Weed Technology

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Research Article

Cite this article: Osipitan OA, Yildiz-Kutman B, Watkins S, Brown PH, Hanson BD (2020) Impacts of repeated glyphosate use on growth of orchard crops. Weed Technol. **34**: 888–896. doi: 10.1017/wet.2020.85

Received: 29 April 2020
Revised: 6 July 2020
Accepted: 23 July 2020
First published online: 18 August 2020

Associate Editor:
Darren Robinson, University of Guelph

Keywords:
Perennial specialty crop; plant nutrition; chlorophyll content; growth; trunk diameter; glyphosate nutrient interaction

Nomenclature:
Glyphosate; almond; *Prunus dulcis* (Mill.) D.A. Webb; cherry; *Prunus avium* (L.) L.; plum; *Prunus domestica* L.

Author for correspondence:
Bradley D. Hanson, Department of Plant Sciences, One Shields Avenue, University of California, Davis, CA 95616
(Email: bhanson@ucdavis.edu)

Impacts of repeated glyphosate use on growth of orchard crops

O. Adewale Osipitan[1], Bahar Yildiz-Kutman[2], Seth Watkins[3], Patrick H. Brown[4] and Bradley D. Hanson[5]

[1]Postdoctoral Researcher, Department of Plant Sciences, University of California, Davis, CA, USA; [2]Assistant Professor, Institute of Biotechnology, Gebze Technical University, Kocaeli, Turkey; [3]Staff Research Associate, Department of Plant Sciences, University of California, Davis, CA, USA; [4]Professor, Department of Plant Sciences, University of California, Davis, CA, USA and [5]Cooperative Extension Specialist, Department of Plant Sciences, University of California, Davis, CA, USA

Abstract

Glyphosate is an important component of herbicide programs in orchard crops in California. It can be applied alone or in tank-mix combinations under the crop rows or to the entire field and often is used multiple times each year. There has been speculation about the potential impacts of repeated use of glyphosate in perennial crop systems, because of uptake from shallow root systems or indirectly because of effects on nutrient availability in soil. To address these concerns, research was conducted from 2013 to 2020 on key orchard crops to evaluate tree response to glyphosate regimens. Almond, cherry, and prune were evaluated in separate experiments. In each crop, the experimental design was a factorial arrangement of two soil types, four glyphosate rates (0, 1.1, 2.2, and 4.4 kg ae ha^{-1}, applied three times annually), and two post-glyphosate application irrigation treatments. In the first 2 yr of the study, there was no clear impact of the glyphosate regimens on shikimate accumulation or leaf chlorophyll content, which suggested no direct effect on the crop. In the seventh year of the study, after six consecutive years of glyphosate application to the orchard floors, there were no negative impacts of glyphosate application on leaf nutrient concentration or on cumulative trunk growth in any of the three orchard crops. Lack of a negative growth impact even at the highest treatment rate, which included 18 applications of glyphosate totaling nearly 80 kg ae ha^{-1} glyphosate over the course of the experiment suggest there is not likely a significant risk to tree health of judicious use of the herbicide in these production systems. Given the economic importance of orchard crops in California, and grower and industry concerns about pesticides generally and specifically about glyphosate, these findings are timely contributions to weed management concerns in perennial specialty crops.

Introduction

Tree fruit, tree nut, and vineyard production systems are economically important in California. In 2017, orchards and vineyards accounted for more than 1.6 million irrigated ha in the state and had an aggregate farm-gate value of more than $21 billion (CDFA 2018). Of the top 10 commodity groupings in California in 2017, grape (*Vitis vinifera* L.), almond, pistachio (*Pistacia vera* L.), oranges [*Citrus x sinensis* (L.) Osbeck] alone had a collective value of $16 billion (CDFA 2018). Although production practices in orchard systems vary among crops and growing regions, weed management is an important component in these intensely farmed high-value crops. As in most crops, weeds can directly compete with trees for limited resources, especially during the establishment period. In addition to direct competition, weeds can interfere with cultural operations such as irrigation, pruning, harvesting, and application of fertilizers and pesticides. In some crops, understory vegetation is managed to reduce the risk of frost during critical periods in the spring. Because of planting arrangements and irrigation infrastructure, weed management in these crops often uses different approaches within the tree row compared with the area between crop rows (Hanson et al. 2014). Commonly, California orchards are managed using relatively intense herbicide programs to manage weeds in the “strips” within the tree row, whereas less-intense chemical and/or physical methods are used in the “middles.” The width of the herbicide-treated strip varies by crop and among growers but can range from as narrow as 0.5 m in grapevines and young trees to 5.5 m in large-tree crops such as walnut. Although less common, some growers use full orchard-floor herbicide programs instead of mowing or tillage to reduce management time and dust generated by these mechanical weed-control operations.

In most orchard crops in California, herbicide programs are highly dependent on glyphosate as part of a tank mix applied with PRE herbicides and for POST weed control at multiple times during the year. In all tree crops for which data are available, glyphosate is by far the most widely used herbicide (CDPR 2020). Based on treated acres and gross crop acres, the average orchard is treated two to three times each year with glyphosate and potentially more often if PRE herbicides

Table 1. Planting, glyphosate application, and trunk diameter measurement dates in three orchard experiments conducted to evaluate the cumulative effects of glyphosate over six growing seasons in California.

	Year							
Activity	2013	2014	2015	2016	2017	2018	2019	2020
Planting	Mar 13							
Glyphosate application								
First		Apr 15	Apr 22	Apr 29	Jun 6	Jun 21	May 2	
Second		Jun 10	Jun 12	Jul 20	Jul 20	Sep 7	Jul 19	
Third		Aug 5	Aug 11	Aug 31	Nov 1	Oct 24	Nov 13	
Leaf sampling								
		April 29	May 21				Oct 24	
		May 13	July 11					
		June 24	Sep 9					
		July 9						
		Aug 19						
		Sep 2						
Trunk diameter measurement		Mar 27	Mar 6	Feb 12	Feb 7	Mar 7	Feb 22	Feb 5

are not used as part of the year-round weed control program. For several years in the late 2000s, several publications suggested there are nontarget effects of glyphosate, including interactions with crop nutrient status, plant disease interactions, and soil microbial-community effects (reviewed by Duke et al. 2012). Although these reports were largely in the context of glyphosate-tolerant soybean systems, California orchard-crop growers and industries expressed concern about micronutrient availability issues or direct glyphosate effects in orchard systems in which glyphosate is regularly used. In particular, one trade publication (Huber 2007) was widely distributed among the crop-input supply chain and used to support sales of manganese and other micronutrient products in tree nut crops.

