NMWRRI Final Report

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2. **Project title:** Herbicide Phytotoxicity Under Drought Conditions in Warm and Cool Season Turfgrass

3. **Introduction**

Water management is one of the most pressing issues turfgrass managers face in the arid regions of the world and periodically in temperate regions. In response to reduced water supplies, governments enacted policies that restrict potable water use for non-essential uses (Cisar, 2004; Nagourney, 2015). Although turfgrass managers are generally well-adapted in reducing water consumption and showcasing their water conservation efforts, additional water restrictions will continue to limit turfgrass water use in the future (Throssell et al., 2009). In addition, regional and local climate change impacts will result in increased evaporation and decreased soil moisture available for turfgrass growth (Diffenbaugh, 2015). To reduce the appearance of drought stressed turfgrass, turfgrass managers will need to further modify primary cultural management practices for short- and long-term drought periods to maintain healthy and playable turfgrass swards. Although turfgrass managers utilize cultural management practices, including proper fertilization and correct species selection, weeds will be present under highly maintained swards (Busey, 2003).

Changes in turfgrass water status results in altered physiological growth, and thereby impact herbicide tolerances of desired turfgrasses. Leaf stomata closure increases with reduced water content (Mansfield and Atkinson, 1990), which will ultimately result in reduced photosynthesis and subsequent plant growth. In addition, longer periods of drought can reduce chlorophyll content of cool-season turfgrasses by 30-40% (Jiang and Huang, 2001). Lastly, plant-water relations are disrupted and may reduce turfgrass leaf water potential to -3.5 to 4.0 MPa, severely reducing uptake and translocation of water and nutrients (Qian and Fry, 1997). Ultimately, plants with reduced water status will have reduced physiological activity and may not effectively respond to biotic or abiotic stresses.

These same physiological changes may also influence the herbicide turfgrass tolerance and weed control efficacy. Physiological water status can impact herbicide uptake, translocation and site of action activity of target weeds. Milkweed (*Asclepias syriaca* L.*)* absorption of glyphosate is reduced from 44% to 29% when the soil moisture content changes from 25% to 13% (Waldecker and Wyse, 1985). Fenoxaprop crabgrass control in cool season turfgrass was reduced in non-irrigated sites compared to well-water sites (Neal et al., 1990). Flauzifop-p control of green foxtail (*Setaria viridis* (L.) P. Beauv.) was reduced by 40-57% when plants were drought stressed before application (Boydston, 1990). Green foxtail control was reduced to as low as 23% when plants were drought stressed before and after application of four herbicides (Boydston, 1992).

One method used to subject turfgrass plants to varying water statuses has been a linear gradient irrigation system (LGIS). This system has been used to determine the minimum irrigation requirement for acceptable turfgrass quality and evaluate cultivar performance under varying water statuses (Ow et al., 2017; Zhang et al., 2015). Herbicide treatments were not evaluated. Research is needed to determine how herbicide turfgrass phytotoxicity is influenced by varying water statuses and if herbicide efficacy varies with water status.

3a. **Research Objectives**

The objectives of this research are to determine 1) the severity of herbicide turfgrass phytotoxicity at differing water statuses and 2) if these differing turfgrass water statuses effect herbicide efficacy.

4. **Description of methodology employed.**

Two experiments were conducted to meet these objectives.

4a. **Field Experiment**

The field experiment was conducted at the Fabian Garcia Research Science Center in Las Cruces, NM from August-September 2019. A Linear Gradient Irrigation System will be used to determine the interaction of precise water statuses use and herbicide application responses. A LGIS is a single row of sprinkler heads arranged to provide an irrigation continuum from none to excessive applied-water. After establishment, experiment will be initiated and LGIS will used to differentially irrigate. Plots will run along irrigation gradient so that one herbicide treatment is exposed to the entire continuum of irrigation amounts. Irrigation was measured along the irrigation gradient at every irrigation event and precipitation event to determine total applied water amounts. Irrigation was scheduled to irrigate twice weekly to replace 100% ET at 1.5 m (5 ft) from LGIS as measured with nearby NMSU weather station. A total of five experimental areas were established, each with their own independent LGIS.

