

## **EVALUATING GLYPHOSATE-MICRONUTRIENT INTERACTIONS IN DRIED PLUM**

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Herbicides are an important part of orchard floor management in dried plum orchards as well as in nearby cropping systems. Dried plum growers occasionally observe tree injury from inadvertent exposure to herbicides drifting from adjacent crop fields or from within-orchard applications. For several years, we have addressed the short- and longer-term effects of simulated herbicide drift on established and young plum trees.

In 2013-15, experiments were conducted to address questions about glyphosate soil residues in the soil. Although glyphosate is generally considered to have little to no soil activity and few negative impacts on the soil environment, there have recently been questions raised about whether high use rates and frequent applications of glyphosate herbicide are having unexpected effects on orchard crops. These questions are usually along the lines of either 1) can glyphosate be taken up by tree roots close to the soil surface? Or 2) does glyphosate in the soil affect micronutrient availability as rumored in some trade magazines?

Prune herbicide research objectives addressed during FY13-15:

1. Evaluate the visual symptoms and growth effects of repeated applications of glyphosate on young orchard trees.
2. Determine how soil type and irrigation regime affects potential glyphosate exposure to young trees via root uptake.

### **PROCEDURES**

An experimental orchard was established at UC Davis in 2013. Half of the French prune trees were planted in the soil native to the field in Yolo County (eg Rincon silty clay loam) and half were planted in soil imported from Merced County (eg. Delhi sandy loam). Prior to planting dormant nursery stock in February 2013, the site was prepared by augering each tree site with a 30-inch diameter auger and refilling with either the native soil or the imported sandy soil. The test orchard contains 64 prune trees planted in a 10 by 20 spacing and irrigated with microsprinklers at each tree.

No herbicide treatments were imposed in 2013 in order to ensure good tree establishment. Beginning in spring 2014, the orchard was subjected to various treatment combinations of glyphosate and irrigation regimes (Table 1). The experimental design is a factorial arrangement of two soils, four glyphosate rates, and two post-treatment irrigation regimes and each treatment is replicated four times in single-tree plots.

Glyphosate was applied three times in 2014 and three times in 2015 by research personnel using CO<sub>2</sub>-pressurized backpack sprayer and a 2-nozzle boom calibrated to deliver 20 GPA spray solution. Treatments were applied to the soil at the base of the trees in order to mimic a “strip” application; spray boom orientation and tree protectors minimized any direct herbicide exposure to the tree bark or leaves. Immediately after each herbicide application, the post treatment drench treatments were imposed by applying an equivalent of one acre-inch of water in the immediate area of the tree. Water was contained with an earthen berm in order to force percolation into soil immediately surrounding the tree.

Data collection in the first year of the trial included evaluations of tree growth and chlorophyll content at six time points during the growing season and shikimate accumulation after each treatment. Specifically, tree growth was evaluated by comparing canopy area as determined by digital photography and image analysis using ImageJ software and plotting changes over the season. Chlorophyll content was determined on representative new and older leaves from each tree using a SPAD meter; this provides an approximation of the “greenness” of the leaves which indicates overall health and function of the photosynthetic apparatus. Shikimate accumulation, which provides a measurement of whether glyphosate is inhibiting the target enzyme (EPSPS) in the shikimic acid pathway, was measured 14 days after each application. In this assay, multiple young leaves were collected from each tree, frozen and ground to a powder in liquid nitrogen and shikimate levels determined using a laboratory spectrophotometric assay. As a supplement to this field research, a greenhouse pot experiment was conducted using the same soils to evaluate the potential effects of glyphosate on soil micronutrient availability, namely zinc, iron, copper, manganese, nickel, calcium, and magnesium. Soils were treated with 0, 1x, or 32x glyphosate doses, the herbicide was thoroughly mixed in the soil, and pots were incubated under irrigated conditions. Soil samples were extracted using both DTPA and Mehlich III methods and the extractant samples were sent to the UC Davis Analytical Lab for nutrient analyses. After leaf-fall in 2014, trunk diameter was compared to the trunk diameter of each tree prior to treatment initiation in spring 2014 to assess relative growth rates.

