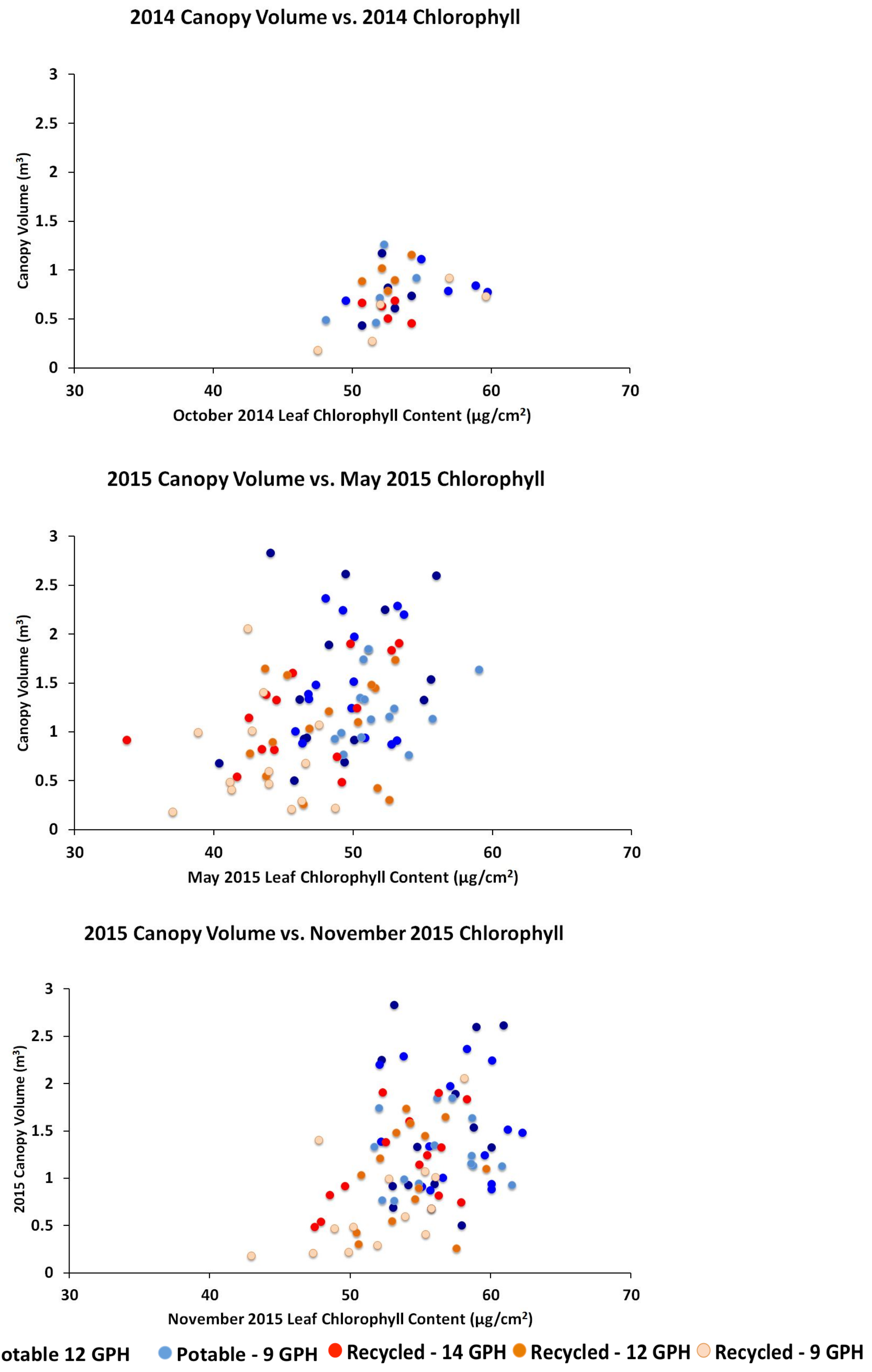


Figure 13 . Canopy Volume vs. Leaf Chlorophyll Content. Top: October 2014 canopy volume vs. October 2014 chlorophyll content. **Middle:** 2015 canopy volume vs. May 2015 leaf chlorophyll content. **Bottom:** May 2015 canopy volume vs. November 2015 leaf chlorophyll content.

Tree Trunk Diameter

Tree trunk diameters were measured using calipers just above the graft. Average tree trunk diameters for Potable – 14 GPH and Potable – 12 GPH were significantly larger than Potable – 9 GPH, Recycled – 14 GPH, and Recycled – 12 GPH, while the average Recycled – 9 GPH trunk diameters were significantly smaller than those of the rest of the treatments in October 2014 (Figure 14). However, in June 2015 (data not shown), the trees irrigated with recycled water were similar in diameter regardless of volume of water applied, and all trees in recycled water treatments were much thicker than trees irrigated with potable water. There was not a significant increase in trunk diameter in any of the treatments irrigated with potable water. It is uncertain why tree trunk diameter increased dramatically in trees irrigated with recycled water. It was noted that trees irrigated with recycled water had a higher occurrence of lodging; that is, these trees had a higher tendency for trunks above ground to fall over while roots remained intact. The lodged trees were alive, however they did not remain upright. It is possible that trunks were enlarged to support a less stable canopy. By March 2016, the only major significant difference among tree trunk diameters was in the Recycled – 9 GPH. These trees had much smaller tree trunk diameters than any of the other treatments.

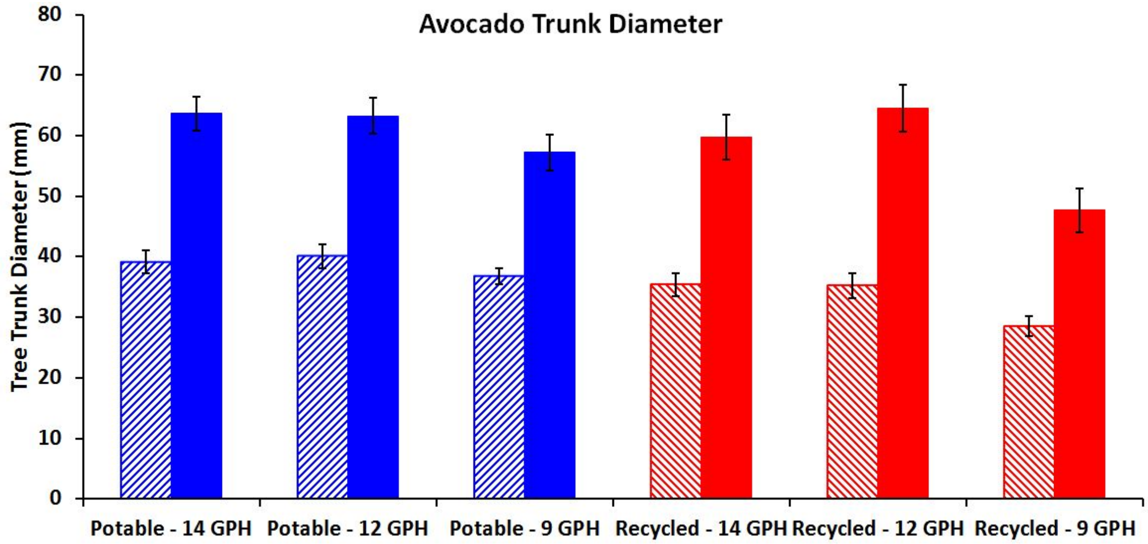


Figure 14. Tree trunk diameter. Vertical error bars indicate standard error values. Average values shown in table below. $P < 0.05$

Trunk Diameter (mm)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
October 2014	39.2	40.1	36.8	35.3	35.2	28.5
March 2016	63.6	63.3	57.2	59.7	64.6	47.7

Trunk Diameter ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**14 GPH**	**12 GPH**	**14 GPH**	**12 GPH**
October 2014	a	a	a	a	a	a
March 2016	b	b	bc	bc	b	ac

Leaf Drop

In February 2015, a significant increase in leaf drop was noted for trees irrigated with recycled water (Table 4). Leaf drop affects fruit yield as there is less photosynthetic surface area available for carbon fixation and energy production. Severe leaf drop can also lead to sunburn on the trunk and fruit caused by direct prolonged exposure to the sunlight. Exposed trunks should be painted white to protect from sunburn, particularly on young trees. Sunburnt fruit develop red or brown spots on the peel (Whiley, 2002).

Table 4. Average severity of leaf drop associated with defoliation caused by salinity. A score of 0 indicates no leaves present at the base of the tree, 5 indicates trees with near complete defoliation.

Leaf Drop	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**14 GPH**	**12 GPH**	**14 GPH**	**12 GPH**
February 2015	0.27	0	0	0.67	1.10	2.07

Salt burn and Leaf Damage

High soil salinity contributes to higher levels of salt in plant tissue, particularly in salt-sensitive crops such as avocado. Salt burn presents itself as dead brown tissue at leaf tips. As leaf salt burn becomes more severe, the brown necrotic leaf tissue progresses along leaf margins towards the leaf petiole and inward towards the center of the leaf. Trees were inspected to estimate the salt burn damage; no damage was assigned a 0 rating, whereas severe damage covering at least 50% of the leaf tissue on 75% or more of the canopy was assigned a rating of 5. In 2014, Salt burn damage was minimal on Potable – 12 GPH trees, while Potable 14 – GPH, Potable – 9 GPH, Recycled – 14 GPH and Recycled –12 GPH had some salt damage but showed no significant difference between each other (Figure 16). Recycled – 9 GPH treatment showed the highest salt burn damage to the leaf tissue.

In June 2015, salt burn was negligible on most treatments due to the spring leaf flush and pruning. Minor salt burn was noted on Recycled – 12 GPH and 9 GPH. However, in November and December 2015, salt burn was clearly evident. In Fall 2015, trees in the Potable – 14 GPH treatment were showing signs of salt burn and chloride toxicity in the leaves. Initially, it was thought that this was due to hypoxic soil conditions induced by excessive irrigation. Inadequate oxygen availability facilitates chloride uptake by plants, since roots cannot obtain adequate energy via aerobic respiration to prevent chloride uptake. The high amount of salt burn on Potable – 14 GPH was unexpected, due to the higher quality water and presumed adequate drainage. Upon further investigation, it was discovered that the region of the orchard where the Potable – 14 GPH trees were planted had a high clay content in the soil (see Figure 7). Conversely, the trees irrigated with recycled water showed less severe symptoms of salt burn. These results may be explained by the variation in clay content and associated co-accumulation of salts and chloride in the soil profile. Representative pictures from each treatment are shown in Figure 15.

Potable – 14 GPH **Potable 12 GPH** **Potable 9 GPH**

Recycled – 14 GPH **Recycled – 12 GPH** **Recycled 9 GPH**

Figure 15. Leaf salt-burn damage. Pictures shown were taken of trees representative of the average appearance in regards to leaf salt burn damage. Photographs were taken on December 13, 2015.

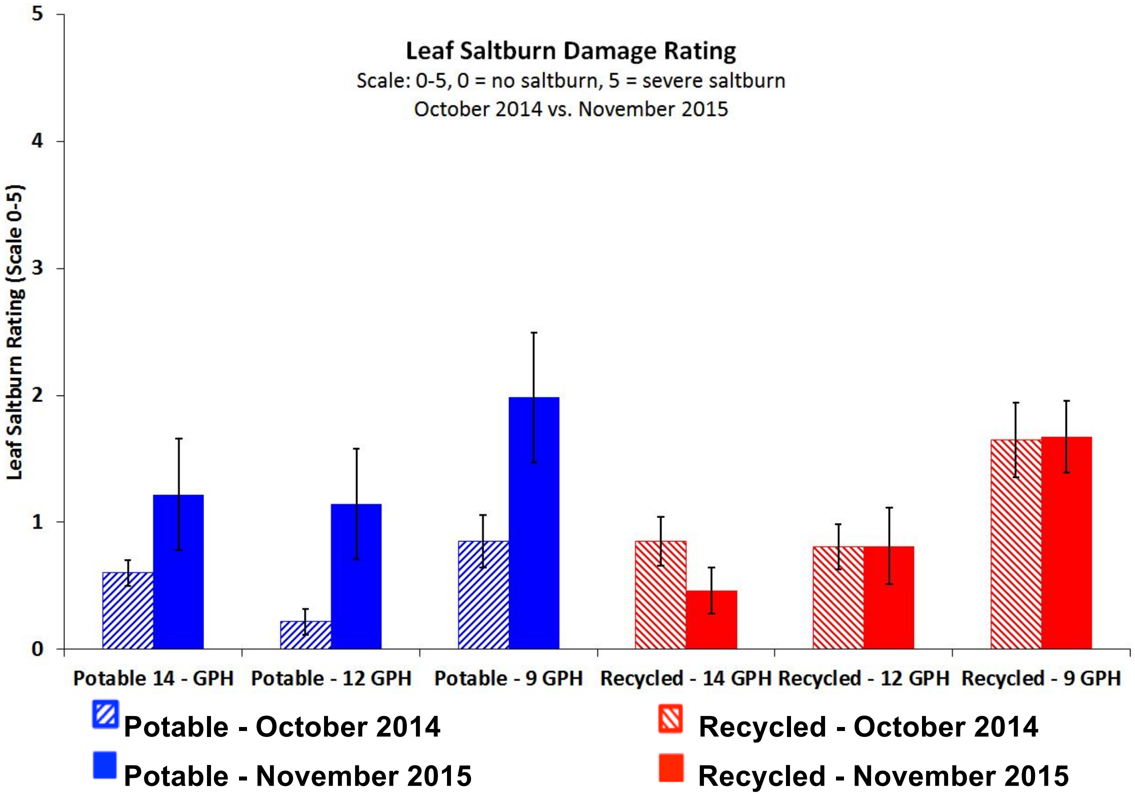


Figure 16. Leaf Salt-burn severity. Development of salt burn symptoms on the foliage of avocado trees as estimated visually for trees irrigated with potable water or recycled wastewater at different application rates. 0 = no leaf salt-burn symptoms, 5 = severe leaf salt-burn symptoms. Vertical error bars indicate standard error values. P < 0.05

Leaf Saltburn	**POTABLE**			**RECYCLED**		
(0-5)	**14 GPH**	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
October 2014	0.3	0.0	0.0	0.7	1.1	2.1
November 2015	1.5	1.2	2.0	0.4	0.8	1.8

Leaf Saltburn	**POTABLE**			**RECYCLED**		
ANOVA groups	**14 GPH**	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
October 2014	a	a	a	ac	c	b
November 2015	cd	c	b	a	ac	bd

Leaf Chlorophyll Content

Between October 2013 and October 2014, leaf chlorophyll contents were not statistically different among the potable water treatments, with the exception of the trees in the Potable – 12 GPH treatment, which showed a reduction from 56.0 μg/cm^2 in October 2014 to 49.6 μg/cm^2 in May 2015. However, all recycled water treatments showed a significant reduction in leaf chlorophyll concentration between October 2014 and May 2015 (see Figure 17). By November 2015, chlorophyll content had increased among all treatments, but chlorophyll content was still lower in trees irrigated with recycled water in comparison to their counterparts irrigated with potable water.

Reduced chlorophyll content in trees irrigated with recycled water may be best explained by low concentrations of iron and magnesium in leaf tissue. Recycled water tends to elevate soil pH due to higher mineral content, and basic soils reduce iron availability for plant uptake (AP Figure 7). Iron is involved in the biosynthesis of chlorophyll molecules, and iron is also a component of cytochromes, which are important for photosynthesis and cellular respiration (Heldt and Heldt, 2005). A deficiency in iron thus can directly reduce leaf chlorophyll content and interfere with energy production and consumption. A tree with low energy is not likely to appear in good health, and a tree in poor health is not likely to yield much fruit. As evidenced in the graphs in Figure 18, leaf iron content decreased over time in trees irrigated with recycled water, and this decline was associated with a drop in leaf chlorophyll content. Magnesium is part of the chlorophyll molecule itself; it is situated at the center of the organic ring and serves as the coordinating ion, binding the organic arms together (Heldt and Heldt, 2005). Leaf magnesium concentrations were lower in trees irrigated with recycled water, and this was also correlated with a decline in leaf chlorophyll content (Figure 19). In soils irrigated with recycled water, the presence of ions such as chloride compete for ion uptake; this reduces the probability that magnesium ions will be assimilated in adequate quantities and can lead to reduced leaf magnesium content, and thus reduced chlorophyll content. Data in Figures 17-19 are displayed in a map in AP Figure 27 and AP Figure 31.

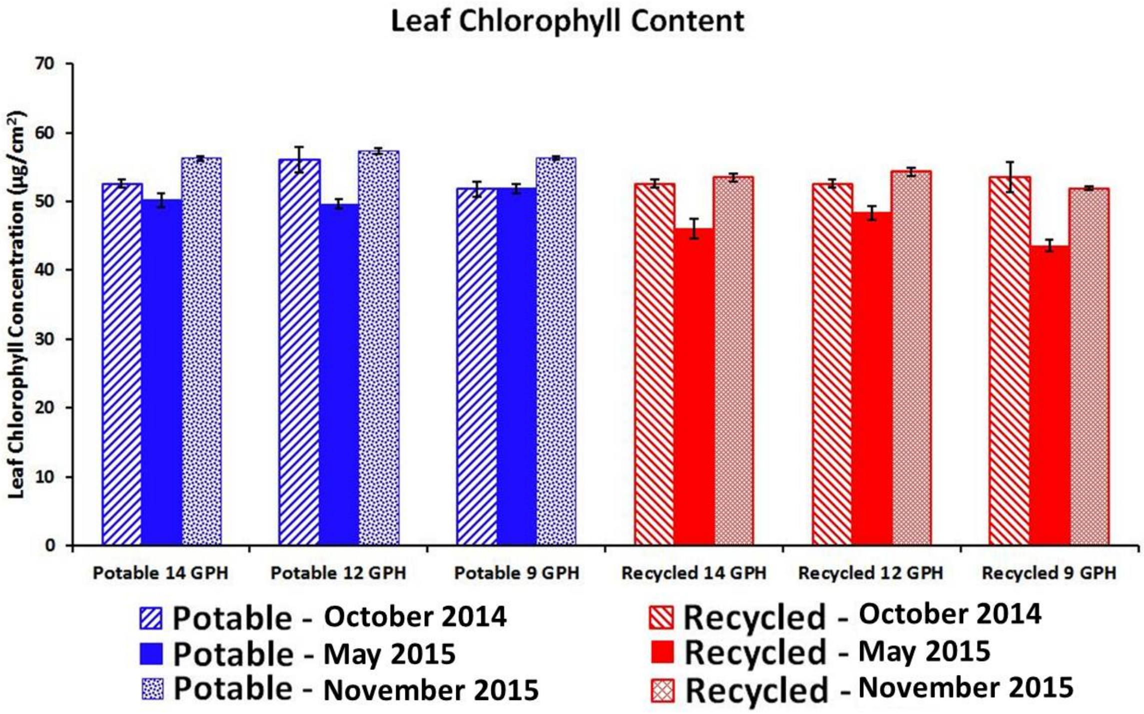


Figure 17. Leaf chlorophyll content. Vertical error bars indicate standard error values. Average values are shown in table below.

Leaf Chlorophyll (µg/cm^2)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
October 2014	52.6	56.0	50.8	55.6	58.7	53.5
May 2015	50.1	49.6	52.0	45.6	48.4	43.6
November 2015	56.2	57.4	56.3	53.5	54.3	51.9

Leaf Chlorophyll ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
October 2014	a	a	ab	a	a	a
May 2015	ab	ab	ab	b	ab	b
November 2015	a	a	a	a	a	ab

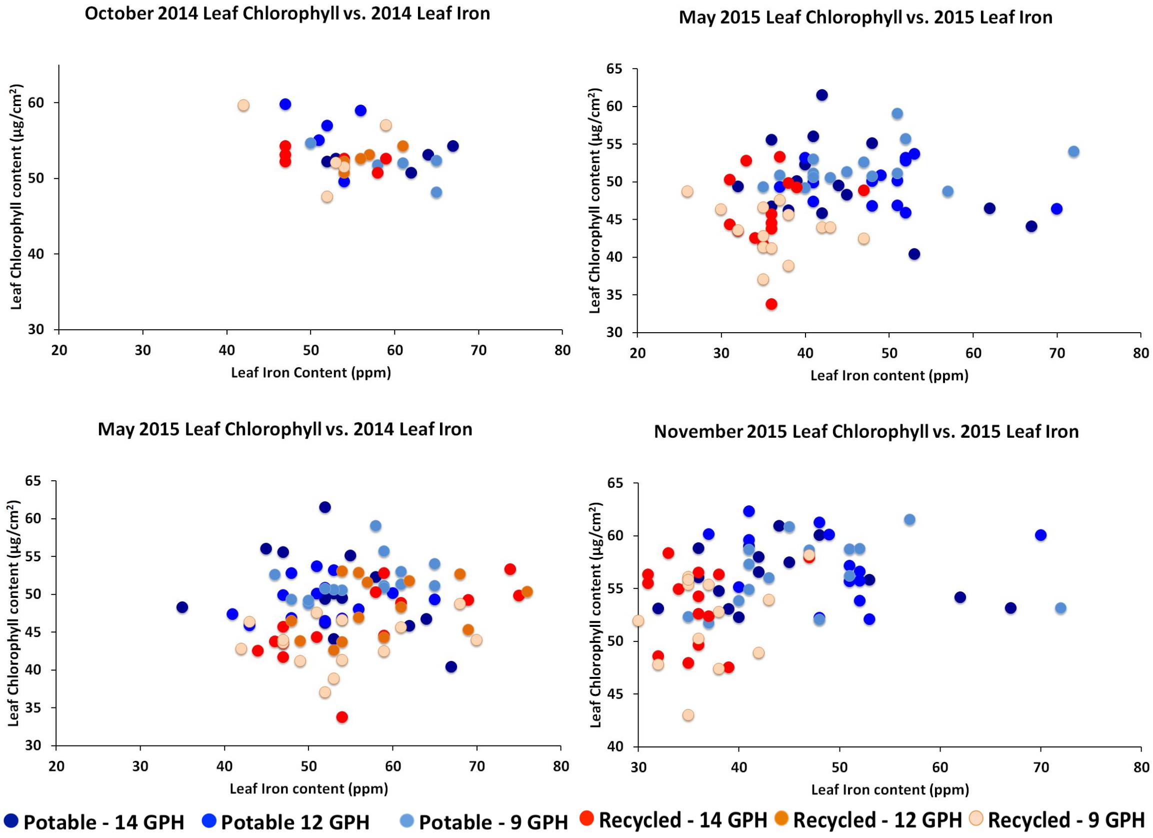


Figure 18. Leaf chlorophyll concentration vs. leaf iron concentration. Top left: October 2014 leaf chlorophyll vs. October 2014 leaf iron content. **Bottom left:** May 2015 leaf chlorophyll content vs. 2014 leaf iron content. **Top right:** May 2015 leaf chlorophyll content vs. 2015 leaf iron content. **Bottom right:** November 2015 leaf chlorophyll content vs. 2015 leaf iron content.

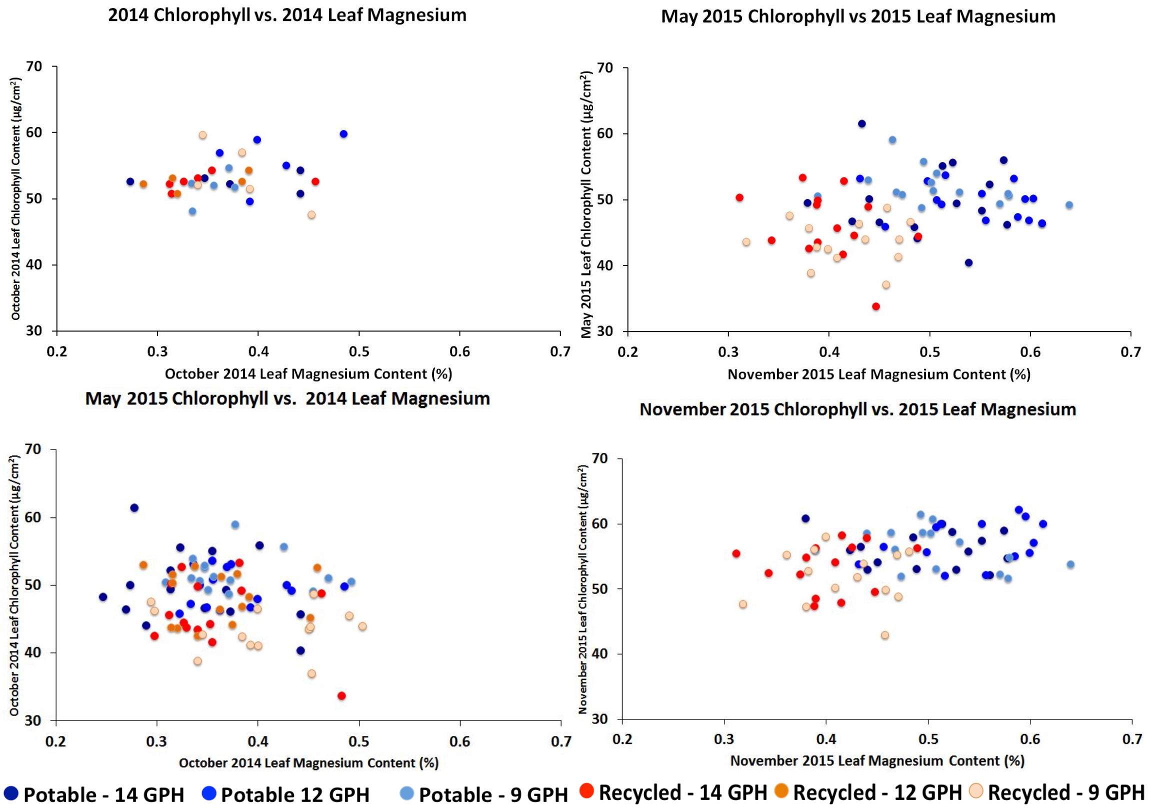


Figure 19. Leaf chlorophyll concentration vs. leaf magnesium concentration. Top left: October 2014 leaf chlorophyll vs. October 2014 leaf magnesium content. **Bottom left:** May 2015 leaf chlorophyll content vs. October 2014 leaf magnesium content. **Top right:** May 2015 leaf chlorophyll content vs. November 2015 leaf magnesium content. **Bottom right:** November 2015 leaf chlorophyll content vs. November 2015 leaf magnesium content.

CHAPTER 4

LEAF AND SOIL NUTRITION

MATERIALS AND METHODS

Leaf Analysis

Leaves were collected in Fall of each year for leaf analysis. 20 young, fully-expanded leaves were collected from each tree. Leaves were placed in labeled paper bags; bags were rolled closed and kept in the shade. Leaves were placed in cold storage overnight at 4^{o}C and transported to Fruit Growers' Lab Inc. in Santa Paula, CA. Due to limited resources in 2013, leaves were only collected from 2 trees per potable irrigation treatment and 3 trees per recycled water irrigation treatment. In 2014 and 2015, leaves were collected from all trees in the 90-tree plot. Leaves were analyzed by Fruit Growers' Lab staff for nitrogen, phosphorus, potassium, calcium, magnesium, chloride, sodium, manganese, boron, iron, and zinc. Statistical significance was calculated via 1-way ANOVA in SPSS, using a p-value of $P < 0.05$.

Soil Analysis

Soil samples were also collected at the same time. Soil samples were collected from 3 trees per irrigation treatment at the surface, half a meter away from the tree trunk on the northeast side. Soils were placed in wax-lined paper bags. Bags were labeled with their tree numbers, rolled shut and placed in the shade while the rest of the samples were collected. Soil samples were delivered to Fruit Growers' Lab in Santa Paula and analyzed for pH, salinity, SAR, nitrate, phosphorus, sulfate, zinc, iron, copper, boron, chloride, exchangeable potassium, calcium, magnesium, and sodium, soluble potassium, calcium, magnesium, and sodium. Cation Exchange Capacity (CEC) and the proportion of calcium, magnesium, potassium, sodium and hydrogen ions occupying cation exchange sites were measured.

RESULTS

Leaf Chloride Content

Chloride is an essential nutrient in small quantities but usually presents more of an injury hazard to leaves and reduced yield. Leaf chloride content above 0.50% is considered to be excessive; less than 0.25% leaf chloride is considered optimal (AP Table 2). Leaf necrosis associated with chloride accumulation results in reduced photosynthetic area (Whiley, 2002). Elevated leaf chloride concentrations contribute to leaf necrosis and leaf drop; less leaf surface area reduces energy resources available for fruit production and thus diminishes bloom and thus fruit yield in the following spring season (Whiley, 2002). High chloride concentrations also decrease photosynthetic rates. In addition to effects on the leaf tissues, root growth is also affected with decreased root growth occurring before the appearance of chloride toxicity symptoms as leaf burn. This hinders the ability for plants to take up water and nutrients (Whiley, 2002).

Studies have also shown that high soil nitrate levels suppress boron, and chloride uptake in avocado (Bar et. *al*, 1997). The authors suggest that providing a constant supply of nitrogen to trees can offset effects of irrigation water with high boron and chloride levels (Bar et *al,* 1997). Fortunately, this is what already inevitably happens in orchards irrigated with recycled water. Unfortunately, the level of nitrogen needed to suppress chloride assimilation will also reduce flowering, fruit quality, fruit yield, invite attacks from pests and pathogens, and cause a whole host of problems (Dreistadt, 2008). As shown in Figure 20, trees irrigated with recycled water had a higher level of chloride in leaves in 2014 than trees irrigated with potable water, as might be expected. Surprisingly, in 2015, leaf chloride content actually decreased in trees irrigated with recycled water both in relation to their

2014 chloride levels in and in relation to 2015 chloride levels for trees irrigated with potable water. This may be attributed to three factors: high levels of nitrate supplied frequently can suppress chloride uptake; the salinity of the potable water source increased in 2015 due to a change in water source; and trees irrigated with potable water were situated on soils containing higher amounts of clay. Additionally, the differences between leaf chloride values in 2014 and 2015 may be due to soil and trees moving towards a new equilibrium with the water supply. As shown in Figure 21, the drop in 2015 in leaf chloride content in trees irrigated with recycled water was associated with high leaf nitrogen in 2014.

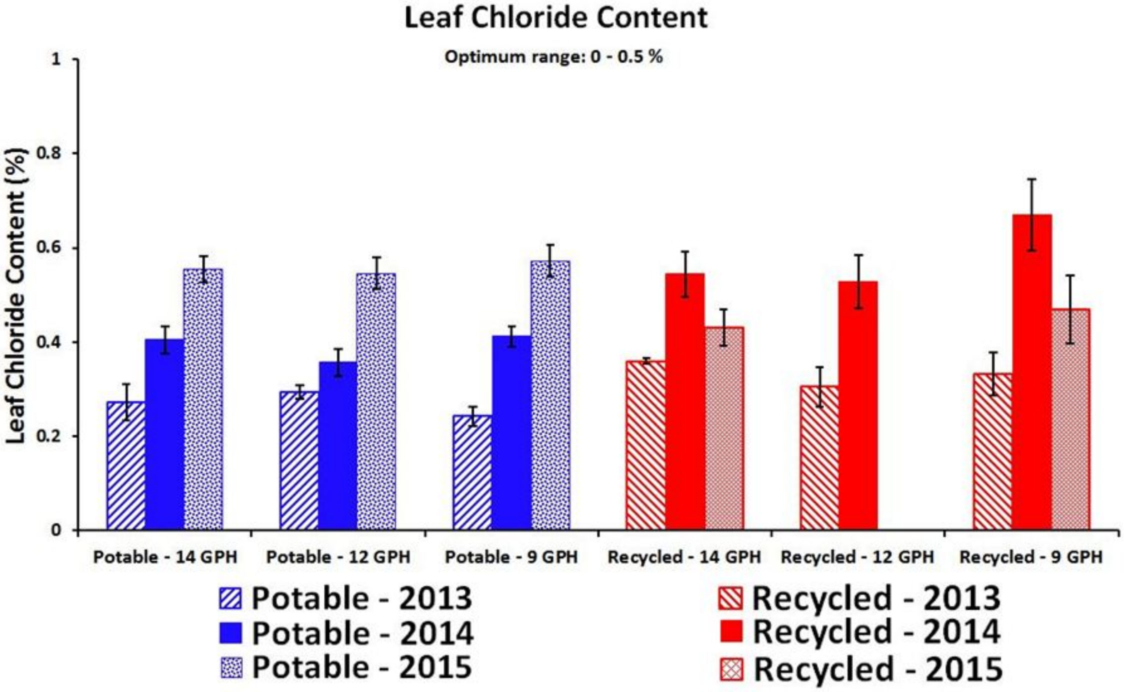


Figure 20. Leaf chloride content. 2015 data not available for Recycled – 12 GPH. Vertical error bars indicate standard error values. Average values are shown in table below. P < 0.05

Leaf Chloride (%)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	0.27	0.29	0.24	.036	0.31	0.33
2014	0.40	0.36	0.41	.054	0.53	0.67
2015	0.55	0.54	0.57	0.43	No Data	0.47

Leaf Chloride ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	ac	a	ac	ac	ac	bc
2015	ac	ac	ac	ac	No Data	ac

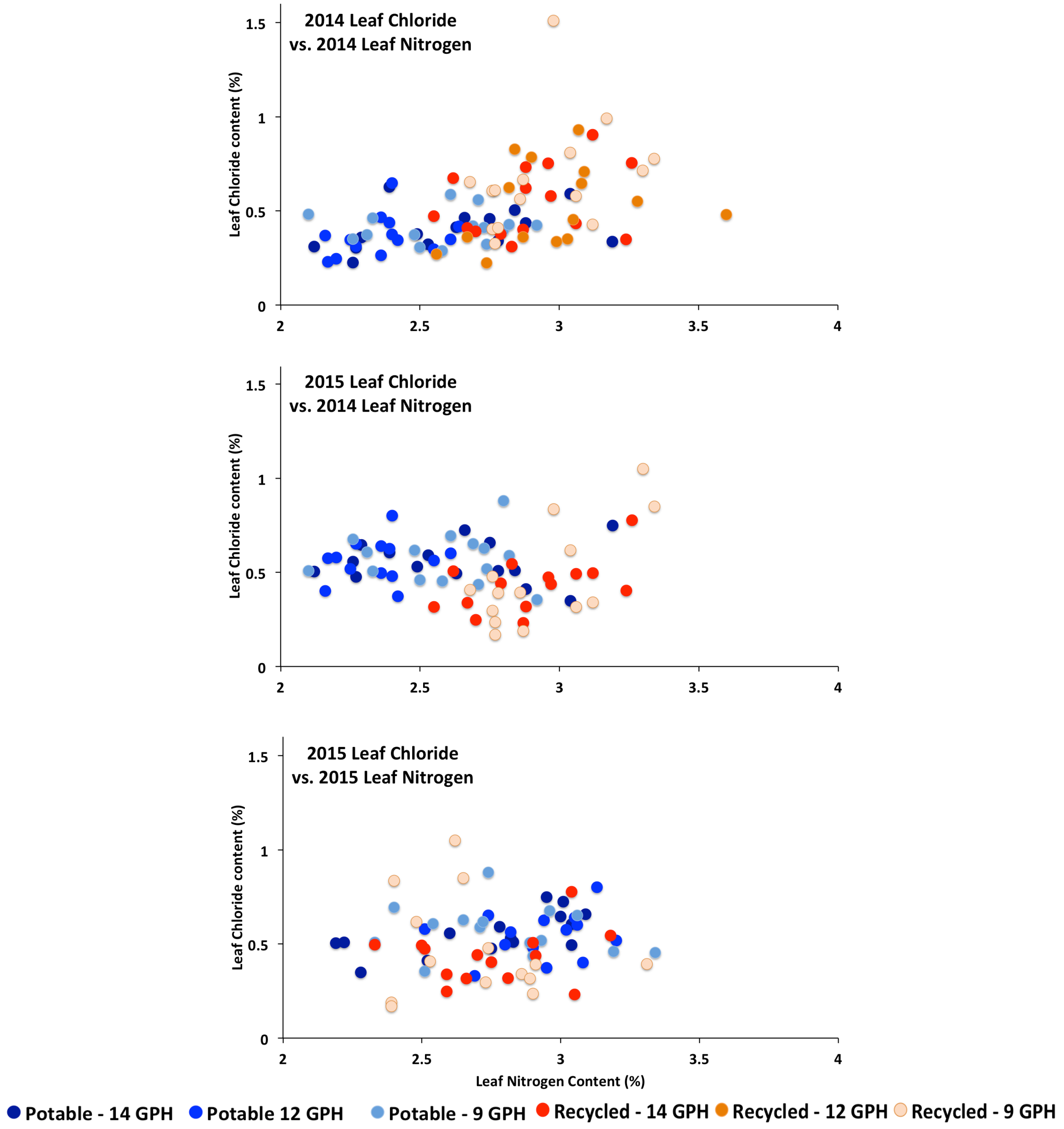


Figure 21. Leaf chloride contents of individual avocado trees. Top: 2014 leaf chloride vs. 2014 leaf nitrogen. **Middle:** 2015 leaf chloride content in relation to 2014 leaf nitrogen content. **Bottom:** 2015 leaf chloride content vs. 2015 leaf nitrogen. Leaf chloride concentrations below 0.25% is considered ideal, above 0.5% is considered excessive.

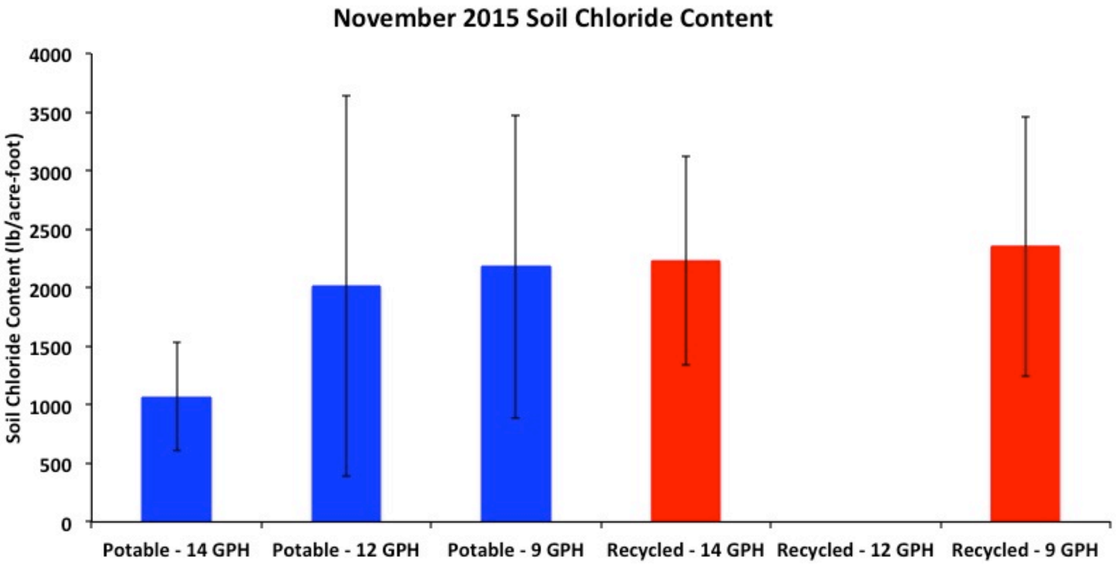


Figure 22. November 2015 Soil Chloride Content. 2015 data is not available for Recycled – 12 GPH. Error bars indicate standard error values.