Two warm season experimental areas have been established with bermudagrass (*Cynodon dactylon* L.). Three unique cool-season experimental areas were established with perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.), and a mixture of perennial ryegrass, Kentucky bluegrass and tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.). Four weed species were introduced into each experimental area after establishment. Green foxtail (*Setaria viridis* L. P. Beauv.), annual bluegrass (*Poa annua* L.), dandelion (*Taraxacum officinale* F.H. Wigg*),* and white clover (*Trifolium repens* L.) were inter-seeded in each experimental area (0.5 lbs/1000 ft^2). These weeds were selected based on the high economic impact each of these weeds have in turfgrass systems in the Las Cruces area. All experimental areas will be maintained as golf course rough and mowed at 5 cm (2 in). Other cultural practices and pest management strategies will occur as needed to prevent stress. The LGIS systems used for this experiment were one Bermudagrass LGIS, the Kentucky bluegrass LGIS, and the perennial ryegrass LGIS.

Each plot was irrigated on a gradient of applied water through LGIS for two weeks and received a combination of herbicide applications arranged in a 2 (application rates) x 9 (herbicides) factorial treatment structure. Two rates of each herbicide will be applied: maximum label rate and 2x maximum label rate to mimic overlapping of herbicides. Plots will be visually rated for turfgrass and weed phytotoxicity, turfgrass and weed quality, and percent turfgrass green cover on 0.3m (1-foot) intervals along irrigation gradient 0, 1, 3, 7, 14, 21, 28 and 60 days after treatment. NDVI ratings and volumetric soil moisture will be taken weekly along these same gradients. Proc glimmix in SAS 9.4 was used to conduct the statistical analysis.

4b. **Greenhouse Experiment**

The greenhouse experiment was conducted at the research greenhouses in Fabian Garcia Research Science Center in Las Cruces, NM. The first experimental run was from April-June 2019 (Experiment 1) and the second trial was from January-March 2020 (Experiment 2). 'Princess-77' synthetic hybrid common bermudagrass and 'Spitfire' Kentucky bluegrass were grown in 3.8 L black nursery greenhouse pots filled with a soil mixture of 80% United States Golf Association (USGA) #2 sand (Chaparral Sand & Gravel, Chaparral, NM) and 20% by weight Premier Tech (Quakertown, PA) Horticulture peat moss. Helena (Collierville, TN) monoammonium phosphate 11-52-0 fertilizer was mixed in the soil with a Stone Construction (Honeoye, NY) cement mixer at a rate of 32 g m^{-3}. Bermudagrass was asexually propagated by planting 12-14 rhizomes per pot. These rhizomes were obtained from a nearby research plot at Fabian Garcia Research Center (Las Cruces, NM). Dead rhizomes were replaced throughout establishment. Kentucky bluegrass was sexually propagated by seed. Pots were initially seeded in each pot using a mixture of 1 g of seed and 99 g of USGA #2 sand to ensure uniform distribution. Kentucky bluegrass pots with low germination were over seeded throughout establishment. Pots were uniformly irrigated to field capacity daily during establishment. All turfgrasses were mowed weekly at a height of cut of 5 cm with grass shears (Makita XMU04Z 18V LXT Lithium-Ion Cordless Grass Shears, La Mirada, CA).

For both Experiment 1 and 2, the experimental design was a randomized complete block design with three to four replications for both species. When available, the fourth replicate of each treatment was assigned to plants that had 70-90% turfgrass cover. All other replicates had 100% turfgrass cover. After establishment, plants were exposed to four decreasing irrigation amounts. Reference evapotranspiration (ET_0) was used to determine irrigation amounts as this measurement is used as a tool by golf course superintendents to help schedule irrigation amounts (McPherson, 2010). ET_0 was estimated using an ETgage (Model A, Loveland, CO) with #30 green canvas covers for turfgrass ET_0 placed within the greenhouse. Plants were exposed to four different irrigation amounts (80, 60, 40, 20% ET_0) until uniform visual drought stress was achieved. Irrigation was scheduled twice weekly to replace ET_o lost from the previous week. The soil of each plant was saturated at each irrigation event. Uniform visual drought stress was observed 14 and 21 days after differential irrigation initiation for Experiment 1 and 2, respectively. Turfgrasses were then sprayed with a combination of herbicide applications arranged in a two application rates and eight herbicides (See Table 2.2). Herbicides were sprayed with their label recommended non-ionic surfactant adjuvant (See Table 2.2). Two rates of each herbicide were applied: maximum label rate and 2x maximum label rate to mimic overlapping of herbicides. A single nozzle CO_2 pressurized backpack sprayer with a Teejet 8002VS flat spray tip calibrated to 561 L ha^{-1} with distilled water as a carrier was used to apply herbicide treatments at 207 kPa. Spray to completion method was used to spray each treatment where each pot in a treatment was placed in a nearby outdoor plot measuring 1.5 m X 1.5 m and the entire spray volume was sprayed in two different directions until empty.