In their review, which focused on annual cropping systems, Duke et al. (2012) suggested that "significant effects of glyphosate on soil mineral content or availability to plants are highly unlikely." Although relatively less well explored in perennial cropping systems, available crop production statistics in California do not suggest there are negative indirect effects of glyphosate on orchard crops despite decades of use (CDFA 2018). However, given the significant investment involved in establishing and maintaining these long-lived orchard crops, their high value, and the conflicting information available from trade channels, many growers remain concerned about the potential for subtle cumulative direct or indirect effects of glyphosate use in orchard crops. To address these concerns, a 7-yr study was conducted on three orchard crops to evaluate growth and various plant-health metrics related to glyphosate treatments. To create a worst-case scenario, the experimental design included rates up to 4.4 kg ae ha^{-1}, multiple applications per year, a coarse soil in some planting sites, and, in the first 2 yr of the study, herbicide treatments were immediately followed with a simulated flood irrigation event to facilitate downward movement of the herbicide into the root zone.

Materials and Methods

Experimental Description and Design

This research was conducted for the 7 yr during 2013 to 2020. Experimental orchards were planted at the University of California, Davis Plant Sciences Field Facility (38.5382°N, 121.7617°W) for almond, cherry, and prune in spring 2013 (Table 1). Half of the trees in each orchard were planted in the soil native to the field in Yolo County (Rincon silty clay loam), with 1.74% organic matter (OM), pH of 7.7, and 28.1 mEq 100 g^{-1} cation exchange capacity (CEC) (Andrews 1972); and half were planted in soil imported from Merced County (Delhi sandy loam) with 0.87% organic matter (OM), pH of 6.9, and 3.8 mEq 100 g^{-1} CEC (Qin et al. 2013). Before planting, each tree site was prepared using a tractor-mounted auger to create a 90-cm diam by 60-cm deep hole; every second hole was refilled with either the native silty clay loam soil or the imported sandy loam soil; these were considered paired plots. The orchard was planted in March 2013 with dormant, bareroot nursery stock in a 3 by 6 m spacing irrigated with microsprinklers at each tree.

Each experiment was managed according to local practices (e.g., Micke et al. 1996) with microsprinkler irrigation, fertilization, insect and disease management, and pruning practices. Weeds were managed with glufosinate several times each season in addition to the experimental treatment regimens. In 2017, the entire almond experiment was inadvertently treated with one application of glyphosate in addition to the glyphosate and no-glyphosate treatments in the experimental design.

Beginning in spring 2014, glyphosate (Roundup PowerMAX®; Monsanto, St. Louis, MO) was applied at 0, 1.1, 2.2, and 4.4 kg ae ha^{-1} (equivalent to 0X, 1X, 2X, and 4X, respectively, of a common use rate in orchard crops) three times each growing season (Table 1). Ammonium sulfate (1% vol/vol, Bronc® Max; Wilbur-Ellis Agribusiness, San Francisco, CA) was included with each glyphosate treatment. Glyphosate was applied to a 2 by 2 m area around the base of each tree using two passes (one on either side of the tree to simulate a grower "strip" treatment) with a CO_2-pressurized backpack sprayer at 186 kPa, equipped with two flat-fan XR11002 nozzles (TeeJet® Technologies; Spraying Systems Co., Wheaton, IL), calibrated to deliver 187 L ha^{-1} of spray solution. In the first season, the trunks were protected with wax-paper cartons, but during the remainder of the experiment, the lower 20 cm of trunk was exposed to spray solution when the spray patterns overlapped from the two passes, which is consistent with production practices in the region. Herbicide-application equipment setup, lower-limb pruning, and sucker-removal practices minimized the risk of foliar exposure.

In the first 2 yr of glyphosate treatment, a post-glyphosate irrigation treatment (drench vs. none) was included as a split-plot factor. In the drench treatments, a shallow earthen berm was built around the tree and, immediately after each glyphosate application, 20 L of water was poured into the basin—a volume that approximated a 2.5 ha-cm irrigation within the confined area.

https://doi.org/10.1017/wet.2020.85 Published online by Cambridge University Press

Table 2. Treatment structure in three California orchard experiments conducted to evaluate the cumulative effects of three annual glyphosate applications over six consecutive years.

No.	Soil type[a]	Glyphosate rate[b]	Postapplication irrigation[c]
		kg ae ha^{-1}	
1	Silty clay loam	0	None
2	Silty clay loam	1.1	None
3	Silty clay loam	2.2	None
4	Silty clay loam	4.4	None
5	Silty clay loam	0	Drench
6	Silty clay loam	1.1	Drench
7	Silty clay loam	2.2	Drench
8	Silty clay loam	4.4	Drench
9	Sandy loam	0	None
10	Sandy loam	1.1	None
11	Sandy loam	2.2	None
12	Sandy loam	4.4	None
13	Sandy loam	0	Drench
14	Sandy loam	1.1	Drench
15	Sandy loam	2.2	Drench
16	Sandy loam	4.4	Drench

[a]Experiments were conducted in a site with a silty clay loam soil, but a split-plot factor included half of the trees planted in a 90-cm diam planting site filled with imported sandy loam soil.
[b]Each treatment was applied three times each year during 2014–2019. Ammonium sulfate was used as adjuvant.
[c]In the first 2 yr of the experiments, an additional split-plot factor included a 20-L flush of water immediately after each glyphosate application to facilitate movement of the herbicide into the tree root zone.

The intent of this drench treatment was to increase the potential for leaching of the just-applied herbicide into the root zone of the young tree.

The experimental design was a factorial arrangement of two planting site soils, four glyphosate rates, and two post-treatment irrigation regimens (Table 2). Each treatment was replicated four times in single-tree plots, making a total of 64 experimental units. The almond, cherry, and prune crops were managed separately and considered independent experiments.

Data Collection and Analysis

In 2014, the first year of glyphosate application, shikimate assays were conducted 14 d after each glyphosate application to evaluate direct herbicidal effects of glyphosate. Five young leaves were collected from different parts of each tree and this composite leaf sample was prepared for shikimate accumulation assays using the procedure described by Ozturk et al. (2008) and Hanson et al. (2009). Additionally, in the first 2 yr of glyphosate application (2014 and 2015), the relative chlorophyll content in six randomly selected, youngest, fully expanded leaves from each tree was measured at 30 d after each glyphosate application using a SPAD-502 meter (Spectrum Technologies Inc., Plainfield, IL). These values provided a general approximation of the health of the photosynthetic apparatus and would be affected by either direct herbicidal effect of glyphosate or by indirect effects of micronutrient limitations. On the basis of the initial results, which indicated no treatment-related effects, the shikimate and chlorophyll analyses were discontinued after the first and second growing seasons, respectively. In October 2019, after 6 yr of glyphosate treatments, five young, fully expanded leaves were collected from different parts of each tree to measure the following nutrients: nitrogen (N); phosphorus (P); potassium (K); calcium (Ca); magnesium (Mg); sulfur (S); boron (B); iron (Fe); zinc (Zn); manganese (Mn); and copper (Cu). Leaf samples were collected from all trees but composited over soil type and soil drench split plots to represent only the glyphosate rate main effect in the four experimental replicates in each orchard crop. The nutrients were quantitatively determined by the University of California Davis Analytical Laboratory using combustion (AOAC, 2005) or nitric acid digestion (Sah and Miller, 1992) methods as appropriate for each element.

