Title
Influence of California Tree Nut Orchard Management Practices on Herbicide Residues in Soil

Author
Martin, Katie

Publication Date
2022

Peer reviewed|Thesis/dissertation

Influence of California Tree Nut Orchard Management Practices on Herbicide Residues in Soil

By

MARY CATHERINE MARTIN

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural and Environmental Chemistry

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Bradley D Hanson, Chair

Matt Hengel

Kassim Al-Khatib

Committee in Charge

2022

Acknowledgements

There are so many people who made this degree possible. First, I would like to thank my advisor, Brad Hanson, for taking me on as a student. Working in a challenging, positive, and interdisciplinary environment has made me a better scientist. I am proud of the work I produced during my time in Brad's lab and I could not have done it without his encouragement, mentorship, and countless hours of editing. I would also like to thank professors Matt Hengel and Kassim Al-Khatib for serving on my thesis committee and providing constructive feedback.

My lab members, turned lifetime friends, were some of my biggest cheerleaders throughout the last five years. This group spent many hours helping me process almonds, extract soil samples, and set up field trials. They believed in me when maybe sometimes I didn't believe in myself, they are truly fantastic people.

My biggest pillar of support has been my family and friends. I could not have done this without them. My family encouraged me to chase my dreams of pursuing my PhD at UC Davis during a time that was difficult for us all, their unwavering support was a selfless act of love. I want to give a special thank you to my mother, Peggy Martin, who always encouraged me to pursue education.

This dissertation is dedicated in loving memory to my father, Stan Martin. Although he was not able to see me on this journey, I know he would be proud.

Abstract

California is nation's leader in tree nut production. Weed control in orchards is essential for production and the most common form of weed control in commercial production is the use of herbicides. How growers manage their orchards, and weeds, can have an impact on herbicide residues in the environment. This work aims to answer questions that intersect environmental chemistry, weed science, and orchard management practices. We hypothesized that orchard management practices would influence herbicide residue in soil and herbicide transfer to almond kernels. Chapter 1 examines how irrigation water pH and salinity influence the partitioning of three weak acid herbicides between soil and soil solution. A modified method of a traditional K_d experiment was used to quantify herbicide concentrations eluted from treated soil after a series of flushes. We demonstrated that pH and salinity did not have a significant effect on the partitioning of saflufenacil, indaziflam, or penoxsulam out of soil. Chapters 2 and 3 focus on herbicide transfer to almonds via herbicide-bound on soil particles. Low levels of glyphosate and glufosinate residues in almonds have become a concern to the commodity board, growers, and chemical companies because of changing regulations and low maximum residue limits in kernels. Over the course of two field seasons, we measured herbicide residue in unharvested almond fractions, harvested almond fractions, and soil at various timepoints in the field before and during almond harvest as well as at the huller/sheller processing facility. The data addresses the questions of whether residues were coming from herbicide residues in the almonds or from herbicide on soil particles on almonds. It was established that herbicide treated soil could contribute to residues on almonds by being loosely attached to the almond (i.e., dust on almonds). We discovered that a glufosinate metabolite, MPP, is a major contributor to glufosinate residue in almonds and is of concern to the maximum residue limit in processed

almond exports to the European Union market. Furthermore, the metabolite was found in unharvested almonds, sampled directly from the tree without contact with soil, at surprisingly elevated levels which leads us to suspect that the translocation of the glufosinate metabolite into the tree is contributing to residue levels. Glyphosate and its common metabolites were not found in kernels at the end stage of processing.

Table of Contents

Introduction

California produces 99% of the nation's almonds, walnuts, and pistachios which contributed over $9.3 billion to the United States agricultural economy in 2019, these nuts are in the top 10 most valuable commodities in the state (CDFA 2020a). Tree nut exports contribute significantly to the California Gross Domestic Product, almond exports alone generated $4.9 billion in 2019 (CDFA 2020b). Tree nut orchards are planted on nearly 750,000 hectares in California; almonds account for 650,000 hectares (CDFA 2020a).

Weed control in orchards is an essential part of tree nut production because weeds can compete for resources with young trees, serve as a habitat for other orchard pests, interfere with irrigation, and hinder harvest operations (Roncoroni et al. 2019). There are various ways to control weeds in orchards such as mowing, flaming, and weeder animals like ducks and goats, but by far the most common form of weed control in commercial orchards is chemical control using herbicides (Connell 2001, UCANR 1996 & 2002). In 2018, nearly 3 million total hectares of California tree nuts were treated with herbicides, this means most orchards are treated with herbicide multiple times a year (CDPR 2018).

The frequent use of herbicides in orchard systems creates a space for questions surrounding food, crop, and environmental safety. Typically, tree nut orchards receive an application in the winter while the trees are dormant, in the spring, and an application before harvest (UCANR 1996 & 2002). Orchard herbicides can be preemergent, meaning they are applied ideally before seed germination, or postemergent, meaning they are applied after the seed has germinated and become a seedling (Stephenson et al. 2006). Preemergent herbicides have soil activity, therefore, it is important to consider application timing and irrigation events so the herbicide can move into the weed germination zone but not further down into the crop root zone

(Roncoroni et al. 2019). For the most effective control, postemergent herbicides are applied when the weed is small and actively growing (Roncoroni et al. 2019).

In the California Central Valley, irrigation water comes from combination of groundwater and surface water (Faunt et al. 2009). In years of drought, surface water supplies are reduced and there is a greater reliance on groundwater resources for orchard irrigation to maintain crop health and productivity. Groundwater quality is dependent upon the region which it is drawn from and where it is drawn within the water table (Faunt et al. 2009). Irrigation water quality parameters such as pH, salinity, absorption ration (SAR), and ion toxicity have a great effect on crop production by limiting water availability at the root zone, reducing infiltration, and causing nutrient imbalance (Ayers and Wescot 1985). Information on how water quality affects the crop is abundant but there is space to explore how water quality influences herbicide partitioning into soil solution, particularly ionizable herbicides.

The purpose of the preharvest burndown herbicide treatment is to remove as much vegetation as possible to allow the harvest equipment to move cleanly and efficiently through the orchard, usually the treatment is done with one or more broad-spectrum herbicides relatively close to the planned harvest date (Connell 2001, UCANR 1996 & 2002). As harvest equipment navigates through the orchard the top layer of soil on the orchard floor is disturbed by the equipment, generating dust. Every year there are detections of herbicide residues above maximum residue limits in almonds and it is hypothesized that interactions between freshly harvested almonds and recently treated soil may be contributing to these residues.

Orchards are a complex system and there are moving parts to make the system work effectively and efficiently. Because herbicides are applied over the entire course of the growing season, herbicide use intersects with irrigation events and harvest operations. This body of work

focused on the influence of these orchard management practices on herbicide residues in soil and almonds.

References

Ayers RS, Wescot DW (1985) Water quality for agriculture. Rome (IT): Food and Agriculture Organization of the United Nations. Available from: https://www.fao.org/3/t0234e/t0234e00.htm

[CDFA] California Department of Food and Agriculture (2020a) California Agricultural Production Statistics 2019 Crop Year Report. 4 p. https://www.cdfa.ca.gov/Statistics/PDFs/2020_Ag_Stats_Review.pdf. Accessed August 18, 2021

[CDFA] California Department of Food and Agriculture (2020b) California Agricultural Exports 2019-2020. 6-9 p. https://www.cdfa.ca.gov/Statistics/PDFs/2020_Exports_Publication.pdf. Accessed August 18, 2021

[CDPR] California Department of Pesticide Regulation (2018) 2018 Annual Statewide Pesticide Use Report Indexed by Commodity. https://www.cdpr.ca.gov/docs/pur/pur18rep/statewide_site_2018.htm. Accessed September 26, 2021

Connell JH, Colbert F, Kreuger W, Cudney D, Gast R, Bettner T, Dallman S (2001) Vegetation management options in almond orchards. HortTechnology 11(2):254-257. https://doi.org/10.21273/HORTTECH.11.2.254

Faunt CC, Belitz K, Hanson RT (2009) Groundwater availability in California's Central Valley. In:Faunt CC, editor. Groundwater availability of the Central Valley Aquifer, California. Reston (VA): U.S. Department of the Interior and U.S. Geological Survey; 2009. p. 59-116. Available from: https://pubs.usgs.gov/pp/1766/PP_1766.pdf

Roncoroni JR, Hanson BD, Hembree KJ (2019) Weeds. In: DeBiase R, Rosa F, editors. Almond pest management guidelines for agriculture. Richmond (CA): University of California Agriculture and Natural Resources, 110-140 p.

Stephenson GR, Ferris IG, Holland PT, Nordberg M (2006) Glossary of Terms Related to Pesticides. Pure Appl. Chem. 78(11): 2075-2154 p. https://doi.org/10.1351/pac200678112075

[UCANR] University of California Agriculture and Natural Resources (1996) Almond Production Manual. Oakland, CA: Reagents of the University of California Division of Agriculture and Natural Resources

[UCANR] University of California Agriculture and Natural Resources (2002) Integrated Pest Management for Almonds. 2nd edition. Oakland, CA: Regents of the University of California Division of Agriculture and Natural Resources, 160-166 p

Evaluation of Irrigation Water Salinity and pH on the Partitioning of Saflufenacil, Indaziflam, and Penoxsulam in Soil

Formatted for *Pest Management Science* (submitted)

Abstract

Background

Ongoing drought in California has resulted in greater reliance on groundwater for irrigation in many areas of California's Central Valley. The quality of groundwater can vary among regions and where it is drawn from within the water table. Salinity and pH are two water quality parameters that can be affected by changes in the water table levels; these parameters can also affect herbicide partitioning in the soil solution. Saflufenacil, indaziflam, and penoxsulam are three weak acid herbicides commonly used in orchard crops whose leaching potential could be affected by changes in salinity and pH which could, in turn, affect weed control efficacy, orchard crop safety, and environmental quality.

Results

Herbicide-spiked soil samples were extracted with different saline and pH water treatments then analyzed using high performance liquid chromatography with a diode array detector. Increasing water salinity slightly decreased herbicide partitioning in saflufenacil- and penoxsulam-treated soil samples. About 70% of saflufenacil and 25% of penoxsulam was removed by flushing soil with a low salinity water treatment and this decreased to 65% and 22%, respectively, when treated with water adjusted to moderate to high salinity. Surprisingly, increasing pH also decreased saflufenacil and penoxsulam partitioning but this is likely due to degradation of the

herbicide by alkaline hydrolysis. Indaziflam was not detected in any water sample extracted from treated soil.

Conclusions

The salinity and pH of irrigation water does not drastically affect the partitioning of the weak acid herbicides saflufenacil, indaziflam, and penoxsulam in orchard soil conditions.

Keywords: salinity, pH, irrigation, saflufenacil, indaziflam, penoxsulam

1. Introduction

California produces 99% of the nation's almonds, pistachios, and walnuts which contributed over $9.3 billion to the United States agricultural economy in 2019[1]. Most tree nuts in the state are grown in the Central Valley, a region with a Mediterranean climate receiving 12.7 to 50.8 cm of annual precipitation[2], primarily during winter. Almond, pistachio, and walnut crops have an average water requirement of 0.49-, 0.41-, and 0.41-hectare meters of water per year, respectively[3-5] which is mostly met with irrigation during the growing season.

Irrigation water in the Central Valley comes from a combination of surface water distributed from reservoirs that impound winter runoff and snowmelt and from groundwater sources. In years of drought, surface water allocations may be curtailed which, in turn, leads to greater reliance on groundwater for orchard irrigation to maintain short-term crop productivity and long-term orchard health. Groundwater quality can vary among regions of the Central Valley and often is dependent upon where it is drawn from in the water table. In particular, some areas have substantial depth-related differences in salinity and pH[6]. Irrigation water quality plays an important role in crop safety and crop yield. Factors such as salinity, sodium absorption ratio (SAR), ion toxicity, and pH can drastically affect crop production by limiting water availability at the root zone, reducing water infiltration, damaging the crop, and causing nutritional imbalance[7]. The Food and Agriculture Organization of the United Nations have set guidelines for these parameters; a modified summary of salinity and pH guidelines is presented in Table 1.1.

Weed control in orchard systems is an essential part of pest management as weeds can compete for resources, interfere with irrigation, serve as a habitat for pests, and disrupt harvest operations[8]. Chemical control with herbicides is the most common form of weed control in

commercial tree nut production systems and applications of herbicide often occur multiple times throughout the growing season.

Saflufenacil, indaziflam, and penoxsulam (Figure 1.1) are three weakly acidic herbicides commonly used in orchard herbicide programs[9-10]. In 2018, a cumulative total of 485,000 tree nut orchard hectares were treated with saflufenacil, indaziflam, or penoxsulam[11]. Weak acid herbicides are partially ionized within the normal range of soil pH, and this affects their reactivity and partitioning between the soil surface and soil solution[12]. The pKa of saflufenacil, indaziflam, and penoxsulam is 4.41, 3.5, and 5.1 respectively[13-15]. As the pH of the soil or soil solution nears the pKa of the herbicide, more of the herbicide will be in its neutral form which could promote binding to the negatively charged soil surface[12]. Irrigation delivery via sprinklers, microsprinklers, or drip lines typically overlap with areas of the field that have been treated with herbicides. Water with high salinity or pH could lead to transient changes in herbicide-soil interactions in surface soil causing concerns about herbicide performance and environmental fate of the herbicides.

The influence of soil properties on herbicide efficacy has been widely studied as well as the influence of spray water quality on herbicide performance. However, limited studies on the effects of irrigation water quality on herbicide dissipation have been completed. This study was conducted to evaluate the effects of water pH and salinity on the dissipation of saflufenacil, indaziflam, and penoxsulam in two representative California orchard soils.

2. Materials and Methods

2.1 Soil

Two soils were obtained from different locations in California. Yolo silt loam[16] was collected from the University of California, Davis Department of Plant Sciences Field Research Facility in Davis, CA (38.54°N, 121.79°W). The loam soil had a bulk density of 1.08 g $cm^{3\ -1}$, field capacity of 22.7%, pH of 7.90, and 1.62% organic matter. Delhi sand[16] was collected from Delhi, CA (37.43°N, 120.68°W). The sand soil had a bulk density of 1.22 g $cm^{3\ -1}$, field capacity of 12.8%, pH of 6.55, and 1.00% organic matter. Both soils were sifted using a 2 mm sieve before experimental use.

2.2 Water Treatments

Hydrochloric acid, sodium hydroxide, sodium chloride, calcium chloride, magnesium sulfate, and sodium bicarbonate were all purchased from Sigma Aldrich.

The range of pH values in irrigation water in California span from the extremes of pH 5 to pH 8[17]; four water treatments were made within this range. To make water treatments of pH 5, 6, 7, and 8, 0.1 M hydrochloric acid and 0.1 M sodium hydroxide was added dropwise to deionized water until the desired pH was reached. The pH of each solution was measured using a Pinnacle 530 pH meter (Corning Inc., 275 River St., Oneota, New York, United States).

Salinity values were chosen to span the low, medium, and high EC_w values found in irrigation water[7]. Saline water treatments of EC_w 0.5, 1.5, and 3.5 dS m^{-1} were made by adding a combination of salts to deionized water. The amount of salts added were determined based on the equation: total dissolved solids (mg L^{-1}) = EC_w (dS m^{-1}) * 640, the requirement of the SAR to be less than 3 for the given range of salinity treatments, as well as the concentration of the

individual salt ions to be within a normal range as defined by the FAO Irrigation Water Quality Guidelines[7]. The salt mixture of each treatment was composed of 40% sodium chloride, 30% calcium chloride, 20% magnesium sulfate, and 10% sodium bicarbonate to fulfill the requirements described above.

2.3 Herbicide Solutions

The three active ingredients used in this study were saflufenacil (applied as Treevix®)[18], indaziflam (applied as Alion®)[19], and penoxsulam (applied as Pindar® GT)[20]. The herbicide concentration of saflufenacil, indaziflam, and penoxsulam applied to soil was 12.25 ppm, 17.39 ppm, and 13.75 ppm, respectively. All herbicide solutions were made using deionized water and the appropriate amount of commercial product; the herbicide rate was calculated based on a field rate applied to 25 cm^2 spray area but in a 1 mL carrier volume to ensure even distribution of chemical.

2.4 Experimental Setup

The experimental protocol is a modified version of the method published by Sheppard et al.[21]. The experiment was a completely randomized design with each herbicide being tested in both soil types at every water treatment and replicated three times. The amount of soil used in this experiment was determined by the bulk density of the soil and the assumption of a 25 cm^2 spray area and a 2 mm soil depth. Each loam experimental unit contained 5.4 g of soil and each sand replicate contained 6.1 g of soil.

Soil was first treated with herbicide by weighing appropriate amounts of each soil into a weigh boat, pipetting 1 mL of herbicide solution onto the soil, homogenizing by vigorous

mixing, then letting the mixture sit for 24 hours until completely dry. The treated soil was then transferred into a 50 mL centrifuge tube equipped with a 0.22 µm Nylon filter (Thermo Scientific, 168 Third Ave., Waltham, Massachusetts, United States). Soil was brought to field capacity by adding 1.220 mL of water treatment to loam soil or 0.780 mL of water treatment to sand soil, covered with parafilm, and left in the dark, at room temperature (21°C) for seven days.