In the second year of the study, 2015, data collection was limited to SPAD measurements and visual injury assessments approximately 30 days after each glyphosate treatment and water drench. Shikimate analyses were deemed too laborious to continue given that we saw no significant shikimate accumulation from any glyphosate treatment during the first year of the trial. The trees were too large to effectively use the digital image canopy analysis used in 2014.

## RESULTS AND DISCUSSION

**Prune tree growth 2014:** Glyphosate treatments as high as 4 lb ae/A applied three times during the 2014 growing season did not reduce tree growth (Figure 1) as measured by digital image analysis of prune canopy size. In fact, the slowest growing trees tended to be in the untreated plots; probably due to an early infestation of field bindweed that was well-controlled by glyphosate but not by the contact-only program used in the zero glyphosate plots. This trend was also observed in trunk caliper changes during the 2014 growing season.

**SPAD measurements 2014:** There were no statistical differences in the chlorophyll leaf content among the three glyphosate treatments (1, 2, and 4 lb ae/A) in the two soils or in the post

treatment drench application (Figure 2) in the first year of the study. On several rating dates, the no-glyphosate treatments tended to have slightly elevated SPAD values but this was attributed to the fact that the slower growing trees (shown in Figure 1) tended to have fewer young leaves; older leaves are typically “greener” than expanding young leaves.

**Shikimate accumulation 2014:** There were no obvious trends in shikimate accumulation that could be associated with either glyphosate rate, coarse soil, or the post treatment drench (Figure 3). These results suggest that there is not a significant effect of glyphosate taken up by prune roots in this study, even a 4x use rate applied three times during the growing season.

**Soil micronutrient analysis 2014-15:** The soil nutrient analysis conducted in the greenhouse using the same soils as in the field study are presented in Figure 4. As expected, there were very large differences in available nutrients between the two soil types for all nutrients analyzed. However, in terms of glyphosate effects, there were no differences in extractable zinc, iron, copper among the 0, 1x, or 32x glyphosate doses. There was a slight, but not statistically significant, reduction in extractable manganese in the clay loam soil as measured by the DTPA extraction but it was not as apparent in the data from the Mehlich III technique.

**SPAD measurements 2015:** Leaf chlorophyll content was evaluated 30 days after each treatment in 2015. Similar to the 2014 results, there were no statistical differences among treatments in SPAD values among soil type or glyphosate treatment (Figure 5). SPAD values were higher overall during the July evaluation, but this was the case for all treatments and was likely a function of environment and water status in the orchard rather than a treatment effect. Regular visual observations of old and young leaves did not suggest any herbicide-related injury at any time during the 2015 season (data not shown).

## CONCLUSIONS

Overall, this two-year experiment did not reveal any measurable negative effect of glyphosate on French prune trees. In the expected “worst case” treatment, soil at the base of the trees planted in sandy, low CEC soil were treated six times with a 4x rate of glyphosate over two growing seasons; however even in these treatments no visual injury was observed, there was no accumulation of shikimate in young leaves (evaluated only in year 1), and no differences in chlorophyll content was observed. While this does not rule out the possibility that glyphosate injury could occur via root uptake, it suggests that the phenomena is not easy or probably very common in California prune orchards. These prune results mirror the observations conducted concurrently in almond and cherries at the same site.

## VALUE TO INDUSTRY:

In the most recent pesticide use reports (2013) glyphosate herbicides were applied to 46,650 acres of prunes in California. This is the most widely used herbicide in the cropping system by more than double the next most important herbicide. Additionally, given that herbicide in orchards are usually applied to strips under the tree row (approximately 20-50% of the orchard floor), these data suggest that most of the ~49,0000 acres of prune trees are exposed to glyphosate at least twice per season. While this research does not provide a direct economic

benefit to the industry, it directly addresses important grower concerns about their most important chemical weed management tool.

Table 1. Glyphosate treatments to address the effect of glyphosate in the soil on young prune trees at the Davis site.

trt	soil	Glyphosate rate (lb ae/A)*	post treatment irrigation
1	clay loam	0	no extra
2	clay loam	1	no extra
3	clay loam	2	no extra
4	clay loam	4	no extra
5	clay loam	0	post trt drench
6	clay loam	1	post trt drench
7	clay loam	2	post trt drench
8	clay loam	4	post trt drench
9	sandy loam	0	no extra
10	sandy loam	1	no extra
11	sandy loam	2	no extra
12	sandy loam	4	no extra
13	sandy loam	0	post trt drench
14	sandy loam	1	post trt drench
15	sandy loam	2	post trt drench
16	sandy loam	4	post trt drench

\* One lb ae/A is equivalent to 28 fl oz of Roundup Powermax and is in the mid-range of glyphosate application rates commonly used in orchard crops. Post treatment drench was intended to mimics 1-acre inch irrigation immediately following the herbicide application.

