PEANUT SEEDLING DEVELOPMENT AND PHYSIOLOGICAL RESPONSE TO FLUMIOXAZIN

by

NICHOLAS LANE HURDLE

(Under the Direction of Timothy L. Grey)

ABSTRACT

Over 50% of U.S. peanut production can be credited to Georgia. The growing season for peanut can extend up to 150 days, making it essential to manage weeds in such a manner to achieve maximum yield potential. This includes applications of preemergence (PRE) herbicides. Numerous PRE herbicides are registered for peanut production including pendimethalin, diclosulam, and flumioxazin. Emerging peanut will inevitably contact these PRE applied herbicides. It has been observed by numerous peanut growers across Georgia that in unfavorable weather conditions at peanut emergence, flumioxazin can cause crop injury. As over 65% of U.S. peanut growers use flumioxazin, studies were performed to quantify the effects of flumioxazin applied directly to peanut seed, as well as, its effects on emerging peanut plants. Though injury was noted, it was transient across the growing season and yield was not affected. Peanut germination was also not affected at rates equivalent to soil concentrations of flumioxazin.

INDEX WORDS: Weed management, peanut, diclosulam, pendimethalin, flumioxazin, linear regression, peanut germination

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DEDICATION

I dedicate this work to my parent, Jeff and Elaine Hurdle. Without their support and motivation, I would not be in the position I am today. To dad, thank you for always encouraging me to be better than I was the day before and showing me what hard work is. The long days in the garden pulling corn were always about more than just pulling corn, it was about learning from you. Thank you for the countless nights coaching baseball when you went a step above and coached about life. To mom, thank you for always making me take the extra step to help others and pushing me to do my best in school. Thank you for those late nights studying, it has led me to where I am today. Also, to my brother and sister for the encouragement throughout this degree.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Developed by Valent U.S.A Corporation, flumioxazin {2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione; V-53482, S-53482} was introduced for research in 1989 as a selective herbicide for use in peanuts and soybeans (Shaner 2014). A selective herbicide is classified as an herbicide that is toxic to only a certain classification of plants (Yiesla 2004). Flumioxazin is applied to control troublesome broadleaf weeds such as common ragweed (*Ambrosia artemisiifolia* L.), pigweeds (*Amaranthus* spp.), prickly sida (*Sida spinosa* L.), eastern night blackshade (*Solanum ptycanthum* Dunal), and suppress Pennsylvania smartweed (*Polygonym pensylvanicum* L.), velvetleaf (*Abutilon theophrasti* Medik), and Florida beggarweed (*Desmodium tortuosum* Sw.) (Lovell et al. 2002). Flumioxazin provides little to no control of annual and perennial grass species, sicklepod (*Senna obtusifolia* L.) or nutsedge (*Cyperus* spp. L.) (Grey et al. 2002).

Broad spectrum residual weed control has made flumioxazin a popular choice among producers, along with the wide range of broadleaf weed management. Flumioxazin can be utilized in numerous crop categories such as legumes, cereals, and fibrous crops. The recommended application rates are from 36 to 432 g/ha, with rotational intervals dependent on the rate applied and the specific crop (Anonymous 2017). Classified as a group 14 herbicide (Anonymous 2011), flumioxazin affects the protoporphyrinogen oxidase (PPO) enzyme causing an oxidation of protoporphyrinogen IX to protoporphyrin IX. This oxidation reaction causes a buildup of singlet, radical O^- which will result in lipid peroxidation, followed by lipids attacking the cell membrane causing membrane degradation and eventual cellular leakage (Shaner 2014). Other herbicides in this group include the diphenylethers, oxadiazoles, phenylpyrazoles, and triazolinones.

Flumioxazin is a preemergence (PRE) herbicide with considerable residual activity. Classified as a light-dependent peroxidizing herbicide (LDPH), flumioxazin becomes activated as the plant absorbs light through photosynthesis (Federoff et al. 2003). Some plant species are not affected by flumioxazin such as soybean (*Glycine max* L.) and peanut (*Arachis hypogaea* L.) due to flumioxazin's selective properties. Peanut and soybean are naturally tolerant to flumioxazin PRE applications due to rapid metabolic processes where cleavage occurs at the imide and amide linkage, each producing an independent metabolite (Kwon et al. 2004). When flumioxazin is applied postemergence (POST) to peanut or soybean, severe crop injury will result due to contact with photosynthetic plant matter (Johnson 2006). Less than favorable growing conditions, such as cool temperatures or excessive moisture, may intensify crop damage even in tolerant plant species. Environmental conditions such as cool and wet soil can reduce herbicide metabolism and lead to herbicide injury in plants (Legleiter et al. 2014). If flumioxazin comes into contact with emerged peanut plants, symptoms of foliar toxicity include chlorosis and necrosis where the herbicide contacted the plant tissue. One immediate concern of foliar toxicity would be that of yield impact. The earlier this contact occurs in the peanut growing season, the longer the crop has to recover from this injury as suggested by Johnson et al. (2006). These researchers were concerned about the level of phytotoxicity from late applied flumioxazin in peanuts up to 10 days after planting. They noted that flumioxazin applied at 71 g/ha 8 or 10 days after planting (DAP), caused visual growth injury up to 39% at a midseason rating taken 34 d after treatment in 2002. Flumioxazin applied at 105 g/ha 10 DAP could result in up to 59% stunting 26 DAP. No effects were noted by the different application timing or rates on peanut yield (Johnson et al. 2006). Their findings supported an earlier study conducted in Florida that peanut canopy width and stunting were negatively impacted by increasing flumioxazin rate, but pod yield was not affected (Main et al. 2003). Though yield was not different, the ratio of jumbo size to medium size to Number one size peanut seed may have been affected by a single size outnumbering the other two sizes. This effect would result in a lower peanut grading, therefore reducing grower income. Wilcut et al. (2001) noted that the peanut grades were not adversely affected by flumioxazin applications.

As a soil applied herbicide, flumioxazin must be absorbed by roots to enter the plant or it will be ineffective. Rainfall must leach flumioxazin into the rhizosphere or the herbicide will not penetrate the soil layer and reach the roots. Studies have been performed in order to determine the method of flumioxazin absorption, but has yet to be determined. Price et al. (2004) examined the root absorption rate of flumioxazin of peanut, ivyleaf morningglory (*Ipomoea hederaceae* L.), and sicklepod. The investigators applied 1,850 Bq of ^{14}C-flumioxazin contained in 3 mL of a 50% Hoagland solution to ivyleaf morningglory and sicklepod roots at the cotyledon stage. At 4 hr after treatment (HAT), 42% and 35% ^{14}C-flumioxazin was absorbed by ivyleaf morningglory and sicklepod, respectively. At 72 HAT, the absorption rate increased to 57% and 46% respectively, indicating that only 15% and 11% more ^{14}C-flumioxazin was absorbed after the 4 hr mark. For peanut, two different plant sizes were used, 3.8 and 7.6 cm tall. The 3.8 cm peanut absorbed 24% of the applied ^{14}C-flumioxazin 4 HAT and increased to 72% at 72 HAT. The 7.6cm tall peanut absorbed over 2 times the amount of ^{14}C-flumioxazin as the 3.8 cm plant at 74% at 4 HAT. The 72 HAT absorption level was 99%. The investigators suggested that the differing absorption levels may be due to the higher levels of transpiration resulting in higher water uptake in the taller plant. In that same study, translocation throughout the plant was measured. In both peanut sizes, the ^{14}C-flumioxazin predominately stayed in the roots with only 10% translocated into the leaves and 6% to the hypocotyl and cotyledon 72 HAT. They also noted metabolism for each plant species. Peanut had metabolized ^{14}C-flumioxazin by 66% while sicklepod metabolized 60% at 4 HAT. Alternatively, the ivyleaf morningglory had 49% of the applied ^{14}C-flumioxazin remain as the parent herbicide 72 HAT. Peanut contained only 11% ^{14}C-flumioxazin remaining unmetabolized 72 HAT. These metabolism rates indicate that injury from flumioxazin applications imply that the injury results from rainfall after peanut emergence, rather than root absorbed flumioxazin injury.

The potential for leaching or long soil persistence is a concern for environmental protection and subsequent rotational crop choices for the specific field with any herbicide. A study performed by Ferrell et al. (2003) concluded that flumioxazin will reach its half-life approximately 13 to 18 days in Georgia soil types (Greenville sandy clay loam, Dothan sandy loam, Cecil sandy loam, and Tifton loamy sand) at 15 and 25 C. Having such a short half-life is beneficial in the sense that weeds are controlled for an adequate period of time and rotational crop retraction are minimal. Ferrell et al. (2005) noted that flumioxazin will adsorb to soil organic matter in the soil profile due to its low water solubility and will also be rapidly degraded through microbial breakdown. This implies that there is low concern of long-term environmental persistence or harmful effects from leaching. Flumioxazin has a low vapor pressure at 3.21×10^{-4} Pa compared to dicamba at 4.5×10^{6} Pa at 25 C (Nishimura et al. 2015). Once flumioxazin has made contact with the soil, it is adsorbed making it not susceptible to volatilization (Shaner 2014). Typical soil residues at the 108 g/ha rate were less than 0.01 ppm from 9 tests with 18 samples of each peanut vine and hay at 97 to 152 DAT, along with vines harvested 14 to 28 DAT from 2 tests (Dotson 2001). Additionally, 13 tests were also performed on the peanut nutmeat with 26 samples and 9 tests with 18 samples of hulls and resulted in less than 0.01 ppm of flumioxazin collected. The 540 g/ha applications were also performed on 8 nutmeat and 6 hull samples with the same results recorded, except 2 hull samples contained 0.04 ppm of flumioxazin.

As the end of harvest draws to a close, the grower must now decide whether to sell their crop, or save a portion as seed stock. One factor a grower must consider is the manner in which their saved seed will be stored. Many options of seed storage exist, such as a warehouse or in bulk bags, but environmental conditions (relative humidity, temperature, and microflora) the stored seeds are subjected to can affect the physiological characteristics in the form of deterioration (McDonald 2004). Seed deterioration is a problem that all storage facilities face, yet no facility can completely halt the process, but only control the rate at which the seeds deteriorate (McDonald 2004). Peanuts may be stored in large bulk bags and stacked on one another, or may be stored in wagons, exposed to the environment. Storage facilities may incorporate several techniques to decrease the rate at which the stored seeds deteriorate such as the type of warehouse constructed, and the use of aeration and ventilation. Storage facility construction specifications and techniques may be found in the American Peanut Shellers Association's publication titled: Handling and Storage of Farmer Stock Peanuts (Smith Jr. 2015). Factors such as relative humidity and temperature play a crucial role in slowing the deterioration process. Peanut stored in controlled, cool/dry conditions will have greater vigor and germination the following season, compared to seed stored out of shell and under variable, warm/moist conditions.

The hypothesis of the proposed study is that seed cost represents a significant expense to growers, herbicide injury can result in poor stand establishment, causing growers to purchase replacement seed and reducing profits. The objective of this study is to quantify the interactions of flumioxazin in peanut germination and stand establishment of field and lab experiments.

Georgia-16HO peanut seed with varying levels of germination (artificially altered to via stresses using heat to low or high) will be tested by replication and herbicide treatment. Seed were tested for germination by the Georgia Seed Association, using growth chamber and temperature gradient table experiments. Seedling development and response to flumioxazin was evaluated on a temperature gradient table. Herbicide solutions corresponding to soil concentrations: 10.0 parts per billion (ppb), 1.0 ppb, 0.1 ppb, 0.01 ppb, and a non-treated control were used to expose the seed upon experiment initiation and during seedling development. Twenty Petri dishes were placed at each temperature: 20, 23, 26, and 29 °C simultaneously for a total of 4 replications per concentration per temperature. Beginning 120 hr. after seeding, peanut germination were counted when the radicle extends 5mm or more from the seed, and pictures were taken as the seedlings developed over time to generate a time lapse record. From this photographic record, measures of growth in mm can be counted from each seedling. To activate the herbicides, lights mounted within the cover of the temperature gradient table will be switched five days after initiation. This will simulate a seedling emerging over time through the soil. The goal is to determine if there is an interaction between temperature and residual herbicide effects on peanut seed germination and seedling development and if this can be correlated to seed vigor.

