Prohexadione Calcium for Turfgrass Management and *Poa annua* Control and Molecular Assessment of the Acetolactate Synthase Gene in *Poa annua*

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Dissertation submitted to the Faculty of the Virginia Polytechnical Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

PLANT PATHOLOGY, PHYSIOLOGY, AND WEED SCIENCE

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May 03, 2004

Blacksburg, Virginia

Keywords: Gene sequencing, radioactive assay, turfgrass growth regulation, and weed control

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ABSTRACT

Managing turf for high aesthetic value is costly. Such management usually involves mowing, disease prevention, insect control, and weed control. Mowing is the most expensive practice on golf courses and annual bluegrass (*Poa annua* L) is the most challenging weed problem in professional turf. The plant growth regulators trinexapac-ethyl and paclobutrazol are commonly used in VA for these two costly and challenging jobs. Prohexadione calcium (PC) is an experimental chemical that inhibits the same enzyme (3ß-hydroxyalase) as trinexapac-ethyl and may selectively suppress annual bluegrass. Experiments were conducted at the Virginia Tech Turfgrass Research Center and Glade Road Research Facility to determine the PC rate required to reduce clipping biomass of four turfgrass species as effectively as trinexapac-ethyl. Prohexadione calcium reduced clipping biomass of bermudagrass (*Cynodon dactylon* (L.) Pers.), Kentucky bluegrass (*Poa pratenis* L.), perennial ryegrass (*Lolium perenne* L.), and zoysiagrass (*Zoysia japonica* Steud.) equivalent to trinexapac-ethyl at 0.70, 0.22, 0.60, and 0.27 kg a.i. ha^{-1}, respectively. Further experiments conducted at three locations across Virginia determined that PC was comparable to paclobutrazol for annual bluegrass suppression. Since turfgrass response to PC was different between annual bluegrass, Kentucky bluegrass, and perennial ryegrass, ^{14}C labeled PC was used to assess absorption, translocation, and metabolism of PC between annual and Kentucky bluegrass, creeping bentgrass (*Agrostis stolonifera* L.), and perennial ryegrass. Annual and Kentucky bluegrass absorbed more PC than creeping bentgrass or perennial ryegrass and partially explained the selectivity between these species. Translocation and metabolism of PC did not differ between species. Our final objective launched experiments characterizing possible resistance to acetolactate synthase (ALS) inhibiting herbicides in annual bluegrass. Several selective herbicides for annual bluegrass control inhibit ALS. Since many weeds have developed resistance to ALS-inhibiting herbicides, the ALS gene in annual bluegrass was sequenced and derived amino acid sequences were at least 87% similar to other previously sequenced grass species. This sequencing data will be used in future experiments to predict the likelihood of ALS resistance in annual bluegrass.

DEDICATION

This dissertation is dedicated to my father, Charles H. Beam, who taught me everything I really needed to know.

AUTHOR'S ACKNOWLEDGEMENTS

Appreciation is expressed to Shawn Askew for granting me the opportunity for this degree and working with me to see it through. To the other graduate and undergraduates, who have worked with me along the way with many projects and have made me laugh in the face of adversity. To my mom and family for their support and love. To my wife and best friend, Gay, for her support, love, and good spirit that have kept me going forward when I did not know if I could. Finally, to God above who makes all things possible to those that believe.

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Chapter I. Literature Review

Managing turfgrass height is vital to the turfgrass industry. Mowing turfgrass to manage height constitutes a large amount of turfgrass professionals' time and resources. Plant growth regulators (PGRs) have been assessed as an alternative to mowing since the energy crisis of the 1970s and many have been labeled for use in turfgrass. Maintaining high turf quality and color are crucial in the turfgrass industry because any change in the aesthetic value could upset patrons and reduce revenue. Paclobutrazol and trinexapac-ethyl (structure and chemical name are in Figure 1) suppress vegetative growth of bentgrass (*Agrostis stonlonifera* L.), bermudagrass (*Cynodon dactylon* (L.) Pers.), Kentucky bluegrass (*Poa pratensis* L.), tall fescue (*Festuca arundinacea* Schred.), perennial ryegrass (*Lolium perenne* L.), and zoysiagrass (*Zoysia japonica* Steud) (Ervin and Ok 2001; Fagerness et al. 2000; Fagerness and Penner 1998; Johnson 1989, 1992, and 1994; Marcum and Jiang 1997; Razmjoo et al. 1994; Steir et al. 2000; Qian et al. 1998) and may injure bermudagrass and bentgrass maintained at green height (Fagerness et al. 2000; Johnson 1992, 1994). Applying iron with trinexapac-ethyl reduced injury after application (Johnson 1997; Wiecko and Couillard 1997).

