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Reassessing the role of exogenously applied iodine in agriculture:
Lessons from almond, pistachio, and avocado field trials

By

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THESIS

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Abstract

Iodine's impact on plant growth and development remains incompletely understood due to its volatility and its ubiquity in the environment that complicates experimental control. These attributes of iodine experimentation have led to a wide diversity of results that are often circumstantial to species, experimental growing conditions, and the rate, form, and timing of iodine application. Iodine has been demonstrated to improve the overall productivity of some plants in certain conditions when applied as a fertilizer, to influence bloom timing and fruiting, and to mitigate negative effects of salt and heavy metals in stress treatments. This study investigated iodine's potential as a biostimulant and beneficial nutrient in almond, pistachio, and avocado orchards. Field trials were conducted using a commercial potassium nitrate, iodine containing product (UltrasolINE, SQM) treatment applied via drip irrigation ($0.154 \text{ kg I ha}^{-1}$) and as a foliar spray (3.5 g I ha^{-1}). Control treatments received identical rates of potassium nitrate without iodine (Ultrasol, SQM). Results showed no significant effects on plant tissue iodine concentration, leaf nutrient content, vegetation indices, or yield across all species. Iodine applied at pink bud in almond did not influence bloom timing at the individual spur or at the whole-tree level or have any yield impacts. Naturally occurring root iodine concentrations in almond and avocado were found to be higher than in leaves, consistent with observations in other species. This study contributes to the understanding of iodine's role in plant phenology and physiology, concluding that low-rate iodine application did not demonstrate agricultural benefits in the studied tree crops.

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Chapter 1: A review of iodine in plants and humans

1.1 Introduction

The importance of iodine in human diets is well understood, but despite over 100 years of research, there is no consensus on if, why, and most importantly, how iodine impacts plant physiology and phenology. This literature review seeks to explore the diversity of work done to date on the impact of exogenously applied iodine on plant physiological processes and phenological patterns. Iodine is an essential element for humans, and as such, there is a large body of work exploring the potential for iodine biofortification that will not be specifically reviewed here. Iodine biofortification studies have been, and continue to be, carried out to ameliorate iodine deficiency disorders (IDD). These biofortification studies are foundational in describing iodine treatment effects on plants despite these effects often being unrelated to their biofortification goals and hypotheses. The results of these studies will be reviewed independent of biofortification results as the data covers a wider diversity of rates, application methods, and species than studies done without a biofortification intent. Research on iodine in plants began with observational studies and then attempts to prove iodine essentiality in the 1940s, biofortification efforts starting in the 2000s, and has now come full circle with a new modern-day discussion of essentiality. However, the dialog surrounding plant nutrient essentiality has changed, and as such, the understanding of exogenous iodine's impacts on plants has followed. In this new era of plant nutrients, should iodine be considered a beneficial micronutrient, biostimulant, or possibly something in between?

1.2 Iodine in the natural environment

Iodine, atomic number 53, is a chemical element with a large ionic radius of 220pm that precludes it from entering the crystal lattice of rock-forming minerals in the soil (Fuge, 2013). Due to this elemental property, it is estimated that the oceans contain around 70% of all iodine on the planet as its terrestrial fate is transitory (Fuge & Johnson, 2015). The majority of iodine in the ocean exists as iodate and is relatively uniform in distribution, except in the uppermost layer where biological activity depletes the free concentration (Tsunogai, 1971; Wong & Cheng, 2001). This depletion mainly occurs due to the sequestration iodine by seaweeds at often high levels – in one study, commercially available seaweeds were found to have iodine concentrations from 16 to over 8165 µg/g (Teas et al., 2004).

Brown kelp, *L. digitata,* has been found to accumulate a 30,000x concentration when compared to the seawater in which it grows (Leblanc et al., 2006). Yet despite this clear trend towards purposeful accumulation, the biological function of this iodine is still uncertain. It has been proposed that "Iodide accumulation leads to an apoplastic antioxidant reservoir that can be mobilized during oxidative stress" (Küpper et al., 2008). This was determined in an experiment that exposed kelp to stress and monitored the release of iodide into the medium (Küpper et al., 2008). This work has been largely corroborated in a study that found kelp had tightly regulated transcriptional responses and iodine metabolism in response to pathogens that largely resemble Pathogen Associated Molecular Patterns (PAMPs) in land plants (Cosse et al., 2009). This work in kelp is important because similarities between PAMPs in kelp and land plants suggest that some elements of pathogen response and antioxidant processes may be conserved as ancient mechanisms. Iodine's function as an inorganic antioxidant in kelp might inform our understanding of stress reduction-associated responses in plants.

The biological release of iodine into the atmosphere plays a crucial role in terrestrial deposition, although it is currently considered less significant than non-biological photochemical release (Fuge & Johnson, 2015; Saiz-Lopez et al., 2012). Nevertheless, transect studies of marine boundary layers have demonstrated that soluble organic iodine can be a major component—and sometimes even a dominant one—of marine aerosols (Baker, 2005). It has been suggested that the volatilization of organic iodine serves as the primary source of atmospheric iodine, which subsequently undergoes photooxidation to form inorganic iodine (Carpenter, 2003). However, a global precipitation study found that most iodine in precipitation is associated with organic compounds, indicating that not all atmospheric iodine undergoes photooxidation before deposition (Gilfedder et al., 2007). Once in the atmosphere, iodine can deposit into the terrestrial environment through both dry and wet precipitation processes.

Iodine present in terrestrial soil is most often associated with deposition from the sea, either current or historical, and especially so in area that were once oceans (Fuge & Johnson, 2015). Rainfall, fog, and direct spray deposit iodine, but the terrestrial iodine content has not been shown to directly correlate with distance from the sea coast (Johnson, 2003). Samples closer to the sea are often associated with higher deposition, but variability is still often extreme, and coastal precipitation leaching iodine in these areas can also skew results as the increased deposition is offset by the increased precipitation (Jensen et al., 2019). Research mapping soil iodine concentration on large islands such as New Zealand and Ireland often shows a strong association between distance to coast and concentration, but this is less prevalent in continental countries (Hansford Shacklette & Boerngen, 1984a; Jensen et al., 2019; Smyth & Johnson, 2011). In China, soil type, organic matter (OM) percentage, climate, and precipitation amount

were more indicative of soil iodine content compared to the distance to the coast (Liu et al., 2024).

1.2.1 Iodine in soils

Once iodine is deposited onto a soil, its fate is most associated with the organic matter content of the soil (Fuge & Johnson, 1986; Hu et al., 2012). This is due to the affinity for inorganic iodine to associate with organic matter and to a lesser extent, aluminum and/or iron oxides. Inorganic iodine (I^- and IO_3^-) was found to strongly associate with all sizes of humic acids to produce organoiodine (Bowley et al., 2016). In soils with low organic matter, I^- and IO_3^- remained unchanged after 60 days compared to OM-sufficient soils where inorganic iodine was transformed into organoiodine (Yamaguchi et al., 2010). It was also found that soil microbial populations were not essential for IO_3^- to make the transformation into organoiodine, but occurred faster (1d) with microbial activity. The conversion of I^- to organoiodine in terrestrial environments is likely explained by microbial activity (Yamaguchi et al., 2010).

In studies employing methods to reduce biological activity (use of antibiotics, autoclaving, fumigating, desiccating, γ-irradiation, anaerobic treatments, and heat treating) There was reduced iodide sorption (Behrens, 1982; Bors, 1992; Fukui et al., 1996). Suzuki et al. (2012) found that an alphaproteobacterium was able to oxidize iodide (I^-) to molecular iodine (I_2) through the use of a multicopper enzyme laccase (Suzuki et al., 2012). Seki et al. (2012) found that, indeed, bacterially derived laccase strongly increased sorption of iodide (Seki et al., 2013). Iodine is held relatively strongly in the soil with an estimated 10% remaining water-soluble (Fuge & Johnson, 1986). This is strongly influenced by soil type and one experiment with soils from a variety of areas in Slovakia found on average: 38 % of iodine was associated with organic matter, 37.7 % was mineral-bound and only 4.4 % of the total soil iodine was water-

soluble (Duborská et al., 2020). However, in flooded conditions, iodine can become extremely available and cause toxicity symptoms known as "Akagare reclamation disease" in Japan. This is thought to occur because of the increase in reduction of IO_3^- to I^- and I_2 in flooded and anaerobic rice fields (Sheppard & Motycka, 1997; Yamaguchi et al., 2006).

1.2.2 Loss from soils

Iodine is known to volatilize from soils, with rates of volatilization depending on the soil type and the specific form of iodine present (ex: Weng et al., 2009). Additionally, plants can release methyl iodide (CH_3I), which is hypothesized to be a detoxification response (Saini et al., 1995). Both soil and plant volatilization contribute to reduced soil iodine levels and help explain differences in soil iodine content across geographic regions. Methyl iodide volatilization has gained attention due to its role in degrading ozone molecules in the troposphere (Davis et al., 1996). In response to climate change, efforts are underway to map and quantify the global iodine budget, aiming to minimize iodine release from terrestrial sources. Soil and plant volatilization are particularly important because they are more manageable through plant breeding and land-use practices compared to marine iodine volatilization.

Although the majority of global iodine resides in the oceans, terrestrial iodine is transient and can be re-volatilized, allowing it to travel far from marine sources (Fuge, 1990). Terrestrial iodine sources, often tied to agricultural activities, are primarily mediated by soil bacteria. Bacterial activity has been linked to methyl iodide release, although the underlying mechanisms and purposes remain unclear (Amachi et al., 2003). In controlled laboratory experiments, fungi also volatilized CH_3I (Ban-nai et al., 2006). These biologically driven processes contribute significantly to the global iodine flux and provide insight into regional variations in soil iodine concentrations.

Plant release of CH_3I occurs in a many situations and has been most heavily studied in rice production systems where it is estimated that 5% of global atmospheric methyl iodide arises (Redeker et al., 2000). Rice plants release the majority of the methyl iodide as a theorized detoxification mechanism to deal with the high availability of iodine in flooded environments, but this release varies by rice variety (Redeker et al., 2000). Targeted knockout of a specific (*OsHOL1*) gene in rice led to a drastic reduction in methyl iodide production. The function of the HOL gene is uncertain, though it has been proposed that HOL genes are responsible for glucosinolate metabolism and defense against phytopathogens (Carlessi et al., 2021; Nagatoshi & Nakamura, 2009). Different varieties of rice have been shown to have variable tolerance to iodine, but the tolerance mechanism is still being understood. Rice plants under a high IO_3^- treatment had increased reduction activity and reduced iodine concentrations in the stem due to methyl iodide production. However, counterintuitively reduced shoot iodine concentration had no relationship to variety tolerance as increased reduction activity and lower shoot concentrations were linked to decreased tolerance rather than increased tolerance (Kato et al., 2013). This is an important finding as it shows that rice plants able to tolerate high iodine are passively tolerating it rather than actively detoxifying their environment. Though rice may not be indicative of all plants, understanding the mechanisms behind methyl iodide production in this species can help to improve the understanding of global iodine cycling at large. This knowledge can also add to the understanding of plant interaction with iodine more broadly.

1.3 Plant uptake
1.3.1 Roots

Iodine is taken up by plant roots in the IO_3^- and I^- forms, though there is evidence that there is preferential uptake of the I^- form. As discussed above, Kato et al., 2013 found that rice

roots actively reduce IO_3^- to I^-, and in spinach it was observed that the uptake of I^- was greater than IO_3^- (Humphrey et al., 2019; Kato et al., 2013). Borst Pauwels, 1961 found that plants are better able to tolerate IO_3^- compared to I^- and this is likely because the intermediary reduction step slows down the uptake and prevents phytotoxicity. However, Weng et al., (2008) showed that iodine form uptake preference was concentration-dependent, with low concentrations favoring IO_3^- uptake (Weng et al., 2008). With the use of metabolic inhibitors that limited ATP synthase function to effectively limit symplastic uptake, both Weng and Humphrey showed low uptake at low concentrations. This transitioned to primarily passive apoplastic movement as external iodine concentration increased, regardless of form. Both theorized this was due to the saturation of active transport sites. However, if organic forms of iodine are also considered, different species display a diversity of "preferred forms," whether in sweet corn, lettuce, barley, pea, spinach, or whether grown hydroponically or in soil (Grzanka et al., 2021; Halka et al., 2019; Smoleń et al., 2021; Umaly & Poel, 1971; Weng et al., 2008).

Iodine uptake studies are frequently performed on different species of plants, and the results are often unique to the species used. A study looking at root iodate reduction activity found that rice and barley were nearly identical in activity. In contrast, soybeans showed almost no iodine reduction activity and yet maintained low root and shoot concentrations (Kato et al., 2013). Hydroponically grown wheat, lettuce, and tomato all showed uptake of iodine and incorporation into proteins in shoots and leaves, while maize in the same experiment showed no iodinated proteins in its organs (Kiferle et al., 2021). This non-uniform response is observed in different varieties of the same species of plant that show marked differences in iodine tolerance. It was demonstrated that iodine sensitive rice variety *"Nipponbare"* had increased root iodine reduction activity and reduced shoot concentration, but increased foliar toxicity symptoms when

compared to the less sensitive variety "*Gohyakumangoku*" (Kato et al., 2013)*.* The diversity in passive and active uptake of different species in the same iodine environment indicates that some varieties and some species of plants actively take in iodine, but active uptake is not dose responsive. This hints at the idea that plants can utilize iodine, but at an incredibly low rate that, once exceeded, turns into an element needing exclusion or active removal via volatilization.

Rice and barley appear to deal with high iodine concentrations in an active manner, whereas soybean appears to utilize an exclusion strategy (Kato et al., 2013). Kato et al. (2013) concluded that species' differences in reduction activity or decreased shoot concentration do not necessarily correlate with increased tolerance. Only one other soybean study has been carried out with exogenous iodine with the focus of that study being to understand stress response rather than iodine uptake. This study found that all iodine treatments caused a significant increase in SOD (~4x rise in mol./mg protein/min) while heat stress alone did not raise SOD (Gupta et al., 2016). Stress response will be discussed in detail later, but these findings suggest that patterns of iodine uptake, utilization, and detoxification vary among species. They also indicate that the proposed benefit of iodine application for stress mitigation could be species-dependent or as a byproduct of a stress response to iodine rather than a direct benefit from the element itself.

1.3.2 Leaves/Shoots

Iodine is deposited onto terrestrial environments in both wet and dry forms. The primary form of iodine in rain and snow was found to be organically associated, accounting for 56% of total deposited iodine (Gilfedder et al., 2007). In the air, gaseous iodine is most often in the form of I_2, but due to the highly reactive nature of iodine, the composition and species of iodine vary spatially and temporally around the globe (Baccarini et al., 2020; Koenig et al., 2020; Puentedura et al., 2012). Iodine is readily absorbed by stomata and cuticular waxes during deposition- a trait

that has been studied to better understand the impact of nuclear fallout and the release of radionuclides (Shaw et al., 2007; Tschiersch et al., 2009; Yanai et al., 2022).

The sorption of iodine is most often highly localized, though in some cases, such as tomatoes, foliar iodine has been found to move between organs (Landini et al., 2011). Deposition from radioactively labelled iodine vapor in a controlled environment was found to readily be absorbed by leafy vegetables such as endive, curly kale, spinach, and leaf lettuce (Tschiersch et al., 2009). The rates of sorption differed by species, with spinach having 3x the iodine concentration than leaf lettuce and curly kale, 4x more than endive, 9x more than head lettuce and over 30x more than white cabbage. The authors suggest this marked difference in absorption was due to a mix of leaf area index, stomatal conductance, and plant morphology, as each factor independently did not explain the differences. Absorbed iodine was only decreased by 10% when washed with deionized water, indicating that it was effectively bound to the cuticular waxes on the leaf surface or was rapidly incorporated within the leaves. Differentiating between adsorbed and absorbed is important to understand the possible function of iodine though no studies to our knowledge have addressed this issue.

In biofortification studies where foliar applied iodine was directly applied to nectarines, there was no significant decrease in iodine concentration when the peels were removed, indicating that the iodine application was not bound to the exterior of the fruit but was transported into the interior (Humphrey et al., 2019). However, in apples, the exact opposite was found, with the majority of the iodine being adhered to or within the peel and not in the flesh (Budke et al., 2021). In globally dispersed field trials looking at biofortification potential in wheat, rice, and maize, iodine was of higher concentration in the bran and embryo than in the endosperm (Cakmak et al., 2017). These trials were all sprayed 2-3x with KIO_3, but there was no

indication in the paper about the timing of these sprays and if the KIO$_3$ came in direct contact with the fully formed edible portion or if it was transported, either actively or passively, to the developing grain. Spinach was found to absorb and store foliar iodine treatments within the sprayed leaves with <2% of applied iodine moving to younger leaves after 9 days (Humphrey et al., 2019). In most cases, foliar-applied iodine or environmentally deposited iodine appears to become fixed to the external surfaces of plants-though it is not known if it is simply sorbed or actively iodinated by an unknown mechanism.

Shaw et al. (2007) found that iodine applied as KI was strongly sorbed into the cuticle of broad bean (*Vicia faba L.*) with a retention coefficient 5.5x higher than expected based on previous work in ryegrass and clover (Angeletti & Levi, 1977; Muller & Prohl, 1993; Shaw et al., 2007). The expected leaf sorption was based on simple anion exchange of I^0 from KI, but the authors theorized that the I^0 might need to be oxidized to IO$_3^-$, which has a stronger tendency to sorb to the leaf cuticles (Ashworth & Shaw, 2006). It's also important to note that broad bean (*Vicia faba L.*) has 85% of its stomatal density on the adaxial (upper) surface of the leaf, so high accumulation might be, once again, species-specific.

1.4 Mobility in plants

The mobility of iodine in plants is dependent on the plant organ where it enters, and specific plant species appear to behave differently. Once iodine has entered the plant through the roots, it moves upwards in xylem vessels (Blasco et al., 2008; Caffagni et al., 2012; Landini et al., 2011; Smoleń et al., 2014; Zhu et al., 2003). Iodine is also of low mobility in many species since it is often preferentially highest in the organ where it enters the plant (Gonzali et al., 2017). In tomatoes, iodine is slightly mobile in the phloem as leaf applications lead to increased

concentrations in fruit (Landini et al., 2011). It was also found that lettuce leaves treated with iodine had higher concentrations of iodine in the roots than the control, reaffirming that some phloem mobility is possible (Smoleń et al., 2014). However, iodine mobility seems to be highly influenced by the growing system. Medrano-Macias, (2016) found that growing plants in a hydroponic environment with high iodine availability led to high uptake, which could be responsible for the differences seen between studies.

It has also been demonstrated that the rate of methyl iodide production varies by tissue and species (Itoh et al., 2009). Unfortunately, many studies have not considered loss of iodine from the tissue. The assumption of iodine accumulation used by all studies to date fails to account for known methyl iodide production that varies widely by plant organ, species, and growth environment.

1.5 Effects of iodine on plants

The main challenge in ascertaining the effects of iodine on plant growth and development is the wide diversity of responses that differ between species and growth systems. Species, timing, and the method and form of iodine applied all seem to have a significant impact on the outcomes. The traditional nutrient essentiality paradigm that restricts plant nutrients to those that can be demonstrated to be essential is reductionist and lacks the nuance needed. Brown et al, (2022) proposed an expansion of the definition to include both essential elements and beneficial nutrients (Brown et al., 2022). Research to date on exogenous iodine application to plants appears to fit far more neatly in this expanded definition due to its unique elemental properties and diverse experimental outcomes.

1.5.1 Plant physiology and phenology

In studies of the role and function of iodine, the choice of species of plant being studied and growth system is highly relevant to the results observed. Iodine is also strongly bound to organic matter in the soil, and as such, the availability of applications in hydroponic systems differs greatly from that in soil/field culture. There are a few comprehensive reviews of plant response to iodine applications with the best summarization being offered by Medrano-Macias et al. (2016). Studies thus far have noted a diversity of responses but without any explanation for the cause of these differences. For example, Borst Pauwels (1961) found a 10-18% increase in dry matter yield of tomatoes treated with various rates and forms of iodine, but could not differentiate whether this was a "stimulatory" effect or evidence of "exercise{ing} a specific function". Borst Pauwels (1961) also noted that response varied greatly between species, with different rates and forms, between years in their experiments, and found that their control plants were "relatively high in iodine content". This experiment foreshadows many of the challenges and observations made by other researchers between 1961 and the present day.

1.5.2 Stimulatory effect

Responses measured in plant metrics, such as dry weight, are hard to disentangle without an understanding of the function or mechanism of iodine action. Most authors to date have applied iodine and then measured differential response to elucidate mechanisms or to biofortify crops for human consumption. The application of exogenous iodine might also have cascading impacts that complicate interpretation. For example, Hageman et al., (1942) found an increase in fruit number but a significant decrease in fruit size in tomato plants treated with iodine (overall significant decrease in total dry weight). Kiferle et al. (2022) in contrast found an increase in tomato fruit yield dry weight as well as an increase in fruit number with plants treated with KIO_3,

indicating that small fruit size was not a consistent consequence of iodine application. The iodine literature is full of these conflicting results that need to be individually explained in the context of each experiment.

