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Short title: Tetflupyrolimet drift

Grapevine, stone fruit, and tree nut crop response to simulated tetflupyrolimet drift

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Abstract

Off-target rice herbicide drift is historically a concern in California, where susceptible crops such as orchards and vineyards are nearby. Tetflupyrolimet is a potent inhibitor of dihydroorotate dehydrogenase which provides excellent grass weed control in rice cropping systems. In efforts to steward tetflupyrolimet before its registration in California, this research was conducted to compare the onset of foliar symptoms from tetflupyrolimet applications onto almond, grapevine, peach, pistachio, plum, and walnut. Tetflupyrolimet was applied to these tree and vine crops at fractional rates of 1/200×, 1/100×, 1/33×, and 1/10× of the 125 g ai ha^{-1} rice use rate. Almond, pistachio, and walnut trees also received the 1× of the rice use rate. Tetflupyrolimet treatments were applied on one side of 3- to 4-yr-old almond, peach, pistachio, plum, and walnut trees and on one side of 25- to 26-yr-old grapevines in 2022 and 2023. Visible injury ratings were carried out weekly to assess symptomology throughout the growing seasons and at leaf out the following springs. Tree trunk diameter was recorded before and after herbicide applications. No injury was observed on any crops tested, regardless of the tetflupyrolimet application rate. In all orchard crops, tree trunk diameter was not affected by tetflupyrolimet treatments. Likewise, grape yield was not reduced even at the 1/10× tetflupyrolimet fractional rate. Since no injury symptoms were recorded, this research suggested that tetflupyrolimet can be safely used at nearby rice fields and might be a target for future registration consideration in orchard and vineyard crops.

Nomenclature: tetflupyrolimet; almond, *Prunus dulcis* (Mill.) D.A. Webb; grapevine, *Vitis vinifera* L.; peach, *Prunus persica* (L.) Batsch; pistachio, *Pistacia vera* L.; plum, *Prunus domestica* L.; rice, *Oryza sativa* L.; walnut, *Juglans regia* L.

Keywords: aryl pyrrolidinone anilide, *de novo* pyrimidine nucleotide biosynthesis, dihydroorotate dehydrogenase inhibitor, nontarget crop, off-target drift, rice herbicide

Introduction

Tetflupyrolimet [CAS: 2053901–33–8; (3S,4S)-*N*-(2-fluorophenyl)-1-methyl-2-oxo-4-[3-(trifluoromethyl)phenyl]pyrrolidine-3-carboxamide] is an aryl pyrrolidinone anilide herbicide that was developed for selective control of grass, sedge, and broadleaf weeds in rice cropping systems. As the first novel mode-of-action, Herbicide Resistance Action Committee/Weed Science Society of America Group 28 herbicide, in the last three decades, tetflupyrolimet is a potent inhibitor of dihydroorotate dehydrogenase (DHODH) which is involved in the *de novo* pyrimidine nucleotide biosynthesis (Maienfisch and Mangelinckx 2021). The DHODH is the fourth enzyme of the pyrimidine *de novo* biosynthesis pathway that is localized to the mitochondria and catalyzes the conversion of dihydroorotate to orotate (Zrenner et al. 2006). In plants, the *de novo* pyrimidine nucleotide biosynthesis pathway is a vital process for metabolism; gene expression; and the production of substrates for DNA, RNA, and multiple biosynthesis pathways such as polysaccharides, glycoproteins, and phospholipids (Kang et al. 2023; Zrenner et al. 2006).

Owing to the central role of nucleotides, inhibition of the DHODH is lethal to most organisms (Dayan 2019). Tetflupyrolimet has been shown to have very high levels of activity against grass species such as watergrasses (*Echinochloa* spp. P.Beauv.), sprangletops (*Leptochloa* spp. P.Beauv.) (Lombardi and Al-Khatib 2024), giant foxtail (*Setaria faberi* R.A.W.Herrm.), and hairy crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Selby et al. 2023). Moreover, tetflupyrolimet's activity is at least 10-fold greater on weeds such as foxtail than rice, which suggests it is selective in rice because the crop can metabolize tetflupyrolimet (Dayan 2019; Selby et al. 2023). Therefore, the discovery of tetflupyrolimet is important and promising for weed management in rice cropping systems.