Gibberellins are responsible for cell elongation, induction of hydrolytic enzymes in germinating seeds, and the induction of bolting in plants (Rademacher 2000; Taiz and Zeiger 1998; Figure 2). Paclobutrazol inhibits cell elongation by blocking the ent-kaurene oxidase enzyme, which converts ent-kaurene to ent-kaurenoic acid and therefore prevents gibberellin formation (Rademacher 2000). Trinexapac-ethyl inhibits cell elongation by blocking the 3ß-hydoxylase enzyme, which converts GA_{20} to growth-active

GA_1. Paclobutrazol and trinexapac-ethyl also increase cytokinin and abscisic acid, and decrease ethylene concentration in responsive tissues (Rademacher 2000).

While PGRs suppress turfgrass, amount of suppression is dependant on species and PGR. Fagerness and Penner (1998) reported trinexapac-ethyl at 0.38 kg ai/ha suppressed perennial ryegrass 58%, Kentucky bluegrass 54%, tall fescue 60%, creeping bentgrass 31%, and creeping red fescue (*Festuca rubra* L.) 25 % 2 weeks after treatment (WAT). Razmjoo et al. (1994) reported paclobutrazol reduced Kentucky bluegrass growth more than dryland bentgrass (*Agrostis castellana* Boiss. & Reuter), hard fescue (*Festuca ovina* L. Koch.), perennial ryegrass, rough bluegrass (*Poa trivialis* L.), creeping bentgrass, tall fescue, and creeping red fescue. Christians and Nau (1984) reported mefluidide [N-[2,4-dimethyl-5-[[(triflurомethyl) sulfonyl] amino] phenyl] acetamide] at 0.56 kg ai/ha reduced clipping yield of Kentucky bluegrass and hard fescue more than tall fescue.

In addition to species specificity, PGRs also vary in their effects on turfgrass phenology and carbohydrate partitioning throughout the plant. Mefluidide decreased photosynthate partitioning to expanding leaves one WAT, increased photosynthate partitioned to roots and crowns, and stimulated root growth (Cooper et al. 1988; Danneberger et al. 1987; Hanson and Branham 1987). In contrast, paclobutrazol did not change photosynthate partitioning to the crown tissue, reduced translocation to the roots, and decreased total root length and maximum rooting depth (Hanson and Branham 1987; Marcum and Jiang 1997). Likewise, maximum root density and total root length were decreased by ethephon [(2-chloroethyl phosphonic acid] (Marcum and Jiang 1997). Trinexapac-ethyl has minimal effects on rooting, increases tillers per plant, cell density, and chlorophyll concentration, and reduces evapotranspiration rates equivalent to

mefluidide and ethephon (Marcum and Jiang 1997, Ervin and Koski 1998; Ervin and Koski 2001a, b). Since trinexapac-ethyl effectively reduces turfgrass height, increases tillers per plant, does not effect rooting, and reduces evapotranspiration rates, it is commonly used on golf courses to manage turfgrass growth and improve aesthetics.

Miyazawa et al. (1991) suggested that prohexadione calcium (Ca) (Structure and chemical name are in Figure 1) could be used to suppress turfgrass growth. Prohexadione Ca suppresses vegetative growth of apples (*Malus* spp.), rice (*Oryza sativa* L.), tomato (*Lycopersicon esculentum* Mill.), grain sorghum [*Sorghum bicolor* (L.) Moench], wheat (*Triticum aestivum* L.), peanut (*Arachis hypogaea* L.), and oilseed rape (*Brassica Napus* L.) (Byers and Yoder 1999; Culpepper et al. 1997; Grossman et al. 1994; Lee et al. 1998; Nakayama et al. 1992; Yamaji et al. 1991). Like trinexapac-ethyl, prohexadione Ca inhibits gibberellin biosynthesis by blocking the 3ß-hydroxylation of GA_{20} to GA_1 (Nakayama et al. 1992; Figure 2). Prohexadione Ca and trinexapac-ethyl are classified as acylcyclohexadiones and display similar degrees of activity when applied in appropriate formulation to turfgrass (Rademacher 2000).

