ANNUAL REPORT COMPREHENSIVE RESEARCH ON RICE

January 1, 2017 – December 31, 2017

PROJECT TITLE

Continued research on the effects of bispyribac-sodium and other rice herbicide drift on walnut in the Sacramento Valley.

PROJECT LEADER

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LEVEL OF 2017 FUNDING

$ 30,000

OBJECTIVES AND EXPERIMENTS CONDUCTED, BY LOCATION, TO ACCOMPLISH OBJECTIVES

The overall objective of this work is to determine the effects of rice herbicide drift on walnuts, an issue that appears to becoming more widespread as walnut acreage increases in the Sacramento Valley and as new, low use-rate rice herbicide enter the market.

Particularly, the aim is to:

- Evaluate the symptoms and growth effects of simulated herbicide drift on young walnut trees in a field study
- Compare symptoms and growth response of young walnuts exposed to single or multiple exposures to simulated herbicide drift
- Evaluate the effects of simulated herbicide drift on walnut quality and yield
- Determine the effect of herbicide droplet application on walnut leaves and flower buds
- Determine bispyribac persistence in walnut leaves and the minimum herbicide concentration required to cause visible injury to walnut leaves

SUMMARY OF 2017 RESEARCH (major accomplishments), BY OBJECTIVE:

1. Evaluate the symptoms and growth effects of simulated herbicide drift on young walnut trees in a field study

The first run of these studies was conducted during 2015 in an experimental walnut orchard planted in winter 2014 at UC Davis. In June 2016, validation experiments (2nd run) were conducted on another walnut orchard block planted in 2015.

The herbicides tested were: bispyribac-sodium, bensulfuron and propanil. Each herbicide was applied at four rates resembling a plausible drift rate: 0.5%, 1%, 3% and 10% of the high product use rate in rice (44.8, 70.2, and 6725.1 g ai/ha for bispyribac sodium, bensulfuron and propanil, respectively).

The trial was established as a randomized complete block with four replicates, experimental units were single trees. Herbicides were applied from the ground, to one side of the canopy, using a hand held, CO2-pressurized boom at a volume of 93 L/ha with a fine spray quality. Crop oil concentrate (0.5% v/v) was added to all treatments.

Typically, in the Sacramento Valley the majority of rice herbicides are applied between late May and early July. Therefore, in order to conduct the field experiment under the right environmental conditions and at the most applicable walnut growth stage, treatments were applied on May 31, 2016.

Injury estimates and SPAD values were collected one, two, three, four and eight week after herbicide application. Injury estimates were obtained using the following procedure: at each assessment, two injured leaflet per tree were collected and scanned using a desktop scanner. Injured areas of the leaf were separated from the green area using picture thresholding software and the percentage of injured area was calculated.

Prior to treatment application three actively growing shoots per tree were marked and the number of leaves per shoot was counted. Leaf counts were subsequently made four, eight and twelve weeks after treatments were applied.

During the first year of the experiment, nut production was relatively low and highly variable among single tree plots; thus, yield was not evaluated in the year of simulated drift treatment. However, these trees were used for evaluating the effect of simulated drift the year subsequent simulated drift exposure. Thus, walnut yield from trees treated in 2015 was collected on October 13, 2016, and trees treated in summer 2016 were harvested on October 20, 2017.

The effect of simulated drift on same year yield was evaluated on two separate batches of trees in the orchard transplanted in 2015. Due to the low number of available trees, based on visual symptoms and growth data collected in this study in 2015 and 2016, this experiment included only bispyribac-sodium. Three drift rates were tested: 0.5, 1 and 3% of the maximum product rate in rice.

Bispyribac-sodium and bensulfuron-methyl may damage nearby walnut orchards if they drift at significant amounts. The severity of the visual injury symptoms caused by bispyribac-sodium and bensulfuron-methyl may peak approximately one month after exposure but recover in approximately 2 months. No yield, average nut size or quality reduction was observed either the year of simulated drift exposure or the year after simulated drift exposure.

