MANAGEMENT OF ANNUAL BLUEGRASS (*Poa annua* L.) USING POST-EMERGENCE HERBICIDES AND PLANT GROWTH REGULATORS

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CHAPTER 1

Literature Review

Introduction

Annual bluegrass (*Poa annua* L.) is recognized as a troublesome weed in turfgrass communities. It can exist in many turfgrass stands including home lawns, sports fields, and golf courses. Although annual bluegrass is seldom intentionally planted (Beard 1970), it can outcompete desired turfgrass species and disrupt the aesthetic and playability of the turf (Cox et al. 2003). It is a troublesome weed due to its ability to adapt to a wide range of mowing heights. Even on a golf course putting green, annual bluegrass can survive and reproduce at mowing heights as low as one tenth of an inch.

Playability of a golf course is the smoothness of ball roll over the turfgrass surface. Playability is often the direct result of turfgrass quality, the higher quality, the better playability. Factors that influence turf quality are: density, texture, uniformity, color, growth habit, and smoothness. Density is a measure of the number of aerial shoots per unit area. Texture is a measure of the width of the leaf blades. Uniformity is an estimate of the even appearance of a turf. Color is a measure of the light reflected by turfgrass, which varies with species and cultivars. Growth habit describes the type of shoot growth evident in a particular turfgrass; three basic types are bunch type, rhizomatous, and stoloniferous. Smoothness is a surface feature of turf that affects visual quality and playability.

In the transition zone, creeping bentgrass (*Agrostis stolonifera* L.) turf creates the best quality putting greens. The transition zone is an area that lies between the cool region and warm region of the United States. When annual bluegrass encroaches on creeping bentgrass putting greens, turf quality is drastically reduced. Annual bluegrass is undesirable on creeping bentgrass putting greens, because of its bunch-type growth habit, lighter green color, prolific seedhead production, poor disease, drought, and wear tolerances that creates unsightly patches (Beard et al. 1978, Johnson and White 1997, 1998, Lush 1989, Martin and Wehner 1987). Diseases that attack annual bluegrass include anthracnose (*Colletotrichum graminicola*), brown patch (*Rhizoctonia solani*), dollar spot (*Sclerotinia homoeocarpa*), pink snow mold (*Microdochium nivale*), pythium blight (*Pythium aphanidermatum*), red thread (*Laetisaria fuciformis*), and gray snow mold (*Typhual incarnate*) (Beard et al. 1978). Heat stress begins as temperatures reach 80 F and above, causing reduction in root growth and even death. Cold temperature stress is also a concern with annual bluegrass, which is more susceptible to death compared to creeping bentgrass (Beard et al. 1978). Spring green up is also another disadvantage of having an annual bluegrass infestation on a putting green. Annual bluegrass initiates growth at about 55 F, while creeping bentgrass starts growth at lower temperatures (Beard 1978). To avoid the patchy appearances, superintendents are forced to apply more water and fungicides, especially in summer months, to annual bluegrass infested putting greens to maintain acceptable quality (McCullough and Hart 2010).

Expectations for weed control on golf courses are very high; therefore herbicides are often applied for control. Unfortunately, there are very few herbicides labeled for annual bluegrass control on putting greens, especially for post emergent control. Bensulide and bensulide plus oxadiazon are pre mergence herbicides currently registered for creeping bentgrass golf greens (McCarty 1999). Plant growth regulators (PGRs) are being used for differentially suppressing the growth of annual bluegrass in mixed stands of creeping bentgrass. While PGRs do show some promise for control, they do not completely eliminate annual bluegrass. New chemistries, such as bispyribac-sodium, amicarbazone, and methiozolin, have recently been developed that show promise for annual bluegrass control in creeping bentgrass turf (McCullough and Hart 2010, McCullough et al. 2010). Bispyribac-sodium is currently only labeled for creeping bentgrass maintained at fairway height of cut (12 mm) and not for use on putting green height (≤3 mm). Amicarbazone and methiozolin have yet to be registered.

The most effective post emergence control of annual bluegrass in creeping bentgrass putting greens at this time is achieved after applications of paclobutrazol (Bell et al. 2004, Johnson and Murphy 1995, Woosely et al. 2004, McCullough et al. 2005). Paclobutrazol is a PGR that interrupts the gibberellic acid (GA) synthesis (Rademacher 2000). This results in reduced cell elongation, therefore reduces shoot growth when applied on turfgrass plants. Paclobutrazol tends to affect annual bluegrass for a longer period of time compared to other GA inhibitors, such as trinexapac-ethyl, resulting in a competitive growth advantage to creeping bentgrass (Isgrigg et al. 1998, Johnson and Murphy. 1995, Neylan et al. 1997). While paclobutrazol applications show promise for selective annual bluegrass suppression, risk of turf injury is a concern (Johnson and Murphy 1995).

With few options for annual bluegrass control, herbicide resistance will become a concern once a new herbicide is introduced. There are currently 234 herbicide resistant weed species (138 dicots and 96 monocots) in the world (Heap 2014). Annual bluegrass has already shown resistance to triazine herbicides (Hutto et al. 2004, Kelly et al. 1999, Yelverton and Isgrigg 1998), and most recently glyphosate (Binkholder, 2010, Brosnan et al. 2012). It has been found that the mechanism of triazine resistance in annual bluegrass was due to an altered site of action, where a single nucleotide change occurred in the *psbA* gene(Kelly et al.1999). By the continual use of triazine herbicides and also glyphosate on golf courses, selection for resistance in annual bluegrass will be a major concern. Resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (WSSA. 2010).

***Poa* Biology**

The *Poa* genus comprises over 500 species that are widely distributed throughout the cool, humid climatic regions of the world (Hurley 2003, Christians 2007, Turgeon 2008). The growth habit of the *Poa* genus ranges from an erect bunch type, prostrate stoloniferous type, to an extremely dense sod-forming type which produces vigorous rhizome system (Hitchcock 1950). All bluegrasses (*Poa*) have two structural characteristics in common that can be useful clues to identification. They all have boat-shaped leaf tips and folded vernation; beyond that, their size and appearance varies widely (Christians 2007). Of the 500 species, there are only eight used primarily as a turfgrass, and three that are widely used. Kentucky bluegrass (*Poa pratensis* L.) is the most widely used cool-season turfgrass in the United States (Christians 2007). This species is planted for golf courses, athletic fields, home lawns and pastures. Rough bluegrass (*Poa trivialis* L.) is also becoming more widely accepted and even propagated as a turfgrass, mostly due to its ability to adapt to wet, shaded conditions (Liskey 1999, Christians 2007, Hurley 2003).

The third most common *Poa* species is annual bluegrass. Annual bluegrass is native to Europe and has spread as a contaminant in seed mixtures (Zontek 1973). It can be found in all climate zones in the United States, and nearly everywhere in the world where turfgrass is grown (Christians 2007). Annual bluegrass is generally considered to be a weed and is seldom intentionally planted, however under frequent irrigation, close cut, and high fertility conditions common on golf course fairways, tees and greens, annual bluegrass will tend to invade, persist and become the dominant species (Beard 1970). Under highly managed turfgrass conditions, annual bluegrass is capable of forming a dense, uniform turf of intermediate leaf texture (Beard 1973, Piper 1927, Beard et al. 1978). Some of the highest quality putting surfaces in the United States consists of annual bluegrass. Annual bluegrass can be identified using three major characteristics: boat-shaped leaf tips, folded vernation, and wrinkled leaves. Vernation is a characteristic of a grass plant that describes how a new leaf blade emerges as growth occurs. In turfgrass, this is either rolled or folded. These three characteristics alone allow differentiation between annual bluegrass and creeping bentgrass (Christians 2007). Poa plants may be bunch-type or weakly stoloniferous, with a prominent “train-track” midrib on the adaxial leaf surface. The ligule is 0.8 to 3mm long acute, auricles absent, broad-divided collar. Panicle-shaped inflorescences generally are apparent throughout most of the growing season, but particularly abundant in the spring (Huff 2003). The convex surface of the lemma is five-nerved and pubescent along the lower portions of the nerves, and completely dominates the abaxial side of the floret (Vargas and Turgeon 2004). Annual bluegrass is a winter annual, which germinates in late summer, fall and winter, and matures in spring or early summer (Ross and Lembi 1999). This cycle is one of the reasons that annual bluegrass is so competitive with other turf species. In the late summer when cool-season grasses are weak, annual bluegrass can often fill in the voids that were left due to the summer stress. Similarly, when warm-season grasses are dormant in the fall, annual bluegrass often emerges in open spaces, where seed has previously been dispersed.

Annual bluegrass has the unique ability to survive as both a true annual or as a perennial. Tutin (1957) observed considerable morphological variability within annual bluegrass, including upright-growing types with light green leaves and darker low-growing types that root at the nodes of decumbent shoots. In examining plants obtained from various locations in the United States and abroad, Hovin (1957) discovered that compared with the upright-growing, relatively short-lived types of annual bluegrass, which he classified as annuals, the spreading, stoloniferous types, classified as perennials; produce more tillers per plant, contributing to the formation of denser turfs. Youngner (1959) also recognized the two general types of annual bluegrass, adding that perennials are likely to be found in greens, often forming distinct patches, while annuals are typically found in open fields and meadows. Currently, the annual biotype of annual bluegrass is referred to as *Poa annua* L. var. *annua* Timm, or simply as *Poa annua* L., while the perennial biotype was designated *Poa annua* L. f. *reptans* (Hauskins) T. Koyama (GRIN 1996).

Creeping Bentgrass (*Agrostis stolonifera* L.)

Creeping bentgrass is a cool-season grass best known for its fine texture and adaptation to mowing heights as low as 3 mm (Warnke 2003). It is the most widely used cool-season grass for golf and bowing greens, and is primarily adapted to cool, humid regions. Creeping bentgrass tolerates a wide range of soil types, but is best adapted to fertile, fine-textured soils of moderate acidity and good water holding capacity (Beard

1973). This species is highly stoloniferous, with rolled vernation and pointed leaf tips, and prominent, membranous ligules and prominent veins on the upper side of its leaves (Warnke 2003, Christians 2007, Turgeon 2002). At low mowing heights, creeping bentgrass is susceptible to environmental stresses that can leave voids and opportunity for weeds (Beard 2003).

Annual Bluegrass Control

Annual bluegrass populations are best controlled by maximizing the turfgrass species employed for greens, tees, or fairways to its invasion and subsequent growth (Vargas and Turgeon 2004). Annual bluegrass growing in the transition zone is thought of and treated as a weed. In the transition zone, winter air temperatures make it difficult to maintain perennial stands of many warm-season grasses; however air temperatures are sufficient in summer to cause heat stress to many cool-season grasses (Christians 2007). Most cool-season and warm season grasses are not well adapted to the transition zone. The perennial type of annual bluegrass is the most troublesome weed in golf turf in the transition zone (Woosley et al. 2002). There are a number of cultural practices proven to reduce the incidence of annual bluegrass in putting greens. These include manual removal, adjusting irrigation and fertility, mowing practices, cultivation, and also seeding of desired turfgrasses (Vargas and Turgeon 2004). One of the earliest reports for control of annual bluegrass was in 1922, prior to development of selective herbicides. The article published in the Bulletin of the Green Section of the USGA (Alexander 1922) recommended mechanical control by removing annual bluegrass with a knife. Over a 10 year period of mechanic removal, annual bluegrass was eradicated from the putting greens. Zontek (1973) reported hand techniques including a knife and cup cutter, which are still used today.

The first step in a total annual bluegrass management program is to shift fertility practices to those that favor creeping bentgrass growth. A high available nitrogen supply encourages profuse annual bluegrass germination, growth and tillering. Research indicated that lower annual nitrogen rates decrease the competition of annual bluegrass to creeping bentgrass (McCarty 1999). Phosphorus is another nutrient that increases the ability of annual bluegrass to persist. Annual bluegrass prefers high phosphorus levels and will out-compete creeping bentgrass under these conditions (McCarty 1999).

Managing irrigation to favor creeping bentgrass will decrease the competition from annual bluegrass. Annual bluegrass is more tolerant of persistently wet soils (Vargas and Turgeon 2004); therefore deep and infrequent irrigation will discourage the growth of annual bluegrass. Since annual bluegrass seeds can germinate throughout the growing season, frequent irrigation is undesirable. Musser (1962) cautioned that water should be applied sparingly in early fall when annual bluegrass seed is germinating.

Annual bluegrass thrives under wet and compacted soil conditions, due to its shallow root system and tolerance to low soil-oxygen levels (McCarty 1999). The practice of cultivation is recommended to alleviate these problems. Aerification is most commonly used as the means for cultivation. Cultivation in the turf industry refers to a variety of mechanical processes that are used to loosen the soil and reduce compaction, control thatch, or to groom the surface (Christians 2007). Aerification allows for water and oxygen to move deeper into the soil profile where the desired turfgrass is growing (Beard et al. 1978, Turgeon, A. J. 2002, Christians, N. E. 2007).

Plant Growth Regulators

Plant growth regulators are chemicals that can reduce the growth of plants (Christians 2007). As used on turfgrass, PGRs are divided into five major classes. Class-A and B (Formerly Type-II) PGRs suppress plant growth by inhibiting gibberellin biosynthesis and thus causing reductions in cell elongation and associated expansive growth of plants. Class-A PGRs (trinexapac-ethyl) inhibit gibberellin production late in the biosynthetic pathway while class-B PGRs (paclobutrazol and flurprimidol) inhibitory effects occur earlier in the pathway. One of the most promising uses of class-B PGRs is for suppressing annual bluegrass in mixed stands with creeping bentgrass (Turgeon 2002). Class-C (Formerly Type-I) PGRs inhibit or suppress the growth of plants by causing a cessation of cell division and differentiation in meristematic regions of the plant (Turgeon 2002). Class-C PGRs consist of maleic hydrozide and mefluidide and are used to suppress seedheads. Class-D PGRs are considered herbicides and include glyphosate and ethofumesate. At low rates class-D PGRs will suppress plant growth and allow different species to be established. Class-E PGRs include phytohormones as gibberellic acid for enhancing fall color retention and auxin formulated with GA for stimulating turfgrass growth (Turgeon 2002). Flurprimidol, paclobutrazol, and trinexapac-ethyl have all shown abilities to reduce annual bluegrass in mixed stands with creeping bentgrass (Johnson and Murphy 1995, Biaelow et al. 2007). Isgrigg (1998) reported that applying paclobutrazol reduced annual bluegrass biomass 40%, compared to a 30% reduction of creeping bentgrass. Photosynthetic rates were also reduced for 5 weeks after treatment to annual bluegrass, and 3 weeks to creeping bentgrass. Johnson and Murphy (1995) reported that greatest suppression of annual bluegrass in creeping bentgrass putting greens was achieved following two spring and two fall applications of paclobutrazol. Over a two year period, 4 applications totaling 1.8 kg ha^{-1} reduced annual bluegrass by 28% (Johnson and Murphy, 1995). Woosley et al. (2003) demonstrated annual bluegrass control up to 85% after spring and summer applications of paclobutrazol at rates of 0.28 and 0.14 kg ha^{-1}. There have also been reports of limited control with trinexapac-ethyl (Neylan 1997). Branham (1991) reported mixed results using PGRs for the suppression of annual bluegrass, but observed a general increase in bentgrass populations after applications of flurprimidol, paclobutrazol, and ethofumesate.

Plant growth regulators are also used to suppress seedhead formation of annual bluegrass. Seedhead production of annual bluegrass in spring reduces aesthetic quality and disrupts the uniformity of the turf (Borger 2008, Danneberger and Vargas 1984). Since the 1980's, PGRs, such as ethephon, mefluidide, trinexapac-ethyl, paclobutrazol, mefluidide and flurprimidol, have been tested for suppression of annual bluegrass seedheads (Zontek 1987). Successful seedhead suppression depends on application timing and repeat applications of PGRs (McCarty 2008). Annual bluegrass begins to form seedheads in the spring. Therefore, PGRs must be applied prior to any seedhead emergences. Applications after seedhead emergence will not suppress seedhead development. At the "boot" stage, a small stem will be evident growing from the crown of the plant. The annual bluegrass plant may also have a noticeable bulge at the base; this is the seedhead forming and also considered the "boot" stage of development (Borger et al. 2004). PGRs should be applied at this growth stage to maximize seedhead suppression (Borger 2008).

To predict seedhead emergence, Danneberger and Vargas (1984) developed a cumulative growing degree day (GDD) model for annual bluegrass. GDDs are calculated by adding the daily maximum and minimum temperatures, dividing the sum by 2, and subtracting a base temperature value. Only positive numbers are added to the total, negative values are calculated as zero (Vargas and Turgeon 2004). Applications of PGRs were most successful when GDDs reached between 25 and 50 (Stahnke and Bembenek 2008). The peak in seedhead production occurred at 363 to 433 degree-days. The authors concluded that calculating GDDs provided a more reliable estimate of seedhead emergence than simply following the calendar.

