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Effects of Glyphosate Application on Preharvest Fruit Drop and Yield in 'Valencia' Citrus

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Abstract. **The use of glyphosate as a post-emergent weed management tool is crucial in Florida citrus production. However, extensive and nonjudicious application of glyphosate has drawn increasing concerns about its inadvertent effects on citrus, mainly linked to its possible impacts on preharvest fruit drop. Our study investigated the effect of applying glyphosate in the tree rows near the fruit harvesting window on fruit drop and yield in 'Valencia' sweet orange. Field trials were conducted at Southwest Florida Research and Education Center, Immokalee, FL. The experiments had a randomized complete block design with four replications. Three different doses of glyphosate within the labeled range of rates in citrus (i.e., low, medium, and high at 0.84, 2.10, and 4.20 kg acid equivalents of glyphosate per hectare, respectively) along with a water control treatment were sprayed in 'Valencia' citrus tree rows close to the harvesting period and assessed for their effects on preharvest fruit drop and yield. Our findings show that glyphosate application near the harvesting window may influence the fruit detachment force (FDF) in Valencia citrus; however, no significant effect on increasing fruit drop or reducing yield was observed during this 2-year study.**

Citrus growers in Florida face weed management problems throughout the year because of conditions that favor the rapid growth of weeds in groves. A weed-free tree row is desired in a citrus grove to minimize weed competition with the trees. Hence, chemical weed control using post-emergent, systemic herbicides like glyphosate has become a vital production practice in the Florida citrus industry. After its introduction in the 1970s, glyphosate became very popular among citrus growers for its broad-spectrum weed control under trees and in the area between the rows. The use of glyphosate as a "burndown" application, alone or in combination with other herbicides, became a standard practice in citrus groves. Recent usage data indicate glyphosate as a primary herbicide option for citrus production in the state (USDA, 2017).

In spite of positive assessments of weed control effectiveness, an increasing number of more recent observations suggest a relationship between extensive glyphosate application and adverse nontargeted effects in agroecosystems (Ferreira et al., 2017; Kanissery et al., 2019). Similarly, there is an ongoing debate on whether glyphosate used for weed management in the citrus grove has unintended consequences on the trees (Gravena et al., 2012). In particular, there are growing concerns among the citrus growers associated with the extensive use of this herbicide, its persistence in the soil, and the potential effects on fruit drop and productivity in citrus (Hest, 1996; Kanissery et al., 2018).

Although several studies discuss the adverse effects of glyphosate exposure on diverse crops (Combellack, 1984), only a few reports on the impact of glyphosate in citrus are available, and many of them are limited to visual evaluations of the herbicide injury resulting from glyphosate application. For instance, Gravena et al. (2012) and Tucker (1977) evaluated the glyphosate-induced damage in citrus and noticed that the damage levels are dependent on tree age, application rate, and contact area exposed to herbicide. Foliar contact to glyphosate sprayings was more harmful in citrus trees compared with trunk-directed spray. In addition, young trees are more sensitive to glyphosate sprayings than mature trees (Tucker, 1977). Most impacts of glyphosate in citrus are temporary (Gravena et al., 2009, 2012); however, in certain instances, trees can take up to 2 years to recover from herbicide injury and resume normal growth (Toth and Morrison, 1977).

Fruit drop in citrus is a natural phenomenon following activation of the abscission process and is significantly exacerbated by HLB, the disease associated with the bacterium *Candidatus* Liberibacter asiaticus (*C*Las) that has drastically reduced citrus fruit production in the past 15 years in Florida (Chen et al., 2016; Tang et al., 2019). Some research reports have suggested that the sensitivity of fruit drop to glyphosate increases as fruit approaches maturity. For instance, glyphosate sprayed at 6-week-old citrus fruit did not result in damage and drop; however, a massive drop was observed when the herbicide came in contact with 5-month-old fruit (Tucker, 1977). Another prior study reported increased fruit drop and yield reduction in early-season oranges and grapefruit following late summer and fall glyphosate applications (Hest, 1996). The reason for this glyphosate-linked fruit drop is not well understood, but the contact of spray with the developing fruits likely plays a major role in promoting the observed fruit drop (Kanissery et al., 2018). Heavy fruit-bearing or low-lying branches are more likely to be affected by the herbicide spray because of direct contact of the fruits to glyphosate. The potential of glyphosate to injure fruits and affect productivity through spray drift and the consequential fruit-herbicide contact has been documented on several other fruit trees, including cherries and apples (Rosenberger et al., 2013; Rothwell et al., 2008).