The same seed lots of Geogia-16HO (low and high used in laboratory experiments) were tested to flumioxazin at 107 g ai/ha, diclosulam at 27 g ai/ha, and a nontreated control in field experiments at the Southwest Georgia Research and Education Center in Plains, GA and at the Ponder Farm near Ty Ty, GA. Three planting dates around April 9th and 23rd, and May 7th were used to promote the interaction effects of cold soil temperatures. Monitoring of peanut emergence and stand development was conducted beginning within one week after each planting date and continued until flowering. Stand counts, peanut injury, plant width, and other parameters were taken. Plant physiological aspects will be measured to quantify peanut response to flumioxazin and environmental variables. Data gathered will include gas exchange and fluorescence that measure net photosynthesis, stomatal conductance to water vapor, electron transport rate, and actual quantum yield of photosystem II, using the LI-6800 (LI-COR, Inc., Lincoln, NE) in leaves at various growth stages (V3 to V6) whenever possible. Measurements taken will be conducted on the outermost fully expanded leaflet of each plant for every reading. Photosynthesis is measured by recording CO_2 differences flowing to the leaf from the LI-COR using the following parameters: CO_2 concentration of 400 μmol^{-1} at a flow rate of 500 $\mu mol\ s^{-1}$ and photosynthetic radiation at 1,200 $\mu mol\ m^{-2}\ s^{-1}$ from blue and red light emitting diodes, in a closed leaf chamber (Cutts 2011). Once the chamber has been sealed with the leaf enclosed, time will be allowed for the measurements to stabilize before being taken. Measurements will be conducted at solar noon. Plots will then be maintained weed free and monitored for the entire season and then mechanically harvested to determine yield. The harvests will occur based upon pod maturity and harvested by herbicide treatment and seed germination rate.

Data collected during the lab study was subjected to linear regression using SAS PROCREG analysis. The field study is arranged in a split-split plot design with the main plot as the planting dates, and the subplots as herbicide treatment and germination rate. Means will be separated using Tukey's HSD set at an alpha of 0.05. Data from the field study will be analyzed by growing degree days within each planting date of each location and year.

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CHAPTER 2

PEANUT SEED GERMINATION AND RADICLE DEVELOPMENT RESPONSE TO DIRECT EXPOSURE OF FLUMIOXAZIN ACROSS MULTIPLE TEMPERATURES[1]

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"Peanut Seed Germination and Radicle Development Response to Direct Exposure of Flumioxazin Across Multiple Temperatures"

Abstract

Peanut injury in the field can occur from flumioxazin applied PRE, but this is associated with plants that have emerged, or are about to, emerge from soil. The direct effect of flumioxazin on peanut seed germination and radicle development has not been evaluated. Therefore, research was conducted to determine peanut seed radicle development response to flumioxazin at different concentrations (0.0, 0.01, 0.1, 1.0 and 10.0 ppb) when tested at multiple temperatures (20, 23, 26, and 29 C) in laboratory experiments on a thermogradient table. Data analysis indicated that flumioxazin concentration was not different from the nontreated control (0.0 ppb) for 0.01, 0.1, and 1.0 ppb for peanut germination. Flumioxazin at 10.0 ppb was different from all other treatments and the nontreated control. However, comparing linear regression models for each flumioxazin concentration across all temperatures indicated no differences for slope. These data indicate that when there is direct peanut seed exposure to flumioxazin at field application rates, there is no impact on germination and radicle development. Temperature was noted to affect radicle development greater than field application rates of flumioxazin. As temperature decreased, germination and radicle length was inhibited or decreased, respectively.

Introduction

Georgia produced 51% of all U.S. peanut (*Arachis hypogaea* L.) in 2019 (Neal 2019). Georgia peanut growers achieve high yields by utilizing seeds that have been tested for quality and vigor. Excellent seed quality is crucial in establishing peanut stands of 20 plants/m of row (Monfort 2019), and healthy plants early in the season. Healthy emerging plant characteristics include undamaged cotyledons on a thick hypocotyl narrowing above the root, an epicotyl with more than one primary leaf, and radicle that has adventitious roots (Peterson *et al*. 2018). To be classified as certified peanut seed in Georgia, Georgia Department of Agriculture Lab testing must indicate at least 70% germination (Black 2018).

Peanut seed storage techniques are an important factor in seed deterioration. The relative humidity (RH) and temperature of the storage environment are important due to influencing seed moisture and rate of seed cellular metabolism (McDonald 2004). Optimal peanut seed storage environment is 10 C at 65% RH, once peanuts have been dried to 6% moisture content (Ketring 1992). Under controlled storage conditions, Navarro *et al*. (1989) concluded that as humidity increased, peanut seed germination decreased. The researchers placed in-shell peanut at 15 C at 79 to 83% RH, with subsequent testing resulting in greater than 80% germination 150 d after storage initiation. When RH was increased from 85 to 89%, peanut seed germination decreased to 30% at 80 d of storage. The investigators then increased temperature to 26 C and used the same RH, this resulted in germination less than 50% at 90 d of storage for both humidity levels. Morton *et al*. (2008) indicated that germination rate and in-field stand establishment may be acceptable to growers, but poor seedling vigor may result in suboptimal plant performance.

To achieve high yield in peanut, proper weed control must be maintained. Weed species compete with peanut for light, nutrients, space, and water (Grichar and Dotray 2011). The critical period of weed control for maximum peanut yield will vary by broadleaf and grass species (Everman *et al*. 2008 a; 2008 b). Critical period is defined as the maximum time the crop and weed can compete in the early season without hindering crop performance and minimum weed-free time that will prevent yield reductions (Everman 2008b). The critical period of weed control for peanut can vary from 3 to 8 wk after planting (Everman *et al*. 2008 b; Webster *et al*. 2007). Growers maintain this period weed free through numerous techniques of integrated weed management practices. These include chemical (herbicides), physical, cultural, and biological methods (Harker and O'Donovan 2013), though herbicides are the dominant tool. Proper herbicide mode of action rotation is essential to prevent weed resistance to herbicides.

Georgia peanut growers use diclosulam and flumioxazin for weed control in peanut (Monfort *et al*. 2019). In 2018 according to the USDA chemical use database, 45, 14, and 25% of Alabama, Florida, and Georgia peanut hectares, respectively, were treated with diclosulam (NASS 2019). Diclosulam is an acetolactate synthase inhibitor (ALS) WSSA Group 2 herbicide belonging to the triazolopyrimidines family (WSSA). The ALS group of herbicides prevent weed growth by inhibiting the production of valine, leucine, and isoleucine amino acids. The ALS herbicides have at least 162 resistant weed biotypes across the globe (Heap 2019). The primary mode of resistance found in ALS resistant biotypes is target site mutation in which the herbicide binding position is altered preventing proper herbicide binding (Heap 2013). Wise *et al.* (2009) noted ALS-resistant Palmer amaranth (*Amaranthus palmeri)* populations in Georgia. When herbicide resistant weed populations are established, growers must switch to alternative mechanism of action for successful weed control. Flumioxazin is a protoporphyrinogen IX oxidase inhibitor, WSSA Group 14 herbicide that disrupts proper chloroplast formation by preventing protoporphyrinogen IX from oxidizing into protoporphyrin IX and produces reactive oxygen species (ROS) (Green and Owen 2011; Shaner 2014). The ROS will cause lipid peroxidation, damaging cell membrane and cell walls, resulting in cellular leaking and eventual plant death.

Since registration in 2001, peanut seedling injury from flumioxazin has been noted in field research. Johnson III *et al.* (2006) reported flumioxazin visual injury and stunting on peanut when applied 8 to 10 days after planting (DAP) at 71 g/ha at midseason. The researchers also noted that peanut treated with 107 g/ha of flumioxazin at 4 DAP were injured up to 49% and stunted at midseason. Similar conclusions were noted by Main *et al.* (2003) in which the researchers applied 0, 71, 105, or 211 g/ha of flumioxazin and observed stunting and visual injury. They reported that all treatments caused up to 25% stunting 28 DAP, while the 211 g/ha rate caused stunting up to 56 DAP compared to the non-treated control (NTC). In laboratory research, Williams *et al.* (2015) indicated that peanut radicle length decreased as flumioxazin rate increased with decreasing temperature. There were no differences noted in relation to germination and rate of flumioxazin. Peanut seedling mass was also negatively affected by both flumioxazin rate and temperature. The 2018 USDA chemical use database indicated that 62, 62, and 74% of Alabama, Florida, and Georgia peanut hectares, respectively, were treated with flumioxazin (NASS 2019), emphasizing the importance of this herbicide.

With improved tomato spotted wilt resistant cultivars, growers are now planting peanut in mid to late April (Grey *et al*. 2017). April peanut planting allows growers to spread out their risk associated with biotic and environmental concerns. With the acceptance of new cultivars and planting in April when soil temperatures may be highly variable, the need to ensure consistent stand establishment is always a concern. Peanut injury from flumioxazin PRE applied has not been evaluated at the initiation of seed germination and radicle development stage.

To identify the effects of flumioxazin on peanut seed germination and radicle development, laboratory experiments were conducted to test seed of the same cultivar with varying germination across a range of temperatures using a thermogradient table. Peanut seed germination and radicle development were also measured across varying rates of flumioxazin.

Materials and Methods

Experiments were conducted in 2018 using two certified Georgia Department of Agriculture Lab seed lots. All peanut seed samples had been stored from the September 2017 harvest until the following March 2018 in a warehouse at a constant temperature and relative humidity. After processing at a commercial facility, seed were tested by an independent laboratory to confirm viability. Multiple 22.6 kg bags were obtained from the warehouse and sub-samples taken for testing. Seed lots were tested for germination under varying temperatures to support the recorded germination rates from the seed suppliers.

Peanut seed germination response to flumioxazin and temperature were then evaluated on a thermogradient table. The thermogradient table was created using solid aluminum blocks measuring 2.4 m long by 0.9 m wide and 7.6 cm in height. Each side has a 1.0 cm drilled hole allowing fluid to flow across each side of the table from either a cooling or warming unit (Anova Model A40, Anova Industries Inc., Stafford, TX) through a hose. The fluids consisted of a 1:10 ethylene glycol to water mixture flowing at a rate of 3.8 L per minute that were independent of each other. The units were set to achieve temperatures ranging between 20 C and 30 C across the entire table. Thermocouples consisted of duplex insulated PR-T-24 wire (Omega Engineering, Stamford, CT) and were placed under the table in holes measured 8 mm by 7 cm deep, allowing the couples to be within 5 mm of the table surface in 10 cm intervals. Temperature at each thermocouple was recorded every 30 min. using a Graphtec data logger (MicroDAQ.com Ltd., Contoocook, NH). The temperature was regularly checked to insure proper temperature range was achieved.

Multiple solutions of flumioxazin were prepared in 1000 mL Erlenmeyer flasks. Initially a 10,000 ppb solution was prepared by dissolving 19.7 mg of commercial flumioxazin (Valor SX, 51 WG, Valent Corp., Walnut Creek, CA) in 1L distilled water. A serial dilution was performed to achieve solution concentrations of 0.01, 0.10, 1.0, and 10.0 ppb, and included a nontreated control. These concentrations were selected based upon previous research, in which the researchers noted a linear decrease in radicle length as flumioxazin concentration increased from 0.01 to 10 ppm (Williams *et al.* 2015). Ten whole peanut seed were evenly distributed on germination paper (SDB 86 mm, Anchor Paper Co., St. Paul, MN) and placed in a 100 by 15 mm sterile Petri-dish (Fisher Scientific Education, Hanover Park, IL). Petri dishes with seed were placed on the thermogradient table within a row of cells with temperatures of 20, 23, 26, or 29 C.

The flumioxazin solutions were applied to each Petri dish on the table using a syringe (Fisher Scientific Education, Hanover Park, IL) beginning with the lowest (0.0 ppb) to the highest concentration (10.0 ppb). For each Petri dish with 10 peanut seed, 10 mL of solution was added initially. For each germination rate, there were four replications per concentration per temperature and replicated three times per temperature for all concentrations. During the course of the study, the Petri dish would be replenished with the respective concentration as needed to maintain constant moisture levels and flumioxazin concentration. Seeds of the same germination rate were tested at the same time as to not mix high germination with low germination. When applying the initial treatments, a green light was used to prevent herbicide activation. Upon trial initiation, all seed were kept in complete darkness for 5 days after initial application (DAA).

Seed were then exposed to 8 hours of light via cool, white fluorescent lamps at 10 μmol/m^2/s^1 for an additional 5 days, for a total of 10 days in solution. Seeds were then removed from the thermal gradient table, germination counts taken, and radicle lengths measured. Seeds were considered germinated when the radicle length was greater than 5 mm in length (Grey *et al.* 2011). Fresh weight was obtained by weighing all seeds per dish as well as only germinated seeds.

