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Characterization of Florpyrauxifen-benzyl Herbicide in California Water-Seeded Rice

By

DENIZ INCI
DISSERTATION

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Abstract

Rice (*Oryza sativa* L.) is a staple crop globally and California is the second-largest rice producer in the United States with more than 200,000 hectares of rice planted in the Sacramento Valley and the Sacramento-San Joaquin River Delta. Weeds have historically been one of the biggest challenges of California rice production systems, where herbicide-resistant weeds have increased the complexity of the weed management. Florpyrauxifen-benzyl (FPB) is a new auxin-mimic herbicide with a novel binding site of action for selective grass, sedge, and broadleaf weed control in rice. The objectives of this research were to 1) characterize the effects of FPB on rice crop safety and weed control when applied alone or in mixture with other partner herbicides; 2) determine optimum FPB application timing to control smallflower umbrella sedge; 3) elucidate the response of late FPB applications on rice flower sterility; 4) determine the effects of FPB on nontarget crops including almond, grape, peach, pistachio, plum, and walnut; and 5) compare the onset of foliar symptoms resulting from simulated FPB drift with residues in almond, pistachio, and walnut leaves at several timepoints after exposure.

In the first study, FPB was applied at 1/2X, 1X, and 2X field use rates based on 30 g ai ha⁻¹ alone as well as in mixture combinations with bispyribac-sodium, penoxsulam, and propanil in fields near Arbuckle, Biggs, and Willows, CA. FPB applied alone at 30 g ai ha⁻¹ on 4-5-leaf rice stage controlled more than 80% of watergrasses, ricefield bulrush, and smallflower umbrella sedge (SMF) as well as more than 99% of all broadleaf weeds including duck salad, redstem, waterhyssop, and arrowhead at 28 days after treatment (DAT). The highest rice yield was observed with FPB plus propanil in Arbuckle and Willows, CA. At Biggs, the highest yield (4,626 kg ha⁻¹) was achieved with FPB applied alone at 60 g ai ha⁻¹.

In the second study, FPB at maximum use rate of 40 g ai ha⁻¹ was applied to SMF at 1-leaf, 10-, 15-, 20-, and 25-cm tall growth stages. SMF was controlled by 95%, 86%, 89%, 87%, and 85% when FPB applied on 1-leaf, 10-, 15-, 20-, and 25-cm tall growth stage at 42 DAT, respectively.

In the third study, FPB at 40 and 80 g ai ha⁻¹ rates were applied at rice panicle initiation (PI) and 50% flowering (FL) growth stages, respectively. While the weed control was more than 90% at 42 DAT for all applications, the FL application caused 26% and 35% rice sterility at the 40 and 80 g ai ha⁻¹ rates, respectively.

In the first off-target drift study, fractional rates were 1/200X, 1/100X, 1/33X, and 1/10X of the FPB use rate of 29.4 g ai ha⁻¹ used in 2020 and 2021 on almond, pistachio, and walnut trees treated early in the growing season. Herbicide treatments were applied directly to one side of the canopy of one- to two-year-old almond, pistachio, and walnut trees. The general symptoms were chlorosis, chlorotic spots, leaf curling, leaf narrowing, leaf distortion, leaf malformation, leaf crinkling, shoot curling, stem coloring, stunting, terminal bud death, and twisting. Most symptoms peaked between 14 through 28 DAT with the 1/10X FPB rate, maximum visible injury was 16%, 49%, and 79% on almond, walnut, and pistachio, respectively. The 1/10X FPB treated pistachio trees did not recover as fully as almond and walnut, and injury symptoms persisted for the remainder of the 2021-2022 growing seasons on pistachio.

In the second drift study, FPB was applied to one side of the canopy of one- and two-year-old almond, pistachio, and walnut trees at 1/100X and 1/33X of the field use rate of 29.4 g ai ha⁻¹ in 2020 and 2021. Leaf samples were randomly collected for residue analysis at 7, 14, and 28 DAT. Seven DAT with the 1/33X rate, almond, pistachio, and walnut leaves had FPB at 6.06, 5.95, and 13.12 ng g⁻¹ (fresh weight; FW) leaf, respectively. By 28 DAT, all samples from all crops tested

with the 1/33X drift rate had FPB at less than 0.25 ng g⁻¹ FW leaf. This study showed that the ideal time frame to collect leaf tissues from trees should be within 14 days after exposure; chemical analysis after this time may underestimate actual exposure.

In the third drift study with grapevine, peach, and plum, the fractional rates were 1/200X, 1/100X, 1/33X, and 1/10X of FPB based on 29.4 g ai ha⁻¹. Herbicides treatments were applied on one- to two-year-old peach and plum trees as well as on established grapevines in 2020 and 2021. The general symptoms were chlorosis, chlorotic spots, leaf curling, leaf distortion, leaf malformation, leaf crinkling, necrosis, necrotic spots, and twisting on leaves. Most symptoms appeared at 1/10X FPB rate and peaked from 14 through 42 DAT depending on the species. At 1/10X rate, visible injury was 5%, 50%, and 71% for plum, peach, and grapevine, respectively, at 14 DAT. Some grapevine clusters showed deformation, asymmetrical growth, and fruit dropping. Foliage of all treated crops gradually recovered throughout the growing season regardless of the application rate. Because of low injury symptoms and rapid recovery from herbicide injury in almond, peach, plum, and walnut trees, the proper herbicide drift management, and application precautions are likely to reduce the risk of crop injury from florpyrauxifen-benzyl drift for these crops; however, extra precaution should be taken if there are nearby grapevine vineyards or pistachio orchards because of their greater sensitivity.

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Chapter 1

Assessment of florpyrauxifen-benzyl in California water-seeded rice systems as affected by application timing

Deniz Inci and Kassim Al-Khatib

In preparation of submission to Weed Technology

Abstract

California rice weeds are well-adapted to the semiaquatic environments, are highly competitive, and have evolved widespread resistance to the limited herbicides available. Florpyrauxifen-benzyl (FPB) is a novel picolinate-type auxin-mimic herbicide used to control selected grass, sedge, and broadleaf weeds in rice. Field experiments were conducted in the Sacramento Valley of Northern California in 2020-2022 growing seasons to 1) characterize the effects of FPB on rice crop safety and weed control when applied alone or in a mixture with partner herbicides, 2) determine the FPB application timing to optimize smallflower umbrella sedge (SMF) control, and 3) elucidate the response of late season application on rice flower sterility. In the first study, FPB was applied alone at 1/2X, 1X, and 2X field use rates based on 30 g ai ha⁻¹ as well as in mixture combinations with bispyribac-sodium, penoxsulam, and propanil at three sites. FPB applied alone at 30 g ai ha⁻¹ at 4-5-LF rice stage controlled more than 80% of watergrasses, ricefield bulrush, and SMF as well as more than 99% of all broadleaf weeds at 28 DAT. The mixture treatments of FPB plus bispyribac-sodium applied at maximum use rate had the greatest grasses control whereas, FPB plus propanil at the maximum use rate provided the greatest sedge control. Although, rice had initial injury symptoms after FPB treatments, rice plants gradually recovered from symptoms. In all sites, the highest yield was observed with FPB plus propanil at 30 and 6,734 g ai ha⁻¹; and FPB applied alone at 60 g ai ha⁻¹. In the second study, FPB at 40 g ai ha⁻¹ was applied at 1-leaf, 10-, 15-, 20-, and 25-cm tall SMF, where treatments controlled 95%, 86%, 89%, 87%, and 85% of SMF at 42 DAT, respectively. In the third study, FPB at 40 and 80 g ai ha⁻¹ rates were applied at rice panicle initiation (PI) and mid-flowering (FL) growth stages. While the weed control was more than 90% at 42 DAT for all applications, the FL stage application caused 26% and 35% rice panicle blanking at 40 and 80 g ai ha⁻¹ rate, respectively. This research suggests, the window of

FPB application should be from 2-leaf rice to rice PI stage to ensure crop safety and highest weed control.

Nomenclature: Florpyrauxifen-benzyl, benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate; smallflower umbrella sedge, *Cyperus difformis* L. CYDI4; rice, *Oryza sativa* L. ORSA; watergrass, *Echinochloa* P. Beauv. ECHIN4

Keywords: Blanking; continuous flood; crop safety; rice yield; panicle sterility; weed control

Introduction

Rice (*Oryza sativa* L.) is the most widely consumed staple crop globally and it is a principal nutrition source of food for more than half of the world population (Chauhan 2012). California is the second-largest rice producer in the United States with more than 200,000-hectares (ha) planted in the Sacramento Valley and Sacramento-San Joaquin River Delta of Northern California [California Department of Food and Agriculture (CDFA) 2024; Salvato et al. 2024]. More than 97% of California rice is water-seeded, where pregerminated rice seeds are aerially broadcast into permanently flooded rice paddies (Galvin et al. 2022). Rice seeds settle on the soil surface and peg down their roots and ultimately the seedling emerge from the flood water in a few days (Ceskeski et al. 2022). The water depth is generally maintained from 10 to 15 cm throughout the growing season and the fields are drained approximately one- to two-months before the harvest [University of California Agriculture and Natural Resources (UCANR) 2023]. Water seeding and the continuously flooding practice was widely adopted in California in the 1920s to suppress grass weeds (Kennedy 1923) and since then it has been the primary method of rice production (Hill et al. 2006).

A century long continuous flood practices in California rice and lack of crop rotation have resulted in the selection of semiaquatic competitive weeds including *Echinochloa* complex (watergrasses, hereafter ‘WTG’) such as *Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass, ‘BYG’), *E. phyllopogon* (Stapf) Koso-Pol. (late watergrass, ‘LWG’), *E. oryzoides* (Ard.) Fritsch (early watergrass, ‘EWG’), and *Leptochloa fusca* (L.) Kunth ssp. *fascicularis* (Lam.) N. Snow (bearded sprangletop, ‘SPG’); sedges such as *Cyperus difformis* L. (smallflower umbrella sedge, ‘SMF’) and *Schoenoplectiella mucronata* (L.) J.Jung & H.K.Choi (ricefield bulrush, ‘BLR’); and broadleaves such as *Ammannia* spp. (redstem, ‘RDS’), *Bacopa* spp. (waterhyssops, ‘WHS’),

Heteranthera limosa (Sw.) Willd. *Heteranthera* Ruiz & Pav. (ducksalad, ‘DKS’), and *Sagittaria* spp. (arrowhead, ‘ARW’) (Brim-DeForest et al. 2017a; Hill et al. 2006). Among those weeds, BYG and SMF are also among the world’s worst weeds (Holm et al. 1977). Moreover, aquatic weeds are favored by the continuously flood and can shade submerged rice seedlings in the early season, which delays or inhibits rice emergence (Ceskeski and Al-Khatib 2021; Hill et al. 1994).

Weed competition can dramatically reduce rice yields and quality (Hill et al. 2006; Oerke 2006; Webster et al. 2012) and weeds further cause harvest difficulties, host pests and diseases, restrict the flow of irrigation water, reduce land values, and increase energy consumption (Hill et al. 1994; Strand 2013). Moreover, weeds are the major limiting factor of early establishment of rice seedlings (Kumar et al. 2023). Approximately 60% combined weed coverage can cause up to 85% rice yield reduction in water-seeded rice systems (Brim-DeForest et al. 2017b). Furthermore, when the composition of weeds are primarily grasses, only ~20% of relative grass coverage can reduce rice yields up to 85% (Brim-DeForest et al. 2017b). Smith (1983) reported as few as 11 barnyardgrass plants m⁻¹ can cause 25% yield reduction at season-long competition. Direct and indirect rice yield losses by weeds are estimated at 10–15% worldwide (Baltazar and De Datta 2023). Alongside the cultural management methods such as using certified rice seed free from weed seeds, high rice seeding rate, proper water management, controlling weeds on levees and irrigation canals, and land preparation, herbicides are considered the most important part of the rice weed management.

In California rice production systems, weed management programs almost entirely rely on the use of herbicides (UCANR 2023). Once the rice fields are flooded, herbicides are generally applied at the day of seeding or within the two leaf rice growth stages. Most of the California rice growers follow up with at least one more post-emergent herbicide application

during the season usually before or at the mid-tiller rice growing stages. However, to maintain a season-long weed management, a cleanup application later in the season may be necessary to control weeds that have escaped from the earlier herbicide applications. Moreover, the number of available herbicides is limited in California rice due to the cost of development and registration (Hill et al. 1994), restrictive regulations, and concerns of off-target herbicide drift, particularly from the aerial applications (Galla et al. 2019; UCANR 2023). Due to the use of herbicides from the limited modes of action (MoA), a widespread herbicide resistance has developed in multiple weeds to different MoA such as ACCase inhibitors, ALS inhibitors, DXPS inhibitors, PSII inhibitors, VLCFA inhibitors, and synthetic auxins in California (Becerra-Alvarez et al. 2023). Herbicide resistance has become one of the biggest challenges for the sustainability of California's rice-based agroecosystems (Linguist et al. 2008). Herbicide resistance management efforts have primarily focused on the rotation of the limited herbicides.

Florpyrauxifen-benzyl [FPB; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate; CAS: 1390661-72-9; EPA: 62719-743] is a new auxin mimic (HRAC/WSSA Group IV) herbicide with a novel binding site of action, which FPB exhibits strong binding affinity in the auxin signaling system, auxin receptor, and/or auxin-signaling F-Box (AFB) proteins, instead of favoring the Transport Inhibitor Response1 (TIR1) compared to other auxin mimics (Miller and Norsworthy 2018b). The 6-aryl-picolinate auxin herbicides such as FPB have a carboxylic acid functional group, which allow them to pass through the lipophilic walls of the phloem, be excessively concentrated, and translocated (Epp et al. 2016). The carboxylic acid functional group is highly involved in binding interactions at the TIR1 and AFB1–5 receptors and mimics indole-3-acetic acid (IAA) to fill between the receptor and the co-repressor proteins at the cell nucleus (Grossmann 2010; Busi et al. 2018). Important

features of FPB are low use rates and wide application windows from 2-leaf rice stage until 60-d before harvest (Baltazar and De Datta 2023). Therefore, FPB can be used for late season cleanup applications and/or in a sequential application program between 14-d apart (Wright et al. 2021). However, a 60-d before harvest application may coincide with flowering stage of rice and auxin mimics are known for flower sterility when applied at flower initiation stages (Rehm 1952). Hence, it is important to understand potential adverse effects of late-season FPB applications. Additionally, FPB, unlike the other auxin-type herbicides, has a broader weed control spectrum including grasses, thus can be used to manage selective post-emergence grass, sedge, and broadleaf weeds in rice. Moreover, FPB may be used to control weeds which have evolved resistance to ALS and EPSP synthase inhibitors in rice systems (Baltazar and De Datta 2023). Therefore, FPB could be a useful herbicide in rice fields, considering the increase in herbicide resistance (Norsworthy et al. 2012; Teló et al. 2018). The objectives of this research were to 1) characterize the effects of FPB on rice crop safety and weed control when applied alone or in mixture with other partner herbicides, 2) determine the FPB application timing to optimize SMF control, and 3) elucidate the response of late applications on rice flower sterility.

Materials and Methods

Rice Response and Weed Control

This study was conducted during the 2020 growing season to elucidate FPB crop safety, weed control spectrum, and mixture compatibility with other commonly applied rice herbicides. Three field sites across the Sacramento Valley (Butte, Colusa, and Glenn counties) were selected for this study, where more than 60% of rice is grown in the state. The rice fields were at Arbuckle, CA (lat. 39°01'08.8"N, long. 121°55'52.2"W), Biggs, CA (lat. 39°27'03.6"N, long. 121°43'07.5"W), and Willows, CA (lat. 39°33'54.5"N, long. 122°03'48.6"W). The field

experiment in Biggs was conducted at the California Rice Experiment Station (hereafter ‘RES’). At Arbuckle, the soil is classified as a Clear Lake (Vertisols: fine, smectitic, thermic, Xeric Endoaquerts) with an average of NO₃-N: 10 ppm, Olsen-P: 21 ppm, K: 288 ppm, Na: 106 ppm, Ca: 18 meq/100 g, Mg: 14 meq/100 g, CEC: 34 meq/100 g, OM: 5%, and pH: 6.4. At Biggs, the soil is classified as an Esquon-Neerdobe (Vertisols: fine, smectitic, thermic, Xeric Epiaquerts or Duraquerts) with an average of NO₃-N: 19 ppm, Olsen-P: 28 ppm, K: 115 ppm, Na: 35 ppm, Ca: 8 meq/100 g, Mg: 5 meq/100 g, CEC: 14 meq/100 g, OM: 3%, and pH: 4.9. At Willows, the soil is classified as a Castro (Vertisols: fine, thermic, Typic Calciaquolls) with an average of NO₃-N: 7 ppm, Olsen-P: 3 ppm, K: 125 ppm, Na: 41 ppm, Ca: 15 meq/100 g, Mg: 10 meq/100 g, CEC: 25 meq/100 g, OM: 4%, and pH: 6.5. Average air temperatures in the Arbuckle, Biggs, and Willows fields for the 2020 (May 1 to October 31) growing season were 22.6 C, 22.3 C, and 21.5 C, respectively (CADWR 2024; Figure 1). Field preparation and cultural practices followed the University of California (UC) rice production guidelines (UCANR 2023).

In all fields, a pass with a single offset stubble disk was done after the 2019 rice harvest, then the fields were flooded to ~10-cm during the winter season to encourage straw decomposition (Linquist et al. 2006) and drained in early spring in 2020. Field preparation in spring included one pass with a chisel plow and two passes with a single-offset disk, followed by a land plane to smooth the soil surface. Aqua-ammonia, the primary N source, was injected about 10 cm below the soil surface (Cornelio and Linquist 2023) and then a corrugated roller was used to pack the soil and prevent large clods on the surface (Becerra-Alvarez et al. 2022). Rice seeds were placed in steel bins and filled with water containing 5% sodium hypochlorite solution for one hour to control *Bakanae* (*Fusarium fujikuroi*) disease, then the water was drained and filled with fresh water for rest of the 24-h period prior to seeding operations in all sites. The ‘M-209’

rice cultivar, a Calrose-type medium grain and smooth hulled (glabrous) *Japonica* rice variety with 92-d to 50% heading, was planted on May 3, 2020, at Arbuckle field. The seeding rate was 225 kg ha⁻¹. The cultivar planted in Biggs and Willows fields was ‘M-206’, a Calrose-type medium grain and glabrous *Japonica* rice variety with 86-d to 50% heading, that is a common cultivar in California [California Cooperative Rice Research Foundation (CCRRF) 2024]. Both rice varieties are high yielding, early maturing, and semi-dwarf cultivars. The pregerminated rice seeds were broadcast onto 10-cm flooded paddies with a rate of 200 kg ha⁻¹, in the Biggs and Willows fields, on May 23, 2020, and May 16, 2020, respectively.

Experiments were conducted as a randomized complete block (RCB) design, with four replicates, where a 3-m by 6-m rice plot (hereinafter referred to as ‘plots’) was an experimental unit. Biggs field plots were surrounded by a 1.8-m-wide buffer zone including levees to allow independent flush-irrigation and flooding of each plot and prevent herbicide contamination from adjacent treatments. Due to the inability to make plot-level levees in Arbuckle and Willows fields, herbicide plots and blocks were separated from each other by 3-m- and 6-m-wide buffers, respectively.

Prior to herbicide application, relative cover of weeds and rice as percentage of soil covered by each species were visually determined (Brim-DeForest et al. 2017b). Species with less than 1% cover were eliminated from the assessment. The weed composition was approximately 55% SMF, 15% combined broadleaves, and 5% WTG in Arbuckle; 35% WTG, 15% SMF, 15% BLR, and 5% combined broadleaves in Biggs; and 50% combined broadleaves, 15% combined sedges and 5% WTG in Willows.

Nine herbicide treatments were applied (Table 1), plus an untreated check (UTC). Herbicide applications were applied to rice at the 4–5 leaf (LS) stage as early postemergence

(EPOST). Herbicides were applied with a CO₂-pressurized boom sprayer with six XR 8003-VS flat-fan nozzles (TeeJet Technologies, Springfield, IL), placed 50 cm apart and calibrated to deliver 187 L ha⁻¹ at 206 kPa. All plots except UTC received a FPB [Loyant[®] CA, 25 g active ingredient (ai) L⁻¹, Corteva Agriscience, Indianapolis, IN, USA] application alone or in mixture combinations with propanil (Super WHAM![®] CA, 479 g ai L⁻¹, UPL NA INC., King of Prussia, PA, USA), penoxsulam (Granite[®] SC, 240 g ai L⁻¹, Corteva Agriscience, Indianapolis, IN, USA), or bispyribac-sodium (Regiment[®] CA, 80% ai by wt, Valent LLC, Walnut Creek, CA, USA) herbicides (Table 1). All foliar herbicide applications included methylated seed oil (hereafter ‘MSO’; Super Spread[®] MSO, Wilbur-Ellis, Fresno, CA, USA) at 584 ml ha⁻¹.

The Arbuckle and Willows fields were drained before the herbicide application to allow at least 70% foliar coverage and fields were reflooded ~48-h after treatments. In Biggs, the field was drained using a powered water pump immediately before the application and the water was brought back at ~48-h after application. At all sites, water was maintained at a depth of 10-cm throughout the growing season. Environmental conditions at the time of application were 20 C air temperature (temp), 60% relative humidity (RH), and 0.8 m s⁻¹ wind speed at Arbuckle; 19 C air temp, 60% RH, and 1 m s⁻¹ wind speed at Biggs; and 21 C air temp, 40% RH, and 0.6 m s⁻¹ wind speed at Willows, CA.