## BUDGET SUMMARY:

The relatively modest budget for this project was used to provide approximately 10% FTE support for a postdoctoral researcher who conducted a series of plant and soil experiments on glyphosate soil and foliar uptake and possible interactions between glyphosate and soil or foliar micronutrients. The budget for the project will be expended by the end of the contract period with about \$3300 spent on supplies and field expenses and 4311 spent on support scientist salary and benefits. Although travel funds were budgeted in this proposal, other funding sources were used for local and regional travel instead.

## WEED SCIENCE PROGRAM:

Support from the dried plum board complements our statewide research and extension program that focuses on weed management in orchard and vineyard cropping systems. Our research covers several broad areas applicable to most California tree and vine systems: methyl bromide alternatives in nursery and orchard replant situations, chemical and non-chemical weed control in orchards and vineyards, biology and management of herbicide resistant weeds, and herbicide fate in plants and the environment. Results are routinely presented to growers and industry stakeholders in extension presentations, field days, and scientific meetings as appropriate as well as extended through the cooperative extension network.

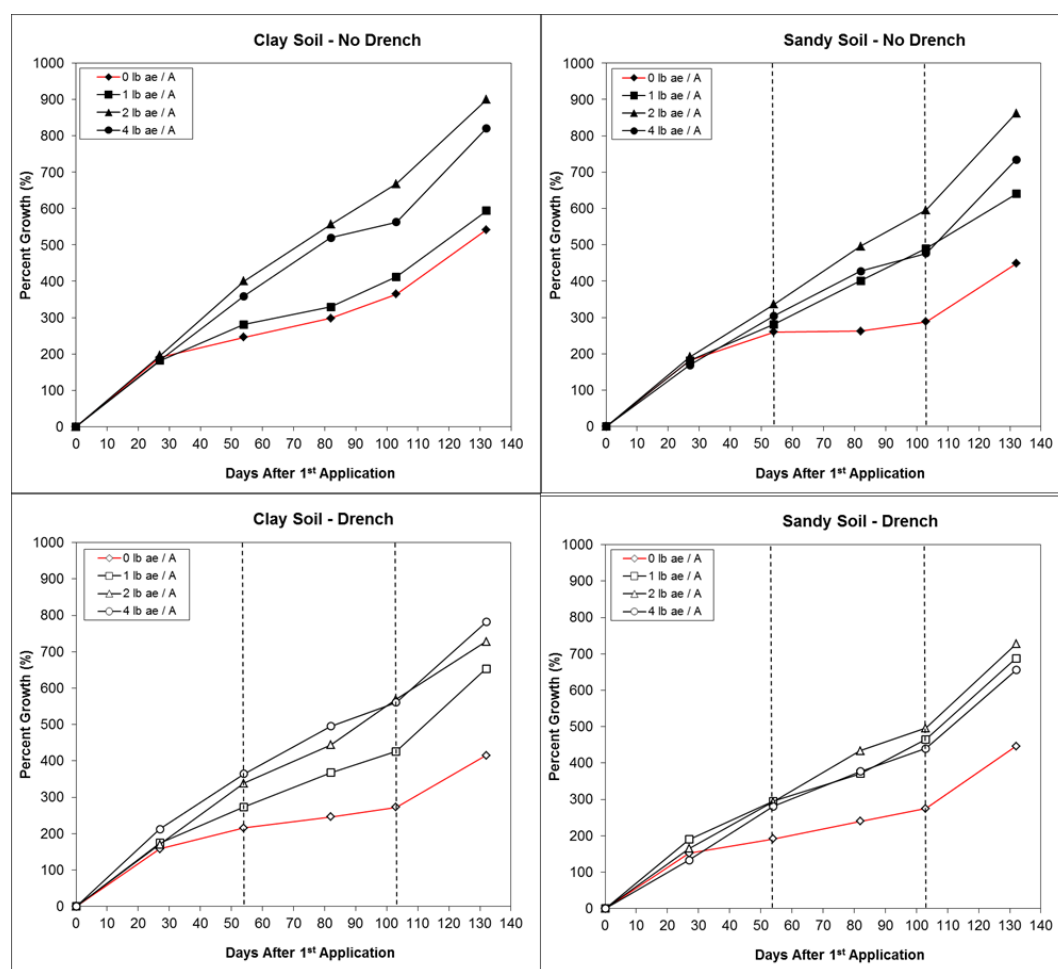


Figure 1. Relative growth rates of French prune on Mariana 2624 rootstocks following three applications of glyphosate at 1, 2, and 4x normal use rates in a UC Davis study. Trees were in their second growing season and were planted in native clay soils or in tree sites amended with sandy soils. Drench treatments received a simulated 1 acre-inch irrigation within five minutes of each glyphosate application.

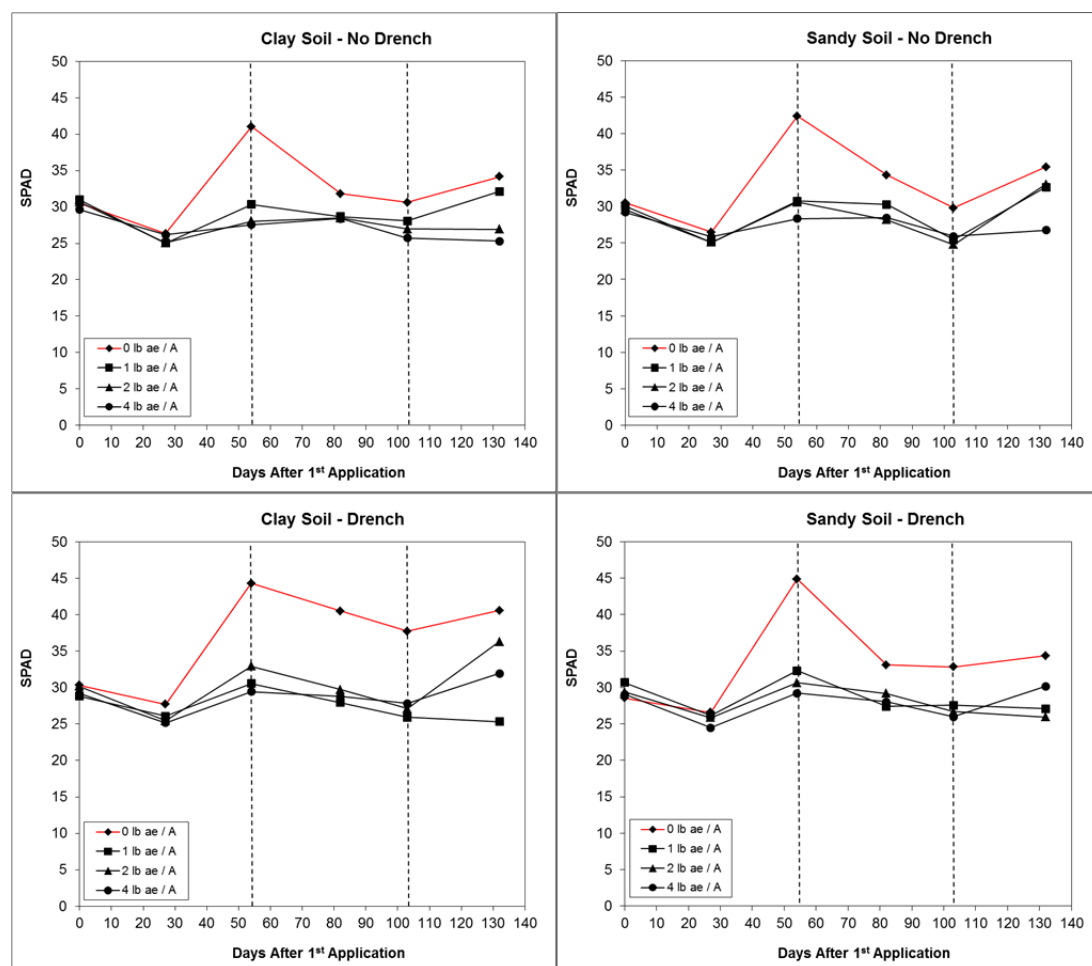


Figure 2. SPAD values as a measure of leaf chlorophyll content of French prune on Mariana 2624 rootstocks following three applications of glyphosate at 1, 2, and 4x normal use rates in a UC Davis study. Trees were in their second growing season and were planted in native clay soils or in tree sites amended with sandy soils. Drench treatments received a simulated 1 acre-inch irrigation within five minutes of each glyphosate application.