The experimental design was a randomized complete block with a split-plot, with flumioxazin concentration as the whole plot (blocking factor), and seedling germination and temperature as the sub plots. Flumioxazin concentration, seed germination and temperature were considered as fixed effects, whereas replication and replication x flumioxazin concentration were considered as random effects. Data was also analyzed using ANOVA in SAS 9.4 (SAS Institute Inc., Cary NC). Linear regression analysis was utilized to evaluate the parameter estimates. The intent was to determine if the response could be described by using linear regression

$$y = B1(x) + B0 \qquad [1]$$

where y is peanut radicle length in mm, B0 is the y-intercept, B1 is the slope, and x is temperature. Where there were no interactions, data for peanut radicle length in mm was analyzed to establish the effects of concentration across temperatures. Conversely, data was further analyzed to determine temperature effects across each concentration. Data for the linear regression equations were subjected to ANOVA using the REG procedure in SAS 9.4 (SAS Institute Inc., Cary NC) with mean separation of parameter estimates. The different seed lots (75% and 90%) were not significant, therefore, data was combined for analysis with respect to seed lot.

Results and Discussion

Peanut radicle development response to temperature was not affected by flumioxazin (Table 1). Comparing the slopes of each concentration regression line, there were no differences noted, with r^2 values ranging from 0.55 to 0.80 (Table 2.1). The field rate of flumioxazin is 107 g ai/ha, in terms of soil concentration based on a hectare furrow slice (top 15 cm of soil), which corresponds to 46 ppb. Previous research indicated that the concentration applied to the field was too high for proper radicle development, when in direct contact with peanut seed (Williams *et al.* 2015). Due to flumioxazin being applied on the soil surface and not incorporated, the actual flumioxazin concentration contacting the seed is lower than 46 ppb as it moves through the soil profile. These results indicate that the levels of flumioxazin in the soil profile at the concentrations tested in this study, will not affect germination or radicle development up to 10 days after treatment (DAT).

Temperature affected peanut germination more than flumioxazin. Peanut seed germination was analyzed (Table 2.2) and effect of temperature was observed (P=0.0009), while flumioxazin concentration and temperature by concentration had no differences in germination, P=0.61 and P=0.39, respectively. As temperatures decreased, radicle length was inhibited or development was minimal. (Figure 2.1). This indicates that regardless of temperature, flumioxazin did not adversely affect peanut germination and radicle development. These data are supported by Williams *et al*. (2015) in which the researchers noted that as temperatures decreased, both germination decreased and radicle development was reduced. It was noted that the 29 C temperature seeds had the highest germination of the studied temperatures, followed by 23, 26, and 20 C and is supported by Grey *et al.* (2011). Prasad *et al.* (2006) noted that planting early, which coincides with cooler soil temperatures, results in lower peanut seed germination; whereas the later planting dates with higher soil temperatures resulted in higher germination rates. Though flumioxazin may cause physiological injury in seedlings following emergence, this injury is not caused by root absorption. This is supported by Price *et al*. (2004) in which the authors concluded that root-absorbed flumioxazin is quickly metabolized and that injury is likely caused from water splashing onto green plant matter during a rain event while peanuts are emerging.

McNaughton *et al.* (2014) reported that flumioxazin at 107 g ai/ha applied on emerged soybean cotyledons resulted up to 18% injury, primarily consisting of leaf necrosis and stunting at 7 days after treatment. At 28 days after treatment, injury from that same rate of flumioxazin was 8%. Lovell *et al.* (2001) noted that soybean stand counts were reduced and visible plant injury increased as temperatures decreased during crop emergence. The researchers suggested that this was due to reduced metabolism, with some varieties having up to 50% stand reduction with 107 g ai/ha flumioxazin.

Proper planting date, herbicide application, and herbicide activation timings are crucial in establishing a healthy peanut population early in the season. The herbicide label states that peanut may be severely injured if applications are made more than 2 days after planting (Anonymous 2019). Flumioxazin has been noted to cause peanut injury, but has been concluded that this injury does not come from radicle absorption, but rather through splash during a rain event. This injury is transient and typically will not affect yield. Planting peanuts at 10 cm in depth when soil temperatures are >20 C for 3 consecutive days, avoids risk of future cold temperatures to achieve maximum seedling emergence (Monfort *et al.* 2019). These research data support the previous statement by indicating that decrease in temperature result in lower peanut germination and radicle development.

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Table 2.1. Peanut radicle development response to different flumioxazin concentrations across temperature when evaluated on a thermal gradient table[a].

Flumioxazin (ppb)	slope[b]		y-intercept	t value	Pr > t	r^2
0	0.96	a[c]	-1.13	5.23	<0.0001	0.55
0.01	1.11	a	-5.37	6.04	<0.0001	0.80
0.1	1.29	a	-9.23	7.01	<0.0001	0.77
1.0	0.87	a	0.75	4.74	<0.0001	0.62
10.0	1.01	a	-4.64	5.49	<0.0001	0.75
Standard error	0.184		4.54			

[a]Each concentration was evaluated at 20, 23, 26 and 29 C on the thermogradient table with 4 replications, experiments were conducted twice and combined for presentation (n = 320 seed per flumioxazin concentration).

[b]Slopes were calculated by linear regression for flumioxazin concentration as a response of temperature (20, 23, 26 and 29 C).

[c]Values for each concentration slope within a column followed by the same letter are not significantly different at $P < 0.05$ probability level using the REG Procedure in SAS 9.4.

Table 2.2: ANOVA of total peanut germination to different flumioxazin rates across multiple temperatures when evaluated on a thermal gradient table [a].

Effect	Df	Sum Sq.	Mean Sq.	F Value	Pr(>F)
Temperature	3	44.8	44.83	11.19	0.0009[b]
Concentration	4	1.0	1.04	0.26	0.61
Temperature by Concentration	6	2.9	2.92	0.73	0.39
Residuals	476	1907.1	4.01		

[a]Each concentration (0, 0.01, 0.1, 1.0, 10.0 ppb) was evaluated at 20, 23, 26 and 29 C on the thermogradient table with 4 replications, experiments were conducted twice and combined for presentation (n = 320 seed per flumioxazin concentration).

[b]Indicates temperature differences at an alpha level of 0.05.

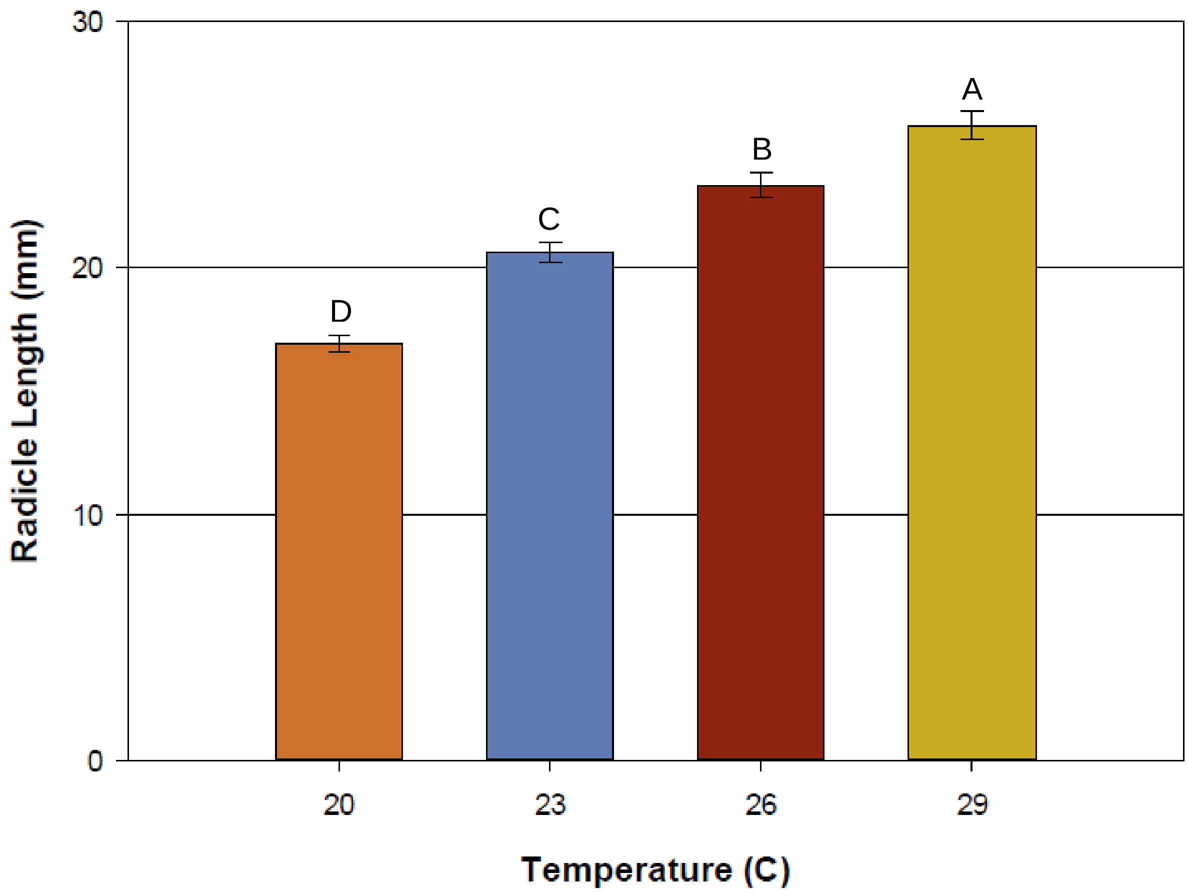

Figure 2.1: Peanut radicle length response to different temperatures across multiple flumioxazin rates when evaluated on a thermal gradient table[a]. Data was subjected to Tukey-Kramer HSD set at an alpha of 0.05.

[a]Each temperature contains radicle length measures from flumioxazin concentrations 0, 0.01, 0.1, 1.0, 10.0 ppb with 4 replications. Error bars represent standard error.

CHAPTER 3

INTERACTION OF SEEDLING GERMINATION, PLANTING DATE, AND FLUMIOXAZIN ON PEANUT PHYSIOLOGY UNDER IRRIGATED CONDITIONS

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“Interaction of Seedling Germination, Planting Date, and Flumioxazin on Peanut Physiology”

Abstract

Diclosulam and flumioxazin PRE applied results in direct peanut exposure to these herbicides prior to seedling emergence. Flumioxazin has been reported to induce injury in adverse weather (i.e. cool-wet soil conditions) at crop emergence. Research at Ty Ty and Plains, Georgia evaluated the physiological effects of PRE herbicides to emerging peanut in 2018 and 2019. Peanut seed with variable germination and planting date were evaluated as additional factors. Peanut plant physiological measurements included electron transport (ETR), net assimilation rate (A_{net}), quantum yield of PSII (Φ_{PSII}), and stomatal conductance to water vapor (GSW). Data were obtained multiple times from V3 to R1 peanut growth stages using a LiCOR 6800, along with stand counts and plant width measures. In 2018, differences were noted in which peanut ETR was reduced by diclosulam when measured across multiple growing degree days (GDD) after planting, as compared to the nontreated control (NTC). Flumioxazin reduced peanut ETR compared to the NTC, at several sample timings for each planting date. In 2019, similar differences were noted, but flumioxazin treated peanut plants had higher ETR. In 2018 and 2019 at both locations, peanut plants had A_{net} with fewer differences than ETR, yet flumioxazin treated peanut was consistently similar to/or greater than the NTC. Peanut Φ_{PSII} responded similarly to A_{net} at each location and year. GSW varied in both years as flumioxazin treated plants noted higher GSW rates than other treated plants, while the next measurement indicated the same plants had a lower GSW compared to the other treated plants. Peanut stand counts, plant widths, and pod yields noted minimal differences. Though peanut plant physiological differences were noted when measured at varying GDD's after planting with the different PRE treatments, planting date, and seed vigor, there were no specific trends noted. Growers will often

observe peanut injury from flumioxazin early in the season. However, it is transient and does not affect yield.

Introduction

Originating from the Bolivia region of South America, peanut (*Arachis hypogaea* L.) is a major crop in the southeastern United States (Putnam et al. 1991). Peanuts were first introduced in the United States in the 1770's by Portuguese or Spanish traders via the African slave trade, but did not gain popularity until the 1800's when they were primarily used as livestock feed, oil, and food for the poor (Anonymous a. 2019; Valentine 2019). The Civil War and cotton (*Gossypium hirisutum* L.) destruction by the boll weevil (*Anthonomus grandis* Boheman) assisted in spreading peanut across the United States and increasing production (Valentine 2019). Currently, 85% of US peanut hectares are planted in the southeastern states (Alabama, Florida, Georgia, Mississippi, North Carolina, South Carolina, and Virginia) with Georgia leading with 47% (Anonymous b 2019). In order to maintain this profitability, peanut growers need to be able to start the growing season clean with high quality seed, and establish an adequate population.

