# ASSESSING GLYPHOSATE INJURY AND FORAGE BERMUDAGRASS REGROWTH USING CANOPEO

By

LUCAS FREIRES ABREU

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Centro Universitário de Mineiros - UNIFIMES

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Thesis Approved:

Dr. Alex Rocateli

Thesis Adviser

Dr. Brian Arnall

Dr. Misha Manuchehri

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Abstract: Applications of glyphosate for weed control in bermudagrass pastures are permitted; however, product labels are vague regarding use after spring green-up. In-season weed control with glyphosate could be a feasible option if bermudagrass tolerance to glyphosate was better understood. Also, a quantitative method might improve glyphosate injury assessment. The objective of this study was to evaluate the tolerance of bermudagrass cultivars Goodwell and Greenfield to varying glyphosate rates and develop a quantitative methodology to determine glyphosate injury based on relative green canopy cover (RGCC). The experimental design was a completely randomized factorial containing the two bermudagrass cultivars and five glyphosate rates (0.4, 0.5, 1.1, 1.5, and 3.1 kg a.i. $ha^{-1}$) plus a nontreated control. Visual green canopy cover (VGCC) and RGCC ratings were collected at 8, 16, 24, 37, and 56 days after glyphosate application (DAG), and biomass from canopy regrowth following applications was collected at 56 DAG. The root mean square difference (RMSD) values indicated that agreement between methodologies decreased at later days after glyphosate application. The visual capability to estimate glyphosate injury was not as precise as the RGCC method, which measures green color at the pixel level. The fractional green canopy cover (FGCC) results indicated greater glyphosate injury of Greenfield than Goodwell at 24 DAG. At 56 DAG, canopy regrowth of Greenfield and Goodwell was lower than the control at glyphosate rates of 1.1 and 3.1 kg a.i. $ha^{-1}$, respectively. Thus, improved glyphosate recommendations for bermudagrass could be cultivar specific.

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# CHAPTER I

# REVIEW OF LITERATURE

## Bermudagrass description

Originally from Africa, bermudagrass [*Cynodon dactylon* (L.) Pers.] is the primary perennial warm-season grass feedstock in beef farms in the southern United States (Christians, et al., 2011, Hill, et al., 2001, Collins, et al., 2017). Main morphological characteristics of bermudagrass are hairy ligules of 1 to 3 mm long, smooth leaf blades of 1.5 to 4 mm width, rhizomes and stolons with uneven internodes, and digitate panicles (Christians et al., 2011, Duble, 2001). In addition, bermudagrass can reach 61 cm tall and develop deep-rooted systems of up to 0.9 m (Ball et al., 2007, Christians et al., 2011). Established bermudagrass growth consists of producing new stems, leaves, and roots from rhizomes (belowground) and stolons (aboveground) every spring. In other words, bermudagrass has the ability of creating new clonal plants from its original tillers (Cudley, 2007).

Bermudagrass is considered a drought tolerant plant (Christians, et al., 2011, Huang et al., 2019, Shi et al., 2012,) which has potential to produce dry forage yields as high as 19.8 Mg $ha^{-1}$ $yr^{-1}$ (Osborne et al., 1999). It also has a satisfactory grazing tolerance due to its high proportion of rhizomes and stolons (Christians, et al., 2011, Collins, et al., 2017). Bermudagrass cultivars developed for forage production are classified as either grazing or hay types (Silva, et al., 2015). Grazing types are short plants with steady growth through the season, which produces a dense sod. On the other hand, hay types are taller plants with fast growth, consequently producing a lesser dense sod. Moreover, hay types have greater yield potential. However, they mature faster, which decreases forage quality earlier in the season than grazing types (Stone et al., 2012).

Its deep-rooted system (up to 0.9 m) is another bermudagrass trait which allows the plant to retain color, density and resistance to drought periods (Christians et al., 2011). However, drought resistance varies among cultivars (Christians et al., 2011). The grazing types tend to have lower water requirements and be more drought resistant than most hay types, which tend to thin during extended drought periods (Christians et al., 2011). In addition, bermudagrass is highly responsive to rainfall, where a minimum of 700 mm yearly precipitation followed with proper fertilization is considered appropriate to maximize forage production (Christians et al., 2011).

Dormancy is a physiological stage where bermudagrass growth ceases temporarily due to frost and sustained soil temperatures below 10 °C (Christians et al., 2011) and growth ceases when temperature is lower than 0 °C (Mirabile et al., 2016). During dormancy, bermudagrass loses its chlorophyll content, and carbohydrates are translocated to underground reserve organs, i.e., rhizomes and roots (Christians et al., 2011). Bermudagrass breaks dormancy during mid to late spring when soil temperature persists for several days above 10 °C (Duble, 2001).

Bermudagrass is physiologically active when air temperature is above 4 °C; optimum growth is achieved when temperatures are within 27 and 35 °C. At temperatures higher than 35 °C, plant growth is detrimentally affected due to low photosynthesis, and high respiration rates (Beard, 1973, Munshaw et al., 2006, Unruh et al., 1996).

Bermudagrass, like most tropical grasses, is a C4 plant, flowering is photoperiod dependent. A study conducted by Keeley and Thullen (1989) in Kern county, California, reported that bermudagrass planted from March to May flowered 10 to 15 weeks after planting; June through September planted bermudagrass flowered within 4 to 8 weeks after; and August planted while flowered within 4 to 5 weeks after. Bermudagrass emergence (green up for established fields) will affect flowering, since the longer the plant takes to flower, the higher the amount of seeds produced (Keeley and Thullen, 1989).

**Bermudagrass types**

The essential traits for selecting an appropriate bermudagrass cultivar to a specific location are winter hardiness, site-specific adaptations (e.g.: disease, low pH resistance), growth morphology (grazing vs. haying types), and yield potential (Dexter, 1956; Patton et al., 2008). The winter hardiness, which is the first concerning trait, is the capacity of plants to survive under freezing conditions (Abreu and Rocateli, 2019; Patton et al., 2008). Moreover, winter-hardy cultivars are less affected by stress in the fall and break dormancy in better growing condition than cold-sensitive cultivars. (Munshaw et al., 2006).

The majority of bermudagrass cultivars are adapted to slightly acidic soils - 5.0 to 5.5 (Collins et al., 2017), sandy soils, and moderate to heavy grazing pressure (Hill, et al., 2001). Hybrid bermudagrass, which is the offspring of two different cultivars (Fehr, 1991), are more popular for hay production since they present higher quality, response to N fertilization, yield potential, and faster field curing than non-hybrid bermudagrass (Hansen, 2000, Collins et al., 2017). However, their clonal sprigs do not produce viable seeds and must be vegetatively planted (Fehr, 1991). ‘Goodwell’ and ‘Greenfield’ hybrids are some of the commercial bermudagrass cultivars available for the state of Oklahoma (Abreu and Rocateli, 2019). ‘Goodwell’ is the most recently released dual purpose (hay and grazing) hybrid type. ‘Goodwell’ is a hybrid with larger stems and wider leaves among grazing types and presents a denser sod than usual hay cultivars (Redfearn et al., 2005). ‘Greenfield’ is a steady yielding grazing type that establishes easily and performs well on poor soils (Abreu and Rocateli, 2005).

Bermudagrass management changes according to its use (hay or grazing). For hay meadows, the first harvest must be completed when forage height reaches 35 to 40 cm in most hybrids, which takes around 4 weeks of vegetative growth. Four-week cut intervals are recommended to maintain high forage nutritive value, digestibility, and crude protein (CP); leading to an increase in hay yield and quality (Newman et al., 2014). For grazing pastures, appropriate N fertilization is recommended after each grazing in order to stimulate forage regrowth with high nutritive value, leading to an increase in animal performance (Newman, 2014). In grazing rotational system, during bermudagrass peak growth rate, haying is recommended to remove and store forage surplus. This operation will not only avoid losses in forage quality (maturity), but also allow the use of the excess during periods of forage shortage (Newman, 2014).

**Bermudagrass fertilization**

For successful bermudagrass establishment, soil must have a minimum pH of 5.0, and P (Phosphorus) and K (Potassium) must be applied according to soil test recommendations. Slaton et al. (2008) found that P levels in soil tend to reduce after two years of forage production on bermudagrass fields without P fertilization. A long-term experiment demonstrated that applications of 58 kg P $ha^{-1}$ and 235 kg K $ha^{-1}$ are recommended to maintain minimum levels for proper bermudagrass growth (Guretzky et al., 2010). Research by Silveira et al. (2017) observed that warm-season grasses responded positively to P and K fertilization. Several authors (Yarborough et al., 2017; Silveira et al., 2017) found that K fertilization is crucial for bermudagrass production and persistence, especially under continuous aboveground removal. However, high costs of these fertilizers limits the extent of their use (Silveira et al., 2017).

Different Nitrogen fertilization levels have been extensively evaluated for bermudagrass production systems (Osbourne et al., 1999). Several authors reported positive bermudagrass forage yield responses to N fertilization. Nitrogen is a dynamic nutrient in the soil that can be easily lost to the atmosphere or leached to deeper soil layers. Therefore its application frequency and time is different from other nutrients such as P and K (Nevens and Rehuel, 2003, Power, 1980). Nitrogen might be applied when plants are physiologically active since the nutrient can be easily lost by different biochemical processes including volatilization, denitrification, leaching, and immobilization (Campbell et al., 1986, Nevens and Rehuel, 2003). Additionally, N can be taken up by undesired plants, increasing weed invasion early in the season (Christians et al., 2011, Green and Martin 1998, Collins, et al., 2017). During establishment, 33 to 56 kg N $ha^{-1}$ is recommended when stolons reach 7.5 to 15 cm, then a topdress application of 56 kg N $ha^{-1}$ is recommended thirty days later (Jennings and Boyd, 2013, and Redfearn 1995).

