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PLANT GROWTH REGULATORS AND HERBICIDES FOR MANAGEMENT OF POA ANNUA: IMPACT OF BIOTYPES AND BEHAVIOR OF FLURPRIMIDOL IN TURFGRASS SPECIES

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DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in
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at the University of Kentucky

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2014

ABSTRACT OF DISSERTATION

PLANT GROWTH REGULATORS AND HERBICIDES FOR MANAGEMENT OF *POA ANNUA:* IMPACT OF BIOTYPES AND BEHAVIOR OF FLURPRIMIDOL IN TURFGRASS SPECIES

In 2011, *Poa annua* L. (*Poa*) biotypes were collected from greens of two golf courses in Lexington, Kentucky: 1.) The Lexington Country Club (LCC) and 2.) The University Club (UC). The samples were collected based on exhibiting one of two appearances while on the same green: 1.) dark green, with few to no flower heads (dark biotype) or 2.) light green, with numerous flower heads (light biotype). Two PGRs, paclobutrazol and flurprimidol, and two herbicides, bispyribac-sodium and amicarbazone, were applied to the plants both in the field and the greenhouse. Quality ratings were recorded weekly in both the field and greenhouse and grass clippings were collected and measured weekly in the greenhouse. Flurprimidol controlled the dark biotypes and paclobutrazol controlled the light biotypes in the field in 2011. However, only location by treatment interactions were in 2012; flurprimidol, bispyribac-sodium, and amicarbazone controlled the biotypes from the LCC while paclobutrazol controlled the biotypes from UC. In the greenhouse study there was a significant three way interaction between color, location, and treatment for quality. PGRs controlled the light biotypes from LCC and the dark biotypes from UC. Herbicides controlled the light biotypes more than the dark, however, the light biotypes recovered after amicarbazone treatments. PGRs reduced clipping weights of the dark biotypes more than the light and herbicides reduced clipping weights of the light biotypes more than the dark. Both PGRs and herbicides reduced clipping weights of the *Poa* collected from the LCC more than UC. These results demonstrate both the potential for differential responses between *Poa* biotypes to PGRs and herbicides and that these differences, like all things about *Poa*, may be complex. A laboratory experiment was also designed to examine the absorption and potential metabolism of ^{14}C-labeled flurprimidol in creeping bentgrass (*Agrostis stolonifera* (L.)), bermudagrass (*Cynodon dactylon* (L.)), Kentucky bluegrass (*Poa pratensis* (L.)), perennial ryegrass (*Lolium perenne* (L.)), tall fescue (*Festuca arundinacea* (Schreb.)), and zoysiagrass (*Zoysia japonica* (Steud.)) and light and dark *Poa* biotypes collected from golf greens. Flurprimidol absorption and translocation was greater for warm season grasses than cool season grasses. Only parent flurprimidol was detected in all turf species.

Keywords: Biotype, flurprimidol, *Poa annua* (L.), herbicide, plant growth regulator

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Chapter One

Poa annua (L.): A Review of its Biology and Management

Poa annua (L.) is a member of the *Poaceae* grass family, *Poodieae* subfamily, *Poodae* supertribe, *Poeae* tribe, and *Poa* genus (Chapman and Peat 1992; Watson and Dallwitz 1992). In North America, *Poa* common names include six-weeks grass, annual June grass, spear grass, annual spear grass, low spear grass, or walk grass (Hendry 1931; Gleason and Cronquist 1963; Vargas and Turgeon 2004). In Europe, *Poa* is referred to as meadowgrass, annual meadowgrass, dwarf meadow-grass, and Suffolk-grass. In Australia and Asia, it is known as wintergrass (Kellerman and Kellerman 1900; Curtis et al. 1824; Mao and Huff 2012). Other common names recorded for *Poa annua* include cause-way grass and, possibly, gemein grass which was mentioned in a 16th century book written by the herbalist, Jerome Bock (Arber 1934). *Poa* is Greek for grass or fodder and *annua*, naturally, refers to a plant with an annual life cycle (Gledhill 1985; Hyam and Pankhurst 1995). According to the Weed Science Society of America (2010), *Poa annua* L. is most commonly referred to as annual bluegrass, although this name has contradictions in reality as will be discussed in this review.

The term “bluegrass” first appeared in Christopher Gist’s Journals in 1751. Christopher Gist, an English settler, wrote describing the land of America, “full of beautiful meadows covered with wild Rye, blue grass and Clover" (Ahlstrom 2004). In 1879, Sir George Campbell published White and Black; the Outcome of a Visit to the United States. In this document he wrote, "The blue-grass of Kentucky is famous; though it is not blue at all, but green, and like our common natural grass" (Simpson and Weiner 1989). In 1889, Scribner, an American botanist, published a list of the grasses he

encountered while exploring the Roane Mountain (found between Tennessee and North Carolina) of which *Poa annua* was described to have been found "everywhere along the road and walks and about the hotel." Kellerman and Kellerman (1900), botanists from Ohio, listed *Poa annua* as an indifferent, non-indigenous grass species. In a 1911 publication, Brenchley described *Poa annua* as a weedy species in England. In a 1917 publication from Oregon, *Poa annua* was listed as an introduced weed species found "abundant on lawns and waste places" (Nelson 1917). One of the earliest published accounts of *Poa annua* on golf courses was in 1917 where it was described first as a weedy winter annual but later as "when abundant it makes excellent putting-greens" (Piper and Oakley 1917). In 1931, George Hendry examined the sun-dried bricks of Spanish adobe walls found in California. In his accounts, he confirmed finding *Poa annua*, an "alien weed", which he believed arrived during the Spanish Mission Period sometime between 1769-1824.

Today, *Poa annua* is the most problematic weedy grass species in managed turfgrass as well as uncultivated habitats (Beard et al. 1978; Warwick 1979; La Mantia and Huff 2011; McCarty 2011). *Poa annua* grows in most soil-types ranging from sands to clays (Warwick 1979), even taking root in concrete crevices. It is among the top 10 weed species studied in seedbank and seedling emergence experiments (Gardarin et al. 2009). *Poa annua* is often referred to as a "cosmopolitan" grass because it can be found on all seven continents, including Antarctica where it is believed to have been transferred from the shoes of explorers (Heide 2001; Chwedorzewska 2008). Initially believed to be native to Europe or Eurasia (Tutin 1952, 1957; Darmency and Gasquez 1997; Heide 2001), this world-wide naturalized grass species (Heide 2001; Chwedorzewska and

Bednarek 2012) can be found inhabiting polar regions in the Arctic Circle, temperate regions, Mediterranean climates, scorching hot deserts, and Tierra del Fuego, Argentina (Warwick 1979; Darmency and Gasquez 1981; Ruemmele 1989; Frenot et al. 2001). *Poa annua* has long interested scientists and scholars and has more recently gained interest among golf course superintendents.

Poa annua Genetics and Plasticity

Poa annua plants exhibit tremendous phenotypic variability, even within the same environment. Since the 1930's, numerous researchers have investigated the basis for this diversity. Nannfeldt (1937) proposed that *Poa annua* was an allotetraploid formed from crossing the morphologically similar grasses, *Poa infirma* H.B. Kunth and *Poa supina* Schrader. However, he was unable to successfully cross the two diploid species. Tutin (1952, 1957) and Hovin (1957a, b) investigated Nannfeldt's hypothesis by examining *Poa annua* at the morphological and cellular level. Tutin (1957) proposed that *Poa supina* was the female parent of *Poa annua* and that the morphological plasticity of this species was likely due to multiple hybridizations between *Poa supina* and another grass species. Karyotype studies conducted by Koshy (1968) suggested that *Poa annua* could have originated from either *Poa supina* or *Poa exilis* (also referred to as *Poa infirma*) and another unknown grass species. Ellis et al (1971) compared the genetic variation of seven populations of *Poa annua* and concluded that the "breeding system of *P. annua* remains an enigma." Pietsch (1989), believed that *Poa annua* could have arisen from a cross between *Poa supina* and *Poa trivialis* (rough bluegrass). Darmency and Gasquez (1997) were able to cross *Poa supina* and *Poa infirma* spontaneously with a 0-2% success rate; nearly all of the crosses survived and grew normally, but were sterile. They found

that the plants, like their parents, were diploid, but had features of the allotetraploid *Poa annua.*

Mao and Huff (2012) identified the evolutionary origin of *Poa annua* by using DNA fingerprinting and the nuclear sequences *trx* and CDO504 and chloroplast sequences *ndh*F and *trn* TLF. *Poa annua* was shown to be a polyploid species with the genomes of *Poa infirma* H.B. Kunth and *Poa supina* Schrader (Mao and Huff 2012). Hybridization of the diploids *Poa infirma* and *Poa supina* generates a sterile dihaploid plant (1n=2x=14 chromosomes) (Vargas and Turgeon, 2004; Mao and Huff 2012). Although sterile, fertility is restored after DNA doubling to give the allotetraploid *Poa annua* (2n=4x=28 chromosomes) (Huff, 1999). An allotetraploid species contains two sets of chromosomes from two different genomes; in the case for *Poa annua*, one genome is from *Poa infirma* while the other is from *Poa supina.* The hybridization and DNA doubling events lead to the extreme genotypic and phenotypic variability found in the *Poa annua* species.

Poa infirma is a grass species originating in the Mediterranean regions and Atlantic borders of Europe (Heide 2001). *Poa infirma* is a short-lived grass with an erect growing habit that produces flowers early in the year (Heide 2001). *Poa supina* is a grass species originating in the Alpine regions of central Europe and Scandinavia (Heide 2001). *Poa supina* plants have a prostrate growth habit and flower late in the year (Heide 2001). The cross between these two species is thought to be a natural one (Vargas and Turgeon 2004) although it is very curious how these two species came into close proximity. Predictions of when the initial cross occurred range from 10,000 to 2.4 million years ago when glaciers likely shifted *Poa supina* from the alpine region to the

Mediterranean regions where *Poa infirma* is found (Mao and Huff 2012; Tutin 1957; Clayton et al. 2006; Hobbs 1946). Mao and Huff (2012) suggest that further studies need to be conducted to explain the adaptability of *Poa annua* to extremely diverse environments.

Poa annua Morphology and Physiology

Poa annua L. is a cool season C3 grass (Vargas and Turgeon 2004). The seedhead was described by Hitchcock and Chase (1950) as follows:

> "Panicle pyramidal, open, 3 to 7 cm long; spikelets crowded, 3- to 6-flowered, about 4 mm long; first bloom 1.5 to 2 mm, the second 2 to 2.5 mm long; lemma not webbed at base, distinctly 5-nerved, more or less pubescent on the lower part of the keel sometimes simulating a web; anthers 0.5 to 1 mm long."

Poa annua is estimated to produce between 150,000 and 650,000 seeds per meter^2 per year (Lush 1988). *Poa annua* seeds collected from putting greens are known to germinate quickly even in varying temperatures and soil conditions (Wu et al. 1992; Rossi 2001). At 68 F, Wu et al. (1992) found that greater than 80% of the *Poa annua* seeds collected from California putting greens had germinated while only 20% of those collected from the rough germinated. However, at 47 F, there were no differences in germination between the seeds collected from the roughs or the putting greens. When mowed at a low height of cut, *Poa annua* inflorescences are often not as visible, therefore, vegetative identification is important (Beard et al. 1978). The vegetative characteristics of *Poa annua* were described by Beard (1978) as follows:

"vernation folded; sheath distinctly compressed, glabrous, whitish at base, keeled, split with over-lapping hyaline margins; ligule membranous, 1-3 mm long, thin, white, acute, entire; collar conspicuous, medium broad, glabrous, divided by the midribs; auricles absent; blades V-shaped, 2-3 mm wide, usually light green, glabrous, soft, boat-shaped apex, transparent lines on each midrib, parallel sided, flexuous transversely, margins slightly scabrous and hyaline; stems flat, erect to spreading sometimes rooting at the nodes and forming stolons."

Poa annua can be described as an annual or short-lived perennial (Warwick 1979). Among biotypes and environments, consistent characteristics of *Poa annua* include a "boat-shaped" leaf tip, a double mid-rib, a membranous ligule, and a panicle inflorescence.

Timm (1965) subdivided *Poa annua* into two subspecies according to their growth habit and life cycle: erect annual types are ssp. *annua* and the prostrate perennial types are ssp. *reptans* meaning "creeping." Full names are *Poa annua* ssp. *annua* (L.) Timm. and *Poa annua* (L.) ssp. *reptans* (Hausskn,) Timm., respectively. *Poa annua* spp. *annua* is common in disturbed land whereas *Poa annua* spp. *reptans* can be found mostly in lawns, grasslands, and in alpine snow beds (Cordukes 1977; Warwick and Briggs 1978*a*, *b*; Heide 2001). Law et al (1977) studied the life histories of 28 populations of *Poa annua* in England and Wales. They found that *Poa annua* populations located in pastures and older lawns were perennial (Law et al. 1977). *Poa annua* spp. *reptans* plants have the tendency to produce fewer flowerheads and more tillers than their annual counterpart.

Poa annua flowers are cleistogamous, meaning that the species propagates primarily using non-opening self-fertilizing flowers (Ellis 1973). The morphology of the flower limits outcrossing. However, outcrossing was between 0-15% (Ellis 1973) and as high as 22% when plants were exposed to a stressful environment (Mengistu et al. 2000; Chen et al. 2003). *Poa annua* var. *reptans* tends to have closed flowering structures and var. *annua* tends to have more open flowering structures. *Poa annua* was also reported to be apomictic (Johnson et al. 1993). Researchers testing *Poa annua* populations from America, Norway, and Australia confirmed divergent flowering responses among their collection of plants (Johnson and White 1997*a*, *b*; Heide 2001). Life cycles reported ranged from both summer and winter annuals in addition to biennial and perennial cycles (Heide 2001). Heide wrote that, "Virtually any kind of photoperiodic and vernalization responses can be found within the *Poa annua* complex" (2001). Other research has documented extensive morphological and physiological characteristics as well as both genetic and epigenetic mechanisms among *Poa annua* populations (Tutin 1957; Ellis 1974; Law et al. 1977).

Poa annua var. *reptans* plants have the tendency to flower in the spring and occasionally in the fall and are also often referred to as "seasonal-types" (Johnson et al. 1993). Conversely, *Poa annua* var. *annua* plants will often flower repeatedly throughout the growing season and are often referred to as "continual-types" (Johnson et al. 1993). Despite their many differences, the two subspecies are frequently found together growing together. Given that *Poa annua* is pandemic in many landscapes (Chwedorzewska 2008; Chwedorzewska and Bednarek 2012; Cline et al 1993; Cordukes 1977; Frenot et al 2001;

Lush 1989; Mengistu et al 2000; Scriber 1889; Woosley 2002), the occurrence of *Poa annua* eco or biotypes is not surprising (Heide 2001).

Poa annua on the Golf Course

In highly maintained turf, *Poa annua* is an undesirable grass because of its characteristic light color, course texture, profuse flowering habit, and susceptibility to heat, drought, and disease stresses (Beard 1970; Gibeault 1966). Superintendents and researchers have observed that *Poa annua* biotypes that are finer-textured and have higher density, slower growing vegetation become dominant and plants which are more coarse-textured, less dense, and are slower growing become less common (Cook 1996). On golf courses, a trained eye can often distinguish between multiple *Poa annua* forms on the same putting green whether the course is newly established or much older (Cook 1996). Variations in *Poa annua* populations found on creeping bentgrass golf greens are attributed to the inherent genetic and physiological processes combined with environmental conditions (Cline 1993). In a 2013 *Poa annua* survey conducted by the University of Kentucky, different *Poa annua* forms were reported by golf course superintendents to exist on putting greens, collars, fairways, and roughs. Golf superintendents have learned to work with the high phenotypic diversity of *Poa annua* on golf courses.

Poa annua hybridization, followed by selection pressure, has developed biotypes that respond differently to various environmental conditions (Vargas and Turgeon 2004). Gibeault (1971) examined morphological characteristics of *Poa annua* annual and perennial forms. Due to greater seed production, annual biotypes colonize open areas rapidly. However, they will often die in the summer due to low tolerance to heat,

drought, insect, and disease stresses. However, at the same time, greens are often maintained (i.e. frequent irrigation, fungicide application, and high fertility regimes) so well that these grasses can often survive the stresses of summer. The slower growing perennial biotype is less susceptible to environmental stresses but can be weakened by certain diseases or insects (Vargas and Turgeon 2004). In greenhouse studies, McNeilly (1981) examined the differences of *Poa annua* populations from open and closed habitats in response to competition with *Lolium perenne* and cutting. He found that *Poa annua* competitive ability is not a fixed characteristic but was dependent on the environment in which it is found. Huff (1999) reported that *Poa annua* "variability is reduced by 50% within a particular strain after every generation of self-pollination," such that the first selfing reduces diversity by 50%, the second by 25%, the third by 12.5%, and so forth.

Turf on golf courses with high populations of *Poa annua* requires high amounts of inputs to maintain acceptable quality, particularly in the summer (McCullough et al. 2010). Some of the practices employed by superintendents to maintain *Poa annua* quality are on-going surface disturbance (i.e. verticutting or core cultivation), green smoothing practices (i.e. topdressing, vertical mowing, and rolling), a well-adjusted fertility regime, regular irrigation (being careful to not allow extreme wet and dry periods), regular chemical control of insects and diseases, and finally, a high frequency of mowing events at a low height of cut (Cook 1996; Wu et al. 1992).

The weed species found on greens at the majority of the most highly acclaimed golf courses in the world is partially or sometimes even 100% *Poa annua* (Vargas and Turgeon 2004). *Poa annua* on the golf course is both a robust annual (*r*) (Law et al. 1977) and a perennial, often referred to as a "greens-type" phenotype (*K*) because of its

high turf quality and utility (Law et al. 1977; La Mantia and Huff, 2011). McNeilly (1981) wrote that *Poa annua* plants that are considered *r*-strategists can be found in "open" environments similar to a "garden or horticultural weed," while *K*-strategists are associated with "closed" habitats like "lawns, amenity, and agricultural grasslands, particularly those subject to heavy mowing, trampling, or grazing." Gibeault and Goetze (1972) observed that, among annual and perennial types of *Poa annua*, there is significant morphological variation that is specifically unique to ecological niches. La Mantia and Huff (2011) suggested that the greens-type phenotype is not stable and they hypothesized that this type is regulated by an epigenetic mechanism other than modifications in the core DNA sequence. When *Poa annua* plants that were collected from golf courses because they varied morphologically were transferred to a greenhouse, all plants eventually converted to the *Poa annua* var. *annua* phenotype (personal observation, 2013).

Poa annua growing in areas of minimal maintenance (i.e. golf course roughs) is an annual whereas populations that grow in a highly maintained environment (i.e. putting greens) are perennials (Johnson and Murphy 1995). Seedlings of *Poa annua* establish most readily in the type of environment/turf area from which the seeds were collected (Lush 1989). In the 1970's and 80's, genetic differences between *Poa annua* plants were detected in plants as close as 3 meters (Law 1977; McNeilly 1981; Warwick and Briggs 1978). Although, distinctively different phenotypic variations of *Poa annua* can be seen in adjacent plants on the same golf green (personal observation).

Lush (1989) found that *Poa annua* populations on putting greens are very different than those in fairways and roughs. California *Poa annua* populations growing

in dry, low maintenance areas (i.e. roughs) were annual, whereas those growing in a well irrigated, highly maintained area (i.e. putting greens) were perennial (Wu et al. 1992; Wu and Harivandi 1993). Woolsey (2002) conducted studies on *Poa annua* from three different environments (putting greens, fairways, and roughs). Using random amplified polymorphic DNA (RAPD) analysis, he found less genetic diversity in *Poa annua* plants from putting greens than in those collected from fairways and roughs. Some of the individual genotypes from roughs and fairways were similar to those of putting greens. Woolsey's (2002) results showed a stronger selection pressure (individuals with more adaptive traits, contributing to more offspring to the succeeding generation) in putting greens when compared to fairways and roughs.

Management of Golf Course *Poa annua* with Plant Growth Regulators and/or Herbicides

Plant Growth Regulators

It is very difficult to selectively remove a weedy grass species growing with a desired grass species. In addition, it is even more difficult to slowly remove the weed from highly maintained landscapes to avoid voids in the turf following death of the weed. The majority of the putting greens in the transition zone are planted with creeping bentgrass, although there are bermudagrass greens in this region as well. Ideally, an effective control measure on golf greens would cause *Poa annua* to slowly disappear from the green, unbeknownst to the golfer, and allowing time for the desirable grass to fill the resulting void. This is the challenge that golf superintendents face when considering approaches to reduce *Poa annua*. The weapons of choice are often gibberellic acid synthesis inhibiting plant growth regulators (PGRs) for use in fine turfgrasses (Murphy et al. 2000). There are two main types of plant growth regulators

applied to fine turf: Type I and Type II. Type I PGRs are primarily absorbed through the foliage and inhibit cell division in the plant meristem (Christians 2001). Mefluidide is an example of a Type I PGR that inhibits cell division (Murphy et al. 2000). Type II plant growth regulators are generally crown and root absorbed; they suppress growth by inhibiting gibberellic acid (GA) synthesis (Christians 2001). Under the Type II category, there are Class A and Class B PGRs. Class A (trinexapac-ethyl) interferes very late in GA biosynthesis and Class B inhibit GA production earlier in the biosynthetic pathway (Christians 2001). Type II Class B (flurprimidol and paclobutrazol) PGRs are the most effective for *Poa annua* control. These PGRs suppress *Poa annua* growth and allow the desirable grass species to outgrow the weed.

Suppression of *Poa annua* on putting greens using PGRs was reported in several studies. Breuninger (1993) found that flurprimidol effectively inhibited the growth of the *Poa annua* var. *reptans* while stimulating lateral growth in creeping bentgrass. Flurprimidol rates ranged from 0.14 to 0.28 kg/ha and it was applied beginning in the spring and repeated at 3 to 6 week intervals until early fall. Flurprimidol treatment resulted in a 20% *Poa annua* population reduction. Johnson and Murphy (1995), however, found that applying flurprimidol twice in the spring and twice in the fall (totaling 1.8 kg/ha for two consecutive years) did not reduce *Poa annua* populations. However, paclobutrazol applied at the same timings and same rates as flurprimidol reduced *Poa annua* populations 52% one month after all treatments. But, 4 months after the final treatment, *Poa annua* populations were reduced only 28%, indicating that additional paclobutrazol treatments were required (1995). Johnson and Murphy (1996) later confirmed that paclobutrazol controlled the perennial subspecies of *Poa annua*

better than flurprimidol. Extended suppression of *Poa annua* with paclobutrazol resulted in creeping bentgrass dominance (Woosley et al. 2003). Paclobutrazol applications in the spring and summer provided a more uniform creeping bentgrass turf and optimum *Poa annua* control in fairways was achieved with paclobutrazol applications every 2-3 weeks.