After the resting period, the parafilm was removed and the samples were centrifuged at 6000 m s^{-1} for 15 minutes using a Sorvall Legend XTR centrifuge (Thermo Scientific, 168 Third Ave., Waltham, Massachusetts, United States). After the initial centrifugation, an additional 1 mL of the respective water treatment was added to each sample then samples were centrifuged again at 6000 m s^{-1} for 15 minutes. This process was repeated once more for a total of two 1 mL water treatment aliquots washed over every sample after the field capacity water was removed. A separate pilot study completed to establish the number of water treatment washes needed to remove the unbound herbicide from the sample indicated that two 1 mL washes was adequate for the purpose of the experiment (Supplemental Figures S1.1).

The centrifuge filter was removed and discarded while all water from initial incubation plus the two 1 mL aliquots were collected from the centrifuge tube and filtered using a 0.22 µm Nylon syringe filter. The filtered solution was collected in an HPLC vial and analyzed using high performance liquid chromatography (HPLC).

2.5 Sample Analysis

Analyses were performed with an Agilent C-18 Poroshell 120 column (2.1 x 100 mm x 2.7 µm) in an HPLC system (1220 Infinity LC, Agilent Technologies, Santa Clara, California,

United States) equipped with a diode array detector. Mobile phase A consisted of ultrapure water and mobile phase B consisted of acetonitrile with 0.1% formic acid. Chromatography was accomplished using an isocratic elution of 60% mobile phase A and 40% mobile phase B. The method run time was 9 minutes. All samples were observed at 270, 268, and 205 nm which corresponded to the absorbance of saflufenacil, indaziflam, and penoxsulam, respectively[13-14,22]. The approximate retention time of saflufenacil, indaziflam, and penoxsulam were 4.9, 2.5, and 2.6 minutes, respectively (Supplemental Figures S1.2-S1.4). Samples were background corrected and converted into units of percent removal from soil using 5-point calibration curves (Supplemental Figures S1.5).

2.6 Statistical Analysis

The experimental data were subjected to ANOVA using R statistical analysis software (2020) and multiple comparisons were performed with Tukey's HSD with $\alpha = 0.05$.

3. Results

3.1 Soil Type Effects

Statistical analysis of the data revealed that soil type was not a significant factor in the amount of herbicide recovered from the samples (data not shown) and therefore, the loam and sand data were pooled for each herbicide/water treatment combination, resulting in a sample size of n=6.

3.2 Water pH

It was hypothesized that, as the water treatment pH increased, there would be greater removal of herbicide from the soil in the rinsate. As pH of the solution increased, the equilibrium of the weak acid herbicide would be pushed towards the anionic herbicide form resulting in lower sorption to the soil and greater removal in the rinsate. However, the opposite trend occurred for two of the herbicides (Figure 1.2). As water treatment pH increased, the concentration of saflufenacil and penoxsulam decreased in the aqueous solution extracted from the soil. Saflufenacil removal in the rinsate was greatest at pH 5 with about 78% and lowest at pH 8 with about 64%. At pH 5, about 35% of the penoxsulam was removal from soil and this decreased to about 22% removal at pH 8 (Figure 1.2). Indaziflam was below the detection limit of the instrumentation in all rinsate samples, regardless of pH.

3.3 Water Salinity

The results of the EC water treatments show that, as EC increased, herbicide removal decreased slightly (Figure 1.3). The effect of ionic strength on ionizable pesticide adsorption to soil has been well documented[12]; the common trend is that as ionic strength increases, the pesticide adsorption also increases (to a point). These data support that trend as well. The greatest amount of saflufenacil and penoxsulam was removed from soil in the 0.5 dS m^{-1} water solution rinse; about 70% of saflufenacil was removed from soil and about 25% of penoxsulam was removed. Meanwhile, in the 1.5 dS m^{-1} and 3.5 dS m^{-1} solutions, approximately 65% of saflufenacil and 22% of penoxsulam was removed (Figure 1.3). Indaziflam was below the detection limit of the instrumentation in all rinsate samples regardless of ionic strength of the rinse solution.

4. Discussion

Indaziflam was below the detection limit in all samples; however, it is not clear if this is due to strong sorption to soil or to degradation processes. While indaziflam is considered moderately mobile to mobile in soil[14], it does have a higher K_{oc} range than saflufenacil or penoxsulam[23] meaning indaziflam would be more strongly sorbed to soil than the other herbicides in this study. Indaziflam has been reported to undergo photolysis in aqueous solutions rather quickly ($t_{1/2}$ less than five days)[14]; samples were stored in the dark for much of the duration of the experiment. A brief follow-up experiment confirmed the laboratory lights did not cause photolysis of the chemical in aqueous solution under the conditions of the experiments (Supplemental Table S1.6).

Saflufenacil dissipates relatively quickly in the environment[23]. The herbicide has biotic and abiotic degradation pathways but the most relevant pathway to this study would be hydrolysis in alkaline water[13]. The data set shows a significant decrease in herbicide removal from soil from pH 5 to pH 6 and 8 (Figure 1.2). The pH 7 data point was not statistically different from the other pH water treatments.

There have been differing reports on penoxsulam hydrolysis. The Environmental Protection Agency states that penoxsulam is stable under hydrolysis conditions[15] while Jabusch and Tjeerdema report triazolopyrimidine sulfonamide (TSA) herbicides do undergo hydrolysis and the rate is dependent on pH[24]. There have been studies completed on two other herbicides in the TSA class which support pH dependent hydrolysis rates[25-26]. Given that the experimental samples were held at field capacity for seven days in this study, pH dependent hydrolysis could explain why penoxsulam concentrations were decreasing from 34% removal from soil at pH 5 to 22% removal at pH 8 (Figure 1.2).

Adsorption mechanisms of pesticides are difficult to define because of the complex interactions between the soil surface, soil solution, and pesticide. Additionally, it is likely more than one adsorption mechanism occurs. There are several mechanisms by which weak acid pesticide adsorption could be positively influenced by ionic strength - cations could displace hydrogen atoms from the soil surface resulting in a slight pH decrease that would favor a neutral pesticide form, more cations could be available to bridge the anionic form of the pesticide to the negatively charged soil surface, or cations could bond with the anionic pesticide resulting in a neutral form[12].

A recent study on the adsorption-desorption properties of penoxsulam narrowed down the possible sorption mechanisms to H-bonding, cation bridging, and surface complexation with transition metals[27]. The data set presented here supports the cation bridging mechanism. As ionic strength of the water treatment was increased, cation concentration increased resulting in the greater likelihood to bridge the anionic form of penoxsulam to the negatively charged soil surface. Figure 1.3 shows no statistical significance between EC_w 1.5 dS m^{-1} and EC_w 3.5 dS m^{-1}, this likely indicates most of the available binding sites of the soil were occupied close to EC_w value 1.5 dS m^{-1}. Due to the similarity in size and ionizable functional group to penoxsulam, it is likely that saflufenacil is undergoing the same phenomena.

The water treatments representing different irrigation water quality parameters did have a slight effect on saflufenacil and penoxsulam sorption to soil. The pH treatments indicated that both herbicides likely experience pH-dependent hydrolysis; saflufenacil and penoxsulam showed a decreasing trend in herbicide removal with increasing pH, the opposite of what the hypothesized pH effect would be. This indicates that even if irrigation water has relatively high pH, it is unlikely to substantially change the availability or movement of saflufenacil or

penoxsulam in California orchard soils. Results from the EC_w treatments showed that flushing soil with a solution with moderate ionic strength could help saflufenacil and penoxsulam bind to soil versus low ionic strength. While there were statistically significant differences between water treatments, the overall effect on herbicide dissipation was minimal; the observed difference between the highest and lowest EC_w treatment was only about 10% for each herbicide.

5. Conclusion

In conclusion, irrigation water quality likely does not significantly affect leaching potential of saflufenacil, indaziflam, and penoxsulam within the larger scope of farming practices. While the experiment did not directly address weed control efficacy, the lack of differential effects of EC or pH treatments on herbicide availability of the three herbicides suggests there is likely little impact on weed control or crop safety. Although poor irrigation water quality can directly affect crop productivity as well as soil physical and chemical properties[7], it does not appear to have a significant impact on the fate of these weak acid herbicides used in tree nut orchard production systems. The minimal effects of water pH or salinity on these herbicides indicates that weed control efficacy, orchard crop safety, and risk of groundwater contamination would not be differentially affected by the quality of the irrigation water applied.

Acknowledgements

The authors would like to thank the California Department of Agriculture for funding this research (grant CDFA-SCBG #29380). Additional funding and support were provided by the Almond Board of California and the Pistachio Research Board.

Conflict of Interest

No conflicts of interest have been declared.

Works Cited

1 California Department of Agriculture. California agricultural statistics review 2019-2020. Sacramento (CA): CDFA;2020. 157p. Available from: https://www.cdfa.ca.gov/Statistics/PDFs/2020_Ag_Stats_Review.pdf

2 California Central Valley [Internet]. [place unknown]; American Museum of Natural History; 2021. Available from: https://www.amnh.org/learn-teach/curriculum-collections/grace/grace-tracking-water-from-space/california-central-valley

3 Schwankl L, Prichard T, Fulton A, Almond irrigation improvement continuum 2017. https://www.almonds.com/sites/default/files/2020-04/Almond-Irrigation-Improvement-Continuum.pdf [accessed December 12, 2021]

4 Goldhamer DA, Tree water requirements and regulated deficit irrigation. In: Ferguson L, Haviland DR, editors. Pistachio Production Manual. Richmond (CA): University of California Agriculture and Natural Resources; 2016. p. 103-116.

5 Fulton A, Evaluating requirements of developing walnut orchards in the Sacramento Valley. [place unknown]; California Walnut Board; 2013. p. 113-119. Available from: http://walnutresearch.ucdavis.edu/2013/2013_113.pdf

6 Faunt CC, Belitz K, Hanson RT, Groundwater availability in California's Central Valley. In: Faunt CC, editor. Groundwater availability of the Central Valley Aquifer, California. Reston (VA): U.S. Department of the Interior and U.S. Geological Survey; 2009. p. 59-116. Available from: https://pubs.usgs.gov/pp/1766/PP_1766.pdf

7 Ayers RS, Wescot DW, Water quality for agriculture. Rome (IT): Food and Agriculture Organization of the United Nations; 1985. Available from: https://www.fao.org/3/t0234e/t0234e00.htm

8 Roncoroni JR, Hanson BD, Hembree KJ, Weeds. In: DeBiase R, Rosa F, editors. Almond pest management guidelines for agriculture. Richmond (CA): University of California Agriculture and Natural Resources; 2019. p. 110-140.

9 Brunharo CACG, Watkins S, Hanson BD, Season-long weed control with sequential herbicide programs in California tree nut crops. *Weed Technol* **34**:834-842 (2020).

10 Moretti ML, Shrestha A, Hembree KJ, Hanson BD, Postemergence control of glyphosate/paraquat-resistant hairy fleabane *(Conyza bonariensis)* in tree nut orchards in the Central Valley of California. *Weed Technol* **29:**501-508 (2015).

11 California Department of Pesticide Regulation, 2018 Annual statewide pesticide use report indexed by commodity. Sacramento (CA): California Department of Pesticide Regulation; 2018. Available from: https://www.cdpr.ca.gov/docs/pur/pur18rep/statewide_site_2018.htm

12 Kah M, Brown CD, Adsorption of ionisable pesticides in soils. *Rev Environ Contam Toxicol* **188**:149-217 (2006).

13 United States Environmental Protection Agency, Pesticide fact sheet saflufenacil. Washington, D.C.: United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; 2009. 92 p. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-118203_01-Aug-09.pdf

14 United States Environmental Protection Agency, Pesticide fact sheet indaziflam. Washington, D.C.: United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; 2010. 108 p. Available from:

https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-080818_26-Jul-10.pdf

15 United States Environmental Protection Agency, Pesticide fact sheet penoxsulam. Washington, D.C.: United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; 2004. 11 p. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-119031_27-Sep-04.pdf

16 California Soil Resource Lab [Internet]. [place unknown]: University of Californian Davis; [date unknown]. Soil Web; [date unknown]. Available from: https://casoilresource.lawr.ucdavis.edu/gmap/

17 Cahn M, Evaluating salinity in irrigation water. Davis (CA): UC Vegetable Research and Information Center; [year unknown]. 6 p. Available from: https://vric.ucdavis.edu/pdf/irrigation/EvaluatingSalinityinIrrigationWater.pdf

18 BASF Corporation. Treevix® [herbicide label]. Triangle Park (NC): BASF Corporation; 2020

19 Bayer CropScience. Alion® [herbicide label]. St. Louis (MO): Bayer CropScience LP; 2019

20 Corteva Agriscience. Pindar® GT [herbicide label]. Indianapolis (IN): Dow AgroSciences LLC; 2020.

21 Sheppard S, Long J, Sanipelli B, Sohlenius G, Solid/liquid partition coefficients for selected soils and sediments at Forsmark and Laxemar-Simpevarp. Stockholm (SE): Swedish Nuclear Fuel and Waste Management Co.; 2009 Mar. 72 p. Report No.: SKB Rapport R-09-27.

22 Chachada A, Marques C, Pereira R, Goncalves F, Duarte AC, Development and application of an off-line SPE-LC-UV methodology for the determination of penoxsulam in aquatic

systems adjacent to rice fields. *Chromatographia* **71**:347-350 (2010).

23 Shaner DL, editor. Herbicide handbook. 10th ed. Lawrence (KS): Weed Science Society of America; 2014.

24 Jabusch TW, Tjeerdema RS, Chemistry and fate of triazolopyrimidine sulfonamide herbicides. *Rev Environ Contam Toxicol* **193**:31-52 (2008).

25 van Wesenbeeck IJ, Peacock AJ, Havens PL, Measurement and modeling of dicolsulam runoff under the influence of simulated severe rainfall. *J Environ Qual* **30**:553-560 (2001).

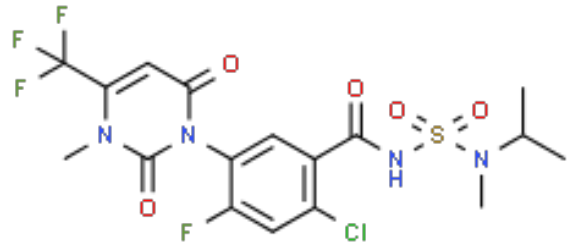
26 United States Environmental Protection Agency, Pesticide fact sheet cloransulam-methyl. Washington D.C.: United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; 1997. 19 p. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-129116_29-Oct-97.pdf

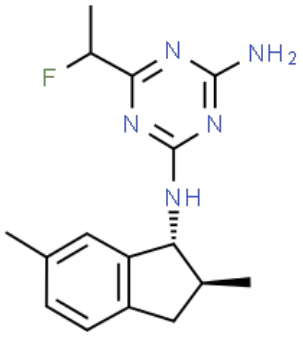
27 Pandey V, Srivastava A, Srivastava PC, Adsorption-desorption behavior of penoxsulam herbicide on soils with different characteristics. *Soil and Sediment Contamination: An International Journal* **30**:107-121 (2021).

Table 1.1. Summary of the salinity and pH irrigation quality guidelines provided by the Food and Agriculture Organization of the United Nations (FAO).

	Low	Moderate	High
Salinity (dS/m)	< 0.7	0.7 - 3.0	> 3.0
pH	< 6.5	6.5 - 8.4	> 8.4

Figure 1.1. Chemical structures of saflufenacil (top), indaziflam (center), and penoxsulam (bottom). All structures from www.ChemSpider.com.





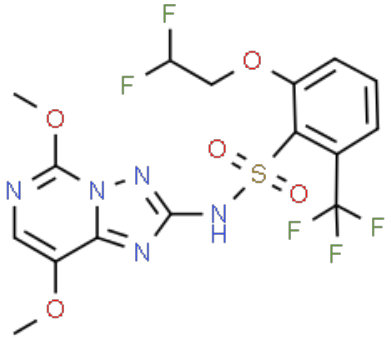


Figure 1.2. Percent removal of indaziflam, penoxsulam, and saflufenacil from soil samples at varying water treatment pH values. Loam and sand samples were not statistically different from one another and therefore results were pooled over soil type (n=6). Indaziflam was below the detection limits (ND) in all samples. The pH values of 5, 6, 7, and 8 span the low and moderate values of irrigation water quality as defined by the FAO.

