

**ALTERNATIVE CONTROL OPTIONS FOR ANNUAL BLUEGRASS:
RESPONSE TO ZINC**

by

Caleb Lowell Bristow

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Approved by

Elizabeth A. Guertal, Co-Chair, Professor of Agronomy and Soils
J. Scott McElroy, Co-Chair, Associate Professor of Agronomy and Soils
Charles W. Wood, Professor of Agronomy and Soils
Julie A. Howe, Assistant Professor of Agronomy and Soils

Abstract

Annual bluegrass (*Poa annua* L.) is the most problematic winter weed that turfgrass managers face in the southeast U.S. Herbicides are often used to control annual bluegrass, however this weed quickly invades most turf areas, and can become the dominant turf species over time. With the existence of both annual and perennial biotypes of annual bluegrass, as well as an increase in herbicide-resistant populations throughout the world, the incorporation of cultural control methods could be vital for controlling annual bluegrass. Objectives of this study were to: 1. determine the tolerance of different cool-season turfgrass seedlings to various zinc (Zn) rates at three soil pH levels, 2. evaluate annual bluegrass control following Zn applications in non-overseeded bermudagrass, and 3. evaluate the effects of Zn on annual bluegrass and bermudagrass in a bermudagrass putting green.

The addition of Zn at any rate significantly reduced plant growth of turfgrass species in greenhouse studies. This reduction was observed in plant height, plant dry matter (shoot and root), and germination. Although the effect of soil pH was not as significant as Zn rate, there was a significant increase in plant height as soil pH increased for creeping bentgrass (*Agrostis stolonifera* L.) and perennial ryegrass (*Lolium perenne* L.). In field studies, when Zn was applied at a rate of 179 kg ha⁻¹, annual bluegrass populations were significantly reduced. Although lower Zn rates

did not provide adequate control of annual bluegrass, there was a linear increase in control as Zn rate increased. Zn reduced clipping yield, increased Zn content in leaf tissue, and increased Zn levels in the thatch layer of the bermudagrass-annual bluegrass putting green community.

This research indicates that applications of Zn inhibit germination and significantly reduce the growth of annual bluegrass, as well as other cool-season turfgrasses. Based on this research, when applied in the fall, Zn applications could provide some annual bluegrass control in areas where it is considered a weed. However, further research is needed to better understand the long term effects of Zn applications on bermudagrass and the underlying soil. Additional research should also be conducted to further evaluate various application timings, as well as combining Zn applications with currently labeled herbicides to extend annual bluegrass control.

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I. Literature Review

Introduction

In the Southeast, hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *Cynodon transvalensis* Burt-Davy) is the most commonly chosen turfgrass due to its vigorous growth habit, drought tolerance, and strong adaptation to the Southeast. However, bermudagrass goes dormant during the fall and winter, and as a result winter annual weeds often dominate the turf making it aesthetically unpleasing to the eye (Johnson, 1980). Annual bluegrass (*Poa annua* L.) is one of the most widely distributed winter weeds found in bermudagrass (Johnson, 1983), and its bright green color stands out against a dormant, brown bermudagrass turf. Despite efforts that have been put into controlling annual bluegrass, this weed quickly invades most turf areas, and can become the dominant turf species over time (Xu and Mancino, 2001).

Annual bluegrass is a serious weed on golf greens, fairways, and tees, as well as lawns and other turfgrass areas where frequent watering encourages its establishment (Juska and Hanson, 1969; Goss et al., 1975). Annual bluegrass is capable of producing seedheads at low mowing heights (Juska and Hanson, 1969), which leaves greens bumpy (Toler, 2007) and disrupts the true roll of a ball (Ferguson, 1936). It also competes for soil nutrients, moisture, and sunlight (Hall

and Carey, 1992). Annual bluegrass is a more serious problem when bermudagrass is overseeded with cool-season grasses such as perennial ryegrass (*Lolium perenne* L.) or rough-stalk bluegrass (*Poa trivialis* L.) during the fall (Johnson, 1983).

In addition, annual bluegrass exists as both annual (*Poa annua* var. *annua* L. Timm) and perennial (*Poa annua* var. *reptans* (Hauskn) Timm) biotypes (Tutin, 1957). The annual biotype is short-lived with an erect growth habit (Warwick, 1979) and can be found in areas that receive minimal precipitation, such as rough areas on golf courses (Gibeault and Goetze, 1972). It also produces many more seedheads than the perennial biotype, and thus more seed (McElroy et al., 2004). The perennial variety is more prostrate and produces fewer flowers (McElroy et al., 2004). It is more prominent where precipitation and moisture are adequately supplied, such as on putting greens (Gibeault and Goetze, 1972).

The annual versus perennial nature of annual bluegrass, coupled with application timing issues with pre-emergent herbicides, often results in inconsistent chemical control of annual bluegrass (Callahan and McDonald, 1992). Inconsistent control often leads to a less than desirable stand of turfgrass. The incorporation of cultural practices for annual bluegrass control could lead to a more uniform and aesthetically pleasing stand of turfgrass.

Chemical Control of Annual Bluegrass

Preemergence

Preemergence herbicides are applied prior to annual weeds germinating. These herbicides form a chemical barrier in the top 2.5 cm of soil that inhibits root growth of germinating seedlings (Baldwin, 1993). Since annual bluegrass relies

heavily on seed production for its survival, the preemergence stage provides an opportunity for effective herbicide treatment (Baldwin, 1993). When applied at the right time, preemergence herbicides can provide adequate control of annual bluegrass. Applications of oxadiazon, dithiopyr, prodiamine, and pendimethalin in early October have been shown to result in >86% control of annual bluegrass (Lewis, 1994). Pronamide applied at the same time in October provided more than 90% control (Johnson, 1975). Metribuzin and benefin have also been found to provide >80% control (Johnson, 1976b).

However, preemergence herbicides sometimes provide less than acceptable control of annual bluegrass. In the Southeast single applications in the fall are often ineffective against the flush of plants that germinate in the spring (Gibson et al., 1998). Lewis (1994) found that DCPA (dimethyl tetrachloroterephthalate) applied in October was not effective in controlling annual bluegrass, and Johnson (1976b) found that DCPA applied in late September provided less than 60% control. Also, the presence of perennial biotypes can result in ineffective control of annual bluegrass (Callahan and McDonald, 1992). This is due to a longer period of vegetative growth (Lush, 1989), which does not provide an effective window for the use of preemergence herbicides.

Annual bluegrass has also been found to be resistant to some of the preemergence herbicides. In North Carolina annual bluegrass populations have exhibited resistance to prodiamine, following eight consecutive years of application, resulting in less than 40% control (Isgrigg et al., 2002). These populations exhibited a 105-fold resistance to prodiamine when compared to susceptible populations.

Postemergence

Postemergence herbicides are applied after weeds have germinated. Applications are found to be most effective when they are applied to young, active growing plants due to greater adsorption and translocation in the plant (Camacho and Moshier, 1991). Trifloxysulfuron, flazasulfuron, foramsulfuron, and pronamide have been shown to provide more than 90% control of annual bluegrass (Toler, 2007). In a field study, simazine provided 80% control while atrazine and sulfosulfuron only provided 70% and 50% control of annual bluegrass, respectively (Toler, 2007). Non-selective herbicides such as paraquat and glyphosate, which can only be applied to dormant, non-overseeded turf, have also been shown to provide up to 90% control of annual bluegrass (Johnson, 1976a; Toler, 2007).

The success of these herbicides not only depends on effective control against target weeds, but it also depends on the safety to desirable turfgrasses following application (Baldwin, 1993). Paraquat and glyphosate applied to dormant bermudagrass in January and February did not reduce green-up in the spring (Johnson, 1976a). Similarly, Toler (2007) did not see any reduction in green-up following applications of trifloxysulfuron or pronamide. However, in other trials ethofumesate delayed bermudagrass green-up in the spring when applications exceeded 1.1 kg ha^{-1} , regardless of application timing (Coats and Krans, 1986).

One of the main problems that turf managers face with postemergence herbicides is developed resistance. The amino acid synthesis inhibitors (ALS) inhibiting herbicides, such as trifloxysulfuron, are among some of the most popular herbicides used, but they are also best known for their resistance issues (Tranel and

Wright, 2002). Resistance has also been found in the triazine herbicides, such as atrazine and simazine. In Mississippi, annual bluegrass populations were found to be resistant to both simazine and atrazine (Kelly and Coats, 1998). Cold temperatures can also inhibit the absorption and translocation of herbicides that are applied during the winter (Pline et al. 1999).

Control of Annual Bluegrass in Overseeded Bermudagrass

In regions where bermudagrass goes into dormancy during the winter months, bermudagrass is commonly overseeded with perennial ryegrass in order to maintain green color throughout the winter months. However, both annual bluegrass and perennial ryegrass are cool-season grasses, which make the management of annual bluegrass in overseeded systems more difficult (Yelverton and McCarty, 2001). Most preemergence herbicides for annual bluegrass control must be applied 2-3 months prior to overseeding (Johnson, 1983), and they are often ineffective in controlling annual bluegrass (Johnson, 1975). Benefin, bensulide, and dithiopyr applied 45 days before overseeding reduced annual bluegrass populations by less than 50% in bermudagrass overseeded with perennial ryegrass (Gibson et al., 1998). Prodiamine applied 6 weeks before overseeding (WBO) resulted in 78% control of annual bluegrass (Yelverton and McCarty, 2001), while oxadiazon applied 45 days prior to overseeding resulted in greater than 90% control of annual bluegrass (Gibson et al., 1998). Fenarimol applied 30 days before overseeding resulted in less than 50% control (Gibson et al., 1998). Foramsulfuron applied 2 weeks before overseeding provided 63% control of annual bluegrass, and

ethofumesate and bispyribac-sodium applied 12 weeks after overseeding resulted in 83% and 82% control, respectively (McElroy et al., 2011).

The main concern with herbicide applications in overseeded situations is injury to the overseeded turfgrass. Oxadiazon applied 8 WBO resulted in 80% annual bluegrass control, without any significant injury to perennial ryegrass (Yelverton and McCarty, 2001). However, there was a reduction in perennial ryegrass cover when oxadiazon was applied 4 and 6 WBO (Yelverton and McCarty, 2001). Johnson (1983) found that ethofumesate applied at 2.2 kg ai ha⁻¹ at the time of overseeding significantly reduced the quality of the overseeded turf. In another field study proflaminate applied at 0.56 and 0.84 kg ha⁻¹ 6 WBO reduced perennial ryegrass cover (Yelverton and McCarty, 2001).

Cultural Control of Annual Bluegrass

Soil pH

Soil pH is one of the most important factors in determining availability of both macronutrients and micronutrients. The effect of soil pH on annual bluegrass has been documented (Sprague and Burton, 1937; Juska and Hanson, 1969). Sprague and Burton (1937) reported that annual bluegrass plants grown in sand at a pH of 6.5 had much stronger growth and root development, when compared to plants grown at a pH of 5.0. Juska and Hanson (1969) found that annual bluegrass produced twice as many roots and leaf tissue at a soil pH of 6.5, when compared to a pH of 4.5, when grown in a loamy sand soil. Annual bluegrass plants also produced four times as many seed heads at a pH of 6.5, when compared to a pH of 4.5, on a

loamy sand soil (Juska and Hanson, 1969). Ferguson (1936) found that annual bluegrass (seeded on an acidic soil surface) did not germinate at pH 3.6, while 66% of the seed germinated at pH 5.2. The findings of this study led Ferguson to state that it might be possible to control annual bluegrass by creating unfavorable conditions at time of germination. He also noted that mature annual bluegrass plants grew better under neutral, rather than acidic, soil conditions (Ferguson, 1936). However, when the pH of the soil decreases aluminum solubilizes (Delhaize and Ryan, 1995) and Al toxicity becomes a concern (Kuo, 1993). The availability of essential macronutrients also decreases as soil pH decreases (Kuo, 1993). Because of these reasons, reducing soil pH could be detrimental to desirable turfgrass such as bermudagrass.