1.5.2.A Biomass and yield

Leafy vegetables generally have an increase in biomass with the addition of low rates of iodine in soilless culture (Blasco et al., 2013; Weng et al., 2008; Zhu et al., 2003). Tomatoes are noted to have a variety of responses that are often not reproducible with variations in growing method and iodine form and rate. Halka et al. (2019) found no difference in yield or mass of tomato plants grown in soilless culture (peat/vermiculite) when treated with KIO_3 or KI applied at the beginning of florescence. Kiferle et al. (2022) on the other hand, found a marked increase in plant height and shoot fresh weight of tomato plants grown with 10 μM of KIO_3 added in a hydroponic system following transplant. A possible explanation was that all iodine was bound to the peat in the Halka study. Caffagni et al, (2011) found in a similar experimental design, a 50-70% decrease in tomato biomass and fruit biomass in all treatments with both KIO_3 and KI ranging from 2.34-23.36 μM. Smolen et al. (2015) found no increase or decrease in fruit yield with the addition of 7.88 μM of KIO_3 or KI in a hydroponic system starting at the beginning of florescence. In a soilless culture (sand) experiment, Hageman et al. (1942) found a 24 μM rate of KI had no impact on plant dry weight but significantly reduced average fruit weight from 5.8 to 4.9 grams. In wheat, Caffagni et al (2011) found a 40-55% decrease in biomass in greenhouse grown wheat and Mao et al. (2014) found an 8% reduction in grain yield of field grown wheat treated with iodine. On the other hand, in a global field study, Cakmak et al. (2017) found no reduction in grain yield up to a rate of 5 mg I kg $^{-1}$ with both KI and KIO_3, but did find a

reduction in yield above 5 mg I kg $^{-1}$ with KI, but not KIO$_3$. Each study taken alone often presents interesting data that is rarely corroborated between studies, growing systems, plant species, or plant variety. For this reason, a possible indirect effect of exogenous iodine application has been proposed as an explanation for disparate and non-replicable results.

Lehr et al. (1958) theorized that iodine enhanced the rate of nitrification in the soil after observing high nitrate accumulation in plants with burnt leaves. This would imply that the increase in dry matter seen in some iodine studies was an indirect effect. The addition of KIO$_3$ or KI to hydroponically grown spinach significantly increased the NO$_3$ percentage in the leaf sample dry weight but not the dry matter yield (Smoleń & Sady, 2012). Blasco et al. (2010) found that IO$_3^-$ causes stimulation of nitrate reductase in plant roots, but in lettuce, this did not lead to a higher accumulation of NO$_3^-$ in leaf tissue but only to higher biomass. In hydroponic strawberry, Li et al. (2017) found that IO$_3^-$ led to a significant increase in above root biomass but also a 5%-15% increase in fruit nitrate, a negative quality metric for strawberry.

1.5.2.B Stress response

Summarizing physiological responses to iodine from observational studies is difficult due to the diversity of responses. More recently, gene expression and plant metabolite analysis have been used to better understand the effects an iodine application elicits in a plant. To determine if iodine can help mitigate stress response from NaCl, Kiferle et al., (2022) monitored the relative expression of genes commonly associated with salt stress- particularly in ABA-related responses and in oxidation management. In the ABA response category, *AREEB1, lptg2, LEA, and Hat9* were monitored. In oxidation mitigation, *CAT, SOD, cAPX, and GR* were measured. In this study, no measurement of plant stress associated with the NaCl treatments was measured, and the

stress was inferred. Measurements were made at 72hrs and also at 8 weeks after treatments were applied. It was found that, generally, iodine reduced the expression of stress-related genes at 72 hours after treatment with NaCl. However, at 8 weeks after treatment, this trend was less pronounced. Additionally, at 8 weeks, the plants receiving iodine but not receiving NaCl treatments showed a higher expression of stress-related genes, indicating that iodine alone could be stress-inducing, even at 0.2 μM and 10 μM rates. The relative expression of SOD was 2.5X that of the untreated control in the 10 μM KIO$_3$ treatment (Kiferle et al., 2022). This could be assumed given the well-documented toxicity levels experienced by many researchers, but at 10 μM, this is far below what is considered a phytotoxic dose. This rise in SOD in response to IO$_3^-$ was also seen in hydroponic lettuce not under a stress treatment but only at the higher rates of 40 μM and 80 μM (Blasco et al., 2011). Iodine concentration "tolerance" variation has been established by many authors including (Borst Pauwels, 1961), so variable response to iodine alone is expected.

In other studies looking at iodine impact on antioxidant production under stress, control treatments were entirely untreated and made no measure of iodine impacts without the stressors (Gupta et al., 2015; Leyva et al., 2011). Without this important check, it's impossible to disentangle if iodine also impacted antioxidants like was demonstrated in Kiferle et al (2022). Using NaCl as a stressor, Leyva et al. (2011) concluded that the addition of iodine alleviated some of the stress due to an overall decrease in the loss of dry weight biomass and boosted the activity of antioxidant enzymes such as *SOD, APX, DHAR* and *GR* when compared to the treatments only receiving NaCl. This impact on biomass was then replicated and confirmed by Blasco et al. (2013). Using cadmium as a stressor, Gupta et al, (2015) did not measure plant biomass or plant yield, but inferred that an increase in SOD in cadmium and iodine-treated plants

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and a decrease in hydrogen peroxide and lipid peroxidation supported their hypothesis that iodine could alleviate cadmium stress.

These collective results indicate that iodine does alter the stress response of the species studied, but alone, iodine can also cause a stress response, even at low doses in some species. This particular mechanism could be interpreted as stress priming or biostimulantory rather than as a beneficial element. More work would need to be done to show this effect since a criterion of stress priming is postulated to be that the initial stimulus is stored as a 'memory' until a repeated exposure induces an alternate response (Martinez-Medina et al., 2016). The timing of the stressor, as well as the timing of iodine application, would also be important to better understand as most studies apply both stressor and iodine at the same time, making a priming effect delusive.

1.5.2.C Floral attributes

Flower formation, bloom timing, and successful fruit development are all vital in many crops where the fruit or seed is the product of interest. Understanding the mechanisms of floral emergence and resulting set success opens the possibility of agronomic intervention for more productivity. In Borst Pauwels (1961), the author noted that an iodine dose of 0.1 ppm advanced the earing of wheat and oats "by a few days"**.** (Lehr et al., 1958) noted that iodine-treated plants flowered earlier and ascribed it to the "more rapid growth of the experimental plants" seen in that study. These early studies suggested a potential impact of iodine on flowering timing, but since then, very few authors have worked to better understand if iodine impacts bloom. Some authors apply iodine at bloom time, so any potential impacts on flowering timing would not be seen (Ex: Caffagni et al., 2012). While others apply iodine significantly before flowering and many fail to gather floral attributes such as first inflorescence, number of inflorescences, set percentage, etc.,

as they were not an expressed goal of the study despite fruit number (yield) often being reported (Ex: Hageman et al., 1942; Landini et al., 2011).

Kiferle et al. (2021) found a hastening of bloom in arabidopsis plants treated with iodine, as well as an increase in the number of siliques/plant. This led to a remarkable 35-50% increase in seed production for KIO_3 treated plants. The authors theorized that because plant biomass production increased in iodine-treated plants in addition to an earlier flowering time, iodine had a "flowering-promoting" effect and did not induce a stress response that led to earlier flowering as a stress evasion mechanism. In *Pelargonium hortorum L.,* both foliar, soil and combined applications of KI increased the number and diameter of inflorescences (EL-Sayed, 2019). This finding is counter to most studies where the use of KI is often associated with negative responses from plants. In addition to positive flowering impacts, this study also found increases in total chlorophyll, leaf Nitrogen %, leaf phosphorus %, and an increase in plant dry weight. The role of iodine in flowering dynamics is presently unknown, and the form of iodine, the species of plant being studied, and the timing of application are likely to be critical to elucidate the nuances of iodine's potential impact on flowering. For example, in hydroponic lettuce, Smolen et al. (2023) studied the relative expression of the *Adagio 3* gene, which is known for its role in flowering initiation. They found that the addition of KIO_3 did not induce the *Adagio 3* gene, and iodine-treated lettuce did not show any differences in flowering dynamics counter to expectations. Particularly since the same author found that the organic iodine compound 5-ISA caused a differential stimulation of lettuce inflorescences (though possibly unpublished) (Smoleń et al., 2019).

1.5.3 Exercising a specific function

Traditionally, the idea of a nutrient serving a 'specific function' would have been constrained to the idea of nutrient essentiality to complete a plant's lifecycle. However, as discussed above, many different mineral elements and nutrients are often present in plants and influence their growth and behavior despite not being labeled essential.

Plants actively transport iodine from the soil into their roots, but this transport also transitions to majority passive at a slightly increased soil concentration (Humphrey et al., 2019; Weng et al., 2008). This and many studies showing decreased benefits as rates of iodine increase are all evidence that any benefit from iodine is accomplished with incredibly low rates. This is typically referred to as a biphasic dose response or hormesis where a toxic substance can be beneficial at low doses (Jalal et al., 2021; Mattson, 2008). Incredibly small rates themselves do not rule out the functional effect of iodine, as the element molybdenum is essential for many redox reactions in plants but is needed at an incredibly low rate when compared to other micronutrients (Kaiser et al., 2005). There is also a possibility that the iodine response in plants is multifaceted, with low doses serving in an antioxidant or biostimulant capacity and higher doses acting as a stress primer. A spectrum of responses based on rate might explain some of the variability seen across the literature- including in experiments with similar designs.

1.5.3.A Inorganic antioxidant

If iodine were to function as an inorganic antioxidant as demonstrated in kelp, would this be considered as serving a specific function or simply a strategy to deal with stress? In the presence of species-specific low concentrations of iodine, plants could utilize iodine's antioxidant capacity to reduce ROS at a limited energetic cost. However, in the absence of iodine, alternative methods for dealing with ROS would be utilized. This might explain why

Kiferle et al. (2022) found SOD relative expression in high NaCl environments 72 hours after treatment was higher in control treatments when compared to those treated with iodine at low rates (0.2 μM and 10 μM) (Kiferle et al., 2022). Iodine could have reduced the burden of antioxidant requirement by the plant. In the same study at 8 weeks after treatment, the expression of SOD was no different than the control indicating that under continuous salinity stress, iodine alone did not mitigate all ROS. In Leyva et al. (2011), the relative expression of SOD after 21 days following treatment was tested and confirmed the Kiferle et al. (2022) findings even when the rates of IO_3^- were far higher (20 μM, 40 μM, and 80 μM).

1.6 Iodine in the human diet

Iodine is an essential element for humans because of its role in the thyroid. Thyroid function is regulated, or even throttled, by thyroid-stimulating hormone (TSH), and the presence of TSH causes increased mobilization of iodine to the thyroid. Iodine is an important component of two hormones in the thyroid: thyroxine and triiodothyronine. These hormones are critical for the creation of proteins and enzyme activity and for regulating metabolism. Additionally, during fetus development and in early childhood, iodine is especially important for skeletal and nervous system development (National Institutes of Health, 2024) The need for a constant but small supply of iodine means humans must consume 100+mcg for infants and adults and >220mcg for pregnant and lactating women each day to be sufficient (The Institute of Medicine, Food, and Nutrition Board, 2001).

Humans receive all their dietary iodine from consumed food, but the iodine content of this food is typically, and naturally, extremely low. Seaweed and seafood such as fish from the ocean have the highest levels of naturally occurring iodine as these organisms typically concentrate iodine from the seawater (Zimmermann, 2009). Iodine is present in some bread

dough conditioners and cleaning agents used in the food industry, and some food products have higher iodine content because of this (National Institutes of Health, 2024). Animal feed is sometimes supplemented with seaweed or iodine supplements, and iodine can also be used as a cleaning agent in the dairy process. The inconsistency of use across producers means the concentration in food products is extremely variable; one study of US food products found dairy products had between 28% - 231% of daily iodine requirement (Pennington & Young, 1990). Soil iodine is often extremely low in areas not receiving direct deposition from the ocean, and plant uptake and allocation into consumed parts reflect this (Fuge & Johnson, 2015).

A lifelong daily requirement paired with low availability in most diets gives rise to human deficiency and the resultant disorders termed iodine deficiency disorders (IDD).

1.7 Introduction to human iodine deficiency disorders (IDD)

As explained in detail above, iodine is a human essential element because of its role in the synthesis of hormones in the thyroid. Iodine deficiency disorders (IDD) refer to a collection of human diseases and disorders caused by a deficiency of iodine intake. There can be functional and developmental abnormalities caused by deficiency that can cause: "endemic goiter and cretinism, endemic mental retardation, decreased fertility rate, increased perinatal death, and infant mortality"(Delange, 1994). It has also been labeled as one of "the most common preventable causes of mental deficiency in the world today"(Delange, 1994).

A meta-analysis of 18 studies in 1994 found children with iodine deficiency averaged 13.5 points lower on IQ tests (Walker et al., 2007). In countries with low levels of iodine deficiency, children with urinary iodine higher than recommended had higher IQs than those with lower iodine, albeit not deficient. Indicating even a mild deficiency has outsized consequences on IQ (Santiago-Fernandez et al., 2004). However, with many human nutrition

studies, it is hard to draw a direct correlation between diet and outcomes. For instance, the authors of this second study noted that those children with higher IQ scores and high iodine intake also consumed dairy products in a higher amount. The dairy was likely a source of much of the iodine, but also an indicator of a more nutritious or sufficient diet, which are both likely to influence IQ. Despite these difficulties in ascribing a correlation for the benefits of iodine *beyond* sufficiency, there is a dearth of research showing the negative impacts of iodine deficiency.

1.7.1 Iodine deficiency – Global disparities

Iodine is a unique, essential dietary element because improvements in socio-economic status and "development" do not necessarily decrease levels of human deficiency (Biban & Lichiardopol, 2017). In 2007, 52% of people in Europe were iodine deficient, and though this number has decreased, there is still a high prevalence of iodine deficiency in Europe (Ittermann et al., 2020; World Health Organization, 2007b). This is due in large part to the natural variation of iodine concentration in soils that impacts the resulting iodine concentration in the food produced in those soils (Zimmermann, 2010). Iodine concentration in soils varies widely by region and is influenced by geomorphology, climate, propinquity to an ocean, and many other factors (Fuge & Johnson, 2015; Shetaya et al., 2012). The presence of iodine in soils and water is important for passive iodine sufficiency, but what people choose to eat is also incredibly important.

The iodine status of the people in a region is heavily influenced by the food culture, what foods are consumed most, and from what areas they are produced. For instance, in 2021, Korean school-aged children had an excessive median urinary iodine concentration (UIC) of 449 µg/L (The USA's median UIC was 190 µg/L) as a result of high seafood intake associated with their

food culture (The Iodine Global Network, 2021). The rise of vegan diets in many areas has also led to speculation of increased iodine deficiency since a vegan diet eliminates most of the foods with the highest natural iodine content (meat, dairy, and seafood). A study conducted in Germany found "in one-third of the vegans {studied}, iodine excretion was lower than the WHO threshold value (<20 µg/L) for severe iodine deficiency"(Weikert et al., 2020). A similar study in Norway found that the vegans (n=115) and vegetarians (n=55) studied were, on average, moderately deficient in iodine, and the Pescatarians (n=35) were mildly iodine deficient (Groufh-Jacobsen et al., 2020). In addition to food preference, the use and intake of iodized salt varies widely by population and for many different reasons, including governmental public health policy, availability, preference for non-iodized sea salt, and increased consumption of processed foods.

1.7.2 Fortification of salt

Public health officials discovered in the early 1900s that iodine deficiency was the cause of many disorders, such as goiter, which has plagued humans for all of time. However, 3600 years before, the Chinese recognized that seaweed and burnt sea sponges could be used to treat goiter (Rosenfeld, 2000). In the early 1900s, many regions in the US, including the Great Lakes, Appalachians, and Northwest, had a high incidence of goiter, and this area was referred to as the "Goiter Belt". It was estimated that between 26%-70% of children had "clinically apparent goiter"(Pearce, 2007). Collective and global understanding of iodine's importance led to a push by public health officials to encourage higher iodine intake. A French chemist, Boussingault, working in Bogota in 1825, had noted that Andean peoples were treating goiter with marine salt deposits and noted that peoples moving between regions were sometimes cured of their goiters

simply by residing in certain areas. He didn't quite make the jump to understand that it was the ingestion of the iodine-rich marine deposits, but it did give rise to the idea of salt as a source of iodine (Zimmermann, 2008). In the 1920s in Switzerland and then the United States shortly after, table salt was voluntarily fortified with iodine (Leung et al., 2012). This saw a drastic reduction in goiter and the elimination of the "Goiter Belt". Iodizing salt is a useful approach for targeting IDD as "It is a universal foodstuff, intake is seasonally consistent, costs are relatively small, and it is easily distributed"(Leung et al., 2012).

Since the initial successes of iodized salt in treating IDD, many countries around the world have legislation requiring salt iodization. In 2020, 124 countries require it, and as a result, it is estimated that 88% of the global population uses iodized salt (Zimmermann & Andersson, 2021).

1.7.3 Current trends

An enormous amount of effort has been exerted to try and decrease the incidence of iodine deficiency since iodine was discovered to be a human essential element and a common deficiency in the early 1900s. The World Health Organization adopted a resolution in 2007 to report on the status of iodine deficiency in the global population every 3 years (World Health Organization, 2007a). In the most recent World Health Assembly (75th) in 2022, it was reported that efforts to eliminate iodine deficiency had "stalled" and that in 2020, 21 countries had insufficient iodine intakes. This was up from 19 countries in 2017 and then the preceding downward trend of 25 in 2015, 32 in 2012, 47 in 2007, 54 in 2003, and 110 in 1993(World Health Organization, 2022). In 2006, 30% of people in 193 WHO member states still had urinary iodine concentrations below the recommended level (Zimmermann, 2013). Most notable was the re-emergence of deficiency in countries previously thought to have sufficient iodine

consumption, such as Finland, Germany, Norway, and the Russian Federation (The Iodine Global Network, 2021). In the United States, median urinary iodine concentration decreased more than 50% from 1970 to 1990, as found in the National Health and Nutrition Examination Survey (NHANES)(Niwattisaiwong et al., 2017). In this same period, the percentage of women of childbearing age with moderate iodine deficiency increased from 4% to 15%. It is important to note that during this time, and continuing today, the overall population is still, on average, iodine sufficient. At-risk populations, such as women of reproductive age, are of most concern because their iodine requirement is higher, and the outcomes of IDD during child development are most problematic. This emerging trend of increased iodine deficiency in nations previously thought "cured" has brought increased attention to the issue (Hatch-McChesney & Lieberman, 2022).

The decline in iodine status in areas previously sufficient has been theorized about, and many, mostly cultural, reasons have been highlighted. Of most agreed upon are the transition of cleaning products in the dairy industry, use of non-iodized salt by the processed food industry and coincident increase in consumption of processed foods, and the decrease in iodized salt consumption either as decreased overall salt consumption or transition to alternate salt sources such as sea salt (Hatch-McChesney & Lieberman, 2022; Niwattisaiwong et al., 2017). The increased consumption of processed foods without iodine fortification has led to the call by some to begin requiring the food industry to fortify these foods with iodine (Ohlhorst et al., 2012). It is estimated that currently, 90% of salt consumed in industrialized economies comes from processed foods (Zimmermann, 2013). The initial success seen with salt fortification has seemed to wane as cultural food habits change and fortification efforts have not adapted to remain in step. Alternative methods need to be explored to ensure populations receive adequate iodine, and the biofortification of plants is an alternative approach.

1.8 Biofortification efforts

The fortification of food is done with many types of food products, but in the case of unprocessed food sources such as vegetables, biofortification is used to accumulate nutrients during the plant's life cycle. This differs from fortification where the desired nutrients are added to the processed food product before consumption. Biofortification can be seen as an attempt to try and grow the most nutritionally dense food products, whereas fortification is a means to make up for nutrient deficiency or go beyond the natural capacity to supply a nutrient (Yadav et al., 2020). In the case of plants, biofortification can be accomplished through a variety of methods. Supplemental biofortification would be the introduction of a nutrient beneficial for human health during the plant's natural lifecycle to try and increase the concentration in the consumed plant part. Biofortification can also be accomplished through plant breeding, traditional or transgenic, to select varieties that naturally accumulate higher concentrations of human beneficial elements in their consumed parts. In both cases, our bio/fortification interventions are entirely anthropocentric.

Biofortification has advantages over fortification, depending on the method used. In the case of variety development, natural concentrations of beneficial elements can be increased with minimal capital investment or buy-in from governments/producers (Yadav et al., 2020). It should be noted that seen as an advantage in one light; it can be an Achilles heel in another if implementation turns off those likely to benefit from the biofortification. Such was the case in the Gates Foundation funded "Golden Rice" project seeking to increase Vitamin A content in rice through the genetic modification and addition of two genes from other species' genomes (Paine et al., 2005; Ye et al., 2000). This has sparked rigorous debate centering with the biotechnical camp highlighting a single, one-time solution and the socio-systemic camp arguing

for a more systemic and contextual understanding of Vitamin A deficiency (Kettenburg et al., 2018). In the case of further biofortification efforts, this public debate has highly influenced many current methods and efforts for biofortification; including in the case of iodine.

Unlike in other agricultural crop species where plant breeders can select or genetically modify them to sequester higher nutrient contents, the low natural availability of iodine in the landscape (as discussed above) makes this approach with iodine less practical. If the iodine is not present in the soil or the water that the plant is growing in, having strong genetics for accumulation does not influence the final iodine content. Instead, plant scientists and researchers have demonstrated that supplying iodine externally to many agricultural species has resulted in anthropocentrically meaningful concentrations in consumed plant parts. Pea, pumpkin, buckwheat, wheat, sweet basil, spinach, alfalfa, basil, lettuce, pakchoi, kohlrabi, pepper, radish, carrot, tomato, apple, and strawberries have all been shown to be successfully biofortified with iodine addition (Zhang et al., 2023). These successes seem to appear as a viable solution to target IDDs, but disparate implementation potential and regulatory challenges make this unlikely to be implemented.

Biofortification efforts using this method are reliant on individual producers to carry out biofortification with a spray or fertilizer application containing iodine in addition to their normal practices. Without market incentives or government legislation, there is likely to be inconsistent implementation relying solely on farmer altruism. The market incentive for iodine content is unlikely to arise as It has already been demonstrated that there is wide diversity in the nutritional density of agricultural products and that the farming practices used to grow those products are what influence that density (Montgomery et al., 2022; Montgomery & Biklé, 2021). Despite this variability, consumers are unable to find out the nutrient density of the crops they purchase, and

farmers do not directly measure this component of their crops before selling them. Some consumers have begun to demand higher quality produce that aligns with their social and environmental views and introduces nuance into the view of crops. For example, purchasing a certified Organic tomato over one produced with synthetic pesticides and fertilizers? However, there is usually an assumption that one is healthier and more nutritionally dense than the other, and that may or may not be confirmed with lab analysis. If iodine biofortification is to be implemented, consumer preference would need to support the addition of iodine during the growing stage of the plants, and this is extremely unlikely to happen.