Soil Chloride (lb/acre-foot)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2015	1,070	2,012	2,180	2,230	No Data	2,354

While the suppression of chloride uptake by high nitrogen levels appears to be a beneficial side effect of recycled water use, there is still a drastic reduction in yield and fruit quality that may likely be in part due to high nitrogen levels in the irrigation water. Trees irrigated with potable water showed a higher rate of salt burn than might be expected, while trees irrigated with recycled water at 14 GPH and 12 GPH showed almost no signs of saltburn. Avocado leaves have the ability to accumulate chloride in their leaves and show no signs of chloride toxicity until suffering suboptimal soil moisture conditions, either via drought or flooding (Ferreyra, 2007). The high rates of saltburn in Potable – 14 GPH and Potable – 9 GPH trees are correlated with the amount of clay present in the soil. Clay retains chloride ions more readily than sand due to its high surface area and reduced permeability. Thus more chloride is bioavailable for uptake in the clay soils irrigated with potable water, even though the water applied to these soils contains less than half the amount of chloride in the recycled water. Clay soils tend to drain more slowly, allowing for hypoxic soil conditions to occur, and facilitating higher rates of chloride assimilation. Chloride is normally sequestered in cell vacuoles, but hypoxia encourages the release of chloride from the vacuoles. As shown in AP Figure 17, leaf chloride content was positively correlated with yield, which was unexpected. Since excessive chloride is known to reduce yield, this suggests that a) trees irrigated with potable water may have reached even higher yields if they had been planted on soils low in clay, and b) other factors associated with recycled water had more of an influence on yield reduction than chloride content. The data in AP Figure 17 should not be misinterpreted to suggest that chloride content in the recycled water does not need to be reduced.

Leaf Sodium Content

Leaf analyses show that sodium content is currently within optimal ranges among all treatments, however the data shows that there is a strong trend for increasing sodium concentrations in the leaves in trees irrigated with recycled water (Figure 23). Sodium damage appears as gold necrotic spots throughout the leaf (Whiley, 2002). Sodium toxicity symptoms were negligible throughout the orchard; only 2 trees were observed exhibiting slight symptoms of sodium toxicity on a few leaves in the summer of 2015, and these two trees were in the Recycled – 9 GPH treatment. However, if the trend of increasing leaf sodium content were to continue, it could soon cross the maximum threshold of 0.25% within approximately 5 years. In contrast, trees irrigated with potable water are less than 0.01% and show no increase in sodium content over time. These data demonstrate that reducing sodium content of the irrigation water may be necessary. Furthermore, the high soil SAR in soils irrigated with recycled water necessitates a reduction in sodium.

As shown in Figure 24, the higher levels of leaf sodium were strongly correlated with reduced yield. It is unlikely that this is a causative factor of reduced fruit yield and more likely simply associated with a different causative factor. Sodium content in the soil was not significantly higher in November 2015 (Figure 25). However this is only a snapshot of the soil in time and place, as only 3 soil samples were collected per irrigation treatment. Data shown in Figures 23 and 24 are displayed as a map in AP Figure 28.

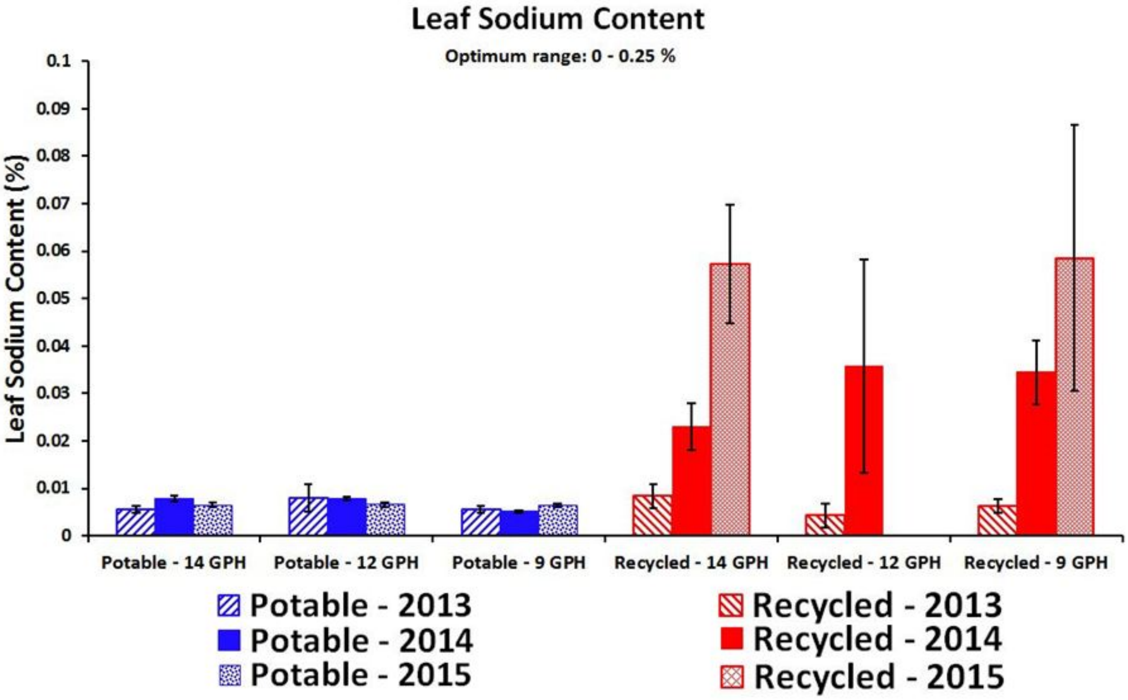


Figure 23. Leaf sodium content. 2015 data not available for Recycled – 12 GPH. Vertical error bars indicate standard error values. Average values are shown in table below. $P < 0.05$

Leaf Sodium (%)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	0.0055	0.0080	0.0055	0.0083	0.0043	0.0063
2014	0.0079	0.0079	0.0051	0.0230	0.0358	0.0345
2015	0.0064	0.0065	0.0064	0.0574	No Data	0.0585

Leaf Sodium	**POTABLE**			**RECYCLED**		
ANOVA groups	**14 GPH**	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	a	a	b	abc	abc	c
2015	a	a	a	c	No Data	c

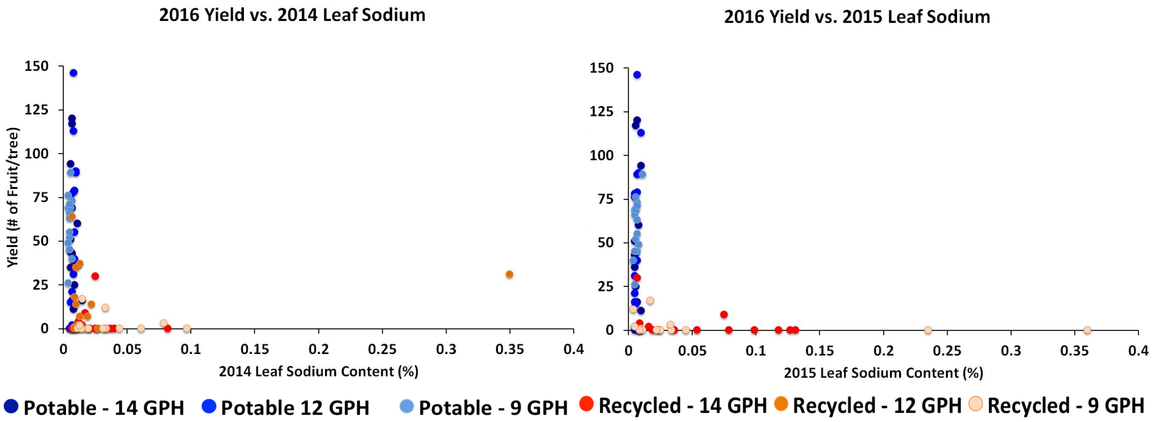


Figure 24. 2016 fruit yield vs. 2014 (left) and 2015 (right) leaf sodium concentrations for individual trees. In 2015, data is not available for Recycled – 12 GPH.

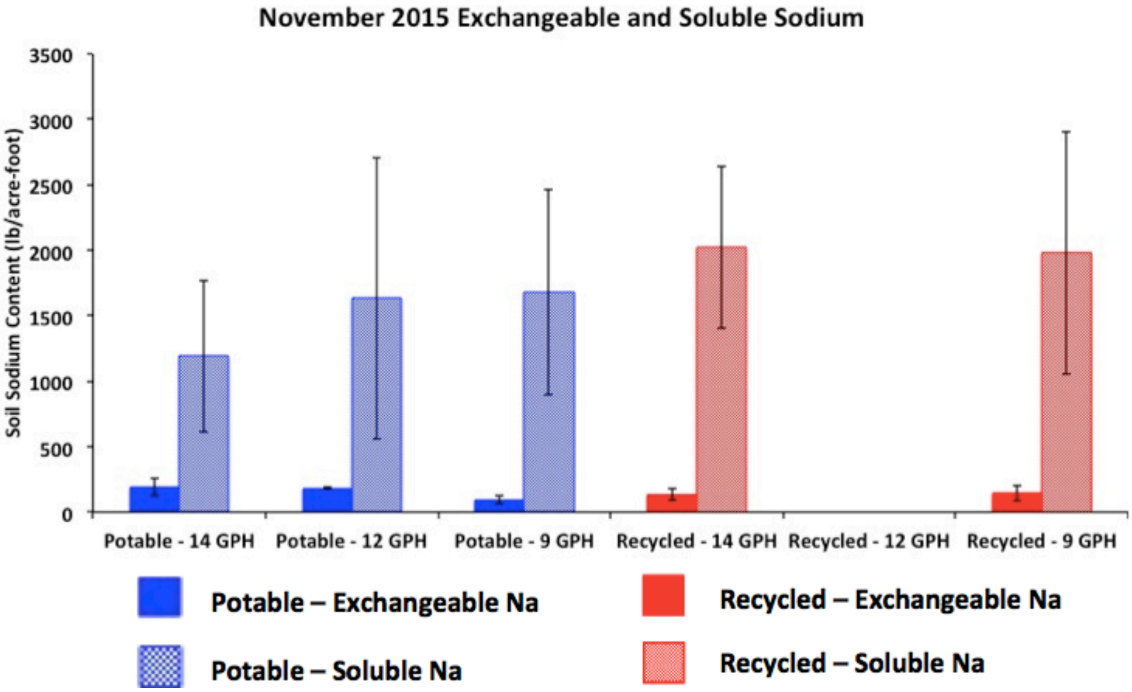


Figure 25. Exchangeable and soluble sodium in the soil from November 2015. Data for Recycled – 12 GPH not available. Error bars indicate standard error values.

Soil Sodium (lb/acre-foot)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Exchangeable Na	193	183	93	133		147
Soluble Na	1,194	1,635	1,677	2,023		1,981

Leaf Boron Content

Boron is an essential micronutrient for plants. In avocado, boron contributes to cell wall and plasma membrane structure, auxin metabolism, development of floral organs pollen viability, germination, and pollen tube growth (Whiley, et *al*. 1996). Boron deficiency is indicated by abnormally small, young, pale green to yellow leaves with holes that could be mistaken for insect damage (Whiley et. *al*. 1996). Trees exhibit shoot dieback, swelling at the nodes, and a horizontal growth pattern that is caused by the loss of apical dominance (Whiley, 2002). Fruit is sickle-shaped with a bumpy peel and lesions (Whiley, 2002). Boron toxicity is typically the threat in soils irrigated with recycled water. In avocado, boron toxicity symptoms present as necrotic leaf margins and yellow tissue behind that (Dreistadt, 2008). There is no sign of boron accumulating over time in trees irrigated with recycled water (Figure 26), however it may take several years before boron concentration in the soil reaches a point where leaf tissue. Boron bioavailability is reduced at moderately high pH values of 7.5-8.5 (AP Figure AP 7). The probability of boron deficiency due to high pH may be partially offset by the excess of boron present in recycled water.

As shown in Figure 27, November 2015 soil concentrations of boron were higher at higher rates of irrigation. There was no difference between potable and recycled irrigation treatments at equivalent irrigation rates. AP Figure 18 shows that the highest levels of boron in leaves were associated with low yields; however since the highest boron levels were still within optimal range, this observation is more likely an association of another factor in reduced yield rather than a causation of reduced yield. Concentrations of copper were not deficient in the present study, however, leaf analyses from Witman Ranch show sub-optimal concentrations of copper in leaf tissue, likely due to higher soil pH (Table 2).

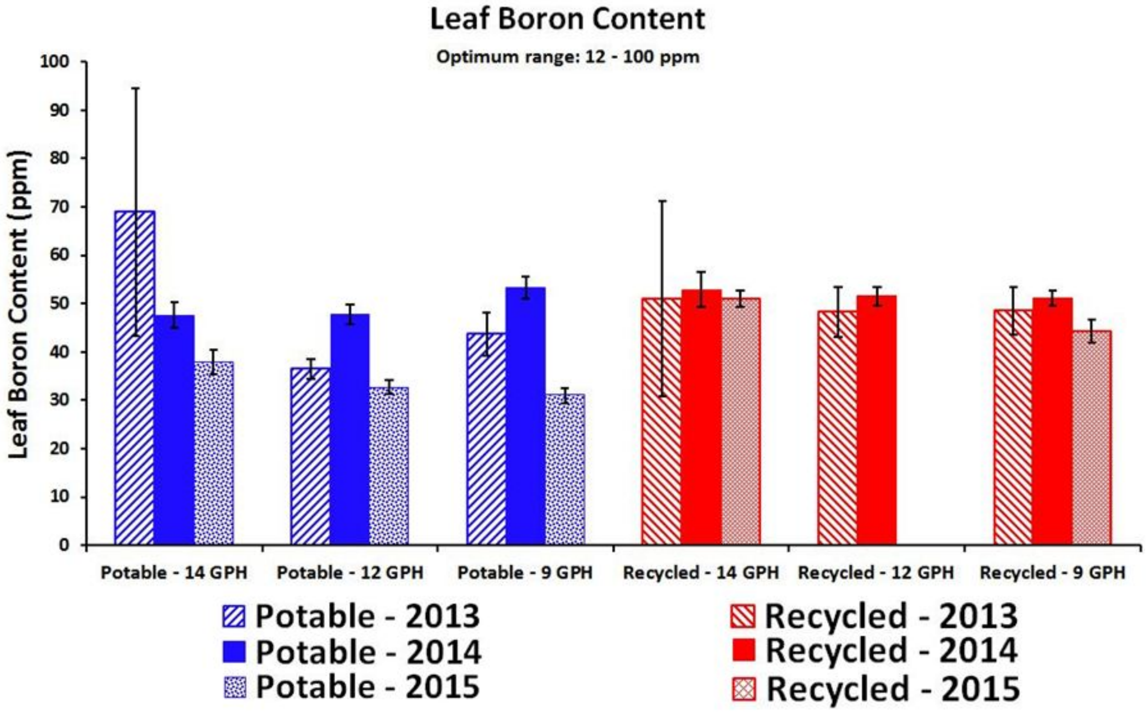


Figure 26. Leaf boron content. 2015 data not available for Recycled – 12 GPH. Vertical error bars indicate standard error values. Average values are shown in table below.

Leaf Boron (ppm)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	68.9	36.6	43.8	51.0	48.3	48.5
2014	47.6	47.8	53.2	52.9	51.5	51.0
2015	37.9	32.7	31.0	51.0	No Data	44.3

Leaf Boron ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	a	a	ac	ac	ac	ac
2015	ab	b	b	ac	No Data	a

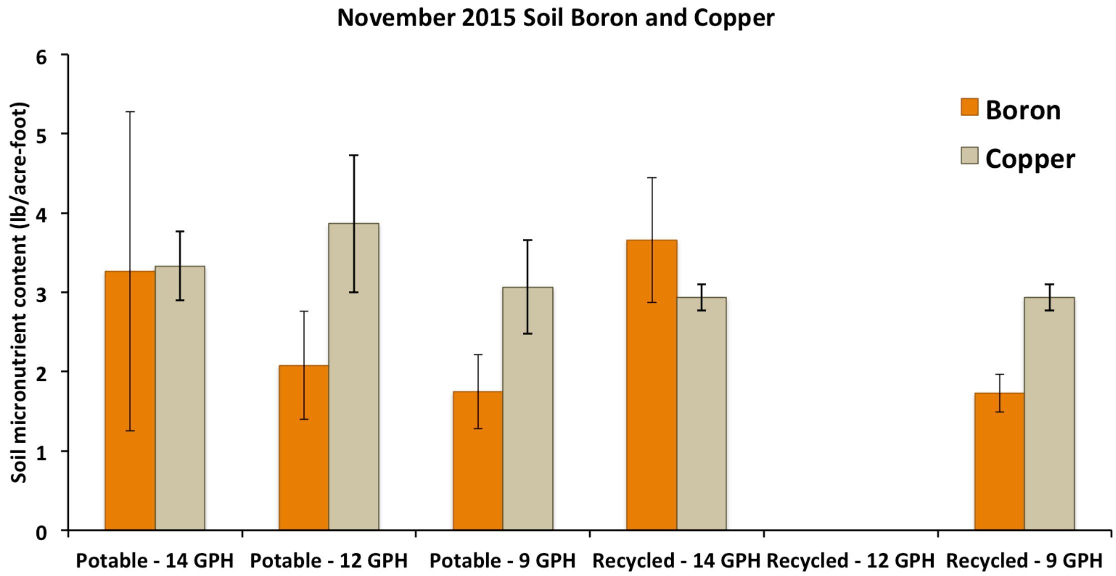


Figure 27. November 2015 soil boron and copper content. 2015 data not available for Recycled – 12 GPH. Error bars indicate standard error values.

Soil Boron and Copper (lb/acre-foot)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Boron	3.27	2.08	1.75	3.66	No Data	1.73
Copper	3.33	3.87	3.07	2.93	No Data	2.93

Leaf Nitrogen Content

Nitrogen is an essential macronutrient and often the most limiting nutrient in agriculture. Nitrogen is a major component of proteins, which are ubiquitous in nearly every plant cell (Taiz and Zeiger, 2006). Proteins are essential cellular machinery involved in every aspect of plant growth and survival, from the construction of cell walls, to photosynthesis, hormone signaling, and DNA replication (Taiz and Zeiger, 2006). Nitrogen is also a part of DNA and RNA base pairs, anti-nutritive defense compounds, as well as a number of other molecules (Taiz and Zeiger, 2006). In avocado, nitrogen deficiency is characterized by small, uniformly yellow leaves, premature leaf drop, reduced fruit yield, and small fruit (Dreistadt, 2008). The symptoms of yellow leaves first appear in older foliage, while newer leaves are a pale green (Taiz and Zeiger, 2006).

Agriculture irrigated with recycled water is unique in that nitrogen is plentiful. Excess nitrogen turns leaf margins a dark green color, sometimes with gray or brown margins, which is known as fertilizer burn (Dreistadt, 2008). Excess nitrogen is associated with reduced shelf life in post-harvest storage and decreased fruit quality in avocado (Arpaia et. *al*, 1996). Elevated nitrogen concentrations typically equate to a higher protein content in plant tissue, which signifies high-quality food for insects. Insects have a low carbon to nitrogen ratio, which means they have a high demand for nitrogen sources such as protein. Thus above-optimal nitrogen levels in avocado are more likely to encourage pest outbreaks (Dreistadt, 2008). Managing nitrogen levels in avocado is imperative to a healthy orchard and a profitable yield.

While excess nitrogen appears to reduce damage from toxic ions, research also suggests that high nitrogen availability may reduce essential macro- and micronutrient uptake as well. High nitrogen conditions have been shown to reduce potassium content of avocado leaves (Lahav, 1990, Loupasaki 1998) or have no effect (Embleton 1958a; Embleton 1958b). The effect of excess nitrogen on phosphorus uptake in avocado is also under debate. A number of studies point to evidence for increased phosphorus uptake under increased nitrogen availability (Haas et *al.* 1947; Lynch et. *al,* 1954; Embleton 1958b). Conversely, Bar et. *al,* 1997 noted that phosphorus assimilation was inhibited by nitrogen application on 1-year old trees in sand culture. Nitrogen applied as ammonium reduced calcium and magnesium leaf content, while nitrogen applied as nitrate in calcium nitrate or ammonium nitrate increased calcium and magnesium concentrations in the leaves (Embleton 1958b). Differing environmental conditions may likely explain the variation in results among the listed studies. The pH of the water and soil can have a significant effect on nutrient availability for plants (AP Figure 7).

In the present trial, leaf nitrogen content was elevated in trees irrigated with recycled water relative to trees irrigated with potable water in 2014 (Figure 28). The optimal leaf nitrogen content for Hass avocado is 2.2-2.4% (Fruit Growers' Lab, 2014). Leaf nitrogen content fell within this optimal range for the trees irrigated with potable water at 12 GPH in 2014. However, both 14 GPH and 9 GPH treatments with potable water had leaf nitrogen concentrations above the optimal range. The elevated nitrogen concentrations in the Potable 14 GPH and 9 GPH treatments correlate with increased clay content in these soils. Higher clay content would allow for more retention of nitrogen in the soil, thus making more nitrogen bioavailable for the trees in these irrigation treatments. Although Potable-14 GPH and Potable-9 GPH had elevated nitrogen content, it does not appear to be so excessive so that it significantly affected fruit yield.

In contrast, the trees irrigated with recycled water, regardless of irrigation treatment, had much higher nitrogen values, reaching closer to 3% leaf nitrogen in October 2014. Fruit production in these trees is reduced, despite healthy appearance in comparison to the Potable – 14 GPH and Potable – 9 GPH trees (Figure 29). This suggests that the reduced fruit production in trees irrigated with recycled water cannot be attributed to elevated leaf chloride or higher soil salinity. In many crops, elevated nitrogen encourages vegetative growth, while inhibiting fruit production (Pettymeyer and Ashano, 1984). It is possible that this phenomenon is the explanation for what we are observing in this avocado study. During a nitrogen fertilization trial on avocado, Arpaia et. *al* 1996 noted that elevated nitrogen content in leaf tissue elevated leaf concentrations of manganese and zinc, while depressing potassium and boron content in the leaves. In fact, nitrogen fertilization is sometimes strategically used to mitigate boron toxicity in waters containing marginally to moderately high amounts of boron (Arpaia et. *al,* 1996).

Deviations beyond the optimal range for leaf nitrogen elicit susceptibility to attacks from insects and pathogens, and elevated nitrogen also encourages vegetative growth while inhibiting fruit production (UCANR, 2008). In the present study, in trees irrigated with potable water, leaf nitrogen content was within the optimum range or slightly above; in these treatments, fruit production closely paralleled canopy volume. In trees irrigated with recycled water, leaf nitrogen was elevated in 2014, reaching close to 3%, and there was a trade-off between leaf canopy growth and fruit production that is not explained with an increase in soil salinity or leaf chloride. This suggests that monitoring nitrogen of the water, soil, and leaf tissue is essential to ensure optimal conditions for fruit productivity and fruit quality. Monitoring nitrogen levels also allows the farmer to minimize pest outbreaks and reduce costs on pesticide applications. If the trees irrigated with recycled water were situated on soils with higher clay content, it is possible that more nitrogen would be retained in the soil profile, thus further exacerbating excess nitrogen availability and fruit yield reduction. Yield in relation to canopy volume is shown in AP Figure 25.

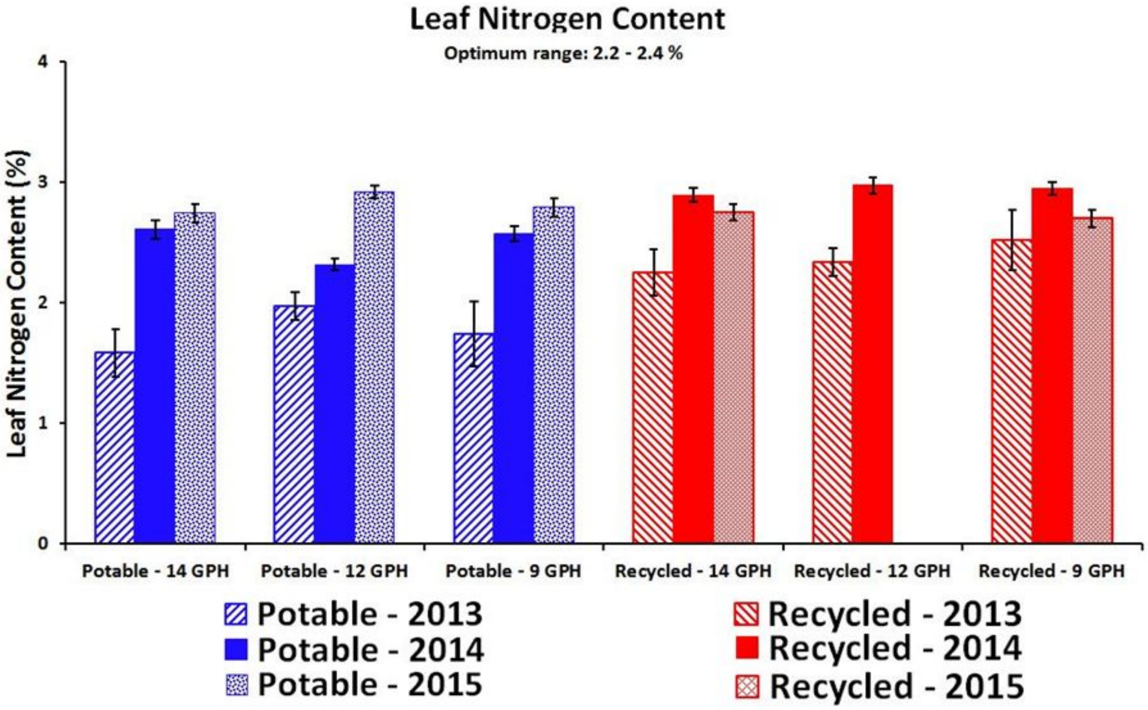


Figure 28. Leaf nitrogen content. 2015 data is not available for Recycled – 12 GPH. Vertical error bars indicate standard error values.

Leaf Nitrogen (%)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	1.59	1.98	1.74	2.25	2.34	2.52
2014	2.61	2.34	2.57	2.89	2.97	2.95
2015	2.74	2.92	2.79	2.75	No Data	2.70

Leaf Nitrogen ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	af	ac	af	adef	bdef	bdef
2015	aef	bef	aef	aef		aef

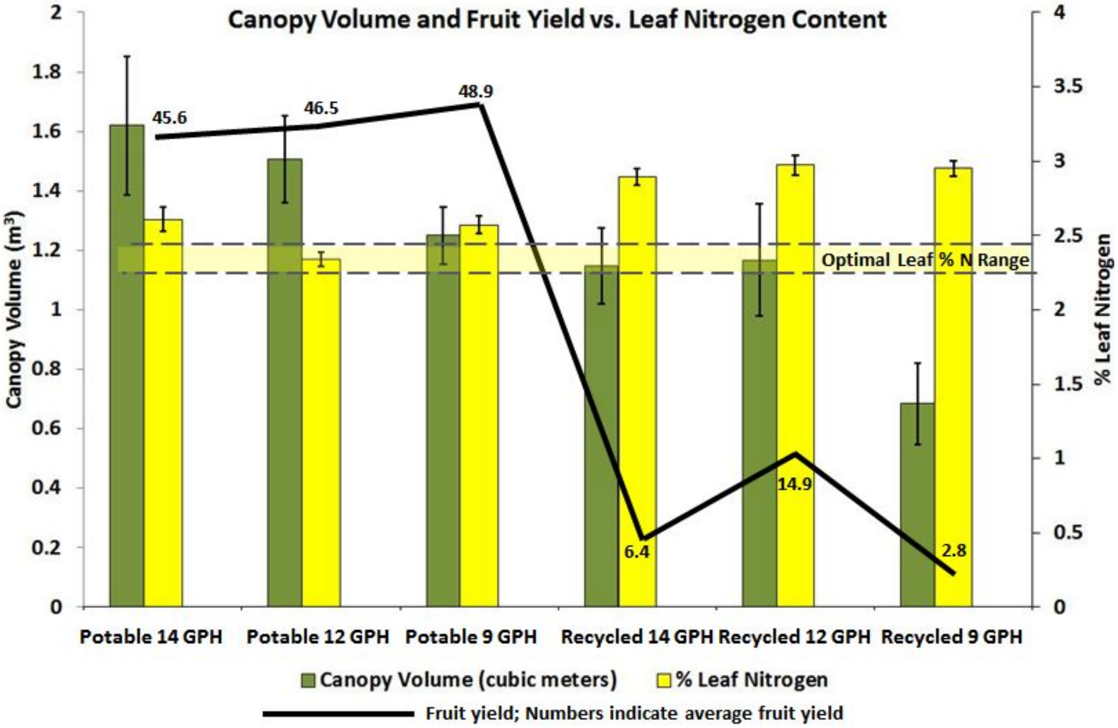


Figure 29. 2014 Leaf nitrogen content, 2015 canopy volume, and 2016 estimated yield. Dashed lines indicate optimal leaf N range of 2.2-2.4%. Vertical error bars indicate standard error values.

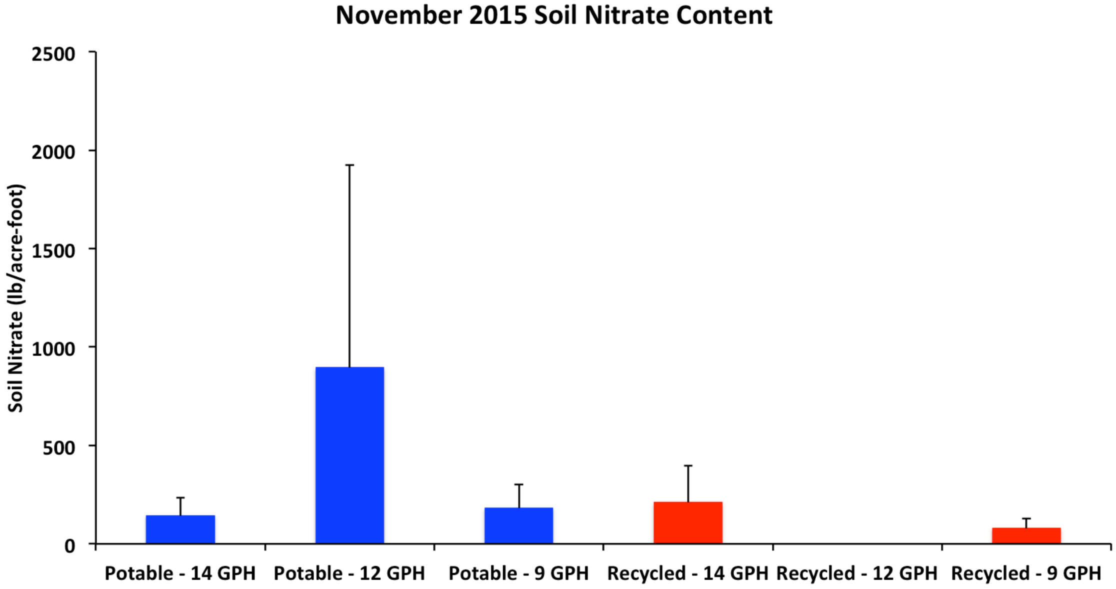


Figure 30. November 2015 Soil nitrate content. 2015 data not available for Recycled – 12 GPH. Error bars indicate standard error values.

Soil nitrate (lb/acre-foot)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Soil nitrate	142	894	182	211		79

Leaf Phosphorus Content

Phosphorus is generally the second most limiting nutrient for plants, after nitrogen. Phosphorus is an essential component DNA molecules and adenosine triphosphate (ATP), which is the molecular currency for energy (Taiz and Zeiger, 2006). Although usually abundant in soils, phosphorus is highly immobile, thus the concentration of soluble phosphorus in the soil solution is typically low compared to the reserve in the soil (Taiz and Zeiger, 2006). Excessive phosphorus has been shown to increase leaf concentrations of calcium, magnesium, and chloride, while reducing potassium content (Embleton, 1958b). Labanauskas et. *al* (1958a) reported an increase in manganese leaf concentration, and a reduction in zinc, copper, and boron leaf concentrations in association with high P levels.

Leaf phosphorus content was generally higher in trees irrigated with recycled water in 2013 and 2014, although not quite excessive, as shown in Figure 31. According to Fruit Growers' Lab, Inc, the optimum range for phosphorus content in avocado leaves is 0.08 – 0.44 %. UC IPM specifies that 0.08 – 0.25 is optimal, while anything above 0.3% is considered excessive (Dreistadt, 2008). Fertilization is recommended only if leaf concentrations fall below 0.14%.

As shown in Figure 32, soil phosphorus content decreases with irrigation rate in the potable water treatments, while soil phosphorus content in Recycled – 14 GPH is approximately the same as Recycled – 9 GPH. High phosphorus availability in Potable – 14 is likely due to lower soil pH and higher clay content, which can retain the ions. Soils irrigated with recycled water were not significantly higher in phosphorus than soils irrigated with potable water, suggesting it was leached out in these soils; this would also explain the modest leaf phosphorus content in trees irrigated with recycled water.

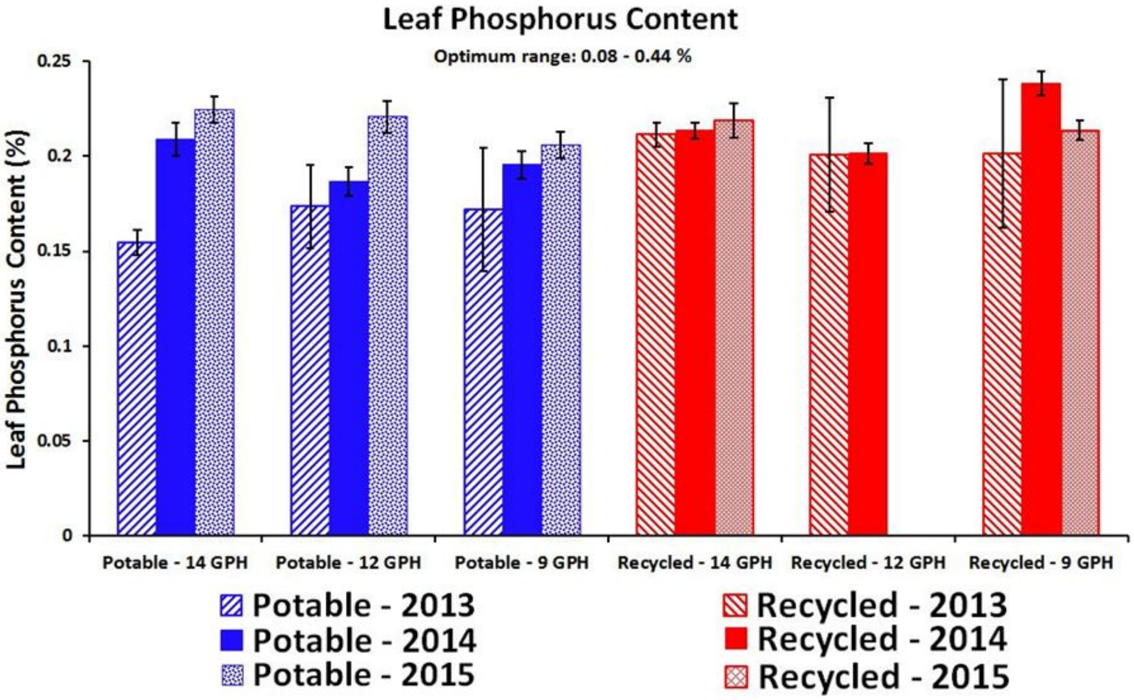


Figure 31. Leaf phosphorus content. 2015 data is not available for Recycled – 12 GPH. Vertical error bars indicate standard error values.

Leaf Phosphorus (%)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	0.155	0.174	0.172	0.211	0.201	0.201
2014	0.209	0.187	0.196	0.214	0.202	0.238
2015	0.225	0.221	0.209	0.219	No Data	0.214

Leaf Phosphorus ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	a	ad	a	a	a	abc
2015	ab	ab	a	ab	No Data	a

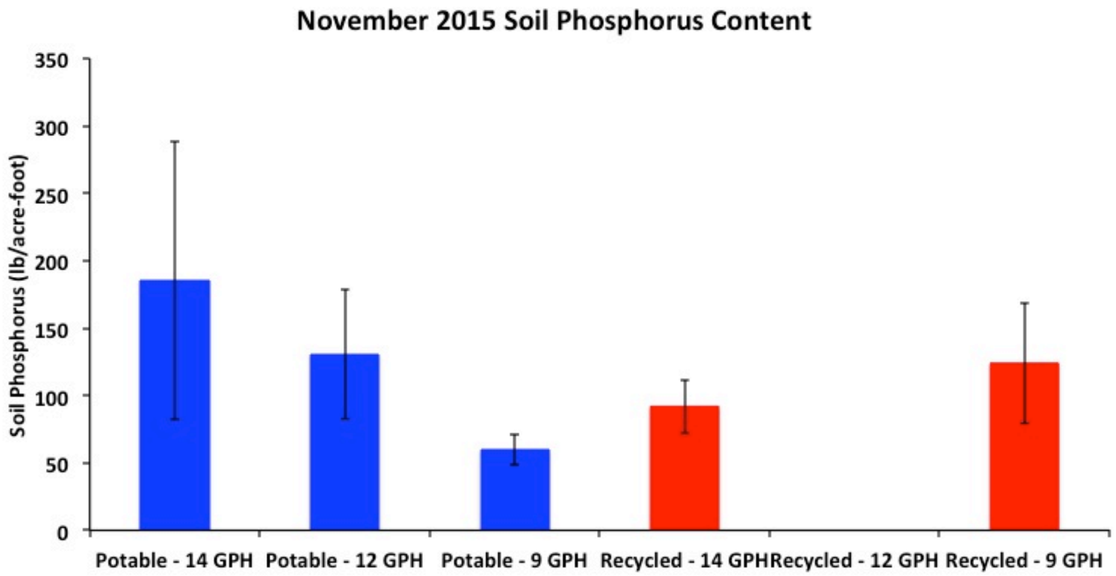


Figure 32. November 2015 Soil Phosphorus content. 2015 data not available for Recycled – 12 GPH. Error bars indicate standard error values.

Soil Phosphorus	**POTABLE**			**RECYCLED**		
(lb/acre-foot)	**14 GPH**	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2015	185	131	60	92	No Data	124

Trees irrigated with recycled water initially had leaf phosphorus levels on the higher end of the spectrum in 2014, and at that point, leaf iron content in those trees was not reduced relative to leaf iron content of trees irrigated with potable water (Figure 33). However, in 2015, leaf iron content decreased in trees irrigated with potable water, possibly due to higher soil pH and antagonistic effects from excessive phosphorus levels (Figure 33). Phosphorus content was not available for either water source.