A one-year experiment evaluated common bermudagrass yield under cumulative applications of four N rates (0, 56, 112 and 168 kg $ha^{-1}$) applied through the season (Coblentz et al., 2004). The results found linear increases on total dry matter (DM) content of 5.2, 7.0, 7.8, 9.2 kg $ha^{-1}$ at Stephens site, and 11, 12.4, 13.8 and 14.2 kg $ha^{-1}$ at Latta site for the respective N rates. The same study evaluated the influence of the four different N rates on bermudagrass P uptake and found linear increases of 20.9, 27.4, 26.9 and 27.9 kg P $ha^{-1}$ at Stephens and 41.8, 47.3, 48.7 and 50 kg P $ha^{-1}$ at Latta sites for N rates of 0, 56, 112, and 168 kg $ha^{-1}$, respectively. Both Stephens and Latta sites were located close to Lincoln, AR. Alderman et al. (2011) reported yields of 3.9, 8.5, 9.6 and 10.5 kg $ha^{-1}$ when fertilizing N on bermudagrass 'Tifton 85' at rates of 0, 45, 90, and 135 kg N $ha^{-1}$ $yr^{-1}$ in Gainesville, FL, respectively. In addition, the authors concluded that N fertilization had an impact only on aboveground production such as leaves and stems. No greater production of belowground structures such as rhizomes and roots were found.

Another research study developed in Ardmore, OK by Guretzky et al. (2010) evaluated the effects of N fertilizer and yield stability at rates of 0, 112, 224, 336, and 448 kg $ha^{-1}$ from 2002 to 2007 on 'Midland' bermudagrass. Average DM yields across all years were reported as 10.6, 11.5, 9.5, 10.1 and 10.4 Mg $ha^{-1}$ from the lowest to the highest evaluated N rates. According to the authors, the plots fertilized with 112 kg $ha^{-1}$ had the highest yield stability throughout the years, with the greatest yield mean across the years presenting the lowest standard error (SE = 0.04). The linear regression of treatment means were not significant ($p > 0.10$) indicating that forage yields did not increase or decrease with different fertilizer rates. However, rates of 224 and 336 kg $ha^{-1}$ indicated high yields when growing conditions were favorable such as higher rainfall events. Thus, under unfavorable weather conditions, N rates of 112 kg $ha^{-1}$ might be more cost efficient than higher rates due to extensive losses to the atmosphere, leaching, or volatilization.

Osborne et al. (1999) evaluated the effects of 0, 112, 672 and 1,344 kg N $ha^{-1}$ $yr^{-1}$ and 0, 224, 448, 672 and 1,344 kg N $ha^{-1}$ $yr^{-1}$ of urea fertilizer during early-spring and late-summer, respectively, on ‘Midland’ bermudagrass plots in Burneyville, OK and Ardmore, OK. The early-spring applications in Burneyville denoted DM yields of 10.9, 12.1, 14.9 and 14.4 Mg $ha^{-1}$ in 1994 and 5.8, 7.3, 10.5 and 10.6 Mg $ha^{-1}$ in the 1995 year for the respective N rates. The late-summer application denoted DM yields of 6.3, 9.2, 10.7, 11.6 and 13.2 Mg $ha^{-1}$ during 1994 and 4.9, 6.6, 5.0, 7 and 7.7 Mg $ha^{-1}$ during the 1996 year. In Ardmore, the early-spring applications denoted DM yields of 4.6, 8.9, 17.8 and 17.2 Mg $ha^{-1}$ in 1994 and DM yields of 3.4, 4.6, 8.2 and 8.3 Mg $ha^{-1}$ in 1995. The late-summer applications denoted DM yields of 4.7, 7.9, 8.7, 9.2 and 12.4 Mg $ha^{-1}$ in 1994; 4.7, 6.8, 9.8, 10.4 and 11.5 Mg $ha^{-1}$ in 1995; and 1, 3.1, 4.3, 6.1 and 6.9 Mg $ha^{-1}$ in 1996. Based on the authors’ findings, it was concluded that, under conditions of high rainfall, N fertilizer should be applied in early-spring. For the late-summer applications, urea should be avoided due to increased $NH_3$ volatilization losses.

Differences in bermudagrass forage yield at similar N rates were found in different locations throughout the southern U.S (Coblentz et al., 2004; Guretzky et al., 2010). Initial soil fertility and seasonal precipitation were the main factors leading to forage yield variations within similar N rates applied. The variable N responses from previous research may be justified due to N fertilization being highly dependent on weather conditions (especially rainfall). Overall, an optimum N fertilization and efficiency depends on environmental factors, economic constraints, soil conditions, and producer goals (Franzluebbers et al., 2004, Silveira et al., 2007).

### Beef and livestock enterprise in Oklahoma

Beef cattle production plays an important role in the Oklahoma agricultural enterprise (Rogers et al., 2012). Total Oklahoma pastureland area, including introduced and native pastures for cattle production accounted for 56.6% of the total state agricultural land (NASS, 2012). In 2018, agricultural land devoted to cattle production in Oklahoma sustained a cattle inventory, i.e.; all cattle including calves, heifers, steers, and bulls raised on pastures and feedlots, of 5.10 million head, making Oklahoma the 5th biggest cattle producer of the United States with to a total income of $5.61 billion (NASS, 2018). Livestock sales (including poultry, swine, hogs, sheep, dairy, horses and other animal products) represented 74% of all the agricultural products sold in Oklahoma, while only the cattle industry signified 47.73% of the total agricultural income in 2012 for the state (NASS, 2012).

### Bermudagrass use in livestock

In the Southern Great Plains, including the state of Oklahoma, most of the cattle are raised on introduced warm-season grasses such as bermudagrass [*Cynodon dactylon* (L.) Pers.] during late spring and summer; and forage-grain wheat (*Triticum aestivum* L.) through the winter and early spring (Peel 2003, Rao et al., 2002). Bermudagrass is a major forage used on cattle operations in the USA, mainly cow-calf types, which is a consequence of its high forage yield potential on different soil types, resistance to drought and the its resilience to pests (Redfearn et al., 2005). Bermudagrass genotypes have been widely used for either hay or grazing production systems in the southern USA (Silva, et al., 2015). Bermudagrass can be used during the spring-summer or early fall season, as it can be stockpiled for late-fall and winter grazing (Lalman et al., 2017). Bermudagrass provides fair to satisfactory pasture and hay if well managed since quality is dependent on maturity and soil fertility (USDA-NRCS, 2000). A fair and satisfactory quality hay must have around 55% Total Digestible Nutrients (TDN) and ≥9% of CP to meet most cattle categories nutrient requirements (Hall, et al., 2005). Research of Kloppenburg et al. (1995), which evaluated the chemical composition of 'Hardie' bermudagrass from May 30 to October 3 under an irrigation system and application of 306 kg $ha^{-1}$ N in 3 split applications, found CP and TDN levels of 17 and 78%, respectively, which met most cattle categories requirements according to Hall et al. (2005). Research conducted by Lalman et al. (2017), where fall fertilized bermudagrass was evaluated to measure nutritive value and cattle allocation, it was concluded that CP content of the bermudagrass harvested in November was able to meet the demand for gestating cows (13.1 and 15.2% CP for the years of 1997-98 and 1998-99, respectively). On the other hand, a stockpiled bermudagrass forage evaluated from September to December in a 3-year experiment had from 9 to 13.6% CP (Lalman et al., 2017).

Research of Utley et al. (1974) compared yearling steer performance following one forage bahiagrass (*Paspalum notatum*) 'Pensacola', and two cultivars of bermudagrass, 'Coastal' and 'Coastcross', and found animal gains of 249, 372 and 527 kg $ha^{-1}$ $yr^{-1}$, respectively. These findings showed bermudagrass animal gain superiority over bahiagrass. A grazing trial with the bermudagrass cultivars 'Callie', 'Coastal', 'Brazos', and experimental hybrids 'S-54' and 'S-16', evaluated weight gains on Santa Gertrudis

(*Bos taurus*) steers in a 151 day period, and found gains of 881, 686, 673, 655 and 613 kg $ha^{-1}$ for 'Callie', 'Brazos', 'S-16', 'Coastal', and 'S-54', respectively (Bransby, et al., 1988). Work of Pedreira et al. (2016) evaluated yearling beef cattle performance on 'Tifton 85' and 'Florakirk' bermudagrass during a 3 year stocking period. The authors reported gains of 638 and 358 kg $ha^{-1}$, respectively, which demonstrated a better potential for grazing of 'Tifton 85'.

A grazing and supplement trial with early weaned calves were evaluated on 'Tifton 85' bermudagrass and live weight gains of 700, 1,080, 1,450 and 1,550 kg $ha^{-1}$ following 0, 10, 15, and 20 g $kg^{-1}$ BW (body weight) concentrate supplement were found, respectively (Vendramini et al., 2007). A grazing trial with heifers on 'Jiggs' bermudagrass found gains of 692, 975, and 1.064 kg $ha^{-1}$ ($P < 0.01$), in 3, 7.5 and 12 animal units $ha^{-1}$ (AU = 450 kg live weight), all receiving 10 g $kg^{-1}$ Live weight (LW) of concentrate (Aguiar et al., 2014). Furthermore, it was found that 'Jiggs' bermudagrass cannot be grazed under 17 cm stubble height to maintain good stand during the growing season (Aguiar et al., 2014). A grazing trial with yearling steers on 'Coastal', 'Tifton 78', and 'Tifton 85' found an Average Daily Gain (ADG) of 0.65, 0.74, and 0.72 kg BW, respectively (Hill et al., 2001). Another grazing trial with Angus (*Bos Taurus*) steers on 'Coastal' and 'Tifton 44' bermudagrass under three different nitrogen rates: 101, 202, and 303 kg N $ha^{-1}$, found ADG of 0.53, 0.55, and 0.63 kg BW and 0.60, 0.64 and 0.65 kg BW, for 'Coastal' and for 'Tifton 44', respectively, as N rates increased (Burns et al., 2009).

The bermudagrass cultivars mentioned above have shown to be an appropriate feedstock for grazing different cattle categories (Bransby et al., 1988, Burns et al., 2009, Hill et al., 2001, Pedreira et al., 2016). In addition, cattle performance was improved when concentrate supplementation was present (Aguiar, et al., 2014, Vendramini et al., 2007).

**Rotational vs continuous grazing**

Bermudagrass, similar to other forages can be managed for cattle on either continuous, rotational, and, strip grazing. However, a conclusion for which is the best system is yet ambiguous. According to Heady (1961), continuous stocking includes yearlong and a whole season grazing on the same area, or as long as the weather permits. On rotational grazing, the animals graze on different paddocks, allowing the vegetation to rest (Heady, 1961).