California is the second largest rice producer in the United States with approximately 220,000 ha under cultivation (Galvin et al. 2022), which accounts for more than US$1 billion in farmgate value (CDFA 2024). The primary rice production area is in the Sacramento and Northern San Joaquin Valleys and the crop typically is water-seeded and grown under continuously flooded conditions during the growing season (Inci et al. 2024d). Competitive grass weeds in rice systems such as barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], early watergrass [*E. oryzoides* (Ard.) Fritsch], late watergrass [*E. phyllopogon* (Stapf) Koso-Pol.], and bearded sprangletop [*Leptochloa fusca* (L.) Kunth ssp. *fascicularis* (Lam.) N. Snow] can significantly reduce rice yields (Brim-DeForest et al. 2017). Alongside cultural management methods such as planting certified weed-free rice seed, high seeding rates, and continuous water management, herbicides are crucial for weed management in rice (Inci and Al-Khatib 2024).

Beyond rice, California is the primary producer of many specialty crop commodities in the United States including production of more than 99% of the nation's almonds, nectarines, peaches, pistachios, plums, raisin grapes, and walnuts (CDFA 2024). Among those, grape is the most valued crop in California, generating more than US$5.5 billion in cash receipts from 350,000 ha of wine, table, and raisin grapes. Tree nuts are produced on 1 million ha in the Sacramento and San Joaquin Valleys and these regions account for 85% of the world's almond production. Collectively, grapes, stone fruits, and tree nuts are significant crops grown on ~1.5 million ha with a value of more than US$12 billion. With such diverse cropping systems in California, rice is often grown adjacent to orchards and vineyards (Inci et al. 2024b, 2024c).

California's unique crop diversity, paired with strict regulatory structures, has limited the number of herbicide active ingredients available to rice growers because of the potential for off-target herbicide drift to nearby orchards and vineyards (Hill et al. 2006). Research on tetflupyrolimet in the complex herbicidal programs of California rice suggests utility as both a preemergence and early postemergence herbicide (Lombardi and Al-Khatib 2024). The application timing of tetflupyrolimet is from the day-of-seeding to 2-leaf rice growth stage. This herbicide application timing usually occurs from May to June depending on the planting date, rice variety, and environmental conditions.

During May and June, orchard crops and grapes are in relatively vulnerable growth stages for off-target herbicide exposures. Almond, pistachio, and walnut trees are in vigorous growth periods and actively developing terminal and lateral buds, leaves, spurs, and shoots and accumulating assimilates for kernels (Galla et al. 2018a, 2018b). Grapevine phenological stages at this time range from bloom to veraison (Bettiga 2013), and stone fruits are at a stage when the endocarp (pit) hardening process begins, and the fruit size increases (Strand 1999).

Herbicide drift is the physical movement of spray droplets through the air at the time of application or soon thereafter to any site other than the intended target (Whithaus 2016). Under most circumstances, off-target herbicide exposures are similar to herbicide fractional rates from below 1/100× up to 1/33× of the field application rates of herbicides (Al-Khatib and Peterson 1999). Significant drift events are most frequently associated with relatively high air temperature and wind speed, low relative humidity, small spray droplet size, and short distances to nearby nontarget crops (Whithaus 2016). The concerns of rice herbicide drift to off-target crops in the Sacramento and Northern San Joaquin Valleys are common among growers, crop consultants, and researchers.