Seedhead suppression of 75 to 93 percent was achieved using mefluidide in a mixed stand of annual bluegrass and creeping bentgrass (Watschke and Borger 2000, Borger and Naedel 2007), while trinexapac-ethyl provides little annual bluegrass seedhead suppression. Trinexapac-ethyl inhibits gibberellic acid biosynthesis (cell elongation), but has little effect on suppression of seedhead production. Seedhead production is primarily driven by cell division (Borger et al. 2004). However, when trinexapac-ethyl is tank mixed with ethephon, greater suppression of annual bluegrass seedheads compared to ethephon alone have been reported (Bigelow and Hardebeck 2006).

Herbicides

Eradication of annual bluegrass is more desirable than simply suppressing seedhead formation, but damage to turfgrass is a concern. As putting greens are maintained at extremely low mowing heights, the potential of phytotoxicity associated with herbicides usage is greater, and hence herbicide usage is far more limited on putting greens than it is on fairways or higher mowed turf (Vargas and Turgeon 2004). Additionally, rapid removal of annual bluegrass, especially when plants occupy a large area, will leave open spaces, therefore, should be avoided (Vargas and Turgeon 2004). An ideal herbicide program would provide a smooth transition by slowly eradicating annual bluegrass, and allowing the creeping bentgrass to fill in.

The most effective herbicides labeled for creeping bentgrass putting greens are pre-emergence herbicides, specifically bensulide. This material is applied in late summer to spring for control of annual bluegrass emergence; however, control is often unsatisfactory in greens where annual bluegrass is a well-established component of the turfgrass community (Dernoeden 2000). Bensulide has successfully controlled the annual subspecies of annual bluegrass up to 97% after an application of 11kg ha^{-1} in January, followed by 6 kg ha^{-1} in February and March (Callahan and McDonald 1992). While this shows promise, the perennial type is not controlled by pre-emergence herbicides.

Few post-emergence herbicides can be used on putting greens, often because of the increased phytotoxicity that follows their use. Dating back as far as the 1930's when lead arsenate was found to discourage annual bluegrass (Sprague and Burton 1937), chlorosis on closely mowed turf was observed. Since then, all arsenic compounds were removed from the market for risk of human exposure (Christians 1996).

In recent years, amicarbazone has shown potential to selectively control annual bluegrass in creeping bentgrass. Amicarbazone is a photosystem II-inhibiting herbicide applicable for post-emergent broadleaf weed control with residual activity (Senseman 2007). Although potentially useful as a herbicide, turfgrass injuries of up to 50% have been reported from fall applications (Yelverton 2008). Early spring applications might be safer and could provide an alternative to other herbicides. Yelverton (2008) suggested that sequential applications of amicarbazone at 0.1 kg ha^{-1} in April provide excellent annual bluegrass control without significantly injuring creeping bentgrass. As air temperatures increase, the injury to creeping bentgrass following application of amicarbazone also increases (McCullough et al. 2010). Turfgrass and other plants generally have greater translocation of photosynthate from leaves to stems in fall than spring, which increases herbicide movement to stolons and rhizomes (Gesch et al. 2007, Wilson and Michaels 2003). This would explain the increased turf injury for fall compared to spring applications. As of now amicarbazone shows potential to offer a new mode of action for annual bluegrass control in cool-season turf, but more research is needed to evaluate timing and application rates (McCullough et al. 2010).

Bispyribac-sodium has the greatest potential to selectively control annual bluegrass on creeping bentgrass putting greens. Bispyribac-sodium was introduced in 2004 by Valent U.S.A. Corporation under brand name Velocity®. It belongs to the pyrimidinyl carboxy herbicide family and controls weeds by inhibiting acetolactate synthesis (ALS), similar to sulfonylureas (Shimizu et al. 2002). Bispyribac-sodium has been used for post-emergence control of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], gooseweed [*Sphenoclea zeylanica*], and other weeds in rice (*Oryza sativa* L.) (Schmidt et al. 1999, Webster et al. 1999). Currently, bispyribac-sodium is registered to control annual bluegrass in creeping bentgrass and perennial ryegrass golf course fairways and roughs (Anonymous 2010). A label for use on putting green mowing height (3mm) creeping bentgrass has not been released.

A number of research experiments have demonstrated the efficacy of bispyribac-sodium on *Poa* control in creeping bentgrass fairways. Lycan and Hart (2006) evaluated bispyribac-sodium use on creeping bentgrass fairways to determine seasonal (May, August or October) effects to turf injury. August applications did not reduce bentgrass quality, whereas May and October applications reduced turf quality by three weeks after treatment (Lycan and Hart 2006). Treatments applied in August were also more effective than May or October applications in reducing annual bluegrass coverage. After application, plots treated at the August timing resulted in ~40% and 32% less annual bluegrass coverage, compared to untreated control and May application, respectively (Lycan and Hart 2006). This study demonstrated that bispyribac-sodium was safer on creeping bentgrass when applied during summer compared to spring or fall. Similar results were reported by McCullough and Hart (2006) who conducted greenhouse experiments and found warmer temperatures (20 and 30 C) increase bispyribac-sodium efficacy with minimal creeping bentgrass discoloration. Cooler temperatures (10 C) have minimal efficacy on annual bluegrass and increase bentgrass chlorosis (McCullough and Hart 2006).

Experiments have also been conducted to explore alternative practices to reduce the potential discoloration on creeping bentgrass following bispyribac-sodium applications. One practice was to provide addition of plant nutrients. Applications of bispyribac-sodium at 49, 74, and 111g ai ha^{-1} all caused discoloration within one week and generally persisted for 14 to 21 days (McDonald and Kaminski 2006). After the addition of iron and nitrogen, the discoloration to creeping bentgrass was reduced while control of annual bluegrass was 82%. Similar results were reported by McCullough and

Hart (2009) who found tank-mixing chelated iron with bispyribac-sodium decreased creeping bentgrass discoloration.

Reducing the rate of bispyribac-sodium reduces creeping bentgrass discoloration (Teuton et al.2007, McCullough and Hart 2010). Spray adjuvants with bispyribac-sodium may allow turfgrass managers to reduce application rates while enhancing efficacy for annual bluegrass control. McCullough and Hart (2008) conducted field and laboratory experiments to investigate the influence of spray adjuvants on foliar absorption and efficacy of bispyribac-sodium on annual bluegrass. Sequential applications of bispyribac-sodium at 37g ai ha^{-1} with spray adjuvants controlled annual bluegrass similarly to 74g ai ha^{-1} applied sequentially without adjuvants (McCullough and Hart 2008).

With such success at controlling annual bluegrass in creeping bentgrass fairways, scientists have been working to create a regime that can be used on creeping bentgrass putting greens. McCullough and Hart (2009) discovered creeping bentgrass discoloration from bispyribac-sodium was exacerbated by reductions in mowing height from 24 to 3 mm. Teuton et al. (2007) found that applying bispyribac-sodium at 12 and 24g ai ha^{-1} weekly to a creeping bentgrass putting green in Tennessee controlled annual bluegrass 86%. However, creeping bentgrass injury up to 85% was observed by eight weeks after the initial treatment.. This study showed that managing annual bluegrass in creeping bentgrass putting greens is possible with bispyribac-sodium, but excessive injury may occur (Teuton et al. 2007). McCullough and Hart (2010) evaluated bispyribac-sodium application regimes for annual bluegrass control on creeping bentgrass putting greens and concluded that the most effective regime was 24.6g ha^{-1} applied weekly, which controlled

90% annual bluegrass after eight weeks with acceptable levels of creeping bentgrass discoloration (McCullough and Hart 2010)

Objectives

Currently, the safety of bispyribac-sodium use on creeping bentgrass putting greens in the Midwestern United States is unknown. The addition of plant growth regulators in combination of bispyribac-sodium are hypothesized to minimize the discoloration of creeping bentgrass. Optimal timing and repeat application of plant growth regulators or herbicides for annual bluegrass seedhead suppression is lacking repeated experimental data.

Studies will be conducted under both greenhouse and field conditions. Objectives of field studies will include: 1) determine effects of tank mixing plant growth regulators with bispyribac-sodium to minimize discoloration to creeping bentgrass putting greens; while evaluating the control of annual bluegrass; and 2) determine the greatest suppression of annual bluegrass seedheads from plant growth regulators applied on a mixed stand of creeping bentgrass and annual bluegrass putting green. Objectives of a greenhouse study will be to determine if annual bluegrass seed production and viability are impacted by plant growth regulators at different plant growth stages.

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CHAPTER II

Creeping Bentgrass (*Agrostis stolonifera*) Golf Green Tolerance to Bispyribac-sodium Tank Mixed with Paclobutrazol

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Abstract. Annual bluegrass is a troublesome weed in golf course putting greens. The objective of this research was to evaluate creeping bentgrass putting green tolerance to bispyribac-sodium tank mixed with paclobutrazol in the transition zone. Field trials with four replications were conducted in Oklahoma during 2009 and 2010 and in Missouri during 2010. Treatments were as follows: 1) untreated control; 2) bispyribac-sodium at 12.4 g ai ha^{-1}; 3) bispyribac-sodium at 24.8 g ai ha^{-1}; 4) bispyribac-sodium at 12.4 g ai ha^{-1} + trinexapac-ethyl at 57 g ai ha^{-1}; 5) bispyribac-sodium at 24.8 g ai ha^{-1} + trinexapac-ethyl at 57 g ai ha^{-1}; 6) bispyribac-sodium at 12.4 g ai ha^{-1} + paclobutrazol at 224 g ai ha^{-1}; and 7) bispyribac-sodium at 12.4 g ai ha^{-1} + paclobutrazol at 224 g ai ha^{-1}. Plots were evaluated for visual turfgrass quality (1-9 scale where 1 = dead, 6 = acceptable, and 9 = excellent) and visual phytotoxicity (1-9 scale where 1 = dead, 6 = acceptable, and 9 = no phytotoxicity). The results of this study suggest that tank mixing bispyribac-sodium with

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paclobutrazol may increase creeping bentgrass putting green discoloration due to phytotoxicity for up to 2 weeks after the initial treatment but will not reduce turf quality below acceptable levels. Although bispyribac-sodium is not currently labeled for use on creeping bentgrass putting greens, under the parameters of this research, weekly application of bispyribac-sodium at 12.4 g ai ha^{-1} or bi-weekly application at 24.8 g ai ha^{-1} alone or with monthly applications of paclobutrazol at 224 g ai ha^{-1} appear to be safe to apply to creeping bentgrass putting greens during the spring in the transition zone.

Nomenclature: Bispyribac-sodium {2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy] benzoic acid}; paclobutrazol; trinexapac-ethyl; annual bluegrass, *Poa annua* L. POAAN; creeping bentgrass, *Agrostis stolonifera* L. AGSST, ‘Penncross’.

Key words: phytotoxicity, turfgrass, putting green, plant growth regulator.

Introduction

Annual bluegrass is a troublesome weed in creeping bentgrass golf course putting greens. Annual bluegrass can withstand mowing heights of 3 mm and is a prolific seedhead producer which enables it to effectively compete with creeping bentgrass maintained at putting green height of cut (Vargis and Turgeon, 2004). However, during the warm summer months in the transition zone, substantial annual bluegrass losses may occur due to its annual life cycle, poor heat and drought tolerance (Vargis and Turgeon, 2004), and disease (Vargas, 2004) and insect damage (Vittum et al., 1999). In addition, there are annual and perennial biotypes of annual bluegrass which can exacerbate control measures. Annual bluegrass infestations can reduce overall turfgrass quality and negatively affect golf ball roll (Beard, 2002) making it an undesirable weed on creeping bentgrass putting greens.

Bispyribac-sodium targets the enzyme acetolactate synthase (ALS; EC 2.2.1.6), thus disrupting branched-chain amino acid biosynthesis of isoleucine, valine, and leucine in target weeds (Umbarger, 1978; Shimizu et al., 1994). Bispyribac-sodium is labeled for use on creeping bentgrass golf course fairways but is not yet labeled for use on creeping bentgrass greens (Anonymous, 2004). Bispyribac-sodium has been shown to effectively control annual bluegrass on creeping bentgrass fairways (Lycan and Hart, 2006; McDonald et al., 2006) and on creeping bentgrass golf greens, but creeping bentgrass injury is a concern at greens height of cut (McCullough and Hart, 2010a; Teuton et al., 2007).

Park et al. (2002) applied 60 g ai ha^{-1} of bispyribac-sodium to 'Penncross' creeping bentgrass greens maintained at 4 mm height of cut in Japan. The researchers reported slight injury to creeping bentgrass and noted that treated plots recovered from injury four weeks after initial treatment (WAIT). Teuton et al. (2007) assessed annual bluegrass control on ‘Penncross’ creeping bentgrass greens mowed at 3 mm with bispyribac-sodium applied weekly or biweekly at rates of 12 and 24 g ai ha^{-1} or monthly at a rate of 48 g ai ha^{-1} in Tennessee. The researchers found that eight weekly applications of bispyribac-sodium at 24 g ai ha^{-1} produced 96 and 99% annual bluegrass control at 16 WAIT in their 2003 and 2005 studies, respectively. However, these treatments also induced 50% creeping bentgrass injury at four WAIT and 85% creeping bentgrass injury at eight WAIT in the 2003 and 2005 studies, respectively. The researchers concluded that although bispyribac-sodium can effectively remove annual bluegrass in creeping bentgrass greens, it will likely cause unacceptable injury to creeping bentgrass mowed at putting green height.

McCullough and Hart (2009) conducted greenhouse and field trials to examine creeping bentgrass putting green tolerance to bispyribac-sodium applications. In the greenhouse trial, the researchers studied the influence of a 3 mm mowing height on ‘L-93’ creeping bentgrass tolerance to bispyribac-sodium when applied at rates of 0, 74, 148, and 296 g ai ha^{-1}. The 74 g ai ha^{-1} treatment produced approximately 35% ‘L-93’ turf discoloration at one week after treatment (WAT). The 74 g ai ha^{-1} rate also reduced ‘L-93’ clipping yield by 37% and 6% compared to the untreated control plants at 10 and 20 days after treatment respectively. At 30 days after treatment, there was no ‘L-93’ turf discoloration or clipping yield difference in the 74 g ai ha^{-1} treated plots compared to the untreated control plants. In the field trials, four bispyribac-sodium treatment regimes were utilized over a 60 day period, each with a cumulative treatment total of 222 g ai ha^{-}

^{1}. The bispyribac-sodium treatments included: 37 g ai ha^{-1} every 10 days; 74 g ai/ha^{-1} every 20 days; 111 g ai ha^{-1} every 30 days; and one application at 222 g ai ha^{-1}. All treatments produced significant turf discoloration compared to untreated control plots for 5 to 10 days following treatment application. The researchers also tank mixed the bispyribac-sodium treatments with chelated iron and trinexapac-ethyl and evaluated if the combination of treatments would reduce turf discoloration. The researchers found that tank mixing chelated iron with bispyribac-sodium reduced turf discoloration on 11 of 12 rating dates, and tank mixes with trinexapac-ethyl reduced turf discoloration on 7 of 12 rating dates. The researchers concluded that creeping bentgrass putting greens mowed at 3.0 to 3.2 mm are less tolerant of bispyribac-sodium applications compared to fairway mowing heights. The researchers also reported that either chelated iron or trinexapac-ethyl may help to reduce turf discoloration related to bispyribac-sodium applications to putting greens, thus increasing the potential for safe application while maintaining turf quality. McCullough and Hart (2010b) also reported that trinexapac-ethyl may help to reduce creeping bentgrass discoloration. The researchers also found that co-application of bispyribac-sodium and trinexapac-ethyl may improve foliar absorption of bispyribac-sodium while reducing creeping bentgrass chlorosis.

Plant growth regulators (PGR) are often used by golf course managers to improve putting green turf quality, reduce mowing frequency, and reduce annual bluegrass seedhead production (Christians, 2007). Paclobutrazol is a PGR that inhibits gibberellic acid (GA) biosynthesis in plants thus reducing vertical shoot growth and seedhead production in annual bluegrass (Johnson and Murphy, 1995). Woosley et al. (2003) reported that repeated applications of paclobutrazol to creeping bentgrass greens provided

85% control of annual bluegrass at rates of either 0.14 or 0.28 kg ai ha^{-1}. Paclobutrazol is labeled for use on golf course putting greens for annual bluegrass suppression (Anonymous, 2009). However, no research concerning co-application of bispyribac-sodium with paclobutrazol has been reported.

Bispyribac-sodium and paclobutrazol have been reported to be effective for suppressing and/or reducing annual bluegrass populations in creeping bentgrass golf greens. However, no work has been reported evaluating creeping bentgrass golf green tolerance to tank mixes of bispyribac-sodium with paclobutrazol. We hypothesized that applying paclobutrazol to suppress annual bluegrass seedhead production and tank-mixing with bispyribac-sodium to control annual bluegrass in creeping bentgrass greens could be an effective annual bluegrass management tool for golf course managers. Therefore, the objective of this research was to evaluate creeping bentgrass golf green tolerance to various tank mixes of bispyribac-sodium and paclobutrazol at rates suitable for annual bluegrass control or suppression.