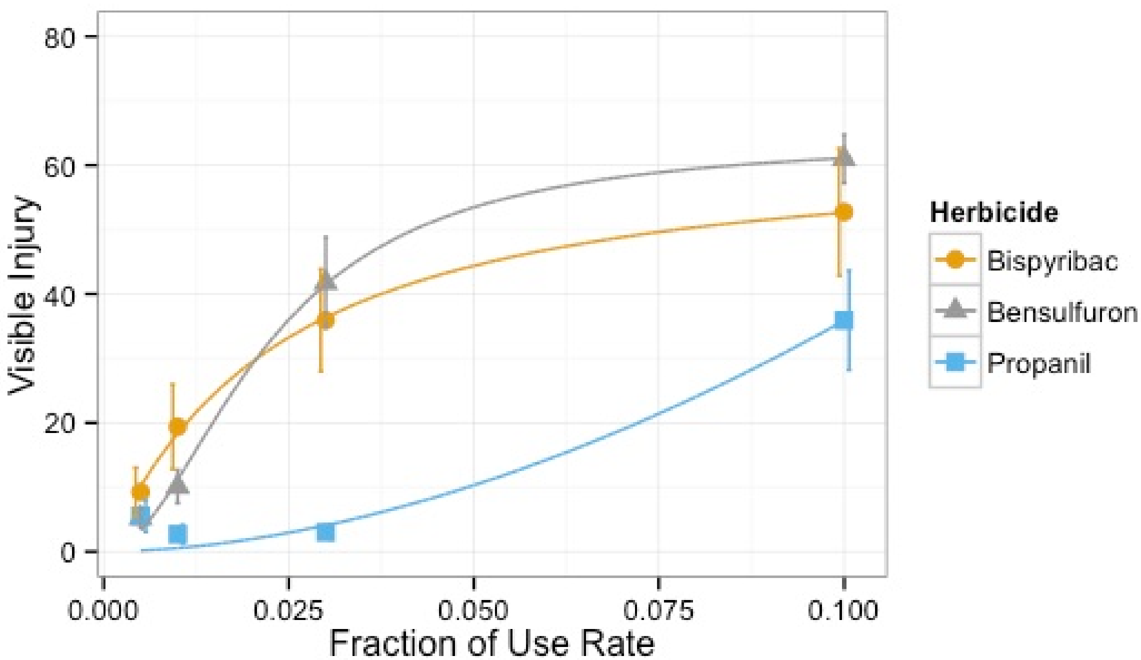

Figure 1 Visual herbicide symptom ratings of walnut trees as affected by simulated herbicide drift rates of bispyribac-sodium, bensulfuron-methyl and propanil

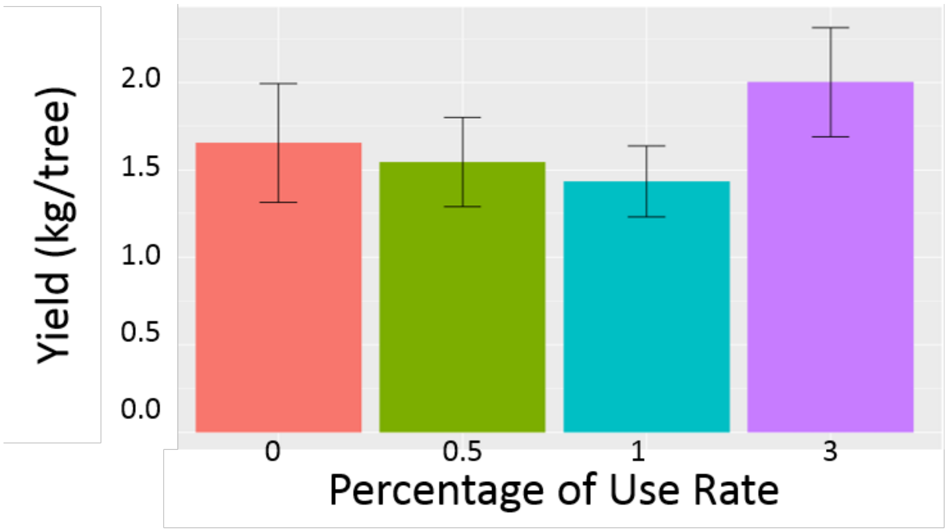

Figure 2 Walnut yield as affected by simulated herbicide drift rates of bispyribac-sodium applied the year of harvest