Trunk diameter was used as a measure of growth of each orchard crop. Trunk diameter 45 cm above the soil surface was measured before the first glyphosate application in 2014 and then in each subsequent year during the dormant season (between January and March of each year), from 2015 to 2020, accounting for 6 yr of post-glyphosate observation. A three-parameter sigmoidal regression model was used to characterize the growth of the orchard crops over 6 yr for the different glyphosate treatments; Equation 1 was used:

$$T = \frac{a}{\{1 + \exp[-(x - h)/b]\}} \qquad [1]$$

where T was the trunk diameter; a was the trunk diameter at the final observation (i.e., sixth year after glyphosate application); x was the year of observation; h was the year at which half of the observed final trunk diameter was achieved, as a measure of growth speed at the log stage; and b was the slope around h. ANOVA was used to determine the effects of soil type, glyphosate rate, post-glyphosate application irrigation, and their interactions on the orchard crop response. A treatment was considered to be significant if $P \leq 0.050$, and this was followed by the Tukey honestly significant difference test for mean comparison. All statistical analyses and graphs were performed with SigmaPlot® 14 software (Systat Software Inc., San Jose, CA).

Results and Discussion

Shikimate, Chlorophyll, and Plant Nutrients

There were no differences in shikimate accumulation among the glyphosate-treated and nontreated plants in the almond experiment irrespective of the soil type or postapplication irrigation (Tables 3 and 4). For example, the shikimate accumulation in untreated almond trees (0.20 µmol g^{-1}) 14 d after the third glyphosate application was not significantly different from the 0.28, 0.14, and 0.15 µmol g^{-1} accumulated shikimate in trees grown in a coarse soil (sandy loam) treated with glyphosate rates of 1.1, 2.2, and 4.4 kg ae ha^{-1}, respectively, with postapplication drench (Tables 3 and 4). Similar shikimate accumulation results were observed in the cherry and prune experiments, except for a significant interaction between soil type ($P = 0.035$) or postapplication irrigation ($P = 0.047$) and glyphosate rate, 14 d after the first glyphosate application in cherry (Tables 3 and 4). However, because there was no clear association with the higher-risk treatments (i.e., high application rates, coarse soil, post-treatment drench), these few significant interactions in cherry may be spurious rather than an indication of cherry sensitivity.

When absorbed and translocated in a sensitive plant, glyphosate binds with the 5-enolpyruvylshikimate 3-phosphate (EPSP) enzyme in the shikimate pathway, thereby inhibiting the conversion of shikimate-3-phosphate (and phosphoenolpyruvate) to EPSP and inhibiting the biosynthesis of important aromatic acids (Duke and Powles 2008; Wiersma et al. 2015). A relative increase in shikimate accumulation has been widely used as a measure of the presence and phytotoxicity of glyphosate in a plant (Gaines et al.

Table 3. Mean shikimate concentrations by soil type, postapplication irrigation (drenched vs. none), and glyphosate rate in 2014.

			Shikimate concentration								
			Almond			Cherry			Prune		
Soil type[a]	Postapplication irrigation	Glyphosate[b] rate[b]	14 DAT1[c]	14 DAT2	14 DAT3	14 DAT1	14 DAT2	14 DAT3	14 DAT1	14 DAT2	14 DAT3
		kg ae ha^{-1}	μmol g^{-1} FW ± SE								
Silty clay loam	None	0	0.15 ± 0.14	0.79 ± 0.23	0.11 ± 0.15	3.42 ± 0.83	3.51 ± 1.29	2.75 ± 0.70	1.99 ± 1.57	2.71 ± 2.13	2.00 ± 1.70
		1.1	0.62 ± 0.41	0.43 ± 0.25	0.08 ± 0.19	3.39 ± 0.98	4.24 ± 0.47	2.60 ± 0.78	3.84 ± 0.88	3.57 ± 3.18	4.34 ± 2.06
		2.2	0.17 ± 0.26	0.97 ± 0.09	0.05 ± 0.13	2.94 ± 0.49	3.91 ± 0.98	2.59 ± 0.56	2.31 ± 1.30	4.29 ± 2.96	4.12 ± 1.35
		4.4	0.34 ± 0.40	0.82 ± 0.47	0.17 ± 0.13	4.21 ± 0.75	4.47 ± 1.63	2.46 ± 0.75	3.29 ± 1.06	4.12 ± 2.84	2.72 ± 0.59
	Drench	0	0.28 ± 0.33	0.31 ± 0.21	0.05 ± 0.09	2.92 ± 0.97	5.06 ± 1.49	2.65 ± 0.13	3.77 ± 2.31	3.05 ± 1.86	3.94 ± 3.37
		1.1	0.01 ± 0.03	1.07 ± 0.42	0.12 ± 0.14	2.73 ± 0.40	4.18 ± 1.33	2.84 ± 0.82	3.43 ± 1.03	4.0 ± 2.42	3.41 ± 2.59
		2.2	0.12 ± 0.17	0.57 ± 0.20	0.01 ± 0.02	2.97 ± 0.44	4.08 ± 1.20	2.49 ± 0.61	4.23 ± 1.13	6.19 ± 0.66	3.74 ± 1.81
		4.4	0.22 ± 0.19	1.02 ± 0.47	0.02 ± 0.03	3.21 ± 0.82	5.02 ± 1.63	2.32 ± 0.38	3.57 ± 1.16	3.22 ± 2.70	3.06 ± 2.05
Sandy loam	None	0	0.21 ± 0.18	0.91 ± 0.61	0.15 ± 0.22	3.57 ± 0.67	3.85 ± 1.25	3.51 ± 0.60	2.67 ± 1.25	2.02 ± 1.39	2.81 ± 0.99
		1.1	0.16 ± 0.32	0.68 ± 0.14	0.24 ± 0.34	2.44 ± 1.33	3.49 ± 0.72	2.85 ± 0.71	3.56 ± 0.35	4.39 ± 2.14	1.96 ± 1.46
		2.2	0.03 ± 0.07	0.96 ± 0.39	0.04 ± 0.03	2.88 ± 1.14	3.38 ± 0.55	3.17 ± 0.60	2.12 ± 1.85	6.03 ± 1.36	2.98 ± 2.02
		4.4	0.29 ± 0.25	0.85 ± 0.43	0.15 ± 0.18	2.48 ± 0.79	3.42 ± 1.66	2.94 ± 0.86	3.26 ± 0.73	3.85 ± 1.50	2.46 ± 2.01
	Drench	0	0.05 ± 0.09	0.78 ± 0.78	0.20 ± 0.29	1.85 ± 1.29	5.07 ± 1.77	3.05 ± 0.66	2.83 ± 0.73	3.52 ± 1.80	3.82 ± 1.91
		1.1	0.58 ± 0.67	0.46 ± 0.35	0.28 ± 0.20	2.37 ± 0.86	3.31 ± 0.57	2.82 ± 0.72	3.72 ± 2.04	2.24 ± 1.29	3.53 ± 3.34
		2.2	0.22 ± 0.15	0.72 ± 0.44	0.14 ± 0.21	4.36 ± 1.76	3.97 ± 1.45	3.07 ± 0.47	4.29 ± 1.21	5.31 ± 0.88	4.02 ± 2.58
		4.4	0.11 ± 0.13	0.75 ± 0.11	0.15 ± 0.15	2.14 ± 0.73	3.88 ± 0.62	3.12 ± 0.94	2.69 ± 0.33	4.14 ± 2.83	4.34 ± 1.07

[a]Experiments were conducted in a site with a silty clay loam soil, but a split-plot factor included half of the trees planted in a 90-cm diam planting site filled with imported sandy loam soil.
[b]Each treatment was applied three times each year during 2014–2019.
[c]Abbreviations: DAT1, days after first glyphosate treatment; DAT2, days after second glyphosate treatment; DAT3, days after third glyphosate treatment; FW, fresh weight.