Materials and Methods

This research was conducted on creeping bentgrass golf course greens in 2009 and 2010 at the Oklahoma State University Turfgrass Research Center in Stillwater, OK (OK); and in 2010 at the Country Club of Missouri in Columbia, MO (MO) (Table 2.1). The OK putting green was 'Penncross' creeping bentgrass and was constructed according to modified USGA specifications. The MO putting green was 'Penncross' creeping bentgrass and was constructed to USGA specifications. Treatments were applied in OK with a CO_2 bicycle-type research sprayer (R&D Sprayers, Opelousas, LA) calibrated to deliver 842 L ha^{-1} at 220 kPa. Treatments were applied in MO with a CO_2 backpack-type research sprayer (R&D Sprayers, Opelousas, LA) calibrated to deliver 215 L ha^{-1} at 283 kPa for herbicide treatments and 430 L ha^{-1} at 283 kPa for PGR treatments. Plots (1.5 x 1.5 m) were arranged in a randomized complete block design with four replications for each treatment at each location. All plots received fertilizer weekly at the rate of 5 kg N ha^{-1} with 1 kg $FeSO_4$ ha^{-1}. Plots were mowed five days per week at 3.8 mm height of cut at OK, and six days per week at 3.2 mm height of cut at MO. Plots were evaluated for visual turfgrass quality (1-9 scale where 1 = dead, 6 = acceptable, and 9 = excellent) (Morris and Shearman, n.d.) and visual phytotoxicity (1-9 scale where 1 = dead, 6 = acceptable, and 9 = no phytotoxicity) in OK and MO.

The lowest labeled treatment rate of bispyribac-sodium for transitional or slow removal of annual bluegrass in fairway height creeping bentgrass is 24.8 g ai ha^{-1} applied on 7 or 14 day intervals depending on severity of annual bluegrass infestation and time of year (Anonymous, 2004). Therefore, the 24.8 g ai ha^{-1} was chosen as the highest single application rate for this creeping bentgrass golf green study. Treatments were as follows: 1) untreated control; 2) bispyribac-sodium at 12.4 g ai ha^{-1}; 3) bispyribac-sodium at 24.8 g ai ha^{-1}; 4) bispyribac-sodium at 12.4 g ai ha^{-1} + trinexapac-ethyl at 57 g ai ha^{-1}; 5) bispyribac-sodium at 24.8 g ai ha^{-1} + trinexapac-ethyl at 57 g ai ha^{-1}; 6) bispyribac-sodium at 12.4 g ai ha^{-1} + paclobutrazol at 224 g ai ha^{-1}; and 7) bispyribac-sodium at 24.8 g ai ha^{-1} + paclobutrazol at 224 g ai ha^{-1}. Treatment intervals during each study period were as follows: 1) bispyribac-sodium at 12.4 g ai ha^{-1} treatments were applied once every 14 d; 2) bispyribac-sodium at 24.8 g ai ha^{-1} treatments were applied once every 28 d; 3) trinexapac-ethyl at 57 g ai ha^{-1} treatments were applied once every 28 d; and 4) paclobutrazol at 224 g ai ha^{-1} treatments were applied once every 28 d (Table 2.2).

Bispyribac-sodium with paclobutrazol treatments were watered in with 6.3 mm of irrigation water 15 hours following treatment application according to labeled recommendations (Anonymous, 2004; Anonymous 2009). Initial treatments were applied on 14 April 2009 and 29 April 2010 in Oklahoma. Initial treatments were applied on 21 June 2010 in Missouri.

Analysis of variance was performed using SAS 9.1 (SAS Institute, Cary, NC) PROC GLM at the 0.05 probability level. Mean separation test was performed using Duncan's multiple range test at the 0.05 probability level. There was a significant location by treatment interaction and data is presented separately for each location. In Oklahoma, there was significant year by treatment interaction and data is presented separately for each year.

Results and Discussion

Stillwater, OK – 2009: All plots were at 100% turf coverage prior to beginning this study and average turf quality was 6.0. At 1 WAIT, there was no difference in turf quality among treatments and average turf quality was 6.2 (Table 2.2). At 1 WAIT, there was no difference in phytotoxicity ratings among treatments with an average of 9.0 (Table 2.3). At 2 WAIT, there was no difference in turf quality among treatments and average turf quality was 6.6 (Table 2.2). At 2 WAIT, there was a difference in phytotoxicity among treated plots (Table 2.3). Treated plots exhibited greater phytotoxicity compared to untreated control at 2 WAIT, but all treatments were at visually acceptable levels (Table 2.3). There were no differences in turf quality at 4 WAIT with an average rating of 6.3 (Table 2.2). At 4 WAIT, treated plots showed greater phytotoxicity compared to untreated control (Table 2.3), but all plots were at visually acceptable levels. At 6 WAIT, there were no differences in turf quality or phytotoxicity among treatments with an average turf quality rating of 6.2 and average phytotoxicity rating of 9.0 (Tables 2.2 and 2.3).

Stillwater, OK – 2010: All plots were at 100% turf coverage prior to beginning this study and average turf quality was 7.0. There was no difference in turf quality at 1 WAIT and mean plot turf quality was 6.6 (Table 2.2). There was a difference in phytotoxicity ratings at 1 WAIT where treated plots showed greater phytotoxicity compared to untreated control plots, however all ratings were visually acceptable (Table 2.3). At 2 WAIT, there was no difference in turf quality ratings among treatments and mean turf quality was 6.6 (Table 2.2). At 2 WAIT, there was a difference in phytotoxicity ratings where both bispyribac-sodium co-applied with paclobutrazol treatments showed greater phytotoxicity compared to untreated control plots (Table 2.3). The bispyribac-sodium at 24.8 g ai ha^{-1} tank-mixed with paclobutrazol at 224 g ai ha^{-1} had an average phytotoxicity rating of 6.6 which exhibited noticeable yellowing but was still a visually acceptable rating (Table 2.3). At 4 WAIT, there was noticeable recovery and all treated plots showed visually acceptable turf quality (6.2 average) and phytotoxicity ratings compared to untreated control with phytotoxicity ratings ranging from 8.4 to 9.0 (Tables 2.2 and 2.3). There was no difference in turf quality at 6 WAIT with an average of 6.8 among all treatments. There was a difference in phytotoxicity ratings at 6 WAIT where all treatments exhibited greater phytotoxicity compared to untreated control plots, but all ratings were visually acceptable (Table 2.3).

Columbia, MO: All plots were at 100% turf coverage prior to beginning the study and average turf quality was 8.0. There was a difference among treatments in turf quality and phytotoxicity ratings at 1 WAIT (Tables 2.4 and 2.5). Bispyribac-sodium tank mixed with paclobutrazol treatments exhibited decreased turf quality (Table 2.4) and greater phytotoxicity (Table 2.5) compared to untreated control plots. Although noticeable discoloration compared to control plots occurred, both turf quality and phytotoxicity ratings were at or above acceptable levels in all treated plots. At 2 WAIT, there was a difference in both turf quality and phytotoxicity ratings (Tables 2.4 and 2.5). Turf quality was reduced in the bispyribac-sodium at 24.4 g ai ha^{-1} treatment, both bispyribac-sodium tank-mixed with trinexapac-ethyl treatments, and both bispyribac-sodium tank-mixed with paclobutrazol treatments (Table 2.4). Both of the bispyribac-sodium tank-mixed with paclobutrazol treatments caused significant phytotoxicity at 2 WAIT (Table 2.5), however, mean turf quality ratings were still at an acceptable level (Table 2.4). At 4 WAIT, there was no difference in turf quality (8.0 average) or phytotoxicity ratings (9.0 average) (Tables 2.4 and 2.5). There was a difference in phytotoxicity ratings at 6 WAIT. Bispyribac-sodium at 24.8 g ai ha^{-1} tank-mixed with trinexapac-ethyl and both bispyribac-sodium tank-mixed with paclobutrazol treatments exhibited greater phytotoxicity compared to untreated control plots, but all ratings were visually acceptable (Table 2.5). There was no difference in turf quality ratings at 6 WAIT and average plot turf quality was 7.5. Lastly, at 8 WAIT, there was no difference in turf quality ratings (6.8 average) or phytotoxicity ratings (9.0 average) (Tables 2.4 and 2.5).

Bispyribac-sodium is not currently labeled for use on golf course greens due to the susceptibility of herbicide injury to creeping bentgrass (Teuton, 2007). The results of these studies suggest that bispyribac-sodium can be safely used on creeping bentgrass greens in the transition zone at weekly of 12.4 g ai ha^{-1} or bi-weekly rates 24.8 g ai ha^{-1}

during the spring season without producing unacceptable visual turf quality. Teuton et al. (2007) reported that the application of bispyribac-sodium at 12 g ai ha^{-1} weekly resulted in 86% and 92% annual bluegrass control in their 2003 and 2005 studies, respectively. Teuton et al. (2007) also reported that the application of bispyribac-sodium at 24 g ai ha^{-1} bi-weekly resulted in 42% and 73% annual bluegrass control in their 2003 and 2005 studies, respectively. Additionally, the researchers reported that weekly application of bispyribac-sodium at 12 g ai ha^{-1} resulted in 15% and 2% creeping bentgrass injury at 4 WAIT in 2003 and 2005, respectively, while the bi-weekly application at 24 g ai ha^{-1} resulted in 10% and 2% at 4 WAIT in 2003 and 2005, respectively (Teuton et al., 2007). In this study, bispyribac-sodium applied alone did not cause unacceptable turf quality at any rating date but did cause visual phytotoxicity compared to untreated control plots. However, phytotoxicity was rated as acceptable at each rating date (Tables 2.2-2.5). These results suggest that the weekly application of bispyribac-sodium at 12.4 g ai ha^{-1} or the bi-weekly application at 24.8 g ai ha^{-1} may be safe on creeping bentgrass greens during the spring before daily maximum temperatures reach 29.4º C. This also corresponds to current labeled recommendations that state bispyribac-sodium should be applied to creeping bentgrass fairways at air temperatures above 29.4º C (Anonymous, 2004).The findings of this research are similar to those of McCullough and Hart (2009) where the researchers determined that application of bispyribac-sodium to creeping bentgrass maintained at putting green mowing height resulted in some turf discoloration. Similar to the results of McCullough and Hart (2009), the creeping bentgrass recovered from the bispyribac-sodium induced discoloration within 14 to 28 d after initial treatments (Tables 2.3 and 2.5).

Co-application of bispyribac-sodium at 12.4 g ai ha^{-1} weekly or 24.8 g ai ha^{-1} bi-weekly with trinexapac-ethyl appears safe on creeping bentgrass golf greens as it did not cause unacceptable turf quality on any rating dates (Tables 2.2-2.5). This corresponds to work reported by McCullough and Hart (2009 and 2010b). The researchers reported that co-application of trinexapac-ethyl with bispyribac-sodium helped to reduce creeping bentgrass discoloration on 7 of 12 rating dates (McCullough and Hart, 2009). In an additional study, the researchers again reported that co-application of trinexapac-ethyl with bispyribac-sodium increased creeping bentgrass green color by up to 15% compared to bispyribac-sodium only treated plots (McCullough and Hart, 2010b).

Paclobutrazol has been shown to effectively suppress annual bluegrass growth in creeping bentgrass greens (Woosley et al., 2003) and is currently labeled for use on creeping bentgrass mowed at greens height of cut (Anonymous, 2009). The results of this study suggest that bispyribac-sodium tank-mixed with paclobutrazol can be applied to creeping bentgrass golf greens without producing unacceptable turf quality (Tables 2.2 and 2.4), but phytotoxicity symptoms may occur. In Missouri, application of bispyribac-sodium with paclobutrazol resulted in significantly greater phytotoxicity compared to all other treatments in this study (Table 2.5). The 12.4 g a.i ha^{-1} and the 24.8 g a.i ha^{-1} bispyribac-sodium tank-mixed with paclobutrazol treatments resulted in phytotoxicity ratings of 5.9 and 5.6, respectively, due to yellowing/browning of the turfgrass leaves. Potential herbicide phytotoxicity and herbicide efficacy of bispyribac-sodium is highly temperature dependent (McCullough and Hart, 2006) and may vary by season (Lycan and Hart, 2006). In the Missouri study (initial treatment on 21 June 2010), the average maximum daily air temperature was 31.5° C during the study period which may help to explain the increased visual phytotoxicity ratings at 2 WAIT (Table 2.5). In addition, the creeping bentgrass in MO was mowed at 3.2 mm and the creeping bentgrass in OK was mowed at 3.8 mm and reduced creeping bentgrass mowing heights are reported to increase potential injury from bispyribac-sodium applications (McCullough and Hart, 2010a; Teuton et al., 2007). However, even though there was increased visual phytotoxicity due to bispyribac-sodium with paclobutrazol treatments, no treatments resulted in unacceptable turf quality at any rating date in MO (Table 2.4). In Oklahoma, application of bispyribac-sodium with paclobutrazol did not result in unacceptable turf quality, but resulted in significant phytotoxicity compared to untreated control plots due to slight yellowing and/or browning of turfgrass plants in the treated plots (Tables 2.2 and 2.3). Although bispyribac sodium and bispyribac sodium tank-mixed with paclobutrazol did not cause unacceptable visual phytotoxicity in the Oklahoma trials, results could vary if applied later in the spring or early summer once maximum daily air temperatures increase to 29.4 C or above or if the mowing height is reduced (McCullough and Hart, 2006; Anonymous, 2004).

The results of this study suggest that co-application of bispyribac-sodium and paclobutrazol on creeping bentgrass putting greens may significantly reduce turf quality due to increased yellowing and/or browning of creeping bentgrass due to phytotoxicity, but will not reduce turf quality below visually acceptable levels. However, prior research suggest that if these treatments are applied during the warmer summer months in the transition zone, significant injury could occur due to temperature and seasonal effects of bispyribac-sodium on creeping bentgrass turf (Lycan and Hart, 2006; McCullough and Hart, 2006; Teuton et al., 2007).

In addition to the possible benefit of increased annual bluegrass control with combinations of bispyribac-sodium and paclobutrazol, the dual use of these two products could help to prevent ALS herbicide resistance in annual bluegrass plants. ALS herbicide resistance is a concern in several weed species (Tranel and Wright, 2002). Paclobutrazol is a PGR that inhibits GA biosynthesis in plants thus reducing vertical shoot growth and seedhead production in annual bluegrass (Johnson and Murphy, 1995). If plants treated with combinations of paclobutrazol and bispyribac-sodium do not produce seedheads, there is reduced probability of annual bluegrass plants developing ALS resistance compared to plants treated with low end labeled rates of bispyribac-sodium alone, provided some of those plants are able to produce viable seedheads. However, future work is needed to collect data to confirm possible reduction of ALS resistance in annual bluegrass treated with bispyribac-sodium alone versus annual bluegrass plants treated with combinations of bispyribac-sodium and paclobutrazol.

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Table 2.1. Oklahoma and Missouri research site characteristics[a].

	Oklahoma	Oklahoma	Missouri
Site Characteristics	2009	2010	2010
Location	Stillwater	Stillwater	Columbia
Creeping bentgrass cultivar	Penncross	Penncross	Penncross
Soil type	Sand	Sand	Sand
Soil pH	7.2	7.1	6.3
Mowing height	3.8 mm	3.8 mm	3.0 mm
Mowing frequency	5 days/week	5 days/week	6 days/week
Average daily minimum	11.7° C	15.5° C	21.1° C
Total rainfall	179.1 mm	230.4 mm	212.3 mm

[a] This research was conducted at the Oklahoma State University Turfgrass Research Center in Stillwater, OK in 2009 and 2010 and at the Country Club of Missouri in Columbia, MO in 2010. Initial treatments were applied on 14 April 2009 and 29 April 2010 in Oklahoma and data was collected for six weeks after initial treatments. Initial treatments were applied on 21 June 2010 in Missouri and data was collected for eight weeks after initial treatments.

[b] Average daily maximum temperature, average daily minimum temperature, and total rainfall is reported for each site during each study period.

Table 2.2. Turf quality ratings[a] of creeping bentgrass greens treated with bispyribac-sodium alone or tank-mixed with either trinexapac-ethyl or paclobutrazol in Oklahoma at 1, 2, 4, and 6 weeks after initial treatment (WAIT).