A comparison between continuous, strip and rotational grazing found no significant difference of ADG at equal grazing pressures for steers grazing 'Coastal' bermudagrass in a 3-year experiment (Hart et al., 1976). No significant difference of ADG was observed after a two-year study among cattle on strip grazing and continuous management, although higher ADG was found on rotational grazing (Volesky et al., 1994). After evaluating milk yield on rotational vs. continuous grazing systems in a perennial ryegrass (*Lolium perenne*) pasture, no significant difference was found between managements and the hypothesis that rotational grazing increases milk yield per cow could not be supported (Pulido and Leaver, 2003). In a research study conducted by Heitschmidt et al. (1987), no significance for growth dynamics was found on grasses from rotational and continuous grazing systems. However, higher CP was found on rotational systems due to the amount of senesced forage on the continuous system. In work of Hart et al. (1989), weight gain of calves, cows, and heifers did not differ significantly when comparing rotational and continuous grazing systems. The same work observed an increase of 60% of forage utilization for areas surrounded by water sources and a decrease of 30% for areas greater than 4 km from water sources. In another study, the performance of steers was evaluated in a mixed pasture of bromegrass (*Bromus madritensis*), alfalfa (*Medicago sativa*), and red fescue (*Festuca rubra*) on rotational and continuous regimes, and found ADGs of 218 vs. 119 kg $ha^{-1}$, respectively, (Walton et al., 1981). Work of Conway (1963) found significantly higher gains on rotational grazing on high stocking rates compared to continuous grazing. Work of Matthews et al. (1994), evaluated three grazing methods, including rotational with short grazing periods, rotational with long grazing periods, and continuous for two years in 'Callie' bermudagrass on Holstein heifers (*Bos taurus*) and found no significant difference was found among the treatment means. However, 'Callie' bermudagrass outcompeted by 'Common' bermudagrass and Bahiagrass (invasive species) on the continuous stocking system, which may have decreased cattle performance over time (Mathews et al., 1994).

Several research studies comparing both rotational and continuous grazing systems have found divergent conclusions on which system is best (Conway, 1963; Hart et al., 1976; Hart et al., 1989; Heitschmidt et al., 1987; Pulido and Leaver, 2003; Volesky et al., 1994; Walton et al., 1981). Nonetheless, some research shows no statistical differences on animal gains between continuous and rotational grazing systems (Hart et al., 1976, Heady 1961, Pulido and Leaver, 2003, Volesky et al., 1994). However, rotational grazing can be more advantageous when intensive grazing around water surroundings could lead to overgrazing. In addition, continuous grazing system increases selectivity for specific plants and grazing pressure close to cattle feeders, and consequently increases prevalence of invasive species. Conversely, rotational systems force animals to graze the paddock uniformly (Hart et al., 1989, Heady, 1961, Mathews et al., 1994, Walton et al., 1981), allowing the entire paddock to regrow evenly. However, costs associated with rotational stocking is higher due to the need for more fencing, water sources, and feeders (Heady, 1961).

**Control of weeds in bermudagrass**

Weeds can be a problem as they compete with target plants for nutrients, sunlight and water, and result decreased forage yields. Methods of weed control in pasture include mechanical, biological, chemical, cultural (prescribed fire, fertility, cultivar selection etc.) are available.

The mostly used techniques to suppress weeds are mechanical and chemical methods (Smith, 2017). Mechanical methods aim to remove the entire plant or part of the plant from the vegetation, through the use of mowing or cultivation (Smith, 2017). The chemical method consists of the application of herbicides, mainly by liquid spraying, or pellets broadcasting (Smith, 2017). Biological weed control is another available option that has been used for several years (McFadyen, 1998). However, compared to other methods, biological control usually takes longer to establish, and become effective; furthermore, its management is work intensive. Biological control consists of the use of natural predators to cause stress and injury to target weeds (Smith, 2017). Livestock can even be used as biological weed control. Addition of different species of animals in a pasture, such as goats and sheep, or by increasing cattle stocking rates can result in weed control (Popay and Field, 1996).  Insects (e.g., weevils), mites, and pathogens are other options for weed biocontrol (McFadyen, 1998). However, in case of success, efficacy may not last for long (Smith, 2017). In addition, one of the major challenges reported by biological control users is potential damage to non-targeted plants (McFadyen, 1998).

Soil fertilization also is considered as a weed control method (Green and Martin, 1998). Bermudagrass is highly responsive to N fertilization (Christians, et al., 2011), as previously mentioned, thus enhancing soil fertility consequently decreases weed population as bermudagrass competes with undesired species. Work of Eytcheson (2011) in Oklahoma evaluated different N rates and herbicide treatments, and found that the addition of N fertilizer increased bermudagrass yield and consequently increased field sandbur (*Cenchrus echinatus*) control. In Oklahoma, common difficult-to-control weeds in bermudagrass pastures include field sandbur, western ragweed, (*Ambrosia psilostachya*) (New, 1997; WSSA, 2004), large crabgrass, (*Digitaria sanguinalis*) (Kering et al., 2012), johnsongrass (*Sorghum halepense*) (Mack, 1991), pigweeds (*Amaranthus spp.*) (Bond et al. 2006), and horseweed (*Conyza canadensis*) (Heap, 2018).

There are several herbicides, preemergence and post emergence, available for controlling the weeds mentioned above. In the case of the crabgrass, field work of Butler et al. (2006) used different herbicides and different timings on newly established 'Coastal' bermudagrass to evaluate their effects on large crabgrass and bermudagrass injury. The same authors applied 0.26 kg a.e. (acid equivalent) $ha^{-1}$ of glyphosate 14 days after planting (DAP) on newly established 'Coastal' bermudagrass, and found injury rates of about 8% and 86 to 90% control of crabgrass. Field work of Walker et al. (1998), observed the control of sandbur and crabgrass following applications of diuron as PRE and POST on two bermudagrass. The PRE application of diuron resulted in an increase of 38% and 62% of herbage mass on the bermudagrass + crabgrass plots and the bermudagrass + sandbur, respectively. In this same experiment, there were no effective results for POST applications of diuron. A study by Matocha et al. (2010) tested a mix of nicosulfuron + metsulfuron applied when field sandbur was 2.5 to 7.5 cm tall and another application at 7.5 to 10 cm tall to evaluate control of the sandbur on bermudagrass fields. Control of 90% of field sandbur in ‘Tifton 85’ and ‘Jiggs’ bermudagrass pastures was observed at 30 and 90 days after treatment (DAT). The same study also found that ‘Tifton 85’ had a higher tolerance to the herbicide than ‘Jiggs’ as the injury ranged from 0 to 15% and 7 to 22%, respectively.

Work of Grichar et al. (2008) evaluated the combination of 2,4-D plus imazapic at rates of 40 kg a.e. $ha^{-1}$ + 70 g a.i. $ha^{-1}$, 70 kg a.e. $ha^{-1}$ + 140 g a.i. $ha^{-1}$, 110 kg a.e. $ha^{-1}$ + 210 g a.i. $ha^{-1}$, 140 kg a.e. $ha^{-1}$ + 280 g a.i. $ha^{-1}$, 200 kg a.e. $ha^{-1}$ + 350 g a.i. $ha^{-1}$, 240 kg a.e. $ha^{-1}$ + 420 g a.i. $ha^{-1}$, respectively, to control field sandbur and johnsongrass on ‘Coastal’ and ‘Tifton 85’ bermudagrass fields. More than 80% of field sandbur was controlled at all rates and more than 96% of control was observed when imazapic rates were over 70 g a.i. $ha^{-1}$. Johnsongrass was controlled more than 80% with imazapic plus 2,4-D at 110 g a.i. $ha^{-1}$ + 210 g a.e. $ha^{-1}$ or higher. In addition, the yield reduction of both varieties was observed when compared to the nontreated control. On the other hand, ‘Tifton 85’ was severely affected by the herbicides at all rates on the first harvest. For the second harvest, yield was reduced by 49% at rates of 200 kg a.e. $ha^{-1}$ + 350 g a.i. $ha^{-1}$ or greater in comparison to the control. At the third harvest, no difference was observed. Overall, ‘Tifton 85’ can be less tolerant to the following combination of herbicides than ‘Coastal’ bermudagrass and rates of 110 kg a.e. $ha^{-1}$ + 210 g a.i. $ha^{-1}$ imazapic plus 2,4-D, respectively, showed 98 and 82% of control on sandbur and johnsongrass, respectively; with ≤51% injury on the selected bermudagrasses. Sellers and Ferrell (2012) imply that, under established bermudagrass, rates of 0.07 to 0.11 kg a.e. $ha^{-1}$ of metsulfuron + nicosulfuron can provide effective control against seedlings of crabgrass, sandbur, and established johnsongrass.

Research of Funderburg et al. (2014) evaluated the control of western ragweed in heavily infested bermudagrass plots in Ardmore, OK. POST applications of 2,4-D plus aminopyralid at rates of 0.457 + 0.057 kg a.e.$^{-1}$ controlled 100% of ragweed when applied in April and May. Preemergence applications with aminopyralid at rates of 0.133 kg a.e. ha$^{-1}$ controlled 95% of ragweed plants when applied in February and March. Thus, PRE and POST applications were effective controlling western ragweed in bermudagrass pasture. In addition, the PRE application could be more beneficial if the bermudagrass was dormant at the time of application, and the application cost was lower due to a single chemical application. Another study of Matocha et al. (2013) evaluated western ragweed control and injury of 'Tifton 85' bermudagrass under applications of picloram plus diflufenzopyr, triclopyr plus diflufenzopyr, dicamba plus diflufenzopyr and picloram, triclopyr and diflufenzopyr alone at different rates. The most successful rates were picloram at 0.28 and 0.56 kg a.e ha$^{-1}$ with or without diflufenzopyr, which resulted in 95% control of ragweed at 94 days after treatment (DAT) in 2003; and picloram alone at 0.56 kg a.e ha$^{-1}$ or picloram plus diflufenzopyr at 0.28 and 0.112 kg a.e.$^{-1}$ with control rates of 96 and 97%, respectively, at 95 DAT in 2004. 'Tifton 85' bermudagrass had a growth reduction of 17.5% and 23.8% under applications of picloram at rates of 0.28 and 0.56 kg a.e. ha$^{-1}$; and a growth reduction of 57.5% and 66.3% for the combination of picloram plus diflufenzopyr at rates of 0.28 + 0.0112 and 0.56 + 0.112 kg a.e. ha$^{-1}$, respectively. Thus, it was concluded that picloram alone could have similar ragweed control to picloram plus diflufenzopyr, however, with lower bermudagrass injury.