Nitrogen and Annual Bluegrass

Of all the macronutrients that are commonly applied, nitrogen (N) influences turfgrass growth and quality the most (Schlossberg and Schmidt, 2007). Early studies indicated that N applications of 34 kg ha⁻¹ were enough to reduce annual bluegrass seedhead production (Sprague and Burton, 1937). Applications of N at 148 kg/ha significantly increased top growth of annual bluegrass growing in a loamy sand soil (Juska and Hanson, 1969). This increase in top growth was also identified with the decrease in seedhead production noted in earlier studies (Sprague and Burton, 1937). In later studies, the timing of N applications was found to have an effect on seedhead production (Dest and Allinson, 1981). In one of two field studies, they found that altering N rates and application timings reduced the number of panicles produced.

Many turf managers manage annual bluegrass as a desirable cool-season turfgrass, especially when the annual bluegrass is found co-inhabiting with bentgrass (*Agrostis stolonifera* L.). In this dual species scenario annual bluegrass is often studied as a part of an annual bluegrass/bentgrass ecosystem, and thus control is not automatically assumed to be the outcome. For example, the effect of N on annual bluegrass/bentgrass putting greens has been studied. Goss et al. (1975) found that N applications reduced annual bluegrass populations in bentgrass, when compared to no application of N. It was noted that a high rate of N (976 kg ha⁻¹) and low rate of N (293 kg ha⁻¹) reduced annual bluegrass populations more than medium rates of N (Goss et al., 1975). In another study, plots receiving no N had a higher density of bentgrass than annual bluegrass as compared to plots that received 48 kg N ha⁻¹ (Dest and Guillard, 1987). It was also found that annual bluegrass plants had more tillers in the plot receiving 48 kg N ha⁻¹, as compared to treatments receiving 0 N. In a separate study, applications of N, especially those comprised of ammonium (NH₄), increased clipping yield, color, and leaf N content of an annual bluegrass/bentgrass putting green. However, this study did not distinguish between annual bluegrass and bentgrass species (Schlossberg and Schmidt, 2007).

Phosphorus

Phosphorus (P) is another essential macronutrient for plants, and its effect on annual bluegrass has been heavily studied. In a greenhouse study, annual bluegrass clippings increased with an increase in P applications (up to 120 kg ha⁻¹), which illustrates the high P requirement of annual bluegrass (Varco and Sartain,

1986). Dest and Allinson (1981) reported an increase in annual bluegrass density with increasing P rates ranging from 0-176 kg ha⁻¹. Leaf tissue phosphorus was found to be adequate (ranging from 0.42-0.44%) across all P treatments, including areas with only native P levels (Dest and Allinson, 1981).

As with N studies, some P studies have been conducted in mixed annual bluegrass/bentgrass swards to help better understand community dynamics. Phosphorus applications have been found to significantly increase annual bluegrass populations within a bentgrass stand (Goss et al. 1975). However, Dest and Guillard (1987) found that P did not have an impact on annual bluegrass growing within bentgrass. In a conflicting study, high P treatments were found to benefit annual bluegrass more than bentgrass (Kuo, 1993). When grown in acidic soils, bentgrass had a leaf tissue concentration of 1.78 mg P g⁻¹ while annual bluegrass only contained 1.48 mg P g⁻¹ (Kuo et al., 1992).

Sulfur

Applications of sulfur (S) have been reported by several authors to reduce annual bluegrass populations (Sprague and Burton, 1937; Goss, 1974). In early studies, S applications of 340 kg ha⁻¹ yr⁻¹ were found to reduce annual bluegrass coverage to only 7% in a bentgrass putting green, compared to 30% coverage in treatments not containing S (Sprague and Burton, 1937). In another study, sulfur applied at 168 kg ha⁻¹ yr⁻¹ significantly reduced annual bluegrass populations when compared to plots that did not receive S (Goss et al., 1975; Goss, 1974). However, low S applications (56 kg ha⁻¹) have been shown to increase annual bluegrass populations (Goss et al., 1975). In another experiment, it was determined that S

applications acidified only the top 0-2.5 cm of the soil, resulting in decreased annual bluegrass emergence and establishment (Varco and Sartain, 1986). Some authors have also found that the magnitude of annual bluegrass reduction following S applications is dependent upon phosphorus levels (Varco and Sartain, 1986; Goss, 1974).

Zinc

Zinc (Zn) is the 23rd most abundant element on earth and the second most abundant transition metal found in organisms (Broadley et. al, 2007). Zinc is found in numerous minerals including sulfides (ZnS), sulfates (ZnSO₄), oxides (ZnO), carbonates (ZnCO₃), phosphates (Zn₃(PO₄)₂), and silicates (Zn₂SiO₄) (Barak and Helmke, 1993). For most soils, a common range for total zinc concentration ranges from 10-300 mg kg⁻¹ with an average concentration of 50 mg kg⁻¹ (Kiekens, 1995). The primary input of Zn to soils is from the chemical and physical weathering of parent rocks (Broadley et al., 2007). The amount of quartz, which dilutes most elements, can also affect the concentration of zinc present in soil (Barak and Helmke, 1993). In addition to parent materials present in the soil, zinc inputs in the soil can come from sewage sludge applications, which often contain large amounts of zinc (Kiekens, 1995).

Zinc in Soils

Soil Zn occurs in the following forms: (i) free ions (Zn²⁺) and organic complexes in the soil solution; (ii) adsorbed and exchangeable Zn in the colloidal fraction (clay particles, humic compounds, and iron and aluminum oxides; and (iii) secondary minerals and insoluble complexes (Kiekens, 1995). More than 90 percent of the soil

zinc is in an insoluble form and unavailable for plant uptake (Broadley et al., 2007). Based on activity (a) as a function of pH, the dominant zinc species found below a pH 7.7 is Zn^{2+} (Kiekens, 1995). The cationic form of zinc (Zn^{2+}), which is the plant available form of zinc, usually accounts for 50% of the plant-available zinc (Broadley et al., 2007). Factors such as soil composition/texture and soil pH can affect Zn adsorption in the soil. Divalent cations, such as zinc, are subject to strong adsorption when in the presence of clay and humus particles, which are negatively charged (Viets, 1962). Shuman (1975) found that the adsorptive capacity of Zn was higher for soils with a high clay and organic matter content than for sandy soils that were low in organic matter. In addition to clay minerals and organic matter, soil pH has been identified as one of the most important factors in determining soil availability of micronutrients (Sims, 1986). In a laboratory experiment comparing four soils, Sims (1986) found that above a soil pH of 5.2 most zinc in the soil was organically bound.

In addition to soil properties, studies show that macronutrients such as phosphorus and nitrogen can affect Zn uptake in plants. The P-Zn interaction is usually designated as a P-induced Zn deficiency (Olsen, 1972). This is commonly encountered when both Zn and P levels are limiting, and the addition of P promotes growth, which leads to the dilution of Zn in the leaf tissue inducing a Zn deficiency (Boawn et al., 1954). As with phosphorus, studies have shown that addition of N results in plant growth, which can dilute Zn levels in the plant as well (Chaudhry and Loneragan, 1970). Other studies have also indicated that excess micronutrients

such as copper and iron can induce zinc deficiencies as well (Chaudhry and Lonergan, 1970; Zhang et al., 1991).

Soil samples are often analyzed for either available zinc, or total zinc. Total Zn analysis in the soil requires that all forms of Zn in the soil be solubilized (Reed and Martens, 1996). Jackson (1958) originally developed the HF-HNO₃-HClO₄-H₂SO₄ method, which breaks down all organic and inorganic soil components, allowing for total Zn analysis. Simplicity of this procedure has led to its common use, but precautions must be taken due to caustic reagents and explosive reactions that can occur during the process (Reed and Martens, 1996). A safer alternative to this method is the microwave assisted acid digestion method (USEPA, 1994).

Extractants used for determining available zinc include chelating agents, inorganic acids, and a combination of both (Reed and Martens, 1996). Two chelating agents commonly used to test for available zinc in soils are EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriaminepentaacetic acid). The DTPA method was developed to test for insufficient levels of copper, iron, manganese, and zinc (Lindsay and Norvell, 1978). The Mehlich-I method, originally used to test for available phosphorus (Nelson et al., 1953), has been used as a method to test for available zinc (Perkins, 1970). More recently, the Mehlich-III method, which is similar to the Mehlich-I method, has been used to evaluate micronutrient levels in the Southeast (Mehlich, 1984). The dilute hydrochloric acid method was first used by Wear and Sommer (1948) to determine zinc deficiencies in corn. However, this method does not provide accurate results for calcareous soils. (Reed and Martens, 1996).

Zinc Fertility

Although Zn was found to be essential for higher plants in 1926 (Sommer and Lipman, 1926), Zn deficiency is still one of the most widespread micronutrient deficiencies worldwide (Cakmak, 1999). Field trial results indicating Zn deficiencies have been reported for almost all soil types (Takkar and Walker, 1993).

Approximately 50% of the soils in India and Turkey, one-third of the soils in China, and most soils in Western Australia are classified as being zinc deficient (Broadley et al., 2007). Zinc deficiencies have been reported in corn (Lingle and Holmberg, 1957), rice (Wissuwa, 2006), wheat (Graham et al., 1992), and many other crops throughout the world. These deficiencies are often associated with high pH soils, wetland soils, soils very low or high in organic matter, and/or sandy soils (Takkar and Walker, 1993).

For this reason, Zn fertilizers are commonly applied to many crops (Mortvedt and Gilkes, 1993). Zinc fertilizers are available in various formulations (i.e., powders, granules, and liquids) that vary in chemical composition and physical properties (Slaton et al., 2005). Zinc sulfate (ZnSO_4) and zinc oxide (ZnO) are the two most commonly used inorganic zinc sources in research studies (Murphy and Walsh, 1972). However, due to the low water solubility of ZnO , applications may not be as effective as ZnSO_4 (Mortvedt and Gilkes, 1993). Smith (1967) found that after the first year of application, ZnSO_4 was more effective than ZnO for orange production. However, after three years, ZnO was equally effective in supplying Zn. One of the most widely used chelates for soil applications is ZnEDTA (Murphy and Walsh, 1972). Chelates are usually the most effective zinc sources (Mortvedt and

Gilkes, 1993). However, chelates such as ZnEDTA may cost up to 5-10 times more than sources such as ZnSO₄ (Mortvedt and Gilkes, 1993).

In a single growing season a plant can only absorb 2-3% of Zn added to the soil (Viets, 1962). Thus, large soil applications of Zn have been found to influence the soil for relatively long periods of time (Murphy and Walsh, 1972). Application rates of 11 and 22 kg ha⁻¹ as ZnSO₄ have provided residual zinc for up to two years (Ellis et al., 1969). Some reports have demonstrated that crops can respond to zinc applications up to 5 years after treatment (Mortvedt and Gilkes, 1993). However, these residual effects can be detected and monitored with continual soil analysis (Murphy and Walsh, 1972).

Zinc in Plants

Zinc enters plants primarily by root absorption of Zn²⁺ from the soil solution (Kochian, 1993). Once absorbed by the roots of the plant, the zinc is then transported to the shoots (Riceman and Jones, 1958). The transport of zinc within the plant is complex and many mechanisms of transport remain unknown (Longnecker and Robson, 1993). It is known that zinc is transported from the roots to the shoots by the xylem (White et al., 1981). Once zinc has entered the plant, translocation by the phloem is variable (Robson and Pitman, 1983) and depends on Zn supply to the plant (Longnecker and Robson, 1993). Studies conducted by Peterson (1969) showed that 58-91% of the zinc in plants exists in a soluble form.

Plants use zinc as a functional, structural, or regulatory factor for a large number of enzymes (Kiekens, 1995). These enzymes play an important role in carbohydrate metabolism, protein metabolism, maintaining membrane stability,

and auxin metabolism (Brown et al., 1993). Studies show that Zn deficiencies can reduce the activity of RuBPC (ribulose-1,5-bisphosphate carboxylase), which catalyzes the first step in CO₂ fixation in plants (Jyung et al., 1972). Other studies report that Zn deficiencies have led to the reduction of RNA and the deformation of ribosomes (Prask and Plocke, 1971). Skoog (1940) reported that indole acetic acid (IAA) was reduced in tomato plants when Zn was limiting. He found that adding zinc resulted in an increase in IAA. Later studies indicated that Zn was more specifically required for the production of tryptophan, the precursor for IAA (Tsui, 1948). Zn has also been noted as playing an important role in flowering and seed production (Reed, 1941).