Visual ratings for all weed species were conducted at 7, 14, 21, 28, and 60-d after treatment (DAT). Weed control evaluations were based on a percentage scale relative to the UTC, ranging from 0 (no control) to 100 (complete control). Due to the difficulty of young seedling differentiation of *Echinochloa* species (WTG), they were grouped together. Weed density in plots was estimated at 28 DAT by counting plants in 30-cm by 30-cm quadrat samples, with three randomized samples in each plot.

Rice growth parameters and response to herbicide treatments such as chlorosis, necrosis, stand reduction, and stunting were measured when each weed control rating was conducted throughout the season. Crop injury evaluations were made based on a percentage scale relative to the UTC, ranging from 0 (absence of symptoms) to 100 (plant death). Days to 50% heading was estimated visually. Plant height was determined at ~120-d after seeding by measuring plant height from the soil to the extended panicle. At all sites, rice was hand harvested from two 1-m² quadrats in each plot and mechanically threshed (Large Vogel Plot Thresher, Almaco, Nevada, IA, USA). Due to no rice yield in UTC plots in the Biggs experiments, UTC plots were not harvested. Rice grain yield for all sites were adjusted to 14% moisture.

All data were subjected to analysis of variance (ANOVA) using the ‘agricolae’ (Mendiburu 2024), ‘emmeans’ (Lenth 2024), ‘lme4’ (Bolker 2024), and ‘lmerTest’ (Christensen 2024) packages in RStudio Version 2023.12.1+402 (R Core Team 2023). Means were separated using Tukey’s honestly significant difference (HSD) test at significance level of $\alpha = 0.05$. The ‘multcomp’ (Hothorn 2024) package was used to generate multiple comparisons among means. Treatment by location interactions were observed for the rice yield data; therefore, these data were analyzed and presented individually by location. The herbicide rates and application timing were considered fixed factors, while location and replication were considered random factors. There was no significant treatment by location interaction for rice injury or weed control data; therefore, all locations’ data in this study were combined.

Timing of FPB Application for Smallflower Umbrella Sedge Control

A field experiment was conducted at the RES in Biggs, CA, (lat. 39°27'03.6"N, long. 121°43'07.5"W; elev. 30-m) in 2021 and 2022 to determine optimum FPB application timing to control SMF in water-seeded rice systems. Soils at the site were classified as Esquon-Neerdobe

(Vertisols: fine, smectitic, thermic, Xeric Epiaquerts or Duraquerts), with an average of $\text{NO}_3\text{-N}$: 14 ppm, Olsen-P: 26 ppm, K: 98 ppm, Na: 37 ppm, Ca: 7 meq/100 g, Mg: 5 meq/100 g, CEC: 13 meq/100 g, OM: 2.5%, and pH: 5. Average air temperatures for 2021 and 2022 (May 1 to October 31) were 22.1 C and 22.4 C, respectively (CADWR 2024; Figure 2). Cultural practices followed the UC rice production guidelines in both years (UCANR 2023).

Prior to 2021 and 2022 growing seasons, the remaining rice straw was incorporated into the soil by a pass with a single offset stubble disc and then field was flooded to 10 cm above the soil throughout the winter. In early spring, the water was drained, and the field preparation was conducted as described in the previous study. A granule fertilizer starter mixture of ammonium sulfate and potassium sulfate (34% N, 17% P, 0% K) was applied by airplane at 336 kg ha^{-1} prior to the corrugated roller (Becerra-Alvarez and Al-Khatib 2024). The rice cultivar ‘M-209’ (features described above) were pregerminated as described earlier. The rice seed were seeded by aircraft at 190 kg ha^{-1} on May 30, 2021, and May 22, 2022, onto the field with a 10-cm standing flood.

Experiments were conducted as an RCB design with four replications in 2021, and three replications in 2022. The plots were 3 X 6-m as described in previous study. Copper sulfate (Copper Sulfate Crystals MUP, Quimag Quimicos Aguila, Jalisco, MX) at 17 kg ha^{-1} was broadcast onto fields three days after seeding for algae control in both years.

Five herbicide treatments were applied (Table 2), plus an UTC. Foliar herbicide applications were timed based on SMF development stages as they reached the first leaf (1-LF), 10-, 15-, 20-, and 25-cm tall SMF. Herbicides were applied as described in the previous study. All plots including UTC received a base application of clomazone (Cerano[®] 5 MEG, 5% ai by wt, Wilbur-Ellis, Fresno, CA, USA) at a rate of 672 g ai ha^{-1} at the day of seeding (DOS) to

control grass species. The FPB was applied at the maximum field use rate of 40 g ai ha⁻¹ with MSO at 584 ml ha⁻¹ (Table 2).

In both the 2021 and 2022 experiments, fields were drained using a powered water pump the day before the foliar application to allow at least 70% foliar coverage and fields were reflooded ~48-h after application. Water was maintained at a depth of 10-cm throughout the remainder of the growing season. Environmental conditions at the time of application was 20 C temp, 60% RH, and 0.8 m s⁻¹ wind speed in 2021; and 19 C air temp, 60% RH, and 1 m s⁻¹ wind speed in 2022, respectively.

Visual ratings for SMF control were conducted at 7, 14, 21, 28, 35, and 42 DAT, and weed density was determined 28 DAT as described in the previous study. Rice growth parameters and responses to herbicide treatments such as chlorosis and necrosis were measured with the same methodology as described above. Whole plots were mechanically harvested, and yields were determined using a small-plot combine harvester (SPC40, Almaco, Nevada, IA, USA) with a swath width of 2.3-m. Yield was adjusted to 14% moisture content.

All data were subjected to ANOVA using the ‘agricolae’ (Mendiburu 2024), ‘emmeans’ (Lenth 2024), and ‘lme4’ (Bolker 2024) packages in RStudio Version 2023.12.1+402 (R Core Team 2023). Means were separated using Tukey’s HSD test at significance level of $\alpha = 0.05$. In 2021 and 2022 experiments, no significant interactions were found between treatment and year; therefore, data were averaged over two years.

Rice Sterility as Affected by FPB Application at Different Timing

The study was conducted at the RES in Biggs, CA, (lat. 39°27'03.6"N, long. 121°43'07.5"W; elev. 30-m) in 2021 and 2022 to determine the potential adverse effects of late FPB applications on rice flower sterility. Soils at the site were classified as an Esquon-Neerdobe (Vertisols: fine,

smectitic, thermic, Xeric Epiaquerts or Duraquerts), with an average of $\text{NO}_3\text{-N}$: 3 ppm, Olsen-P: 22 ppm, K: 288 ppm, Na: 522 ppm, Ca: 14 meq/100 g, Mg: 11 meq/100 g, CEC: 28 meq/100 g, OM: 2.7%, and pH: 5.6. Average air temperatures for 2021 and 2022 (May 1 to October 31) were 22.1 C and 22.4 C, respectively (CADWR 2024; Figure 2). Cultural practices followed the UC rice production guidelines in both years (UCANR 2023).

In 2021 and 2022, field practices were the same as described in the previous study. Rice cultivar ‘M-209’ were pregerminated and broadcast by aircraft at a 190 kg ha^{-1} seeding rate on May 30, 2021, and May 26, 2022, onto the field with a 10-cm standing flood. Experiments were conducted as an RCB design with four replications in 2021, and three replications in 2022. The 3-m wide by 6-m long plots were encased by 1.8-m-wide buffer zone including levees to allow independent flush-irrigation and flooding of each plot and prevent herbicide contamination from adjacent treatments.

Four herbicide treatments were applied (Table 3), plus an UTC. Foliar herbicide applications were based on rice development stages as they were reached 4-unfolded-leaf, rice panicle initiation (PI), and rice 50% flowering (FL) growing stage at ~90-d after seeding (Jordan et al. 1998). Herbicides were applied as described in the previous study. Plots received clomazone at a rate of 672 g ai ha^{-1} at the DOS to control WTG species. Additional herbicides were applied later in the season for broadleaf and sedge weed control in treated plots, which included SMF, BLR, DKS, and RDS. These herbicide treatments were cyhalofop-butyl plus penoxsulam (RebelEX[®] CA, 213 + 30 g ai L⁻¹, Corteva Agriscience, Indianapolis, IN, USA) applied at $312 \text{ plus } 44 \text{ g ai ha}^{-1}$ at 4-LS rice, followed by FPB applied at 40 g ha^{-1} or 80 g ha^{-1} at PI growth stage. Two treatments received FPB applications of 40 g ha^{-1} and 80 g ha^{-1} rates at FL growing stage, respectively (Table 3).

Field operations regarding water management and weed control measurements were as described in the previous study. Environmental conditions at the time of PI applications were 20 C temp, 60% RH, and 0.8 m s⁻¹ wind speed in 2021; and 19 C air temp, 60% RH, and 1 m s⁻¹ wind speed in 2022, respectively.

Prior to the field harvest in both years, 20 panicles per plot were randomly selected, hand-harvested, and dried for three d at 50 C. Sterility ratio was determined by counting filled and unfilled rice florets per panicle. Grain yield per panicle and 1,000-grain weight were measured and adjusted to 14% grain moisture content. Whole plots were mechanically harvested, and yields were determined using a small-plot harvester (SPC40, Almaco, Nevada, IA, USA) with a swath width of 2.3-m. Plot yield were adjusted to 14% moisture content.

All data recorded were subjected to ANOVA using the ‘agricolae’ (Mendiburu 2024), ‘lme4’ (Bolker 2024), and ‘lmerTest’ (Christensen 2024) packages in RStudio Version 2023.12.1+402 (R Core Team 2023). Means were separated using Tukey’s HSD test at significance level of $\alpha = 0.05$. The ‘multcomp’ (Hothorn 2024) package was used to generate multiple comparisons among means. In 2021 and 2022 experiments, no significant interactions were found between treatment and year; therefore, data were averaged over two years.

Results and Discussion

Rice Response and Weed Control

At all sites, WTG were the major grass species. FPB applied alone at 15, 30, and 60 g ha⁻¹ resulted in WTG control of 68%, 75%, and 85% at 14 DAT, respectively (Table 4). When FPB applied at 30 g ha⁻¹ in mixture combinations with partner herbicides, greater weed control was observed. FPB plus bispyribac-sodium gave the greatest weed control, 96%, at 14 DAT. Similarly, FPB plus penoxsulam, FPB plus propanil, and FPB plus bispyribac-sodium controlled

92%, 91%, and 90% of WTG, respectively at 14 DAT. Weed counts at 28 DAT showed a reduction in WTG with all FPB applications. The WTG density was 8 and 28 plants m⁻² with FPB applied alone at 60 g ha⁻¹ and FPB plus propanil, respectively, whereas UTC had an average of 52 plants m⁻². Our results are similar to previous research (Miller and Norsworthy 2018; Miller et al. 2018; Sanders et al. 2020). Miller and Norsworthy (2018) reported that FPB applied at 30 g ha⁻¹ provided from 91% to 93% control of BYG, FPB plus bispyribac-sodium at 30 plus 22 g ha⁻¹ gave 97%, and FPB plus penoxsulam at 30 plus 40 g ha⁻¹ provides 98% control of BYG at 14 DAT. In another study, Miller et al. (2018) found FPB applied at 30 g ha⁻¹ controlled approximately 96% of 152 BYG accessions at 21 DAT. Sanders et al. (2020) also reported that FPB applied at 29 g ha⁻¹ achieved 98% control of BYG at 28 DAT.

The SMF and BLR infestations were dense with up to ~55% of plots infested with these two sedges prior to herbicide treatments. When FPB was applied alone at 15, 30, and 60 g ha⁻¹, BLR and SMF control ranged from 66% to 73% at 14 DAT (Table 4). The greatest sedge control was 73% for both BLR and SMF when FPB was applied alone at the highest rate of 60 g ha⁻¹ at 14 DAT. When FPB was applied with propanil, the control increased to 87% and 88% for BLR and SMF at 14 DAT, respectively. However, BLR and SMF control with other mixture combinations did not differ from FPB applied alone. At 28 DAT, sedge densities ranged from 20 to 40 BLR and 20 to 36 SMF plants m⁻² with the greatest reduction with FPB plus propanil and lowest reduction with FPB applied alone at 15 g ha⁻¹, respectively, whereas UTC plots had an average of 52 and 44 BLR and SMF plants m⁻², respectively.

The DKS was by far the most common broadleaf weed species in this study and followed by RDS, WHS, and ARW, respectively. At 14 DAT, broadleaf weed control ranged from 92% to 100%, across all weed species when FPB applied alone (Table 4). Moreover, DKS control was

the greatest with FPB applied at 60 g ha⁻¹ and FPB plus bispyribac-sodium. Similarly, all FPB treatments gave superior control of RDS ranging from 97% to 100% at 14 DAT. The WHS and ARW were 100% controlled with all FPB treatments including the lowest rate of FPB.

Furthermore, DKS and RDS plant count at 28 DAT showed more than 95% reduction with all FPB treatments. The excellent broadleaf weed control in our study agrees with earlier research that show FPB applied at 15 g ha⁻¹ rate caused 100% reduction in waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] at 35 DAT (Mudge et al. 2021). Beets and Netherland (2018) reported that the aquatic weeds, floating heart [*Nymphoides cristata* (Roxb.) Kuntze] and hydrilla [*Hydrilla verticillata* (L.f.) Royle], were completely controlled with FPB.

All FPB treatments caused up to 34% injury symptoms on rice and the severity of symptoms increased as the rates increased at 14 DAT; however, the severity of symptoms decreased at 28 DAT and rice gradually recovered from FPB injury symptoms (Table 5). When FPB applied alone at 30 g ha⁻¹ caused 10% chlorosis, 18% necrosis, 15% stand reduction, and 22% stunting compared to the UTC plots at 14 DAT. The highest rice injury was observed when FPB applied at 60 g ha⁻¹ and in mixture with penoxsulam, which caused 14% chlorosis, 25% necrosis, 30% stand reduction, and 34% stunting. All symptoms dissipated and rice appeared normal at 60 DAT. Velásquez et al. (2021) reported that 30 g ha⁻¹ FPB applied at 2-LS and 6-LS rice caused up to 20% and 10% rice injury at 21 DAT, respectively. Wright et al. (2021) reported that sequential applications of FPB at 30 g ha⁻¹ applied 14-d apart resulted in 15% rice injury at 21 DAT. Nevertheless, plant height and 50% heading did not differ among treatments (data not shown). These results indicate that ‘M-206’ and ‘M-209’ rice cultivars potentially recover from the early-season FPB injury.

At all sites, FPB applied alone resulted in greater yield compared to UTC (Table 6). FPB applied at 15 g ha⁻¹ resulted in the lowest yield among FPB treatments at the Arbuckle, Biggs, and Willows fields; however, this treatment still resulted in 40% more rice yield than the UTC (data not shown). When FPB was applied alone, yields increased as the FPB application rate increased at all sites. When FPB at 30 and 60 g ha⁻¹ applied alone resulted in 60% and 97% more yield than the UTC, respectively (data not shown). Moreover, the highest yield, 4,626 kg ha⁻¹, was achieved when FPB was applied at 60 g ha⁻¹ rate in the Biggs field.

Mixture combinations of FPB with propanil increased average yields up to 55% and 95% with FPB plus propanil at 3,367 g ha⁻¹ and 6,734 g ha⁻¹ rates, respectively (Table 6). When compared to FPB applied alone at 30 g ha⁻¹, FPB plus propanil at 6,734 g ha⁻¹ rate also resulted in ~22% higher yields at all three sites. Similarly, the mixture of FPB with penoxsulam applied at 40.2 g ha⁻¹ and 49 g ha⁻¹ resulted in 82% and 99% greater yield than UTC, respectively. In addition, the FPB plus penoxsulam at 49 g ha⁻¹ resulted in ~25% greater average yield than FPB applied alone. Finally, FPB plus bispyribac-sodium at 22.4 g ha⁻¹ and 37.5 g ha⁻¹ yielded 63% and 73% more than the UTC; however, 2% and 8% more than FPB applied alone at 30 g ha⁻¹. The yield differences among the three sites should be caused by the prior weed pressure. The Biggs field had an average of up to 35% WTG density, which explains the high yield reductions as well as no yield in the UTC plots (Smith 1983). At the Willows field, the prior broadleaf and sedge weeds pressure were up to 50% and the low UTC yield confirmed the adverse impacts of weed rice competition. All FPB applications with maximum use rate of various partner herbicides improved yield compared to FPB applied alone at 30 g ha⁻¹. This is largely due to the greater weed control compared to FPB applied alone.

Timing of FPB Application for Smallflower Umbrella Sedge Control

When FPB applied at 1-LF stage gave 95% SMF control at 42 DAT in 2021 and 2022 (data not shown). The FPB applied on 10-, 15-, 20-, and 25-cm tall SMF controlled 86%, 89%, 87%, and 85% SMF at 42 DAT, respectively. Moreover, the weed count at 28 DAT showed a significant reduction in SMF density ranging from 4 to 8 SMF m⁻², while UTC had 12 SMF m⁻² (data not shown). Miller and Norsworthy (2018) reported that FPB applied at 30 g ha⁻¹ controlled 95% of SMF when applied at 10 to 15-cm tall plants in a greenhouse study. In addition, Yin et al. (2023) found that 100% control was achieved in a greenhouse study when FPB applied at 36 g ha⁻¹ at 10-cm tall SMF.

In both 2021 and 2022, rice injury from all herbicide treatments was minimal. At 1-LF and 10-cm tall SMF applications 3% and 2% chlorosis, and 2% and 1% necrosis were observed at 14 DAT, respectively (data not shown). All FPB injury symptoms on rice dissipated by 28 DAT. The ranking of yield was 7,086, 7,050, 6,977, 6,900, and 5,997 kg ha⁻¹ with, FPB applied at 40 g ha⁻¹ on 20-cm, 10-cm, 1-LF, 25-cm, and 15-cm tall SMF, respectively (data not shown). All treatments had more rice yield compared to UTC which only yielded 3,658 kg ha⁻¹. This study indicates FPB at 40 g ha⁻¹ can control SMF 85% to 95% when applied at up to 25-cm tall SMF stages, caused almost no rice injury, and increased yields up to 94% compared to the UTC.

Rice Sterility as Affected by FPB Application at Different Timing

When FPB applied at 40 and 80 g ha⁻¹ on rice at PI growth stage resulted in 91% and 95% control of WTG at 42 DAT, respectively (Table 7). Similarly, BLR was controlled 99% with applications at PI, whereas SMF was controlled 97% and 98% with FPB at 40 and 80 g ha⁻¹ rates, respectively. Likewise, weed control with FPB applied at the FL growth stage was similar to applications at the PI growth stage, which might suggest a late season application of FPB

could provide good weed control. Regardless of the treatments and application timings, all broadleaf weed species were controlled by 100% at 42 DAT (Table 7).

Rice injury was 10% chlorosis at 14 DAT for PI growth stage applications (Table 8). However, symptoms gradually dissipated, and rice appeared normal by 28 DAT. The FL stage applications did not cause rice injury symptoms at any rating time; however, there was an inverse correlation between injury symptoms and the rice spikelet sterility at harvest (Table 8). When FPB applied at 40 and 80 g ha⁻¹ at the FL growth stage resulted in 26% and 35% rice spikelet sterility, respectively. At the PI stage applications, sterility was 9% and 15% for FPB applied at 40 and 80 g ha⁻¹, respectively. In contrast, seeds per panicle and 1,000-grain weight for all treatments were similar including UTC. Regardless of the sterility at the FL application timing, all treatments resulted in greater yield than the UTC plots. The highest yield was 8,710 kg ha⁻¹ with FPB applied at 40 g ha⁻¹ at the rice PI growth stage, whereas the UTC yielded 5,101 kg ha⁻¹. Together this research suggests that FPB at 40 g ha⁻¹ can be safely applied to water-seeded rice with adequate weed control up to the rice PI growth stage.

Practical Implications

This research showed that FPB applied alone at 30 g ha⁻¹ at 4-5-LF rice stage controlled more than 80% of WTG, BLR, and SMF as well as more than 99% of all broadleaf weeds including DKS, RDS, WTS, and ARW at 28 DAT. Grass and sedge weed control increased when FPB was applied within herbicide combinations. The mixture treatments of FPB plus bispyribac-sodium applied at maximum use rate had the greatest grass control. On the other hand, FPB plus propanil at the maximum use rate provided the greatest sedges control. Even though rice had initial injury symptoms after FPB treatments, plants gradually recovered, and yield was not reduced. This

study suggests that FPB weed control can be improved when FPB is applied with an appropriate mixture partner.

This study also showed that SMF can be effectively controlled with FPB at 40 g ha⁻¹ rate when applied at 1-LF, 10-, 15-, 20-, and 25-cm tall SMF growth stages without causing significant rice injury. If a cleanup treatment required later in the growing season, a sequential application of FPB can be used to control late emerged weeds (unpublished data). When rice is large enough (1- to 2-tiller), the injury caused by FPB is minimal, and rice recovered from injury symptoms. This response is not surprising and is likely due to an increase in FPB metabolism (Wright et al. 2021). However, any application after the rice PI growth stage may increase the potential for rice spikelet sterility especially at FL applications.

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Competing Interests

The authors declare none.

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Tables

Table 1. Florpyrauxifen-benzyl treatments applied on rice in 2020 at Arbuckle, Biggs, and Willows, California.