Annual bluegrass (*Poa annua* L.) is a troublesome weed in turf that produces abundant seeds and adapts to close mowing and compacted soils (Watschke et al. 1979; Wu et al. 1992). Herbicide options are limited for postemergence control of annual bluegrass in Kentucky bluegrass and creeping bentgrass turf. Some PGRs affect annual bluegrass more adversely than other turfgrass species, thus enabling the desired turf to compete with annual bluegrass. Eggens and Wright (1985) reported that ethephon reduced growth of annual bluegrass more than Kentucky bluegrass. Paclobutrazol suppressed annual bluegrass more than creeping bentgrass and controlled annual

bluegrass 85% (Gibson et al. 1998; Isgrigg et al. 1998**;** Johnson and Murphy 1995, 1996). Isgrigg and Yelverton (1999) reported paclobutrazol or flurprimidol applied twice in the fall and twice in the spring reduced stands of annual bluegrass at least 80% by the end of year two. Isgrigg and Yelverton (1999) also reported trinexapac-ethyl did not reduce annual bluegrass populations. Yelverton et al. (1999) reported paclobutrazol at 0.28 and 0.56 kg ai/ha applied twice in fall and twice in spring controlled annual bluegrass 50 and 85%, respectively. Woosley et al. (2003) reported paclobutrazol at 0.28 and 0.14 kg ai/ha controlled annual bluegrass 85%.

Plant growth regulators are also used to reduce annual bluegrass seedhead production. Annual bluegrass seedheads are especially detrimental on golf course greens by affecting ball roll and aesthetic value. Ethephon and mefluidide reduced annual bluegrass seedhead formation in numerous studies (Cooper et al. 1987 and 1988; Danneberger et al. 1987; Watschke et al. 1979).

In warm-season turfgrass such as bermudagrass, additional options are available for selective chemical control of annual bluegrass. Herbicides that inhibit acetolactate synthase (ALS) have been registered for use in turfgrass since 1988 (Gaul and Christians 1988; Larocque and Christians 1985; US EPA Pesticide Product Label System). The ALS enzyme is the committed step for formation of branched chain amino acids isoleucine, leucine, and valine (Buchanan et al. 2000). Acetolactate synthase-inhibiting herbicides will selectively control annual bluegrass in warm-season turfgrass, such as bermudagrass. These herbicides were first used in wheat, corn (*Zea mays* L.), and soybean (*Glycine max* (L.) Merr.), and resistant weeds was observed in these crops within five years (Primiani et al. 1990).

Heap (1997) reported that new cases of weeds resistant to ALS-inhibiting herbicides have increased faster than any other herbicide group. Currently, 83 weed species are resistant to ALS-inhibiting herbicides (Heap 1997, 2004). Herbicide resistance can develop from a mutation in gene sequence that codes for a different amino acid in the target enzyme (Devine et al. 1993). This change leads to reduced or no herbicide binding in resistant plants. Ultimately, resistant plant populations will replace susceptible populations after continued use of the herbicide. Five domains have been identified in the ALS amino acid sequence that contain single amino acid changes that confer resistance to ALS-inhibiting herbicides in altered species (Wright et al. 1998).

Phenological and morphological variation abounds in annual bluegrass (Wu et al. 1992). Currently, annual bluegrass populations resistant to ALS-inhibiting herbicides have not been reported. However, annual bluegrass populations resistant to triazine, bipyridilium, thiocarbamate, urea, ethofumesate, and dinitroaniline herbicides have been reported (Bulcke and Callens 1998; Dyer and Bowman 1986; Heap 1997, 2003; Himme et al. 1984; Kelly et al. 1999; Mengistu et al. 2000; Struve et al. 1987; Vaughn and Gasquez 1987).

As of 2004, nine ALS-inhibiting herbicides were marketed for use in turf in the United States. While chlorsulfuron, halosulfuron, imazaquin, and metsulfuron have been registered for use in turfgrass for many years, their primary use was not for annual bluegrass control. Foramsulfuron, trifloxysulfuron, rimsulfuron, bispyribac sodium, and sulfosulfuron were first used in turfgrass in the past three years, and are labeled for annual bluegrass control or suppression. As such, the selection pressure for developing annual bluegrass populations resistant to ALS-inhibiting herbicides has increased.