Turfgrasses were visually rated for phytotoxicity, quality, canopy green cover, and turfgrass coverage at 0, 1, 3, 7, 14, 21, and 28 DAT. Phytotoxicity was visually measured on a scale where 0 = no phytotoxicity, 30 = minimum level for unacceptable turfgrass quality, and 100 = completely brown necrotic tissue. Turfgrass quality was judged on a 1-9 scale, where 1 = brown, dead turfgrass, 6 = minimum acceptable quality, and 9 = the optimum (Morris and Sherman, 1998). Canopy green cover was taken on a scale where 0 = no visible green cover, 70 = minimum level for acceptable turfgrass quality, and 100 = the optimum. Turfgrass coverage was taken on a scale where 0 = no turfgrass cover in pots and 100 = complete turfgrass coverage in pots. After 28 days after treatment (DAT), uniform irrigation was initiated twice weekly to measure recovery after herbicide application. Each pot was watered to full field capacity. At 60 DAT after a period of adequate irrigation, all turfgrass was visually rated for green canopy cover and relative shoot growth as a means to determine each treatment's recovery from drought stress. Relative growth was the increase in plant height after being mowed to 5 cm at 28 DAT. Relative growth was calculated by (60 DAT height – 5 cm). Height was measured with a ruler beginning at soil surface. Negative values were converted to zero. Green cover was calculated as (60 DAT green cover – 28 DAT green cover). Negative values were converted to zero.

Data for each species and data collection date were analyzed separately using Proc Glimmix in SAS 9.4 (SAS Institute Inc., Cary, N.C.). Before ANOVA testing, homogeneity of variance was tested using Hartley's Fmax test (p = 0.05) on each dependent variable for each collection date for both trials of the experiment (Hartley, 1950). Each test for each collection date failed to reject the null hypothesis that variance was homogenous for the dependent variables of turfgrass phytotoxicity, quality, canopy green cover, and recovery canopy green cover in both trials of the experiment, so data from both trials were pooled and analyzed. However, relative growth data for each trial (2019 and 2020) was analyzed separately because data failed Harley's F-max test. For all measurements and collection dates, significant ANOVA differences were separated by Fisher's Protected Least Significant Difference (LSD; p=0.05).