Tetflupyrolimet is anticipated to be a useful and widely used herbicide in rice cropping systems (Lombardi and Al-Khatib 2024). It is important to understand the relative sensitivity of crops subjected to fractional rates of tetflupyrolimet, particularly considering the economic impact of California grape, stone fruit, and tree nut industries. To steward tetflupyrolimet prior to its registration, this research was conducted to compare the onset of foliar symptoms from fractional tetflupyrolimet rates onto six tree and vine crops. We analyzed the growth response of almond, peach, pistachio, plum, and walnut trees to tetflupyrolimet at different rates, and evaluated grapevine yield in response to tetflupyrolimet exposure.

Materials and Methods

Study Sites

Six herbicide experiments were conducted in 2022 and 2023 in a 3- to 4-yr-old almond (38.539°N, 121.794°W), peach (38.539°N, 121.794°W), pistachio (38.539°N, 121.793°W), plum (38°538N, 121°794W), and walnut (38°539N, 121°794W) orchards (elev. 18 m) at the University of California, Davis Plant Sciences Field Facility orchards; and in a 25- to 26-yr-old wine grape vineyard (38.525°N, 121.788°W) at the University of California, Davis Department of Viticulture and Enology Tyree Vineyard near Davis, CA. The orchards were established in March 2020 with 'Nonpareil' almond on 'Empyrean 1', 'Coralstar' peach on 'Krymsk 86', 'Kerman' pistachio on 'UCB 1', 'French Improved' plum on 'Krymsk 86', and 'Chandler' walnut on 'clonal RX1'. All almond, peach, plum, and walnut trees were planted 6 m apart within rows and 4.2 m apart between rows, while pistachio trees were 6 m apart within rows and 7 m apart between rows. The vineyard was established in 1998 with a bi-lateral double-cordon-trained 'Grenache' wine grape planted 1.8 m apart within rows and 3.6 m apart between rows.

The soil was classified as a Yolo silt loam with NO_3–N 57 ppm, Olsen-P 26 ppm, K 351 ppm, Na 21 ppm, Ca 8 meq 100 g^{-1}, Mg 10 meq 100 g^{-1}, cation exchange capacity (CEC) 19 meq 100 g^{-1}, organic matter (OM) 2.7%, and pH 6.7 in the orchards; and NO_3–N 23 ppm, Olsen-P 12 ppm, K 288 ppm, Na 12 ppm, Ca 11 meq 100 g^{-1}, Mg 9 meq 100 g^{-1}, CEC 21 meq 100 g^{-1}, OM 2.5%, and pH 7.1 in the vineyard (University of California, Davis Analytical Lab). Irrigation was applied in all crops through a single-line drip irrigation system with emitters spaced every 30 cm during the growing seasons. All trees and vines were maintained free of diseases and insects following standard commercial practices (Bettiga 2013; Strand 1999). In all experiments, weeds between rows were managed with regular mowing and within rows with a mixture 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.

Herbicide Applications

Tetflupyrolimet (Dodhylex™, 400 g ai L^{-1}, FMC Corporation, Philadelphia, PA, USA) herbicide as a suspension concentrate (SC) formulation was applied on June 6, 2022, in all experiments. Tetflupyrolimet was applied at concentrations of 1/200×, 1/100×, 1/33×, 1/10×, and 1× the rice use rate of 125 g ai ha^{-1} on almond, pistachio, and walnut trees. The concentrations actually represent percentages of the use rate as 0.5%, 1%, 3%, 10%, and 100% (Inci et al. 2024b). Due to a limited number of trees and vines, the 1× tetflupyrolimet treatment was not included for grapevines, peach and plum trees. Plots with nontreated trees or vines were also included for comparison in each experiment.