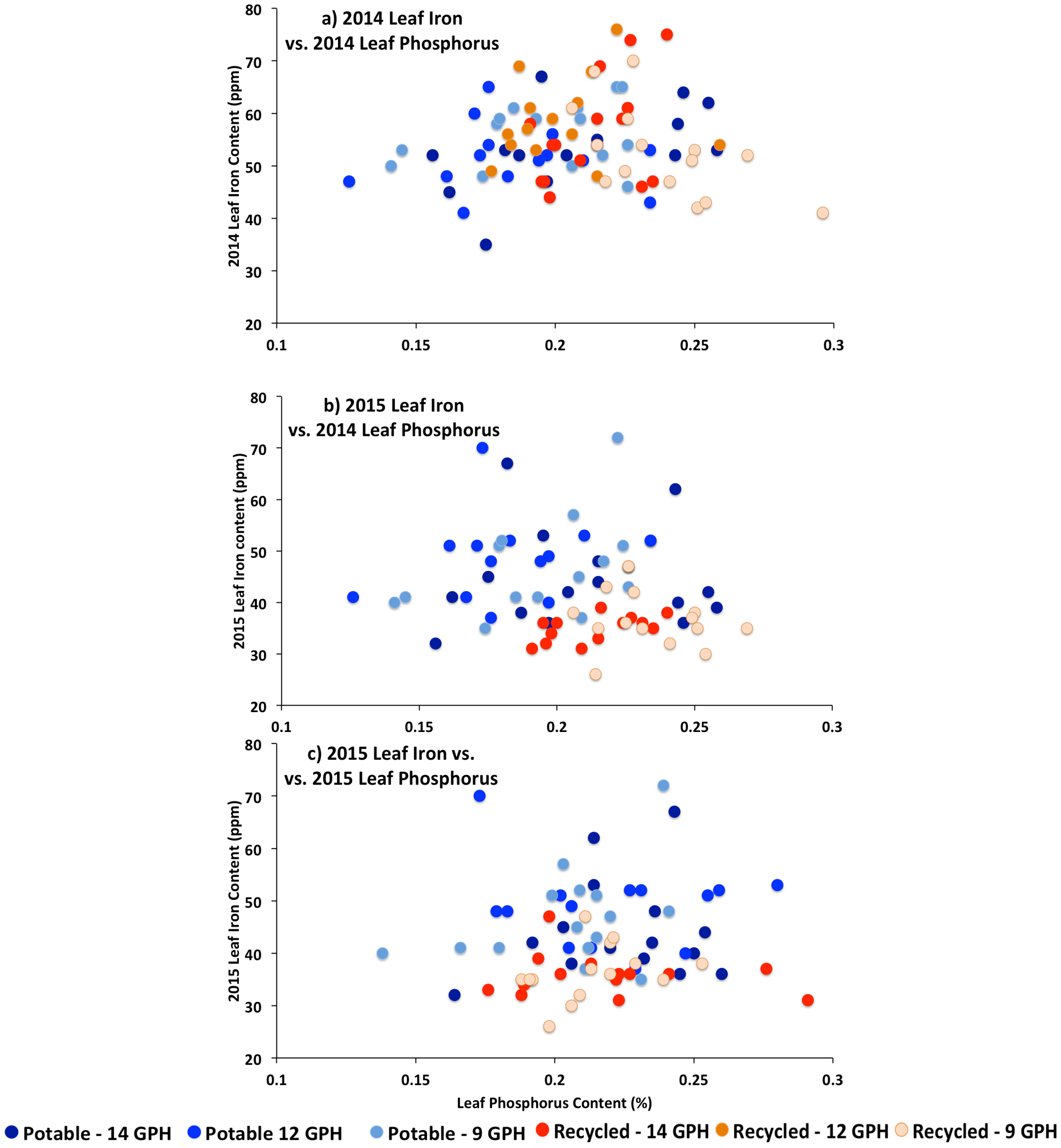


Figure 33. Leaf concentrations of Phosphorus vs Iron, 2014 and 2015. 2015 data is not available for Recycled – 12 GPH.

Leaf Potassium Content

After nitrogen and phosphorus, potassium is typically the third most limiting nutrient for plants. Potassium ions regulate the opening and closing of stomata for the uptake of carbon dioxide and release of water (Heldt and Heldt, 2005). Avocado trees deficient in potassium present new foliage with narrow leaves pale green or yellow in color, while older leaves develop interveinal chlorosis (Dreistadt, 2008). Leaf curling and discolored or necrotic spots are also common symptoms, and yield and fruit size may be reduced (Dreistadt, 2008). An excess of potassium may cause deficiencies in calcium and magnesium (Dreistadt, 2008). Optimal leaf potassium levels fall in the range of 0.75 – 2%. The UC IPM guide published that a concentration less than 0.35% is considered deficient, and greater than 3% is considered excessive (Dreistadt, 2008). In cases of potassium deficiency, potassium chloride should not be used as fertilizer as it adds undesirable chloride ions to the soil solution.

A deficiency in potassium is associated with grey discoloration in the pulp of avocado fruit, while on the contrary potassium sufficiency reduces likelihood for brown discoloration and pulp spot (Du Plessis and Koen, 1992). Potassium sufficiency also helps to ensure higher yields (Bender and Faber).

Levels of potassium in leaves were not different from each other in 2014, however in 2015, potassium content was higher in trees irrigated with recycled water than in trees irrigated with potable water (Figure 34). Potassium content did not change between 2014 and 2015, in trees irrigated with recycled water, while it dropped in trees irrigated with potable water. This is in contrast to previous studies that found a decline in leaf potassium content in trees grown in soils with higher phosphorus levels; this suggests there is some other interaction between nutrients or soil pH that is enhancing potassium uptake in the trees irrigated with recycled water. As indicated in AP Figure 19, leaf potassium content was generally higher in trees irrigated with recycled water, but these trees still consistently had the lowest yields. Since potassium has been shown to increase yield, the data suggest that other factors, such as osmotic stress, zinc and iron deficiency, reduced chlorophyll and smaller tree size over-ride the positive effects of potassium on yield.

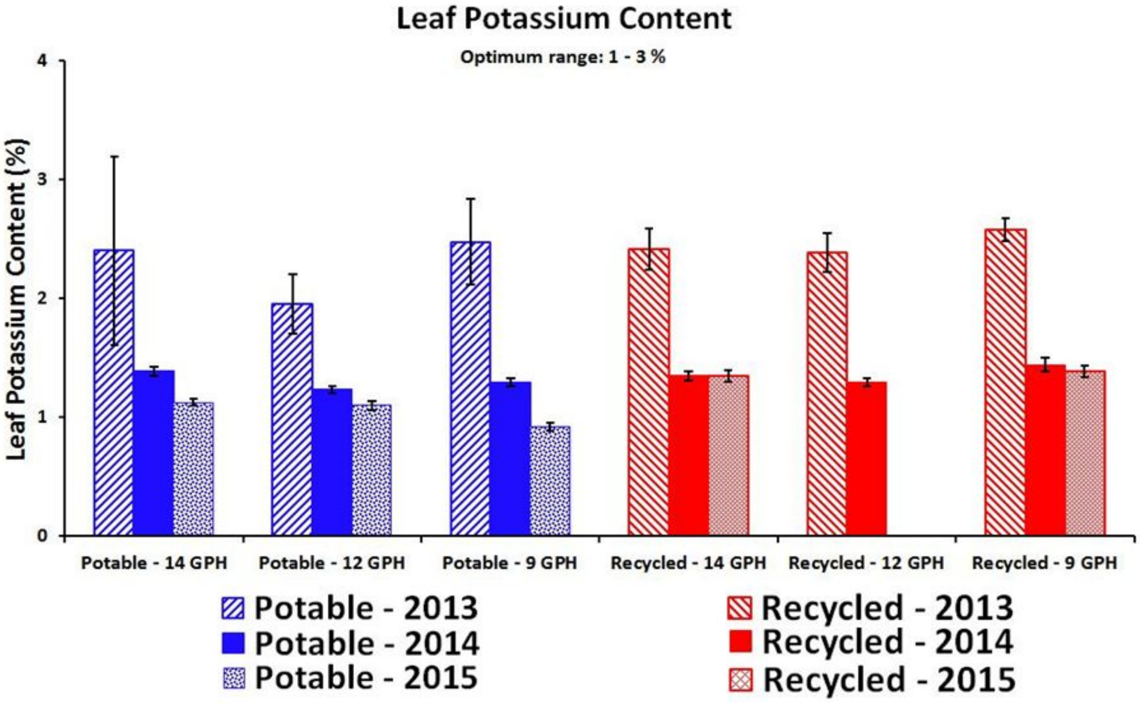


Figure 34. Leaf Potassium content. 2015 Data not available for Recycled – 12 GPH. Error bars indicate standard error values.

Leaf Potassium (%)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	2.40	1.96	2.48	2.41	2.39	2.58
2014	1.40	1.23	1.30	1.35	1.29	1.44
2015	1.12	1.10	0.92	1.33	No Data	1.39

Leaf Potassium ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	a	ae	ae	ae	a	ad
2015	be	be	bc	a	No Data	a

Relative to potable water treatments, calcium and magnesium were reduced in recycled water treatments, according to 2015 leaf analysis, and associated with higher levels of K. Potassium content of the soil decreased with decreasing irrigation rates, irrespective of type of water source (Figure 35). Thus, soil availability of potassium is not responsible for the higher potassium in leaves from trees irrigated with recycled water.

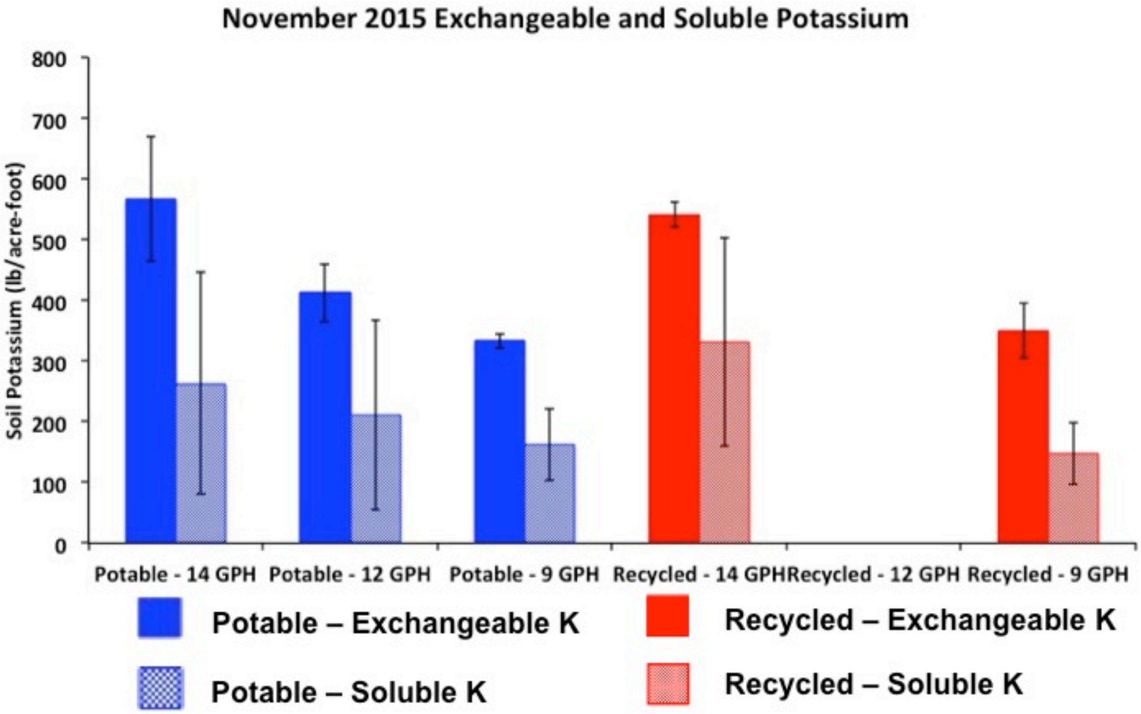


Figure 35. November 2015 exchangeable and soluble potassium in the soil. Error bars indicate standard error. Data not available for Recycled – 12 GPH.

Soil Potassium (lb/acre-foot)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Exchangeable K	567	413	333	541	No Data	350
Soluble K	264	211	163	331	No Data	147

Leaf Zinc Content

Zinc is an essential micronutrient for plants, and is needed for proper enzyme function, hormone production, and internode elongation (Hafeez et. *al.,* 2013). Zinc deficiency reduces yield in a number of crops (Hafeez et. *al,* 2013). The minimum threshold for zinc concentrations in avocado leaf tissue is 15 ppm; optimal range is 30-250 ppm (Fruit Growers' Lab, 2014). Symptoms of zinc deficiency affect the young leaves and appear as interveinal chlorosis, compact rosette-like foliage, decreased leaf surface area with necrotic margins, and round red fruit (Whiley, 2002). Zinc deficiency also reduces pollen production and fertility; a decline in pollen count and pollen viability drastically limits chances for high or even modest yields (Hafeez et. *al*., 2013). Reduced yield was correlated with low levels of zinc in the present study (AP Figure 20; AP Figure 29). Zinc deficiency is common in avocado, particularly in soils with high pH (Ruehle, 1940). It is likely that pH may increase in soils irrigated with recycled water due to the presence of minerals in the water, or the presence of calcareous parent material in the soil, more commonly found in more arid climates.

Symptoms of zinc deficiency were not fully quantified in this study, but mild symptoms of zinc deficiency presented themselves more frequently and more dramatically in trees irrigated with recycled water in comparison to trees irrigated with potable water. In trees irrigated with recycled water, a higher incidence of reduced leaf size and reduced leaf internode distance were observed. Shorter distances between leaves results in a more compact canopy, which inhibits air flow. This provides an inviting environment for pests and pathogens, while simultaneously reducing light penetration into the tree canopy. Although zinc concentrations measured in 2014 were not deficient, they were not optimal

(Figure 36). In late 2015, trees irrigated with recycled water exhibited mild symptoms of zinc deficiency, particularly compact foliage and small leaves.

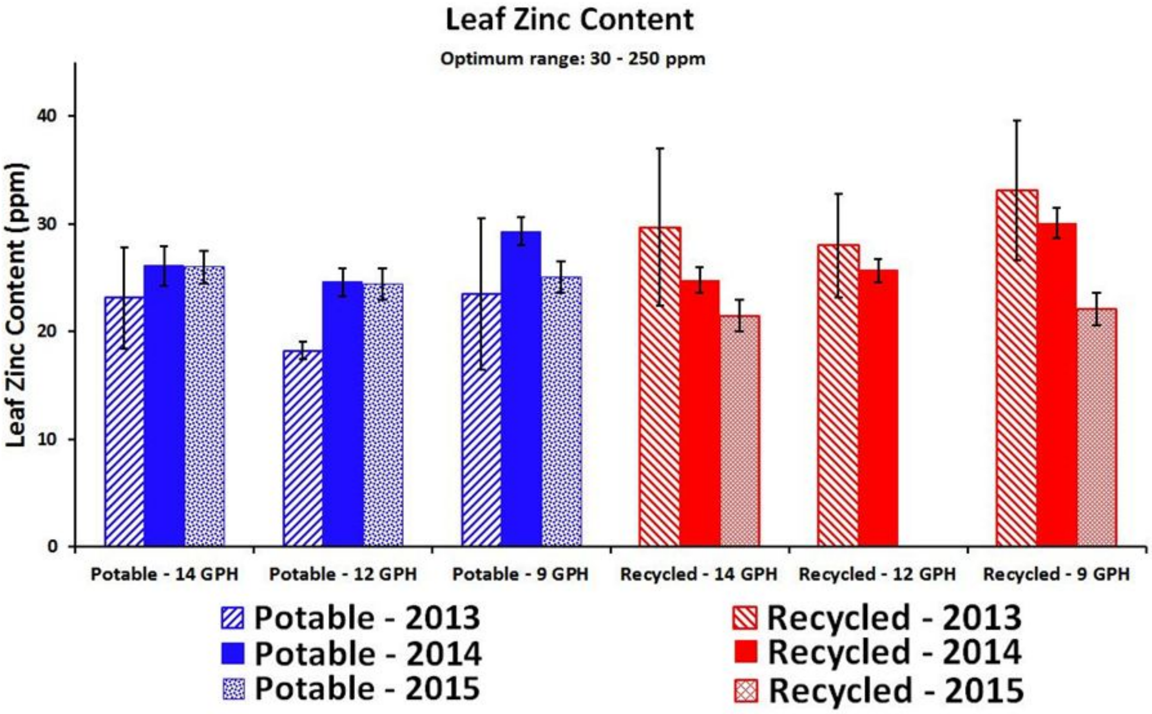


Figure 36. Leaf zinc content. 2015 data is not available for Recycled – 12 GPH. Vertical error bars indicate standard error values.

Leaf Zinc (ppm)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	23.1	18.2	23.5	29.7	28	33.1
2014	26.1	24.6	29.3	24.8	25.7	30.1
2015	26.0	24.4	25.0	21.5	No Data	22.1

Leaf Zinc ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	a	a	ab	a	a	ab
2015	a	a	a	ac	No Data	ac

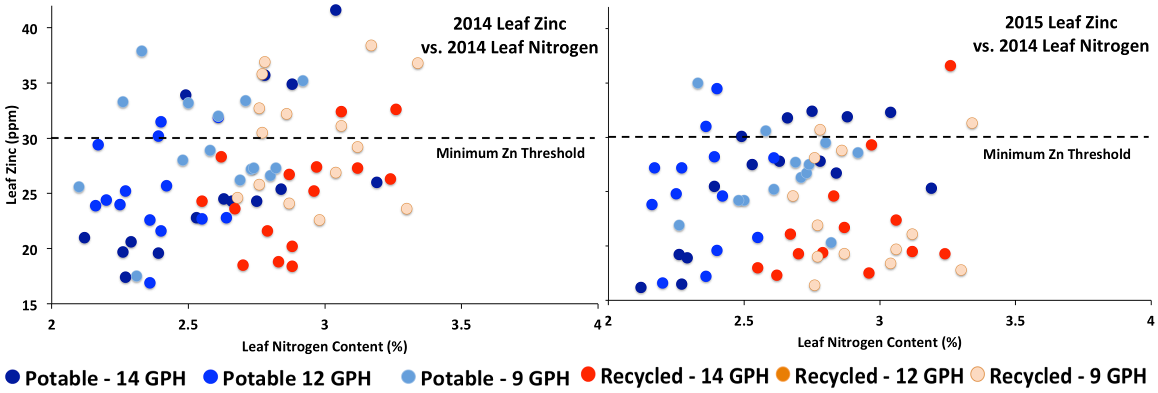


Figure 37. Leaf zinc content vs. leaf nitrogen content. Leaf zinc contents of individual avocado trees for 2014 (left) and 2015 (right) in relation to 2014 leaf nitrogen content. Leaf zinc concentrations above 30 ppm are considered optimal, while zinc concentrations below 15 ppm is considered deficient.

Leaf Magnesium Content

Magnesium is an essential element that is integral to photosynthesis due to its role as the coordinating ion at the center of chlorophyll, holding the organic chains of the molecule together (Heldt and Heldt, 2005). Magnesium deficiency is identified is as interveinal chlorosis (loss of green color) in older foliage, while an excess of magnesium reduces the potassium content in avocado (Dreistadt, 2008). Since magnesium is needed to complete the chlorophyll structure, lower levels of magnesium directly reduce chlorophyll content, and this can lead to a chain reaction of reduced photosynthetic capacity, reduced growth, and reduced yield. These observations were observed in the present study in association with reduced leaf magnesium content in trees irrigated with recycled water (Figure 38). Soil magnesium concentrations were reduced in soils irrigated with recycled water, perhaps due to displacement from cation exchange sites by sodium (Figure 39). This would subsequently allow leaching of magnesium from the soil. 2016 yield vs. leaf magnesium content is displayed in AP Figure 21.

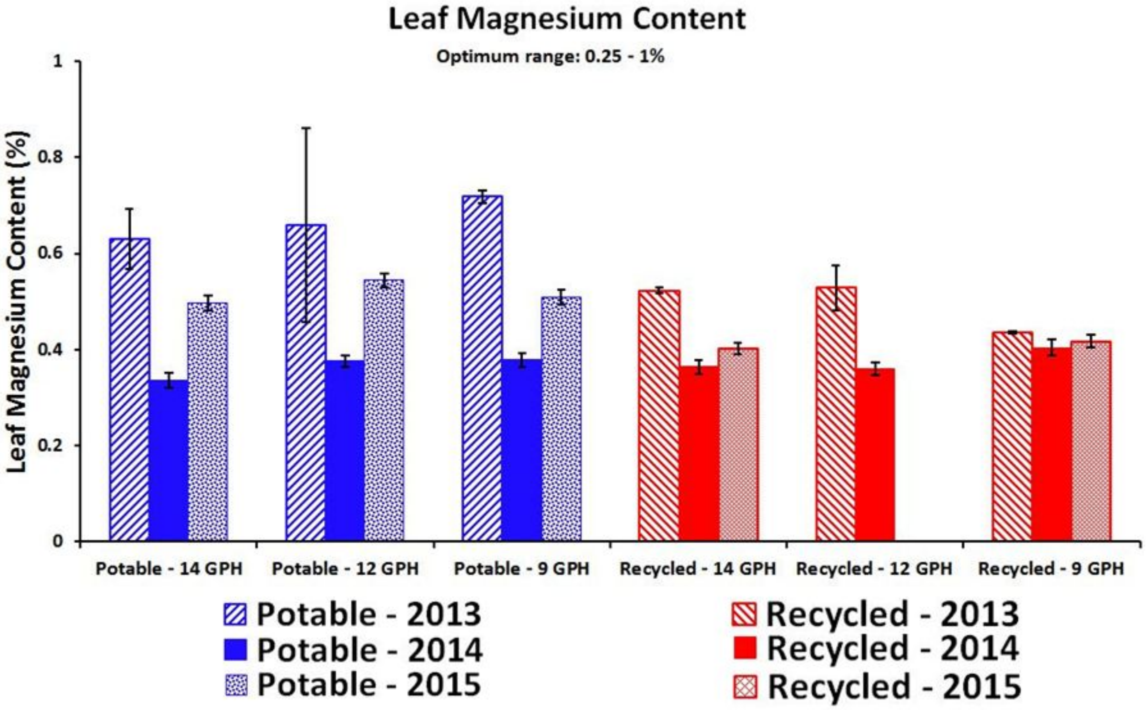


Figure 38. Leaf magnesium content. 2013 data sample size was limited: All potable treatment averages were based on results from 2 trees, all recycled treatment averages were based on results from 3 trees. 2015 data is not available for Recycled – 12 GPH. Vertical error bars indicate standard error values.

Leaf Magnesium (%)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	0.631	0.659	0.718	0.523	0.528	0.436
2014	0.335	0.375	0.379	0.364	0.359	0.404
2015	0.498	0.544	0.508	0.401	No Data	0.417

Leaf Magnesium ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	a	a	a	a	a	ac
2015	bc	bd	bd	ac	No Data	ac

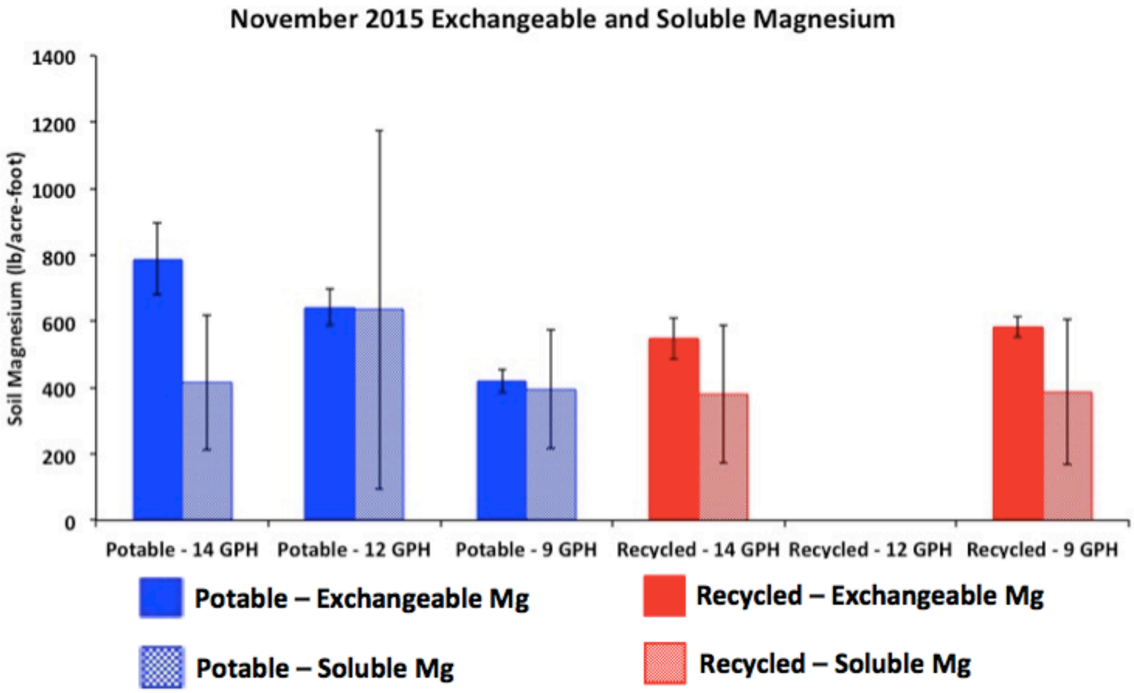


Figure 39. November 2015 exchangeable and soluble magnesium in the soil. Data not available for Recycled – 12 GPH. Error bars indicate standard error values.

Soil Magnesium (lb/acre-foot)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	789	643	421	549	No Data	583
2015	415	635	396	381	No Data	386

Leaf Calcium Content

Calcium is incorporated into plant cell walls and is needed for cell division and growth (Bender and Faber). Low calcium is associated with poor quality fruit and accelerated ripening due to more rapid breakdown of cell walls in fruit. (Witney et. *al*, 1990). Calcium uptake is reduced in trees irrigated with recycled water and may thus explain poorer fruit quality in these irrigation treatments (Figure 40). In the present study, low calcium content in leaf tissue was strongly correlated with reduced growth (Figure 42, AP Figure 30) and reduced yield (AP Figure 22) in trees irrigated with recycled water. This may be due to the reduction in growth potential, as calcium is needed for cell division and growth. If growth cannot occur at the cellular level, it cannot occur at the organismal level. As stated earlier, smaller trees are unable to physically, or nutritionally support fruit. As was the case for soil magnesium content, soil calcium content was reduced in soils irrigated with recycled water, likely due to sodium ions displacing calcium ions from cation exchange sites.

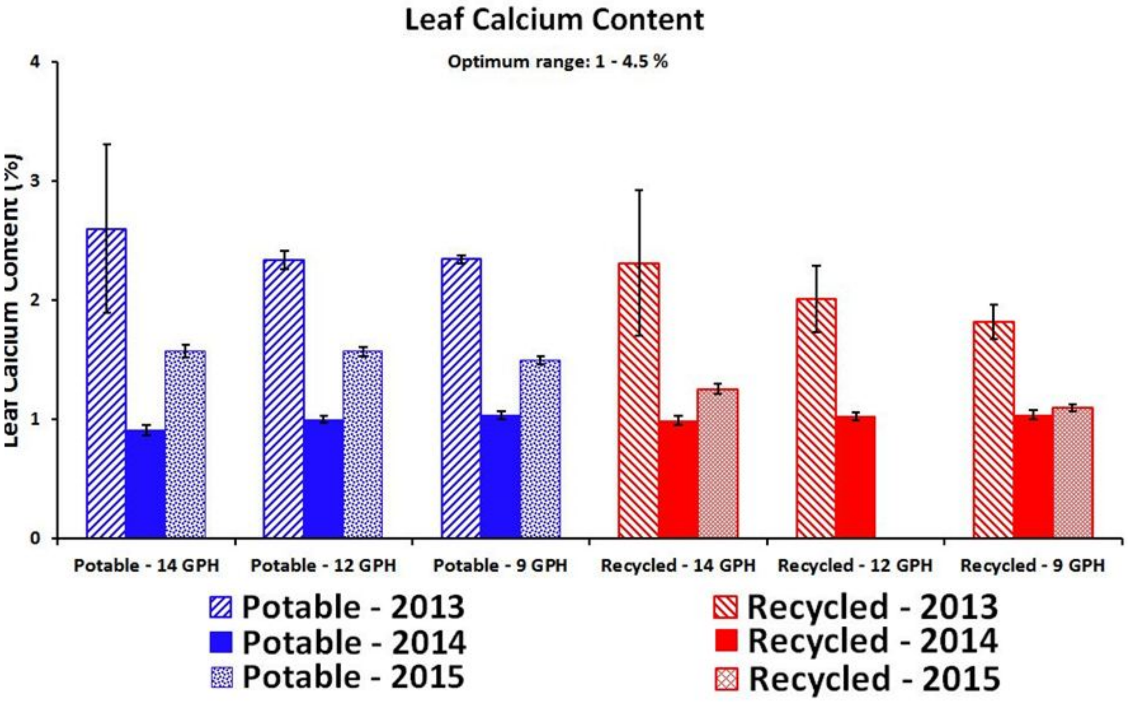


Figure 40. Leaf calcium content. 2013 data sample size was limited: All potable treatment averages were based on results from 2 trees, all recycled treatment averages were based on results from 3 trees. 2015 data is not available for Recycled – 12 GPH. Vertical error bars indicate standard error values.

Leaf Calcium (%)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	2.60	2.34	2.35	2.31	2.01	1.82
2014	0.91	1.00	1.04	0.99	1.02	1.04
2015	1.57	1.57	1.50	1.26	No Data	1.10

Leaf Calcium ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	a	ad	ad	ad	ad	ad

2015	bc	bc	bc	bcd	No Data	d

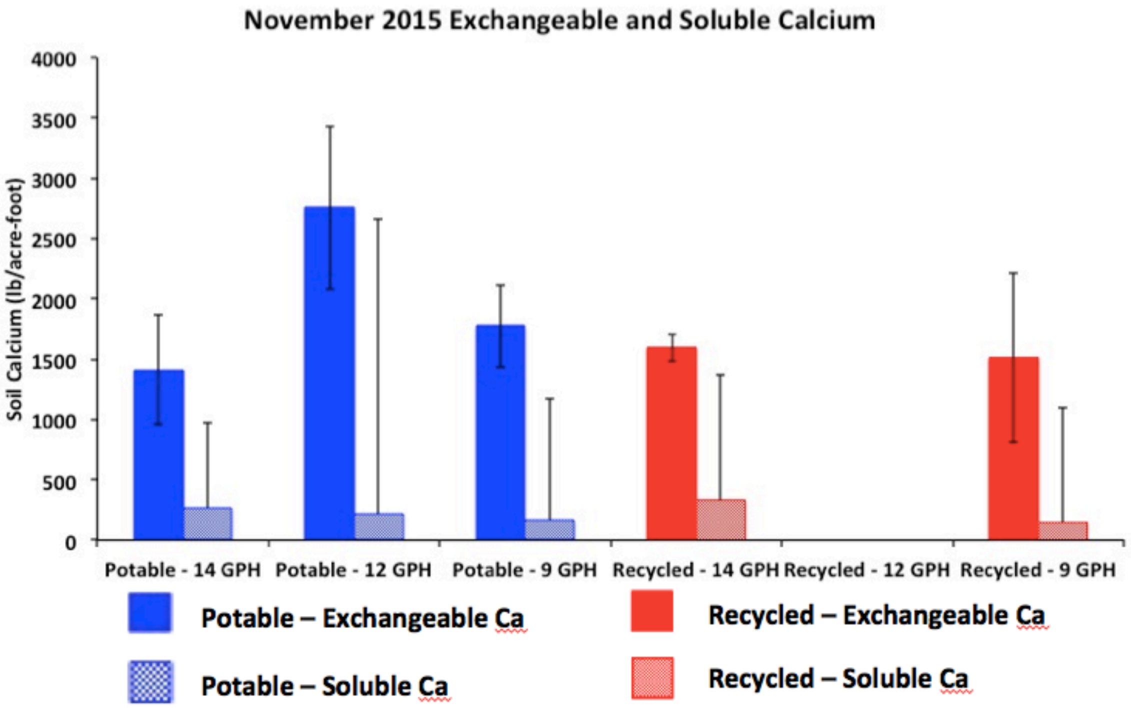


Figure 41. November 2015 exchangeable and soluble calcium in the soil. Error bars represent standard error.

Soil Calcium (lb/acre-foot)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Exchangeable Ca	5103	5477	3610	4730	No Data	4277
Soluble Ca	1414	2754	1776	1596	No Data	1511

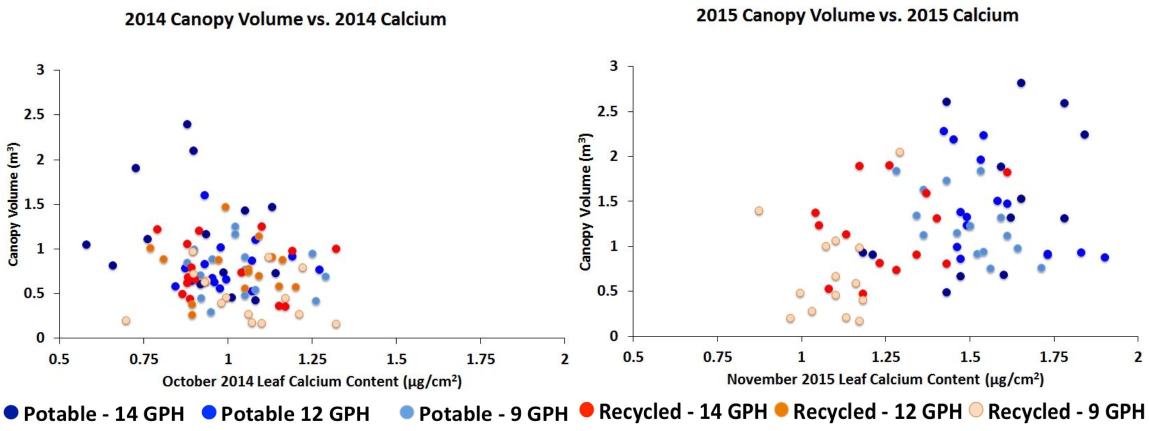


Figure 42. Tree canopy volume vs. leaf calcium content. Left: 2014 Canopy volume vs. 2014 leaf calcium concentrations. **Right:** 2015 Canopy volume vs. 2015 leaf calcium concentrations. 2015 data not available for Recycled – 12 GPH.

Leaf Iron Content

Iron is incorporated into cytochromes in mitochondria and chloroplasts, and is also involved in the synthesis of chlorophyll (Heldt and Heldt, 2005). Avocado trees deficient in iron have young leaves are white to yellow in color between the veins, with necrotic leaf tips and leaf margins (Whiley, 2002). Leaves drop and branches die back, while fruit is light green in color (Whiley, 2002).

Avocado trees at Witman Ranch were suffering from iron deficiency prior to the installation of a reverse osmosis system at the Ramona wastewater treatment plant and this deficiency could not be corrected via fertilization (Witman, personal communication, 2016). However, upon switching to potable water for 3 months, the trees recovered and roots grew prolifically (Witman, personal communication, 2016).

Leaf analyses from the present study site in Escondido show that iron deficiency is also present. In 2014, iron concentrations in the leaves hovered just above the minimum threshold of 50 ppm. However in 2015, leaf iron concentrations dropped, and the change was much more significant in trees irrigated with recycled water. Iron deficiency among trees irrigated with recycled water was correlated with decreased leaf chlorophyll content (Figure 18). Along with other factors, a drop in chlorophyll content likely contributes to the decreased growth rate of trees and decreased fruit yield in irrigated with recycled water.

Although not relevant in this study, an excess of iron induces manganese deficiency, and ironically, manganese deficiency exhibits the same symptoms as iron deficiency (Dreistadt, 2008). An avocado grower might notice such symptoms and attempt to correct it with the application of iron, which would only exacerbate the problem. Thus soil and leaf testing are necessary to identify best courses of action. Manganese was not deficient in the trees in this study, as would be expected since iron was deficient rather than excessive.

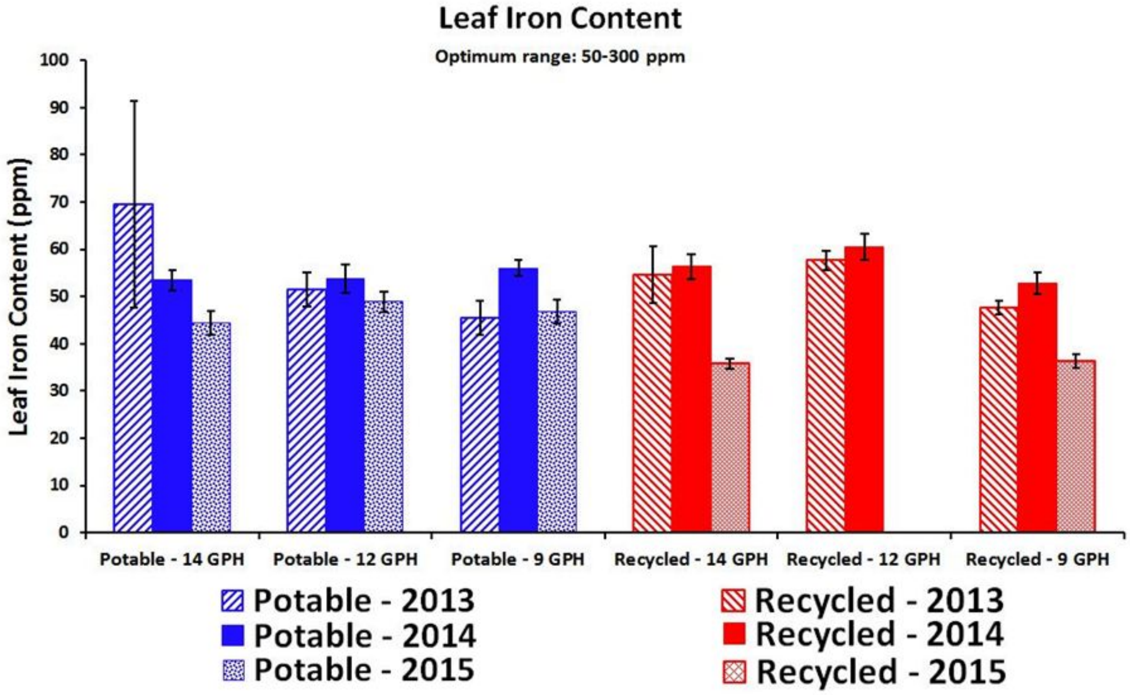


Figure 43. Leaf iron contents. 2015 data is not available for Recycled – 12 GPH. Vertical error bars indicate standard error values. Data from analyses provided by Fruit Growers' Lab Inc., Santa Paula, CA.