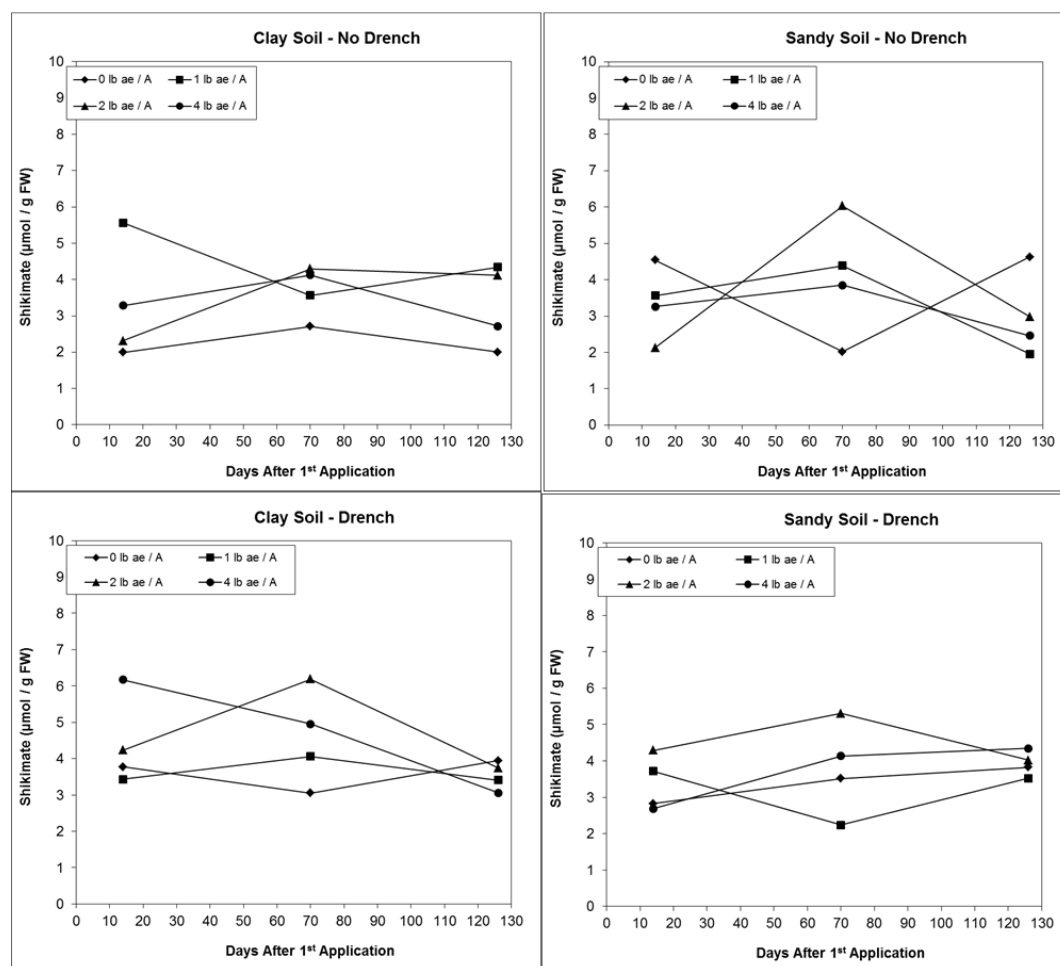


Figure 3. Shikimate accumulation of new leaves on French prune on Mariana 2624 rootstocks following three applications of glyphosate at 1, 2, and 4x normal use rates in a UC Davis study. Shikimate accumulation indicates the activity of glyphosate on the target enzyme EPSPS. Trees were in their second growing season and were planted in native clay soils or in tree sites amended with sandy soils. Drench treatments received a simulated 1 acre-inch irrigation within five minutes of each glyphosate application.