Table 4. P values based on ANOVA of soil type, post-application irrigation (drenched vs. none), glyphosate rate, and their interactions on orchard crops treated with glyphosate three times in 2014.

	Almond			Cherry			Prune		
Factor	14 DAT1[d]	14 DAT2	14 DAT3	14 DAT1	14 DAT2	14 DAT3	14 DAT1	14 DAT2	14 DAT3
Soil[a]	0.775	0.591	0.095	0.061	0.113	0.007	0.654	0.961	0.738
Drench[b]	0.875	0.295	0.867	0.159	0.091	0.708	0.053	0.864	0.130
Rate[c]	0.219	0.497	0.235	0.436	0.498	0.677	0.431	0.008	0.846
Soil × drench	0.231	0.623	0.168	0.449	0.963	0.806	0.553	0.501	0.285
Soil × rate	0.960	0.260	0.829	0.035*	0.493	0.661	0.970	0.927	0.672
Drench × rate	0.617	0.530	0.817	0.047*	0.393	0.867	0.094	0.646	0.825
Soil × drench × rate	0.086	0.351	0.684	0.258	0.978	0.887	0.657	0.381	0.709

[a]Sandy or clay loam soil in original 2013 planting site.
[b]Post-glyphosate irrigation treatment conducted in the first 2 yr of the experiments.
[c]Glyphosate applied three times y^{-1} at 0, 1.1, 2.2, or 4.4 kg ae ha^{-1}.
[d]Abbreviations: DAT1, days after first glyphosate treatment; DAT2, days after second glyphosate treatment; DAT3, days after third glyphosate treatment.
*$P \leq 0.050$.

Table 5. Mean chlorophyll content (SPAD values) based on soil type, postapplication irrigation, and glyphosate rates in 2014 and 2015.

Soil type[a]	Postapplication irrigation	Rate[b]	2014			2015		
			30 DAT1[c]	30 DAT2	30 DAT3	30 DAT1	30 DAT2	30 DAT3
		kg ae ha^{-1}	chlorophyll (SPAD value) ± SE					
Silty clay loam	None	0	30 ± 1	30 ± 5	34 ± 5	31 ± 1	36 ± 1	37 ± 2
		1.1	30 ± 1	28 ± 4	32 ± 1	31 ± 2	34 ± 4	36 ± 1
		2.2	30 ± 1	25 ± 3	34 ± 1	31 ± 1	36 ± 1	34 ± 6
		4.4	30 ± 1	27 ± 5	33 ± 3	31 ± 1	35 ± 1	37 ± 2
	Drench	0	30 ± 1	30 ± 3	31 ± 3	31 ± 2	35 ± 2	38 ± 1
		1.1	30 ± 1	29 ± 1	32 ± 2	30 ± 1	34 ± 1	36 ± 3
		2.2	29 ± 1	28 ± 5	34 ± 1	32 ± 1	34 ± 1	38 ± 1
		4.4	29 ± 1	27 ± 3	31 ± 1	32 ± 2	34 ± 1	34 ± 2
Sandy loam	None	0	29 ± 1	32 ± 3	34 ± 3	31 ± 1	36 ± 0	37 ± 2
		1.1	29 ± 0	27 ± 0	34 ± 1	31 ± 2	36 ± 5	37 ± 1
		2.2	30 ± 1	27 ± 4	35 ± 1	32 ± 2	35 ± 2	35 ± 3
		4.4	30 ± 2	30 ± 6	32 ± 2	30 ± 2	35 ± 1	37 ± 3
	Drench	0	30 ± 1	31 ± 3	34 ± 3	31 ± 1	33 ± 3	38 ± 3
		1.1	30 ± 0	32 ± 4	35 ± 1	31 ± 1	35 ± 1	34 ± 5
		2.2	30 ± 1	28 ± 3	33 ± 2	32 ± 1	36 ± 2	38 ± 2
		4.4	30 ± 1	31 ± 3	32 ± 2	32 ± 1	34 ± 1	36 ± 2

[a]Experiments were conducted in a site with a silty clay loam soil, but a split-plot factor included half of the trees planted in a 90-cm diam planting site filled with imported sandy loam soil.
[b]Each treatment was applied three times each year during 2014–2019.
[c]Abbreviations: DAT1, days after first glyphosate treatment; DAT2, days after second glyphosate treatment; DAT3, days after third glyphosate treatment; SPAD, Soil Plant Analysis Development.

2019; Hanson et al. 2009; Hernandez et al. 1999; Osipitan and Dille 2017). At sublethal levels, shikimate accumulation would be expected to increase with increase in glyphosate dose (Shaner et al. 2005; Wilson et al. 2020). In this study, similar shikimate levels in trees grown in glyphosate-treated and untreated plots and no dose-response trend suggest little or no direct herbicidal impact of glyphosate due to root uptake and movement into the aboveground portions of the tree. This is consistent with previous reports of extremely low soil activity of glyphosate due to its strong binding to soil particles (Duke et al. 2012). Even in the worst-case scenario in these three orchard experiments, which had young trees growing in sandy soil, treated three times with 4.4 kg ae ha^{-1} glyphosate, and immediately followed with a flood event (postapplication irrigation), no detectable differences in shikimate accumulation were observed (Tables 3 and 4).

Once taken up by a sensitive plant, glyphosate reduces chlorophyll content, with a consequential effect on photosynthesis and growth in plants (Gomes et al. 2016; Reddy et al. 2001; Ye et al. 2019). Chlorophyll content of the orchard trees was evaluated 30 d after each of the three glyphosate applications in 2014 and 2015, accounting for six observation times for each orchard experiment. Only one of these six observations showed a treatment-related influence on chlorophyll content in each of the three orchard experiments; however, timing and form (increase or decrease) of the treatment effect were inconsistent across experiments (Tables 5–10). In almond, there was a significant interaction ($P = 0.043$) between postapplication irrigation and glyphosate rates, whereas in cherry, glyphosate rate was significant as a main effect ($P = 0.006$) 30 d after third glyphosate application in 2015 (Tables 5–8). Meanwhile, in prune, the significant influence ($P = 0.031$) of glyphosate rate was at 30 d after third glyphosate application in 2014 (Tables 9 and 10). If there was a biologically meaningful effect, a greater response would be expected from treatments likely to result in the greatest amount of herbicide in the tree root zone. The inconsistent pattern with regard to the highest glyphosate rate, coarse soil, and postapplication irrigation suggest the relatively few statistically significant results may be due to random variation or experimental artifacts.