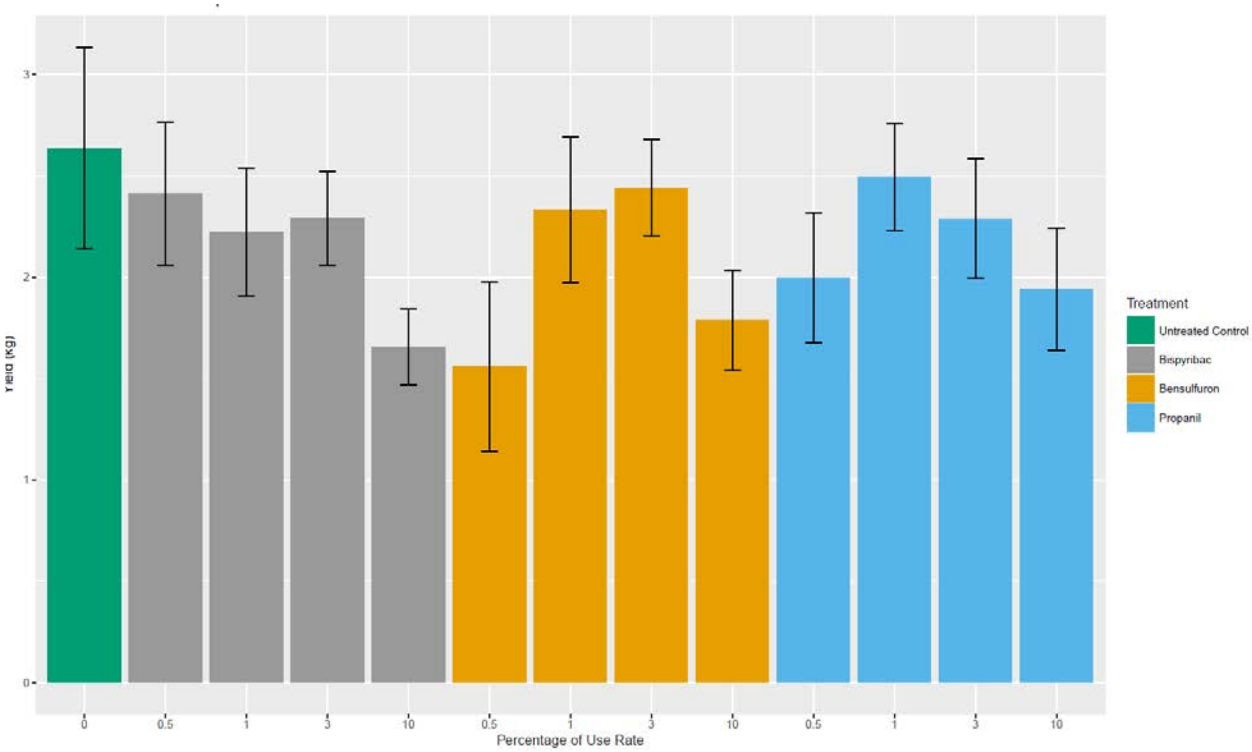

Figure 3 . Walnut yield as affected by simulated herbicide drift rates of bispyribac-sodium, bensulfuron-methyl and propanil applied one year before harvest

2. Compare symptoms and growth response of young walnuts exposed to single or multiple exposures to simulated herbicide drift

A field trial was established in a two year-old chandler walnut orchard at the UC Davis experimental station. In this case, the objective was to simulate a scenario in which a walnut orchard is exposed multiple times in a season to herbicide drift. The first run of these studies was conducted during 2015 in an experimental walnut orchard planted in winter 2014. In June 2016, validation experiments (2nd run) were conducted on another walnut orchard block planted in 2015.

Bispyribac sodium was applied four times at weekly intervals at two different rates: 0.5% and 3% of the rice use rate (44.8 g ai/ha). The first application was made on June 1, 2016.

The trial was established as a randomized complete block with four replicates and experimental units were single trees. Herbicides were applied from the ground, to one side of the canopy, with a hand held, CO2-pressurized boom at a volume of 93 L/ha with a fine spray quality. Crop oil concentrate (0.5% v/v) was added to all treatments. Injury estimates and SPAD values were collected at each treatment application and one, two, three, four and seven weeks after the last application.

Prior to the first treatment application three actively growing shoots per tree were marked and the number of leaves per shoot was counted. Leaf counts were conducted at each assessment time.