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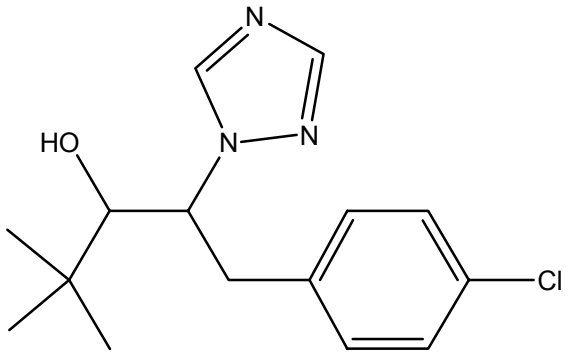
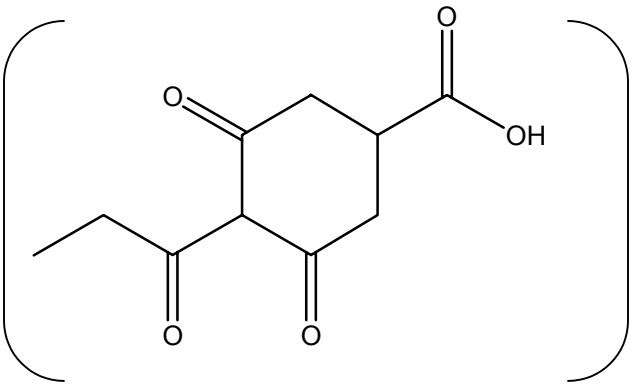


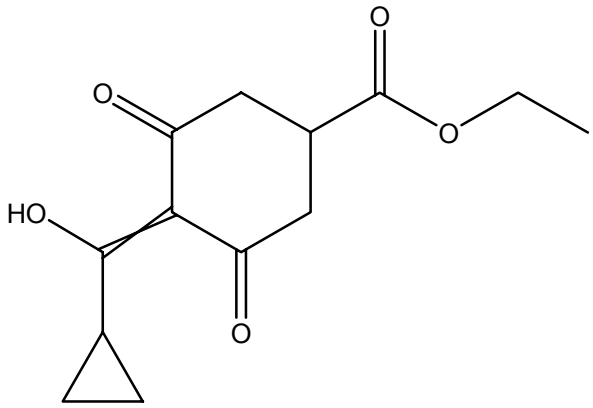
a. (±)-(R*,R*) B-[(4-chlorophenyl)-methyl]-α-(1,1dimethylethyl)-1H-1,2,4-triazole-1-ethanol



2-

Ca^{2++}

b. Calcium salt of 3,5-dioxo-4 propionylcyclohexane-carboxylic acid



c.4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane-carboxylic acid ethyl ester

Figure 1. Chemical structure and name of paclobutrazol (a.), prohexadione calcium (b.), and trinexapac-ethyl (c.).