Table 6. P values based on ANOVA of soil type, postapplication irrigation, glyphosate rate, and their interactions on almond treated with glyphosate three times in 2014 and 2015.

Factor	2014			2015		
	30 DAT1[c]	30 DAT2	30 DAT3	30 DAT1	30 DAT2	30 DAT3
Soil[a]	0.764	0.102	0.144	0.858	0.454	0.523
Drench[b]	0.758	0.203	0.259	0.393	0.098	0.849
Rate[c]	0.458	0.075	0.155	0.423	0.423	0.163
Soil × drench	0.100	0.679	0.119	0.615	0.615	0.997
Soil × rate	0.215	0.741	0.336	0.649	0.649	0.810
Drench × rate	0.842	0.496	0.667	0.778	0.778	0.043*
Soil × drench × rate	0.862	0.781	0.345	0.582	0.582	0.774

[a]Sandy or clay loam soil in original 2013 planting site.
[b]Postglyphosate irrigation treatment conducted in the first 2 yr of the experiments.
[c]Glyphosate applied three times yr^{-1} at 0, 1.1, 2.2, or 4.4 kg ae ha^{-1}.
[d]Abbreviations: DAT1, days after first glyphosate treatment; DAT2, days after second glyphosate treatment; DAT3, days after third glyphosate treatment.
*$P \leq 0.050$.

It has been hypothesized that cumulative effects of glyphosate use in orchard production systems could affect plant growth indirectly by limiting micronutrient availability in the soil and, ultimately, the mineral nutrition of plants (Duke et al. 2012). However, evaluation of leaf nutrients (N, P, K, Ca, Mg, S, B, Fe, Zn, Mn, and Cu) of the orchard crops after 6 yr of repeated application of glyphosate at extreme rates did not provide evidence of negative impacts of the treatments on crop nutrient status in three orchard crop experiments (Table 11). This may be explained by the relatively weak chelation of these nutrients by glyphosate (Duke et al. 2012; Mertens et al. 2018) by relatively rapid degradation in the soil environment (Zablotowicz et al. 2009) and by the relatively small amount of glyphosate applied compared with the concentration of the nutrients in soil (Duke et al. 2012).

Trunk Diameter Growth

The use of trunk diameter has been widely used as a robust measure of orchard crop growth (Hernandez-Santana et al. 2017; Martín-Palomo et al. 2019; Moriana et al. 2003). From 2014 to

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Table 7. Mean chlorophyll content (SPAD values) based on soil type, postapplication irrigation (drenched vs. none), glyphosate rate, and their interactions on cherry treated with glyphosate three times in 2014 and 2015.

Soil type[a]	Postapplication irrigation	Rate[b]	2014			2015		
			30 DAT1[c]	30 DAT2	30 DAT3	30 DAT1	30 DAT2	30 DAT3
		kg ae ha^{-1}	chlorophyll (SPAD value) ± SE					
Silty clay loam	None	0	19 ± 1	21 ± 2	21 ± 2	25 ± 1	44 ± 1	42 ± 2
		1.1	21 ± 2	21 ± 2	22 ± 1	25 ± 1	45 ± 2	45 ± 3
		2.2	18 ± 2	21 ± 1	21 ± 0	25 ± 1	44 ± 2	44 ± 3
		4.4	20 ± 2	23 ± 2	21 ± 2	26 ± 3	43 ± 2	45 ± 2
	Drench	0	21 ± 1	21 ± 1	22 ± 1	25 ± 1	43 ± 4	43 ± 3
		1.1	19 ± 0	21 ± 2	21 ± 2	25 ± 1	45 ± 2	45 ± 6
		2.2	20 ± 1	21 ± 0	23 ± 2	27 ± 2	45 ± 2	44 ± 2
		4.4	20 ± 1	20 ± 1	21 ± 1	25 ± 2	46 ± 2	45 ± 3
Sandy loam	None	0	19 ± 1	20 ± 1	21 ± 1	24 ± 2	44 ± 2	42 ± 4
		1.1	19 ± 1	20 ± 1	20 ± 1	25 ± 2	46 ± 2	46 ± 3
		2.2	19 ± 1	19 ± 2	21 ± 1	23 ± 3	44 ± 3	42 ± 1
		4.4	19 ± 1	20 ± 1	20 ± 1	24 ± 1	46 ± 2	46 ± 2
	Drench	0	19 ± 1	20 ± 2	20 ± 1	25 ± 3	46 ± 1	41 ± 5
		1.1	19 ± 2	20 ± 1	25 ± 8	25 ± 2	45 ± 3	47 ± 1
		2.2	19 ± 1	20 ± 1	20 ± 1	26 ± 2	46 ± 3	45 ± 5
		4.4	20 ± 1	21 ± 2	22 ± 2	24 ± 2	46 ± 1	44 ± 3

[a]Experiments were conducted in a site with a silty clay loam soil, but a split-plot factor included half of the trees planted in a 90-cm diam planting site filled with imported sandy loam soil.
[b]Each treatment was applied three times each year during 2014–2019.
[c]Abbreviations: DAT1, days after first glyphosate treatment; DAT2, days after second glyphosate treatment; DAT3, days after third glyphosate treatment; SPAD, Soil Plant Analysis Development.

Table 8. P values based on ANOVA of soil type, postapplication irrigation (drenched vs. none), glyphosate rate, and their interactions on cherry treated with glyphosate three times in 2014 and 2015

Factor	2014			2015		
	30 DAT1[c]	30 DAT2	30 DAT3	30 DAT1	30 DAT2	30 DAT3
Soil[a]	0.140	0.007*	0.874	0.062	0.101	0.815
Drench[b]	0.372	0.742	0.191	0.448	0.135	0.738
Rate[c]	0.254	0.684	0.479	0.859	0.483	0.006*
Soil × drench	0.780	0.351	0.908	0.303	0.982	0.644
Soil × rate	0.691	0.936	0.662	0.584	0.919	0.686
Drench × rate	0.057	0.715	0.489	0.229	0.524	0.700
Soil × drench × rate	0.378	0.356	0.060	0.951	0.499	0.588

[a]Sandy or clay loam soil in original 2013 planting site.
[b]Postglyphosate irrigation treatment conducted in the first 2 yr of the experiments.
[c]Glyphosate applied three times yr^{-1} at 0, 1.1, 2.2, or 4.4 kg ae ha^{-1}.
[d]Abbreviations: DAT1, days after first glyphosate treatment; DAT2, days after second glyphosate treatment; DAT3, days after third glyphosate treatment.
*$P \leq 0.050$.