5a **Greenhouse Experiment Results**

Figure 1 – Bermudagrass phytotoxicity 7 days after treatment

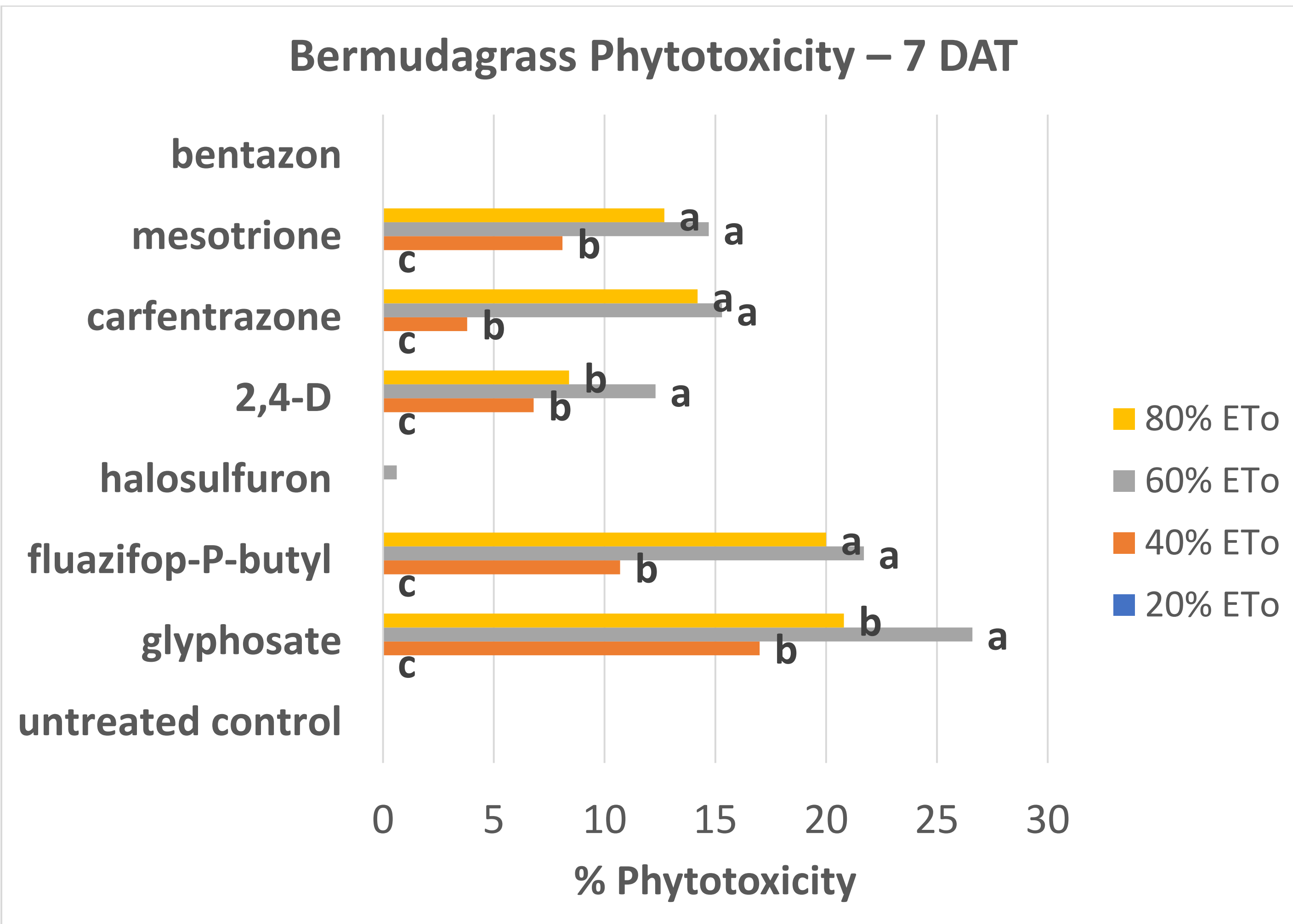

Figure 2 – Kentucky bluegrass phytotoxicity 7 days after treatment

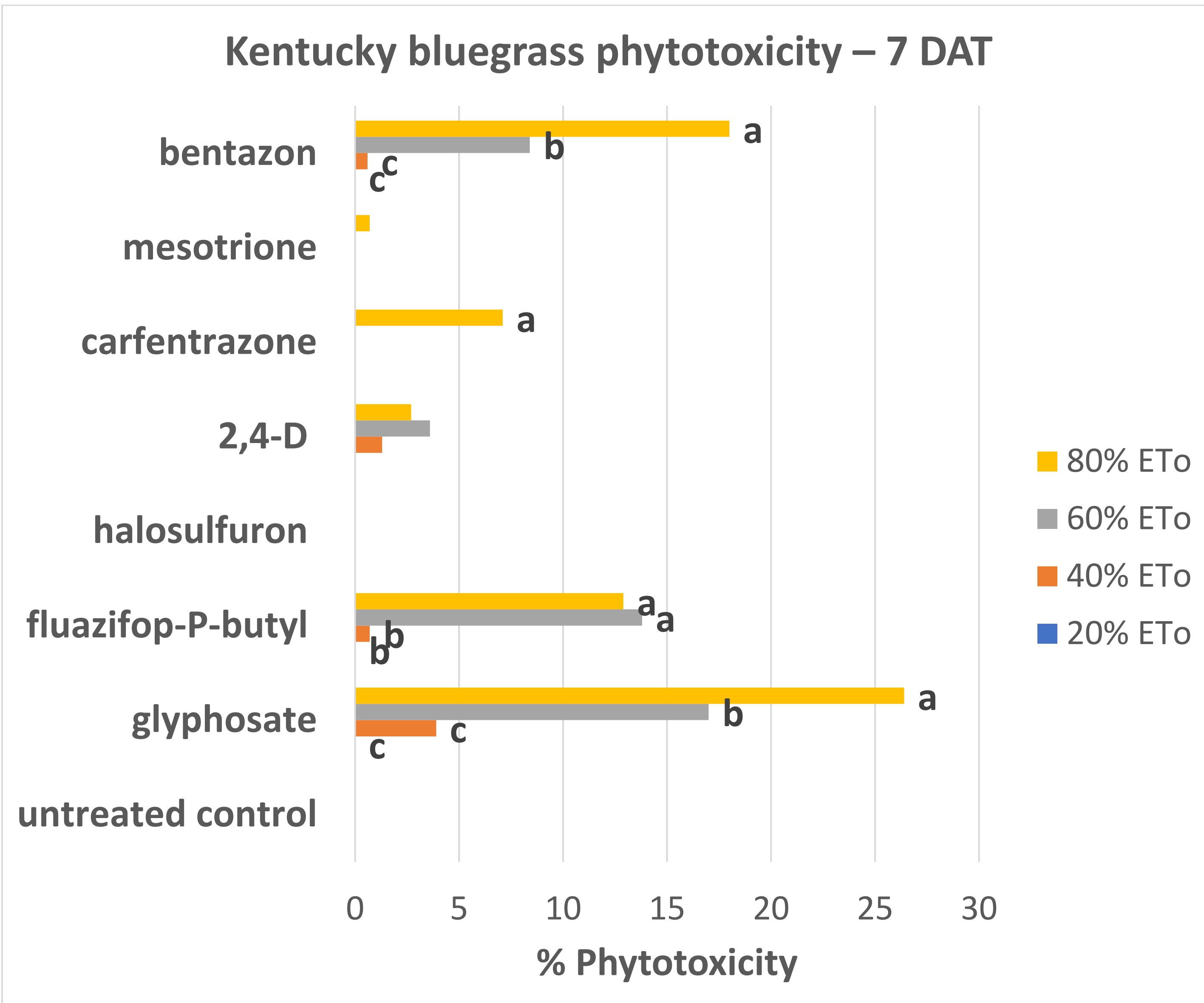

Figure 3 – Bermudagrass canopy green cover 28 days after treatment

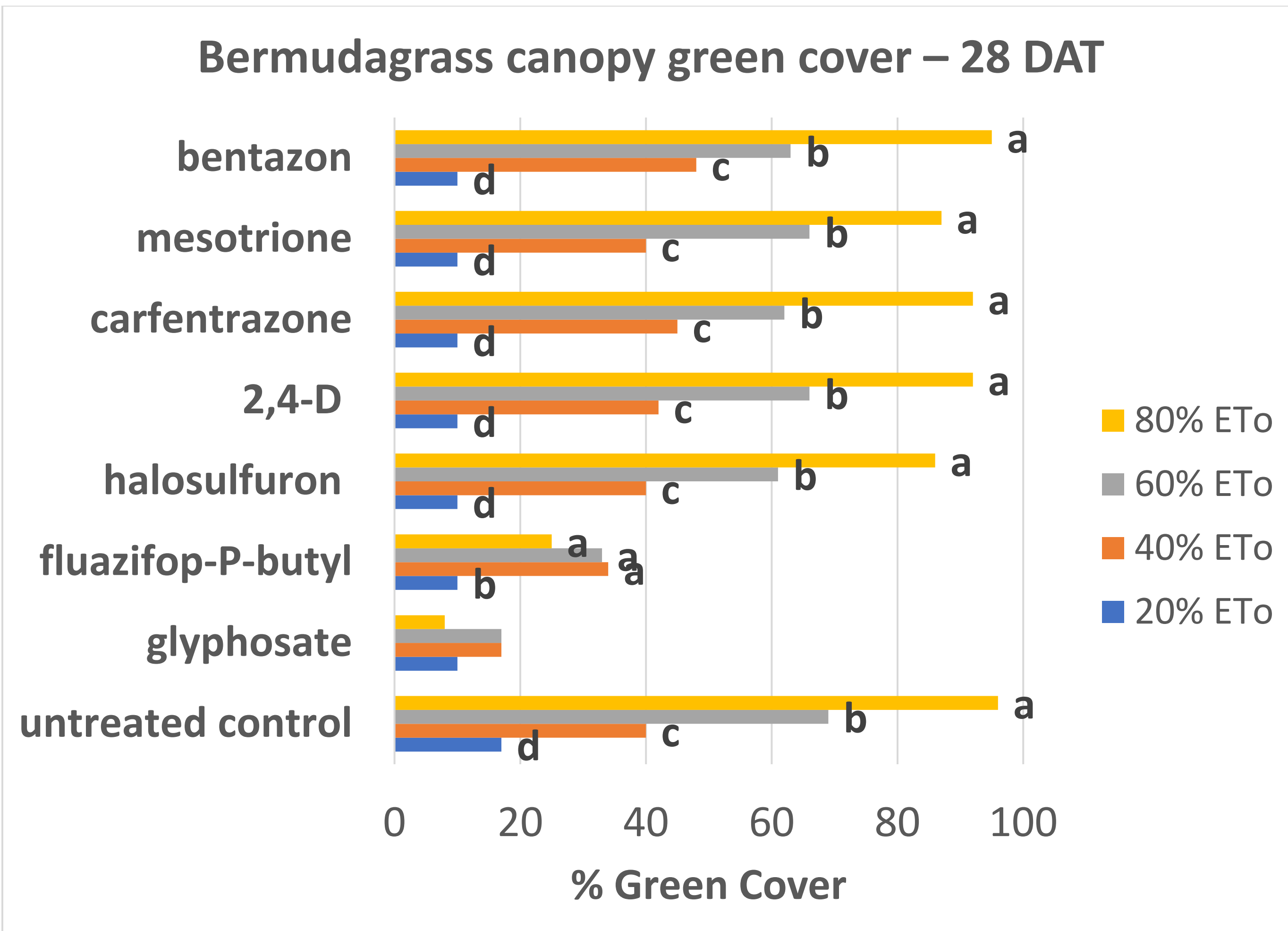

Figure 4 – Kentucky bluegrass canopy green cover 28 days after treatment

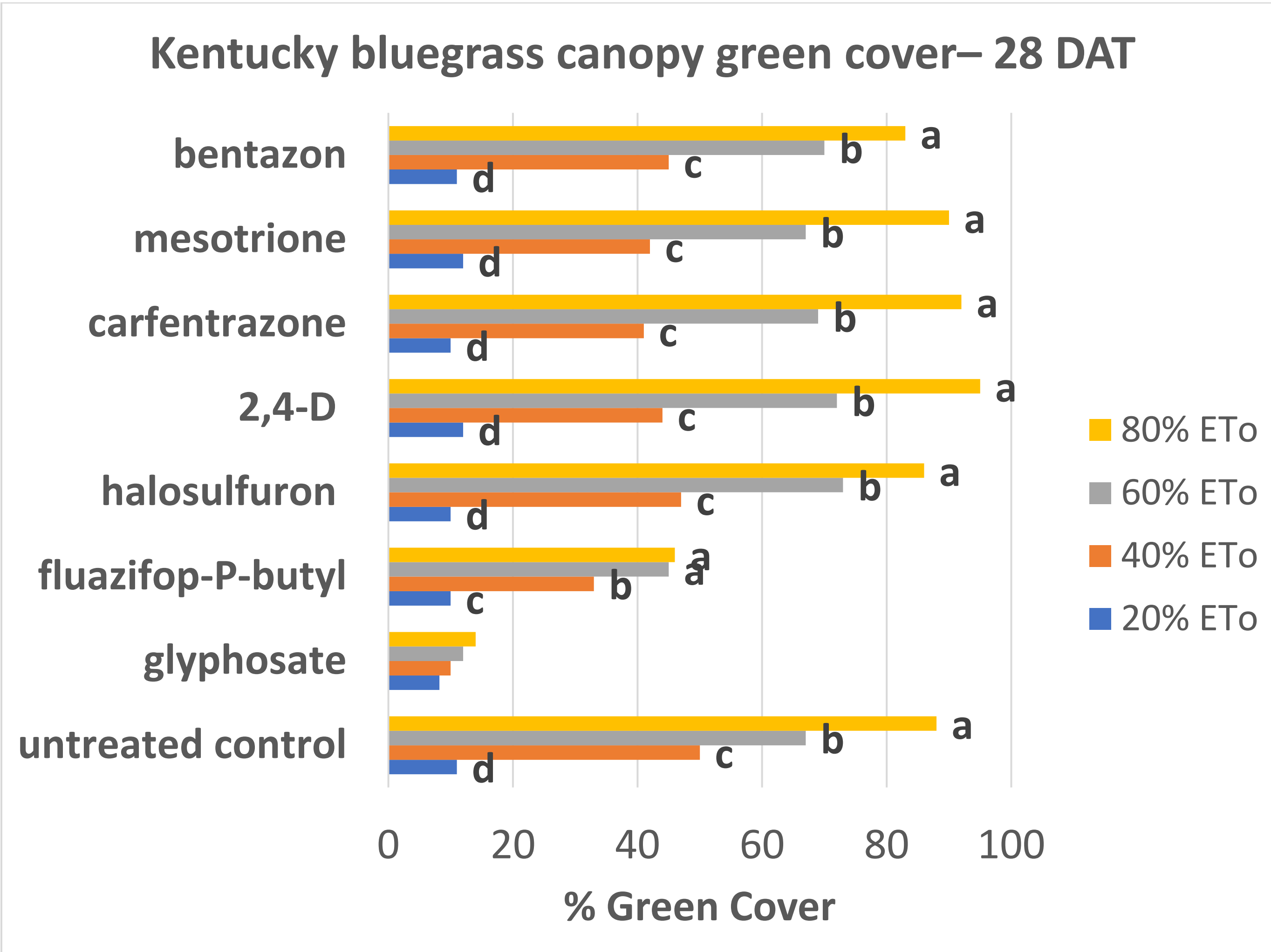

Table 1 – Safety of herbicides when applied to bermudagrass

	Initial Injury?	Recovery with regular irrigation?	Recovery with increased irrigation?	Safe to use?
glyphosate	YES	NO	NO	NO
fluazifop-P-butyl	YES	NO	NO	NO
halosulfuron	NO	YES	YES	YES
2,4-D	YES	YES	YES	YES*
carfentrazone	YES	YES	YES	YES
mesotrione	YES	YES	YES	YES
bentazon	NO	YES	YES	YES
*Safe if no overlap occurs				

Table 2 – Safety of herbicides when applied to Kentucky bluegrass

	Initial Injury?	Recovery with regular irrigation?	Recovery with increased irrigation?	Safe to use?