Field work of Kruger et al. (2010) noted successful controlled ≥90% of a glyphosate resistant horseweed population under applications of dicamba and 2,4-D at rates of 280 g a.e. $ha^{-1}$ applied at 0-7, 7-15, 15-30, and >30 cm height. In addition, no differences in horseweed height or dry weight were observed between any of the herbicide treatments applied. However, Kruger et al. (2010) recommends that horseweed should be controlled at a maximum of 30 cm height since latter herbicide application decreases control effectiveness. A study by Wiese et al. (1995) found ≥95% control of horseweed under applications of 2,4-D at 560 g a.e. $ha^{-1}$, dicamba at 280 g a.e. $ha^{-1}$, atrazine + 2,4-D at 3,360 + 1,120 g a.e. $ha^{-1}$, chlorsulfuron at 13 g a.e. $ha^{-1}$, metsulfuron at 5 g a.e. $ha^{-1}$, metsulfuron + 2,4-D at 5 + 560 g a.e. $ha^{-1}$, trialsulfuron at 13 g a.e. $ha^{-1}$, and thifensulfuron at 13 g a.e. $ha^{-1}$. In addition, weed species such as pigweed can be controlled with metsulfuron + aminopyralid at rates of 0.14 to 0.23 kg a.e. $ha^{-1}$ with a non-ionic surfactant at 0.17 to 0.35 ml L $ha^{-1}$ (Sellers and Ferrell, 2012).

The herbicides presented above have effective weed control on bermudagrass pastures. Overall, it is important to maintain consistent weed control throughout the seasons. Moreover, the manipulation of chemicals, crop rotation and use of cover crops are important agronomic practices (Abouziena and Haggag, 2016) able to diminish the propagation of resistant weed biotypes and some weed control. A useful tool that can be used on bermudagrass weed control, is the application of herbicides during dormant season, which can avoid weed infestations during the spring (White, 1994). Another important factor for maintaining weeds controlled in a pasture, is to start clean during establishment, which consists of removing all the weeds from a field prior to planting (Abouziena and Haggag, 2016).

CHAPTER II

# ASSESSING GLYPHOSATE INJURY AND FORAGE BERMUDAGRASS REGROWTH USING CANOPEO

## INTRODUCTION

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is the most common introduced summer forage in the U.S. Southern Great Plains (SGP), which is primarily used as beef and dairy cattle feedstock (Christians et al., 2011; Collins et al., 2017; Hill et al., 2001). Bermudagrass breaks dormancy and starts green-up during mid-spring when soil temperature persists for several days above 10°C. Plants then achieve maximum growth during mid-summer at air temperatures within 27 and 35°C and stay physiologically active until mid-fall as long as air temperature is above 4°C (Christians, et al., 2011; Duble, 2001; Beard, 1973, Munshaw, et al., 2006, Unruh, et al., 1996). The mid-spring bermudagrass green-up combined with its initial submaximal growth until mid-summer allows weeds to grow with minor suppression. These weeds are usually undesirable in pastures because they are unpalatable, sometimes toxic to animals, and decrease overall forage yield and/or quality. Furthermore, some of those plants are other grasses, such as Italian ryegrass (*Lolium multiflorum* Lam.), crabgrass (*Digitaria spp.*], johnsongrass [*Sorghum halapense* (L.) Pers.], and sandbur (*Cenchrus spp.*), which are not easily controlled in bermudagrass with selective herbicides. Consequently, the control of undesirable grasses relies on the use of non-selective herbicides, such as glyphosate, which injures bermudagrass. To avoid injury, glyphosate applications on bermudagrass pastures are highly recommended during dormancy to control winter weeds, whereas its application after green-up is cautioned and vaguely described on product labels.

However, previous research indicated that physiologically active bermudagrass is tolerant to glyphosate at certain rates and application frequencies. Johnson (1988) reported that established common-type (cv. 'Ormond') and hybrid bermudagrass (cv. 'Tifway', 'Tifgreen', and 'Tifdwar') were not successfully terminated with two glyphosate applications, spaced 30 days apart, at rates of 2.2, 3.3, and 4.5 kg a.i. $ha^{-1}$ in Griffin, GA. Single applications were only tested at 4.5 kg a.i. $ha^{-1}$ because lower rates could not effectively injure bermudagrass with single applications. Thus, the highest visual injury achieved by single and double glyphosate applications were 38 and 70%, respectively, when following a rate of 4.5 kg a.i. $ha^{-1}$ rate. Other authors stated that genetic variations among bermudagrass cultivars might play a role in injury responses to glyphosate. Bryson and Wills (1985) reported different glyphosate injury responses among 16 bermudagrass biotypes. In general, the differences among biotypes were greater with the lower glyphosate rates (≤1.12 kg a.i. $ha^{-1}$). For instance, significant variations in visual injury (38 – 87%) among biotypes were observed at 1.12 kg a.i. $ha^{-1}$ rate. In another study, a glyphosate application at a rate of 1.10 kg a.i. $ha^{-1}$ effectively terminated recently sprigged hybrid bermudagrass (cv. 'Tifton-10', 'TifSport', and 'TifEagle'). However, common bermudagrass (cultivar unknown) had its diameter size reduced by 52% and survived following a 1.10 kg a.i. $ha^{-1}$ glyphosate application (Webster et al., 2003). Furthermore, Webster et al. (2004) reported that hybrid bermudagrass tended to be easily injured by glyphosate compared to common bermudagrass after testing 12 bermudagrass cultivars.

Previous research indicated that undesirable plants can be effectively controlled by glyphosate without substantial bermudagrass injury after green-up. Control of 99% of johnsongrass was reported following glyphosate applications of 0.92 kg a.i. $ha^{-1}$ (Brown et al., 1987). At those rates, based on previous discussion, bermudagrass could be minimally injured and able to recover quickly. Crabgrass and broadleaf signalgrass (*Urochloa platyphylla* Munroe ex C. Wright) were controlled 76 and 91%, respectively, and hybrid bermudagrass (cv. 'Tifton 85') was injured 9% when a glyphosate rate of 0.28 kg a.i. $ha^{-1}$ was applied (Butler et al., 2006). The authors also reported that bermudagrass establishment was improved after weeds were controlled. The cited studies indicated that glyphosate application on bermudagrass pastures after green-up might be a viable management option for controlling undesirable plants; however, glyphosate injury magnitude may vary among bermudagrass forage varieties. The cultivars 'Greenfield' and 'Goodwell' are commonly used in the SGP, and no information on their tolerance to glyphosate is available.

Furthermore, the previously cited studies assessing bermudagrass injury to glyphosate used a visual rating system. This commonly used method entails a scale of 0 to 100 where 0 = no visual injury compared to the nontreated control and 100 = complete kill. This method relies on the observer's judgement and is a subjective, qualitative measurement (Johnson, 1975). Although the visual rating system has been accepted as a standard method, visual estimations have been criticized for their subjective nature and the need of properly trained observers (Leinauer et al., 2014; Richardson et al., 2001).

Furthermore, visual rating protocols and standards may vary among researchers; consequently, the lack of normalization of this method makes data comparison among different studies nearly impossible (Krans and Morris, 2007).

Affordable digital cameras and mobile devices have popularized the use of digital images. Moreover, interactive, simple, and accurate tools capable of quantifying fractional green canopy cover (FGCC), such as the app Canopeo (http://www.canopeoapp.com), are available to the public at no cost. Fractional green canopy cover is a non-destructive measurement that estimates canopy cover development. Its use has extended to forest land cover, green and senescing fraction of soybean canopy [*Glycine max* (L.) Merr.], percent land cover in turf, and weed growth rates after tillage, etc. (Karcher and Richardson, 2005, Korhonen et al., 2006, Purcell, 2000; Rasmussen et al., 2010; and Richardson et al., 2001). In practice, herbicide injury evaluation partly consists of monitoring green canopy cover discoloration. Therefore, Canopeo might be a useful standardized and quantifiable tool for this task. Thus, the objectives of this study were to (1) contrast the RGCC quantitative method against the commonly adopted visual rating system, while (2) evaluating the tolerance of 'Greenfield' and 'Goodwell' forage bermudagrass cultivars to different glyphosate rates.

## MATERIALS AND METHODS

### Experimental Conditions

A greenhouse study was conducted at the Controlled Environmental Research Lab (CERL), Oklahoma State University, Stillwater, OK (36.12 °N, 97.06 °W). During the fall of 2017, bermudagrass cv. 'Greenfield' and 'Goodwell' were sprigged into co-extruded polypropylene pots (0.15 m tall, 0.17 m diameter), which were filled with appropriate potting mix, fertilized, watered daily, and clipped monthly until a 100% sod cover was present in every pot. During the experiment, the applied photoperiod was extended to 14 hours using supplemental lighting provided by a combination of metal halide and high-pressure sodium lamps. Temperature maintained inside of the greenhouse was controlled by a wall-mounted evaporative cooling pad system (Acme's Koll-Cel, USGR, Houston, TX), and monitored by a data logger (TP425, The Dickson Company, Addison, IL). The average day and night temperatures observed during the experiment period were 29.0±7.8 and 21.3±5.1°C, respectively.

**Glyphosate Treatments and Experimental Design**

In the early spring of 2018, the following five rates of glyphosate (Roundup PowerMAX, Monsanto, St. Louis, MO) plus a nontreated control were applied to bermudagrass pots three weeks after clipping (2.5 cm stubble height) using a spray chamber: 0.4, 0.5, 1.1, 1.5, and 3.1 kg a.i. (active ingredient) $ha^{-1}$ (Generation III Research Sprayer, DeVries Mfg., Hollandale, MN). The sprayer chamber was equipped with an 80001 EVS nozzle calibrated to deliver 140 L $ha^{-1}$ in order to achieve appropriate spray coverage. Five pots of each cultivar were sprayed at once for each glyphosate rate, and two runs were conducted for a total of 10 pots per rate per cultivar. Four hours after glyphosate application, once leaves were dry, pots were returned to the CERL, where moisture was monitored daily, and pots were watered as needed.