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 cordon-trained vines) with a handheld, carbon dioxide-propelled backpack sprayer calibrated to deliver 187 L ha^{-1} at 206 kPa pressure through XR 8004-VS nozzle tips (TeeJet® Technologies, Wheaton, IL, USA). The sprayer boom had two nozzles spaced 50 cm apart and spray was delivered based on a 3-s pass per tree or vine. Plots were sprayed early in the morning when the weather conditions were calm to avoid herbicide cross contamination to adjacent trees or vines. Environmental conditions at the time of the orchard and vineyard applications were 16 C air temperature, 58% relative humidity (RH), and 0.4 m s^{-1} wind speed on June 6, 2022. All experiments were repeated on May 31, 2023, with a different set of trees or vines in the same orchards and vineyard. Environmental conditions at the time of second-year application were 18 C air temperature, 50% RH, and 0.6 m s^{-1} wind speed. Because the trees were relatively young, no yield data were taken for orchard crops; however, grapevines had 5–10 mm berries present at the time of herbicide application in both 2022 and 2023.

Data Collection and Experimental Design

Experiments were arranged in a randomized complete block design with four replications, where an individual tree or vine was an experimental unit. Trees and vines were observed for visible injury symptoms at 6, 12, 24, 48, and 72 hours after herbicide treatment as well as 7, 14, 21, 28, 35, 42, and 90 d after treatment (DAT). Visible injury was rated on a scale where 0 = no injury and 100 = plant dead.

Tetflupyrolimet treated trees and vines were compared with nontreated control plots at each observation. Furthermore, trunk diameters from almond, peach, pistachio, plum, and walnut trees were measured using a digital caliper with ± 25 µm accuracy at approximately 25 cm above the ground on April 15, October 20, 2022, and April 23, October 20, 2023. The timing of these measurements' correspondence with before the spring growth starts in April (pretreatment) measurements and after the growing season ends in October (post treatment) measurements (Inci et al. 2024b, 2024c). To maintain the consistency of assessments, all trees' trunk diameter were measured four times regardless of whether they were treated in 2022 or 2023 experiments. Tree growth was expressed through trunk diameter growth as a percent increase based on the following formula:

$$Y=\{[(x_{n+1}/x_n)-1]\times 100\}+100$$

where Y is the percent relative change of trunk diameter, x_n = trunk diameter at pretreatment measurements in spring, x_{n+1} = trunk diameter at post treatment measurements ~140 DAT in fall. Thereby, the relative change in herbicide treated trees' trunk diameter was compared to nontreated control trees' trunk diameter change.

Grapes were hand harvested when berries in nontreated control vines reached ~20°Bx (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 nontreated control and weighed for total fruit yield and sugar content from a fruit subsample determined with a handheld refractometer.

Statistical Analysis

Trunk diameter data were subjected to analysis of covariance using AGRICOLAE (de Mendiburu 2024) and DPLYR (Wickham et al. 2024a, 2024b) packages to characterize the growth of the orchard crops with equation $Y = A + BX$, where Y is the predicted value, A is the y-intercept; B is the slope of the line, and X is the observation time in RStudio Version 2024.09.1+394 (R Core

Team 2024). Means were separated using Tukey's honestly significant difference (HSD) post-hoc test at significance level of $P \leq 0.05$, when applicable. The MULTCOMP (Bretz et al. 2010) package was used to generate multiple comparisons among means. Tetflupyrolimet fractional rates were considered fixed factors, while crop, year, and replication were considered random factors. Grape yield and brix were analyzed with analysis of variance (ANOVA) at alpha = 0.05 (Kniss and Streibig 2018). The Type II Wald *F* tests with the Kenward-Roger degrees-of-freedom method and Type III with Satterthwaite's method were used, when the confidence level was 0.95 for both ANOVA types. Graphical illustration was generated using GGPLOT2 package version 3.5.1 in RStudio (Wickham et al. 2024c).