glyphosate	YES	NO	NO	NO
fluazifop-P-butyl	YES	NO	NO	NO
halosulfuron	NO	YES	YES	YES
2,4-D	NO	YES	YES	YES
carfentrazone	YES	YES	YES	YES
mesotrione	NO	YES	YES	YES
bentazon	YES	YES	YES	YES

5b. **Description of Greenhouse Experiment Results**

At 7 DAT, a significant two-way interaction between herbicide and water level turfgrass phytotoxicity of bermudagrass. Visual herbicide phytotoxicity was found in plants maintained at 60 and 80% ETo than in plants maintained at 40 and 20% ET_o. (**Figure 1**). Glyphosate, fluazifop-P-butyl, 2,4-D, carfentrazone, and mesotrione phytotoxicity varied across irrigation amounts, while the remaining herbicides did not respond differentially to irrigation amount. Glyphosate phytotoxicity was higher in plants maintained at 60% ET_o (26.6%) compared to plants maintained at 80, 40, and 20% ET_o (20.8, 17, and 0%, respectively). Fluazifop-P-butyl phytotoxicity was higher in plants maintained at 60 and 80% ET_o (21.7 and 20%, respectively) compared to plants maintained at 40 and 20% ET_o (10.7 and 0%, respectively). 2,4-D phytotoxicity was highest in plants maintained at 60% ET_o (12.3%) compared to plants maintained at 80, 40, and 20% ET_o (8.4, 6.8, and 0%, respectively). Carfentrazone phytotoxicity was higher in plants maintained at 60 and 80% ET_o (15.3 and 14.2%, respectively) compared to plants maintained at 40 and 20% ET_o (3.8 and 0%, respectively). Mesotrione phytotoxicity was higher in plants maintained at 60 and 80% ET_o (14.7 and 12.7%, respectively) compared to plants maintained at 40 and 20% ET_o (8.1 and 0%, respectively). Phytotoxicity of all other herbicides were not influenced by water level.

A significant two-way interaction between herbicide and water level turfgrass on turfgrass phytotoxicity of Kentucky bluegrass. Visual herbicide phytotoxicity was found in plants maintained at 60 and 80% ET_o than in plants maintained at 40 and 20% ET_o. (**Figure 2**). Glyphosate, fluazifop-P-butyl, carfentrazone, and bentazon phytotoxicity varied across irrigation amounts, while the remaining herbicides did not respond differentially to irrigation amount. Glyphosate phytotoxicity was higher in plants maintained at 80% ET_o (26.4%) compared plants maintained at 60, 40, and 20% ET_o (17, 3.9, and 0%, respectively). Fluazifop-P-butyl phytotoxicity was higher in plants maintained at 60 and 80% ET_o (13.8% and 12.9%, respectively) compared to plants maintained at 40 and 20% ET_o (0.7% and 0%, respectively). Carfentrazone phytotoxicity symptoms were only present in plants maintained at 80% ET_o (7.1%). Bentazon phytotoxicity was higher in plants maintained at 80% ET_o (18%) than in plants maintained at 60, 40 and 20% ET_o (8.4, 0.6, and 0%, respectively). Phytotoxicity of all other herbicides were not influenced by water level. Phytotoxicity of all other herbicides were not influenced by water level.