In most plants, a leaf Zn concentration of 15-20 mg kg⁻¹ (dry weight) is adequate for proper growth (Marschner, 1995). However, some studies show that turfgrass species and cultivars differ in their ability to acquire and utilize Zn (Xu and Mancino, 2001). It has been reported that a Zn level of 1500 mg kg⁻¹ had no negative effects on Penncross creeping bentgrass (Spear and Christians, 1991). Merlin red fescue (*Festuca rubra* L.) has also been found to tolerate higher shoot tissue concentrations of zinc than other red fescue cultivars (Chaney, 1993). Davis and Beckett (1977) found that for perennial ryegrass a shoot concentration of 221 mg kg⁻¹ Zn caused phytotoxicity. Xu and Mancino (2001) concluded that shoot concentrations of zinc exceeding 200 mg kg⁻¹ could result in reduced color and growth of annual bluegrass. They reported that chlorosis of annual bluegrass and creeping bentgrass first appeared on younger leaves and then moved throughout the plant.

Two of the most common procedures for plant tissue analysis are wet and dry ashing. However, dry ashing cannot be used for plants containing high amounts of silica (Ryan et al., 2001). Dry ashing and wet ashing details can be found in Piper (1942) and Johnson and Ulrich (1959). Comparisons have been made between the two techniques, and differences have been found for nutrients such as calcium, iron, and Zn (Jones, 1972). Studies were conducted by Sahrawat (2002) to compare the two methods. He found that the two methods were equally effective in determining potassium, magnesium, manganese, and Zn. The wet ashing method has been commonly used to determine Zn concentrations in grass plants (Xu and Mancino, 2001; Boawn and Rasmussen, 1971; Wu et al., 1981). The microwave assisted acid digestion method, as previously discussed for soil analysis, has also become a common method for plant tissue analysis. Rechcigl and Payne (1990) compared this method with other commonly used methods for plant tissue analysis and concluded that the microwave digestion technique was effective for plant tissue analysis.

Summary and Objectives

With an increase in herbicide resistance, cultural weed control methods could provide turfgrass managers with alternative control options. In addition, these cultural control methods could reduce the number of herbicide applications, which could be vital in reducing the occurrence of herbicide resistance. Research examining the use of zinc as a cultural control method for annual bluegrass control is limited. Previous research has primarily looked at the response of annual bluegrass/bentgrass communities to zinc applications, and control of annual

bluegrass has not been the primary focus of these studies. Research is lacking which evaluates zinc fertilization effects on annual bluegrass growth, and on subsequent possible soil accumulation. Thus, the objectives of this project are to:

- i) Determine the tolerance of different turfgrass seedlings to various zinc rates at three soil pH levels.
- ii) Evaluate annual bluegrass control following zinc applications in a non-overseeded bermudagrass putting green.
- iii) Evaluate the effects of pH and zinc on annual bluegrass in a bermudagrass putting green.

II. Evaluation of the Response of Cool-Season Turfgrass Species to Zinc at Three Soil pH Levels

Materials and Methods

Greenhouse experiments were conducted from July to December 2011 to evaluate the response of various cool-season turfgrass species to Zn at different soil pH levels. All experiments were conducted at the Auburn University Plant Science Research Center (PSRC) in Auburn, AL. Four grass species were evaluated in this study: annual bluegrass (commercial population), roughstalk bluegrass ('Havana'), perennial ryegrass ('Caddieshack'), and creeping bentgrass ('T-1'). The soil medium used for this study was a Marvyn loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludult) with an initial pH of 5.0. Background P, K, Ca, and Mg were 27, 65, 693, and 113 kg ha⁻¹, respectively.

Three soil pH levels (5.5, 6.0, 6.5) were obtained by thoroughly mixing reagent grade calcium hydroxide (Ca(OH)₂) with the soil in a soil-mixing machine. The Ca(OH)₂ was mixed with approximately 35 kg of soil at rates of 7, 15, and 21 g to achieve a desired soil pH of 5.5, 6.0, and 6.5, respectively. Three bulk batches of soil were mixed throughout the experimental period in order to provide sufficient soil for all the experiments. Once mixed, bulk soil was placed in the greenhouse for one week to allow for pH adjustment before seeding and applying Zn. Prior to

experiment initiation, pH of the bulk soil was checked to ensure that desired pHs had been reached. After pH adjustment pots with a diameter of 15 cm were filled with soil (to a bulk density of $\sim 1.3 \text{ g cm}^{-3}$) and seeded at a rate of 50 seed per pot. Fifty seeds of each grass species were planted at depth of $\sim 3 \text{ mm}$, without adjustment to a Pure Live Seed basis. Granular Zn was applied at five rates (0, 33, 67, 100, and $134 \text{ kg Zn ha}^{-1}$) using reagent grade zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$). These rates were based on preliminary experiments in which higher Zn rates caused toxicity to annual bluegrass. Zinc was uniformly applied to the soil surface at the time of seeding using a shaker can. After seeding and then applying zinc, the pots were covered with germination cloth to maintain adequate soil moisture (at approximately 80% of field capacity) for germination. After one week, the germination cloth was removed. Pots were watered throughout the experiment as needed to maintain adequate soil moisture for seedling survival. Daytime and nighttime temperatures in the greenhouse were approximately 24 and 20°C, respectively. Each experiment was a 3×5 factorial (3 soil pH levels and 5 zinc rates), with four replications of each treatment. Treatments were arranged in a completely randomized design. Experiments were repeated twice for each grass species. Each grass species was conducted as a separate experiment. Each experiment was conducted for 28 days.

Data collected included plant counts taken 7, 14, 21, and 28 days after seeding (DAS) to determine seedling germination and survival. Any seedling with a visible hypocotyl was counted as a living seedling. At the conclusion of the 4-week period, plant height was recorded from three randomly selected plants in each pot.

Root and shoot dry matter production were also collected at the end of each experiment. Shoots were harvested by clipping all plants at the crown and drying in a forced-air oven at 70°C for 72 hours. Following shoot harvest, roots were collected by washing all sand free from the roots and drying in a forced-air oven at 70°C for 72 hours. Soil samples were collected at the end of each experiment to determine soil pH. A 10 gram sample was taken from each pot and mixed with 20 mL of deionized water. After resting for 30 minutes, soil pH was determined.

Results and Discussion

Soil samples collected at the end of each experiment showed that desired soil pH levels of 5.5, 6.0, and 6.5 were an average of 5.5, 5.9, and 6.3, respectively. Analysis of variance for each data set revealed that, except for roughstalk bluegrass, the experimental run was almost always significant (Table 1). Therefore, data was not pooled across experimental runs for analysis. Main effects of pH and Zn rate, and their interaction, were also frequently significant (Table 1). For any species, the interaction of Zn and soil pH was never significant for seed germination, although the main effect of Zn always affected germination, and the main effect of soil pH sometimes did ($\alpha=0.05$) (Table 1).

For every grass species, as Zn rates increased from 0 to 134 kg ha⁻¹, germination decreased (Figures 1 and 2). Germination of all grasses was affected similarly, with an average germination percentage of 82% when no Zn was added (Runs 1 and 2), and an average germination of 53% when Zn was applied at the highest rate of 134 kg ha⁻¹. In previous studies, Zn applied at similar rates (up to 181

kg ha⁻¹) had no phytotoxic effect on 'Penncross' creeping bentgrass (Spear and Christians, 1991). However, the pH of the soil was 7.5, which is significantly higher than the highest pH level (6.5) used in this study. In other work with Zn rate and grasses, tall fescue (*Festuca arundinacea* Schreb.) was grown in soil to which Zn at rates of 125 to 1,000 mg kg⁻¹ had been added (Bryson and Barker, 2007). Regardless of Zn rate, fescue germinated and grew at all Zn levels (Bryson and Barker, 2007). It should be noted that the lowest rate of Zn used in that study (~114 kg ha⁻¹) was roughly equal to the highest rate used in our work. Others have shown a limited effect of Zn on germination of pearl millet (*Pennisetum glaucum* (L.) R.Br.) in a nutrient solution when increasing Zn rates were studied. This work also included a pH variable, and found best growth when pH was between 5 and 7 (Davis et al., 1993). Roots were more sensitive than shoots to increasing Zn, but only when concentrations far exceeded those found in native soil (Davis et al., 1993). Since germination studies that include Zn are often part of a heavy metal study, other metals such as copper (Cu), nickel (Ni), and cadmium (Cd) are often included in the work. Often, Zn was found to have less of an effect on seed germination, with lower concentrations of Cu (Davis et al., 1993) and Cd (Souza et al., 2005) causing reduced germination and greater plant damage than observed with Zn, especially in nutrient solution studies.

Since the number of emerged seedlings was counted every week for four weeks, differences in time to emergence could be determined. For every species the maximum number of emerged plants had occurred by week 1, and no new seedlings emerged in subsequent weeks. Additionally, seedlings did not die in subsequent

weeks – if Zn or pH did affect them it affected their height (Table 1). Basically, Zn and pH separately affected germination of grass seeds, but this effect was created by the seed failing to germinate, and not by delaying its germination. Soil pH did affect germination of creeping bentgrass and perennial ryegrass, but it did not affect germination of roughstalk bluegrass or annual bluegrass (data not shown). In previous research, annual bluegrass in particular has been shown to tolerate a lower soil pH (Juska and Hanson, 1969). The effects of pH on the germination of creeping bentgrass and perennial ryegrass were not consistent between runs, with significantly lower germination at a pH of 6.0 in Run 1, and a slight linear increase in germination as pH increased in Run 2. The overall lack of a pH effect on grass germination is likely due to the generally suitable range of pHs used in this study. The desired pH ranges in this work (5.5 to 6.5) are typical for southeastern soils, and only the lowest pH of 5.5 might (variable with crop) result in a lime recommendation.

Dry weight of harvested roots was always significantly affected by Zn rate, sometimes by soil pH, but never by their interaction (Table 1). As Zn rate increased, the dry weight of harvested roots significantly decreased. Zinc applied at 134 kg ha⁻¹ decreased dry root weight by >90% for all grass species (Figures 3 and 4). Across both runs the root weights of perennial ryegrass and roughstalk bluegrass were most affected by increasing soil Zn, while creeping bentgrass and annual bluegrass root weights were less affected (Figures 3 and 4). Soil pH also had an effect on root dry weight for all grass species, except for roughstalk bluegrass (Table 1). As pH increased, the dry weight of harvested roots generally increased (Table 2). In other

work, increasing soil pH to any level above 5.0 improved root-length and root-hair length of several perennial pasture species (Haling et al., 2010). Calcareous soils were shown to improve the ability of cool-season turfgrasses to revegetate in Zn contaminated soils (15,500 mg Zn kg⁻¹) (Li et al., 2000). When soils with two pH levels (6.5 and 6.0) were treated with Zn (0 to 1200 mg kg⁻¹) soil pH had little effect on sorghum sudan (*Sorghum vulgare* var. sudanese) biomass yield, while increasing Zn decreased biomass yield (Sonmez et al., 2009).

The main effects of Zn and soil pH also significantly affected the dry weight of harvested shoots, and for perennial ryegrass their interaction was also significant ($\alpha=0.05$) (Table 1). Similar to root dry weight, as Zn rate increased the dry weight of harvested shoots significantly decreased. The highest Zn rate of 134 kg ha⁻¹ reduced the dry shoot weight of roughstalk bluegrass by 30%, while the dry shoot weights of the remaining three grass species were reduced to a far greater extent (>60%) at the same Zn rate (Figures 5 and 6). Soil pH also had a significant effect on harvested dry shoot weights (Table 1). As pH increased, the dry weight of harvested shoots generally increased across all species (Table 3). The exception to this was perennial ryegrass in Run 1, where greatest shoot dry weight was measured at the lowest soil pH of 5.5 (Table 3).