In order to establish adequate populations, planting peanut at the optimal timing is critical. Factors such as soil temperature, air temperature, and soil moisture play an important role in acceptable stand establishment. Kvien *et al.* (2019) indicated that optimal soil temperature for peanut germination ranges from 20 to 35 °C at a depth of 10 cm for 3 consecutive days. Typically, peanut emerges from the soil within 6 to 11 days after planting, depending upon soil and air temperatures (Canavar and Kaynak 2010), optimally growing between air temperatures of 27 to 32 °C (Boote *et al.* 1989). This optimal germinating condition will typically fall between late April and May for southern Georgia, yet some varieties allow for early planting due to a resistance to Tomato spotted wilt virus (Family: Bunyaviridae Genus: *Tospovirus*) transmitted by the western flower thrip (*Frankliniella occidentalis* Pergande) and tobacco thrips (*Frankliniella fusca* Hinds) (Sundaraj *et al.* 2014). Prasad *et al.* (2006) reported that cooler soil temperatures resulted in smaller seedlings and lower plant populations in peanut. These sub-optimal soil temperature conditions may also impact physiological factors in early season peanut growth.

Photosynthesis is a process utilized by plants to grow and produce glucose and oxygen from water and carbon dioxide (Evans 2013 and Kirschbaum 2011), driven by photosynthetically active radiation (PAR). PAR are light wavelengths between 400 and 700 nm (Möttus 2012). Within the chloroplast, two reactions take place that make up photosynthesis, light and dark, but for the purposes of this paper, the focus will be only on light reactions. Virk *et al.* (2019) determined the physiological effects of multiple planting dates on peanut photosynthetic efficiency of first true leaves. The investigators noted that net photosynthesis was unaffected by planting date for any cultivar tested, though numerous photosynthetic reactions were affected. Parameters such as electron transport rate and stomatal conductance to water vapor were all affected by temperatures subjected to the crop by each planting date. These parameters may affect early season growth and development, potentially reducing crop biomass and yield.

Beginning the season weed-free includes using herbicides to allow the crop to outcompete weed species. Maintaining a weed free peanut crop from week 3 to 8 is essential, allowing peanut to achieve a maximum yield (Everman 2008). Georgia growers typically apply PRE herbicides including diclosulam and flumioxazin (Monfort 2020). Diclosulam has been used as a PRE herbicide in peanut since 2000 (Grey et al. 2003). Diclosulam affects growing plants by inhibiting the acetolactate synthase enzyme (ALS) from properly functioning, which will prevent the formation of the branch chain amino acids valine, leucine, and isoleucine. This occurs through diclosulam binding to the ALS enzyme active site channel, preventing substrates from entering the active site (Pang 2002; Zhou 2007). This reduction in branch chain amino acids will result in disruption of the G1 or G2 phase of mitosis, preventing cell division and plant growth (Jachetta 2011). One major concern for Georgia growers when using diclosulam is carryover into the following crop. Diclosulam at the recommended labeled rate has 10 and 18 months rotation restrictions for cotton and corn, respectively (Anonymous d 2010). As Georgia produced the 2^{nd} highest amount of cotton and cottonseed in the United States in 2018, this carryover could potentially cause early season injury issues (Anonymous e 2018). If growers plan on rotating to corn or cotton following a peanut crop, as recommended by the University of Georgia, other herbicides should be applied in the peanut growing season (Tubbs 2019).

Another PRE herbicide that can be used in peanut production is flumioxazin. Since 2001, growers have been able to use flumioxazin as an additional alternative to peanut weed control when the Environmental Protection Agency granted Valent U.S.A. a full flumioxazin peanut registration (Grichar 2004). Flumioxazin is an *N*-phenylpthalimide and is classified as a PPO-inhibiting herbicide (Shaner 2018). In a normal functioning chloroplast, protoporphyrinogen IX oxidase will oxidize protoporphyrinogen IX (PPGIX) into protoporphyrin IX (PPIX) to eventually become chlorophyll or cytochromes. Once a PPO-inhibiting herbicide is absorbed, the oxidation process will not occur. This will result in a buildup of PPGIX that will undergo extraplastidic oxidation (Grossmann et al. 2010). After oxidation, the newly formed PPIX will begin absorbing light, resulting in radical singlet oxygen species that will cause cell membrane and pigment destruction, tissue decay, and eventual plant death. Opposite of diclosulam, flumioxazin has only a 14 day and 2 month rotation interval to corn and cotton, respectively (Anonymous f 2010). Though flumioxazin can provide excellent early season weed control, adverse environmental conditions may cause flumioxazin injury on desired crops. Numerous studies have established that cotton and peanut may sustain injury after emergence from flumioxazin applications (Berger et al. 2012; Jordan et al. 2003; Price et al. 2004; Johnson III et al. 2006; Wilcut et al. 2001).

Other crops have shown sensitivity to POST flumioxazin applications. Wilcut *et al.* (2001) reported rainfall splash bouncing up to 15 cm reaching green plant matter, did not cause yield loss or fiber quality reduction in cotton. Bigot et al. (2007) performed a study measuring the physiological response of grapevine (*Vitis vinifera* L.) to flumioxazin applications. The investigators noted that net photosynthesis rate was significantly decreased when 5 mM and 50 mM concentrations were applied at only one day after soil application. At the 0.5mM concentration, the net photosynthesis decreased 3 days after application. Other physiological parameters such as stomatal conductance and transpiration rate were also reduced in 1 to 3 days. Overtime, the higher rate of flumioxazin resulted in a net photosynthesis rate of 0, indicating plant death. Other PPO herbicides have been shown to cause injury when plants are subjected to adverse conditions during emergence. Miller *et al.* (2012) reported that soybean sustained injury from saflufenacil when emerging during cold, wet conditions.

Though injury seen is transient and should not not affect yield, physiological injury has not been measured in peanut. The purpose of this study is to quantify flumioxazin interactions in emergence, stand establishment, and photosynthetic efficiency in leaves of peanut seedlings.

Materials and Methods

Irrigated peanut field trials were conducted at the University of Georgia Ponder Farm in Ty Ty, GA (31.51N, 83.65 W) and the Southwest Georgia Research and Education Center in Plains, GA

(32.04 N, 84.38 W) in 2018 and 2019. Soil properties in Ty Ty consisted of 100% Tifton loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with 24.5% clay, 66.7% sand, 8.8 % silt, and 0.28% organic matter in the 2018 location. The 2019 location consisted of Dothan loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults) and Fuquay loamy sand (Loamy, Kaolinitic, thermic Arenic Plinthic Kandiudults) for a soil composition of 22.1% clay, 70% sand, 7.9% silt, and 0.44% organic matter. The 2018 Plains location consisted of Faceville sandy loam (Fine, kaolinitic, thermic Typic Kandiudults), Ochlockonee local alluvium (Coarse-loamy, siliceous, acid, thermic Typic Udifluvents), and Tifton sandy loam (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults) for a soil composition of 26% clay, 62% sand, 12% silt, and 0.44 % organic matter. The 2019 location consisted of a Greenville sandy loam (clayey, kaolinitic, thermic Rhodic Kandiudults) and Tifton sandy loam (Fine-loamy kaolinitic, thermic Plinthic Kandiudults) for a composition of 34.7% clay, 57% sand, 8.3% silt, and 0.24 organic matter.

Experimental design was a split-split-plot with 4 replications. Main plots were planting dates to simulate one early planting and 2 on-time plantings. One subplot is seed with 90% or 75% germination, while the herbicide treatment sub-plots were flumioxazin at 107 g ai/ha (Valor SX, 51 WG, Valent Corp., Walnut Creek, CA), diclosulam at 27 g ai/ha (Corteva Agriscience, Wilmington, DE, 80705) and a nontreated control (NTC). Herbicide treatments were applied immediately after planting using TeeJet (TeeJet, Wheaton, IL) AIXR 11002 nozzles at 187 L/ha^{-1} and 207 kpa. Herbicides were irrigated within 3 days of applications to insure proper herbicide activation if no rainfall had occurred within those 3 days. Planting dates in Ty Ty for 2018 were April 9th, April 25th, and May 8th. The 2019 plantings were conducted on April 12th, April 25th, and May 9th. The planting dates for Plains in 2018 were on April 20th, May 3rd, and May 14th.

Planting dates in 2019 were on April 17th, May 1st, and May 14th. The Plains location was planted in a single-row manner for a population of 18 seed/m, while Ty Ty was planted in twin rows for 18 seed/m for both twin rows in each year (Monfort *et al.* 2019). All plots at both locations and both years received a PRE blanket application of pendimethalin at 1067 g ai/ha. All plots at Ty Ty received an application of phorate at 2945 g/ha both years. The Plains location did not receive a PRE insecticide treatment, but did receive a POST treatment of dicrotophos at 140 g/ha. All plots were maintained weed-free and under University of Georgia agronomic recommendations (Monfort 2019).

Numerous steps of photosynthesis can be measured using specialized instruments. The infrared gas analyzer LiCOR 6800 (LiCOR Biosciences, Lincoln, NE) can measure multiple physiological parameters, including net photosynthesis assimilation (A_{net}), electron transport efficiency (ETR), quantum yield of PSII (Φ_{PSII}), and stomatal conductance to water vapor, all of which were collected in this study. The LiCOR compares the mass flow per time of these gases and determines the net assimilation rate using:

$$Anet = \frac{\mu_0[c_0 - c_a(\frac{1-\omega_0}{1-\omega_a})}{s}$$

where Anet represents net carbon assimilation, μ_0 is the flow rate entering the leaf chamber, C_0 is the CO_2 concentration entering the chamber, ω_0 is H_2O entering the chamber, μ_a is the flow rate of air leaving the chamber, C_a is the CO_2 concentration leaving the chamber, ω_a is the H_2O leaving the chamber, and s represents the leaf area. Quantum yield of PSII can be described by using: [2]

$$\Phi_{PSII} = \frac{[Fm' - Fs]}{Fm'}$$

where Fm' represents the maximum fluorescence yield of PSII and Fs represents the minimum fluorescence yield of PSII. ETR is determined by:

$$ETR\colon\ \Phi_{PSII} f Q \alpha_{leaf}$$

where Φ_{PSII} represents the quantum yield of PSII, f is the fraction of photons going to PSII, and $\alpha_{leaf\backslash}$ represents absorption at measurement wavelengths. Finally, the stomatal conductance to water vapor is represented as GSW and is calculated by:

$$\frac{2}{\left(\frac{1}{gtw}-\frac{1}{gbw}\right)+\sqrt{\left(\frac{1}{gtw}-\frac{1}{gbw}\right)^2+\frac{4K}{(K+1)^2}\left(2\frac{1}{gtw}-\frac{1}{gbw}\right)\frac{1}{gbw}}} \quad [3]$$

Where gtw is the total conductance to water vapor, gbw is the boundary layer conductance to water vapor, and K is the stomatal ratio (Anonymous c 2017).

The gas analyzer was equipped with a MultiPhase Flash™ Fluorometer set to control the flow rate to leaf chamber at 400 µmol s^{-1}, temperature set at leaf temperature, and set to measure 2 cm^2 of the clamped leaf. Readings were taken within 2 hours of solar noon in full sun conditions and between growth stages of V3 to R1 in 2018, and V3 to V6 in 2019 (Boote 1982). This measurement timing was chosen as ETR rates are at maximum rate and stability (Snider *et al.* 2013). Physiological data collection timings can be found on Table 3.1 for both years and all planting dates. Readings were collected when measured parameters stabilized, or 2 minutes after leaf chamber was clamped onto the outermost, fully expanded top leaf. Stand counts were taken twice per planting date at each location in 2018 and three times per planting date at each location in 2019. Stand counts were collected at a length of 1 m and each row was counted per plot. Twin row plots had both sides of the row counted as one row. Plant width was collected by measuring the widest leaf-tip to leaf-tip of the plant, with 3 plants per plot being measured. Inversion of each planting date was determined by the Hull-Scrape Method described in Williams and

Drexler (1981). In 2018, Ty Ty plant date 1 was inverted 2,295 growing degree days (GDD), 2,387 GDD for plant date 2, and 2,409 GDD for plant date 3. Plains GDD for harvesting plant date 1, 2, and 3 were 2,208, 2,387, and 2,522, respectively. In 2019, Ty Ty peanuts at planting date 1, 2, and 3 were harvested at 2,335, 2,423, and 2,472 GDD respectively. Plains plant date 1 peanuts were harvested at 2,395, GDD, followed by plant date 2 peanuts at 2,549 GDD, and finally, plant date 3 peanuts were harvested at 2,512 GDD. Several days above soil drying time was allowed for harvest with a small plot combine. At harvest, peanut pod yield was collected.

