



Agronomic implications of diflufenzopyr application in peanut: transient physiological responses and sustained yield performance

Erika R. Bucior^{1,2} · Ronald B. Sorensen¹ · Joseph S. McIntyre¹ · Amanda A. Cardoso² · Matthew J. Taggart² · Marshall C. Lamb¹

Received: 9 July 2025 / Accepted: 29 October 2025 / Published online: 24 November 2025
© The Author(s) 2025

Abstract

Low-dose applications of certain herbicides have been hypothesized to induce physiological responses that conserve water and reduce aflatoxin contamination risk in peanut (*Arachis hypogaea* L.). Motivated by reported leaf rolling following diflufenzopyr application, this study evaluated whether herbicide-induced stomatal closure and biomechanical changes could serve as a water-saving mechanism during late-season stress. Farm field, rainout shelter plot, and greenhouse experiments were conducted under irrigated and drought conditions to quantify the impacts of herbicide treatment on gas exchange, soil water potential, stem water potential, and leaf biomechanics. Herbicide application in well irrigated plots unexpectedly led to a short-term increase in transpiration, stomatal conductance, and photosynthesis without impacting plant physiological capacity or soil drying rate. However, herbicide application under drought conditions accelerated soil drying and promoted a more rapid physiological decline of plants in drier plots. However, stem water potential and leaf inclination data suggest only temporary disruption in stomatal regulation post-treatment, and after leaves had mechanically compensated to the stress by folding, physiological recovery was seen. Despite these physiological interruptions, responses were ultimately transient and not sustained more than a few days, and there was no significant yield or aflatoxin impacts measured in any treatments. These results revise the initial hypothesis of herbicide-induced water conservation by revealing a more complex, context-dependent interaction between plant water status herbicide response, and agronomic implications.

Keywords Peanut (*Arachis hypogaea*) · Herbicide response · Stomatal regulation · Physiological plasticity

Introduction

Arachis hypogaea (peanut) is a globally important oilseed and food crop, integral to both human nutrition and agricultural economies, particularly in the southeastern United States where it forms a cornerstone of regional farming systems. Despite its agronomic and economic significance, peanut production faces persistent challenges from abiotic stresses, notably drought. Drought stress disrupts physiological homeostasis and carbon assimilation, causing yield

reductions and quality degradation (Qiao et al. 2024). A particularly severe late-season consequence of drought is increased susceptibility to infection by the soilborne fungus *Aspergillus flavus*, which produces aflatoxins in infected pods (Payne 1998; Zakaria 2024). Aflatoxins produce toxic and carcinogenic effects in humans and animals (Dhakal et al. 2023). Aflatoxin contamination represents a critical food safety and economic threat worldwide, with regulatory limits imposing strict penalties on contaminated produce. Because *A. flavus* thrives under dry soil conditions and during pod development stages characterized by indeterminate flowering, agronomic strategies aimed at preserving soil moisture and promoting timely pod maturation are central to reducing aflatoxin risk (Torres et al. 2014).

In this context, the application of herbicides to modulate plant developmental processes and water use presents a potentially innovative approach to aflatoxin mitigation. Diflufenzopyr, an auxin transport inhibitor herbicide, has

✉ Erika R. Bucior
erbucior@ncsu.edu

¹ USDA-ARS National Peanut Research Laboratory, Dawson, GA, USA

² Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA

been demonstrated to induce late-season flower termination in peanut when applied at sublethal doses (Lamb et al. 2017). Auxin inhibitors interfere with the polar transport of indole-3-acetic acid (IAA), a key plant hormone regulating cell elongation, division, and organogenesis, including flower initiation and development (Gomes et al. 2021). By disrupting auxin gradients, diflufenzopyr can alter growth patterns without causing overt phytotoxicity, as seen by the absence of leaf necrosis or tissue damage in treated plants at recommended dosages (Pakdaman et al. 2018).

One notable physiological response observed following diflufenzopyr application is leaf rolling, a hydronastic movement that reduces leaf surface area exposed to sunlight and air, thereby diminishing transpiration and photodamage. Leaf rolling is a well-characterized drought-adaptive mechanism that modulates leaf water status by reducing evaporative demand and minimizing water loss during periods of limited soil moisture (Kadioglu et al. 2012). This response, while energetically costly due to alterations in leaf turgor and morphology, serves as a short-term buffer that helps maintain internal water balance and protect photosynthetic tissues from excess radiation and desiccation.

Building on these physiological observations, we hypothesized that diflufenzopyr's transient induction of leaf rolling and possible stomatal closure could contribute to reduced transpiration rates, conserving soil moisture and thereby limiting the favorable dry conditions for *A. flavus* colonization. The initial premise was that such modulation of plant water relations might serve as an indirect, non-chemical strategy to suppress fungal proliferation and subsequent aflatoxin production during critical late-season drought periods.

However, plant responses to herbicide application are complex and multifaceted, involving not only transient physiological changes but also potential downstream effects on carbon assimilation, growth, and reproductive output. Therefore, a comprehensive evaluation of diflufenzopyr's net impact on peanut physiology and agronomic performance is necessary to assess its practical viability as a management tool. This study thus pivots from focusing solely on early physiological indicators to a more integrative assessment encompassing transpiration dynamics measured hourly via load cell systems in greenhouse experiments, alongside gas exchange parameters (net carbon assimilation, stomatal conductance, and transpiration)—recorded in rainout shelter plots. This investigation also incorporates multi-year field trials evaluating yield responses under both irrigated and dryland regimes. Yield remains the paramount agronomic endpoint, reflecting the ultimate balance of physiological effects and environmental interactions. Determining whether diflufenzopyr application adversely impacts yield is essential to ensuring that any potential benefits in

water conservation or aflatoxin risk reduction do not come at the cost of production losses.