Widespread use of iodine-enriched fertilizers is unlikely due to regulatory challenges, the risk of iodine loss to the atmosphere contributing to ozone depletion, and potential toxicity to plants at higher concentrations. While passive iodine fortification through fertilizers has minor potential, its effectiveness and environmental impact remain largely unstudied, making it a low-priority strategy for addressing iodine deficiency disorders.

1.9 Summary

In reading literature reviews on the impact of iodine in plants, most authors conclude that iodine is beneficial to plants, might be an essential element, and that more information is needed to be certain (Fuge & Johnson, 2015; Medrano-Macías et al., 2016; Nascimento et al., 2022; Riyazuddin et al., 2023; Zhang et al., 2023). This despite over 100 years of study with incredibly inconsistent responses across species, cultivation practices, application timings, rates, and iodine form, as discussed in this review. Part of the difficulty in defining iodine's role comes from the limited categories that are presented to sort elements and nutrients into.

Given the options of an essential element or a biostimulant, there seems to be a collective emphasis on working to prove that iodine is an essential element. Work by many authors

proceeds down a path seeking to isolate its essentiality rather than acknowledging results that do not support this hypothesis. Disparate responses temporally, between species- and even varieties, as well as known volatilization pathways, point to a plant relationship with iodine different than any known essential element (Kato et al., 2013; Medrano-Macías et al., 2016; Saini et al., 1995). A variety of studies have made it clear that there can be a beneficial response to iodine applied at the right time and in the right form and concentration. This aligns more with the definition of a biostimulant or beneficial nutrient rather than as an essential micronutrient.

Chapter 2: The impact of iodine on plant dynamics in almond, pistachio and avocado

2.1 Introduction

Exogenously applied iodine has been studied in a diversity of crops, but the majority have been annuals grown in a controlled environment setting. Documented benefits for stress mitigation, as well as having a potential flowering impact, highlight the potential suitability for iodine's use in perennial crops with complex fruit set dynamics that are often exposed to stress during their most vulnerable fruit setting period. In California, almond, avocado, and pistachio are all economically important crops with large acreage dedicated to their production. In each of these crops, the success of flowering and subsequent seed/fruit set is the primary determinant of yield and economic viability. Adverse conditions due to water, nutrient, salt, and carbohydrate depletion can lead to stress during the period of bud formation. Fruit yield can be further reduced due to adverse temperature, wind, water and/or heat stress during the spring flowering period (February in almond, March/April in avocado and pistachio). In almond, year-to-year yield

variations of 25-45% are normal (approx. +/- 225kg). In pistachio and avocado, yield

fluctuations may be substantially greater at 40-75%. Fluctuations in yield and failure to reach full

yield potential are due to a combination of physiological characteristics and environmental

stresses, and this fluctuation frequently represents a substantial portion of grower profitability

(Muhammad et al., 2015, 2018; Saa et al., 2017).

In almond, pistachio, and avocado, the periods of flowering, fruit set, and subsequent-

year bud formation are logically critical to tree productivity as without buds, there will be no

blooms, and without blooms, there will be no fruit. Unfavorable conditions at flowering can

reduce the number of flowers that set fruit and the number of fruitlets that are retained by the

tree, while unfavorable conditions such as water stress, drought stress, and within-tree

competition for carbon or nutrients during bud formation can reduce the number of viable buds

for the subsequent years fruit production (Valdebenito et al., 2017). Due to the evidence

presented in Chapter 1, iodine likely functions as a biostimulant rather than an essential element.

A targeted intervention of a biostimulant to mitigate stress during these crucial points mean

plants would likely have improved yields in that season and others. However, mitigating stress

by relying on an element functioning as a biostimulant with an unknown mode of action means

applications would need to be made at theoretically ideal times and by correct application

methods. Applying iodine when it is theoretically ideal for perceived future stress is a

challenging undertaking that will be explored in this thesis. Without an underlying understanding

of iodine's mechanism, an approach covering a diversity of timings and delivery methods is

needed. In almonds, iodine would theoretically need to be introduced at the pink-bud stage of

floral development to have a potentially direct antioxidant effect during bloom and be present at

mid-summer bud formation for stress mitigation to free up carbohydrate reserves. In pistachio,

iodine would likely need to be present during mid-summer bud formation for multiple years to understand if decreased stress during this time leads to a decrease in year-to-year alternate bearing. In avocado, the introduction and availability of iodine in late Summer/early Fall during bud formation would also likely target a period critical for subsequent yield by mitigating potential stressors.

2.1.1 Almond bloom

Bloom in almonds is important as the resulting yield is entirely dependent on the success of that bloom. An unsuccessful bloom or fruit set cannot be overcome through any agronomic practices thereafter. Each crop has unique bloom characteristics to be understood and potentially managed, but many aspects of bloom timing and set are not well understood. This is especially true in almonds due to the limited geographical cultivation range of this crop. Cultivar to cultivar, field to field, and season to season, differences in blooming date and success do not often follow a linear trend easily assigned to a single variable. Bloom is often influenced by several variables whose weight can often seem unpredictable. Temperature, for example, is very influential, but also highly variable across space and time. Warm single days, for example, can be followed by weeks of cold weather and be variable across microclimates. The study of bloom is further complicated by a changing global climate as long-term datasets meant to capture the range of variability are often presenting new trajectories in response to changing climate. For example, In work studying phenological data from cherry trees for 732 years, bloom on average was 10 days earlier from the 1950s till now when compared to the mean for all previous data (Vitasse et al., 2022). Using historical averages to inform current bloom projections are less valuable when new benchmarks are being established.

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The economic importance of almond production in California has led to an increase in effort to better understand and manage almond bloom, but for all the effort, there has been little research resulting in an implementable practice to effectively control or alter bloom. This research has however, increased our understanding of bloom-specific nutrient requirements, overwintering carbohydrate storage, and chill requirements, and how these influence flower count and quantity (Tombesi et al., 2016). However, this information is often descriptive of trends rather than resulting in a prescriptive or implementable practice for agronomic decision-making. Temperature is viewed as the single most influential factor on almond bloom, and as such, a number of models have been created (Egea et al., 2003; Gaeta et al., 2018). These models are useful for a coarse understanding of bloom timing, but are not specific enough for individual tree treatment differentiation. The application of iodine as a bloom-time-altering intervention means that a more nuanced understanding of individual tree bloom is needed.

To study if a treatment influences the bloom of a single tree in an orchard, a different technique needs to be employed that is more localized. Counting individual blooms is moderately useful to better understand a tree's blooming progression, but time limitations and extreme canopy variability mean statistical census requirements are often too burdensome. Remotely sensed data is robust in capturing bloom characteristics in almond orchards, but has yet to be shown to aid in differentiating treatment effects during bloom (Chakraborty et al., 2023; Chen et al., 2019). To understand individual tree responses, a novel approach is required that utilizes multiple approaches used in tandem: a) high frequency and high resolution remotely sensed data paired with differential treatments to allow statistical comparison and b) localized and hyper-monitored treatments tracking flower progression.

2.1.2 Pistachio alternate bearing

Pistachio trees are known for their tolerance of challenging soil conditions and drought when compared to almond. Additionally, pistachio trees are wind pollinated, so they do not need the supplementation of insect pollinators as almond do. Pistachio has become an economically viable and important crop in California, especially in the Southern San Joaquin valley, where these characteristics are beneficial. However, while pistachios are more agronomically viable in these regions, the 'alternate bearing' phenology of these trees mean yields are less consistent than almonds.

The mechanisms or causes of alternate bearing have not been fully described, and hypotheses can be divided into two broad categories: varietal characteristics and external factors such as environment. Within internal factors, genetic variability, hormonal fluctuations, and resource availability can each play a part in year-to-year yield. External factors such as stressors, both abiotic and biotic, and triggering events have also been shown to influence tree bearing. Agronomic cultural practices such as variety selection, rootstock selection, and pruning have all been demonstrated to greatly reduce alternate bearing, but why they do so is still open to debate. Like many agricultural challenges, it is unlikely that a single factor is responsible for this result, and it is more likely due a combination of many different factors.

Research that has demonstrated iodine's ability to reduce stress make it a potential intervention to target both resource availability as well as hormonal signaling that could impact nut abscission. The application of iodine following bloom and continuing through the growing season could potentially be beneficial in mitigating the impacts of stressor as they occur.

2.1.3 Avocado bloom

Avocado trees have complex bloom dynamics when compared to most other agronomic crops that make their monitoring difficult. At the root of this complexity is a form of flowering behavior called "synchronous dichogamy"- when individual flowers alternate being male and female. This is further complicated as different varieties display different orders of male and female phase presentation- alternating which part of the flower opens at which time of the day (Alcaraz et al., 2013). It has also been estimated that mature avocado trees can have over a million flowers during peak bloom, and a grove of avocados of the same variety will likely not bloom synchronously (Arpaia et al., 2023). Monitoring if a treatment of iodine impacts the timing of flowers is extremely challenging, given the natural variation between trees. Remotely sensed imagery has been used in avocado for yield estimation as well as to identify phenological stages, but not for treatment differentiation (Bianchi, 2012; Robson et al., 2017). On the ground visual comparison systems have been established for floral stage and bloom intensity that have been demonstrated to work to differentiate between varieties (Alcaraz et al., 2013; Salazar-García et al., 1998). Although more coarse than high-resolution imagery, these two methods offer the potential to differentiate between treatments if the effect of iodine on bloom stage or intensity is pronounced.

2.1.4 Study intent

This study was conducted to understand the capacity for exogenously applied iodine to impact plant stress response when applied in field trials in almond, pistachio and avocado. Based on the literature reviewed, iodine has a potential to influence almond fruitset when applied as an inorganic antioxidant at the onset of bloom. This capacity is theoretically not unique to bloom, and this study also seeks to understand if iodine can influence stress response at other times

when applied in almond, pistachio and avocado. The methods selected to monitor iodine's potential effect reflect our current understanding of iodine's role as a potential antioxidant and are intended to monitor this response while also gathering baseline data about naturally occurring iodine concentrations.

2.2 Materials and Methods

2.2.1 Preliminary study on soil iodine concentrations in California

A literature review was carried out to source geolocated soil analyses that included iodine. Due to the analytical difficulty in quantifying iodine concentration and the relatively limited focus on iodine when compared to more agronomically important elements, only two studies were found. One by the United States Geological Survey (Hansford Shacklette & Boerngen, 1984b), and one by the University of California Agriculture and Natural Resources (Bradford et al., 1996). These data were aggregated with an unpublished dataset from Patrick Brown's lab at the University of California, Davis, of 11 California locations (Supplementary Data 2). In total, this analysis included 126 geolocated soil samples.

These soil samples were mapped using ArcGIS Pro, and an Empirical Bayesian Kriging analysis was performed to interpolate between the sample locations.

2.2.2 Field trial locations and characteristics

Trials were conducted in four different fields in California that were each owned and managed by different farmers and managers. Locations were selected based on the preliminary study of iodine content in California soils, showing that there are generally low soil iodine concentrations throughout the Central Valley agricultural region of California.

The majority of trials were laid out in a systematic block design to account for variations in irrigation efficiencies as distances from irrigation source increase. At trial locations, irrigation risers were in the center of the field, creating a gradient moving outwards. This variation in irrigation and fertigation over time creates linear gradients along orchard rows that need to be accounted for. Repeating blocks of trees that were spatially close allowed for a more accurate comparison of localized and historical conditions.

Almond: Westwind Ranch located near Woodland, CA
- Variety: Nonpareil and Monterrey
- Age: 13 years old
- 308 trees/ha
- 2022 Yield: Nonpareil 2000 kg/ha and Wood Colony 2185 kg/ha
- Micro sprinkler
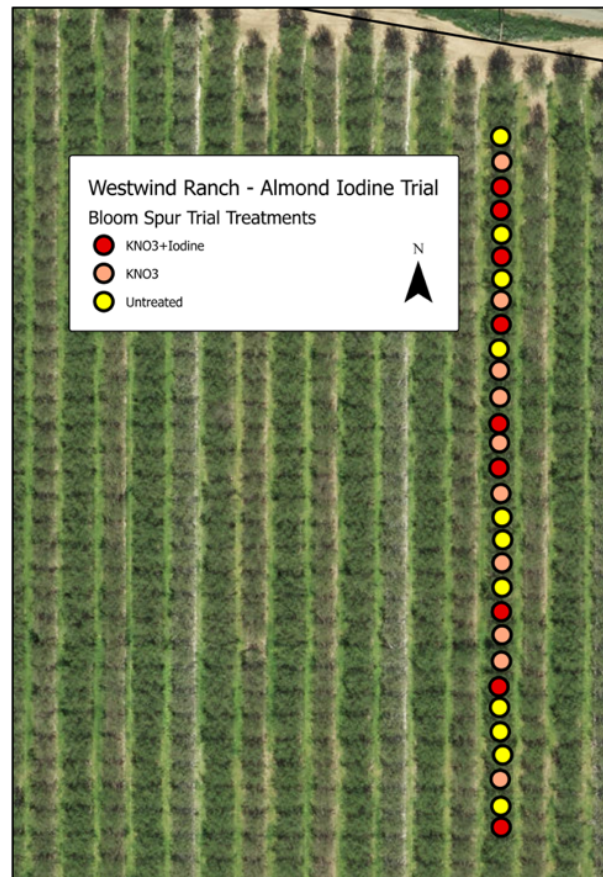- Bloom spur trial was laid out with a randomized design

Figure 1: Map of Westwind Ranch in Woodland, California, where the 2024 bloom trial was located. Each colored point is a tree in a single row of nonpareil almonds.

Almond: KG Ranch located near Madera, CA
- Variety: Nonpareil and Wood Colony
- Age: 9 years old
- 326 trees/ha
- 2022 Yield: Nonpareil 1100 kg/ha and Monterey 3740 kg/ha
- Double drip
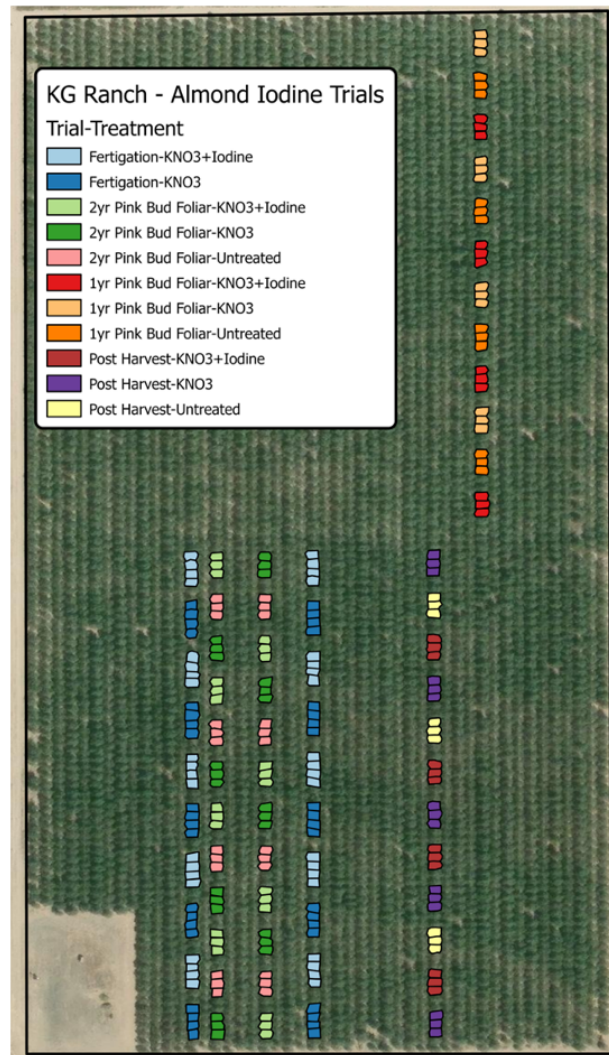- Trials were laid out in a systematic block design with two buffer trees between each grouping of trees.

Figure 2: Map of KG ranch rear Firebaugh, California, where a variety of Iodine trials from this study were located. Trials and treatments are differentiated on the map by different colors with a breakdown in the key.

Avocado: Cornell Ranch located near Temecula, CA
- Top worked with GEM variety in 2022
- Trials were laid out in a randomized complete block design with one buffer tree between treatments.

Pistachio: Maricopa Ranch/ATB Growers located near Firebaugh, CA

- Variety: Kerman
- Rootstock: ucb1
- Pollinators: 5% Male
- Spacing: 5.8m between rows and 5.2m between trees - 331 trees/ha
- Irrigation: Double drip
- Trials were laid out in a systematic block design with one buffer tree between each two-tree group.
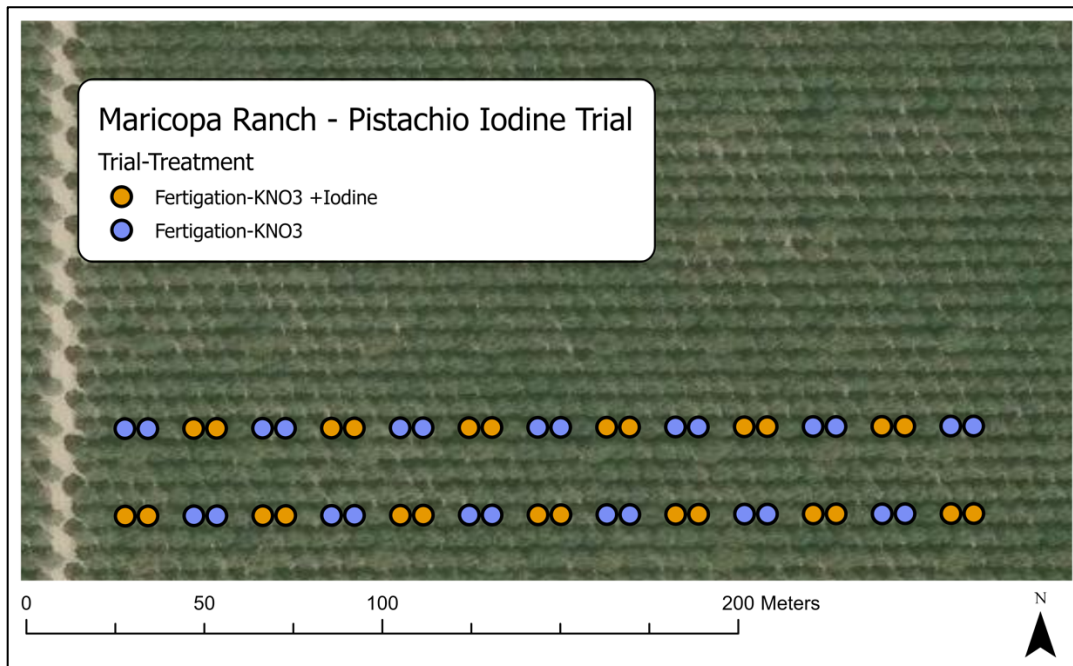

Figure 3: Map of Maricopa Ranch/ATB Growers pistachio orchard near Madera, California. The fertigation trial occurred in two rows of trees, and the treatments can be differentiated by the color-coded key on the map

2.2.3 Almond – KG Ranch - Hail event 2023

On March 12, 2023, a storm system moved over the Central Valley of California and a large amount of hail fell on the KG ranch in Madera (Supplementary Data 1). Peak bloom for the block containing the research block was around March 6th, when a bloom survey/count was conducted. This hailstorm systematically removed the majority of the flowers and emerging buds from the orchard, resulting in an extremely light fruit year. A flower survey had already been conducted for this orchard prior to the hail event and was followed up on to establish a set

percentage. Figure 4 shows the interpolated set percentage over the orchard based on these numbers that was around 3-8% in the study area. For reference, this orchard averaged 32% fruitset in 2022.
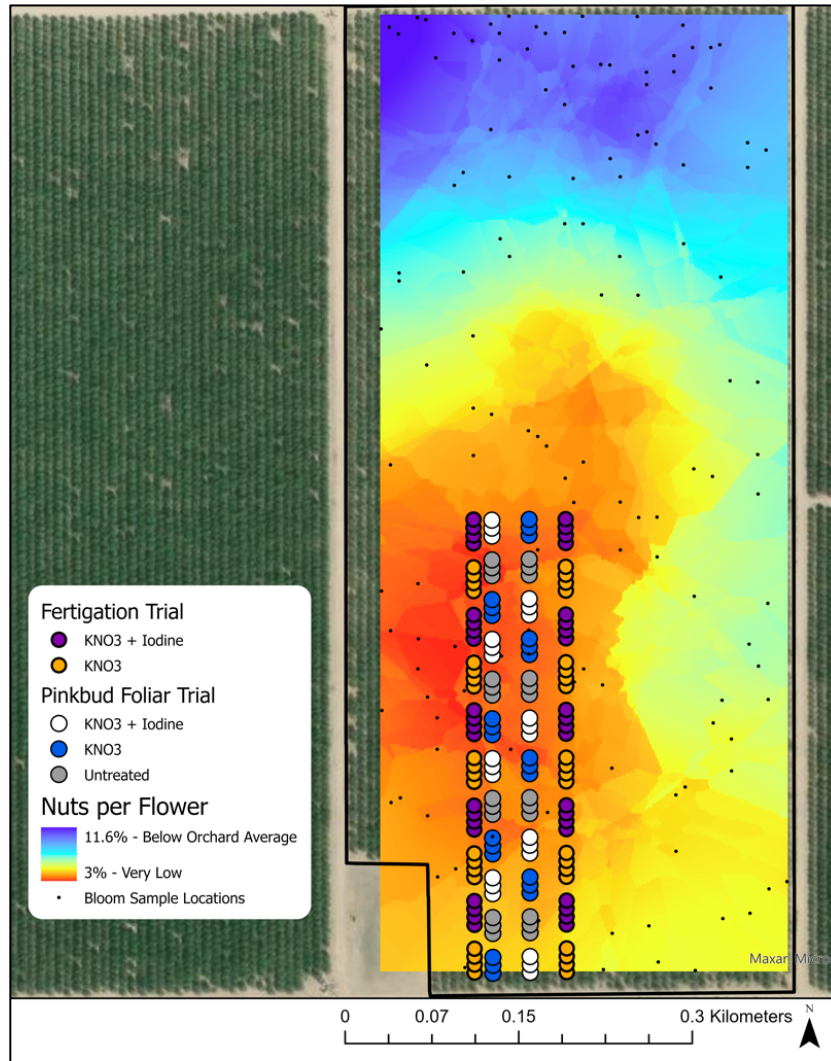

Figure 4: Map of hail damage to KG ranch block 26 near Firebaugh, California. Hail damage was assessed using a bloom sample where the number of nuts was divided by the number of flowers on a particular branch taken at the points indicated on the map. These data were used to construct an interpolated raster surface to visualize the variation in hail damage in the orchard. 2023 trial locations are displayed on the map and are located in the area most impacted by the storm.