During the first year of the experiment, nut production was relatively low and highly variable among single tree plots; thus, yield was not evaluated in the year of simulated drift treatment. However, walnuts were harvested the year after simulated drift exposure. In order to evaluate the effect of simulated drift on reproductive structures that contribute to subsequent year yield, no additional drift simulation treatments were made the year of harvest. Trees were harvested on October 13, 2016 and on October 20, 2017, respectively. The effect of simulated drift on walnut yield and nut quality the year of drift simulation exposure was evaluated in a separate set of trees. In order to reduce variability among single trees, drift simulation treatments were evaluated on trees three years after transplant.

Multiple exposure of bispyribac-sodium drift can cause visual injury and slow growth of young walnut shoots. Growth of shoots treated with the 0.5% rate was somewhat delayed during the treatment regime but appeared to recover after treatments ended; however, walnut shoots exposed to the high rate had fewer internodes than nontreated walnuts at the end of the season, one month after the last application. Although bispyribac-sodium simulated drift showed the potential of causing visual symptoms and also reducing or slowing shoot growth, it did not cause measurable reduction in walnut yield or average nut weight either in the year of exposure or in the subsequent one.

Table 1 Average number of internodes per walnut shoot as affected by four exposures of simulated herbicide drift rates of bispyribac-sodium. Means followed by same letter within a column are not statistically different according to Tukey's test (P < 0.05)

Treatments	Rate	Average number of internodes per shoot							
		7DALA		14DALA		21DALA		28DALA	
	%	----------------------Number of internodes----------------------							
Nontreated control	0	11	a	12	a	13	a	13	a
Bispyribac-sodium	0.5	9	b	10	ab	11	b	11	ab
Bispyribac-sodium	3	8	b	8	b	9	b	10	b

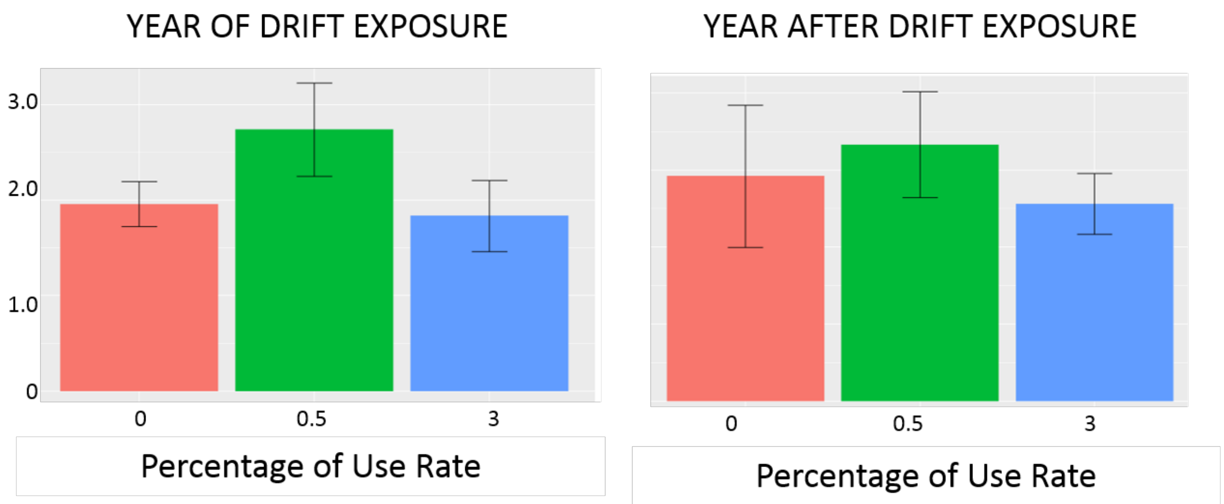

Figure 4 Average nut weight and nut yield per tree as effected by four exposures of simulated drift rates of bispyribac-sodium

3. Evaluate the effects of simulated herbicide drift on walnut quality and yield

Field research was established to evaluate the effect of bispyribac-sodium simulated drift on walnut kernel color, an important quality parameter of walnut nuts. In order to obtain a higher yield and to reduce variability between trees, treatments were applied to three-year-old walnuts.