Key questions addressed in this study include: (1) Does diflufenzopyr application result in sustained reductions in transpiration beyond initial transient responses? (2) How do changes in leaf morphology and stomatal behavior observed shortly after treatment relate to longer-term whole-plant water use patterns? (3) Does the application negatively influence yield or biomass accumulation in both water-limited and irrigated environments? By integrating detailed physiological measurements with robust agronomic outcomes, this work seeks to provide insights into the safety and utility of diflufenzopyr beyond its established role in flower termination. The current study aims to investigate the complex interactions between herbicide application, plant water relations, and fungal contamination risk under variable environmental conditions. A clearer understanding of these dynamics will contribute to the development of improved strategies for peanut production and aflatoxin management.

Materials & methods

Farm field experiment (1)

The peanut cultivar Georgia 06G (GA06G) was utilized for the study. This cultivar has been the predominate cultivar used by growers across most acres in the South-eastern United States (Georgia, Alabama, Florida) because of its yield and disease resistance genetics. This trial was conducted at the USDA-ARS National Peanut Research Laboratory Bolton Irrigation Research Farm, 7 km (kilometers) west of Dawson, Georgia (31°47'35"N 84°30'50"W) on a Red Bay sandy loam. The farm is equipped with an overhead lateral irrigation system. The farm fields are divided into six independent irrigation tiers where two tiers are non-irrigated, and four tiers are irrigated. The irrigated tiers have a four-year rotation of cotton, corn, corn, and peanut. The two dry land tiers have an alternate year peanut-fallow rotation.

Traditional field equipment was used to prepare, plant, maintain, and harvest each peanut crop at the farm site. All sprinkler irrigated areas were fall tilled using a disk harrow, chisel plow, deep rip chisel, field cultivator, and other tillage equipment as needed depending on soil condition and time of year. Dry land areas underwent the same tillage practices. Soil samples were taken each spring for fertility and other soil amendment recommendations. In the spring, fertilizer (P, K, and lime) was applied prior to tillage to aid fertilizer incorporation in each farm field plot and treatment depending on soil analysis. Peanut was planted using a 6-row

vacuum planter at 20 seeds/m at a depth of 15.2 cm (6 in). Planting was done between 01 to 15 May as recommended.

Individual farm field plots are 5.5 m wide (6 crop rows planted on a 0.91 m row spacing) and 6.1 m long with a 2.1 m border between each plot. Peanut was planted in a randomized complete block within each irrigation treatment regimes (dryland, irrigation until 90 days, full season irrigation) over three years (2021–2023). There were a minimum of three and up to six plot replications depending on total number of treatments selected per crop year. Peanut was irrigated using an overhead sprinkler irrigation system. Irrigation events were scheduled using a combination of soil water potential sensors installed at 25 and 50 cm soil depth (MPS-2, ZL6 dataloggers, Meter Group, Pullman, WA). An irrigation event occurred when the average soil water potential of both sensors was approximately -60 kPa. Irrigation treatments consisted of dry land all year, irrigation stopped 90 days after planting, and full year irrigation. These three irrigation treatments allowed for a more practical comparison of common scenarios that occur in grower's fields.

Treatment groups receiving doses of herbicide diflufenzopyr (2-(1-[[[3,5-difluorophenylamino] carbonyl] hydrazono]ethyl)-3-pyridinecarboxylic acid) were sprayed in 2022 and 2023. Diflufenzopyr was applied at a rate of 17 g of active ingredient per hectare (g ai/ha) as described by Lamb et al. (2017). The herbicide was applied once per growing season using a compressed CO₂ powered backpack sprayer at 90 days after planting at reproductive stage R6 (Boote 1982). This treatment was applied in all three irrigation regimes. Peanut was harvested at the recommended time (Williams and Drexler 1981) using a traditional digger but combined with a small experimental plot peanut picker. Peanut from each individual plot was collected, dried, weighed, and processed to determine yield, farmer stock, grade, and kernel distribution. While soil physical and chemical properties vary across production regions and could influence herbicide uptake or water dynamics, the experimental design in this study minimized those variables to isolate physiological responses.

Rainout shelter plot experiment (2)

GA 06G Peanuts were planted in environmentally controlled plots equipped with rain-out shelters (6.1 × 12.2 m)

(Blankenship et al. 1989) on 1 May 2023, at NPRL, Dawson, GA, USA. Peanut was planted in a single row plot of 5.5 m long and 0.91 m wide with three replications using seed rate of 20 seeds m⁻¹ at a depth of 15.2 cm. The rain-out shelter plots were irrigated before and after planting to provide uniform soil moisture to promote germination. Irrigation and agronomic management inputs were applied according to University of Georgia best management practices for peanut. Timing and amount of irrigation was determined using MPS2 dielectric water potential sensors (Meter Environment, Pullman, Washington, USA) placed at 25 and 50 cm depths and interrogated hourly and data compiled daily. Rainout shelter plots were irrigated at -60 kPa, based on an average moisture reading of both 25 and 50 cm depths.

In situ stem psychrometers (PSY 1, ICT International, Armidale, NSW), were installed in treatments 3 and 4 (see Table 1.) and time-lapse cameras (ATL-200S, Afidus, New Taipei City, Taiwan) were set up in front of those same rows prior to herbicide application. Psychrometers took a stem water potential measurement every 10 min, and the time-lapse cameras recorded images at 1-min intervals. Diflufenzopyr was applied 14 Aug 2023 using a CO₂ back-pack sprayer at a rate of 17 g ai/ha.