2.2.4 Iodine source

A commercially available potassium nitrate fertilizer was used to apply all iodine treatments as part of a research agreement with the manufacturer of the product. This potassium nitrate (KNO_3) fertilizer contains iodine as potassium iodate. The commercially available products used are branded as 'Ultrasol' and 'UltrasolINE' manufactured by Sociedad Química y Minera (SQM). For this report, Ultrasol will be referred to as KNO_3, and UltrasolINE will be referred to as KNO_3+iodine.

Spectrophotometric analysis was done to determine the iodine content of the fertilizer applied. The analytical method employed was adapted from one used to determine potassium iodate in table salt by Silva et al. (1998). A description of the adapted method can be found in the supplementary documents section.

Ultrasol/UltrasolINE fertilizer is commercially available in 50 lb (USA) bags. Each bag has the potential to contain different amounts of iodine and for that iodine to be heterogeneously distributed in the fertilizer. For this reason, two separate bags were sampled, and two subsamples of 50g were taken from different locations in each bag that was then dissolved in Milli-Q water. Five aliquots were removed from each fertilizer solution and independently analyzed spectrophotometrically.

A large variation in iodine content was found between bags, between subsamples of fertilizer, as well as some variation in spectrophotometric analysis. All samples were below the expected 1000 mg/kg iodine content of UltrasolINE (Figure 5).
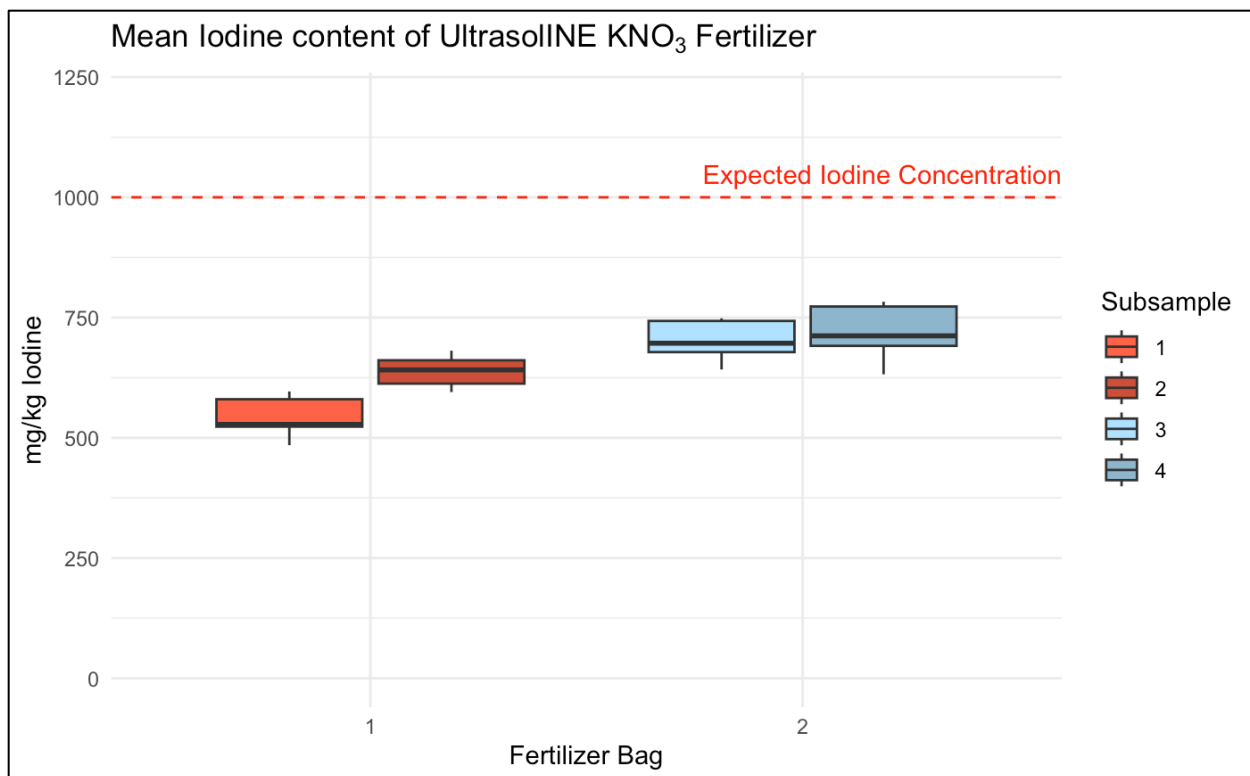
Figure 5: Spectrophotometric analysis of Iodine concentration in commercially available 'UltrasolINE' potassium nitrate fertilizer.

2.2.5 Field trials

2.2.5.A Almond – KG Ranch, Madera, CA – Whole tree – Two-year pink bud foliar

In 2023, this trial was established to monitor the impact of foliar-applied KNO_3+iodine when compared to KNO_3 without iodine. This trial was particularly focused on understanding if iodine applied at the pink-bud stage led to any change in floral emergence and, ultimately, fruit set when applied at the full tree level.

The trial was laid out starting with two buffer trees to the field edge and then groups of three trees separated by two buffer trees. This was repeated for the entirety of the irrigation block (60 trees) and duplicated in two rows. This led to a total of 72 trees in the experiment, with 24 in each specific treatment.

Applications of treatments were made using a Stihl backpack sprayer. Rates of carrier water were meant to model a grower standard of 935 l/ha used during bloom applications. A timer was used for each tree to ensure rates were identical and applications were made to both sides of the row. An untreated control was included, and a grower standard surfactant of Miller brand "Exit" was included at a rate of 0.3ml/L. Rates of KNO_3 and KNO_3+iodine fertilizers were 5.6 kg/ha. This amount of fertilizer should contain between 3.0 and 4.2 grams of iodine based on bag variability (Figure 5).

A pink bud application was made when the ranch manager assessed that the orchard aligned with their expectations of being in the pink bud stage. An application was made on February 15th in 2023, and on February 9th in 2024.

In 2023, four branches per tree were flagged at locations along their length with approximately 50 flowers. The exact number of flowers was then counted and recorded. The majority of the flowers were knocked off in the hail event described above, but a follow-up count of fruit was made. Trees were harvested in 2023 in groups of three corresponding to their treatment due to the incredibly low yields. The total weight was recorded and then cracked to collect quality data.

In 2024, high-frequency CERES multispectral imagery was collected during bloom, and individual tree yield data was collected using a UC Davis experimental yield monitoring device. This device utilizes a laser to measure the volume of nuts passing over a belt within the harvest machine.

2.2.5.B Almond – KG Ranch, Madera, CA – Whole tree – One-year pink bud foliar

This trial was identical to the two-year trial but was only a single row (60 trees), and trees only received treatments for the 2024 season. This row was also located on the North part of the almond block, whereas the two-year trial had both rows in the South part of that block.

2.2.5.C Almond – Westwind Ranch, Woodland, CA – Spur – Foliar

In 2024, a trial was established to monitor the impact of foliar-applied KNO_3+iodine when compared to KNO_3 without iodine. This trial was particularly focused on understanding if iodine applied at the pink bud stage led to any change in floral emergence and, ultimately, fruit set.

A row of trees was selected based on NDVI analysis of the previous year's growth that showed low variability between trees. Two trees were left as a buffer in the North along a roadway, and the trial had 30 trees in it. Prior to the green tip stage (2/10/24), six spurs per tree - three on the East and three on the West - were tagged and labelled. At visual pink-bud, treatments were applied by hand using sprayer bottles. The carrier volume of water was meant to model the grower standard of 935 l/ha. The rate of KNO_3 and KNO_3+iodine was 5.6 kg/ha, and this amount of fertilizer should contain between 3.0 and 4.2 grams of iodine based on bag variability (Figure 5). A surfactant, Miller brand "Exit" was added at a rate of 0.3ml/L. A third treatment of surfactant and water was included to ensure surfactant alone was not a factor.

Spurs were monitored daily from the date of application until the complete petal drop. Each day, the number of each category of developmental bud was counted. Stages used for this assessment were: Green tip, Pink Bud, Popcorn, Full Bloom, and Petal Fall.

A follow-up count of fruit was made in May, and all fruit was harvested on 8/5/24. Fruit was dried in an oven and then cracked out to collect quality data.

2.2.5.D Almond – KG Ranch, Madera, CA – Whole tree – Two-year fertigation

In 2023, a trial was established to understand if the addition of KNO_3 and KNO_3+iodine in drip irrigation at high frequency during the growing season had any effect. The trial was laid out starting with two buffer trees to the field edge and then groups of four trees separated by two buffer trees. This was repeated for the entirety of the irrigation block (60 trees) and duplicated in two rows. This led to a total of 80 trees in the experiment, with 40 in each specific treatment.

To apply the treatments in this design, a novel injector setup was designed and built (see Figure 6). Duplicate irrigation lines were laid out for each of the two treatments in the block, and emitters were added to create the blocks. Emitters were placed to align with the existing irrigation emitters to ensure treatments were made to existing wetted zones. These irrigation lines were then attached to the injection board that utilized Dosatrons to inject the treatments. These boards were controlled using a Spherag Atmos 2 cellularly-linked controller. Injection events were made weekly and occurred ¾ of the way through the irrigation event.

Fertilizer corresponding to each treatment was injected into the lines to a total of 220kg/ha of fertilizer that contained 110 and 165 total grams of iodine based on bag variability (Figure 5). At 326 trees/ha plant spacing in this orchard, this amounts to each tree received between 0.33 to 0.5 g of iodine throughout the treatment.

In 2023, four branches per tree were flagged at locations along their length with approximately 50 flowers. The exact number of flowers was then counted and recorded. The majority of the flowers were knocked off in the hail event described above, but a follow-up count of fruit was made.

Trees were harvested in 2023 in groups of three corresponding to their treatment due to the incredibly low yields. Total weight was recorded and then cracked-out to collect quality data.

In 2024, high-frequency CERES multispectral imagery was collected during bloom, and individual tree yield data was collected using a UC Davis experimental yield monitoring device.

Figure 6: Fertigation system designed to operate without grid power in the center of an irrigation run and be operated remotely. Two valves are actuated via a SIM-enabled controller that also broadcasts data from the two flow sensors. Each dosatron injector handles a treatment and feeds the corresponding lines to the treatment layout.

2.2.5.E Almond – KG Ranch, Madera, CA – Whole tree – Foliar post-harvest

In Fall 2023, a single post-harvest foliar application of KNO_3 and KNO_3+iodine was made to a row of nonpareil almond trees in Madera. This trial was carried out to explore if iodine

could improve overwintering bud status, leading to an improved flowering and set the following year.

The trial was laid out similarly to the other foliar trials, starting with two buffer trees at the field edge and then groups of three trees separated by two buffer trees. This was repeated for the entirety of the irrigation block (60 trees). This led to a total of 36 trees in the experiment, with 12 in each specific treatment.

Applications of treatments were made using a Stihl backpack sprayer. Rates of carrier water were meant to model "normal" field applications of 935 l/ha used during post-harvest applications. A timer was used for each tree to ensure rates were identical and applications were made to both sides of the row. A grower standard surfactant of Miller Brand "Exit" was included at a rate of 0.3ml/L. Rates of KNO_3 and KNO_3+iodine fertilizers were 5.6 kg/ha. This amount of fertilizer should contain between 3.0 and 4.2 grams of iodine based on bag variability (Figure 5).

2.2.5.F Pistachio – Firebaugh, CA – Whole tree – Fertigation

In 2023, a trial was established in a pistachio block to understand if the addition of KNO_3 and KNO_3+iodine in drip irrigation at high frequency during the growing season had any effect- with special emphasis on alternate bearing. The trial was laid out starting with two buffer trees to the field edge and then groups of two trees separated by a buffer tree. This was repeated for the entirety of the irrigation block (40 trees) and duplicated in two rows. This led to a total of 52 trees in the experiment, with 26 in each specific treatment.

Irrigation was duplicated similarly to almond fertigation trials, except that an in-line emitter hose was used. On each side of the tree, one line of emitter hose and one line of blank hose was laid. It was then cut at each buffer tree, and the sections were alternated to create the

treatment blocks. This was a far superior method to the thousands of drip emitters that were hand-installed in the almond treatments. Identical fertigation injector boards were installed and used to make applications (Figure 6).

Fertilizer corresponding to each treatment was injected into the lines to total 220kg/ha of fertilizer that contained 110 and 165 total grams of iodine based on bag variability (Figure 5). At 331 trees/ha plant spacing in this orchard, this amounts to each tree received between 0.32 to 0.49 g of iodine over the course of the treatment.

Trees were tissue sampled in June for comprehensive nutrient analysis as well as iodine analysis. In July, fruits/cluster were counted for seven clusters per tree in a circle around each treatment tree.

During the 2023 harvest, a 38cm x 63.5cm mesh bag of fruit was collected for each set of two trees in the trial. These 26 samples were sent to Wonderful Laboratories for quality assessment.

The 2024 harvest was during a pistachio off-year, so trees were harvested using pole pruners and by hand into buckets. These samples were then dried in an oven and then processed to remove sticks, leaves and other non-nut "trash" to establish total tree yield.

2.2.5.G Avocado – Temecula - Fertigation

In the Fall of 2023, a trial was installed in an avocado grove in Temecula, CA. This grove had been top worked with Gem variety two years prior. A similar trial design to the pistachios and almond was used, but due to the slope of the site, some modifications were made. A total of 28 trees were used, with 14 being assigned to each treatment (

Supplementary Data 3). These trees were in two side-by-side rows, with blocks of four trees being partnered to ensure any water movement downslope did not carry between treatments. Identical fertigation injector boards were installed and used to make applications (Figure 6).

Applications commenced July 27th, 2023, and were made weekly for 14 weeks. These applications applied a total rate of 168 kg/ha of KNO_3 alone or KNO_3 +iodine. The timing was targeted to make iodine available during important flower bud development time.

Observations of phenology were made by a UC Riverside collaborator, Marllon Soares dos Santos, according to the methodology proposed by Salazar et al. and Alcaraz et al (Alcaraz et al., 2013; Salazar-García et al., 1998). A description of this assessment scale can be found in supplementary materials.

Due to the cost of labor, the market price at the time of maturity, and the projected yield, the grower decided not to harvest this block of avocados, so no yield or quality data was collected for this trial.

Leaf tissue and root samples were taken in August 2024 to determine the iodine content of plant tissue. Twenty-five leaves were taken from each tree while walking 360 degrees around the tree. Feeder root samples were taken at two locations for each tree and combined into tree groups of two for trees in the same row and growing next to each other.

2.2.6 CERES fixed-wing aircraft multispectral imagery analysis

CERES Imaging provided fixed-wing multispectral images of the almond and pistachio sites at temporal frequencies conducive to the desired analyses. Thermal, and multispectral (550nm, 670nm, 717nm, and 800nm) images were captured each flight.

In 2023, only two images were taken during the almond bloom. These images were not alone enough to understand bloom progression, so in 2024, nine images were captured from 2/12/24 to 3/14/24 to better analyze variation. For both years, monthly images were captured throughout the growing season and into Fall until leaf drop.

2.2.6.A Individual tree segmentation

Polygons were hand-drawn around each blooming tree in the respective trials. Hand drawing was necessary because of overlap in the tree canopy that was not easily discerned by methods used by other workers such as YOLOV5, object segmentation, deep learning tools in ArcGIS with ArcGIS Pro Image Analyst, or Meta's Segment Anything Model (SAM). Tree centroid point-to-polygon conversion was tried to determine if various shapes could also capture each tree accurately. Circles of differing diameters, as well as "donuts" (circles without centers), were analyzed and compared with hand-drawn polygons. Ultimately, it was determined that hand-drawn polygons most accurately captured each tree, as the centroid of a tree trunk might not be the centroid of the canopy.

2.2.6.B Almond bloom analysis

Previous work has established that almond blooms reflect the majority of the visible light spectrum (Chen et al., 2019). This attribute assists with determining bloom variation in early season imagery as blooming almonds are easily differentiated from the soil and any weeds surrounding them. Using ESRI ArcGIS Pro, a histogram of pixel values was created for each 550nm band image, and a bloom threshold was selected. These values differed slightly from image to image due to minor variations, but this difference was never large. Once a threshold value was established, the Raster Calculator geoprocessing tool was used to set any values below

that threshold to <NULL>. Using null values allowed any following analysis to easily

differentiate the blooming tree from the background (Figure 7). This variation in reflectance is

the overall whiteness of the tree in the image with the higher reflectance due to greater amount of

overall whiteness.
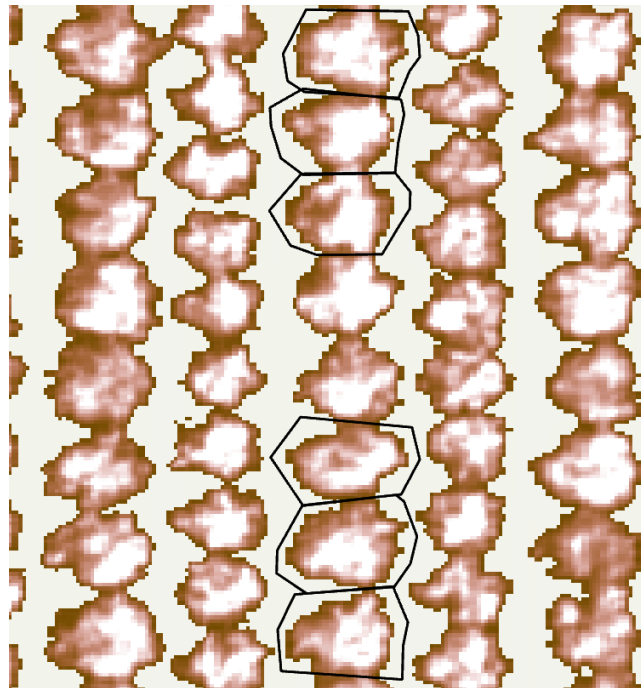
Figure 7: Example image taken from ArcGIS Pro showing the ability to differentiate between blooming almond trees and the soil/weeds/etc. surrounding them. Trees within the trial are encircled by polygons and summary statistics are calculated for the pixels of that raster image.

2.2.6.C In-season reflectance comparison

CERES multi-spectral imagery was processed using ArcGIS Pro using the Raster

Calculator geoprocessing tool for two vegetation indices: NDRE and NDVI. These indices were

selected as they are very commonly used, well understood, and good indicators of in-season tree

status. NDVI was used to compare treatments early in the growing season as trees are

accumulating chlorophyll, and NDRE was used later in the season as trees likely reach a peak

canopy cover/chlorophyll content. The equations for these indices are as following:

$$NDVI = \frac{NIR - Red}{NIR + Red} \qquad NDRE = \frac{NIR - Red\ Edge}{NIR + Red\ Edge}$$

The imagery was overlaid with individual tree polygons corresponding to different trials and treatments. The ArcGIS Pro geoprocessing tool "Zonal Statistics as Table" was used to calculate descriptive statistics for each tree polygon.

2.2.7 Statistical software and analysis

RStudio version 2023.12.1+402 was used for all statistical analysis and graph and figure generation. Packages used: emmeans, ggplot2, lme4, tidyverse, dplyr, purr, lubridate, scales, multcomp, broom, ggeffects, ggpubr, rstatix, ez, bspline, splines, and splines2.

Statistical analysis was done using an analysis of variance (ANOVA) in R on the fit of linear models for each experiment's results. In most cases, a single predictor was used in linear model creation and in all cases, models were checked using a plot of residuals.

To analyze timescale aerial imagery, a repeated measures analysis was done using a linear mixed-effects model in R- *lmer()*. Date of the image and treatment were used as independent variables and metric of interest (ex: mean whiteness) as the dependent variable. A random effects variable was introduced to account for individual tree variability.