The experimental design was a factorial arranged in a completely randomized design where the two forage bermudagrass cultivars 'Greenfield' and 'Goodwell' were assigned as the main plots, while the five glyphosate application rates plus the nontreated control were assigned as the subplots.

**Estimating Relative Green Canopy Cover with Canopeo**

The Canopeo app (Oklahoma State University, Stillwater, OK) is an ACT image analysis software based on the red-green-blue (RGB) color system. Using this system, all pixels of a digital image are analyzed and classified according to the ratios of red/green, blue/green, and excess green index resulting in a binary black and white image. Pixels classified as white are predominantly in the green band (~500 – 750 nm) which correspond to green canopy; and pixels classified as black correspond to non-green canopy. Then, FGCC is calculated based on the white/black pixel ratio ranging from 0 (no green canopy) to 1 (100% green canopy) (Patrignani and Ochsner, 2015).

Each bermudagrass pot was placed at the center of a white square panel (0.5 m x 0.5 m), and pictures were taken at a 1.5-m height parallel to the ground using a 12-megapixel camera (iPhone 7, Apple Inc., Cupertineto, CA). Then, pictures' excess borders were precisely cropped at the edges of the white panel to a standardized size of 1000 x 1000 pixels using Adobe Photoshop CC 2018 (Adobe Inc., San Jose, CA). These image treatments standardized the relative plant size in all pictures making possible FGCC comparisons among different images of the same pot at different time periods. Then, FGCC for each individual picture was calculated using the Canopeo Matlab app.

Finally, relative green canopy cover (RGCC) was calculated using Eq. [1]:

$$\left(\frac{\mathrm{FCC}\ _{n}}{\mathrm{FCC}\ _{0}}\right) \mathrm{x}\ 100 = \%\mathrm{RC}\ \ \mathrm{C}_{n} \qquad [1]$$

where: $FGCC_0$ = FGCC at day zero (initial conditions), $FGCC_n$= FGCC at a given day *n*, and $\%RGCC_n$ is the percentage of green cover at day *n* relative to its initial FGCC at day zero. The $RGCC_n$ values <100% reflect reduction in green canopy area; while values >100% reflect increase in green canopy area cover relative to day zero.

**Data Collection**

Based on the methodology described above, pictures of each bermudagrass pot was taken on the same day but prior to glyphosate application (0 DAG), then pictures were taken at 8, 16, and 24a DAG. Following pictures taken 24a DAG, aboveground bermudagrass biomass was clipped at a stubble height <2 cm using electronic shears (18V LXT® Grass Shear, Makita® U.S.A Inc., La Miranda, CA), and a second round of pictures were taken after removal on the same day (24b DAG). Finally, pictures were also taken on 37 and 56 DAG.

Relative green canopy cover was calculated for each pot for all days evaluated based on calculated FGCC using the Canopeo software. For a given bermudagrass pot, $FGCC_0$ was calculated using the picture taken at 0 DAG, and the $FGCC_n$ was calculated using the picture taken on that specific *n* day (*n* =0, 8, 16, 24a, 24b, 37, and 56 DAG). The RGCC values calculated for days prior to clipping (8, 16, and 24a DAG) reflects the glyphosate injury to the bermudagrass canopy. This is based on the assumption that canopy discoloration (i.e., deviation from green color band, ~500 – 750 nm) was caused by glyphosate application. In this case, the lower the RGCC, the higher the glyphosate injury. However, the RGCC values calculated after clipping (2b, 37 and 56 DAG) reflect the percentage of canopy cover regrowth relative to coverage at 0 DAG. In this case, the higher the RGCC, the higher the bermudagrass recovery after glyphosate application.

A visual injury rating for each bermudagrass pot was performed simultaneously with pictures at 8, 16, and 24a DAG. Rates were based on a scale of 0 to 100, where 0 = no herbicide injury, and 100 = complete plant death or necrosis. Then, the visual green canopy rating (VGCC) was calculated by subtracting visual injury ratings from 100.

Finally, all pots were re-clipped at a stubble height <2 cm in the last sampling day (56 DAG) after taking pictures. The collected aboveground dry biomass regrowth (ADMR) in each pot was dried in a forced-air oven maintained at 55°C to a constant weight. Data were used to determine final forage dry matter production (g $m^{-2}$) regrown from 24b to 56 DAG.

**Experimental Design and Statistical Analysis**

The RMSD method was used to compare the RGCC method against the standard VGCC method based on measurements recorded at 0, 8, 16, 24a DAG. The RMSD was estimated using Eq. [2]:

$$RMSD = \sqrt{\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{n}} \qquad [2]$$

where: $n$ was the number of observations, $P_i$ was the calculated RGCC value for the $i$th measurement, and $O_i$ was the observed (visually rated) value for the $i$th measurement.

The statistical analysis for evaluating the effect of the glyphosate treatment on ‘Greenfield’ and ‘Goodwell’ cultivars was conducted using the Statistical Analysis System (SAS V9.4; SAS Institute, Cary, NC). The RGCC dataset for 0, 8, 16, 24a, 24b, 37, and 56 DAG was subjected to repeated measurement by ANOVA using PROC GLM at $\alpha = 0.05$ (Littell et al., 1996). Finally, the ADMR dataset collected on 56 DAG was also analyzed by ANOVA using PROC GLM; however, treatment means were separated by the LSMEANS procedure when protected by F-tests significant at $\alpha = 0.05$.

## RESULTS AND DISCUSSION

### Visual Rating vs. Relative Green Canopy Cover

Across all rates and DAGs, the agreement between VGCC and RGCC was higher between Goodwell than Greenfield cultivar values. The RMSD values were 28.70% for Goodwell, and 40.78% for Greenfield. Moreover, the latter the green canopy estimation, the lower the agreement between methods. For cultivar Goodwell, the RMSD values were 27.73%, 29.96%, and 33.40% for 8, 16, and 24 DAG, respectively. The same trend was found for the Greenfield cultivar, where the RMSD values were 31.95%, 40.32%, and 49.37% for 8, 16, and 24a DAG, respectively (Fig. 1A – F).

The low agreement between VGCC and RGCC relies on how the methods estimate glyphosate injury in bermudagrass. Figure 2 illustrates both methods. Glyphosate acts in plants by altering different physiologic processes, such as photosynthesis, chlorophyll biosynthesis, photochemical reactions, plant mineral nutrition, etc., leading to gradual wilting and yellowing (chlorosis), which advances to necrosis (Fernandez and Bayer, 1976; Gomez et al., 2014; Mahakhode and Somkuwar, 2015). These color variations (green – brown) fall into the hue range from 5G to 5YR in the Munsell color system; where yellowing is classified as the intermediate hues 10 GY and 5 GY (Malacara, 2011). Greater difficulty in visually assessing yellowish canopies resulted in inaccurate VGCC estimations in latter periods, i.e., at 16 and 24a DAG, when chlorosis was more accentuated (Abreu, *unpublished data*, 2018). This issue was also mentioned by Webster et al. (2000) when studying human color hue identification among 51 individuals. The authors argued that ambient lighting can drastically affect an individual's color hue rating; and that human color rating is subjective due to different proportions of eye cone receptors among individuals. Conversely, Canopeo eliminates subjectiveness associated with visual ratings, because it estimates canopy green cover based on the RGB system where pixels predominantly in the green band (~500 – 750 nm) are classified as green canopy (Patrignani and Ochsner, 2015). Thus, the RGCC seems to be a more precise and reliable method to estimate the effects of discoloration caused by glyphosate in plant canopies. Therefore, the rest of this manuscript will focus on RGCC and not discuss VGCC.

**Glyphosate Effect on Bermudagrass Canopy**

Greenfield had lower RGCC values (higher glyphosate injury) than Goodwell at 8 to 24a DAG; however, RGCC differences were significant at 16 and 24a DAG, only (Table 1). Glyphosate rate was significant at all evaluation periods. Overall, the higher the glyphosate rate, the lower the RGCC. Furthermore, the interaction of cultivar × rate was not significant at any period. Thus, the effect of glyphosate rate over time was analyzed separately by cultivar.

At 8 DAG, no Goodwell RGCC differences were observed among the control (91%) and plants sprayed with glyphosate at 0.4 (72.0%) and 0.5 (73.1%) kg a.i. $ha^{-1}$ (Fig. 3A). Relative green canopy cover for Goodwell following the 1.1 kg a.i. $ha^{-1}$ rate was 57.7% and was lower than the control, but not different from RGCC values for Goodwell following 0.4 and 0.5 kg a.i. $ha^{-1}$ rates. Moreover, Goodwell following the two highest rates (1.5 and 3.1 kg a.i. $ha^{-1}$) had lower RGCC values than all other treatments. Goodwell RGCC values for 1.5 and 3.1 kg a.i. $ha^{-1}$ rates were 13.4% and 6.2%, respectively; and they were not different from each other. A similar RGCC trend was observed for Greenfield at 8

DAG. The nontreated control had a RGCC value of 90.9%, while Greenfield following rates of 0.4 and 0.5 kg a.i. $ha^{-1}$ had similar values of 83.1 and 71.8%, respectively (Fig. 3B). The RGCC for Greenfield following the 1.1 kg a.i. $ha^{-1}$ rate was 49.6% and was lower than values for the nontreated control and 0.4 kg a.i. $ha^{-1}$ glyphosate rate; and not different from either 0.5 or 1.5 kg a.i. $ha^{-1}$ rates. The highest applied rate (3.1 kg a.i. $ha^{-1}$) had a RGCC of 21.7% which was similar to the RGCC value following the 1.5 kg a.i. $ha^{-1}$ rate, but lower than values for the nontreated control and those following rates of 0.4, 0.5, and 1.1 kg a.i. kg $ha^{-1}$.

For both Greenfield and Goodwell cultivars, a slight RGCC decrease was observed for the control at 16 DAG. After five weeks of unsuppressed growth, nontreated controls produced large amounts of stems and leaves which demanded more water and other nutrients than pot-confined roots could supply resulting in the observed RGCC reduction of ~9%. At 16 DAG, Goodwell RGCC following glyphosate rates of 0.4, 0.5, and 1.1 kg a.i. $ha^{-1}$ were similar (~27%) and higher than RGCC values for 1.5 and 3.1 kg a.i. $ha^{-1}$ rates. Goodwell plants following glyphosate rates of 1.5 and 3.1 kg a.i. $ha^{-1}$ had aboveground biomass (i.e., leaves and stems) that was completely necrotic (RGCC ~ 0%). Although substantial glyphosate injury was reported for Goodwell, glyphosate injury for Greenfield was even more severe at 16 DAG. No RGCC differences were found among all glyphosate rates when applied to Greenfield; and they were substantially lower than the control. Greenfield RGCC values were ~10% for 0.4, 0.5, and 1.1 kg a.i. $ha^{-1}$ and ~0% for 1.5 and 3.1 kg a.i. $ha^{-1}$ rates.