Drought and herbicide treatments were applied simultaneously, 90 days after planting at R6. Treatments were broken up into 4 groups: (1) Drought and herbicide spray, (2) Drought no herbicide spray, (3) Irrigated and herbicide spray, (4) Irrigated no herbicide spray (control). “Droughted” plots had an automated rainout shelter system set to close upon any precipitation detected, however there was no rain during the duration of the experiment. Irrigated plots were watered when the soil water potential fell below -60 kPa. Two days after application of treatments, physiological measurements were taken every two hours from 9am to 5 pm for five consecutive days (Cobb 2022). Net carbon assimilation (A), stomatal conductance water (g_{sw}), chlorophyll fluorescence (Fv/Fm) and transpiration (E) were measured on the youngest fully expanded leaf on the apical meristem using the LI-6800 Portable photosynthesis system (LI-COR Biosciences, Lincoln, NE).

To investigate differences in response to low doses of Diflufenzopyr, time-lapse video images of growing peanut plants were recorded. The resulting videos were edited using Shotcut editing software (Melttech 2022). The software made it possible to synchronize video frames of treatment and control peanut plant leaf images for comparison. The synchronized video frames were labeled and then saved as MP4 video files with the individual frames in turn were saved as JPEG files. A Windows script was then used to copy and save frames of the synchronized video images at 30-min intervals. The Windows script was programmed to copy frames at the beginning and middle of each hour of

Table 1 Treatment groups and associated numerical codes/symbols

○1	Drought and herbicide spray
△2	Drought no spray
●3	Irrigated and herbicide spray
▲4	Irrigated no spray (control)

Ranked in order from most to least significant.

recording so the time would coincide with measurements of peanut plant and soil water potential. The synchronized 30 min interval frame images were then imported as an image stack into ImageJ software (Schneider 2012). The images in the stack were surveyed to find peanut plant meristems with petioles extending from the meristems in a plane parallel to the image plane. The images used for analysis were those where image plane parallelism existed for the entire series of images for both treatment and control peanut plants. The angle between the meristem and the petioles was then measured in each frame using the ImageJ Angle Tool. Beginning with the first image of the treatment plants, the first measurement line was from the tip of the meristem down along the meristem to the base of the petiole. The second measurement line was then along the petiole to the first leaflet and degrees of the formed angle were recorded. The image stack was advanced to the next image and the endpoints of the lines were adjusted to realign them to the meristem and petiole. Degrees of the formed angle were again recorded. Degrees of the formed angle for each image were saved in a CSV file. On selected control plants, the measurement procedures were repeated, and degrees of the formed angles were recorded.

The measured angles with frame date and time, and elapsed time since beginning of recording were organized in a spreadsheet for plotting and analysis. A time interval of more than 30-min occurred after 21 elapsed hours due to a camera problem. A 30 min interval between measurements was restored beginning at 30 h of lapse time.

Greenhouse experiment (3)

In addition to the outdoor measurements, individual plants of Ga06G were sown in a greenhouse environment at North Carolina State University. Plants were grown in 5L plastic pots, filled with potting mix (Professional Growing Mix, Sun Gro Horticulture, Agawam) for 90 days. A total of 12 pots were propagated, 6 control plants and 6 sprayed. Plants were grown and maintained in a greenhouse under semi-controlled conditions from November 2024 to March 2025. Conditions were kept at mean day/night temperatures of $35\text{ }^{\circ}\text{C}/21\text{ }^{\circ}\text{C} \pm 4.5\text{ }^{\circ}\text{C} / 1.7\text{ }^{\circ}\text{C}$, with natural sunlight and $450\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ supplemental lighting on a 12 h photoperiod. Plants were irrigated daily, fertilized once a month with all-purpose fertilizer (Miracle-Gro® Water Soluble All Purpose Plant Food, 24–8–16). Before the experiment began and the herbicide was sprayed, the soil surface of each pot was covered with 0.64 cm thick black open cell polyurethane foam to prevent soil transpiration.

At 90 days (R6) plants were sprayed within an enclosed spray chamber (Generation III track sprayer, DeVries Manufacturing) equipped with two nozzles, 48 cm apart, and

placed at 50 cm height from the plants, delivering 17 g ai/ha . Evapotranspiration was quantified using the mass balance method by placing each pot on individual custom-built 10 kg balance (YB6-B-10 KG, Sentran, LLC., Ontario, CA, USA). Each balance was calibrated using a three-point calibration prior to the experiment. A CR1000x data logger (Campbell Scientific, Inc., Logan, UT, USA) controlled the balances and logged mass data at 5 min intervals. Biomass accumulation during the short experiment period was assumed to be negligible and daily evapotranspiration was calculated as the difference in mass for a 24 h period. Transpiration values for each plant were normalized by measuring the whole plant leaf area after 7 days (LI-3100, LI-COR, Inc., Lincoln, NE, USA). During the experiment g_{sw} (LI-600, LI-COR, Inc., Lincoln, NE, USA) was also monitored at 9am for 5 days.