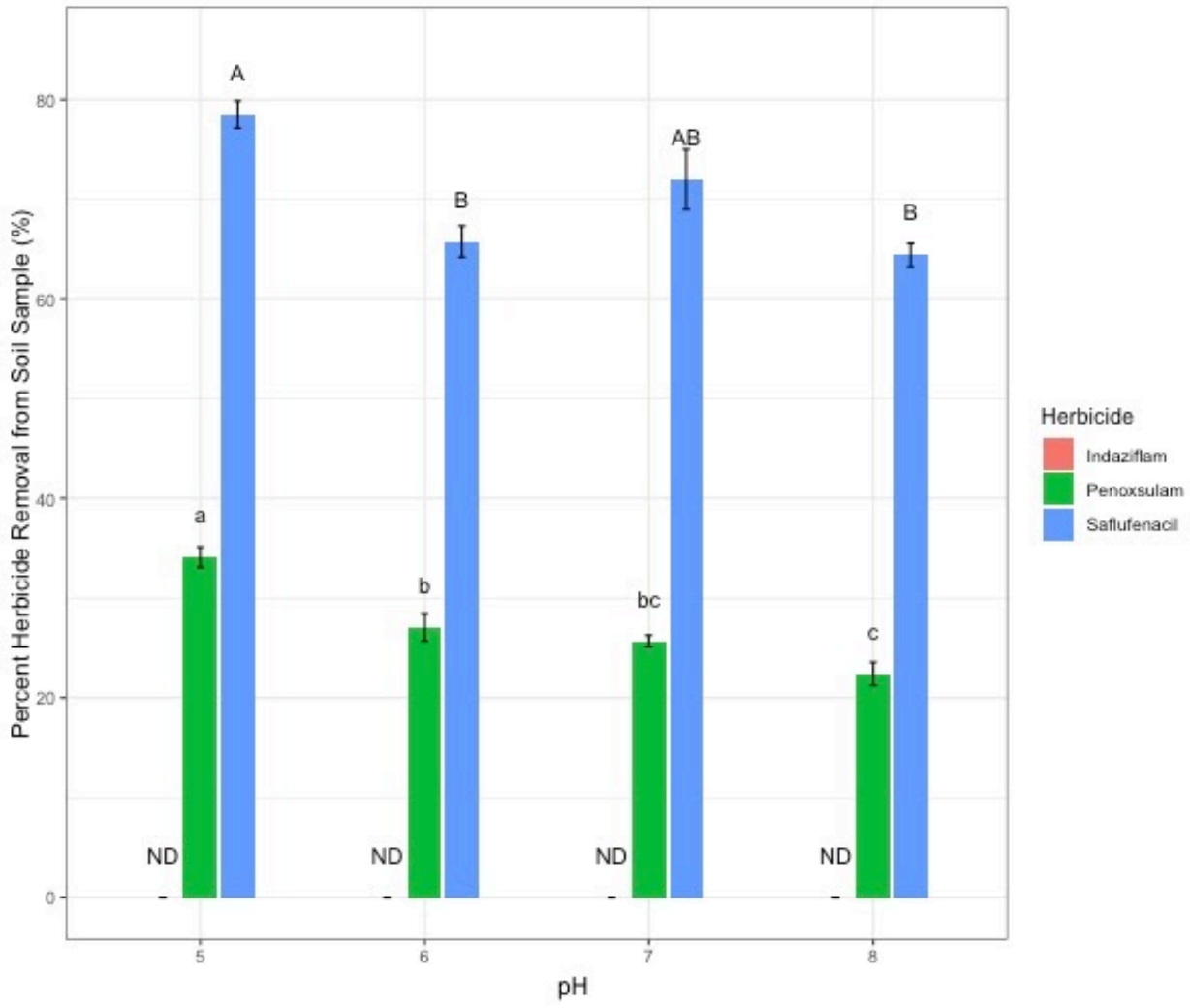
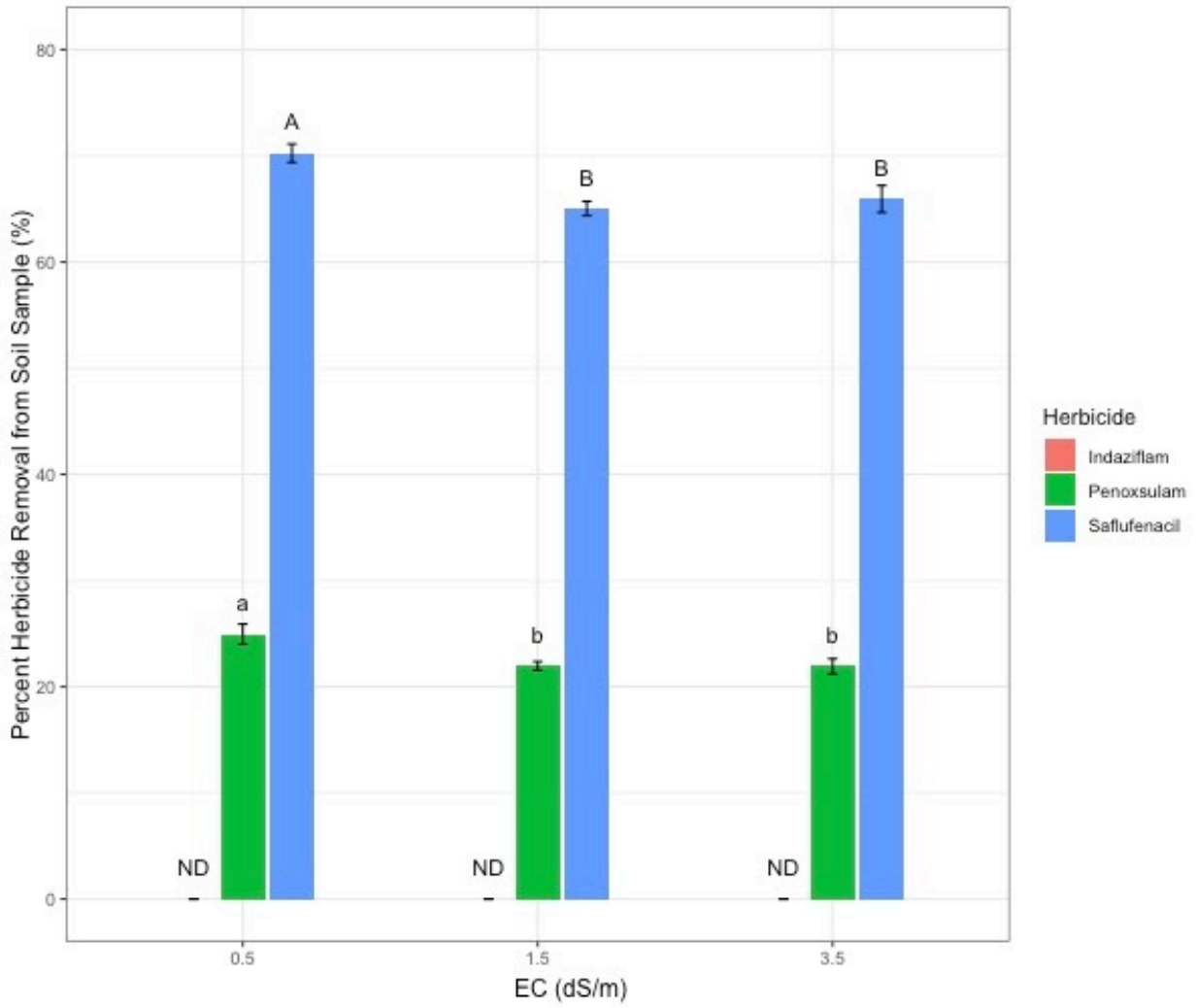


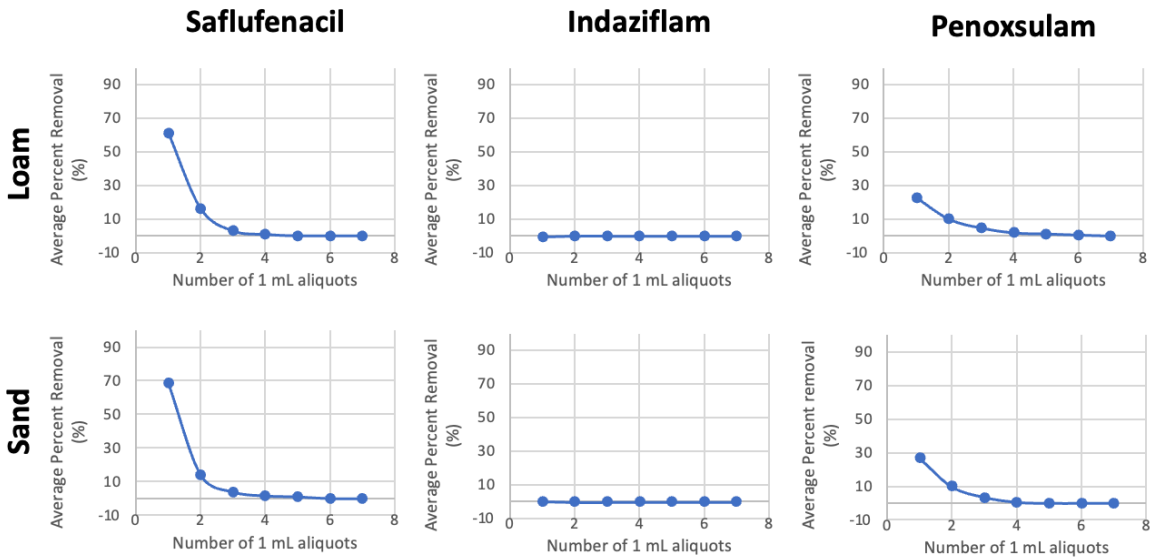
Figure 1.3. Percent removal of indaziflam, penoxsulam, and saflufenacil from soil samples at varying water treatment EC values. Loam and sand samples were not statistically different from one another and therefore data were pooled over soil type (n=6). Indaziflam was not detected (ND) in any sample. ECw values of 0.5, 1.5, and 3.5 dS/m represent the low, moderate, and high values of irrigation water quality as defined by the FAO.



Supplemental Material

Supplemental Figures S1.1

A preliminary study to determine the number of 1 mL aliquots of water treatment needed to remove the majority of herbicide that was not tightly bound to soil was conducted. The procedure follows the experiment outlined in the methods section of the manuscript; however, after each 1 mL aliquot, the eluent was collected individually and analyzed up to seven 1 mL aliquots. The procedure was repeated three times in both loam and sand soils using deionized water. The samples were analyzed using HPLC as described in the methods section.

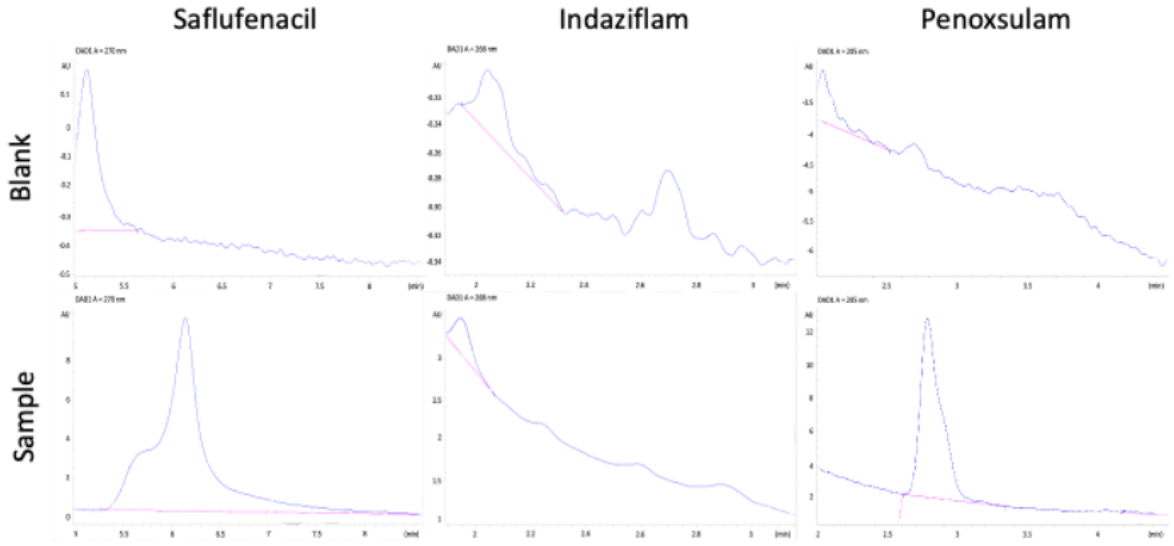


Supplemental Figures S1.2

Chromatograms of saflufenacil, indaziflam, and penoxsulam standards. (Left) Saflufenacil is shown at 270 nm and has a retention time of approximately 5 minutes; the standard is representative of 50% herbicide removal from soil or 6.125 ppm. (Center) Indaziflam is shown at 268 nm and has a retention time of approximately 2.5 minutes; the standard is representative of 2.5% herbicide removal from soil or 0.43 ppm. (Right) Penoxsulam is shown at 205 nm and has a retention time of approximately 2.6 minutes; the standard is representative of 50% herbicide removal from soil or 6.875 ppm.

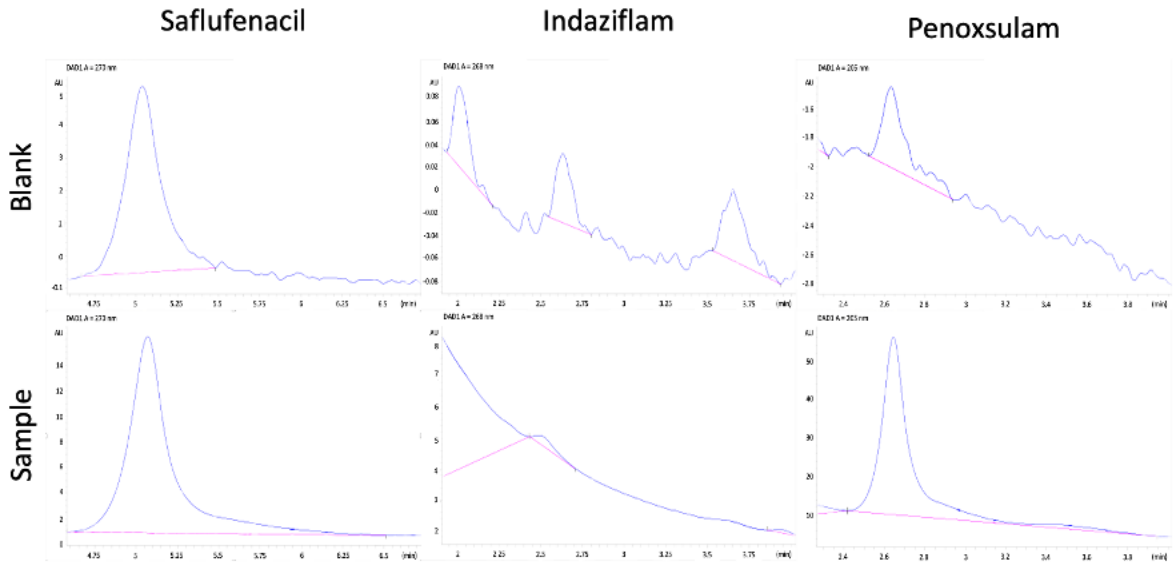
Supplemental Figures S1.3

Chromatograms of each herbicide in loam soil. (Left) Top: saflufenacil loam blank shown at 270 nm. Bottom: pH 5 saflufenacil sample shown at 270 nm; retention time is approximately 6 minutes. (Center) Top: indaziflam loam blank shown at 268 nm. Bottom: pH 5 indaziflam sample shown at 268 nm. (Right) Top: penoxsulam loam blank shown at 205 nm. Bottom: pH 5 penoxsulam sample shown at 205 nm; retention time is approximately 3.1 minutes.



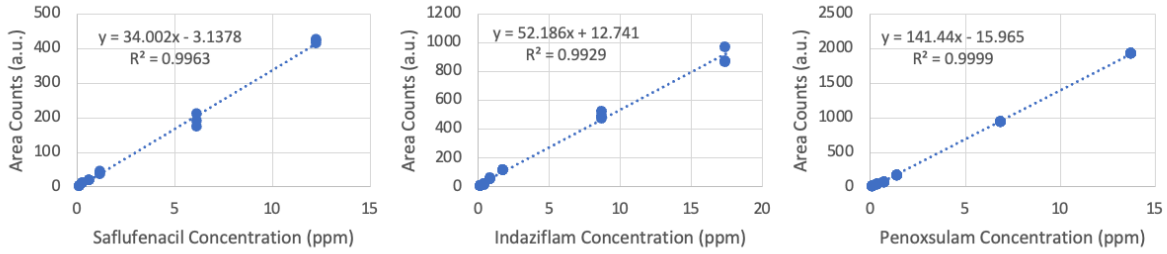
Supplemental Figures S1.4

Chromatograms of each herbicide in sand soil. (A) Top: saflufenacil sand blank shown at 270 nm. Bottom: pH5 saflufenacil sample shown at 270 nm; approximate retention time is 5 minutes. (B) Top: indaziflam sand blank shown at 268 nm. Bottom: pH 5 indaziflam sample shown at 268 nm. (C) Top: penoxsulam sand blank shown at 205 nm. Bottom: pH 5 penoxsulam sample shown at 205 nm; approximate retention time is 2.6 min.



Supplemental Figures S1.5

Calibration curves of saflufenacil (left), indaziflam (center), and penoxsulam (right).



Supplemental Table S1.6

A brief follow-up experiment was conducted to determine if indaziflam was not detected in samples due to photolysis of the parent compound by laboratory lights. One mL aliquots of 17.39 ppm indaziflam solution were pipetted into sixteen 2 mL centrifuge tubes. Seven tubes were stored in the dark, seven tubes were stored on the lab bench and lights were kept on for the duration of the experiment, and two tubes were collected for initial concentration. Every 24 hours a one sample tube was collected and analyzed for indaziflam using the methods described in the manuscript. The results show parent compound was present in samples stored both in the light or in the dark after seven days.

Day	Indaziflam Sample Stored in Light (ppm)	Indaziflam Sample Stored in Dark (ppm)
0	19.16	18.00
1	13.29	13.45
2	15.60	12.91
3	16.96	13.83
4	17.15	14.22
5	14.47	13.68
6	17.76	16.47
7	17.67	17.43

These are unreplicated samples.