Plant height was significantly affected by a pH \times Zn rate interaction for all grass species (Table 1). Increasing rates of Zn significantly reduced plant height across all soil pH levels, regardless of turfgrass species (Tables 4 and 5). Plants were more sensitive to increasing Zn at lower soil pHs, and plant height was most often greatest at a soil pH of 6.5. This effect of increasing pH was almost always a linear

response, and a similar linear decrease in plant height was observed as Zn rate increased (Tables 4 and 5). A Zn rate of 134 kg ha⁻¹ reduced plant height up to 70% across all turfgrass species. As soil pH increased, plant height generally increased (Tables 4 and 5). Exceptions to this were found for perennial ryegrass, roughstalk bluegrass, and creeping bentgrass in Run 1, where pH often did not affect plant height, especially when no Zn or lower Zn rates were applied.

Conclusion

For a loamy sand soil, such as the one used in this study, the addition of any amount of Zn significantly reduced growth of the cool season grasses studied. Reductions were observed at lower levels of Zn addition than studied in previous research, as most previous work focused on Zn as a byproduct of waste application. This reduction was observed in plant height, plant dry matter (shoot and root), and germination. As Zn was added to the soil, there was a decrease in germination across all grass species. Of the plants that did germinate, there was a significant reduction in plant height, root weight, and shoot weight when Zn was applied. The highest Zn rate of 134 kg ha⁻¹ reduced root mass by >90%, while reducing shoot mass up to 80%, varying with grass species. In areas where any of these grass species are desirable, applications of Zn could reduce turf quality. However, in areas where grasses such as annual bluegrass are considered a weed, applications of Zn could be beneficial, especially when cool-season annual bluegrass is an undesirable species in warm-season bermudagrass. Not only could Zn applications reduce plant vigor and health, but they could also prevent these annual weeds from germinating.

Thus, applications of Zn to cool season grasses as a weed control option might be possible, as this work demonstrates the negative effect of Zn on cool season turfgrass species. However, further work of Zn effects on warm season species is needed.

III. Evaluation of Annual Bluegrass Control Using Applications of Zinc and Other Herbicides Used for Annual Bluegrass Control

Materials and Methods

Field experiments were conducted from 2009-2012 to evaluate the control of annual bluegrass using applications of zinc and commercial herbicides used for annual bluegrass control. All experiments were conducted at the Auburn University Turfgrass Research Unit in Auburn, AL. In the first year (September 2009 - April 2010), research was conducted on a TifDwarf bermudagrass putting green. After completing the first year of research, the green was renovated as follows. The existing TifDwarf bermudagrass was removed in May 2010 using two sequential applications of glyphosate (3.3 kg ai ha⁻¹ per application). After 21 days, the green was lightly tilled and sprigged with TifEagle in June 2010. Experiments conducted from September 2010 - April 2012 were conducted on the new TifEagle bermudagrass green. Both putting greens were maintained at a height of 5 mm throughout the study and fertilized with 195 kg N ha⁻¹ annually (May-August). Soil type for the putting green was a Marvyn loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludult) with a pH of 5.6. Background P, K, Mg, and Ca were 32, 41, 58, and 421 kg ha⁻¹, respectively.

Treatments included Zn applied at 22, 45, 90, 179 kg ha⁻¹, fenarimol applied both singly and sequentially (2.2 kg ai ha⁻¹ per application), and fenarimol (2.2 kg ai ha⁻¹) + zinc (22 or 90 kg Zn ha⁻¹). The treated check was a combination of prodiamine (1.1 kg ai ha⁻¹) and trifloxysulfuron (29.4 g ai ha⁻¹). A non-treated check was also included for a total of 10 treatments in this study. Initial applications of all materials in all three years were made on September 15, with the second application of fenarimol being made approximately 14 days later. Reagent grade zinc sulfate (ZnSO₄) was used as the Zn source. All Zn was applied as granular treatments to the turf surface using a shaker can in 2010 and 2011. However, Zn treatments were applied with a CO₂-pressurized sprayer using 8006 flat fan nozzles calibrated to deliver 935 L ha⁻¹ in 2009. All other treatments were applied with a CO₂-pressurized sprayer using 8002 flat fan nozzles calibrated to deliver 280 L ha⁻¹ (2009-2011). Treatments were watered in (~5 mm) following application. Experimental units were 1.5 m × 1.5 m, arranged in a randomized complete block design with 3 replications.

Data collected included both visual ratings as well as plant density determination. Visual ratings were taken for both bermudagrass injury and annual bluegrass control. Bermudagrass injury was rated on a 0 to 100% scale, where 0 indicated no injury and 100 indicated complete death. Injury ratings were taken until the bermudagrass entered dormancy. Annual bluegrass control was also rated on a 0 to 100% scale with 0 indicating no control and 100 indicating complete control. During the months of February and April, annual bluegrass populations

were determined by counting the total number of living annual bluegrass plants within each plot.

Results and Discussion

Bermudagrass Injury

Fenarimol applied in September never caused injury or delay in spring green up of either Tifdwarf (2009) or TifEagle (2010 and 2011). Previous studies have also shown that fenarimol applied at rates up to 3.2 kg ai ha⁻¹ caused no injury or reduction in turf color of TifEagle bermudagrass (McCullough et al., 2006). It is important to note that in the same study, increasing rates of fenarimol did linearly decrease root mass. However, root length was not affected by fenarimol applications (McCullough et al., 2006). The combination of prodiamine and trifloxysulfuron never had a visual effect on either bermudagrass cultivar. However, some studies have shown that prodiamine reduced root growth of Tifgreen bermudagrass at rates as low as 4 µg kg⁻¹ (Fishel and Coats, 1993).

Bermudagrass injury occurred in September 2009 following the application of Zn. However, visual injury ratings never exceeded 30%, and turf fully recovered before entering dormancy. Bermudagrass injury did not occur following Zn applications in 2010 or 2011. The reason for injury occurring in 2009 is most likely attributed to the ZnSO₄ being applied as foliar treatments, unlike the next two years in which the ZnSO₄ was applied as a granular treatment. Although direct injury from application was not noticed in 2010 or 2011, there was evidence of 'tracking'. After applying all Zn treatments, the fenarimol and prodiamine treatments were then

applied in their appropriate plots. Most of the 'tracking' was noted where footsteps had been taken in the plots where Zn had been applied. Again, injury never exceeded 20%, and the turf fully recovered within one month.

Annual Bluegrass Control

Analysis of variance revealed that there was not a significant run or run × month interaction ($P \geq 0.05$), therefore data was pooled across experimental runs for analysis. Results for annual bluegrass control, both visual and population data, are presented separately for all rating dates (Tables 6 and 7). All treatments were subjected to means separation using Fishers Protected LSD at $\alpha = 0.05$ (Tables 6 and 7). In addition to means separation, regression analysis was conducted for all Zn rates, except when combined with fenarimol (Figure 7).

One application of fenarimol ($2.2 \text{ kg ai ha}^{-1}$) in September provided 45% control of annual bluegrass in April. Making a repeat application of fenarimol approximately 6 weeks after the initial treatment ($2.2 \text{ kg ai ha}^{-1} + 2.2 \text{ kg ai ha}^{-1}$) did not increase the control of annual bluegrass, when compared to a single application of fenarimol. Similarly, adding 22 kg Zn ha^{-1} to a single fenarimol treatment in September had no effect on annual bluegrass control either. However, adding 90 kg Zn ha^{-1} to a single fenarimol treatment in September increased annual bluegrass control by approximately 15%, when compared to a single application of fenarimol alone. In greenhouse studies, fenarimol applied at rates up to $6.1 \text{ kg ai ha}^{-1}$ did not reduce quality or clipping weight of annual bluegrass (Gaul and Christians, 1988). In field studies, fenarimol applied at the same rate did not reduce annual bluegrass density in a creeping bentgrass putting green (Gaul and Christians, 1988).

As Zn rate increased, annual bluegrass control also increased (Figure 7). When applied at rates below 90 kg ha⁻¹, Zn provided ≤50% control of annual bluegrass throughout the year. Although control was ≤50%, Zn applied at 22 and 45 kg ha⁻¹ provided control equivalent to that of fenarimol applied at 2.2 kg ai ha⁻¹. The highest Zn rate (179 kg ha⁻¹) provided approximately 70% control of annual bluegrass, regardless of rating date, which was greater than all other treatments in this study, except for prodiamine (Table 6). Using the response equation ($y = -0.003x^2 + 0.89x + 1.67$), it was determined that 70% control could be achieved by applying 148 kg Zn ha⁻¹. A combination of prodiamine and trifloxysulfuron applied in September provided 99% control of annual bluegrass throughout the year (Tables 6 and 7). Other studies have shown a similar response of annual bluegrass to prodiamine (Lewis, 1994).

Conclusion

Although prodiamine provided exceptional control in this study, it is important to remember that this study was conducted on a putting green. Prodiamine, which is widely used for annual bluegrass control in bermudagrass, is not labeled for use on putting greens due to evidence that prodiamine can reduce root production (Fishel and Coats, 1993). In this study as Zn rates increased annual bluegrass control also increased. Applying Zn at a rate of 148 kg ha⁻¹ could provide up to 70% control of annual bluegrass throughout the year. Considering that there are a number of herbicides, such as prodiamine, that are not labeled for annual bluegrass control on putting greens, Zn could provide an alternative option.

Additionally, the use of reduced rates of prodiamine, coupled with Zn, might provide affective annual bluegrass control without root damage. However, further research is needed to not only evaluate the tolerance of warm season grasses to Zn, but also to examine the effect of different Zn rates and timing of applications.

IV. Effects of Zinc Applications on an Annual Bluegrass-Bermudagrass Putting Green Community

Materials and Methods

Field experiments were conducted on a bermudagrass putting green from August 2010 -April 2012 to evaluate the effects of Zn on annual bluegrass and bermudagrass. All experiments were conducted at the Auburn University Turfgrass Research Unit (TGRU) in Auburn, AL on a 6 year old Mini-Verde bermudagrass putting green. The putting green was maintained at a height of 5 mm and fertilized with 195 kg N ha⁻¹ annually (May-August). Soil type for the putting green was a USGA sand-peat mix (85:15 sand:peat ratio) with a pH of 5.1. Background P, K, Mg, and Ca were 36, 58, 41, and 353 kg ha⁻¹. Lime (CaCO₃) was applied at 1 metric ton ha⁻¹ in June of each year to reach a more desirable soil pH level (~6.0). The experiment was moved to a new location on the same putting green in the second year of the study.

Treatments were: Zn alone at 180 kg ha⁻¹, calcium (Ca) alone at 280 kg ha⁻¹, 180 kg Zn ha⁻¹ + 280 kg Ca ha⁻¹, and a control. Reagent grade zinc sulfate (ZnSO₄·7H₂O) was used as the zinc source, and gypsum (CaSO₄) was used as the Ca source. The Zn was split into four monthly granular applications of 45 kg Zn ha⁻¹ each. Initial Zn applications, as well as the gypsum application, were applied as

granular applications on August 2 of each year. Treatments were watered in (~5 mm) following application. Experimental units were 1.2 m × 2.4 m, arranged in a randomized complete block design with 3 replications.

Data included annual bluegrass cover (percent cover), clipping yield, Zn content in leaf tissue, and total soil Zn at varying depths. Annual bluegrass percent cover was evaluated during the months of February and April and was determined using the line-intersect method (Laycock and Canaway, 1980; Kershaw, 1973). To do this two line-intersect subsamples were taken from each plot using a 15 cm² grid which contained 25 intersections (2.5 cm spacing). Any intersection at which annual bluegrass occurred was counted. The total number of counted intersections was divided by the total number of intersections (25) to determine percent cover of annual bluegrass.

At the conclusion of each experiment (April 2010, 2011), clippings were harvested and placed in a forced-air oven at 70°C for 72 hours. Clipping dry weight was recorded, and the clippings were then dry ashed and analyzed for Zn content (Piper, 1942). Clippings were harvested after the bermudagrass had initiated spring green-up and therefore analyzed as a bermudagrass-annual bluegrass community. Soil samples (5 per plot, 2.5 cm diameter soil probe) were also collected at the conclusion of each experiment. Samples were collected at the following depths, with 0 representing the bottom of the thatch layer: thatch layer, 0-2.5 cm, 0-7.6 cm, and 7.6-15.2 cm. All samples were extracted for total Zn content using microwave assisted acid digestion (USEPA, 1994) and analyzed for Zn using standard ICP techniques.