		Turf Quality Ratings By Year (WAIT)							
	Rate	2009[c]				2010			
Treatment[b]	g a.i. ha^{-1}	1	2	4	6	1	2	4	6
Untreated Control	—	6.5 a[d]	6.8 a	6.5 a	6.3 a	7.0 a	6.6 a	6.1 a	7.0 a
Bispyribac-sodium	12.4	6.3 a	6.7 a	6.0 a	6.0 a	7.0 a	6.7 a	6.2 a	7.0 a
Bispyribac-sodium	24.8	—	—	—	—	7.0 a	6.7 a	6.3 a	6.8 a
Bispyribac-sodium + trinexapac-ethyl	12.4 + 57	6.0 a	6.5 a	6.0 a	6.0 a	7.0 a	7.0 a	6.0 a	7.0 a
Bispyribac-sodium + trinexapac-ethyl	24.8 + 57	—	—	—	—	7.0 a	7.0 a	6.0 a	6.8 a
Bispyribac-sodium + paclobutrazol	12.4 + 224	6.0 a	6.5 a	6.5 a	6.5 a	6.7 a	6.3 a	6.3 a	6.8 a
Bispyribac-sodium + paclobutrazol	24.8 + 224	—	—	—	—	6.7 a	6.2 a	6.6 a	6.5 a

[a] Turf quality ratings on a 1-9 scale where 1 = dead, 6 = acceptable, and 9 = excellent.

[b] Treatment intervals during each study period were as follows: 1) bispyribac-sodium at 12.4 g a.i. ha^{-1} treatments applied once every 14 d; 2) bispyribac-sodium at 24.8 g a.i. ha^{-1} treatments applied once every 28 d; 3) trinexapac-ethyl at 57 g a.i. ha^{-1} treatments applied once every 28 d; and 4) paclobutrazol at 224 g a.i. ha^{-1} treatments applied once every 28 d.

[c] The following treatments were not applied in 2009: bispyribac-sodium at 24.8 g a.i. ha^{-1}; bispyribac-sodium at 24.8 g a.i. ha^{-1} + trinexapac-ethyl at 57 g a.i. ha^{-1}; and bispyribac-sodium at 24.8 g a.i. ha^{-1} + paclobutrazol at 224 g a.i. ha^{-1}.

[d] Means within columns followed by the same letter are not statistically different at the 5% significance level according to Duncan's multiple range test.

Table 2.3. Phytotoxicity ratings[a] of creeping bentgrass greens treated with bispyribac-sodium alone or tank-mixed with either trinexapac-ethyl or paclobutrazol in Oklahoma at 1, 2, 4, and 6 weeks after initial treatment (WAIT).

		Phytotoxicity Ratings By Year (WAIT)							
	Rate	2009[c]				2010			
Treatment[b]	g a.i. ha^{-1}	1	2	4	6	1	2	4	6
Untreated Control	—	9.0 a[d]	8.8 a	8.8 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a
Bispyribac-sodium	12.4	9.0 a	7.0 b	7.0 b	9.0 a	8.0 b	8.2 ab	8.8 ab	8.0 b
Bispyribac-sodium	24.8	—	—	—	—	8.0 b	7.9 abc	8.8 ab	7.8 b
Bispyribac-sodium + trinexapac-ethyl	12.4 + 57	9.0 a	7.0 b	7.0 b	9.0 a	8.0 b	8.0 ab	8.4 b	8.0 b
Bispyribac-sodium + trinexapac-ethyl	24.8 + 57	—	—	—	—	8.0 b	8.0 ab	8.5 ab	7.8 b
Bispyribac-sodium + paclobutrazol	12.4 + 224	9.0 a	7.5 b	7.5 b	9.0 a	7.5 bc	7.1 bc	8.6 ab	7.8 b
Bispyribac-sodium + paclobutrazol	24.8 + 224	—	—	—	—	7.1 c	6.6 c	8.7 ab	7.5 b

[a] Phytotoxicity ratings on a 1-9 scale where 1 = dead, 6 = acceptable, and 9 = no phytotoxicity.

[b] Treatment intervals during each study period were as follows: 1) bispyribac-sodium at 12.4 g a.i. ha^{-1} treatments applied once every 14 d; 2) bispyribac-sodium at 24.8 g a.i. ha^{-1} treatments applied once every 28 d; 3) trinexapac-ethyl at 57 g a.i. ha^{-1} treatments applied once every 28 d; and 4) paclobutrazol at 224 g a.i. ha^{-1} treatments applied once every 28 d.

[c] The following treatments were not applied in 2009: bispyribac-sodium at 24.8 g a.i. ha^{-1}; bispyribac-sodium at 24.8 g a.i. ha^{-1} + trinexapac-ethyl at 57 g a.i. ha^{-1}; and bispyribac-sodium at 24.8 g a.i. ha^{-1} + paclobutrazol at 224 g a.i. ha^{-1}.

[d] Means within columns followed by the same letter are not statistically different at the 5% significance level according to Duncan's multiple range test.

Table 2.4. Turf quality ratings[a] of creeping bentgrass greens treated with bispyribac-sodium alone or tank-mixed with either trinexapac-ethyl or paclobutrazol in Missouri at 1, 2, 4, 6, and 8 weeks after initial treatment (WAIT).

	Rate	Turf Quality Ratings (WAIT)				
		Missouri				
Treatment[b]	g a.i. ha^{-1}	1	2	4	6	8
Untreated Control	—	8.0 a[c]	8.0 a	8.0 a	7.5 a	7.1 a
Bispyribac-sodium	12.4	7.6 ab	8.0 a	8.0 a	7.5 a	6.5 a
Bispyribac-sodium	24.8	7.6 ab	7.5 b	8.0 a	7.5 a	7.0 a
Bispyribac-sodium + trinexapac-ethyl	12.4 + 57	7.0 bc	7.5 b	8.0 a	7.5 a	6.5 a
Bispyribac-sodium + trinexapac-ethyl	24.8 + 57	7.0 bc	7.3 b	8.0 a	7.5 a	6.8 a
Bispyribac-sodium + paclobutrazol	12.4 + 224	6.9 c	6.1 c	8.0 a	7.5 a	6.8 a
Bispyribac-sodium + paclobutrazol	24.8 + 224	6.4 c	6.1 c	8.0 a	7.5 a	6.9 a

[a] Phytotoxicity ratings on a 1-9 scale where 1 = dead, 6 = acceptable, and 9 = no phytotoxicity.

[b] Treatment intervals during each study period were as follows: 1) bispyribac-sodium at 12.4 g a.i. ha^{-1} treatments applied once every 14 d; 2) bispyribac-sodium at 24.8 g a.i. ha^{-1} treatments applied once every 28 d; 3) trinexapac-ethyl at 57 g a.i. ha^{-1} treatments applied once every 28 d; and 4) paclobutrazol at 224 g a.i. ha^{-1} treatments applied once every 28 d.

[c] Means within columns followed by the same letter are not statistically different at the 5% significance level according to Duncan's multiple range test.

Table 2.5. Phytotoxicity ratings[a] of a creeping bentgrass green treated with bispyribac-sodium alone or tank-mixed with either trinexapac-ethyl or paclobutrazol in Missouri at 1, 2, 4, 6, and 8 weeks after initial treatment (WAIT).

	Rate	Phytotoxicity Ratings (WAIT)				
		Missouri				
Treatment[b]	g a.i. ha^{-1}	1	2	4	6	8
Untreated Control	—	8.8 a[c]	8.9 a	9.0 a	9.0 a	9.0 a
Bispyribac-sodium	12.4	8.4 a	8.9 a	9.0 a	9.0 a	9.0 a
Bispyribac-sodium	24.8	7.6 b	8.5 ab	9.0 a	9.0 a	9.0 a
Bispyribac-sodium + trinexapac-ethyl	12.4 + 57	7.5 bc	7.8 c	9.0 a	9.0 a	9.0 a
Bispyribac-sodium + trinexapac-ethyl	24.8 + 57	7.0 cd	8.1 bc	9.0 a	8.5 b	9.0 a
Bispyribac-sodium + paclobutrazol	12.4 + 224	6.5 d	5.9 d	9.0 a	8.0 c	9.0 a
Bispyribac-sodium + paclobutrazol	24.8 + 224	6.5 d	5.6 d	9.0 a	8.0 c	9.0 a

[a] Phytotoxicity ratings on a 1-9 scale where 1 = dead, 6 = acceptable, and 9 = no phytotoxicity.

[b] Treatment intervals during each study period were as follows: 1) bispyribac-sodium at 12.4 g a.i. ha^{-1} treatments applied once every 14 d; 2) bispyribac-sodium at 24.8 g a.i. ha^{-1} treatments applied once every 28 d; 3) trinexapac-ethyl at 57 g a.i. ha^{-1} treatments applied once every 28 d; and 4) paclobutrazol at 224 g a.i. ha^{-1} treatments applied once every 28 d.

[c] Means within columns followed by the same letter are not statistically different at the 5% significance level according to Duncan's multiple range test.

CHAPTER III

Suppression of Annual Bluegrass Seedheads with Mefluidide, Ethephon, and Ethephon plus Trinexapac-ethyl on Creeping Bentgrass Greens

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Abstract. Annual bluegrass (*Poa annua* L.) is a problematic weed on creeping bentgrass (*Agrostis stolonifera* L.) putting greens. Annual bluegrass produces extensive seedheads in spring, and plant growth regulators (PGRs) are frequently used to suppress seedhead formation. This study investigated the effects of single and sequential applications of mefluidide, maleic hydrazide, and ethephon alone or in combination with trinexapac-ethyl (TE), on annual bluegrass seedhead formation. A secondary objective evaluated whether addition of TE enhanced the efficacy of ethephon while improving the safety on creeping bentgrass. Three independent studies were established on golf course greens during a two-year period, with initial PGRs application prior to annual bluegrass seedhead formation. Regardless of PGR treatment, single or sequential applications in all studies showed no differences in annual bluegrass seedhead suppression. Ethephon-containing treatments provided the best seedhead suppression with up to 95% reduction.

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No PGR treatments caused visible phytotoxicity to creeping bentgrass following single applications. Sequential applications of both mefluidide and maleic hydrazide caused significant phytotoxicity to creeping bentgrass for up to three weeks after initial treatment (WAIT). With the exception of one evaluation date in the third study, tank mixture of TE with ethephon had little effect on annual bluegrass seedhead suppression. Tank-mixing TE with ethephon marginally enhanced turf quality of creeping bentgrass up to 6% compared to ethephon alone.

Abbreviations: GDD, growing degree-days; NDVI, normalized difference vegetation index; PGR, plant growth regulators; TE, trinexapac-ethyl; WAIT, weeks after initial treatment.

Introduction

Annual bluegrass is a major weed of creeping bentgrass putting greens (Beard, 1973). The encroachment of annual bluegrass into creeping bentgrass is undesirable due to its poor stress tolerance, susceptibility to most cool-season turfgrass diseases (Watschke et al., 1979), and unsightly light green color compared to creeping bentgrass. Annual bluegrass is a prolific seed producer and a single plant can produce up to 2200 seeds per season (McCarty, 1999). Almost all mature tillers produce an inflorescence (Ong and Marshall, 1975), further affecting turf playability (Cooper et al., 1987).

Herbicide options for selective control of annual bluegrass on creeping bentgrass putting greens are limited. As a result, PGRs are often used to shift populations and/or suppress seedhead formation (Inguagiato el al., 2010; Petrovic et al., 1985; Watschke et al., 1979). Commonly used PGRs for seedhead inhibition in turf include mefluidide (N-[2, 4-dimethyl-5-[[(trifluoromethyl) sulfonyl] amino] phenyl] acetamide), ethephon ([(2-Chloroethyl) phosphonic acid]), and a combination of ethephon and TE (4-(cyclopropyl-a- hydroxymethylene)-3, 5-dioxo-cyclohexanecarboxylic acid ethylester). Mefluidide inhibits cell elongation and division (Tautvydas, 1984). When applied prior to or during the early stages of forming floral primordia, mefluidide effectively inhibits annual bluegrass seedhead formation (Cooper et al., 1987; Danneberger et al., 1987; Inguagiato el al., 2010; Petrovic et al., 1985). Ethephon, which converts to ethylene in the presence of water, selectively inhibits annual bluegrass versus creeping bentgrass shoot growth (Eggens et al., 1989), and can inhibit annual bluegrass seedheads up to 90% (Gelernter and Stowell, 2001). As a late-stage gibberellin synthesis inhibitor, TE exhibits limited effectiveness on annual bluegrass when applied alone (Fagerness and Penner, 1998). When tank-mixed with ethephon, addition of TE was reported to enhance seedhead suppression of annual bluegrass by 20%, while concomitantly reducing discoloration of creeping bentgrass (Gelernter and Stowell, 2001). However, this seedhead suppression was only an estimation, and the authors provided no research-based data to support this statement. Inguagiato et al. (2010) observed that TE applied alone actually increased annual bluegrass seedhead cover from 14 to 99% depending upon re-application interval. When TE was applied within 7 to 14 days to the same plots receiving ethephon, no additional seedhead suppression was observed compared to plots receiving ethephon alone (Inguagiato et al., 2010). Similarly, reports of ethephon plus TE on enhancement of creeping bentgrass color are inconsistent (McCullough et al., 2005a; McCullough et al., 2006). To date, no research-based data have proven that adding TE to ethephon increases annual bluegrass seedhead suppression.

Sequential application of the same PGR for suppression of annual bluegrass seedheads has also generated variable results. For example, suppression of annual bluegrass seedheads following mefluidide application has been reported to be 12 to 15% (Inguagiato et al., 2010), 50 to 76% (Cooper et al., 1987), or 64 to 99% (Petrovic et al., 1985). Similarly, repeated application of ethephon reduced annual bluegrass seedhead formation by 12 to 47% (Inguagiato et al., 2010), or 70 to 90% (Gelernter and Stowell, 2001).

Variation in biotypes between annual (*P. annua* L. var. *annua*) and perennial (*P. annua* var. *reptans* Hausskn.) annual bluegrass likely contributes to inconsistencies in seedhead suppression. For example, the formation of annual bluegrass seedheads, primarily in spring (Danneberger and Vargas, 1984a), may or may not require vernalization depending on biotype (Johnson and White, 1997). The influence of biotype on seedhead productivity is unclear, as Danneberger and Vargas (1984a) indicated that seedhead counts for annual and perennial biotypes were similar, while Slavens et al. (2011) reported that annual types produced six-fold more seedheads than perennial types. Geographic location and management intensity affect the distribution and selection of annual or perennial types; however, in most cases, the two biotypes co-exist (Slavens et al., 2011). Besides genetic variation, other factors that contribute to the inconsistencies in seedhead control include PGR application rate, application timing (Watschke et al., 1979), and seedhead evaluation methodologies (Danneberger and Vargas, 1984a; Inguagiato et al., 2010).

Research outcomes for determining effectiveness of single or sequential PGR applications on annual bluegrass seedhead control are also inconclusive. Multiple applications of ethephon were suggested by Mahad (2001), who reported that three sequential applications of ethephon at 3.8 or 7.6 kg ha^{-1} reduced annual bluegrass seedheads by 72 or 83%, respectively. However, Mahad (2001) applied treatments to an annual bluegrass putting green with no creeping bentgrass. When the same rates of ethephon were applied to creeping bentgrass at a re-application interval of 3 weeks, McCullough et al. (2005b) reported that treatments caused significant injury to creeping bentgrass, evidenced by turf quality decreases for 8 of 9 evaluations and root mass reductions up to 35% over a 9-week period. This result was supported by Dernoeden (1984), who indicated that two applications of mefluidide and ethephon should be avoided on intensively managed turf because of adverse impacts on turf quality and density.

Few researchers have documented the influence of single versus sequential applications of mefluidide and ethephon alone, or in combination with TE, on annual bluegrass seedhead formation on creeping bentgrass maintained as putting greens. Therefore, the objectives of this study were to: 1) determine if sequential versus single applications of select PGRs enhance suppression of annual bluegrass seedhead formation; and 2) assess whether addition of TE in tank-mixes with ethephon enhances efficacy of ethephon on annual bluegrass while improving the safety on creeping bentgrass.

Materials and Methods

Field experiments were conducted on creeping bentgrass greens with natural infestations of annual bluegrass at three different golf courses in Missouri during 2011 and 2012. The first study (Study 1) was conducted on a chipping green at Columbia Country Club in Columbia in 2011. The green was constructed from native soil (Mexico silt loam, udollic Ochraqualf) in a push-up style with sand incorporation through regular topdressing to the upper soil profile. Soil test revealed that rootzone soil was a sandy loam containing 65% sand, 31% silt and 4% clay. The soil pH was 6.3 and organic matter was 1.6%. The green was established with a seed mixture of 'Cato/Crenshaw' creeping bentgrass. During the experiment, the green was mowed at a height of 3.2 mm, with adequate irrigation and approximately 7.5 kg N ha^{-1} applied bi-weekly for a total of 120 kg N ha^{-1} throughout each growing season. Prior to the initial application of treatments, annual bluegrass encroachment, primarily annual type, was evenly distributed in the plot area and ranged from 19 to 23% in cover.