Treatment ^a	Herbicide	Application rate (g ai ha ⁻¹)
Trt1	Florpyrauxifen-benzyl	15
Trt2	Florpyrauxifen-benzyl	30
Trt3	Florpyrauxifen-benzyl	60
Trt4	Florpyrauxifen-benzyl	30
	Propanil	3,367
Trt5	Florpyrauxifen-benzyl	30
	Propanil	6,734
Trt6	Florpyrauxifen-benzyl	30
	Penoxsulam	40.2
Trt7	Florpyrauxifen-benzyl	30
	Penoxsulam	49
Trt8	Florpyrauxifen-benzyl	30
	Bispyribac-sodium	22.4
Trt9	Florpyrauxifen-benzyl	30
	Bispyribac-sodium	37.5
UTC	—	—

^aAbbreviations: ai, active ingredient; Trt, treatment; UTC, untreated check.

Table 2. Florpyrauxifen-benzyl treatments applied on smallflower umbrella sedge (SMF) at different growth stages in 2021 and 2022 at Biggs, California.

Treatment ^a	Herbicide	Application rate (g ai ha ⁻¹)	Rice growth stage timing	SMF timing
Trt1	Clomazone	672	DOS	DOS
	Florpyrauxifen-benzyl	40	2–3 LS	1-LF
Trt2	Clomazone	672	DOS	DOS
	Florpyrauxifen-benzyl	40	4 LS	10-cm SMF
Trt3	Clomazone	672	DOS	DOS
	Florpyrauxifen-benzyl	40	4–5 LS	15-cm SMF
Trt4	Clomazone	672	DOS	DOS
	Florpyrauxifen-benzyl	40	5 LS	20-cm SMF
Trt5	Clomazone	672	DOS	DOS
	Florpyrauxifen-benzyl	40	5–6 LS	25-cm SMF
UTC	Clomazone	672	DOS	DOS

^aAbbreviations: ai, active ingredient; DOS, day of seeding; LF, SMF-leaf stage; LS, rice-leaf stage; SMF, smallflower umbrella sedge; Trt, treatment; UTC, untreated check.

Table 3. Herbicide treatments applied on rice at different growth stages in 2021 and 2022 at Biggs, California.

Treatment ^a	Herbicide	Application rate (g ai ha ⁻¹)	Rice timing
Trt1	Clomazone	672	DOS
	Cyhalofop-butyl	313	4-LS
	Penoxsulam	44	4-LS
	Florpyrauxifen-benzyl	40	PI
Trt2	Clomazone	672	DOS
	Cyhalofop-butyl	313	4-LS
	Penoxsulam	44	4-LS
	Florpyrauxifen-benzyl	80	PI
Trt3	Clomazone	672	DOS
	Cyhalofop-butyl	313	4-LS
	Penoxsulam	44	4-LS
	Florpyrauxifen-benzyl	40	FL
Trt4	Clomazone	672	DOS
	Cyhalofop-butyl	313	4-LS
	Penoxsulam	44	4-LS
	Florpyrauxifen-benzyl	80	FL
UTC	—	—	—

^aAbbreviations: ai, active ingredient; DOS, day of seeding; FL, 50% flowering stage; LS, rice-leaf stage; PI, panicle initiation; Trt, treatment; UTC, untreated check.

Table 4. Average weed control ratings at 14 and 28 days after florypyrauxifen-benzyl applied alone or in mixture combinations in 2020 at Arbuckle, Biggs, and Willows, California.

Treatment	Grasses ^a		Sedges				Broadleaves				
	WTG ^b		BLR		SMF		DKS		RDS		
	Days after treatment										
	14	28	14	28	14	28	14	28	14	28	
	% of untreated check										
Trt1 ^c	68	a ^d 77	a 66	a 76	a 63	a 72	a 92	b 96	a 97	b 99	
Trt2	75	b 81	ab 73	a 81	ab 70	a 80	a 98	a 99	a 98	a 100	
Trt3	85	bc 89	ab 73	a 80	ab 73	a 78	a 99	a 99	a 98	a 100	
Trt4	86	bc 86	b 81	a 88	bc 77	a 87	a 94	a 99	a 99	a 100	
Trt5	91	c 92	c 87	a 91	c 88	a 88	a 95	a 99	a 99	a 100	
Trt6	84	bc 90	ab 73	a 82	ab 72	a 79	a 98	a 99	a 99	a 100	
Trt7	92	c 95	b 78	a 82	bc 77	a 86	a 95	a 100	a 99	a 100	
Trt8	90	c 93	b 77	a 84	ab 73	a 83	a 98	a 100	a 99	a 100	
Trt9	96	c 97	b 81	a 86	bc 81	a 87	a 99	a 100	a 100	a 100	

^aAbbreviations: WTG, watergrasses; BLR, ricefield bulrush; SMF, smallflower umbrella sedge; DKS, ducksalad; RDS, redstem; Trt, treatment.

^bWTG species including barnyardgrass, early watergrass, and late watergrass.

^cTreatments were timed to rice 4-5-leaf growing stages.

^dMeans with the same letters are not different at a 5% significance level, according to Tukey's HSD test.

Table 5. Average rice chlorosis, necrosis, stand reduction, and stunting as affected by florpyrauxifen-benzyl applied alone and in mixture combinations with partner herbicides applied in 2020 at Arbuckle, Biggs, and Willows, California.

Treatment ^a	Chlorosis		Necrosis		Stand reduction		Stunting	
	Days after treatment							
	14	28	14	28	14	28	14	28
	%							
Trt1 ^b	4 a ^c	2 a	15 ab	12 b	10 a	8 a	16 b	15 b
Trt2	10 b	6 b	18 b	15 bc	15 ab	13 b	22 cd	21 c
Trt3	14 b	9 b	20 bc	16 bc	16 ab	15 bc	19 bc	18 b
Trt4	7 ab	3 a	13 a	7 a	15 ab	10 b	10 a	7 a
Trt5	9 b	4 a	24 c	13 b	26 cd	25 d	24 cd	21 c
Trt6	10 b	7 b	17 b	15 bc	27 cd	26 d	26 de	21 c
Trt7	7 ab	4 ab	25 d	21 c	30 d	26 d	34 e	30 d
Trt8	10 b	7 b	18 b	15 bc	19 c	17 cd	31 e	29 d
Trt9	11 b	8 b	20 bc	17 bc	24 cd	22 d	29 e	27 d

^aAbbreviations: Trt, treatment.

^bTreatments were timed to rice 4-5-leaf growing stages.

^cMeans with the same letters are not different at a 5% significance level, according to Tukey's HSD test.

Table 6. Rice grain yield as affected by florpiauxifen-benzyl applied alone and in mixture combinations with partner herbicides in 2020 at Arbuckle, Biggs, and Willows, California.

Treatment ^a	Arbuckle		Biggs		Willows	
	kg ha ⁻¹					
Trt1 ^b	7,357 ^c	ab ^d	1,861	cd	1,666	ab
Trt2	7,396	ab	2,990	abc	2,055	ab
Trt3	8,573	ab	4,626	a	2,152	ab
Trt4	7,368	ab	2,201	bcd	2,495	ab
Trt5	9,367	a	2,511	abc	3,324	a
Trt6	7,867	ab	3,952	abc	2,368	ab
Trt7	8,527	ab	4,404	ab	2,574	ab
Trt8	8,889	ab	1,930	cd	1,858	ab
Trt9	9,204	ab	2,047	cd	2,210	ab
UTC	6,835	b	0	d	955	b

^aAbbreviations: Trt, treatment; UTC, untreated check.

^bTreatments were timed to rice 4-5-leaf growing stages.

^cYields were adjusted to 14% moisture contents.

^dMeans with the same letters are not different at a 5% significance level, according to Tukey's

HSD test.

Table 7. Average weed control ratings at 14, 28, and 42 days after florpurauxifen-benzyl applied in 2021 and 2022 at Biggs, California.

Trt	Grasses						Sedges						Broadleaves															
	SPG ^a			WTG ^b			BLR			SMF			DKS	RDS														
	Days after treatment																											
	14	28	42	14	28	42	14	28	42	14	28	42	42	42														
	% of untreated check																											
Trt1	96	a ^c	96	a	99	a	82	b	88	b	91	b	96	a	99	a	99	a	85	a	87	a	97	a	100	a	100	a
Trt2	99	a	99	a	100	a	94	a	95	ab	95	ab	95	a	97	a	99	a	82	a	92	a	98	a	100	a	100	a
Trt3	100	a	100	a	100	a	99	a	99	a	99	a	100	a	100	a	100	a	70	a	84	a	96	a	100	a	100	a
Trt4	100	a	100	a	100	a	99	a	99	a	100	a	100	a	100	a	100	a	80	a	96	a	97	a	100	a	100	a

^aAbbreviations: SPG, bearded sprangletop; WTG, watergrasses; BLR, ricefield bulrush; SMF, smallflower umbrella sedge; DKS, ducksalad; RDS, redstem; Trt, treatment.

^bWTG species including barnyardgrass, early watergrass, and late watergrass.

^cMeans with the same letters are not different at a 5% significance level, according to Tukey’s HSD test.

Table 8. Rice yield components and grain yield as affected by florpyrauxifen-benzyl applied at different rice growth stages in 2021 and 2022 at Biggs, California.

Treatment ^a	Sterility ^b	Total seeds	Grain weight	Yield
	%	seed panicle ⁻¹	g 1,000-seed ⁻¹	kg ha ⁻¹
Trt1	9 b ^c	86 a	38 a	8,710 ^d a
Trt2	15 ab	83 a	36 a	7,915 a
Trt3	26 ab	84 a	42 a	6,574 ab
Trt4	35 a	80 a	38 a	6,557 ab
UTC	14 ab	82 a	42 a	5,101 b

^aAbbreviations: Trt, treatment; UTC, untreated check.

^bSterility is reported as the percentage of unfilled florets of the total florets per panicle.

^cMeans with the same letters are not different at a 5% significance level, according to Tukey's HSD test.

^dYields were adjusted to 14% moisture content.

Figures



Figure 1. Daily temperature extremes and daily rainfall for 2020 growing season. Solid and dashed lines are daily maximum and minimum temperatures (in degrees C), respectively. Bars are daily precipitation (mm). Vertical dashed lines are planting dates of May 3, 16, and 23, for the Arbuckle, Willows, and Biggs fields, respectively.

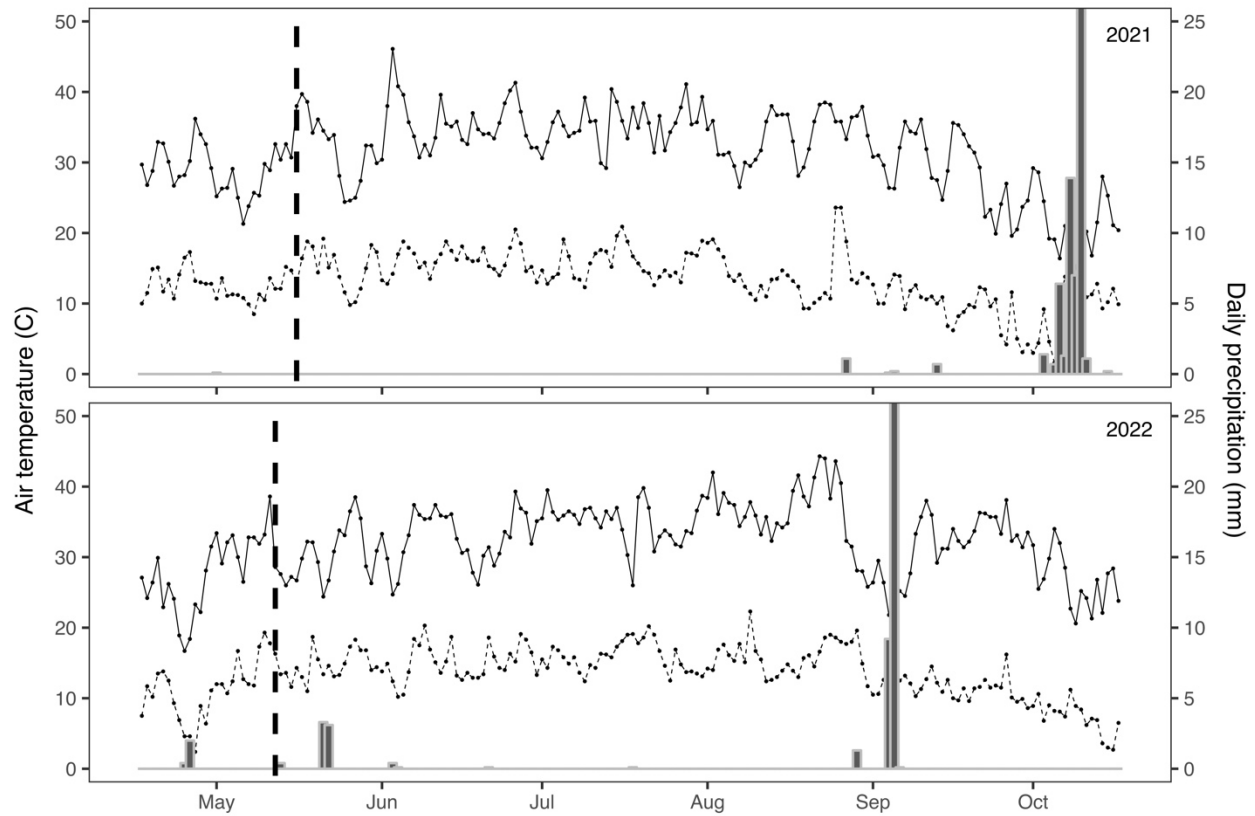


Figure 2. Daily temperature extremes and daily rainfall for 2021 and 2022 growing seasons. Solid and dashed lines are daily maximum and minimum temperatures (in degrees C), respectively. Bars are daily precipitation (mm). Vertical dashed lines are the planting dates of May 30, 2021, and May 26, 2022.

Chapter 2

Tree nut crop response to simulated florpyrauxifen-benzyl and triclopyr herbicide drift

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Abstract

California is the nation's primary producer of almonds, pistachios, and walnuts. Because of California's diverse cropping systems, off-target herbicide drift can be a considerable problem. Florpyrauxifen-benzyl is a newly registered auxin-mimic rice herbicide. This research was conducted in 2020 and 2021 to determine the relative sensitivity of almond, pistachio, and walnut trees to simulated drift rates of florpyrauxifen-benzyl. Triclopyr is another synthetic auxin type rice herbicide that is commonly used in California rice production systems. Although typical auxin herbicide symptoms are similar each other and be recognized on trees, it is important to understand differences and comparison between florpyrauxifen-benzyl and triclopyr symptoms. Therefore, we characterized and compared the symptoms caused by florpyrauxifen-benzyl and triclopyr. The fractional herbicide rates tested were 1/200X, 1/100X, 1/33X, and 1/10X of the florpyrauxifen-benzyl use rate of $29.4 \text{ g} \cdot \text{ha}^{-1} \text{ ai}$ and 1/200X, 1/100X, and 1/33X of the triclopyr use rate of $420 \text{ g} \cdot \text{ha}^{-1} \text{ ae}$. Florpyrauxifen-benzyl and triclopyr herbicides were applied directly to one side of the canopy of one- to two-year-old almond, pistachio, and walnut trees. The general symptoms of florpyrauxifen-benzyl and triclopyr were chlorosis, chlorotic spots, leaf curling, leaf narrowing, leaf distortion, leaf malformation, leaf crinkling, shoot curling, stem coloring, stunting, terminal bud death, and twisting. The florpyrauxifen-benzyl and triclopyr injury symptoms were compared at the same simulated drift rates and found to be similar each other. The herbicide injury was distinguishable at the entire pistachio canopy, particularly on developing leaves and terminal buds. In contrast, injury symptoms on almond and walnut were more apparent on the side to which herbicides were applied. Most symptoms peaked at 14 days after treatment with the 1/10X florpyrauxifen-benzyl rate, when the visible injury was 16%, 48%, and 78% on almond, walnut, and pistachio, respectively. Although almond and

walnut symptoms from the 1/10X florasulfuron-benzyl rate remained visible longer, all trees gradually recovered throughout the growing season. In contrast, pistachio trees did not recover as fully as almond and walnut, and injury symptoms persisted for the remainder of the 2021-2022 growing seasons on pistachio. Anticipated drift rates below 1/100X up to 1/33X of herbicide use rates. Because of low injury symptoms at the anticipated drift rates and rapid herbicide injury recovery in almond and walnut trees, this research suggested that proper herbicide drift management practices currently used by rice growers and application precautions are likely to minimize the risk of significant injury from the potential florasulfuron-benzyl off-target drift to almond and walnut, but extra precautions may be needed if there are nearby pistachio orchards.

Keywords: Herbicide symptomology; multiple exposures; off-target movement

Introduction

California is the major producer of almonds [*Prunus dulcis* (Mill.) D.A. Webb], pistachios (*Pistacia vera* L.), and walnuts (*Juglans regia* L.) in the United States (US), where they are grown on 1M hectares (ha) and added more than \$11.5B to the US economy in 2021 [US Department of Agriculture National Agricultural Statistics Service (USDA-NASS) 2024]. Among these tree nuts, almond is particularly important in California and accounts for approximately 82% of the global almond production and being the highest-valued California agricultural export commodity, with a value of more than \$4.6B in 2021 [California Department of Food and Agriculture (CDFA) 2024].

The Sacramento Valley of California is also the second largest rice (*Oryza sativa* L.) production region in the US with more than 200,000 ha of rice (Galvin et al. 2022) with a farmgate value of nearly \$1B (CDFA 2024). California rice systems are favored by a Mediterranean climate with high solar radiation and relatively cold nighttime temperatures, leading to 20% higher rice yields than the average of all other rice producing regions in the US (Hill et al. 2006). Approximately 97% of California rice fields are water-seeded, where pregerminated rice seeds are aerially spread into 10 to 15 cm water, and the fields are continuously flooded throughout the growing season (Brim-DeForest et al. 2017a; Hill et al. 2006). This unique water-seeded and continuously flooding growing practice was adopted to suppress weedy grasses (Kennedy 1923).

Weeds are considered a major problem in California rice production (Brim-DeForest et al. 2017). Continuous flood in California rice and lack of crop rotation have resulted in the selection of semiaquatic weeds including grasses such as *Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass), *E. oryzicola* (Vasinger) Vasinger (late watergrass), *E. oryzoides* (Ard.) Fritsch

(early watergrass), and *Leptochloa fusca* (L.) Kunth ssp. *fascicularis* (Lam.) N. Snow (bearded sprangletop); sedges such as *Schoenoplectiella mucronata* (L.) J.Jung & H.K.Choi (ricefield bulrush) and *Cyperus difformis* L. (smallflower umbrella sedge); and broadleaves such as *Ammannia* spp. (redstem) and *Heteranthera limosa* (Sw.) Willd. *Heteranthera* Ruiz & Pav. (ducksalad) (Brim-DeForest et al. 2017; Hill et al. 2006).

Weed competition may reduce yield by 90% in water-seeded rice systems (Brim-DeForest et al. 2017; Hill et al. 2006) if left uncontrolled. Alongside cultural management methods such as using certified weed-free seeds, land leveling to maintain uniform water depth, and water depth management, herbicides are critical components of rice weed management [University of California Agriculture and Natural Resources (UCANR) 2023]. Most California rice herbicides have relatively narrow activity spectrums and require proper selection, and application timing to achieve adequate weed control (Strand 2013; UCANR 2023). To maintain season-long and broad-spectrum rice weed control, tank mix combinations and sequential herbicide applications are commonly used in California rice fields, which results in a broad application window of herbicides (Espino et al. 2023; Hill et al. 2006).

In the diverse cropping systems of the Sacramento Valley, rice is often grown in close proximity to almond, pistachio, and walnut orchards. Water-seeded rice cropping systems in California highly favor aerial applications of herbicides (Espino et al. 2023), where off-target rice herbicide drift is a growing concern for tree nut crops (Galla et al. 2019). Most common California rice weed management programs rely on herbicide applications at the day-of-seeding or within up to two-leaf-rice growth stage and followed by at least one application of post-emergence herbicide later in the growing season (Hill et al. 2006), generally from May to mid-July (UCANR 2023). At this time, almond trees are actively growing from terminal and lateral

buds, spurs, and shoots are emerging, and photosynthates are translocating from leaves to kernels (Micke 1996). Likewise, pistachios are vigorously growing from shoots and buds, when apical and terminal buds are developing approximately from late May to early July (Ferguson and Haviland 2016). Similarly, walnuts are initiating and differentiating buds, developing leaves, hull, and the shell size between late May and early July as well as accumulation of assimilates and proteins in kernels are developing (Galla et al. 2018a, 2018b, 2019; Ramos 1998). In the Sacramento Valley, a substantial portion of rice herbicide applications coincide with vigorous and susceptible growth stages of almond, pistachio, and walnut leading to greater risk of crop injury from herbicide exposure.

During the herbicide application process, high wind speed, low relative humidity, high air temperature, and small droplet size may result in the physical movement of herbicides off target, also known as herbicide drift [UC Integrated Pest Management (UCIPM) 2016]. Under most herbicide application circumstances, non-target plant exposure to off-target herbicide drift is low with actual rates estimated from below 1/100X up to 1/33X of the field use rates (Al-Khatib and Peterson 1999; Al-Khatib et al. 2003). However, even at these low exposure levels, herbicide can injure or kill highly sensitive plants depending on the species, herbicide, actual rate, and growth stage (Egan et al. 2014). Therefore, concerns over the exposure of herbicides to off-target crops by either drift or accidental direct application are common among growers, crop consultants, and researchers (UCANR 2023).