Statistics

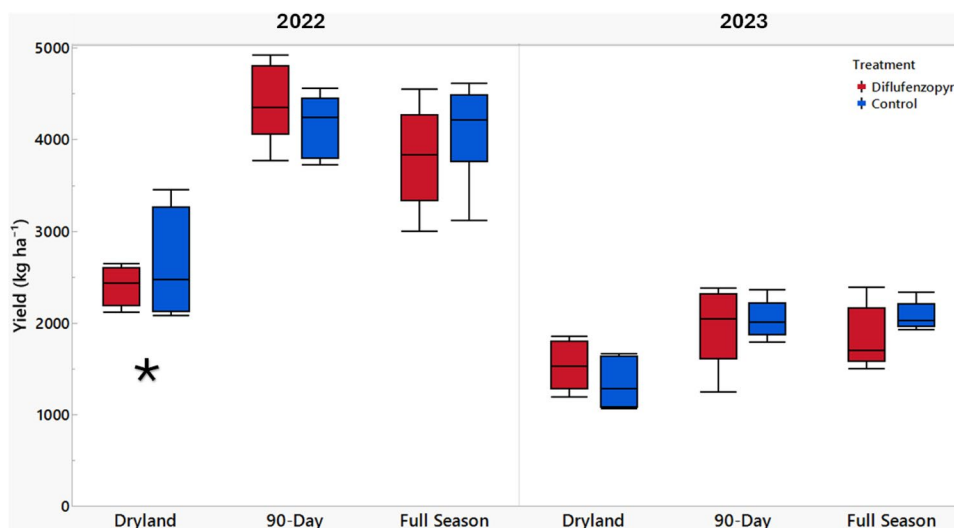
Trait values were tested for normality and equal variance using a Shapiro–Wilk test in R, and all values met those assumptions. ANOVAs were run for each measured variable across treatments and means were compared using both Tukey and Student T-tests. Bivariate trait relationships were analyzed with Pearson product–moment correlation using the Hmisc package in R. Figures were generated in both R and JMP (R Studio Team (2020) version 4.3.0; JMP, Version 17.2. SAS Institute Inc.)

Results

Field experiment: peanut yield

Peanut yield was measured across the 2022 and 2023 growing seasons under three irrigation regimes: dryland, irrigation through 90 days after planting, and full-season irrigation. In each irrigation tier, diflufenzopyr-treated plots and untreated controls were compared for yield (Fig. 1). In 2022, yield increased with irrigation in both treatment groups. Both treated and untreated plots showed the highest yields under full-season irrigation, with 2022 yields ranging between approximately 3700 and 5000 kg ha^{-1} . Under dryland conditions, yields ranged from approximately 2000 to 3300 kg ha^{-1} . In 2023, overall yield values were lower than in 2022 across all treatments and irrigation levels. The full-season irrigation group again produced the highest yields, followed by the 90 days cutoff group, and then dryland. Yield ranges for 2023 spanned approximately 1200 to 2300 kg ha^{-1} across treatments. Across both years, yield variability was present within each irrigation level and treatment group, as shown by the distribution of boxplots

Fig. 1 Peanut yield (kg ha^{-1}) response to diflufenzopyr treatment under varying irrigation regimes across two growing seasons. Boxplots show yield distributions for diflufenzopyr-treated (red) and control (blue) peanut plants under dryland, 90-day, and full-season irrigation in 2022 and 2023. (*) denote statistical differences between irrigation treatments



in Fig. 1. Corresponding soil and peanut pod samples collected from each treatment group showed no measurable differences in aflatoxin contamination.

Rainout shelter experiment: physiology measurements

Significant differences were observed in soil water potential (SWP), transpiration (E), photosynthesis (A), and stomatal conductance (g_{sw}) across treatments combining irrigation and herbicide application (Fig. 4). Throughout the 5-day measurement period, treatment 3 (irrigated + sprayed) maintained the highest average SWP, remaining near field capacity. Treatment 4 (irrigated + unsprayed) exhibited a moderate decline, while treatment 1 (drought + sprayed) showed the most rapid and significant reduction in SWP. SWP was not measured for treatment 2 (drought + unsprayed).

Transpiration rates varied significantly across treatments. Treatment 3 exhibited the highest average daily E values, more than double that of treatment 1 and over three times greater than treatment 2. Treatment 3 also had significantly higher E than treatment 4. Corresponding patterns were observed for g_{sw} , with treatment 3 maintaining elevated values relative to all other treatments for the first five days. Treatment 1 exhibited a marginal increase in g_{sw} over treatment 2, but this difference was not statistically significant.

Photosynthetic assimilation rates followed a similar pattern. Treatments 1 and 2 did not differ significantly from each other but had lower values compared to treatments 3 and 4. Treatment 3 maintained significantly higher A values than treatment 4. Pearson correlation analysis revealed consistent associations between g_{sw} , E, and SWP within treatment 4, with most correlations highly significant ($p < 0.001$). Treatments 1 and 3 showed a partial decoupling of E and

soil water potential associations, while treatment 2 exhibited a general loss of trait correlations (Fig. 2).

Rainout shelter: leaf angle and stem mechanics

Significant treatment differences were observed in stem water potential and leaf angle inclination over the 5-day measurement period (Fig. 3). Diurnal stem water potential measurements in the irrigated treatments showed that unsprayed plants (treatment 4) followed a typical pattern, with midday values reaching approximately -5 MPa and recovering to around -2 MPa by predawn. In contrast, irrigated plants that received herbicide treatment (treatment 3) exhibited less negative overnight stem water potential values across all five days, indicating more rapid recovery of the water column during the nighttime period. By day six, stem water potentials between treatments 3 and 4 had converged. Leaf angle inclination measurements showed significant differences between sprayed and unsprayed plants in both irrigated and droughted conditions. Herbicide sprayed plants (treatments 1 and 3) exhibited more acute and folded leaf positions throughout the 5 days measurement period compared to their unsprayed counterparts. This effect was most pronounced in treatment 3, where leaf angle remained approximately 50% more closed than the control during both daytime and nighttime intervals. By 140 h post-treatment, leaf angle values in all treatments had returned to baseline levels.