The second study (Study 2) was conducted on a practice green at the Heritage Hills Golf Course in Moberly in 2011. The green was a native soil (Mexico silt loam, udollic Ochraqualf) push-up green with sand incorporated through topdressing, which subsequently resulted in a sandy loam rootzone with 63% sand, 32% silt, and 6% clay. The soil pH was 6.3 and organic matter content was 3.6%. The turf consisted of 'Penncross' creeping bentgrass, with 13 to 18% annual bluegrass cover before the initial application. The annual bluegrass population was comprised of approximately 75% perennial and 25% annual biotypes. The green was maintained at a height of 3.2 mm, with adequate irrigation and approximately 7.5 kg N ha^{-1} applied bi-weekly for a total of 120 kg N ha^{-1} throughout each growing season.

The third study (Study 3) was conducted in 2012 on the 18th green of the Lake of the Woods Golf Course in Columbia. The green was built as a native soil (Mexico silt loam, udollic Ochraqualf) push-up style with sand incorporated by topdressing. The rootzone soil contained 66% sand, 29% silt, and 6% clay, with a pH of 6.8 and organic matter content of 1.5%. The green consisted of 'Penncross' creeping bentgrass with 30 to 37% annual bluegrass cover prior to treatment application. The annual bluegrass population was comprised of approximately 80% of the perennial biotype and 20% of the annual biotype. The green was mowed at a height of 3.2 mm and maintained with adequate irrigation and approximately 12.2 kg N ha^{-1} applied monthly for a total of 110 kg N ha^{-1} throughout each growing season.

Treatments for each study included: ethephon at 3.8 kg ai ha^{-1}, ethephon tank-mixed with TE at 3.8 and 0.05 kg ai ha^{-1}, respectively, mefluidide at 0.07 ai kg ha^{-1}, and an untreated control. Treatments were made as single or sequential applications spaced 2 weeks apart. Study 3 also contained an additional treatment of maleic hydrazide (1, 2-dihydro-3, 6-pyridazinedione) at 1.12 kg ai ha^{-1} as both single and sequential applications. All applications were made with a CO_2 pressurized backpack sprayer calibrated to deliver 411 L ha^{-1} at a spray pressure of 303 kPa with TeeJet XR8004 flat fan spray tips (Spraying Systems Co., Wheaton, IL).

For all studies, initial PGR applications were made 3 to 4 weeks prior to the annual bluegrass seedheads becoming visible. Timing was determined by a combination of historical records, field scouting in adjacent areas at higher mowing heights (Inguagiato et al., 2010), and determining the accumulation of growing degree-days (GGD) at 50 with 10 C as the base temperature (Danneberger and Vargas, 1984b) starting in January 1st of each year. For Studies 1 and 2, initial applications were made on 21 Mar. 2011, and sequential applications were made 4 Apr. 2011. For Study 3, initial and sequential applications were made on 13 and 27 Mar. 2012, respectively.

Treatment effect on annual bluegrass seedhead formation was determined by counting the number of visible seedheads at the time of initial application, and every 2 WAIT for a period of 12 weeks. Counts were obtained by randomly placing a 30 × 30 cm frame four times in each plot, and counting the number of annual bluegrass seedheads within the frame. The four counts from each plot were expressed as mean annual bluegrass seedhead counts per 900 cm^2. Phytotoxicity on creeping bentgrass was evaluated weekly on a 1 to 9 scale with 1= plant death, 9 = no phytotoxicity, and 6 = minimally acceptable injury. Treatment effect on turf quality was measured on the same dates by Normalized Difference Vegetation Index (NDVI) using a GreenSeeker handheld sensor (NTech Industries, Ukiah, CA) (Bell et al., 2000; Moss et al., 2012).

Treatments were arranged in a split-plot design with four replications for all three studies. The whole plot variable consisted of the PGRs, and the subplot variable was single or sequential application. In all studies, the whole plot size was 1.6 by 3.3 m with a 30 cm buffer between each plot, and the subplot measured 1.6 by 1.6 m. Analysis of variance was conducted using the Proc Mixed procedure in SAS 9.2 (SAS Institute, 2008). For annual bluegrass seedhead count and NDVI data, no significant main effects or interactions of single versus sequential applications were found. Therefore, seedhead counts and NDVI data were pooled across the number of applications for further analysis. Since three way interactions among studies, PGRs, and time of measurements were detected, seedhead counts and NDVI results were presented separately for each study. Single and sequential applications of PGRs produced various degrees of creeping bentgrass phytotoxicity; therefore, the interaction of treatment by application time was analyzed for each study. To simplify the discussion, phytotoxicity results presented only include the evaluations when phytotoxicity occurred. Treatment means were separated using Fisher's Protected LSD at $P = 0.05$.

Results and Discussion

Annual bluegrass seedhead formation varied among studies and PGRs applied. Single or sequential applications did not result in differences in seedhead formation; therefore, the influence of time of application is not presented. Seedhead formation in the untreated control plots increased rapidly in the three studies, and reached peak production at 6 WAIT, ranging from 40 to 1,891 counts per 900 cm^2. Variation in annual bluegrass seedhead formation among studies is associated with differences in annual bluegrass weed pressure and biotype at the experimental sites. Seedhead formation in all studies decreased from 6 to 8 WAIT, and by 12 WAIT, counts were reduced overall by 80 to 97%. The rapid decline in seedhead formation reflected seasonal effects on annual bluegrass peak seedhead production, which was reported previously by Danneberger and Vargas (1984a).

Application of PGRs resulted in significant impacts to seedhead formation of annual bluegrass in Study 1 (Table 3.1). Prior to 8 WAIT, PGR treatments did not significantly affect annual bluegrass seedhead formation. At 8 WAIT or later, ethephon or ethephon plus TE reduced annual bluegrass seedheads by 73 to 83% compared to the untreated control. The presence or absence of TE along with ethephon did not influence annual bluegrass seedhead suppression. These results are in agreement with those of Inguagiato et al. (2010). At 8 WAIT, mefluidide reduced annual bluegrass seedheads by 52% compared to the untreated control. However, this effect was short-lived and no differences were observed at 10 or 12 WAIT.

The influence of PGR treatments on annual bluegrass in Study 2 was similar to that observed in Study 1. Annual bluegrass seedhead counts in the untreated control plots reached a maximum of 152 per 900 cm^2 at 6 WAIT (Table 3.2). Despite changes in seedhead counts throughout the growing season, differences in seedhead counts among treatments were significant at all evaluation timings. The only exception was mefluidide, which showed no effect at 10 WAIT, and 54 to 76% seedhead suppressions at the other evaluation dates. Results with mefluidide were similar to seedhead reduction reported by Cooper et al. (1987), who reported a 50 to 76% reduction following mefluidide application at either 0.07 or 0.14 kg ai ha^{-1}. Overall, the most effective PGR treatments were ethephon or ethephon plus TE. Up to 100% suppression in annual bluegrass seedheads was found in plots receiving these two treatments. Ethephon alone or ethephon plus TE reduced 83 or 95% of the seedheads, respectively, at the peak seedhead formation timing (6 WAIT). These results are in contrast with those of Gelernter and Stowell (2001) who estimated a 20% increase in seedhead suppression when TE is tank mixed with ethephon compared to ethephon alone. However, our results support those of Inguagiato et al. (2010), who indicated the TE applications within 7 to 14 days of applying ethephon did not improve annual bluegrass seedhead suppression.

Annual bluegrass seedhead densities were up to 47-fold higher in Study 3 compared to Studies 1 and 2 (Table 3.3). Despite high annual bluegrass pressure (30 to 37% annual bluegrass cover prior to initial treatment), treated plots exhibited significantly fewer seedheads throughout this experiment than the untreated control. The exceptions were plots treated with mefluidide at 10 WAIT, and maleic hydrazide at 10 and 12 WAIT. In the first 6 WAIT, reductions in seedhead formation with mefluidide were comparable to ethephon-containing treatments. From 8 to 12 WAIT, mefluidide treated plots only resulted in seedhead reductions up to 44%, compared with up to 83% seedhead suppression from ethephon-containing treatments. A diminishing effect through time following mefluidide application was reported by Cooper et al. (1987), who found that mefluidide effects on seedhead suppression were temporal. Addition of TE to ethephon resulted in similar levels of seedhead suppression compared with ethephon alone. The only exception was seedhead counts at 4 WAIT, where ethephon alone resulted in 34% suppression while ethephon plus TE reduced seedhead formation by 59%. At other recording time periods during this experiment, ethephon alone or tank-mixed with TE resulted in similar levels of annual bluegrass seedhead suppression, up to 83 or 92% seedheads, respectively.

Creeping bentgrass phytotoxicity

Phytotoxicity caused by PGR applications on turfgrass plants has been documented (Dernoeden, 1984; Watschke at al., 1979). Injury symptoms to sensitive plants include discoloration of leaf tissues and stunted shoot and root growth (Cooper et al., 1987; Eggens et al., 1989; and McCullough et al., 2005b). Phytotoxicity caused by PGR treatments in our study mainly appeared to be discoloration. No symptoms of discoloration were observed until 3 WAIT, and the symptoms lasted for less than 3 weeks in all studies (Table 3.4). Creeping bentgrass injury was influenced by both PGR treatment and the number of applications. One application of PGR treatments did not cause visible phytotoxicity, compared to the untreated control. This result supports Dernoeden (1984), who has suggested that no more than one application of a PGR, such as mefluidide and ethephon, should be made to intensively managed turf.

Ethephon alone did not cause any injury to creeping bentgrass in all three studies, regardless of the number of applications. Addition of TE to ethephon did not improve the safety of ethephon; rather, significant phytotoxicity was observed at 3 WAIT in Study 2 following sequential applications of ethephon and TE. This phytotoxicity, however, was acceptable (8 out of 9) and only lasted for 1 week. McCullough et al. (2005a) reported that sequential applications of ethephon at 3.8 kg ha^{-1} caused significant injury to creeping bentgrass maintained as a putting green. However, this study was conducted in May in South Carolina, when air temperature was higher and treatments were applied only 6 days apart. In a separate study conducted by McCullough et al. (2005b), injury to creeping bentgrass following ethephon treatment was also reported. Ethephon, however, was applied to juvenile plants at 3 weeks after turf establishment. Our study also disagrees with results of Dernoeden and Pigati (2009), who reported that two applications of ethephon alone or in a tank-mix with TE reduced turf quality. The differences in mowing height (4 mm vs. 3.2 mm), mowing frequency (5 times vs. 6 times per week) and mowing equipment (triplex vs. walk-behind mower) compared to our study might contribute to the difference in turf quality.

In our study, creeping bentgrass was sensitive to mefluidide, and sequential applications of mefluidide in all three studies caused significant phytotoxicity. Similarly, Cooper et al. (1987) reported that between 3 to 4 weeks after application, mefluidide caused leaf tip yellowing of sensitive plants. Sequential applications of maleic hydrazide in Study 3 caused significant injury to creeping bentgrass for 3 weeks. This result supported an early report by Engel and Aldrich (1960), where maleic hydrazide application reduced creeping bentgrass cover by 29%.

Creeping bentgrass turf quality

Treatment effect on creeping bentgrass turf quality as evaluated by NDVI provides an objective assessment of overall turf performance (Bell et al., 2000; Moss et al., 2012). Similar to results with seedhead suppression, treatments applied singly or sequentially did not interact with other factors. Therefore, data were pooled across application time and are presented separately for individual studies (Fig. 3.1-3.3). NDVI collected in these studies varied between 0.71 and 0.93, which reflected the seasonal effect on creeping bentgrass turf quality (Xiong et al., 2007).

In Study 1, NDVI responses indicated that plots receiving PGRs showed either the same or higher turf qualities compared to the untreated control (Fig. 3.1). No differences between ethephon alone or ethephon plus TE were found. The only exception was at 6 WAIT, when addition of TE resulted in a 4% increase in NDVI compared to ethephon alone. This effect, however, was short-lived and no differences were detected after this date. A similar observation was reported by McCullough et al. (2005c), who concluded that addition of TE to PGRs did not enhance turf quality of creeping bentgrass turf.

Similarly, NDVI data collected in Study 2 showed that treated plots had the same turf quality at most evaluation dates compared to the untreated control (Fig 3.2). The only exceptions were at 2 and 4 WAIT, when treated plots resulted in up to a 2% NDVI reduction compared to the untreated control.

In Study 3, NDVI responses showed more variation among treatments, partially due to the addition of maleic hydrazide (Fig 3.3). Compared to the untreated control, plots treated with maleic hydrazide showed either similar or lower turf quality. The significant reduction in quality following maleic hydrazide application occurred at 6 WAIT, when a reduction in NDVI of 4% was observed. Besides maleic hydrazide, other PGR treatments maintained or improved turf quality compared to the untreated control. Plots receiving mefluidide decreased turf quality for the first 6 weeks, but the NDVI responses improved at 8 WAIT or later compared to the untreated control. Application of ethephon had no effect on turf quality compared to the untreated control, but addition of TE improved the turf quality consistently with an increase of up to 6% of NDVI.

Conclusions

Our study suggests that annual bluegrass seedhead formation can be suppressed by a single application of PGR, if the application is timed before seedheads become visible. A single application appeared safe to creeping bentgrass turf, as none of the treatments evaluated caused phytotoxicity. Sequential applications spaced at 2 week intervals did not enhance suppression of annual bluegrass seedheads compared to single applications. Sequential applications did increase the probability of phytotoxicity to creeping bentgrass, especially for mefluidide and maleic hydrazide. Overall, treatments containing ethephon effectively suppressed seedhead formation, regardless of whether TE was added. Addition of TE to ethephon, however, may marginally improve creeping bentgrass turf quality. Future study on PGR effects on annual bluegrass seed viability would be of interest.

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Figure 3.1. The effect of plant growth regulators (PGRs) on normalized difference vegetation index (NDVI) for Study 1 in Columbia, MO in 2011. Single or sequential applications did not interact with PGRs; therefore, data were pooled across the application time. Abbreviations: trinexapac-ethyl, TE.

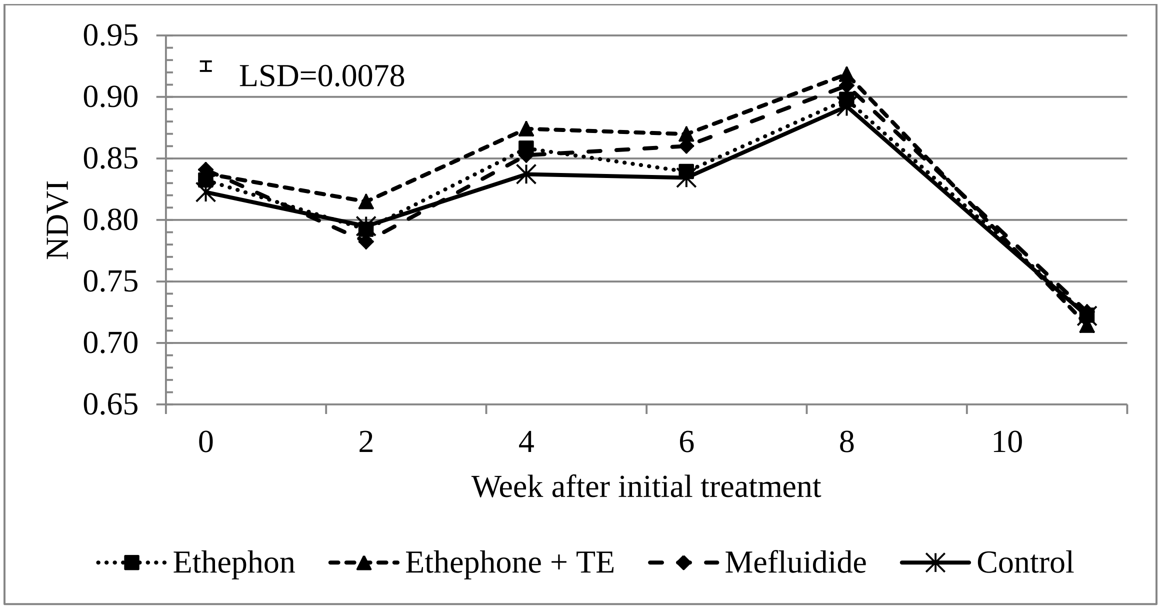

Figure 3.2. The effect of plant growth regulators (PGRs) on normalized difference vegetation index (NDVI) for Study 2 in Moberly, MO in 2011. Single or sequential applications did not interact with PGRs; therefore, data were pooled across the application time. Abbreviations: trinexapac-ethyl, TE. Vertical bar represents the Fisher's Protected LSD at *P*=0.05.