Leaf Iron (ppm)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2013	69.5	51.5	45.5	54.6	57.6	47.6
2014	53.4	53.9	56.0	56.3	60.5	52.7
2015	44.3	48.9	46.7	35.8	No Data	36.4

Leaf Iron ANOVA groups	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2014	a	a	ac	ac	ac	a
2015	ab	a	a	b		b

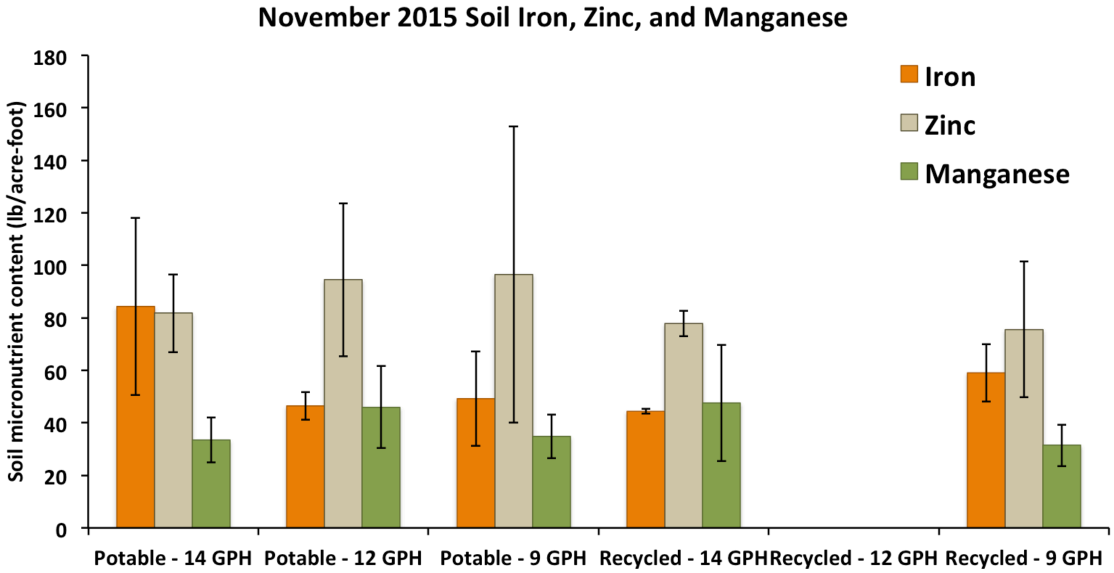


Figure 44. November 2015 soil concentrations of iron, zinc, and manganese. 2015 Data not available for Recycled – 12 GPH. Error bars indicate standard error values.

Soil micronutrients	**POTABLE**			**RECYCLED**		
(lb/acre-foot)	**14 GPH**	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Iron	84.3	46.4	49.2	44.4	No Data	59.1
Zinc	81.7	94.5	96.5	77.9	No Data	75.5
Manganese	33.5	46.0	34.8	47.6	No Data	31.5

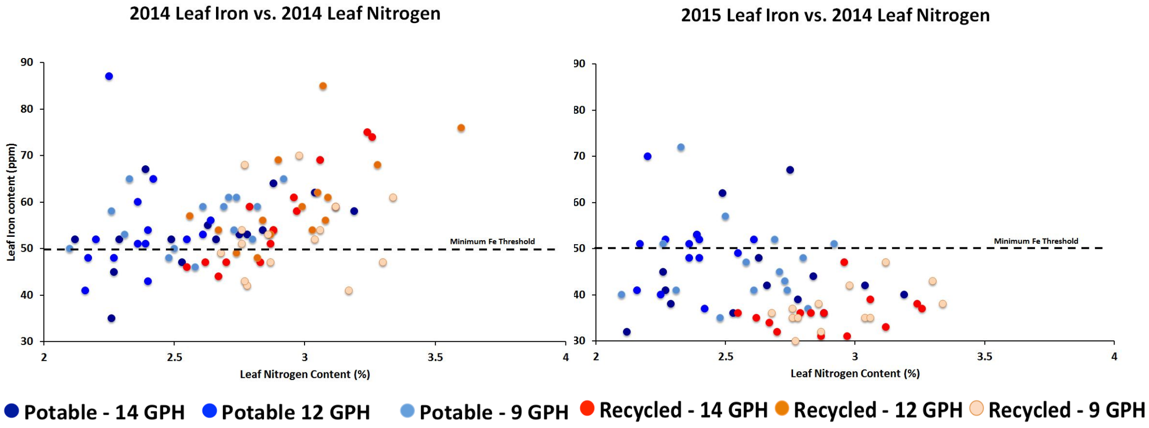


Figure 45. Leaf Iron content vs. Leaf Nitrogen content of individual trees. 2014 leaf N vs. 2014 leaf Fe (left), 2014 leaf N vs. 2015 leaf Fe (right).

Excess nitrogen in the form of nitrate reduces iron availability and/or assimilation. While iron levels were lower in all treatments in 2015 than in 2014, the decline in leaf iron content was much more drastic in the trees irrigated with recycled water. Avocado exhibited iron deficiency under high nitrate conditions in previous work by Bar and Kafkaki (1992). Ruehle (1940) noted zinc deficiency in avocado trees grown on high-pH soils. These data can be explained by reduced bioavailability of micronutrients in high pH soil conditions (AP Figure 7). Recycled water is often rich in minerals, resulting in a high pH. The Escondido wastewater has a pH of 7.5 and an abundance of minerals (Escondido, 2014). Furthermore, plant uptake of nitrate facilitates an increase in pH due to bicarbonate and hydroxide ions being released to the soil for anion exchange (Kirkby, 1969).

CHAPTER 5

FRUIT QUALITY

Fruit Yield and Quality Analysis

Fruit was harvested on March 30, 2016. Fruit was cut from the trees using Corona Tools clippers designed specifically for harvesting avocado fruits (Corona Tools, Corona, CA). Stems were cut just above the fruit to provide protection against fruit rot. During the harvest process, fruit was stored in paper bags in the shade (for trees with low yield). Fruit from trees with high yields was stored in plastic harvesting bins in the shade. The fruit was then taken to a weigh station, where yields from individual trees were counted and weighed. Approximately 3 fruit per tree were selected for fruit quality analysis and placed in paper bags until they could be placed in cold storage. Fruit was stored in plastic Ziploc bags left slightly open, and kept in cold storage for 5 days. After 5 days, 5 fruit per irrigation treatment were analyzed for oil content, while the remaining fruit were transferred to ripening chambers, which were maintained at 85% humidity and 18.4^{o}C. After 5 days in the ripening chambers, golden delicious apples were added to speed ripening via release of ethylene. The ripening chamber was checked daily for ripe fruit and these fruit were removed and placed in cold storage until the taste quality survey (April 14, 2016).

To determine the fruit oil content, core samples were taken from the pulp and weighed. The peel and, if applicable, the seed coat, were sliced off from the pulp core. Core weights were recorded as fresh core weights. Core samples were then microwaves at 20% power for 35 minutes, after which time their weights were again recorded. Core samples were microwaved for an additional 5 minutes at 20% power, and re-weighed. Core samples were allowed to sit overnight, and were then microwaved in the morning for an additional 5 minutes at 20% power. The final dry weight was subtracted from initial fresh core weights and divided by initial weight. This calculation gives the oil content of the fruit, as any difference in mass was due to water lost via evaporation in the microwave.

The day of the fruit quality survey, avocados were sliced, rated on appearance, odor, and ease of preparation according to the following categories: ease of peeling, presence and amount of slime between peel and fruit, fruit pulp color, peel appearance, pulp appearance, percentage of rot, presence and intensity of pockmarks on the surface of the fruit internal to the peel, odor, and an overall rating. The fruits were then sliced into 1-inch cubed pieces and stored in Tupperware containers according to their irrigation treatment until the fruit was served to survey participants. Approximately 6 fruit were used per irrigation treatment. 60 people participated in the survey. Each participant received a tray with 6 avocado slices in small paper cups, 1 slice per irrigation treatment. In order to facilitate a double-blind study, the order of fruit on trays were randomized. For example, one individual's tray may have been ordered as: Potable – 9 GPH, Potable – 14 GPH, Recycled 12 – GPH, Potable – 9 GPH, Recycled – 14 GPH, Recycled – 9 GPH, while another person's tray could be: Recycled – 14 GPH, Recycled – 9 GPH, Potable – 12 GPH, Potable – 9 GPH, Recycled – 12 GPH, Potable – 14 GPH. In addition, the cups were labeled with random 3-digit numbers obtained via a randomization function in Excel. These numbers corresponded to the 3-digit numbers on the individual participant's survey response form; no two survey forms had the same numbers.

RESULTS

Fruit Production

Since the trees were young, fruit production data should be viewed conservatively. In the first year after planting, trees in the both Potable – 14 GPH and Recycled – 14 GPH treatments had almost no fruit production, while trees in the Potable – 12 GPH and Potable – 9 GPH treatments produced an average of 0.66 fruits per tree. Recycled – 12 GPH trees produced an average of 1.25 fruits per tree, while Recycled – 9 GPH trees produced an average of almost 2 fruits per tree. In the second year, fruit production was higher in all treatments over the previous year, and was much higher in trees irrigated with potable water in comparison to trees irrigated with recycled water (Figure 46). At first glance, this appears to suggest irrigation with recycled water is not a viable option; however, further investigation via leaf nutrient analyses suggest that if nutrient levels can be brought into ideal ranges via additional water treatment and strategic orchard management, recycled water may indeed be a useful source of water for avocado irrigation. Leaf nitrogen content is of particular significance, and the correlation between leaf nitrogen content, water nitrogen concentrations, and fruit yield are discussed in further detail in the Leaf Nitrogen Content section.

Leaf chlorophyll content was associated with reduced yield (Figure 47). This is to be expected, as reduced chlorophyll content reduces a tree's ability to photosynthesize efficiently, and thus reduces energy production of a tree. Less energy production means fewer resources available to invest in fruit production. As shown in Figure 47, trees irrigated with recycled water (red symbols) dominate the lower range of chlorophyll concentrations in May 2015 (middle graph) and November 2015 (bottom graph). The graphs also show that fruit yield was significantly lower from trees irrigated with recycled water, particularly for those trees with lower leaf chlorophyll content. Conversely, trees irrigated with potable water (represented by blue dots), had higher levels of chlorophyll content in leaves and this was associated higher yields (Figure 47).

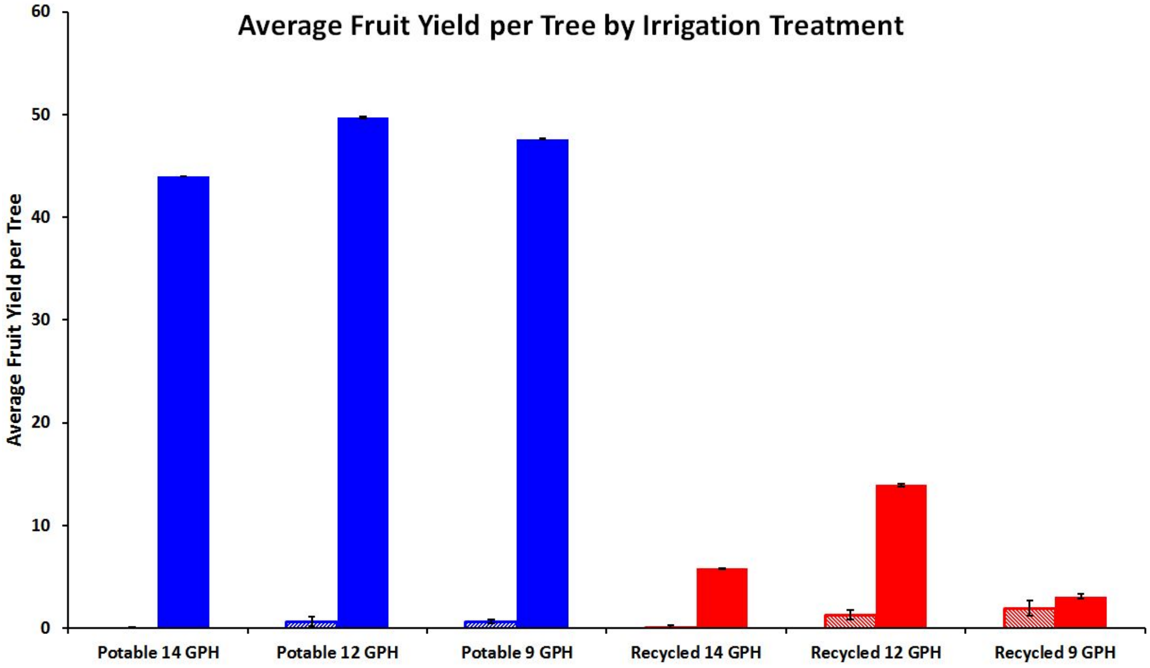


Figure 46. Fruit yields for avocado trees provided with potable water or recycled wastewater at different application rates. Fruit yield was counted in Winter 2014, and for the second year in December 2015. Vertical error bars indicate standard error values. Average values are shown in table below.

Yield	**POTABLE**			**RECYCLED**		
(# Fruits/tree)	**14 GPH**	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
March 2015	0.1	0.7	0.7	0.1	1.3	1.9
March 2016	51.1	56.3	59.2	3.0	15.4	2.3

Yield	**POTABLE**			**RECYCLED**		
ANOVA groups	**14 GPH**	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
March 2015	a	a	a	a	a	a
March 2016	b	b	b	a	c	a

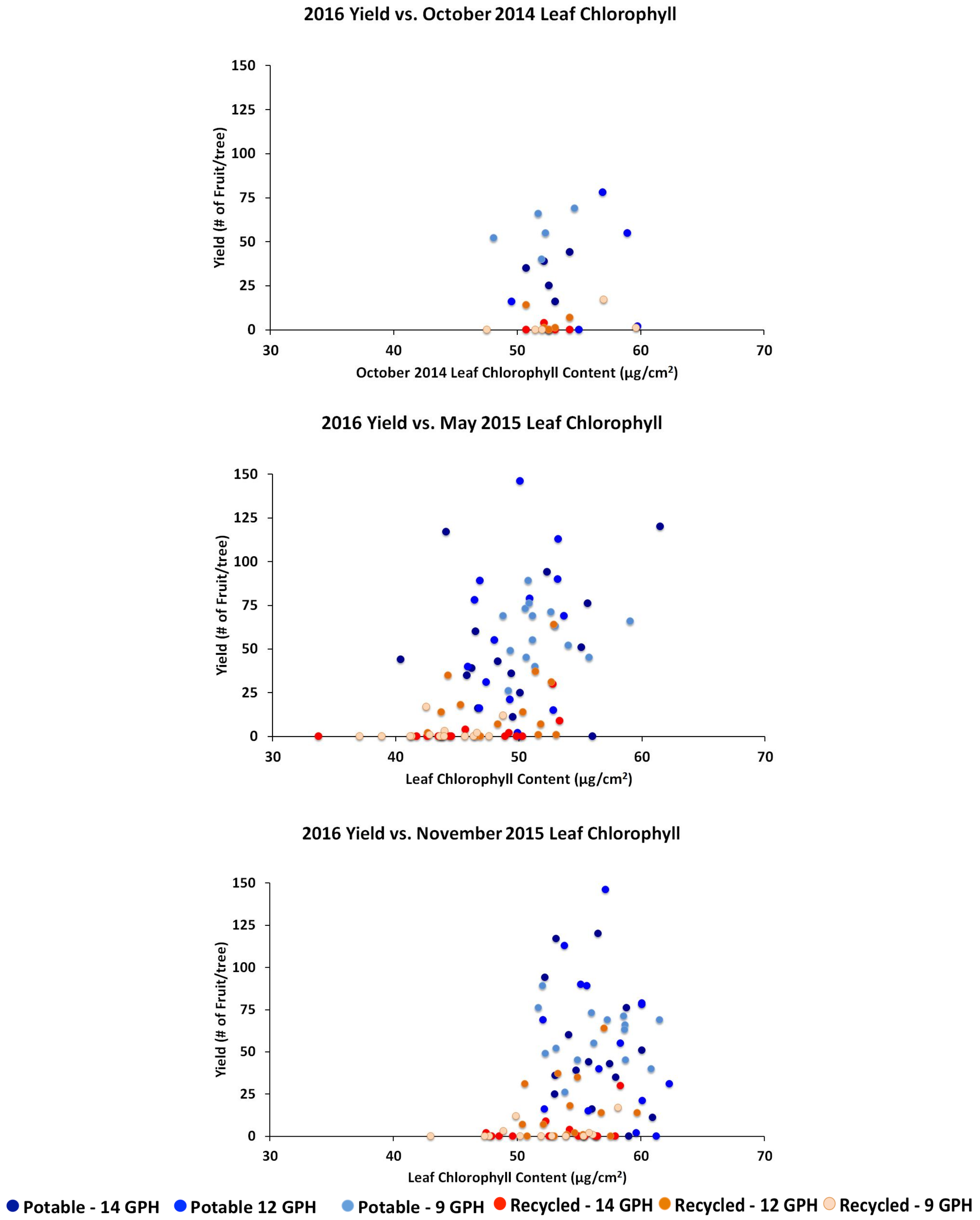


Figure 47. Leaf Chlorophyll Content vs. Yield. Top: March 2016 yield vs. October 2014 leaf chlorophyll content. **Middle:** March 2016 yield vs. May 2015 leaf chlorophyll content. **Bottom:** March 2016 yield vs. November 2015 leaf chlorophyll content.

Yield efficiency

Yield efficiency is calculated as number of fruit divided by canopy volume; results are shown in Figure 48. Trees irrigated with recycled water had significantly lower yield efficiencies, and as explained in the Leaf Nitrogen Content section, this is likely due to excessive nitrogen causing excessive tree growth at the expense of fruit yield.

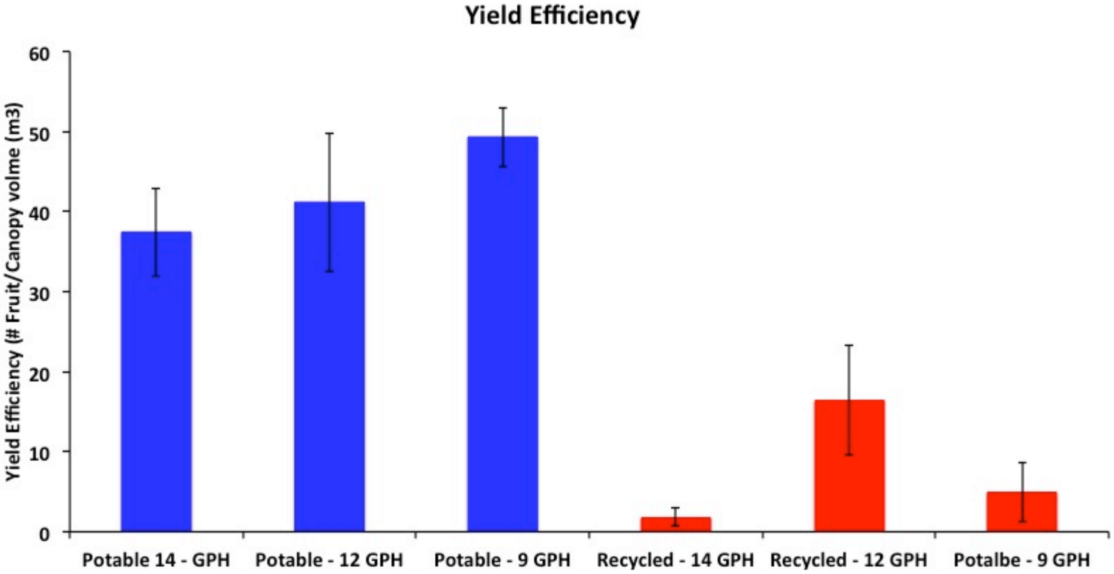


Figure 48. Yield efficiency. Yield efficiency is calculated as yield (number of fruit) divided by canopy volume (m^3).

Yield Efficiency (Yield/Canopy volume)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Yield Efficiency	37.4	41.2	49.3	1.8	16.4	4.9
ANOVA groups	ab	b	b	c	bc	c

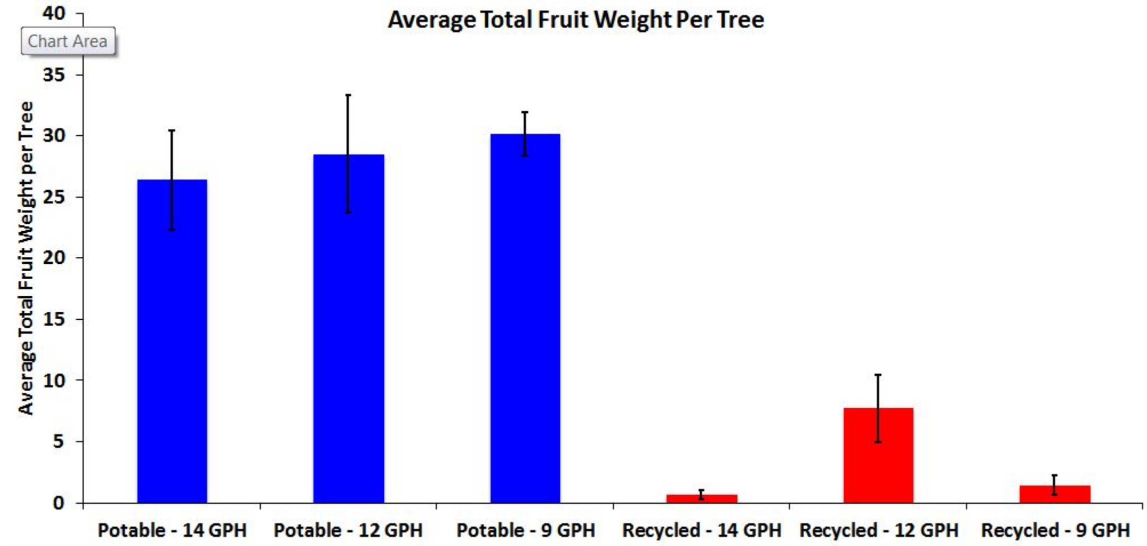


Figure 49. Average Total Fruit Weight per Tree, 2016 harvest. Error bars show standard error values.

Average Fruit Aftertaste Rating	POTABLE			RECYCLED		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Fruit Aftertaste	26.4	28.5	30.2	0.6	7.7	1.4
ANOVA groups	a	a	a	b	bc	b

Average individual fruit weight

When selling to avocado distributors, growers are typically paid for their harvest by the pound. The breakeven point is $0.65/pound. Fruit size also impacts grower profitability; fruits weighing between 7.5 and 8.75 ounces receive the highest dollar per pound value (Grangetto, personal communication). Fruits from Potable – 9 GPH and Recycled – 14 GPH fell in this high-value range (Figure 49). Every other irrigation treatment had fruits with higher than optimal individual fruit weight. Trees irrigated with lowest volumes of recycled water had highest individual fruit weight, but they also had very low yields. Low-yielding trees are able to yield larger individual fruits because there is less competition among fruits for available resources.

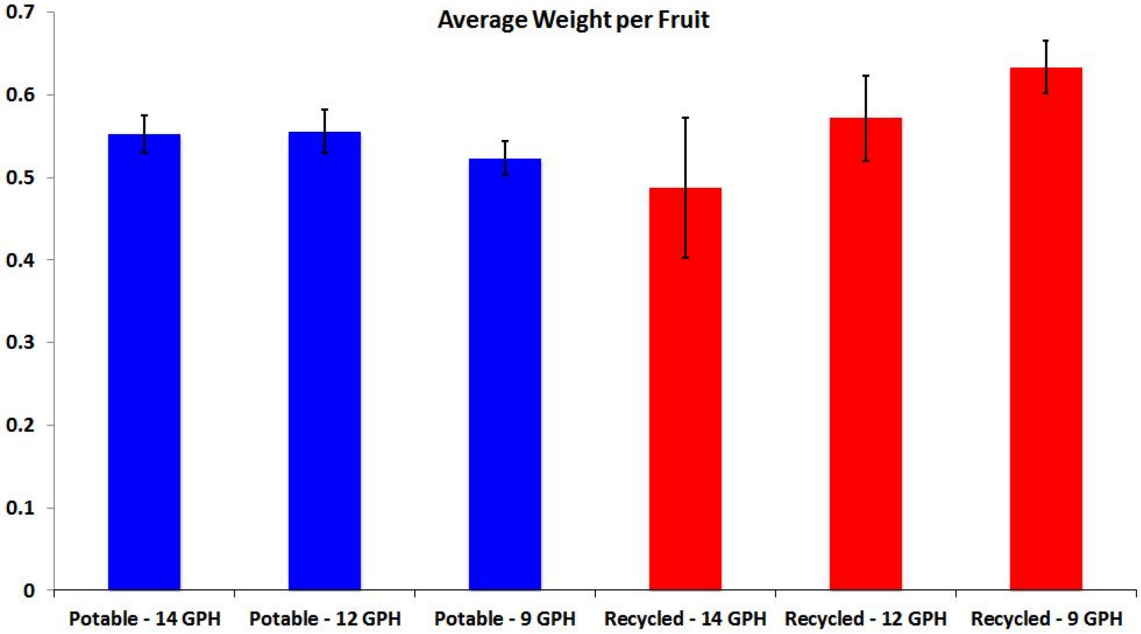


Figure 50. Average weight per individual fruit. Error bars show standard error values.

Average Individual Fruit Weight	POTABLE			RECYCLED		
	14 GPH	12 GPH	9 GPH	14 GPH	12 GPH	9 GPH
Fruit weight (oz)	8.8 oz	8.9 oz	8.4 oz	7.8 oz	9.2 oz	10.1 oz
ANOVA groups	a	a	a	b	abc	bc

Post-harvest fruit rot

A high rate of fruit rot was observed in this study. The March 2015 harvest, although small due to the young age of the trees, had a very high rate of fruit rot among trees irrigated with recycled water. Almost all of the fruit from trees irrigated with recycled water exhibited severe internal fruit rot, while almost none of the fruits from tress irrigated with potable water showed such symptoms. The fruit appeared normal externally, but upon slicing the fruit open longitudinally, the peels of each half detached from the fruit, leaving the fruit pulp intact. The pulp itself was entirely coated with a brown slime 2 mm thick. Some of this slime remained attached to the peel as well. Scraping off the slimy coat revealed an otherwise healthy looking fruit. Unfortunately, fruit rot data from 2015 was not fully quantified and pictures were not taken. It was thought that the high prevalence of fruit rot might be due to an excess of nitrogen. Fertilizer of all trees in the orchard was applied uniformly until July 2015, at which point additional fertilization was halted on trees irrigated with recycled water since the water supply provides necessary nutrients. As demonstrated by Fall 2015 leaf analyses, leaf nitrogen content subsequently declined to optimal or near-optimal ranges in most of the trees irrigated with recycled water. Fruit quality also improved significantly in the following year.

Fruit from March 2016 exhibited symptoms that appear similar to anthracnose and bacterial soft rot. The infectious agent that causes anthracnose is *Colletotrichum gloeosporioides* and *Colletotrichum acutatum,* while *Erwinia caratovora* is the microbe responsible for bacterial soft rot (Whiley, 2002). Anthracnose causes rotten brown spots on fruit pulp after harvest, and *Erwinia caratovora* causes the pulp to turn soft and brown (Whiley, 2002). High-quality fruit were not limited to trees irrigated with potable water, and poor-quality fruit was not limited to trees irrigated with recycled water. The following images are pictures of fruit from the March 2016 harvest, but are not necessarily representative of their respective treatments. Figure 60 graphs average visual fruit quality.

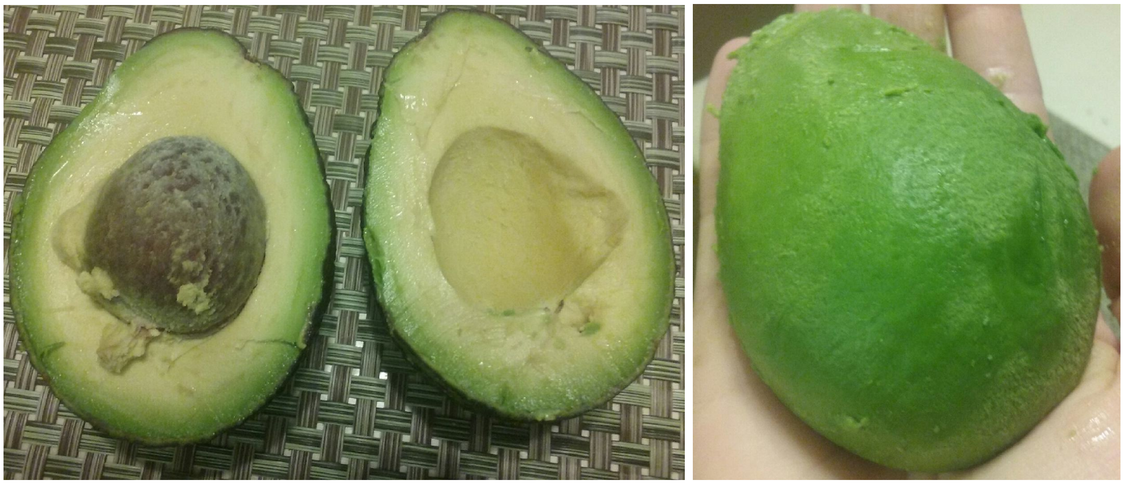


Figure 51. Potable – 9 GPH – Tree 4. High-quality fruit, representative of fruit with an appearance rating of 4.5-5.

Figure 52. Potable – 14 GPH- Tree 8. This fruit received a rating of 2.85 due to rotten spots on the pulp and a slimy coating on the pulp just inside the peel. The right image shows circular rotten spots on the peel, a characteristic anthracnose symptom.

Figure 53. Potable – 9 GPH – Tree 5. This fruit received a 4 rating. Overall it looked good, but had some brown slime on the pulp just inside the peel.

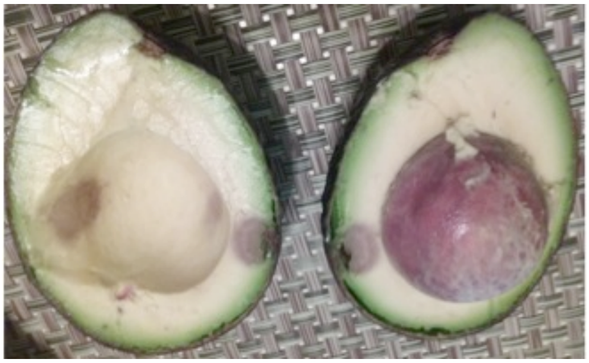


Figure 54. Potable – 12 GPH – Tree 10. characteristic anthracnose symptoms with circular brown rot.

Figure 55. Recycled – 12 GPH – Tree 7. This fruit received a 1 rating. This picture does not capture the severity of the rot, or the associated foul odor. The fruit pulp was almost entirely coated in a thick dark brown slime inside the peel.

Figure 56. Recycled – 9 GPH – Tree 12. This fruit also received a rating of 1 due to extreme rot. However, the upper green part of the fruit had good flavor, texture and aftertaste.

Figure 57. Potable – 12 GPH – Tree 13. This fruit had moderate rot on the peel and was rated a 2.

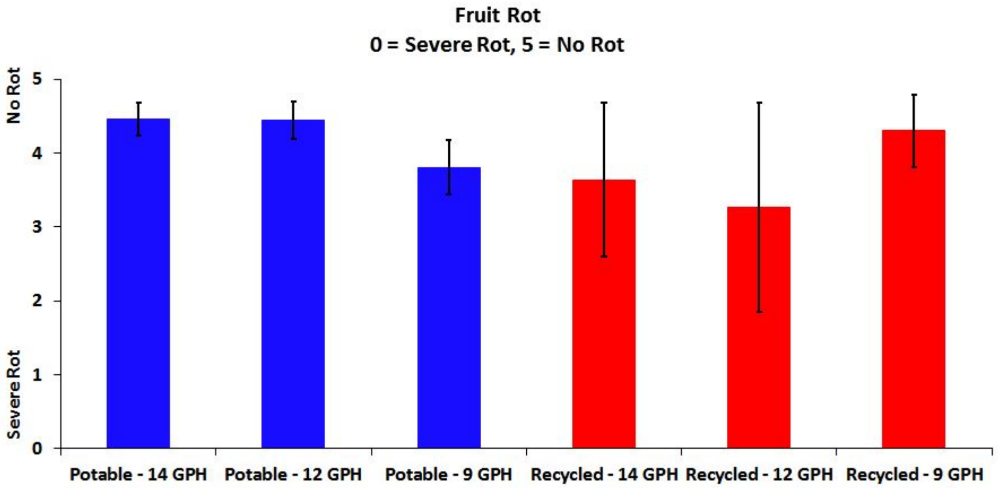


Figure 58. Frequency of fruit rot in March 2016 harvest. Lower values indicate more severe fruit rot. Error bars indicate standard error values.

As indicated in Figure 58 and 59, there is a lot of variance in the fruit rot and slime data, however, fruit rot and slime occurred most frequently in the Potable – 9 GPH, Recycled – 14 GPH and Recycled – 12 GPH irrigation treatments. Fruit quality was consistently more severe in Potable – 14 GPH, Potable – 12 GPH, and Recycled – 9 GPH. With the exception of one fruit, all fruit in the Recycled – 9 GPH were of high quality. Unfortunately, high-quality fruit does not make up for the drastic yield reduction or poor tree health.

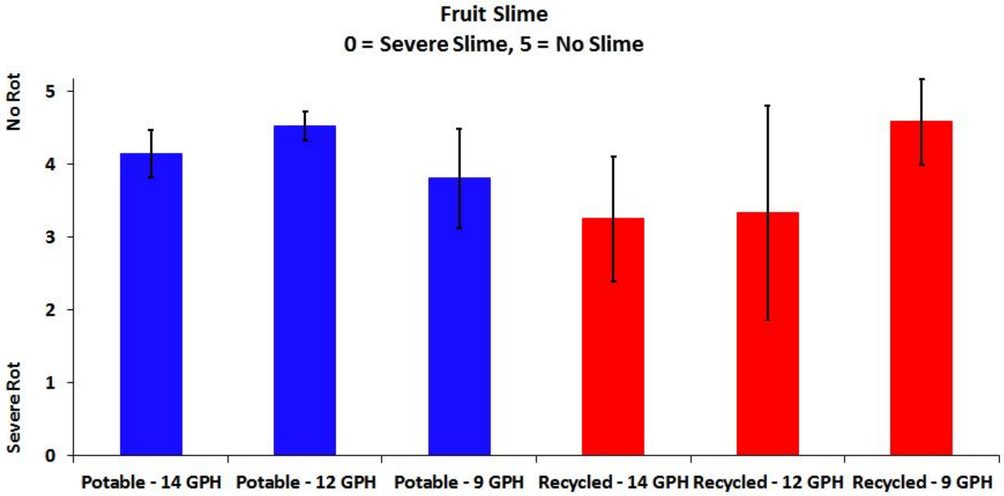


Figure 59. Frequency of fruit slime in March 2016 harvest. Lower values indicate more severe fruit slime. Error bars indicate standard error values.

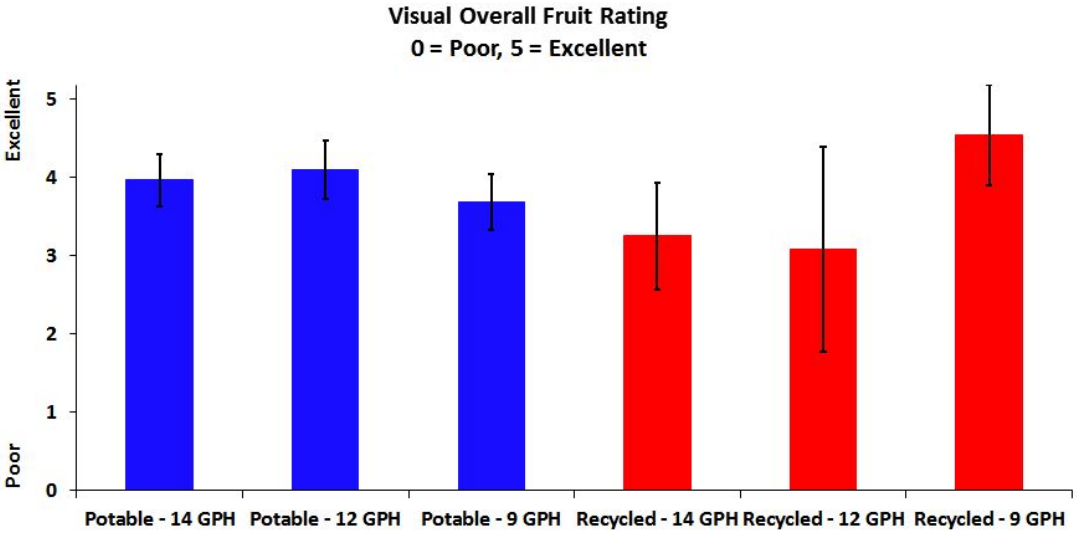


Figure 60. Visual overall fruit appearance from March 2016 harvest. Lower values indicate poor fruit quality, while higher values indicate higher fruit quality.

Post-harvest ripening time

Results from Arpaia et. *al* 1996 show that an increase in nitrogen beyond the optimum range decreases the time to ripeness after harvest (Arpaia et. *al,* 1996). This constrains the flexibility of the avocado grower and distributors by reducing the amount of time available to get the fruit from tree to market and increasing time needed for cold storage prior to ripening. In this field study, the same results were observed (Figure 61).

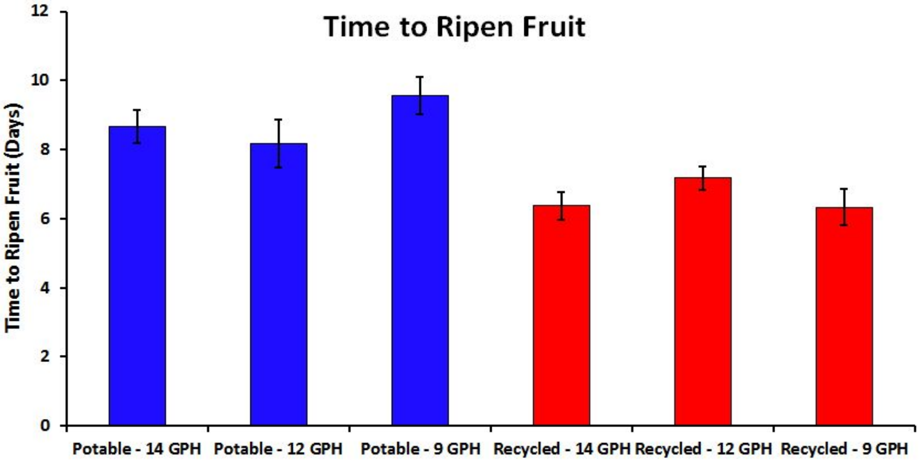


Figure 61. Time until fruit ripens (days). Error bars represent standard error values.