Florpyrauxifen-benzyl [benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate; CAS: 1390661-72-9] is a new synthetic auxin (HRAC/WSSA Group IV) herbicide with a novel site of action for selective post-emergence grass, sedge, and broadleaf weed control in rice. The 6-aryl-picolinate auxin herbicides such as

florpyrauxifen-benzyl have a carboxylic acid functional group, which allow them to pass through the lipophilic walls of the phloem, be concentrated, and translocated (Epp et al. 2016). Moreover, the carboxylic acid functional group of florpyrauxifen-benzyl involves a key binding interaction at the site of action and mimics indole-3-acetic acid (IAA) to fill between the receptor and the co-repressor proteins in the cell nucleus (Grossmann 2010).

Triclopyr [2-(3,5,6-trichloropyridin-2-yl)oxyacetic acid; CAS: 55335-06-3] is another auxin herbicide widely used to control sedges and broadleaf weeds in rice fields. Triclopyr is a pyridyloxy-carboxylate auxin-type herbicide available as in triethylamine salt and butoxyethyl ester formulations. Similar to other synthetic auxins, triclopyr interacts with the auxin receptor complex and binds to Aux/IAA proteins. Inherently, synthetic auxins are much more stable than natural auxins in plants (Epp et al. 2016) and show a strong auxin effect in susceptible plants, stimulating plant cell elongation even at a concentration as low as 1 μ M. Synthetic auxins have been used as herbicides to manage dicotyledonous weeds for more than 80 years (Grossmann 2010; Peterson et al. 2016). In susceptible plants, synthetic auxins can deregulate normal patterns of growth through distorted cell division and expansion; moreover, can cause disruption and senescence, leaf epinasty, tissue swelling, stem curling, excessive chloroplast damage, membrane and vascular system damage, wilting, and necrosis may be observed, ultimately causing plant death and the collapse of plant tissue (Grossmann 2010).

Synthetic auxins can be active at very low exposure levels and cause visible injury symptoms on sensitive broadleaf plants. Particularly with the commercialization of auxin-tolerant broadleaf crops, auxin-mimic herbicides off-target injuries have been widely reported across the US in recent years on vegetables, fruit and nut trees, field and forage crops, ornamentals, and vines (Egan et al. 2014; Haring et al. 2022; Warmund et al. 2022). A new

auxin-type herbicide, florpyrauxifen-benzyl, was recently registered for use in rice. Given the importance of tree nuts in the Sacramento Valley where rice is widely grown, it is important to elucidate relative sensitivity of tree nuts to florpyrauxifen-benzyl simulated drift and compare the florpyrauxifen-benzyl injury symptoms to triclopyr injury symptoms which has been widely used in California rice for many years. Therefore, the overall objective of this research was to characterize the symptoms of florpyrauxifen-benzyl at different simulated drift rates on young almond, pistachio, and walnut trees and evaluate growth responses to florpyrauxifen-benzyl. We also evaluated almond, pistachio, and walnut responses, including herbicide symptomology and growth response to triclopyr simulated drift rates; hence, compare florpyrauxifen-benzyl to triclopyr.

Materials and Methods

Study Site

Four simulated off-target drift experiments were conducted in 2020 and 2021 in newly planted almond (lat. 38°32'18.8" N, long. 121°47'40.3" W), pistachio (lat. 38°32'19.5" N, long. 121°47'37.5" W), and walnut (lat. 38°32'19.6" N, long. 121°47'38.9" W) orchards (elev. 18 m) in UC Davis Plant Sciences Field Facility orchards near Davis, CA; and in an established walnut (lat. 38°30'27.4"N, long. 121°58'17.2"W, elev. 44 m) orchard at the UC Davis Wolfskill Experimental Orchard near Winters, CA, USA. The Davis orchards were established in March 2020 with 'Nonpareil' almond scion on 'Empyrean 1' rootstock, 'Kerman' pistachio scion on 'UCB 1 rootstock', and 'Chandler' walnut scion on 'clonal RX1' rootstock. Almonds and walnuts were planted with 6 m intra-row spacing and 4.2 m between rows, while pistachios were planted with 6 m intra-row spacing and 7 m between rows in Davis. The Winters orchard was

established in February 2018 with ‘Chandler’ walnut scion on ‘clonal Vlach’ rootstock, with trees planted 5.4 m apart within the row and with 5.1 m between rows.

The soil was classified as Yolo silt loam with NO₃-N: 56 ppm, Olsen-P: 25 ppm, K: 348 ppm, Na: 15 ppm, Ca: 8 meq/100 g, Mg: 10 meq/100 g, CEC: 19 meq/100 g, OM: 2.7%, and pH: 6.7 in the Davis orchards, and as Yolo silt loam with NO₃-N: 38 ppm, Olsen-P: 44 ppm, K: 447 ppm, Na: 29 ppm, Ca: 9 meq/100 g, Mg: 10 meq/100 g, CEC: 21 meq/100 g, OM: 2.7%, and pH: 7.3 in the Winters orchard. All trees were maintained free of diseases and insects as recommended by the UCIPM Guidelines (Ferguson and Haviland 2016; Strand 2002, 2003). In all experiments, weeds between rows were regularly mowed, and the intra-row strip was treated with a tank mix of rimsulfuron at 70 g·ha⁻¹ active ingredient (ai), indaziflam at 50 g·ha⁻¹ ai, oxyfluorfen at 560 g·ha⁻¹ ai, and glufosinate-ammonium at 450 g·ha⁻¹ ai plus manufacturer recommended surfactants.

Herbicide Applications

Florpyrauxifen-benzyl [benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate; Loyant[®] CA, 25 g·L⁻¹ ai, Corteva Agriscience, Indianapolis, IN, USA] and triclopyr [2-(3,5,6-trichloropyridin-2-yl)oxyacetic acid, triethylamine salt; Grandstand[™] CA, 359 g·L⁻¹ acid equivalent (ae), Corteva Agriscience, Indianapolis, IN, USA] were applied on 9 June 2020 in the Davis orchards and on 18 June 2020 in the Winters orchard during calm weather conditions to avoid off-target movement to nearby trees. In all experiments, florpyrauxifen-benzyl was applied at four rates resembling a plausible drift rate of 1/200X (0.5% drift), 1/100X (1% drift), 1/33X (3% drift), and 1/10X (10% drift) of the use rate of 29.4 g·ha⁻¹ ai. Due to the limited number of available trees, triclopyr was applied at only at three plausible drift rates of 1/200X, 1/100X, and 1/33X of the use rate of 420.3 g·ha⁻¹ ae (Al-Khatib et al.

1992a; Galla et al. 2019). Four untreated check (UTC) plots also were included for comparison. Florpyrauxifen-benzyl spray mixtures included methylated seed oil (Super Spread MSO, Wilbur-Ellis, Fresno, CA, USA) at 584 ml·ha⁻¹, and triclopyr spray mixtures included crop oil concentrate (MOR-ACT, Wilbur-Ellis, Fresno, CA, USA) at 1% v·v⁻¹ rate.

All herbicide treatments were applied to one side of the tree canopy as one pass from top to bottom with a handheld, carbon dioxide-propelled backpack sprayer calibrated to deliver 187 L·ha⁻¹ at 206 kPa pressure through AIXR8004 nozzle tip (TeeJet Technologies, Wheaton, IL, USA). The sprayer boom had two nozzle tips spaced 50 cm apart, and the spray treatments were calibrated based on a three-second pass from top to bottom per tree. Environmental conditions at the time of application were 16°C air temperature, 58% relative humidity (RH), and 0.4 m·s⁻¹ wind speed on 9 June 2020 in Davis; and 14°C air temperature, 65% RH, and 0.6 m·s⁻¹ wind speed on 18 June 2020 in Winters. No in-season auxin-type herbicides were used to avoid the potential confusion with florpyrauxifen-benzyl and triclopyr drift symptoms on crops. Irrigation was made through a single-line drip irrigation system with surface emitters spaced every 30 cm in Davis and with micro-sprinklers in Winters during the growing seasons.

Studies were repeated on 31 May 2021 with almond, pistachio, and walnut trees that were used as buffers during 2020 experiments (one-year exposure study, where $n = 8$). In addition, the trees that were treated with florpyrauxifen-benzyl and triclopyr in 2020 were also retreated in 2021 with the same treatments as in the previous year's protocols to distinguish trees two-year exposure response (two-year exposure study, where $n = 4$) from one-year exposure response (Bhatti et al. 1995). However, due to unavailability of trees, two-year exposure treatments were not repeated during the 2022 growing season. The methodology for the two-year exposure study was the same as previously described for the one-year exposure study. Environmental conditions

at the time of second-year application were 18°C air temperature, 50% RH, and 0.6 m·s⁻¹ wind speed in Davis and 20°C air temperature, 50% RH, and 0.6 m·s⁻¹ wind speed in Winters.

Data Collection and Experimental Design

Experiments were conducted using a randomized complete block design with four replicates, where an individual tree was an experimental unit. An untreated tree between treated plots was included as a buffer to reduce herbicide contamination in 2020.

Trees were observed for visible symptoms at 6, 12, 24, 48, and 72 hours after herbicide treatment as well as 7, 14, 21, 28, 35, 42, and 90 days after treatment (DAT). Symptomology descriptions of the treated foliage were made according to the UCIPM Herbicide Symptoms guideline (UCIPM Herbicide Symptoms 2024). Injury was rated on a scale where 0 = no injury and 100 = death (Al-Khatib et al. 1992a; Bhatti et al. 1995) according to the following scale:

- 0% = Normal size growth; green pigmentation of all leaves; and identical appearance to UTC trees.
- 1–4% = Normal-sized leaves; less than 5% of the leaves have only one discernible chlorotic spot, and the overall canopy has an indistinct injury appearance.
- 5–9% = Slight reduction in leaf size; 2 to 5 diffuse chlorotic spots clearly visible on 5 to 10% of the leaves; adjacent chlorotic spots coalesce and result in puckering, usually at the leaf margin or interveinal areas; up to 5% of leaf curling and crinkling at only young leaves.
- 10–29% = Reduction in leaf size up to 5%; growth restriction and chlorosis at interveinal tissue; symptoms moderate to severe on 10 to 30% of the leaves; less than 30% of the leaf surface chlorotic; from 5 to 10% of necrosis, leaf curling, and crinkling at mostly

young leaves; adjacent chlorotic areas merge and result in necrosis, usually at the interveinal areas; and shoot and stem curling up to 5% of the canopy.

- 30–49% = Reduction in leaf size from 5 to 10%; shoot tip growth restricted; symptoms severe on 30 to 50% of the leaves; up to 50% of the leaves with chlorosis; from 10 to 25% necrosis, leaf curling, and crinkling; adjacent necrotic areas coalesce and result in holes, usually at the interveinal areas; and moderate to severe curling at shoots and stems from 5 to 10% of the canopy.
- 50–69% = Reduction in leaf size from 10 to 25%; growth significantly restricted; symptoms very severe on 50 to 70% of the leaves; up to 70% of leaf surface chlorotic; from 25 to 50% necrosis, leaf curling, and crinkling; up to 10% stunting and irregular growth at the overall canopy; interveinal tissue-restricted and containing little green pigment; noticeable stem discoloring with dark red-brown spots up to 15% of the young branches; and terminal bud twisting and death.
- 70–89% = Reduction in leaf size from 25 to 50%; growth severely restricted; symptoms very severe on 70 to 90% of the leaves; up to 90% of leaf surface chlorotic; from 50 to 75% necrosis, leaf curling, and crinkling; distinguishable leaf loss; from 10 to 50% stunting at the overall canopy; interveinal tissue-restricted and containing little green pigment; severe leaf distortion and malformation; very obvious stem discoloring with dark red-brown-black spots up to 50% of the young and old branches; and terminal bud twisting and death.
- 90–99% = Almost no development of leaf and interveinal tissues; symptoms extremely severe on all the leaves; leaves 100% chlorotic; severe leaf curling, narrowing, distortion,

malformation, and crinkling; necrosis at leaf margins and shoot tips dead; more than 50% of stunting overall; and extremely damaged appearance.

- 100% = Plant dead.

Florpyrauxifen-benzyl and triclopyr-treated sides of canopies from almond, pistachio, and walnut trees were compared with UTC trees at each observation time. Photos of trees were taken from the treated side of the canopy throughout the growing season to ensure consistency in evaluations. Furthermore, four randomly selected branches from the herbicide-treated side of each tree were measured to determine shoot growth. The number of nodes on the shoots were counted from the terminal buds through trunks for approximately 30 cm prior to the simulated drift treatments and at 90 DAT. The number of nodes in treated trees were compared with the UTC trees. In addition, trunk diameters were measured at approximately 25 cm above ground before the spring growth started in April (spring-data) and at the end of the summer (fall-data) approximately 140 DAT (Abit and Hanson 2013; Al-Khatib et al. 1992a). Tree growth was expressed through trunk diameter growth as a percent increase based on the following formula (Equation 1):

$$Y = \left[\left(\frac{X_f}{X_s} \right) - 1 \right] \times 100$$

where Y is the percent increase of trunk diameter, X_f = trunk diameter at the fall measurement, X_s = trunk diameter at the spring measurement. The relative change in herbicide treated tree trunk diameter was compared to UTC tree trunk diameter change.

Statistical Analysis

Visual injury, number of nodes, and trunk diameter data for all trees were subjected to analysis of variance using ‘agricolae’, ‘emmeans’, and ‘lme4’ packages in RStudio Version

2023.06.0+421 (R Core Team 2023), and Tukey's honestly significant difference (HSD) were used for means separation at $\alpha = 0.05$, when applicable. The injury data for the walnut trees from the Davis and Winters orchards were combined because there was no significant interaction between site and treatment.

Results and Discussion

Because of no significant interactions occurred between year and treatment for one-year exposure study, the visible injury ratings data for the 2020 and 2021 were combined ($n = 8$). In general, floryrauxifen-benzyl and triclopyr injury symptoms were apparent on all treated trees, and symptoms increased as herbicide rates increased. Initial floryrauxifen-benzyl and triclopyr symptoms were similar on almond, pistachio, and walnut. However, the symptoms were more severe on pistachio compared to almond and walnut. Additionally, the time required to develop symptoms was shorter with pistachio than with almond and walnut at all rates.

Visible injury symptoms in both one-year exposure ($n = 8$) and two-year exposure ($n = 4$) studies were observed at seven DAT for almond, and generally peaked at 14 DAT (Figure 1). Injury symptoms on almond were more noticeable on the treated side of the tree. However, slight injury symptoms were apparent on developing leaves on the nontreated side of the canopy. In addition, developing leaves and shoots showed more injury symptoms at any rate compared to developed leaves and shoots. Herbicide injury symptoms on almond from both herbicides were chlorosis, chlorotic spot, epinasty, leaf narrowing, leaf crinkling, necrosis, necrotic spots, shoot curling, terminal bud death, and twisting (Figure 1). Initial chlorosis symptoms turned to necrosis within seven to 14 days and eventually to necrotic spots. At 1/10X floryrauxifen-benzyl rate some necrotic spots turned to holes on the leaf surfaces. Leaf curling, necrosis, and necrotic spots were characteristic on almonds for both herbicides at higher rates. The 1/10X floryrauxifen-

benzyl drift caused the most severe symptoms on almond. However, even at this high simulated drift rate, these trees recovered from the initial injury symptoms and appeared normal at 90 DAT.

Florpyrauxifen-benzyl visible injury on almonds in the one-year exposure study ($n = 8$) was less than 7% at all rates at 14 DAT (Table 1). Similarly, triclopyr visible injury on almond was less than 2% at all rates at 14 DAT. The injury symptoms on almonds were visible through 42 DAT and florpyrauxifen-benzyl visible injury was less than 8% at all rates. Similarly, triclopyr visible injury was less than 1% at all rates 42 DAT. These results suggest that almond injury recovery from both herbicides was rapid with the exception of 1/10X florpyrauxifen-benzyl rate, which still had 8% injury 42 DAT. Florpyrauxifen-benzyl and triclopyr injury symptoms on almonds from the two-year exposure study ($n = 4$) were similar to one-year exposure study ($n = 8$) symptoms (Table 1). Two-year treated almonds showed slightly more necrotic spots and leaf curling. The greatest herbicide injury on almonds was 16% at 1/10X florpyrauxifen-benzyl rate 14 DAT, which gradually decreased throughout the growing season. Except florpyrauxifen-benzyl at 1/10X rate, none of the florpyrauxifen-benzyl and triclopyr visible injury rating was statistically significant and less than 2% at 42 DAT.

Florpyrauxifen-benzyl and triclopyr injury symptoms on pistachios in both one- and two-year exposure studies were observed at three DAT, and generally peaked at from 14 through 28 DAT (Table 2). The injury was distinguishable at the entire pistachio canopy, but most notable on developing leaves and terminal buds. Herbicide injury symptoms on pistachio caused by both herbicides were similar to the injury symptoms on almond and initially appeared as chlorosis and leaf curling (Figure 2). Florpyrauxifen-benzyl and triclopyr at 1/33X and higher rates resulted in some maroon-brown lesions on stems and stunting on pistachio. By 14 DAT, leaf epinasty

became more apparent and leaf epinasty symptoms became more severe through 42 DAT. Stems developed dark color lesion spots on new branches and these lesions persisted throughout the growing season in trees treated with the 1/10X florpyrauxifen-benzyl rate. The highest herbicide injury on pistachio was observed with florpyrauxifen-benzyl at 1/10X rate treatment as 56% injury at 28 DAT in the one-year exposure study, whereas two-year exposure study resulted 79% injury with 1/10X florpyrauxifen-benzyl treatment at 14 DAT. Shoot curling, stem coloring, stunting, and twisting symptoms on pistachio were more apparent at higher rates of both florpyrauxifen-benzyl and triclopyr. Herbicide injury symptoms on pistachio slightly and gradually decreased, although injury symptoms from the 1/10X florpyrauxifen-benzyl rate treatment remained throughout the same growing season and were still present at leaf out the following season (data not shown). In the following spring observations, stunting was the only symptom that remained from the 1/10X florpyrauxifen-benzyl drift treatments. In all other rates, both florpyrauxifen-benzyl- and triclopyr-treated pistachio trees appeared normal at leaf out in the following spring.

Florpyrauxifen-benzyl and triclopyr injury symptoms on walnut were initially observed at seven DAT and generally peaked at 14 DAT (Table 3). Florpyrauxifen-benzyl at the 1/10X rate 45% and 49% injury on treated walnut trees at 14 DAT in the one- and two-year exposure studies, respectively. Although, both florpyrauxifen-benzyl and triclopyr injury symptoms on walnuts were more distinguishable on the treated side of the tree, symptoms on developing leaves were apparent at the entire canopy (Figure 3). Symptoms of florpyrauxifen-benzyl and triclopyr injury on walnuts were similar to the symptoms on almond. However, compared to almond and pistachio, herbicide injury symptoms on walnut were only apparent on the developing leaves and no symptoms were observed on leaves that had already developed before

the herbicide treatments. This is different than previous research which indicated that actively growing and developing walnut leaves and shoots are susceptible to acetolactate synthase (ALS) inhibitor herbicides bispyribac-sodium and bensulfuron-methyl exposure even at 1/200X field use rates (Galla et al. 2018a). ALS inhibitors can be lethal at a very low concentration (Zhou et al. 2007) and plant responses are likely to differ among modes-of-action such as auxin-mimics. Walnut recovery from injury symptoms was similar to almond recovery. At 42 DAT, herbicide injury symptoms on walnut were less than 10% at all rates with both florpyrauxifen-benzyl and triclopyr in the both one- and two-year exposure studies.

In previous research, Serim and Patterson (2024) reported florpyrauxifen-benzyl and quinclorac at 1/32X rates caused 88% and 40% visible injury, respectively, on sunflower 28 DAT. Miller and Norsworthy (2018) reported that a 1/10X florpyrauxifen-benzyl rate caused 96% injury symptoms on soybeans 14 DAT and the injury increased to 99% at 28 DAT. Moreover, Schwartz-Lazaro et al. (2017) reported that florpyrauxifen-benzyl injury symptoms were more severe on soybean compared to ALS inhibitors injury symptoms such as bispyribac-sodium, penoxsulam, halosulfuron, orthosulfamuron, and imazosulfuron at drift rates. The greater florpyrauxifen-benzyl injury in annual crops is likely due to their higher growth rate (Taiz et al. 2022).

Tree trunk diameter change for almond, pistachio, and walnut was variable and showed no significant interactions between herbicide treatment, exposure, and year. Results showed that the herbicide drift rates did not significantly effect relative trunk growth rate of any of the crop trees, in spite of foliar injury symptoms (data not shown). These results suggest that almond, pistachio, and walnut can recover from florpyrauxifen-benzyl and triclopyr herbicide drift rates.

Moreover, the average number of nodes per shoot at 90 DAT was similar in all treatments and was not affected even by the highest simulated drift rate (data not shown).

Conclusions

The research showed that the newly-registered rice herbicide, florpyrauxifen-benzyl, can cause visible injury on almond, pistachio, and walnut trees at simulated drift rates. As an auxin-mimic, florpyrauxifen-benzyl injury symptoms are very similar to symptoms from another registered auxin-mimic herbicide, triclopyr, on all crops. The 1/10X florpyrauxifen-benzyl treatment resulted in greater injury on all crops and delayed growth on pistachio, which suggests that pistachio was the most susceptible tree nut crop to florpyrauxifen-benzyl. Moreover, pistachio injury from the 1/10X florpyrauxifen-benzyl treatment was greater after two-years of exposure, which may suggest a cumulative injury from repeated exposure.