2020, average trunk diameter (across treatments) increased from 28 to 169 mm in almond, 28 to 217 mm in cherry, and 32 to 127 mm in prune (Figure 1). There were no differences in final trunk diameter among treatments in the almond or prune experiments; in cherry, there was a slight but significant increase in trunk diameter with the highest glyphosate rate. ANOVA revealed no significant impact of soil type in the planting hole, post-glyphosate application irrigation, or their interactions on cumulative trunk-diameter change in three orchard crops over 7 yr of observation that included 6 yr of glyphosate treatment (Table 12). Similarly, the ANOVA results suggested that glyphosate rates applied on the soil had no negative impact on growth of the orchard crops and, in the case of cherry, a slight increase in trunk diameter in the highest glyphosate rate (Table 13). The regression model adequately fitted the cumulative trunk growth data over the 6-yr period of observation (Figure 1) with small root mean square error (≤6%) in all three experiments. In terms of growth rate, it took 2 to 3 yr for the orchard crops to attain half of their final trunk diameter, but these growth-rate data were not influenced by glyphosate regimens (Table 8).

In apple (*Malus* spp.), glyphosate has been linked to trunk injury and scaffold death (e.g., Rosenberger et al. 2013). However, in these California experiments, no trunk cankers or other trunk and limb malformations were observed in almond, cherry, or prune during the 7-yr evaluation period (data not shown). The lack of negative impact of glyphosate on the growth rate or total growth of the orchard trees over time supports the finding of no measurable effects on shikimate accumulation in the first year, leaf chlorophyll content in the first 2 yr, or leaf nutrient levels after 6 yr of treatment. The highest glyphosate rate (4.4 kg ae ha^{-1}) evaluated in this study resulted in a total annual soil glyphosate load of 13.3 kg ae ha^{-1}, or nearly 80 kg ae ha^{-1} over the life of the experiment, which is well beyond what would typically occur in a commercial orchard in California.

Several studies that simulated drift cases in sensitive plants or at above-label doses in glyphosate-tolerant plants have shown that foliar-applied glyphosate can have negative impacts on plant nutrient uptake, photosynthetic apparatus, and plant productivity (Al-Khatib et al. 1992; Cakmak et al. 2009; Foshee et al. 2008; Gomes et al. 2016; Huang et al. 2012; Su et al. 2009). In a glyphosate-resistant soybean, foliar application of glyphosate at 2.4 kg ae ha^{-1} substantially reduced chlorophyll content (Soil Plant Analysis Development (value), photosynthetic rate, plant nutrients and growth of the plant (Zobiole et al. 2012). Root uptake of glyphosate, with resulting impacts on plant nutrient status, is possible, such as reported by Ozturk et al. (2008),

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Table 9. Mean chlorophyll content (SPAD values) based on soil type, postapplication irrigation (drenched vs. none), glyphosate rate and their interactions on prune treated with glyphosate three times in 2014 and 2015.

Soil type[a]	Postapplication irrigation	Rate[b]	2014			2015		
			30 DAT1[c]	30 DAT2	30 DAT3	30 DAT1	30 DAT2	30 DAT3
		kg ae ha^{-1}	chlorophyll (SPAD value) ± SE					
Silty clay loam	None	0	26 ± 1	26 ± 1	30 ± 5	41 ± 4	53 ± 5	46 ± 9
		1.1	25 ± 3	29 ± 3	26 ± 1	41 ± 2	57 ± 2	53 ± 9
		2.2	25 ± 2	28 ± 3	27 ± 1	42 ± 1	54 ± 3	55 ± 4
		4.4	26 ± 1	28 ± 2	25 ± 1	43 ± 2	54 ± 5	55 ± 4
	Drench	0	28 ± 2	27 ± 1	30 ± 3	42 ± 2	56 ± 3	52 ± 3
		1.1	26 ± 1	28 ± 2	25 ± 2	41 ± 2	57 ± 2	53 ± 4
		2.2	25 ± 2	30 ± 1	28 ± 4	41 ± 3	57 ± 3	57 ± 5
		4.4	25 ± 2	29 ± 2	25 ± 3	43 ± 3	58 ± 3	49 ± 7
Sandy loam	None	0	26 ± 1	29 ± 3	26 ± 1	41 ± 2	54 ± 3	51 ± 4
		1.1	25 ± 2	30 ± 1	26 ± 2	42 ± 3	57 ± 1	50 ± 8
		2.2	25 ± 2	28 ± 2	27 ± 0	43 ± 2	54 ± 1	49 ± 9
		4.4	26 ± 1	28 ± 2	27 ± 1	40 ± 2	54 ± 3	50 ± 8
	Drench	0	27 ± 2	26 ± 2	29 ± 4	41 ± 1	56 ± 4	51 ± 5
		1.1	26 ± 1	27 ± 1	27 ± 1	41 ± 3	55 ± 4	55 ± 4
		2.2	26 ± 2	29 ± 3	26 ± 2	40 ± 2	56 ± 2	56 ± 5
		4.4	24 ± 2	28 ± 1	26 ± 1	40 ± 1	54 ± 3	54 ± 6

[a]Experiments were conducted in a site with a silty clay loam soil, but a split-plot factor included half of the trees planted in a 90-cm diam planting site filled with imported sandy loam soil.
[b]Each treatment was applied three times each year during 2014–2019.
[c]Abbreviations: DAT1, days after first glyphosate treatment; DAT2, days after second glyphosate treatment; DAT3, days after third glyphosate treatment; SPAD, Soil Plant Analysis Development.

Table 10. P values based on ANOVA of soil type, postapplication irrigation (drenched vs. none), glyphosate rate, and their interactions on prune treated with glyphosate three times in 2014 and 2015.

Factor	2014			2015		
	30 DAT1[d]	30 DAT2	30 DAT3	30 DAT1	30 DAT2	30 DAT3
Soil[a]	0.680	0.594	0.521	0.346	0.298	0.767
Drench[b]	0.370	0.476	0.559	0.460	0.028*	0.124
Rate[c]	0.061	0.133	0.031*	0.875	0.408	0.304
Soil × drench	0.662	0.078	0.625	0.576	0.138	0.271
Soil × rate	0.891	0.628	0.289	0.250	0.600	0.640
Drench × rate	0.229	0.221	0.690	0.584	0.380	0.563
Soil × drench × rate	0.910	0.565	0.656	0.877	0.927	0.287

[a]Sandy or clay loam soil in original 2013 planting site.
[b]Postglyphosate irrigation treatment conducted in the first 2 yr of the experiments.
[c]Glyphosate applied three times yr^{-1} at 0, 1.1, 2.2, or 4.4 kg ae ha^{-1}.
[d]Abbreviations: DAT1, days after first glyphosate treatment; DAT2, days after second glyphosate treatment; DAT3, days after third glyphosate treatment.
*$P \leq 0.050$.