Average Days to Ripe	POTABLE			RECYCLED		
	14 GPH	12 GPH	9 GPH	14 GPH	12 GPH	9 GPH
Days to Ripe	9.1	8.7	9.9	6.8	7.6	7.2
ANOVA groups	ab	ab	a	b	ab	ab

Taste survey participants had a slight preference for fruits from trees irrigated with recycled water. In fact, the trees in poorest health yielded some of the most desirable fruit. This data is somewhat skewed because rotten fruits were not included in the taste survey. It is likely that fruits from trees irrigated with recycled water have higher protein content due to the availability of more nitrogen, thus imparting a more desirable flavor, texture, and aftertaste. Protein content of the fruit was not chemically measured in this study. Many people prefer to add salt to their avocados; fruit from trees irrigated with recycled water likely had higher salt content and were "pre-salted".

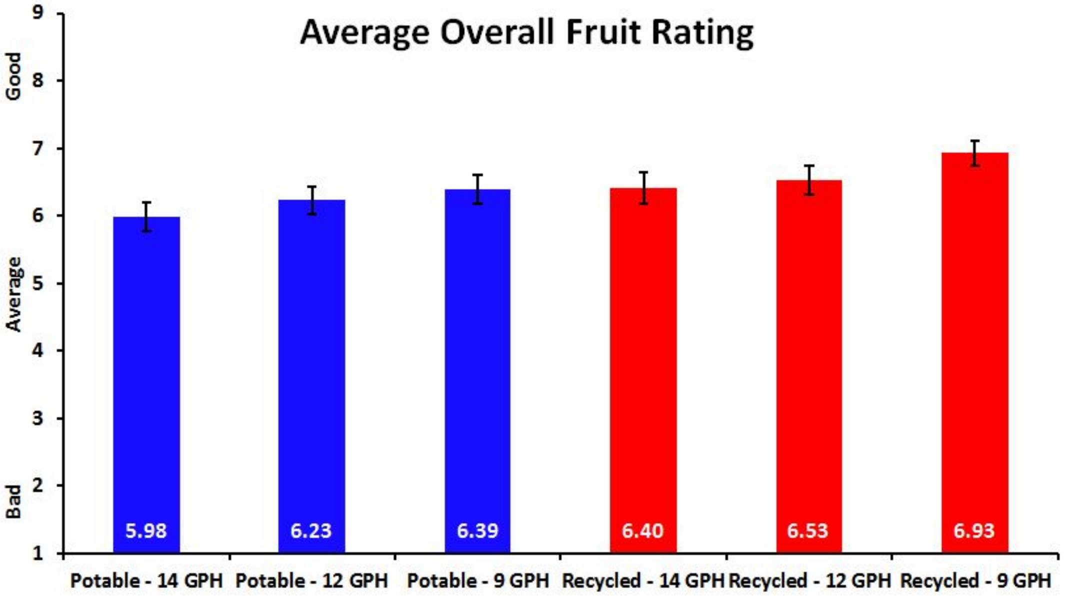


Figure 62. Average Overall Fruit Taste Rating as rated by taste survey participants. Rating scale ranged from 1-9, with 1 = "extreme dislike" and 9 = "like extremely".

Overall Avocado Taste Rating	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Days to Ripe	5.98	6.23	6.39	6.40	6.53	6.93
ANOVA groups	a	ab	ab	ab	ab	b

The average fruit ratings generally fell within the 6-7 range (Potable – 14 GPH was just below 6 at 5.98). Overall appeal increased for both sources of irrigation water as volume of water applied via irrigation decreased, with fruits from trees irrigated with recycled water earning higher ratings than their counterparts receiving equal volumes of potable water. Fruits from trees irrigated with potable water earned an average rating closer to the 6 end of this range, which means survey participants "slightly like" the fruits from this category. Tasters gave the highest ratings to fruits trees irrigated with the lowest volume of recycled water (Recycled – 9 GPH) with an average rating of 6.96. The higher oil content in the fruits irrigated with recycled water likely improved favorability among tasters, as flavor among these groups was rated closer to the "buttery/nutty" side of the flavor scale. Fruits from the Recycled – 9 GPH also had the most pleasant aftertaste, on average ranking between "slightly to moderately pleasant" (Figure 65).

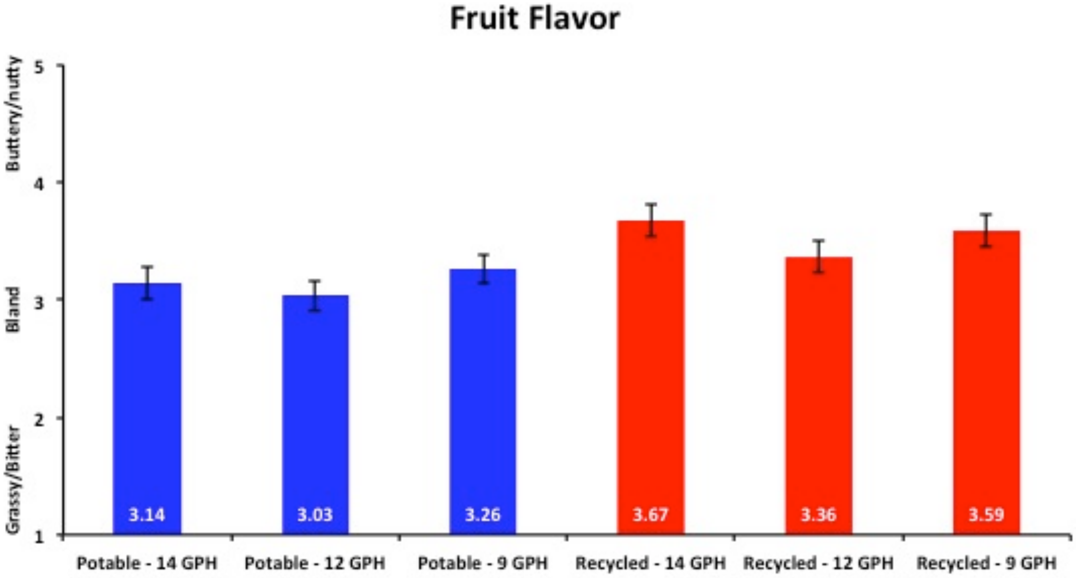


Figure 63. Average Flavor Rating as rated by taste survey participants. Flavor was rated on a scale of 1-5, with 1 = grassy/bitter, 3 = bland, and 5 = buttery/nutty. Error bars represent standard error values.

Average Fruit Flavor Rating	POTABLE			RECYCLED		
	14 GPH	12 GPH	9 GPH	14 GPH	12 GPH	9 GPH
Days to Ripe	3.14	3.03	3.26	3.67	3.36	3.59
ANOVA groups	a	a	ab	b	ab	b

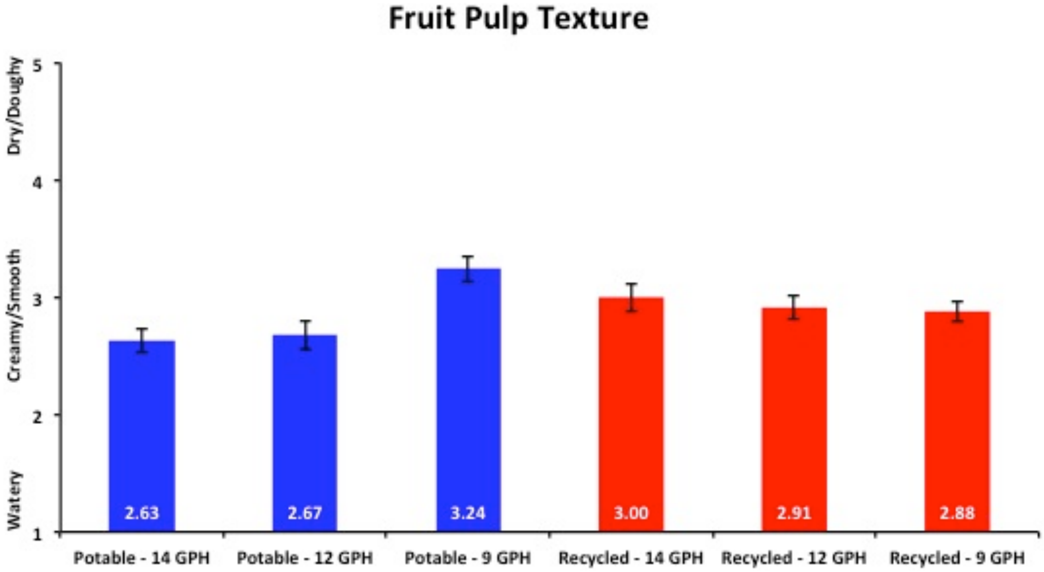


Figure 64. Average Texture Rating as rated by taste survey participants. Texture was rated on a scale of 1-5, with 1 = watery, 2 = slightly watery, 3 = creamy/smooth, 4 = slightly dry/doughy, and 5 = dry/doughy. Error bars indicate standard error values.

Average Fruit Texture Rating	POTABLE			RECYCLED		
	14 GPH	12 GPH	9 GPH	14 GPH	12 GPH	9 GPH
Texture rating	2.63	2.67	3.24	3.00	2.91	2.88
ANOVA groups	a	a	bc	ac	a	b

For optimal texture, fruits should fall into the creamy/smooth category with an average texture rating close to 3. Ratings below three indicate a watery texture; a 1 rating represents a watery texture, and 2 rating represents slightly watery. Ratings above 3 indicate a dry or doughy texture, reminiscent of bread dough prior to baking. Potable -14 GPH and Potable – 12 GPH ratings were 2.65 and 2.64, respectively, leaning slightly towards a watery texture. The rest of the irrigation treatments yielded fruits were closer to an average rating of 3 (Figure 64).

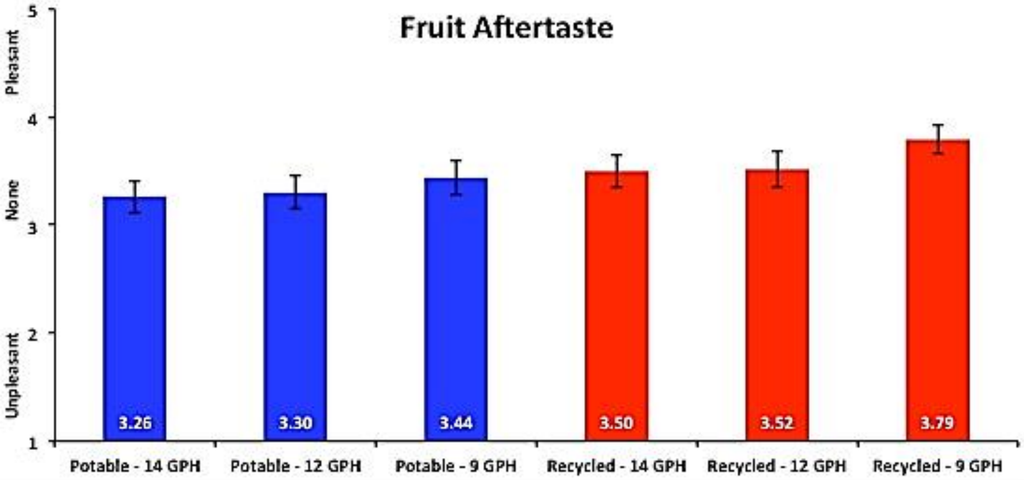


Figure 65. Fruit Aftertaste. Aftertaste was rated on a scale of 1-5, with 1 = very unpleasant; 2 = slightly unpleasant; 3 = no aftertaste; 4 = slightly pleasant, and 5 = very pleasant. Error bars indicate standard error values.

Average Fruit Aftertaste Rating	POTABLE			RECYCLED		
	14 GPH	12 GPH	9 GPH	14 GPH	12 GPH	9 GPH
Fruit Aftertaste	3.26	3.30	3.44	3.50	3.52	3.79
ANOVA groups	a	ab	ab	ab	ab	b

Average aftertaste ratings fell between 3-4 for all irrigation treatments (Figure 65). Following a similar trend as the overall rating, Recycled – 9 GPH earned the most favorable rating for aftertaste, which was close to 4, giving these fruits a slightly pleasant aftertaste. All other irrigation treatments had slightly lower average ratings and did not differ significantly from each other.

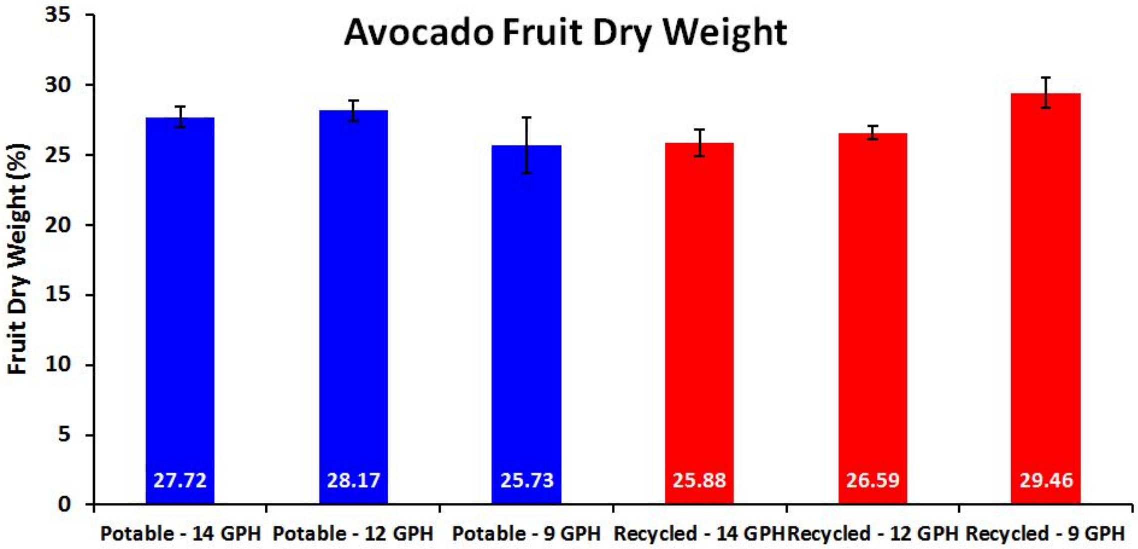


Figure 66. Avocado fruit dry weight, March 2016 harvest. Error bars indicate standard error values.

Average Fruit Dry Weight (%)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Dry weight	27.2	28.17	25.73	25.88	26.59	29.46

Higher calcium to nitrogen ratios in fruit also protect against post-harvest rot (Du Plessis and Koen, 1992). Fruit nutrient concentrations were not measured, so leaf analyses were used as a proxy for estimating nutrient deficiencies in fruits. Fruit harvests from high-yielding trees usually have a lower incidence of post-harvest rot, since these trees act as a stronger sink and are better able to extract nutrients from the soil. (Du Plessis and Koen, 1992).

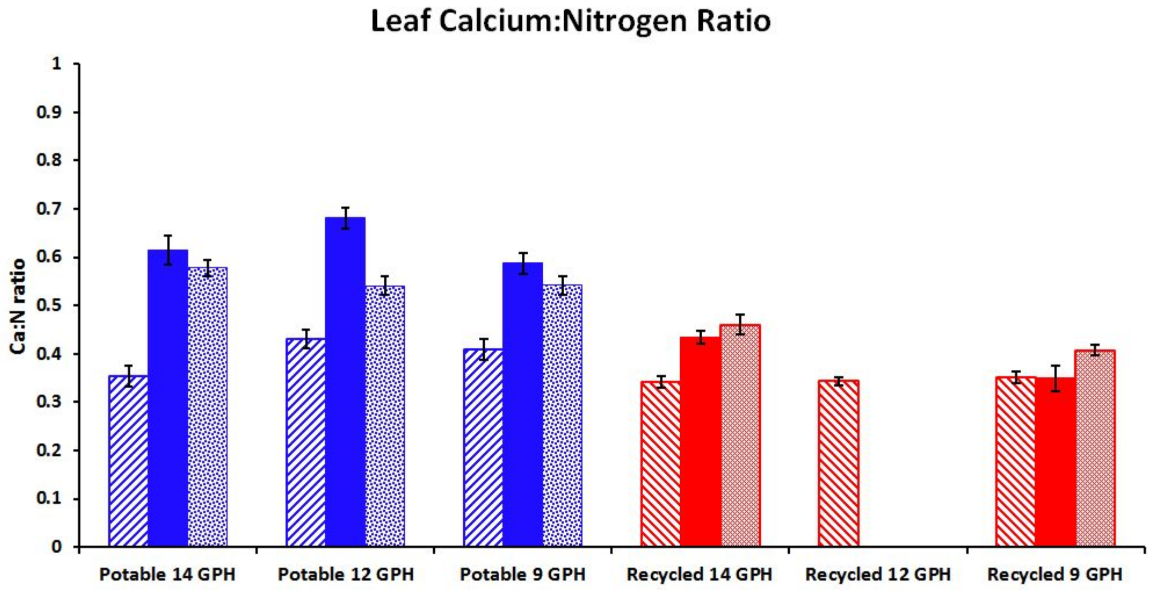


Figure 67. Leaf calcium to nitrogen ratios. Left striped bars = 2014 Ca: 2014 N. Middle solid bars = 2015 Ca: 2014 N. Right dotted bars = 2015 Ca : 2015 N

Leaf Ca:N ratio (2014:2014)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Leaf Ca:N ratio	0.35	0.43	0.41	0.34	0.34	0.35
ANOVA groups	a	b	ab	a	a	a

Leaf Ca:N ratio (2015:2014)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Leaf Ca:N ratio	0.61	0.68	0.59	0.43	No Data	0.35
ANOVA groups	ab	a	b	c	No Data	c

Leaf Ca:N ratio (2015:2015)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
Leaf Ca:N ratio	0.58	0.54	0.54	0.46	No Data	0.41
ANOVA groups	a	a	a	b	No Data	b

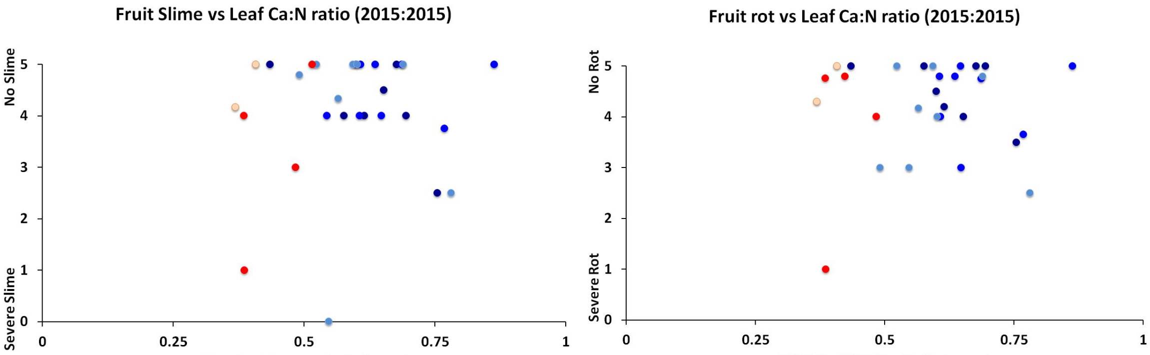


Figure 68. Severity of fruit slime (left) and fruit rot (right) from March 2016 harvest in relation to leaf Ca:N ratios. Leaf nutrient data shown is from November 2015.

The ratio of calcium and magnesium to potassium in the subsoil (25 cm below the soil surface) is a good indicator of potential hazard for fruit rot in avocado (Du Plessis and Koen, 1992). Data was not readily available on nutrient concentrations at this depth since soil samples were taken from the surface, so nutrient concentrations in surface soil samples were used instead. Du Plessis and Koen (1992) indicate that a low (Ca + Mg)/K ratio (less than 6.4) guards against vascular browning and pulp spot, while a (Ca + Mg)/K ratio higher than 6.4 reduces chances for grey pulp. As indicated in Figure 69, none of the irrigation treatments in the present study had (Ca + Mg)/K ratios lower than 6.4, and thus fruit quality may be compromised. Much of the fruit damage appeared to be due to microbial infectious agents. Some fruits exhibited clear symptoms of anthracnose. Higher (Ca + Mg)/K ratios was correlated with higher-quality fruit.

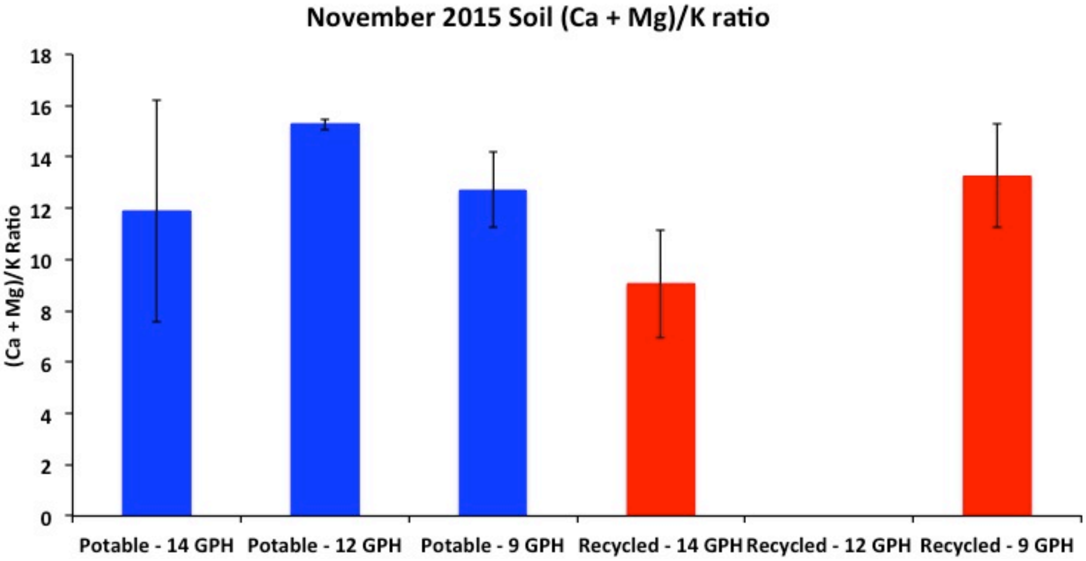


Figure 69. Soil calcium and magnesium to potassium ratio (Ca + Mg)/K. Error bars indicate standard error values. Data not available for Recycled – 12 GPH.

Leaf (Ca +Mg)/K ratio (2015:2015)	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
(Ca +Mg)/K	11.88	15.28	12.71	9.06	No Data	13.25

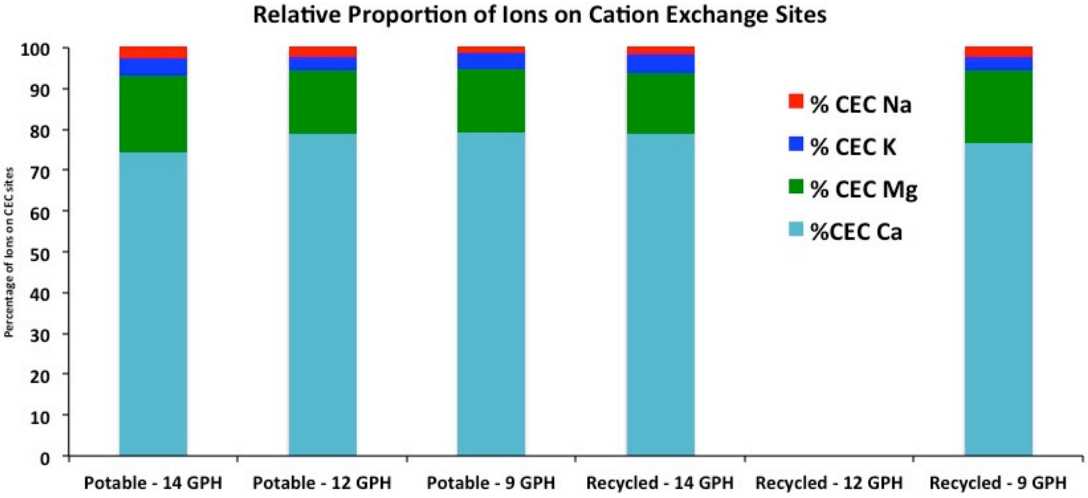


Figure 70. Relative proportions of Na, K, Mg, and Ca on cation exchange sites in soil.

Percent of Ions on Cation Exchange	**POTABLE**			**RECYCLED**		
	14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
% CEC Na	2.60	2.35	1.41	1.53	No Data	2.3
% CEC K	4.19	3.03	3.72	4.60	No Data	3.3
% CEC Mg	19.00	15.63	15.53	15.10	No Data	17.7
% CEC Ca	74.20	79.00	79.33	78.80	No Data	76.8

CHAPTER 6

DISCUSSION AND CONCLUSIONS

SUMMARY OF RESULTS

Recycled water is higher in salinity, and for this reason imparts more osmotic stress on plant roots. Avocado is particularly sensitive to salinity and osmotic stress, thus tree health was compromised and yield was reduced in trees irrigated with recycled water. However, a number of other parameters of tree health were associated with trees irrigated with recycled water, and the reasons behind them might not be as obvious as osmotic stress due to high salt concentrations in the soil.

Trees irrigated with recycled water generally had lower levels of magnesium and calcium in leaves, and were deficient in iron and zinc. Magnesium and iron are involved in chlorophyll production and photosynthesis; deficiencies in these nutrients in trees irrigated with recycled water were correlated with reduced chlorophyll content. The highest leaf sodium levels were strongly correlated with reduced yield.

High calcium to nitrogen ratios in fruit enhance fruit quality. Trees irrigated with recycled water yielded fruit with lower Ca:N ratios, and these fruits were rated more poorly for fruit quality. Unexpectedly, fruit flavor, texture, and aftertaste were rated highest for fruits from trees in the Recycled – 9 GPH treatment. Fruit rot was most severe in Recycled – 14 GPH and Recycled – 12 GPH.

Another factor that adds complexity to the results is the soil clay content, which was not uniform across the study site. Trees irrigated with potable water were situated on soils containing higher proportions of clay, which explains unexpectedly high salinity and leaf injury due to salt-burn and chloride damage. Despite significant leaf damage, these trees yielded large amounts of fruit, although it is likely that they would have produced more fruit in soils with lower clay.

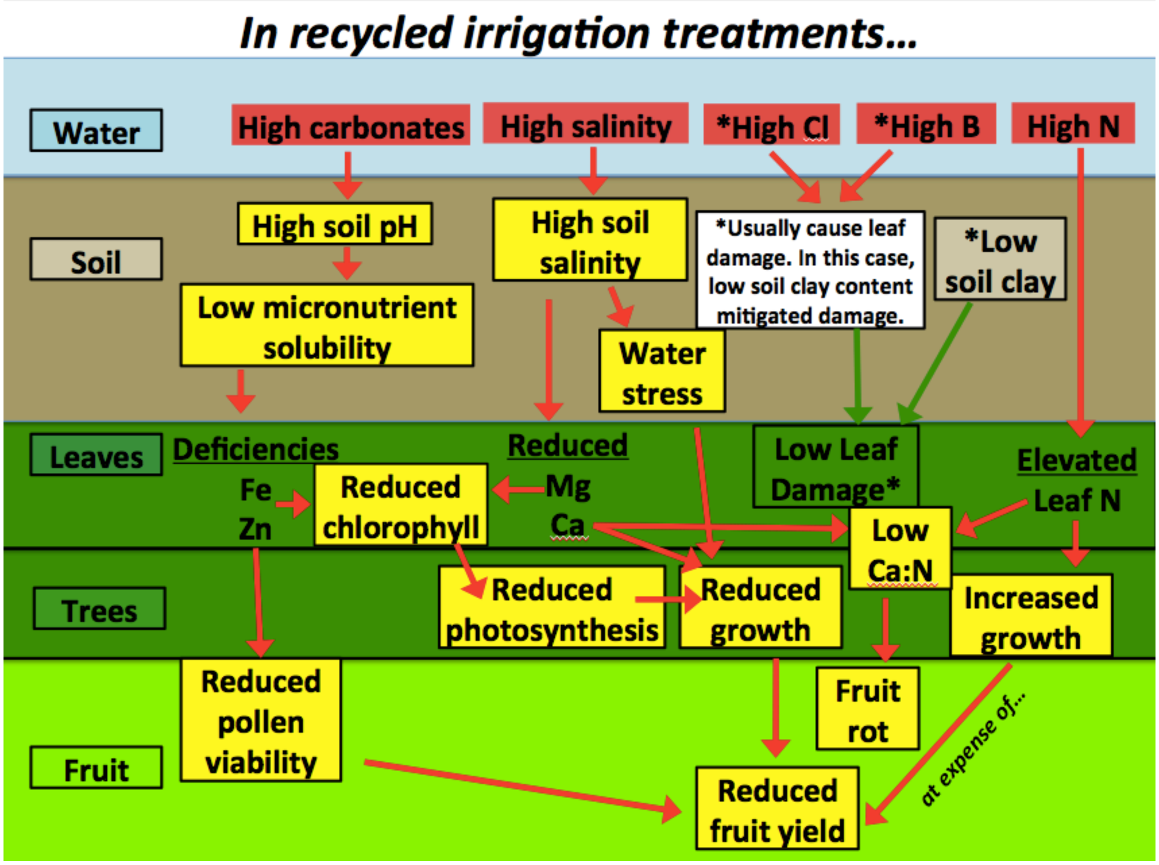


Figure 71. Summary of interactive effects in trees irrigated with recycled water from the present study. Red arrows indicate negative direct or indirect effects of irrigation with recycled water in avocado. Green arrows indicate effects from conditions at the study site that mitigated expected negative effects from irrigation with recycled water.

The above graphic summarizes how avocados were affected by irrigation with recycled water in the present study. Recycled water was higher in salinity, chloride, boron, and nitrogen, and most likely higher in carbonates (although data was not available on carbonate content of the recycled water). However, recycled water is typically higher in carbonates. The pH of the recycled water was slightly less alkaline than the potable water (7.5 vs. 8.0). However carbonates tend to keep soil pH higher. Carbonates also facilitate preferential uptake of calcium instead of iron or zinc, thus inducing iron or zinc deficiencies.

Deficiencies in micronutrients such as iron and zinc are common in California due to calcareous parent material and hot, dry summers, which encourage carbonate formation and high soil pH. Thus iron and zinc were deficient in trees irrigated with potable and recycled water, however the deficiency was more severe in the recycled water treatments.

Zinc is needed for internode elongation and pollen viability. Trees deficient in zinc are stunted and have compact foliage. This reduces light penetration into the canopy, and thus reduces growth. Reduced pollen production and viability reduces the chances for fertilization of fruit, and this could have contributed to the yield reduction observed in trees irrigated with recycled water.

High soil salinity increased ion competition for root uptake, thus available nutrients such as calcium and magnesium were decreased in proportion to the total ions in the soil solution. This reduced probability that nutrients would be absorbed and increased probability that unwanted ions would be absorbed, such as chloride, boron and sodium. Although magnesium and calcium concentrations were not deficient in trees irrigated with recycled water, ion competition would explain the reduced leaf nutrient concentrations. Iron and magnesium are integral to chlorophyll synthesis; a deficiency in these nutrients will directly reduce chlorophyll content. Reduced chlorophyll naturally leads to reduced photosynthetic rates, which leads to reduced growth, and reduced yield. Trees with inadequate energy production are unable to physically or nutritionally support profitable fruit yields.

High salinity causes also osmotic stress, making it more difficult for plants to extract water from the soil due to water retention tendencies of soluble salts in the soil. Reduced water availability limits growth potential. Plants need to expend additional energy to extract water from the soil, energy that could otherwise have been invested in fruit production. Calcium is needed for cellular growth; a reduction in calcium will result in reduction in growth at the cellular and tree level. This will then reduce yield.

Recycled water was elevated in chloride, sodium, and boron. Typically, these ions cause leaf damage and it is usually more severe with increasing concentrations in the water. However, in the present study, trees irrigated with recycled water at 14 GPH (20% leaching fraction) and 12 GPH (no leaching fraction) were healthier than their counterparts irrigated with potable water. Conversely, trees irrigated with potable water were expected to look very healthy, but showed moderately severe symptoms of chloride toxicity by the end of November 2015. These unexpected results can be explained by variations in soil clay content. Clay inhibits drainage and encourages retention of ions, including chloride. Trees irrigated with potable water at 14 GPH were situated on soils with high clay content (30% at some depths), while the trees irrigated with recycled water were situated on soils low in clay. Despite this, the trees irrigated with potable water still yielded significantly more fruit than trees irrigated with recycled water (in some cases up to 10 times as much fruit). If soil conditions were uniform throughout the study site, this difference would likely be even more dramatic.

Recycled water was also elevated in nitrogen. Excessive nitrogen is known to decrease fruit quality and encourage vegetative growth at the expense of fruit production for a number of crops. These statements are consistent with observations from the present study. Elevated nitrogen content, particularly in combination with decreased calcium content, encourages fruit rot and accelerated ripening time.

DISCUSSION AND CONCLUSIONS

Overall, the results from this experiment suggest that trees can be irrigated with recycled water, pending additional advanced water treatment. Reduced fruit yield and poor tree health were strongly correlated with a number of identifiable and correctable factors. Some of these factors will necessitate improving water quality by reducing constituents in the water; others can likely be addressed via orchard management strategies.

Irrigation management becomes critical when using recycled water. A useful irrigation technique is the application of a leaching fraction. A leaching fraction is calculated as the volume of water applied that exceeds evapotranspiration demand divided by total water volume applied. This leaching fraction requirement will likely vary for different soils, with clay soils requiring a larger leaching fraction. Trees irrigated with potable water had better growth and yield than trees receiving recycled water at the same application rate. Potable – 14 GPH and Potable – 9 GPH irrigation treatments showed unusually high levels of leaf salt burn damage, while Recycled – 14 GPH and Recycled – 12 GPH showed almost no salinity damage. Leaf chloride contents were higher in all recycled water irrigation treatments in comparison to potable irrigation treatments, irrespective of volume of water applied. A higher leaf chloride content would usually equate with more salt burn damage; however, salt burn symptoms appear when the trees are subject to water stress events caused by soil drying or potentially during hypoxia when the roots are submerged. Further analysis suggested that the trees with unexpectedly high salt burn damage in the Potable – 14 GPH and Potable – 9 GPH were situated on soils having greater clay content in the B horizon. Thus, the soil salinity values and salt damage to the leaves were more closely correlated with clay content of the soil rather than the irrigation water source or leaf chloride content. Clay soils tend to have poor drainage. These results highlight the importance of tailoring irrigation management for soils with different texture classes. Soil water monitoring also becomes increasingly important for monitoring plant-soil water relations and designing effective leaching programs.

While several years of data are required to assess the effects of recycled water on fruit yields, the preliminary data for the second harvest year suggest that chloride and nitrogen levels were too high in the treatments receiving recycled water. Trees irrigated with recycled water had significantly higher levels of leaf nitrogen, reaching close to 3%. The optimum range for avocado leaf nitrogen is 2.2-2.4%. The trees irrigated with potable water at 14 GPH and 9 GPH were also slightly above this level, indicating nitrogen requirements needs to be carefully calculated. The results show that nitrogen from recycled water needs to be accounted for in fertilization programs. Recycled water can reduce and potentially eliminate the need for fertilization.

A number of factors most likely contributed to reduced yield via a domino effect. As evidenced by the preceding summary, the intricacies between avocado health and yield in relation to water quality, soil conditions, and climate is very complex. Some plant nutrients affect other nutrients via synergistic, positive-feedback, while other nutrients act antagonistically against other nutrients. Soil pH has a significant effect on nutrient availability. The following summarizes the general chain reaction in avocado orchards irrigated with recycled water.

Treated wastewater typically differs from potable water sources by having higher levels of salinity, nitrogen, phosphorus, boron, chloride, and sodium. Recycled water tends to have a higher mineral content, which elevates soil pH. As soil pH becomes more basic, the solubility of micronutrients such as zinc and iron is reduced (AP Figure 7). High nitrogen availability and high leaf nitrogen content in avocado may either increase or decrease phosphorus assimilation. High phosphorus levels in leaves act synergistically to increase leaf concentrations of calcium and magnesium. High nitrate in the soil elevates soil pH and thus increases availability of calcium and magnesium for plant uptake. A high ammonium concentration in the soil lowers soil pH and thus decreases availability of calcium and magnesium. High phosphorus availability tends to increase chloride concentrations in leaves, while high nitrogen levels reduce chloride and boron assimilation. Nitrogen fertilization is sometimes strategically used as mitigation for irrigation with waters high in boron and/or chloride. Unfortunately, in avocado, the nitrogen level at which this benefit occurs usually brings other unwanted consequences, thus reduction of chloride and boron in the water supply are more desirable options. High phosphorus reduces zinc and copper uptake, while increasing manganese.

In addition to antagonistic effects from other nutrients, essential plant nutrients such as magnesium are in competition with toxic ions such as chloride for root uptake, effectively lowering the concentrations of these nutrients in plant tissue relative to trees irrigated with potable water. Magnesium and iron are integral to photosynthesis; magnesium is the coordinating ion in the chlorophyll molecule, while iron is a component of cytochromes (Heldt and Heldt, 2005). Zinc is responsible for internodal elongation (Whiley, 2002). A deficiency in magnesium will result in reduced chlorophyll content, which will in turn reduce photosynthetic rates and tree growth. Deficiencies in iron or zinc will also reduce photosynthetic rates; iron will do so because it is directly involved in the photosynthetic process, while a zinc deficiency will limit the ability of a plant to expand its branches for increased leaf surface area exposed to sunlight. Since these nutrient deficiencies directly or indirectly reduce photosynthetic rates, tree growth and canopy volume will be reduced. Smaller trees cannot capture as much sunlight and convert it to energy for fruit production, thus yield is reduced. Unfortunately, due to various constraints, photosynthetic measurements were not performed on these trees.

Trees irrigated with recycled water had lower concentrations of zinc, iron, and magnesium in their leaves in relation to trees irrigated with potable water (AP Figures 8 - 16). The ratio of calcium to nitrogen impacts post-harvest fruit quality. Fruit higher in calcium tend to have lower rates of rot, while elevated in nitrogen and potassium content in fruit tends to increase the incidence of post-harvest rot (Hofman, et. *al*., 2005). Trees irrigated with recycled water had reduced concentrations of calcium in their leaves and increased leaf nitrogen content. Leaf nitrogen content decreased subsequent to the discontinuation of granular fertilization on trees irrigated with recycled water, as signified in the reduced nitrogen content in 2015 in comparison to 2014 in these trees.

The data from this experiment suggest that current levels of treatment for recycled water from the HARRF wastewater treatment plant could be further adjusted to prevent chloride injury to avocado trees. Projected boron levels of 0.3 mg/L are acceptable, however soil and leaf boron should be monitored closely for waters having elevated boron concentrations. Additional treatment of recycled water to lower chloride levels to 80-100 ppm will likely be necessary to be improve tree growth and fruit yields. Lowering the SAR to <2 is also recommended, as this is the preferred SAR level for avocado. Extensive soil analysis should be performed prior to changing the source of irrigation water. Soil clay content is particularly important, and soil should be assessed for drainage or the presence of impermeable subsurface layers. A preliminary soil assessment can be performed via NCRS Web Soil Survey, (http://websoilsurvey.sc.egov.usda.gov/). This data from the web soil survey should be further verified with samples collected throughout the orchard. The new proposed targets for the recycled water (Appendix Table AP2) are overall a good starting point. Further research and data collection on yields can guide additional recommendations for use of recycled water. If soil conditions were uniform at this site, we would expect that the share of toxic ions in plant tissues would increase in trees irrigated with recycled water relative to trees irrigated with potable water at equivalent irrigation rates. Ideally, additional data collection would include root density with depth, photosynthetic rates, fruit nutrient concentrations and their impact on fruit quality, and a 2-D or 3-D salinity profiles of the soil using Super STING equipment.