It is well known that glyphosate enhances ethylene production in plant tissues (Abu-Irmaileh et al., 1979; Lee and Dumas, 1983). Ethylene may regulate abscission in citrus (Goren, 1993) and is associated with regulating different maturation processes in citrus, ultimately leading to senescence, abscission, fruit drop, carotenoid synthesis, and chlorophyll degradation (Alferez et al., 2021; Goldschmidt et al., 1993). In addition, exposure of mature citrus fruit to ethylene may result in abscission and fruit drop (Alferez et al., 2006;

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Lipe and Morgan, 1972). FDF, the parameter used in this study to assess the likelihood of fruits to abscise from the peduncle, is an indicator of the occurrence of such abscission and has been used previously to determine the fruit drop potential in citrus (Alferez et al., 2006; Chen et al., 2016; Khefifi et al., 2020). Although glyphosate has been found to dissipate relatively quickly from the topsoil following application, its potential to persist in a citrus root zone in Florida's citrus production systems has been reported previously (Biwek et al., 2021). Although the uptake of glyphosate residue through roots and its subsequent impact on citrus crops has not been established, such possibilities have been observed in several crop production systems (Cornish and Burgin, 2005; Tong et al., 2017). Hence, the main objective of the current study is to evaluate the potential of glyphosate in promoting preharvest fruit drop when applied in 'Valencia' sweet orange tree rows near the harvesting timeline. The information generated from the study will be used for fine-tuning glyphosate's crop-safe usage in citrus.

Materials and Methods

Two field trials were conducted in 2017 and 2018 in the experimental citrus orchard at the University of Florida Southwest Florida Research and Education Center (Immokalee, FL). Citrus trees included in this study were 10-year-old trees of 'Valencia' grafted on 'Swingle' rootstock. All trees in this study were symptomatic for HLB throughout the 2-year trial. The trees were spaced 3.0 to 3.6 × 6.7 m within and between rows. Standard orchard management practices were followed throughout the study for nutrition, irrigation, disease, and pest management (University of Florida, 2021).

Experimental design and treatments. The experimental design was a randomized complete block with four replications. The treatments consisted of three rates of glyphosate, low [0.84 kg·ha^{-1} acid equivalent (a.e.)], medium (2.10 kg·ha^{-1} a.e.), and high (4.20 kg·ha^{-1} a.e.), in addition to untreated control consisting of water spray. The rates selected were within the recommended range of glyphosate rates in citrus (Kanissery et al., 2021). Each replicate consisted of 10 trees in the 2017 trial and five trees in the 2018 trial. One tree was left as a buffer space between the experimental plots.

Herbicide application. Herbicide spray solutions were made from the glyphosate product (Roundup Powermax, 48.7% glyphosate in the form of its potassium salt, Environmental Protection Agency registration number 524–549) according to manufacturer recommendation. The different rates were prepared by mixing the herbicide with water, and application was made from an herbicide boom mounted on the front of a tractor with the spray carrier volume at 280 L·ha^{-1}. The boom height was 0.45 m, and the spray bandwidth was 3.96 m, including both sides of the tree. Treatments were applied once into the tree rows as an early spring season spray, ≈10 weeks before the scheduled fruit harvesting. Injury on the fruit from direct