Table 11. Leaf nutrient analysis in three California orchard crops in October of 2019 after 18 glyphosate applications made during 2014–2019.[a]

Crop	Rate[b]	N[c]	P	K	Ca	Mg	S	B	Fe	Zn	Mn	Cu
	kg ae ha^{-1}	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm
Almond	0	1.60 ± 0.10	0.17 ± 0.01	1.31 ± 0.16	3.04 ± 0.12	1.83 ± 0.06	1388 ± 37.1	34.4 ± 2.56	274.6 ± 15.6	10.5 ± 1.13	25.7 ± 1.13	3.20 ± 0.11
	1.1	1.52 ± 0.06	0.15 ± 0.01	1.25 ± 0.10	2.99 ± 0.09	1.75 ± 0.03	1350 ± 30.3	29.9 ± 0.65	274.4 ± 7.12	12.2 ± 0.60	29.2 ± 2.13	3.00 ± 0.14
	2.2	1.74 ± 0.09	0.14 ± 0.02	1.28 ± 0.06	2.96 ± 0.11	1.76 ± 0.02	1468 ± 65.7	34.1 ± 3.76	257.6 ± 20.9	13.4 ± 0.46	27.3 ± 1.18	3.28 ± 0.23
	4.4	1.51 ± 0.06	0.15 ± 0.02	1.15 ± 0.09	3.08 ± 0.10	1.80 ± 0.03	1425 ± 37.1	30.7 ± 1.14	280.2 ± 14.5	12.9 ± 1.50	26.9 ± 2.58	3.03 ± 0.05
	P	0.102	0.416	0.366	0.230	0.264	0.087	0.345	0.310	0.207	0.548	0.594
Cherry	0	1.58 ± 0.03	0.27 ± 0.02	0.79 ± 0.11	2.09 ± 0.13	1.48 ± 0.03	943 ± 9.50	92.4 ± 5.40	407.2 ± 25.7	13.7 ± 0.39	37.4 ± 0.82	5.88 ± 0.13
	1.1	1.34 ± 0.33	0.22 ± 0.05	0.56 ± 0.13	1.71 ± 0.39	1.23 ± 0.30	1023 ± 14.7	71.7 ± 16.9	308.5 ± 73.6	9.6 ± 2.34	29.4 ± 7.26	4.63 ± 1.14
	2.2	1.63 ± 0.02	0.28 ± 0.01	0.67 ± 0.06	2.00 ± 0.05	1.49 ± 0.01	963 ± 11.1	91.7 ± 4.91	384.4 ± 17.7	13.9 ± 0.29	35.8 ± 1.45	5.73 ± 0.09
	4.4	1.66 ± 0.01	0.25 ± 0.04	0.70 ± 0.09	1.98 ± 0.12	1.48 ± 0.06	963 ± 25.3	90.2 ± 4.69	384.9 ± 21.2	12.9 ± 0.42	34.3 ± 2.10	5.83 ± 0.23
	P	0.113	0.884	0.368	0.699	0.457	0.019*	0.710	0.688	0.016*	0.637	0.852
Prune	0	1.52 ± 0.09	0.31 ± 0.05	0.96 ± 0.21	2.32 ± 0.04	1.62 ± 0.04	1340 ± 42.0	50.1 ± 2.15	433.4 ± 7.60	9.0 ± 0.33	24.2 ± 1.48	4.63 ± 0.19
	1.1	1.65 ± 0.10	0.26 ± 0.04	0.97 ± 0.26	2.34 ± 0.03	1.54 ± 0.10	1370 ± 49.3	53.2 ± 4.47	390.1 ± 11.4	9.0 ± 0.43	25.4 ± 1.06	4.45 ± 0.16
	2.2	1.61 ± 0.05	0.23 ± 0.03	0.94 ± 0.21	2.21 ± 0.10	1.55 ± 0.09	1375 ± 52.4	51.7 ± 2.49	413.1 ± 9.98	8.9 ± 0.52	24.5 ± 1.65	4.48 ± 0.39
	4.4	1.69 ± 0.04	0.23 ± 0.02	0.74 ± 0.12	2.12 ± 0.11	1.55 ± 0.11	1440 ± 30.8	45.0 ± 1.20	368.7 ± 36.4	8.9 ± 0.31	21.6 ± 1.58	4.08 ± 0.28
	P	0.441	0.129	0.189	0.177	0.701	0.125	0.067	0.166	0.986	0.112	0.216

[a]Data are reported as mean ± SE; n = 4 for each rate and crop. Within-crop and block composite leaf samples included two soil types in the original planting site and two postapplication irrigation treatments for each glyphosate application rate.
[b]Treatments were applied three times each year during 2014–2019.
[c]Abbreviations: B, boron; Ca, calcium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; N, nitrogen; P, phosphorus; S, sulfur; Zn, zinc.
*$P \leq 0.050$.

Table 12. P values based on ANOVA of soil type, postapplication irrigation (drenched vs. none), glyphosate rate, and their interactions on trunk diameter of orchard crops in 2014 to 2020.

	Almond						Cherry						Prune					
	Years						Years						Years					
Factor[a]	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Soil[a]	0.45	0.51	0.72	0.92	0.81	0.91	0.40	0.41	0.44	0.70	0.96	0.95	0.71	0.81	0.27	0.85	0.86	0.95
Drench[b]	0.25	0.08	0.30	0.22	0.40	0.58	0.18	0.31	0.45	0.49	0.49	0.65	0.89	0.33	0.57	0.72	0.80	0.52
Rate[c]	0.87	0.87	0.61	0.56	0.56	0.88	0.51	0.21	0.31	0.27	0.15	0.05*	045	0.48	0.23	0.67	0.71	0.85
Soil × drench	0.65	0.45	0.81	0.89	0.51	0.61	0.06	0.44	0.30	0.18	0.16	0.20	0.25	0.65	0.52	0.92	0.86	0.81
Soil × rate	0.97	0.92	0.70	0.27	0.36	0.22	0.94	0.96	0.76	0.88	0.84	0.88	0.39	0.62	0.56	0.60	0.65	0.53
Drench × rate	0.53	0.58	0.23	0.57	0.80	0.66	0.42	0.26	0.38	0.59	0.79	0.92	0.29	0.27	0.29	0.90	0.93	0.65
Soil × drench × rate	0.07	0.33	0.87	0.97	0.91	0.99	0.27	0.33	0.33	0.37	0.29	0.38	0.15	0.35	0.20	0.37	0.56	0.45

[a]Sandy or clay loam soil in original 2013 planting site.
[b]Postglyphosate irrigation treatment conducted in the first 2 yr of the experiments.
[c]Glyphosate applied three times yr^{-1} at 0, 1.1, 2.2, or 4.4 kg ae ha^{-1}.
[d]Abbreviations: DAT1, days after first glyphosate treatment; DAT2, days after second glyphosate treatment; DAT3, days after third glyphosate treatment.
*$P \leq 0.050$.