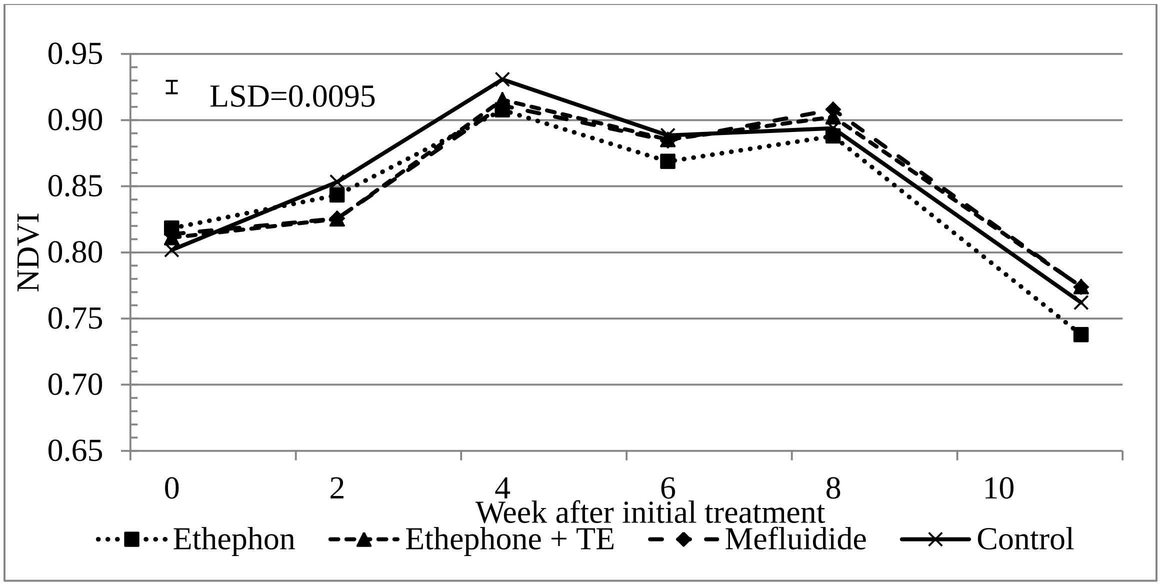

Figure 3.3. The effect of plant growth regulators (PGRs) on normalized difference vegetation index (NDVI) for Study 3 in Columbia, MO in 2012. Single or sequential applications did not interact with PGRs; therefore, data were pooled across the application time. Abbreviations: trinexapac-ethyl, TE. Vertical bar represents the Fisher's Protected LSD at *P*=0.05.

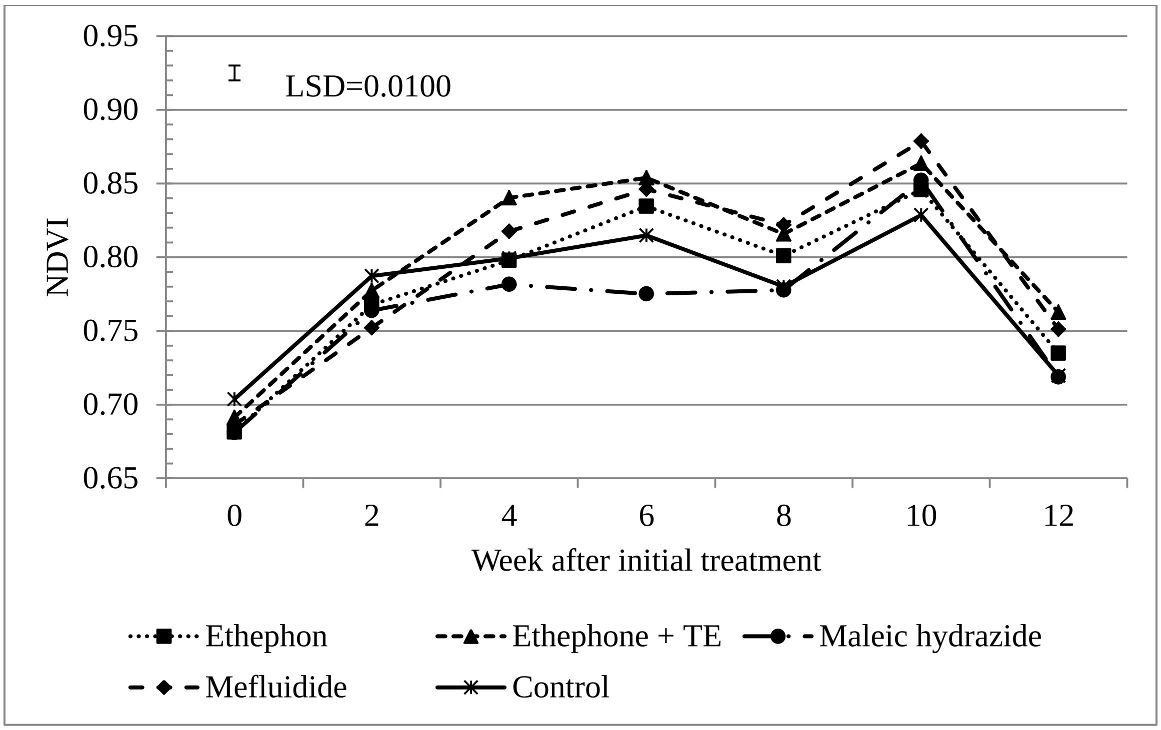

Table 3.1. Influence of plant growth regulators (PGRs) on annual bluegrass seedhead formation per 900 cm^2 area when evaluated at 2, 4, 6, 8, 10, and 12 weeks after initial treatment (WAIT) from Study 1 conducted in 2011 at Columbia Country Club in Columbia, MO. The number of applications did not significantly affect seedhead production, and hence data were pooled together.

Treatment†	Rate	Weeks after initial treatment						LSD
		2	4	6	8	10	12	
	kg ai ha^{-1}	seedhead counts per 900 cm^2						(P=0.05)
Ethephon	3.8	2.6	26.0	26.1	7.3	6.2	1.4	10.6
Ethephon + TE	3.8 + 0.05	0.8	14.6	27.0	10.5	4.9	1.4	9.1
Mefluidide	0.07	0.1	9.8	22.8	19.9	17.2	5.6	8.7
Control	___	4.5	23.0	40.0	38.2	26.4	8.0	7.2
LSD (P=0.05)		NS‡	NS	NS	10.4	10.7	3.2	

†The initial application was made on 21 Mar., 2011 and the second application was made 2 weeks later. TE: trinexapac-ethyl.

‡NS represents not significantly different by Fisher's Protected LSD (P = 0.05).

Table 3.2. Influence of plant growth regulators (PGRs) on annual bluegrass seedhead formation per 900 cm^2 area when evaluated at 2, 4, 6, 8, 10, and 12 weeks after initial treatment (WAIT) from Study 2 conducted in 2011 at Heritage Hills Golf Course in Moberly, MO. The number of applications did not significantly affect seedhead production, and hence data were pooled together.

Treatment[†]	Rate	Weeks after initial treatment						LSD
		2	4	6	8	10	12	
	kg ai ha^{-1}	seedhead counts per 900 cm^2						(P=0.05)
Ethephon	3.8	0.5	7.6	25.5	7.2	25.1	6.5	9.4
Ethephon + TE	3.8 + 0.05	0.0	1.5	8.0	7.3	13.8	3.0	5.5
Mefluidide	0.07	3.9	14.9	57.3	21.8	35.8	10.1	13.3
Control	___	12.7	62.5	151.5	47.3	46.1	24.3	25.8
LSD (P=0.05)		8.3	15.7	42.0	17.8	17.7	11.6	

[†]The initial application was made on 21 Mar., 2011 and the second application was made 2 weeks later. TE: trinexapac-ethyl.

Table 3.3. Influence of plant growth regulators (PGRs) on annual bluegrass seedhead formation per 900 cm^2 area when evaluated at 2, 4, 6, 8, 10, and 12 weeks after initial treatment (WAIT) from Study 3 conducted in 2012 at Lake of the Woods Golf Course in Columbia, MO. The number of applications did not significantly affect seedhead production, and hence data were pooled together.

Treatment[†]	Rate	Weeks after initial treatment						LSD
		2	4	6	8	10	12	
	kg ai ha^{-1}	seedhead counts per 900 cm^2						(P=0.05)
Ethephon	3.8	67.3	613.1	445.5	24.9	19.8	16.5	257.7
Ethephon + TE	3.8 + 0.05	49.6	376.0	154.4	46.5	20.3	14.3	89.2
Maleic hydrazide	1.12	38.3	464.3	825.2	85.9	71.3	42.4	220.0
Mefluidide	0.07	49.3	347.3	339.2	81.8	48.8	37.9	88.8
Control	___	148.2	921.9	1890.8	147.2	68.1	50.7	230.0
LSD (P=0.05)		32.6	229.2	427.5	39.4	20.0	10.6	

[†]The initial application was made on 13 Mar., 2012 and the second application was made 2 weeks later. TE: trinexapac-ethyl.

Table 3.4. Influence of single or sequential applications of plant growth regulators (PGRs) on creeping bentgrass phytotoxicity[†] at 3, 4 or 5 weeks after initial treatment (WAIT) from three Missouri golf course greens (Study 1, 2, 3). Study 1 and 2 were conducted in Columbia and Moberly, respectively, and Study 3 was conducted in Columbia in 2012.

Treatment[‡]	Rate	Number of Application	—Study 1—		—Study 2—		———Study 3———		
			3 WAIT	4 WAIT	3 WAIT	4 WAIT	3 WAIT	4 WAIT	5WAIT
	kg ai ha^{-1}		Phytotoxicity (1-9)						
Ethephon	3.8	1	9.0a[§]	9.0a	9.0a	9.0[¶]	9.0a	9.0a	9.0a
Ethephon	3.8	2	9.0a	9.0a	8.8a	8.8	9.0a	9.0a	9.0a
Ethephon + TE	3.8 + 0.05	1	9.0a	9.0a	8.5ab	9.0	9.0a	9.0a	9.0a
Ethephon + TE	3.8 + 0.05	2	9.0a	9.0a	8.0b	9.0	9.0a	9.0a	9.0a
Maleic hydrazide	1.12	1	___[#]	___	___	___	9.0a	9.0a	9.0a
Maleic hydrazide	1.12	2	___	___	___	___	8.3b	8.0c	7.0b
Mefluidide	0.07	1	9.0a	9.0a	8.5ab	9.0	9.0a	9.0a	9.0a
Mefluidide	0.07	2	8.0b	8.3b	7.0c	9.0	8.0c	8.8b	9.0a
Control	___	___	9.0a	9.0a	9.0a	9.0	9.0a	9.0a	9.0a

[†]Phytotoxicity was evaluated on a 1 to 9 scale with 1= plant death, 9 = no phytotoxicity, and 6 = minimally acceptable injury.

[‡]The initial and sequential applications were made on 21 Mar. and 4 Apr., 2011 for Study 1 and 2, and 13 and 27 Mar., 2012 for Study 3. TE: trinexapac-ethyl.

[§]Means in the same column followed by the same letters are not significantly different according to Fisher's Protected LSD (P = 0.05).

[¶]ANOVA was not significant and hence, no mean separation was performed.

[#]Treatment maleic hydrazide was not included in Study 1 and 2.

Chapter IV

Shoot growth and seedhead development of annual bluegrass (*Poa annua* L.) influenced by herbicide, PGR, and application timing

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Abstract: Annual bluegrass (*Poa annua* L.) is the most troublesome weed in creeping bentgrass (*Agrostis stolonifera* L.) golf course putting greens. Plant growth regulators (PGRs) and a few postemergence (POST) herbicides provide limited suppression of annual bluegrass; however, the effectiveness is often influenced by application timing. A greenhouse study was aimed at determining the optimal growth stage for applications of PGRs or herbicides for annual bluegrass shoot and seedhead suppression. Treatments included seven PGRs and two herbicides applied at three stages, pre-boot, boot and flowering stages. Results showed that annual bluegrass shoot growth, including plant height, density, canopy area, and shoot biomass, were influenced by treatments tested. Among the PGRs, paclobutrazol reduced plant height and canopy area by 29 and 33%, respectively, at four weeks after treatments (WAT). Shoot density was increased 12% at 4 WAT following paclobutrazol application. Although no significant differences were observed for seedhead production, applications of ethephon reduced seedhead production by 70% following pre-boot applications. Addition of trinexapac-ethyl (TE) to ethephon did not increase seedhead suppression following applications made at pre-boot or boot stage. For applications made at the flowering stage, addition of TE to ethephon reduced

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seedhead production by 72%, compared to ethephon only treatment. Our results indicate that application timing is essential especially for annual bluegrass seedhead suppression.

Abbreviations: GDD, growing degree days; PGRs, plant growth regulators; POST, post emergence herbicides; TE, trinexapac-ethyl; WAT, weeks after treatment.

Introduction

Annual bluegrass (*Poa annua* L.) is arguably the most troublesome weed on creeping bentgrass (*Agrostis stolonifera* L.) golf course putting greens, fairways and tees. Due to its bunch-type growth habit, light green color, and poor disease and drought tolerance, annual bluegrass is an undesirable species in turf (Beard et al., 1978; Johnson and White, 1997, 1998; Lush, 1989; Martin and Wehner, 1987). Annual bluegrass is also a prolific seedhead producer, with single plants producing up to 2,200 seeds in a growing season (McCarty, 1999). Once established, annul bluegrass is difficult to eradicate. Management using herbicides and plant growth regulators (PGRs) is often inconsistent (Reicher and Gaussoin, 2013) and results in different levels of suppression.

Currently, there are no selective POST herbicides labeled for annual bluegrass control on creeping bentgrass putting greens, due to a risk of turf injury. At fairway mowing heights, bispyribac-sodium, an acetolactate synthase (ALS) inhibiting herbicide, has shown up to 90% control of annual bluegrass in creeping bentgrass (Branham and Calhoun, 2005). However, when applied to creeping bentgrass putting greens, unacceptable turfgrass injury has been observed (Teuton et al., 2007; Lycan and Hart, 2006). Methiozolin, an isoxazoline herbicide, effectively controls annual bluegrass $>$ 85% as POST under greenhouse and field conditions, while demonstrating safety to creeping bentgrass maintained as putting green(McNulty et al., 2011; Han and Kaminski, 2011; Askew and Koo, 2012). However, this herbicide is not yet registered for use in the United States.

PGRs are an alternative to herbicides, but only provide some measure of growth suppression. The most commonly used PGRs for shoot growth suppression include paclobutrazol and flurprimidol. As gibberellic acid (GA) biosynthesis inhibitors, paclobutrazol and flurprimidol reduce cell elongation, and ultimately shoot extension (Murphy et al., 2005). Isgrigg et al. (1998) reported that paclobutrazol suppressed annual bluegrass shoot growth for five weeks, while creeping bentgrass growth was suppressed for only three weeks. This difference results in a slow transition from annual bluegrass to creeping bentgrass in a mixed stand. Similarly, monthly applications of flurprimidol for two growing seasons reduced annual bluegrass population 78% on a creeping bentgrass fairway (Bigelow et al., 2007).

Frequently used PGRs for annual bluegrass seedhead suppression include mefluidide, ethephon, and combinations of ethephon and trinexapac-ethyl (TE). Mefluidide, a mitotic inhibitor, and ethephon, an ethylene promoter, have shown effective suppression of annual bluegrass seedhead when applications were made prior to seedhead formation (Cooper et al., 1987; Danneberger et al., 1987; Eggens et al., 1989; Inguagiato et al., 2010; Petrovic et al., 1985). TE, a late-stage gibberellin synthesis inhibitor, improves creeping bentgrass quality when tank mixed with ethephon (Haguewood et al., 2013), but when applied alone, it exhibits limited efficacy for seedhead suppression (Fagerness and Penner, 1998).

Treatment effect of PGRs for annual bluegrass shoot growth varies significantly when applied at different timings. Johnson and Murphy (1995) reported that spring and fall applications of paclobutrazol achieved 28% reductions in annual bluegrass shoot growth. In contrast, Woosley et al. (2003) found that summer applications of paclobutrazol resulted in the greatest reduction of annual bluegrass at 77%. Similarly, annual bluegrass stand reductions following flurprimidol application varied from 22 to

78% when applications were made between March and October (Baldwin and Brede, 2011; Johnson and Murphy, 1995; Bigelow et al., 2007).

To optimize annual bluegrass seedhead suppression, growing degree day (GDD) models have been developed in an attempt to provide a proper timing for PGR applications (Danneberger and Vargas, 1984; Danneberger et al. 1987). These models suggest PGRs should be applied at 10 GDD at a base temperature of 10°C (10 GDD_{10}) to optimize annual bluegrass seedhead suppression. However, these models were developed based on the use of mefluidide and may or may not be an optimal timing of other PGRs (Cooper et al., 1987; Inguagiato et al., 2010; Petrovic et al., 1985). In Virginia, late winter applications of ethephon alone followed by applications of ethephon + TE applied at 10 GDD_{10} improved annual bluegrass seedhead suppression by 87%, compared to ethephon + TE applied at 10 GDD_{10}, which only provided 32% seedhead suppression (Smith et al, 2013; Askew et al., 2012). A recently developed model for ethephon + TE suggests a base temperature of 0°C and a target GDD between 200 and 500 (Calhoun, 2004). However, the current model is only for the northern and northeastern United States.