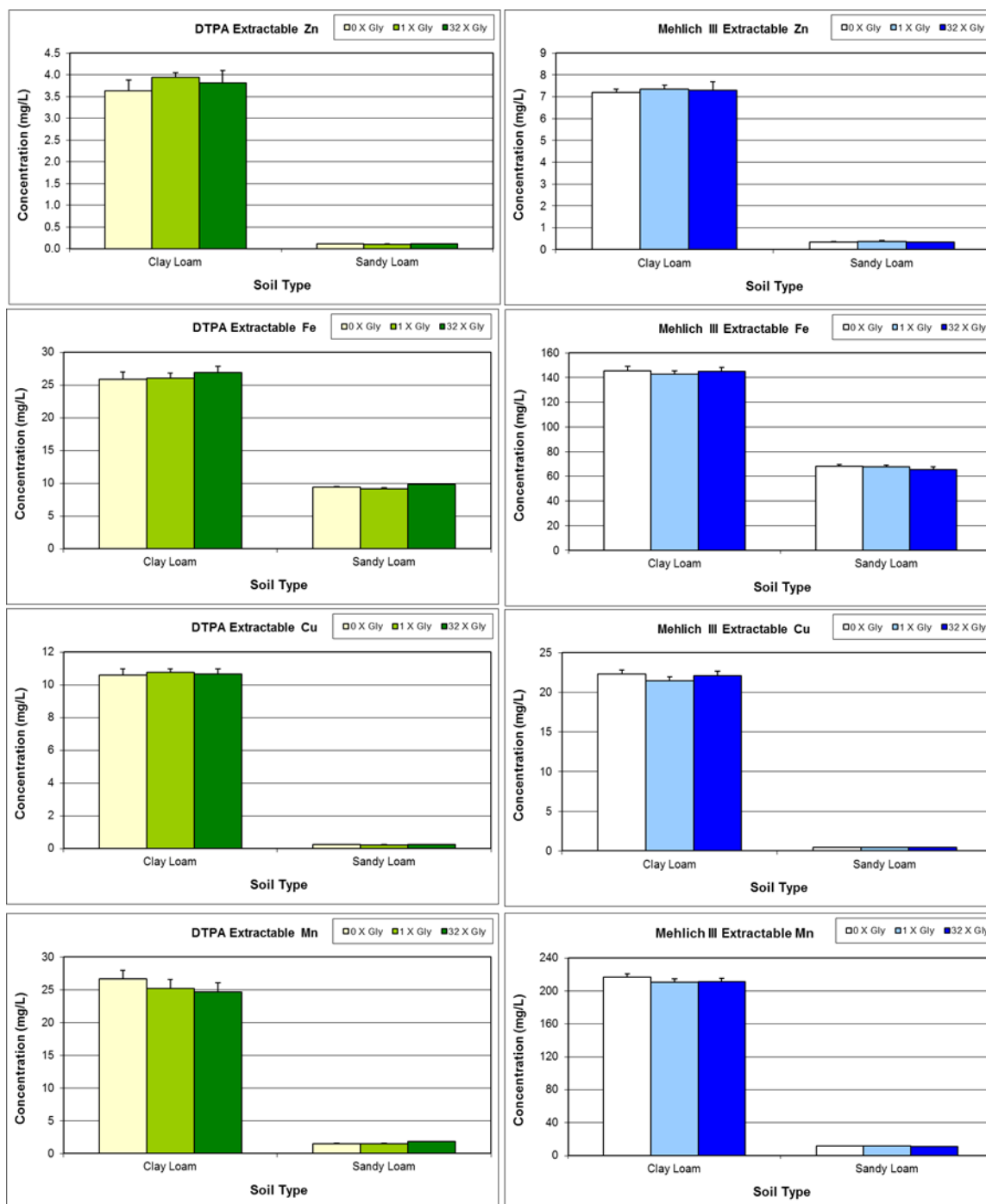
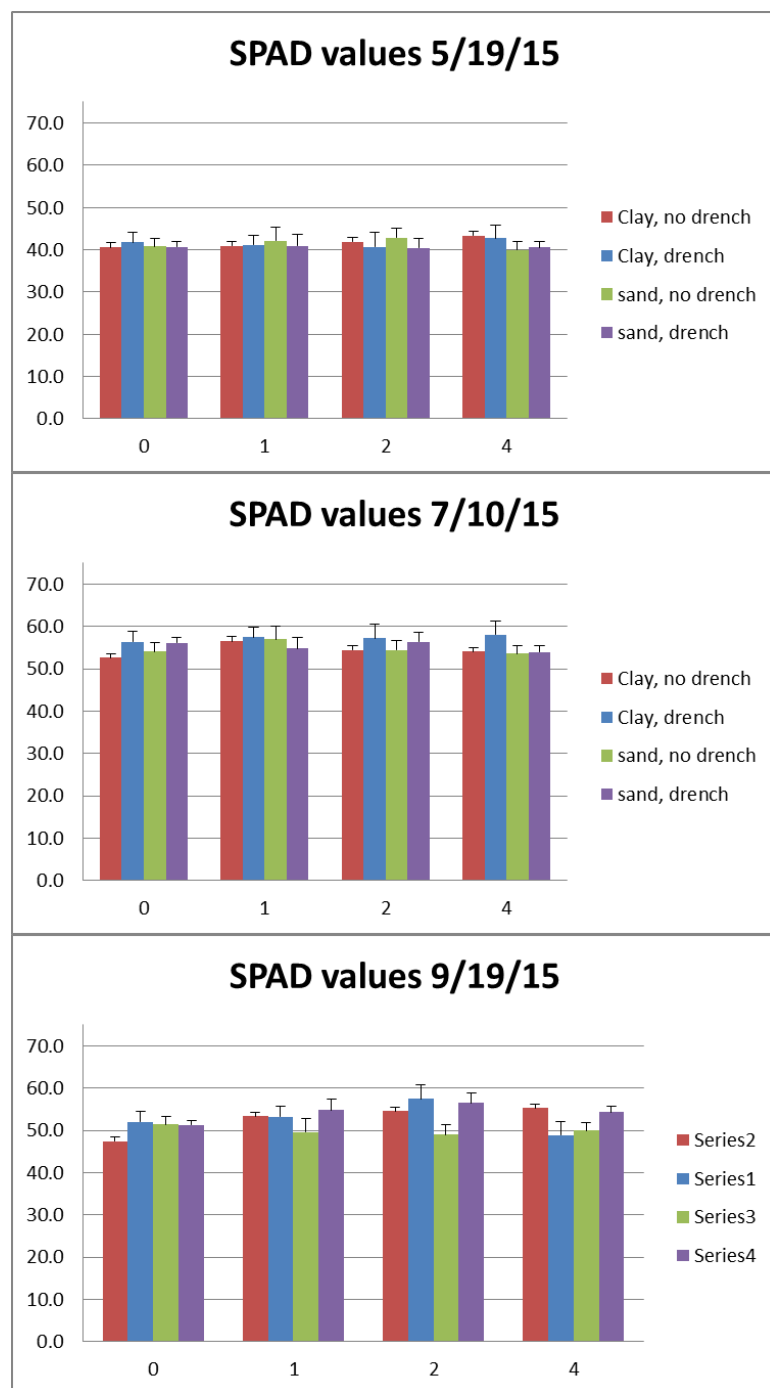


Figure 4. Soil nutrient analysis from field soils treated with glyphosate at doses intended to mimic zero, low, and very high concentrations relative to field applications. These soils were incubated in pots in the greenhouse after being mixed with glyphosate; while the soils were watered regularly, they did not include any growing plants. After the incubation period, micronutrients were extracted using DTPA or Mehlich III protocols to minimize potential interactions between soils and nutrient analyses.





*Figure 5. SPAD values as a measure of leaf chlorophyll content of French prune on Mariana 2624 rootstocks following three applications of glyphosate in 2014 and three in 2015. Glyphosate was applied at 1, 2, and 4x normal use rates and these data were collected approximately 30 days after treatment. Trees were in their third growing season and were planted in native clay soils or in tree sites amended with sandy soils. Drench treatments received a simulated 1 acre-inch irrigation within five minutes of each glyphosate application.*