Relative green canopy cover at 24a DAG slightly increased or were similar to the values at 16 DAG for all glyphosate rates applied to both Greenfield and Goodwell.

Therefore, RGCC results at 24a DAG were similar to 16 DAG. These findings also suggest that peak glyphosate injury to Greenfield and Goodwell bermudagrass aboveground biomass takes no more than 16 d after application when ambient conditions such as light, temperature, soil water, and nutrient conditions are not limiting.

According to Webster et al. (2003), common and hybrid bermudagrass types showed final green canopy ranging from 0 to 13% (i.e., 87 to 100% visual injury) when glyphosate was applied 1.10 kg a.i. $ha^{-1}$. These findings are similar to the Goodwell and Greenfield RGCC findings at tested rates ≥1.5 kg a.i. $ha^{-1}$ at 16 DAG. Conversely, RGCC after glyphosate applications of 1.12 kg a.i. $ha^{-1}$ varied from 13 to 62% (i.e., 38 to 87% visual injury) among sixteen bermudagrass biotypes (Bryson and Wills, 1985). These contrasting findings indicate that glyphosate injury in bermudagrass likely is cultivar-dependent.

Moreover, our findings agreed with Bryson and Wills (1985) who documented that differences among biotypes were greater following lower glyphosate rates (1.12 – 2.24 kg a.i. $ha^{-1}$). Relative green canopy cover differences between Goodwell and Greenfield were more evident at tested rates ≤ 1.1 kg a.i. $ha^{-1}$ (Fig. 3A and 3B). Goodwell cultivar is a high yielding hybrid bermudagrass released in 2007 for haying and grazing purposes, presenting a predominant upright growth, which results in a tall and sparse sod. On the other hand, Greenfield cultivar is a low and steady yielding hybrid released in 1954 for grazing purposes, presenting a predominant horizontal growth with short plants and thick sod (Abreu and Rocateli, 2019). Greenfield canopy architecture could have facilitated increased glyphosate contact/absorption, and its relatively low biomass production may have allowed increased glyphosate injury (less tissue to translocate and desiccate). On the other hand, because glyphosate is a herbicide that moves in both the phloem and xylem, contact is typically not a limiting factor when considering performance. Another factor likely contributing to the differences in tolerance between the two hybrids is genetics (Price et al., 1983; Paris et al. 2008).

**Glyphosate Effect on Bermudagrass Regrowth**

Goodwell had higher RGCC at canopy regrowth than Greenfield at 37 and 56 DAG (Table 2). The effect of glyphosate rate was significant at both regrowth periods. Overall, the higher the glyphosate rate, the lower the RGCC. Moreover, the cultivar x rate interaction was significant at 37 DAG, in spite of the fact that Goodwell's RGCC values were always higher than Greenfield for all tested rates. Consequently, the interaction effect at 37 DAG was ignored because it did not influence the cultivar effect. Thus, glyphosate rates over time were analyzed separately by cultivar.

At 37 DAG, no canopy regrowth differences were observed for Goodwell when comparing the control, 0.4, 0.5, and 1.1 kg a.i. $ha^{-1}$ glyphosate rates, and their RGCC values ranged from 35.2 to 44.6% (Fig. 4A). At the glyphosate rate of 1.5 kg a.i. $ha^{-1}$, canopy regrowth was intermediate (RGCC = 22.8%). Precisely, canopy regrowth following the rate of 1.5 kg a.i. $ha^{-1}$ was lower than previous cited rates, and higher than regrowth following 3.1 kg a.i. $ha^{-1}$ (RGCC = 4.9%). Overall, Goodwell increased its RGCC to ~21% across all glyphosate rates from 37 to 56 DAG. Final RGCC values for Goodwell and plants following applications of 0.4, 0.5, 1.1, and 1.5 kg a.i. $ha^{-1}$ at 56 DAG were similar and ranged from 67.8 to 55.7%. Goodwell following the rate of 1.5 kg a.i. $ha^{-1}$ had a final RGCC of 42.7%, which was lower than nontreated control and following rates of 0.4 and

0.5 kg a.i. $ha^{-1}$, but similar to Goodwell following the 1.1 kg a.i. ha $^{-1}$ rate. Moreover, the rate of 3.1 kg a.i. $ha^{-1}$ had the lowest final canopy regrowth (RGCC = 23.5%).

Greenfield canopy regrowth was lower than Goodwell, especially at higher glyphosate rates. At 32 DAG, Greenfield canopy regrowth for the control and plants following rates of 0.4 and 0.5 kg a.i. $ha^{-1}$ did not differ, with RGCC values ranging from 26.6 to 40.2% (Fig. 4B). Furthermore, the rates of 1.1, 1.5, and 3.1 kg a.i. $ha^{-1}$ resulted in lower canopy regrowth, ranging from 0.1 to 10.3%. At 56 DAG, no differences in final canopy regrowth were found among the nontreated control and Greenfield following rates of 0.4, 0.5, and 1.1 kg a.i. $ha^{-1}$ at 56 DAG, and their final RGCC values ranged from 53.6 to 41.5%. Greenfield following the rate of 1.5 kg a.i. $ha^{-1}$ had a final RGCC of 21.4 %, which was lower than nontreated control, 0.4, 0.5, and 1.1 kg a.i. $ha^{-1}$ rates. Following the rate of 3.1 kg a.i. $ha^{-1}$, Greenfield had the lowest final canopy regrowth (RGCC = 5.8%).

Destructive samples showed similar canopy regrowth findings. Goodwell with 205.4 g $m^{-2}$ produced 20.2% more ADMR than Greenfield (163.9 g $m^{-2}$) across all glyphosate rates at 56 DAG ($P < 0.01$). Similar results were reported from a multi-site-year bermudagrass cultivar performance trial in Oklahoma, where Goodwell produced 15 to 25% more dry biomass than Greenfield (Rocateli et. al, 2019). Aboveground dry matter regrowth differences ($p = 0.03$) were also found among glyphosate treatments. These destructive canopy samples showed a similar trend to the RGCC for the same sampling period (56 DAG): the higher the glyphosate rate, the lower the canopy regrowth. Thus, the ADMR cultivar x rate interaction effect was not significant ($P = 0.16$); and data were analyzed separately by cultivar (Fig. 5A – B).

Goodwell's ADMR following the 3.1 kg a.i. $ha^{-1}$ rate (87.8 g $m^{-2}$) was lower than the control and all other rates (Fig. 5A); and the control (242.8 g $m^{-2}$) and rates ≤1.5 kg a.i. $ha^{-1}$ did not differ. This observation concurred with previous RGCC findings for all glyphosate rates, except 1.5 kg a.i. $ha^{-1}$ rate. Relative green canopy cover values for the 1.5 kg a.i. $ha^{-1}$ rate indicated lower canopy regrowth than rates ≤1.1 kg a.i. $ha^{-1}$. These different results between ADMR and RGCC measurements were explained by visual observations. Goodwell bermudagrass following the glyphosate rate of 1.5 kg a.i. $ha^{-1}$ had detrimental effects on canopy regrowth, such as substantial amounts of chlorotic leaves (Abreu, *unpublished data*, 2018). These chlorotic leaves (not green) were accounted as canopy regrowth in the ADMR, but not accounted by the RGCC method. Therefore, the RGCC method underestimated Goodwell canopy cover regrowth following the 1.5 kg a.i. $ha^{-1}$ glyphosate rate.

As opposed to that found with Goodwell, a slight amount of chlorotic leaves was present in Greenfield canopy regrowth for all evaluated glyphosate rates; therefore, chlorotic leaves were not a confounding variable. However, different findings between ADMR and RGCC were found for Greenfield. At a glyphosate rate of 1.1 kg a.i. $ha^{-1}$ (149.2 g $m^{-2}$), Greenfield had lower ADMR than the control (271.3 g $m^{-2}$) and plants following the 0.4 kg a.i. $ha^{-1}$ rate (248.0 g $m^{-2}$); however, values were not different from the 0.5 (214.4 g $m^{-2}$) and 1.5 kg a.i. $ha^{-1}$ rates (94.8 g $m^{-2}$). Finally, the rate of 3.1 kg a.i. $ha^{-1}$ (5.8 g $m^{-2}$) had the lowest ADMR value. Although ADMR values indicated that glyphosate detrimental effects started at 1.1 kg a.i. $ha^{-1}$, the RGCC values indicated that canopy regrowth started to significantly decrease at the 1.5 kg a.i. $ha^{-1}$ rate. This discrepancy between ADMR and RGCC values was explained by considerable plant stunting, i.e.:

reduction in plant height, following the rate of 1.1 kg a.i. $ha^{-1}$ (Abreu, *unpublished data*, 2018). The plant stunting which substantly decreased ADMR was not detected by the RGCC method.

**CONCLUSION**

The proposed RGCC method was demonstrated to be more precise and less subjective to estimate plant injury and regrowth after herbicide application than the visual rating method. Color identification by human eye is subjective. Variation among individuals vision characteristics, such as proportion of eye cone receptors, makes data normalization impossible from different evaluators; even a single evaluator might have its color rating affected by different ambient lighting. Conversely, the proposed RGCC method is standardized to quantify green canopy at its specific wavelength band rather than relying on variations of the human eye. Thus, the greatest benefit that the adoption of the RGCC method would result in is the development of a standardized method to quantify plant discoloration among scientists. This standardized method would allow the collection of normalized data across different studies, making possible their comparison. One limitation to this method is that it only quantifies discoloration. For injury caused by glyphosate, this system works well, but for other herbicide sites of action, it could be limiting as other symptoms (stunting, epinasty, strapping, cupping, callusing, etc.) could be observed. Because of a potential limitation of the Canopeo app to not record other symptoms, it was assumed that Canopeo data could be assessed with plant height measurements and subjective visual estimates for better accuracy of herbicide injury observations.