Herbicides

Preemergence herbicides (prodiamine and bensulide) can be effective for controlling *Poa annua* seedlings (Callahan and McDonald 1992; Dernoeden 1998; Goss et al. 1980). However, these treatments do not control existing populations. Rossi (2001) observed reduced *Poa annua* control when trinexapac-ethyl was applied in combination with either a preemergence (prodiamine) or postemergence (ethofumesate) herbicide. They hypothesized this antagonism could be caused by "pre-stressing" the *Poa annua* plants.

Developing a post-emergence herbicide to selectively control *Poa annua* without damaging creeping bentgrass is a difficult task since both are cool-season grasses. In 2010, bispyribac-sodium, a postemergence herbicide, was registered to control *Poa annua* in established turf on golf courses, but not for putting greens. Bispyribac-sodium is an acetolactate synthase inhibiting herbicide and a member of the pyrimidinyl carboxy herbicide family (Shimizu et al. 2002). Symptoms of bispyribac-sodium toxicity on susceptible species includes: vein reddening, chlorosis, and a brown and weak growing point (McCarty and Estes 2005). Bispyribac-sodium can selectively control *Poa annua* in creeping bentgrass, perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*) and chewings fine fescue (*Festuca rubra* subsp. *cummutata*), and Kentucky

bluegrass (*Poa pratensis*). Some Kentucky bluegrass cultivars are more tolerant to applications of bispyribac-sodium than others (Lycon and Hart 2005, 2006; Shortell et al. 2007). The principal use for bispyribac-sodium is to control *Poa annua* on creeping bentgrass tees and fairways and overseeded perennial ryegrass fairways (Branham and Calhoun 2005). Studies with bispyribac-sodium suggest that *Poa annua* infestations in creeping bentgrass can be controlled using rates ranging between 60 and 140 g ha^{-1} (Askew et al. 2004; Borger and Watschke 2005; Lycan et al. 2003; Lycan and Hart 2005). Lycan and Hart (2006) demonstrated that two applications of bispyribac-sodium at 74 g a.i. /ha in the summer can reduce *Poa annua* cover while reducing creeping bentgrass injury. McCullough and Hart (2010) found the most successful bispyribac-sodium regime for *Poa annua* control in creeping bentgrass was 24.6 g a.i. ha^{-1} applied weekly for up to 2 months.

Temperature influences bispyribac-sodium activity. McCullough and Hart (2006) found that temperatures ranging between 20 and 30 C increased *Poa annua* control with bispyribac-sodium in the growth chamber. At low temperatures (10 C), there was little *Poa annua* control. Bispyribac-sodium is not labelled for use on putting greens; however, studies have shown that creeping bentgrass maintained at 3 mm has the potential to tolerate the herbicide (Teuton et al. 2007). The use of spray adjuvants with bispyribac-sodium applications can increase *Poa annua* control at reduced application rates (McCullough and Hart 2008). Although, bispyribac-sodium is primarily root absorbed and then translocated to the shoots (Lycan and Hart 2006).

Amicarbazone, also a post-emergence herbicide, was registered in 2012 for the selective control of *Poa annua* on the golf courses. Amicarbazone is a triazolinone

photosystem II (PS II) inhibiting herbicide (Dayan et al. 2009). Symptoms of amicarbazone toxicity on susceptible plants include: chlorosis, stunting, necrosis initiating at leaf margins, and eventually advancing throughout shoot tissue, leading to the eventual death of the plant (Senseman 2007). Amicarbazone is rapidly absorbed and translocated within sensitive plants (Dayan et al. 2009). Amicarbazone is primarily absorbed by *Poa annua* roots (Perry et al. 2011). Amicarbazone was absorbed and translocated more in *Poa annua* than creeping bentgrass and tall fescue (Perry et al. 2011). Amicarbazone was also metabolized less in *Poa annua* than creeping bentgrass and tall fescue (Yu et al. 2013). McCullough et al. (2010) found that fall-applied amicarbazone treatments injured creeping bentgrass and Kentucky bluegrass stands more than spring applications, however, fall treatments were also more effective for the control of *Poa annua.*

Methiozolin, belonging to the isoxazoline chemistry class, is a herbicide under development for the control of *Poa annua* and other annual grass species (Brosnan et al. 2013; Han and Kaminski 2011; Hwang and Koo 2009; McCullough and Gomez de Barreda 2012; McNulty and Askew 2011; Nam et al. 2012; Norsworthy et al. 2011). It is currently not registered in the United States but is used in Korea. Methiozolin provided both pre-emergence and post-emergence control of *Poa annua* (Haguewood et al. 2012; Han and Kaminski 2011 and 2012; Hoyle et al. 2012; Hwang and Koo 2009; McNulty and Askew 2011; Nam et al. 2012; Trappe et al. 2012). Flessnor et al (2013) found that methiozolin was absorbed by both shoots and roots. In contrast, Brosnan et al (2013) concluded that methiozolin is primarily a root absorbed herbicide. They reported that soil-only and foliar-plus-soil methiozolin application resulted in better *Poa annua* control

than foliar-only application (Brosnan et al. 2013). Because of reported limited translocation, methiozolin should be applied to both the leaves and the roots of *Poa annua* (Flessnor et al. 2013). McCullough et al (2013) found no methiozolin metabolites in either *Poa annua* or creeping bentgrass, suggesting that methiozolin selectivity may be attributed to differential absorption, translocation, or target site sensitivity rather than metabolism.

Poa annua Resistance to Chemical Control

The repeated application of the same herbicide to a weed population can select resistant biotypes (Gringnac 1978). Gringnac (1978) reported that annual biotypes of *Poa annua* can rapidly develop herbicide resistance. Resistance to atrazine (Barros and Dyer 1988), simazine (Yelverton and Isgrigg III 1998) dinitroaniline chemistries (Isgrigg III et al. 2002), glyphosate (Binkholder et al. 2011), haloxyfop, clethodim, paraquat, diuron and ethofumesate (Eelen et al. 1999) in *Poa annua* have all been reported. According to the International Survey of Herbicide Resistant Weeds, there are 25 reported cases of herbicide resistance in *Poa annua* as of 2013 (Heap 2012). Of those reported, six were found on golf courses and half had displayed PS II triazine resistance (Heap 2012). Perry et al (2012) found that *Poa annua* populations that are triazine resistant are also resistant to amicarbazone. *Poa annua* biotypes resistant to ALS inhibiting herbicides on golf courses were reported in both Georgia and South Carolina (Cross et al. 2013). Brosnan et al (2014) reported that a putative prodiamine-resistant *Poa annua* population from a golf course in Tennessee was controlled by indaziflam. Although new chemistries are being developed for the control of *Poa annua* in turfgrass systems, recent history suggests that the continuous control of these populations should involve a diversity of techniques.

Chapter Two

Investigating *Poa annua* Biotypes Collected from Golf Greens

Poa annua (L.), commonly known as annual bluegrass, is the most widespread weedy grass species found in managed turfgrass (Warwick 1979; La Mantia and Huff 2011). Contrary to its common name, both annual (*Poa annua* ssp. *annua* (L.) Timm.) and perennial (*Poa annua* ssp. *reptans* (L.) (Hausskn.) Timm.) subspecies of *Poa annua* exist (Timm 1965; Vargas and Turgeon 2004). *Poa annua* spp. *reptans* has fewer flowerheads and more tillers than its annual counterpart (Timm 1965). *Poa annua* var. *reptans* flowers in the spring and occasionally in the fall; whereas *Poa annua* var. *annua* will often flower repeatedly throughout the growing season (Johnson et al. 1993). Despite their opposite natures, the two subspecies are commonly found coexisting, sometimes contiguously, on golf course putting greens and fairways.

Poa annua decreases turfgrass quality on the golf course because it creates an uneven playing surface and decreases the aesthetic quality due to the light green color and high levels of seedhead production (Beard et al. 1978; Goss and Zook 1971). Superintendents, scientists, and industry researchers have tried for many years to find a chemical solution to control *Poa annua* on the golf course; however, it continues to be a challenge to manage. Plant growth regulators (PGRs) and herbicides are currently both employed for the management of this weed. Flurprimidol and paclobutrazol are Type II PGRs that are used by golf course superintendents to reduce *Poa annua* infestations on fairways and greens (Branham 1991). The effectiveness of PGRs to control *Poa annua* depends on the rate and timing of applications (Johnson and Murphy 1995). PGRs can gradually reduce *Poa annua* populations and with little or no damage to creeping

bentgrass *(Agrostis stolonifera* (L.)), a common turf species used on golf course greens in the transition zone (Branham 1991). Type II PGRs are known to have a greater inhibitory effect on *Poa annua* than on creeping bentgrass (Christians 2001).

Within the last few years, herbicides have been developed for the control of *Poa annua.* Development of a herbicide to selectively control *Poa annua* without damaging creeping bentgrass is a difficult task since these are both cool-season grasses. For this reason, PGRs are often seen as more acceptable tools for managing *Poa annua* than herbicides, particularly on putting greens. Bispyribac-sodium, a postemergence herbicide, was labelled in 2010 to control *Poa annua* on golf courses but not on putting greens. Bispyribac-sodium is an acetolactate synthase (ALS) inhibiting herbicide and part of the pyrimidinyl carboxy herbicide family (Shimizu et al. 2002). Amicarbazone, also a post-emergence herbicide, was registered in 2012 for selective control of *Poa annua* on the golf course. Amicarbazone is a triazolinone photosystem II (PS II) inhibiting herbicide (Dayan et al. 2009).

Herbicide resistance can be a problem with biotypes of *Poa annua*. According to the International Survey of Herbicide Resistant Weeds (Heap 2012), there are 25 reported cases of *Poa annua* herbicide resistance as of 2013. Of those, seven were found on golf courses (Heap 2012). Perry et al (2012) found that triazine resistant *Poa annua* populations were also resistant to amicarbazone. *Poa annua* biotypes resistant to ALS inhibiting herbicides on the golf course were also reported in Georgia and South Carolina (Cross et al. 2013).

Differential responses between *Poa annua* subspecies, ecotypes, biotypes, and populations to control treatments have been reported. *Poa annua* spp. *reptans* was

successfully controlled using paclobutrazol at both putting green (Johnson and Murphy 1995) and fairway height (Woosley et al. 2003). Adams and Bryan (1980) collected *Poa annua* from seven turfgrass sites. They grew these plants under three levels of nitrogen and three different heights of cut. They found that *Poa annua* types from soccer and cricket fields produced more biomass regardless of management regime than those types collected from golf and bowling greens. Wu et al. (1992) collected biotypes from both roughs and greens and found that, when exposed to nine combinations of paclobutrazol and light intensities, both biotypes produced more dry biomass under low light than under high light intensities. However, they also found that plants from golf greens produced greater total dry biomass than rough biotypes under all treatment combinations. Callahan and McDonald (1992) found that bensulide (a pre-emergence herbicide) controlled the annual subspecies but not the perennial subspecies of *Poa annua*. McElroy et al (2004) conducted laboratory studies to evaluate the variation in germination of eight *Poa annua* ecotypes to photoperiod, temperature, and fenarimol (a fungicide-herbicide used for pre and post-emergence *Poa annua*). They found significant ecotype by environment interactions affecting *Poa annua* germination. Some ecotypes germinated better at higher temperatures and had variable shoot length responses to applications of fenarimol.

Despite the aforementioned studies, there is limited research comparing differential responses of *Poa annua* biotypes to PGR and postemergence herbicide applications. Based on recent reports and our own observations, we hypothesize that *Poa annua* biotypes will respond differently to chemical treatments. Therefore, the objectives of this research were to compare the responses of *Poa annua* biotypes collected from

Kentucky golf course putting greens to applications of flurprimidol, paclobutrazol, bispyribac-sodium, and amicarbazone.

Materials and Methods

Plant Material

In the spring of 2011, *Poa annua* types were collected from two different golf courses in Lexington, Kentucky: 1.) The Lexington Country Club (2550 Paris Pike Lexington, KY 40511) and 2.) The University Club of Kentucky (4850 Leestown Road Lexington, KY 40511) (Figure 2.1). The Lexington Country Club is an older golf course where putting greens are maintained as to favour the health of *Poa annua* while The University Club of Kentucky putting greens are managed for creeping bentgrass including applications of products to reduce *Poa annua* populations. *Poa annua* biotypes were selected based on having one of two physical criteria (Figure 2.2): 1.) a light-green colored plant with many flowerheads and coarse-texture and 2.) a dark -green colored plant with few to no flowerheads and fine-texture. Using a cup cutter, these light and dark biotypes were collected from multiple putting greens from each golf course. The putting greens that were utilized for collections had both light and dark populations within the same green, often side by side. *Poa annua* plugs were placed in a container and carefully labelled as from which golf course and putting green it was collected and whether or not it was a light or dark biotype. After collecting, the plugs were taken to the greenhouse where they were quartered and planted in 10 cm x 10 cm plastic pots filled with 50% Maury silt loam soil (fine, mixed, mesic typic Paleudalfs), collected from Spindletop Farm, Lexington, KY and 50% coarse builder's sand (Clay Ingels Co. LLC. 914 Delaware Ave, Lexington, KY). Osmocote® Slow Release fertilizer (19-19-19, 2

g/0.001 m^3, The Scotts Company LLC. Marysville, OH) was added to the sand/soil mix. Plants were clipped using scissors to 1.6 cm weekly.

Field Study

Once plants were established, they were transferred from the greenhouse to the A.J. Powell Jr. Research Center at Spindletop Farm in Lexington, KY in early June 2011. The research site was in an existing stand of 'L-93' creeping bentgrass (*Agrostis stolonifera* L.) maintained at fairway height (1.6 cm) and mowed 3 times per week during the normal growing season. The study was organized in a split plot design with herbicide and PGR treatments serving as the main plots and the biotypes were the split plots. There were four replications and main plot size was 1.5 m by 1.5 m. Approximately one week prior to planting the plugs, each split plot was sprayed with glyphosate (560 g a.i./ha^{-1} Mad Dog® Plus, Loveland Products, INC. P.O. Box 1286, Greeley, Colorado 80632-1286) was applied in each split plot to kill a circular area (approximately 20 cm^2) of grass. This area created separation between the existing bentgrass and the *Poa annua* plugs. To maintain this separation, paraquat (280 g a.i./ha^{-1} Gramoxone® SL, Syngenta Crop Protection, LLC P. O. Box 18300 Greensboro, NC 24719-8300) was sprayed carefully around the plugs (while covered) once per month. Mesotrione (Tenacity™, Syngenta Crop Protection, Inc. P.O. Box 18300 Greensboro, North Carolina 27419-8300) at 168 g a.i./ha^{-1} was applied monthly to the *Poa annua* plugs to suppress any creeping bentgrass that may have been collected with the *Poa annua* from the golf courses.

There were 5 treatments in this study, two commonly used Type II PGRs (flurprimidol [Cutless® MEC Turf Growth Regulator SePro Corporation 11550 North Meridian Street, Suite 600 Carmel, IN 46032] and paclobutrazol [Trimmit® 2SC Plant

Growth Regulator Syngenta Crop Protection, LLC P.O. Box 18300 Greensboro, North Carolina 27419-8300]), two herbicides (bispyribac-sodium [Velocity® SG Herbicide Valent U.S.A. Corporation P.O. Box 8025 Walnut Creek, CA 94946] and amicarbazone [Xonerate® Herbicide Arysta LifeScience North America, LLC 15401 Weston Parkway, Suite 150 Cary, NC 27513]), and an untreated control. Flurprimidol (490 g a.i./ha^{-1}) and paclobutrazol (270 g a.i./ha^{-1}) were applied every three weeks, bispyribac sodium (25 g a.i./ha^{-1})was applied once in the summer and once in the fall, and amicarbazone (49 g a.i./ha^{-1}) was applied four times weekly commencing at the beginning of the study (Table 2.1). All treatments were made using a CO_2 pressurized 1.8 m wide boom sprayer with 4 TeeJet® 8004 flat fan tip nozzles (P.O. Box 7900 Wheaton, IL 60187) spaced 48 cm apart. The carrier volume was 496 L/ha^{-1} at 207 kPa spray pressure. These treatments were applied in 2011 and again, to the same plugs, in 2012.

Turf quality of the *Poa annua* plugs was rated visually on a scale from 1 to 9, where 1 = poor and 9 = excellent quality. Data was subjected to analysis of variance and analyzed using PROC GLM in SAS® Statistical Software using a repeated measures statement. Means were separated using Fischer's Protected LSD with P= 0.05. Interactions were separated using LSMeans. Results from 2011 and 2012 were significantly different from each other and are, therefore, reported separately.

Greenhouse Studies

Two separate greenhouse studies were conducted at the University of Kentucky Greenhouse Facility in Lexington, KY between the spring and fall of 2011 and the spring and fall of 2012. The same collection of light and dark biotypes used in the field study was used in these experiments and planted as previously described. To suppress any

creeping bentgrass present in the plugs, mesotrione at 168 g. a.i./ha^{-1} was applied prior to the experiments. Plants were maintained with a 16 h photoperiod and temperatures ranged between 21 to 30 C.

Once plants were well-established, the same treatments were applied at the same rates and frequencies used in the field study (Table 2.1). All treatments were made using a CO_2 pressurized spray chamber. The carrier rate volume 280 L/ha^{-1} at 207 kPa spray pressure. Plants were arranged in a completely randomized design and there were 4 replications.

Clipping weights and *Poa annua* quality ratings were collected weekly. Turf quality was rated visually on a scale from 1 to 9, where 1 = poor and 9 = excellent quality. Both fresh and dry weights were measured for this study, but only fresh weights are reported. Treatment effects were similar for dry and fresh weight responses. Data was subjected to analysis of variance and analyzed using PROC GLM in SAS® Statistical Software using a repeated measures statement. Means were separated using Fischer's Protected LSD with p= 0.05. Interactions were separated using LSMeans. Results for 2011 and 2012 were significantly different from each other and are, therefore, reported separately.

Results and Discussion

Field Study

In 2011, there were significant biotype by treatment interactions (Table 2.2). There was no difference in quality between the color types in the control. Paclobutrazol reduced the quality of the light biotypes over the dark biotypes 8, 9, and 10 weeks after initial treatment (WAIT). Conversely, flurprimidol reduced the quality of the dark

biotypes over the light biotypes 3, 4, 5, 6, and 7 WAIT. These were the same results noted by the University Club golf course superintendent where he found the light types were controlled by paclobutrazol and the dark controlled by flurprimidol. There were no significant differences in responses between the biotypes to bispyribac-sodium or amicarbazone treatments. It appears that flurprimidol treatments could selectively control dark biotypes over light and thus result in a mostly light colored *Poa annua* putting green. Whereas, paclobutrazol could selectively control the light colored biotypes and, thus, result in a mostly dark colored *Poa annua* putting green. Assuming that the dark biotypes represent the perennial subspecies of *Poa annua,* these results are contradictory to those found by Johnson and Murphy (1996) where they found that paclobutrazol controlled the perennial subspecies of *Poa annua* better than flurprimidol. It is also interesting to note bispyribac-sodium did not control either color types.

In 2011, there were also *Poa annua* turf quality differences in the plants collected for the two locations (Table 2.3). Over all treatments and biotypes, *Poa annua* from the Lexington Country Club had higher quality ratings 1, 2, 3, 4, and 9 WAIT than *Poa annua* collected from the University Club. This may indicate that the Lexington Country Club biotypes may be more resistant to these treatments or simply have higher inherent quality. *Poa annua* collected from the Lexington Country Club was found on putting greens that had mostly converted to populations of *Poa annua*, while the University Club's putting greens are managed for creeping bentgrass.

In 2012, only location main effects and location by treatment interactions were significant. *Poa annua* from the University Club had higher quality ratings than *Poa annua* from the Lexington Country Club 3 and 7 WAIT (Table 2.3). This is in contrast to

2011, when *Poa annua* from the Lexington Country Club had higher quality than that from the University Club in 2012. In Kentucky, 2011 had above average rainfall and milder temperatures June through October compared to 2012 which had higher temperatures in July and very little precipitation June, August, and October (Table 2.4).

Lexington Country Club *Poa annua* had lower quality at some sampling times than the *Poa annua* from the University Club in the paclobutrazol, bispyribac-sodium, or amicarbazone treatments (Table 2.5). Paclobutrazol treatments reduced Lexington Country Club *Poa annua* quality at 6, 7, and 12 WAIT. The University Club applies paclobutrazol and flurprimidol on a rotational program every two to three weeks during the summer. Even though paclobutrazol is used to decrease *Poa annua* populations within creeping bentgrass, without the competition, perhaps the *Poa annua* collected from the University Club is resistant to paclobutrazol while the Lexington Country Club is more susceptible to flurprimidol. Bispyribac-sodium treatments reduced the Lexington Country Club *Poa annua* quality more than the University Club *Poa annua* quality at 3 and 12 WAIT. Amicarbazone reduced the quality of the Lexington Country Club *Poa annua* more than the University Club *Poa annua* at 5, 6, 7, 8, and 12 WAIT. It should be noted that, 3 and 4 weeks after the amicarbazone initial treatment, *Poa annua* collected from both locations had low (< 6) quality ratings. *Poa annua* collected from the University Club recovered from amicarbazone injury 4 weeks faster than Lexington Country *Poa annua.* Flurprimidol treatments reduced University Club *Poa annua* quality more than Lexington Country Club *Poa annua* at 6, 9, and 11 WAIT. This is in contrast to paclobutrazol treatments which injured University Club *Poa annua* less than Lexington Country Club *Poa annua.*

Greenhouse Studies

There was a three way interaction between biotype, location, and treatment for quality ratings in 2011. For *Poa annua* from the Lexington Country Club (Table 2.6), in a few instances paclobutrazol (2 WAIT) and flurprimidol (7 and 10 WAIT) reduced quality of the light biotypes more than the dark biotypes. There were also a few differences in biotype responses to herbicide treatments. For the bispyribac-sodium treatment, the light biotypes had lower quality 1 and 6 WAIT. Amicarbazone reduced the quality of the light biotype more than the dark biotype 1 and 2 WAIT.

Treatment and color differences from the University Club were also observed (Table 2.7). Paclobutrazol initially reduced the light biotype collected from the University Club more than the dark biotype collected from the same location (Table 2.7). But, the University Club dark was injured more than the light 5, 6, and 7 WAIT. The University Club dark biotype quality was reduced more by flurprimidol than was the Lexington Country Club light biotype quality 4 to 9 WAIT (Table 2.6). This means that the light biotypes had a more rapid recovery than the dark biotypes over time. Our data suggests that PGRs initially effect light biotypes more than the dark biotypes, however, light biotypes recover quicker from the flurprimidol than dark biotypes. The biotypes from The University Club responded similarly to the treatments. Lexington Country Club biotypes treated with amicarbazone reduced the light biotypes 1 and 2 WAIT. The University Club biotypes treated with amicarbazone also reduced the light biotypes 1 and 2 WAIT, however, 10 WAIT the dark biotypes had lower quality, indicating that the light biotypes recovered faster than the dark biotypes with this treatment.