Inconsistent suppression of annual bluegrass shoot growth and/or seedhead production from applications made at different timings warrants a need for detailed research. Therefore, the objective of this study was to evaluate various PGRs and herbicides on annual bluegrass shoot growth and seedhead development following applications at pre-boot, boot and flowering stages.

Materials and Methods

Annual bluegrass seed was collected from an annual biotype (*Poa annua* var. *annua*) population growing in cool season turf maintained as a home lawn in Columbia, Mo in the fall of 2012. Seeds were allowed to germinate, grow, and flower in a commercial potting mixture (Pro-mix, Premier Tech Horticulture, Quakertwon, PA) under greenhouse conditions prior to collecting the seeds. Collected seed (30% germination rate) were planted in a greenhouse spring 2013. After germination, individual annual bluegrass seedlings were transplanted into 10 cm diameter and depth polypropylene pots containing a commercial potting mixture (Pro-mix, Premier Tech Horticulture, Quakertown, PA). Plants were fertilized weekly with Miracle-Gro® All Purpose Pant Food (The Scotts Company LLC, Marysville, OH) at the rate equivalent to 12.25 kg N ha^{-1} and irrigated as needed to promote growth. Air temperatures during the experiment were maintained at 29 +/- 7°C, with supplemental lighting provided by high-pressure sodium lights for a 10/14 day/night photoperiod with light intensity of ~ 400 µmol m^{-2} s^{-1}.

Chemical treatments were applied to annual bluegrass plants at three different growth stages: pre-boot, boot, and flowering stages (Table 4.1). The pre-boot stage was defined as plants with 8 to 10 tillers. Boot stage was characterized when the stem apex for flower production was formed and a swelling at the base of the stem was observed, but prior to seedhead emergence. Plants entered the flowering stage when the first spikelet was visible, but prior to the release of pollen (Binkholder et al. 2010). Chemical treatments were made using an air-driven hydraulic sprayer calibrated to deliver 187 L ha^{-1} at a spray pressure of 167 kPa using a XR8001E flat fan nozzle (TeeJet Technologies, Springfield, Illinois).

Plants were evaluated for color, density, natural height, and canopy area at 0, 4 and 8 weeks after treatment (WAT). Annual bluegrass color and density were visually assessed using a 1 to 9 scale were 1= poorest color or density, 9= best possible color or density, and 6 = minimally acceptable color or density. Poorest color was defined as necrotic or brown plant tissue, best possible color indicates dark green, and minimally acceptable was light green color. Density was determined visually according to parameters established by the national turfgrass evaluation program (NTEP) (Morris, 2014). Plant natural height was measured as the distance of the plant from the furthest tip of the plant to the soil surface. Canopy area was determined by analyzing a digital image captured from the top of each plant, followed by calculating the total area for green pixels with comparison to a target with known area that was included in each image. The analysis was carried out by using WinRHIZO (Regent Instruments Inc., Quebec, Canada). At 8 WAT, the experiment was terminated and above ground plant tissues were harvested and dried in an oven to constant weights before the shoot dry biomasses were determined.

At 4 and 8 WAT, formed seedheads were recorded. Throughout the experiment, mature seedheads were harvested to determine total seed production and viability. Seedheads were deemed mature when the inflorescence from the point of the panicle tip to leaf sheath was golden brown in color, but prior to the release of seed from the inflorescence. Seeds were separated and cleaned by threshing seedheads and removing the extra debris, before the total seed weight was determined for each plant.

Annual bluegrass seed viability was evaluated using tetrazolium (2,3,5-triphenyl tetrazolium chloride) assays following the procedure described by Binkholder et al. (2010). Fifty seeds from each plant were soaked overnight between two pieces of deionized water-moistened filter papers. The testa of each seed was then pierced using a needle to facilitate tetrazolium uptake. Seeds were submerged in a tetrazolium salt solution (1% w/v) four hours at 38°C (Grabe and Peters 1998, Peters 2000). The seeds were then removed from the tetrazolium solution, and rinsed twice in deionized-water before exposure to 0.25 mL of an 85% lactic acid solution for 30 minutes. The lactic acid solution removed stains from the palea and lemma, allowing stained embryo to be clearly visible. Seeds were then visually assessed under a dissecting microscope at 10X magnification. Viable seed was defined as a seed with red-stained embryo outlined by a uniformly clear edge. Seeds lacking the red staining or clarity of the embryo outlines were considered to be non-viable (Fig. 4.1).

Factorial treatment combinations, including 10 chemical compounds and 3 annual bluegrass growth stages, were arranged in a randomized complete block design with 3 replications and two experimental runs. The variances of the experimental runs were homogeneous as determined by Levene's test and therefore, data were combined for ANOVA using Proc Mixed analysis in SAS 9.2 (SAS Institute, Cary, NC). Results for plant height, color, density, canopy area, and shoot biomass showed no interactions between chemical treatment and growth stage, therefore only main effects are presented. A significant interaction between chemical treatment and plant growth stage was found for seedhead counts; therefore data were analyzed separately for each growth stage. For the pre-boot and boot stages, no interactions between chemical treatment and evaluation time were found; therefore, only main effects are presented. For the flowering stage, significant treatment by evaluation timing was found; hence, data were presented separately for 4 and 8 WAT. Seed weight per seedhead, expressed as a percent to the untreated control, showed a significant treatment by growth stage interaction; and hence the interaction was analyzed by multiple comparison. Seed viability showed no interaction between treatment and growth stage, and hence, only treatment main effect was presented. Significant means were separated using Fisher's Protected LSD at P=0.05.

Results and Discussion

Treatment effects on shoot growth

Chemical treatments influenced plant height, color, density, canopy area, and shoot biomass. Annual bluegrass plants were uniform for height, color and density prior to treatment application (Table 4.2). When compared with the control, only annual bluegrass plants treated with paclobutrazol or bispyribac-sodium exhibited significant height reductions. The greatest height reduction followed applications of paclobutrazol, where annual bluegrass plant height was reduced 29 and 26% at 4 and 8 WAT, respectively. This result agrees with Beasley et al. (2007), who reported up to 59% height reductions from Kentucky bluegrass following applications of paclobutrazol. Bispyribac-sodium at 0.01 kg ai ha^{-1} reduced plant height by 13% at 8 WAT compared with the control. These results agree with regression curves configured by McCullough and Hart (2006), who showed that 0.03 kg ai ha^{-1} of bispyribac-sodium reduced annual bluegrass clipping production by 50%.

During the 8 week period, no significant differences in plant color were observed among treatments, compared with the untreated control (Table 4.2). However, differences were observed among treated plants. Bispyribac-sodium and ethephon both resulted in 10% color reductions when compared to maleic hydrazide or mefluidide at 8 WAT. Annual bluegrass chlorosis of up to 20% was reported following bispyribac-sodium applications at 0.028 kg ai ha^{-1} (McCullough and Hart, 2006). Ethephon has also been reported to significantly reduce creeping bentgrass quality (McCullough et al., 2005; Dernodeden and Pigati, 2009); however, no reports have demonstrated quality reduction on annual bluegrass alone. Our findings coincide with those of Gaussoin et al. (1997), who reported a 38% increase in leaf chlorophyll content of annual bluegrass following a mefluidide application of 0.14 kg ai ha^{-1}.

None of the treatments applied reduced annual bluegrass shoot density compared with the untreated control (Table 4.2). The increase in density was found in plants following applications of ethephon + TE and paclobutrazol. At 4 WAT, plant density was increased by 15 and 12% following treatments of ethephon + TE and paclobutrazol, respectively. By 8 WAT, only paclobutrazol treated plants demonstrated a significant increase in density compared with the control. No significant increases in plant density were found in plants that received only TE. These results are supported, partially, by Beasley and Branham (2007), who reported 27 and 47% increases in the number of Kentucky bluegrass tillers at 8 WAT following applications of TE or paclobutrazol, respectively.

Prior to chemical applications, no difference in canopy area was found for all annual bluegrass plants (Table 4.3). Annual bluegrass canopy area was reduced by 37, 33, 17 and 24% following applications of ethephon + TE, paclobutrazol, bispyribac-sodium, and maleic hydrazide 4 WAT, respectively. Treatment effects were short-lived, however, with the only exception of paclobutrazol treated plants which exhibited a significant canopy area reduction (26%) 8 WAT, compared with the control (Table 4.3; Fig. 4.2). Collectively, these results indicate that paclobutrazol provided the greatest residual activity for annual bluegrass shoot suppression among the chemical treatments included in this study. Similarly, Woosley et al. (2003) reported a 5.3 fold longer growth suppressions to annual bluegrass following application of paclobutrazol at 0.28 kg ai ha^{-1} compared to TE at 0.1 kg ai ha^{-1}.

Significant differences in shoot biomass harvested at 8 WAT were found among treatments (Fig. 4.3). Applications of ethephon + TE and paclobutrazol significantly reduced shoot biomass production. Compared with the control, shoot biomass of plants treated with ethephon + TE or paclobutrazol was reduced 19 and 31%, respectively. No other treatment significantly affected shoot biomass production. Our result agrees with Isgrigg et al. (1998), who reported a 40% biomass reduction of annual bluegrass following paclobutrazol applications 6 WAT.

Treatment effects on seedhead development

A significant interaction between chemical treatment and plant growth stage was found for seedhead production per plant. Therefore, data were analyzed separately for each growth stage. For pre-boot and boot growth stages, there were no interactions between chemical treatment and evaluation time, therefore, chemical treatment and evaluation timing main effects are presented plant (Table 4.4). Annual bluegrass plants treated at the pre-boot and boot growth stages showed a 4-fold increase in seedhead production from 4 to 8 WAT. No chemical treatments applied at pre-boot or boot growth stages significantly reduced seedhead production compared with the untreated control. However, numerically, pre-boot stage applications of ethephon and ethephon + TE reduced seedhead production 70 and 59%, respectively. These results support those of Haguewood et al. (2013), who found that ethephon and ethephon + TE reduced seedhead production up to 83 and 95%, respectively, under field conditions when applications were made equivalent to pre-boot stage annual bluegrass. Conversely, when applied at boot stage, seedhead production following applications of ethephon and ethephon + TE increased by 48% and 42%, respectively, compared with the control. These results are consistent with Smith et al. (2013), who demonstrated that applications of ethephon are more effective when applied earlier in the calendar year, which would imply a pre-boot growth stage. Although not significant, annual bluegrass plants treated with mefluidide had 63 and 13% more seedheads than the control when applied at the pre-boot and boot growth stages, respectively. When applied at the boot stage, paclobutrazol numerically reduced seedhead production by 35%. However, seedhead production was increased by 37% when applied at the pre-boot stage.

Prior to chemical applications at the flowering stage, annual bluegrass plants were statistically similar for the number of seedheads, ranging from 9.8 to 16.7 per plant. At 4 and 8 WAT, untreated plants produced 102 and 178 seedheads, respectively (Table 4.5). At 4 WAT, only maleic hydrazide significantly reduced seedhead development. At 8 WAT, both ethephon + TE and maleic hydrazide significantly reduced seedhead development, compared with the untreated control. Applications of maleic hydrazide at the flowering growth stage resulted in 67 and 53% seedhead reduction at 4 and 8 WAT, respectively. While these results are promising for suppression of annual bluegrass seedheads, injury to desired turfgrass species is a concern with maleic hydrazide applications (Engle and Aldrich, 1960; Haguewood et al., 2012). Annual bluegrass treated with ethephon alone had 41 and 43% more seedheads than the control at 4 and 8 WAT, respectively. However, when TE was added to ethephone (ethephon + TE) seedhead development was reduced 52 and 48% at 4 and 8 WAT, respectively (Table 4.5). Our results contrast those of Inguagiato et al. (2010) and Haguewood et al. (2013), who reported the addition of TE to ethephon applications did not reduce seedhead development compared to ethephon alone, and agree with Gelernter and Stowell (2001), who estimated a 20% reduction in seedhead development when TE is tank mixed with ethephon compared to ethephon alone. These differences in seedhead productions with or without addition of TE to ethephon are mainly due to the application timing. Treatments in studies conducted by Inguagiato et al. (2010) and Haguewood et al. (2013) were applied prior to flowering stage. In our study, for applications made at pre-boot and boot stages, no differences in seedhead counts were found in plants treated with ethephon alone or ethephon + TE (Table 4.4). Collectively, our results indicate that application timing is essential for PGRs when the objective is to suppress the annual bluegrass seedhead.

Seed weight per seedhead was presented as a percent relative to the untreated control (Table 4.6). Treatments applied at the pre-boot stage resulted in a wide range of seed weight per plant from 16 to 133% relative to the untreated control. When treated with ethephon and ethephon + TE, seed weight was reduced 45.4 and 40.9% compared with the control, respectively. Seed weight per seedhead was reduced 84.2 and 83.5%

following applications of maleic hydrazide and TE, respectively. When applied at the boot stage, chemical treatments increased seed weight per seedhead from 8 to 202%. The only exception was treatment of paclobutrazol, which decreased seed weight per seedhead 19%, compared with the control. A similar result was reported by Hampton and Hebblethwaite (1985), who observed a 4% reduction in seed weight following paclobutrazol applications to perennial ryegrass when applied at a similar growth stage. When applied at flowering, chemical applications reduced seed weight per seed head 14 to 60%; however, there were no significant differences among treatments.

Viability of annual bluegrass seed was not affected by any treatment tested, compared to the untreated control (Table 4.7). The average viability of the annual bluegrass seed was 28%, which coincides with that from McElroy et al. (2002), who reported a purchased annual bluegrass seed had a germination rate of 17.7%.

Collectively, our results indicate that the chemical treatments tested influence annual bluegrass height, color, density, canopy area and shoot biomass similarly when applied at pre-boot, boot, or flowering growth stages. Treatments of paclobutrazol suppressed annual bluegrass height the greatest among chemical treatments included. Treatments of ethephon + TE and paclobutrazol caused an increase in plant density and a reduction in canopy area and shoot biomass. Although no significant differences were observed, seedhead production was numerically reduced following pre-boot applications of ethephon and ethephon + TE. At flowering stage, however, an increase in seedhead production was observed following application of ethephon. Addition of TE to ethephon significantly reduced seedhead production compared to ethephon alone at flowering stage. These results clarify the importance of application timing of ethephon containing treatments for annual bluegrass seedhead development.

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Table 4.1. Chemical treatments and rates applied at pre-boot, boot and flowering growth stage of annual bluegrass in greenhouse study.

Entry	Treatment	Rate (kg ai ha^{-1})
1	Untreated	----
2	TE	0.06
3	Ethephon	3.8
4	Ethephon + TE[†]	3.8 + 0.06
5	Methizolin	0.5
6	Mefluidide	0.07
7	Flurprimidol	0.14
8	Paclobutrazol	0.224
9	Bispyribac-sodium	0.0124
10	Maleic hydrazide	1.12

[†]TE: trinexapac-ethyl

Table 4.2. Influence of chemical compound and application at pre-boot, boot and flowering growth stages on annual bluegrass plant height (cm), color (1-9) and density (1-9) at 0, 4 and 8 weeks after treatment (WAT).

		Weeks after treatment								
Treatment	Rate	0	4	8	0	4	8	0	4	8
	kg ai ha^{-1}	----------Height (cm)-----------			-----------Color (1-9)-----------			----------Density (1-9)----------		
Untreated	---	11.4	13.2	16.3	6.9	6.7	6.2	5.3	5.7	6.0
Bispyribac-sodium	0.01	11.2	12.9	14.2	6.8	6.3	5.7	5.1	5.6	5.8
Ethephon	3.80	11.9	17.3	15.2	6.9	6.2	5.7	5.4	5.7	5.3
Ethephon + TE†	3.80 + 0.06	11.5	12.6	16.3	6.9	7.2	6.1	5.2	6.7	5.8
Flurprimidol	0.14	10.7	12.2	14.4	6.8	6.7	6.1	5.1	5.5	6.2
Maleic hydrazide	1.12	11.6	12.3	17.3	6.9	7.1	6.4	5.1	6.1	6.4
Mefluidide	0.07	11.4	13.8	15.4	6.9	6.3	6.4	5.1	5.5	6.2
Methiozolin	0.50	11.1	14.0	14.5	6.9	6.2	5.8	5.2	5.2	5.3
Paclobutrazol	0.22	10.8	9.4	12.1	6.9	7.4	6.9	5.1	6.5	7.1
TE	0.06	11.5	12.9	15.9	6.9	6.8	6.6	5.2	6.3	6.3
LSD (P=0.05)		NS‡	1.5	2.0	NS	NS	0.7	NS	0.7	0.8
Growth stage										
Pre-boot		8.4	13.9	17.3	7.0	6.6	6.7	4.9	5.2	6.6
Boot		12.4	15.5	17.4	7.0	6.9	6.3	5.5	6.2	6.0
Flowering		13.2	9.7	10.8	6.7	6.6	5.6	5.3	6.2	5.5
LSD (P=0.05)		0.8	0.8	1.1	NS	NS	0.4	0.3	0.4	0.4

†TE: trinexapac-ethyl

‡Not significant at P=0.05 level.