2.3 Results
2.3.1 Soil iodine concentrations in California

Soil iodine was found to be variable across California, but all areas had low concentrations when compared to a metadata analysis global average of 5.1 mg/kg (Johnson, 2003). Though a possible factor when comparing studies between broad geographical regions, in

California, the soil concentration variability is not expected to impact results for the three study

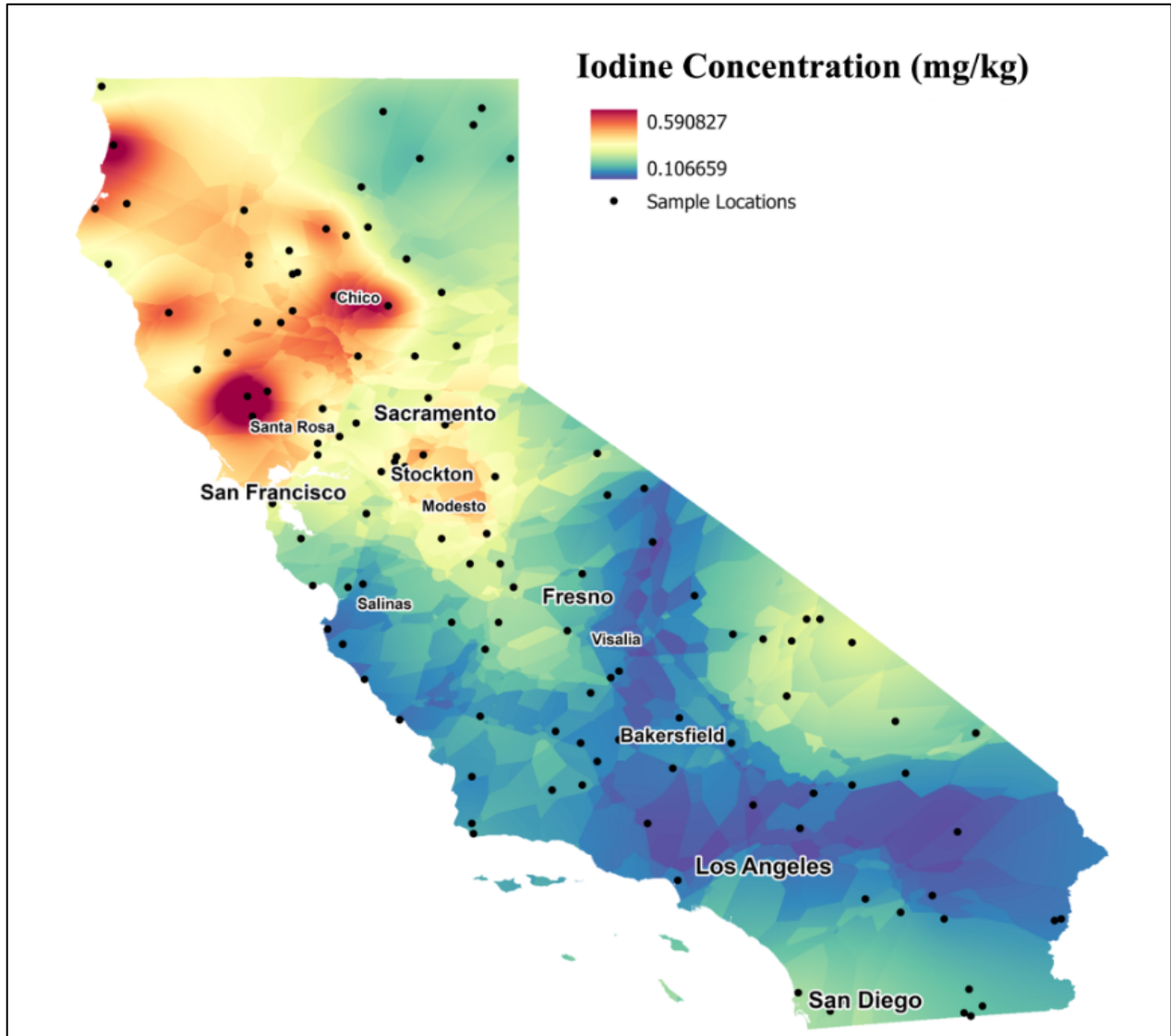areas and cropping systems in this study.

Figure 8: Soil iodine concentration map showing spatial variability within California. Variability exists between the different regions of California, but in general, iodine concetrations are low and trial location is unlikely to be influenced by natural iodine levels.

2.3.2 Almond – Leaf tissue comprehensive nutrient analysis

No difference between treatments was found for any element measured for leaf samples taken in July of 2023.

Table 1: Mean nutrient concentration of almond leaf samples following 2023 fertigation treatment. r=40

	unit	KNO_3 + Iodine	KNO_3	p- value
Nitrogen	%	3.25	3.24	0.69
Phosphorus	%	0.17	0.17	0.83
Potassium	%	2.07	2.02	0.51
Calcium	%	2.43	2.46	0.66
Magnesium	%	0.56	0.57	0.41
Zinc	ppm	16.56	16.35	0.62
Manganese	ppm	62.98	63.7	0.74
Iron	ppm	91.28	92.68	0.47
Copper	ppm	5.93	5.58	0.2
Boron	ppm	30.63	31.16	0.26
Sodium	%	0.02	0.02	0.44

2.3.3 Pistachio - Leaf tissue comprehensive nutrient analysis

No difference between treatments was found for any element measured for leaf samples taken in late July of 2023.

Table 2: Mean nutrient concentration of pistachio leaf samples following 2023 fertigation treatment. r = 26

	unit	KNO_3+ Iodine	KNO_3	p- value
Nitrogen	%	2.10	2.08	0.56
Phosphorus	%	0.11	0.11	0.99
Potassium	%	1.59	1.62	0.53
Calcium	%	2.11	2.11	0.99
Magnesium	%	0.54	0.53	0.32
Zinc	ppm	45.68	43.67	0.24
Manganese	ppm	60.56	61.62	0.75
Iron	ppm	112.64	111.27	0.77
Copper	ppm	20.96	19.69	0.1
Boron	ppm	82.06	79.32	0.28
Sodium	%	0.01	0.01	0.32

2.3.4 Almond - Floral progression bloom assessment – Spurs at Westwind Ranch

Results from the daily monitoring of individual spurs showed logical progression through the developmental stages: Green Tip →Pink Bud →Popcorn →Full Bloom →Petal Fall (Figure 9). Slight differences between the treatments can be observed, but further analysis was needed to understand if there were statistical differences in the timing or amount of blooming that occurred by treatment.
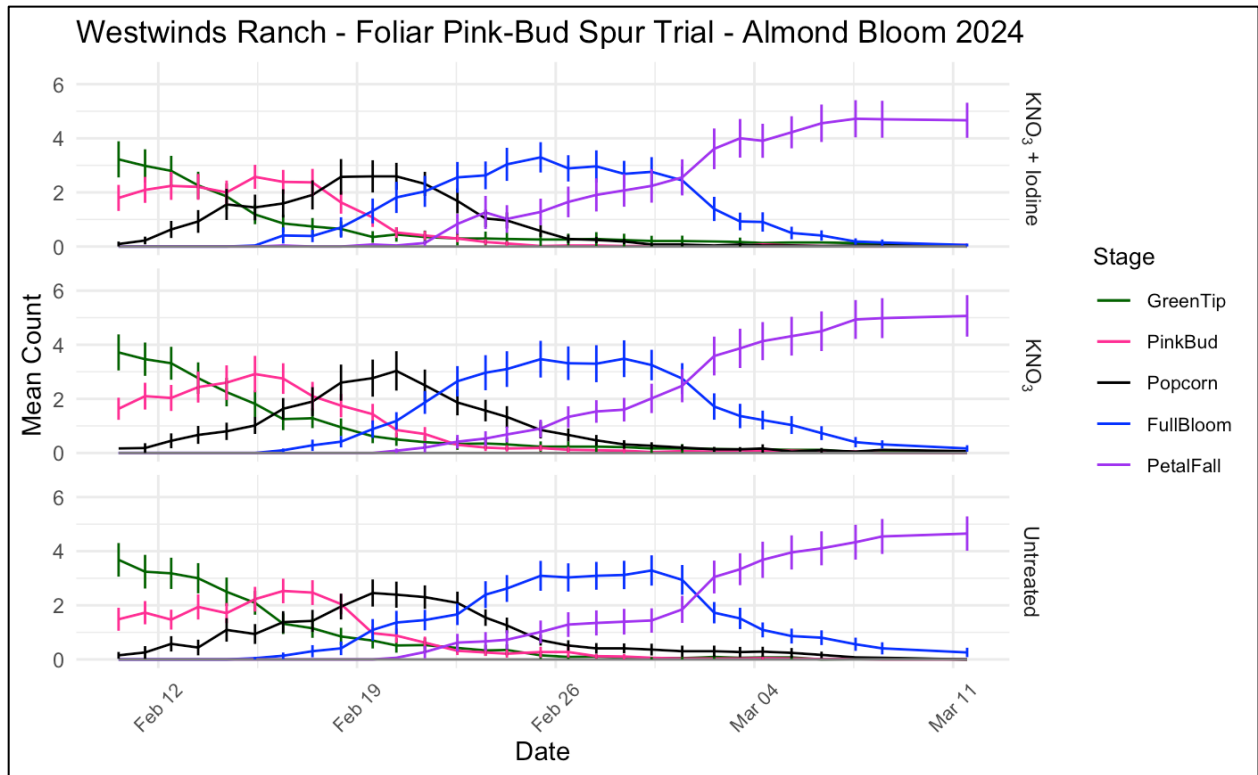

Figure 9: Bloom progression of spurs receiving treatments of KNO₃, KNO₃+iodine, or untreated. The number of buds/flowers in each stage is displayed in its corresponding color to visualize progression over the bloom timeframe from green tip to petal fall. This graph is important for visualization, but no statistics can be calculated to understand if the treatments impacted the bloom development.

The mean flowering percentage was used to standardize the number of flowers per spur since spurs were selected pre-bud break and not selected for a uniform number of flowers. This

ratio for each day and for each spur was generated from the number of buds in full bloom and dividing it by the total number of buds in all stages. This number represents the percentage of flowers flowering at each time step out of that spur's potential total number of flowering buds. A number of "1" would indicate that all buds on that spur are in flower. A low number could indicate that peak bloom had not occurred, had already occurred, or the spur had a non-uniform/synchronized floral emergence. Given the nature of buds proceeding along a progression of development and then declining after peak bloom, plotting values along a time scale elucidates the meaning of a low value. In Figure 10, a shift in blooming for KNO_3 +iodine-treated spurs can be observed, followed by a more abrupt transition to post-flowering when compared to the KNO_3 alone and the Untreated treatments.

The predicted values along a linear progression appear to confirm the trend that iodine application leads to an earlier bloom with a more abrupt decline, but confidence intervals overlap in many regions (Figure 11). When the standard deviation for the six spurs on each tree is factored in to this trend, the uniformity of bloom on a tree did not differ significantly between treatments, but KNO_3+iodine-treated trees had a slightly earlier increase in the standard deviation than the other two treatments (Figure 12). Given the nature of highly variable bloom dynamics at the spur level, this trend is not statistically significant, but suggests a trend needing further investigation through a more precise method.
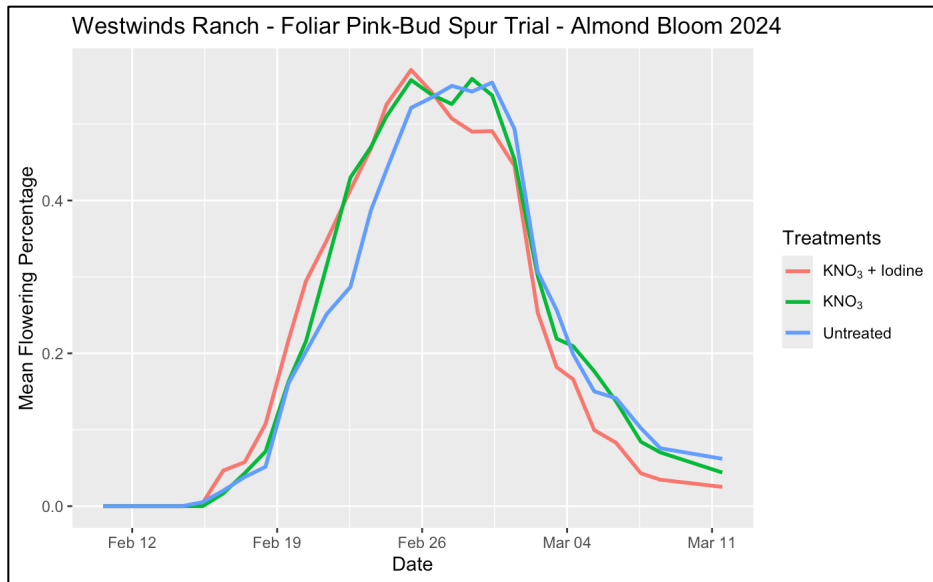
Figure 10: Ratio of buds in flowering to the total number of buds that eventually flowered on each spur (Mean flowering Percentage) over the total bloom time.
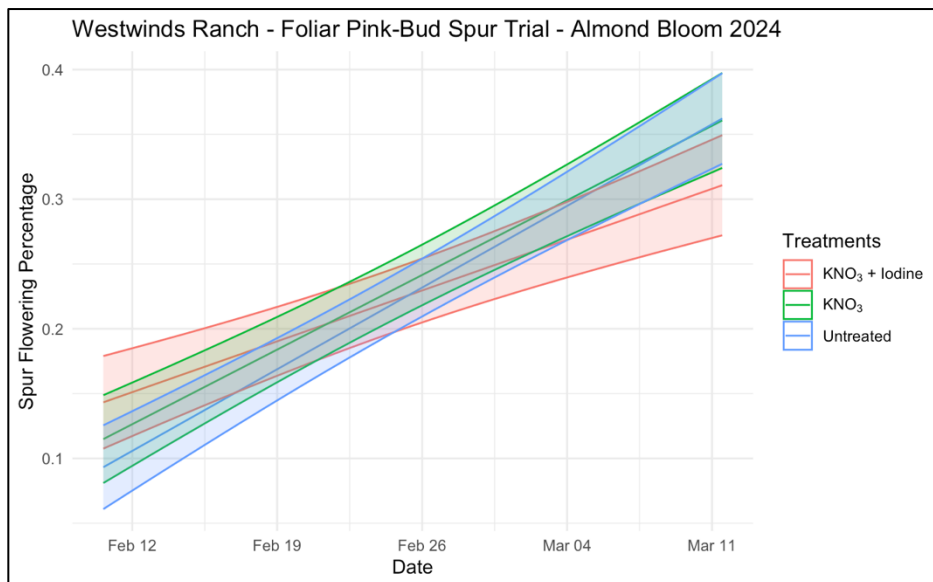

Figure 11: Results of a linear mixed effects model on the spur bloom data accounting for spur variability show slight divergence of iodine treated spurs from other treatments. This divergence is not significant given the 95% confidence interval. Spur flowering percentage is the ratio of bloomed flowers at each time step, given the known potential of that spur for flowering at the end of the flowering cycle.
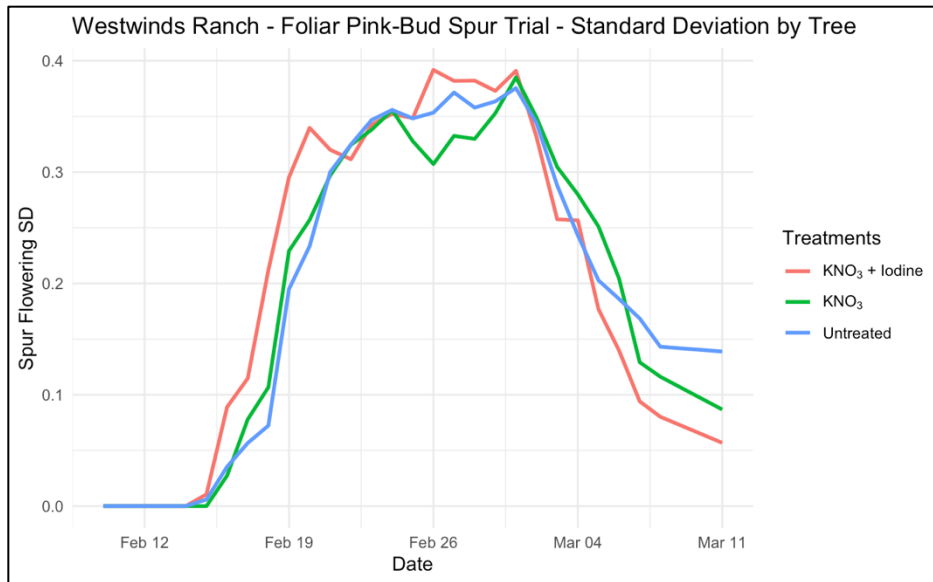
Figure 12: The standard deviation of spur flowering percentage for each day along the progression of almond bloom showing slight differnces between treatments not significant due to variance..

2.3.4.A Nonlinear least squares fit regression analysis of bloom data

Individual almond spur blooming data at this orchard closely resembled a normal Gaussian distribution as a set number of floral buds bloomed and declined without any interruption or secondary release of buds/flowers. A nonlinear least squares fit regression analysis could be performed for each tree and the six spurs being monitored on that tree. The same ratio of the total in full flowering divided by the sum of all stages- "*Flowering*" – was summed for all six spurs and plotted across time. The *Flowering* values for each tree were summed as a way to better represent the collective stage of that tree. A mean of these six spurs would indicate the average stage of blooming but could diminish the differences seen between trees if the variation in the bloom stage of a single tree was high. To check for variation, standard deviation of each tree at each time point was used, as seen in Figure 12. These results show that the standard deviation varies slightly between each treatment but does not vary enough to

preclude the combination of the six spur observations on each tree. It is interesting to note that the KNO$_3$+iodine treated trees had a higher initial standard deviation than the other treatments, implying that not all spurs or trees were equally impacted by the treatment.

The nonlinear curves permitted the use of descriptive calculus to calculate the derivatives, peak, and area under the curve along the progression of bloom (Figure 13). These calculations were carried out for each of the 30 trees as an estimate of that tree's bloom progression (Figure 14). The mean R^2 for this model was 0.93 – well within an acceptable fit for the majority of trees. The results of these analyses found no significant differences between treatments for any of the metrics based on the model (Table 3 and Table 4).The presence of iodine at a crucial period of bloom had no impact on any measure of bloom dynamics when compared to the control.
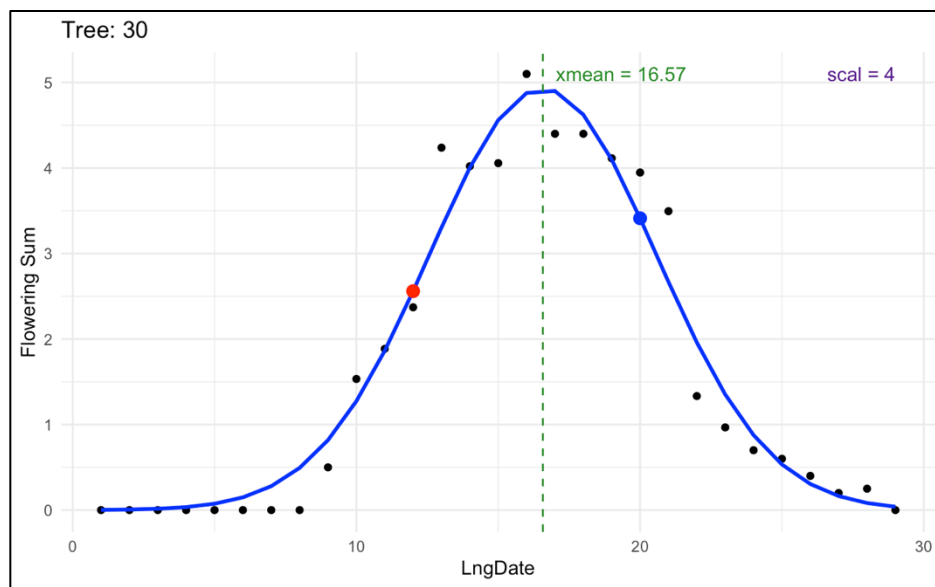

Figure 13: Resulting model fit for a single example tree (tree 30) showing the alignment of the curve as well as the location of the various metrics of interest. The model follows the same progression of time but is displayed as "LngDate" to enable calculation and display of data originating from 0,0 with "1" being February 10th, 2024 and "30" being March 11th, 2024.
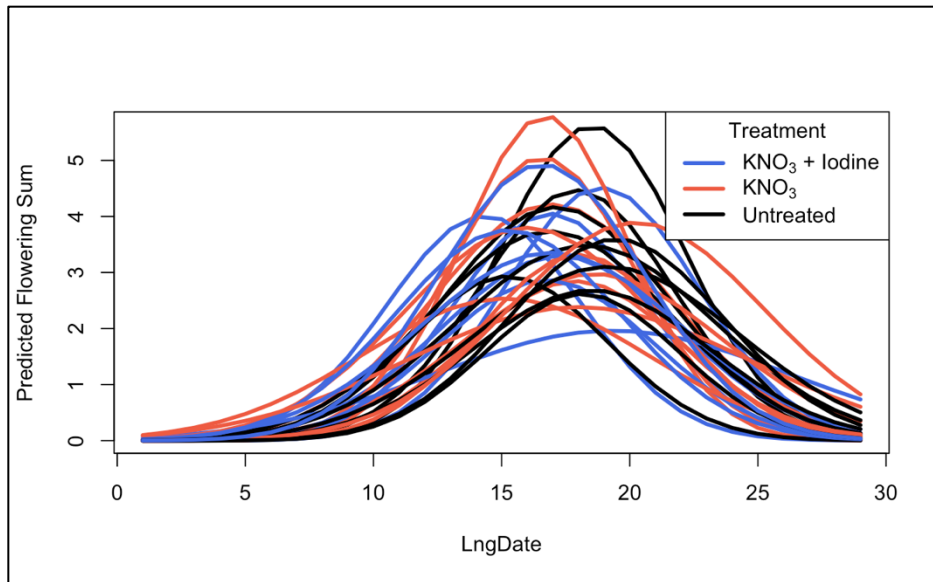
Figure 14: Bloom model for each tree in the trial showing variability in bloom dynamics.

Table 3: Names and explanations for the metrics used to understand each tree's fitted model curve of bloom progression.

area	Total amount of flowering for the tree
first and second inflection points	Aspects of timing: when does flowering start and end, what is the length of time between these intervals
xmean	Timing of peak flowering
scal	The standard deviation of the distribution. It controls the spread of the flowering over time. A smaller scal value would result in a narrower peak, indicating that the flowering occurs over a shorter period, while a larger scal value would result in a broader peak, indicating that the flowering occurs over a longer period.