Interestingly, there was a strong interaction between the origin of the *Poa annua,* averaged across biotypes, and the response to PGR and herbicide treatments (Table 2.8). Paclobutrazol, flurprimidol, and bispyribac-sodium treatments injured Lexington Country Club *Poa annua* more than University Club *Poa annua.* Even the untreated University Club *Poa annua* had higher quality than Lexington Country Club *Poa annua*. Bispyribac-sodium treatment resulted in the greatest differences in injury between University Club *Poa annua* and Lexington Country Club *Poa annua*. Amicarbazone affected *Poa annua* from both locations equally.

There was a biotype by treatment interaction for clipping weights in 2011. The untreated light colored biotype had higher clipping weights 2, 3, 8, and 11 WAIT. The lower clipping weight for the dark biotypes is to be expected since the light biotypes tend to produce more vegetative and seed growth (Table 2.9). Paclobutrazol reduced the clipping weights of the dark biotypes more than the light biotypes 8, 10, and 14 WAIT. Flurprimidol reduced the growth of the dark biotypes more than the light biotypes 8 WAIT. Amicarbazone treatments effected the growth of the biotypes equally soon after treatment but the light biotypes resumed growth by the end of the measurements. With this in mind, amicarbazone treatments produced contrasting results and reduced the biomass of the light biotype, resulting in higher clipping weights for the dark biotypes 2 WAIT. Bispyribac-sodium also caused lower clipping weights for light versus dark biotypes 11 and 12 WAIT. This suggests that herbicide treatments affect light colored biotypes more than dark biotypes recover more after initial herbicide injury than the light biotypes.

There was a location by treatment interaction in 2011 for clipping weights (Table 2.10). The untreated Lexington Country Club *Poa annua* had higher clipping weights than the University Club from 2 to 13 WAIT. With paclobutrazol treatment, the only differences between *Poa annua* from the two locations was 10 WAIT. The Lexington Country Club *Poa annua* had less growth than the University Club *Poa annua*. The Lexington Country Club *Poa annua* had lower clipping weights than the University Club *Poa annua* with flurprimidol treatment 5, 8, and 10 WAIT. Also, bispyribac-sodium reduced clipping weights for Lexington Country Club *Poa annua* 5 to 13 WAIT more than the University Club. Amicarbazone did the same for 1, 2, and 14 WAIT.

University Club *Poa annua* had consistently higher quality than Lexington Country Club *Poa annua* in 2011 and 2012 (Table 2.11). Unfortunately, excessive greenhouse temperatures in 2012 resulted in *Poa annua* death and early termination of the study.

These results demonstrate different responses between *Poa annua* biotypes to PGRs and herbicides and that these differences, like all things about *Poa annua*, are complex. The factors that contributed to the differential responses of *Poa annua* biotypes include the location from which they were collected, the color of the biotype, and yearly climatic differences. The biggest differences recorded in these studies were found in *Poa annua* collected from two locations. Adams and Bryan (1980) concluded in their biotype studies that both the genetic variability of *Poa annua* in the sports turf and the selection within this variability are imposed by turfgrass culture and usage. Even though the *Poa annua* in our study was all collected from golf greens, these plants still had differential responses to the chemical treatments. Our studies only evaluated two basic phenotypes of

Poa annua biotypes collected from putting greens from two golf courses. Future studies should assess *Poa annua* collected from more golf courses as well as determining the differential uptake, absorption, translocation, and potential metabolism of these PGR and herbicide chemistries in these different *Poa annua* collections.

Table 2.1. *Poa annua* control, treatments, and application dates.

Treatment	Rate g a.i. ha^{-1}	Application dates	
		2011	2012
Control	n\a	n\a	n\a
Paclobutrazol	270	6/28; 7/19; 8/09; 8/30; 9/20	6/21; 7/12; 8/02; 8/23; 9/13
Flurprimidol	490	6/28; 7/19; 8/09; 8/30; 9/20	6/21; 7/12; 8/02; 8/23; 9/13
Bispyribac-sodium	25	6/28; 10/04	6/21; 10/11
Amicarbazone	49	6/28; 7/05; 7/12; 7/19	6/12; 6/28; 7/05; 7/12

Table 2.2. The effects of plant growth regulators and herbicides on *Poa annua* biotype turf quality in the 2011 field trial.[abc]

		Weeks after initial treatment									
		1	2	3	4	5	6	7	8	9	10
Treatment	Biotype	*Poa annua* turf quality									
Control	Light	8.4	7.6	7.6	8.5	7.9	7.0	6.6	6.4	5.5	5.9
	Dark	8.8	8.1	8.4	8.0	7.8	5.6	7.1	8.0	6.8	6.9
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Paclobutrazol	Light	7.5	5.0	6.5	5.6	5.6	6.5	7.1	4.9	4.3	4.4
	Dark	8.1	5.5	7.1	6.5	6.5	7.3	8.5	7.3	7.8	7.6
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	*	**	*
Flurprimidol	Light	7.9	6.0	7.9	7.0	6.3	7.4	7.2	6.5	5.2	6.4
	Dark	7.0	4.9	5.5	4.3	4.0	3.9	4.6	4.9	3.8	4.0
	Sig. Level	NS	NS	**	**	*	**	*	NS	NS	NS
Bispyribac Sodium	Light	8.0	6.4	7.0	7.1	7.0	7.4	8.0	6.8	5.5	5.5
	Dark	7.5	5.9	6.6	6.1	6.3	5.3	6.6	7.0	5.4	5.4
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Amicarbazone	Light	6.5	3.6	2.5	2.1	2.0	1.8	4.5	7.5	6.0	6.5
	Dark	6.5	3.9	2.5	2.3	2.0	1.9	4.6	7.1	4.6	4.6
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Quality is on a scale from 1 -9, with 1 = dead and 9 = excellent turf. [b] Abbreviations: Sig. Level, significance level; NS, not significant
[c] All data are least significant means. * and ** denote significant at the $P < 0.05$ and 0.01 levels, respectively

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Table 2.3. The effects of location on turf quality of *Poa annua* collected from Lexington Country Club and University Club in the field trial.[abc]

	2011										2012									
	Weeks after initial treatment																			
Collection	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Location	*Poa annua* turf quality																			
Lexington CC	8.1	6.1	6.6	6.4	5.9	5.3	6.6	7.1	6.2	6.2	8.1	7.3	6.4	6.2	7.1	7.3	7.1	7.1	7.4	7.5
University Club	7.2	5.3	5.7	5.1	5.2	5.5	6.4	6.2	4.8	5.2	8.3	7.5	7.0	6.6	7.3	7.6	7.6	7.4	7.5	7.7
Sig. level	*	*	*	*	NS	NS	NS	NS	*	NS	NS	NS	*	NS	NS	NS	*	NS	NS	NS

[a] Quality is on a scale from 1 -9, with 1 = dead and 9 = excellent turf.
[b] Abbreviations: Lexington CC, Lexington Country Club; NS, not significant
[c] * denote significant at the $P < 0.05$ level

Table 2.4. Average high and low temperatures and precipitation in Lexington, KY during the field data collection period.

	2011			2012			Average in Kentucky		
	Temperature			Temperature			Temperature		
Month	High	Low	Precipitation	High	Low	Precipitation	High	Low	Precipitation
June	28.5 C	17.4 C	8.1 cm	29.6 C	15.3 C	4.1 cm	28 C	17 C	11.3 cm
July	31.7 C	20.8 C	12.5 cm	33.5 C	20.4 C	20.3 cm	30 C	19 C	11.8 cm
August	29.9 C	18.1 C	9.2 cm	29.6 C	17.3 C	5.5 cm	30 C	18 C	8.3 cm
September	23.8 C	13.8 C	17.7 cm	24.8 C	13.5 C	13.8 cm	26 C	14 C	7.4 cm
October	18.7 C	6.5 C	11.2 cm	17.4 C	7.3 C	3.3 cm	20 C	8 C	8.0 cm

Table 2.5. The effects of plant growth regulators and herbicides on *Poa annua,* collected from course locations, turf quality in 2012 field trial.[abc]

		Weeks after initial treatment											
Collection		1	2	3	4	5	6	7	8	9	10	11	12
Treatment	Location	*Poa annua* turf quality											
Control	LCC	7.8	7.6	7.0	6.8	7.1	6.4	7.3	6.8	7.6	8.1	7.5	7.3
	UC	8.1	7.4	6.4	7.0	7.1	7.4	7.5	7.1	7.9	7.6	7.6	8.0
	Sig. Level	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
Paclobutrazol	LCC	7.9	7.3	6.4	5.5	7.4	6.9	6.6	7.4	7.0	7.6	7.9	6.8
	UC	8.4	8.0	7.1	6.5	8.0	8.3	7.9	7.8	7.4	8.0	8.0	8.0
	Sig. Level	NS	NS	NS	NS	NS	**	**	NS	NS	NS	NS	*
Flurprimidol	LCC	8.6	7.8	7.0	5.5	7.3	8.4	8.4	7.6	8.0	8.0	8.1	7.1
	UC	8.0	7.4	6.9	5.9	7.0	6.8	6.8	6.4	6.5	7.4	6.9	6.9
	Sig. Level	NS	NS	NS	NS	NS	**	NS	NS	*	NS	*	NS
Bispyribac Sodium	LCC	8.1	7.1	6.1	7.9	7.6	7.8	7.8	6.5	7.0	7.6	7.4	6.9
	UC	8.3	7.5	7.5	7.9	7.1	7.4	7.4	7.3	7.4	7.8	7.5	8.4
	Sig. Level	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	**
Amicarbazone	LCC	8.0	6.6	5.5	5.0	5.9	6.8	6.8	6.9	7.4	8.3	7.3	6.0
	UC	8.5	7.0	5.8	5.5	7.3	8.0	8.0	8.6	8.4	8.6	8.4	7.6
	Sig. Level	NS	NS	NS	NS	**	**	**	*	NS	NS	NS	**

[a] Quality is on a scale from 1 -9, with 1 = dead and 9 = excellent turf. [b] Abbreviations: LCC, Lexington Country Club; UC, University Club; NS, not significant [c] All data are least significant means. * and ** denotes significant at $P < 0.05$ and 0.01, respectively

Table 2.6. The effects of plant growth regulators and herbicides on *Poa annua*, collected from Lexington Country Club, in the greenhouse in 2011.[abc]

		Weeks after initial treatment									
Collection		1	2	3	4	5	6	7	8	9	10
Treatment	Biotype	*Poa annua* turf quality									
Control	Light	6.8	6.8	7.0	7.5	7.0	7.3	7.0	7.3	7.0	7.0
	Dark	7.8	7.8	7.3	8.0	8.3	8.3	7.3	8.3	7.5	8.0
	Sig. Level	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
Paclobutrazol	Light	5.0	3.3	4.5	5.5	5.5	6.5	6.3	6.3	6.0	6.5
	Dark	6.0	4.8	4.8	5.0	5.0	5.3	5.3	5.5	5.8	6.0
	Sig. Level	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
Flurprimidol	Light	3.3	3.5	4.8	3.5	3.3	4.0	4.0	5.0	5.3	5.5
	Dark	4.3	4.5	5.8	4.8	4.5	5.0	6.0	6.3	6.5	7.5
	Sig. Level	NS	NS	NS	NS	NS	NS	*	NS	NS	*
Bispyribac Sodium	Light	4.0	3.5	4.0	4.5	3.8	3.8	4.3	5.0	5.5	4.8
	Dark	5.3	4.0	4.5	4.3	5.0	5.5	5.3	5.0	5.3	5.6
	Sig. Level	*	NS	NS	NS	NS	*	NS	NS	NS	NS
Amicarbazone	Light	4.3	3.0	2.3	1.3	1.8	2.0	2.3	3.5	2.8	3.5
	Dark	6.0	4.5	2.0	1.3	1.0	1.0	1.8	1.8	2.0	2.5
	Sig. Level	**	*	NS	NS	NS	NS	NS	NS	NS	NS

[a] Quality is on a scale from 1 -9, with 1 = dead and 9 = excellent turf. [b] Abbreviations: LCC, Lexington Country Club; UC, University Club; NS, not significant; Sig. Level, significance level. [c] All data are least significant means. *, **, and *** denote significant at the $P < 0.05$, 0.01, and 0.001 levels, respective

Table 2.7. The effects of plant growth regulators and herbicides on *Poa annua*, collected from the University Club, in the greenhouse in 2011.[abc]

		Weeks after initial treatment									
Collection		1	2	3	4	5	6	7	8	9	10
Treatment	Biotype	*Poa annua* turf quality									
Control	Light	8.3	8.3	8.0	9.0	8.8	9.0	9.0	9.0	9.0	7.8
	Dark	8.0	8.0	7.8	8.8	8.8	8.8	8.5	8.8	8.8	8.8
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Paclobutrazol	Light	5.8	5.0	7.0	6.8	7.3	8.0	8.5	9.0	9.0	8.5
	Dark	7.5	5.5	6.3	5.5	5.5	6.3	6.3	7.5	8.8	7.8
	Sig. Level	**	NS	NS	NS	*	*	**	NS	NS	NS
Flurprimidol	Light	4.8	5.0	6.3	6.8	7.3	8.0	8.5	8.8	8.5	8.5
	Dark	5.5	4.85	7.0	4.0	3.8	5.3	5.5	6.5	6.5	7.0
	Sig. Level	NS	NS	NS	**	***	**	**	*	*	NS
Bispyribac Sodium	Light	5.8	5.3	6.5	7.5	8.0	8.3	8.3	9.0	8.5	7.3
	Dark	5.8	5.8	6.3	7.5	7.8	7.8	8.0	8.3	8.3	7.8
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Amicarbazone	Light	4.3	3.3	2.5	1.3	1.3	1.5	2.0	3.5	3.3	4.0
	Dark	7.3	4.8	3.0	1.5	1.3	1.0	1.8	2.3	2.3	2.3
	Sig. Level	***	*	NS	NS	NS	NS	NS	NS	NS	*

[a] Quality is on a scale from 1 -9, with 1 = dead and 9 = excellent turf.
[b] Abbreviations: LCC, Lexington Country Club; UC, University Club; NS, not significant; Sig. Level, significance level
[c] All data are least significant means. *, **, and *** denote significant at the $P < 0.05$, 0.01, and 0.001 levels, respectivel

Table 2.8. The effect of plant growth regulators and herbicides in the greenhouse on the quality of *Poa annua* collected from two golf courses.[abc]

		Weeks after initial treatment									
Collection		1	2	3	4	5	6	7	8	9	10
Treatment	Location	*Poa annua* turf quality									
Control	LCC	7.3	7.3	7.1	7.8	7.6	7.8	7.1	7.8	7.3	7.5
	UC	8.1	8.1	7.9	8.9	8.8	8.9	8.8	8.9	8.9	8.3
	Sig. Level	NS	NS	NS	*	*	NS	*	NS	*	NS
Paclobutrazol	LCC	5.5	4.0	4.6	5.3	5.3	5.9	5.8	5.9	5.9	6.3
	UC	6.6	5.3	6.6	6.1	6.4	7.1	7.4	8.3	8.4	8.1
	Sig. Level	*	*	**	NS	*	*	*	**	**	*
Flurprimidol	LCC	3.8	4.0	5.3	4.1	3.9	4.8	5.0	5.6	5.9	6.5
	UC	5.1	4.9	6.6	5.4	5.5	6.6	7.0	7.6	7.5	7.8
	Sig. Level	**	NS	**	*	**	**	*	*	*	*
Bispyribac Sodium	LCC	4.6	3.8	4.3	4.4	4.4	4.6	4.8	5.0	5.4	5.3
	UC	5.8	5.5	6.4	7.5	7.9	8.0	8.1	8.6	8.4	7.5
	Sig. Level	*	**	***	***	***	***	***	***	***	**
Amicarbazone	LCC	5.1	3.8	2.1	1.3	1.4	1.5	2.0	2.6	2.4	3.0
	UC	5.8	4.0	2.8	1.4	1.3	1.3	1.9	2.9	2.8	3.1
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Quality is on a scale from 1 -9, with 1 = dead and 9 = excellent turf.
[b] Abbreviations: LCC, Lexington Country Club; UC, University Club; NS, not significant; Sig. level, significance level
[c] All data are least significant means. *, **, and *** denote significant at the $P < 0.05$, 0.01, and 0.001 levels, respectively

Table 2.9. The effects of plant growth regulators and herbicides in the greenhouse on the clipping weights of *Poa annua* biotypes in 2011.[ab]

		Weeks after initial treatment													
Treatment	Color	1	2	3	4	5	6	7	8	9	10	11	12	13	14
		Weekly clipping weight (g)													
Control	Light	0.28	0.35	0.22	0.36	0.72	0.64	0.97	0.84	0.61	0.40	0.66	0.75	0.94	0.62
	Dark	0.36	0.23	0.16	0.35	0.76	0.69	0.82	0.64	0.55	0.39	0.79	0.76	0.87	0.52
	Sig. Level	NS	***	*	NS	NS	NS	NS	*	NS	NS	*	NS	NS	NS
Paclobutrazol	Light	0.23	0.16	0.05	0.12	0.19	0.22	0.40	0.44	0.20	0.21	0.17	0.22	0.42	0.32
	Dark	0.24	0.14	0.04	0.06	0.12	0.14	0.18	0.19	0.14	0.11	0.10	0.12	0.23	0.16
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	**	NS	*	NS	NS	NS	*
Flurprimidol	Light	0.24	0.17	0.10	0.17	0.34	0.23	0.35	0.51	0.33	0.20	0.35	0.32	0.33	0.31
	Dark	0.25	0.18	0.07	0.16	0.22	0.17	0.25	0.29	0.21	0.13	0.26	0.23	0.30	0.24
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
Bispyribac Sodium	Light	0.20	0.20	0.12	0.27	0.48	0.44	0.74	0.73	0.48	0.29	0.52	0.50	0.89	0.73
	Dark	0.18	0.18	0.10	0.34	0.55	0.42	0.94	0.74	0.56	0.31	0.73	0.63	1.04	0.81
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	*	NS	NS
Amicarbazone	Light	0.23	0.16	0.04	0.003	0.00	0.015	0.04	0.09	0.11	0.11	0.21	0.15	0.45	0.50
	Dark	0.26	0.23	0.04	0.004	0.00	0.001	0.01	0.02	0.05	0.04	0.08	0.05	0.13	0.16
	Sig. Level	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	***

[a] Abbreviations: NS, not significant; Sig. Level, significance level
[b] All data are least significant means. *, **, and *** denote significant at the $P < 0.05$, 0.01, and 0.001 levels, respectively

Table 2.10. The effects of plant growth regulators and herbicides in the greenhouse on the clipping weights of *Poa annua* collected from two golf courses in 2011.[ab]

Treatment	Location	Weeks after initial treatment													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
		-Weekly clipping weight (g)													
Control	LCC	0.27	0.24	0.17	0.29	0.64	0.60	0.65	0.64	0.45	0.28	0.58	0.55	0.74	0.51
	UC	0.17	0.35	0.21	0.41	0.84	0.73	1.14	0.84	0.70	0.51	0.86	0.95	1.08	0.63
	Sig. Level	*	**	*	*	*	*	**	*	**	***	***	***	**	NS
Paclobutrazol	LCC	0.24	0.14	0.04	0.07	0.14	0.14	0.21	0.26	0.15	0.10	0.09	0.13	0.31	0.22
	UC	0.36	0.16	0.05	0.10	0.17	0.22	0.37	0.37	0.19	0.23	0.17	0.21	0.34	0.26
	Sig. Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS
Flurprimidol	LCC	0.24	0.17	0.07	0.15	0.18	0.16	0.23	0.30	0.28	0.12	0.27	0.22	0.28	0.21
	UC	0.25	0.18	0.10	0.18	0.38	0.24	0.37	0.50	0.26	0.20	0.34	0.33	0.35	0.34
	Sig. Level	NS	NS	NS	NS	*	NS	NS	*	NS	*	NS	NS	NS	NS
Bispyribac-S.	LCC	0.16	0.18	0.09	0.26	0.33	0.28	0.60	0.60	0.42	0.23	0.52	0.45	0.80	0.73
	UC	0.22	0.21	0.12	0.35	0.70	0.58	1.08	0.87	0.62	0.37	0.73	0.69	1.13	0.81
	Sig. Level	NS	NS	NS	NS	***	***	**	**	**	**	**	**	**	NS
Amicarbazone	LCC	0.17	0.16	0.02	0.003	0.00	0.011	0.04	0.06	0.08	0.06	0.11	0.07	0.19	0.25
	UC	0.32	0.23	0.06	0.004	0.00	0.005	0.02	0.04	0.08	0.10	0.18	0.14	0.39	0.41
	Sig. Level	**	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*

[a] Abbreviations: LCC, Lexington Country Club; UC, University Club; NS, not significant; Sig. Level, significance level

[b] All data are least significant means. *, **, and *** denote significant at the $P < 0.05$, 0.01, and 0.001 levels, respectively

Table 2.11. Turf quality of *Poa annua* collected from two golf courses.[ab]

	2011										2012			
	Weeks after initial treatment													
Collection	1	2	3	4	5	6	7	8	9	10	1	2	3	4
Location	*Poa annua* turf quality													
Lexington CC	6.3	4.6	4.7	4.6	4.5	4.9	4.9	5.4	5.4	5.7	7.1	4.4	3.8	3.4
University Club	5.3	5.6	6.1	5.9	6.0	6.4	6.6	7.3	7.2	7.0	7.8	5.7	4.6	4.5
Sig. Level	*	*	*	*	*	*	*	*	*	*	*	*	*	*

[a] Quality is on a scale from 1 -9, with 1 = dead and 9 = excellent turf.
[b] Abbreviations: Lexington CC, Lexington Country Club; Sig. Level, significance level
[c] * denote significant at the $P < 0.05$ level

Figure 2.1. Examples of *Poa annua* biotypes found on putting greens at The University Club (a.) and The Lexington Country Club (b.), respectively.