Two field studies were conducted. In the first study bispyribac-sodium was applied at three rates: 0.5%, 1% and 3% of the use rate in rice. The second study was designed to simulate the worst case scenario. In this case, bispyribac-sodium was applied four times at weekly intervals at two different rates: 0.5% and 3% of the use rate in rice (44.8 g ai/ha).

Both the studies were established as a randomized complete block with four replicates and experimental units were single trees. Herbicides were applied from the ground, to one side of the canopy, with a hand held, CO2-pressurized boom at a volume of 93 L/ha with a fine spray quality.

A 40 nut subsample was taken from each tree and carefully unshelled by hand. Kernels were visually assessed and subdivided into four color-classes as defined by the Dried Fruit of California Association: extra light, light, light amber and amber (DFA, 2016). Because light-colored nuts are considered the best quality and preferred by the market, data are presented as percentage of light-colored nuts (extra light and light classes). In addition, each kernel was rated in a 1 to 4 score where 1 is extra light and 4 is amber and the average color score is presented.

A single exposure of bispyribac-sodium simulated drift did not significantly affect the color of nut kernels. A significant positive liner correlation, however, was found between drift rates and average color score. Higher herbicide drift rates tended to be associated with darker kernel color. On the other hand, in the second study, nuts harvested from trees exposed to four drift simulation events were significantly darker than nuts collected from nontreated trees.

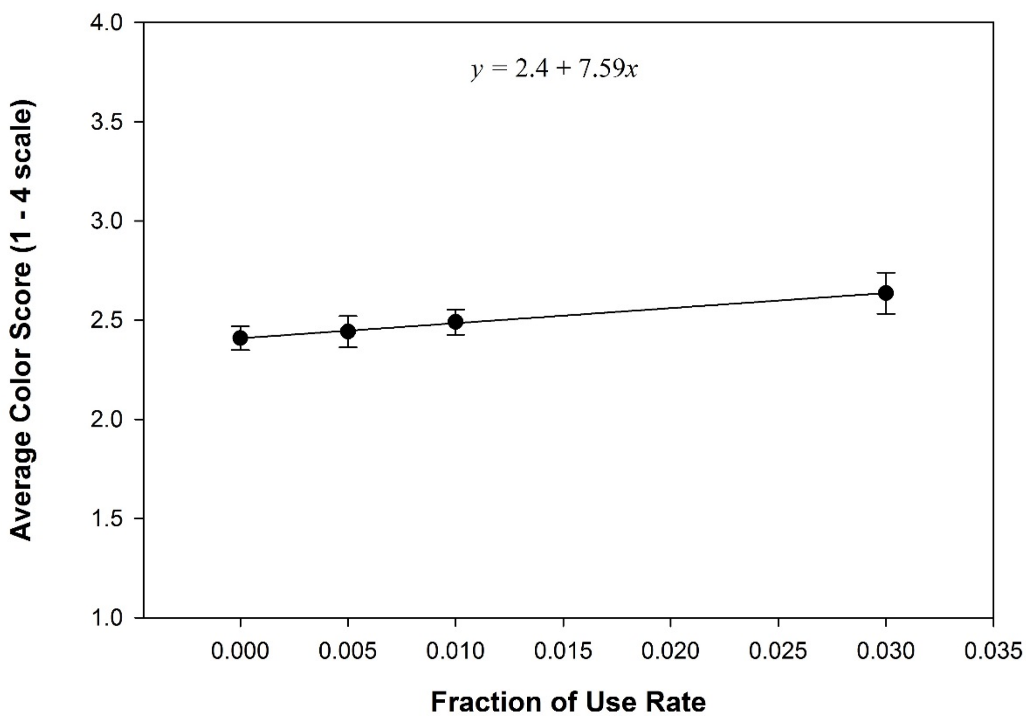

Figure 5 Walnut kernel color as affected by bispyribac-sodium drift. (1 to 4 score where 1 is extra light and 4 is amber)