Table 13. Trunk diameter increase in California orchard crops after 18 glyphosate applications made during 2014–2019.

Rate[a]	Almond[bc]			Cherry[b]			Prune[b]		
kg ae ha^{-1}	*a* (mm)	*b*	*h* (yr)	*a* (mm)	*b*	*h* (yr)	*a* (mm)	*b*	*h* (yr)
0	163.3 ± 5.15	1.08 ± 0.18	2.7 ± 0.24	210.6 ± 8.94	1.05 ± 0.14	2.8 ± 0.19	127.6 ± 1.07	0.89 ± 0.14	2.1 ± 0.18
1.1	169.9 ± 7.89	1.17 ± 0.23	2.8 ± 0.32	213.5 ± 9.89	1.00 ± 0.13	2.8 ± 0.16	127.5 ± 1.64	0.92 ± 0.15	2.1 ± 0.18
2.2	175.9 ± 10.6	1.09 ± 0.19	2.7 ± 0.26	207.7 ± 8.62	0.98 ± 0.13	2.7 ± 0.17	127.9 ± 1.86	0.85 ± 0.13	2.0 ± 0.15
4.4	165.5 ± 7.74	1.16 ± 0.24	2.7 ± 0.32	217.3 ± 6.40	1.02 ± 0.14	2.8 ± 0.19	127.0 ± 2.06	0.81 ± 0.12	2.0 ± 0.14

[a]Each treatment was applied three times each year during 2014–2019.
[b]Regression parameters are final trunk diameter (*a*), slope (*b*), and number of years (h) to achieve half of the final trunk diameter for different rates of soil-applied glyphosate in three orchard crops.
[c]Data are reported as mean ± SE.

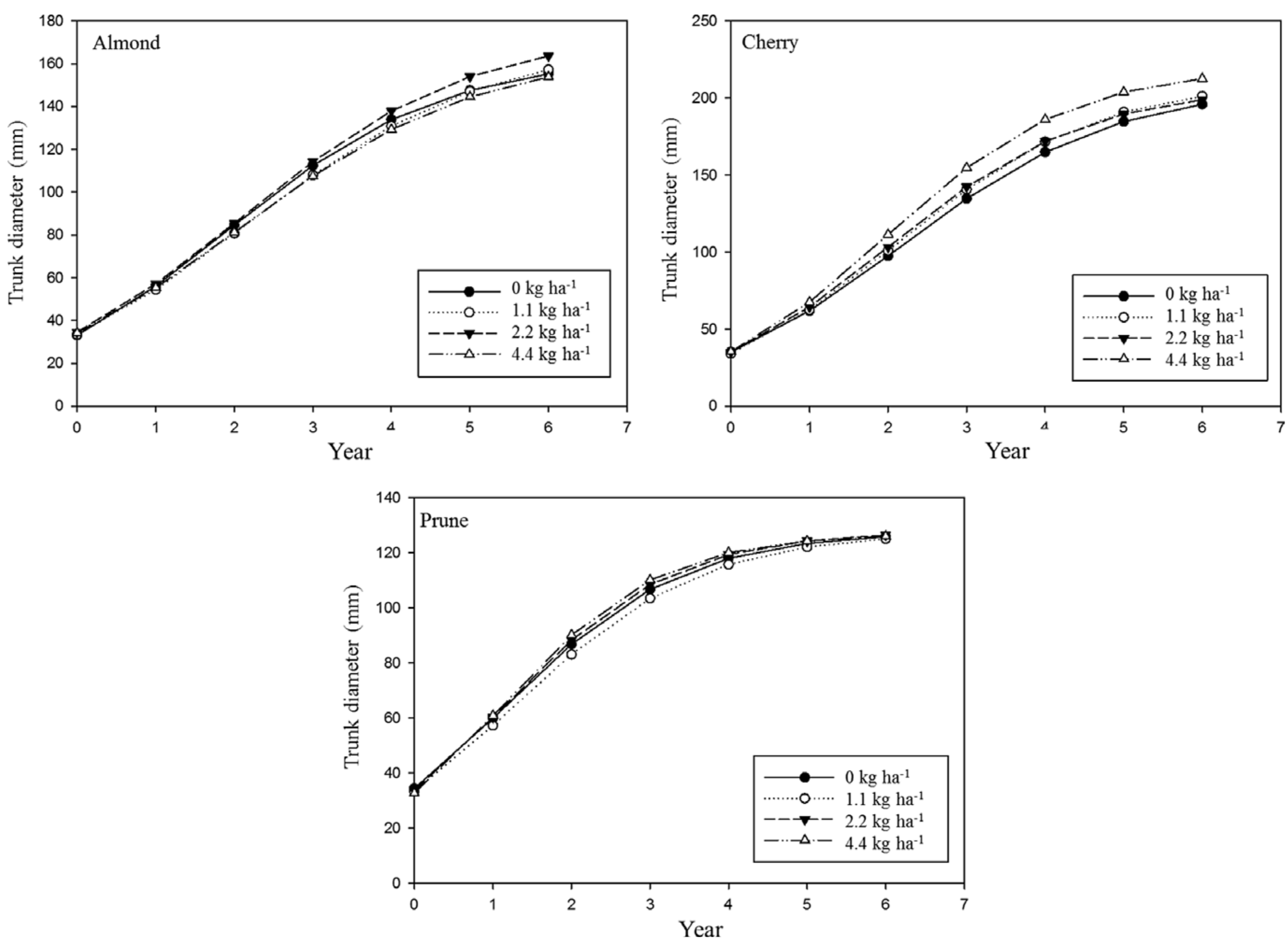

Figure 1. Increase in almond, cherry, and prune trunk diameter over time with or without glyphosate applied around the base of the tree three times per year at up to 4.4 kg ha^{-1} over 6 yr. Data were averaged over the soil type in the original planting hole and a postapplication irrigation conducted after each glyphosate treatment in the first two growing seasons.

who reported reduced ferric reductase activity in sunflower exposed to glyphosate in soil-free hydroponic conditions. However, this has not been observed from glyphosate residues in soil under field conditions (Bromilow et al. 1996; Duke et al. 2012; Liphadzi et al. 2005) and does not appear to be the case in California orchard crops in the current study.

California orchard-production systems are unique compared with many other U.S. crops in terms of potential for direct or indirect impacts of glyphosate use on crop safety, due to the long lifespan of the crop, potential for repeated and high-rate applications of the herbicide, minimal soil disturbance, and the Mediterranean climate conditions. Three experiments with treatments intended to create a worst-case scenario for cumulative glyphosate soil residues to directly or indirectly affect the growth of California orchard crops over 7 yr did not suggest any negative impact. These findings are timely and add value to current conversations about glyphosate use in perennial specialty crops.

Acknowledgements. This research was partially supported with funding from the Almond Board of California and the California Dried Plum Board, and a donation of orchard nursery stock from Sierra Gold Nurseries. No conflicts of interest have been declared

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https://doi.org/10.1017/wet.2020.85 Published online by Cambridge University Press