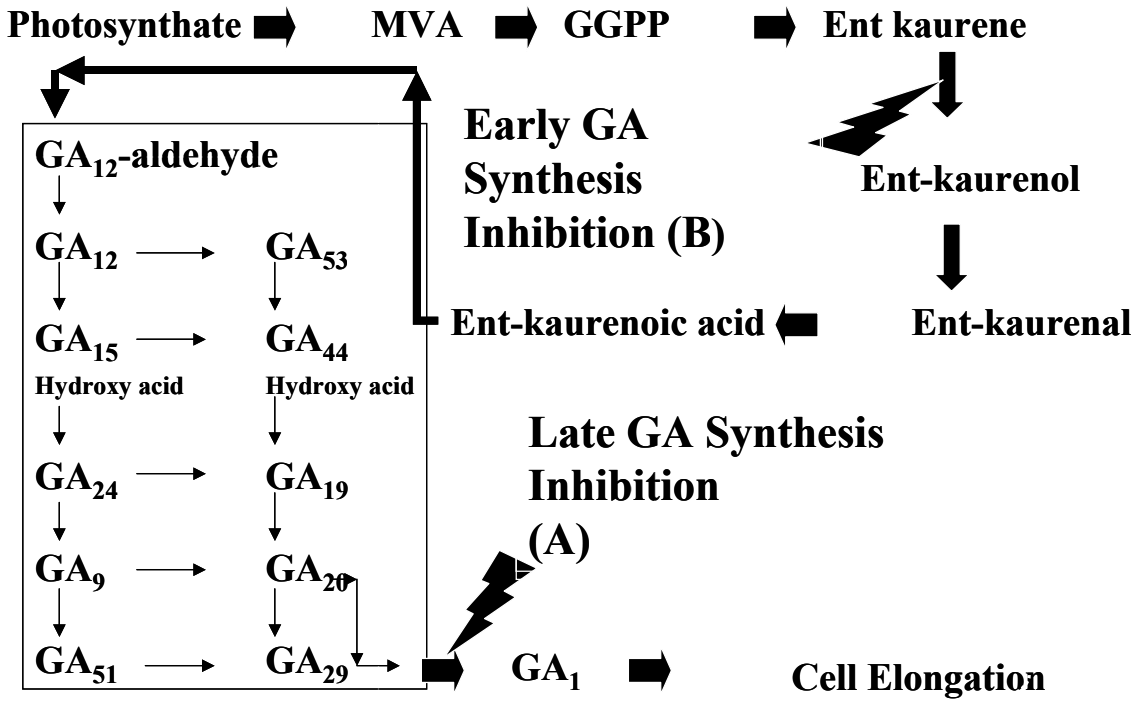


Figure 2. Simplified gibberellin biosynthesis in plants with points of inhibition by growth retardants paclobutrazol (B) and prohexadione calcium and trinexapac-ethyl (A). Melavonic acid is represented by MVA and geranylgeranyl pyrophosphate is represented by GGPP.

Chapter II. A Comparison of Trinexapac-ethyl and Prohexadione Calcium for Turfgrass Growth Suppression

Abbreviations. PGR, plant growth regulator; WAIT, weeks after initial treatment.

This chapter is formatted for publication in the International Turfgrass Research Journal.

ABSTRACT

Trinexapac-ethyl [4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane-carboxylic acid ethyl ester] blocks cell elongation and suppresses turfgrass growth. Prohexadione Ca (calcium salt of 3,5-dioxo-4 propionylcyclohexane-carboxylic acid) inhibits the same enzyme as trinexapac-ethyl and was evaluated for turfgrass growth suppression. Effects of prohexadione Ca rate on percent clipping biomass reduction based on average of nontreated plots of bermudagrass [*Cynodon dactylon* (L.) Pers. cv. Vamont], Kentucky bluegrass (*Poa pratensis* L. cv. Kelly), perennial ryegrass (*Lolium perenne* L. cv. Prosport), and zoysiagrass (*Zoysia japonica* Steud. cvs. Korean Common and Meyer) and equivalent rate to trinexapac-ethyl for each species was assessed for a six-week period during three experiments in 2001 and 2002. Prohexadione Ca was applied at 0, 0.14, 0.27, 0.41, 0.54, and 0.67 kg a.i. ha^{-1} with a non-ionic surfactant at 0.70 L ha^{-1}. Sequential treatments of prohexadione Ca were applied three weeks after initial treatment (WAIT). Trinexapac-ethyl was applied at 0.10, 0.22, 0.29, and 0.38 kg a.i. ha^{-1} on zoysiagrass, Kentucky bluegrass, bermudagrass, and perennial ryegrass, respectively, and sequential treatments were made four WAIT. Percent clipping biomass reduction increased in all species with increasing prohexadione Ca rate. When averaged over prohexadione Ca rate, percent clipping biomass reduction peaked at two and five WAIT for perennial ryegrass and zoysiagrass, five WAIT for Kentucky bluegrass, and at one WAIT for bermudagrass. Prohexadione Ca reduced clipping biomass of bermudagrass, Kentucky bluegrass, perennial ryegrass, and zoysiagrass equivalent to trinexapac-ethyl at 0.70, 0.22, 0.60, and 0.27 kg a.i. ha^{-1}, respectively. These data

suggest prohexadione Ca effectively suppresses turfgrass clipping biomass comparable to trinexapac-ethyl**.**

Managing turfgrass height is vital to the turfgrass industry. Mowing turfgrass to manage height constitutes a large amount of turfgrass professionals' time and resources. Use of plant growth regulators (PGR) on turfgrass can reduce mowing requirements while maintaining turf quality. Trinexapac-ethyl suppresses bentgrass (*Agrostis* sp.), bermudagrass, Kentucky bluegrass, perennial ryegrass, tall fescue (*Festuca arundinacea* Schreb.), and zoysiagrass growth (Ervin and Ok, 2001; Fagerness and Penner, 1998; Fagerness and Yelverton, 2000; Jiang and Fry, 1998; Johnson, 1990; Johnson, 1994; Qian et al., 1998), with minimal injury to turfgrass (Johnson, 1997; Wiecko and Couillard, 1997). In recent years, trinexapac-ethyl has become a widely used PGR in fine turfgrass (Gaussoin 1998).