Greenhouse experiment: transpiration measurements

Average hourly transpiration rates were recorded for seven days following herbicide application using a high-resolution load cell system under greenhouse conditions. Three

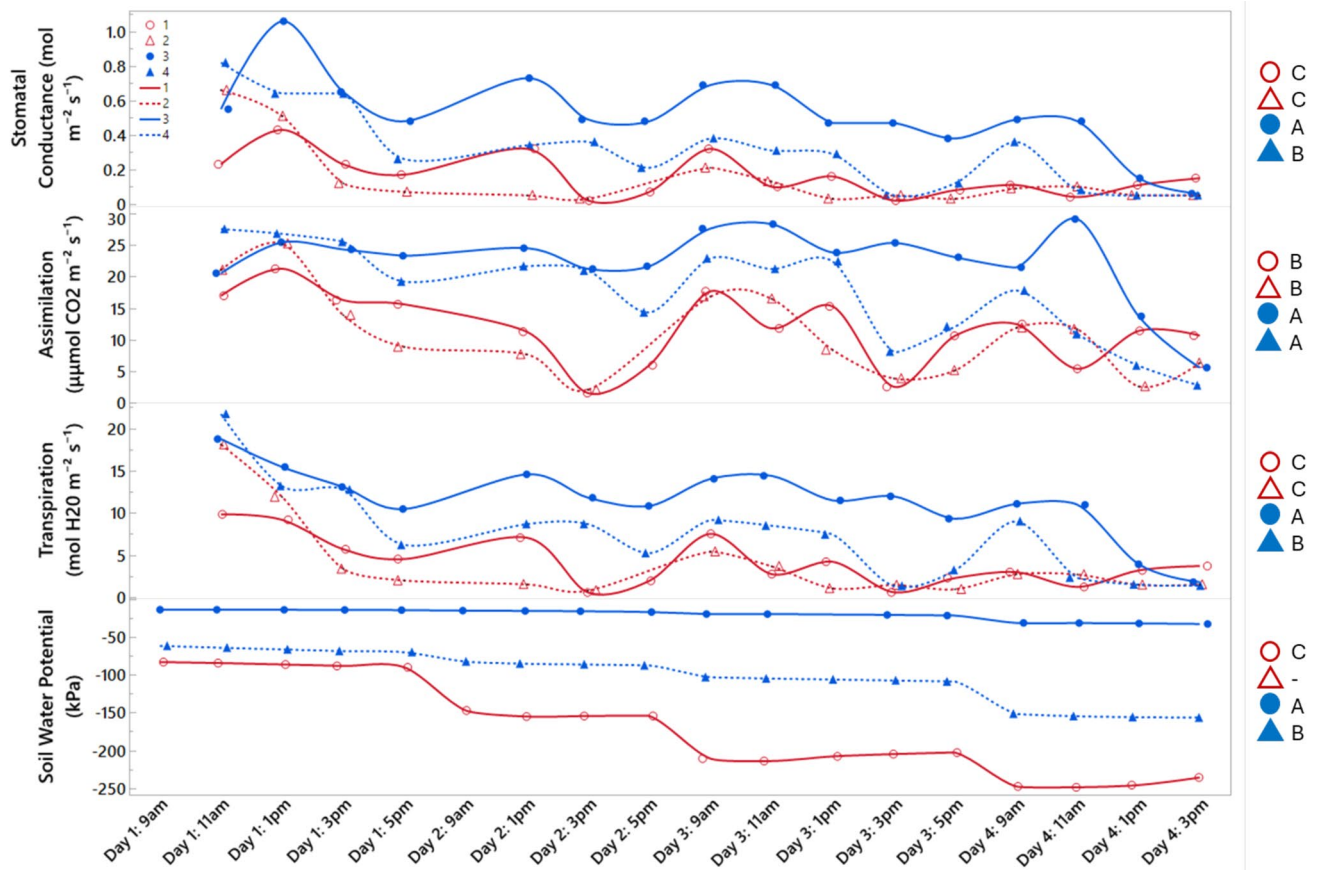


Fig. 2 Top: Stomatal conductance readings (g_{sw} , $\text{mol m}^{-2} \text{s}^{-1}$) for droughted sprayed (Treatment 1, red open circles, red solid line) droughted control (Treatment 2, red open triangles, red dashed line) irrigated sprayed (Treatment 3, blue filled circles, blue solid line) and irrigated control plants (Treatment 4, blue open triangles, dashed line).

Second: Carbon assimilation rates (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) for all treatments. Third: Transpiration rate (E , $\text{mol m}^{-2} \text{s}^{-1}$) for all treatments. Last: Soil Water potential (kPa) for all treatments. Letters beside each measurement type denote significant mean differences between each treatment

control and three diflufenzopyr-treated GA06G variety peanut plants were monitored at 5 min intervals, and transpiration values were normalized to measured whole-plant leaf area. Figure 4. Displays the average transpiration per hour over time for each treatment group. Distinct daily cycles were observed with repeatable peak transpiration occurring during the mid-day hours and minimum occurring at night. Patterns were consistent across the seven-day measurement period. A linear regression of the hourly average transpiration values for treated plants versus control plants (Fig. 4, inset) yielded an R^2 of 0.957. The regression slope was $1.037 (\pm 0.019)$, with an intercept of $-0.00015 (\pm 0.00019)$. The slope term was statistically significant ($p < 0.0001$), while the intercept was not ($p = 0.44$). A total of 131 paired hourly data points were included in the analysis. Stomatal

conductance (g_{sw}) measurements taken at 9:00 AM over five consecutive days showed no statistically significant differences between control and treated plants on any of the days.

Discussion

This study investigated the physiological response of peanut cultivar Georgia 06G to sub-lethal application of the auxin-mimicking herbicide diflufenzopyr under varying water availability regimes across field, greenhouse, and controlled drought conditions. Across environments, we consistently found no measurable reductions in yield or aflatoxin contamination, but observed distinct shifts in water use patterns, particularly in irrigated plants.