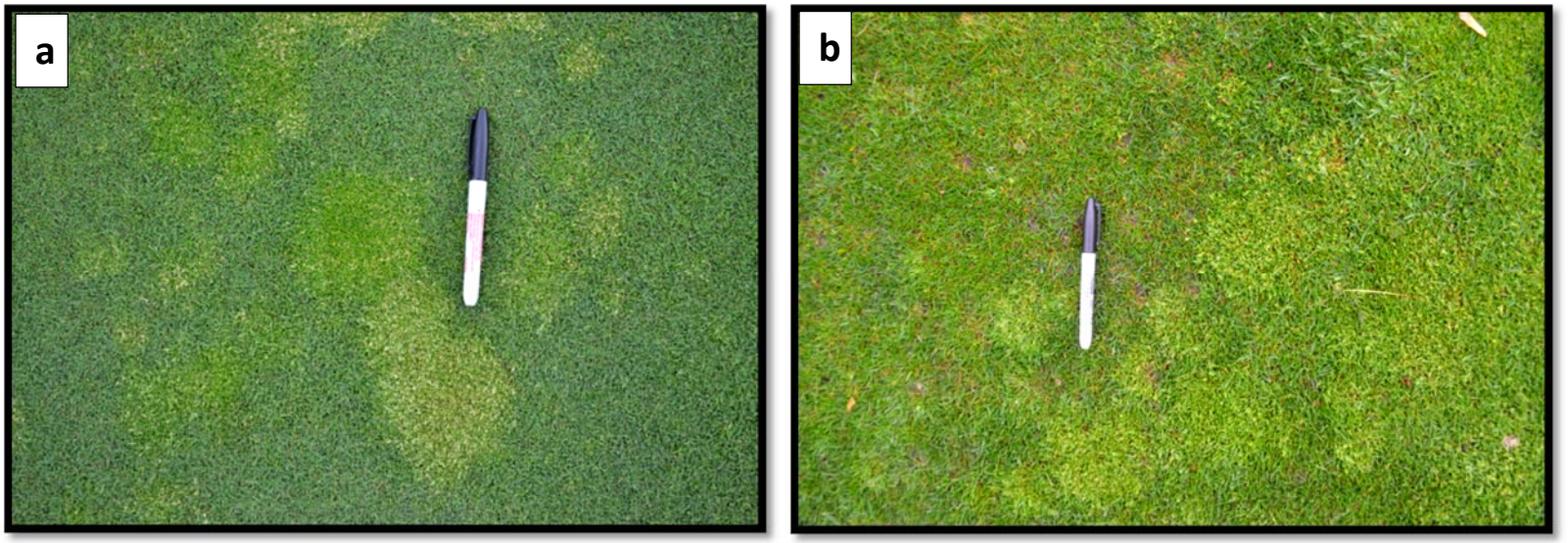

Figure 2.2 Example of light (left) and dark (right) plugs collected with a cup cutter for this study.

Chapter Three

Flurprimidol Uptake and Metabolism in Six Turf Species

Plant growth regulators (PGRs) are effective tools used by managers of highly maintained turfgrass. The primary use of PGRs is to reduce grass growth and decrease mowing frequencies in the spring and fall for cool-season grasses and the summer for warm season grasses (Christians 2001). The use of PGRs can potentially reduce the overall cost and time needed for turf maintenance (DiPaola 1986). Flurprimidol [alpha-(1-methylethyl)-alpha-(4-(trifluoromethoxyphenyl)-5-pyrimidine-methanol] is a commonly used PGR to control turf growth, improve quality, and manage *Poa annua* (L.) (Bigelow et al. 2007; Christians 2001). It is currently labelled for perennial cool season grasses: creeping bentgrass (*Agrostis stolonifera*), tall fescue (*Festuca arundinacea*), perennial ryegrass (*Lolium perenne*), and Kentucky bluegrass (*Poa pratensis*) and warm season turfgrasses: bermudagrass (*Cynodon dactylon*), seashore paspalum (*Paspalum vaginatum*), and zoysiagrass (*Zoysia japonica*). According to the Cutless® MEC Turf Regulator (SePro Corporation 11550 N. St. Ste. 600, Carmel, IN 46032 U.S.A.) label, rate recommendations for flurprimidol range depending on turf type and species, turf location and utility, turf management, and, in some conditions, turf cultivar (Table 3.1). Flurprimidol effectiveness depends on a number of factors including PGR concentration, method of application, their persistence, and plant response (Wroblewska 2013). These distinctive recommendations indicate that different turf species response to flurprimidol may stem from differing flurprimidol behavior in the turf species.

Flurprimidol is a nitrogen-containing heterocycle and organofluorine compound belonging to the pyrimidine chemical class (Bunnell and Cockreham 2005; Key et al.

1997; Leroux et al. 2005). Flurprimidol action is similar to that of inhibitors of sterol synthesis (Totten et al. 2006). Like most (54%) of the organoflurorine compounds created for agricultural use, flurprimidol is a trifluoromethyl-substituted aromatic (Key et al. 1997). Although lacking the OCF_3 group, there are other PGRs that are similar to flurprimidol: ancymidol, which also belongs to the pyrimidine class of chemistry, paclobutrazol and uniconazole, both triazoles, and trinexapac-ethyl (Bunnell and Cockreham 2005; Christians 2001; Pinhero and Fletcher 1994).

Both trinexapac-ethyl and flurprimidol are classified as a Type II, Class B plant growth regulators (Watschke et al. 1992; Watschke and DiPaola 1995). Type II plant growth regulators are generally crown and root absorbed (Christians 2001). Class B refers to plant growth regulators that inhibit gibberellic acid (GA) production early in the biosynthesis pathway (Christians 2001). In particular, flurprimidol inhibits *ent*-kaurene oxidase, a cytochrome P_{450} monooxygenase, blocking the formation of *ent*-kaurenoic acid which is a precursor to active creating gibberellic acids (Bunnell and Cockreham 2005; March et al. 2013).GAs are an assembly of diterpene compounds that regulate plant processes such as seed germination, shoot elongation, and seed development (Yamaguchi et al. 1998). More than 110 forms of GAs exist in plants, however, only a few of these (i.e. GA_1, GA_3, GA_4, and GA_7) function as bioactive hormones (Asrar 2012; Taiz 1991). Throughout the lifetime of a turfgrass, gibberellins continue to promote the elongation of stems and, therefore, without GAs elongation of shoot internodes is decreased (March et al. 2013; Wroblewski 2013).

While flurprimidol is widely used, there is little published information concerning its behavior in plants. Previous studies examining flurprimidol translocation and/or

metabolism in plants used stem injections into trees (Sterrett and Tworkoski, 1987) and determined methods for extraction from Eurasian watermilfoil (*Myriophyllum spicatum*), soil, and water (Chand and Lembi, 1991). Sterrett and Tworkoski (1987) injected 2.5 mg ^{14}C-labeled flurprimidol into the stocks of 1-year-old apple trees (*Malus domestica* Borkh.). After 35 days, 10% had moved into the new shoots, 1.5% into the scion phloem, and 80% remained near the injection site. A high percentage of the ^{14}C activity was unmetabolized flurprimidol.

The behavior of other PGRs have been examined in turfgrasses. Branham and Beasley (2007) studied paclobutrazol and trinexapac-ethyl behavior in Kentucky bluegrass in field studies. Paclobutrazol half-lives were 15.4 and 11.5 days in the spring and summer, respectively. Trinexapac-ethyl half-lives were 5.8 and 4.3 days in the spring and summer, respectively. They also conducted growth chamber studies with Kentucky bluegrass and creeping bentgrass. For Kentucky bluegrass, when temperatures were 18 C, paclobutrazol half-life was 11-15 days and trinexapac-ethyl half-life was 5.3 days. When temperatures were 30 C, paclobutrazol half-life was 7-9 days and trinexapac-ethyl half-life was 3.4 days. For creeping bentgrass, when temperatures were 18 C, paclobutrazol half-life was 9-11 days and trinexapac-ethyl half-life was 6.4 days. When temperatures were 30 C, paclobutrazol half-life was 6-8 days and trinexapac-ethyl half-life was 3.1 days.

Because there is little published information concerning its behavior in plants, the objectives of this study were to determine flurprimidol absorption, translocation, andmetabolism in creeping bentgrass, bermudagrass, Kentucky bluegrass, perennial

ryegrass, tall fescue and zoysiagrass to help explain their differential tolerance to this PGR.

Materials and Methods

Plant Material

Plugs of "Pencross" creeping bentgrass, "Riviera" bermudagrass, "Falcon V" tall fescue, "Palmer V" perennial ryegrass, "Midnight II" Kentucky bluegrass, and "Meyer" zoysiagrass were harvested using a cup cutter from existing stands at the University of Kentucky Research Farm in Lexington, KY in the fall of 2012. The plugs were transferred from the field to the greenhouse where they were separated into individual tillers and planted into a 50% Maury silt loam soil (fine, mixed, mesic typic Paleudalfs) and 50% coarse builder's sand (Clay Ingels Co. LLC. 914 Delaware Ave, Lexington, KY) mixture. Osmocote® Slow Release (The Scotts Company LLC. Marysville, OH) fertilizer (19-19-19, 2 g/0.001 m^3) was added to the sand/soil mixture. Plants were clipped to approximately 5 cm using scissors once a week and sprayed with fungicides and insecticides when necessary to maintain plant health.

When the plants became of sufficient size, they were transferred from the greenhouse to the lab for acclimation. The tiller numbers were as follows: creeping bentgrass 18-20, bermudagrass 4-5, tall fescue 8-10, perennial ryegrass 18-20, Kentucky bluegrass 3-5, and zoysiagrass 4-5. Roots were gently washed 4-5 times with distilled water to remove residual growing media. Once rinsed, the roots were placed in 50 ml polypropylene tubes (Fisher Scientific, 300 Industry Drive Pittsburgh, PA 15275) filled with 45 ml of ¼ strength Hoagland's solution (Hoagland and Arnon, 1950). The tubes were covered with aluminium foil to exclude light. When needed, a foam plug (Identi-

plugs®, Jaece Industries, INC. 908 Niagra Fall Blvd. North Tonawanda, NY) was used to support the plants. Solution lost to transpiration and evaporation was replenished daily with distilled water. Tubes were arranged in a randomized block design with time of harvest used as a blocking factor (Figure 3.1). There were six grass species with four replications and four harvest times. Each tube was aerated using a glass Pasteur pipette (Corning® Incorporated, Corning, NY 14831) connected to air tubing, which was powered by an aquarium air pump system. Supplemental light was provided using fluorescent bulbs (0.25 μmol m^{-2} sec^{-1}) with a 16 h photoperiod. Temperatures were maintained at approximately 28 C. The plants were acclimated between 6 and 7 days in these conditions before initiation of the experiment (Figure 3.2).

Flurprimidol Treatment

After acclimation, the individual plants were transferred into new 50 ml polypropylene tubes filled with a 25μL methanol solution with 1.56 μM ^{14}C-uniformly benzene ring labelled flurprimidol (Figure 3.3) in 45 ml of ¼ strength Hoagland's solution. The specific activity of ^{14}C flurprimidol was 0.78 mCi/mmol. This concentration replicated a flurprimidol rate of 558 g a.i. / ha^{-1} in approximately 15 cm of soil with 50% available in the soil water. The plants were under continuous illumination during the ^{14}C flurprimidol treatment period. After 48 hours, the plants were removed from the treatment solution and the roots were rinsed with distilled water. The plants were harvested at four different intervals. One set of plants were harvested at the end of the 48 hour treatment period (Time 0) while the others were transferred to fresh vials of ¼ strength Hoagland's solution. After the first harvest collection, subsequent harvests were at 24, 72, and 120 hours. At each harvest, roots were rinsed with distilled water and

gently blotted dry with a paper towel. The plants were separated into roots and shoots, weighed, and then frozen (-20 C) until extracted.

Radioactivity Extraction and Analysis

Plant material was ground into a fine powder in liquid nitrogen using a pestle and mortar; 10 ml of methanol was then added to the powder and the mixture was transferred to a centrifuge tube. The mixture was centrifuged at 7650 relative centrifugal force (RCF) for 5 minutes. The supernatant was decanted and a second 10 ml of methanol was added to the pellet, vortexed, and centrifuged again. The second supernatant was added to the first and then brought up to 25 ml.

The extract was concentrated to 1 ml in a rotovap. The sample was transferred to a microfuge vial and centrifuged at 4550 RCF for 2 minutes. The samples were then filtered through a sterile, nylon 0.45 µum filter (Fisher Scientific, 300 Industry Drive Pittsburgh, PA 15275) into a 1.5 ml autosampler vial. Preliminary trials established full ^{14}C flurprimidol recovery using this method. To quantify the total radioactivity in each extract, a 50 µl aliquot was mixed with 15 ml of scintillation cocktail (Bio-Safe II™, Research Products International Corp. 410 N Business Center Drive, Mount Prospect, IL 60056). The radioactivity was quantified using a scintillation counter (TriCarb® 2200CA, Perkin Elmer™ Life Sciences, 2200 Warrenville Rd, Downers Grove, IL 60515).

To quantify unextracted radioactivity, the residue remaining after the extraction was oxidized (Packard Sample Oxidizer model #307, Perkin Elmer™ Life Sciences, 940 Winter Street Waltham, MA 02451) after air drying and released $^{14}CO_2$ was measured in a scintillation counter.

^{14}C in the extracts was analyzed using a high performance liquid chromatography (HPLC) (Prominence UFLC, Shimadzu, 1,Nishinokyo-Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan) system coupled to a radioactivity detector (Radiomatic FLO-ONE® Beta Series A-500, Canberra Industries, Inc. 800 Research Parkway, Meriden, CT 06450). The HPLC was equipped with a C18 5µm 4.6 x 250 mm reverse phase column (GL Sciences Inc. Shinjuku Square Tower 30F, 6-22-1 Nishi Shinjuku, Shinjuku-ku, Tokyo, 163-1130 Japan). Elution was accomplished using a gradient beginning with 80% water and 20% acetonitrile (ACN) (Table 3.2). The flow rate through the HPLC was 1 ml/minute. Flurprimidol standards eluted at 23.2 minutes (Figure 3.4). ^{14}C flurprimidol concentrations were expressed as a ratio where:

$$\frac{\text{Shoot or Root or Total DPM}}{\text{Shoot or Root or Total Weight}}$$

Concentrations were then expressed as nmol ^{14}C flurprimidol/gram of plant weight. Data were subjected to analysis of variance (ANOVA) and means were separated using Fisher's Protected LSD test at $\alpha = 0.05$; LSMeans was used to calculate for means separation for interactions at $\alpha = 0.05$. The experiment was repeated and, as no significant differences were detected between experimental runs, the data was combined for analysis.

Results and Discussion

Flurprimidol Uptake and Translocation

There was no harvest time by species interaction for extraction efficiency so the data was averaged over harvest times. ^{14}C flurprimidol extraction efficiencies were calculated for each turfgrass species (Figure 3.5.). Bentgrass, bermudagrass, Kentucky

bluegrass, perennial ryegrass, tall fescue, and zoysiagrass had a 70, 69, 78, 60, 63, and 67 % extraction efficiency, respectively. These extraction efficiencies were comparable and sometimes lower than those of previous studies that did not use ^{14}C-labeled flurprimidol. Chand and Lembi (1991) had mean flurprimidol recoveries of 86.8% from watermilfoil shoots and 85.2% from roots. West and Rutherford (1986) recovered 80% of the flurprimidol in soil and 78% in a soil-grass mixture. Reed (1988) recovered 83.6% of the flurprimidol from peach leaves. Extraction efficiency declined with time (Figure 3.6). At 0 hours after treatment (HAT), approximately 72% of the ^{14}C was extracted compared to approximated 65% 120 HAT. The ^{14}C-flurprimidol may be incorporated into plant tissue (for example, bound to plant tissue via a protein) so that over time, it makes it more difficult to extract. Bound residues in plant tissues eventually are recycled into natural polymeric products via metabolic pathways (Khan 1982). Alternatively, as the plants were growing, extraction could have been less efficient.

There was not an interaction between time and grass species for flurprimidol tissue concentrations. However, there were differences between species and over time in the flurprimidol concentration in the plants. Zoysiagrass and bermudagrass, both warm season grasses, had the highest flurprimidol concentrations (Figure 3.7). A contrast test revealed that the warm season grasses (bermudagrass and zoysiagrass) had significantly higher ($p < 0.001$) flurprimidol concentration within the total plant tissue than cool season grasses (creeping bentgrass, Kentucky bluegrass, perennial ryegrass, and tall fescue). Flurprimidol concentration declined in the plants over time (Figure 3.8). At 0 HAT, flurprimidol concentration across all grass species was 13.8 nmol/g and 7. 9 nmol/g

at 120 HAT. The plants used for were actively growing over the study time. The growth could have caused the decrease in flurprimidol concentrations with time.

Root concentrations of flurprimidol differed between grasses and over time. Flurprimidol concentrations in the roots were higher for zoysiagrass and bermudagrass than the cool season turfgrass species (Figure 3.9). A contrast test showed that the warm season grasses (bermudagrass and zoysiagrass) had a higher ($p < 0.001$) flurprimidol concentration in the roots than the cool season grasses (creeping bentgrass, Kentucky bluegrass, perennial ryegrass, and tall fescue). Flurprimidol concentration in the roots declined over time (Figure 3.10). The root concentration 0 HAT was 5.7 nmol/g across all turfgrass species and 2.1 nmol/g 120 HAT. The decrease in flurprimidol root concentration over time indicates that the PGR is moving from the roots to the shoots. Root concentration of flurprimidol decreased over time for all species (Figure 3.11). Warm season grasses have higher flurprimidol concentrations in the roots than cool season grasses. All turf species had significantly less flurprimidol in root tissues from 0 HAT to 120 HAT.

Flurprimidol shoot concentrations differed between grass species and over time. Despite the higher amount of initial flurprimidol concentration uptake in the zoysiagrass root system, bermudagrass had higher shoot concentrations than zoysiagrass (Figure 3.12). Bermudagrass, Kentucky bluegrass, tall fescue, and zoysiagrass had higher flurprimidol shoot concentrations than creeping bentgrass and perennial ryegrass (Figure 3.12). Flurprimidol shoot concentration averaged across all turfgrass declined over time (Figure 3.13). As with the total plant concentrations, this decline was likely a result of

actively growing plants, and therefore increased shoot biomass and over the 120 h period the growth rate exceeded the rate of flurprimidol transfer from the roots.

In these studies, the rate of ^{14}C flurprimidol used was based on the highest labelled rate for bentgrass at fairway height, Kentucky bluegrass, and perennial ryegrass (558 g a.i. / ha^{-1}). However, this rate was higher than the highest labelled rate for both bermudagrass and zoysiagrass (424 g a.i. / ha^{-1}) (Table 3.1). Our results indicate that the warm season grasses absorb more flurprimidol than cool season grasses. The greater flurprimidol absorption by the warm season grasses may explain why lower rates are used on these species compared to cool season grasses. Higher rates may be unnecessary or may cause damage to the warm season species.

Metabolism

No flurprimidol metabolites were detected in any of the turfgrass species. Only the parent flurprimidol material was detected in the extracts (Figure 3.14). Sterrett and Tworkoski (1987) found only 20% of the ^{14}C-labeled flurprimidol in apple trees had been translocated and a high percentage of the activity was present was flurprimidol after 35 days. Similarly ^{14}C-labeled paclobutrazol was metabolized 27 days after stem injections into trees (Sterrett 1988). However, Branham and Beasley (2007) found paclobutrazol half-lives in creeping bentgrass and Kentucky bluegrass were between 6 and 15 days, depending on growth chamber temperatures. The Kentucky bluegrass paclobutrazol half-lives were approximately 15 and 12 days in the spring and summer, respectively.

In these studies, flurprimidol was absorbed by the roots of all the turf species. Flurprimidol uptake was higher in warm season grasses (bermudagrass and zoysiagrass) than in cool season grasses (Kentucky bluegrass, tall fescue, creeping bentgrass, and

perennial ryegrass). As a result, both bermudagrass and zoysiagrass had higher flurprimidol concentrations in all plant tissues versus the cool season grasses. Our studies only evaluated the translocation and metabolism of flurprimidol for up to 5 days after the initial treatment. Future studies should evaluate flurprimidol translocation and metabolism after longer periods.

Table 3.1. Rate ranges for growth regulation of perennial turfgrass species with Cutless MEC using a multiple application program.

Turf Species	Initial spring application[a] (g a.i. flurprimidol)	Repeat Applications[a]	
		(g a.i. flurprimidol)	Treatment interval
Cool season grasses			
Bentgrass (golf course fairway)	280 to 560	140 to 560	2 to 6 weeks
Bentgrass putting green	140 to 280	69.5 to 280	2 to 4 weeks
Kentucky bluegrass /Perennial ryegrass	420 to 560	280 to 560	2 to 6 weeks
Warm season grasses			
Seashore paspalum	140 to 560	140 to 560	3 to 6 weeks
Tifway, TifSport, and GN-1 Bermudagrass	140 to 420	140 to 420	3 to 6 weeks
Zoysiagrass	140 to 420	140 to 420	3 to 6 weeks not in late summer or fall

[a]Apply in early spring following resumption of active growth of the grass. Fall applications must be discontinued 4 weeks before the onset of inactive grass growth or winter dormancy.

Table 3.2. HPLC solvent gradient used for flurprimidol analysis.

Time (minutes)	Flow (ml/min)	Water (%)	Acetonitrile (%)
2.00	1.00	80	20
15.00	1.00	40	60
20.00	1.00	40	60
22.00	1.00	10	90
27.00	1.00	10	90
30.00	1.00	80	20
35.00	1.00	80	20

Figure 3.1. Laboratory experimental design. Here, the grass plants are acclimating to the laboratory conditions.

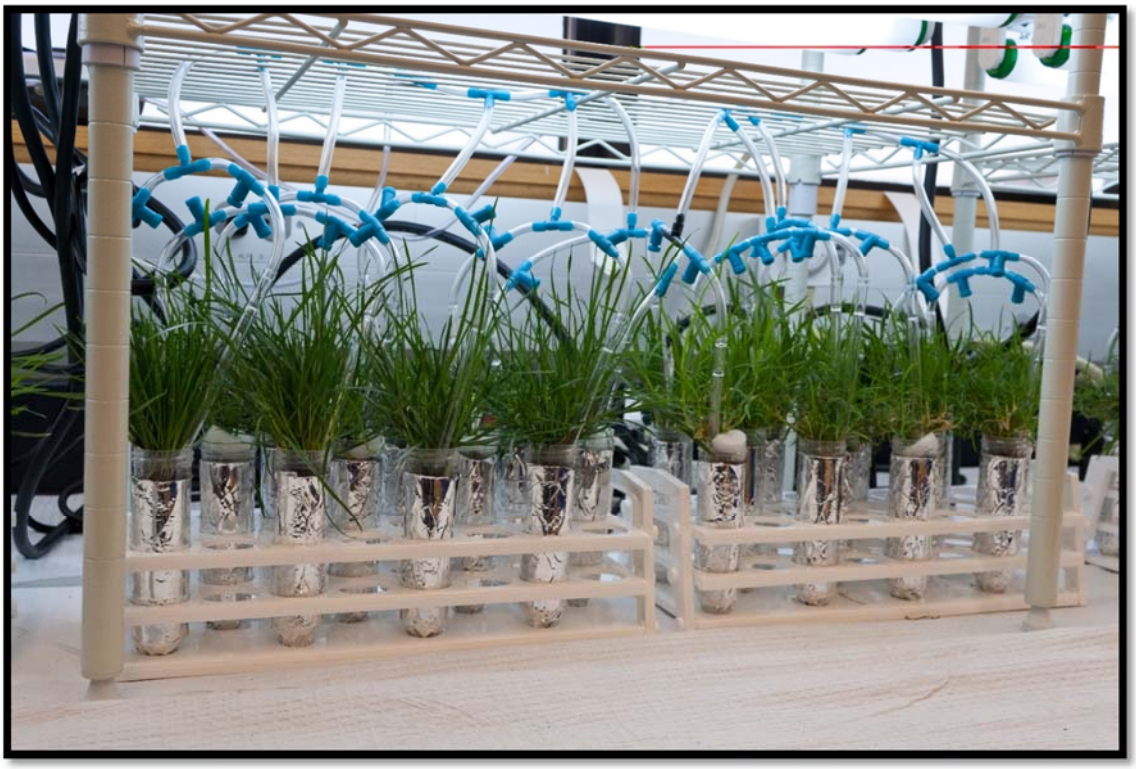

Figure 3.2. A more detailed picture of the grass plants acclimating to the laboratory conditions.