The visible herbicide injury ratings from the simulated florpyrauxifen-benzyl and triclopyr drift rates differences between almond, pistachio, and walnut are not surprising because the absorption, translocation, and metabolism of herbicides are expected to vary among plant species (Al-Khatib et al. 1992b). Although, the anticipated herbicide drift rates under normal field conditions generally are from 1/100X to 1/33X of the field use rates (Al-Khatib and Peterson 1999), the 1/10X florpyrauxifen-benzyl rate in this study was added to simulate a worst-case scenario, considering consecutive drift events in a short interval of time or an accidental herbicide application, events that are unlikely to commonly occur in a typical drift situation. This research suggests that almond and walnut have rapid recovery from florpyrauxifen-benzyl and triclopyr at the expected drift rates.

Due to its selective grass activity and good control of broadleaves and sedges, florpyrauxifen-benzyl is expected to be widely used by growers. California growers are familiar

with management programs for triclopyr, which has been registered for use in rice for many years. Florpyrauxifen-benzyl and triclopyr herbicides might cause significant damage if they drift onto pistachio trees at sufficient amounts. The risk of off-target movement of rice herbicides is greatest for aerial applications (UCANR 2023). Although triclopyr drift mitigation strategies allow aerial applications, the current florpyrauxifen-benzyl label limits the herbicide to ground applications which further reduces the risk of significant injury to nearby tree nut crops and imparts an extra level of precaution. Off-target drift management programs in accordance to label for florpyrauxifen-benzyl applications are likely to be effective to reduce the risk of significant crop injury from florpyrauxifen-benzyl drift on almonds and walnuts, but extra precautions may be needed if there are nearby pistachio orchards.

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Tables

Table 1. Almond injury following simulated drift rates of florpyrauxifen-benzyl and triclopyr in 2020 and 2021.

Herbicide	Rate ²	One-year exposure ¹			Two-year exposure		
		14 DAT ³	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT
		Visible injury (%)					
FPB	1/200x	1 a ⁴	2 b	1 a	1 b	3 b	2 b
FPB	1/100x	2 a	2 b	1 a	4 b	3 b	2 b
FPB	1/33x	2 a	4 b	2 a	4 b	7 b	2 b
FPB	1/10x	7 a	12 a	8 a	16 a	15 a	11 a
TRC	1/200x	1 a	3 b	1 a	1 b	3 b	1 b
TRC	1/100x	2 a	3 b	1 a	1 b	4 b	1 b
TRC	1/33x	2 a	3 b	1 a	4 b	3 b	2 b

¹One-year exposure: Trees were treated in 2020 and the study was repeated in 2021, where sample size $n = 8$. Two-year exposure: The same trees which were treated in 2020 were retreated in 2021, where sample size $n = 4$.

²Florpyrauxifen-benzyl rate is expressed as a percent of rice use rate, 29.4 g·ha⁻¹ ai. Triclopyr rate is expressed as a percent of the rice use rate of 420.3 g·ha⁻¹ ae.

³DAT = days after treatment; FPB = florpyrauxifen-benzyl; TRC = triclopyr.

⁴Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test.

Table 2. Pistachio injury following simulated drift rates of florpyrauxifen-benzyl and triclopyr in 2020 and 2021.

Herbicide	Rate ²	One-year exposure ¹			Two-year exposure			
		14 DAT ³	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT	
		Visible injury (%)						
FPB	1/200x	8 bc ⁴	8 b	10 b	19 b	20 bc	21 b	
FPB	1/100x	13 bc	25 ab	23 ab	22 b	22 bc	29 b	
FPB	1/33x	38 ab	38 ab	36 ab	55 a	36 b	34 b	
FPB	1/10x	52 a	56 a	48 a	79 a	71 a	69 a	
TRC	1/200x	3 c	5 b	10 b	3 b	6 c	8 b	
TRC	1/100x	5 bc	8 b	10 b	9 b	10 c	12 b	
TRC	1/33x	10 bc	10 b	20 ab	14 b	12 c	16 b	

¹One-year exposure: Trees were treated in 2020 and the study was repeated in 2021, where sample size $n = 8$. Two-year exposure: The same trees which were treated in 2020 were retreated in 2021, where sample size $n = 4$.

²Florpyrauxifen-benzyl rate is expressed as a percent of rice use rate, 29.4 g·ha⁻¹ ai. Triclopyr rate is expressed as a percent of rice use rate of 420.3 g·ha⁻¹ ae.

³DAT = days after treatment; FPB = florpyrauxifen-benzyl; TRC = triclopyr.

⁴Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test.

Table 3. Walnut injury following simulated drift rates of florpyrauxifen-benzyl and triclopyr in 2020 and 2021.

Herbicide	Rate ²	One-year exposure ¹			Two-year exposure		
		14 DAT ³	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT
		Visible injury (%)					
FPB	1/200x	10 ab ⁴	22 a	5 a	7 b	22 ab	5 a
FPB	1/100x	12 ab	27 a	6 a	15 b	27 ab	5 a
FPB	1/33x	18 ab	30 a	7 a	21 b	29 ab	6 a
FPB	1/10x	45 a	40 a	8 a	49 a	46 a	10 a
TRC	1/200x	6 b	11 a	5 a	7 b	14 b	5 a
TRC	1/100x	8 ab	13 a	5 a	10 b	14 b	6 a
TRC	1/33x	17 ab	12 a	8 a	14 b	24 ab	6 a

¹One-year exposure: Trees were treated in 2020 and the study was repeated in 2021, where sample size $n = 8$. Two-year exposure: The same trees which were treated in 2020 were retreated in 2021, where sample size $n = 4$.

²Florpyrauxifen-benzyl rate is expressed as a percent of rice use rate, 29.4 g·ha⁻¹ ai. Triclopyr rate is expressed as a percent of rice use rate of 420.3 g·ha⁻¹ ae.

³DAT = days after treatment; FPB = florpyrauxifen-benzyl; TRC = triclopyr.

⁴Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test.

Figures

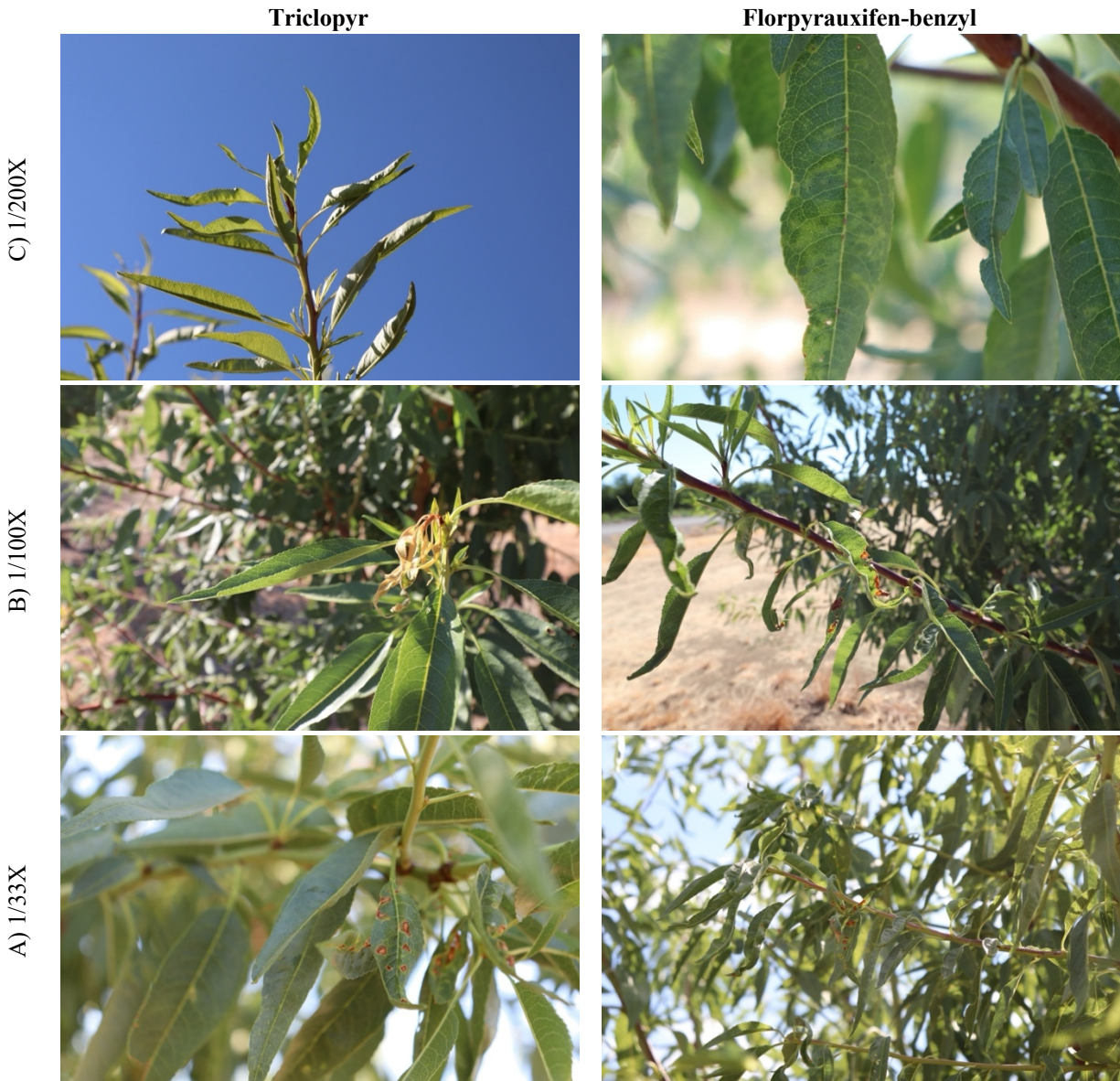


Figure 1. Chlorosis, epinasty, leaf crinkling, leaf narrowing, and necrosis symptoms of florpyrauxifen-benzyl and triclopyr applied on almond at 1/200X, 1/100X, and 1/33X simulated drift rates of the field use rate at 28 days after treatments. Florpyrauxifen-benzyl and triclopyr use rates are $29.4 \text{ g}\cdot\text{ha}^{-1} \text{ ai}$ and $420.3 \text{ g}\cdot\text{ha}^{-1} \text{ ae}$, respectively. Photos were taken on 28 June 2021, in the two-year exposure study.

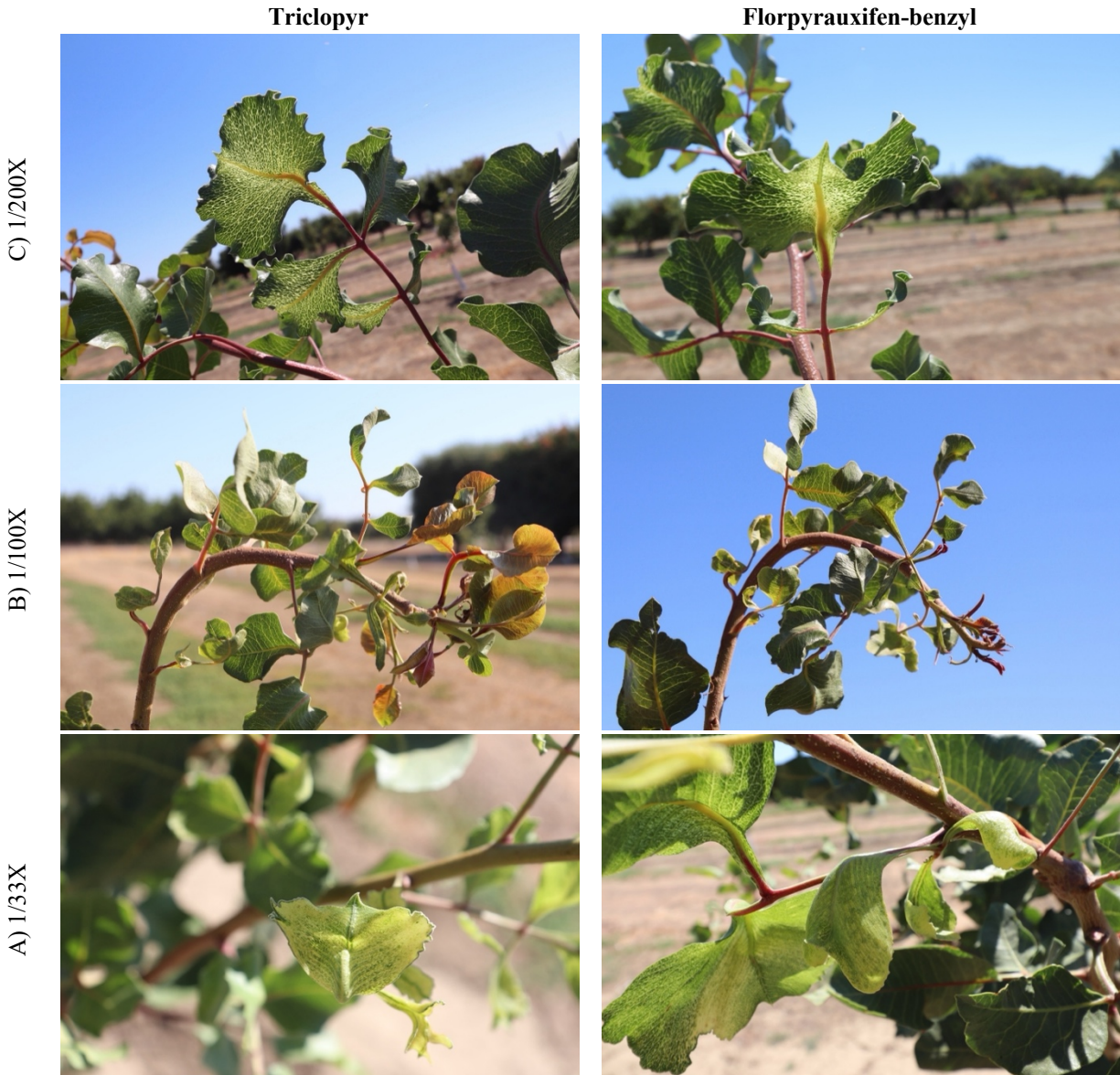


Figure 2. Chlorosis, epinasty, leaf crinkling, leaf narrowing, shoot curling, and twisting symptoms of florpyrauxifen-benzyl and triclopyr applied on pistachio at 1/200X, 1/100X, and 1/33X simulated drift rates of the field use rate at 28 days after treatments. Florpyrauxifen-benzyl and triclopyr use rates are $29.4 \text{ g}\cdot\text{ha}^{-1} \text{ ai}$ and $420.3 \text{ g}\cdot\text{ha}^{-1} \text{ ae}$, respectively. Photos were taken on 28 June 2021, in the two-year exposure study.

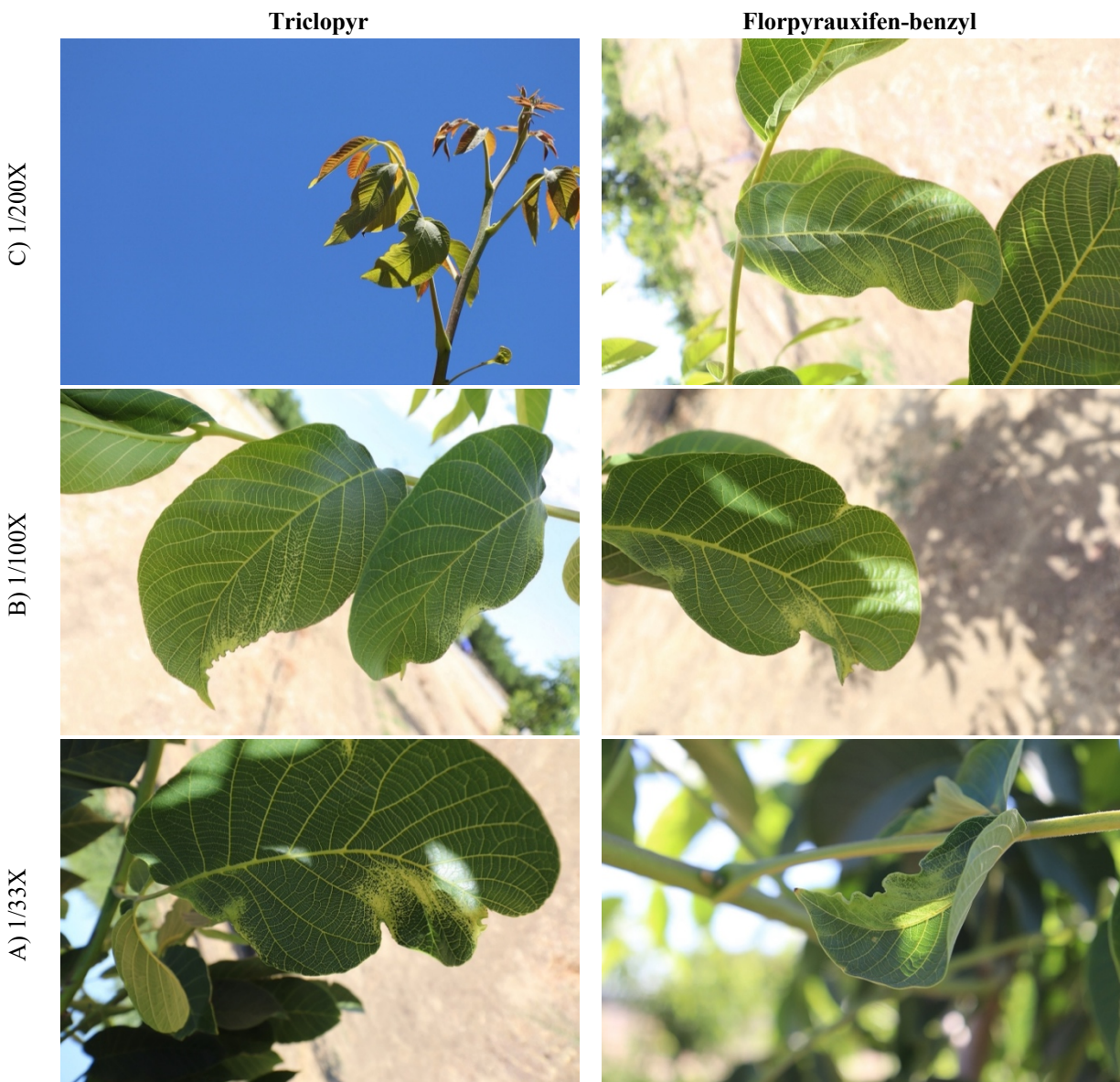


Figure 3. Chlorosis, epinasty, leaf crinkling, leaf narrowing, and twisting symptoms of florpyrauxifen-benzyl and triclopyr applied on walnut at 1/200X, 1/100X, and 1/33X simulated drift rates of the field use rate at 28 days after treatments. Florpyrauxifen-benzyl and triclopyr use rates are $29.4 \text{ g}\cdot\text{ha}^{-1} \text{ ai}$ and $420.3 \text{ g}\cdot\text{ha}^{-1} \text{ ae}$, respectively. Photos were taken on 28 June 2021, in the two-year exposure study.

Chapter 3

Detection of florpyrauxifen-benzyl residues in tree nut crop leaves after simulated drift treatment

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Submitted to Weed Technology

Abstract

California produces many of the nation's high-value orchard crops, including almond, pistachio, and walnut, and is also the second largest rice producer in the United States. Rice herbicide drift poses a significant challenge where rice fields are near almond, pistachio, and walnut orchards. This research was conducted as part of a stewardship program for a newly-registered rice herbicide and specifically aimed to compare the onset of foliar symptoms resulting from simulated florpyrauxifen-benzyl drift with residues in almond, pistachio, and walnut leaves at several timepoints after exposure. Treatments were applied to one side of the canopy of one- and two-year-old trees at 1/100X and 1/33X of the florpyrauxifen-benzyl rice field use rate of 29.4 g ai ha⁻¹ in 2020 and 2021. Symptoms were observed three days after treatments (DAT) for pistachio and seven DAT for almond and walnut, with peak severity around 14 DAT. While almond and walnut symptoms gradually dissipated throughout the growing season, pistachio still had symptoms at leaf out in the following spring. Leaf samples were randomly collected from each tree for residue analysis at 7, 14, and 28 DAT. Seven DAT with the 1/33X rate, almond, pistachio, and walnut leaves had florpyrauxifen-benzyl at 6.06, 5.95, and 13.12 ng g⁻¹ (fresh weight; FW) leaf, respectively. By 28 DAT, all samples from all crops treated with the 1/33X drift rate had florpyrauxifen-benzyl at less than 0.25 ng g⁻¹ FW leaf. At the 1/100X rate, pistachio, almond, and walnut residues were 1.78, 2.31, and 3.58 ng g⁻¹ FW leaf at 7 DAT, respectively. At 28 DAT with the 1/100X rate, pistachio, and almond samples had florpyrauxifen-benzyl at 0.1 and 0.04 ng g⁻¹ FW leaf, respectively, but walnut leaves did not have detectable residues. Together, these data suggest that residue analysis from leaf samples collected after severe symptoms are observed may substantially underestimate actual exposure due to the relatively rapid dissipation of florpyrauxifen-benzyl in nut tree foliage.

Nomenclature: Almond, *Prunus dulcis* (Mill.) D.A. Webb; florpyrauxifen-benzyl, benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate; pistachio, *Pistacia vera* L.; walnut, *Juglans regia* L.