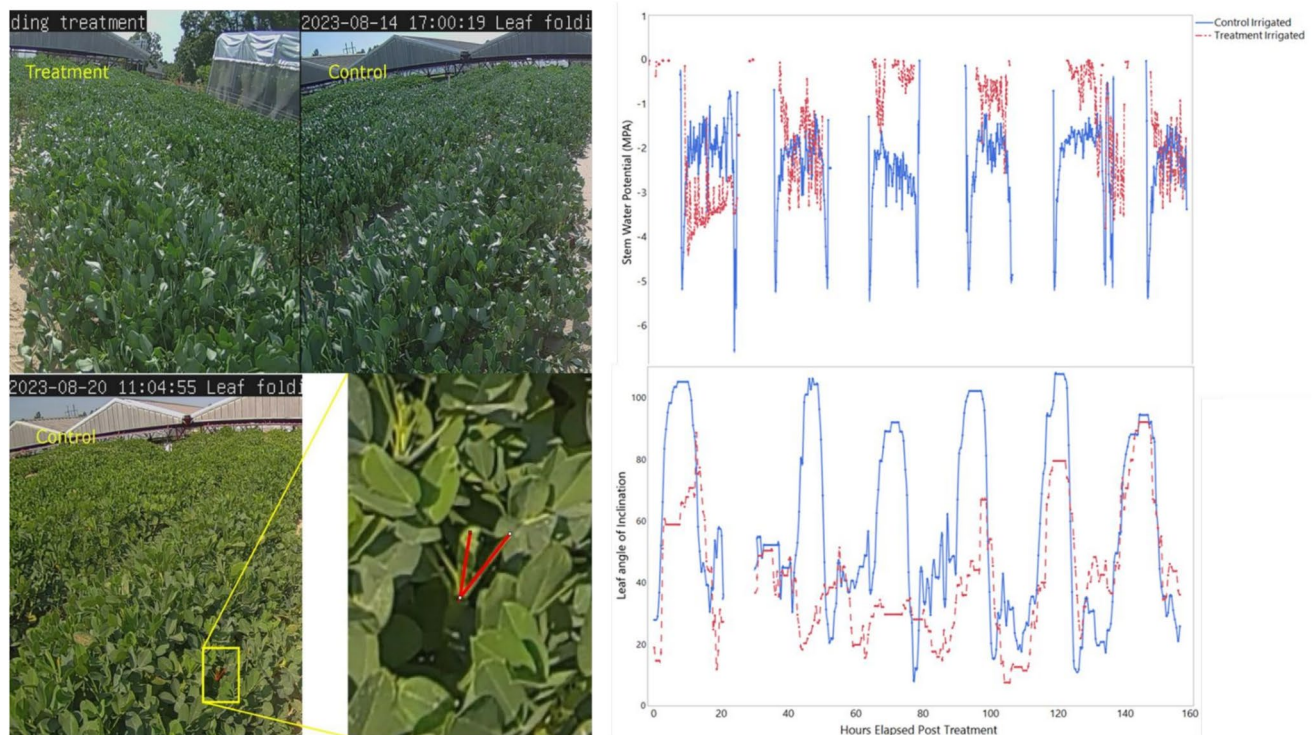


Fig. 3 Left: Time lapse methods for measuring leaf angle of inclination between meristem and petiole for sprayed and unsprayed irrigated treatments. Right: Diurnal stem water potentials for treatments 3 and 4 (irrigated treatment, and irrigated control, respectively). Gaps in measurements were due to high condensation levels in psychrometer

chambers. Bottom: Diurnal angle of inclination for apical meristem leaves in treatments 3 and 4. Significant differences were observed in both stem water potential and leaf angle of inclination between hours 40 and 120

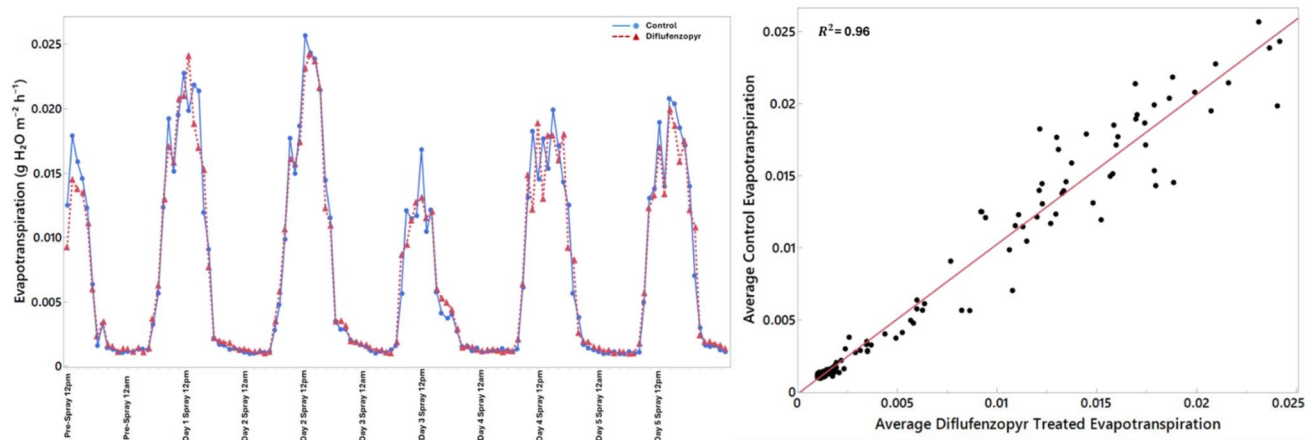


Fig. 4 Evapotranspiration response to diflufenzopyr treatment in greenhouse-grown peanut plants. Left: Diurnal evapotranspiration rates (g H₂O m⁻² h⁻¹) of control (blue, solid line) and diflufenzopyr-treated (red, dashed line) plants over a 5-day period post-application.