At 28 DAT a significant two-way interaction between herbicide and water occurred on bermudagrass canopy green cover. At 80% ETo all treatments were above the green cover threshold except for glyphosate and fluazifop-P-butyl. Across all water levels there was a variable response in canopy green cover. (**Figure 3**). The ranking for canopy green cover for each herbicide treatment was 80% ET_o > 60% ET_o > 40% ET_o > 20% ET_o, except for glyphosate and fluazifop-P-butyl. There were no differences in canopy green cover across all water levels for glyphosate, and the ranking for fluazifop was 80% ET_o = 60% ET_o > 40% ET_o > 20% ET_o. All treatments were below the acceptable green cover threshold at 20% ET_o and no differences existed between herbicides. At 40% ET_o, all herbicides were below the acceptable green cover threshold with glyphosate (25%) being the only treatment lower than the untreated control. At 60% ET_o, all herbicides were below the acceptable green cover threshold with glyphosate and fluazifop-P-butyl (17% and 33%, respectively) being the only treatments lower than the untreated control. At 80% ET_o, all treatments were above the acceptable green cover threshold except for glyphosate and fluazifop-P-butyl (8% and 25%, respectively).

Similar to bermudagrass, a significant two-way interaction between herbicide and water occurred on Kentucky bluegrass canopy green cover percentage. At 80% ET_o, all treatments were above the acceptable green cover threshold except for glyphosate and fluazifop-P-butyl. Across all water levels there was a variable response in canopy green cover (**Figure 4**). The ranking for canopy green cover for each herbicide treatment was 80% ET_o > 60% ET_o > 40% ET_o > 20% ET_o, except for glyphosate and fluazifop-P-butyl. There were no differences in canopy green cover across all water levels for glyphosate, and the ranking for fluazifop was 80% ET_o = 60% ET_o > 40% ET_o > 20% ET_o. All treatments were below the acceptable green cover threshold at 20% ET_o and no differences existed between herbicides. At 40% ET_o, all herbicides were below the acceptable green cover threshold with glyphosate and fluazifop-P-butyl (12% and 45%, respectively) being the only treatments lower than the untreated control. At 60% ET_o plants treated with glyphosate and fluazifop-P-butyl (12 and 45%, respectively) were lower than the untreated control. At 80% ET_o, all treatments were above the acceptable green cover threshold except for glyphosate and fluazifop-P-butyl (14% and 46%, respectively).

5b. **Description of Field Experiment Results**

At the time of this report, there are no new results from the field experiment. Before COVID-19, a second run of the field experiment was scheduled for March/April 2020. The tentative date for the second run is sometime during the summer of 2020, assuming that quarantine measures will be relaxed by then. Once completed, data from both trails will be run and analyzed.

6. Discussion

Based on the results of the experiment, special care must be taken when considering an herbicide application on turfgrass systems during periods of drought. These results can be used to develop a decision-making tool that turfgrass managers can use when considering an herbicide application is safe during periods of drought. Fluazifop-P-butyl and glyphosate would not be safe for use on bermudagrass systems as these herbicides cause initial injury and recovery to acceptable canopy green cover levels was not achieved with regular and excessive irrigation (**Table 1**). 2,4-D, carfentrazone, and mesotrione caused initial injury to bermudagrass, but recovery was achieved through normal or excessive irrigation. 2,4-D phytotoxicity occurred when applied at the 2x rate, so precautions must be taken to not overlap when applying 2,4-D to ensure no phytotoxicity occurs. Similar to bermudagrass, fluazifop-P-butyl and glyphosate would not be safe for use on Kentucky bluegrass systems as these herbicides cause initial injury and recovery to acceptable canopy green cover levels was not achieved with regular and excessive irrigation (**Table 2**). Carfentrazone and bentazon caused initial injury to Kentucky bluegrass but recovery was achieved through normal or excessive irrigation.