Keywords: Auxinic herbicide; herbicide residue; off-target movement; quantification; residue analysis; symptomology

Introduction

California produces the majority of the almonds [*Prunus dulcis* (Mill.) D.A. Webb], pistachios (*Pistacia vera* L.), and walnuts (*Juglans regia* L.) in the United States (US) and over 80% of the global almond production [California Department of Food and Agriculture (CDFA) 2024; US Department of Agriculture, National Agricultural Statistics Service (USDA-NASS) 2024].

Almond, pistachio, and walnut are planted on 1M hectares (ha) in California with a gross value of more than \$5B (CDFA 2024). Moreover, the Sacramento Valley of Northern California is the second largest rice (*Oryza sativa* L.) production region in the US, with more than 0.2M ha of premium quality water-seeded rice (USDA-NASS 2024). California rice systems have unique advantages such as the Mediterranean climate, high solar radiation, and highly mechanized, developed, and precise production practices, which result in ~20% higher yields than the US average (Hill et al. 2006). In the complex cropping systems of California's Sacramento Valley, rice is often planted adjacent to almond, pistachio, and walnut orchards.

Most California rice is pregerminated, aerially seeded into 10 to 15 cm standing water, and maintained under continuous flooding until ~1 month before harvest (Brim-DeForest et al. 2017a, 2017b; Hill et al. 2006). This water-seeded system was initially developed to suppress weeds that pose a significant challenge for California rice growers (Hill et al. 2006). In general, many of the most problematic rice weeds are well-adapted to continuously-flooded growing systems (Brim-DeForest et al. 2017b; Galvin et al. 2022) and capable of reducing rice yields by up to 90% unless successfully controlled (Brim-DeForest et al. 2017a). Nearly all California rice production heavily depends on complex herbicide programs to control weeds and because of the continuous flood conditions, these are mostly applied by aircraft (Espino et al. 2023).

California rice growers use herbicides at planting and typically also apply at least one additional post-emergence herbicide later in the season, between May and mid-July (Galla et al. 2018a). During this time of year, almond trees are actively growing from terminal and lateral buds, spurs and shoots emerge, nut and kernel growth is occurring, and translocation of photosynthates from the leaves to kernels begins (Kester et al. 1996). In addition, pistachio trees begin shoot growth at this time of year, and buds form and extend from late May to early July (Ferguson and Kallsen 2016). Simultaneously, walnut trees in the Sacramento Valley are actively growing and the nuts generally reach their final hull and shell size and the accumulation of assimilates such as alcohol-soluble sugars and proteins in the kernels begin (Galla et al. 2018b, 2019; Pinney et al. 1998). Consequently, most rice herbicide applications coincide with important and sensitive growth stage of almonds, pistachios, and walnuts, when these crops are highly vulnerable to off-target foliar herbicide exposure.

Concerns over off-target crop exposure to herbicides by either drift or accidental direct application have been stated among growers, crop consultants, and researchers [Al-Khatib et al. 2003; Bhatti et al. 1995; Egan et al. 2014; University of California Integrated Pest Management (UCIPM) Herbicide Symptoms 2024]. Factors that affect off-target herbicide drift include wind speed and direction, relative humidity, air temperature, droplet size, applicator distance from the edges of the treatment area, and release height of the herbicides (UCIPM 2016). Under most circumstances, off-target herbicide drift occurs below 1/100X to 1/33X of the field use rates (Al-Khatib and Peterson 1999; UCIPM 2016). Even at these low levels of drift, some rice herbicides such as PSII inhibitors (Galla et al. 2018a), acetolactate synthase inhibitors (Galla et al. 2018a, 2018b, 2019), and growth regulators (Haring et al. 2022) used in rice can be of concern due to their widespread use and potential for injury to highly sensitive tree and vine crops.

In plants, auxins are generally responsible for cell division, elongation, and growth as well as the development of vascular tissue, floral meristem, leaf initiation, apical dominance, shoot and root formation (Grossmann 2010). The small quantities of auxins, such as 5 to 1,000 pg mg^{-1} plant tissue, impact the growth and development processes in higher plants such as almond, pistachio, and walnut (Ferguson and Kallsen 2016; Kester et al. 1996; Pinney et al. 1998). However, auxins are toxic at high cellular concentrations with limited or lack of homeostatic control, such as degradation, conjugation, transport, and sequestration (Taiz et al. 2022). Synthetic auxins are more stable in plants than natural auxins, such as indole-3-acetic acid (IAA), indole-3-butyric acid, 4-chloroindole-3-acetic acid, and phenylacetic acid (Bishop et al. 2015; Epp et al. 2016). Due to their stable structures (Epp et al. 2016) and being much less subject to homeostatic control than natural auxins (Taiz et al. 2022), synthetic auxins cause herbicidal damage such as tissue swelling, growth inhibition, and epinasty, that can be highly injurious or lethal to susceptible plants. Developing plants are expected to be more sensitive to synthetic herbicides as compared to developed plants (Taiz et al. 2022).

Florpyrauxifen-benzyl (benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate; CAS: 1390661-72-9) is a synthetic auxin-type herbicide with a novel site of action for weed control in rice (Epp et al. 2016). Picolinate auxin-type herbicides such as florpyrauxifen-benzyl have a carboxylic acid functional group, which involves a key binding interaction at the site of action (Epp et al. 2016) and mimics IAA to fill between the receptor and the co-repressor proteins at the cell nucleus. When exogenously applied to susceptible plants, growth disruption, leaf epinasty, tissue swelling, stem curling, excessive chloroplast damage, membrane and vascular system damage, wilting, and necrosis are commonly observed, ultimately leading to plant death (Grossmann 2010).

Since the modern era of herbicides began after the commercialization of 2,4-D in the 1940s and dicamba in the 1960s, auxin-type herbicides became important tools, which have been widely used on 2,4-D- and dicamba-resistant crops such as corn, soybean, and cotton (Egan et al. 2014). Due to extensive use, auxin herbicides are historically well known for their off-target injuries onto soybean, cotton, sunflower, vegetables, fruit and nut trees, field and forage crops, ornamentals, and vines (Al-Khatib et al. 1993; Bhatti et al. 1997; Dittmar et al. 2016; Haring et al. 2022; Marple et al. 2007; Miller and Norsworthy 2017; Nunes et al. 2023; Ramos et al. 2021; Sciumbato et al. 2004; Serim and Patterson 2024; Sharkey et al. 2021; Smith et al. 2017; Warmund et al. 2022; Wells et al. 2019). The overall objectives of this research were to study the correlation between symptoms and residue of florpyrauxifen-benzyl in the leaf tissue of nut tree crops after plausible drift rates and to determine if florpyrauxifen-benzyl residue can be used as an indicator of the level of florpyrauxifen-benzyl exposure.

Materials and Methods

Study Site

Three simulated off-target drift experiments were conducted in 2020 and 2021 in newly planted almond (lat. 38°32'18.8"N, long. 121°47'40.3"W), pistachio (lat. 38°32'19.5"N, long. 121°47'37.5"W), and walnut (lat. 38°32'19.6"N, long. 121°47'38.9"W) orchards (elev. 18 m) at the UC Davis Plant Sciences Research Facility near Davis, CA, USA. The orchards were established in March 2020 with 'Nonpareil' almond scion on 'Empyrean 1' rootstock, 'Kerman' pistachio scion on 'UCB 1' rootstock, and 'Chandler' walnut scion on 'clonal RX1' rootstock. Almonds and walnuts were planted between 6 m apart within rows and with 4.2 m between rows, while pistachios were 6 m apart within rows and 7 m between rows. The soil was classified as Yolo silt loam with NO₃-N: 56 ppm, Olsen-P: 25 ppm, K: 348 ppm, Na: 15 ppm, Ca: 8 meq/100

g, Mg: 10 meq/100 g, CEC: 19 meq/100 g, OM: 2.7%, and pH: 6.7 at all experiments. Trees were maintained free of diseases and insects as recommended by the UCIPM Guidelines (Ferguson and Haviland 2016; Micke 1996; Ramos 1998; Strand 2002, 2003). Irrigation was made through a single-line drip irrigation system with emitters spaced every 30 cm. In all experiments, weeds between rows were managed with regular mowing and within rows with a tank mix of rimsulfuron at 70 g ai ha⁻¹, indaziflam at 50 g ai ha⁻¹, oxyfluorfen at 560 g ai ha⁻¹, and glufosinate-ammonium at 450 g ai ha⁻¹. In addition, the spray solution for maintenance sprays included methylated seed oil (MSO) at 0.25% v v⁻¹ and polyvinyl polymer drift control agent at 0.5% v v⁻¹.

Herbicide Applications

Florpyrauxifen-benzyl (Loyant[®] CA, 25 g ai L⁻¹, Corteva Agriscience, Indianapolis, IN, USA) was applied on 9 June 2020 during calm weather conditions to avoid off-target movement, in all three experiments at plausible drift rates of 1/100X (1% drift) and 1/33X (3% drift) of the rice use rate of 29.4 g ai ha⁻¹ (Espino et al. 2023; Galla et al. 2019). Four untreated check (UTC) plots were also included for comparison. All spray mixtures included MSO (Super Spread[®] MSO, Wilbur-Ellis, Fresno, CA, USA) at 584 ml ha⁻¹. Environmental conditions at the time of application were 16°C air temperature, 58% relative humidity, and 0.4 m s⁻¹ wind speed. No in-season auxin-type herbicides were used to avoid the potential confusion with florpyrauxifen-benzyl drift experiments.

Experimental Design and Data Collection

Experiments were set up in a randomized complete block design with four replicates, where an individual tree was an experimental unit. An untreated tree between treated trees was included as a buffer to prevent any herbicide contamination. All florpyrauxifen-benzyl treatments were

applied to one side of the tree canopy with a handheld, CO₂-pressured backpack sprayer calibrated to spray 187 L ha⁻¹ at 206 kPa pressure through AIXR8004 nozzles (TeeJet Technologies, Wheaton, IL, USA). The spray boom had two nozzles spaced 50 cm apart, and treatments were applied in a single three-second pass from top to bottom per tree. All experiments were repeated on 31 May 2021 using the trees that were buffer trees during 2020 growing season in same orchards using the previously described methods. Environmental conditions at the time of the second-year applications were 18°C air temperature, 50% relative humidity, and 0.6 m s⁻¹ wind speed.

Trees were observed for visual symptoms at 6, 12, 24, 48, and 72 hours after herbicide treatments as well as 7, 14, 21, 28, 35, 42, and 90 DAT. Symptomology descriptions of the treated foliage were made according to UCIPM Herbicide Symptoms guideline (UCIPM Herbicide Symptoms 2024). The floryprauxifen-benzyl treated side of almond, pistachio, and walnut trees were compared with UTC trees at each observation. Evaluations were made early in the morning and photos of trees were taken from the treated side of the canopy throughout the growing season for consistency.

Analytical Methods

Randomly selected leaves from 1/100X and 1/33X floryprauxifen-benzyl treated almond, pistachio, and walnut leaves from the treated side of the canopy and from the UTC were harvested at 7, 14, and 28 DAT. The approximately 50 g samples of harvested leaves were immediately rinsed in 50% methanol solution (SIGALD 439193, Sigma-Aldrich, St. Louis, MO, USA, CAS: 67-56-1) in the field to remove soil dust and unabsorbed floryprauxifen-benzyl residues on the leaf surface (Al-Khatib et al. 1992). The leaf samples were double-bagged and brought to the laboratory on dry ice, then frozen in liquid nitrogen, and stored in an ultra-low

temperature freezer (MDF-DU901VHA, PHCbi Corporation of North America, Wood Dale, IL, USA) until the leaf samples were processed.

To recover and quantify florpyrauxifen-benzyl from 7 and 14 DAT samples, leaf tissues were ground in liquid nitrogen to ~5 mm size pieces, and 500 mg of ground tissue was placed in 7-mL tubes on dry ice. Tissues were spiked with an internal standard of similar hydrophobicity (SPEXQuE™ AOAC Internal Standard Mix, Fisher Scientific, Waltham, MA, USA) to check stability of instrument response to ensure the recover accuracy, and 10-15 metal homogenizing beads were added to the tubes. Florpyrauxifen-benzyl was extracted by adding 2.5 mL 90% w v⁻¹ acetonitrile (CAS: 75-05-8, Sigma-Aldrich, St. Louis, MO, USA), and homogenized at 4400 g_n for 16 x 30 seconds cycles. The extracts were centrifuged at 1100 g_n for 5 minutes and 500 uL supernatant transferred to 2-mL tubes containing 300 mg QuE Verde dSPE (Supel QuE Verde Tube #55442-U, Sigma-Aldrich, St. Louis, MO, USA). Extracts with dSPE were shaken on rotary shaker at 0.5 g_n for 15 minutes and centrifuged again at 17700 g_n for 5 minutes. The supernatant was directly analyzed using an ultra-high-performance liquid chromatography coupled to an orbitrap fusion tribrid mass spectrometry (UltiMate 3000 UHPLC, Thermo Fisher Scientific, Waltham, MA, USA). The LC-MS/MS method was optimized to determine the quantity of florpyrauxifen-benzyl in the samples using accurate mass and fragmentation pattern matching to a reference analytical standard [US Environmental Protection Agency (USEPA) 2020]. The concentration of florpyrauxifen-benzyl in each sample was quantified based on an external calibration curve of the analytical standard (#684721, HPC Standards Inc, Atlanta, GA, USA) for florpyrauxifen-benzyl. Method validation was performed to determine the limit of florpyrauxifen-benzyl detection (LOD) and limit of quantitation (LOQ). To recover and quantify florpyrauxifen-benzyl from 28 DAT samples, the same protocols from 7 and 14 DAT were

followed with increased method limits of quantitation (900 mg Supel QuE Verde Tubes) while maintaining acceptable method recoveries in leaf tissues due to the decreased florpyrauxifen-benzyl residue.

Statistical Analysis

Data for florpyrauxifen-benzyl residues were subjected to ANOVA using ‘AGRICOLAE’ package (Mendiburu 2024) in RStudio Version 2023.12.1+402 (R Core Team 2024), and means were separated using Tukey’s honestly significant difference (HSD) at $\alpha = 0.05$, where applicable.

Results and Discussion

Florpyrauxifen-benzyl symptoms were apparent on all three nut tree species, and severity of symptoms increased as herbicide rates increased; however, the symptoms were more pronounced on pistachio than on almond and walnut at similar rates. Additionally, the time to develop symptoms was shorter with pistachio than almond and walnut.

Symptoms on almond and walnut were initially observed at seven DAT, and severity generally peaked at 14 DAT (data not shown). Although, almond and walnut symptoms were mainly observed on the treated side of the tree, some young walnut leaves on the non-treated side of the canopy also showed minor symptoms. Symptoms were most apparent on young leaves and shoots at all rates. Almond and walnut symptoms included chlorosis, chlorotic spot, epinasty, leaf curling, leaf narrowing, leaf crinkling, necrosis, necrotic spots, shoot curling, and twisting (Figure 1). Leaf curling, necrosis, and necrotic spots were more apparent at 1/33X rate for almond than walnut. Walnut symptoms were most apparent on young leaves, while old leaves were free of visual symptoms at any rate. Conversely, symptoms on almond leaves could be found throughout the treated part of the canopy regardless of leaf age. At the 1/100X and 1/33X

rates, almond and walnut symptoms gradually dissipated, and trees appeared normal at the end of the growing season. Furthermore, almond appeared to recover more quickly from florpyrauxifen-benzyl drift rates than pistachio and walnut (data not shown).

Pistachio was considerably more susceptible to florpyrauxifen-benzyl compared to almond and walnut. Florpyrauxifen-benzyl symptoms were visible at 3 DAT for 1/100X and 1/33X treated pistachio, and generally peaked around 14 DAT. Pistachio symptoms were observed throughout the canopy and included chlorosis, chlorotic spot, leaf curling, leaf narrowing, leaf distortion, leaf malformation, leaf crinkling, shoot curling, stem coloring with dark maroon-brown spots, stunting, terminal bud twisting and death (Figure 1). Shoot curling, stem coloring, stunting, and twisting were more apparent at the 1/33X rate than the 1/100X rate. Pistachio symptoms slightly dissipated over time but remained visible throughout the growing season. Injury symptoms persisted into the following spring when the trees leafed out during the 2021 and 2022 growing seasons. Stem curling, stunting, and twisting were most noticeable at the 1/33X rate for pistachio in the year after treatment.

Chemical analyses showed that the recovery of florpyrauxifen-benzyl residues from leaf samples was within the acceptable range: 82.2% for almond, 103.6% for pistachio, and 104.4% for walnut at 14 DAT; and 74% for almond, 79% for pistachio, and 92% for walnut at 28 DAT. In quantification tests, no residues were detected in any of the UTC leaf tissues sampled. At 7 DAT with the 1/100X rate, florpyrauxifen-benzyl residues were 2.31, 1.78, and 3.58 ng g⁻¹ FW in almond, pistachio, and walnut, respectively. The residues in the 1/100X treated plots were to 1.10, 0.68, and 2.05 ng g⁻¹ FW at 14 DAT; and to 0.04, 0.10, and 0 ng g⁻¹ FW at 28 DAT for almond, pistachio, and walnut, respectively (Figure 2, 3, 4).

In almonds treated with the 1/33X rate, florpyrauxifen-benzyl residues were 6.06, 2.21, and 0.25 ng g⁻¹ FW at 7, 14, and 28 DAT, respectively (Figure 2). In pistachio, the 1/33X florpyrauxifen-benzyl treated leaf samples had 5.95, 1.69, and 0.06 ng g⁻¹ FW at 7, 14, and 28 DAT (Figure 3). In walnut, the 1/33X treated leaf samples had 13.12, 8.93, and 0 ng g⁻¹ FW at 7, 14, and 28 DAT, respectively (Figure 4). Walnut leaves had the highest residues at 7 and 14 DAT, but by 28 DAT the residue was below the LOQ (Figure 4).

The results of this research suggest that the ideal time frame to quantify florpyrauxifen-benzyl residues is less than 14 days after a drift event. Florpyrauxifen-benzyl symptoms on nut crop trees generally started to appear within three to 14 days of exposure, and were most severe from 14 to 21 DAT. Therefore, if florpyrauxifen-benzyl drift happens in an almond, pistachio, or walnut orchard, the symptoms may not be recognized until at least 14 days after herbicide drift event occurred but by this point detectable residues likely are decreasing. While a crop consultant, farm advisor, or grower with an auxin-type herbicide symptomology experience on trees may readily identify the symptoms, it may be too late for accurate residue quantification from the leaf tissues unless the drift exposure is extremely high. Under normal circumstances, drift rates are below 1/100X to 1/33X of the field use rate of an herbicide (Al-Khatib and Peterson 1999). The 1/100X drift rate of florpyrauxifen-benzyl caused symptoms on all crops tested in this research in the days and weeks after treatment, but the symptoms decreased gradually during the season for all crops and ultimately disappeared in almond and walnut, but not pistachio. Florpyrauxifen-benzyl residues were not detectable in the leaf tissue at 28 DAT for walnut at any drift rates. Moreover, florpyrauxifen-benzyl at 1/100X rate was only detectable at near or below the lowest quantifiable standard concentration (0.2 ng mL⁻¹ on the instrument) out of one sample for almond and pistachio at 28 DAT. This observation suggests that investigations

of suspected florpyrauxifen-benzyl drift on almond, pistachio, and walnut should not be based entirely on florpyrauxifen-benzyl leaf residue especially when tissue samples are taken 14 days or longer after suspected exposure.

Practical Implications

Increasing herbicide resistance has led to the necessity of complex herbicide programs with different modes of action in California rice. Florpyrauxifen-benzyl is becoming an important herbicide in season-long weed management programs due to its activity on grass, sedge, and broadleaf weeds as well as a broad application window. Florpyrauxifen-benzyl can be applied up to two foliar applications from two-leaf-rice growing stage to 60-days prior to harvest at 40 g ai ha⁻¹ within 14 days intervals. In the Sacramento Valley, these application times generally between May and mid-July, when almond, pistachio, and walnut are highly sensitive to herbicide drift. Pesticide applicators should use extra cautions with florpyrauxifen-benzyl applications particularly near pistachio orchards. Results from this research suggest that florpyrauxifen-benzyl residues in leaf tissue may decrease even before leaf symptoms reach peak severity. Therefore, any florpyrauxifen-benzyl drift case investigations need to consider symptomology, weather conditions, and application records in the area and not rely solely on chemical residue analyses.

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Competing Interests

The authors declare none.

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Figures



Figure 1. Characteristic symptoms of florpyrauxifen-benzyl on 1) almond, 2) pistachio, and 3) walnut at 1/100X and 1/33X simulated drift rates of the rice use rate at A) 7, B) 14, and C) 28 days after treatments in 2021.