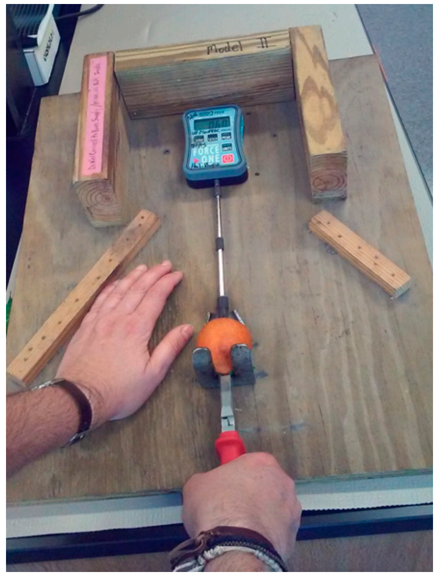

Fig. 1. Digital pull force gauge used for measuring the fruit detachment force of citrus fruits.

spray contact during glyphosate application from the herbicide boom was monitored; however, any substantial damage symptoms on the fruit rind were not observed as a result of the spraying.

FDF measurement. Fruits containing a 5-cm-length peduncle were harvested from each experimental plot biweekly for up to 6 weeks following the herbicide application. A total of four replicates of five fruits from the lower parts of the trees (up to ≈1.5 m aboveground level) were collected from each experimental plot at 2, 4, and 6 weeks after treatments. Collected fruits were transported to the laboratory, where the detachment force of the fruits from the peduncle at the pedicel was assessed. The force was measured in kilogram-force (KgF) using a digital pull force gauge (Force One; Wagner Instruments, Greenwich, CT) (Fig. 1); fruit stems were secured by a clamp connected to the force gauge, and fruits were then pulled to mimic hand harvesting.

Fruit drop and yield data collection. The fruits dropped underneath the trees in each experimental plot were manually counted and cleared weekly beginning on the first day after treatment and continued up to the harvest in both trials. In 2018, the number and weight of the fruits (i.e., yield) in the trees from treated plots were documented during the harvest.

Statistical analysis. The data were tabulated in Microsoft excel and tested using PROC GLM in SAS version 9.4 (SAS Institute, Cary, NC). The data were tested for the assumptions of the linear model and log-transformed whenever applicable before subjecting to analysis of variance for testing statistically significant differences between the means. The means were separated using Tukey's honestly significant difference (α = 0.05). Pearson correlation coefficient was used to measure the strength of a linear association between FDF and glyphosate rates.

Results

Fruit detachment force. The FDFs measured from the citrus fruits in the 2017 and 2018 trials are presented in Fig. 2. Differences in FDF between the untreated control and glyphosate-treated plots were observed at

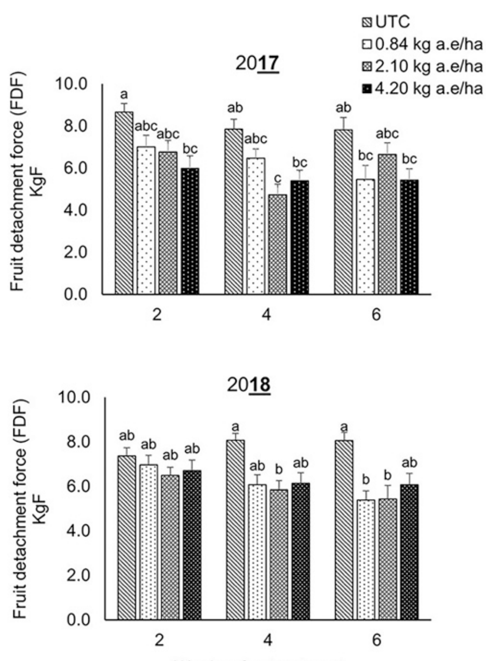

Fig. 2. Fruit detachment force (FDF) in citrus measured from untreated control (UTC) and various glyphosate treatments (kg·ha^{-1} a.e.) in 2017 and 2018 trials. Bars with the same letters do not significantly differ based on Tukey's honestly significant difference (α = 0.05). Error bars indicate SE of 20 observations.