Evaluating the Effects of Extended Preharvest Intervals on Glyphosate and Glufosinate Residues in Almonds

Formatted for *Weed Science* (Submitted)

Abstract

Almonds are grown on nearly 650,000 hectares in California and generate nearly $4.9 billion in export revenue annually, primarily to the European Union (EU). To facilitate harvest operations, broad-spectrum herbicides, such as glyphosate and/or glufosinate, are commonly used to control vegetation prior to harvest. The current minimum preharvest interval (PHI) for glyphosate and glufosinate herbicides registered in the US are three and 14 days, respectively. The maximum residue limit (MRL) for glyphosate and glufosinate in almonds in the EU is 0.1 mg kg^{-1} however, a recent study recommended the glyphosate MRL be reduced to 0.05 mg kg^{-1}. Laboratory and field experiments were conducted to evaluate herbicide transfer from soil to almonds and the effect of longer PHIs on glyphosate and glufosinate residues in harvested almonds. After harvest operations, almonds were dissected into hulls, shells, and kernels for analysis of glyphosate, glufosinate, and their metabolites using LC-MS/MS. In the field experiment, glyphosate and glufosinate were detected at 0.121 to 0.291 mg kg^{-1} in almond hulls and shells. Glyphosate and primary metabolites were below the LOD in almond kernels at all PHIs. Glufosinate was below the LOD but the metabolite 3-(methylphosphinico)propionic acid (MPP) was detected at 0.03 – 0.075 mg kg^{-1} in kernels from some replicate plots. There were no significant differences in either herbicide or any metabolite among PHI treatments. The lab experiment showed decreasing residue levels from hull to shell to kernel; furthermore, rinsing the kernels resulted in a 71% and

46% reduction in [^{14}C]-glyphosate and glufosinate, respectively which suggest much of the herbicide residue may be associated with dust on the kernel surfaces. The results of these experiments indicate very low levels of herbicide transfer from soil to almonds and increasing the PHI within the tested range did not reduce the already low amounts of herbicide or metabolites in almonds.

Key Words: Maximum residue limit

Introduction

In the United States (US) almonds are a $6 billion commodity grown solely in California making almonds the second highest grossing commodity in the state behind only dairy products (CDFA 2020a). As of 2020 there were more than 500,000 bearing hectares of almond trees planted in California which produced 1.3 billion kilograms of almonds (USDA NASS 2020).

Almonds are harvested by mechanically shaking the trees, sweeping the almonds into windrows, and picking the nuts up from the orchard floor. Preharvest herbicide programs and mowing are used to control vegetation that would otherwise reduce harvest efficiency (Connell et al. 2001, UCANR 2002). Glyphosate has been registered in almonds since the early 1990s and glufosinate has been registered since the early 2000s (CDPR 2021); these are commonly used herbicides for preharvest orchard preparations because of their broad spectrum weed control and relatively short preharvest interval (PHI), three and 14 days, respectively. In 2018, over one million kilograms of glyphosate and nearly 300,000 kilograms of glufosinate-ammonium were applied in almond orchards (CDPR 2018). Because of the harvesting process, there is ample opportunity for the almond hulls, shells, and kernels to be in close contact with herbicide-treated soil.

The majority of California's almond crop, about two-thirds, is exported and generated more than $4.9 billion in 2019 (CDFA 2020b). Of the exports, 22% were shipped inshell and 78% were shipped shelled (ABC 2019). Asia is the largest aggregate market for inshell almonds while the majority of shelled almond shipments go to European markets (CDFA 2020b, ABC 2019). Exported shipments of almonds are subject to pesticide residue testing and must be at or below a maximum concentration set by the region's food safety authority.

The maximum residue limit (MRL) for glyphosate and glufosinate in almonds differ by definition as well as concentration between the European Union (EU) and the US. In the United States, both glyphosate and glufosinate MRLs, which are commonly called tolerances, are defined to include the parent compound as well as its primary metabolites (Bryant Christie Inc. 2021). For clarity these MRLs will be referred to as "total glyphosate" or "total glufosinate" if the concentrations of the metabolites are to be summed with the concentration of the parent compound. Total glyphosate is the summation of glyphosate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-acetyl-glyphosate, and N-acetyl-AMPA. Total glufosinate is the summation of glufosinate, N-acetyl-glufosinate, and 3-(methylphosphinico)propionic acid (MPP).

The US MRL for glyphosate in almond hulls is 25 mg kg^{-1} and 1 mg kg^{-1} for kernels. There is not a separate US MRL for inshell almonds because the residue in inshell almonds is determined by shelling the almonds and measuring the residue in only the kernels. The US MRL for total glufosinate in almond hulls and kernels is 0.5 mg kg^{-1}.

In the European Union, the MRL for glyphosate is 0.1 mg kg^{-1} in almond kernels (European Commission 2013). The EU MRL for glufosinate includes its metabolites; the MRL for total glufosinate is 0.1 mg kg^{-1} (European Commission 2016).

Glyphosate is registered in the EU until 2022 (European Commission 2017). A recent review completed by the European Food Safety Authority (EFSA 2019) recommended that the MRL for glyphosate be reduced to 0.05 mg kg^{-1} and an optional total glyphosate MRL for the summation of glyphosate and its primary metabolites, AMPA and N-acetyl-glyphosate, set to 0.2 mg kg^{-1}. Hence, it is anticipated that in upcoming years glyphosate MRLs will be reduced, and it is a possibility that the chemical may not be re-registered. According to statute, if at any time the

safety of a current MRL is reconsidered, the MRL can be reduced to the lowest limit of analytical detection which is 0.01 mg kg^{-1} (European Parliament 2005).

Because of the importance of the European markets to the California almond industry and the importance of glyphosate and glufosinate to preharvest preparations, lab and field studies were conducted to evaluate the herbicide transfer from soil to almonds during harvest. The objectives were to determine if glyphosate and glufosinate residues can transfer to almonds from soil particles or directly sprayed almonds, whether increasing the PHI could substantially reduce the risk of herbicide in or on almond fractions and quantify the concentration of soil-bound herbicide in almond samples.

Materials and Methods

Lab Experiments

Soil Transfer to Whole Almonds

A laboratory experiment was conducted to determine the transfer of glyphosate or glufosinate to different parts of the almond via intimate contact with treated soil particles. The study was carried out using Yolo silt loam (California Soil Resource Lab) soil from the University of California, Davis Department of Plant Sciences Field Research Facility in Davis, CA (38.54°N, 121.79°W). The loam soil had a bulk density of 1.08 g $cm^{3\ -1}$, pH of 7.90, and 1.62% organic matter.

A solution of 1.665 MBq [^{14}C]-glyphosate (50 mCi $mmmol^{-1}$ Glyphosate [phosphono-methyl-^{14}C], American Radiolabeled Chemicals Inc., 101 Arc Drive, St. Louis, Missouri, United States) or 1.665 MBq [^{14}C]-glufosinate (6.35 MBq mg^{-1} [3,4-^{14}C]-Glufosinate Hydrochloride, Bayer Crop Sciences, Alfred-Nobel-Str. 50., Monheim am Rhein, North Rhein-Westphalia,

Germany) in 10 mL HPLC Plus methanol (Sigma Aldrich, 2909 Laclede Avenue, St. Louis, Missouri, United States) was applied to 16.2 g of soil. The soil was air dried until all methanol had evaporated. The mass of soil used for the experiment was calculated based on the assumption that nine almonds occupy an area of 150 cm^2 and 1 mm depth of soil would be swept up by the almond sweeper.

The amount of [^{14}C]-herbicide that was used was based the limit of quantification of the liquid scintillation counter which was 16.67 Bq and the ideal minimum detection being approximately 0.001% herbicide transfer from soil to almond fraction. The total Bq added to the soil was 166,500 for both herbicides. The actual amount of glyphosate and glufosinate added to the soil in these experiments is roughly 6% and 10% of the field rate, respectively. Therefore, the intended use of the data generated is monitoring transfer processes and comparison of residue levels in hulls versus shells versus kernels.

Glyphosate and glufosinate were evaluated in separate experiments. In each experiment, four replicates of nine whole (kernel, shell, hull) almonds were exposed to the herbicide-treated soil. The treatments were carried out in 250 mL Nalgene bottles (Thermo Fisher Scientific, 168 3rd Avenue, Waltham, Massachusetts, United States) containing nuts and soil treated with [^{14}C]-herbicide; the bottles were rotated using a rock tumbler (Dual Drum Rotary Rock Tumbler, 26541 Agoura Road, Chicago Electric Power Tools, Calabasas, California, United States) (Supplemental Figure S2.1). The inside of each bottle had four plastic inserts (9 cm x 1 cm x 1 cm) attached to the wall to help pick up the soil and almonds and create dust during the mixing process. The almonds were tumbled for 15 minutes and let rest for 15 minutes; excess soil was dusted off the almonds before analysis using a tapping method.

Soil Transfer to Almond Kernels

Another experiment to analyze the surface-associated herbicide involved tumbling four almond kernels directly in the [^{14}C]-treated soil. Shelled kernels were tumbled for 15 minutes in the [^{14}C]-treated soil, dusted off, rinsed with water using gentle inverted shaking, and both kernels and rinsate were analyzed for [^{14}C]-herbicide.

Almond-to-Almond Transfer with No Soil Contact

This experiment was conducted to determine glyphosate transfer from directly-treated almonds to non-treated almonds. This was intended to mimic a situation where a small number of almonds fall to the ground very early (e.g. "windfall" nuts) and could conceivably be directly sprayed with preharvest treatments and then contaminate the later-harvested crop during harvest and handling steps. Two almonds were directly treated with 0.8325 MBq [^{14}C]-glyphosate by using a microsyringe to dot the stock solution over the entire almond including the inside of the split hull and exposed shell. The two treated almonds were tumbled with nine non-treated almonds using the apparatus and methods described earlier. The treated almonds were clearly marked so they could be removed after the tumbling process. The almonds were tumbled using a rock tumbler for 15 minutes and let rest for 15 minutes. Before analysis the treated almonds were removed from the bottle, and the untreated almonds were dissected and analyzed for [^{14}C]-glyphosate. This experiment was replicated four times.

[^{14}C]-Herbicide Analysis

The whole almonds from each replicate from both soil transfer experiments and the almond-to-almond transfer experiment were separated for three different analyses: whole almond rinse, herbicide adsorption to almond fractions, and a surface swipe after a post-harvest mimicking process. All samples were analyzed using a liquid scintillation counter (LS6500,

Beckman Coulter, 250 South Kraemer Boulevard, Brea, California, United States). The data were corrected for the background levels of radiation in the scintillation counter.

The rinsate of whole almonds was used to determine how much [^{14}C]-herbicide was loosely associated with the surface of the almonds. Three whole almonds were rinsed with water using gentle inverted shaking. The rinsate was collected into glass scintillation vials and evaporated using a vacuum evaporation system at 30°C (RapidVap, Labconco Corporation, 8811 Prospect Avenue, Kansas City, Missouri, United States). Once the samples were evaporated to near dryness, 10 mL of Ultima Gold™ (PerkinElmer, 940 Winter Street, Waltham, Massachusetts, United States) was added to each vial. The samples were analyzed using the liquid scintillation counter.

To determine how much herbicide was adsorbed to the almond fractions, three almonds were dissected into their hull, shell, and kernel components. Each component was homogenized using a mortar and pestle and liquid nitrogen. Approximately 500 mg of each homogenized almond fraction was collected into a combustion cone (CombustoPad, Perkin Elmer, 940 Winter Street, Waltham, Massachusetts, United States) and combusted using a sample oxidizer (Model 307, PerkinElmer, 940 Winter Street, Waltham, Massachusetts, United States). The combustion product, [$^{14}CO_2$], was collected in 20 mL of scintillation cocktail composed of 10 mL Carbo-Sorb E® (PerkinElmer, 940 Winter Street, Waltham, Massachusetts, United States) and 10 mL Permafluor® (PerkinElmer, 940 Winter Street, Waltham, Massachusetts, United States). Glass scintillation vials containing the [^{14}C]-samples were analyzed using the liquid scintillation counter.

The remaining three almonds went towards a post-harvest mimicking process. The almond hulls were discarded, and the shells were opened by hand cracking through a plastic

barrier then discarded. The plastic was swiped using a filter paper and the swipe was added to a glass scintillation vial with 10 mL Ultima Gold™. The swipes were analyzed using the scintillation counter. The kernels were collected, homogenized and combusted, and the combustion product was mixed with scintillant and analyzed using the scintillation counter as described above.

The four almond kernels (no hull or shell) that were tumbled directly in the [^{14}C]-herbicide treated soil were rinsed with 20 mL of water. The rinsate was collected into glass scintillation vials and evaporated to near dryness using vacuum evaporation. 10 mL of Ultima Gold™ was added to the scintillation vial and analyzed using the liquid scintillation counter. The rinsed kernels were homogenized and combusted; the combustion product was mixed with scintillant and analyzed using the liquid scintillation counter.

Field Experiment

To examine the glyphosate and glufosinate residues in almonds at different pre-harvest intervals (PHI) a field study was conducted in a mature almond orchard at The Nickels Soil Laboratory (38.96°N, 122.07°W) located near Arbuckle, California, United States. The orchard included full rows of nonpareil almonds alternating with rows of several pollinizer varieties; trees were planted 4.9 m apart within the rows and rows were 6.7 m apart.

The experiment was conducted in the nonpareil rows and treatments were organized into a randomized complete block design with four replicates. Herbicide treatments included a single herbicide mix applied at timings that correspond to PHIs of 35, 21, 14, 7, and 3 days before shaking. Each plot was 19.6 m long by 4 m wide and contained four almond trees; the width of each herbicide plot started from one side of the tree trunk and extended 4 m, nearly to the next

tree row (Supplemental Figure S2.2). The herbicide treatment for all plots was a tank mix of commercial glyphosate (Roundup WeatherMAX, Bayer Crop Science, 8400 Hawthorne Road, Kansas City, Missouri, United States) at 1,681 g ae ha^{-1}, commercial glufosinate (Rely280, BASF Corporation, 100 Park Avenue, Florham Park, New Jersey, United States) at 1,681 g ai ha^{-1}, nonionic surfactant at 0.25% v/v (RAINIER-EA, Wilbur-Ellis Company LLC, 16300 Christensen Road #135, Tukwila, Washington, United States), and AMS at 1% v/v (BRONC MAX, Wilbur-Ellis Company LLC, 16300 Christensen Road #135, Tukwila, Washington, United States). Applications were made using a CO_2 pressurized backpack sprayer with a 2 m boom equipped with four air induction extended range nozzles (AIXR 11002, TeeJet® Technologies, 1801 Business Park Drive, Springfield, Illinois, United States) calibrated to deliver 187 L ha^{-1} at a pressure of 207 kPa. At each application date, previously fallen almonds were counted in two 1 sq m areas in each plot.

On the day of harvest, the middle two almond trees of each plot were hand shaken using mallets and poles, then the nuts were left on the orchard floor to dry. Approximately 100 g of surface soil was collected from each plot at this time for herbicide analysis prior to sweeping. Three days after shaking, the nuts were swept into a windrow between tree rows in approximately the center of the herbicide-treated plots using a commercial self-propelled mechanical sweeper. Four days later approximately 500 g of nuts were collected from each plot windrow, separated by hand from the soil and other debris, and stored frozen until further analysis. This timeline corresponds to typical commercial harvest practices. At almond sampling, approximately 100 g of surface soil from each plot was also collected for herbicide analysis post sweeping.

Almond samples from each plot were dissected into hull, shell, and kernel fractions and sent to a commercial laboratory (Safe Food Alliance, 2037 Morgan Drive, Kingsburg, California, United States) for analysis. The laboratory used modified methods from QuPPe v 10 (EURL-SRM 2019) and LC-MS/MS (QTRAP® 5500 LC-MS/MS System, 1201 Radio Road, Sciex, Redwood City, California, United States) equipped with a MicroSolv Congent Diol™ column (4.6 mm x 250 mm x 4 µm, MicroSolv Technology Corporation, 9158 Industrial Boulevard, Leland, North Carolina, United States) to quantify glyphosate, N-Acetyl-glyphosate, AMPA, N-Acetyl-AMPA, glufosinate, N-Acetyl-glufosinate, and MPP. The limit of detection for all analytes in hull and shell samples was 0.040 mg kg^{-1} and in kernel samples was 0.020 mg kg^{-1}.

The same compounds were quantified from an unreplicated composite soil sample from each PHI treatment by the same commercial laboratory. The laboratory used modified methods from Druart et al. (2011) and the same LC-MS/MS instrumentation.

Statistical Analysis

The laboratory and field data were subject to ANOVA using R statistical analysis software (2020) and multiple comparisons were performed with Tukey's HSD with $\alpha = 0.05$.

Results and Discussion

Lab Experiment

Soil Transfer to Whole Almonds

The rinsate analysis of the washed whole almonds showed a removal of herbicide from the surface of the whole almond averaging 6,667 ± 1,782 Bq of [^{14}C]-glyphosate and 6,130 ± 2,319 Bq of [^{14}C]-glufosinate (Supplemental Table S1). The swipe of the plastic barrier used to

crack the almond shells had a residue of 154 ± 36 Bq of [^{14}C]-glyphosate and 109 ± 23 Bq of [^{14}C]-glufosinate (Supplemental Table S2).

The kernels of the almonds used for the post-harvest mimic process contained 0.138 ± 0.035 Bq mg^{-1} of [^{14}C]-glyphosate and 0.093 ± 0.016 Bq mg^{-1} of [^{14}C]-glufosinate (Supplemental Table S3). The amount of herbicide in the kernel samples from the post-harvest mimic process was not significantly different from the amount of herbicide in the kernel samples from the dissection process.