In this study, thanks to its high precision, the RGCC method detected injury and regrowth responses between Goodwell and Greenfield bermudagrass cultivars following the application of various glyphosate rates. Maximum glyphosate injury, regardless of rate, was observed at no more than 16 DAG for both cultivars. However, Goodwell was more tolerant than Greenfield to glyphosate at rates ≤ 1.1 kg a.i. $ha^{-1}$; although both cultivars were severely injured at rates ≥1.5 kg a.i. $ha^{-1}$. After 56 DAG, both cultivars exhibited canopy regrowth at all tested rates. Detrimental effects to the canopy regrowth of Greenfield started at 1.1 kg a.i. $ha^{-1}$ while regrowth was severely suppressed (RGCC = 5.8%) at 3.1 kg $ha^{-1}$. Conversely, Goodwell detrimental effects were only noticeable at the 1.5 kg $ha^{-1}$ rate while regrowth suppression at 3.1 kg $ha^{-1}$ was less severe (RGCC = 23.5%).

Further efforts must be made to evaluate the impact of glyphosate use in non-dormant bermudagrass, including different bermudagrass types. In the future, glyphosate recommendations for in-season bermudagrass management might address a cultivar-specific recommendation or might be fragmented into categories, e.g., grazing and hay types. This approach would provide improved weed management to bermudagrass based pastures.

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APPENDICES

Table 1. P-values for relative green canopy cover on two bermudagrass cultivars at five different rates and control.

| Source of variation | Days after glyphosate application | | |
|---|---|---|---|
| | **8** | **16** | **24a** |
| **Model** | <0.01* | <0.01* | <0.01* |
| **Cultivars** | 0.16 | 0.03* | <0.01* |
| **Rates** | <0.01* | <0.01* | <0.01* |
| **Cultivar x Rates** | 0.80 | 0.51 | 0.06 |

*significant at $\alpha = 0.05$

Table 2. P-values for the effect of glyphosate rate at 37 and 56 days after application on canopy regrowth of two bermudagrass cultivars.

| **Source of variation** | **Days after glyphosate application** | |
|---|---|---|
| | **37** | **56** |
| **Model** | <0.01* | <0.01* |
| **Cultivars** | <0.01* | <0.01* |
| **Rates** | <0.01* | <0.01* |
| **Cultivar x Rates** | <0.01* | 0.75 |

*significant at α = 0.05

Figure 1. A comparison between visual green canopy cover vs. relative green canopy cover collected from Goodwell and Greenfield bermudagrass cultivars at 8 (A and B), 16 (C and D), and 24a (E and F) days after glyphosate applications (DAG). Statistic shown is root mean square difference (RMSD). Dashed lines represent the 1:1 line.

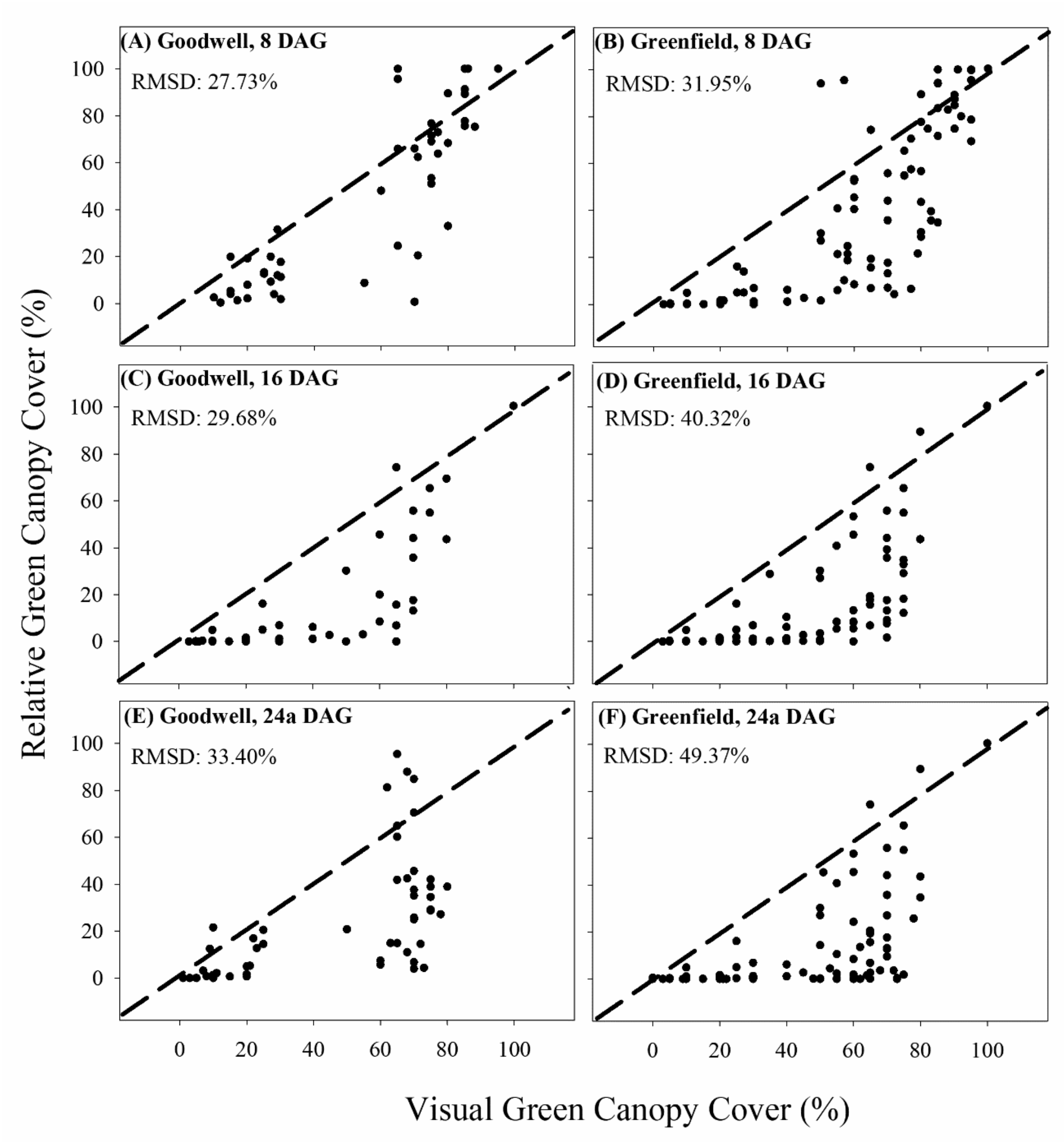


Figure 2. Visual green canopy cover (VGCCn), and relative green canopy cover (RGCCn) ratings of Goodwell bermudagrass treated with 1.1 kg a.i. ha-1 of glyphosate at a given day n. Relative green canopy cover was calculated based on the listed Canopeo output: FGCCn (fractional green canopy cover).

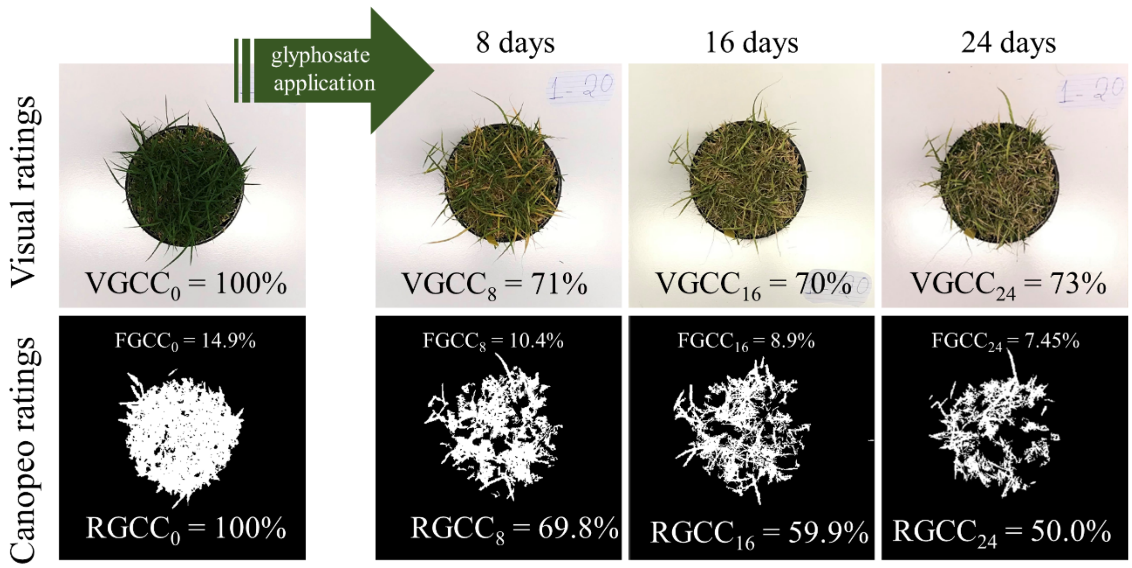


Figure 3. Relative green canopy cover as a proxy for canopy greenness for Goodwell (A) and Greenfield (B) treated with five different glyphosates rates and a control at four different time periods, where 0% = complete herbicide injury (total discoloration), and 100% = no herbicide injury. Error bars denote least significant differences among glyphosate rates within same time period (α =0.05).