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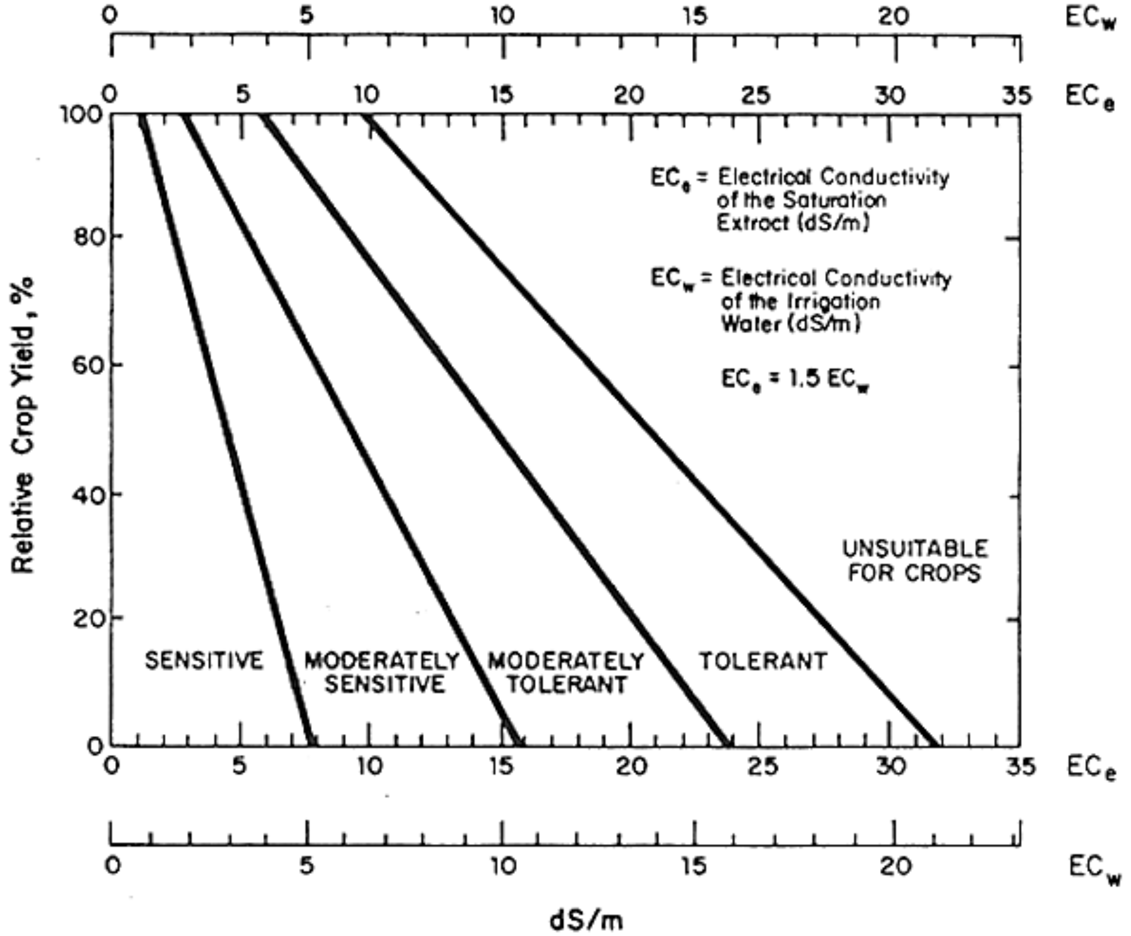
APPENDIX



AP Figure 1. Nomagram illustrating the relationships between irrigation water salinity measured as electrical conductivity in units of decisiemens (dS) and the salinity of a saturated paste extract for soil irrigated with that water supply. Avocado trees are among the most salt-sensitive of all plants studied and are further affected by specific ion toxicities, particularly associated with chloride concentrations above 100 ppm in the irrigation water.

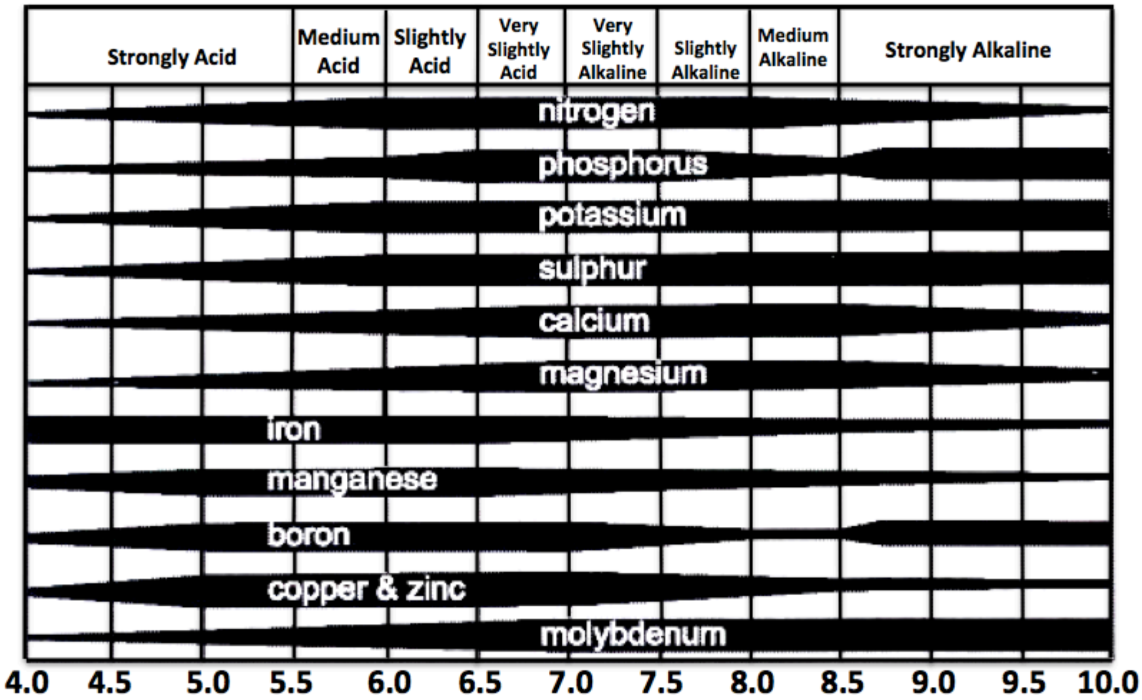
AP Table 1. Cumulative volume of water applied per tree (April 2014 – December 2015) by irrigation treatment.

POTABLE			RECYCLED		
14 GPH	**12 GPH**	**9 GPH**	**14 GPH**	**12 GPH**	**9 GPH**
2,545 gallons	2,181 gallons	1,636 gallons	2,842 gallons	2,436 gallons	1,827 gallons

AP Table 2. Minimum and maximum thresholds and adequate ranges for essential plant nutrients in avocado.

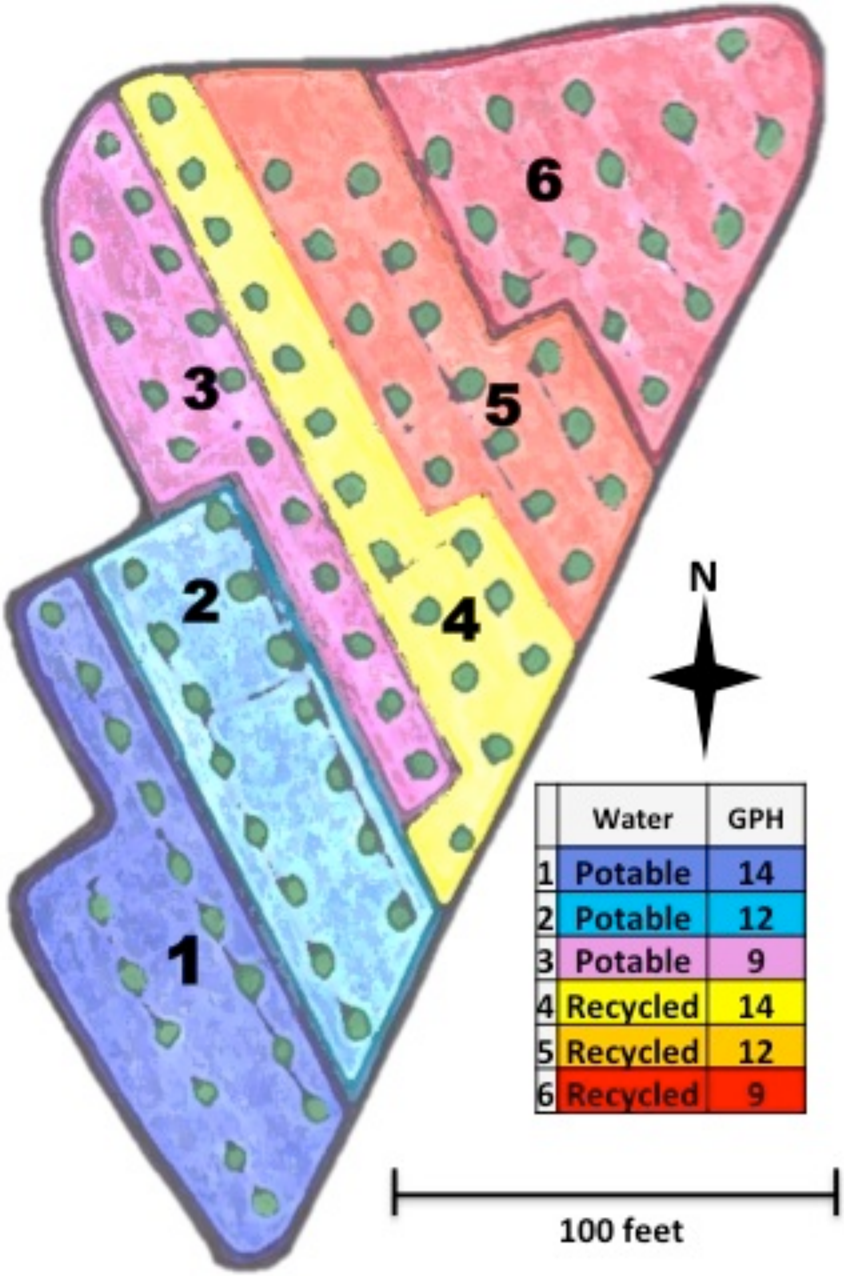
Element Name	Chemical Symbol	Unit of measure	Minimum threshold	Adequate range	Maximum threshold
Nitrogen	N	%	1.6	1.8-2.4	2.8
Phosphorus	P	%	0.05	0.08 – 0.25	0.3
Potassium	K	%	0.35	0.75 – 2.0	3
Calcium	Ca	%	0.5	1.0 – 3.0	4
Magnesium	Mg	%	0.15	0.25 – 0.8	1
Sulfur	S	%	0.05	0.20-0.6	1
Boron	B	ppm	10-20	50-90	100-250
Iron	Fe	ppm	20-40	50-200	?
Manganese	Mn	ppm	10-15	30-599	1,000
Zinc	Zn	ppm	10-20	30-150	300
Copper	Cu	ppm	2-3	5-15	25
Molybdenum	Mo	ppm	0.01	0.05 – 1.0	?
Chloride	Cl	%	?	?	0.25 – 0.5
Sodium	Na	%	N/A	N/A	0.25 – 0.5
Lithium	Li	ppm	N/A	N/A	50-75

Adapted from Lee 1979, 1980; Jones and Embleton 1966.



AP Figure 2. Influence of soil pH on nutrient bioavailability. (Avocadosource.com)

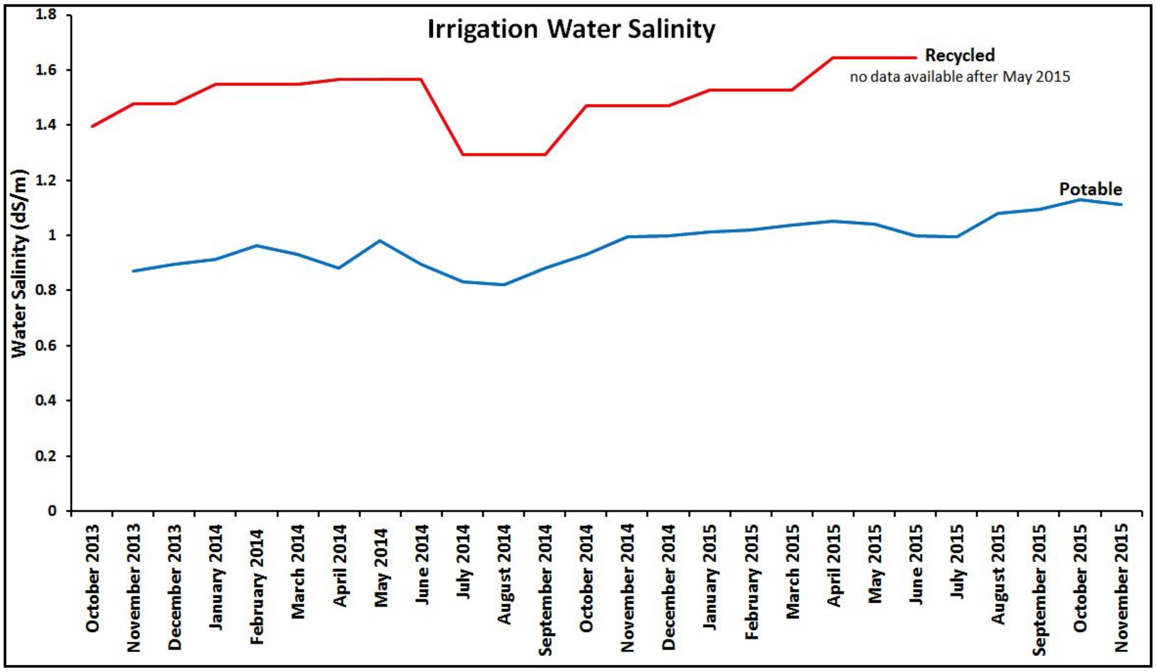
Trial Plot Map



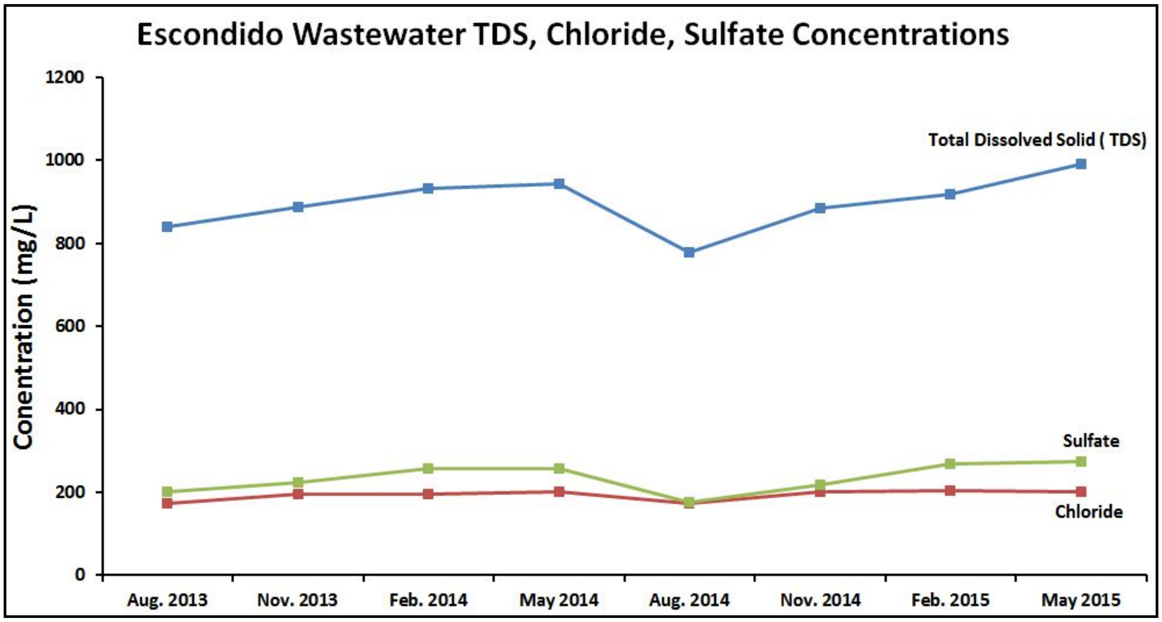
	Water	GPH
1	Potable	14
2	Potable	12
3	Potable	9
4	Recycled	14
5	Recycled	12
6	Recycled	9

AP Figure 3. Trial plot map showing irrigation treatments. Dark blue, light blue and pink represent trees irrigated with potable water. Yellow, orange and red colors represent trees irrigated with recycled water. Dark blue and yellow are irrigated with 14 GPH of their respective water sources, light blue and orange are irrigated with 12 GPH, and pink and red are irrigated with 9 GPH.

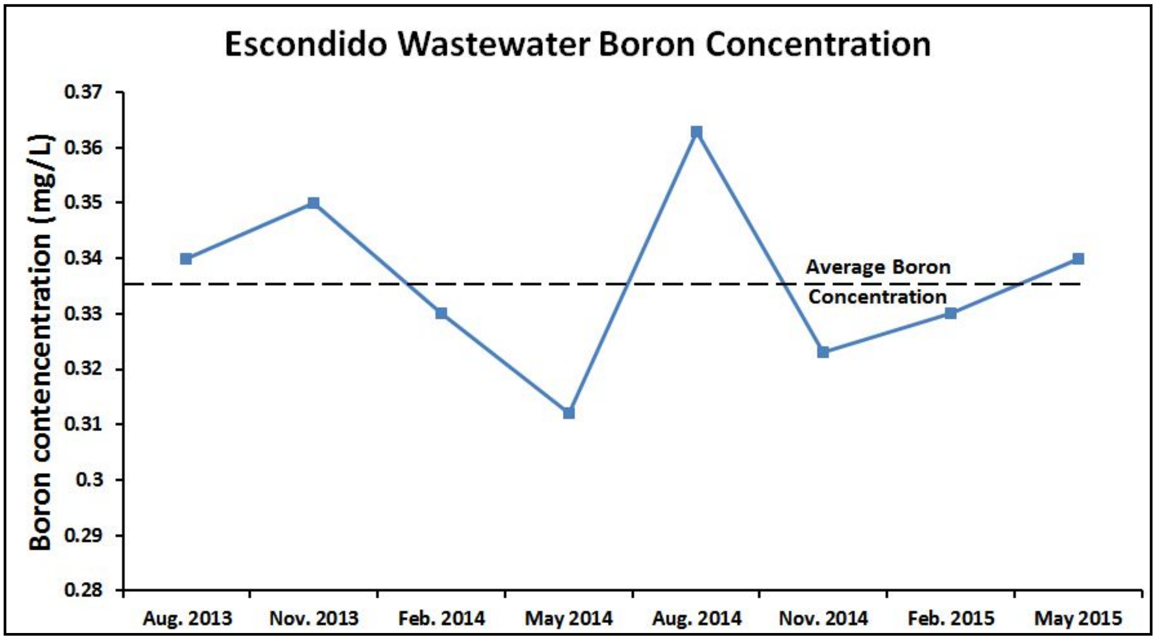
WATER QUALITY



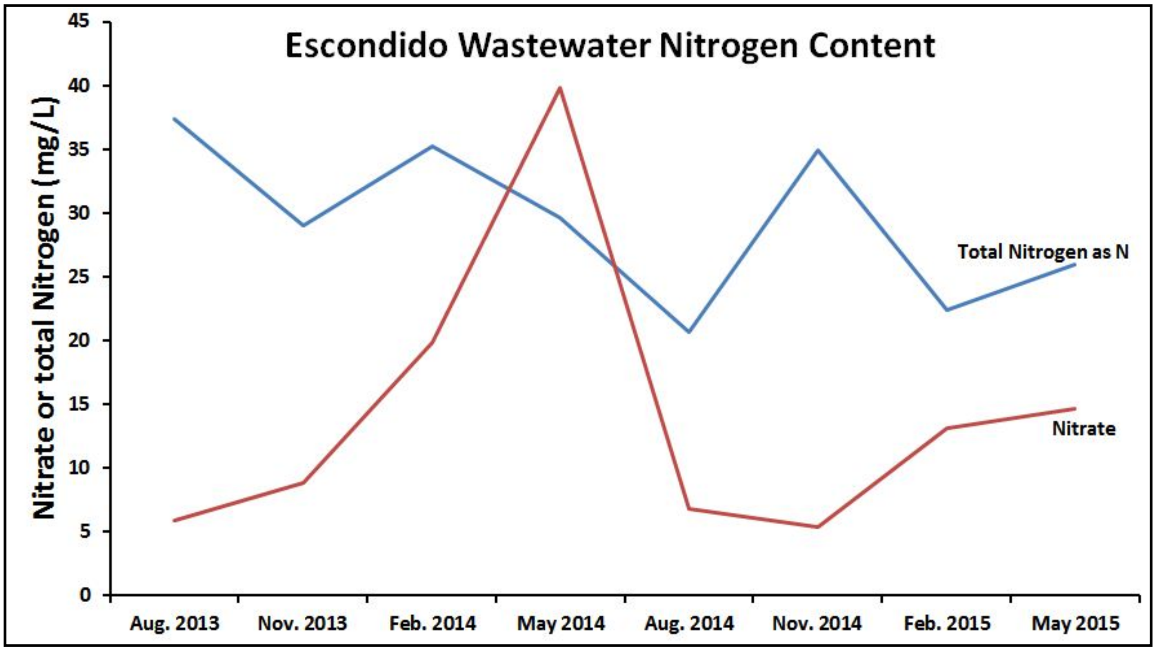
AP Figure 3. Irrigation water salinity for Potable and Recycled water sources. Data not available for recycled water after May 2015 at time of present publication.



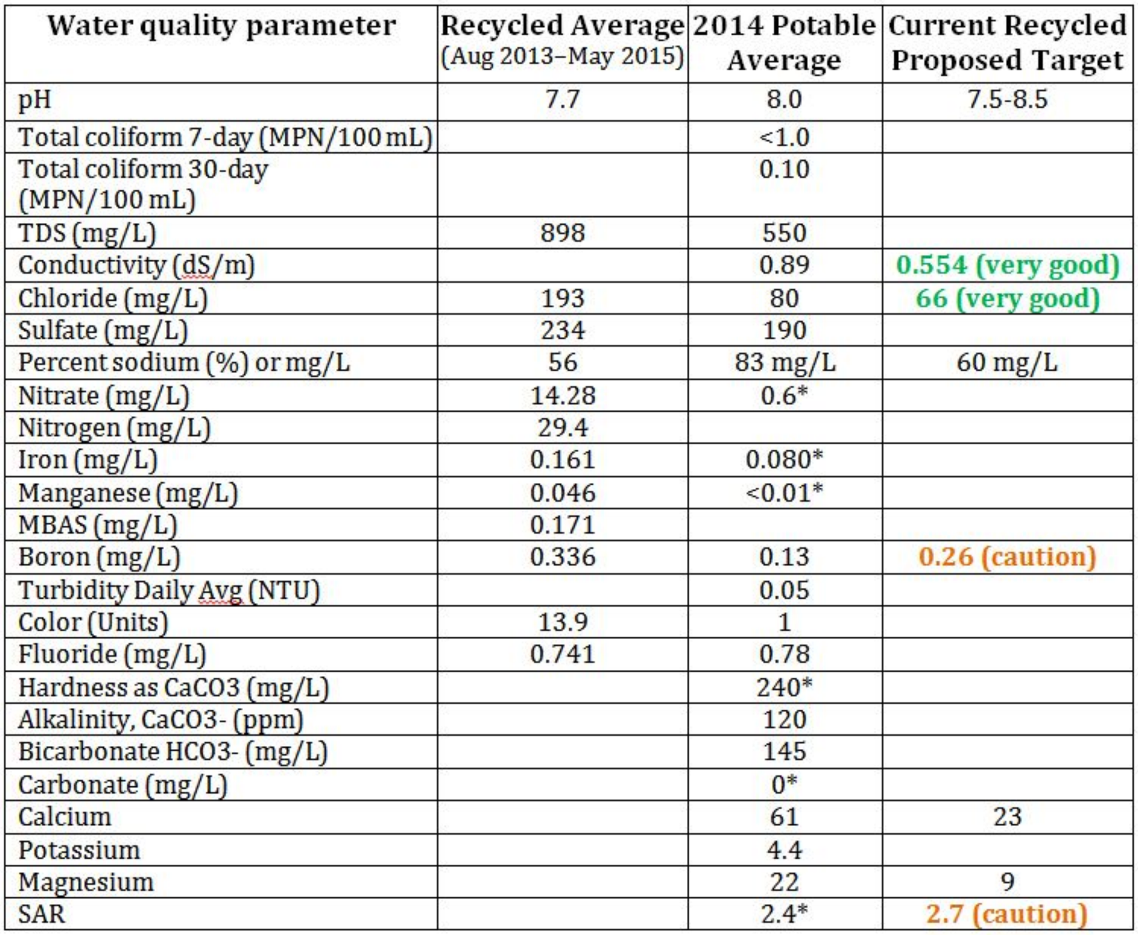
AP Figure 4. Total Dissolved Solids (TDS), Chloride, and Sulfate Concentrations of Recycled water August 2013 – May 2015. Data not available for recycled water after May 2015 at time of present publication.



AP Figure 5. Boron concentration in recycled water (August 2013 – May 2015) Average value = 0.336 mg/L.



AP Figure 6. Nitrogen concentration in recycled water (August 2013 – May 2015).



Water quality parameter	Recycled Average (Aug 2013–May 2015)	2014 Potable Average	Current Recycled Proposed Target
pH	7.7	8.0	7.5-8.5
Total coliform 7-day (MPN/100 mL)		<1.0	
Total coliform 30-day (MPN/100 mL)		0.10	
TDS (mg/L)	898	550	
Conductivity (dS/m)		0.89	0.554 (very good)
Chloride (mg/L)	193	80	66 (very good)
Sulfate (mg/L)	234	190	
Percent sodium (%) or mg/L	56	83 mg/L	60 mg/L
Nitrate (mg/L)	14.28	0.6*	
Nitrogen (mg/L)	29.4		
Iron (mg/L)	0.161	0.080*	
Manganese (mg/L)	0.046	<0.01*	
MBAS (mg/L)	0.171		
Boron (mg/L)	0.336	0.13	0.26 (caution)
Turbidity Daily Avg (NTU)		0.05	
Color (Units)	13.9	1	
Fluoride (mg/L)	0.741	0.78	
Hardness as CaCO3 (mg/L)		240*	
Alkalinity, CaCO3- (ppm)		120	
Bicarbonate HCO3- (mg/L)		145	
Carbonate (mg/L)		0*	
Calcium		61	23
Potassium		4.4	
Magnesium		22	9
SAR		2.4*	2.7 (caution)

* Data obtained from Fruit Grower's lab report water analysis from water collected October 23, 2014. Other data in Potable water column from Escondido.org 2014 Water Quality report. Blank cells indicate data not available at time of publication.

AP Table 3. The main constituents of recycled water that pose a hazard to avocado are: salinity (reported here as conductivity), chloride, boron, and sodium, via Sodium Adsorption Ratio (SAR). The projected salinity and chloride values are excellent for avocado irrigation. The projected boron level is acceptable, with appropriate irrigation, soil and orchard management strategies. Caution should be used when irrigating soils containing clay with recycled water, and boron content of the soil and leaves should be analyzed periodically to determine if boron levels are reaching dangerous levels. Soil should be observed for signs of sodium damage, which presents itself as a cement-like crust on the soil surface. In soils with little clay content, boron and SAR are less likely to pose a concern. Boron levels in the irrigation water above 0.3 mg/L are considered to be risky for avocado irrigation in the long-term. Nitrogen concentrations must be monitored so growers can incorporate this amount into fertilizer application, since an excess of nitrogen will encourage tree growth while simultaneously reducing fruit yield. If possible, the SAR value should be lowered to below 2; this can be accomplished by lowering sodium concentrations and/or increasing calcium and magnesium concentration.

Interpretation of Kohonen Self-Organizing Maps

Kohonen maps are not traditional geographic maps. Instead, Kohonen maps group datasets into similar groups for easier identification of trends and patterns in the data. Peltarion Synapse software was used to create the maps. It should be noted that this form of data analysis are best suited to larger datasets. In the current study, 90 samples, or in the case of soils data, only 18 samples, fall on the small end of the spectrum in regards to dataset size, but the results still provide useful interpretation of data. Red indicates a high value for that variable, yellow is intermediate, and blue represents low value. Kohonen maps do not account for thresholds such as zinc deficiency; they only display data in relation to the data present. Thus, for example, in the case of nitrogen, a data point could be represented as yellow, although it falls in the above-optimal range. In these maps, data is presented in a series of boxes; each box contains a different dataset. Each point in a box corresponds to the same point in every other box for that particular Kohonen map. A viewer to correlate patterns by comparing multiple datasets from multiple boxes, for example, in AP Figure 8, high yield (bottom row, left box) corresponds to irrigation with potable water (top row, left box, AP Figure 8). Data was analyzed separately for 2014 to allow inclusion of Recycled – 12 GPH leaf analysis. 2015 Recycled – 12 GPH leaf data was not available.

The following analyses summarize results from the Kohonen self-organizing maps (AP Figures 8-16). It should be noted that for many of the leaf nutrients, the highest concentrations fall at the lower end of the optimal range. Thus in the following analysis, “high” leaf nutrient concentrations should be interpreted as relative to the other samples in the study and is not necessarily intended to mean higher than maximum threshold for that nutrient. Leaf nitrogen content is an exception, however, in that its lowest values fall within ideal range (2.2-2.4%), while higher values are higher than ideal. It should also be noted that the sample size of the data used in these Kohonen maps is on the small side, as Kohonen maps are better suited to large datasets. However, the Kohonen maps still provide useful information.

Highest saltburn damage to leaf tissue occurred in Potable – 14 GPH and Potable – 9 GPH and was associated with highest canopy volumes (AP Figures 8 – 16). Average fruit weight was generally highest in Recycled – 12 GPH, with fruit from one tree in Recycled – 9 GPH weighing more, thus raising the average fruit weight of Recycled – 9 GPH. In 2014, higher leaf phosphorus content was associated with high leaf potassium and zinc, low leaf calcium, and moderate chlorophyll concentrations in the leaves (AP Figures 8-16). On average, lowest visual ratings for fruit were given to fruits in Recycled – 14 GPH and Recycled – 12 GPH, although the worst individual fruit was from Recycled – 9 GPH, however most of the fruits in this irrigation treatment actually were of good quality. Highest fruit ratings were given to Potable – 12 GPH, some Potable – 14 GPH, and some Potable – 9 GPH, while the rest of fruits from Potable – 14 GPH and Potable – 9 GPH received moderate visual ratings.

Poor fruit quality was associated with high 2014 leaf nitrogen content, high 2015 leaf chloride content, moderate saltburn damage in 2014, low saltburn damage in 2015. Data for 2015 leaf saltburn is not shown in Kohonen maps, but as indicated in the saltburn graph (Figure 17), the Recycled – 14 GPH had little leaf injury due to salt-burn damage. Poor fruit quality was associated with high concentrations of boron, potassium and phosphorus in 2014 and 2015. Leaf calcium concentrations were moderate in 2014 and low in 2015 for trees yielding poor-quality fruit, while leaf zinc content was high for both years. Highest yield was correlated with moderate fruit quality in Potable – 12 GPH, while lowest fruit quality was correlated with low yield. This follows the observation that low-yielding trees tend to bear fruit poorer in quality due to diminished demand for nutrients and therefore reduced ability to assimilate needed nutrients from the soil. However, since fruit nutrition concentrations were not analyzed, it is possible that trees with low nutrient concentrations in the leaves are investing much of their nutrition into the fruit at the expense of the leaves.

Leaf chlorophyll concentrations were reduced in November 2015 and this was correlated with low leaf concentrations of iron, calcium and nitrogen and low to moderate zinc levels in leaves in the same year. Among these trees, yield and yield efficiency were also low. Highest leaf chloride content occurred in Recycled – 9 GPH in 2014. In 2015, highest leaf chloride content was in Potable – 14 GPH. This observation may be explained by the change in water source from low-salinity water to Colorado River water, which is higher in chloride. Frequent rainstorms also which leached out chloride from trees irrigated with recycled water. An additional factor is the presence of high clay fraction in the Potable – 14 GPH section of the orchard, and an absence of clay in the areas of the orchard irrigated with recycled water. Leaf boron concentrations were also actually lower in 2015 in trees irrigated with recycled water than they were in 2014, likely also due to the frequent rainstorms which reduced irrigation demand. Leaf boron was highest in Potable – 14 GPH and Recycled – 14 GPH, which would be expected since larger volumes of water are being applied, and boron does not readily leach from the soil as easily as other nutrients.

Soil pH was highest in Potable – 9 GPH, then Recycled – 14 GPH and Recycled -12 GPH. High soil pH would be expected in soils irrigated with recycled water due to the increased mineral content of the water, while high soil pH in Potable – 9 GPH may be due to the increased clay content of the soil at the soil surface. Potable – 9 GPH actually had the highest clay content at the 0-12" depth, where soil samples were taken. Potable – 14 GPH had the highest clay content at 18-24" below the soil surface. High leaf phosphorus content in 2014 was correlated with high leaf potassium content in both 2014 and 2015 and occurred in the Recycled – 9 GPH treatment. The high phosphorus content in 2014 could be due to limited leaching in 2014 and lower soil pH at the lower water application rate relative to Recycled – 14 GPH and Recycled – 9 GPH. This irrigation treatment also had lowest leaf chlorohyll content for both May 2015 and November 2015. Recycled – 9 GPH trees also had low leaf zinc concentrations in 2014, low iron in 2014 and 2015. Potable – 14 GPH had lowest levels of nitrogen, phosphorus, zinc, and iron in leaves for both 2014 and 2015.

The worst "slime" on fruit occurred in Recycled – 14 GPH and Recycled – 12 GPH, and was associated with high leaf nitrogen and phosphorus content in 2014 and 2015. Potable – 9 GPH also had some severe fruit slime, however, these fruits were from trees with low leaf nitrogen and phosphorus content in 2015. Trees with slimy fruit in the Potable – 9 GPH treatment also had low zinc, moderate chlorophyll, negligible salt-burn leaf injury, high chloride in 2014 and 2015, and high salt-burn in 2015.

High leaf sodium content occurred in Recycled- 12 GPH in 2014 and was associated with high leaf nitrogen, high iron, and low canopy volume. Data is not available for Recycled – 12 GPH, however Recycled – 9 GPH and Recycled – 14 GPH show high sodium content. In Potable – 14 GPH, leaf magnesium was low in 2014 and moderate in 2015, while leaf calcium was low in 2014 and high in 2015. In Recycled – 14 GPH and Recycled – 9 GPH, leaf concentrations of calcium and iron were reduced in 2015 in comparison to trees irrigated with potable water.

Definitions of Abbreviations used in the Kohonen maps:

TotalWeight: total fruit weight harvested per tree.

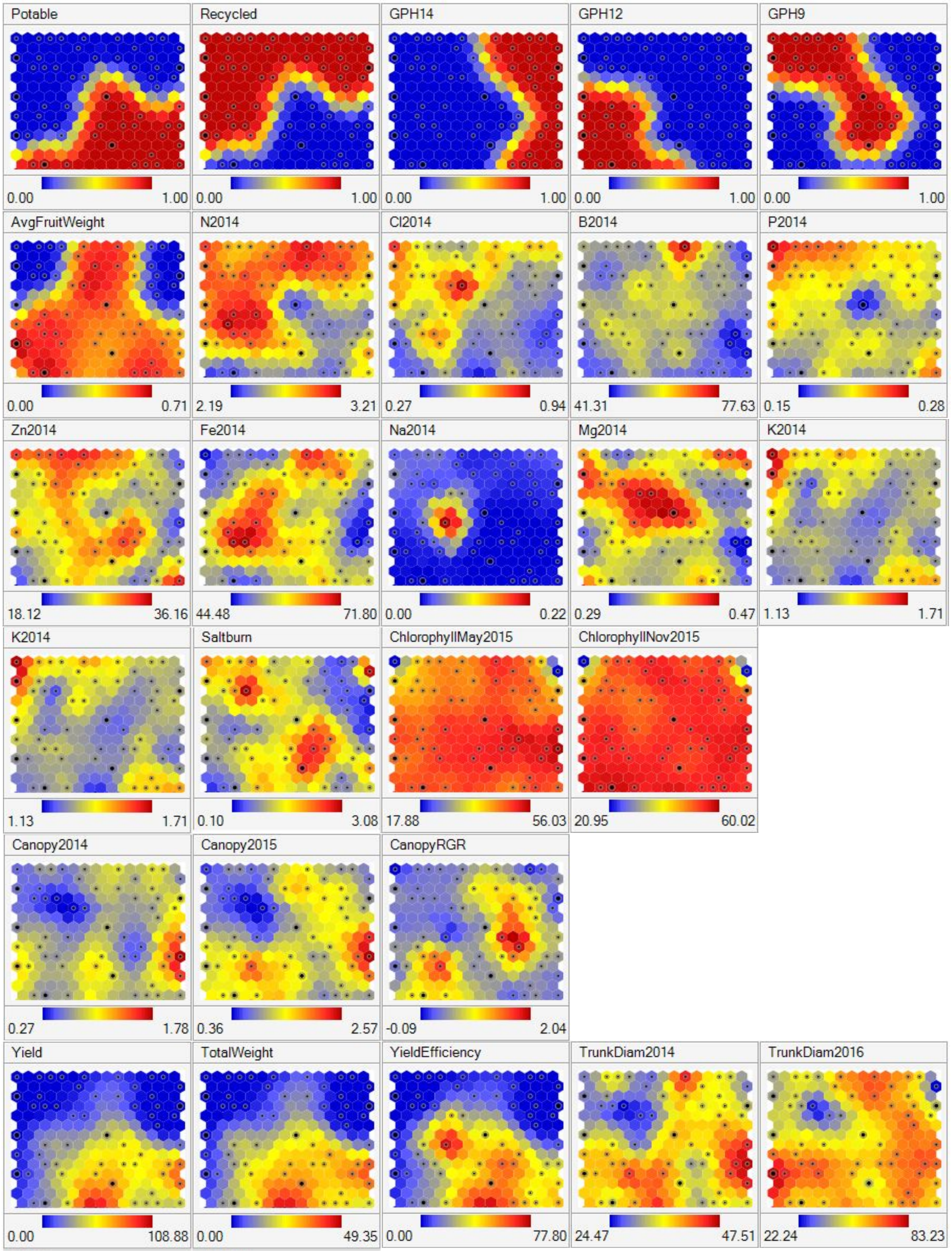
AvgFruitWeight: average weight of individual fruits per tree.