Data were subjected to ANOVA utilizing RStudio Version 3.5.1 (Rstudio, Boston, MA) at an alpha level of 0.05. Data were then subjected to Tukey-Kramer HSD in SAS University Edition (SAS, Cary, NC) separated by year, location, planting date, and measurement. Seedling germination and herbicide treatment for each planting date and measurement were analyzed, including their interactions.

Results and Discussion

Due do differing weather conditions in 2018 and 2019 (Table 3.2 and 3.3), analysis was performed separately by year and location. ANOVA performed by year and location indicated numerous herbicide, germination, or herbicide by germination differences. Peanut of varying germination did respond differently to herbicide treatments at specific growing degree days, but no trend was noted across the entire season. Figures 3.1 and 3.2 indicate herbicide treatment differences per location, year, parameter, and planting date.

Plains: In 2018, a total of 5 measurements per planting date were taken from growth stages V3 to R1 (Boote 1982), while only 4 measurements were taken between V3 and V6 in 2019. Measurements taken at later growth stages in 2018 were due to early season rainfall preventing field entrance. Physiological measurements at multiple planting times have been studied by Virk *et al.* (2019) and noted A_{net} to be within 15 and 25 µmol m^{-2} s^{-1} while ETR was within 97 and 267 µmol m^{-2} s^{-1} for peanut. Peanut physiological measurements recorded were within or near Virk *et al*. findings validating each parameter.

In 2018, quantum yield of PSII was affected at 456 GDD of plant date 1 in which both germination and herbicide by germination differences were indicated. The low germination plants noted a higher PSII rate compared to the high germination plants. The flumioxazin and NTC low germination plants had a significantly higher PSII rate when compared to the NTC high vigor plants, while other germination by herbicides were not different. Germination differences were noted at 925 GDD in which the low germination plants indicated a higher PSII yield than the lower germination plants. In plant date 2 of 2018, germination differences were noted in that the high germination plants indicated a higher PSII yield compared to the lower germination plants at 356 GDD. During plant date 3, the 626 GDD measurement indicated the flumioxazin treated plants noted a higher PSII yield compared to the NTC, yet both herbicide treatments were not different than the diclosulam treated plants. During the 2019 growing season, differences were noted at the 395 GDD in which the flumioxazin treated high germination plants, along with the NTC and diclosulam low germination plants, had a higher PSII yield compared to the NTC high germination plants. The diclosulam treated high germination and flumioxazin treated low plants were not different from any treatment. Finally, the 465 GDD measurement of PD3 indicated that lower germination plants had a higher PSII yield compared to the high germination plants.

In 2018, the ETR rates in plant date 1 at 375 GDD noted a difference in that the low germination plants had a higher ETR compared to the high germination rates. This translated into germination by herbicide interactions in that the high germination plants treated with diclosulam had a lower ETR from all other treatment combinations and germination rates, except for the flumioxazin treated low vigor plants. Differences were also recorded at 404 GDD of plant date 1 with herbicide treatment differences. The flumioxazin treated plants indicated a lower ETR than the other herbicide treatments. This also translated into germination by herbicide interactions, which is indicated in Table 3.4. At 530 GDD of plant date 1, herbicide treatment, germination, and their interaction differences were noted. The diclosulam treated plants indicated a higher ETR than the other herbicide treatments, as well as the lower germination plants had a higher ETR than the high germination plants. Herbicide by germination rate interactions indicated that the diclosulam treated plants with low germination had a higher ETR compared to the flumioxazin treated plants of both germination rates, as well as the NTC high germination seed, yet was not different than the remaining treatment by germination rates. Finally, at 925 GDD, differences were noted with herbicides, germination rates, and interactions. As with the 530 GDD measurement, the diclosulam treated plants were higher than the other herbicide treatments. It was also reported that the high germination plants had a higher ETR than the lower germination plants. The herbicide by germination interaction are noted in Table 3.5. Plant date 2 also noted treatment differences in that the NTC plants had a higher ETR than the diclosulam treated plants, yet both were not different than the flumioxazin treated plants at 282 GDD. Herbicide treatment by germination rate interactions are indicated in Table 3.6. At 356 GDD of plant date 2, treatment and treatment by germination interactions were noted in that the NTC and diclosulam treated plants had a higher ETR compared to the flumioxazin treated plants. It was recorded that only the flumioxazin treated plants had a lower ETR compared to the other herbicide and germination interactions. Differences were noted for all herbicides, germination rates, and interactions at 722 GDD of plant date 2. All herbicide treatments were different from each other, while the low germination plants had a higher ETR than the high germination plants. The diclosulam treated plants with low germination were different from all treatment and germination rates except for the diclosulam treated high germination plants. Diclosulam treated plants from both germination rates were significantly higher than the flumioxazin treated plants with high germination. Finally, at 834 GDD of plant date 2, all treatments were different from with each other, with the flumioxazin treated plants indicating a lower ETR than the other herbicide treated plants. Plants with both high and low germination treated with diclosulam and the NTC high germination plants were all significantly higher than the other herbicide and germination combinations. During plant date 3, differences were noted in that the NTC and diclosulam treatments were different from each other, yet not different from the flumioxazin treated plants, along with the low germination plants being higher than the high germination plants at 465 GDD. The only interaction indicated that the diclosulam treated plants with high germination were lower than all other combinations. At the 576 GDD measurement, the flumioxazin treated plants recorded a lower ETR than all other treated plants. Herbicide by germination interactions noted differences in that the flumioxazin treated plants with low germination were different from all combinations, except for the flumioxazin treated plants with high germination. The flumioxazin treated plants with high germination were also not different from the diclosulam treated plants with high germination, while being different from the remaining herbicide and germination combinations. At the 1159 GDD measurement of plant date 3, only treatment differences were noted as the diclosulam treated plants indicated a higher ETR than the NTC plants, while both were not different from the flumioxazin treated plants. Finally, at the 1192 GDD measurement of plant date 3, treatment differences reported that the diclosulam treated plants had a higher ETR compared to the flumioxazin treated plants, yet both were not different from the NTC plants. Herbicide by germination rate interactions indicated that the flumioxazin treated plants with high germination were different from the diclosulam treated plants of both germination rates and the NTC high germination plants. The other combinations were not different from each other.

Stomatal conductance to water vapor differences were noted at 404 GDD of plant date 1 with the flumioxazin treated plants having a higher GSW than the NTC, yet the diclosulam treated plants were not different from either treatment. These differences were also noted at 456 GDD of plant date 1, but the differences translated into herbicide by germination differences also. These differences are described in Table 3.7. Measurements of plant date 2 at 356 GDD indicated a germination difference in that the high vigor plants had a higher GSW compared to the low germination plants. This difference then translated into herbicide by germination interactions in that the diclosulam treated plants with high germination were significantly higher than the flumioxazin treated low germination plants. All other herbicide and germination combinations were not different from one another. At the 410 GDD measurement, treatment differences were noted as the diclosulam treated plants had a high GSW compared to the flumioxazin treated plants, yet the NTC plants were not different from either. The plant date 3 measurement at 465 GDD noted herbicide and herbicide by germination interactions. The flumioxazin treated plants noted a higher GSW than the NTC plants, yet the diclosulam treated plants were not different from either. The flumioxazin treated plants with low germination had a higher GSW rate than the NTC with high germination. All other treatment and germination combinations were not different from each other. The 2019 growing season noted differences at 252 GDD of plant date 1 in which the NTC and flumioxazin treated plants indicated a higher

GSW than the diclosulam treated plants. Next, differences were noted at 346 GDD of plant date 2. The NTC plants indicated a higher GSW compared to the other herbicide treatments, as well as the low germination plants were higher than the high germination seeds. These differences translated into herbicide by germination interactions in that the NTC plants with low germination were higher than the flumioxazin treated high germination plants and the diclosulam treated high germination plants. All other combinations were not different from each other.

Photosynthetic rate was not nearly as affected by herbicide treatments compared to the amount of ETR differences noted. Differences were first noted for plant date 1 at 404 GDD in that the flumioxazin treated plants had a higher A_{net} than the NTC, yet both were not different than the diclosulam treated plants. Next, differences were noted at the 456 GDD measurement in that the NTC high germination plants indicated a lower A_{net} than all treatment and germination combinations, except for the diclosulam treated higher germination and the flumioxazin treated low germination plants. Differences were also noted for plant date 2 at 356 GDD for germination and herbicide by germination. The high germination plants noted a higher A_{net} compared to the low germination plants, which translated into germination by herbicide interactions. The flumioxazin treated plants with low germination had a lower photosynthetic rate compared to the flumioxazin treated and diclosulam treated plants with high germination. The remaining combinations were not different. Next, plant date 3 only had differences at 465 GDD in that the flumioxazin treated plants noted a higher A_{net} than the other herbicides. This was also noted in the herbicide by germination interactions. The flumioxazin treated plants with low germination were higher than the diclosulam treated plants with high germination and the NTC plants with low germination. All remaining treatments were not different. Finally, in 2019, plant date 1 noted differences at 252 GDD in that the NTC plants had a higher A_{net} than the diclosulam treated plants, while the flumioxazin treated plants were not different from either. Differences were also noted at 372 GDD in that the high germination plants noted a higher photosynthetic rate compared to the low germination plants. Plant date 2 at 346 GDD indicated differences in the NTC plants had a higher A_{net} compared to the flumioxazin treated plants, while the diclosulam treated plants were not different from either.

Stand counts, plant width, and crop yield were also recorded and analyzed. Plant date 3 in 2019 noted a difference as the NTC plants were wider than the flumioxazin treatment, with the diclosulam treated plants were not different from either herbicide treated plants. In 2018 and 2019, stand count and yield had no differences noted. Yield for the 2018 and 2019 growing seasons are described in Table 3.8. Though physiological differences were noted throughout both seasons, no trend was noted and injury was transient in either.

Ty Ty: In 2018, differences in quantum yield of PSII were noted for planting date 1 at 413 GDD in that the high germination plants were higher than the low germination plants, and also indicated herbicide by germination differences. High germination plants treated with flumioxazin indicated a higher yield of PSII compared to the NTC low germination seed, while all other combinations were not different from each other. Differences at 459 GDD were also noted with the flumioxazin treated plants being higher in PSII yield compared to the diclosulam treated plants, while the NTC plants were not different from either herbicide. At 652 GDD of planting date 2, there were herbicide by germination interactions in which the NTC low germination plants were different from the flumioxazin treated high and low germination plants. All other combinations were not different from each other. In 2019, differences were only noted at 487 GDD of planting date 1, in that the high germination plants indicated a higher PSII yield compared to the low germination seed.

Next, ETR differences were first reported at 459 GDD of plant date 1 in 2018. Differences were noted in which the NTC plants had a higher ETR compared to the flumioxazin treated plants, yet the diclosulam treated plants were not different than either treatment. This was also noted in the herbicide by germination interactions in that both germination rates of NTC plants had a higher ETR compared to the low germination rate plants treated with flumioxazin. All other treatment combinations were not different. Next, differences were also reported at 490 GDD of planting date 2, in which the flumioxazin treated plants were lower than the other two herbicide treatments. These treatment differences did indicate treatment by germination interactions which are described in Table 3.9. Differences were noted also at 544 GDD of planting date 2 in which the diclosulam treated plants had a lower ETR than the other treatments. The herbicide by germination interactions indicated a difference between the flumioxazin treated plants with low germination having a higher ETR compared to the diclosulam treated plants with high vigor only. All other treatment by germination combination were not different. During planting date 3, differences were indicated at 331 GDD as the flumioxazin treated plants had a lower ETR than the other 2 herbicide treatments. This was also noted in the herbicide by germination interactions in which the flumioxazin treated low germination plants were different from all combinations, except the flumioxazin on high germination seed. The flumioxazin treated high germination plants were not different from any herbicide and germination rate combination. The final differences for ETR in 2018 were on planting date 3 at 494 GDD. Differences were noted that the NTC plants were higher compared to the diclosulam treated plants, yet the flumioxazin treated plants were not different from either treatment. Interaction differences indicated that the diclosulam treated plants with low germination were different from the NTC plants of both germination rates. Other herbicide and germination rates were not different. The only 2019 season differences were noted in planting date 1 at 487 GDD in which the high germination plants noted a higher ETR compared to the lower germination plants.