Table 4: Statistical summaries for curve fitting to bloom timing model. Mean results for each metric indicate no significant difference in treatments (p>0.05 for all metrics).

	KNO$_3$+ Iodine	KNO$_3$	Untreated	p - value
area	7.25	7.33	7.40	0.91
xmean	16.91	17.41	17.92	0.32
scal	4.50	4.65	4.32	0.71
Inflection1	11.89	12.30	13.10	0.21
Inflection2	20.89	21.50	21.80	0.58
length	9.00	9.20	8.70	0.83

2.3.4.B Westwinds Ranch yield 2024

All fruit was harvested from the foliar pink-bud spur trial in August 2024. The application of iodine at pink-bud did not lead to a significant difference in yield or kernel characteristics (Figures 18, 19, and 20). High variability in natural spur flowering dynamics means no increase in censusing would reduce this variance as the observable variability (mostly 1 or 2 nuts per spur) is not high enough (Figure 17). Trends indicate higher total kernel weight per spur for spurs treated with KNO$_3$+iodine at pink-bud (Figure 15) due to higher nuts per spur at harvest and not by increased kernel weight (Figure 16). However, there was no significant difference between the treatments, indicating that the presence of iodine during bloom had no impact on the yield of those treated spurs.
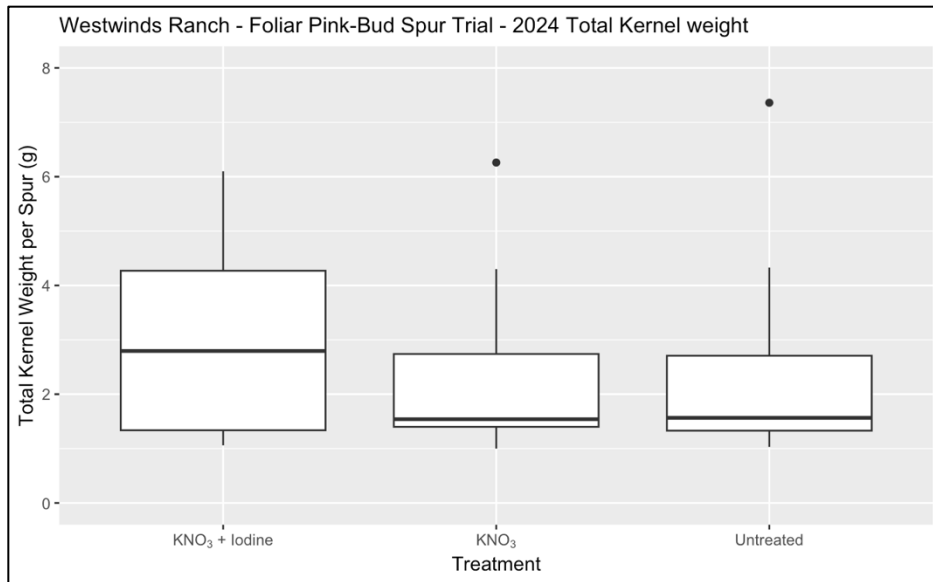
Figure 15: Total almond kernel weight showing a trend towards increased yield for iodine treated spurs that was not significant (p>0.05, df = 26).
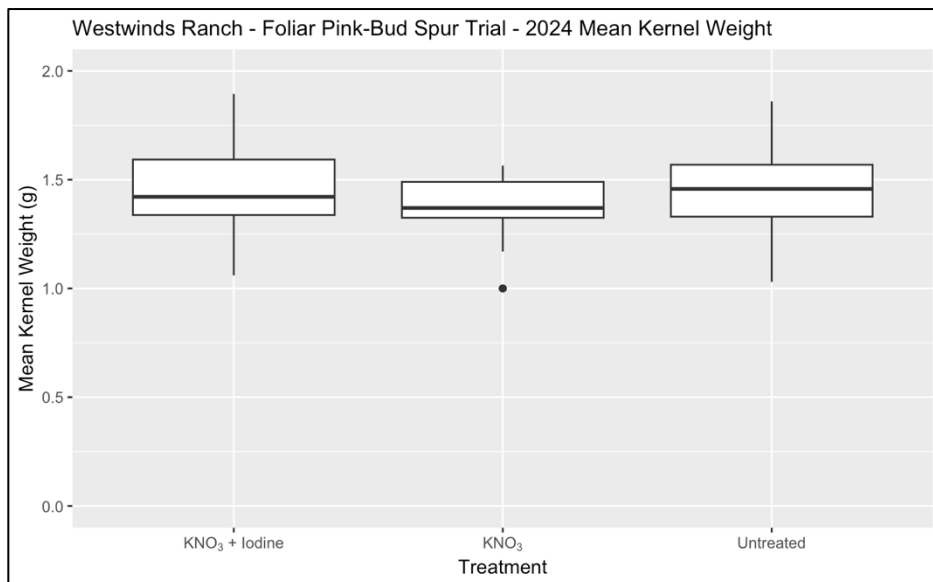

Figure 16: The mean individual kernel weight was not statistically different in response to a folair iodine treatmetn at pink-bud (p>0.05, df = 26).
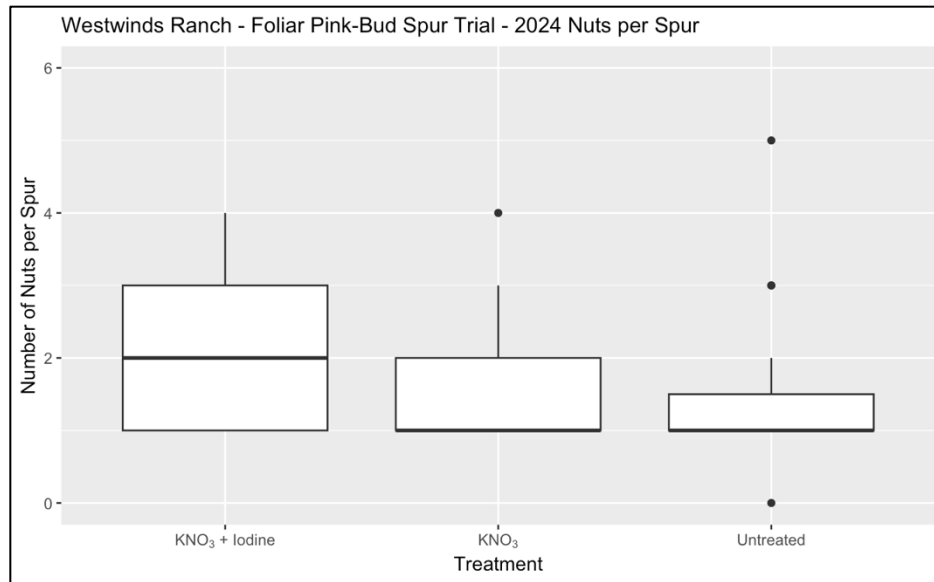
Figure 17: Number of nuts harvested per spur in each treatment showing no improvement in yield for iodine treated trees (p>0.05, df = 26).

2.3.5 Almond - Floral progression bloom assessment – Whole tree at KG Ranch

Individual spur flowering dynamics are often highly temporally and spatially variable. Additionally, prior work has demonstrated that spur return bloom and flower number are influenced by the number of nuts set on that spur during the previous season (Fernandez et al., 2018). Without a multi-year study, results from spur analysis are likely obfuscated by these factors. A whole tree bloom assessment with yield analysis corrects for the high spur variability as measuring the whole tree captures the variance without a need for increased subsamples per tree. Measuring spurs and inferring total tree bloom status would require an enormous number of sub-samples per tree to ensure the variance was captured and represented the many conditions and stages of the tree. However, even a full tree bloom analysis presents challenges as almonds are insect-pollinated, and a highly successful and productive phenological bloom might not result in a high yield due to pollinator effects. A tree blooming during a cold spell might be measurably robust in all bloom aspects but will not have a corresponding yield due to low pollination events.

Similarly, trees exist in a matrix, and the distance to hives influences the number of visitations by honeybees. These factors will all play a role in the ultimate success of a tree, but taken in aggregate, total yield is more related to the total number of flowers as many of these factors are either a) accounted for in experimental design or b) can be averaged out as expected individual variance (Tombesi et al., 2016).

2.3.5.A 2024 CERES aerial imagery bloom analysis

Capturing the blooming status of many different trees in an orchard is most easily accomplished using remotely sensed data that, fortunately, can also be incredibly precise given the spur variance as discussed above. High-frequency (daily when possible) multi-spectral, and thermal images were captured of the KG ranch orchard location leading up to and through bloom. As discussed in the Methods section, the bloom status of each tree in the trial was isolated for each image, and the results are presented below.

There was no difference in mean tree flowering/whiteness by treatment (Figure 18). No difference in the standard deviation in measured tree whiteness by treatment would indicate an inconsistent or individually variable response. In the fertigation trial, trees treated with KNO_3 alone did not have consistently higher mean whiteness values, but did have a numerically higher and earlier peak bloom than trees receiving KNO_3 +iodine through the irrigation (Figure 19). In the post-harvest foliar application trial, the opposite was true, where KNO_3+iodine treated trees bloomed earlier and more intensely than the KNO_3 or Untreated trees (Figure 20).
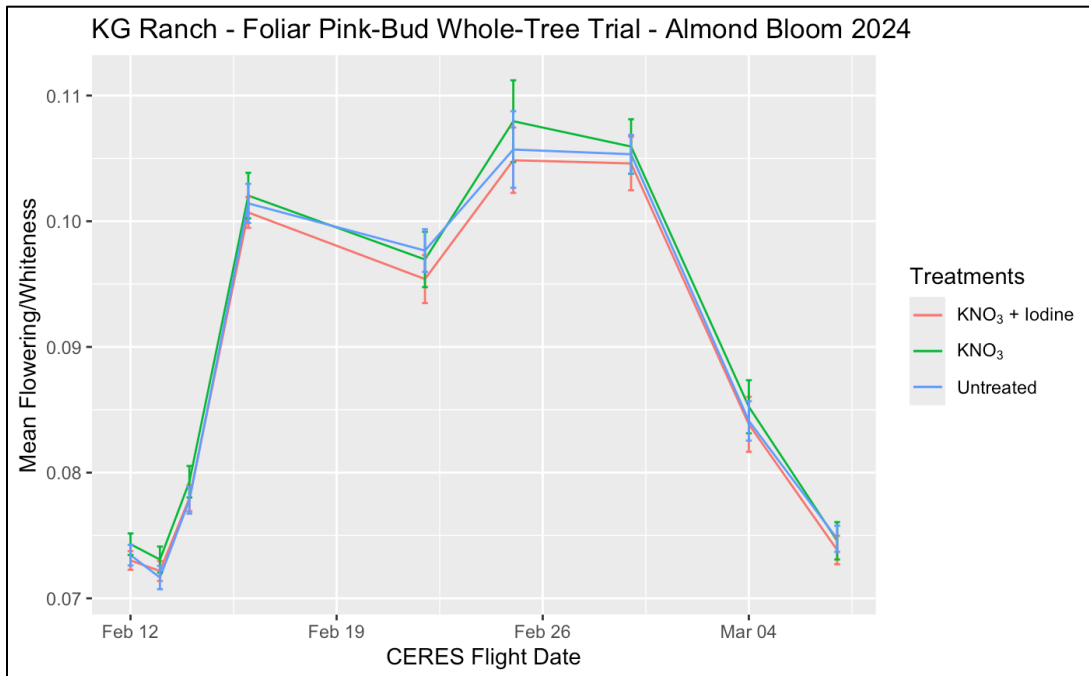
Figure 18: Mean pixel whiteness values derived from remote sensing data for individual trees treated with a foliar application of iodine at pink bud stage. Pixel whiteness serves as a proxy measure for bloom intensity and overall tree flowering status. Error bars represent standard error of the mean (n = 24 trees per treatment group).
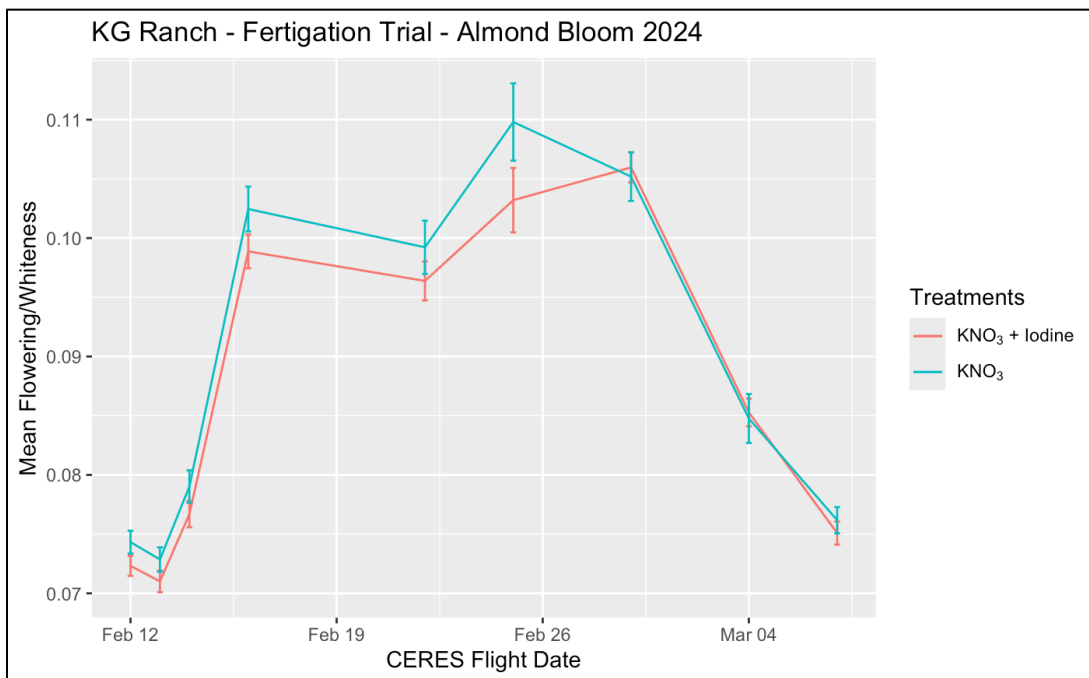

Figure 19: Mean pixel whiteness values derived from remote sensing data for individual trees receiving fertigation treatments. Pixel whiteness serves as a proxy measure for bloom intensity and overall tree flowering status. Error bars represent standard error of the mean (n = 40 trees per treatment group).
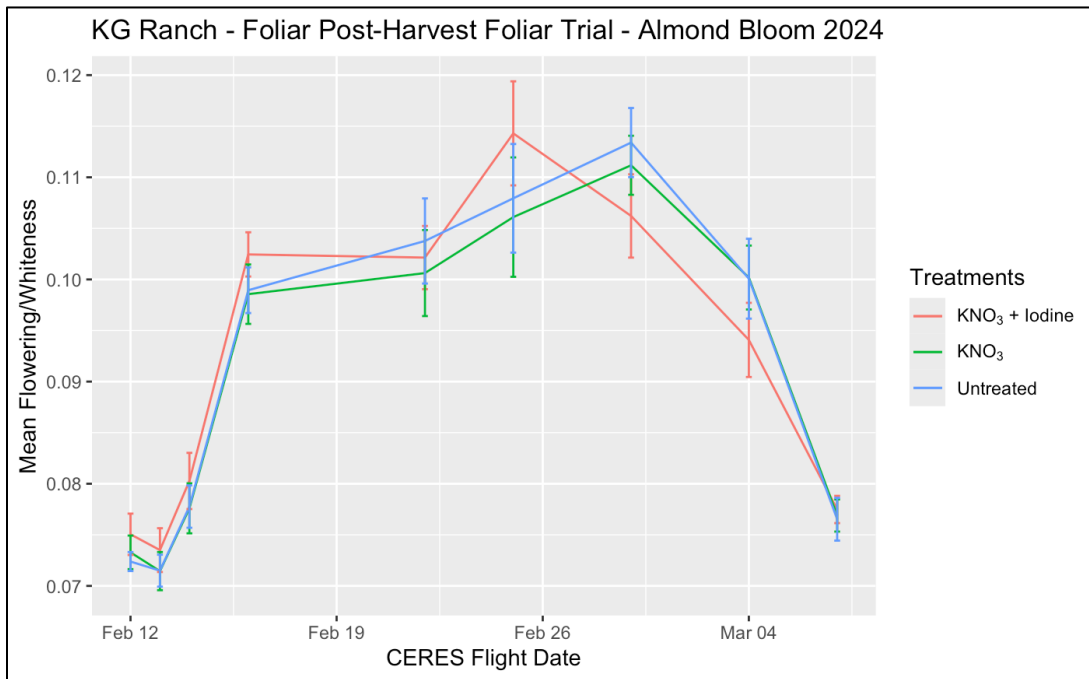
Figure 20: Mean pixel whiteness values derived from remote sensing data for individual trees that received a foliar treatments priot to leaf-fall in the Fall of 2023. Pixel whiteness serves as a proxy measure for bloom intensity and overall tree flowering status. Error bars represent standard error of the mean (n = 12 trees per treatment group).

2.3.5.B KG Ranch 2024 yield for all trials

All fruit from each tree in the two-year foliar pink-bud trial at KG Ranch was harvested, dried, and cracked out to determine yield. There was no statistical difference in kernel weight between the treatments ($p > 0.05$, df = 28) (Figure 21).
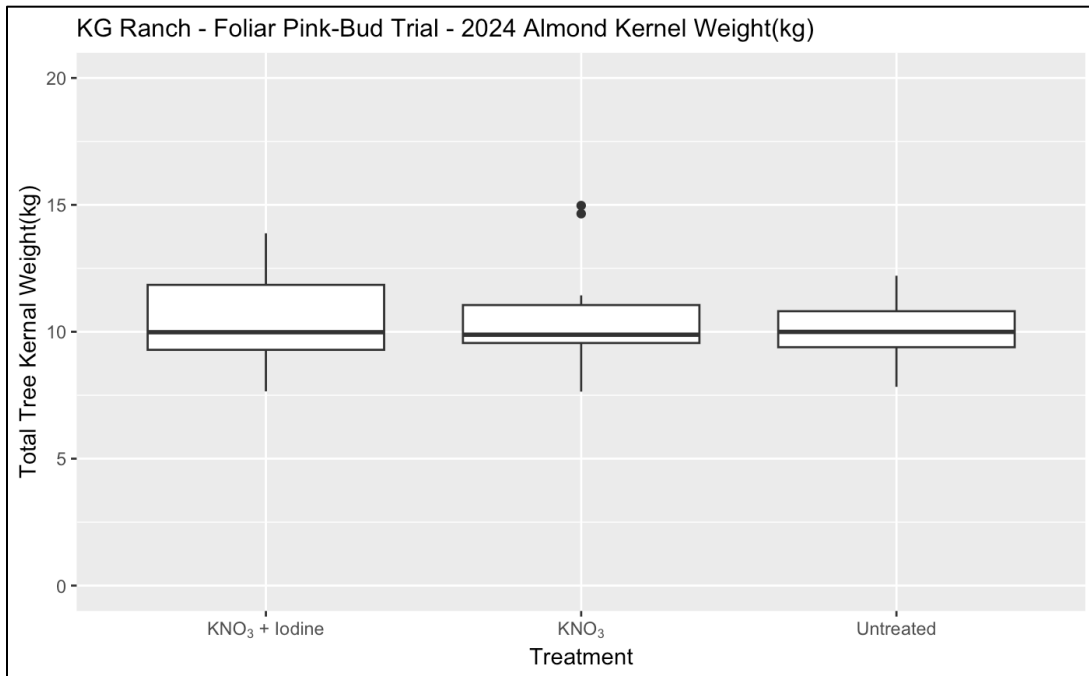
Figure 21: 2024 individual tree yield data for all trees in the whole-tree foliar pink-bud trial at KG ranch.

2.3.6 Pistachio - Fruit counts and 2024 yield

There was no statistical difference in pistachio nuts per cluster between the treatments in 2023 or in 2024 (Figure 22). There was, however, a universally large decrease in nuts per cluster during the 2024 season, but this was also not impacted by the addition of iodine in a fertigation treatment. This large decline in nuts per cluster during the off-year was partially responsible for the overall decline in total yield, but total cluster weight was also lower (Figure 23). In all respects, the addition of iodine did not impact the dynamics of pistachio alternate bearing when applied at this rate and as a fertigation treatment.

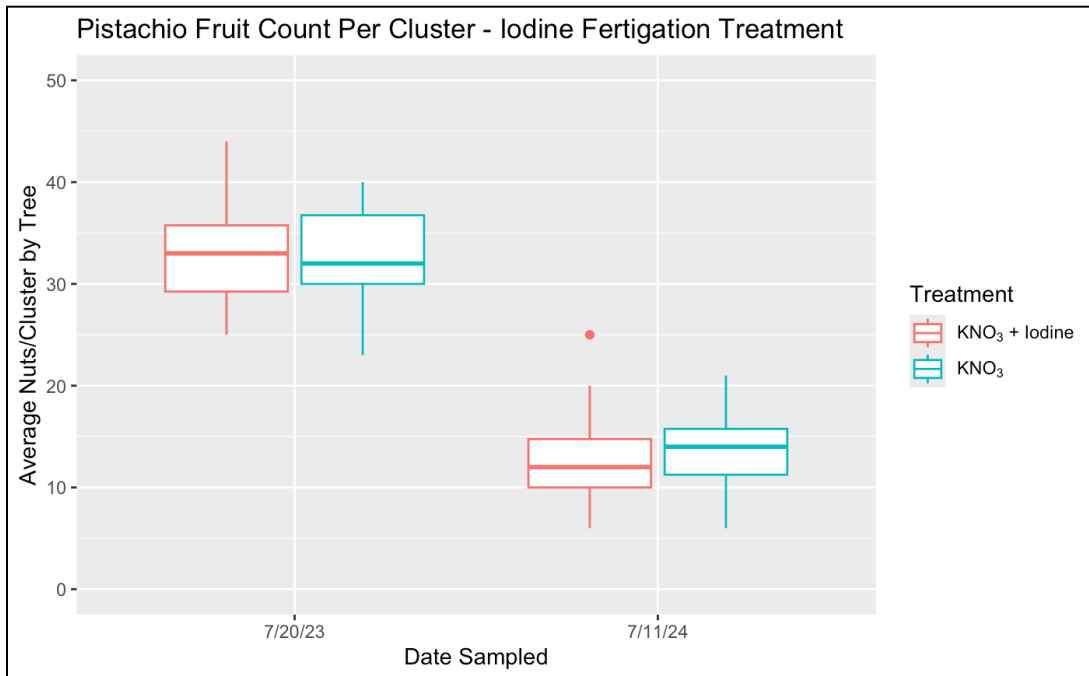*Figure 22: Mean number of fruits per cluster for each treatment in the pistachio fertigation trial. The large decline year to year reflects the alternate bearing tendency of pistachios - especially pronounced in this orchard. (2023: p = 0.9092, and 2024: p= 0.2352)*

Figure 23: Total yield per tree for the 2024 growing season. No statistical difference in treatments was observed. (p = 0.1239, df = 50)

2.3.7 Avocado – Flower counts

There was found to be no difference in flowering stage or bloom intensity between any of the trees in the trial over eight visual assessments from early April to June.