Results and Discussion

In all crops, tetflupyrolimet did not cause any distinguishable injury symptoms at any rating time or at any herbicide treatment including up to the 1× rate of 125 g ai ha^{-1} on almond, pistachio, and walnut (data not shown). The lack of any observed injury could be because established plants are not dependent on the pyrimidine nucleotide biosynthesis as much as developing plants because the pyrimidine nucleotide biosynthesis is energetically expensive, cells utilize pyrimidine nucleotides only if they are rapidly growing and dividing. In higher plants, mature cells can meet their metabolic needs through a salvage pathway, a reutilization mechanism to break down cellular components that are no longer needed and that do not utilize the DHODH enzyme (Zrenner et al. 2006). Tetflupyrolimet, as a DHODH inhibitor, is, therefore, most active against weed seedlings and did not cause any injury symptoms on established trees and vines. No fruit yield data were taken in the orchard crops, fruit that were present appeared normal and consistent among treatments.

Tree trunk diameter change is a common parameter for interpreting orchard crop growth (Inci et al. 2024b, 2024c). The percent of relative change data for 2022 and 2023 were combined ($n = 8$) for tree crops because there were no significant interactions between year and treatment (Wickham et al. 2024a). In all orchard crops, tree trunk diameter change was variable for almond, peach, pistachio, plum, or walnut trees. From April 2022 to October 2023, the relative trunk diameter substantially increased for all tested crops, and the growth was not different ($P < 0.05$) compared to the non-treated control trees (Figures 1 and 2). Almond, peach, and plum trees had an average of ~30 mm trunk diameter increase across all treatments in 2022, whereas walnut and pistachio trees had 22 mm and 12 mm increase, respectively (data not shown). In 2023, tree diameter increases were 17 mm for almond, 10 mm for peach and pistachio, 5 mm for plum, and 7 mm for walnut. At the fall 2023 observations, the total of two seasons of growth, almond, peach, pistachio, plum, and walnut trees, showed cumulative diameter increases of 70 mm, 50 mm, 35 mm, 50 mm, and 45 mm, respectively. Together these data corresponded to an average trunk diameter increase of 230% for almonds, 292% for pistachios, 210% for walnuts (Figure 1), 220% for peaches, and 241% for plums (Figure 2) at the end of two seasons.

The yield response of grapevines treated with tetflupyrolimet fractional rates did not differ ($P < 0.05$) from the nontreated control vines, which was approximately 23.6 kg vine^{-1} in 2022 and 16.6 kg vine^{-1} in 2023 (Table 1). Grape yield was approximately 6.85 kg vine^{-1} lower overall in 2023 than 2022, including nontreated control vines, possibly related to a cooler (average air temperature ~1.5 C lower) season in 2023. Likewise, grape sugar content was similar among all treatments and ranged at approximately 20–21°Bx in 2022 and 2023 harvest (Table 1). The insignificant differences overall indicate that tetflupyrolimet drift events did not cause meaningful impacts on grape yield or brix levels.

Under most conditions, realistic herbicide drift rates range from below 1/100× up to 1/33× of the field use rates of herbicides (Al-Khatib and Peterson 1999; Inci et al. 2024a). This research included tetflupyrolimet at rates up to 1/10× or 1× in these crops to evaluate a worst-case scenario such as consecutive drift events, an accidental herbicide application, or herbicide-contaminated tank, events that are unlikely to happen in a typical drift situation. Even at these exaggerated rates, almond, grape, peach, pistachio, plum, and walnut crops were not injured by tetflupyrolimet exposure. This simulated drift research was conducted using a constant spray volume with variable rates including field use rate in tree nut crops, which is different from actual drift scenarios where both concentration and volume change as herbicides move off-target. Banks and Schroeder (2002) suggested that the droplet concentration can affect crop injury; however, understanding the relative sensitivity of the stone fruit, tree nut, and vine crops species grown near California rice fields is highly relevant to stewardship of tetflupyrolimet in the Sacramento and San Joaquin Valleys (Inci et al. 2024b). Anticipated application advisories for tetflupyrolimet in rice systems should also help prevent the off-target movement of herbicides to nontarget crops (FMC Corporation 2024). Together, these datasets suggest that tetflupyrolimet can be safely used for early-season weed management in rice fields with normal off-target herbicide drift precautions, as noted on the herbicide labels.