AvgOverall: Average overall fruit rating, averaged among all the fruits for that tree

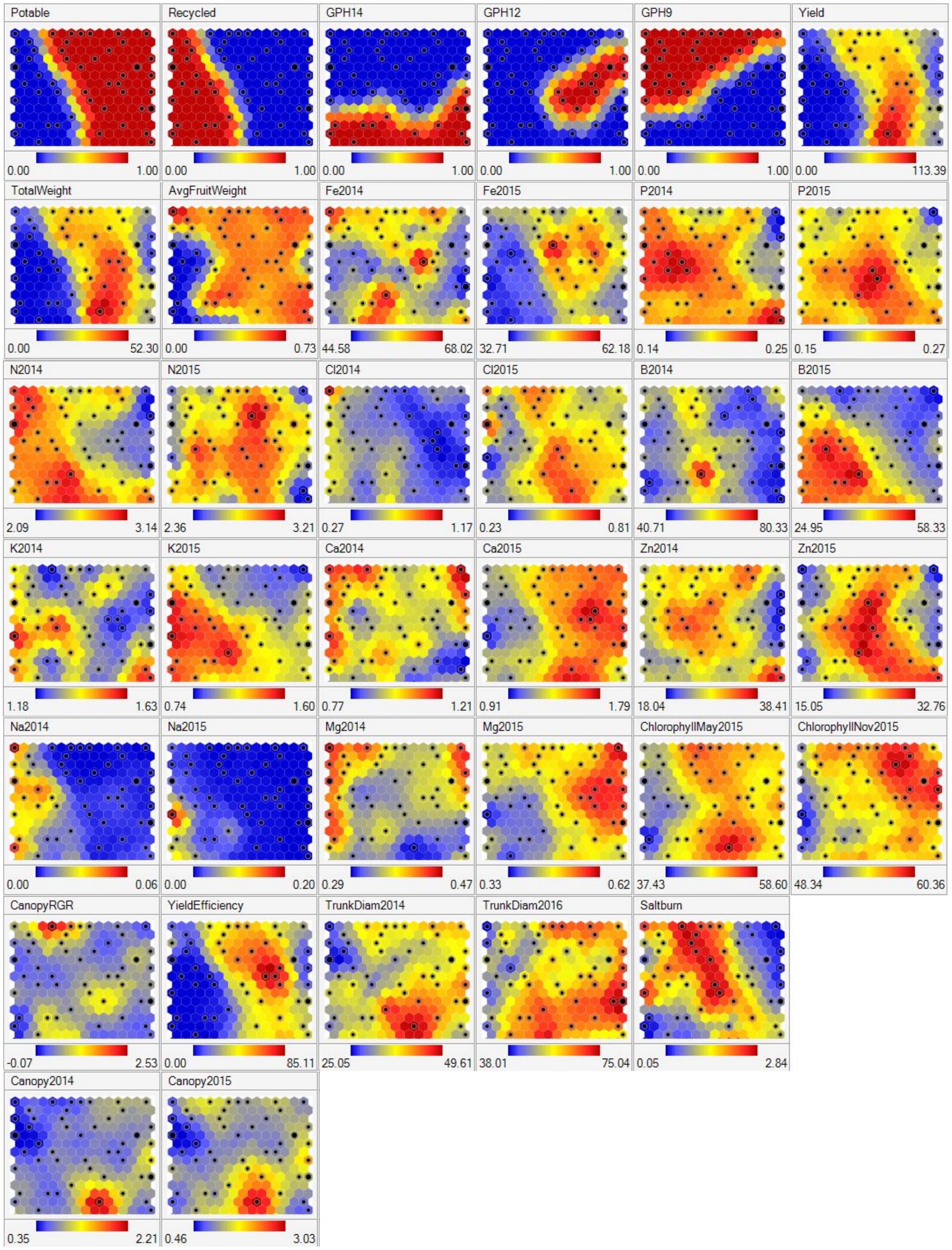
AvgSlimy: Severity of fruit slime (0 or 1 = severe slime, 5 = no slime)

AvgRot: Severity of fruit rot (0 or 1 = severe rot, 5 = no rot)

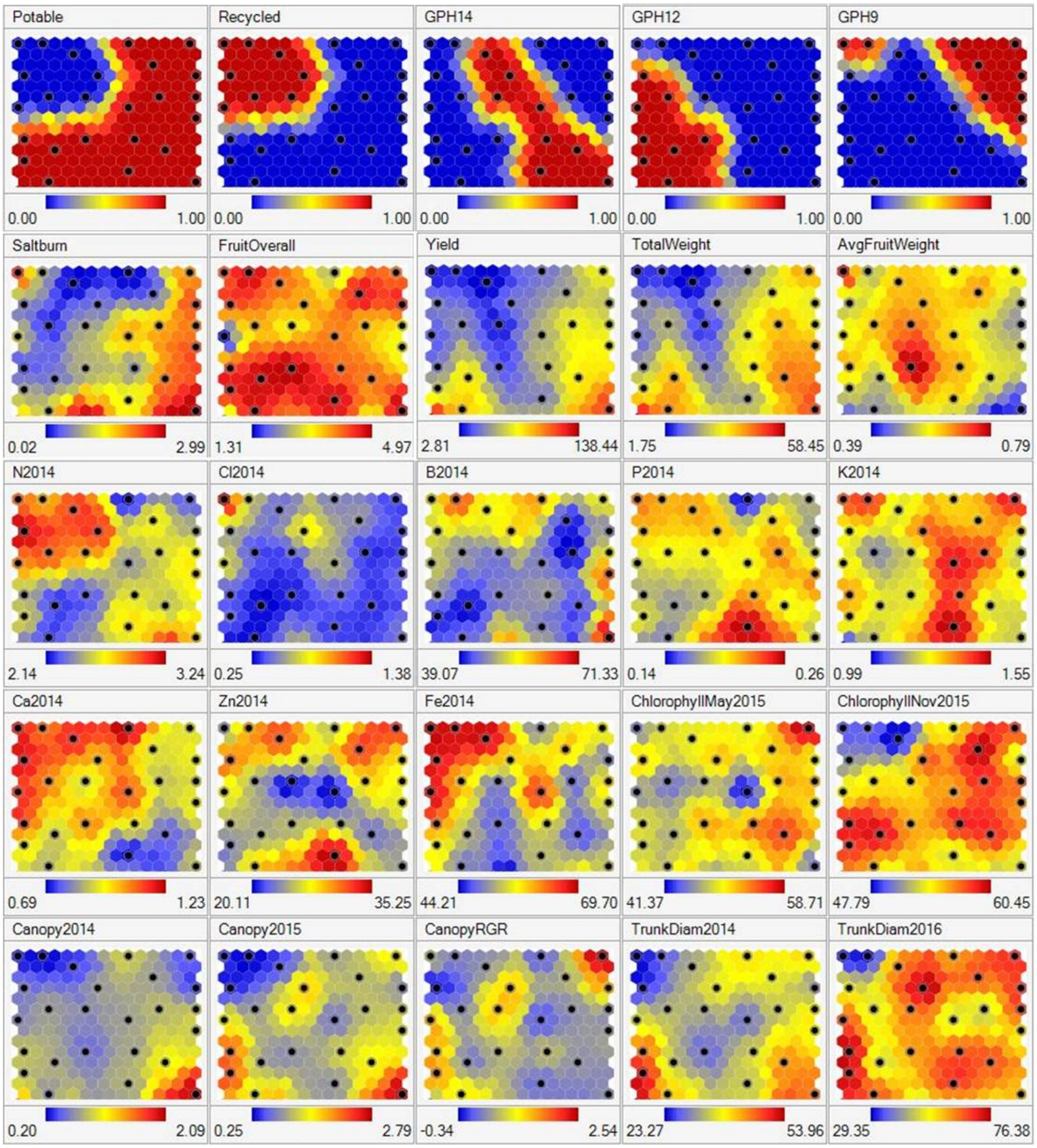
N2014, Cl2014,B2014, N2015, Cl2015,B2015 etc. correspond to nutrient elemental symbol and year.



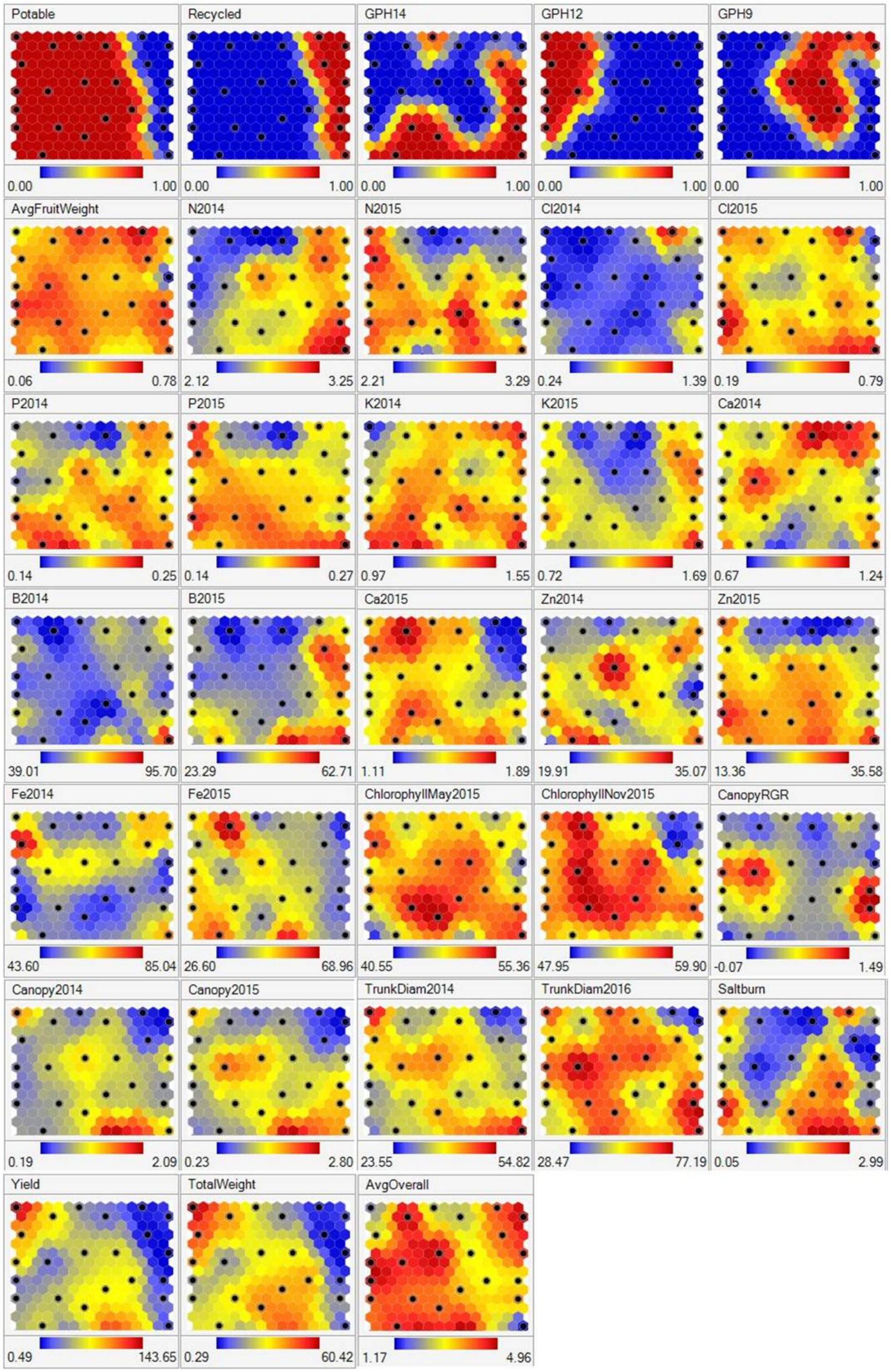
AP Figure 8. Kohonen self-organizing map of tree health and yield in relation to 2014 leaf nutrient concentrations.



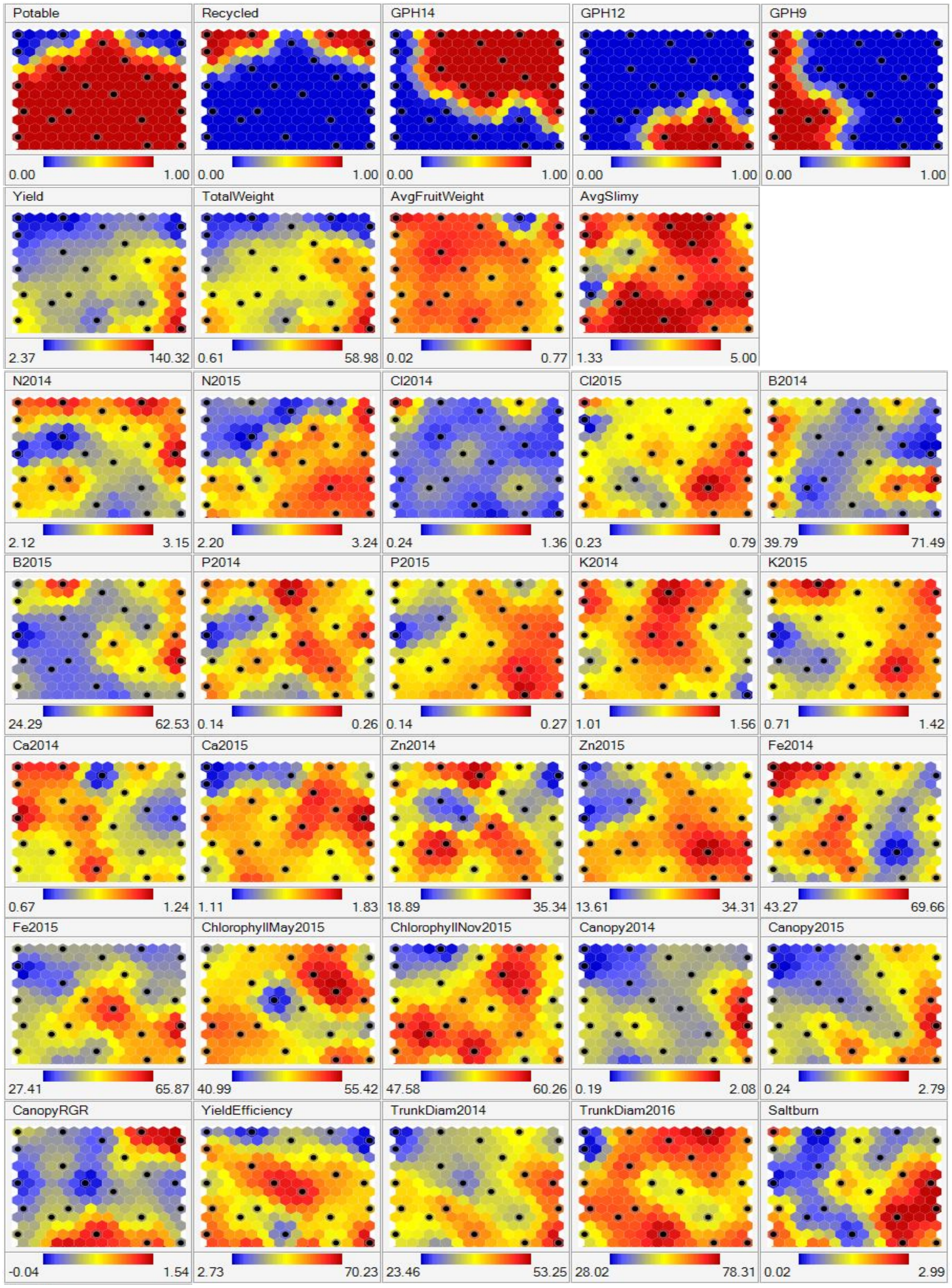
AP Figure 9. Kohonen self-organizing map of tree health and yield in relation to 2014 and 2015 leaf nutrient concentrations.



AP Figure 10. Fruit Quality in relation to 2014 leaf nutrient concentrations. Recycled – 12 GPH data included.



AP Figure 11. Overall fruit appearance rating vs 2014/2015 leaf nutrient content and tree health (Recycled – 12 GPH data not included). “AvgOverall” (bottom right box) = Fruit rating.



AP Figure 12. Fruit "slime" in relation to 2014 and 2015 leaf nutrient concentrations and tree health (Recycled – 12 GPH data not included). "AvgSlimy" (2nd row, right) = Fruit slime. 1 = severe slime, 5 = no slime.

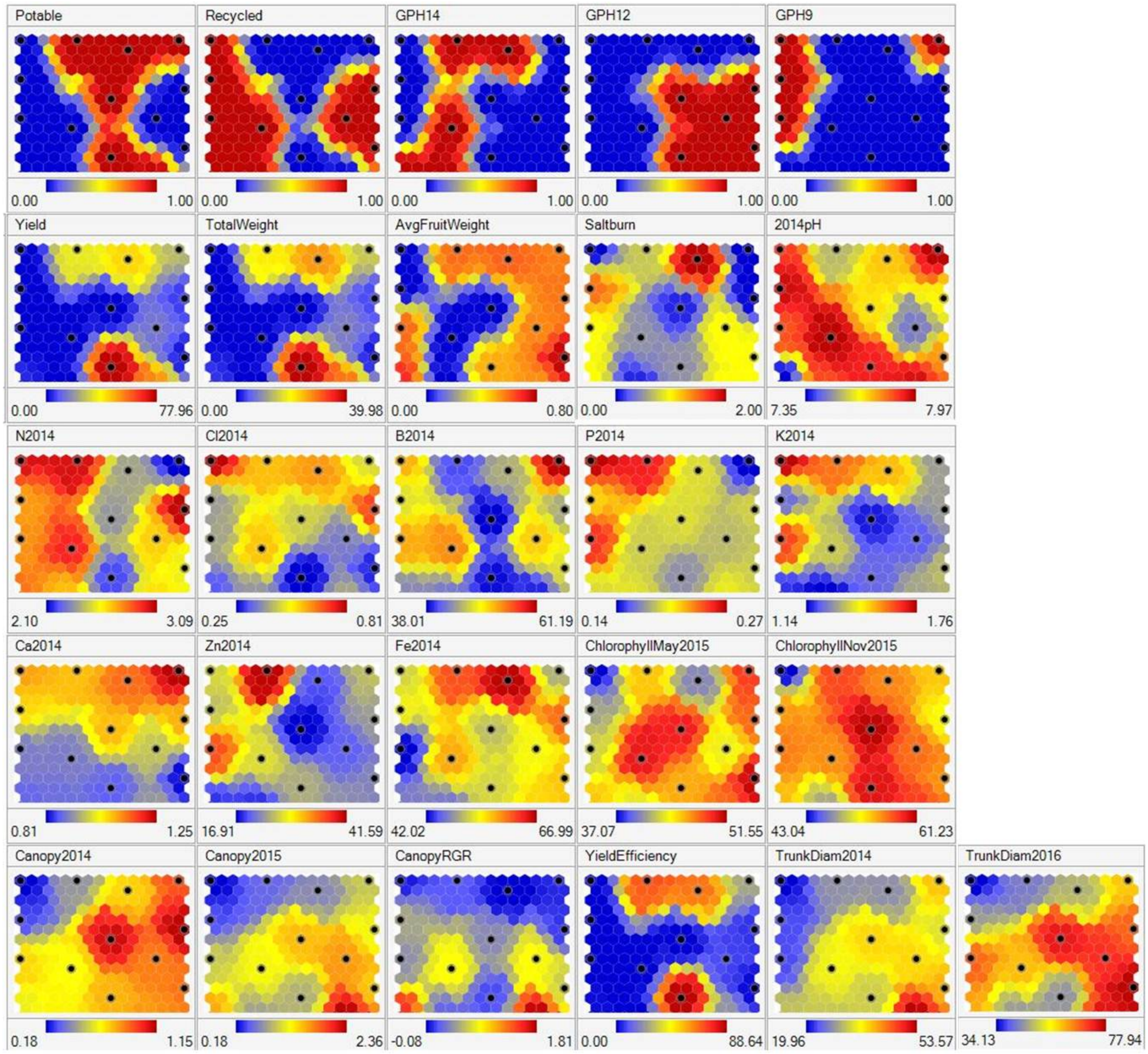
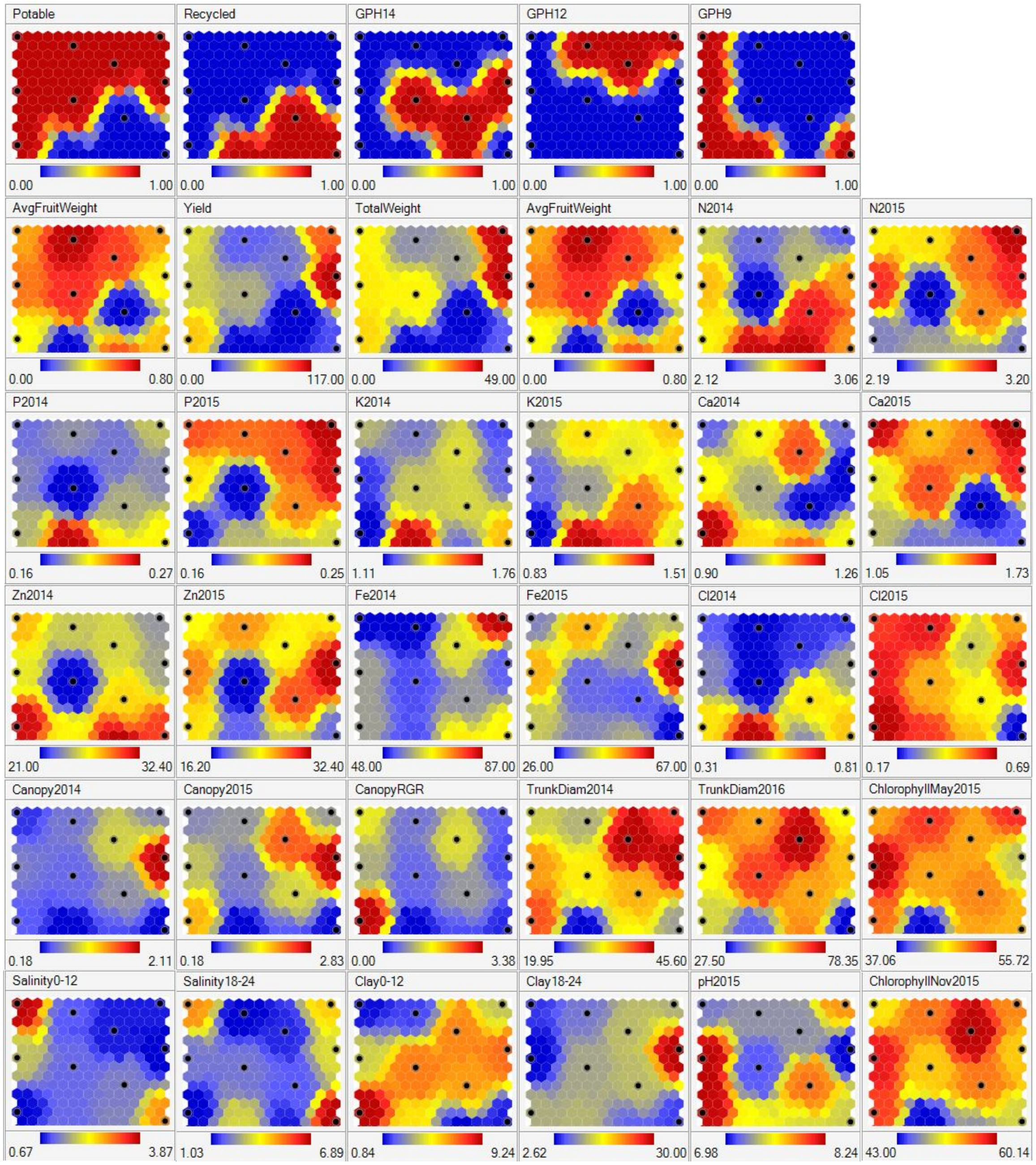
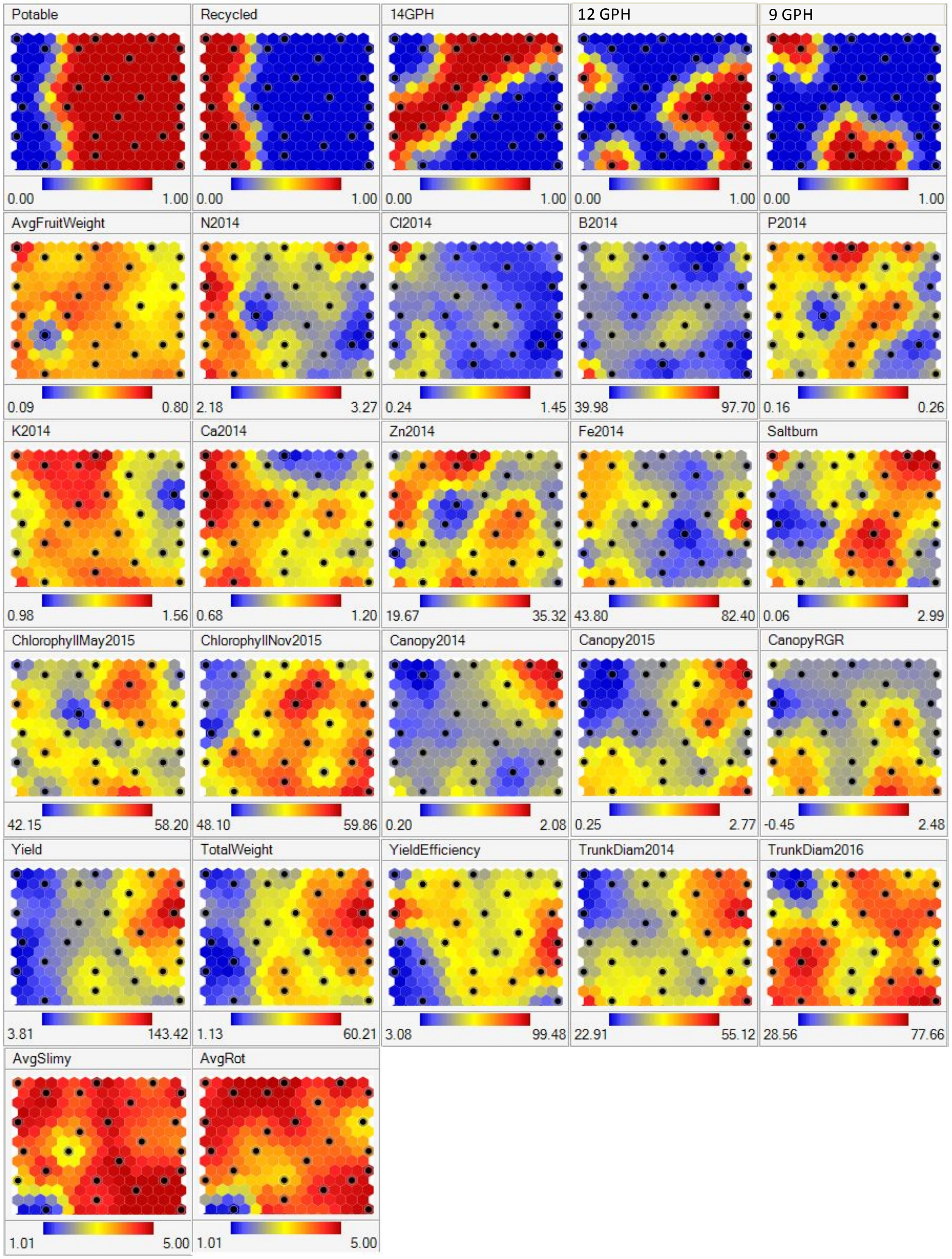


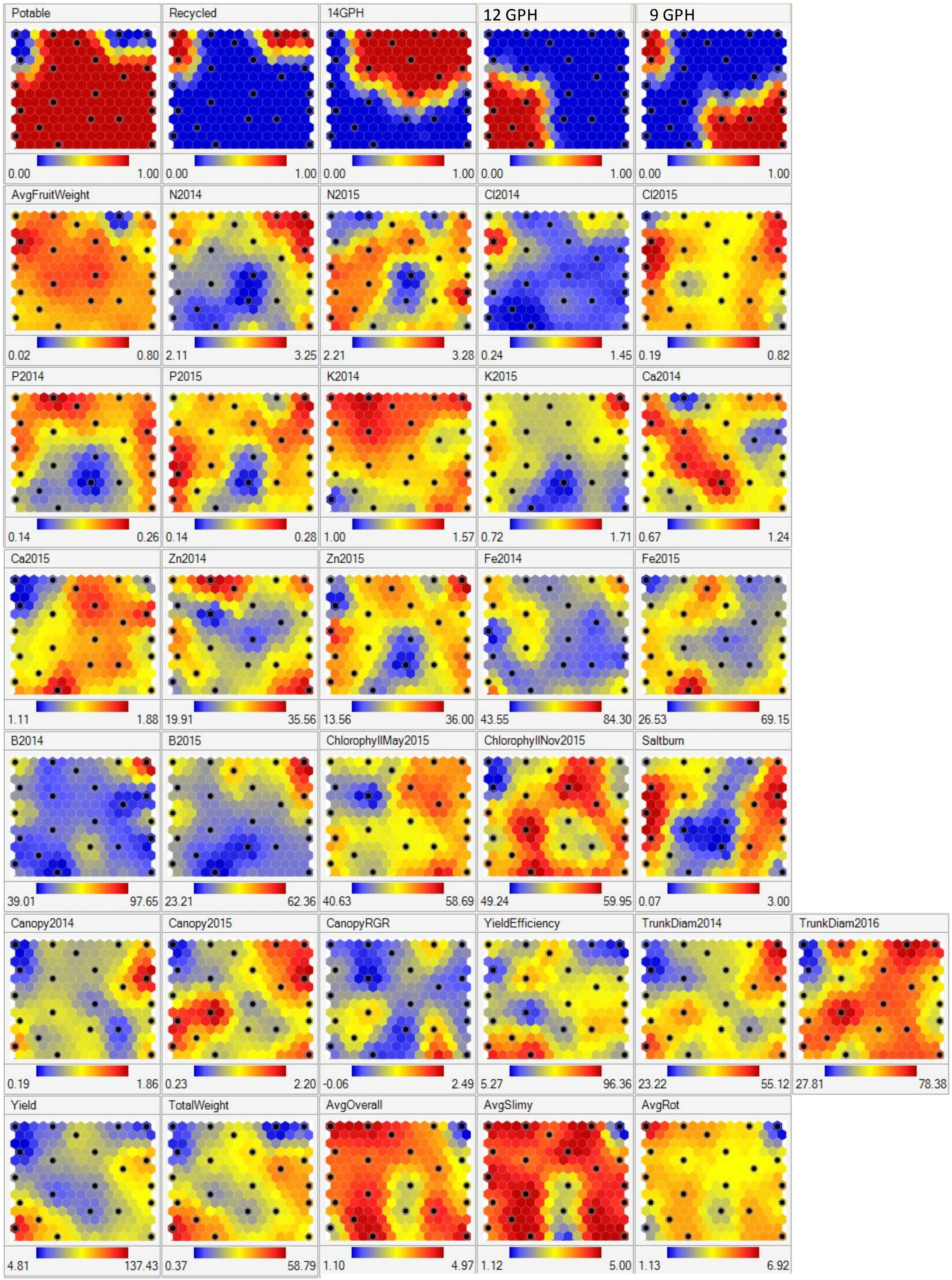
Figure AP 13. AP Figure 13. 2014 leaf nutrients vs 2014 soil pH. 2014 Soil pH = second row, right box.



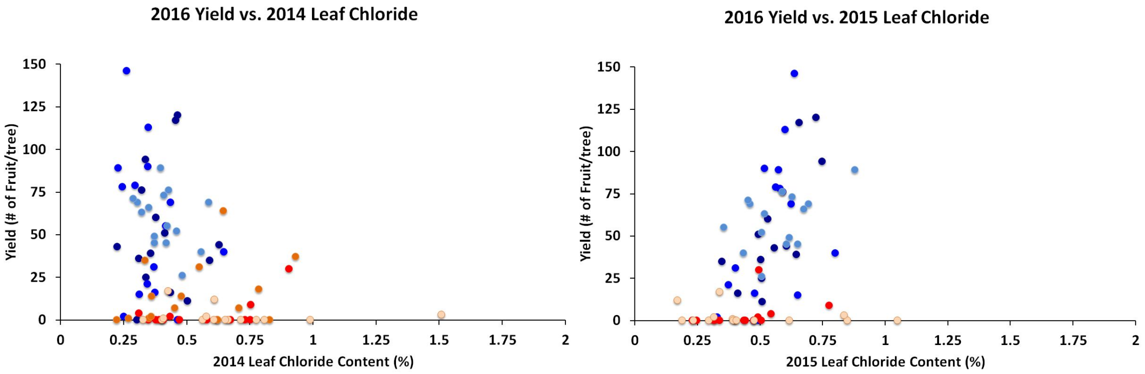
AP Figure 14. 2014 and 2015 leaf nutrients vs. 2015 soil pH. Recycled – 12 GPH data not included. 2015 soil pH = bottom row, second box from right.



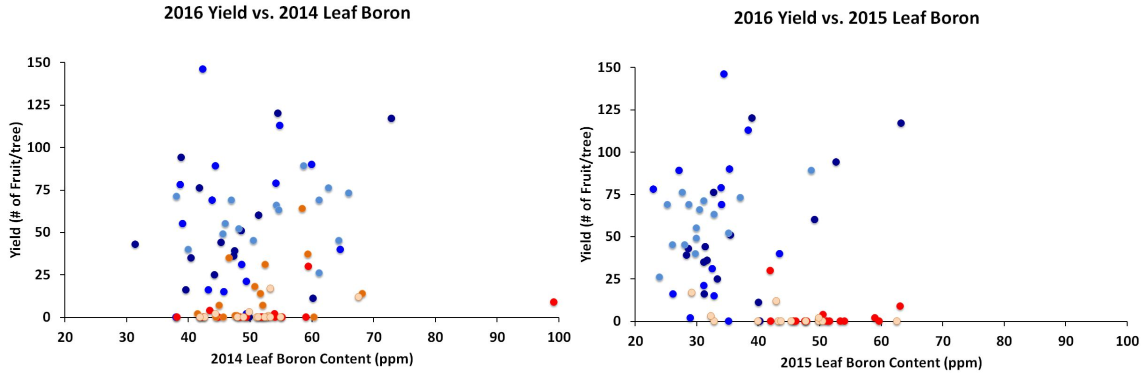
AP Figure 15. Fruit "slime" and fruit rot in relation to 2014 leaf nutrients. (Bottom row)



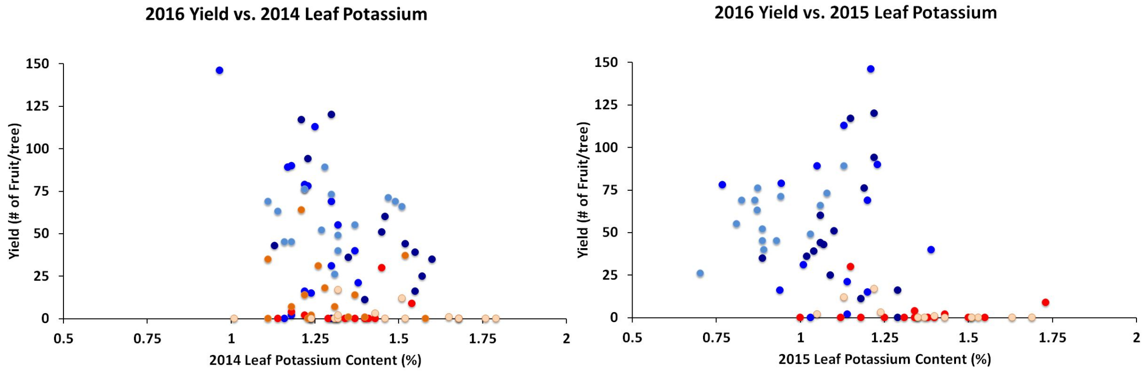
AP Figure 16. Fruit slime and rot in relation to 2014 and 2015 leaf nutrients and tree health. Recycled – 12 GPH not included. Fruit appearance, slime, and rot, bottom right.



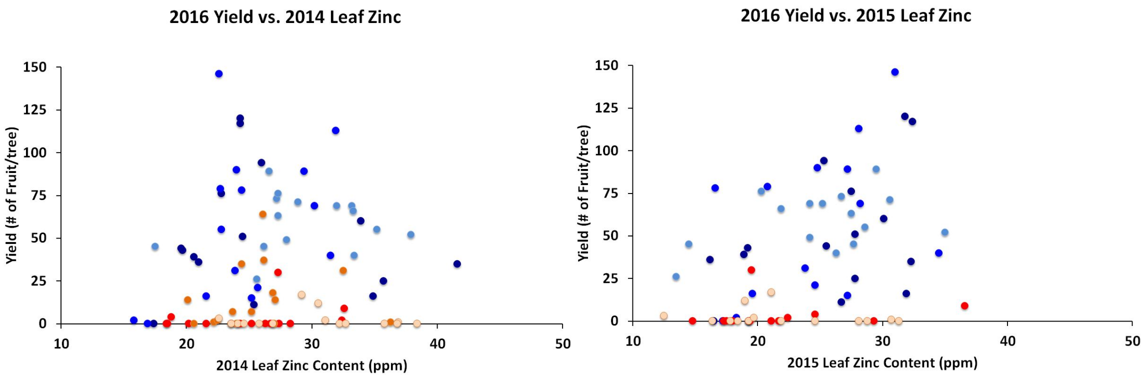
AP Figure 17. March 2016 yield vs. leaf chloride content. Left: March 2016 yield vs. 2014 leaf chloride content. **Right:** March 2016 yield vs. 2015 leaf chloride content .



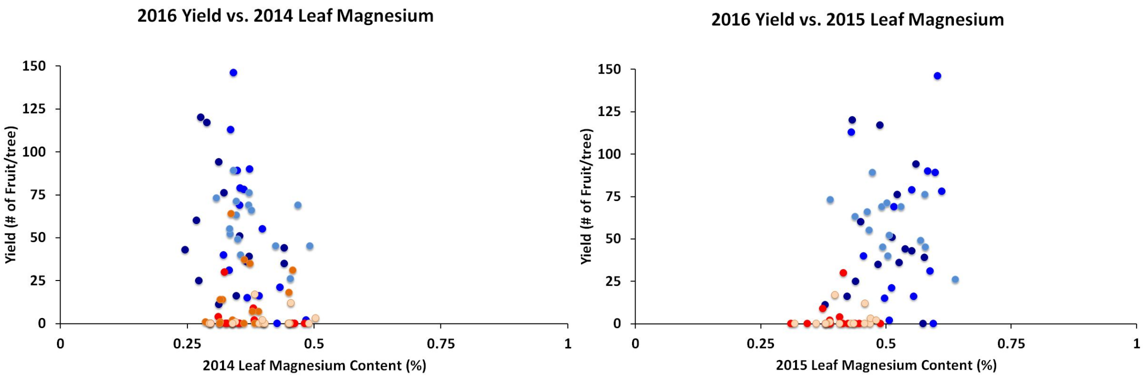
AP Figure 18. March 2016 yield vs. leaf boron content. Left: March 2016 yield vs. 2014 leaf boron content. **Right:** March 2016 yield vs. 2015 leaf boron content .