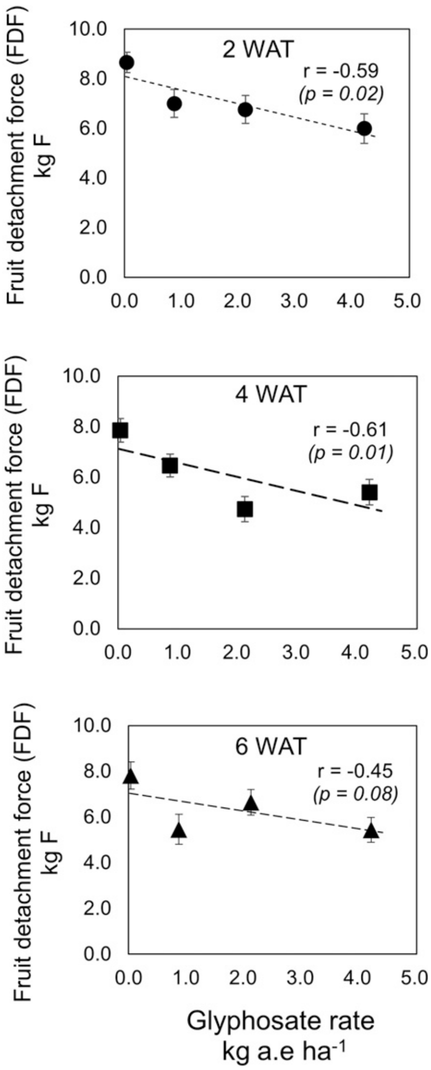

Fig. 3. Citrus fruit detachment force (FDF) measured at different weeks after treatment (WAT) in response to glyphosate application rates (kg·ha^{-1} a.e.); r represents the correlation coefficient between the FDF and glyphosate rates, with the corresponding *P* value. Error bars indicate SE of 20 observations. Data shown from the 2017 trial; a similar trend was observed for the 2018 trial.

2, 4, and 6 weeks following herbicide application in 2017, where the FDF from herbicide-treated experimental plots was lower than that from the untreated control. A similar trend was observed in 2018, particularly at 4 and 6 weeks after applying the treatments. However, significant differences in FDF between the herbicide treatments and untreated controls were not observed at every time point. In both years' trials, a dependence of the FDF reduction on the glyphosate rate was observed from 2 weeks after treatments, as noted from the negative correlation coefficient (r = −0.59; *P* = 0.01) between the FDF and glyphosate rates (Fig. 3). Generally, the reduction in the FDF became less correlated with glyphosate rates as the time progresses, supported by the lower r value in the correlation plots of FDF measurements in response to glyphosate application rates for the final sampling time point.

Fruit drop and yield. Table 1 shows the total preharvest fruit drop per tree and fruit drop per tree as a percentage of initial fruit set in response to different treatments in the two trials. The mean number of fruit drops per tree ranged between 22 and 24 fruits in 2017, whereas the range increased to 33 to 39 fruits per tree in 2018; however, no significant difference was found among treatments in either year. The percentage of fruit drop was calculated based on the total fruits before initiating the trial, and the number of fruits dropped during the observation period before the harvest. After applying the treatments, the average fruit drop per tree was between 34% and 39% in 2017 and 13% and 17% in 2018, with no statistically significant differences. The effects of different treatments on citrus yield (2018 trial) are also shown in Table 1. The mean yields ranged from 127 to 166 fruits per tree in all treatments, with no significant differences among them.