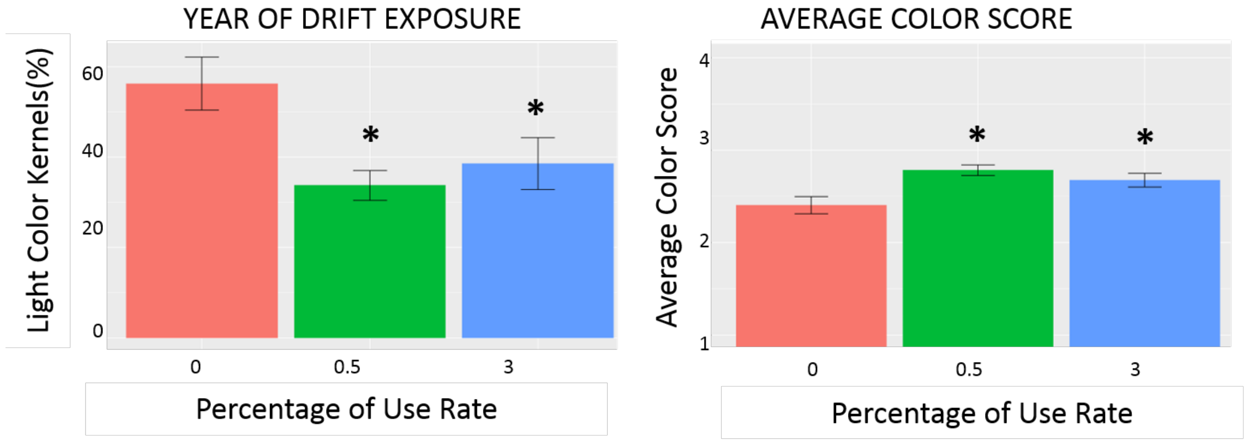

Figure 6 Walnut kernel color as affected by four exposures to simulated drift rates of bispyribac-sodium applied the year of harvest.

4. Determine the effect of herbicide droplet application on walnut leaves and flower buds

The objective of this study is to evaluate the direct impact of micro-droplet application of bispyribac on walnut and compare the effects caused by leaf application with those caused by bud application.

In July 2016, a field study was initiated in a four-year old walnut orchard and replicated in a different location at the UC Davis experimental station. The trial was established as a randomized complete block with four replicates and experimental units were single branches. Flower formation and shoot development on the treated branches was monitored in summer 2017.

Effect of droplet application on leaves. The herbicide was applied at three rates: 1, 3 and 5 one-microliter droplets of rice label aerial rate (0.8 oz/ac at 5 gal/ac volume) solution of bispyribac. In each branch, 10 leaves were selected and the treatment was applied to a maximum of 5 leaflets per leaf.

Effect of droplet application on axillary buds. Spray drift is a dynamic process due to evaporative losses of water in spray droplet as the droplet is carried off target. Water loss would increase the concentration of the herbicide within the droplet. In this experiment, bisypyribac was applied as a 1-microliter droplet directly on the axillary buds of walnut at three different concentrations:

1. 0.8 oz/ac at 5 gal/ac volume (typical aerial application rate)
2. 0.8 oz/ac at 3 gal/ac volume
3. 0.8 oz/ac at 1gal/ac volume

In summer 2017 an additional study was established and conducted in three different sites with the objective to evaluate the direct impact of bispyribac-sodium droplets on walnut fruit.

Bispyribac-sodium was applied as a 1-microliter droplet directly on actively growing fruit at two different concentrations:

1. 0.8 oz/ac at 5 gal/ac volume
2. 0.8 oz/ac at 1gal/ac volume

In general, walnut flower formation and shoot development in 2017 were negatively affected only when bispyribac-sodium droplets were applied to the auxiliary buds at high concentration (1 gal/ac) in summer 2016. However, in summer 2017, all the nuts that received bispyribac-sodium dropped within one week of the treatment.

5. Determine bispyribac persistence in walnut leaves and the minimum herbicide concentration required to cause visible injury to walnut leaves

Field data showed that bispyribac is able to cause injury symptoms on walnuts exposed to rates as low as 0.5% of the use rate in rice. While other common herbicide residues are often found in walnut leaf tissues, analysis of walnut leaf samples with ALS-inhibitor symptoms usually does not find detectable bispyribac-sodium residue levels.

Field research was established to determine if bispyribac-sodium can generate visual symptoms without leaving detectable residues on walnut leaf tissues, to estimate the level of drift exposure necessary to generate detectable bispyribac-sodium residues and to determine if there is a correlation between yield and bispyribac-sodium residues on leaf tissue.