Furthermore, differences were reported for GSW at planting date 1 at 459 GDD in that the flumioxazin treated plants had a higher rate than the diclosulam treated plants, while the NTC plants were not different from either for the 2018 growing season. Interaction differences indicated the diclosulam treated plants with high germination were lower than the flumioxazin treated plants with high germination, yet both were not different from all other herbicide and germination combinations. Differences were also noted at 652 GDD on planting date 2 for germination, herbicide, and their interactions. The low germination plants had a higher GSW compared to the high germination plants, while he NTC plants were higher than the other to herbicide treated plants. These differences resulted in interactions in which the NTC with low germination were higher than the flumioxazin and diclosulam treated plants with high germination, while the remaining combinations were not different. Herbicide differences were also noted at 357 GDD of planting date 3 in which the flumioxazin treated plants were higher than the diclosulam treated plants, while the NTC plants were not different from either. In 2019, GSW differences were noted at 305 GDD of planting date one as the flumioxazin treated plants were higher than the diclosulam treated plants, yet the NTC were not different from either. This was also noted in the herbicide by germination interactions in which the flumioxazin treated plants with low germination were higher than the diclosulam treated plants of both germination rates. Differences were also noted at 485 GDD of planting date 2 in which the NTC with low germination indicated a higher GSW than the NTC plants with high germination, as well as plants treated with flumioxazin with low germination. The other herbicide and germination combinations were not different from either. The final differences were at 420

GDD of planting date 3 in which the flumioxazin treated plants noted a higher GSW compared to the diclosulam treated plants, while the NTC plants were not different from either.

Finally, photosynthetic rate differences were first noted at planting date 1 at 413 GDD of 2018, in which the diclosulam treated plants indicated a lower A_{net} compared to the other herbicide treatments. This was also noted in the herbicide by germination interactions in which diclosulam treated plants with low germination were lower than the flumioxazin treated plants with high germination, as well as the NTC plants with low germination. More differences were also noted at 459 GDD of planting date 1 in that the flumioxazin treated plants were higher than the diclosulam treated plants, yet the NTC was not different from either herbicide treatment. This was also noted in the herbicide by germination interactions in which the flumioxazin treated plants with high germination noted a higher A_{net} compared to the diclosulam treated plants with high germination, while both treatments were not different from any other combination. Next, differences were also noted at 575 GDD of planting date 2 with the low germination plants indicated a higher A_{net} than the high germination plants, which was also noted in the herbicide by germination interactions. The diclosulam treated plants with high germination was different from all combinations, except the NTC on low germination plants and the flumioxazin treated plants with high germination. The last A_{net} differences for the 2018 season were noted at 357 GDD of planting date 3 in which the diclosulam treated plants were lower than the flumioxazin treated plants, while the NTC were not different from either herbicide treatment. The 2019 growing season had differences beginning at 487 GDD of planting date 1 with the high germination plants having a higher A_{net} than the low germination plants. Planting date 2 at 271 GDD noted differences in herbicide treatment, germination, and their interactions. First, the NTC plants were higher than the flumioxazin treated plants, while the diclosulam treated plants were not different from either. The high germinating plants had a higher A_{net} compared to the low germinating plants. The interaction difference was noted to be the NTC on high germinating plants was higher than all other treatment combinations. Differences were also noted at 361 GDD of planting date 3 in which the NTC plants indicated a higher A_{net} than the diclosulam treated plants, while the flumioxazin treated plants were not different from either. The final A_{net} difference was noted with the flumioxazin treated plants were higher than the diclosulam treated plants, while the NTC were not different from either at 420 GDD of planting date 3.

Finally, the GSW of plant date 1 measurement 3 indicated that there were no differences between the flumioxazin treated plants and the NTC. The diclosulam treated plants, though, were noted to be lower than the flumioxazin plants, yet not different than the NTC. In plant date 2 measurement 4, it was recorded that both flumioxazin and diclosulam treated plants had lower GSW when compared to the NTC. In the 2019 season, both plant date 1 measurement 3 and plant date 3 measurement 3 noted flumioxazin treated plants had a higher GSW than the diclosulam treated plants, while the NTC were not different from either treatment.

In 2019, stand counts were unaffected by herbicide treatments. Plant widths in 2019 were affected in plant date 2 and 3. Plant date 2 noted a treatment difference as flumioxazin was different than the remaining treatments by causing some stunting. Plant date 3 recorded that only the NTC was higher than flumioxazin, as both diclosulam and flumioxazin caused stunting. Yields for each plant date noted no differences for both years, indicating that though some injury may be seen early in the growing season, the injury is transient. These data may differ from peanut grown under non-irrigated conditions, warranting to further this study under non-irrigated conditions to collect data for Georgia growers who may not use irrigation.

During both growing seasons, rainfall events occurred during, or shortly after peanut emergence. As the rain impacted the ground, splash bounced onto green leaf matter, while also carrying flumioxazin. The flumioxazin would then cause necrotic and chlorotic injury on the leaves. This injury was noted periodically to occur on the measured leaf, partially causing a reduction in photosynthesis. This was reported by Garry *et al.* (1998) in which the investigators noted a reduction in photosynthesis as necrosis was noted on pea (*Pisum sativum* L.) leaves. It was not directly proportional to injury, though, in that photosynthesis was not noted to decrease as necrotic injury increased. Bigot *et al* (2007) indicated that as stomatal closure occurred from flumioxazin, photosynthesis was reduced, as well as plant transpiration. The investigators also noted that Φ_{PSII} was significantly reduced in grapevine leaves and cutting, due to alterations of other photochemicals, causing a higher number of closed PSII reaction centers. Though numerous physiological injuries occur, this injury was noted to not cause yield loss or reduced fiber quality in cotton (Wilcut *et al* 2001). This supports the findings of this study in that peanut yield for all treatments, with respect to planting dates and locations, were not different in each year.

The 2018 and 2019 growing seasons had drastically different weather events which may have contributed to the varying measurements between years. The increased rainfall during emergence in 2018 played a role in causing increased injury in the 2018 season, and not in the 2019 season as previously discussed. Though numerous differences were noted, no season long trend of injury was noted. The physiological injury noted during the measurements was transient and did not impact yield (data not shown). Though growers may see early season injury from flumioxazin in adverse weather conditions, this injury will likely not cause yield reductions.

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Table 3.1: Physiological data collection timings in 2018 and 2019 for Plains and Ty Ty, GA.

Location and Year		Measurement[a]	Planting Date 1		Planting Date 2		Planting Date 3	
			Date	GDD[b]	Date	GDD	Date	GDD
Plains	2018	1	5/11	375	5/14	282	6/1	493
		2	5/12	404	5/17	356	6/5	599
		3	5/14	456	5/19	410	6/7	654
		4	5/17	530	6/1	750	6/24	1159
		5	6/1	868	6/5	857	6/25	1192
	2019	1	5/1	252	5/14	329	5/24	284
		2	5/6	372	5/15	346	5/28	422
		3	5/7	395	5/16	367	5/29	455
		4	5/14	556	5/21	507	6/3	614
Ty Ty	2018	1	5/4	389	5/16	490	5/19	332
		2	5/5	413	5/18	544	5/20	357
		3	5/7	459	5/19	575	5/22	409
		4	5/10	537	5/22	653	5/25	494
		5	5/16	704	5/25	738	5/30	631
	2019	1	4/27	251	5/6	271	5/22	361
		2	4/28	267	5/7	295	5/23	390
		3	4/29	306	5/8	322	5/24	420
		4	5/6	487	5/15	485	5/30	624

[a] Gas exchange and fluorescence measurements were recorded using the LI-6800 infrared gas analyzer.

[b] GDD is the number of growing degree days from the plant date to the specific measurement date. The GDD formula utilized is: ((Maximum temperature + minimum temperature)/2) – 50 in Fahrenheit.

Table 3.2: Plains, GA weather data for the 2018 and 2019 growing seasons.

	2018			2019		
	Temperature		Rainfall	Temperature		Rainfall
Month	Maximum	Minimum		Maximum	Minimum	
	——C——		—cm—	——C——		—cm—
April	22.9	11.2	7.2	26.6	11.5	3.5
May	29.2	17.9	20.2	31.4	18.4	4.0
June	31.8	20.7	12.5	32.7	20.9	3.7
July	32	21.6	14.1	33	21.5	18.5
August	31.4	20.9	10	33	21.9	10.2
September	32.7	21.1	8.1	33.8	19.9	0.1
October	28.4	16.7	16.9	28	15.8	12.1

[a]Temperature is averaged across the entire month and rainfall is total amount. Collection began on 1st planting data and terminated on final harvest date.

Weather data was collected using the University of Georgia Weather Network.

Table 3.3: Ty Ty, GA weather data for the 2018 and 2019 growing seasons.

	2018			2019		
	Temperature[a]		Rainfall	Temperature		Rainfall
Month	Maximum	Minimum		Maximum	Minimum	
	———C———		—cm—	———C———		—cm—
May	30.4	18.7	34.1	31.9	19.1	4.4
June	32.8	21.6	33	32.2	21.2	14.7
July	32.5	22.3	52.2	33.3	21.8	8.8
August	32.4	21.9	36.1	33.3	22.5	21.5
September	33.1	21.8	3.74	33.3	20.1	0.36
October	31.3	19.9	0	34.3	20.1	0

[a] Temperature is averaged across the entire month and rainfall is total amount. Collection began on 1st planting data and terminated on final harvest date.
Weather Data was collected using the University of Georgia Weather Network.

Figure 3.1: The response of stomatal conductance to water vapor (g_s), net photosynthesis (A_{net}), quantum yield of photosystem II (Φ_{PSII}), and electron transport rate (ETR) during the 2018 and 2019 growing season in Plains, GA. The asterisk indicates significant differences between the treatments at an alpha of 0.05 according to Tukey's HSD. The solid lines indicate each respective planting date with the first being the on the left, second plant date being in the middle, and the third planting date on the right.

2018 2019

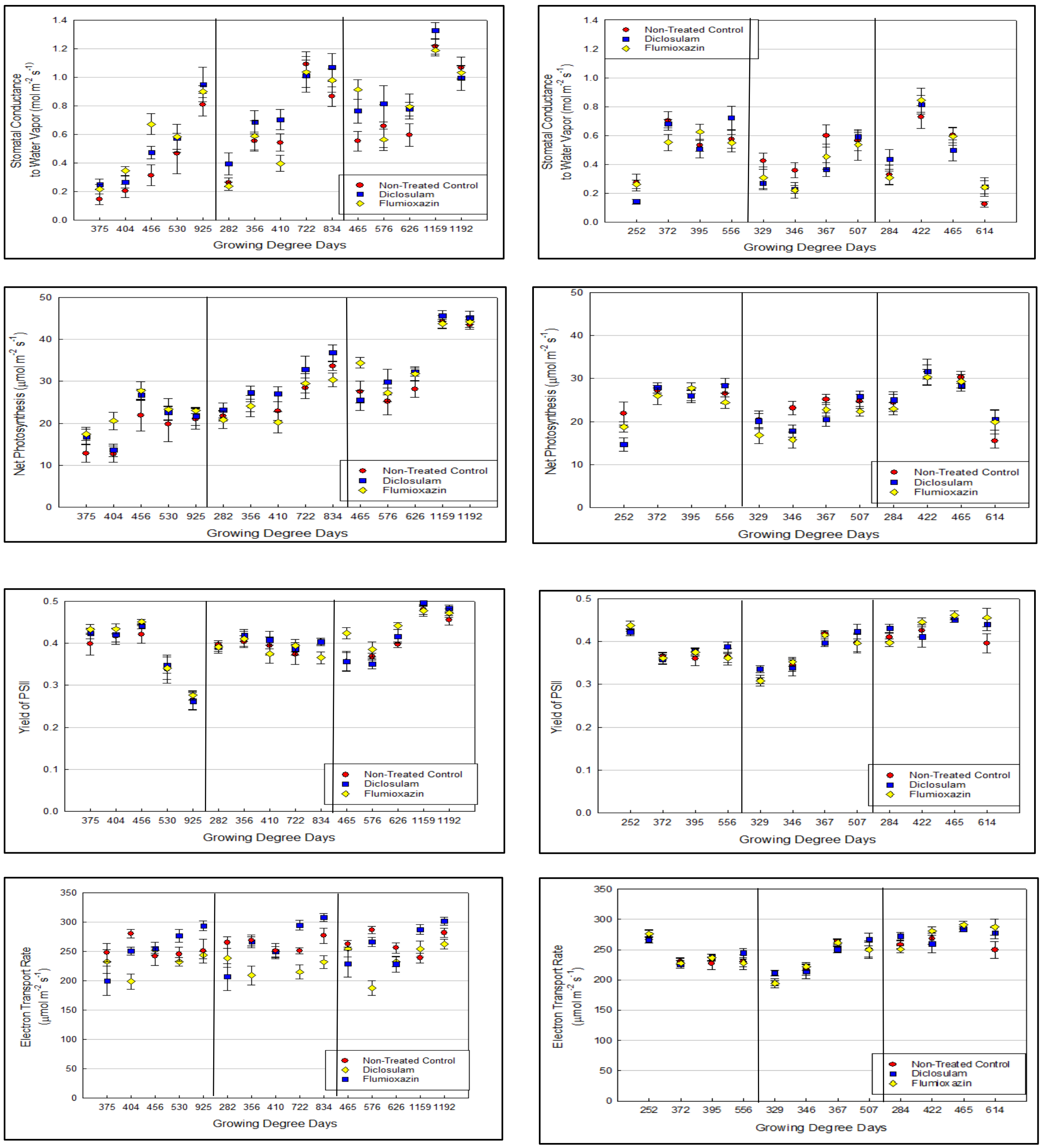

Figure 3.2: The response of stomatal conductance to water vapor (g_s), net photosynthesis (A_{net}), quantum yield of photosystem II (Φ_{PSII}), and electron transport rate (ETR) during the 2018 and 2019 growing season in Ty Ty, GA. The asterisk indicates significant differences between the treatments at an alpha of 0.05 according to Tukey's HSD. The solid lines indicate each respective planting date with the first being the on the left, second plant date being in the middle, and the third planting date on the right.