In farm field trials, there were no statistically significant differences in yield or aflatoxin concentrations between sprayed and unsprayed plots across irrigation treatments. This suggests that under typical grower field conditions, at the tested dosage and application timing, diflufenzopyr did not negatively impact productivity or quality. However,

Data represent hourly averages from load cell balances. Right: Linear regression comparing average hourly evapotranspiration of diflufenzopyr-treated plants to control plants across the full observation period ($R^2=0.96$)

subtle shifts in physiological parameters were evident under more controlled settings.

The greenhouse load cell experiment revealed a distinct, short-lived stimulatory response in water use following herbicide application. Sprayed plants exhibited significantly higher rates of transpiration per unit leaf area over

a five-day period relative to control plants, suggesting that stomatal regulation was temporarily altered. Despite this increased transpiration, stomatal conductance measurements (g_{sw}) taken at 9am did not differ significantly between sprayed and control plants, suggesting the possibility of altered stomatal behavior outside of early morning periods or a decoupling of conductance and whole-plant water loss under these conditions.

Rainout shelter plot results provided a more nuanced picture of physiological compensation. While maximum quantum efficiency of photosystem II (Fv/Fm) remained unaffected across all treatments, indicating no biochemical disruption to light harvesting, herbicide treatment in well-watered conditions led to a clear, statistically significant increase in transpiration, stomatal conductance, and carbon assimilation. This coincided with elevated soil water retention, contrasting with the rapid soil moisture loss observed in the drought+herbicide treatment. In the drought+herbicide treatment, an initial mild hormetic response appeared to compound the effects of water limitation, resulting in greater physiological inhibition than drought alone.

Leaf-level behavior also reflected this divergence. Sprayed plants showed significantly more acute leaf angle inclination over the first four to five days post-treatment, particularly under irrigation. Stem water potential values further supported these patterns: sprayed irrigated plants recovered water potential more rapidly overnight and maintained less negative values than unsprayed counterparts, indicating shifts in xylem water dynamics potentially tied to altered leaf hydraulics or continued transpiration.

Together, these results point toward a transient but reproducible physiological perturbation following diflufenzopyr application under non-stressed conditions. Instead of inducing an water-conserving stress response, the herbicide appears to briefly increase water loss and carbon assimilation in well-watered plants, possibly due to impaired stomatal regulation. In contrast, under existing drought stress, the same dosage may exacerbate hydraulic decline and accelerate soil drying, suggesting a threshold effect beyond which the herbicide no longer stimulates but instead compounds stress. This apparent contrast between irrigated and drought conditions may lie in the mode of action for diflufenzopyr. By disrupting auxin transport, the herbicide may indirectly alter stomatal regulation and leaf hydraulics, producing a short-lived relaxation of stomatal control. Under irrigated conditions, this effect is buffered by ample soil water, allowing plants to sustain elevated transpiration and carbon gain without hydraulic penalty. In contrast, when soil water is limiting, the same shift drives additional evaporative

demand that cannot be met, hastening soil moisture depletion and deepening plant water stress. Thus, the herbicide's stimulatory effect appears conditional, enhancing fluxes when water is abundant but compounding drought inhibition when resources are scarce (Radchenko et al. 2021).

These findings are consistent with prior reports of herbicide-induced hormesis (Belz et al. 2011) and underscore the need for greater mechanistic understanding of how low-dose auxin-mimics modulate physiological traits in crops beyond their intended weed targets. There has been a recent call for studying the concurrence of weed, herbicide and other biotic and abiotic stressors on crop production, after a variety of studies illustrated that in many crops stress combinations that included herbicides resulted in unexpected decline in yields and plant performance (Bagavathiannan et al. 2017). Auxin inhibitors in particular have been found to accelerate leaf senescence (Jiang et al. 2025), increase ABA accumulation (Hansen et al. 2000) and interrupt fruiting or seed set in crops such as rice and maize (Sterling et al. 1997; Grossman et al. 2002). Specifically, they highlight that herbicide–water interactions may be highly context dependent, with implications for crop stress physiology, irrigation efficiency, and long-term plant performance under variable climate regimes. For example, the activation, solubility, availability, uptake metabolism and efficacy of herbicides are highly influence by soil moisture (Diskson et al. 1990) with drought stress conditions reducing the uptake and translocation of foliar-applied herbicides (Bagavathiannan et al. 2017). Therefore, initial drought stress might cause stomata to close, increase cuticle thickness, and/or increase leaf pubescence, thereby reducing herbicide entry into the leaf (Oyarzabal 1991). Future work should investigate the underlying mechanisms, whether hormonal, hydraulic, or biochemical, driving the observed stomatal behavior, and examine whether these patterns hold across genotypes and soil types.

Conclusion

This study was initiated in response to Lamb et al. (2017), who proposed that low-dose diflufenzopyr could induce leaf rolling in peanut and thereby conserve water to reduce late-season aflatoxin risk. Our experiments demonstrated that this mechanism did not hold: rather than triggering a water-saving response, diflufenzopyr produced a short-lived stimulation of transpiration, stomatal conductance, and carbon assimilation under irrigated conditions, while accelerating soil moisture loss and hydraulic decline when combined

with drought stress. Changes in leaf angle inclination and stem water potential confirmed a physiological perturbation following application, but these reflected compensation for greater water loss rather than suppression of fluxes. Crucially, field trials revealed no negative impacts on yield or aflatoxin contamination, indicating that the tested dosage is agronomically safe. Together, these findings refine the narrative posed by Lamb et al., showing that the plant's response to diflufenzopyr is dynamic and context dependent, with outcomes shaped by water availability. From a management perspective, the results suggest that spray timing relative to irrigation is critical, as increased water use following application could reduce soil reserves in sandy soils or during late-season heat events when irrigation capacity is limited.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12892-025-00320-4>.