A summary of the results of the whole almond dissection is presented in Figure 1. Unsurprisingly, the hull fraction contained the most herbicide; [^{14}C]-glufosinate averaged 2.350 ± 0.369 Bq mg^{-1} and [^{14}C]-glyphosate averaged 2.308 ± 0.871 Bq mg^{-1}. Shell samples averaged 1.299 ± 0.230 Bq mg^{-1} [^{14}C]-glyphosate and 1.226 ± 0.145 Bq mg^{-1} [^{14}C]-glufosinate. The average [^{14}C]-herbicide in the kernels was 0.138 ± 0.035 Bq mg^{-1} [^{14}C]-glyphosate and 0.113 ± 0.040 Bq mg^{-1} [^{14}C]-glufosinate. Of the total [^{14}C]-glyphosate found in the almond fractions roughly 62% was in the hull, 35% was in the shell, and 3% was in the kernel; of the [^{14}C]-glufosinate found in the almond fractions 64% was in the hull, 33% was in the shell, and 3% was in the kernel. The data did not show statistically significant differences between the two herbicides. There were significant differences between residues in the hull, shell, and kernel fractions in the samples treated with [^{14}C]-glufosinate. The hull and shell fractions of the [^{14}C]-glyphosate samples had significantly more residue than the kernel fraction.

Soil Transfer to Almond Kernel

The amount of [^{14}C]-glyphosate that remained on the rinsed kernels was 0.040 ± 0.002 Bq mg^{-1} and the amount of [^{14}C]-glufosinate that remained on the rinsed kernel was 0.062 ± 0.004 Bq mg^{-1} (Figure 2). After this brief water rinse there was significantly less herbicide on the

kernels. [^{14}C]-glyphosate was reduced by 71% and [^{14}C]-glufosinate was reduced by 46% in almond kernel samples. There were no statistical differences between herbicides for [^{14}C] in the unrinsed kernels, however, there was less [^{14}C]-glyphosate on rinsed kernels than [^{14}C]-glufosinate. This is unsurprising as the log K_{ow} of glyphosate is lower than that of glufosinate meaning the glufosinate is more attracted to the non-polar almond surface than glyphosate. From these results we can conclude that a large proportion of glyphosate and glufosinate residues in almond samples likely is associated with soil particles on the surface of the kernels.

Almond-to-Almond Transfer

The rinsate analysis of the whole washed almonds showed a removal of glyphosate from the surface of the whole nut averaging 1,534 ± 265 Bq (Supplemental Table S4). The swipe of the plastic barrier used to crack the shells was below the detection limit. The kernels of the almonds used for the swipe test were also below the detection limit of [^{14}C]-glyphosate.

Contact between directly-treated whole almonds and untreated nuts resulted in the untreated hulls having very low levels of herbicide residue. The average untreated hull [^{14}C]-glyphosate residue was 0.136 ± 0.033 Bq mg^{-1} while [^{14}C]-glyphosate was below the limit of quantification in the shells and kernels from the untreated almonds. Therefore, transfer from early fallen nuts directly sprayed during pre-harvest preparations is unlikely a major contributor to herbicide residue in whole sample lots of almonds.

Field Trial

The range of fallen nuts in two 1 sq m quadrats within each plot are shown in Table 1. There was no apparent correlation between the number of early fallen nuts and glyphosate or glufosinate residue levels in the subsequently harvest samples (data not shown).

A summary of the glyphosate residues is presented in Table 2. Total glyphosate concentration is presented as the sum of glyphosate, AMPA, N-acetyl-glyphosate, and N-acetyl-AMPA. There were no statistically significant differences in concentration of glyphosate or total glyphosate found in the hull and shell samples. N-acetyl-AMPA was found only in almond hull samples. There were no detections of glyphosate or its metabolites in any of the almond kernel samples. The almond hulls had the highest detection of glyphosate and its metabolites, averaging 0.174 mg kg^{-1}, while still being well below the US MRL. The almond shell samples were above the EU almond kernel residue limit of 0.1 mg kg^{-1} however, in practice, inshell almonds are shelled before residue analysis. PHI within the tested range did not have a statistically significant effect on glyphosate residues in hull and shell samples.

A summary of the glufosinate residue data is presented in Table 3. Total glufosinate concentration is presented as the sum of glufosinate, N-acetyl-glufosinate, and MPP. There were no significant differences in residues found in hulls, shells, or kernels and these samples were all below the US MRL for total glufosinate. The EU total glufosinate MRL was exceeded in almond shells in at least some replicate plots at 3-, 14-, 21-, and 35-day PHIs. MPP was the only compound detected in almond kernels at PHIs of 3, 14, 21, and 35 days. Although the three- and seven-day PHIs were off-label applications of glufosinate, there were no significant differences in glufosinate residues among the PHI treatments.

Glyphosate and glufosinate are generally considered to have moderate and short soil half-life, respectively (Shaner et al. 2014) and the almond orchard soil samples collected from the orchard floor support that degradation pattern. Total glyphosate concentrations remained consistent, apart from an anomalous 7-day pre-sweep value, across all PHIs and pre- and post-sweep samples; the range of total glyphosate in samples taken prior to sweeping was 2.331 to

2.575 mg kg^{-1} and the range in samples taken after sweeping was 1.536 to 3.554 mg kg^{-1} (Table 4). The half-life of glyphosate in soil ranges between seven and 60 days depending on soil properties (Giesy 2000) and given samples were taken from the soil surface that was dry due to preharvest management practices it is expected the half-life would be closer to the high end of the given range. Total glufosinate concentration in the soil followed a decreasing trend from the PHI of three to 35 days with the majority of the total glufosinate concentration being attributed to MPP (Table 4). Total glufosinate decreased from 5.339 to 0.210 mg kg^{-1} in the pre-sweep samples and from 7.687 mg kg^{-1} to less than the detection limit in the post-sweep samples (Table 4). Glufosinate is rapidly degraded by soil bacteria and has a half-life between three and seven days; the main degradation product is MPP (Gallina and Stephenson1992). The 7-day pre-sweep sample appears anomalous and likely from a sample processing error in the unreplicated sample since there was no correspondingly high values in the almond samples from those plots (Graham et al. 2002).

The current labels state the minimum PHI for glyphosate and glufosinate is three and 14 days, respectively. The field results showed that increasing the PHI up to 35 days before shaking did not appear to substantially reduce the amount of glyphosate or glufosinate in the samples. Total glyphosate residues in kernels from almonds sampled in the windrow were below the limit of detection at every PHI tested (Table 2). At the minimum 14-day PHI total glufosinate residues in kernels from almonds sampled in the windrow were 0.037 mg kg^{-1} while the 35-day PHI residues were 0.089 mg kg^{-1}; these data were not statistically different (Table 3). Based on these data we conclude increasing the PHI of the herbicides within a range of utility for preharvest operations is unlikely to significantly contribute to lower residue levels.

Prior to conducting these experiments, one almond industry concern was windfall nuts that are directly sprayed with herbicide contaminating the whole batch. Windfall nuts typically account for zero to 1% of the total harvest and nuts that fall greater than four weeks prior to harvest are usually of poor quality (Brown 2019) because of immaturity or degradation processes. The number of potentially directly-treated almonds was relatively low (0-46 nuts m^{-2}) in this study and the earliest falling and mostly likely to be directly treated would likely be removed from the batch during processing based on the United States Department of Agriculture grading standards for size, damage, and color (USDA 1997). The almond-to-almond transfer experiment in the lab suggested low transfer of glyphosate or glufosinate from treated to untreated nuts; therefore, the small portion of directly sprayed windfall nuts that make it through the processing facility are unlikely to have high enough residues to elevate the batch residues above the MRL.

Almond hulls, shells, and kernels were below the United States MRLs for both glyphosate and glufosinate as well as their metabolites. If the EU reduces the MRL further based on new hazard and risk assessments, this will pose a challenge to California growers when choosing preharvest herbicides. It is worth noting the almonds in both the field and lab experiments presented here were not commercially processed and, thus were not subjected to mechanical and pneumatic cleaning and sorting operations to remove soil and debris; these steps likely would have more effectively removed the soil particles and soil-associated herbicides compared to these research samples. It is also recognized that the limits of detection of the analytical instrumentation methods used are higher than the recommended new MRLs for glyphosate and its metabolites. Future research will focus on pesticide residues at the later points

in almond processing and include sampling almonds and soil particles at various points within a commercial hulling and shelling facility.

Acknowledgements

This work was supported by the Almond Board of California, grant number HORT54.Hanson. The authors would like to thank the Nickels Soil Laboratory for orchard management, Seth Watkins for chemical application, and Steven Haring and Matthew Fatino for harvest support. No conflicts of interest have been declared.

Works Cited

[ABC] Almond Board of California (2019). Almond Industry Position Report 2019-2020 Crop Year. https://www.almonds.com/sites/default/files/2020-08/2020_07_PosRpt_hg5eg3.pdf. Accessed August 16, 2021.

Bayer CropScience (2018). Rely® 280. Bayer Publication 170522E. St. Louis, MO. 10 p

Bayer Group (2020). Roundup WeatherMAX® Herbicide. Bayer Publication 161018Bv2. St. Louis, MO. 11 p

Brown PH, Camargo R, Cirhigiri G (2019) Windfall – The Off Ground Journey. Slides 24-36. The Almond Conference 2019. https://www.almonds.com/sites/default/files/content/attachments/Journey%20Toward%20Off-Ground%20Harvest.pdf

Bryant Christie Inc. (2021). BC Global Pesticide MRLs. https://bcglobal.bryantchristie.com/db#pesticides/query. Accessed August 16, 2021

California Soil Resource Lab (2019). SoilWeb. https://casoilresource.lawr.ucdavis.edu/gmap/. Accessed August 2, 2021

[CDFA] California Department of Food and Agriculture (2020a) California Agricultural Production Statistics 2019 Crop Year Report. 4p. https://www.cdfa.ca.gov/Statistics/PDFs/2020_Ag_Stats_Review.pdf. Accessed August 18, 2021

[CDFA] California Department of Food and Agriculture (2020b) California Agricultural Exports 2019-2020. 6-9 p.

https://www.cdfa.ca.gov/Statistics/PDFs/2020_Exports_Publication.pdf. Accessed August 18, 2021

[CDPR] California Department of Pesticide Regulation (2018) 2018 Annual Statewide Pesticide Use Report Indexed by Commodity. https://www.cdpr.ca.gov/docs/pur/pur18rep/statewide_site_2018.htm. Accessed September 26, 2021

Connell JH, Colbert F, Kreuger W, Cudney D, Gast R, Bettner T, Dallman S (2001) Vegetation management options in almond orchards. HortTechnology 11(2):254-257. https://doi.org/10.21273/HORTTECH.11.2.254

Druart C, Delhomme D, de Vaufleury A, Ntcho E, Millet M (2011) Optimization of extraction procedure and chromatographic separation of glyphosate, glufosinate, and aminomethylphosphonic acid in soil. Anal Bioanal Chem 399:1725-1732. https://doi.org/10.1007/s00216-010-4468-z

[EURL-SRM] EU Reference Laboratory for Pesticides Requiring Single Residue Methods (2019) Quick Method for the Analysis of Numerous Highly Polar Pesticides in Foods of Plant Prigin via LC-MS/MS Involving Simultaneous Extraction with Methanol (QuPPE-Method). Version 10. Fellbach, Germany

European Commission (2013) Commission Regulation (EU) No 293/2013 of 20 March 2013 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for emamectin benzoate, etofenprox, etoxazole, flutriafol, glyphosate, phosmet, pyraclostrobin, spinosad and spirotetramat in or on certain products. https://eur-lex.europa.eu/eli/reg/2013/293/oj. Accessed August 2, 2021

European Commission (2016) Commission Regulation (EU) 2016/1002 of 17 June 2016 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for AMTT, diquat, dodine, glufosinate and tritosulfuron in or on certain products. https://eur-lex.europa.eu/eli/reg/2016/1002/oj. Accessed August 2, 2021

European Commission (2017) Commission Implementing Regulation (EU) 2017/2324 of 12 December 2017 renewing the approval of the active substance glyphosate in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. https://eur-lex.europa.eu/eli/reg_impl/2017/2324/oj. Accessed August 2, 2021

[EFSA] European Food Safety Authority (2019) Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 17(10):5862

European Parliament, Council of the European Union (2005) Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. https://eur-lex.europa.eu/eli/reg/2005/396/oj. Accessed August 2, 2021

Gallina MA and Stephenson GR (1992) Dissipation of [^{14}C]Glufosinate Ammonium in Two Ontario Soils. J. Agric. Food Chem 40:165-168. https://doi.org/10.1021/jf00013a033

Giesy JP (2000) Ecotoxological risk assessment for Roundup® herbicide. Reviews of Environmental Contamination and Toxicology 167:35-120. https://doi.org/10.1007/978-1-4612-1156-3_2

Graham DG, Clay V, Jackson SH, Jones R (2002) Field Dissipation Studies: The Measurement of Zero-Time Residues. Pages 54-72 *in* Arthur EL, Barefoot AC, Clay VE, eds. Terrestrial Field Dissipation Studies. Washington, DC: American Chemical Society

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://R-project.org/

Shaner DL, ed (2014) Herbicide Handbook. 10th edition. Lawrence, KS: Weed Science Society of America, 238-242 p

[UCANR] University of California Agriculture and Natural Resources (2002) Integrated Pest Management for Almonds. 2nd edition. Oakland, CA: Regents of the University of California Division of Agriculture and Natural Resources, 160-166 p

[USDA] United States Department of Agriculture (1997) United States Standards for Grades of Shelled Almonds, 5-7 p

[USDA NASS] United States Department of Agriculture National Agricultural Statistics Service (2020) 2020 California Almond Objective Measurement Report, 3-7 p. https://www.nass.usda.gov/Statistics_by_State/California/Publications/Specialty_and_Other_Releases/Almond/Objective-Measurement/202007almom.pdf. Accessed August 18, 2021

Table 2.1. The range of nuts on the orchard floor counted in four replicates of two 1 square meter quadrats (n=8) at each preharvest interval.

Date of Application	Preharvest interval	Range of fallen nuts
	— days —	— # of nuts/sq m —
July 6, 2020	35	1 - 35
July 20, 2020	21	1 - 12
July 27, 2020	14	3 - 11
August 3, 2020	7	0 - 7
August 7, 2020	3	8 - 46

Almonds were hand shaken on August 10, swept on August 13, and collected from the windrow on August 17.

Table 2.2. Summary of the concentration of glyphosate and metabolites found in almond hulls, shells, and kernels at each preharvest interval (PHI). Values are represented as mean concentration ± standard error. There were no significant differences in glyphosate or total glyphosate concentrations in the hull or shell fractions. The PHI did not significantly influence the residue levels in hulls, shells, or kernels.

			HULLS		
PHI	Glyphosate	AMPA	N-Acetyl-Glyphosate	N-Acetyl-AMPA	Total Glyphosate
— days —			mg kg^{-1}		
35	0.179 ± 0.044	< LOD	< LOD	0.052 ± 0.002	0.252 ± 0.035
21	0.119 ± 0.021	< LOD	< LOD	0.054 ± 0.004	0.178 ± 0.022
14	0.207 ± 0.047	< LOD	< LOD	0.050 ± 0.003	0.262 ± 0.048
7	0.155 ± 0.027	< LOD	< LOD	0.056 ± 0.006	0.217 ± 0.031
3	0.211 ± 0.030	< LOD	< LOD	0.053 ± 0.005	0.268 ± 0.033
LOD	0.040	0.040	0.040	0.040	
			SHELLS		
PHI	Glyphosate	AMPA	N-Acetyl-Glyphosate	N-Acetyl-AMPA	Total Glyphosate
— days —			mg kg^{-1}		
35	0.055^	< LOD	< LOD	< LOD	0.055^
21	0.225^	< LOD	< LOD	< LOD	0.225 ^
14	0.206 ± 0.003‡	< LOD	< LOD	< LOD	0.206 ± 0.003‡
7	0.058 ± 0.005‡	< LOD	< LOD	< LOD	0.058 ± 0.005‡
3	0.121 ± 0.037	< LOD	< LOD	< LOD	0.121 ± 0.037
LOD	0.040	0.040	0.040	0.040	
			KERNELS		
PHI	Glyphosate	AMPA	N-Acetyl-Glyphosate	N-Acetyl-AMPA	Total Glyphosate
— days —			mg kg^{-1}		
35	< LOD	< LOD	< LOD	< LOD	< LOD
21	< LOD	< LOD	< LOD	< LOD	< LOD
14	< LOD	< LOD	< LOD	< LOD	< LOD
7	< LOD	< LOD	< LOD	< LOD	< LOD
3	< LOD	< LOD	< LOD	< LOD	< LOD
LOD	0.020	0.020	0.020	0.020	

Glyphosate is the concentration of the parent compound and total glyphosate is the sum of the concentrations of glyphosate, AMPA, N-acetyl-glyphosate, and N-acetyl-AMPA. (‡) indicates two replicates were below the limit of detection. (^) indicates three replicates were below the limit of detection.

PHI indicates the preharvest interval prior to hand shaking on August 10. Almond samples were collected from the windrows of each plot on August 17.