2018 2019

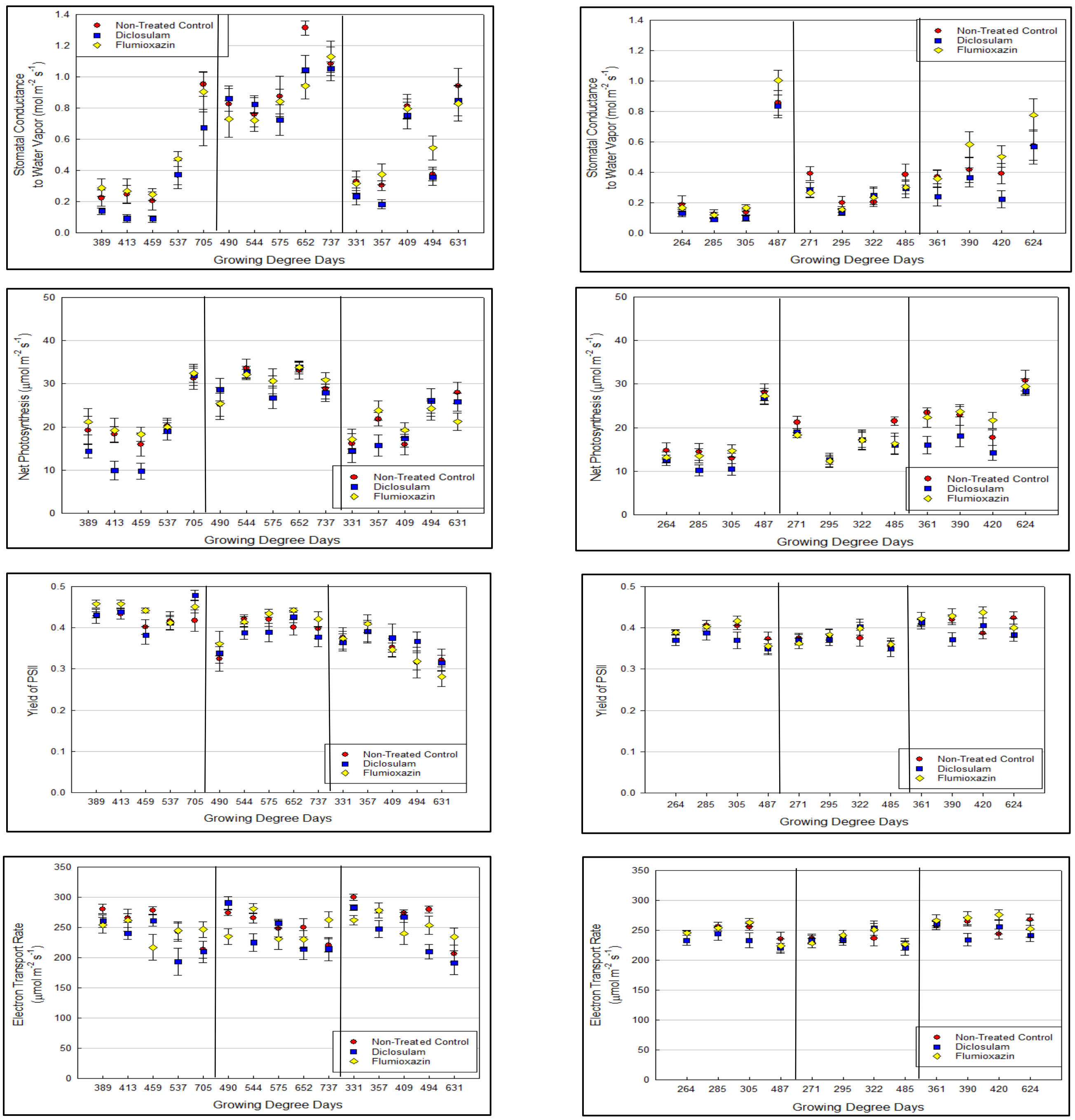

Table 3.4: Herbicide treatment by seedling germination interactions of Plains plant date 1 at 404 GDD for ETR of the 2018 growing season.

Treatment	**Germination**	**Estimate**	
NTC	Low	288	A[a]
NTC	High	273	A
Diclosulam	Low	254	A B
Diclosulam	High	247	A B C
Flumioxazin	High	205	B C
Flumioxazin	Low	193	C

[a] Means with the same letter are not significantly different according to Tukey HSD set at alpha 0.05.

Table 3.5: Herbicide treatment by seedling germination interactions of Plains plant date 1 at 925 GDD for ETR of the 2018 growing season.

Treatment	Germination	Estimate	
Diclosulam	High	304	A[a]
NTC	High	290	A B
Diclosulam	Low	284	A B
Flumioxazin	Low	249	A B
Flumioxazin	High	239	B C
NTC	Low	212	C

[a] Means with the same letter are not significantly different according to Tukey HSD set at alpha 0.05.

Table 3.6: Herbicide treatment by seedling germination interactions of Plains plant date 2 at 282 GDD for ETR of the 2018 growing season.

Treatment	**Germination**	**Estimate**	
Flumioxazin	High	277	A[a]
NTC	High	275	A
Diclosulam	Low	259	A B
NTC	Low	255	A B
Flumioxazin	Low	201	B C
Diclosulam	High	154	C

[a] Means with the same letter are not significantly different according to Tukey HSD set at alpha 0.05.

Table 3.7: Herbicide treatment by seedling germination interactions of Plains plant date 1 at 456 GDD for the stomatal conductance to water vapor of the 2018 growing season.

Treatment	Germination	Estimate	
Flumioxazin	High	0.757	A[a]
Flumioxazin	Low	0.587	A B
Diclosulam	Low	0.574	A B
NTC	Low	0.429	A B C
Diclosulam	High	0.372	B C
NTC	High	0.198	C

[a] Means with the same letter are not significantly different according to Tukey HSD set at alpha 0.05.

Table 3.8: Yield from the 2018 and 2019 growing seasons in Ty Ty and Plains, GA

Location	Year	Treatment	Germination	Planting Date 1	Planting Date 2	Planting Date 3
					kg/ha	
Plains	2018	Diclosulam	High	6136	6285	5743
		Diclosulam	Low	6281	5164	5343
		Flumioxazin	High	6715	6396	6221
		Flumioxazin	Low	6130	5003	6208
		NTC	High	6376	5510	5938
		NTC	Low	6270	5523	6250
	2019	Diclosulam	High	3936	5545	2432
		Diclosulam	Low	4107	5878	2437
		Flumioxazin	High	4303	5551	2450
		Flumioxazin	Low	4283	6140	2223
		NTC	High	3340	5866	2193
		NTC	Low	3598	5247	2450
Ty Ty	2018	Diclosulam	High	6449	6971	7026
		Diclosulam	Low	6473	6826	6423
		Flumioxazin	High	7083	7424	6813
		Flumioxazin	Low	6709	6401	6415
		NTC	High	7473	6948	7449
		NTC	Low	6831	6749	7140
	2019	Diclosulam	High	6483	6808	6141
		Diclosulam	Low	6172	7008	6398
		Flumioxazin	High	6265	6979	6356
		Flumioxazin	Low	6211	6708	6310
		NTC	High	6112	6949	5646
		NTC	Low	6242	6786	5660

Table 3.9: Herbicide treatment by seedling germination interactions of Ty Ty planting date 2 at 490 GDD for ETR of the 2018 growing season.

Treatment	Germination	Estimate	
Diclosulam	High	313	A[a]
NTC	Low	281	A B
Diclosulam	Low	269	A B C
NTC	High	268	A B C
Flumioxazin	Kow	249	B C
Flumioxazin	High	221	C

[a] Means with the same letter are not significantly different.

CHAPTER 4

CONCLUSIONS

Peanut growers across the state of Georgia are seeing injury from flumioxazin applications in adverse weather conditions. The herbicide label indicates that applications must be made within 2 days of planting and prior to peanut emergence. Prior studies have indicated that injury on peanut can be attributed to splash during a rainfall event, rather than root absorption. No data has been collected measuring the peanut response to direct applications of flumioxazin, or the physiological response of emerged peanut to PRE applied flumioxazin.

A lab study was performed measuring the effects of flumioxazin directly applied to peanut seed of differing germination levels on radicle growth and development. Data indicated that flumioxazin directly applied to peanut seed did not affect radicle growth at concentrations similar to field concentrations. Rather, temperature had a greater effect. As temperatures decreased, radicle growth and development did as well, and as temperatures increased, so did radicle growth and development.

Another study was performed to measure the physiological response of peanut to PRE flumioxazin applications in an irrigated setting. Planting dates were selected to simulate an early planting and 2 on time plantings. Injury was noted and did affect the plant physiologically. The injury was transient and did not cause a yield loss under irrigated conditions. The injury was noted to occur after a rainfall event in which water carrying flumioxazin splashed onto green plant matter, causing injury. These data may differ from peanut grown under non-irrigated conditions, indicating more research must be conducted to include all Georgia peanut growers with valuable information.

The information gathered from these studies can be relayed to Georgia growers indicating that flumioxazin will not affect peanut germination or radicle development at concentrations similar to a field setting, and that proper soil temperatures at planting play a greater role in seed germination. Also, although growers may see injury on emerged plants, the injury is transient and should not affect yield. Overall, flumioxazin is a useful tool in weed management for Georgia growers, assisting in grower obtaining maximum yield from their crop.

APPENDIX 1

BERMUDAGRASS TOLERANCE OF INDAZIFLAM PREEMERGENCE APPLICATIONS IN FORAGE PRODUCTION

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Bermudagrass Tolerance of Indaziflam PRE Applications in Forage Production

Abstract

Bermudagrass is a major forage species throughout Georgia and the Southeast. An essential part of achieving high-yielding, top-quality forages is proper weed control. Indaziflam is a residual herbicide that controls many broadleaf and grass species by inhibiting cellulose biosynthesis. Research conducted in Tift and Coulquitt counties in Georgia determined optimal PRE rates for indaziflam for bermudagrass forage production. Treatments applied at spring green up of established 'Alicia' bermudagrass included indaziflam at 47, 77, 155, or 234 g ai ha^{-1} PRE, pendimethalin at 4,480 g ha^{-1} PRE, a split application of indaziflam at 47 g ha^{-1} PRE followed by the same rate applied POST after the first cutting, and a nontreated control (seven treatments in all). Forages were machine harvested three times each year for each location beginning at least 47 d after treatment (DAT) with final cuttings up to 168 DAT. For all treatments, fresh- and dry-weight yields at each harvest and totals for the season did not differ from the nontreated control. Indaziflam at 155 and 234 g ha^{-1} did cause minor stunting at 44 DAT, but this was transient and not observed at the second harvest. Indaziflam applied PRE has the potential to provide residual control of troublesome weeds in bermudagrass forage and hay production, with ephemeral stunting at the recommended application rates.