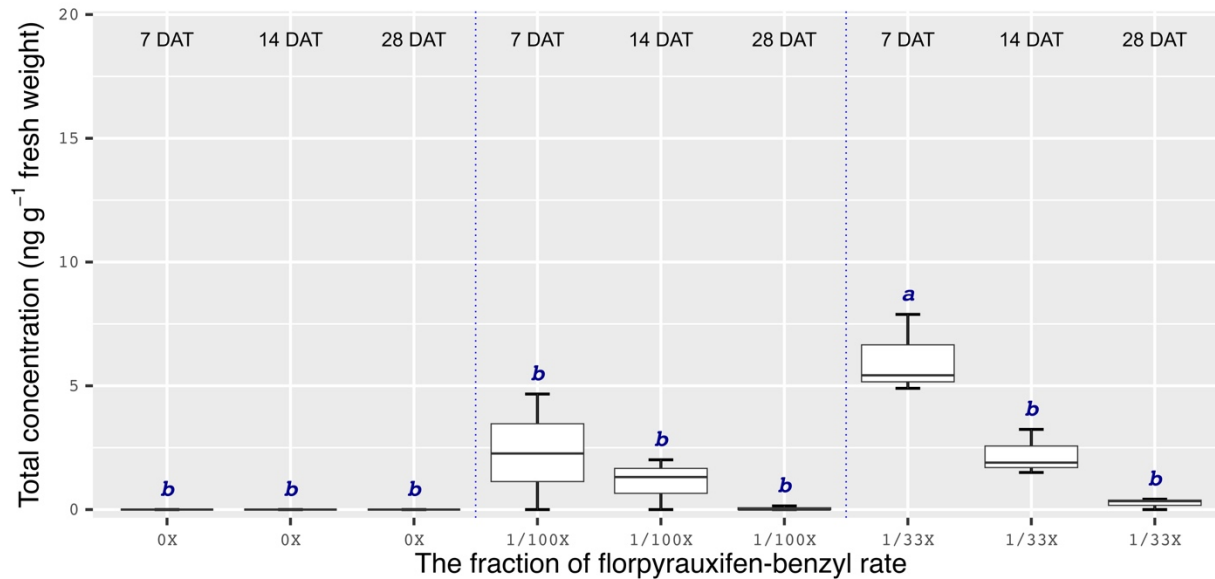


Figure 2. Florpyrauxifen-benzyl residues in almond leaf tissue 7, 14, and 28 DAT with 1/33X and 1/100X simulated drift rates. The rate expressed as the fraction of the use rate in California rice of 29.4 g ai ha⁻¹. Any two means not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test. DAT = days after treatment.

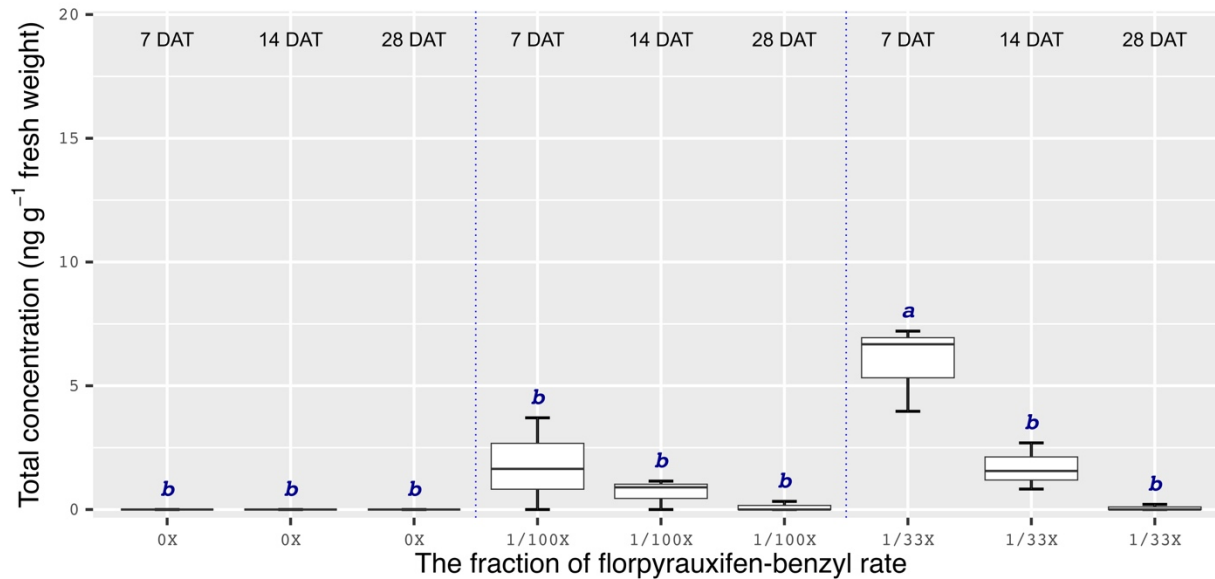


Figure 3. Florpyrauxifen-benzyl residues in pistachio leaf tissue 7, 14, and 28 DAT with 1/33X and 1/100X simulated drift rates. The rate expressed as the fraction of the use rate in California rice of 29.4 g ai ha⁻¹. Any two means not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test. DAT = days after treatment.

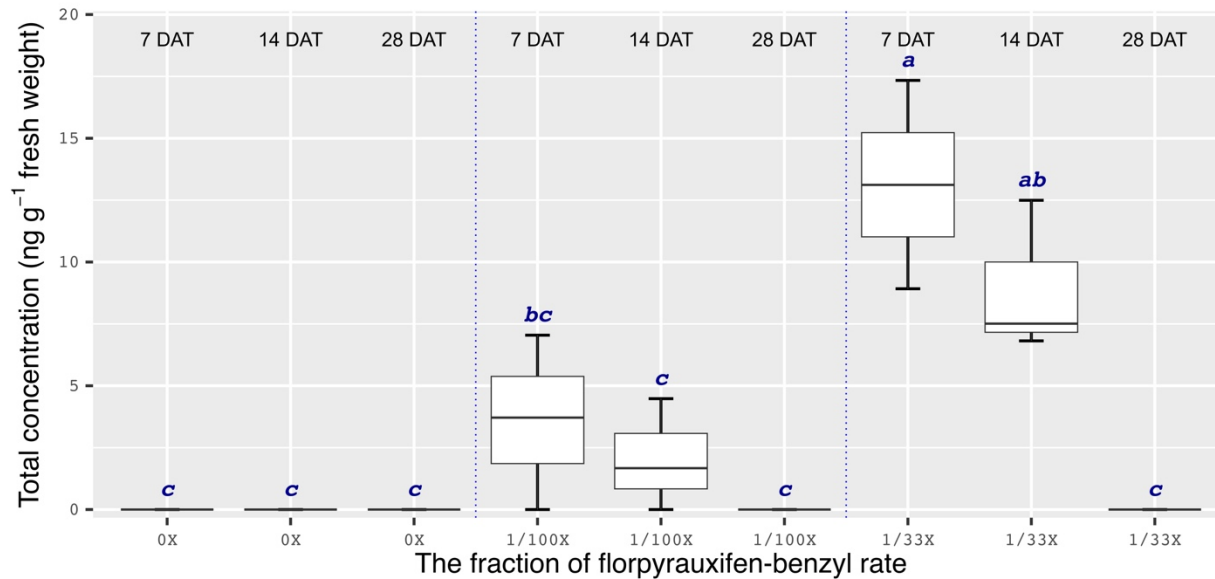


Figure 4. Florpyrauxifen-benzyl residues in walnut leaf tissue 7, 14, and 28 DAT with 1/33X and 1/100X simulated drift rates. The rate expressed as the fraction of the use rate in California rice of 29.4 g ai ha⁻¹. Any two means not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test. DAT = days after treatment.

Chapter 4

Grapevine, peach, and plum responses to simulated florpyrauxifen-benzyl drift

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Abstract

Off-target rice (*Oryza sativa* L.) herbicide drift is a concern in California, where orchards and vineyards are common in the primary rice production region. Synthetic auxin herbicides can cause injury in susceptible non-target crops from relatively low levels of off-target movement; however, this can vary among different crop-herbicide combinations. Florpyrauxifen-benzyl is a new picolinate-type synthetic-auxin herbicide to control selected grass, sedge, and broadleaf weeds in rice. Triclopyr is another synthetic auxin herbicide that has been widely used in California rice. In an effort to steward this new herbicide, research was conducted to compare onset of foliar symptoms from simulated florpyrauxifen-benzyl and triclopyr drift rates onto grapevine (*Vitis vinifera* L.); and simulated florpyrauxifen-benzyl drift onto peach [*Prunus persica* (L.) Batsch], and plum (*Prunus domestica* L.). The simulated drift rates were 1/200X, 1/100X, 1/33X, and 1/10X of the 29.4 g ai ha⁻¹ florpyrauxifen-benzyl and 1/200X, 1/100X, and 1/33X of 420.3 g ae ha⁻¹ triclopyr use rates in rice. Herbicides were applied on one side of one- to two-year-old peach and plum trees as well as on established grapevines in 2020 and 2021. The general symptoms for florpyrauxifen-benzyl and triclopyr were similar and included chlorosis, chlorotic spots, leaf curling, leaf distortion, leaf malformation, leaf crinkling, necrosis, necrotic spots, and twisting on leaves. The florpyrauxifen-benzyl and triclopyr symptoms were distinguishable on the entire grapevine particularly developing leaves, whereas florpyrauxifen-benzyl symptoms on peach and plum were more apparent on the treated side of the tree. Florpyrauxifen-benzyl and triclopyr symptoms developed as early as at three days after treatments (DAT) for grapevines. However, florpyrauxifen-benzyl symptoms developed seven DAT for peach and plum trees; and most symptoms generally persisted through 42 DAT. Some grapevine clusters showed deformation, asymmetrical appearance, and dropping of some berries.

All treated crops gradually recovered throughout the growing season regardless of the application rates. Because symptoms were relatively minor in peach and plum trees, this research suggested that proper herbicide drift management and application precautions are likely to reduce the risk of significant crop injury for peaches and plums; however, grapevines were more sensitive and showed injury symptoms up to 71% at 1/10X florpyrauxifen-benzyl simulated drift rate at 14 DAT. Therefore, an extra precaution should be taken if there are nearby vineyards.

Nomenclature: Florpyrauxifen-benzyl, benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate; plum, *Prunus domestica* L.; peach, *Prunus persica* (L.) Batsch; triclopyr, 2-(3,5,6-trichloropyridin-2-yl)oxyacetic acid; wine grape, *Vitis vinifera* L.

Keywords: Auxinic herbicide; grapevine; multiple exposures; non-target drift

Introduction

California is a major producer of many specialty fruit commodities in the United States (US), including production of more than 99% of the nation's nectarines, plums, prunes, raisins, and table grapes [US Department of Agriculture National Agricultural Statistics Service (USDA-NASS) 2024]. Among those, grape is the most valued crop in California, with more than \$5B in farmgate value from 350,000 hectares (ha) of wine, table, and raisin grapes [California Department of Food and Agriculture (CDFA) 2024]. Today, the state is recognized as the fourth largest wine producing region in the world. Additionally, stone fruits such as peaches, nectarines, plums, and prunes are significant crops grown on 40,000 ha with a value of \$750M (CDFA 2024; USDA-NASS 2024). The Sacramento Valley and the Northern San Joaquin Valley of Northern California are major production regions for these fruit crops.

California also is the second largest rice producer in the US with more than 200,000 ha production (Galvin et al. 2022), which contributes more than \$1B to California economy and 25,000 rice-related jobs in the state (CDFA 2024). California rice is approximately 95% medium-grain and premium quality rice [California Cooperative Rice Research Foundation (CCRRF) 2024]. The primary rice production area is based in the Sacramento and Northern San Joaquin Valleys [University of California Agriculture and Natural Resources (UCANR) 2023]. The majority of California rice is water-seeded and grown in continuously-flooded conditions during growing season (UCANR 2023; Hill et al. 2006).

Weed competition can dramatically reduce rice yields (Hill et al. 2006) and unmanaged weeds also cause harvest difficulties, host pests and diseases, and increase the weed seedbank (Strand 2013). Alongside cultural management methods such as planting certified weed-free rice seed, high seeding rate, and continuous water management, herbicides are crucial for weed

management in rice (UCANR 2023). Once rice fields are flooded, herbicides are generally applied at the day of seeding or before the two leaf rice growth stages. Most California rice growers follow up with at least one more post-emergent herbicide application during the season, usually before or at the mid-tillering stage of rice. These herbicide application times usually occur in May and June depending on the planting date, rice variety and environmental conditions (UCANR 2023).

During May and June when rice herbicides are being applied, grapevine growth stages range from bloom to veraison (Bettiga 2013). Also at this time, stone fruits such as peach and plum are at a growth stage when the endocarp (pit) hardening process begins, and the fruit size increases (LaRue and Johnson 1989). In the Sacramento Valley, a major portion of the rice herbicide application timings coincide with susceptible growth stages of grapevines, peaches, and plums (Bettiga 2013; LaRue and Johnson 1989), which increasing the risk of potential off-target damage to these crops.

Herbicide drift is the physical movement of herbicide droplets through the air, at the time of application or soon thereafter, to any site other than the intended target [UC Integrated Pest Management (UCIPM) Herbicide Symptoms 2024]. Under most herbicide application circumstances, off-target herbicide exposure occurs at rates from below 1/100X up to 1/33X of the field application rate of the herbicide (Galla et al. 2019). Significant drift events are most frequently associated with relatively high air temperature and wind speed, low relative humidity, small spray droplet size, and relatively short distances to nearby nontarget crops (UCIPM 2016). The concerns of rice herbicide drift to off-target crops in the Sacramento Valley have been increasing among growers, crop consultants, and researchers (UCANR 2023).

Florpyrauxifen-benzyl [benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate; CAS: 1390661-72-9] is a synthetic auxin-type (HRAC/WSSA Group IV) rice herbicide recently registered in California with trade name Loyant[®] CA. Florpyrauxifen-benzyl exhibits novel chemical characteristic in the site of action, which result in the selective control of grasses, alongside sedges and broadleaf weeds at low use rates (Miller and Norsworthy 2018). Besides florpyrauxifen-benzyl, triclopyr [2-(3,5,6-trichloropyridin-2-yl)oxyacetic acid; CAS: 55335-06-3] is also widely used to control sedges and broadleaf weeds in rice fields. Triclopyr is a pyridyloxy-carboxylate auxin-type herbicide commercially available as in triethylamine salt and butoxyethyl ester formulations. When synthetic auxins applied exogenously on susceptible plants, growth abnormalities, leaf epinasty, tissue swelling, stem curling, chloroplast damage, membrane and vascular system damage, wilting, and necrosis may be observed, leading to plant death (Grossmann 2010).

Synthetic auxins are known for their off-target injuries on vegetables, fruit and nut trees, field and forage crops, ornamentals, and vines (Egan et al. 2014; Haring et al. 2022; Warmund et al. 2022). The overall objectives of this research were to characterize the effects of florpyrauxifen-benzyl simulated drift rates on stone fruit and vine crops, and to evaluate grapevine responses to florpyrauxifen-benzyl compared to triclopyr.

Materials and Methods

Study Site

Three simulated non-target drift experiments were conducted in 2020 and 2021 in newly planted peach (lat. 38°32'19.5"N, long. 121°47'41.3"W) and plum (lat. 38°32'19.6"N, long. 121°47'40.8"W) orchards at the UC Davis Plant Sciences Field Facility; and in an established wine grape vineyard (lat. 38°31'31.3"N, long. 121°47'18.7"W) at the UC Davis Department of

Viticulture and Enology Tyree Vineyard near Davis, CA, USA. The orchards were established in March 2020 with ‘Coralstar’ peach scion on ‘Krymsk 86’ rootstock and ‘French-prune’ plum scion on ‘Krymsk 86’ rootstock. All peaches and plums were planted with 6 m intra-row spacing and 4.2 m between rows. The vineyard was established in 1998 with a bi-lateral double-cordon-trained ‘Grenache’ wine variety grapevine, which was planted with 1.8 m intra-row spacing and 3.6 m between rows.

The soil in the orchard location was a Yolo silt loam with NO₃-N: 57 ppm, Olsen-P: 26 ppm, K: 351 ppm, Na: 21 ppm, Ca: 8 meq/100 g, Mg: 10 meq/100 g, CEC: 19 meq/100 g, OM: 2.7%, and pH: 6.7 in orchards; and in the vineyard the soil was a Yolo silt loam with NO₃-N: 23 ppm, Olsen-P: 12 ppm, K: 288 ppm, Na: 12 ppm, Ca: 11 meq/100 g, Mg: 9 meq/100 g, CEC: 21 meq/100 g, OM: 2.5%, and pH: 7.1 (UC Davis Analytical Lab, Davis, CA, USA). All trees and grapevines were maintained by farm managers following commercial standard practices to avoid disease and insect infestations as recommended by the UCIPM Guidelines (Strand 1999; Buchner 2012; Bettiga 2013). In all experiments, weeds in the inter-rows were mowed, and intra-rows were treated with a tank-mix of rimsulfuron at 70 g ai ha⁻¹, indaziflam at 50 g ai ha⁻¹, oxyfluorfen at 560 g ai ha⁻¹, and glufosinate-ammonium at 450 g ai ha⁻¹ plus manufacturer recommended surfactants. Irrigation was applied in all crops through a single-line drip irrigation system with emitters spaced every 30 cm during the growing seasons.

Herbicide Applications

Florpyrauxifen-benzyl [Loyant[®] CA, 25 g active ingredient (ai) L⁻¹, Corteva[™] Agriscience, Indianapolis, IN, USA] was applied on 9 June 2020 in the peach and plum orchards and on 11 June 2020 in the vineyard. Triclopyr [Grandstand[™] CA, 359 g acid equivalent (ae) L⁻¹, Corteva Agriscience, Indianapolis, IN, USA] was applied on 11 June 2020 in only grapevines. In all

experiments, florpyrauxifen-benzyl was applied to simulate plausible drift rates of 1/200X (0.5% drift), 1/100X (1% drift), 1/33X (3% drift), and 1/10X (10% drift) of the rice use rate of 29.4 g ai ha⁻¹; the vineyard experiment included triclopyr at three rates simulating a plausible drift rate of 1/200X, 1/100X, and 1/33X of the rice use rate of 420.3 g ae ha⁻¹ (Galla et al. 2019). Untreated check (UTC) plots were also included for comparison in each experiment. The florpyrauxifen-benzyl spray mixtures included methylated seed oil (Super Spread MSO, Wilbur-Ellis, Fresno, CA, USA) at 584 ml ha⁻¹ and triclopyr spray mixtures included crop oil concentrate (MOR-ACT, Wilbur-Ellis, Fresno, CA, USA) at 1% v v⁻¹ rate.

Environmental conditions at the time of the orchard and vineyard applications were 16°C air temperature (temp), 58% relative humidity (RH), and 0.4 m s⁻¹ wind speed on 9 June 2020 and 15°C air temp, 60% RH, and 0.5 m s⁻¹ wind speed on 11 June 2020, respectively. No in-season auxin-type herbicides were used to avoid the potential confusion with florpyrauxifen-benzyl and triclopyr symptoms and injury.

Studies were repeated on 31 May 2021 with a different set of peaches, plums, and grapevines in the same field (one-year exposure study, where $n = 8$). In addition, the trees and vines that were treated with florpyrauxifen-benzyl in 2020 were also retreated in 2021 with the same treatments as in the previous year's protocols to evaluate two-year exposure response (two-year exposure study, where $n = 4$) (Bhatti et al. 1995). However, the two-year exposure experiment was not repeated on the trees initially treated in 2021. All the methodology for two-year exposure study was similar to the one-year exposure study as described above.

Environmental conditions at the time of second-year application were 18°C air temp, 50% RH, and 0.6 m s⁻¹ wind speed.

Data Collection and Experimental Design

Experiments were arranged in a randomized complete block design with four replicates, where an individual tree or vine was an experimental unit. An untreated tree or three consecutive vines between treated plots were included in 2020 experiments in orchards and in both years in vineyard as a buffer to prevent any treatment interaction or contamination. All herbicide treatments were applied to one side of the tree or vine canopy as one pass (top to bottom for trees and side to side for vines) with a handheld, carbon dioxide-propelled backpack sprayer calibrated to deliver 187 L ha⁻¹ at 206 kPa pressure through XR-8004 nozzle tips (TeeJet Technologies, Wheaton, IL, USA). The sprayer boom had two nozzles spaced 50 cm apart and spray was delivered based on a three-second pass per tree or vine. Plots were sprayed early in the morning when the weather conditions were calm and not windy to avoid non-target herbicide drift to nearby trees or vines. Grapevine was the only crop with fruit present at the time of herbicide application; berry diameters were 5 to 10 mm on 11 June 2020 and 31 May 2021, respectively.

Trees and vines were observed for visual injury symptoms at 6, 12, 24, 48, and 72 hours after herbicide treatments as well as 7, 14, 21, 28, 35, 42, and 90 days after treatments (DAT). Symptomology descriptions of the treated foliage were made according to UC IPM Herbicide Symptoms guidelines (UCIPM Herbicide Symptoms 2020). Injury was rated on a scale where 0 = no injury and 100 = death (Al-Khatib et al. 1992; Bhatti et al. 1995; Sciumbato et al. 2004) according to the following scale:

- 0% = Normal size growth; green pigmentation of all leaves; identical appearance to UTC.
- 1–4% = Normal-sized leaves; less than 5% of the leaves have only one discernible chlorotic spot; overall canopy has an indistinct injury appearance.