Table 2.3. Summary of the concentration of glufosinate and metabolites found in almond hulls, shells, and kernels at each preharvest interval (PHI). Values are represented as mean concentration ± standard error. There were no significant differences in residue levels in the almond fractions. The PHI did not significantly influence residue levels in hulls, shells, or kernels.

HULLS				
PHI	Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate
— days —	——— mg kg^{-1} ———			
35	0.103 ± 0.019	< LOD	0.207 ± 0.076†	0.287 ± 0.118
21	0.073 ± 0.010	< LOD	0.118 ± 0.040‡	0.143 ± 0.042
14	0.133 ± 0.048	< LOD	0.178 ± 0.010†	0.291 ± 0.084
7	0.074 ± 0.014	< LOD	0.141 ± 0.033†	0.200 ± 0.050
3	0.133 ± 0.015	< LOD	0.148 ± 0.044†	0.245 ± 0.075
LOD	0.030	0.030	0.030	
SHELLS				
PHI	Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate
— days —	——— mg kg^{-1} ———			
35	0.058 ± 0.009	< LOD	0.076 ± 0.034‡	0.106 ± 0.032
21	0.052 ± 0.011	< LOD	0.080 ± 0.006†	0.123 ± 0.030
14	0.088 ± 0.020†	< LOD	0.080 ± 0.004†	0.154 ± 0.053†
7	0.071 ± 0.011	< LOD	0.042^	0.083 ± 0.019
3	0.087 ± 0.006	< LOD	0.072 ± 0.008	0.173 ± 0.015
LOD	0.030	0.030	0.030	
KERNELS				
PHI	Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate
— days —	——— mg kg^{-1} ———			
35	< LOD	< LOD	0.075 ± 0.030†	0.089 ± 0.036†
21	< LOD	< LOD	0.044 ± 0.026‡	0.052 ± 0.031‡
14	< LOD	< LOD	0.031 ± 0.012‡	0.037 ± 0.014‡
7	< LOD	< LOD	< LOD	< LOD
3	< LOD	< LOD	0.063^	0.075^
LOD	0.015	0.015	0.015	

PHI of 3 and 7 days is an off-label application of the herbicide. Glufosinate is the concentration of the parent compound and total glufosinate is the sum of the concentrations of glufosinate, N-acetyl-glufosinate, and 3-(methylphosphinico)propionic acid (MPP). (†) indicates one replicate was below the limit of detection. (‡) indicates two replicates were below the limit of detection. (^) indicates three replicates were below the limit of detection.

PHI indicates the preharvest interval prior to hand shaking on August 10. Almond samples were collected from the windrows of each plot on August 17.

Table 2.4. Concentrations of total glyphosate, total glufosinate, and MPP found in soil from the Nickels Soil Laboratory field site pre and post orchard sweeping at each preharvest interval (PHI).

	Total Glyphosate		Total Glufosinate		MPP	
PHI	Pre-Sweep	Post-Sweep	Pre-Sweep	Post-Sweep	Pre-Sweep	Post-Sweep
– days –	——— mg kg^{-1} ———		——— mg kg^{-1} ———		——— mg kg^{-1} ———	
3	2.543	3.554	5.339	7.687	3.407	4.875
7	15.205*	3.244	14.096*	4.276	4.469	3.028
14	2.331	2.102	2.780	2.301	1.930	1.521
21	2.400	1.536	0.473	0.306	0.397	0.257
35	2.575	3.056	0.210	< LOD	0.176	< LOD

Total glyphosate represented as the sum of glyphosate, AMPA (α-amino-3-hydroxy5-methyl-4-isoxazolepropionic acid), N-acetyl-glyphosate, and N-acetyl AMPA. Total glufosinate is represented as the sum of glufosinate, N-acetyl-glufosinate, and 3-(methylphosphinico)propionic acid (MPP).

Pre-sweep is the soil sample taken on August 13 prior to the sweeper going through the orchard and post-sweep is the soil sample taken on August 17 after the sweeper has gone through the orchard and almonds are in windrows.

*The 7-day preharvest interval sample appears to be a data anomaly assumed to be from a sample collection or processing error since there were no corresponding high values in the almond samples; however, this cannot be confirmed as the replicated field plot samples were homogenized and analyzed a single unreplicated lab sample. Field dissipation studies have

shown that zero-time soil measurements of various pesticides have resulted in an artificially low residue levels (Graham et al. 2002).

Figure 2.1. [^{14}C]-Glyphosate and glufosinate Becquerels per milligram of almond hull, shell, and kernel detected in samples from the soil transfer experiment. Total Bq applied to the soil was 166,500. Error bars are representative of the 95% confidence interval.

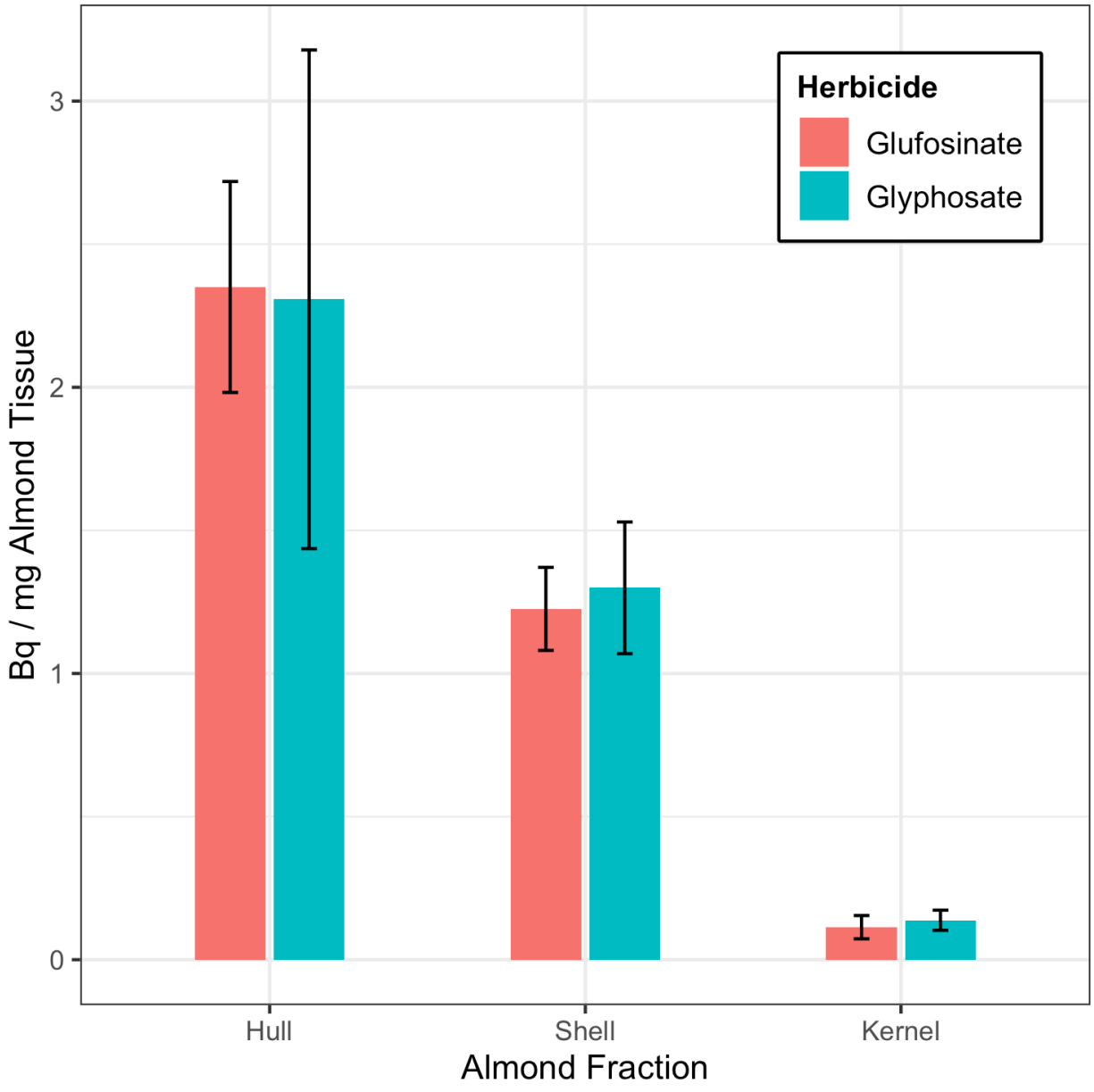
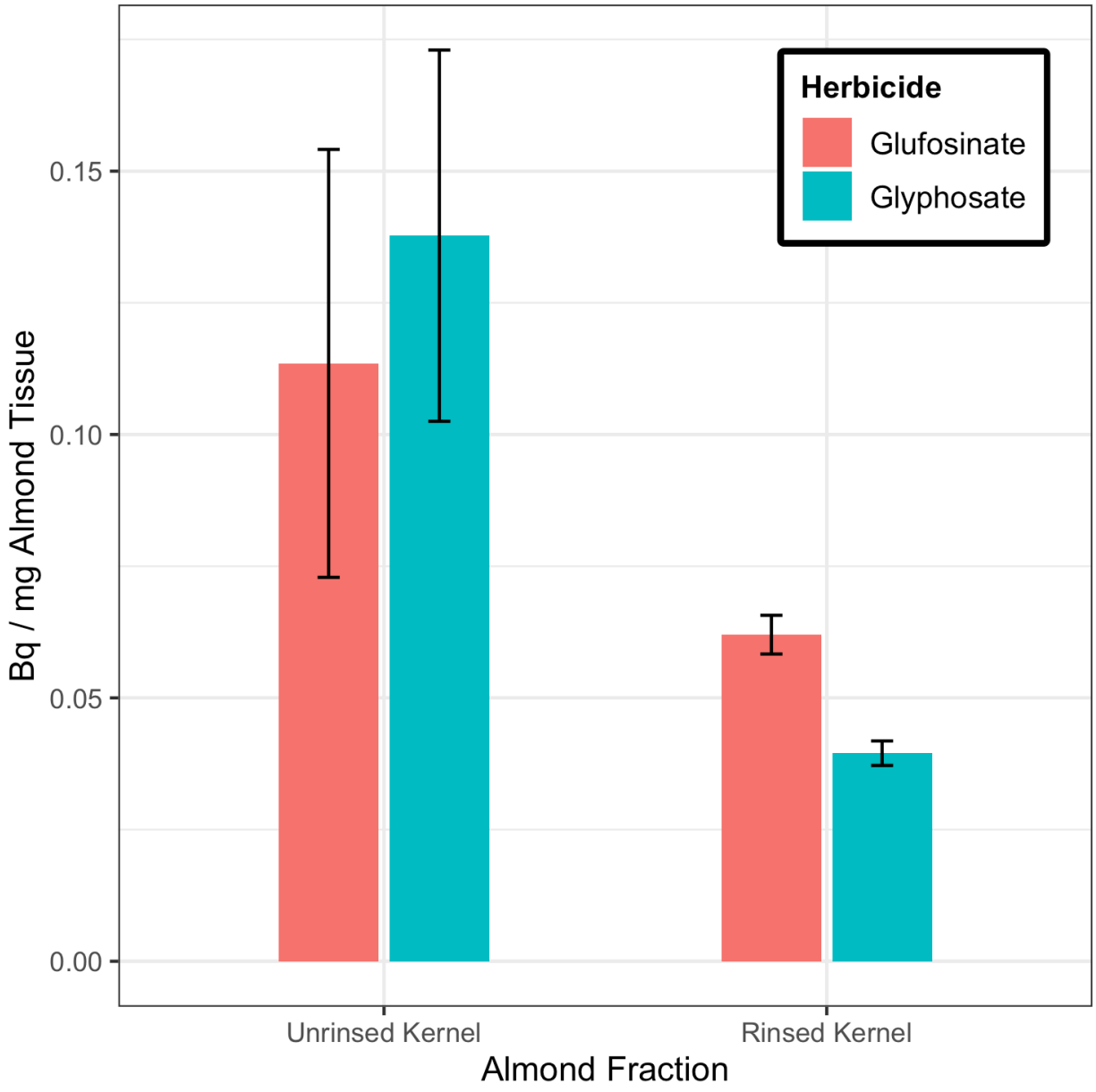


Figure 2.2. [^{14}C]-Glyphosate and glufosinate Becquerels per milligram of unrinsed and rinsed almond kernels from the kernel rinsate experiment. Total Bq added to the soil was 166,500. Error bars are representative of the 95% confidence interval.



Supplemental Materials

Figure S2.1. An image of the sample tumbling setup used for the soil transfer experiment, kernel rinsate experiment, and almond-to-almond transfer experiment.

Figure S2.2. Diagram of the field experiment conducted at Nickels Soil Laboratory. Plots were 19.6 m long by 4 m wide.

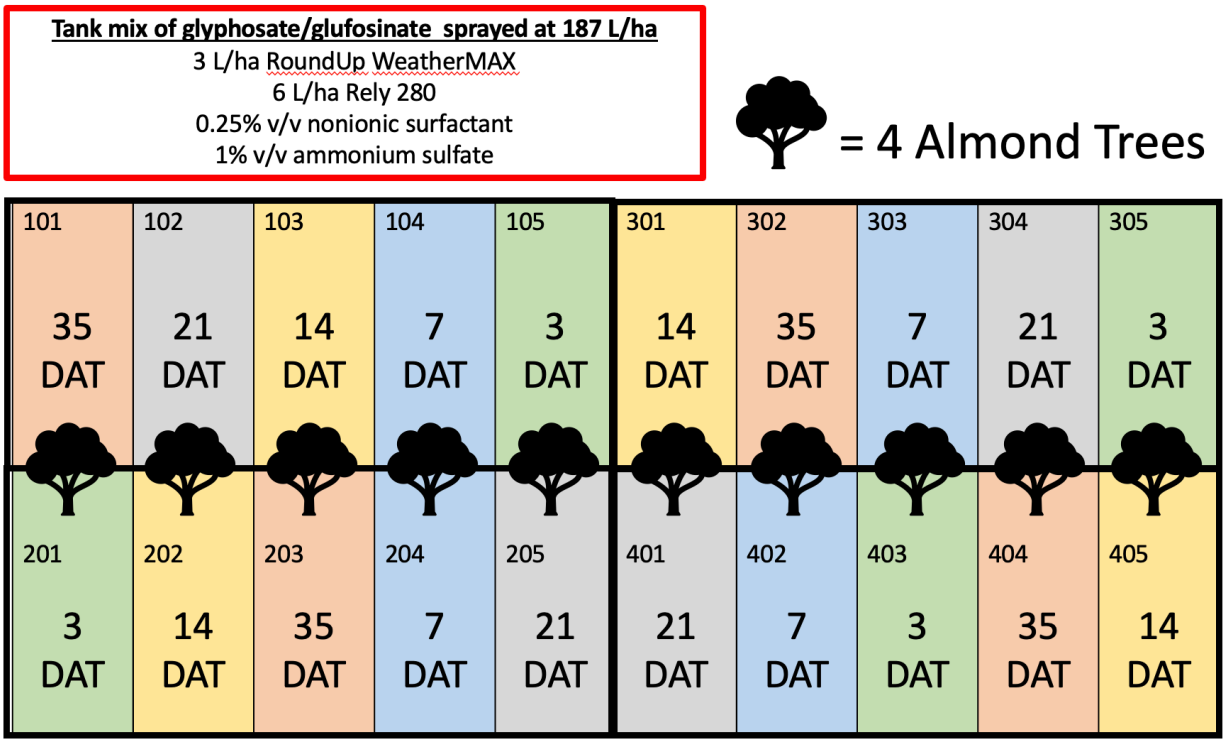


Table S2.1. Results of the rinsate analysis of the washed whole almonds for the soil transfer experiment. Whole almonds tumbled in [^{14}C]-herbicide treated soil were rinsed end-over-end in 20 mL of deionized water. The rinsate was analyzed for [^{14}C]-herbicide using a liquid scintillation counter. A total of 166,500 Bq were added to the soil.

[^{14}C]-Herbicide	Replicate	Bq	Average Bq	Standard Error (Bq)
Glyphosate	1	11,465	6,667	1,782
	2	7,073		
	3	4,888		
	4	3,240		
Glufosinate	1	5,817	6,130	2,319
	2	12,704		
	3	3,899		
	4	2,101		

Table S2.2. Results of the swipe analysis of the plastic barrier used to crack the almond shells in the soil transfer experiment. After whole almonds were tumbled in [^{14}C]-herbicide treated soil, almonds were shelled using a plastic barrier to crack the hard outer shell and expose the kernel. The plastic piece was swiped with filter paper and analyzed for [^{14}C]-herbicide using a liquid scintillation counter. A total of 166,500 Bq were added to the soil.

[^{14}C]-Herbicide	Replicate	Bq	Average Bq	Standard Error (Bq)
Glyphosate	1	165	154	36
	2	248		
	3	128		
	4	75		
Glufosinate	1	121	109	23
	2	85		
	3	166		
	4	62		

Table S2.3. Results of the post-harvest mimic kernels from the soil transfer experiment. Whole almonds were tumbled in [^{14}C]-herbicide treated soil. After tumbling, the whole almonds were hulled and shelled. The kernels were collected and analyzed for [^{14}C]-herbicide using a liquid scintillation counter. A total of 166,500 Bq were added to the soil.