Results and Discussion

Analysis of variance revealed that year was significant ($\alpha=0.05$) for percent annual bluegrass cover and clipping yield, but never significant for total soil Zn or Zn content in leaf tissue. Therefore, percent cover and clipping yield data were analyzed within each year, while total soil Zn and leaf tissue Zn data were pooled across years. The main effect of treatment was always significant for clipping yield, percent annual bluegrass cover, and Zn content in leaf tissue. There was a depth x treatment interaction for total soil Zn.

Line-intersect counts taken in February and April of each year are presented in Table 8 as percent annual bluegrass cover. In 2011-2012, applications of Zn reduced annual bluegrass cover below that measured in the untreated control plots, but in the 2010 trial February annual bluegrass populations were unaffected by application of Zn or Ca. In April 2011, plots that received only Ca had greater populations of annual bluegrass than in any other treatment. In 2012, the applications of Zn, with or without Ca, significantly reduced populations of annual bluegrass as compared to the untreated control. As in 2011 (April) the application of only Ca significantly increased annual bluegrass populations (Table 8). Applications of Ca also reduced the effectiveness of Zn, when compared to Zn applied alone. During the first year of the experiment, perennial biotypes of annual bluegrass (*Poa annua* var. *reptans* (Hauskn) Timm) were known to exist on the putting green where the experiment was being conducted. Therefore, following the completion of the 1st run (May 2011), foramsulfuron was sprayed ($0.02 \text{ kg ai ha}^{-1}$) in an attempt to rid the putting green of these perennial biotypes of annual bluegrass. This, coupled

with preliminary research demonstrating that Zn primarily affects annual bluegrass germination and not perennial plants, could explain the differences in control amongst years.

April clipping yield was also significantly affected by Zn applications (Table 9). Zinc applied at 180 kg Zn ha⁻¹ reduced clipping yield by as much as 50%, with a significant drop in the second year. This effect of Zn on reduced clipping yield occurred regardless of the presence of Ca. There was a trend for applications of Ca, without Zn, to increase clipping yield, when compared to the nontreated. The Ca soil-test for this green was low (350 kg ha⁻¹) and this may have been a response to the added Ca.

Applications of Zn also increased Zn levels in leaf tissue (data not shown). The Zn content in bermudagrass/annual bluegrass tissue receiving Ca (without Zn) and the nontreated plots averaged 170 mg kg⁻¹. Applying Zn at a rate of 180 kg ha⁻¹ significantly increased Zn content in leaf tissue to 420 mg kg⁻¹. Applications of Ca had no effect on Zn content in leaf tissue. Previous research has found that a Zn concentration of 221 mg kg⁻¹ in leaf tissue caused phytotoxicity in perennial ryegrass (Davis and Beckett, 1977). However, other research found that a Zn content of 1500 mg kg⁻¹ in leaf tissue had no negative effects on Penncross creeping bentgrass (Spear and Christians, 1991). Bermudagrass cultivars have also been found to vary in their tolerance to heavy metals such as Zn (Wu et al., 1981).

A treatment x soil depth interaction significantly affected total soil Zn (Table 10). Applications of Zn increased total soil Zn across all depths, when compared to the nontreated. The thatch layer contained significantly more Zn than any other soil

sampling depth across all treatments, except for the nontreated. Adding Zn at a rate of 180 kg ha⁻¹ increased total soil Zn in the thatch layer from ~24 mg kg⁻¹ (nontreated) to >76 mg kg⁻¹. For all treatments, total soil Zn was lowest at the 0-7.6 cm depth, averaging 60% less Zn than that found in the thatch layer. This is likely a dilution effect of sampling a greater soil volume. Studies have found that the average Zn content for most soils is 50 mg kg⁻¹, with a range from 10-300 mg kg⁻¹ (Kiekens, 1995). Research shows that divalent cation such as Zn are subject to strong adsorption when in the presence of organic matter (Shuman, 1975). This could explain total Zn being the highest in the thatch layer.

Conclusion

For a USGA soil-based putting green, as used in this study, applications of Zn can significantly reduce annual bluegrass populations. When applied at a rate of 180 kg ha⁻¹, Zn reduced annual bluegrass cover by 50%. In addition to population number, there was also a significant decrease in clipping yield. However, it is important to keep in mind that Zn applications could potentially reduce the growth of the desired bermudagrass turf. Some research indicates that bermudagrass cultivars vary in their tolerance to Zn (Wu et al., 1981). Therefore, further evaluations should be conducted to gain a better understanding of the tolerance of various turfgrasses to Zn applications. Additionally, research should also be conducted to evaluate the effects that pH, soil organic matter, and other nutrients have on the availability of Zn. Last, the long-term effects of Zn application on turfed soils will need study.

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Table 1. Analysis of variance for growth indices of various turfgrass species, Plant Science Research Center, Auburn, AL, 2011.

Source	Germination		Plant Height	Root Dry Weight	Shoot Dry Weight
	14 DAT	28 DAT			
P > F					
Creeping Bentgrass					
Run †	< 0.0001	0.0339	< 0.0001	< 0.0001	< 0.0001
pH ‡	0.0093	0.0005	< 0.0001	0.0043	0.0001
Zn Rate §	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pH × Zn Rate	0.4308	0.0589	0.0010	0.2232	0.4421
Perennial Ryegrass					
Run	0.0040	0.0476	< 0.0001	< 0.0001	0.2789
pH	0.0106	0.0157	< 0.0001	0.0016	0.0005
Zn Rate	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pH × Zn Rate	0.3472	0.2675	0.0168	0.2315	0.0079
Annual Bluegrass					
Run	0.0068	0.0010	0.0018	0.0814	< 0.0001
pH	0.3766	0.1896	< 0.0001	0.0002	< 0.0001
Zn Rate	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
pH × Zn Rate	0.5213	0.8532	0.0011	0.2474	0.7515
Roughstalk Bluegrass					
Run	0.5643	0.0255	0.7612	0.4532	< 0.0001
pH	0.0874	0.2825	< 0.0001	0.9120	0.2132
Zn Rate	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.6577
pH × Zn Rate	0.8658	0.7152	0.0044	0.9103	0.1705

† The experiment was repeated twice for each grass species.

‡ Three soil pH levels of 5.5, 6.0, and 6.5.

§ Five Zn rates of 0, 34, 68, 100, and 134 kg Zn ha⁻¹.

Table 2. Effect of soil pH on root dry weight of selected turfgrasses, Plant Science Research Center, Auburn, AL, 2011.

Soil pH	Dry Weight (g)			
	Creeping Bentgrass	Perennial Ryegrass	Annual Bluegrass	Roughstalk Bluegrass
	Run 1			
5.5	0.435	0.564	0.053	0.279
6.0	0.225	0.610	0.116	0.154
6.5	0.672	1.253	0.154	0.233
LSD	0.162	0.245	0.063	0.111
	Run 2			
5.5	0.023	0.061	0.101	0.129
6.0	0.058	0.210	0.145	0.259
6.5	0.048	0.187	0.158	0.207
LSD	0.035	0.133	0.022	0.073

Table 3. Effects of soil pH on shoot dry weight of selected turfgrasses, Plant Science Research Center, Auburn, AL, 2011.

Soil pH	Dry Weight			
	Creeping Bentgrass	Perennial Ryegrass	Annual Bluegrass	Roughstalk Bluegrass
	Run 1			
5.5	0.096	0.367	0.032	0.134
6.0	0.043	0.230	0.063	0.155
6.5	0.107	0.280	0.075	0.164
LSD	0.028	0.095	0.024	0.093
	Run 2			
5.5	0.012	0.160	0.108	0.048
6.0	0.020	0.253	0.145	0.085
6.5	0.028	0.613	0.176	0.095
LSD	0.009	0.174	0.042	0.015

Table 4. Interaction of Zn and soil pH on plant height (cm) for selected turfgrass species, Run 1[†], Plant Science Research Center, Auburn, 2011.

Zn Rate	Soil pH			Linear [‡]
	5.5	6.0	6.5	
————— Creeping Bentgrass —————				
0	11.6	7.3	10.6	0.4338
33	7.5	7.8	10.0	0.0020
67	7.0	5.0	8.9	0.0988
100	5.6	2.7	9.0	<0.0001
134	2.4	2.1	6.2	<0.0001
Linear	<0.0001	<0.0001	<0.0001	
Quadratic	0.7292	0.3886	0.1951	
————— Perennial Ryegrass —————				
0	14.3	10.8	13.1	0.1820
33	10.2	10.0	11.1	0.1099
67	9.2	7.6	9.4	0.8277
100	5.7	5.6	8.4	0.0026
134	3.5	3.9	3.9	0.5138
Linear	<0.0001	<0.0001	<0.0001	
Quadratic	0.6013	0.4691	0.0255	
————— Annual Bluegrass —————				
0	6.1	8.7	9.2	<0.0001
33	5.6	7.2	8.7	<0.0001
67	4.6	5.3	7.2	<0.0001
100	2.9	3.8	7.7	<0.0001
134	2.1	3.6	3.9	0.0020
Linear	<0.0001	<0.0001	<0.0001	
Quadratic	0.2394	0.0590	0.0186	
————— Roughstalk Bluegrass —————				
0	10.5	6.4	7.8	<0.0001
33	6.9	6.4	6.7	0.8549
67	5.8	5.1	6.3	0.3487
100	3.6	2.9	6.2	<0.0001
134	1.7	1.9	5.1	<0.0001
Linear	<0.0001	<0.0001	0.0003	
Quadratic	0.1009	0.0064	0.9153	

[†] Experiment was conducted twice for each grass species.

[‡] Significant if P ≤ 0.05.

Table 5. Interaction of Zn and soil pH on plant height (cm) of selected turfgrass species, Run 2[†], Plant Science Research Center, Auburn, 2011.

Zn Rate	Soil pH			Linear [‡]
	5.5	6.0	6.5	
————— Creeping Bentgrass —————				
0	6.3	7.5	9.6	0.0005
33	4.7	8.0	6.4	0.0771
67	4.0	5.6	6.6	0.0003
100	3.5	4.3	6.4	0.0002
134	3.3	3.7	5.0	0.0092
Linear	<0.0001	<0.0001	<0.0001	
Quadratic	0.0261	0.6557	0.1937	
————— Perennial Ryegrass —————				
0	10.1	11.5	13.0	0.0007
33	9.3	11.3	13.8	<0.0001
67	8.2	11.4	11.9	<0.0001
100	7.3	9.1	11.1	<0.0001
134	4.7	8.2	8.1	0.0004
Linear	<0.0001	<0.0001	<0.0001	
Quadratic	0.0811	0.0885	0.0070	
————— Annual Bluegrass —————				
0	6.6	8.2	7.5	0.0887
33	6.8	8.1	7.7	0.1152
67	6.0	6.2	6.5	0.2509
100	4.5	5.3	6.4	0.0002
134	3.5	4.4	5.8	0.0003
Linear	<0.0001	<0.0001	0.0004	
Quadratic	0.0119	0.5873	0.7847	
————— Roughstalk Bluegrass —————				
0	5.6	7.7	7.9	<0.0001
33	5.9	6.8	7.6	0.0028
67	5.3	6.7	5.6	0.6770
100	4.3	4.7	5.5	0.0288
134	3.7	3.8	3.8	0.9707
Linear	<0.0001	<0.0001	<0.0001	
Quadratic	0.0458	0.1729	0.5631	

[†] Experiment was conducted twice for each grass species.

[‡] Significant if P ≤ 0.05.

Table 6. Annual bluegrass control (visual ratings) on a bermudagrass putting green[†] following treatment applications in September, 2009-2012, Auburn University Turfgrass Research Unit, Auburn, AL. Months are averaged over each year.

Treatment	Annual Bluegrass Control (%) [‡]		
	December	February	April
20 kg Zn ha ⁻¹	14 e	37 e	21 e
40 kg Zn ha ⁻¹	18 de	31 ed	35 de
80 kg Zn ha ⁻¹	55 c	51 c	51 c
160 kg Zn ha ⁻¹	76 b	70 b	73 b
1 app fenarimol	33 d	50 cde	40 cd
2 app fenarimol	18 de	40 de	41 cd
fenarimol + 20 kg Zn ha ⁻¹	28 de	50 cd	43 cd
fenarimol + 80 kg Zn ha ⁻¹	59 c	59 bc	53 c
prodiamine + trifloxysulfuron [§]	99 a	99 a	99 a
LSD [¶]	16	18	15

[†] Study conducted on a Tifdwarf putting green in 2009. Green was renovated and study was conducted on a TifEagle putting green in 2010 and 2011.