- 5–9% = Slight reduction in leaf size; 2 to 5 diffuse chlorotic spots visible on 5 to 10% of the leaves; up to 5% of leaf curling and crinkling at only young leaves.
- 10–29% = Reduction in leaf size up to 5%; growth restriction and chlorosis at interveinal tissue; symptoms moderate to severe on 10 to 30% of the leaves; less than 30% of the leaf surface chlorotic; 5 to 10% of necrosis, leaf curling, and crinkling; adjacent chlorotic areas merge and result in necrosis at the interveinal areas; up to 5% shoot curling.
- 30–49% = Reduction in leaf size from 5 to 10%; shoot tip growth restricted; symptoms severe on 30 to 50% of the leaves; up to 50% of the leaves with chlorosis; from 10 to 25% necrosis; from 5 to 10% moderate to severe curling at shoots and stems.
- 50–69% = Reduction in leaf size from 10 to 25%; growth significantly restricted; symptoms very severe on 50 to 70% of the leaves; up to 70% of leaf surface chlorotic; from 25 to 50% necrosis, leaf curling, and crinkling; up to 10% stunting and irregular growth at the overall canopy; interveinal tissue-restricted; noticeable stem discoloring with dark red-brown spots up to 15% of the young branches.
- 70–89% = Growth severely restricted; symptoms very severe on 70 to 90% of the leaves; up to 90% of leaf surface chlorotic; necrosis becomes the primary indicator of plant injury; distinguishable leaf loss; from 10 to 50% stunting at the overall canopy; severe leaf distortion and malformation; obvious stem discoloring with dark red-brown-black spots up to 50% of the branches; terminal bud twisting and death.
- 90–99% = Almost no development of leaf and interveinal tissues; symptoms extremely severe on all the leaves; epinasty is extreme throughout the leaves, chlorosis and necrosis coverage is dominant; more than 50% of stunting; extremely damaged appearance.
- 100% = Plant dead.

Herbicide symptoms on treated grapevines, peach, and plum trees were compared with UTC plants at each observation. Photos were taken from the treated side of the canopy throughout the growing season to ensure consistency in evaluations. Furthermore, the number of internodes were counted and compared to UTC on four randomly selected shoots from each vine or tree side, where simulated herbicide treatments delivered. Number of nodes was recorded prior to the treatments and at 90 DAT on shoots for approximately 30-cm long. Trunk diameters from peach and plum trees were measured at approximately 25 cm above the ground before the spring growth started in April (spring-data) and at the end of the summer (fall-data) approximately 140 DAT (Abit and Hanson 2013). Tree growth was expressed through trunk diameter growth as a percent increase based on the following formula (Equation 1):

$$Y = \left[\left(\frac{X_f}{X_s} \right) - 1 \right] \times 100$$

where Y is the percent increase of trunk diameter from the X_f = fall-data change of X_s = spring-data. The relative change in herbicide treated plots' trunk diameter was compared to UTC plots' trunk diameter change.

Grapes were hand harvested when berries in UTC plots reached ~20°Brix (1% soluble solids), a common practice for the Northern San Joaquin and Sacramento Valleys grapevine industry (Bettiga 2013). Grape clusters were harvested from all treated vines as well as UTC and weighed for total fruit yield and sugar content from a fruit subsample determined with a handheld refractometer (Haring et al. 2022).

Statistical Analysis

Visual injury ratings, number of nodes, and trunk diameter data were subjected to analysis of variance (ANOVA) using 'agricolae', 'emmeans', 'lme4', and 'multcomp' packages in RStudio Version 2023.06.0+421 (R Core Team 2023), and Tukey's honestly significant difference (HSD)

were used at $\alpha = 0.05$ to separate means, when applicable. We used Type II Wald F tests with the Kenward-Roger degrees-of-freedom method and Type III with Satterthwaite's method, when the confidence level at 0.95, and the significance level at $\alpha = 0.05$ for both ANOVA types. Grape yield and °Brix were analyzed with ANOVA at $\alpha = 0.05$ as described above (Kniss and Streibig 2018).

Results and Discussion

Because there were no significant interactions between year and treatment (data not shown), the visual symptom data for 2020 and 2021 were combined for presentation (Table 1, 2, 3).

Generally, florpyrauxifen-benzyl and triclopyr symptoms were apparent on all treated vines (Figure 1, 2) and florpyrauxifen-benzyl symptoms were apparent on all treated trees (Figure 3, 4) with symptoms increasing as herbicide rate increased (Table 1, 2, 3). However, the florpyrauxifen-benzyl symptoms were more severe on grapevine than peach and plum, and the most apparent symptoms were observed in the 1/10X florpyrauxifen-benzyl rate. Furthermore, the time to develop florpyrauxifen-benzyl symptoms at all rates was shorter with grapevine than with peach and plum. All crops had slightly more injury after two years of exposure which may suggest cumulative injury.

Florpyrauxifen-benzyl injury symptoms were observed three DAT for grapevine and gradually peaked by 42 DAT. Grapevine symptoms were noticeable on both the treated and nontreated side of the vines and the developing leaves and shoots showed more symptoms compared to fully developed leaves and shoots. Grapevine symptoms included chlorosis, chlorotic spot, epinasty, leaf curling, leaf narrowing, leaf crinkling, necrosis, necrotic spots, shoot curling, and twisting (Figure 1). Initial chlorosis symptoms turned to necrosis within seven to 14 DAT and eventually to necrotic spots and holes in the leaf. Chlorosis and epinasty,

especially in the interveinal areas, and necrosis were characteristic at the 1/33X and 1/10X rates. The 1/10X floryprauxifen-benzyl drift caused the most severe symptoms on grapevine including deformation of grape clusters and berries which showed an asymmetrical appearance of clusters compared to UTC. Two-year treated grapevine showed more asymmetrical appearance and reduced growth with necrosis throughout the 2021 season. However, even at this high simulated drift rate, these vines gradually recovered and the foliage appeared normal at 90 DAT except at 1/10X floryprauxifen-benzyl rate treated vines, which injury symptoms remained throughout the season (data not shown). Overall, grapevine was more sensitive to floryprauxifen-benzyl than peach or plum trees. Triclopyr injury symptoms were observed at seven DAT for grapevine, and gradually peaked through 42 DAT. In general, grapevine injury symptoms from triclopyr were similar to floryprauxifen-benzyl injury symptoms at the same rates (Figure 2).

Grapevine visible injury ratings was significant at 1/10X rate of floryprauxifen-benzyl throughout the observation period (Table 1). Other floryprauxifen-benzyl treatments caused similar injury levels to one another, except 1/33X rate at 42 DAT. Similar to 1/33X floryprauxifen-benzyl rating at 42 DAT, triclopyr also caused injury of 35% at 1/33X drift rate. Our results indicated lower levels of visible injury to grapevines from triclopyr at 1/100X and 1/33X rates compared with previous research (Haring et al. 2022; Roberto et al. 2021). This variation could be the result of the application timing, adjuvant selection, environmental conditions at the time of application, and the maturity of vines.

Grapevines treated with 1/33X and 1/10X floryprauxifen-benzyl and 1/33X triclopyr rates two years in a row had up to ~50% yield reduction (Table 4) compared to the UTC which was 22.1 kg vine⁻¹ in one-year exposure study and 19.3 kg vine⁻¹ in two-year exposure study. The correlation between floryprauxifen-benzyl and triclopyr drift rates and grape yield was

significant at 1/33X and 1/10X florpyrauxifen-benzyl and triclopyr rates (Table 4) in the both one- and two-year exposure studies as the herbicide rate increase, the yield decrease. However, the grape yield from 1/200X and 1/100X drift rate treated plots for both florpyrauxifen-benzyl and triclopyr was not different than the UTC. Furthermore, grape sugar content increased as florpyrauxifen-benzyl and triclopyr rates increased (Table 4). The higher °Brix level was associated with greater fractional herbicide rates (Table 4). Yet, only florpyrauxifen-benzyl at 1/10X rate in the one-year exposure study and florpyrauxifen-benzyl at 1/33X and 1/10X rates as well as triclopyr at 1/33X rates in two-year exposure study were different than UTC. The greatest drift rate, florpyrauxifen-benzyl at 1/10X, elevated °Brix up to ~20% greater compared to UTC vines that were ~20°Brix at harvest. The higher sugar content with greater herbicide rates were similar with the previous research (Haring et al. 2022).

Florpyrauxifen-benzyl symptoms were observed at seven DAT for peach, and generally peaked at 14 DAT (Table 2). The injury was apparent on the treated side of the peach canopy particularly on developing leaves and terminal buds. Injury symptoms on peach from florpyrauxifen-benzyl were similar to grapevine with addition of stunting at the 1/10X rate (Figure 3). Symptoms initially appeared as chlorosis and leaf curling. At approximately 14 DAT, leaf curling became more severe and young shoots showed curling symptoms. Shoot curling, stunting, and twisting were more apparent at 1/33X and 1/10X rates than at the lower rates. Peach visible injury ratings were up to 50% at 14 DAT with 1/10X florpyrauxifen-benzyl drift rate in the one-year exposure study (Table 2). In the two-year exposure study, florpyrauxifen-benzyl at 1/10X resulted in 61% injury at 28 DAT. However, peach symptoms dissipated through 42 DAT and trees appeared normal at the end of the growing season except trees treated with the 1/10X florpyrauxifen-benzyl rate. Stunting symptom remained throughout the growing

season and was noticeable in the following spring on 1/10X florpyrauxifen-benzyl treated peaches.

Injury symptoms were detectable by seven DAT in plum, and generally peaked at 14 DAT (Table 3). Plum symptoms were only distinguishable on the treated side of the tree and symptoms were more apparent on developing leaves and branches. In general, injury symptoms from florpyrauxifen-benzyl on plum were less than on grapevine and peach (Figure 4).

Florpyrauxifen-benzyl injury symptoms on plum included chlorosis, leaf curling, necrosis, and stem curling. In addition, epinasty symptoms on the tips of developing branches were observed in the following growing seasons. Visible injury ratings on plum were less than 5% at all rates for all observations in both one- and two-year exposure studies (Table 3). The results showed that even at the highest rate, plum rapidly recovered from visible injury compared to grapevine and peach and appeared normal throughout most of the growing season and was the least sensitive crop to florpyrauxifen-benzyl in this study.

Tree trunk diameter change was variable and showed no significant interactions between herbicide treatment, exposure, and year for peach or plum trees. In both crops, the relative trunk diameter growth was not statistically different compared to the UTC trees (data not shown). This indicates that trunk diameter change is not a strong parameter to evaluate the potential florpyrauxifen-benzyl drift impacts despite the foliar symptoms. The results regarding visible injury recovery suggest that peach and plum can recover from florpyrauxifen-benzyl drift exposure. Moreover, the number of nodes on young shoots at 90 DAT was similar in all florpyrauxifen-benzyl treatments compared to UTC trees and was not affected even by the highest simulated drift rate (data not shown). This research suggests that grapevine and peach

can recover from florpyrauxifen-benzyl expected drift rates; and plum's susceptibility level is unlikely to cause long-lasting injury.

Practical Implications

The differences between grapevine, peach, and plum responses to simulated florpyrauxifen-benzyl rates are not surprising because the absorption, translocation, and metabolism of herbicides are expected to vary among plant species (Al-Khatib et al. 1992). In addition, severe symptoms of developing leaves are expected since young leaves are metabolically more active and absorb more herbicide than developed leaves (Al-Khatib et al. 1992). Realistic herbicide drift rates under the most field conditions generally range from below 1/100X up to 1/33X of field use rates (Al-Khatib and Peterson 1999); the 1/10X florpyrauxifen rate in this study was added to simulate a worst-case scenario, considering consecutive drift events in a short interval of time, an accidental herbicide application, or herbicide-contaminated tank, events that are unlikely to happen in a typical drift situation.

Due to its selective grass activity and good control of broadleaves and sedges, florpyrauxifen-benzyl is expected to be widely used in rice fields. California growers are familiar with management programs for triclopyr, another auxin-type herbicide which has been registered for use in rice for many years. This research suggests that florpyrauxifen-benzyl might cause significant damage if drifted onto grapevines at sufficient amounts. However, spray drift advisories for florpyrauxifen-benzyl applications only allow ground applications whereas triclopyr is allowed aerially applied. Likewise, tolerated wind speed at the time of application for florpyrauxifen-benzyl is also more restrictive than triclopyr ground applications, which helps to reduce the risk of significant crop injury from florpyrauxifen-benzyl applications.

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Competing Interests

The authors declare none.

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Tables

Table 1. Grapevine injury following simulated drift rates of florpyrauxifen-benzyl and triclopyr in 2020 and 2021.

Herbicide	Rate ²	One-year exposure ¹			Two-year exposure			
		14 DAT ³	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT	
		Visible injury (%)						
FPB	1/200x	11 b ⁴	10 b	17 b	9 c	9 c	9 b	
FPB	1/100x	15 b	12 b	22 b	12 c	12 bc	20 b	
FPB	1/33x	17 b	15 b	32 ab	37 b	37 bc	55 a	
FPB	1/10x	49 a	46 a	66 a	71 a	66 a	66 a	
TRC	1/200x	6 b	9 b	12 b	8 c	13 bc	19 b	
TRC	1/100x	7 b	14 b	29 b	8 c	16 bc	22 b	
TRC	1/33x	8 b	22 b	35 ab	8 c	34 b	49 a	

¹One-year exposure: Vines were treated in 2020 and the study was repeated in 2021 on different vines in the same vineyard, where sample size $n = 8$. Two-year exposure: The vines treated in 2020 were retreated in 2021, where sample size $n = 4$.

²Florpyrauxifen-benzyl rate is expressed as a percentage of the rice use rate of 29.4 g ai ha⁻¹.

Triclopyr rate is expressed as a percentage of the rice use rate of 420.3 g ae ha⁻¹.

³DAT = days after treatment; FPB = florpyrauxifen-benzyl; TRC = triclopyr.

⁴Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test.

Table 2. Peach injury following simulated drift rates of florpyrauxifen-benzyl in 2020 and 2021.

Rate ²	One-year exposure ¹			Two-year exposure		
	14 DAT ³	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT
	Visible injury (%)					
1/200x	5 b ⁴	4 b	1 b	3 b	3 c	3 b
1/100x	9 b	7 b	3 b	7 b	4 bc	4 b
1/33x	10 b	14 b	12 b	10 b	20 b	4 b
1/10x	50 a	37 a	31 a	42 a	61 a	51 a

¹One-year exposure: Trees were treated in 2020 and the study was repeated in 2021 on different trees in the same orchard, where sample size $n = 8$. Two-year exposure: Trees treated in 2020 were retreated in 2021, where sample size $n = 4$.

²Florpyrauxifen-benzyl rate is expressed as a percentage of the rice use rate of 29.4 g ai ha⁻¹.

³DAT = days after treatment.

⁴Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test.

Table 3. Plum injury following simulated drift rates of florpyrauxifen-benzyl in 2020 and 2021.

Rate ²	One-year exposure ¹			Two-year exposure		
	14 DAT ³	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT
	Visible injury (%)					
1/200x	2 a ⁴	0 b	0 a	1 b	0 b	0 a
1/100x	2 a	0 b	0 a	2 ab	0 b	0 a
1/33x	4 a	0 b	0 a	4 ab	1 ab	0 a
1/10x	4 a	3 a	1 a	5 a	3 a	1 a

¹One-year exposure: Trees were treated in 2020 and the study was repeated in 2021 on different trees in the same orchard, where sample size $n = 8$. Two-year exposure: Trees treated in 2020 were retreated in 2021, where sample size $n = 4$.

²Florpyrauxifen-benzyl rate is expressed as a percentage of the rice use rate of 29.4 g ai ha⁻¹.

³DAT = days after treatment.

⁴Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test.

Table 4. Grape yield and sugar concentration response to florpyrauxifen-benzyl and triclopyr simulated drift rates.

Herbicide	Rate ²	One-year exposure ¹		Two-year exposure	
		Yield	Brix ³	Yield	Brix
		(kg vine ⁻¹)	(1°Bx = 1% sugar)	(kg vine ⁻¹)	(1°Bx = 1% sugar)
FPB ⁴	1/200x	22 a ⁵	20.7 ab	15.7 ab	20.8 bc
FPB	1/100x	13.8 ab	20.8 ab	12.2 ab	22.4 abc
FPB	1/33x	12.3 b	23.9 ab	11.8 b	24.7 ab
FPB	1/10x	11.2 b	24.9 a	11.3 b	25.4 a
TRC	1/200x	21.8 a	20.6 b	17 ab	20.7 bc
TRC	1/100x	14 ab	20.8 ab	12.4 ab	21.5 abc
TRC	1/33x	12.1 b	22.7 ab	11.9 b	24.2 ab
UTC	–	22.1 a	20.3 b	19.3 a	19.9 c

¹One-year exposure: Vines were treated in 2020 and the study was repeated in 2021 on different vines in the same vineyard, where sample size $n = 8$. Two-year exposure: The vines treated in 2020 were retreated in 2021, where sample size $n = 4$.

²Florpyrauxifen-benzyl rate is expressed as a percentage of the rice use rate of 29.4 g ai ha⁻¹.

Triclopyr rate is expressed as a percentage of the rice use rate of 420.3 g ae ha⁻¹.

³One degree Brix is 1 g of sucrose in 100 g of solution (1°Brix = 1% sugar).

⁴FPB = florpyrauxifen-benzyl; TRC = triclopyr; UTC, untreated check.

⁵Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test.

Figures

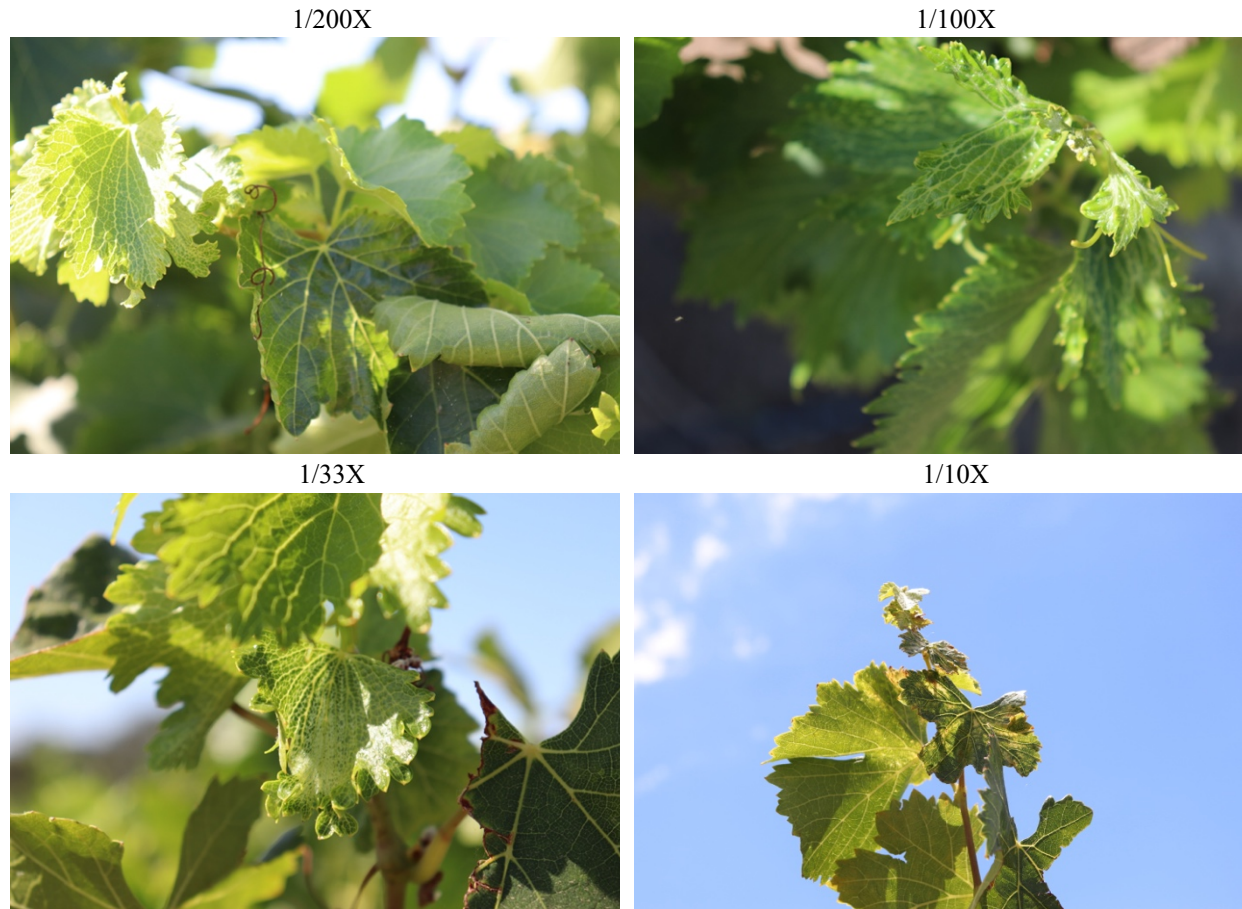


Figure 1. Grapevine chlorosis, epinasty, leaf crinkling, necrosis, and shoot curling symptoms 28 days after treatment with simulated drift treatments of flupyrauxifen-benzyl at 1/200X, 1/100X, 1/33X, and 1/10X of the rice use rate of 29.4 g ai ha⁻¹.

1/200X



1/100X



1/33X



Figure 2. Grapevine chlorosis, epinasty, leaf crinkling, leaf narrowing and twisting symptoms 28 days after treatment with simulated drift treatments of triclopyr at 1/200X, 1/100X, and 1/33X of the rice use rate of 420.3 g ae ha⁻¹.



Figure 3. Peach chlorosis, epinasty, and necrosis symptoms 28 days after treatment with simulated drift treatments of florasulfuron-benzyl at 1/200X, 1/100X, 1/33X, and 1/10X of the rice use rate of 29.4 g ai ha⁻¹.



Figure 4. Plum chlorosis, epinasty, and stem curling symptoms 28 days after treatment with simulated drift treatments of florpyrauxifen-benzyl at 1/200X, 1/100X, 1/33X, and 1/10X of the rice use rate of 29.4 g ai ha⁻¹.