Introduction

Georgia, Mississippi, South Carolina, and Tennessee are southeastern US states that collectively produce over one billion kg of forage annually each (NASS 2018). The Georgia cattle industry had nearly 1.1 million cattle to feed in 2018 (NASS 2018). In 2017, Georgia produced hay and haylage worth over $187 million (NASS 2018). Supplying forages in the form of hay to animals is required during late autumn and winter months to maintain animal health and productivity. During spring and summer months, producers will harvest forage grass species for hay. In the southeastern United States, a common forage species for hay production is bermudagrass. Many weed species are common in bermudagrass hay fields, and if uncontrolled, will reduce the palatability of the forage (Masters et al. 1996), and performance of the animal. Common and troublesome weeds in forages include various species of crabgrass (*Digitaria* spp.), pigweeds (*Amaranthus* sp.), and thistles (*Cirsium* sp.) (Webster 2012). When present in forages, weeds reduce hay value, nutritional quality, and animal productivity.

Many of the common weed control systems in Georgia and southeast forage production include herbicides that share the same mechanism of action. These include WSSA Group 4's 2,4-D, aminopyralid, clopyralid, dicamba, fluroxypyr, and triclopyr; and the Group 2 acetolactate synthase inhibitors chlorosulfuron, halosulfuron, imazapic, metsulfuron, nicosulfuron, and sulfosulfuron (McCullough 2018). Multiple herbicides are registered for bermudagrass forage production, but many have been shown to cause injury (Bhowmik and Bingham 1990; Butler and Muir 2006; Walker et al. 1998). The production of a perennial forage crop, utilizing the same herbicides over multiple years, can result in weed resistance (McCullough et al. 2013; Simmons et al. 2017). Expanding the number of herbicides with different sites of action available in perennial forage crop production can reduce the selection pressure on currently available herbicides, reducing the chances of weeds becoming resistant.

Indaziflam is a WSSA group 29 alkylazine herbicide that inhibits cellulose biosynthesis of susceptible plants (Brabham et al. 2014), allowing it to control numerous weed species with a soil residual half-life of >150 d (Shaner 2014). It is registered in multiple perennial crops (Grey et al 2016; Jhala and Singh 2012; Marble et al. 2016). It is also registered for use in multiple warm season turfgrasses (Anonymous 2011). Indaziflam has pending registration for bermudagrass forage production with a maximum use rate of 94 g ha^{-1} over a 12-mo period. Brosnan and Breeden (2012) noted that indaziflam provided similar control of nontillered smooth crabgrass to dithiopyr (560 g ha^{-1}) and quinclorac (840 g ha^{-1}). Control of annual bluegrass (*Poa annua* L.) was 88 to 100% in Tennessee when applied PRE and 4 and 8 wk after planting at 35 and 54 g ha^{-1} (Brosnan et al. 2012). In Georgia, investigators noted annual bluegrass control was 97 to 100%, as compared to the nontreated control, 30 wk after treatment (Brosnan et al. 2012). In view of the traditional use of herbicides with the same mechanism of action in forage production, the potential use of indaziflam as an alternative for weed control could provide more sustainable weed management systems for bermudagrass forage production in the Southeast, while proactively avoiding the development of herbicide-resistant weeds.

If registered for forage bermudagrass production, indaziflam as a WSSA Group 29 could provide broad-spectrum control of many weed species and offer alternatives to the WSSA Groups 2 and 4 herbicides. The objective of this research was to determine the tolerance to PRE and POST applications of indaziflam on bermudagrass at multiple rates and timings.

Materials and Methods

Field experiments were conducted in separate areas of the same fields in 2017 and 2018 at the UGA Tifton Campus in Tift County, GA (Latitude: 31.503; Longitude: 83.532) at the UGA Research Dairy, and the Sunbelt Agriculture Exposition Center in Colquitt County, GA (Latitude: 31.14500; Longitude 83.71194) (four tests total). 'Alicia' bermudagrass (Hancock et al. 2017) established stands (>10 years) were utilized at both locations. The experimental bermudagrass areas were established and maintained using best management practices. At establishment and throughout the study period, bermudagrass was close to weed free. The few weeds that were observed were removed by hand. The soil at the Sunbelt Ag Expo consisted of a Dothan loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults), 88% sand, 6.2% silt, 6.0 clay, pH 6.0 to 6.2, 2.9 to 3.5% OM content, while at the UGA Research Dairy soil was Cowarts loamy sand (Fine-loamy, kaolinitic, thermic Typic Kanhapludults) 86% sand, 8.2% silt, 5.9% clay, pH 6.9 to 7.0, 3.3 to 3.4% OM content.

Experimental design consisted of a randomized complete block with four replications. Plot size was 1.5 m wide by 6.1 m long. Treatments applied at spring green up to established 'Alicia' bermudagrass included indaziflam at 47, 77, 155, or 234 g ai^{-1} PRE, pendimethalin at 4480 g ha^{-1} PRE, a split application of indaziflam at 47 g ha^{-1} PRE followed by the same rate applied POST after the first forage harvest, and a nontreated control (seven treatments in all). Initial herbicide applications were made at the Tifton UGA Research Dairy on February 24th, 2017 and February 28th, 2018, and at the Sunbelt Agriculture Exposition Center in Colquitt county on February 27th, 2017, and March 2nd, 2018. For the POST treatment after the first forage harvest, applications were made at the Tifton UGA Research Dairy on April 20th, 2017 and May 11th, 2018, and at the Sunbelt Exposition Center in Colquitt County on June 21st, 2017 and May 16th, for 2018. No adjuvants were applied for the POST applications. Herbicides were applied with a CO_2-pressurized backpack sprayer at 187 L ha^{-1} at 165 kPa using Teejet AIXR11002 nozzles (Teejet Technologies LLC, Springfield, IL).

Bermudagrass injury was visually estimated 20 to 30 d after PRE applications, and 15 to 20 d after POST applications on a scale of 0 (no injury) to 100% (crop death). Bermudagrass forage yield was measured by harvesting each plot with a modified mower with a chute attachment. The chute was placed on the open port of the cutting area to vacuum the clippings. The sample bag was placed at the opposite end of the chute to receive the grass clippings. When wet conditions necessitated a hand harvest, a sample size of 1 m long by 0.5 m wide was measured and grass clipped using a hand-held trimmer. All samples were cut to within 5 cm of the soil surface. Forage samples were weighed for fresh weight, and dried to less than 20% moisture (Hancock et al. 2017) with heated-forced-air (50 C) for three d before dry weight was determined. Harvests at the Tifton Research Dairy were 47, 110, and 168 days after treatment (DAT) in 2017, and 72, 107, and 154 DAT in 2018. Harvests at the Sunbelt Agriculture Exposition Center were 79, 107, and 154 DAT in 2017, and 76, 113, and 154 DAT in 2018. Production and pest management practices other than specific treatments were standard for forage bermudagrass production in Georgia to optimize growth and development (Hancock 2017).

Data were subjected to an analysis of variance (ANOVA) using PROC Mixed in SAS 9.4 (SAS Institute Inc, Cary, NC) combined across experiments, location, and year to test for interactions. Treatments were separated with Tukey-Kramer least squares means test ($P \leq 0.05$). Herbicide treatment was considered a fixed effect, and locations (nested within year) and replications were regarded as random factors.

Results and Discussion

Because there was no significant interaction of experiment by location by year for any variable, data were combined for analysis. Data for bermudagrass injury, harvest data for first, second, third, and total biomass for fresh weight, and dry weight were combined. Data for the main effects of herbicide treatment are presented. Average rainfall and temperatures were recorded for both years and locations (data not shown), and resulted in normal forage bermudagrass growth.

Injury: Bermudagrass stunting in response to the PRE treatments 20 to 40 DAT was not observed for 47 and 77 g ha^{-1}, and was less than 30% for indaziflam at 155 or 234 g ha^{-1} (data not shown). All injury was transient, with no stunting after the initial harvest, even at the highest rates (data not shown). No injury was observed 20 to 30 DAT when indaziflam was applied at 47 g ha^{-1} POST after the first harvest. Brosnan et al. (2012) reported no bermudagrass injury in response to indaziflam applied up to 60 g ha^{-1} from PRE or POST applications

Harvest data: There was no indaziflam rate effect at the first harvest based on fresh or dry weight of bermudagrass compared with the nontreated control at 47 to 79 DAT (Table 4.1). The nontreated control fresh weight was 5,050 kg ha^{-1}, with indaziflam treatments resulting in 4,770 to 5,970 kg ha^{-1}. Pendimethalin had no effect on bermudagrass growth based on fresh and dry biomass. Pendimethalin has been previously shown to be non-detrimental to bermudagrass quality (McCullough et al. 2013). After repeated applications of dinitroaniline herbicides, some weed species, such as goosegrass [*Eleusine indica* (L.) Gaertn.], have become resistant (McCullough 2013). Although pendimethalin provided excellent dry matter yield, the use of other herbicides should be considered to prevent resistance development and/or the spread of a resistant population in bermudagrass pasture

Harvest 2 differed from harvest 1 in that treatment effects were significant only for fresh weight (Table 4.1). Bermudagrass treated with indaziflam at 234 g ha^{-1} had statistically the greatest fresh weight yields. Although not different, this rate of indaziflam also provided the highest dry-weight biomass. The high rate of indaziflam was significantly only greater than pendimethalin. Except for the aforementioned fresh biomass differences at harvest 2, dry weight biomass reflected the same as the fresh weights. Additionally, the split application of indaziflam at 47 g ha^{-1} PRE followed by POST yield was similar to all other treatments for fresh- and dry-weight biomass. This indicates the crop safety if a POST application of indaziflam after an initial harvest is needed to extend residual weed control. This is similar to other crop scenarios where indaziflam has been applied as split applications during the season (Grey et al. 2016; 2018). At harvest 3, 154 to 167 DAT, there were no differences in yield for any herbicide treatments for fresh or dry weight biomass.

When combined over the entire season, there were no differences in bermudagrass fresh- or dry-weight biomass for any herbicide treatment compared to the nontreated control (Table 1). Similar dry-weight yields of 8,000 to 10,000 kg ha^{-1} have been noted in other forage bermudagrass research on herbicides (Butler and Muir 2006; Matocha et al. 2010; Walker et al. 1998). These data support the conclusion that indaziflam at suggested registered rates should provide crop safety and additional herbicides for weed control in bermudagrass.

Preventing resistant weed populations is a major concern for all growers, especially those with limited herbicide choices. The repeated usage of the same herbicide only exacerbates this problem. The introduction of indaziflam in bermudagrass forage production has the potential reduce the reliance on limited herbicide options, adding a different mechanism of action for growers to utilize. Though there was some stunting noted, it was at rates exceeding the potential registered rates, was ephemeral, and did not affect fresh- or dry-weight yields. This study supports the use of indaziflam for safe weed management in actively growing bermudagrass with potential short-lived stunting but no long-term crop damage.

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Table 4.1: Fresh and dry biomass for forage *Alecia* bermudagrass comparing herbicide treatments in Georgia, 2017 and 2018[a].

			Fresh biomass				Dry biomass			
Treatment[b]	Rate	Timing	Harvest 1	Harvest 2	Harvest 3	Total	Harvest 1	Harvest 2	Harvest 3	Total
	g ai ha^{-1}		kg ha^{-1}							
Nontreated			5050 a[c]	6800 ab	15,300 a	27,160 a	3030 a	1740 a	4580 a	9350 a
Indaziflam	47[a]	PRE	5930 a	7930 ab	14,530 a	28,390 a	3340 a	2490 a	4820 a	10,650 a
	77	PRE	5440 a	7700 ab	14,600 a	27,750 a	3100 a	2470 a	4610 a	10,180 a
	155	PRE	4770 a	7870 ab	14,730 a	27,320 a	2640 a	2450 a	4620 a	9710 a
	234	PRE	5250 a	8090 a	13,700 a	27,040 a	3080 a	2770 a	4550 a	10,400 a
	47 + 47[a]	PRE + POST[b]	5970 a	7790 ab	14,360 a	28,120 a	3370 a	2640 a	4760 a	10,770 a
Pendimethalin	4480	PRE	5410 a	6320 b	14,050 a	25,780 a	3350 a	1780 a	4630 a	9760 a

[a]First herbicide application were made in Tift county on 24 Feb and 28 Feb, and in Colquitt county on 27 Feb and 2 Mar in 2017 and 2018, respectively.

[b]Second herbicide applications were made in Tift county on 20 Apr and 11 May, and in Colquitt county on 21 June and 16 May for 2017 and 2018, respectively.

[c]Means within a column followed by the same letter are not significantly different from each other according to Tukey-Kramer test at α=0.05.

APPENDIX 2

FLUMIOXAZIN INJURY ON PEANUT

Pictures were captured by Nick Hurdle

Peanut injured by flumioxazin noted as plant stunting, chlorotic and necrotic leaves in Ty Ty, GA.

Non-injured growth stage V-2 peanut in Ty Ty, GA.