[‡] % control is relative to the nontreated plot.

[§] A prodiamine + trifloxysulfuron combination is commonly used in bermudagrass turf for preemergence annual bluegrass control. However, it is not labeled for use on putting greens

[¶] Means followed by common letters are not significantly different at the $\alpha=0.05$ level according to Fishers Protected LSD.

Table 7. Plant density counts and annual bluegrass control ratings on a bermudagrass putting green[†] following treatment applications in September, 2009-2012, Auburn University Turfgrass Research Unit. Months are averaged over each year.

Treatment	Counts		Control (%) [‡]	
	Feb	Apr	Feb	Apr
	— Plants per Plot —			
20 kg Zn ha ⁻¹	153 a	116 a	28 c	22 e
40 kg Zn ha ⁻¹	128 ab	99 ab	38 bc	37 d
80 kg Zn ha ⁻¹	118 ab	66 cd	44 bc	58 bc
160 kg Zn ha ⁻¹	86 b	44 d	59 b	73 b
1 app fenarimol	133 ab	80 bc	37 bc	46 cd
2 app fenarimol	151 a	84 bc	36 bc	46 cd
fenarimol + 20 kg Zn ha ⁻¹	128 ab	81 bc	33 c	45 cd
fenarimol + 80 kg Zn ha ⁻¹	86 b	57 cd	50 bc	58 bc
prodiamine + trifloxysulfuron [§]	1 c	3 e	99 a	98 a
LSD[¶]	53	28	23	15

[†] Study conducted on a Tifdwarf putting green in 2009. Green was renovated and study was conducted on a TifEagle putting green in 2010 and 2011.

[‡] % control was determined by dividing the number of plants in each plot by the number of plants in the nontreated plot.

[§] A prodiamine + trifloxysulfuron combination is commonly used in bermudagrass turf for preemergence annual bluegrass control. However, it is not labeled for use on putting greens

[¶] Means followed by common letters are not significantly different at the $\alpha=0.05$ level according to Fishers Protected LSD.

Table 8. Annual bluegrass percent cover on a nonoverseeded Mini-Verde (bermudagrass) putting green, 2010-2012, Auburn University Turfgrass Research Unit, Auburn AL.

Treatment	Cover (%) [†]	
	February	April
	————— 2011 —————	
Nontreated	35 a	25 b
180 kg Zn ha ⁻¹ ‡	34 a	23 b
280 kg Ca ha ⁻¹	37 a	39 a
180 kg Zn ha ⁻¹ + 280 kg Ca ha ⁻¹	29 a	24 b
LSD [§]	9	11
	————— 2012 —————	
Nontreated	65 b	24 a
180 kg Zn ha ⁻¹	33 c	7 b
280 kg Ca ha ⁻¹	79 a	21 a
180 kg Zn ha ⁻¹ + 280 kg Ca ha ⁻¹	40 c	8 b
LSD	11	5

[†]Percent cover was determined by line intersect counts.

[‡]Zn rate was split into four, monthly applications of 45 kg Zn ha⁻¹.

[§]Means separated using Fishers protected LSD. Means followed by the same letter are not significantly different ($\alpha=0.05$).

Table 9. Effect of treatments on dry weight of clippings in a bermudagrass – annual bluegrass community, Auburn University Turfgrass Research Unit, 2010-2012, Auburn, AL.

Treatment	Experimental Run	
	April 2011	April 2012
	— clipping weight (g) —	
Nontreated	1.01 ab	1.54 a
180 kg Zn ha ⁻¹ †	0.69 bc	0.82 b
280 kg Ca ha ⁻¹	1.16 a	1.61 a
180 kg Zn ha ⁻¹ + 280 kg Ca ha ⁻¹	0.66 c	0.66 b
LSD‡	0.33	0.41

†Zn rate was split into four, monthly applications of 45 kg Zn ha⁻¹.

‡Means separated using Fishers protected LSD. Means followed by the same letter are not significantly different ($\alpha=0.05$).

Table 10. Interaction of soil depth and treatment on total soil Zn content (g kg^{-1}) following treatment applications on a Mini-Verde (bermudagrass) putting green, Auburn University Turfgrass Research Unit, 2010-2012, Auburn AL.† Samples were collected in April 2011 and April 2012. Data shown is averaged over years.

Depth (cm)	Treatment				LSD§
	Nontreated	180 kg Zn ha ⁻¹ ‡	280 kg Ca ha ⁻¹	180 kg Zn ha ⁻¹ + 280 kg Ca ha ⁻¹	
	Zn (g/kg)				
Thatch	28.4 Ab	76.6 Aa	23.8 Ab	93.4 Aa	0.14
0-2.5	34.2 Aa	43.2 Ba	11.8 Ba	28.8 Ba	38.6
0-7.6	8.8 Ab	20.4 Ba	6.2 Bb	17 Ba	7.53
7.6-15.2	11.2 Ab	32.2 Ba	12.6 Bb	25.8 Ba	9.74
LSD	34.5	25.5	10.7	22.8	

† Study was conducted on a separate area of the putting green in the second year.

‡ Four monthly applications were applied at a rate of 45 kg Zn ha⁻¹.

§ Means separated using Fishers Protected LSD. Means followed by the same letter are not significantly different ($\alpha=0.05$). Capital letters indicate separation by depth (down columns). Lower case letters indicate separation by treatment (across rows).

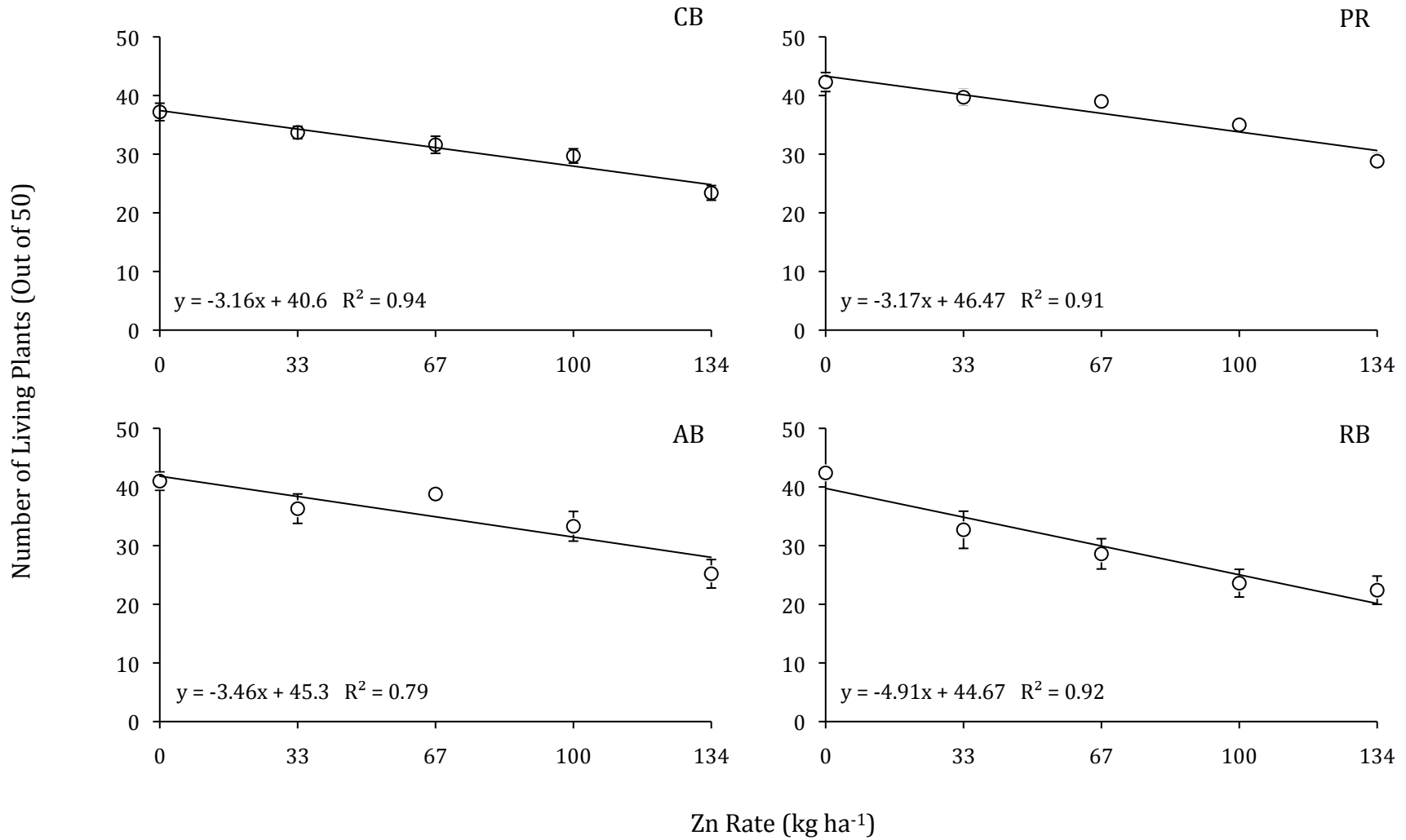


Figure 1. Effect of Zn rate on seedling germination 28 days after seeding, Run 1, Plant Science Research Center, Auburn, AL, 2011. CB: Creeping Bentgrass, PR: Perennial Ryegrass, AB: Annual Bluegrass, RB: Roughstalk Bluegrass.

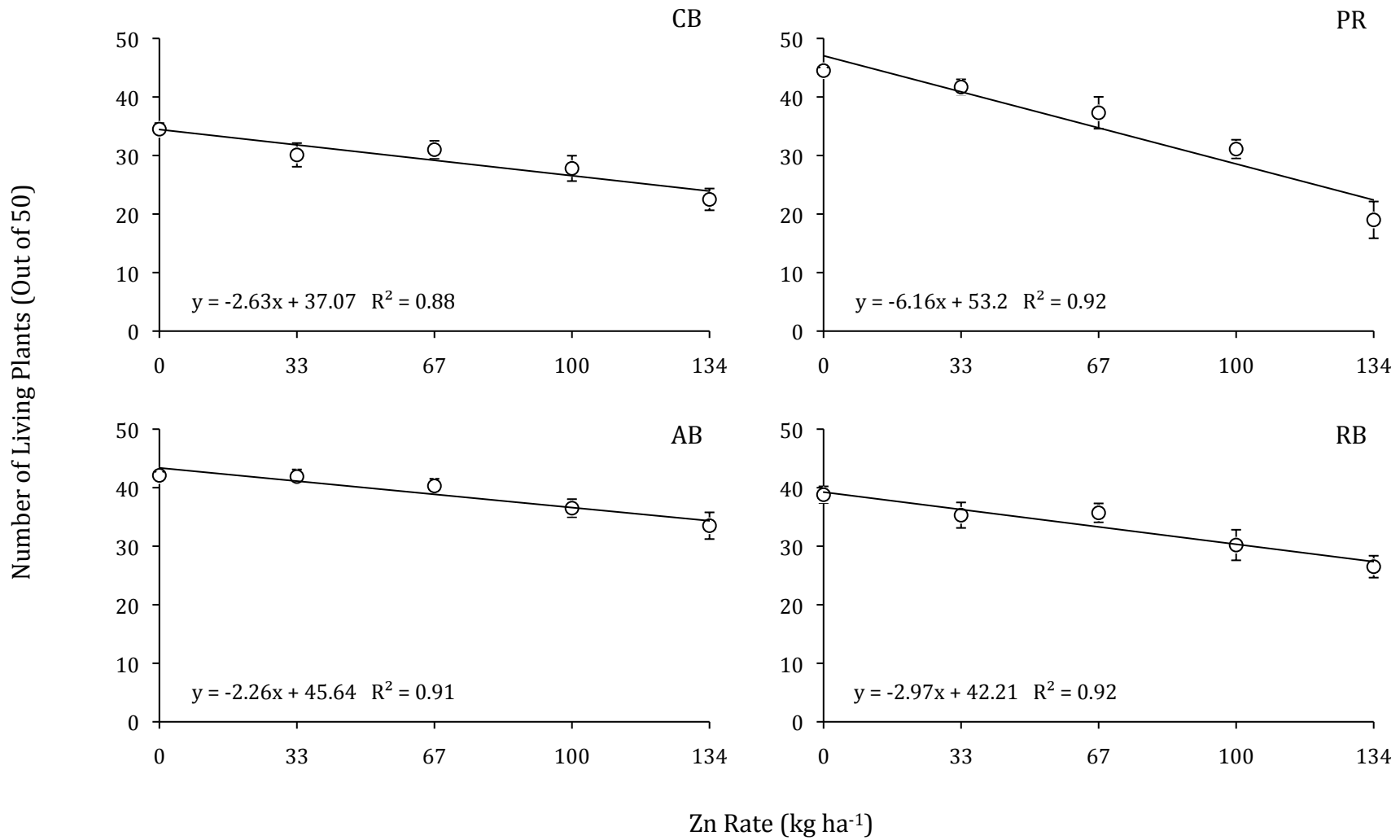


Figure 2. Effect of Zn rate on seedling germination 28 days after seeding, Run 2, Plant Science Research Center, Auburn, AL, 2011. CB: Creeping Bentgrass, PR: Perennial Ryegrass, AB: Annual Bluegrass, RB: Roughstalk Bluegrass.