The research was conducted in two separate areas of the experimental orchard. In the first one bispyribac-sodium was applied at 0.125%, 0.25%, 0.5% and 1% of the normal use rate in rice (44.8 g ai ha^{-1}). In the second one rates were 1%, 3%, 10% and 100% of the use rate.

Bispyribac-sodium caused phytotoxic effects on walnut leaves even at very low concentration, as symptoms were recorded on trees exposed to rates as low as 0.125% of the rice use rate. No residues, however, were detected in walnut leaf tissues sampled from trees exposed to 1% or lower rates. By 10 days after treatment when symptoms were visually apparent, 3% of the rice use rate was the lowest rate at which it was possible to detect bispyribac-sodium residues in symptomatic walnut leaves. In general, visible injury symptoms may remain constant over time or even worsen while bispyribac-sodium residues decrease and are finally not detectable. There was no clear correlation between chemical residue detection and measured yield parameters.

Table 2 Visual herbicide symptom ratings of walnut trees as affected by simulated herbicide drift rates of bispyribac-sodium

Treatments	Rate	Visual Injury 10 DAT		Visual Injury 20 DAT	
	%	------------% ------------			
Bispyribac-sodium	0.125	1	a*	1	a*
Bispyribac-sodium	0.25	2	a	2	a
Bispyribac-sodium	0.5	2	a	3	a
Bispyribac-sodium	1	12	b	15	b

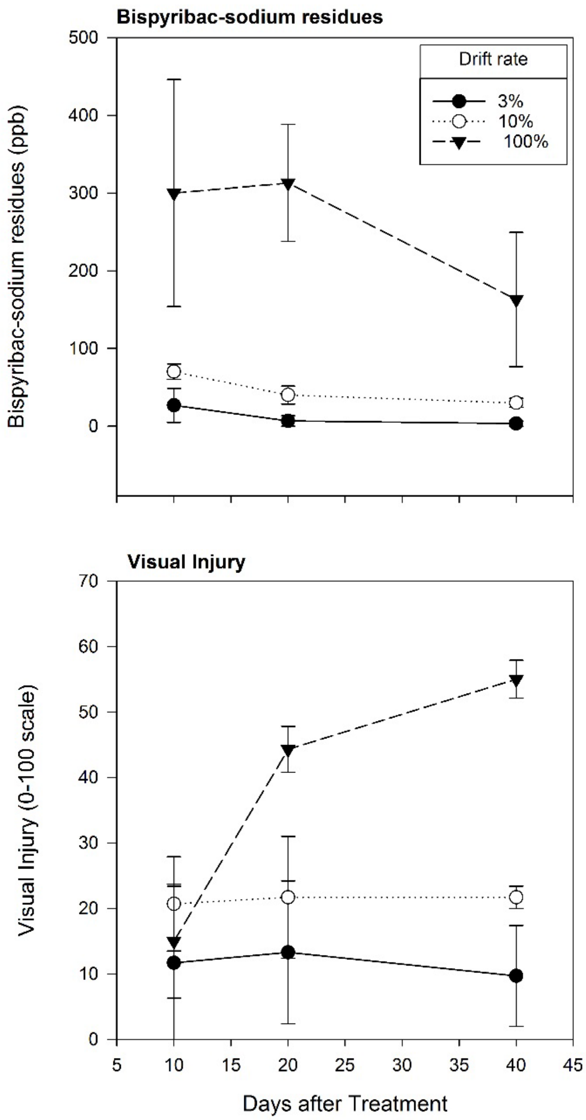

Figure 7 Visual herbicide symptom ratings (bottom) and bispyribac-sodium residues (top) following simulated herbicide drift rates of bispyribac-sodium

PUBLICATIONS OR REPORTS:

Galla MF, Al-Khatib K, and Hanson BD (2017) Response of Walnuts to Simulated Drift Rates of Bispyribac-sodium, Bensulfuron-methyl and Propanil. Submitted for publication in Weed Technology on November 22, 2017

Galla MF, Al-Khatib K, and Hanson BD (2017) Walnut Response to Multiple Exposures to Simulated Drift of Bispyribac-sodium. Submitted for publication in Weed Technology on November 22, 2017

Galla MF, Hanson BD and Al-Khatib K (2017) Injury symptoms and detection of bispyribac-sodium in walnut leaves following simulated drift. Submitted for publication in Environmental Toxicology and Chemistry on December 5, 2017

Galla MF, Al-Khatib K, and Hanson BD (2016) Effects of simulated rice herbicide drift rates on walnuts. Proc. California Weed Science Society. Sacramento, CA, January 14, 2016. 68:82.