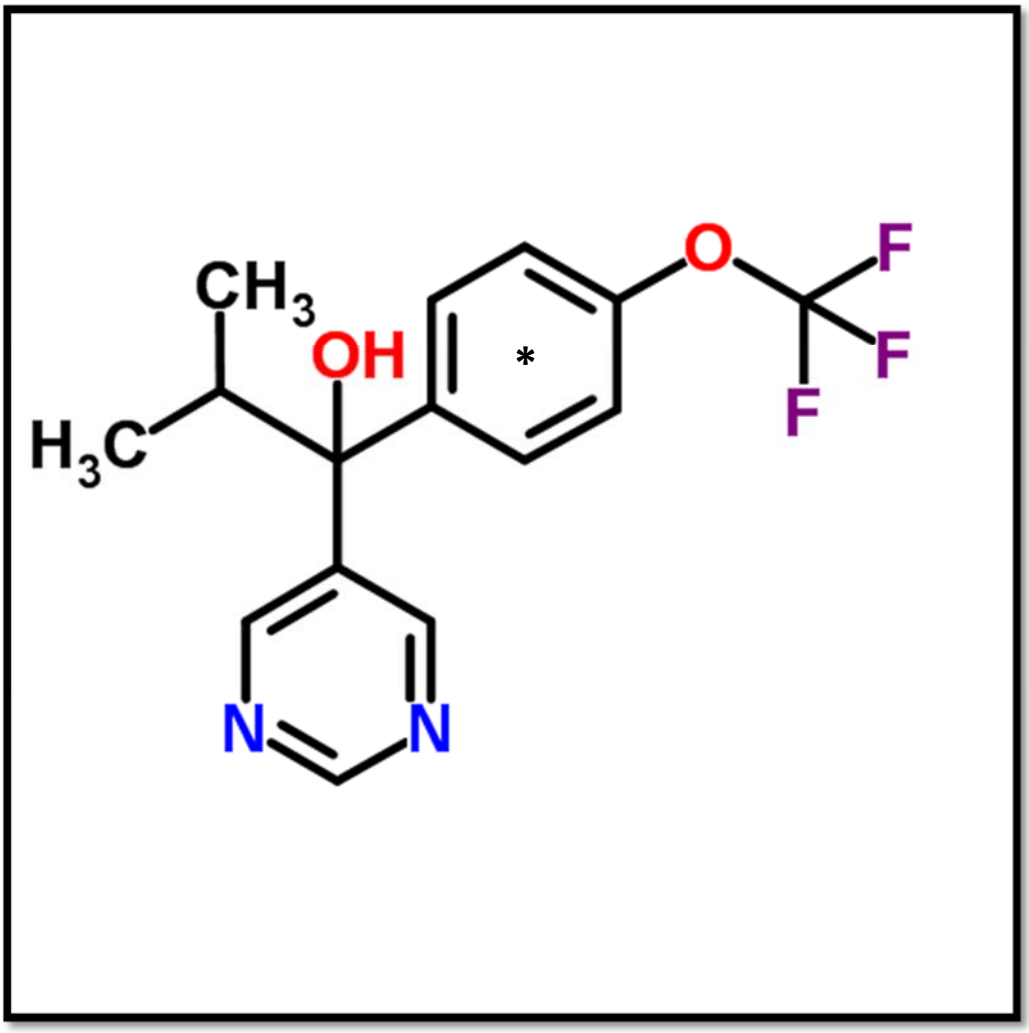

Figure 3.3. Flurprimidol chemical structure. * represents flurprimidol is uniformly labelled in the benzene ring

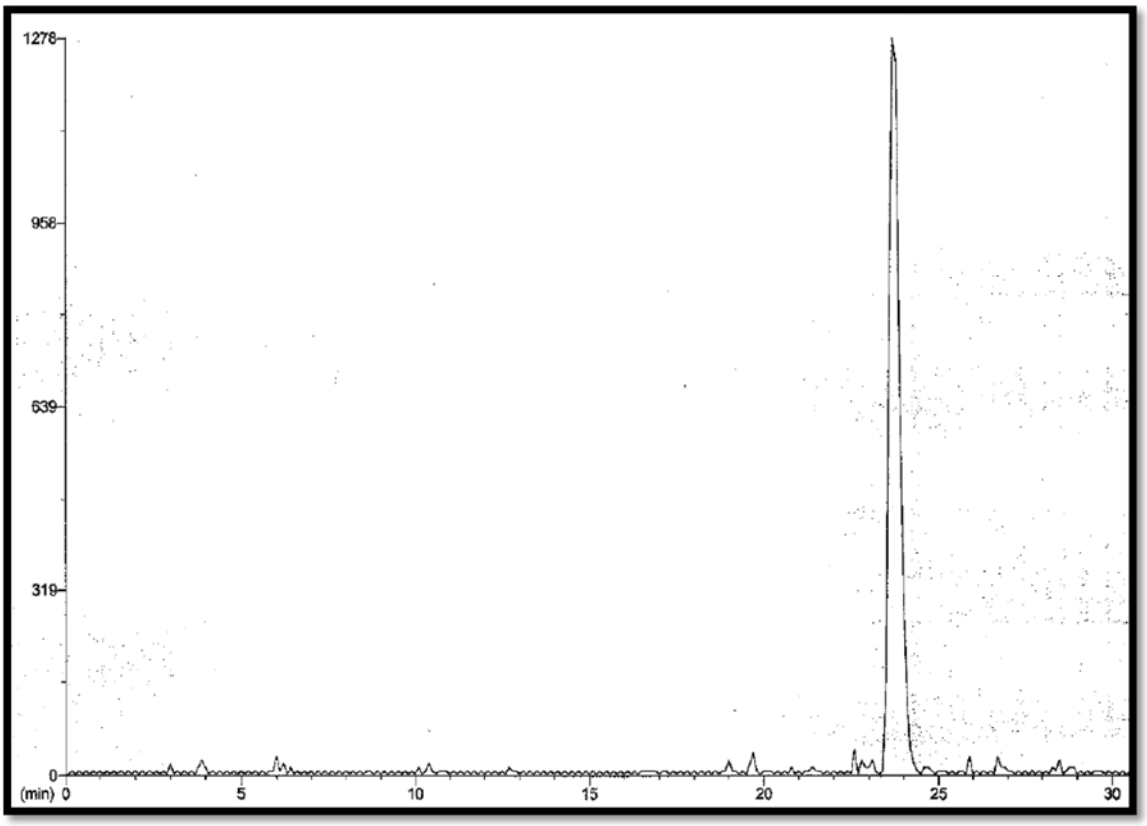

Figure 3.4. Radiochromatograph of the ^{14}C flurprimidol standard. The peak at 23.4 minutes represents the parent peak of ^{14}C-labeled flurprimidol. Units on the y-axis represent CPM (counts per minute).

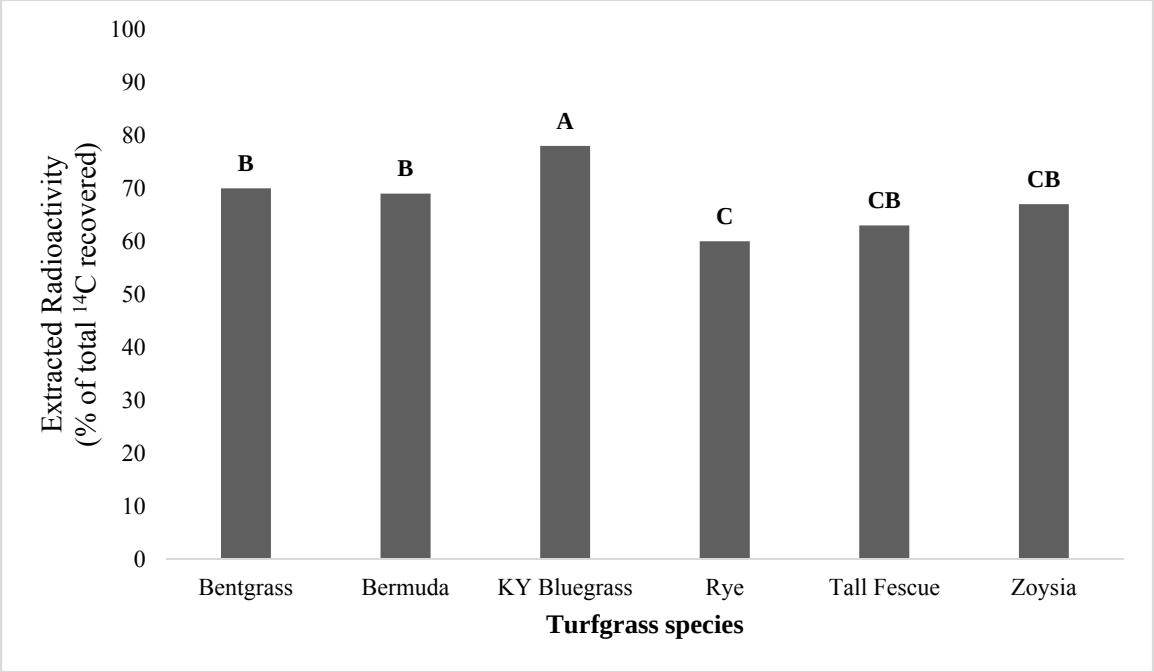

Figure 3.5. Extraction efficiencies for 6 turfgrass species. Bars with the same letter are not statistically different ($p < 0.05$).

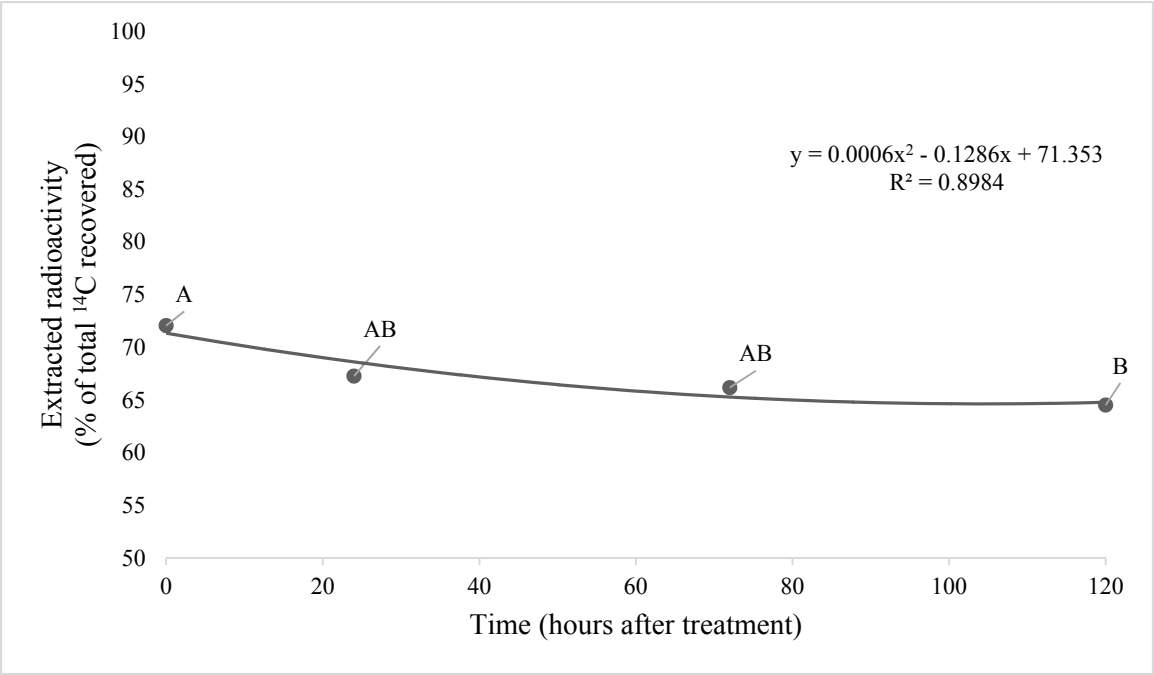

Figure 3.6. Extraction efficiencies for 6 turfgrass species averaged over time. Points with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$) using a polynomial regression model.

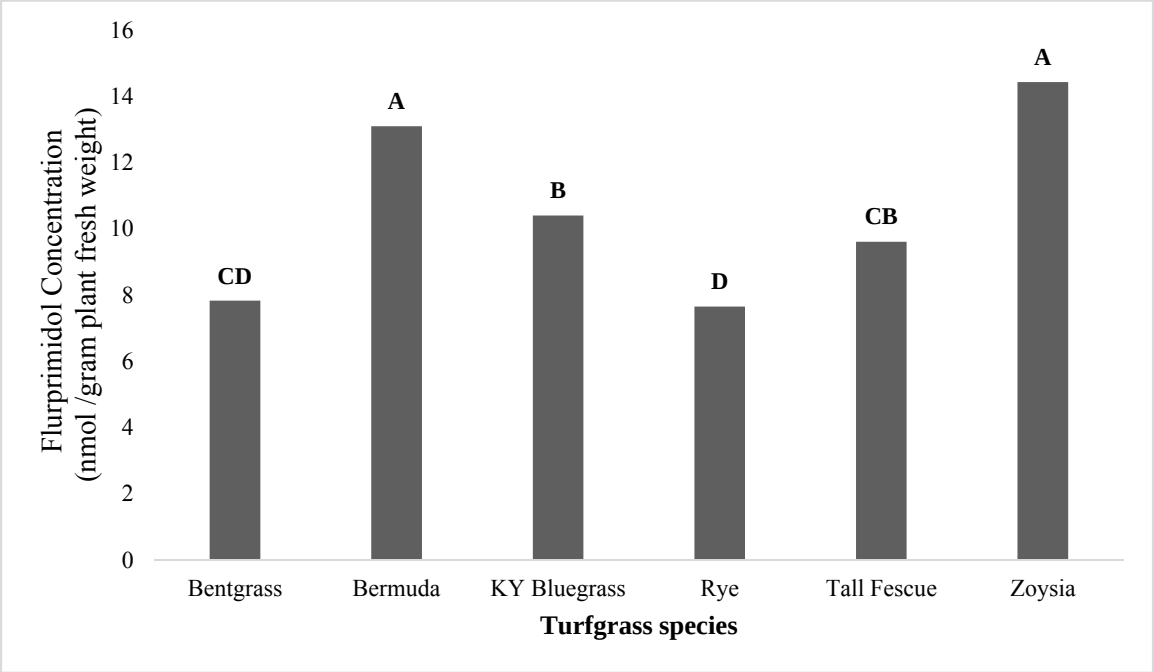

Figure 3.7. Mean total plant concentration of flurprimidol in 6 turfgrasses. Bars with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$).

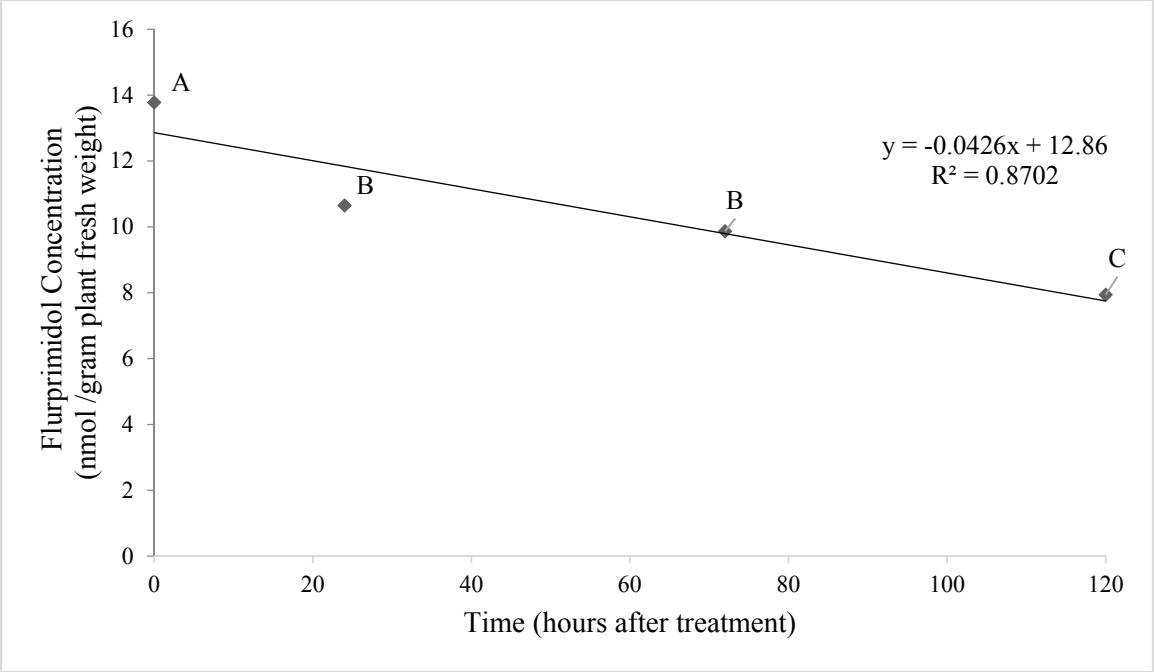

Figure 3.8. Flurprimidol tissue concentrations averaged across turfgrass species 0, 24, 72, and 120 hours after flurprimidol treatments. Points with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$) using a linear regression model.

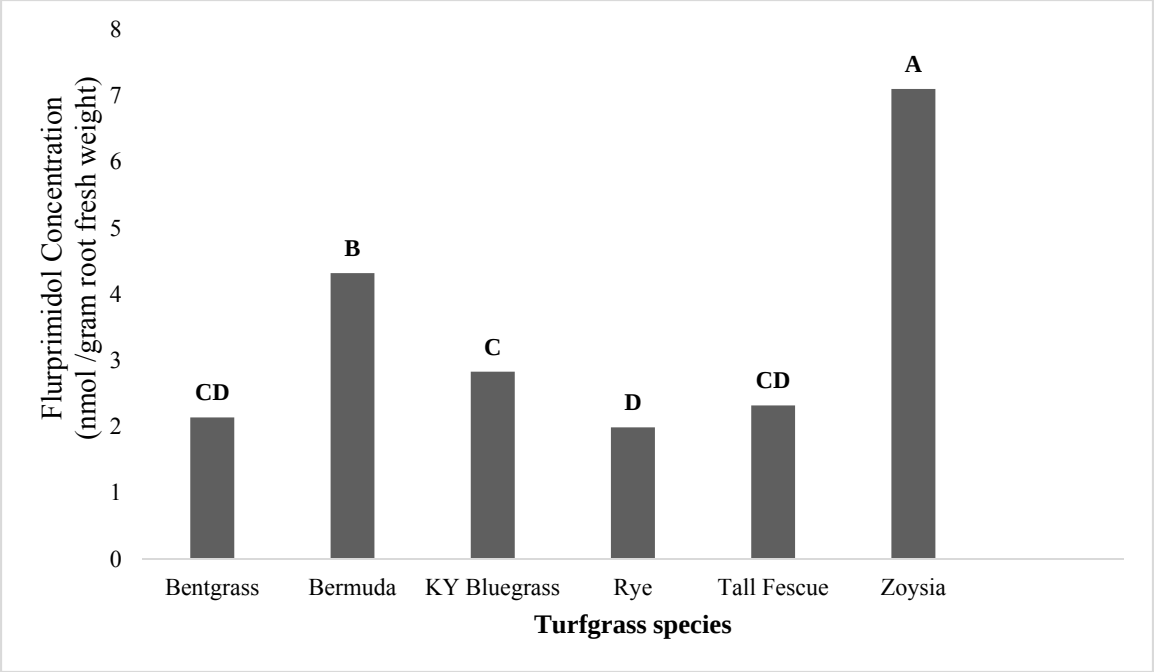

Figure 3.9. Mean flurprimidol concentration in the roots of 6 turfgrasses. Bars with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$).

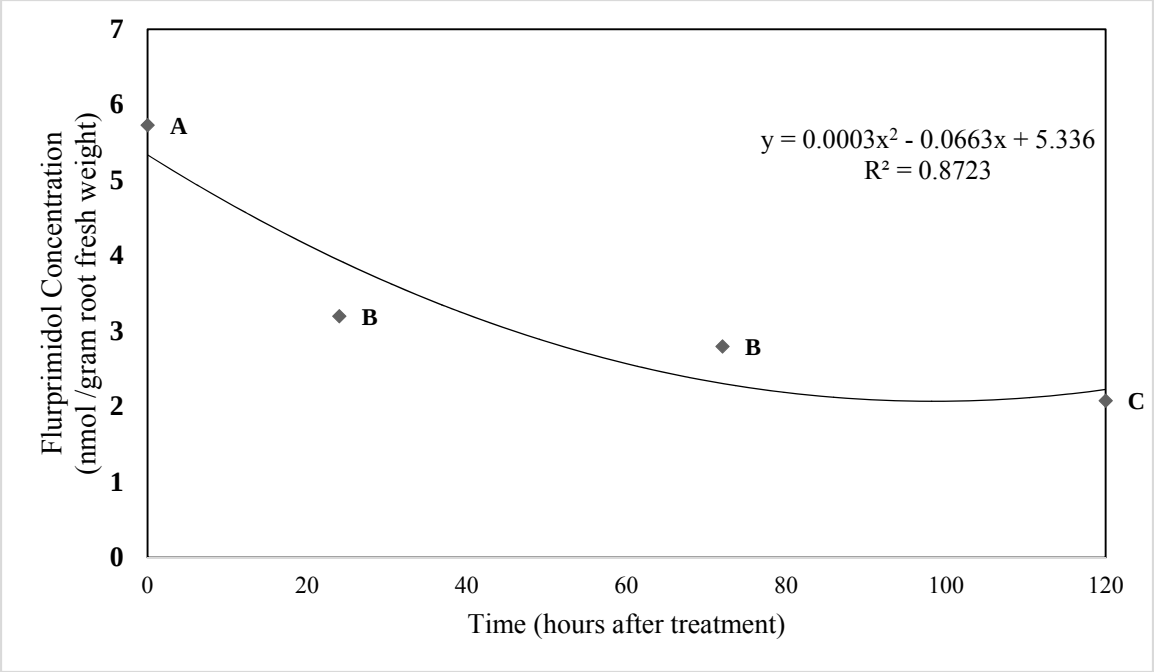

Figure 3.10. Mean root concentration of flurprimidol 0, 24, 72, and 120 hours after treatment. Points with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$) using a polynomial regression model.