Table 4.3. Influence of chemical compound applications at pre-boot, boot and flowing growth stages on annual bluegrass plant canopy area (cm^2).

Treatment	Rate	Weeks After Treatement (WAT)		
		0	4	8
	kg ai ha^{-1}	------------------------ Canopy Area (cm^2) ------------------------		
Untreated	---	277	560	613
Bispyribac-sodium	0.01	275	465	514
Ethephon	3.80	283	528	602
Ethephon + TE[†]	3.80 + 0.06	271	353	511
Flurprimidol	0.14	233	522	570
Maleic hydrazide	1.12	255	429	560
Mefluidide	0.07	277	572	549
Methiozolin	0.50	274	520	544
Paclobutrazol	0.22	245	377	454
TE	0.06	266	625	630
LSD (*P*=0.05)		NS[‡]	81	99
Growth Stage				
Pre-boot		80	468	572
Boot		225	549	777
Flowering		492	469	315
LSD (*P*=0.05)		41	45	54

[†]TE: trinexapac-ethyl

[‡]Not significant at *P*=0.05 level.

Table 4.4. Treatment and evaluation timing main effect on annual bluegrass seedhead counts at pre-boot or boot growth stages.

Treatment	Rate	Growth Stages when sprayed‡	
		Pre-boot	Boot
	kg ai ha^{-1}	---------- Seedhead counts per plant ----------	
Untreated	---	17	14
Bispyribac-sodium	---	36	21
Ethephon	0.01	5	27
Ethephon + TE†	3.80	7	24
Flurprimidol	3.80 + 0.06	16	13
Maleic hydrazide	0.14	10	12
Mefluidide	1.12	46	16
Methiozolin	0.07	13	21
Paclobutrazol	0.50	27	9
TE	0.22	14	6
LSD (*P*=0.05)	0.06	19	11
Evaluation timing			
4 WAT		7	6
8 WAT		31	26
LSD (*P*=0.05)		9	5

†TE: trinexapac-ethyl

‡No seedhead were observed at 0 WAT for pre-boot or boot growth stage.

Table 4.5. Treatment by evaluation timing interaction on annual bluegrass seedhead counts when applications were made at the flowering growth stage. Consistent seedhead counts for the flowering stage were assessed on 0 week after treatment (WAT) and ranged from 9.8 ~ 16.7 per plant.

Treatment	Rate	Weeks after treatment (WAT)	
		4 WAT	8 WAT
	kg ai ha^{-1}	------------- Seedhead counts per plant	---------------
Untreated	---	102 bc1[‡]	178 bc2
Bispyribac-sodium	0.01	103 bc1	197 bc2
Ethephon	3.80	174 d1	314 d2
Ethephon + TE[†]	3.80 + 0.06	49 ab1	92 a2
Flurprimidol	0.14	124 c1	241 c2
Maleic hydrazide	1.12	34 a1	85 a2
Mefluidide	0.07	83 b1	207 bc2
Methiozolin	0.50	106 bc1	225 c2
Paclobutrazol	0.22	108 bc1	241 c2
TE	0.06	84 bc1	175 b2

[†]TE: trinexapac-ethyl

[‡]Means followed by the same letter within the same column are not significantly different at *P*=0.05; Means followed by the same number in each row are not significantly different at the *P*=0.05 level.

Table 4.6. Percent annual bluegrass seed weight per seedhead relative to the control following chemical treatments applied at pre-boot, boot, and flowering growth stages.

Treatment	Rate	Application Growth stage		
		Pre-boot	Boot	Flowering
	kg ai ha^{-1}	--------------- Seed weight per seedhead (% of control) ---------------		
Bispyribac-sodium	0.01	133 b2[‡]	204 b2	59 a1
Ethephon	3.80	55 ab1	302 c2	86 a1
Ethephon + TE[†]	3.80 + 0.06	59 ab1	171 ab2	49 a1
Flurprimidol	0.14	91 ab12	182 b2	65 a1
Maleic hydrazide	1.12	16 a1	210 bc2	40 a1
Mefluidide	0.07	107 ab1	138 ab1	51 a1
Methiozolin	0.50	132 b1	108 ab1	72 a1
Paclobutrazol	0.22	64 ab1	81 a1	70 a1
Trinexapac-ethyl (TE)	0.06	17 a1	176 b2	55 a1

[†]TE: trinexapac-ethyl

[‡]Means followed by the same letter within the same column are not significantly different at *P*=0.05; Means followed by the same number in each row are not significantly different at *P*=0.05.

Table 4.7. Chemical treatment main effects on annual bluegrass seed viability deteminded using tetrazolium assays.

Entry	Treatment	Rate (kg ai ha^{-1})	% Alive Seed
1	Untreated	----	30.8 abc‡
2	TE	0.06	17.7 bc
3	Ethephon	3.8	31.8 abc
4	Ethephon + TE†	3.8 + 0.06	24.8 abc
5	Methizolin	0.5	35.2 ab
6	Mefluidide	0.07	28.4 abc
7	Flurprimidol	0.14	22.5 abc
8	Paclobutrazol	0.224	25.4 abc
9	Bispyribac-sodium	0.0124	37.1 abc
10	Maleic hydrazide	1.12	23.5 c

†TE: trinexapac-ethyl

‡Means followed by the same letter within the same column are not significantly differnet at the *P*=0.05 level.

Figure 4.1. Representative picture of annual bluegrass depicting viable and non-viable seeds, following exposure to tetrazolium. Seeds were considered viable when the embryo was stained red and the outline of the embryo was uiform; seeds lacking this clarity were characterized as non-viable.

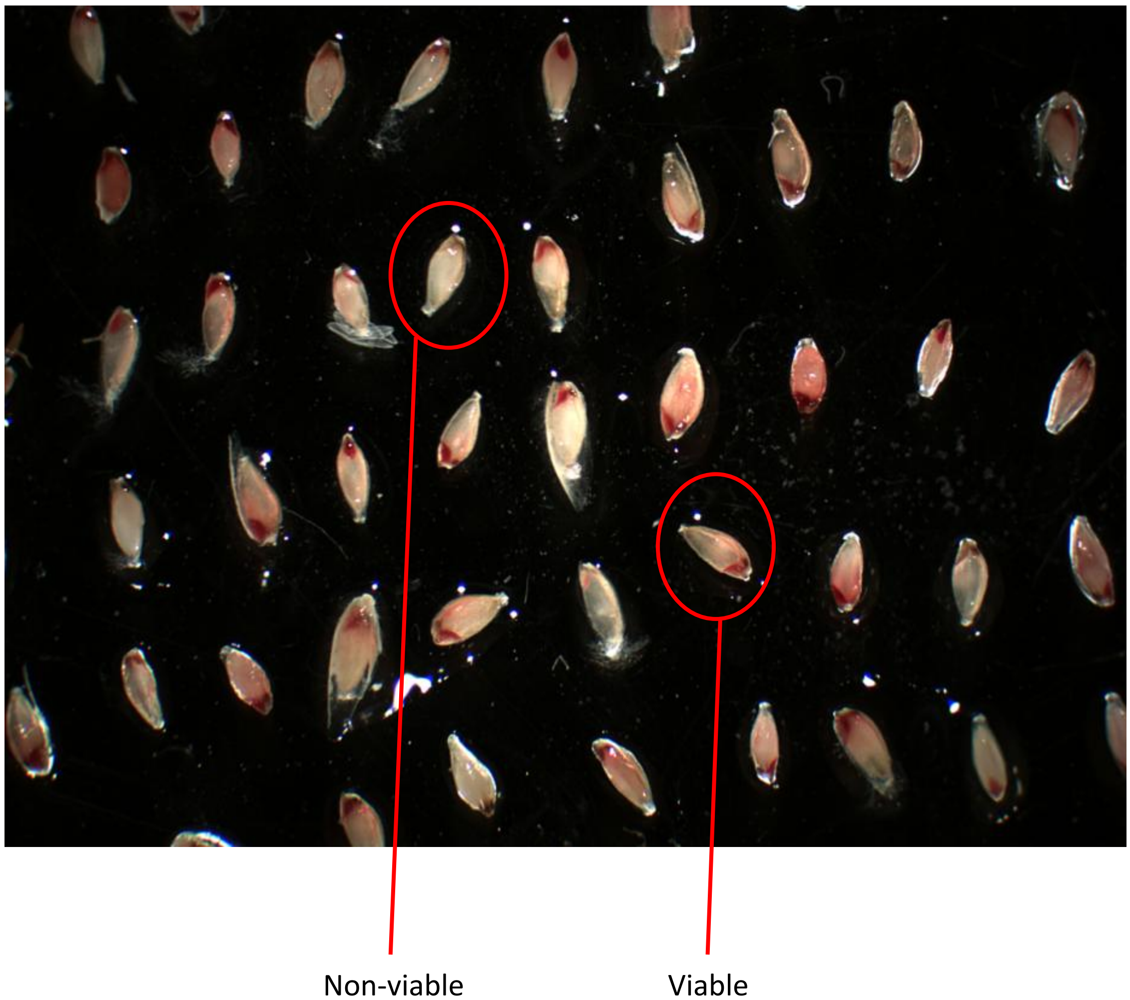
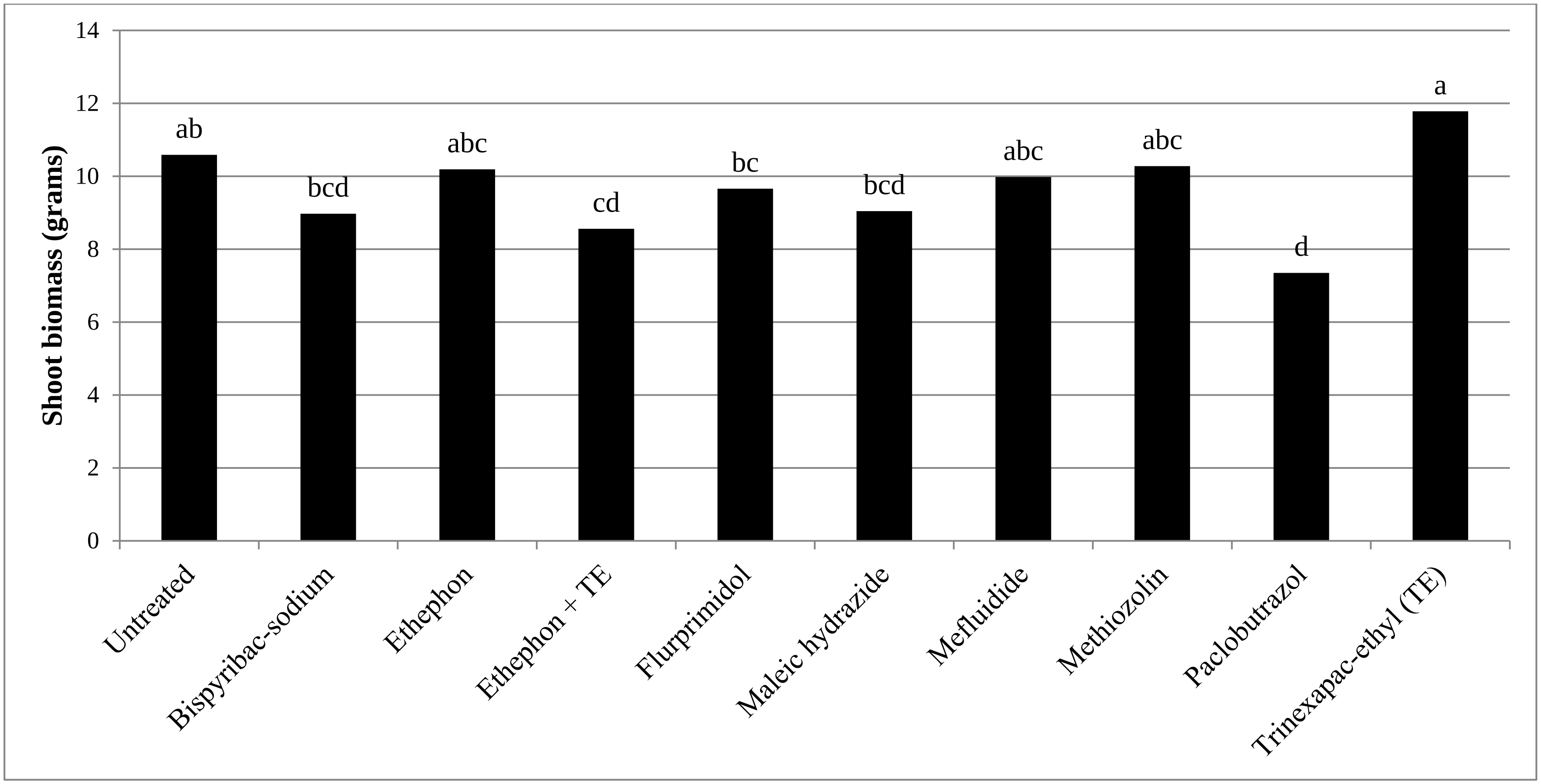


Figure 4.2. Representative annual bluegrass plants response to PGR applications applied at pre-boot growth stage. Rows 1, 2 and 3 represent 2, 4 and 8 weeks after treatment, respectively. Columns from left to right are treatments: untreated, trinexapac-ethyl (TE), ethephone + TE, and paclobutrazol.

Figure 4.3. Treatment main effect for shoot biomass harvested 8 weeks after treatment (WAT). Same letters above bars represent mean values which are not significantly different at the *P*=0.05 level.



APPENDIX

Table A.1. Description of treatment applied at the Country Club of Missouri in 2010 and 2011. Chapter II only includes data collected in 2010 from treatments 1-4 and 8-10. A total of 6, 4, and 3 applications were applied for treatments at 14, 21, and 28 day intervals, respectively. Initial applications were made on 21 June 2010 and 1 June 2011.

Entry	Treatment	Rate ($g\ ai\ ha^{-1}$)	Rate (oz/acre)	Application Interval (days)	PGR (28 days)
1	Control	---	---	---	---
2	BPS[1]	12.4	1	14	---
3	BPS	12.4	1	14	TE[2]
4	BPS	12.4	1	14	PB[3]
5	BPS	12.4	1	21	---
6	BPS	12.4	1	21	TE
7	BPS	12.4	1	21	PB
8	BPS	24.8	2	28	---
9	BPS	24.8	2	28	TE
10	BPS	24.8	2	28	PB

Table A.2. Creeping bentgrass phytotoxicity following treatments with bispyribac-sodium alone or tank-mixed with trinexapac-ethyl (TE) or paclobutrazol (PB) at 1, 2, 4, 6 or 8 weeks after initial treatment in 2010 and 2011.

	Weeks After Initial Treatment (WAIT)									
Entry	1	2	4	6	8	1	2	4	6	8
	------------------------------ 2010 -----------------------------					----------------------------- 2011 -----------------------------				
Control	8.8 a[2]	8.9 a	9.0 a	9.0 a	9.0[3]	9.0 a	9.0 a	9.0	9.0 a	9.0 a
BPS-L-14[1]	8.4 ab	8.9 a	9.0 a	9.0 a	9.0	9.0 a	9.0 a	9.0	8.0 a	9.0 a
BPS-L-TE	7.5 cd	7.8 cd	9.0 a	9.0 a	9.0	8.0 c	9.0 a	9.0	8.0 a	9.0 a
BPS-L-PB	6.5 e	5.9 e	9.0 a	8.0 c	9.0	8.0 c	9.0 a	9.0	9.0 a	9.0 a
BPS-L-21	7.9 bc	8.5 ab	8.5 b	9.0 a	9.0	8.5 b	9.0 a	9.0	8.0 a	9.0 a
BPS-L-TE	7.0 de	7.4 d	8.4 c	9.0 a	9.0	8.0 c	9.0 a	9.0	8.3 a	9.0 a
BPS-L-PB	6.6 e	5.8 e	8.0 d	8.5 b	9.0	8.0 c	5.0 b	9.0	6.3 b	8.0 b
BPS-H-28	7.6 c	8.5 ab	9.0 a	9.0 a	9.0	8.8 ab	9.0 a	9.0	9.0 a	9.0 a
BPS-H-TE	7.0 de	8.1 bc	9.0 a	8.5 b	9.0	8.0 c	5.0 b	9.0	5.3 c	8.0 b
BPS-H-PB	6.5 e	5.6 e	9.0 a	8.0 c	9.0	7.8 c	5.0 b	9.0	7.0 b	8.0 b

[1]Bispryribac-sodium-Low or High Rate (12.4-24.8 g ai ha^{-1})-Reapplication interval (14, 21 or 28 days)

[2]Means within a column followed by the same letter are not significantly different using Fisher's Protected LSD (P≥0.05)

[3]Means followed by no letter are statistically same

Figure A.1. Percent annual bluegrass remaining in plots 10 weeks after final applications from treatments in 2010 and 2011.