Practical Implications

Owing to its outstanding grass activity and crop safety on rice, tetflupyrolimet is expected to be widely used in rice cropping systems (Lombardi and Al-Khatib 2024). Anticipated rice field applications for tetflupyrolimet are as pre-plant, pre-emergence, or early post-emergence up to 3-leaf stage for grasses (FMC Corporation 2024; Lombardi and Al-Khatib 2024). This research suggests that tetflupyrolimet is not likely to cause significant injury to nearby tree and vine crops in the case of off-target drift. Given the apparent low risk of crop injury in tree crops, tetflupyrolimet might be of interest for future registration in these crops. As a new site-of-action, tetflupyrolimet could help manage difficult weeds in orchards and vineyards, such as glyphosate-resistant grasses. Further studies should investigate the use of tetflupyrolimet in floor management of orchard and vineyard production systems.

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Competing Interests. The authors declare they have no competing interests.

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Table 1. Grape yield and sugar concentration response to tetflupyrolimet simulated drift rates in 2022 and 2023 growing seasons near Davis, California.[a,b]

		2022				2023			
Treatment	Rate[c]	Yield[d]		Brix[e]		Yield		Brix	
	g ai ha^{-1}	kg vine^{-1}		°Bx		kg vine^{-1}		°Bx	
NTC	—	23.58	(1.3)	20.67	(0.5)	16.60	(0.2)	20.87	(0.9)
1/200×	0.625	23.34	(1.7)	20.27	(0.4)	16.30	(0.2)	21.25	(0.4)
1/100×	1.25	23.23	(0.8)	20.65	(1.1)	16.37	(0.5)	21.22	(0.7)
1/33×	3.75	23.17	(0.8)	20.82	(1.1)	16.52	(1.1)	21.22	(0.5)
1/10×	12.5	23.01	(1.3)	20.87	(0.9)	16.29	(1.4)	21.05	(0.4)

[a] Abbreviation: NTC, nontreated control treatment.

[b] There were no significant differences within each column at $P < 0.05$ according to Tukey's honestly significant difference post-hoc test.

[c] Tetflupyrolimet rate is expressed as a fraction of the rice use rate, 125 g ai ha^{-1}.

[d] Yield is reported as average mean, where parentheses represent SE.

[e] One degree °Bx is 1 g of sucrose in 100 g of solution, where parentheses represent SE.