Galla MF, Al-Khatib K, and Hanson BD (2016) Response of walnut to simulated drift of rice herbicides. Proc. Western Society of Weed Science. Albuquerque, NM, March 7, 2016. (paper #29).

Galla MF, Al-Khatib K, and Hanson BD (2016) Walnut response to simulated drift rates of selected herbicides. 60th Annual UC Weed Day booklet pg 3-4.

Galla MF, Al-Khatib K, and Hanson BD (2016) Effect of multiple exposures of simulated drift rates of rice herbicides on walnut. 60th Annual UC Weed Day booklet pg 5-6.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS

In 2017, four field studies were conducted and the last harvest data from two more field study were collected. This report presents the final conclusion of a multi-year research project. The overall objective was to determine the effect of herbicide drift on walnut growth, yield and development.

Initially field research was conducted to evaluate symptoms, growth and yield of walnut trees exposed to simulated drift of three rice herbicides selected among the most frequently used in the Sacramento Valley (Objective 1 and 3). Bispyribac-sodium and bensulfuron-methyl, two ALS-inhibitor herbicides, and propanil, a photosystem II inhibitor herbicide, were included in the study. Bispyribac-sodium and bensulfuron-methyl showed more phytotoxic activity than propanil and they may damage nearby walnut orchards if they drifted at significant amounts. In addition, while no yield effect was observed for any of the herbicide tested, the results showed that bispyribac-sodium has the potential to affect walnut kernel color the year of drift exposure and walnut yield the year following drift exposure.

Once bispyribac-sodium was identified as the rice herbicide with higher impact potential on walnuts, the next phase of research was to evaluate more in detail the effect and damaging potential of bispyribac-sodium simulated drift (Objective 2 and 3). Because the majority of rice in the Sacramento Valley are sprayed within a very short window of time it is plausible that a walnut orchard could be exposed to multiple drift events in one season. Bispyribac-sodium was applied four times, at weekly intervals at 0.5% and 3% of the normal use rate in rice. In general, while trees exposed to the lower rate appeared to recover, branches of trees exposed to the 3% rate had fewer internodes than nontreated trees at the end of the growing season. The symptoms observed, however, did not result in measureable yield reduction either the year of drift exposure or the year following drift exposure. Both rates, however, negatively affected walnut kernel color in the year of drift exposure but it was not determined if this was a direct effect of the herbicide or an herbicide-induced stress response. An additional study was established to evaluate the direct impact of bispyribac-sodium droplets in walnut leaves, axillary buds and fruit (Objective 4). Results showed that bispyribac-sodium droplet, at high enough concentration, on axillary buds may affect shoot emergence and growth in the following season. Furthermore, bispyribac-sodium droplets caused actively growing walnut fruit to drop.

While other common herbicide residues are often found in walnut leaf tissues, analysis of walnut leaf samples with ALS-inhibitor symptoms usually does not find detectable bispyribac-sodium residue levels. Therefore, a study was established to determine if bispyribac-sodium can generate visual symptoms without leaving detectable residues on walnut leaf tissues and to determine if there is a correlation between yield and bispyribac-sodium residues in leaf tissue. (Objective 5). Analytical results from symptomatic leaves showed that at low rates (1% or less than the use rate in rice) bispyribac-sodium can generate visual symptoms without leaving detectable levels of chemical residues in the leaf tissue. In general, symptoms may remain constant over time or even worsen while bispyribac-sodium residues decrease and are finally not detectable. No correlation between yield and chemical residues in walnut leaves was found.

In summary, this research shows that bispyribac-sodium drift may potentially be an issue for walnut orchards in the Sacramento Valley. Considering that downwind drift deposits generally range from 1 to 8%, however, it is unlikely that in a field situation bispyribac-sodium would drift at high enough levels to cause significant yield and quality effects observed from in this work