2.3.8 Almond - CERES in-season multispectral imagery
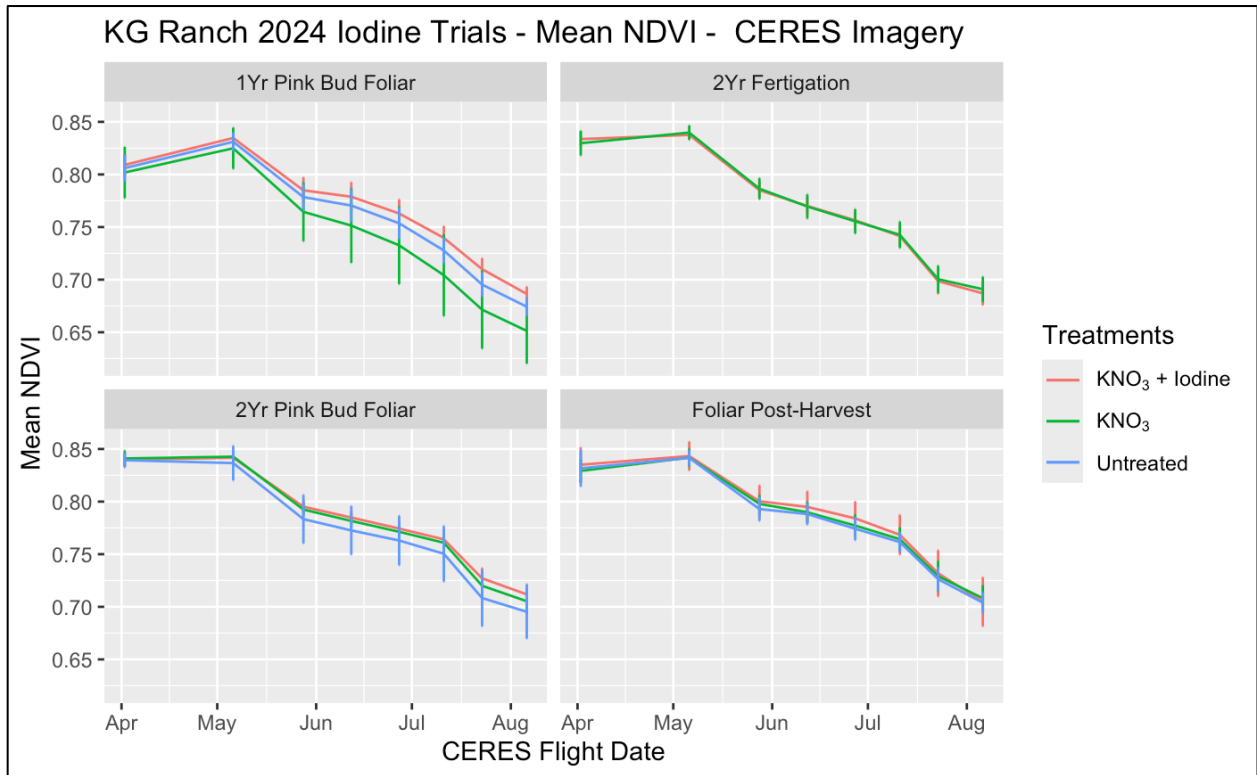
Figure 24: NDVI of each tree within each trial throughout the 2024 growing season. Treatments showed no measurable impact on NDVI.

2.3.9 Iodine analysis in plant tissues and soils

2.3.9.A Almond samples

In root samples taken pre-2024 fertigation treatment, there was no statistical difference between the treatments (Figure 25). Following a month of fertigation with the treatments, there was no statistical difference between the treatments in either the soil (Figure 26) or in the leaf samples (Figure 27).

Figure 25: Iodine content of almond root samples taken prior to 2024 fertigation treatment (p value = 0.1437, df = 30).
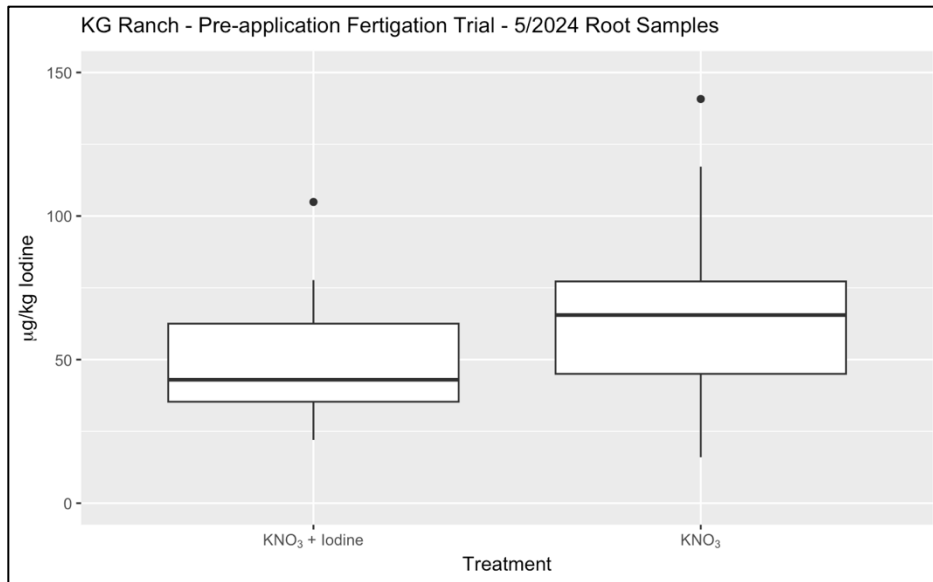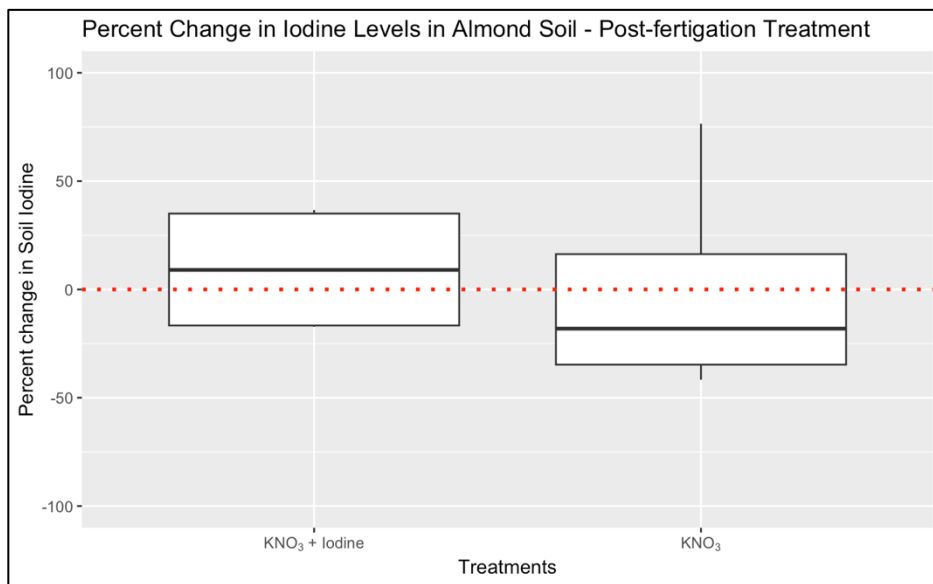

Figure 26: Percent change in soil iodine from samples taken prior to treatment fertigation to those after receiving a month of fertigation treatments. This analysis showed no change in soil iodine in response to the fertigation treatements (p value = 0.7634, df = 14).
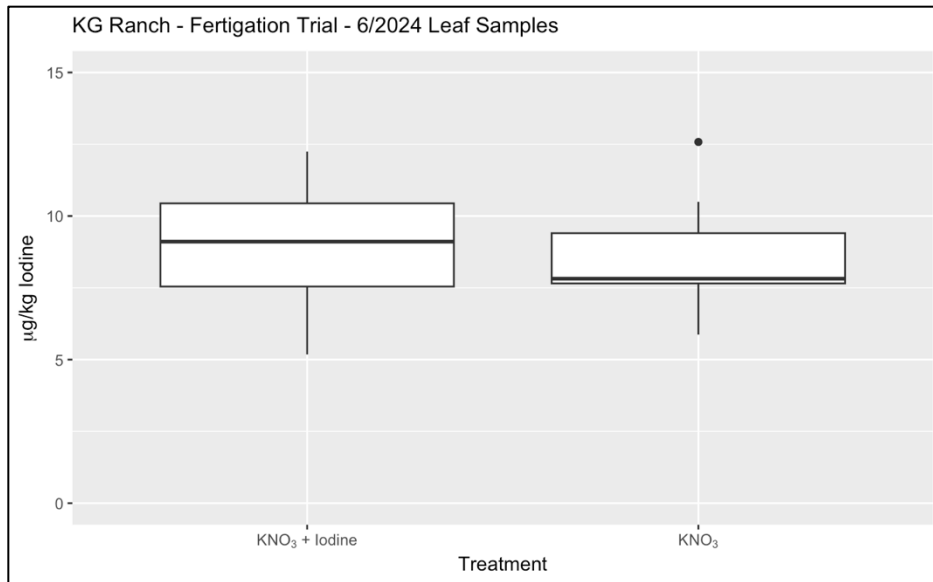
Figure 27: Iodine content of leaf samples taken after fertigation had been applied for 30 days. There was no change in iodine content of leaf tissues due to the treatments (p-value = 0.3977, df= 32).

2.3.9.B Pistachio samples

Pistachio leaves had similar iodine content regardless of treatment and this number was slightly lower than was found in almond leaves (Figure 28).
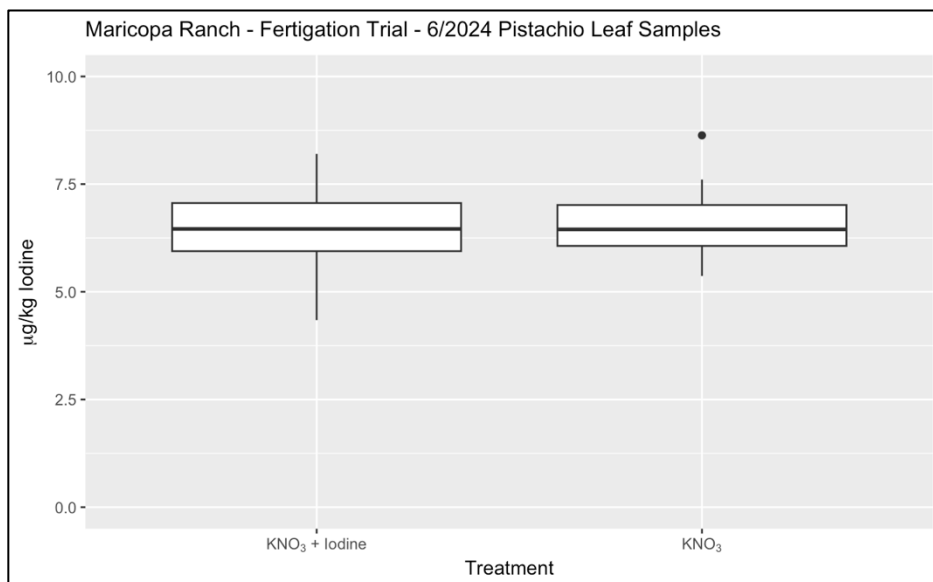
Figure 28: Iodine content of pistachio leaf samples taken prior to fertigation treatments in 2024. (p-value = 0.8396, df=50)

2.3.9.C Avocado samples

Avocado roots and leaves had similar iodine content regardless of treatment and this number was significantly higher than was found in almond or pistachio (Figure 29).
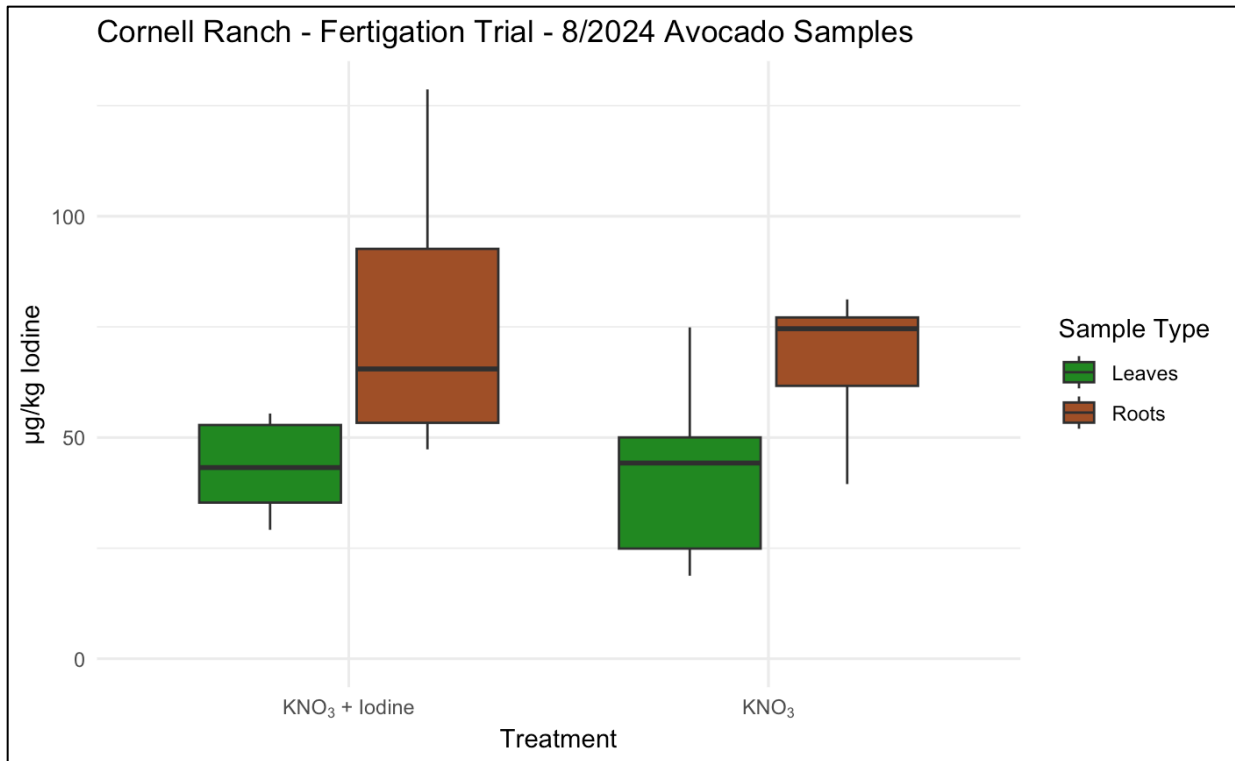
Figure 29: ICP-MS analysis of iodine content of avocado tissues. No difference was found between treatments for roots (p value = 0.5595, df= 10) or leaves (p value = 0.7496, df = 22) and treatment did not impact the distribution between tissues (p value = 0.5886).

2.4 Discussion

Prior research on iodine's role in plants has faced challenges due to iodine's unstable nature, difficulty to analyze, and the inconsistent results often seen between different species and growing conditions. This study also encountered some of these challenges as no work had been done in almond, pistachio, or avocado, or in using fertigated potassium nitrate fertilizer as an iodine delivery source. While the treatments in this study did not lead to significant differences in plant response, valuable and important data was still collected about iodine in these agricultural environments and in three previously unstudied and important agricultural species.

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2.4.1 California soil iodine concentrations

Modeling iodine concentrations for all of California is an important first step in understanding broad trends for soil iodine concentrations based on geologic and atmospheric influences. It was found that California had low concentrations throughout when compared to a global average of 5.1 mg I kg^{-1} (Johnson, 2003). In the Central Valley agricultural region where these trials were located, iodine concentration was found to be 0.1-0.3 mg I kg^{-1} (Figure 8). This analysis is limited by spatial resolution as an interpolation of 126 soil samples is not robust enough to provide adequate certainty of the concentration of iodine at individual locations for the entirety of California. This analysis was, however, useful for ensuring trial locations did not differ significantly. Naturally low concentrations of soil iodine throughout the state mean there is unlikely to be significant interference from naturally present iodine given observed effective rates.

The rates of iodine that have been shown to elicit a plant response are highly variable in the literature. This uncertainty means that there is no known soil concentration that has demonstrated to elicit a response in the orchard crops of this study. California's low soil iodine concentrations could potentially be meeting plant need, but this is unlikely as there is little evidence to support the hypothesis that iodine is an essential microelement. This study focused on iodine's potential role as a beneficial nutrient or biostimulant, and the literature supports the need for higher application rates to elicit a response. The concentration of iodine naturally present in soil is capable of interference, but this is largely dependent on the hypothesized mode of action of iodine.

Continued study of iodine in California agriculture should include localized soil samples as the heterogeneity of iodine at the field-scale is unknown and these samples would refine our

understanding of regional concentration differences. The majority of the data used for this analysis was over 40 years old and there could potentially be changes in soil iodine not captured in a single snapshot in time. Broad geographical iodine concentrations have been shown to be associated with soil parent material and climate for many regions, but have yet to be studied for California (Johnson, 2003; Smyth & Johnson, 2011). These factors often function over geological timeframes, but it is unknown if intensive agricultural practices and the transport of irrigation water over large distances might alter the natural iodine deposition characteristics.

2.4.2 Potassium nitrate as delivery medium for iodine treatments

Using iodine-enriched potassium nitrate fertilizer as a source for a fertigation iodine treatment poses significant limitations for research flexibility in adjusting iodine application rates. To achieve the concentrations of iodine that had been demonstrated effective in prior experimentation using the UltrasolINE product, meant provisioning orchards with unrealistic quantities of nitrogen and potassium. While most studies on exogenous iodine have been conducted in controlled environments, such as soilless systems or hydroponics where maintaining specific iodine concentrations is manageable, applying similar treatments in orchard crops introduces excessive nitrogen and potassium rates (Table 6).

Table 5: Amount of fertigated UltrasolINE needed per tree to match rate from Kiferle et al. (2022).

Kiferle et al. (2022) Iodine rate	Fertigated kg per almond tree				
	UltrasolINE Kg per tree			**Nitrogen** Kg per tree	**Potassium** Kg per tree
0.2 μM	3.38	Which Contains		0.4	1.6
10 μM	168.83			21.9	77.7

Note: This assumes ~8.9×10^6 L ha^{-1} of water (2023 actual usage) applied per season and 700 μg I kg^{-1} in UltrasolINE

Rates for product application were selected to best mirror a 'grower standard' of potassium nitrate fertilizer use given the nutrient supplied and on recommendation from the fertilizer manufacturer SQM based on typical use. Typically, KNO_3 is utilized as a supplemental product during the growing season in response to plant need rather than a pre-season bulk application of potassium, like muriate of potash or sulfate of potash due to price/kg of potassium. The rate of 220 kg/ha fertigated over 8 weeks was selected as a typical use for this product, but the total iodine supplied was minimal at this rate. This was further exacerbated by the lower-than-expected concentration of iodine in the product at 55% to 70% of expected label concentration (Figure 5). Even at 70% of advertised iodine, the total iodine application rate in the fertigation trials was only 0.154 kg I ha^{-1}.

2.4.3 Fertigating iodine: overcoming soil organic matter sorption

Applying trace concentrations of iodine as a fertigation treatment is not likely to be plant-available for an extended period. The majority of iodine in a fertilizer application would be expected to immediately sorb to the organic matter with an estimated 10% remaining in the water soluble fraction theoretically available for plant uptake (Fuge & Johnson, 1986). $CaCl_2$ extraction of peat found that after only 2 hours, peat retained 25% of the IO_3^- applied, and after 2 days, iodine was no longer detectable in the extraction (Budke et al., 2021). California agricultural soils often have <1% organic matter and this level of absorption is unlikely, but each site had compost applications, cover crops, and woody pruning reincorporation that likely tied-up iodine; especially in the case of the avocado site that had large amounts of mulch applied. In these orchard experiment settings, a low rate of iodine application paired with large volumes of irrigation water mean the initial, and then continuous, dilution of iodine in the soil media is also

extreme. To overcome these constraints, a higher concentration of iodine would be required that was impossible to achieve given the fixed concentration in UltrasolINE.

The iodine concentration required is also dependent on soil type, environmental conditions and the agricultural system being fertigated. In a field study of kohlrabi, >5.44 kg/ha of soil applied I was needed to raise the iodine concentration in the kohlrabi, and this large dose did not last beyond a single growing season (Lawson et al., 2015). In a multiple year study of soil applied iodine in potato, and tomato, a rate of 0.125 kg/ha had no impact on leaf iodine concentration until the rates were increased 20x and 40x in the second year of the trial (Caffagni et al., 2012). An orchard system is drastically different than these annual cropping systems in the volume of soil per plant and the total mass of each plant being treated. It is unknown if tree roots preferentially or passively uptake iodine, and this knowledge would drastically change our understanding of the soil concentrations required in an orchard.