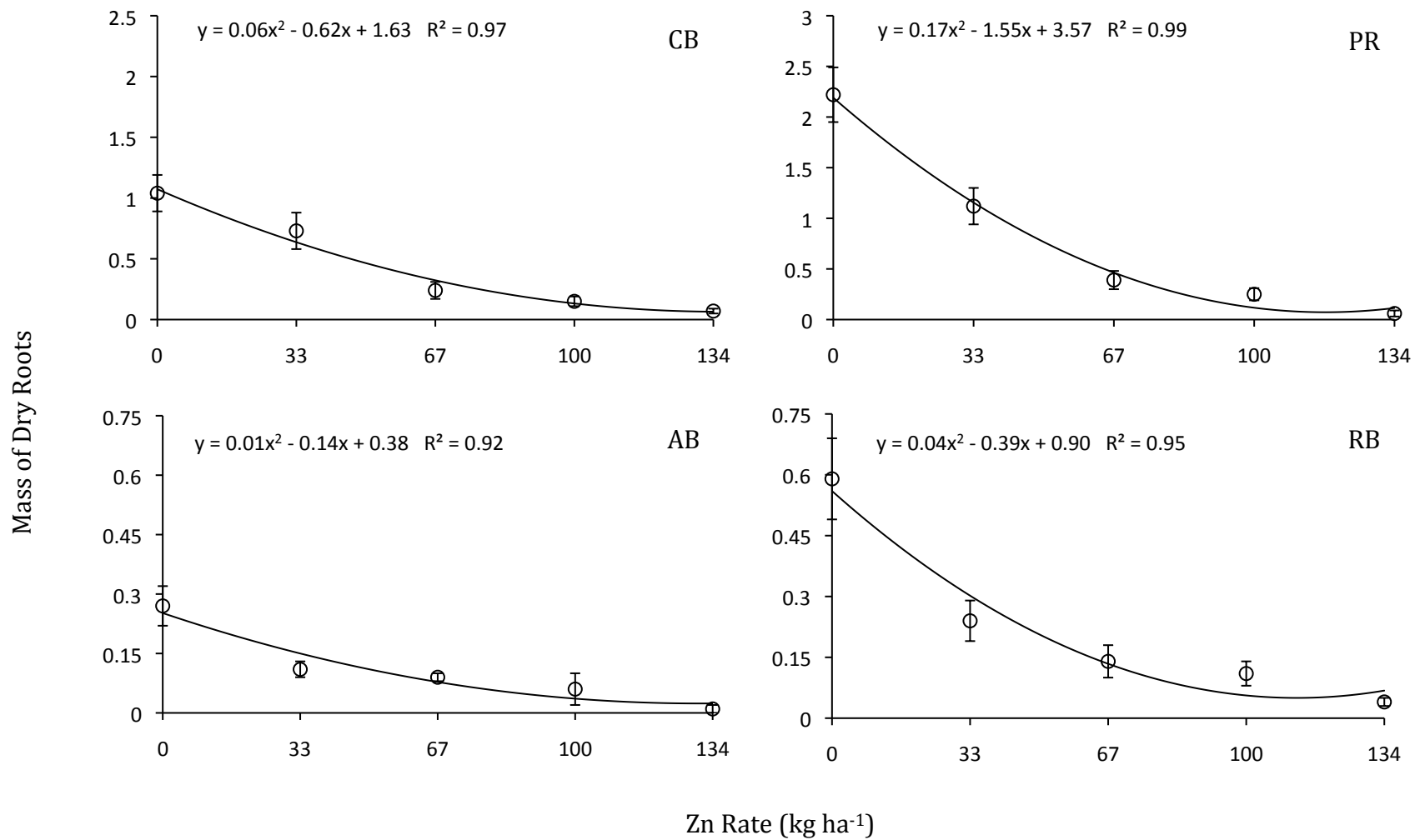


Figure 3. Effect of Zn rate on dry weight of harvested turfgrass roots, Run 1, Plant Science Research Center, Auburn, AL, 2011. CB: Creeping Bentgrass, PR: Perennial Ryegrass, AB: Annual Bluegrass, RB: Roughstalk Bluegrass. Note that scale of y-axis for perennial ryegrass and creeping bentgrass differs from others.

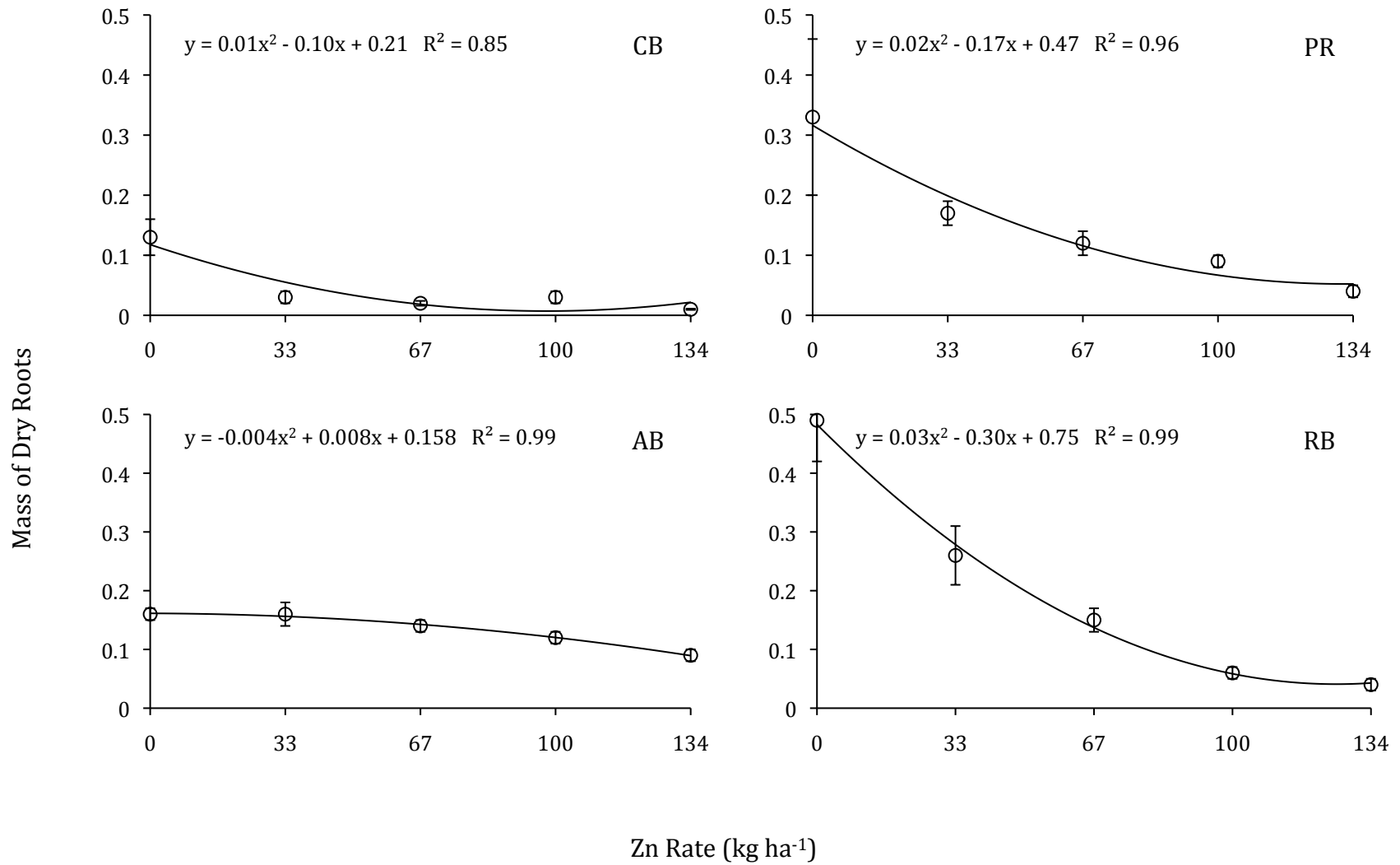


Figure 4. Effect of Zn rate on dry weight of harvested turfgrass roots, Run 2, Plant Science Research Center, Auburn, AL, 2011. CB: Creeping Bentgrass, PR: Perennial Ryegrass, AB: Annual Bluegrass, RB: Roughstalk Bluegrass.

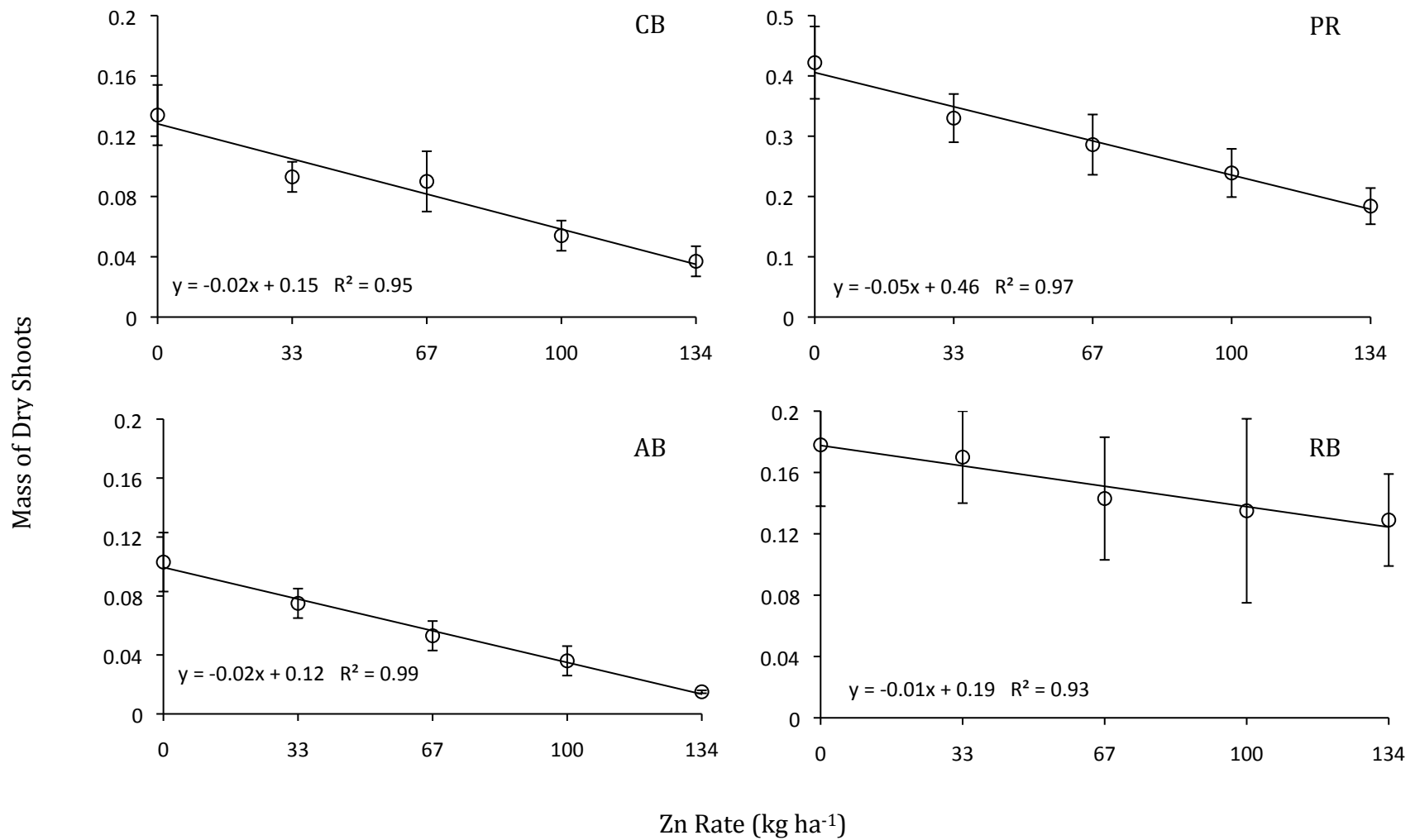


Figure 5. Effect of Zn rate on dry weight of harvested turfgrass shoots, Run 1, Plant Science Research Center, Auburn, AL, 2011. CB: Creeping Bentgrass, PR: Perennial Ryegrass, AB: Annual Bluegrass, RB: Roughstalk Bluegrass. Note that scale of y-axis for perennial ryegrass differs from others.