Prohexadione Ca suppresses vegetative growth of apples (*Malus* spp.), grain sorghum [*Sorghum bicolor* (L.) Moench], oilseed rape (*Brassica Napus* L.), peanut *(Arachis hypogaea* L.), rice (*Oryza sativa* L.), tomato (*Lycopersicon esculentum* Mill.), and wheat (*Triticum aestivum* L.) (Byers and Yoder, 1999; Culpepper et al., 1997; Grossman et al., 1994; Lee et al., 1998; Nakayama et al., 1992; Yamaji et al., 1991). Although not currently registered, Miyazawa et al., (1991) suggested that prohexadione Ca could be used to suppress turfgrass growth. Prohexadione Ca inhibits gibberellin biosynthesis by blocking the 3ß-hydroxylation of GA_{20} to GA_1 (Nakayama et al., 1992). Prohexadione Ca and trinexapac-ethyl are classified as acylcyclohexadiones and display

similar degrees of activity when applied in appropriate formulation to turfgrass (Rademacher, 2000).

Considering the success of trinexapac-ethyl for growth management in fine turfgrass and the similarities between modes of action of prohexadione Ca and trinexapac-ethyl, prohexadione Ca seems a likely candidate for registration as a turfgrass growth suppression chemical. If proven effective, prohexadione Ca may increase management options for turfgrass. Our objectives are to determine effect of prohexadione Ca rate on bermudagrass, Kentucky bluegrass, perennial ryegrass, and zoysiagrass clipping biomass and color and equivalent rates to trinexapac-ethyl for each species.

MATERIALS AND METHODS

Experiments were initiated in August 2001, June 2002, and August 2002, at the Virginia Tech Turfgrass Research Center in Blacksburg, VA in established (>3 yr old stands) 'Vamont' bermudagrass, 'Korean common' (utilized for the August 2001 and June 2002 experiments) and 'Meyer' (utilized for the August 2002 experiment) zoysiagrass, 'Prosport' perennial ryegrass, and 'Kelly' Kentucky bluegrass. Soil at the Turfgrass Research Center was a Groseclose loam (clayey, mixed, mesic, Typic Hapludalfs) with 1.8% organic matter and pH 6.6. Quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) at 0.84 kg a.i. ha^{-1} plus crop oil concentrate (2.8 L ha^{-1}) was applied over all areas four wk before growth regulator treatments to control large crabgrass [*Digitaria sanguinalis* (L.) Scop] and white clover (*Trifolium repens* L.). Plots were 3.34 m^2 with 10-cm edges treated with glyphosate (N-(phosphonomethyl)glycine) at

2.0 kg a.i. ha^{-1}. All plots were mowed weekly for ten wk using a rotary lawnmower equipped to catch clippings. All plots were supplied with supplemental irrigation when needed and 49 kg ha^{-1} of N, P, and K was applied before each experiment. Height of cut was 3.8, 7.6, 5.1, and 3.8 cm for bermudagrass, Kentucky bluegrass, perennial ryegrass, and zoysiagrass, respectively. Clippings were collected and placed in an oven for 48 hrs at 50° C. After drying, clipping mass was recorded.

In each experiment one d after the second weekly mowing, prohexadione Ca was applied at 0.14, 0.27, 0.41, 0.54, and 0.67 kg a.i. ha^{-1} with 0.70 L ha^{-1} nonionic surfactant (Promate Kinetic™; Helena Chemical Company, Collierville, TN). Sequential treatments of prohexadione Ca were applied three wk later (based on BASF recommendation). Trinexapac-ethyl was applied twice at four wk intervals at 0.29, 0.22, 0.38, and 0.10 kg a.i. ha^{-1} on bermudagrass, Kentucky bluegrass, perennial ryegrass, and zoysiagrass, respectively, as specified on the label for commercial turfgrass. A nontreated control was included for comparison. Growth regulators were applied with a CO_2-pressurized sprayer calibrated to deliver 281 L ha^{-1}. Turf color was visually rated two wk after each prohexadione Ca application using a scale of 1 (brown turf) to 9 (darkest green color) where 5 was considered acceptable turfgrass color.

Two greenhouse studies were also conducted at the Glade Road Research Facility in Blacksburg, VA. 'Midnight' Kentucky bluegrass and 'Prosport' perennial ryegrass seed were planted in 15.2-cm diameter pots at 224 kg ha^{-1} in potting mix (Pro-Mix; Premier Horticulture LTEE, Riviere-du-Loup, Quebec, Canada). Grass blades were clipped weekly with scissors at 2.5 cm. As in the field studies, turfgrass was clipped two times at weekly intervals prior to chemical treatment and then weekly following chemical

treatment. The two clipping assessments made prior to growth regulator treatments were used to evaluate uniformity between pots for clipping biomass. Growth regulator rates were identical with the field studies and applied with a track sprayer (Allen Track Sprayer; Allen Machine Works, Midland, MI) at 281 L ha^{-1}, when plants were seven weeks old. Pots were watered daily, fertilized as in field experiments, and average greenhouse temperature was 20 °C.