[^{14}C]-Herbicide	Replicate	Bq mg^{-1}	Average Bq mg^{-1}	Standard Error (Bq mg^{-1})
Glyphosate	1	0.248	0.138	0.035
	2	0.097		
	3	0.149		
	4	0.059		
Glufosinate	1	0.138	0.093	0.016
	2	0.088		
	3	0.063		
	4	0.082		

Table S2.4. Results of the rinsate analysis of the whole almond wash in the almond-to-almond transfer experiment. The experiment was only conducted with glyphosate due to the available amount of [^{14}C]-glufosinate. Two whole almonds were dotted with a total of 166,500 Bq of [^{14}C]-glyphosate and tumbled with whole untreated almonds. The treated almonds were removed and the untreated were rinsed in 20 mL of deionized water. The rinsate was analyzed for [^{14}C]-glyphosate using a liquid scintillation counter.

^{14}C-Herbicide	Replicate	Bq	Average Bq	Standard Error (Bq)
Glyphosate	1	1,853		
	2	1,002		
			1,534	265
	3	1,173		
	4	2,109		

Determining how Almond Harvest and Processing Contributes to Low Levels of Glyphosate and Glufosinate Residues in Almonds

Formatted for submission to *Weed Science*

Abstract

California is the top producer of almonds worldwide, generating over $6 billion in revenue in 2020; the European Union (EU) is the primary importer of California almond exports. Weed control in almond orchards is an important part of the preharvest process because weeds can interfere with harvest equipment and host diseases. Broad-spectrum herbicides, such as glyphosate and glufosinate, are commonly used for preharvest weed control. Global differences in maximum residue limits (MRLs) and regulated compounds can pose a challenge for growers who rely on broad spectrum herbicides such as glyphosate and glufosinate for preharvest weed control. The EU MRL for glyphosate and total glufosinate is currently 0.1 mg kg^{-1}. Meanwhile the United States MRL for total glyphosate is 1 mg kg^{-1} and total glufosinate is 0.5 mg kg^{-1}. An 8-week field experiment, from spray to harvest, was conducted in an 8-hectare commercial orchard to evaluate the potential contribution of the preharvest herbicide treatment to low levels of herbicide residue in almonds. Then, the same batch of almonds were followed through a commercial processing facility to evaluate the potential movement of herbicide residues from soil, debris, and hulls to almond kernels during processing. Glyphosate was not found in any almond kernel samples at the end of processing. MPP, a glufosinate metabolite, was found in kernels at the end of processing at levels above the EU MRL for total glufosinate. The concentration of MPP in almonds sampled directly from the tree, without any contact with soil,

were found to have elevated MPP residues as well indicating glufosinate translocation may be a factor in low level glufosinate residues found in almonds.

Key Words: Maximum residue limit, herbicide, MPP

Introduction

California produces 80% of the world's almonds, and the crop is the most valued export commodity from the state, generating $4.9 billion in export revenue in 2019 (CDFA 2020). Currently there are more than 500,000 ha of bearing almond trees in California producing over 1.3 billion kilograms of almonds annually (USDA NASS 2020).

Almonds are mechanically harvested by shaking the trees, sweeping the nuts into windrows, and finally picking up the nuts from the orchard floor. Weeds on the orchard floor can reduce harvest efficiency by interfering with harvest equipment, so many growers utilize relatively intensive herbicide programs to maintain bare ground prior to harvest (Connell 2001, UCANR 2002). Glyphosate and glufosinate are two commonly used herbicides for preharvest programs because of their broad-spectrum weed control and relatively short preharvest intervals (PHIs), three and 14 days respectively (Bayer CropScience 2018, Bayer Group 2020). In 2018, over one million kg of glyphosate and nearly 300,000 kg of glufosinate-ammonium were applied in California almond orchards (CDPR 2018). Because of the harvest methods there is ample opportunity for whole almonds to come into contact with herbicide-treated soil.

After almonds are collected from the field, they are usually stockpiled under plastic covers before being transported to a processing facility for hulling and possibly shelling. At the huller/sheller, almonds are processed in large batches through rollers and gravity tables as well as pneumatic and sieve separatory equipment to remove dirt, debris, and hulls. These processes produce inshell almonds or include further steps to also remove shells to produce shelled almonds (US EPA 2009). Contact with contaminated processing equipment, almonds, and debris could provide another avenue for pesticide residue contamination.

California exports about two-thirds of its almond production annually (CDFA 2020), with most of the product shipping as shelled almonds (ABC 2019). Historically, the European Union (EU) has been the largest importer of California almonds with over 50% of the shelled product going to the EU whereas the largest importer of inshell almonds is Asia (ABC 2019). Exported shipments of almonds are subject to pesticide residue testing by the importing country's food safety authority, and residues must be at or below the maximum allowable concentration.

The maximum residue limit (MRL), commonly called tolerances in the United States (US), is defined by the Food and Agriculture Organization of the United Nations as the maximum allowable concentration of pesticide residue to be legally permitted in food commodities and animal feed (FAO 2022). In the US, glyphosate and glufosinate MRLs are defined to include the parent compounds (i.e. the herbicides themselves) and the primary metabolites (Bryant Christie Inc. 2021). For clarity, these MRLs will be referred to as "total glyphosate" or "total glufosinate" if the concentrations of the metabolites are to be summed with the concentration of the parent compound. The US MRL for total glyphosate in almond hulls is 25 mg kg^{-1} and 1 mg kg^{-1} for kernels. There is not a separate US MRL for inshell almonds because the residue in inshell almonds is determined by shelling the almonds and measuring the residue in only the kernels. The US MRL for total glufosinate in both almond hulls and kernels is 0.5 mg kg^{-1} (Bryant Christie Inc. 2021).

In the EU, the MRL for glyphosate is 0.1 mg kg^{-1-} in almond kernels (European Commission 2013) but there are not established MRLs for glyphosate metabolites. The EU MRL for glufosinate includes its metabolites N-acetyl glufosinate and 3-(methylphosphinico)propionic acid (MPP) ; the MRL for total glufosinate is 0.1 mg kg^{-1} (European Commission 2016).

Glyphosate is registered in the EU until 2022 (European Commission 2017). A review completed by the European Food Safety Authority (EFSA 2019) recommended that the MRL for parent glyphosate be reduced to 0.05 mg kg^{-1} and an optional total glyphosate MRL for the summation of glyphosate and its primary metabolites, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-acetyl-glyphosate, set to 0.2 mg kg^{-1}. It is anticipated that in upcoming years glyphosate MRLs will be reduced, and it is a possibility that the chemical may not be re-registered. If at any time the safety of a current MRL is reconsidered, the MRL can be reduced to the lowest limit of analytical detection which currently is 0.01 mg kg^{-1}, according to European statute (European Parliament 2005).

An initial field study conducted in 2020 by Martin and Hanson (2022; Ch 2) revealed that very low levels of herbicide can transfer to almonds during harvest operations, and those residues appear to be primarily due to herbicide-bound to soil particles. The European markets are integral to the economic success of the California almond industry; however, glyphosate and glufosinate are also very important to preharvest operations in the almond production system. The work presented here is a continuation of the project aimed to identify possible causes of low herbicide residues in almonds. The objectives of this project were to determine if low herbicide residues are still detectable in almonds throughout the process of commercial harvest operations and while the product moves through the huller/sheller processing facility.

Materials and Methods

Field Location

An 8-hectare, mature, commercial almond orchard with alternating rows of Nonpareil and Aldrich almonds located in the northern San Joaquin Valley near Hughson, California, United States (37°34'40.8"N 120°51'23.5"W) was used to conduct this study. The orchard row spacing was 6.7 meters apart and 5.5 meters between trees; the orchard was irrigated by furrow irrigation. Most of the orchard block is Tujunga loamy sand with some patches of Hanford sandy loam; the sand percentage of the orchard ranges from 70 to 80% (SoilWeb).

Glyphosate and Glufosinate Treatments

The preharvest herbicide treatment was applied on July 30, 2021. A tank mix of commercial glyphosate (Roundup WeatherMAX, Bayer Crop Science, 8400 Hawthorne Road, Kansas City, Missouri, United States) at 1,681 g ae ha^{-1}, commercial glufosinate (Rely 280, BASF Corporation, 100 Park Avenue, Florham Park, New Jersey, United States) at 1,681 g ai ha^{-1}, nonionic surfactant at 0.25% v/v (RAINIER-EA, Wilbur-Ellis Company LLC, 16300 Christensen Road #135, Tukwila, Washington, United States), and ammonium sulfate at 1% v/v (BRONC MAX, Wilbur-Ellis Company LLC, 16300 Christensen Road #135, Tukwila, Washington, United States). The herbicide was applied by the grower as part of regular commercial weed management using an unshielded boom sprayer. The application was made 26 days before the Nonpareil variety was shaken to the orchard floor and 48 days before the Aldrich variety was shaken. A table of all glyphosate and glufosinate applications made during the growing season is presented in Table 3.1.

Field Location Sampling

The field location was divided into three replicates (Figure 3.1) and three subsamples were taken within each replicate to form a composite sample from each variety. Leaves and almonds were sampled directly from both varieties of trees to help compare how much herbicide residue was present in the trees and on soil particles during and after harvest. Mature almond samples, including nuts with hulls, shells, and kernels, were taken from the windrow after undergoing the shaking and sweeping processes of harvest (Table 3.2). Additionally, soil samples were taken at three important timepoints during the harvest process: before the preharvest herbicide treatment, before shaking, and from the windrow after sweeping (Table 3.3). These three timepoints allowed for a snapshot of herbicide movement with soil particles. Both almond varieties were stockpiled at the huller sheller from the date of pick-up until the date of processing (Table 3.3).

Huller/Sheller Sampling

Sampling at the huller/sheller took place on October 28, 2021 (Tables 3.2 and 3.3). Almonds that were sampled during this time were from the same 8-ha field location but did not correspond with the field replicate sampling, because nuts are bulked together during harvest. Sampling within the facility began after the system was full, or after the first kernels from the truckload reached the shipping bin. Two truckloads, containing about 50 metric tons of whole almonds each, took about 1.5 hours to go through the huller/sheller.

The experimental almonds were processed to ship as shelled almonds; sampling occurred at the unloading, hulling, shelling, and shipment steps. First, composite samples were collected from the material stream as it left the truck hopper into the receiving pit of the facility.

Approximately 5 L of material was collected at the beginning, middle, and end of the truck unloading before the almonds entered the preprocess stage where sticks, rocks, excess soil, and other debris were removed using air, sieves, and gravity tables. Meanwhile, a soil sample was collected from the outlet where the fine debris exits the preprocessor at the same timepoints of the truck unloading. Then, while the batch of almonds was going through the hulling and shelling processes, hulls, shells, and kernels were sampled three times over 1.5 hours - at the beginning, middle, and end of the batch. Hulls were separated from the inshell almonds by gravity tables while shells were separated from kernels by gentle cracking and gravity tables. This sampling process was carried out for both Nonpareil and Aldrich varieties on the same day (Table 3.2). Throughout this processing day, three soil samples were taken from the baghouse, which collects the fine dust particles from multiple points in the hulling and shelling equipment, approximately 1.5 hours apart. Additional soil samples were collected directly from the hulling equipment and floor. All samples were brought to the laboratory and stored at room temperature until further hand processing and subsampling.

Sample Processing and Analysis

Almond samples were further processed by hand and dissected into their hull, shell, and kernel fractions, soil samples were sifted using a 2 mm sieve, and leaf samples were dried. From the processed samples, a representative 500-gram sample was sent to a commercial laboratory (Safe Food Alliance, 2037 Morgan Drive, Kingsburg, California, United States) for analysis. The laboratory used modified methods from QuPPe v 10 (EURL-SRM 2019) and high-pressure ion chromatography (HPIC) (Dionex™ Integrion™ HPIC™ System, Dionex, Sunnyvale, California, United States) coupled with a mass spectrometer (Orbitrap™ Exploris™ 120, Thermo Scientific,

Waltham, Massachusetts, United States) to quantify glyphosate, N-acetyl-glyphosate, AMPA, N-acetyl-AMPA, glufosinate, N-acetyl-glufosinate, and MPP in all almond samples. The soil samples were analyzed using the same instrumentation and modified methods from Druart et al. (2011). The limit of detection for each compound was 0.010 mg kg^{-1} for almonds samples and 0.040 mg kg^{-1} for soil samples.

Statistical Analysis

The data were subject to ANOVA using R statistical analysis software (2020) and multiple comparisons were performed with Tukey's HSD with $\alpha = 0.05$.

Results and Discussion

When examining the data presented it is worth noting that the field data (sample locations "Tree" and "Windrow") are treated as a separate data set from the huller/sheller data (sample locations "Truck" and "Huller/Sheller") because it was not possible to follow field replicates through the huller/sheller due to the harvest process resulting in the three replicates being stockpiled together; two truckloads from each stockpile (Aldrich and Nonpareil) were run through the processing facility.

Soil sampling revealed quantities of parent compound and some metabolites at every stage of the experiment (Tables 3.4 and 3.5). Replicate 1 of the "Pre-Sweep" and "Post-Sweep" samples had very high concentrations of glyphosate and glufosinate parent compounds and metabolites. Replicate 1 is at the front of the field so it is suspected that the first subsample sample may have been contaminated by high levels of herbicide from initial equipment testing

before the remainder of the field was sprayed, therefore, replicate 1 was not included in the analysis of those samples.

There were no significant differences between or within any of the samples. The higher levels of herbicide from the baghouse and the floor indicate there is detectable herbicide reside in particulate matter in the processing facility, but the filtration is doing its job by collecting those dust particulates in the baghouse. When comparing the soil data to the almond kernel data (Tables 3.4 and 3.5 to Tables 3.6 and 3.7) it is noteworthy that the major contributor to residues found in almonds is not the parent compound but the metabolites while the soil samples from the field and processing facility all contain detectable levels of parent compound, including the soil samples taken at the time of processing.

Glyphosate was found in Aldrich leaf samples at an average concentration of 0.036 mg kg^{-1}, no glyphosate metabolites were detected. Nonpareil leaves were below the limit of detection of for glyphosate compounds. Glufosinate was found in two Aldrich leaf samples at an average concentration of 0.192 mg kg^{-1}. MPP was found in all Aldrich and Nonpareil leaf samples at an average concentration of 0.348 mg kg^{-1}.

A summary of glyphosate residues found in almond fractions is presented in Table 3.6. Total glyphosate is presented as the sum of glyphosate, AMPA, N-acetyl-glyphosate, and N-acetyl-AMPA. Almond variety was not a significant factor in analysis so the Nonpareil and Aldrich data were pooled for a total of six replicate samples per sample location. The sample location was also not a significant factor in this field or huller/sheller data.

There were no statistically significant differences between glyphosate concentrations in almond hulls and shells; the majority of kernel samples at all sampling stages were below the limit of detection. Glyphosate was not found in any kernels from the final processing step of the

huller/sheller (Table 3.6). AMPA and N-acetyl-AMPA were not found in any almond samples. Therefore, all kernel samples tested would be within the residue limits for total glyphosate within the US and glyphosate within the EU.

There were two kernel replicates with glyphosate compounds detected (Table 3.6), one was sampled from the truck and contained 0.010 mg kg^{-1} of glyphosate and the other was sampled directly from the tree and contained 0.012 mg kg^{-1} of N-acetyl-glyphosate. Both of these detections are at allowable concentrations as determined by the US and EU; additionally, the detection of glyphosate in these individual samples was below the ESFA proposed glyphosate MRL of 0.05 mg kg^{-1}.

A summary of glufosinate residues found in almond fractions is presented in Table 3.5. Total glufosinate is presented as the summation of glufosinate, N-acetyl-glufosinate, and MPP. The majority of glufosinate residues found in almond samples were in the form of the metabolite MPP. Almond variety was a significant factor in the data collected from the field (sample locations “Tree” and “Windrow”) but not in the data collected from the processing steps (sample locations “Truck” and “Huller/Sheller”). Variety being a significant factor in glufosinate analysis is likely due to a physiological trait that is beyond the scope of this study. Both cultivars were grafted to the same peach rootstock, so differences in glufosinate concentration is likely not related to rootstock. The potential interaction between herbicide residue and rootstock and scion cultivars could be explored in future research.

There were no significant differences between total glufosinate concentrations in hulls and shells at any sampling location or within the sampling locations. Sample location was also not a significant factor in total glufosinate concentrations in the Aldrich or Nonpareil kernels. Interestingly, total glufosinate concentrations in some Aldrich and Nonpareil kernel samples

were above the EU MRL of 0.1 mg kg^{-1}, with the major contributor to the summation being MPP (Table 3.7).

The average concentration of MPP in Aldrich kernels coming from the huller/sheller, the last step of processing, was 0.89 mg kg^{-1} and the average concentration of MPP in the Nonpareils from the same sampling location was 0.109 mg kg^{-1}; this is above the EU MRL for total glufosinate (Table 3.7). However, the more surprising data came from the almonds sampled directly from the tree. Aldrich kernels from almonds sampled directly from the tree, having had no contact with the orchard floor, had an average MPP concentration of 0.76 mg kg^{-1} and the Nonpareil kernels had an average concentration of 0.104 mg kg^{-1} (Table 3.7).

A study conducted in apples of ^{14}C-glufosinate-ammonium metabolism and translocation of soil applied chemical revealed that MPP did translocate from into fruits, leaves, and shoots 14 days after application (EFSA 2005, 2015). The study found concentration of MPP in apple fruits at 0.104 mg kg^{-1}, similar to the concentrations observed in almonds in this study; no parent compound was found in the apples. The previous study conducted by Martin and Hanson (2022) found MPP concentrations in almond kernels was roughly 0.05 mg kg^{-1}. These differences are suspected to be due to soil type differences between the 2020 site and the 2021 data in this paper and will be examined further in future studies.