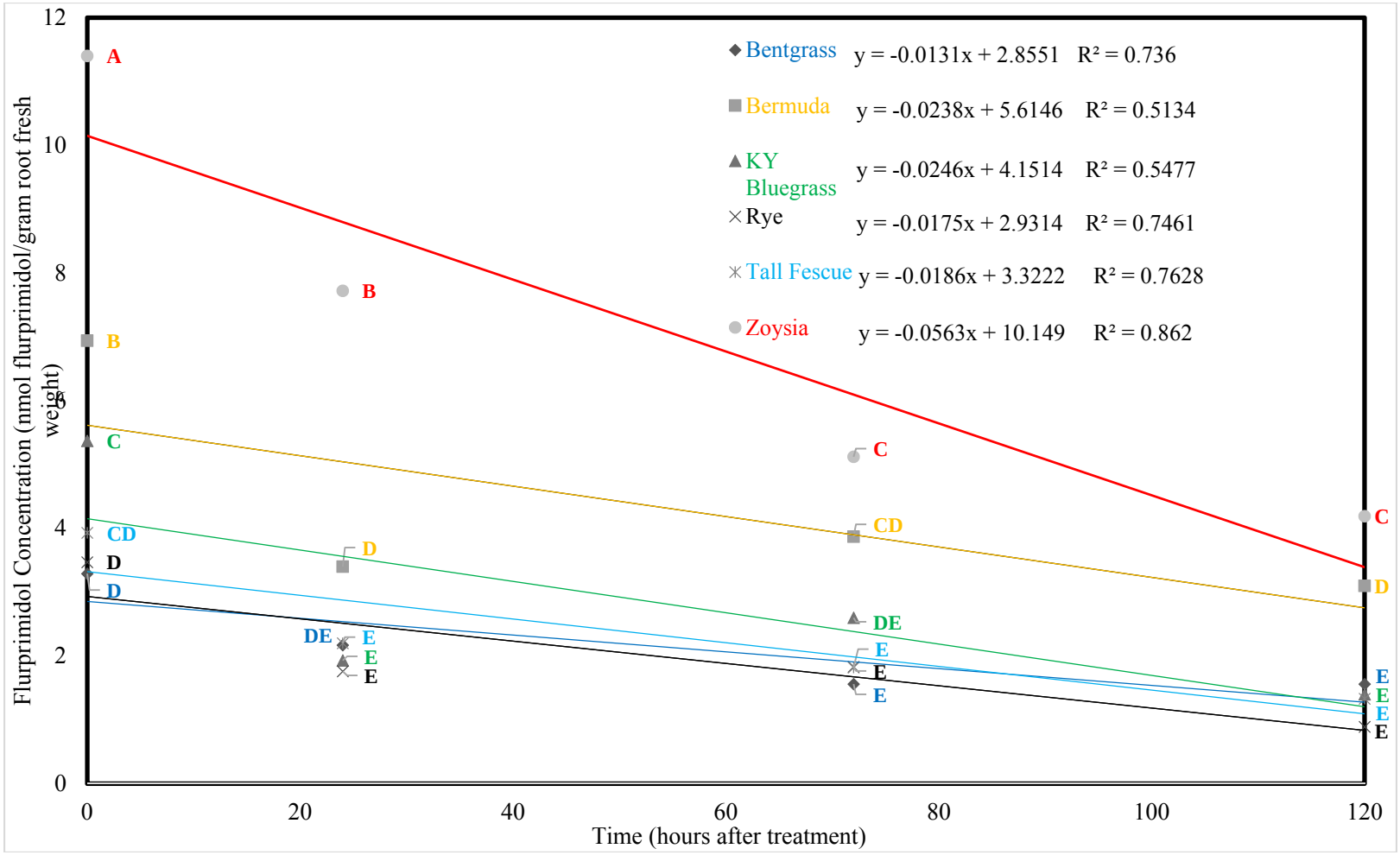

Figure 3.11. Mean root flurprimidol concentration in six turfgrasses 0, 24, 72, and 120 hours after initial flurprimidol exposure. Points with the same letter are not statistically different using LSMeans in SAS and a linear regression model.

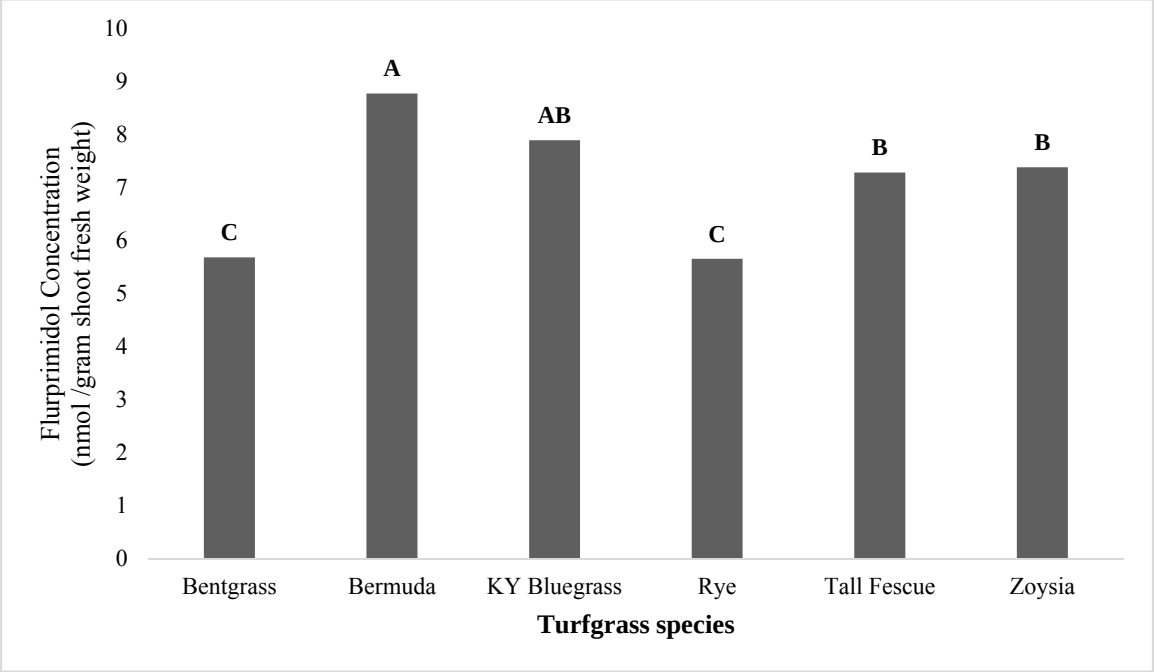

Figure 3.12. Mean shoot concentration for 6 turfgrasses. Bars with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$).

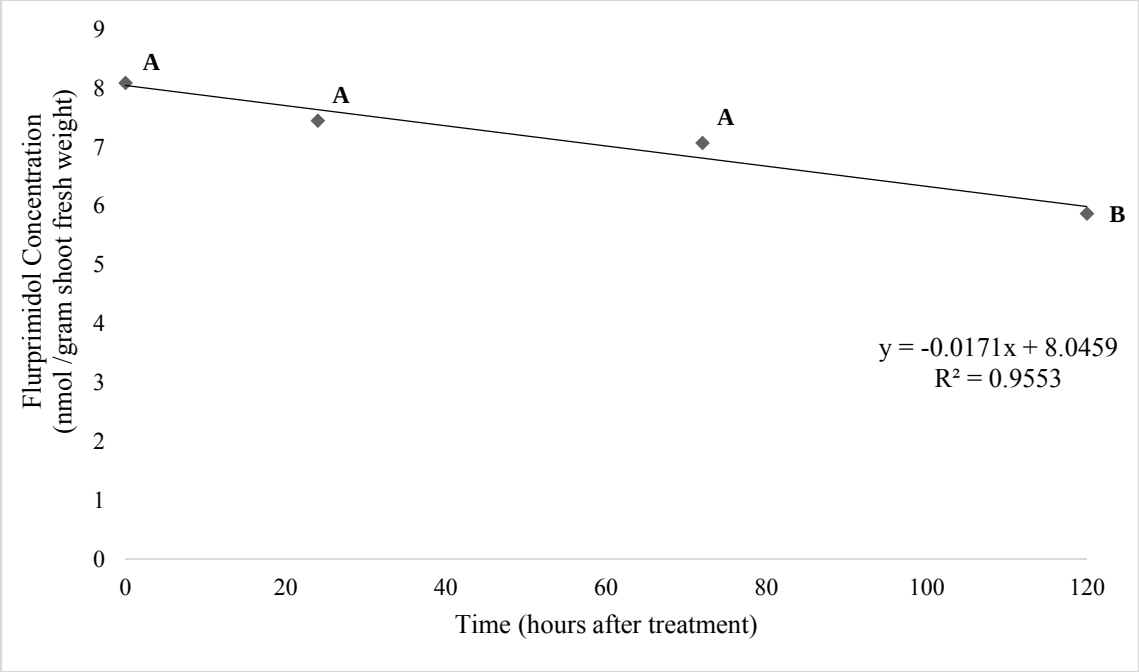

Figure 3.13. Mean shoot concentrations 0, 24, 72, and 120 hours after initial flurprimidol treatments. Points with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$) using a linear regression model.

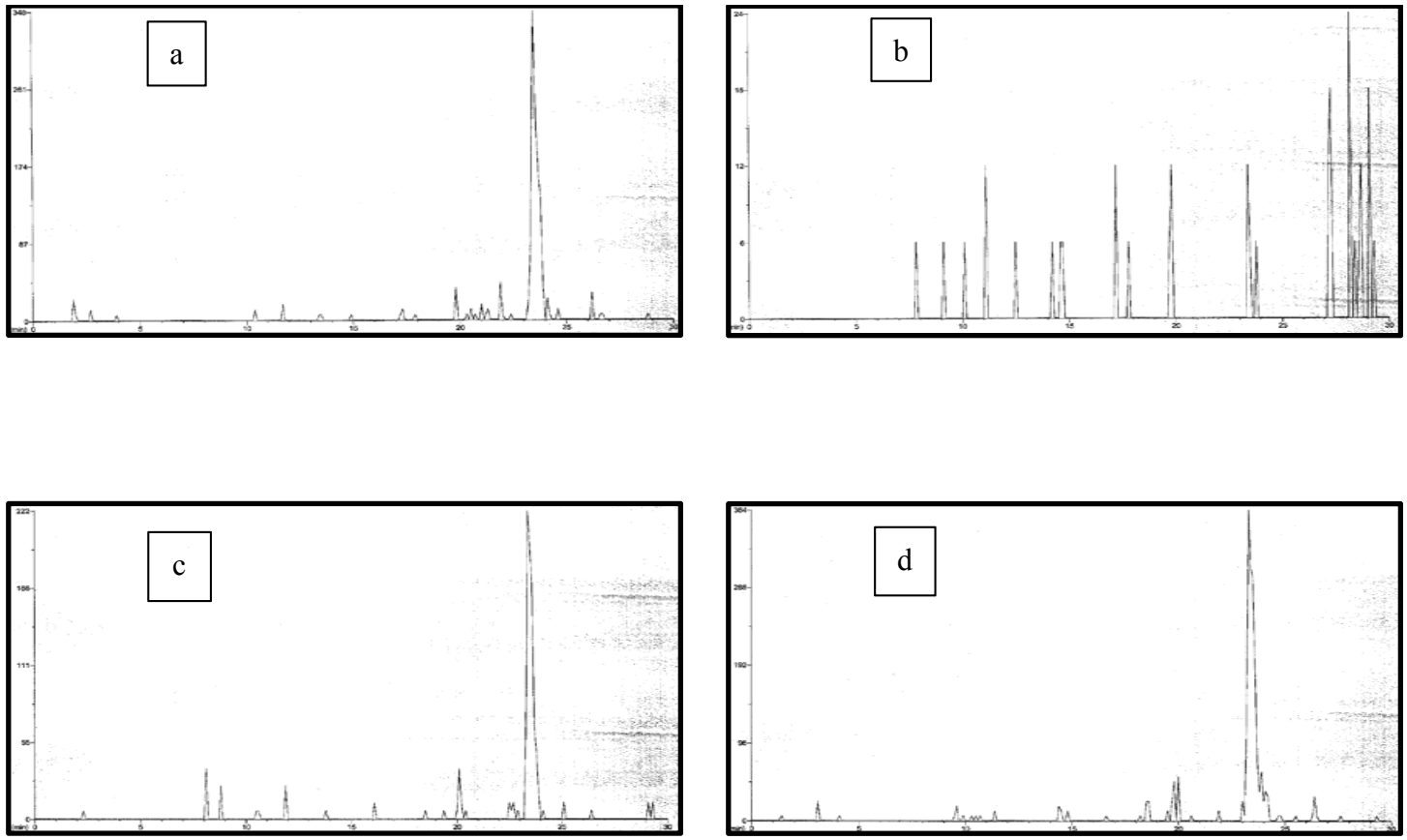

Figure 3.14. Samples of radiochromatographs of extracts from creeping bentgrass roots at 0 (a.) and 120 (b.) hours after treatment and shoots at 0 (c.) and 120 (d.) hours after treatment. Units on the y-axis represent CPM (counts per minute). No metabolites were detected during this study.

Chapter Four

Flurprimidol Uptake and Metabolism in *Poa annua* Biotypes, Kentucky Bluegrass, and Creeping Bentgrass

Flurprimidol [alpha-(1-methylethyl)-alpha-(4-(trifluoromethoxyphenyl)-5-pyrimidine-methanol] is a commonly used plant growth regulator (PGR) to control turf growth, improve turf quality, and manage *Poa annua* (L.) (Bigelow et al. 2007; Christians 2001). *Poa annua*, commonly known as annual bluegrass, is the most widespread weedy grass species found in managed turfgrass (Warwick 1979; La Mantia and Huff 2011). Contrary to its common name, both annual (*Poa annua* ssp. *annua* (L.) Timm.) and perennial (*Poa annua* ssp. *reptans* (L.) (Hausskn.) Timm.) subspecies of *Poa annua* exist (Timm 1965; Vargas and Turgeon 2004). *Poa annua* spp. *reptans* produces less flowerheads and have more tillers than *Poa annua* spp. annua (Timm 1965). *Poa annua* var. *reptans* flowers in the spring and occasionally in the fall; whereas *Poa annua* var. *annua* will often flower repeatedly all through the growing season (Johnson et al. 1993). Despite their opposite natures, the two subspecies are common plants on golf course putting greens and frequently inhabit the same areas. *Poa annua* decreases golf course turfgrass quality because it creates an uneven playing surface and decreases aesthetics due to its light green color and high seedhead production (Beard et al. 1978; Goss and Zook 1971).

Flurprimidol is a nitrogen-containing heterocycle and organofluorine compound belonging to the pyrimidine chemical class (Bunnell and Cockreham 2005; Key et al. 1997; Leroux et al. 2005). Like most (54%) of the organoflurorine compounds created for agricultural use, flurprimidol is a trifluoromethyl-substituted aromatic (Key et al. 1997). Other registered agricultural pesticides that include an OCF_3-group include

indoxacarb, triflumuron, thifluzamide, and flucarbazone-sodium (Leroux et al. 2008). Although lacking the OCF_3-group, there are other PGRs that are similar to flurprimidol: ancymidol, which also belongs to the pyrimidine class of chemistry, paclobutrazol and uniconazole, both triazoles, and trinexapac-ethyl (Bunnell and Cockreham 2005; Christians 2001; Pinhero and Fletcher 1994).

Flurprimidol and trinexapac-ethyl are classified as a Type II, Class B plant growth regulators (Watschke et al. 1992; Warcheke and DiPaola 1995). Type II plant growth regulators are generally crown and root absorbed (Christians 2001). Class B refers to plant growth regulators that inhibit gibberellic acid (GA) production early in the biosynthesis pathway (Christians 2001). Specifically, flurprimidol inhibits *ent*-kaurene oxidase, a cytochrome P_{450} monooxygenase, blocking the formation of *ent*-kaurenoic acid which is a precursor to active creating gibberellic acids (Bunnell and Cockreham 2005; March et al. 2013). GAs are an assembly of diterpene compounds that regulate plant mechanisms like seed germination, shoot elongation, and seed development (Yamaguchi et al. 1998). More than 110 forms of GAs exist in plants, however, only a few of these (i.e. GA_1, GA_3, GA_4, and GA_7) are said to function as bioactive hormones (Asrar 2012; Taiz 1991). Throughout the lifetime of a turfgrass, gibberellins continue to promote the elongation of stems and, therefore, without GAs elongation of shoot internodes is decreased (March et al. 2013; Wroblewski 2013).

While flurprimidol is widely used, there is little published information concerning its behavior in plants. Previous studies examining flurprimidol translocation and/or metabolism used stem injections into trees (Sterrett and Tworkoski 1987) or determined methods for extraction from Eurasian watermilfoil (*Myriophyllum spicatum*), soil, and

water (Chand and Lembi 1991). Sterrett and Tworkoski (1987) injected 2.5 mg ^{14}C-labeled flurprimidol into the stock of a 1-year-old apple tree (*Malus domestica* Borkh.). After 35 days, 10% had moved into the new shoots, 1.5% into the scion phloem, and 80% remained near the injection site. A high percentage of the ^{14}C activity was unmetabolized flurprimidol.

The behavior of other PGRs and herbicides have been studied in *Poa annua*. Branham and Beasley (2007) found that when paclobutrazol is used regularly over an entire growing season growth of *Poa annua* is reduced much more than the growth of creeping bentgrass (*Agrostis stolonifera* L.). This causes the population of *Poa annua* to shrink while creeping bentgrass increases. *Poa annua* spp. *reptans* was successfully controlled using paclobutrazol at both putting green (Johnson and Murphy 1995) and fairway heights (Woosley et al. 2003). Wu et al. (1992) collected *Poa annua* biotypes from both roughs and greens and found that, when exposed to 9 combinations of paclobutrazol and light intensities, both biotypes produced more dry weight under low light than under high light intensities. However, they also found that plants from golf greens produced greater total plant dry weight than rough biotypes under all treatment combinations. Callahan and McDonald (1992) found that bensulide (a pre-emergence herbicide) controlled the annual subspecies but not the perennial subspecies of *Poa annua*. McElroy et al. (2004) conducted laboratory studies to evaluate the variation in germination of 8 *Poa annua* ecotypes in different photoperiods and temperatures with fenarimol (a fungicide-herbicide used for pre-emergence *Poa annua* treatment). They found significant ecotype by environment interactions for the *Poa annua* germination and

that some ecotypes germinated better in higher temperatures and there were differences in the effect of fenarimol on shoot growth.

In Kentucky, the majority of golf course workers use PGRs to control *Poa annua* populations (Figure 4.1). Success with managing *Poa annua* with PGRs, like flurprimidol, depends highly on a golf course superintendent's ability to adapt a PGR regime to their particular golf course (Christians 2001). The efficacy of *Poa annua* management regimes can also vary with the *Poa annua* biotypes adapted to a region (Bingaman et al. 1998; Street and Sherratt 1998). According to the Cutless® MEC Turf Regulator (SePro Corporation 11550 N. St. Ste. 600, Carmel, IN 46032 U.S.A.) label, rate recommendations for flurprimidol range depending on the turf type and species, turf location and use, turf management, and in some conditions, turf cultivar (Table 4.1). These distinctive recommendations suggest that flurprimidol behavior in turfgrasses, such as Kentucky bluegrass (*Poa pratensis*) and creeping bentgrass (maintained at putting green height), may differ. It is recommended on the Cutless® MEC Turf Regulator label that treated areas receive a rain event within 24 hours of application. Because there is little published information concerning its behavior in plants, the objectives of this study were to determine flurprimidol absorption, translocation, and metabolism in *Poa annua* putting green biotypes, creeping bentgrass, and Kentucky bluegrass to help explain their differential tolerance to the PGR.

Materials and Methods

Plant Material

Poa annua plugs were collected using a cup cutter from greens of two different golf courses in Lexington, Kentucky: 1.) The Lexington Country Club (2550 Paris

Pike Lexington, KY 40511) and 2.) The University Club of Kentucky (4850 Leestown Road Lexington, KY 40511) in the fall of 2013. The Lexington Country Club is an older golf course and maintains their putting greens for *Poa annua* while The University Club of Kentucky maintains their putting greens for creeping bentgrass. The biotypes were selected based on one of two physical criteria: 1.) a light-green colored plant with many flowerheads and coarse-textured and 2.) a dark -green colored plant with few to no flowerheads and fine-textured. Plugs were also collected from existing stands of "L-93" creeping bentgrass (maintained at 0.32 cm) and "Midnight II" Kentucky bluegrass (maintained at 5 cm) at the University of Kentucky Research Farm in Lexington, KY in the fall of 2013. The plugs were transferred to the greenhouse where they were separated into tiller groups and planted into a 50% Maury silt loam soil (fine, mixed, mesic typic Paleudalfs) and 50% coarse builder's sand (Clay Ingels Co. LLC. 914 Delaware Ave, Lexington, KY) mixture. Osmocote® Slow Release (The Scotts Company LLC. Marysville, OH) fertilizer (19-19-19, 2 g/0.001 m^3) was added to the sand/soil mixture. Plants were clipped to approximately 0.32 cm for the *Poa annua* and creeping bentgrass and 5 cm for Kentucky bluegrass using scissors once a week. Plants were treated with fungicides and insecticides when necessary to maintain plant health.

Approximately one month later after transfer to the greenhouse, the plants were transferred to the laboratory for acclimation. Roots were gently washed 4-5 times with distilled water to remove residual growing media. Once rinsed, the roots were placed in 50 ml polypropylene tubes (Fisher Scientific, 300 Industry Drive Pittsburgh, PA 15275) filled with 45 ml of ¼ strength Hoagland's solution (Hoagland and Arnon 1950). The tubes were covered with aluminium foil to exclude light. When needed, a foam plug

(Identi-plugs®, Jaece Industries, INC. 908 Niagra Fall Blvd. North Tonawanda, NY) was used to support the plants. Solution lost to transpiration and evaporation was replenished daily with distilled water. Each tube was aerated using a glass Pasteur pipette (Corning® Incorporated, Corning, NY 14831) connected to air tubing, which was powered by an aquarium air pump system. Supplemental light was provided using fluorescent bulbs (0.25 μmol m^{-2} sec^{-1}) with a 16 h photoperiod. Temperatures were maintained at approximately 28 C. The plants were acclimated between 6 and 7 days before initiation of the experiment. Tubes were arranged in a randomized block design with time of harvest used as a blocking factor. There were four replications per treatment and four harvest times.