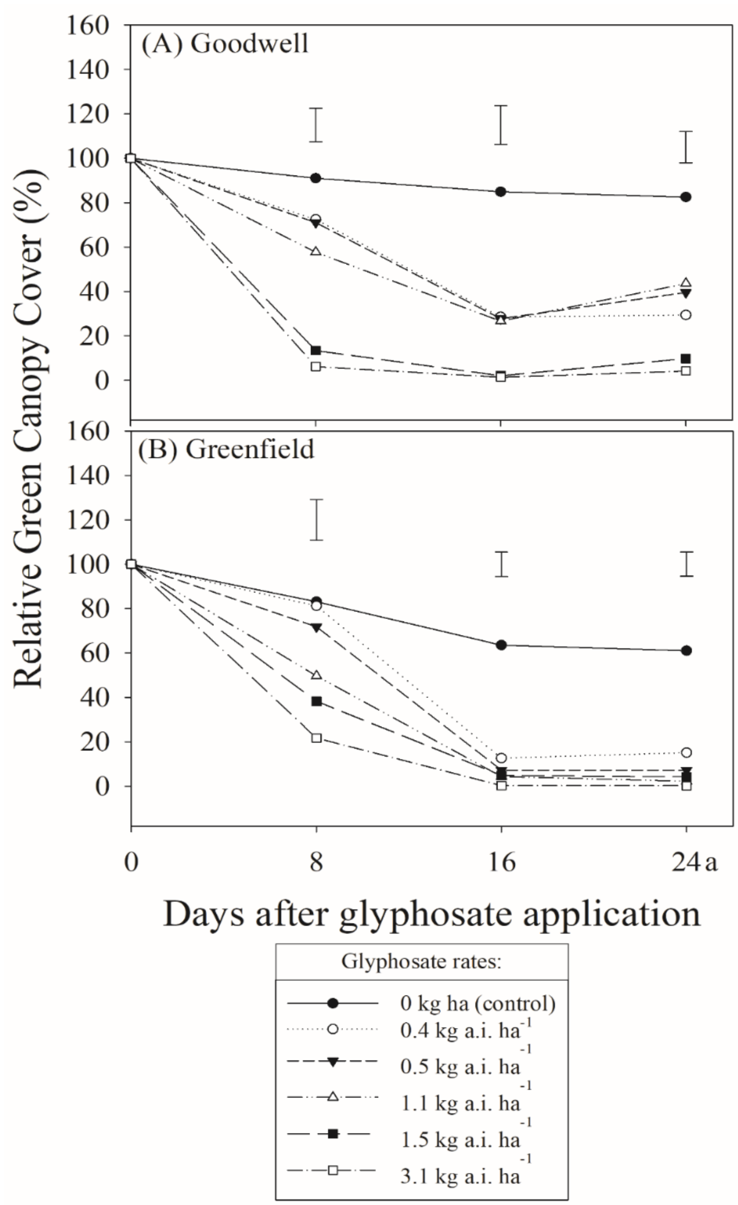


Figure 4. Relative green canopy cover as a proxy for canopy regrowth for Goodwell (A) and Greenfield (B) treated with five different glyphosates rates and a control at three different time periods, where 0% = no regrowth, and 100% = regrowth equals to canopy area at day zero. Injured canopies were removed at time period 24b. Error bars denote least significant differences among glyphosate rates within same time period.

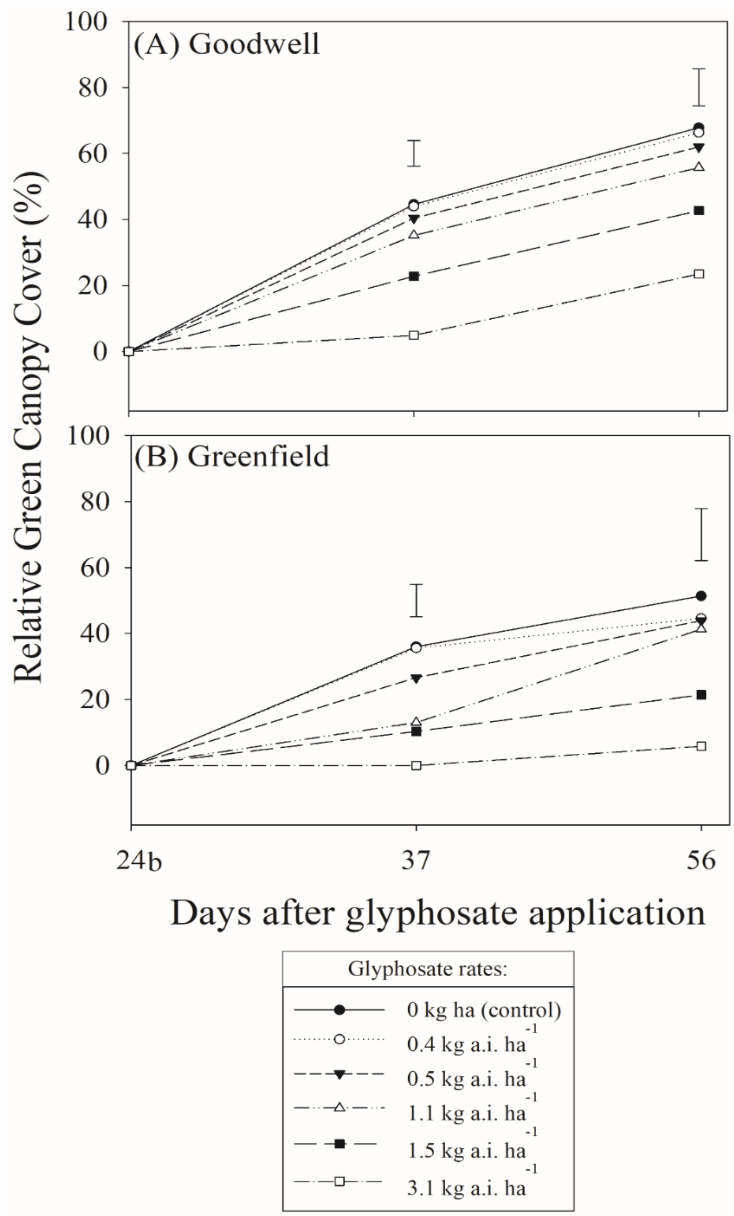
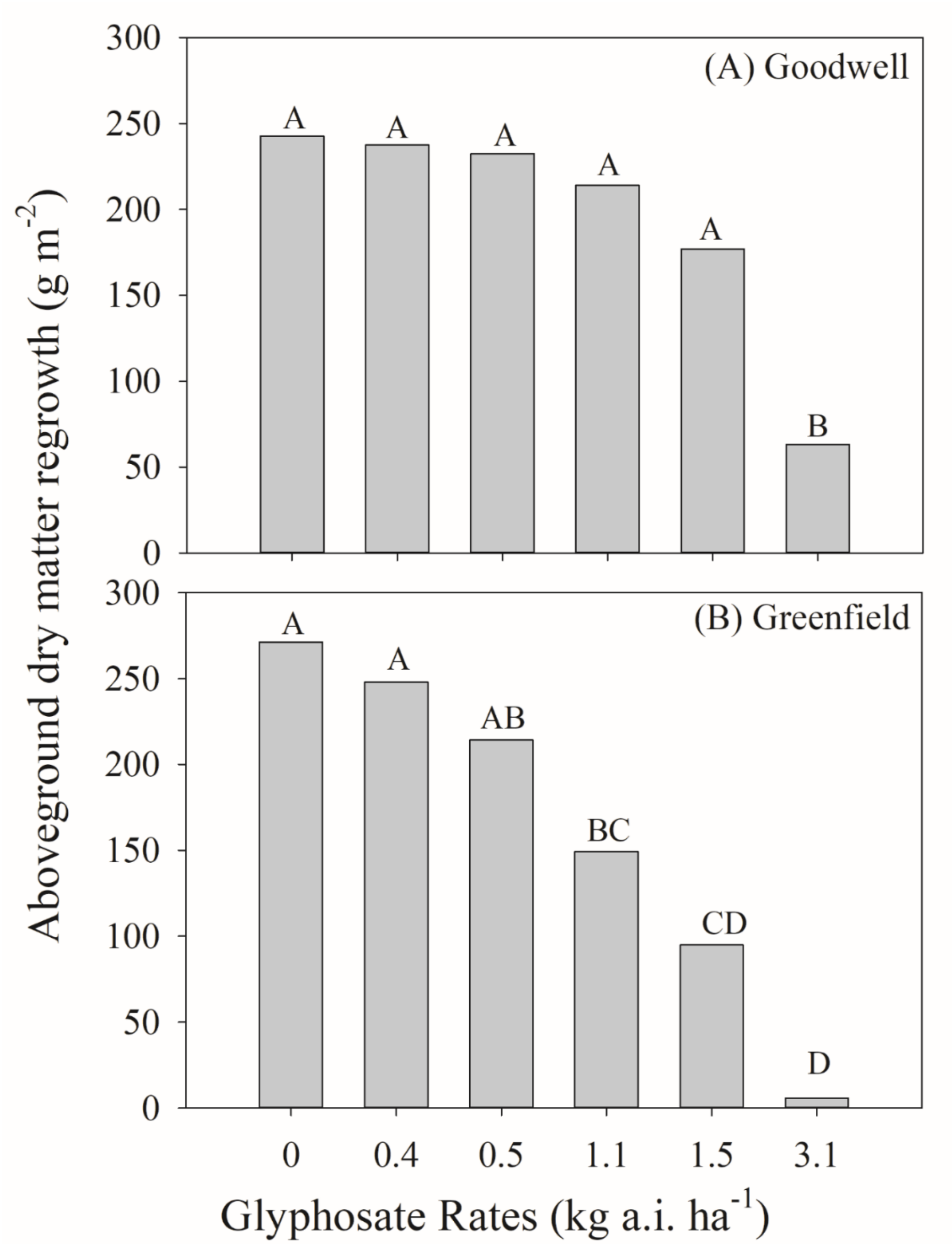


Figure 5. Cumulative aboveground dry matter regrowth from 24b to 56 days after glyphosate application for Goodwell (A) and Greenfield (B) treated with five different glyphosates rates. Treatment means separated by different letters are significantly different (least significant difference, α = 0.05).



VITA

Lucas Freires Abreu

Candidate for the Degree of

Master of Science

Thesis: ASSESSING GLYPHOSATE INJURY AND FORAGE BERMUDAGRASS REGROWTH USING CANOPEO

Major Field: Plant and Soil Sciences

Biographical:

Education:

Completed the requirements for the Master of Sciences in Plant and Soil Sciences at Oklahoma State University, Stillwater, Oklahoma in December, 2019.

Completed the requirements for the Bachelor of Science in Veterinary Medicine at Centro Universitário de Mineiros - UNIFIMES, Mineiros, Goiás/Brazil in 2017.

Experience:

- Graduate Research Assistant, Oklahoma State University, United States, 2018-Current.
- Research Assistant, University of Florida, United States, 2017.
- Dairy Farmer Assistant, Rural Exchange New Zealand (RENZ), New Zealand, 2016.

Professional Memberships:

American Society of Agronomy (ASA), Crop Science Society of America (CSSA) and Soil Science Society of America (SSSA).