The rates of iodine used in these fertigation trials in almond, pistachio, and avocado did not increase soil iodine concentration and there was no increase in iodine concentration in any of the plant tissues measured (roots and leaves) or in the soil immediately surrounding a drip irrigation emitter dispensing the treatment (Figure 26). This finding was contrary to expectations and may have been the consequence of consistent dilution and leaching from irrigation and low iodine application rates due to lower-than-expected iodine concentrations in the UltrasolINE. Drip irrigated and fertigated orchard crops may require greater than the 0.154 kg I ha^{-1} provided.

The findings in this study showed no response when these three orchard crops were fertigated at a low rate of iodine. This is evidence that a larger rate of iodine is required if iodine has any impact on these species,. This alone could be interpreted as evidence that these species are already naturally meeting their iodine 'requirement', or conversely, that insufficient iodine

was applied to elicit a response. In both cases, the supplementation of these crops with fertigation applied iodine at this rate was not beneficial at these trial locations.

2.4.4 Plant iodine concentration differential

In almond, there was approximately 5x more iodine in roots when compared to the leaves, but a less pronounced differential of 2x in avocado (Figures from section 2.3.9). This differential was not a response to fertigation treatment, but rather a normal concentration gradient from baseline iodine present in soil, irrigation water, and other natural deposition sources. Phloem-mediated transport of iodine has been observed in plant species such as tomato and lettuce, and this decreasing gradient from roots to leaves has been observed in most other species studied (Caffagni et al., 2012; Gonzali et al., 2017; Landini et al., 2011; Smoleń et al., 2014; Weng et al., 2008). It has been suggested that iodine follows the transpiration stream since it is often higher in leaf tissue than in fruit, and older yellow cabbage leaves had lower iodine concentration than green leaves, but there has yet to be any direct evidence to support this (Budke et al., 2021; Weng et al., 2009). In both cases, a differential between plant organs could occur for a multitude of reasons not solely transpiration related.

In almond and pistachio, a variable leaf age iodine analysis would need to be done to see if there is canopy segmentation of iodine. Solely based on the low standard deviation seen in iodine leaf samples, this seems unlikely, but these samples were aggregations of multiple leaves collected around the radius of a tree and thus might have averaged out any variability (Table 6). Conducting a study ranking individual leaf characteristics such as age, sun exposure, relationship/distance to fruit. etc. and analyzing them individually for iodine would be beneficial to understand this natural variation. This variation is also worth exploring between a variety of species as the mean concentration of leaf iodine was significantly higher for avocado when

compared to almond and pistachio. The avocado trial was geographically distinct from the almond and pistachio trials and subjected to different conditions such as coastal fog. It is unknown if this differential in plant iodine concentration is due to species specific characteristics or the specific growing conditions and iodine availability.

Table 6: Iodine content in leaf tissue samples

	mean µg/kg	sd	df
almond Leaves	8.67	1.82	32
pistachio Leaves	6.54	0.79	50
avocado leaves	42.43	13.38	22

2.4.5 Inorganic antioxidant - Foliar application timing

The efficacy of stress mitigation strategies hinges on appropriate timing, which is often unpredictable in an agricultural setting. Proactive interventions are ideal, as they can prevent stress-induced damage entirely. Foliar iodine application at the early onset of bloom aims to preemptively address potential stress periods. For iodine, whose stress mitigation mechanisms remain unclear, application near the onset of stress is a logical approach to potentially enhance antioxidant activity.

This hypothesis assumes that plants use iodine as an inorganic antioxidant in a similar way that kelp accumulate iodine during periods of low stress and utilize iodine during high stress, or that an application of iodine functions as a stress primer for future stresses (Küpper et al., 2008). However, research on iodine as a stress mitigator in plants typically introduces it concurrently with stress, not preemptively, and do not include an iodine alone treatment (Blasco et al., 2013; Gupta et al., 2015; Kiferle et al., 2022; Leyva et al., 2011). These studies showed that iodine elicits oxidate stress on plant cells at higher concentrations and in certain forms, and

iodine treated plants have increased activity of key antioxidant enzymes like superoxide dismutase, ascorbate peroxidase, and catalase. Without an iodine only treatment in prior studies, it is unclear if these increases in enzymes are in response to iodine itself, or the stress treatment.

Differential growth responses have been observed with the addition of iodine at the time of stress, but responses are not consistent between species or growth systems. This variable response suggests that species-specific relationship with iodine is important, and that the benefit is not universal. The benefits of iodine are linked to its presence at the appropriate time for two very different mechanisms and further works needs to be done to ensure applications are timed appropriately.

2.4.6 Shifting bloom in almonds

Iodine was demonstrated to synchronize bloom in arabidopsis by Kiferle et al. (2021) through a theorized inorganic antioxidant effect translatable to other species. A synchronization in almond bloom could be either a beneficial or detrimental effect on almond fruit set depending on the year due to the complex blooming dynamics of almond. In years with short weather windows for bloom, a more uniform bloom could enable higher pollination to occur during ideal weather windows. In the case of mismatched emergence in single-field varieties, iodine could be used to better synchronize overlap with the main variety. However, a more synchronous bloom could also be detrimental as it decreases the length of bloom and thus concentrates the flower emergence to a shorter period; potentially resulting in the majority of bloom occurring during unfavorable bloom weather. The concentration of bloom might also saturate the number of available pollinators leading to reduced pollination unless hives/ha was increased to compensate. Though iodine applied as a bloom synchronization agent might not be beneficial, understanding

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if a similar response as seen in arabidopsis would help in understanding the role of iodine in plants.

In these trials, iodine did not have a consistent impact on almond bloom as it did in arabidopsis. In the spur trial, there appeared to be a slight shift towards earlier bloom as well as a trend of ~5% increase in fruit (Figures 10, 11, and 15). However, neither of these trends were corroborated by the data from the whole-tree study (Figure 18 and Figure 21). Almond spurs have naturally high variability in bud number and flower timing, and low, often zero, nuts per spur. This introduces data sparsity issues and low census bias not able to be overcome by increasing the sample size. This high variance and low census number mean statistically it would be impossible to identify a $p < 0.05$ significant result of a treatment measured daily and with minimal expected time shift. There may indeed be a shift in bloom timing, but natural variance prevents divergence of treatment means given the trial design.

In arabidopsis, Kiferle et al, (2021) found that iodine treated plants reached 100% bloom around nine days earlier than the control. This level of bloom shift would likely be detectable in an almond orchard, but the slight 1-2 day shift observed in iodine treated spurs was not statistically significant (Figure 10). The error bars in the arabidopsis study indicate a highly synchronous bloom in that trial that is also not replicable in almond given its natural variance. This may be a species-specific difference, or a challenge associated with replicating growth chamber studies in the field. In both cases, the lack of response seen in iodine treated full trees demonstrate that a meaningful shift in almond bloom is unlikely to occur with the low-rate foliar application used in this trial (Figure 18).

2.4.7 Remote sensing for treatment differentiation

Conducting trials across multiple regions is only made possible through remotely sensed data. This data is valuable for monitoring large areas, but also has important limitations. The greatest limitation is the ability to sample at appropriate temporal and spatial resolution. Studying the shift in bloom dynamics using remotely sensed data provided important insight into the treatments in this trial, but hand counted spur data showed a far more complicated pattern than overall bloom seen remotely (Figure 9 and Figure 18). In almond, individual spurs are extremely variable and have tertiary buds that may or may not bloom depending on the success of primary buds. Trees are complex and 3-dimensional with historical cropping loads in different locations of the canopy as well as differential sunlight affecting each part; all of which influence the productivity of each spur. The challenges of hand counting almond blooms involves selecting a representative branch with a representative quantity of flowers to count. Each branch selected has complex source/sink dynamics that can easily dilute any effects seen between different trees. Aerial imagery of whole tree bloom provides greater understanding of a full tree's bloom rather than a just a spur or branch. Bloom characteristics are theoretically more reliable predictors of overall yield, but due to insect pollination and many other factors, successful bloom may not relate to final yield. Yield does, however, require a successful bloom. Any improvement to bloom and set enhances the possibility of improved yield. Yield is a complex nexus of many different factors each with overlapping impacts on ultimate yield.

Using monthly aerial imagery to compute NDVI and NDRE indices is helpful to understand tree status throughout the growing season and to determine if there were treatment effects. Aerial imagery has been shown to be effective in phenotypic differentiation in many breeding programs and this principal was applied to treatment differentiation in these trials (Rabab et al., 2021). These vegetation indices are indirect measurement tools of plant response to

stress, and therefore, any difference would need additional study to determine the cause. Iodine as a stress-reducing element would likely influence these indices as trees would be more capable of tolerating heat events and other stressor such as saline soils and pest and disease pressure. All prior work of iodine as a stress mitigation antioxidant made direct measurements of antioxidant production and other stress indicators, but also of indirect effect such as seed size and plant dry weight (Blasco et al., 2013; Gupta et al., 2015, 2016; Kiferle et al., 2022; Leyva et al., 2011). No author relied on vegetation indices, but the plant effects observed indicate that chlorophyll production and overall plant size was often influenced and theoretically would be distinguishable with a vegetation index. No difference in treatments was observed in NDVI or NDRE throughout the growing season in response to iodine applied in any of the treatments (Figure 24). In a perennial orchard system, there is a possibility that the benefits of iodine are only seen after multiple seasons of use. In almond, spur fruit set is negatively correlated with leaf area with higher yielding spurs having less leaf area and also decreased nitrogen concentration (Saa et al., 2017; Tombesi et al., 2015). No differences were observed in yield or in vegetation indices, suggesting iodine did not improve in-season plant status (Figure 21 and Figure 23). This indicates that a delayed benefit enhancing production the following year is unlikely.

2.5 Conclusions

This study found no impact of exogenous iodine when provided during bloom or as a beneficial element through fertigation during the growing season in almond, pistachio, or avocado. Trial applications did not supply enough iodine to increase concentrations in any measurable tissues beyond background level which could be responsible for the lack of results. There was no measurable impact of exogenous iodine on plant leaf nutrient content, yield, or NDVI throughout two growing seasons in almond and pistachio and a single growing season in

avocado. In these trials, low-rate exogenous iodine applied as an agricultural benefit ultimately showed no increase in yield.

In almond foliar trials, iodine was not found to be an influential antioxidant when applied at pink bud, but that does not mean it could not work in other circumstances or at other rates. It was also not demonstrated to shift bloom progress forward as was done in arabidopsis. In arabidopsis, it was theorized that because plant biomass production increased in iodine-treated plants in addition to an earlier flowering time, iodine had a "flowering-promoting" effect (Kiferle et al., 2021). However, it is possible that since this original study was done using separate growth chambers with each tray treated as a biological replicate, slight shifts in growing conditions of each chamber could have been responsible for this shift. Additionally, a significant change in plant biomass seen in arabidopsis could have influenced the bloom dynamics in those plants that would not be seen in a perennial orchard crop like almonds. Theoretically reducing reactive oxygen species during bloom should be beneficial, but it has yet to be demonstrated that iodine is capable of doing so in flowers.

The production of methyl iodide is an important factor to consider when studying the movement and allocation of iodine in plant tissues. Current analyses have treated iodine as a mobile element, but failed to account for volatilization that likely alters plant tissue concentrations. A low concentration in a tissue has been viewed as a low preferential movement of iodine. However, this was found not to be the case in rice that had large movement of iodine with proportional volatilization leading to low concentrations in plant tissues (Kato et al., 2013). Other agronomic species have demonstrated similar abilities to volatilize iodine, and the increased study of this ability could drastically alter our current understanding of iodine in

plants. This study made no measurement of methyl iodide production, but given the potential for iodine loss, it should be considered in future work.

In conclusion, there was found to be no agronomic benefit of applying low rates of iodine as fertigation in these three crops, or as a foliar antioxidant treatment in almonds during bloom. This study was the first iodine study in these perennial orchard crops, and one of only a few in commercial production field conditions for any species. Most iodine studies to date have been conducted in controlled environments and the lack of significant results seen in this study indicate that additional study is needed to translate prior work to the field environment. Maintaining soil iodine at concentrations equivalent to those used in hydroponic studies requires a large amount of iodine in orchard settings and a possible alternative application method might need to be explored given the dynamic nature of soil. Foliar application of iodine to green leaf tissue in-season could be a potential alternative for supplying iodine, but additional work is needed to determine appropriate timing and rates.

2.6 Supplementary Documents

Supplementary Data 1: Photo of hail stone from March 13th 2023 hailstorm at KG ranch that removed the majority of flowers in the orchard.

Supplementary Data 2: Results from exploratory soil samples to assess iodine concetration in California agricultural soils.
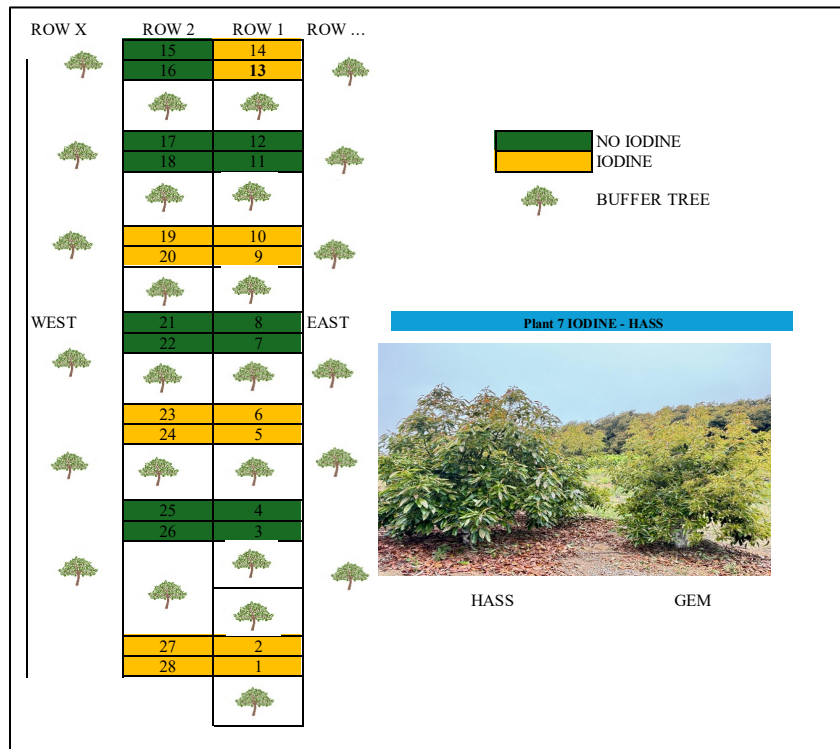
Supplementary Data 3: Layout of avocado field trial near Temecula, Califorrnia.
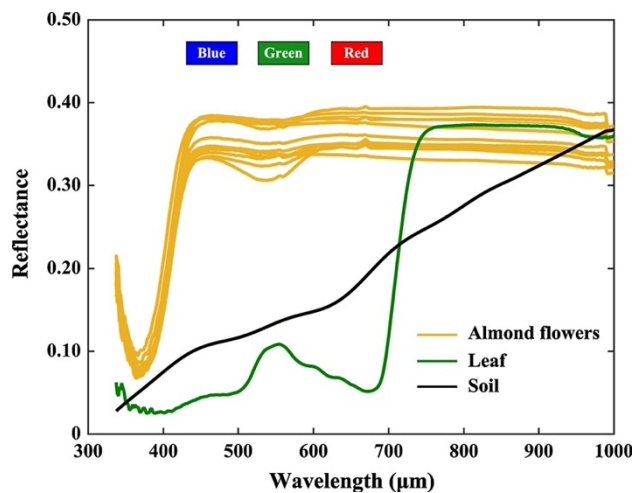
Supplementary Data 4: Figure from "An enhanced bloom index for quantifying floral phenology using multi-scale remote sensing observations" (Chen et al., 2019)

Table 1
Description of the phenological growth stages of avocado (*Persea americana* Mill.) according to the BBCH scale.

BBCH code	Description (mesostage 1)
Principal growth stage 0: vegetative bud development	
010	Vegetative buds dormant
011	Beginning of bud swell
013	End of bud swell
017	Beginning of bud break
019	End of bud break
Principal growth stage 1: primary leaf expansion	
110	First leaves separating
111	First leaf unfolded
112	More leaves unfolded. First leaf at 20% of its full size
113	More leaves unfolded. First leaf at 30% of its full size
11.	Stages continue until . . .
119	All leaves unfolded and fully expanded
Principal growth stage 2: axillary (sylleptic) shoot formation	
210	No sylleptic shoots visible
211	First sylleptic shoot visible
212	Two sylleptic shoots visible
213	Three sylleptic shoots visible
21.	Stages continue until . . .
219	Nine or more sylleptic shoots visible
Principal growth stage 3: primary shoot extension	
310	Beginning of shoot extension
311	10% of final shoot length
312	20% of final shoot length
31.	Stages continue until . . .
319	90% or more of final shoot length
Principal growth stage 5: reproductive development	
510	Reproductive buds dormant
511	Beginning of reproductive bud swell
512	End of reproductive bud swell
513	Reproductive bud break
515	Inflorescences 50% of final length
517	Inflorescences 70% of final length
519	End of inflorescence extension
Principal growth stage 6: flowering	
610	First flowers open
611	10% of flowers opened
612	20% of flowers opened
61.	Stages continue until . . .
619	90% or more of flowers opened
Principal growth stage 7: fruit development	
710	No ovary growth visible
711	Initial ovary growth
712	First fruitlet abscission
715	50% of final fruit size
71.	Stages continue until . . .
719	90% or more of final fruit size

Supplementary Data 5: Description of scale used to assess avocado bloom progression From (Alcaraz et al., 2013)

Supplementary Data 6: UV/Vis Spectrophotometric Method for Detection of Iodine in Fertilizer based on Silva et al., 1997

1. **Preparation of Reagents**:
   o Solution 1:Phosphoric acid
      1. 11.5g phosphoric acid (85%) up to 100mL to make 1.0 mol L^{-1}
   o Solution 2: Potassium Iodide
      1. 3.32g of potassium Iodide plus 1mL of 0.1 mol L^{-1}Sodium Hydroxide to make 100mL
   o Solution 3: Potassium Iodate: To make a solution with 100mg/L of Iodine
      1. Add 168.5mg Potassium Iodate to 100ml water
      2. Add 1ml of that to 9ml of water for 10x dilution
   o Solution 4: Potassium Nitrate Blank solution (Ultrasol)
      1. 5g of fertilizer in 100ml
2. **Preparation of UV Calibration Curve**
   o Standards: .1,1.0,2.5,5.0,10.0 mg I/L
      1. 1ml Solution 2 Potassium Iodide
      2. 2ml of Solution 4 Potassium Nitrate Blank
      3. 1ml Solution 1 Phosphoric acid
      4. Corresponding amounts of solution 3
         1. 1: 0.1ml +9.9ml H$_2$O
         2. 10: 1ml+9 ml H$_2$O
         3. 25: 2.5ml+7.5 ml H$_2$O
         4. 50: 5ml+5ml H$_2$O
         5. 100: 10ml
   o Total volume should be 14ml
   o Then dilute 10x for curve
3. **Procedure**:
   o **For Samples based on volume of Cuvettes**
      1. 1 ml Solution 1:Phosphoric acid
      2. 1ml Solution 2: Potassium Iodide
      3. 2ml sample solution
         1. Solution will turn yellow. Store in dark area for 10 minutes for reaction to occur
      4. Dilute 10x and analyze
4. **Measurement**:
   o Measure the absorbance at 288nm using the UV/VIS spectrophotometer (Silva et al., 1998). Make sure to have correct cuvettes for wavelength. Acrylic or UV-transparent required
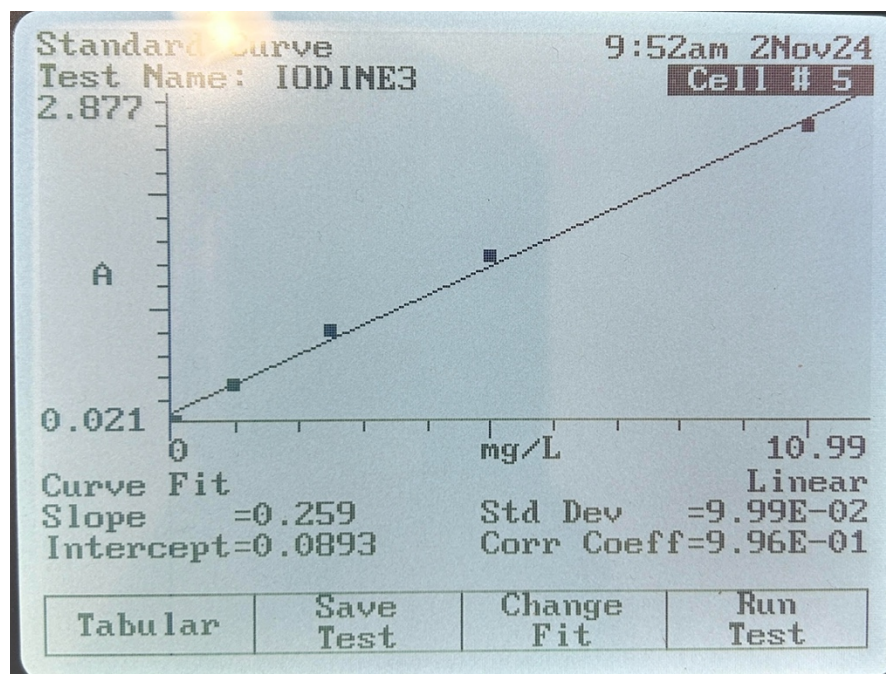
Supplementary Data 7: Standard curve from Thermo Scientific GENESYS 10 UV-Vis Spectrophotometers for detection of iodine in fertilizer.

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