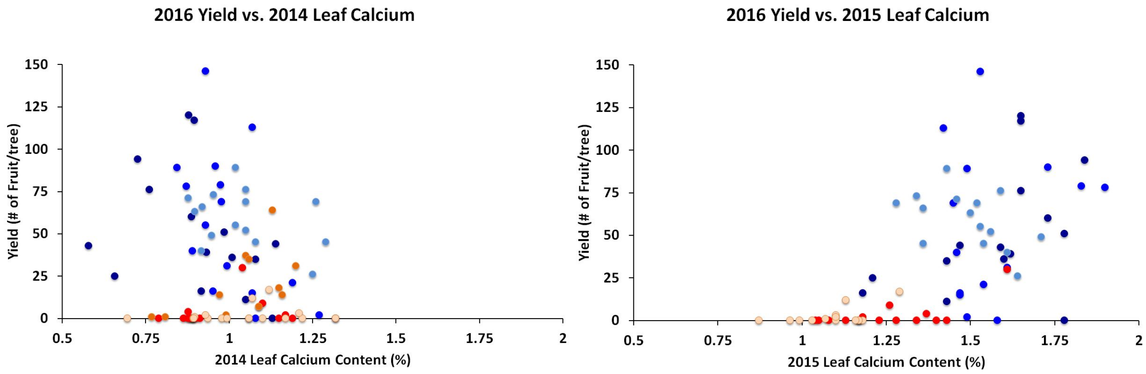
AP Figure 19. March 2016 yield vs. leaf potassium content. Left: March 2016 yield vs. 2014 leaf potassium content. **Right:** March 2016 yield vs. 2015 leaf potassium content .



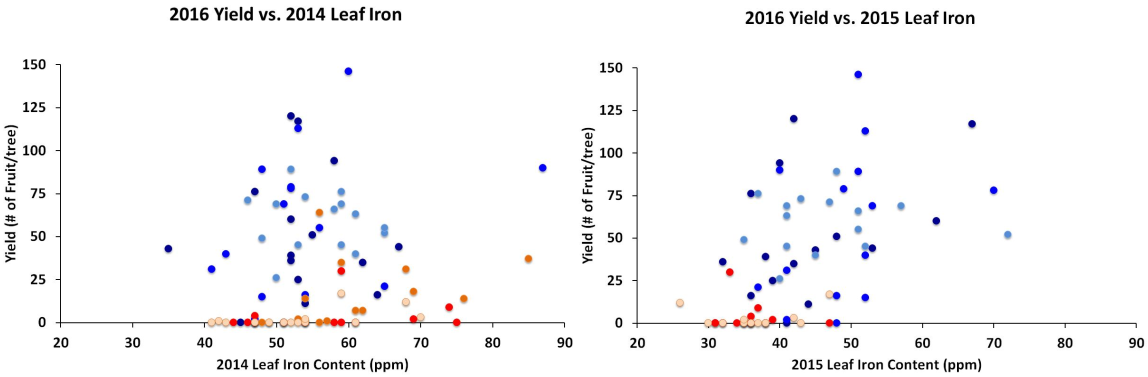
AP Figure 20. March 2016 yield vs. leaf zinc content. Left: March 2016 yield vs. 2014 leaf zinc content. **Right:** March 2016 yield vs. 2015 leaf zinc content .



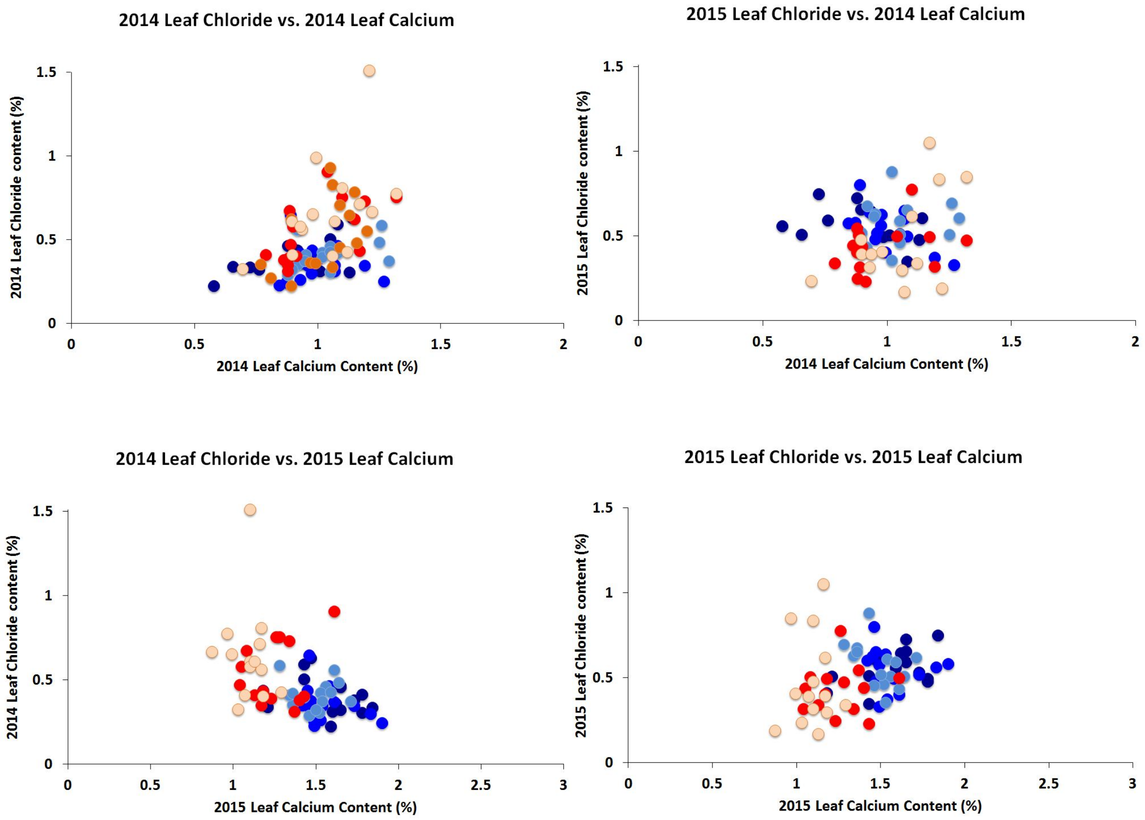
AP Figure 21. March 2016 yield vs. leaf magnesium content. Left: March 2016 yield vs. 2014 leaf magnesium content. **Right:** March 2016 yield vs. 2015 leaf magnesium content .



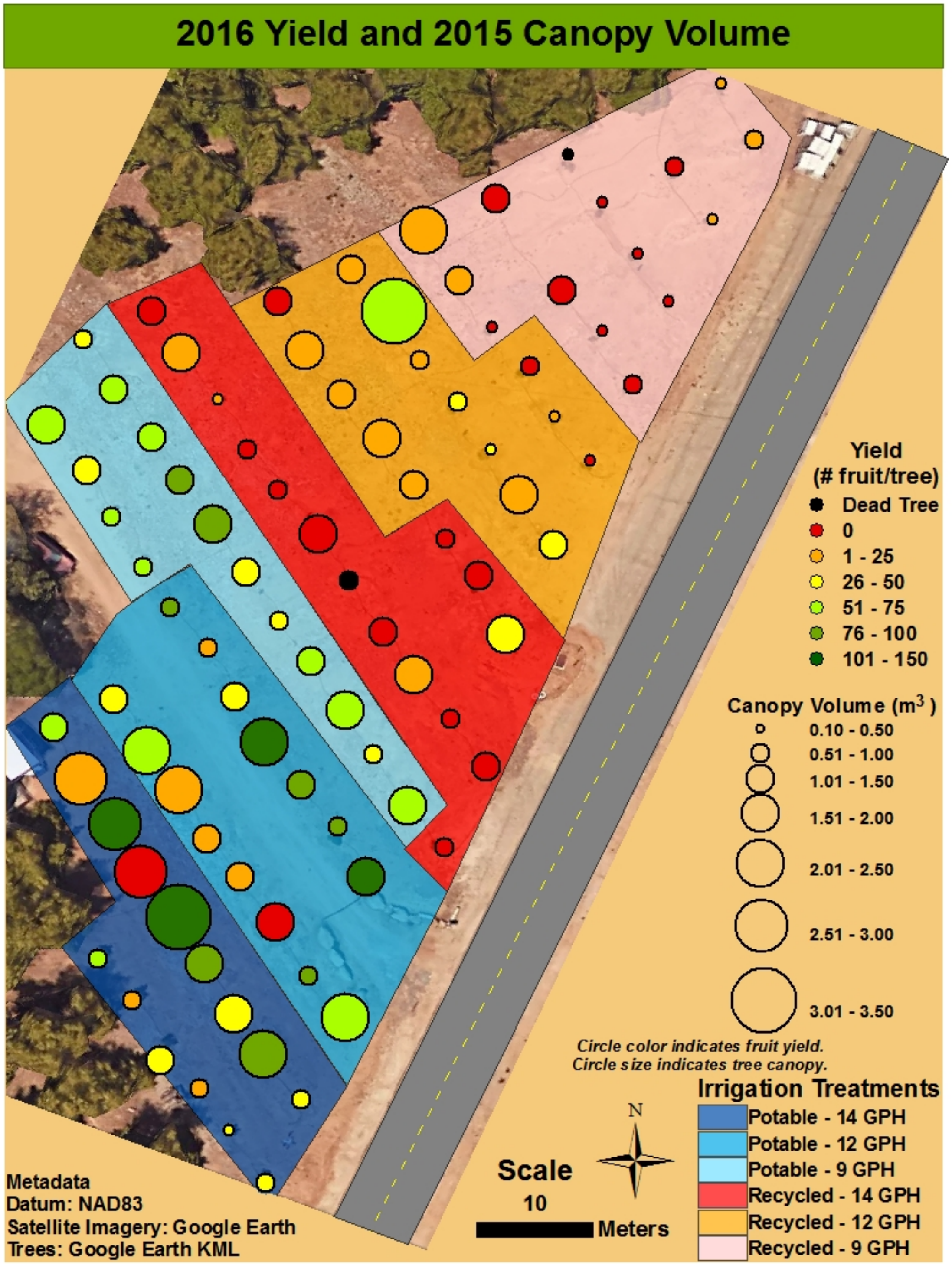
AP Figure 22. March 2016 yield vs. leaf calcium content. Left: March 2016 yield vs. 2014 leaf calcium content. **Right:** March 2016 yield vs. 2015 leaf calcium content.



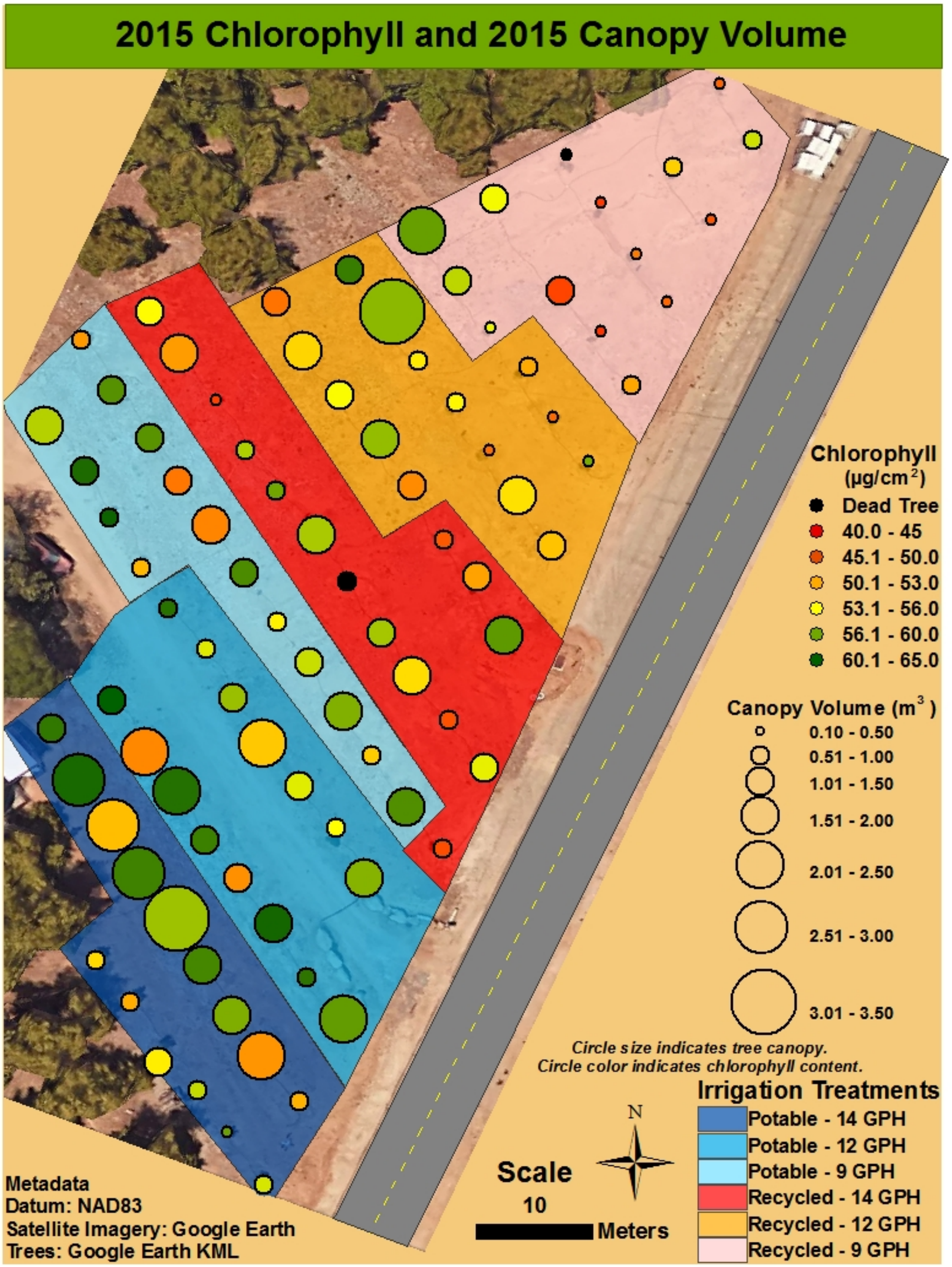
AP Figure 23. March 2016 yield vs. leaf iron content. Left: March 2016 yield vs. 2014 leaf iron content. **Right:** March 2016 yield vs. 2015 leaf iron content.



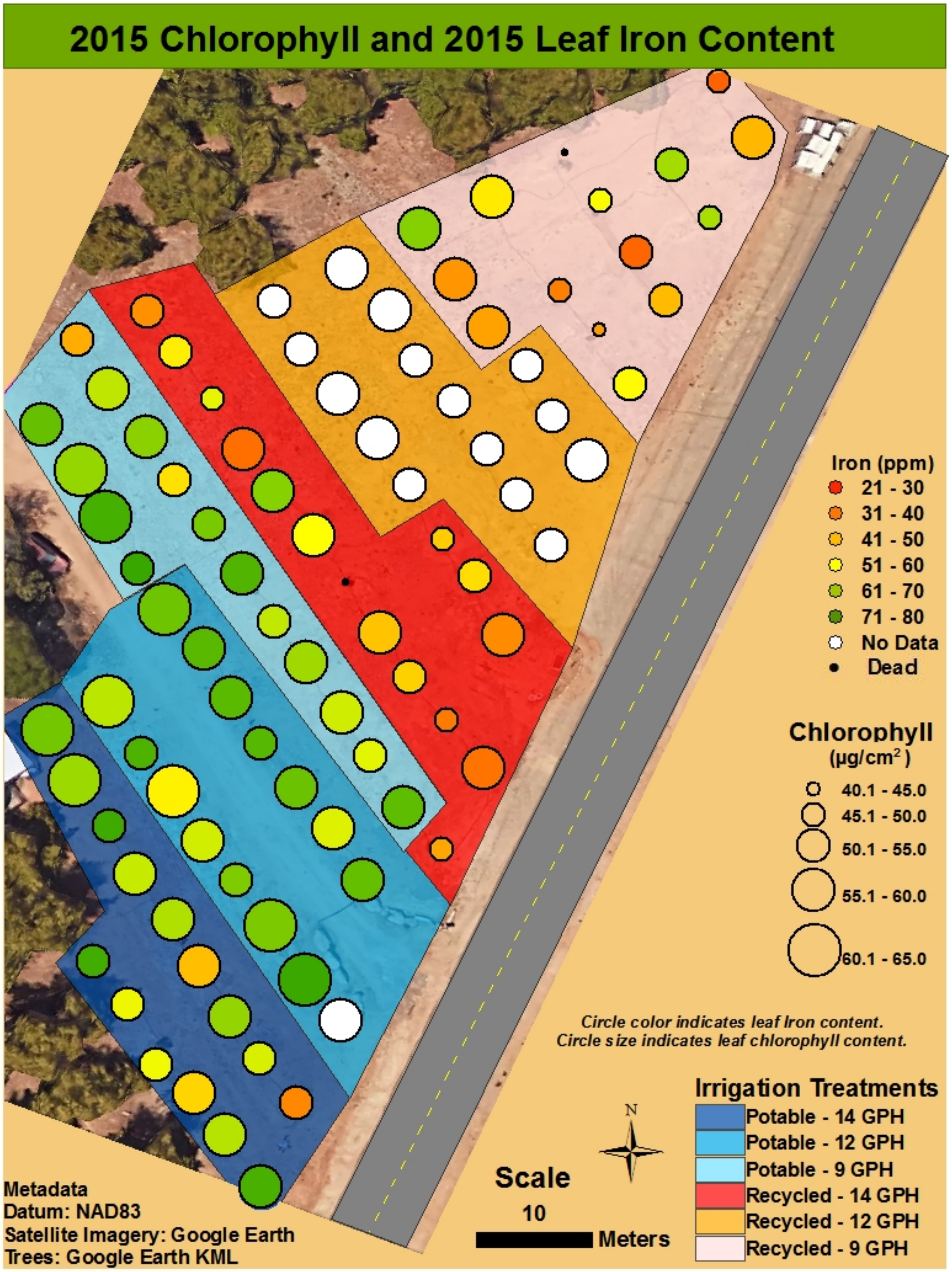
AP Figure 24. Leaf chloride content vs. leaf calcium content. Top left: 2014 leaf chloride vs. 2014 leaf calcium. **Top right:** 2015 leaf chloride vs. 2014 leaf calcium. **Bottom left:** 2014 leaf chloride vs. 2015 leaf calcium. **Bottom right:** 2015 leaf chloride content vs. 2015 leaf calcium.



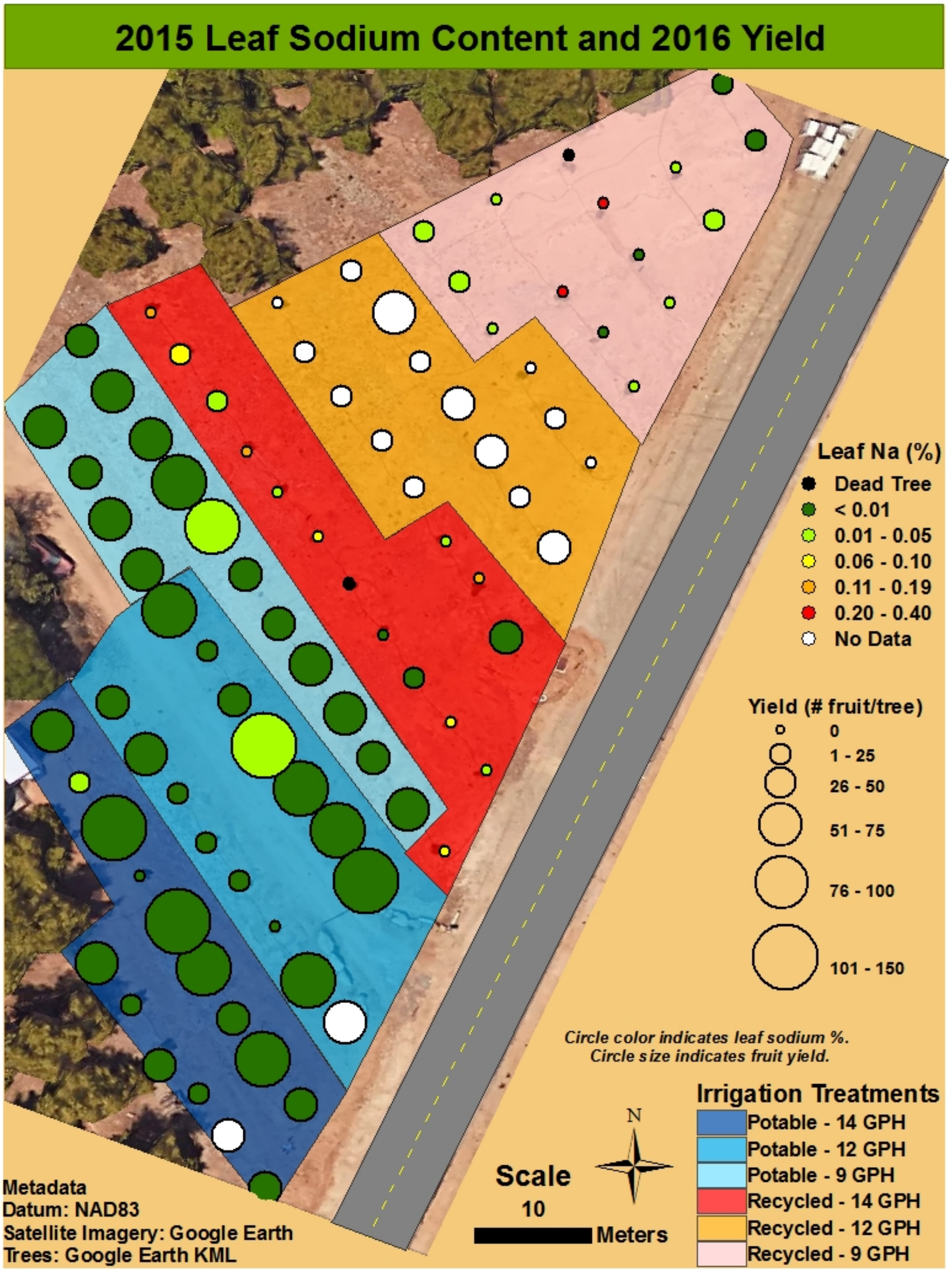
AP Figure 25. Map of fruit yield in relation to canopy volume. Circle color indicates fruit yield; circle size indicates canopy volume.



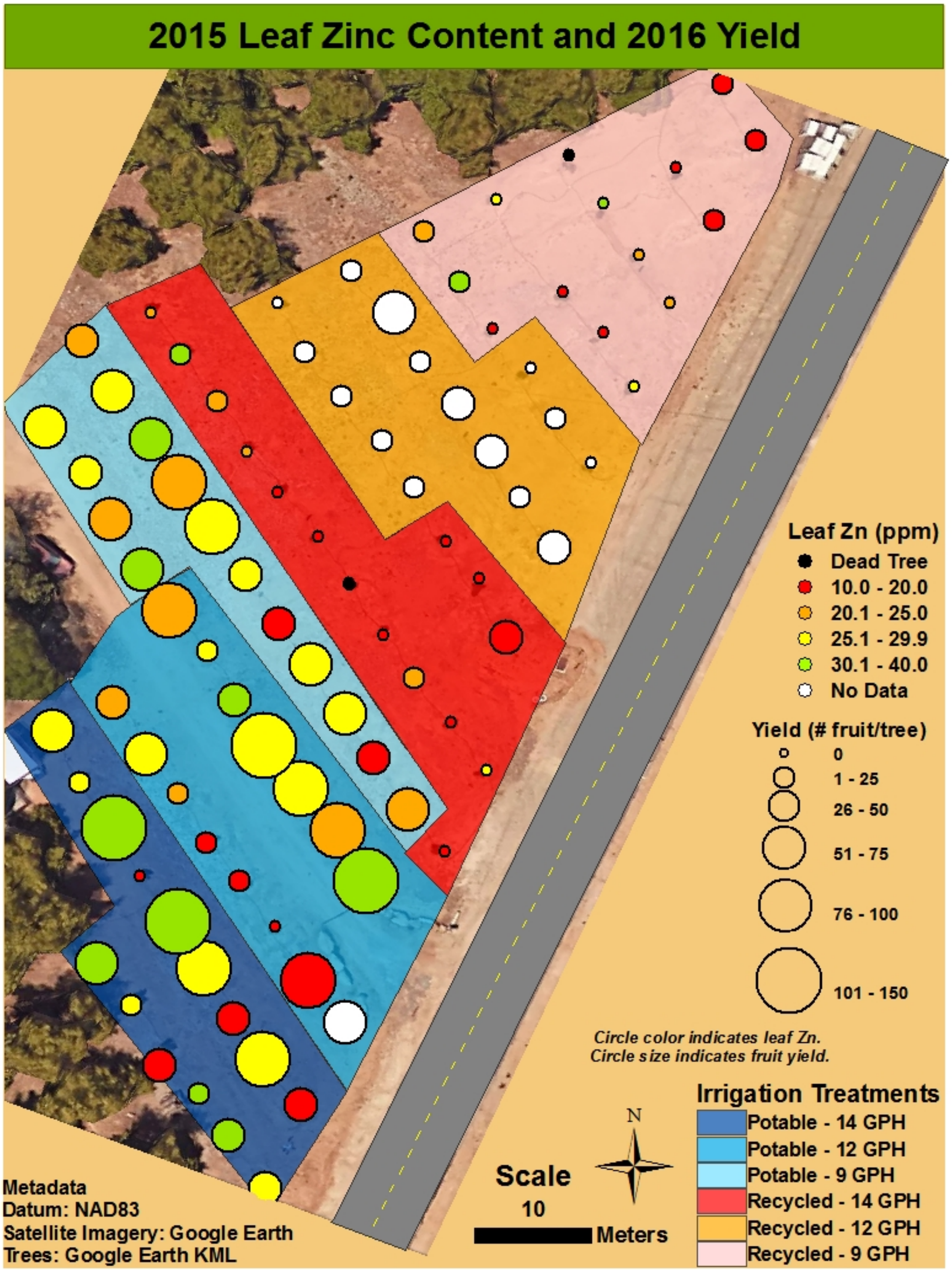
AP Figure 26. Map of leaf chlorophyll content in relation to canopy volume. Circle color indicates chlorophyll content; circle size indicates canopy volume.



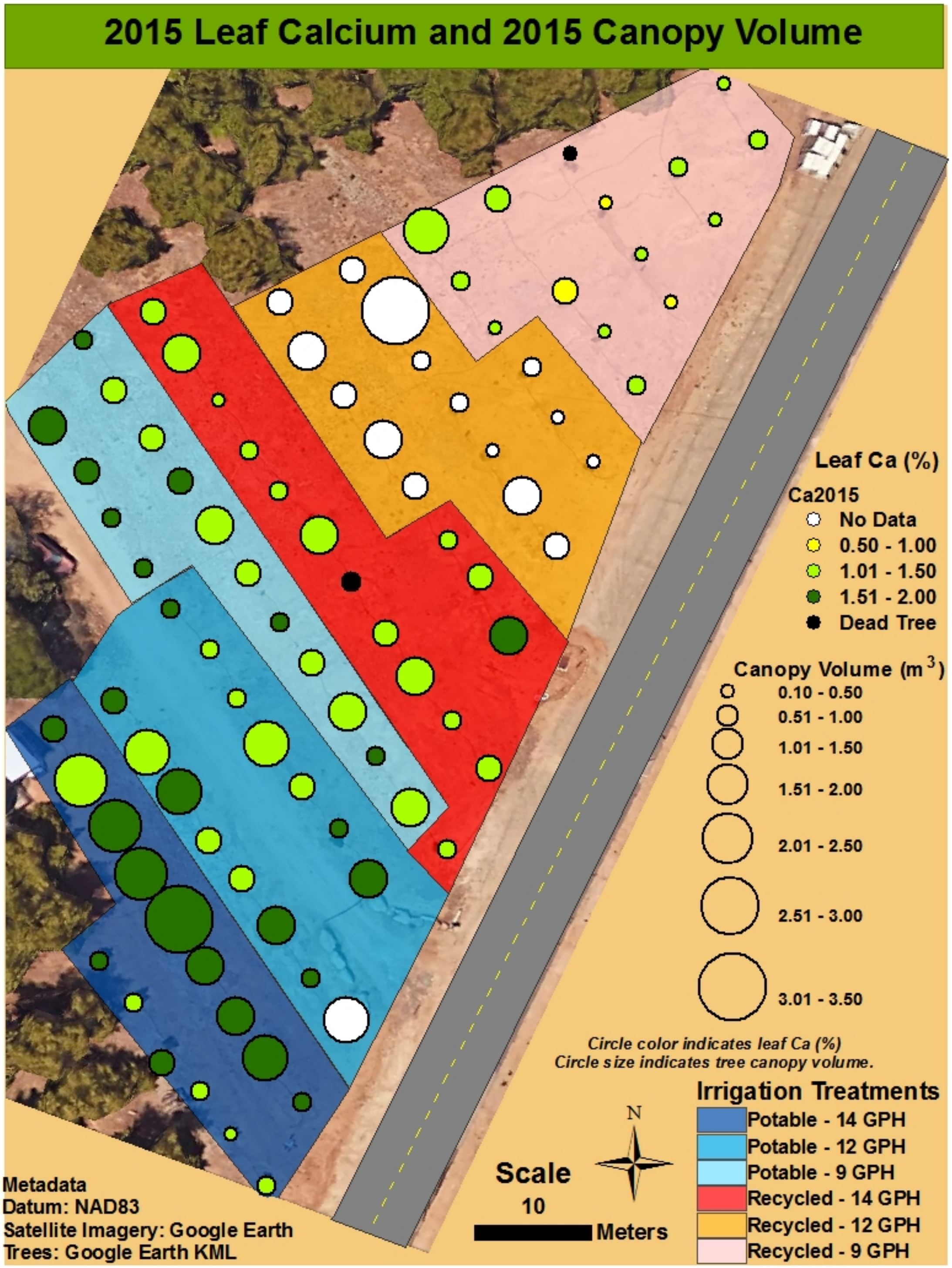
AP Figure 27. Map of leaf chlorophyll content in relation to leaf iron content. Circle color indicates iron content (by dry weight); circle size indicates chlorophyll content.



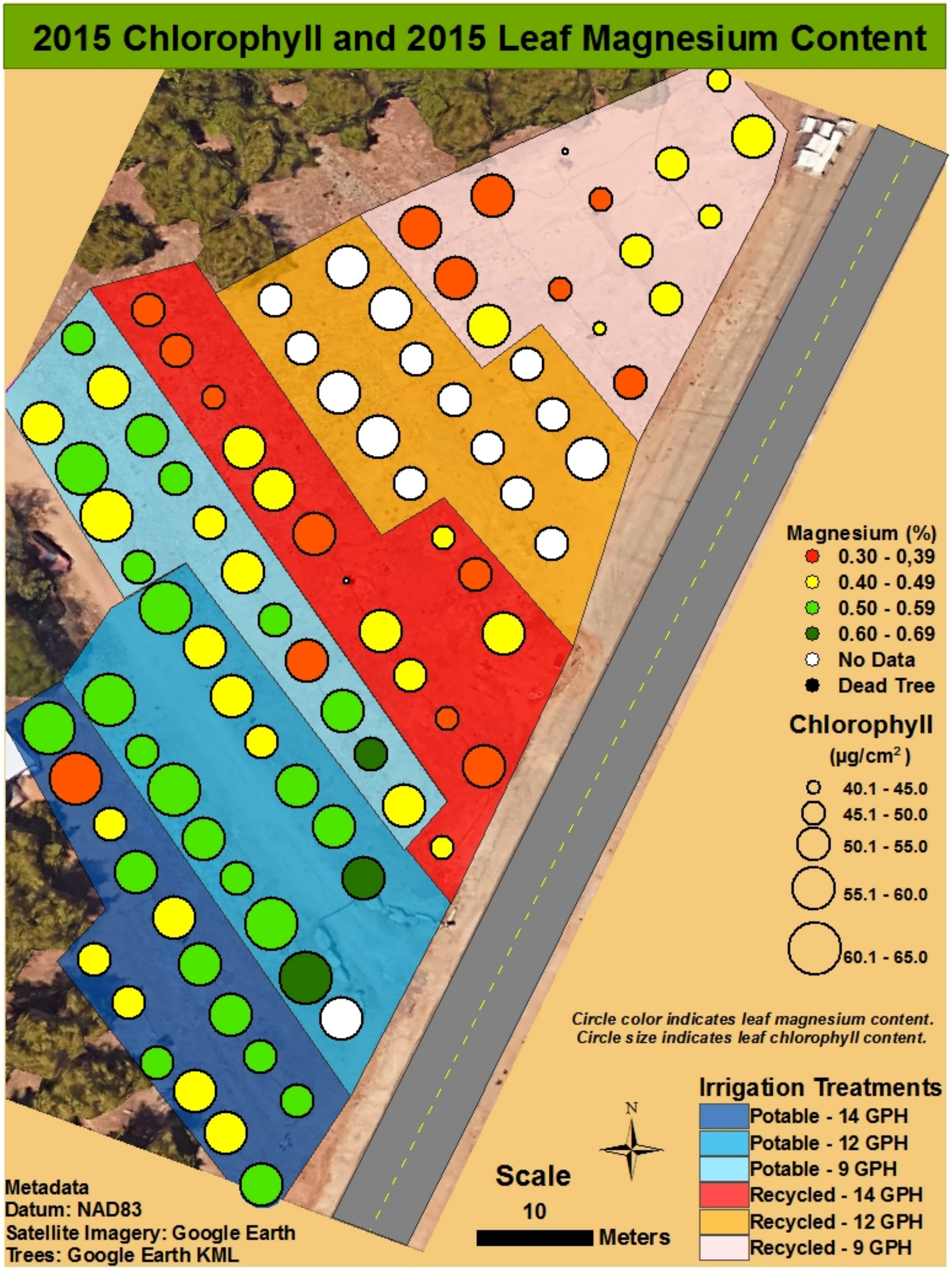
AP Figure 28. Map of leaf sodium content in relation to fruit yield. Circle color indicates leaf sodium content (by dry weight); circle size indicates fruit yield.



AP Figure 29. Map of leaf zinc content in relation to fruit yield. Circle color indicates leaf zinc content (by dry weight); circle size indicates fruit yield.



AP Figure 30. Map of leaf calcium in relation to canopy volume. Circle color indicates leaf calcium (by dry weight); circle size indicates canopy volume.



AP Figure 31. Map of leaf magnesium content in relation to chlorophyll content. Circle color indicates leaf magnesium (by dry weight); circle size indicates leaf chlorophyll content.

Map Interpretation

As demonstrated in the preceding maps, there was considerable variation within irrigation treatments, in some cases due to non-uniform environmental conditions. The most striking variation is in the Potable – 14 GPH irrigation treatment. The trees in the row on the edge of the plot were smaller in size, yielded fewer fruit per tree, and had lower leaf chlorophyll levels. This was likely due to the close proximity to the mature avocado trees to the southwest shading the outermost row of Potable – 14 GPH trees. However, there was additional variation in other datasets, not all of which can be readily explained. This observed variation is most likely due to variation in soil texture, pH, and microsite variations. As described throughout this thesis, nutrient availability and soil conditions interact in a complex fashion to limit or enhance tree vigor. More data would need to be collected to identify all of the factors responsible for within-treatment variations. Despite the variations observed within each treatment, the data still provides compelling evidence that strongly suggests additional water treatment is necessary to improve crop productivity in avocados irrigated with recycled water in Escondido.

AP Figure 25 shows that trees irrigated with recycled water had drastic yield reduction in relation to trees irrigated with potable water at equivalent irrigation rates and volumes; this was correlated with significantly smaller tree canopy volumes. AP Figure 25 shows that trees irrigated with potable water typically had fruit yields in the range of 51-150 fruits/tree, with a few trees yielding less than 50 fruits per tree. In contrast, trees irrigated with recycled water typically yielded fewer than 25 fruits per tree; approximately 60 percent of the trees in the Recycled 14 GPH and Recycled – 9 GPH had no fruit, while 20% of the trees in the Recycled – 12 GPH irrigation treatment yielded no fruit. Further data analysis indicates that the reduction in canopy growth among recycled water treatments was strongly correlated with reduced leaf chlorophyll content. AP Figure 27 demonstrates that a higher rate of iron deficiency occurred in trees irrigated with recycled water, and a reduction in leaf iron content was correlated with reduced chlorophyll content among the recycled water irrigation treatments. Iron levels were on the lower end of the optimal range in trees irrigated with recycled water; recommended iron levels are 50 – 300 ppm by dry weight and the trees irrigated with potable water typically fell between 60-80 ppm, while trees irrigated with recycled water typically had levels in the range of 20 - 50 ppm.

As evidenced in AP Figure 28, trees irrigated with potable water had almost no sodium in their leaves (typically levels of 0.007% by dry weight), while trees irrigated with recycled water had leaf sodium levels between 0.01 to as high as 0.36% by dry weight. The recommended threshold is not to exceed 0.25% leaf sodium by dry weight. While the contrast is significant (as demonstrated even more clearly in Figure), the results are most likely correlation rather than a causation of reduced yield or tree health,, since the trees irrigated with recycled water were generally not near the 0.25% Na threshold. AP Figure 29 displays leaf zinc content in relation to fruit yield. Since zinc is needed for fruit set, a zinc deficiency might help explain a yield reduction in trees irrigated with recycled water. Zinc (and Iron) are both less soluble at higher soil pH and in the presence of carbonates, which are common conditions in soils irrigated with recycled water. As shown in AP Figure 29, there is considerable variation in fruit yield and leaf zinc content among potable irrigation treatments as well; however, the average leaf zinc concentrations are higher in trees irrigated with potable water. The minimum threshold for zinc sufficiency in avocado leaves is considered to be 30 ppm, and many trees in the potable water irrigation treatments had less than 30 ppm, likely due to calcareous soils. Zinc deficiency is common in California avocados and recycled water will only exacerbate the problem via elevating soil pH and the addition of carbonates. This information needs to be factored into orchard management and water treatment. AP Figure 30 shows leaf calcium in relation to leaf canopy volume. The recommended levels of leaf calcium in avocado are 1.0- 4.5% by dry weight. As calcium is responsible for cell elongation and shoot growth, so a deficiency would reasonably contribute to stunted growth. shown in AP Figure 30, this phenomenon was observed; trees irrigated with potable water were more frequently in the 1.51 – 2.00% leaf Ca range by dry weight, while trees irrigated with recycled water fell predominantly within the 1.01- 1.5% leaf Ca range. Although this is still considered sufficient, it is less than trees irrigated with potable water and may partially contribute to reduced canopy growth.