Acknowledgements We'd like to thank Staci Ingram and Larry Hall Jr. for their assistance in the field as well as Brock Dean for his assistance with the greenhouse portion of the experiment.

Author contributions ERB—Project design, experimental setup, data collection, data analysis, manuscript writing; RBS—Project conception, experimental setup, data collection; JSM—data collection, data analysis, manuscript writing; MCL—funding acquisition, project design, analysis feedback; MJT—experimental setup, data collection, data analysis, manuscript writing; AAC—experimental setup.

Funding This study was supported by USDA-ARS project number 6044-21600-001-000D. The authors have no conflict of interest to declare. The use of any name brand herbicides does not indicate endorsement by USDA.

Data availability Data is available upon request.

Declarations

Competing interests No competing interests to report.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Bagavathiannan M, Singh V, Govindasamy P, Abugho SB, Liu R (2017) Impact of concurrent weed or herbicide stress with other biotic and abiotic stressors on crop production. In: Plant tolerance to individual and concurrent stresses. New Delhi: Springer India, pp 33–45
- Belz RG, Cedergreen N, Duke SO (2011) Herbicide hormesis—can it be useful in crop production? *Weed Res* 51(4):321–332
- Blankenship PD, Sanders TH, Dorner JW, Cole RJ, Mitchell BW (1989) Engineering aspects of aflatoxin research in groundnuts: evolution of an environmental control plot facility. In: Aflatoxin contamination of groundnut: proceedings of the international workshop. ICRISAT center, Patancheru, AP vol 502, No. 324, pp 269–278
- Boote K (1982) Growth stages of peanut (*Arachis hypogaea* L.)¹. *Peanut Sci* 9(1):35–40
- Cobb AH (2022) Herbicides and plant physiology. John Wiley & Sons, Hoboken
- Dhakal A, Hashmi MF, Sbar E (2023) Aflatoxin Toxicity. 2023 Feb 19. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. PMID: 32491713.
- Dickson RL, Andrews M, Field RJ, Dickson EL (1990) Effect of water stress, nitrogen, and gibberellic acid on fluzafop and glyphosate activity on oats (*Avena sativa*). *Weed Sci* 38:54–61
- Gomes GLB, Scortecci KC (2021) Auxin and its role in plant development: structure, signaling, regulation and response mechanisms. *Plant Biol* 23(6):894–904
- Grossmann K, Caspar G, Kwiatkowski J, Bowe SJ (2002) On the mechanism of selectivity of the corn herbicide BAS 662H: a combination of the novel auxin transport inhibitor diflufenzopyr and the auxin herbicide dicamba. *Pest Manag Sci Former Pestic Sci* 58(10):1002–1014
- Hansen H, Grossmann K (2000) Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. *Plant Physiol* 124(3):1437–1448
- Jiang N, Wang P, Yang Z, Li C, Xia Y, Rong X, Yang L (2025) Auxin regulates leaf senescence and nitrogen assimilation to promote grain filling in maize (*Zea mays* L.). *Field Crops Res* 323:109787
- Kadioglu A, Terzi R, Saruhan N, Saglam A (2012) Current advances in the investigation of leaf rolling caused by biotic and abiotic stress factors. *Plant Sci* 182:42–48
- Lamb MC, Sorensen RB, Butts CL, Dang PM, Chen CY, Arias RS (2017) Chemical interruption of late season flowering to improve harvested peanut maturity. *Peanut Sci* 44(1):60–65
- Meltytech LLC (2022) Shotcut [Computer Software] ver. 20.12.21
- Oyarzabal ES (1991) Effect of weed water stress on postemergence herbicide activity. Iowa State University, Ames
- Pakdaman Sardrood, B, Mohammadi Goltapeh E (2018) Weeds, herbicides and plant disease management. In: Sustainable agriculture reviews 31: Biocontrol, pp 41–178
- Payne, G. A. (1998). Aflatoxin-producing fungi and their impact on crops. Mycotoxins in agriculture and food safety. In: Sinha KK, Bhatnagar D (eds.) Marcel Dekker, New York, NY, 279
- Qiao M, Hong C, Jiao Y, Hou S, Gao H (2024) Impacts of drought on photosynthesis in major food crops and the related mechanisms of plant responses to drought. *Plants* 13(13):1808
- Radchenko MP, Ponomareva IG, Pozynych IS, Morderer YY (2021) Stress and use of herbicides in field crops. *Agric Sci Pract* 3:50–70
- RStudio Team (2020) RStudio: Integrated Development Environment for R. RStudio, Inc., Boston, MA. URL <http://www.rstudio.com/>

- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. *Nat Methods* 9(7):671–675. <https://doi.org/10.1038/nmeth.2089>
- Sterling TM, Hal JC (1997) Mechanism of action of natural auxins and the auxinic herbicides. *Rev Toxicol* 1:111–142
- Torres AM, Barros GG, Palacios SA, Chulze SN, Battilani P (2014) Review on pre-and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Res Int* 62:11–19
- Williams EJ, Drexler JS (1981) A Non-Destructive Method for Determining Peanut Pod Maturity". *Peanut Sci* 8:134–141
- Zakaria L (2024) An overview of *Aspergillus* species associated with plant diseases. *Pathogens* 13(9):813
- Zaller JG, Brühl CA (2021) Direct herbicide effects on terrestrial non-target organisms belowground and aboveground. In: *Herbicides*. Elsevier, pp 181–229
- Zeng S, Xia J, Chen X, Zou L, Du H, She D (2020) Integrated land-surface hydrological and biogeochemical processes in simulating water, energy and carbon fluxes over two different ecosystems. *J Hydrol* 582:124390

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.