Almond hull, shell, and kernel residues were all below the United States MRLs for glyphosate, glufosinate, and their metabolites. However, the European Union MRLs total glufosinate in almond kernels were exceeded in Nonpareil kernels. Importantly, the concentration of MPP found in almond kernels sampled directly from the tree indicates that movement of the metabolite from the soil to the fruit may be playing a role in elevated residues before the almond touches the orchard floor. Throughout the growing season glufosinate was

used consistently in the orchard (Table 3.1), as is standard practice in many orchards. California growers will face challenges when choosing preharvest herbicide programs if the movement of glufosinate metabolites, specifically MPP, is proven to be a cause of herbicide residue in almonds, in addition to the pressures of potential MRL changes in the European Union.

Future research in MPP residue levels in almonds before and after tree shake across California and across different soil types will help address domestic and export market concerns. Additionally, studies will continue to examine harvest operations for other sources of pesticide residues such as insecticides, fungicides, and other herbicides.

Acknowledgements

This work was supported by the Almond Board of California, grant number HORT12.Hanson with partial grant support provided by Bayer CropScience. The authors would like to thank David Pohl allowing us to use his orchard as well as giving us access to his huller/sheller. No conflicts of interest have been declared.

Works Cited

[ABC] Almond Board of California (2019). Almond Industry Position Report 2019-2020 Crop Year. https://www.almonds.com/sites/default/files/2020-08/2020_07_PosRpt_hg5eg3.pdf. Accessed August 16, 2021.

Bayer CropScience (2018). Rely® 280. Bayer Publication 170522E. St. Louis, MO. 10 p

Bayer Group (2020). Roundup WeatherMAX® Herbicide. Bayer Publication 161018Bv2. St. Louis, MO. 11 p

Bryant Christie Inc. (2021). BC Global Pesticide MRLs. https://bcglobal.bryantchristie.com/db - pesticides/query. Accessed August 16, 2021

California Soil Resource Lab (2019). SoilWeb. https://casoilresource.lawr.ucdavis.edu/gmap/. Accessed December 2, 2021

[CDFA] California Department of Food and Agriculture (2020) California Agricultural Exports 2019-2020. 6-9 p. https://www.cdfa.ca.gov/Statistics/PDFs/2020_Exports_Publication.pdf. Accessed August 18, 2021

[CDPR] California Department of Pesticide Regulation (2018) 2018 Annual Statewide Pesticide Use Report Indexed by Commodity. https://www.cdpr.ca.gov/docs/pur/pur18rep/statewide_site_2018.htm. Accessed September 26, 2021

Connell JH, Colbert F, Kreuger W, Cudney D, Gast R, Bettner T, Dallman S (2001) Vegetation management options in almond orchards. HortTechnology 11(2):254-257. https://doi.org/10.21273/HORTTECH.11.2.254

Druart C, Delhomme D, de Vaufleury A, Ntcho E, Millet M (2011) Optimization of extraction

procedure and chromatographic separation of glyphosate, glufosinate, and aminomethylphosphonic acid in soil. Anal Bioanal Chem 399:1725-1732. https://doi.org/10.1007/s00216-010-4468-z

[EFSA] European Food Safety Authority (2005). Conclusion regarding the peer review of the pesticide risk assessment of active substance glufosinate. EFSA Scientific Report (2005) 27, 1-81. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2005.27r. Accessed February 10, 2022

[EFSA] European Food Safety Authority (2015). Reasoned opinion on the review of the existing maximum residue limits (MRLs) for glufosinate according to Article 12 of Regulation (EC) No 396/2005. Parma:Italy. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2015.3950. Accessed February 10, 2022

[EFSA] European Food Safety Authority (2019) Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 17(10):5862

[EURL-SRM] EU Reference Laboratory for Pesticides Requiring Single Residue Methods (2019) Quick Method for the Analysis of Numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS Involving Simultaneous Extraction with Methanol (QuPPE-Method). Version 10. Fellbach, Germany

European Commission (2013) Commission Regulation (EU) No 293/2013 of 20 March 2013 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for emamectin

benzoate, etofenprox, etoxazole, flutriafol, glyphosate, phosmet, pyraclostrobin, spinosad and spirotetramat in or on certain products. https://eur-lex.europa.eu/eli/reg/2013/293/oj. Accessed August 2, 2021

European Commission (2016) Commission Regulation (EU) 2016/1002 of 17 June 2016 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for AMTT, diquat, dodine, glufosinate and tritosulfuron in or on certain products. https://eur-lex.europa.eu/eli/reg/2016/1002/oj. Accessed August 2, 2021

European Commission (2017) Commission Implementing Regulation (EU) 2017/2324 of 12 December 2017 renewing the approval of the active substance glyphosate in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. https://eur-lex.europa.eu/eli/reg_impl/2017/2324/oj. Accessed August 2, 2021

European Parliament, Council of the European Union (2005) Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. https://eur-lex.europa.eu/eli/reg/2005/396/oj. Accessed August 2, 2021

[FAO] Food and Agriculture Organization of the United Nations (2022) Codex Alimentarius. https://www.fao.org/fao-who-codexalimentarius/codex-texts/maximum-residue-limits/en/#:~:text=A%20maximum%20residue%20limit%20(MRL,accordance%20with%20Good%20Agricultural%20Practice. Accessed February 17, 2022

Martin K, Hanson BD (2022) Evaluating the Effects of Extended Preharvest Intervals on Glyphosate and Glufosinate Residues in Almonds. Weed Science: In Review.

[UCANR] University of California Agriculture and Natural Resources (2002) Integrated Pest Management for Almonds. 2nd edition. Oakland, CA: Regents of the University of California Division of Agriculture and Natural Resources, 160-166 p

[USDA NASS] United States Department of Agriculture National Agricultural Statistics Service (2020) 2020 California Almond Objective Measurement Report, 3-7 p. https://www.nass.usda.gov/Statistics_by_State/California/Publications/Specialty_and_Other_Releases/Almond/Objective-Measurement/202007almom.pdf. Accessed August 18, 2021

[US EPA] United States Environmental Protection Agency (2009) 9.10.2.1 Almond Processing. Washington D.C.: U.S. Environmental Protection Agency AP-42 https://www.epa.gov/air-emissions-factors-and-quantification/ap-42-compilation-air-emissions-factors. Accessed February 10, 2022

Table 3.1. Glyphosate and glufosinate herbicide applications throughout the 2021 growing season.

Date	Active Ingredient	Commercial Product	Application Rate
December 24, 2020	Glufosinate-ammonium	Reckon 280 SL	4.6 L / treated ha
June 9, 2021	Glufosinate-ammonium	Lifeline	4.6 L / treated ha
June 9, 2021	Glyphosate	Honcho K6	3.2 L / treated ha
July 30, 2021	Glufosinate-ammonium	Rely 280	6 L / treated ha
July 30, 2021	Glyphosate	RoundUp PowerMAX	3 L / treated ha

Table 3.2. Dates of significant farming practices for the 2021 growing season.

	Nonpareil	Aldrich
Final Preharvest Herbicide Application	July 30, 2021	July 30, 2021
Leaf and almond samples directly from the tree	August 16, 2021	September 2, 2021
Shake	August 25, 2021	September 16, 2021
Sweep	August 30, 2021	September 18, 2021
Almond sampling from field	September 6, 2021	September 22, 2021
Pickup and stockpile	September 6, 2021	September 24, 2021
Huller/sheller processing	October 28, 2021	October 28, 2021

Table 3.3. Dates and definitions of non-almond samples taken throughout the field and processing facility during the 2021 growing season.

Soil Sample	Date
“Pre-Spray” soil sample - sample was taken before the preharvest application of glyphosate and glufosinate	July 21, 2021
“Pre-Sweep” soil sample - sample was taken after the preharvest but before the almonds were swept into the windrow	August 16, 2021
“Post-Sweep” soil sample - sample was taken from the strips after the almonds had been swept into the windrow	September 6, 2021
“Pre-Process” soil sample - soil and debris was taken from the outlet leading to the debris pile	October 28, 2021
“Baghouse” sample - dust sample was taken from the baghouse outlet leading to the fine dust pile	October 28, 2021
“Huller” sample - sample was directly from the hulling equipment during processing	October 28, 2021
“Floor” soil sample - sample was taken from floor sweepings around the hulling equipment during processing	October 28, 2021

Table 3.4. Summary of the concentration of glyphosate and its metabolites found in soil during the different harvest and processing operations. The values are presented as mean concentration ± standard error (n=3).

	Glyphosate	AMPA	N-Acetyl-Glyphosate	N-Acetyl-AMPA	Total Glyphosate
Soil Sample	mg kg^{-1} ± SE				
Pre-Spray	0.525 ± 0.111	0.192 ± 0.025	< LOD	< LOD	0.715 ± 0.134
Pre-Sweep*	0.408 ± 0.058	0.214 ± 0.070	< LOD	< LOD	0.622 ± 0.128
Post-Sweep*	0.261 ± 0.047	0.146 ± 0.020	< LOD	< LOD	0.407 ± 0.028
Aldrich Pre-Process	0.302 ± 0.102	< LOD	< LOD	< LOD	0.302 ± 0.102
Nonpareil Pre-Process	0.392 ± 0.108	< LOD	< LOD	< LOD	0.392 ± 0.108
Baghouse	0.864 ± 0.072	0.048[1]	< LOD	< LOD	0.892 ± 0.085
Floor**	0.973	0.062	< LOD	< LOD	1.035

[1] Two replicate samples were below the limit of detection

* One replicate sample was omitted from the data set due to an extremely high sample that is an anomaly

** One representative sample from floor dust accumulation was collected due to regular facility cleaning operations

Table 3.5. Summary of the concentration of glufosinate and its metabolites found in soil and debris during the different harvest and processing operations. The values are presented as mean concentration ± standard error.

	Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate
Soil Sample	mg kg^{-1} ± SE			
Pre-Spray	0.441 ± 0.142	0.074 ± 0.011	0.807 ± 0.336	1.322 ± 0.484
Pre-Sweep*	0.224 ± 0.055	0.043 ± 0.002	0.478 ± 0.152	0.745 ± 0.209
Post-Sweep*	0.182 ± 0.028	0.043[1]	0.235 ± 0.002	0.438 ± 0.008
Aldrich Pre-Process	0.142[2]	0.054[2]	0.126 ± 0.049	0.215 ± 0.101
Nonpareil Pre-Process	0.280 ± 0.112	0.047[2]	0.185 ± 0.041	0.494 ± 0.166
Baghouse	0.407 ± 0.058	0.046 ± 0.001[1]	0.322 ± 0.042	0.772 ± 0.100
Floor**	0.477	< LOD	0.374	0.851

[1] One replicate sample was below the limit of detection

[2] Two replicate samples were below the limit of detection

* One replicate sample was omitted from the data set due to an extremely high sample that is an anomaly

** One representative sample was collected due to limited timing in the processing facility and regular cleaning operations

Table 3.6. Summary of the concentration of glyphosate and its metabolites in almond hulls, shells, and kernels at different sampling locations. The values are presented as mean concentration ± standard error (n = 6).

	HULLS				
	Glyphosate	AMPA	N-Acetyl-Glyphosate	N-Acetyl-AMPA	Total Glyphosate
Sampling Location	mg kg^{-1} ± SE				
Tree	0.033 ± 0.005[3]	< LOD	< LOD	< LOD	0.033 ± 0.005[3]
Windrow	0.072 ± 0.031[2]	< LOD	< LOD	< LOD	0.072 ± 0.031[2]
Truck	0.044 ± 0.013	< LOD	< LOD	< LOD	0.044 ± 0.013
Huller/Sheller	0.057 ± 0.030	< LOD	< LOD	< LOD	0.057 ± 0.030
	SHELLS				
	Glyphosate	AMPA	N-Acetyl-Glyphosate	N-Acetyl-AMPA	Total Glyphosate
Sampling Location	mg kg^{-1} ± SE				
Tree	0.015[5]	< LOD	< LOD	< LOD	0.015[5]
Windrow	0.031 ± 0.013[2]	< LOD	< LOD	< LOD	0.031 ± 0.013[2]
Truck	0.027 ± 0.008[3]	< LOD	< LOD	< LOD	0.027 ± 0.008[3]
Huller/Sheller	< LOD	< LOD	< LOD	< LOD	< LOD
	KERNELS				
	Glyphosate	AMPA	N-Acetyl-Glyphosate	N-Acetyl-AMPA	Total Glyphosate
Sampling Location	mg kg^{-1} ± SE				
Tree	< LOD	< LOD	0.012[4]	< LOD	0.018[4]
Windrow	< LOD	< LOD	< LOD	< LOD	< LOD

Truck	0.010[4]	< LOD	< LOD	< LOD	0.010[4]
Huller/Sheller	< LOD	< LOD	< LOD	< LOD	< LOD

[1] One replicate sample was below the limit of detection

[2] Two replicate samples were below the limit of detection

[3] Three replicate samples were below the limit of detection

[4] Five replicate samples were below the limit of detection

Table 3.7. Summary of the concentration of glufosinate and its metabolites in almond hulls, shells, and kernels at different sampling locations. The values are presented as mean concentration ± standard error. Almond variety was a significant factor and was included in analysis.

ALDRICH HULLS					NONPAREIL HULLS				
	Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate		Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate
Sampling Location	mg kg^{-1} ± SE				Sampling Location	mg kg^{-1} ± SE			
Tree	< LOD	< LOD	0.366 ± 0.052	0.366 ± 0.052	Tree	< LOD	< LOD	0.271 ± 0.025	0.271 ± 0.025
Windrow	0.069 ± 0.029[1]	< LOD	0.498 ± 0.043[1]	0.567 ± 0.014[1]	Windrow	< LOD	< LOD	0.205 ± 0.008[1]	0.205 ± 0.008[1]
Truck	< LOD	< LOD	0.227 ± 0.030	0.227 ± 0.030	Truck	< LOD	< LOD	0.218 ± 0.020	0.218 ± 0.020
Huller/Sheller	0.132[2]	< LOD	0.240 ± 0.007	0.284 ± 0.040	Huller/Sheller	< LOD	< LOD	0.225 ± 0.007	0.225 ± 0.007
ALDRICH SHELLS					NONPAREIL SHELLS				
	Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate		Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate
Sampling Location	mg kg^{-1} ± SE				Sampling Location	mg kg^{-1} ± SE			
Tree	< LOD	< LOD	0.251 ± 0.043	0.251 ± 0.043	Tree	< LOD	< LOD	0.197 ± 0.033	0.197 ± 0.033
Windrow	0.055[2]	< LOD	0.494 ± 0.153	0.512 ± 0.151	Windrow	0.020[2]	< LOD	0.232 ± 0.018[1]	0.242 ± 0.008[1]
Truck	< LOD	< LOD	0.291 ± 0.012	0.291 ± 0.012	Truck	< LOD	< LOD	0.214 ± 0.018	0.214 ± 0.018
Huller/Sheller	< LOD	< LOD	0.275 ± 0.009	0.275 ± 0.009	Huller/Sheller	< LOD	< LOD	0.273 ± 0.004	0.273 ± 0.004
ALDRICH KERNELS					NONPAREIL KERNELS				
	Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate		Glufosinate	N-Acetyl-Glufosinate	MPP	Total Glufosinate

Sampling Location	mg kg^{-1} ± SE				Sampling Location	mg kg^{-1} ± SE			
Tree	< LOD	< LOD	0.076 ± 0.008	0.076 ± 0.008	Tree	< LOD	< LOD	0.104 ± 0.004	0.104 ± 0.004
Windrow	< LOD	< LOD	0.111 ± 0.025	0.111 ± 0.025	Windrow	< LOD	< LOD	0.099 ± 0.004[1]	0.099 ± 0.004[1]
Truck	< LOD	< LOD	0.077 ± 0.001	0.077 ± 0.001	Truck	0.016[2]	< LOD	0.122 ± 0.010	0.127 ± 0.012
Huller/Sheller	< LOD	< LOD	0.089 ± 0.003	0.089 ± 0.003	Huller/Sheller	< LOD	< LOD	0.109 ± 0.003	0.109 ± 0.003

[1] One replicate sample was below the limit of detection

[2] Two replicate samples were below the limit of detection

Figure 3.1. Field sampling layout

Concluding Remarks

Part of this work examined how irrigation water quality effects the partitioning of three weak acid herbicides into soil solution. While it is well known that poor irrigation water quality can be harmful to the crop and soil health, this work shows there is no drastic effect on saflufenacil, indaziflam, or penoxsulam partitioning in to soil solution. The minimal effects of pH and salinity on these herbicides indicate crop and groundwater safety as well as herbicide efficacy would not be compromised by irrigation water quality.

Regulatory differences and changes among the United States and European Union pose challenges to California almond growers. This work demonstrated that low levels of glyphosate and glufosinate residue could transfer to almonds via herbicide-bound soil particles during harvest however, the more concerning issue proved to be glufosinate or MPP movement into the almond trees. Field studies revealed that the glufosinate metabolite MPP can be accumulated in the almond tree causing residue levels that exceed the European Union maximum residue limit for total glufosinate.

Herbicide use is an integral part of commercial tree nut orchard production. Continuing herbicide research in orchards helps growers, cooperators, and regulators better understand how the different parts of orchard production interact with each other and contribute to a healthy system.