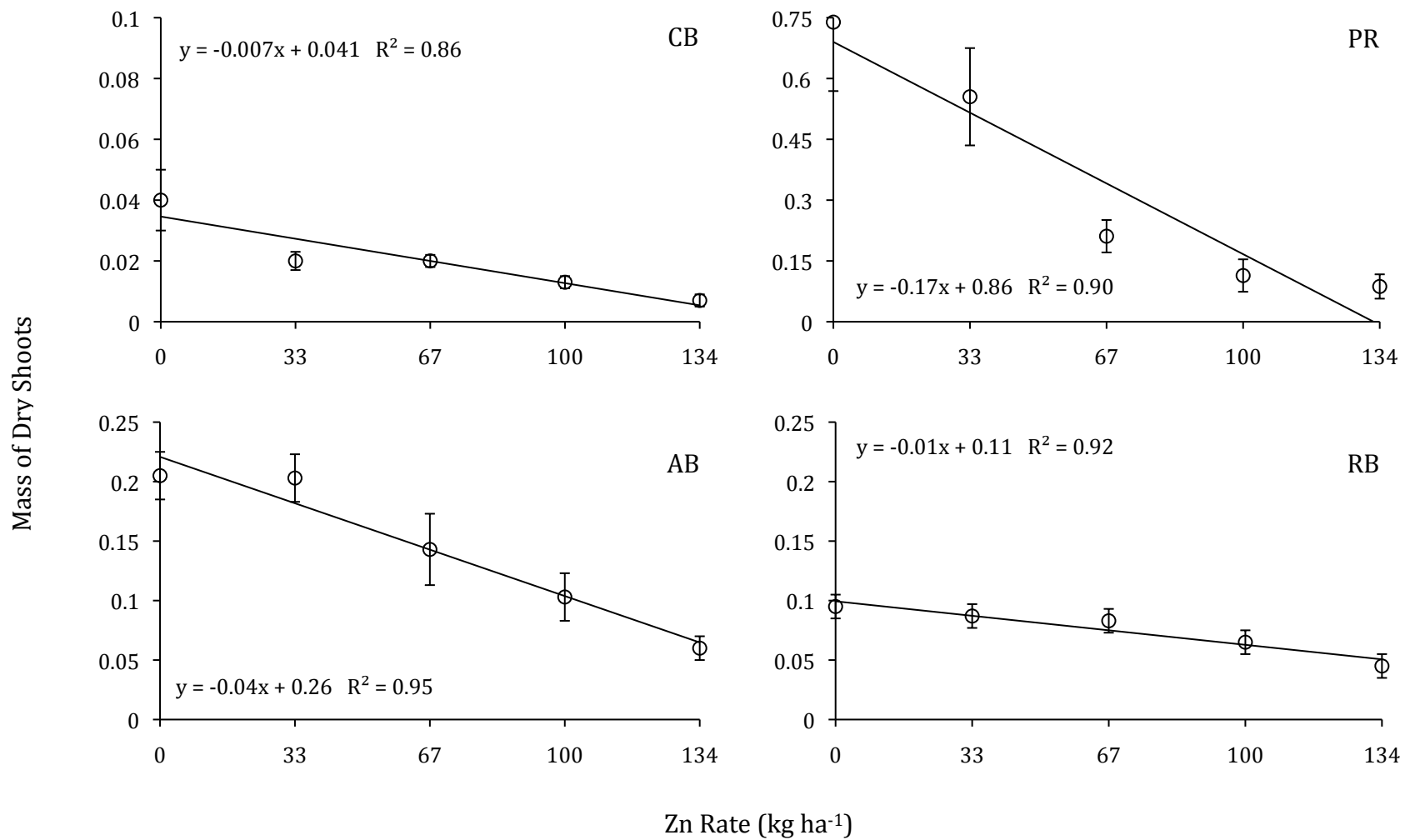


Figure 6. Effect of Zn rate on dry weight of harvested turfgrass shoots, Run 2, Plant Science Research Center, Auburn, AL, 2011. CB: Creeping Bentgrass, PR: Perennial Ryegrass, AB: Annual Bluegrass, RB: Roughstalk Bluegrass. Note that scale of y-axis for perennial ryegrass and creeping bentgrass differs from others.

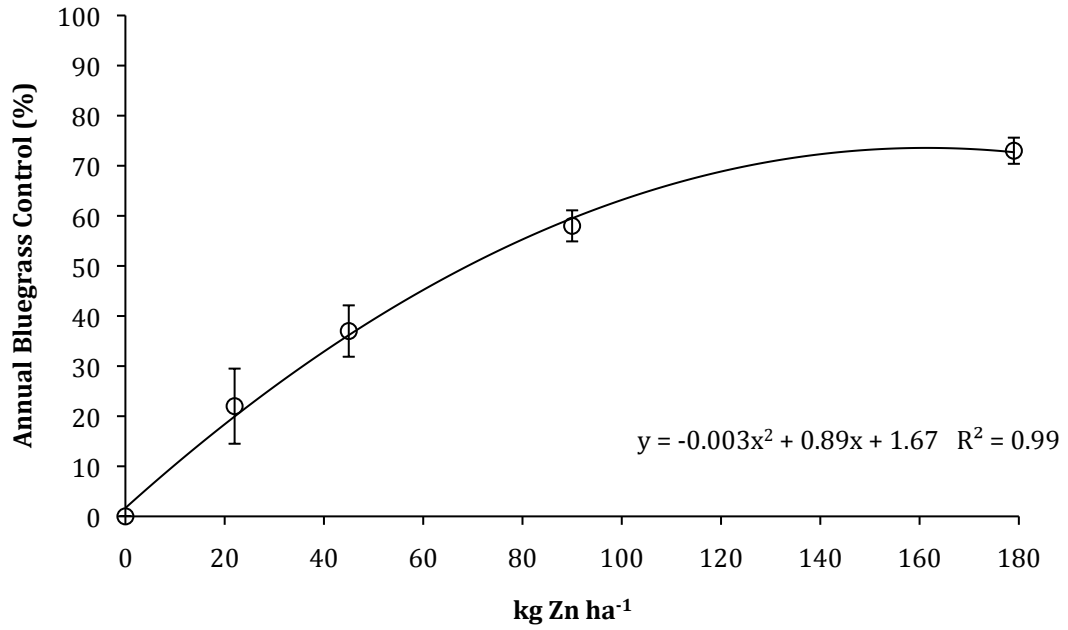


Figure 7. Effect of Zn applications on annual bluegrass control in April, averaged across three experimental runs (2009-2012), Auburn University Turfgrass Research Unit, Auburn, Al.

VI. Appendix

Table 11. Herbicides used for control of annual bluegrass in non-overseeded hybrid bermudagrass.

Common Name	Trade Name	Application Rate (kg a.i. ha ⁻¹)	Manufacturer	Address
Preemergence				
benefin [†]	Balan 2.5G	3.36	Dow AgroSciences LLC	Indianapolis, IN 46268
DCPA	Dacthal Flowable	11.8 – 16.8	Amvac Chemical Corporation	Los Angeles, CA 90023
dithiopyr	Dimension EC	0.28 – 0.56	Dow AgroSciences LLC	Indianapolis, IN 46268
metribuzin [†]	Sencor 75%	0.56	Bayer Environmental Science	Research Triangle Park, NC 27709
oxadiazon [†]	Ronstar G	2.24 – 3.36	Bayer Environmental Science	Research Triangle Park, NC 27709
pendimethalin [†]	Pendulum 3.3 EC	1.65 – 3.35	BASF Corporation	Florham Park, NJ 07932
prodiamine [†]	Barricade 4FL	0.73 – 1.67	Syngenta Crop Protection, Inc.	Greensboro, NC 27419
pronamide	Kerb 50WP	0.56 – 1.68	Dow AgroSciences LLC	Indianapolis, IN 46268
Postemergence				
atrazine ^{†, ‡}	AAtrex 4L	1.2 – 2.24	Syngenta Crop Protection, Inc.	Greensboro, NC 27419
flazasulfuron [†]	Katana	0.025 – 0.1	PBI/Gordon Corp.	Kansas City, MO 64101
foramsulfuron	Revolver	0.014 – 0.029	Bayer Environmental Science	Research Triangle Park, NC 27709
glyphosate ^{†, ‡}	Roundup Pro	0.24 – 2.12	Monsanto Company	St. Louis, MO 63167
paraquat ^{‡, §}	Gramoxone	0.6	Syngenta Crop Protection, Inc.	Greensboro, NC 27419
pronamide	Kerb 50WP	1.12 – 2.24	Dow AgroSciences LLC	Indianapolis, IN 46268
rimsulfuron	TranXit	0.02 – 0.035	DuPont	Wilmington, DE 19898
simazine ^{†, ‡}	Princep Liquid	1.12 – 2.24	Syngenta Crop Protection, Inc.	Greensboro, NC 27419
sulfosulfuron	Certainty	0.065 – 0.105	Monsanto Company	St. Louis, MO 63167
trifloxysulfuron	Monument 75WG	0.017 – 0.029	Syngenta Crop Protection, Inc.	Greensboro, NC 27419

[†] Not labeled for putting greens.

[‡] Apply to dormant bermudagrass only.

[§] Not currently labeled for turfgrass.

Table 12. Herbicides used for control of annual bluegrass in overseeded hybrid bermudagrass.

Common Name	Trade Name	Application Rate (kg a.i. ha ⁻¹)	Manufacturer	Address
Prior to Overseeding				
benefin [†]	Balan 2.5G	3.36	Dow AgroSciences LLC	Indianapolis, IN 46268
bensulide	Bensumec 4 LF	14	PBI/Gordon Corp.	Kansas City, MO 64101
dithiopyr	Dimension EC	0.28 – 0.56	Dow AgroSciences LLC	Indianapolis, IN 46268
fenarimol	Rubigan A.S.	4.57	Gowan Company	Yuma, AZ 85366
foramsulfuron	Revolver	0.014 – 0.029	Bayer Environmental Science	Research Triangle Park, NC 27709
oxadiazon [†]	Ronstar G	2.24 – 3.36	Bayer Environmental Science	Research Triangle Park, NC 27709
prodiamine [†]	Barricade 4FL	0.73 – 1.67	Syngenta Crop Protection, Inc.	Greensboro, NC 27419
rimsulfuron	TranXit	0.02 – 0.035	DuPont	Wilmington, DE 19898
trifloxysulfuron	Monument 75WG	0.017 – 0.029	Syngenta Crop Protection, Inc.	Greensboro, NC 27419
After Overseeding				
bispyribac-sodium [†]	Velocity SG	0.07	Valent USA Corporation	Walnut Creek, CA 94596
ethofumesate [†]	Prograss	0.84 – 2.1	Bayer Environmental Science	Research Triangle Park, NC 27709

[†] Not labeled for putting greens