Flurprimidol Treatment

After acclimation, the individual plants were transferred into new 50 ml polypropylene tubes filled with a 25μL methanol solution with 1.56 μM ^{14}C-uniformly benzene ring labelled flurprimidol (Figure 4.2) in 45 ml of ¼ strength Hoagland's solution. This concentration replicated a flurprimidol rate of 560 g a.i. / ha^{-1} in approximately 15 cm of soil with 50% available in the soil water. This is the highest rate labelled for creeping bentgrass (golf course fairway height) and Kentucky bluegrass initial spring applications (Table 4.1). The plants were under continuous illumination during the ^{14}C flurprimidol treatment period. After 48 hours, the plants were removed from the treatment solution and the roots were rinsed with distilled water. The plants were harvested at two intervals. One set of plants was harvested at the end of the 48 hour treatment period (Time 0) while the others were transferred to fresh vials of ¼ strength Hoagland's solution. After the first harvest collection, a subsequent harvest was

performed 3 weeks later. At each harvest, roots were rinsed with distilled water and gently blotted dry with a paper towel. The plants were separated into roots and shoots, weighed, and then frozen (-20 C) until extracted.

Radioactivity Extraction and Analysis

Plant material was ground into a fine powder in liquid nitrogen using a pestle and mortar; 10 ml of methanol was then added to the powder and the mixture was transferred to a centrifuge tube. The mixture was centrifuged at 7650 relative centrifugal force (RCF) for 5 minutes. The supernatant was decanted and a second 10 ml of methanol was added to the pellet, vortexed, and centrifuged again. The second supernatant was added to the first and then brought up to 25 ml.

The extract was concentrated to 1 ml in a rotovap. The sample was transferred to a microfuge vial and centrifuged at 4550 RCF for 2 minutes. The samples were then filtered through a sterile, nylon 0.45 µum filter (Fisher Scientific, 300 Industry Drive Pittsburgh, PA 15275) into a 1.5 ml autosampler vial. Preliminary trials established full ^{14}C flurprimidol recovery using this method. To quantify the total radioactivity in each extract, a 50 µl aliquot was mixed with 15 ml of scintillation cocktail (Bio-Safe II™, Research Products International Corp. 410 N Business Center Drive, Mount Prospect, IL 60056). The radioactivity was quantified using a scintillation counter (TriCarb® 2200CA, Perkin Elmer™ Life Sciences, 2200 Warrenville Rd, Downers Grove, IL 60515).

To quantify unextracted radioactivity, the residue remaining after the extraction was oxidized (Packard Sample Oxidizer model #307, Perkin Elmer™ Life Sciences, 940

Winter Street Waltham, MA 02451) after air drying and released $^{14}CO_2$ was measured in a scintillation counter.

^{14}C in the extracts was analyzed using a high performance liquid chromatography (HPLC) (Prominence UFLC, Shimadzu, 1, Nishinokyo-Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan) system coupled to a radioactivity detector (Radiomatic FLO-ONE® Beta Series A-500, Canberra Industries, Inc. 800 Research Parkway, Meriden, CT 06450). The HPLC was equipped with a C18 5µm 4.6 x 250 mm reverse phase column (GL Sciences Inc. Shinjuku Square Tower 30F, 6-22-1 Nishi Shinjuku, Shinjuku-ku, Tokyo, 163-1130 Japan). Elution was accomplished using a gradient beginning with 80% water and 20% acetonitrile (ACN) (Table 4.2). The flow rate through the HPLC was 1 ml/minute. Flurprimidol standards eluted at 23.2 minutes.

^{14}C flurprimidol concentrations were expressed as a ratio where:

$$\frac{\text{Shoot or Root or Total DPM}}{\text{Shoot or Root or Total Weight}}$$

Concentrations were then expressed as nmol ^{14}C flurprimidol/gram of fresh plant weight. Data were subjected to analysis of variance (ANOVA) and means were separated using Fisher's Protected LSD test at $\alpha = 0.05$. LSMeans was used to calculate for means separation for interactions at $\alpha = 0.05$. The experiment was repeated and, as no significant differences were detected between experimental runs, the data was combined for analysis.

Results and Discussion

Flurprimidol Uptake and Translocation

There was no difference in the absorption of ^{14}C-flurprimidol between the species. There was also no difference in the total ^{14}C recovered between 0 and 3 WAT (data not shown). Over time, the amount of flurprimidol in the roots declined while shoot concentrations increased (Figure 4.3). The root concentration decreased from 9.2 to 3.4 nmol flurprimidol/g fresh weight and the shoot concentration increased from 11.2 to 18.2 nmol flurprimidol/g fresh weight over the three week period. Thus, even though ^{14}C-flurprimidol absorption ceased, a significant (19%) amount of the flurprimidol was still in the roots 3 weeks after treatment (WAT).

There was no harvest time by species interaction for extraction efficiency. However, extraction efficiency declined between 0 and 3 WAT for both root and shoot tissues (Figure 4.4). A decrease in extraction efficiency over time could indicate that the flurprimidol is being metabolized to unextractable residues or is otherwise being bound within the plant tissues. Or, as the plants grew over the 3 week study period, extraction efficiency could simply be lowered with larger plants. Creeping bentgrass and Kentucky bluegrass had slightly lower extraction efficiencies that the *Poa annua* from the Lexington Country Club (Figure 4.5). There were no differences between the turfgrass species for shoot extraction efficiencies (Figure 4.6). Averaged across all turf species shoot extraction efficiency was approximately 92%. This extraction efficiency was comparable or somewhat higher than those previous flurprimidol studies. Chand and Lembi (1991) had flurprimidol recoveries of 86.8% from watermilfoil shoots and 85.2% from watermilfoil. West and Rutherford (1986) extracted 80% of the flurprimidol from

soil and 78% from a soil-grass mixture flurprimidol recovery using gas chromatography (GC). Reed (1988) recovered 83.6% of the flurprimidol from peach leaves.

According to the Cutless® MEC label, flurprimidol rate recommendations for initial applications to creeping bentgrass putting greens range from 140 to 280 g a.i./ha^{-1} while for Kentucky bluegrass they range from 420 to 560 g a.i./ha^{-1} (Table 4.1). The rate recommendations for repeated applications to creeping bentgrass are also lower than those repeated applications to Kentucky bluegrass. PGRs are generally less phytotoxic than herbicides, however, they can cause grass discoloration (Christians 2001). This is one reason why lower rates are used for creeping bentgrass putting greens.

The turf species in this study all absorbed flurprimidol through the roots. According to the Cutless® MEC label, a 0.64 to 1.3 cm irrigation event is recommended after application to maximize the PGR activity. Absorbed flurprimidol moved from the roots to shoots in all grass species (Figure 4.3). After the initial 48 hour flurprimidol pulse, 45 and 55% of the flurprimidol was found in the root and shoot tissues, respectively. By 3 WAT, the distribution of flurprimidol was 16 and 84% in the roots and shoots, respectively. Turfgrasses in a golf course setting are mowed multiple times a week during the growing season. During each mowing event, flurprimidol within the shoot tissue will be removed. This may be why multiple applications of flurprimidol are recommended as part of a golf course management regime. Future studies should focus on the concentration of flurprimidol in the shoots after multiple "mowing simulation" events in the laboratory.

Metabolism

No metabolites of flurprimidol were detected in any of the turf species at 0 or 3 weeks after the initial flurprimidol treatment. Only parent flurprimidol material was detected in the extracts (Figure 4.7). Previous studies of flurprimidol translocation and/or metabolism in plants include one that employed stem injections of flurprimidol into apple trees (Sterrett and Tworkoski 1987; Redding et al. 1994) and one that determined methods for flurprimidol extraction and quantification in Eurasian watermilfoil (*Myriophyllum spicatum*), soil, and water (Chand and Lembi 1991). Sterrett and Tworkoski (1987) found only 20% of the injected ^{14}C-labeled flurprimidol was translocated and a high percentage of the radioactivity was unmetabolized flurprimidol in trees after 35 days. A similar lack of PGR metabolism was found 27 days after apple tree stem injections with ^{14}C-labeled paclobutrazol (Sterrett 1988).

Our studies evaluated the uptake, translocation, and metabolism of flurprimidol three weeks after the initial treatment of ^{14}C-labeled flurprimidol. It appears that differential responses to flurprimidol in these turf species are not due to differential flurprimidol metabolism. Flurprimidol is stable in these species. Future studies should consider possible differences at the site of flurprimidol action, *ent*-kaurene oxidase (March et al. 2013) between grass species.

Table 4.1. Labeled rates and application timings for growth regulation of perennial turfgrass species with Cutless MEC (flurprimidol) using a multiple application program.

Turf Species	Initial spring application[a] (g a.i. flurprimidol)	Repeat Applications[a]	
		(g a.i. flurprimidol)	Treatment interval
Cool season grasses			
Bentgrass (golf course fairway)	280 to 560	140 to 560	2 to 6 weeks
Bentgrass putting green	140 to 280	69.5 to 280	2 to 4 weeks
Kentucky bluegrass /Perennial ryegrass	420 to 560	280 to 560	2 to 6 weeks
Warm season grasses			
Seashore paspalum	140 to 560	140 to 560	3 to 6 weeks
Tifway, TifSport, and GN-1 Bermudagrass	140 to 420	140 to 420	3 to 6 weeks
Zoysiagrass	140 to 420	140 to 420	3 to 6 weeks not in late summer or fall

[a]Apply in early spring following resumption of active growth of the grass. Fall applications must be discontinued 4 weeks before the onset of inactive grass growth or winter dormancy.

Table 4.2. HPLC solvent gradient used for flurprimidol analysis.

Time (minutes)	Flow (ml/min)	Water (%)	Acetonitrile (%)
2.00	1.00	80	20
15.00	1.00	40	60
20.00	1.00	40	60
22.00	1.00	10	90
27.00	1.00	10	90
30.00	1.00	80	20
35.00	1.00	80	20

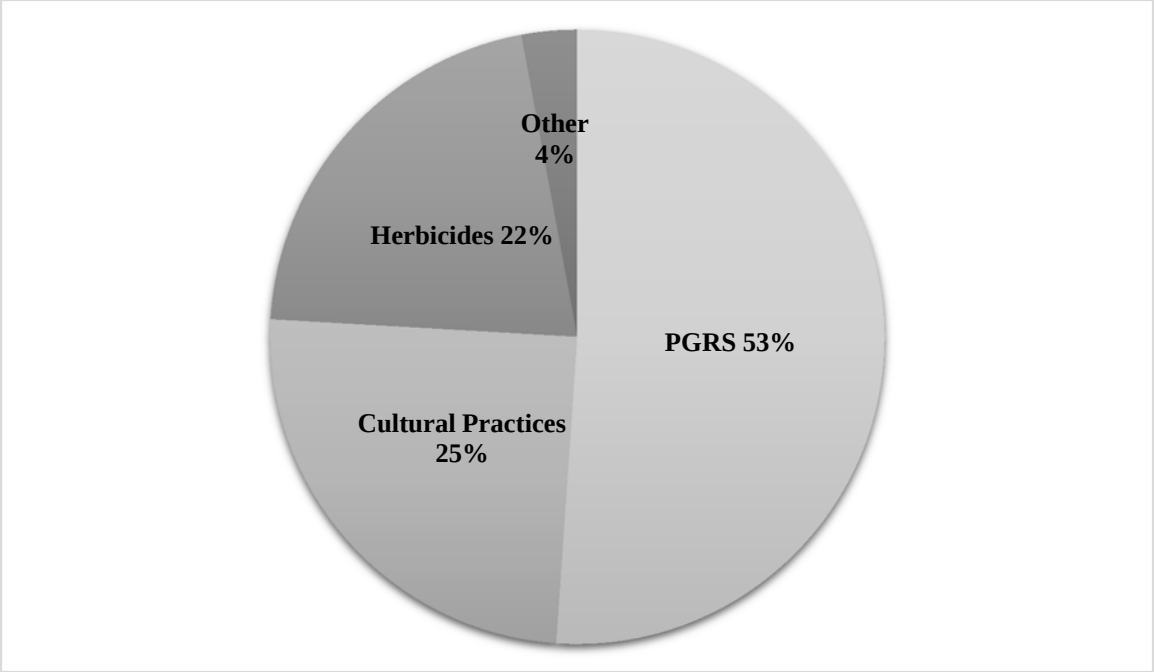

Figure 4.1. 72 golf course superintendents or workers were asked: "If *Poa annua* is a problem, what do you do to control this weed?" Plant growth regulators (PGRs) was the most frequent response. The survey was conducted at the University of Kentucky Turf and Landscape Short Course in February 2013.

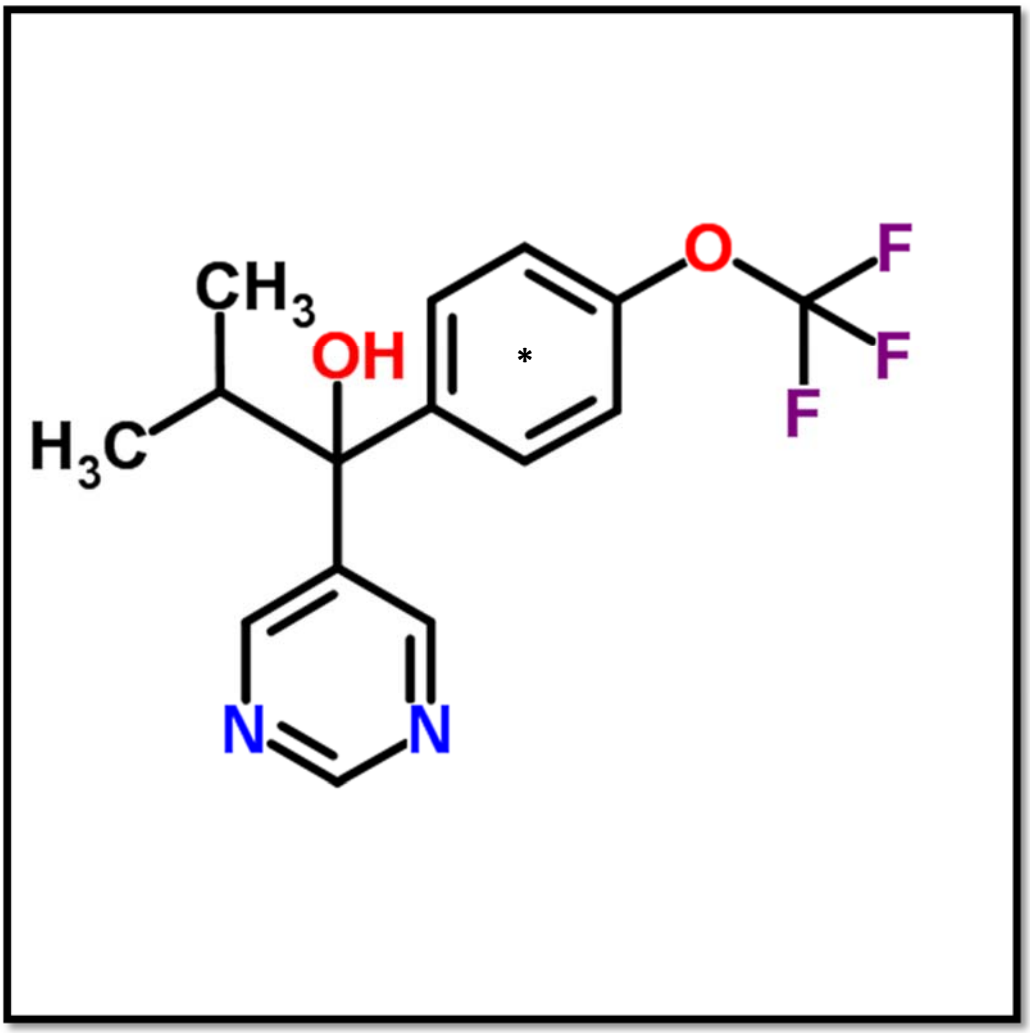

Figure 4.2. Flurprimidol chemical structure. * represents flurprimidol is uniformly labelled in the benzene ring.

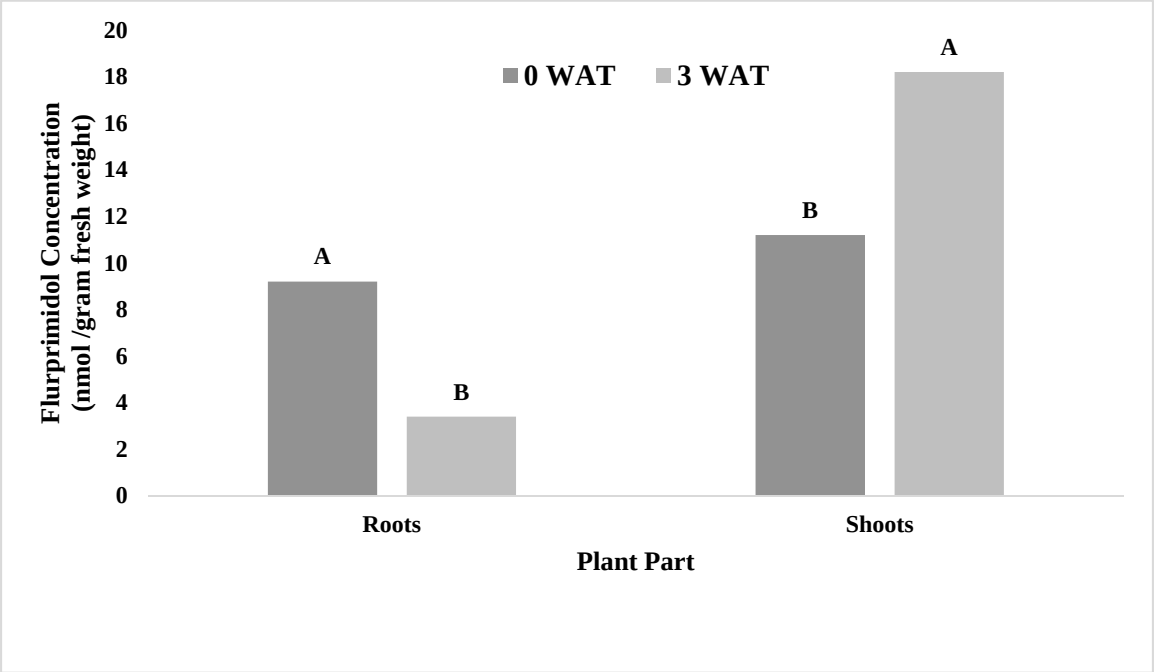

Figure 4.3. Flurprimidol concentrations in the roots and shoots of *Poa annua*, Kentucky bluegrass, and creeping bentgrass (averaged over the three species) at 0 and 3 weeks after treatment (WAT) with ^{14}C-flurprimidol. Bars within plant part with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$).

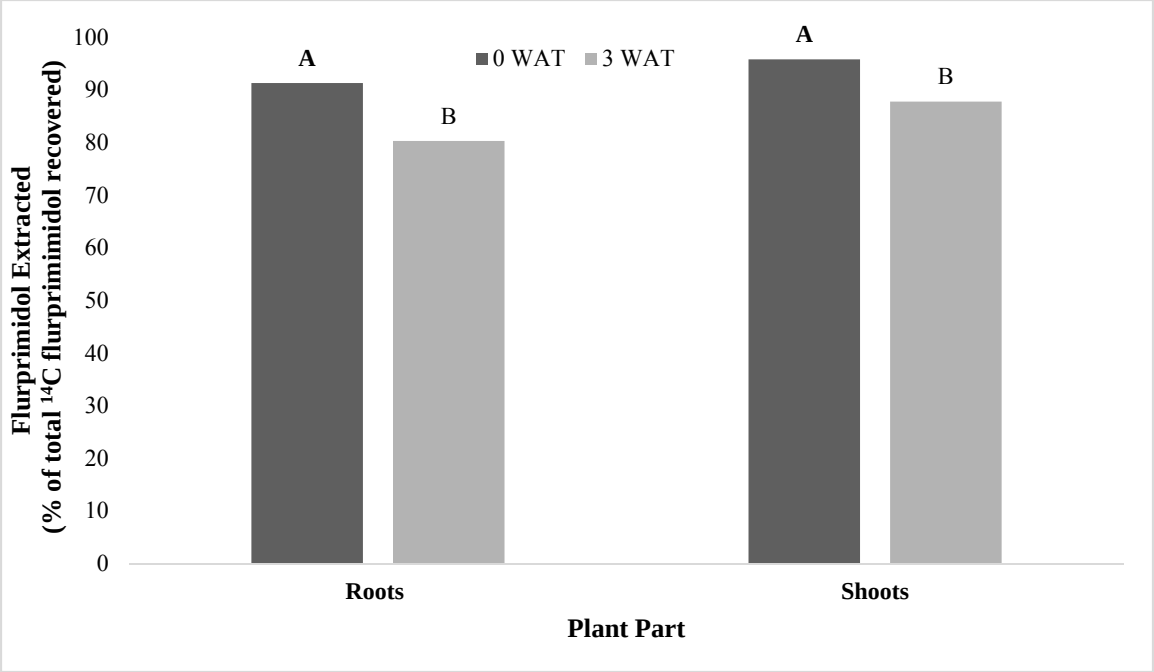

Figure 4.4. Percent of the absorbed radioactivity extracted from *Poa annua*, Kentucky bluegrass, and creeping bentgrass (averaged over the three species); roots and shoots 0 and 3 weeks after the initial root treatment with ^{14}C-flurprimidol. Data analysis was performed using an arcsine of the square root transformation in SAS. Values in graph are back-transformed. Bars within plant part with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$).

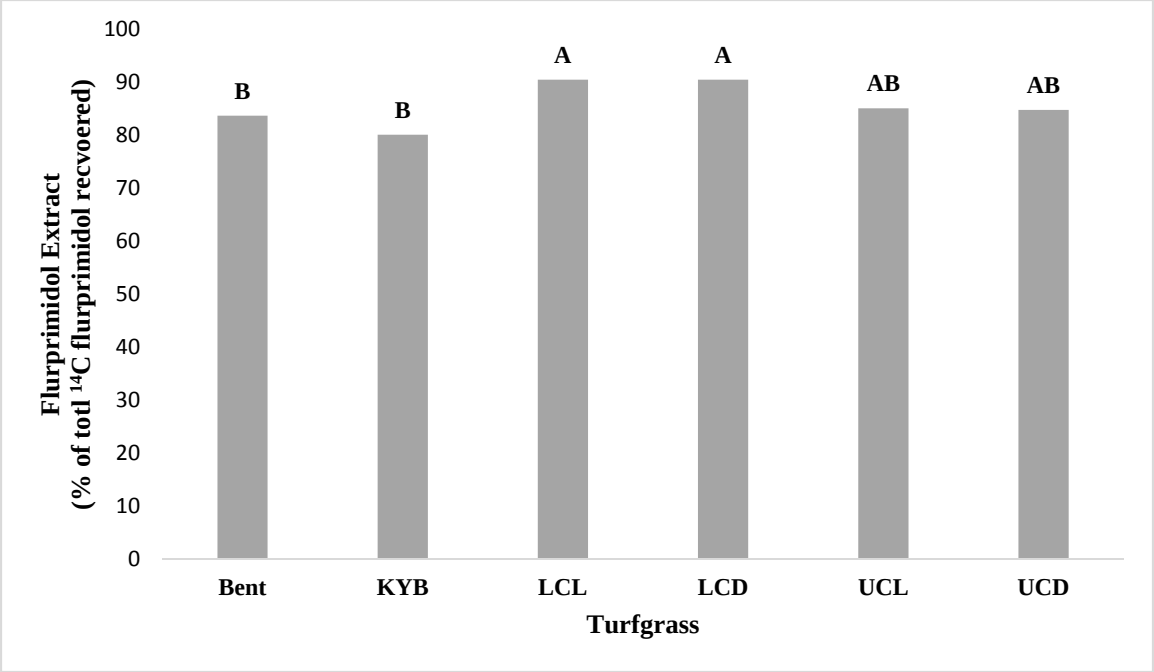

Figure 4.5. Percent of absorbed radioactivity extracted from roots of creeping bentgrass, Kentucky bluegrass, and four *Poa annua* biotypes averaged over harvest times (0 and 3 weeks after root treatment with ^{14}C-flurprimidol). Data analysis was performed using an arcsine of the square root transformation in SAS. Values in the graph are back-transformed. Bars with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$). Abbreviations: Bent, creeping bentgrass; KYB, Kentucky bluegrass; LCL, Lexington Country Club light biotype; LCD, Lexington Country Club dark biotype; UCL, University Club light biotype; UCD, University Club dark biotype.