All experiments were conducted as randomized complete block designs with treatments replicated three times. Accounting for study repetition, field treatments were replicated nine times (three experiments with three replications each) and greenhouse treatments were replicated six times (two experiments with three replications each). Percent reduction of clipping biomass was calculated based on the average biomass of nontreated plots for each species and experiment timing. Data were tested for variance homogeneity prior to analysis. A combined analysis was performed using the PROC GLM procedure in SAS (Statistical Analysis Systems software, SAS Institute, Cary, NC) to test effects of experiment repetition, prohexadione Ca rate, time after treatment, and comparison treatment on percent clipping biomass reduction (McIntosh, 1983). If needed, either arcsine square-root or log transformation was applied prior to ANOVA to improve variance homogeneity. Trial effects were considered random and the mean squares of these effects were used to test treatment effects as is appropriate for a combined analysis (McIntosh, 1983). Polynomial regressions were used to describe the relationship of prohexadione Ca rate on measured responses as indicated by the ANOVA. If second-order effects were more significant, the quadratic curve was applied; otherwise the linear equation was applied. Regression trends were used to predict the

prohexadione Ca rate equivalent to trinexapac-ethyl for each species. Means of significant main effects and interactions were also separated using Fisher's Protected LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Kentucky bluegrass and perennial ryegrass field data were log transformed to improve homogeneity of variance (non-transformed means are presented in the tables for clarity). After log transformation, the interaction of experiment by time after treatment and main effect of prohexadione Ca were significant for percent biomass reduction of perennial ryegrass (Table 1). Percent reduction of perennial ryegrass clipping biomass was higher at two, five, and six WAIT in August 2001, five WAIT in June 2002, and two WAIT in August 2002 (Fig. 1). Warner weather at the start of the June 2002 experiment reduced perennial ryegrass growth in all plots and thus reduced percent reduction. Experiments with trinexapac-ethly have also noted highest growth suppression at two WAIT (Fagerness and Penner, 1998; Jiang and Fry, 1998; Wiecko, 1997).

Increasing prohexadione Ca rate continually reduced clipping biomass of perennial ryegrass (Fig. 2). Prohexadione Ca at 0.60 kg a.i. ha^{-1} reduced perennial ryegrass growth equivalent to trinexapac-ethyl. Fagerness and Penner (1998) also reported trinexapac-ethyl suppressed perennial ryegrass growth. Perennial ryegrass absorbed less prohexadione Ca compared to Kentucky bluegrass and decreased percent reduction of clipping biomass. In lab experiments, perennial ryegrass absorbed less ^{14}C labeled prohexadione Ca than Kentucky bluegrass (Beam, unpublished data).

The main effect of time after treatment and the interaction of experiment by prohexadione Ca rate were significant for reduction of Kentucky bluegrass clipping biomass (Table 1). Kentucky bluegrass clipping biomass continually decreased between one and five WAIT (Fig. 3). In August 2001 and June 2002, increasing prohexadione Ca rate above 0.40 kg a.i. ha^{-1} did not improve suppression of Kentucky bluegrass, while in August 2002 Kentucky bluegrass growth continually decreased with increasing prohexadione Ca rate (Fig 4). In August 2002, Kentucky bluegrass did not grow consistently in all plots compared to the other application timings and led to the interaction. Prohexadione Ca applied at 0.10, 0.10, and 0.51 kg a.i. ha^{-1} reduced Kentucky bluegrass growth equivalent to trinexapac-ethyl in August 2001, June 2002, and August 2002, respectively (Fig. 4). Ervin and Koski (2001) reported greater suppression of Kentucky bluegrass in August application dates relative to May. Fagerness and Penner (1998) also reported that trinexapac-ethyl suppressed Kentucky bluegrass growth.

Based on visual inspection of residuals, log transformations were not needed to improve homogeneity of variance for bermudagrass and zoysiagrass data. The interaction of experiment by time after treatment and experiment by rate were significant for percent reduction of zoysiagrass clipping biomass (Table 1). Regardless of rate, prohexadione Ca inhibited zoysiagrass growth by two WAIT in each experiment (Fig. 5), however, growth reduction was more cyclic in August 2001 when compared to August 2002 and June 2002. Cooler weather in 2001 decreased zoysiagrass growth in all plots and decreased percent reduction of clipping biomass especially in wk four through six. Increasing prohexadione Ca rate reduced clipping biomass of zoysiagrass (Fig. 6).