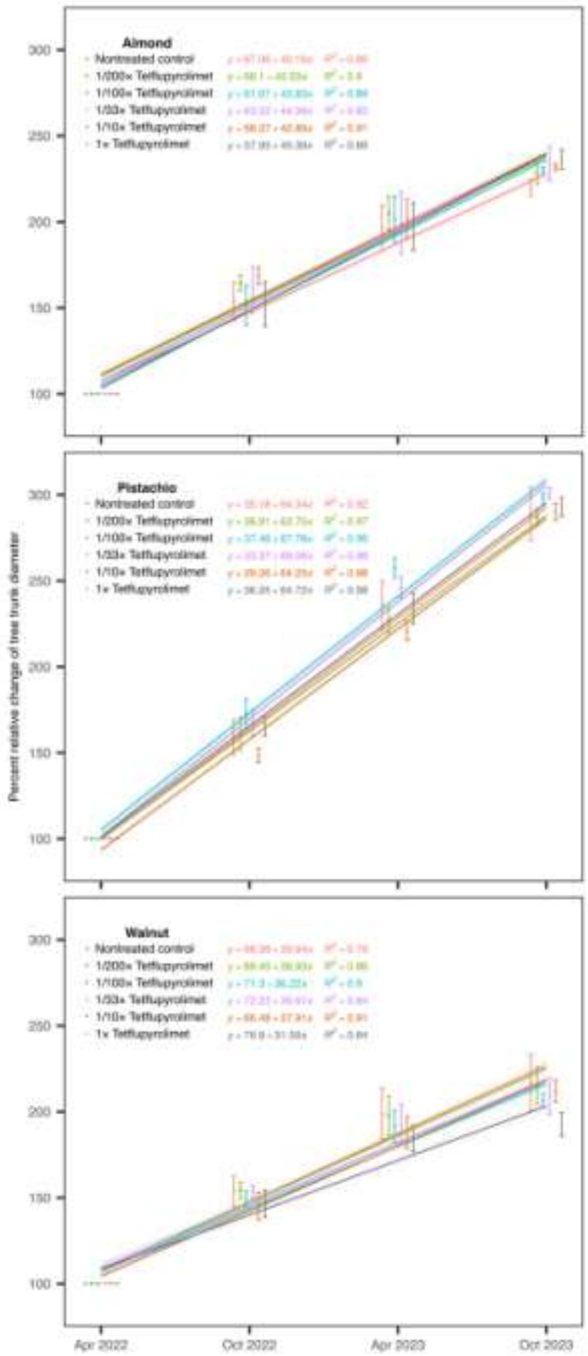

Figure 1. Trunk diameter measurements (n = 8) of almond (top), pistachio (middle), and walnut (bottom) trees before initiation of tetflupyrolimet treatments (April 2022 and 2023) and after treatments (October 2022 and 2023). Tree trunk diameter change was expressed as percent relative change with a linear model $Y = A + BX$, where Y is the predicted value, A is the y-intercept; B is the slope of the line, and X is the observation time. Tetflupyrolimet simulated drift rates were expressed as a fraction of rice use rate of 125 g ai ha^{-1}.

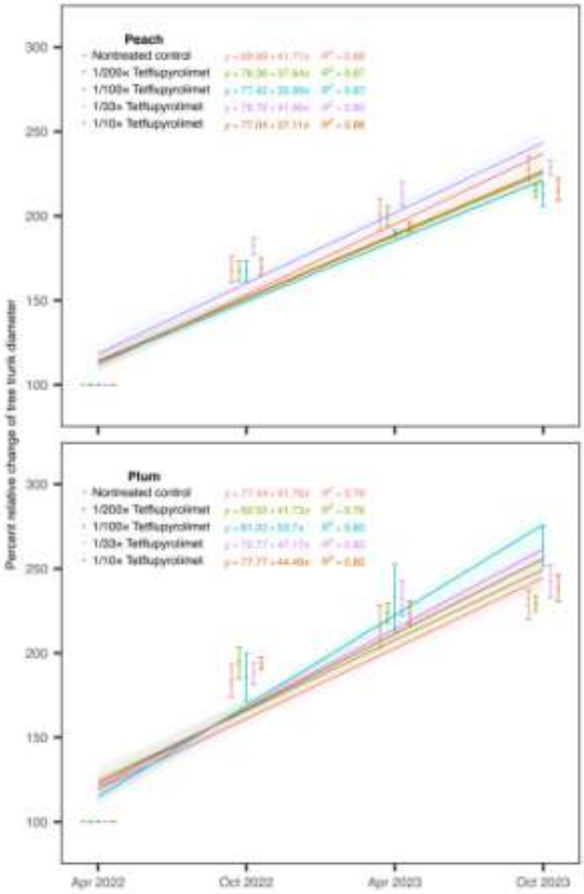

Figure 2. Trunk diameter measurements (*n* = 8) of peach (top) and plum (bottom) trees before initiation of tetflupyrolimet treatments (April 2022 and 2023) and after treatments (October 2022 and 2023). Tree trunk diameter change was expressed as percent relative change with a linear model $Y = A + BX$, where Y is the predicted value, A is the y-intercept; B is the slope of the line, and X is the observation time. Tetflupyrolimet simulated drift rates were expressed as a fraction of rice use rate of 125 g ai ha^{-1}.