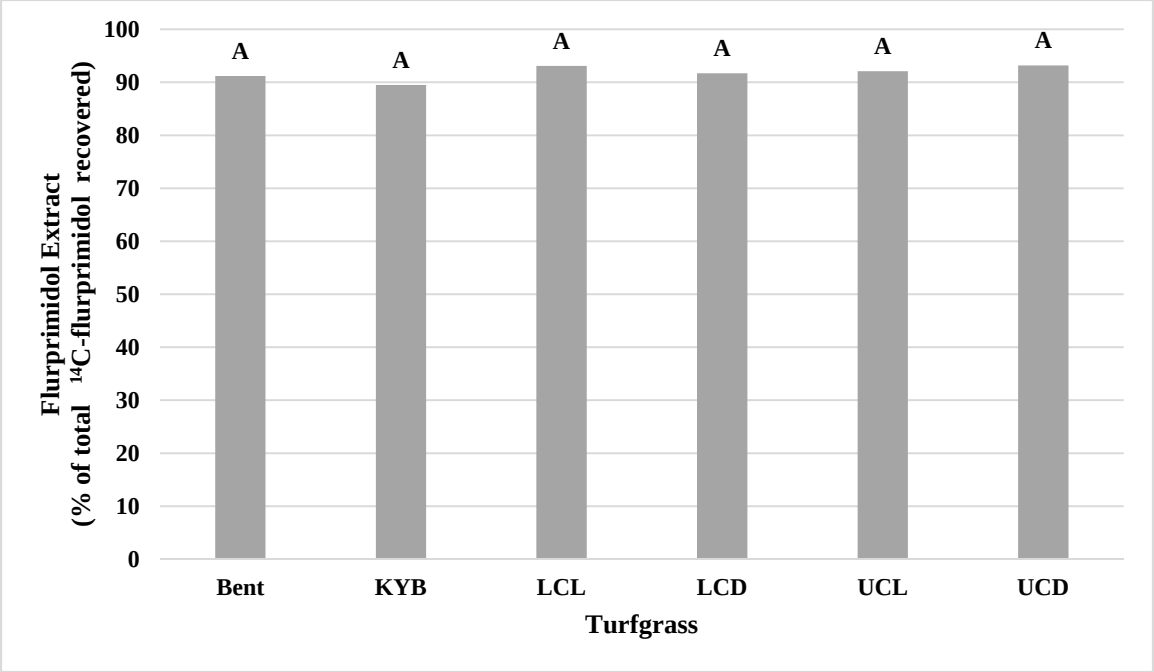

Figure 4.6. Percent of absorbed radioactivity extracted from shoots of creeping bentgrass, Kentucky bluegrass, and four *Poa annua* biotypes averaged over harvest times (0 and 3 weeks after root treatment with ^{14}C-flurprimidol). Data analysis was performed using an arcsine of the square root transformation in SAS. Values in the graph are back-transformed. Bars with the same letter are not statistically different by F-protected Fisher's LSD ($p < 0.05$). Abbreviations: Bent, creeping bentgrass; KYB, Kentucky bluegrass; LCL, Lexington Country Club light biotype; LCD, Lexington Country Club dark biotype; UCL, University Club light biotype; UCD, University Club dark biotype.

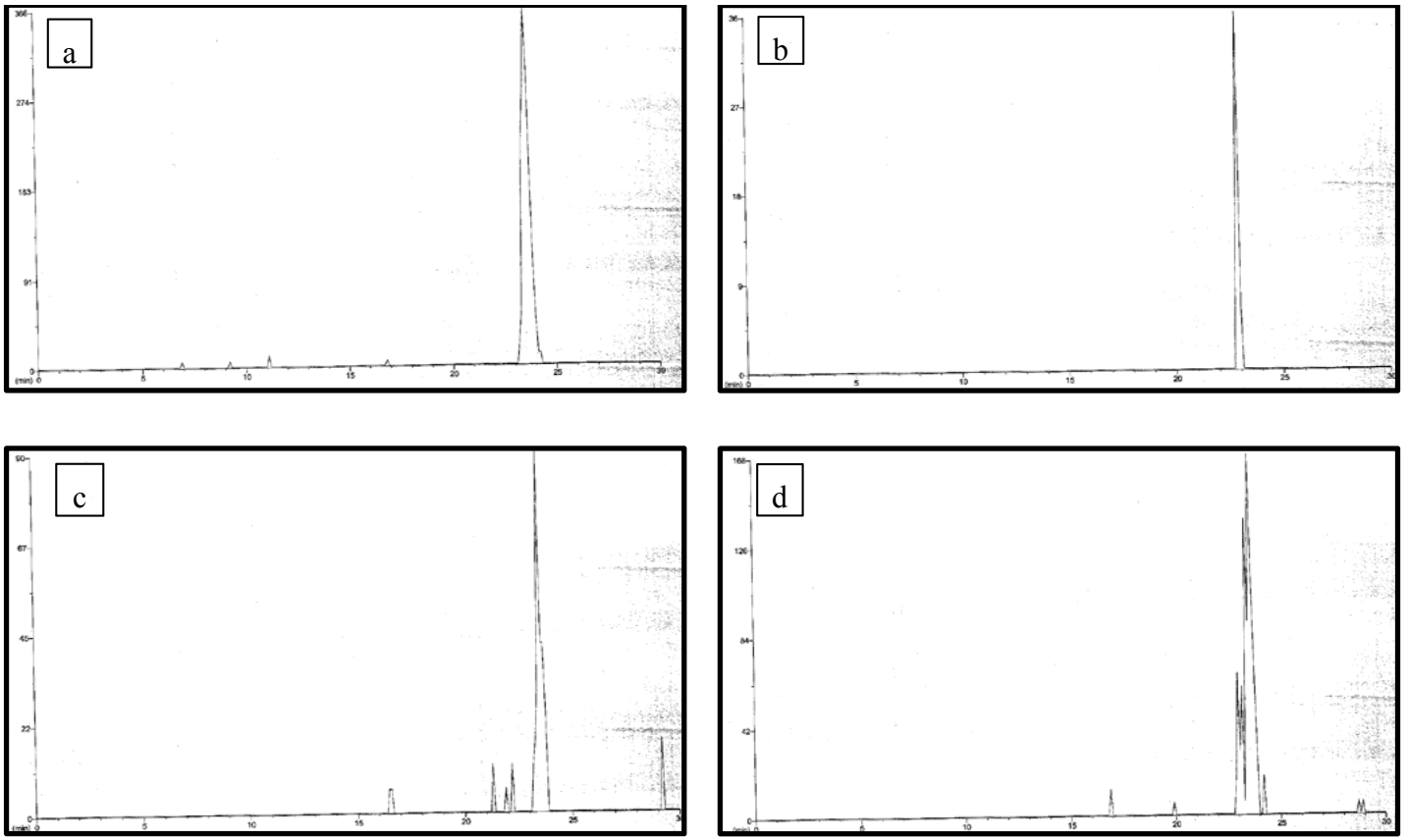

Figure 4.7. Samples of radiochromatographs of extracts from University Club light biotypes at 0 (a.) and 3 (b.) weeks after treatment in the roots and 0 (c.) and 3 (d.) weeks after treatment in the shoots. Units on the y-axis represent CPM (counts per minute). No metabolites were detected during this study.

Chapter Five

Conclusion

In 2010, Dr. David Williams, Dr. Michael Barrett, and I visited the University Club of Kentucky in Lexington, KY. The former superintendent, Jeff Benedict, had observed that his scheduled applications of paclobutrazol and flurprimidol were controlling certain *Poa annua* populations more than others on the same putting greens (Figure 5.1). He referred to these different populations as light and dark to reflect the very visually different colored *Poa annua* patches found on the greens. Furthermore, he observed that paclobutrazol was controlling the light biotypes and flurprimidol was controlling the dark biotypes. These types of anecdotal observations led to our studies.

Chapter One of this dissertation provided a background, explanation of genetic variability, reports of variation on the golf course, and current chemical methods for *Poa annua* control or management. It is intended to provide the reader with suitable information about *Poa annua* before proceeding to the subsequent chapters. Extensive research has been dedicated to this one plant. However, once one question is answered, several other questions seem to arise. In particular, *Poa annua* found growing in sports turf, particularly golf courses, is exhibiting differential responses to management strategies. These different *Poa annua* populations are referred to as biotypes in this dissertation.

The purpose of Chapter Two was to document possible differences between the response of *Poa annua* biotypes to PGRs and herbicides. This research was not intended to demonstrate the best chemical control, but rather document the differential effects of these treatments to selected *Poa annua* biotypes. We found that the *Poa annua* biotypes

collected from different golf courses with a light or dark phenotype responded differently to chemical treatments. The largest differences between biotypes were to PGRs in 2011 where the dark colored *Poa annua* biotypes were controlled by flurprimidol and the light colored biotypes were controlled more by paclobutrazol. These differences are similar to those observed by Jeff Benedict at the University Club in 2010.

In 2012, only location by treatment differences were found. Flurprimidol increased the quality of the biotypes collected from the University Club, while paclobutrazol increased the quality of the biotypes collected from Lexington Country Club. The term "increased" quality is utilized as compared to the control. This increase in quality could be attributed to the lack of competition with creeping bentgrass, allowing *Poa annua* the chance to increase in overall quality. 2011 was also a very different field season compared to 2012. In 2011, the temperatures were less extreme than in 2012, where temperatures were often higher or lower. Also, precipitation in 2011 was more consistent during field trials than in 2012, where precipitation was lower in June, August, and October, but very high in July. As a result of this, PGRs and herbicides absorption could be different as well as different effects on plant growth.

Poa annua biotypes, whether dark or light, were controlled with amicarbazone 2 to 6 WAIT in 2011, with quality ratings as low as 1.8 and 1.9 for light and dark biotypes, respectively. These plants appeared to be dead and turned a straw brown color. However, past this time period, new growth emerged from the *Poa annua* crowns. Interestingly, amicarbazone did not injure these same plugs when applied in 2012 with the lowest quality ratings of only 5.0 and 5.5 for the Lexington Country Club and the University Club 4 WAIT, respectively.

There was a three way interaction between color, location, treatment and *Poa annua* quality in the greenhouse study. Flurprimidol and paclobutrazol controlled the light biotype from the Lexington Country Club but controlled the dark biotype from the University Club. For the herbicides, bispyribac-sodium controlled the light biotypes from the Lexington Country Club but controlled the two biotypes from the University Club equally. Amicarbazone however, controlled the light biotypes from both courses 1 and 2 WAIT. Both flurprimidol and paclobutrazol recued growth of dark biotypes more than the light biotype growth. Bispyribac-sodium controlled the growth of the light biotypes more than the dark 11 and 12 WAIT. Amicarbazone controlled the light biotypes early (2 WAIT), however, 13 and 14 WAIT, the light biotypes recovered. Perhaps the light biotypes have a more rapid recovery compared to the dark biotypes. There was a difference in *Poa annua* growth between the plants collected from the two golf courses. The ones collected from the University Club had higher clipping weights than the Lexington Country Club.

Chapter Three investigates the uptake and metabolism of ^{14}C-labeled flurprimidol in six turfgrass species. Plants were harvest 0, 24, 72, and 120 hours after root exposure to ^{14}C-labeled flurprimidol. Flurprimidol root uptake was higher in warm season grasses, bermudagrass and zoysia, than the cool season grasses, creeping bentgrass, Kentucky bluegrass, perennial rye, and tall fescue. No flurprimidol metabolites were found in any turfgrass species in this study. It was concluded that studies with flurprimidol uptake and metabolism should be evaluated for longer periods of time.

Chapter Four investigates the uptake and metabolism of flurprimidol in *Poa annua* biotypes collected from the Lexington Country Club and the University Club

putting greens plus creeping bentgrass and Kentucky bluegrass. Despite the longer time period (3 weeks) compared to that that used in Chapter Three (5 days) no flurprimidol metabolites were found in the turfgrasses. Flurprimidol concentration in the roots declined between 0 and 3 WAT. Conversely, flurprimidol concentration in the shoots increased between 0 and 3 WAT.

From these studies I discovered many things:

1.) *Poa annua* biotypes are indeed complex. Multiple factors including genetics, plant biology, management strategies, and environmental conditions contribute to the complexity of this plant.
2.) Unintentionally, I found that
    a. *Poa annua* dark biotypes transform into light biotypes in the greenhouse.
    b. *Poa annua* treated with amicarbazone appears to be dead, but later emerges from the same crown in only a matter of days and actually appears to have a dark phenotype with a wider leaf blade (Figure 5.2).
    c. *Poa annua* light biotypes do not die in one year. The same *Poa annua* plugs were used during two different growing seasons for this study.
    d. *Poa annua* can be killed in the greenhouse due to high temperatures (lack of cooling) during the summer months.
3.) Variation in turfgrass responses to flurprimidol is, surprisingly, not due to differential metabolism.

These studies reveal that PGR and herbicide treatments used to control *Poa annua* do not effect all *Poa annua* populations equally. Golf course managers need to test the response of their populations of *Poa annua* before selecting an appropriate management

regime. Furthermore, PGR and herbicide treatments alone will not be a sustainable approach for *Poa annua* management on the golf course.

Figure 5.1. Former superintendent at the University Club of Kentucky, Jeff Benedict (middle), showing the differential responses of *Poa annua* to PGR treatments to Dr. Barrett (left) and Dr. Williams (right). The dark green spots are the response of dark biotypes to Cutless® (flurprimidol).

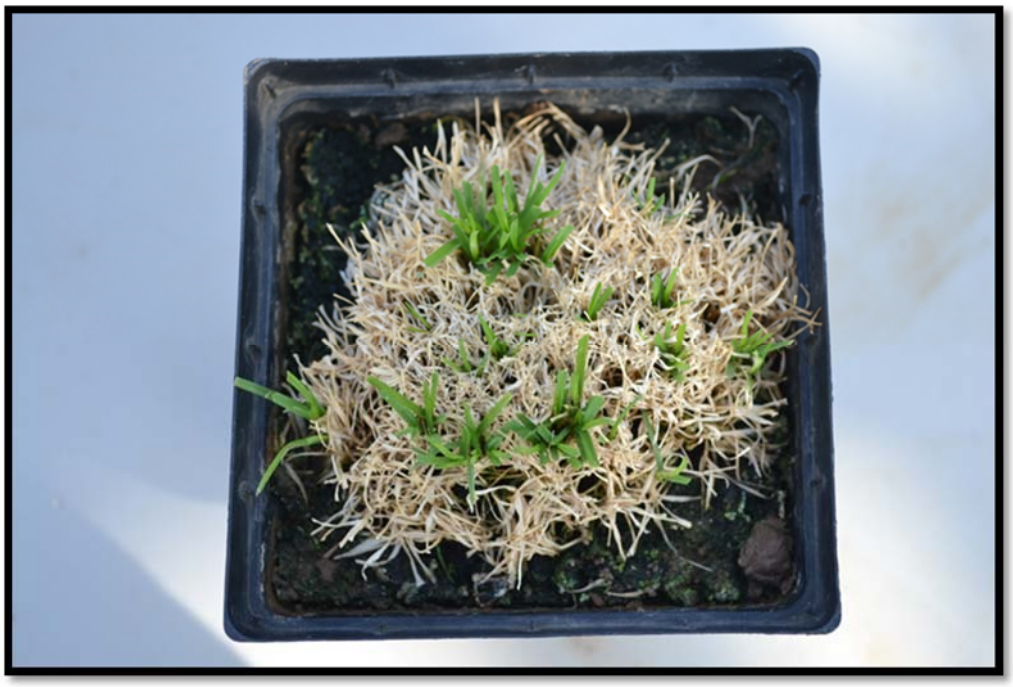

Figure 5.2. Example of *Poa annua* "growing out" of the amicarbazone treatment. Just a week earlier, I had recorded in my observations that this plant was dead.

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Vita

Education:

M.S., Horticulture, University of Georgia, Completed: December 2009, Thesis:

The Influence of Soilless Growing Media on the Movement and Longevity of the Herbicide Dimethenamid-P

B.S., Agriculture, Western Kentucky University, Completed: December 2006

Positions Held:

- **Graduate Research Assistant**, Turf and Weed Lab, University of Kentucky, Department of Plant and Soil Science, 2010-2014
- **Graduate Research Assistant**, Lab of Weed Science, University of Georgia, Department of Horticulture, 2007-2009.
- **Horticulturalist**, Redbud Ridge Gardens, Bowling Green, KY, 2004-2007.

Scholastic and Professional Honors:

- Co-organized Symposium: "January 2014 Dr. Donald Sparks IPSS Graduate Student Research Symposium", Lexington, KY. 2014
- 1st Place Graduate Student Poster Contest – Invasive Species Conference, 2013
- 2nd Place Graduate Student Poster Contest – North Central Weed Science Society, 2013
- 2nd Place Graduate Student Paper/Oral Contest – North Central Weed Science Society, 2013
- 3rd Place Graduate Student Poster Contest – ASA, CSSA, SSSA, 2013

- University of Kentucky Graduate School Travel Support Award, PhD 2013 and 2014
- Lyman T. Johnson Fellowship Award, 2010-2013
- 2nd Place Print Division- Weed Science Society of America Photo Contest, 2009
- Modern Language Superior Achievement Award in Spanish, 2005

Publications:

Williams, A., M. Barrett, D. Williams. Poa annua L.: A Review of its Biology and Management. (In progress)

Williams, A. M. Barrett, D. Williams. Investigating Poa annua L. Biotypes Collected from Putting Greens. (In progress)

Williams, A. M. Barrett, D. Williams. Flurprimidol Uptake, Translocation, and Metabolism in Six Turfgrass Species. (In progress)

Williams, A. M. Barrett, D. Williams. Flurprimidol Uptake, Translocation, and Metabolism in Poa annua Biotypes. (In progress)

Czarnota, M. and A. Williams. Dimethenamid-P Movement and Longevity in Pine Bark Media. (In progress)

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Reicher, Z., M. Sousek, R. Calhoun, A. Hathaway, A. Patton, D. Weisenberger, M. Barrett, L. Williams, and A. Williams. 2011. Controlling Poa annua in Putting Green Height Turf in Indiana, Kentucky, Michigan, and Nebraska. 2010 Annual Report – Purdue University Turfgrass Science Program.

Invited Presentations/Extension Programs:

Williams, A. 2014. Poa annua: Unlocking the Complexity Behind Golf's Worst Weed. Turf and Landscape Managers Short Course. Louisville, KY.

Williams, A. 2014. Herbicide Formulations, Labels, and New Herbicide Products in the Turf Industry. Certified Turf and Landscape Managers Workshop, Lexington, KY.

Williams, A. 2013. Research Update: New Products for the Control of Nimblewill, Poa annua, Crabgrass, Speedwell, and Dandelions in Cool Season Turfgrass. Kentucky Turfgrass Council Meeting. Florence, IN.

Williams, A. 2013. Golf Course Tour: National Turf Evaluation Program Trials and Poa annua Management. University of Kentucky Turf Field Day, Lexington, KY.

Williams, A. 2013. New Products Available for Poa annua Management on the Golf Course. Quad State Turf Association Meeting, Paducah, KY.

Williams, A. 2013. Current Options for Poa annua Control on the Golf Course. Kentucky Turfgrass Council Turf and Landscape Management Short Course, Louisville, KY.

Williams, A. 2012. Research Update: Investigating Poa annua Biotypes on Golf Greens. Kentucky Turfgrass Council Annual Meeting, Florence, IN.

Williams, A. 2012. Differential Responses of Poa annua Biotypes to PGR and Herbicide Treatments. University of Kentucky Turfgrass Field Day, Lexington, KY.

Williams, A. 2012. Investigating the Differential Responses of Poa annua Biotypes to PGR and Herbicide Treatments. Kentucky Turfgrass Council Turf and Landscape Management Short Course, Louisville, KY.

Williams, A. 2011. Research Update: Investigating Poa annua Biotypes on Golf Greens. Kentucky Turfgrass Council Annual Meeting, Florence, IN.

Williams, A. 2011. The Differential Responses of Poa annua Biotypes to Chemical Treatments. University of Kentucky Turfgrass Field Day, Lexington, KY.

Williams, A. 2011. Comparison of Annual Bluegrass Biotype Chemical Responses. Kentucky Turfgrass Council Turf and Landscape Management Short Course, Louisville, KY.

Williams, A. 2010. Research Update: Investigating Poa annua Biotypes on Golf Greens. Kentucky Turfgrass Council Annual Meeting, Bowling Green, KY.

Teaching

Teaching Assistant

PLS 103: Plants, Soils, and People: A Science Perspective, University of Kentucky, 2010-2011/2012 (Teaching Assistant/Recitation Instructor)

PLS 104: Plants, Soil, and People: A Global Perspective, University of Kentucky, 2012 (Laboratory Instructor)

PLS 404: Integrated Weed Management, University of Kentucky, 2011 (Laboratory Instructor)

HORT 2000: Horticulture Science, University of Georgia, 2008 (Teaching Assistant)

HORT 302: Introduction to Landscape Plants Laboratory, Western Kentucky University, 2006 (Laboratory Assistant)

Guest Lecturer

PLS 404: Integrated Weed Management, University of Kentucky, 2014

GEN 300-012: Special Course (Wine Class), University of Kentucky, 2014

PLS 515: Turf Management, University of Kentucky, 2010

Professional Memberships:

- American Society of Agronomy
- Crop Science Society of America
- Weed Science Society of America
- North Central Weed Science Society
- Southern Weed Science Society
- North Eastern Weed Science Society
- Kentucky Turfgrass Council
- Gamma Sigma Delta

- Golden Key International Honor Society