Discussion

A general reduction in FDF was found in glyphosate-treated plots; lower FDF indicates that relatively less force is required to abscise the fruit from the branches. However, an increase in fruit drop compared with untreated control was not observed in any of them. Some authors, including Tucker (1977) and Erickson (1996), have reported that fruit drop is likely to occur, especially when glyphosate sprays contact the fruits. As fruits grow and mature, the fruit load may cause branches to droop, thus increasing the likelihood of fruit exposure to sprays during herbicide application near harvest. Glyphosate may also potentially enter citrus trees through foliage or stem from undirected spray applications or through root absorption from soil residues (Sharma and Singh, 2001). This herbicide has been shown to promote the production of ethylene in plant tissues (Abu-Irmaileh et al., 1979; Grossbard, 1985), which can induce fruit and leaf abscission and affect color development in maturing citrus fruit (Ismail, 1970, 1971; Ritenour et al., 2003). Increased ethylene concentrations resulting from glyphosate reaching the fruit tissues may enhance fruit abscission (Estornell et al., 2013); however, scarce information is available on the amount of glyphosate required to trigger ethylene production that is high enough to induce abscission in citrus fruits. In this study, the evidence of a reduction in FDF in glyphosate-treated plots indicates the possible effect of herbicide application near the harvesting period in the FDF; however, seemingly, the decrease in FDF was not sufficient to make a significant impact on fruit drop. It has been reported for citrus, FDF must be reduced to a threshold of 6 KgF to become detrimental (Tang et al., 2019), but this reduction likely needs to be maintained over time to induce fruit drop. In the absence of deleterious climatic conditions (i.e., heavy rainfall, hail, or wind), a transient reduction in FDF would not trigger fruit drop. In other words, glyphosate may decrease FDF and render the fruits prone to premature drop, but other concomitant factors, such as biotic and abiotic stresses may be necessary for fruit drop to occur.

Even though not statistically significant, the reduction in fruit yield in the untreated control plots could be attributed to the competition between trees and weeds. Tracking the movement of glyphosate within the citrus tree and studying the tree's physiological and metabolic response to this herbicide would better help to understand its impact on citrus. Hence, additional research is needed to understand the possibility of root uptake and translocation of glyphosate in citrus plants and the consequent response of citrus trees to high doses of glyphosate. Furthermore, in future experiments, efforts to measure ethylene production need to be performed while evaluating the herbicide-related fruit drop and yield loss in HLB-affected citrus trees.

Conclusions

Glyphosate is a widely used and essential weed management tool in citrus groves for controlling emerged weeds throughout the

Table 1. Effects of glyphosate application on preharvest fruit drop and yield in citrus.

	Total no. of fruit dropped per tree[z]		Fruit drop per tree as % ofinitial fruit set[z]		Yield per tree[z,y]	
Treatments	2017	2018	2017	2018	No. of fruits	Harvest wt (kg)
Glyphosate at 0.84 kg·ha^{-1} a.e.	22 ± 2 a[x]	39 ± 3 a	36 ± 2 a	16 ± 1 a	166 ± 14 a	26.0 ± 2.16 a
Glyphosate at 2.10 kg·ha^{-1} a.e.	22 ± 2 a	32 ± 2 a	35 ± 2 a	13 ± 1 a	204 ± 15 a	33.5 ± 2.18 a
Glyphosate at 4.20 kg·ha^{-1} a.e.	24 ± 3 a	33 ± 3 a	34 ± 3 a	16 ± 2 a	207 ± 11 a	33.6 ± 1.66 a
Untreated control	22 ± 2 a	33 ± 3 a	39 ± 4 a	17 ± 1 a	180 ± 14 a	31.0 ± 2.25 a

[z]Mean values ± SE.

[y]Yield from the 2018 trial.

[x]Letters represent similarity within columns based on Tukey's honestly significant difference (α = 0.05); data were normalized by log-transformation before analysis.

year. In this study, the glyphosate application in citrus groves during early spring, close to fruit harvest in 'Valencia' citrus, did not cause any significant effect on the preharvest fruit drop; however, a reduction in FDF, an indicator of fruit holding force to the bearing branch, was observed in trees located in glyphosate treatments. Based on our observations, we conclude that by avoiding early spring season sprays of glyphosate close to harvesting date (e.g., within 10 weeks of harvest), in late spring maturing citrus varieties like 'Valencia,' the yield safety of glyphosate-based herbicide programs could be possibly ensured. For instance, using alternative herbicide products to manage weeds in the tree rows and practicing mechanical (e.g., mowing) or cultural (e.g., cover crops) strategies to manage vegetation in interrows will help reduce exposure of glyphosate to citrus trees during the periods near fruit maturity and harvest. In addition, adopting proper spray practices such as maintaining a safe spray boom distance and carefully positioning the angle of off-center spray nozzles to prevent any potential glyphosate exposure to the citrus fruit, foliage, and trunk will also help enhance the crop-safe use of glyphosate in citrus.

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