Consistent with time after treatment, less reduction was recorded in 2001, due to cooler weather and decreased growth of zoysiagrass. Prohexadione Ca at 0.45, 0.10, and 0.25 kg a.i. ha^{-1} reduced zoysiagrass growth equivalent to trinexapac-ethyl in August 2001, June 2002, and August 2002, respectively (Fig. 6). Trinexapac-ethyl reduced clippings 75 and 35% in shade and full sun, respectively (Ervin and Ok 2001; Ervin et al. 2002; Qian et al. 1998). Our experiments were conducted in full sun and consistent with these findings.

The interaction of experiment by time after treatment and experiment by prohexadione Ca rate were significant for bermudagrass clipping biomass reduction (Table 1). Percent reduction of bermudagrass was highest at one and four WAIT for August 2001 and June 2002 (Fig. 7). In August 2002, percent reduction of bermudagrass was highest five WAIT (Fig. 7). In August 2002, quinclorac applications decreased growth of all plots, compared to surrounding nontreated areas, until three WAIT and led to the interaction. Wiecko (1997) reported highest trinexapac-ethyl reductions of bermudagrass two WAIT. Increasing prohexadione Ca rate reduced clipping biomass of bermudagrass (Fig. 8). Increasing trinexapac-ethyl rate has also reduced clipping biomass of bermudagrass (Fagerness and Yelverton 2000; Wiecko 1997). Prohexadione Ca at 0.70 kg a.i. ha^{-1} reduced bermudagrass growth equivalent to trinexapac-ethyl in August 2001, June 2002, and August 2002 (Fig. 8).

In greenhouse experiments, log transformations were not needed to stabilize data variance. The interaction of experiment by time after treatment was significant for perennial ryegrass growth reduction in the greenhouse (Table 2). Percent reduction of perennial ryegrass clipping biomass in the greenhouse was higher at one and four WAIT

(Fig. 9). Jiang and Fry (1998) reported trinexapac-ethyl suppressed perennial ryegrass growth in the greenhouse until four WAIT

The main effect of time after treatment and prohexadione Ca rate were significant for Kentucky bluegrass clipping biomass reduction in the greenhouse (Table 2). Highest clipping biomass reduction for Kentucky bluegrass in the greenhouse was noted two WAIT (Fig. 10). Increasing prohexadione Ca rate up to 0.27 kg a.i. ha^{-1} reduced Kentucky bluegrass growth; above 0.27 kg a.i. ha^{-1} reduction was similar (Fig. 11). Kentucky bluegrass and perennial ryegrass age, soil media, and growing conditions may have influenced greenhouse results compared to field experiments.

Color of all turfgrass species treated with both growth regulators was not different from nontreated two WAIT. The main effect of growth regulator was significant for bermudagrass (P=0.0003), Kentucky bluegrass (P=0.0158), and zoysiagrass (P=0.0216) color five WAIT. Bermudagrass, Kentucky bluegrass, and zoysiagrass decreased in color with the application of prohexadione Ca or trinexapac-ethyl five WAIT. All rates of prohexadione Ca decreased Kentucky bluegrass and zoysiagrass color five WAIT compared to nontreated but not when compared to labeled rate of trinexapac-ethyl for each species (Table 3). Prohexadione Ca at 0.67 kg a.i. ha^{-1} reduced bermudagrass color five WAIT while all other rates did not decrease bermudagrass color. Trinexapac-ethyl reduced bermudagrass color five WAIT compared to nontreated and all prohexadione Ca treatments (Table 3). Color may have decreased because newer leaves were not growing, thus enabling an inspection of older leaf tissue that was not visible in nontreated plots. Bermudagrass discoloration after an application of trinexapac-ethyl has been reported in numerous studies (Johnson, 1994; Johnson, 1997; Wiekco, 1997; Wiekco and Couillard,

1997) and applying Fe with trinexapac-ethyl to bermudagrass decreases this discoloration (Wiekco and Couillard, 1997; Johnson, 1997). Prohexadione Ca at rates between 0.14 and 0.67 kg a.i. ha^{-1} did not differ from trinexapac-ethyl in its effects on turfgrass color on the species tested five WAIT.

When averaged over experiment timings, prohexadione Ca reduced growth of bermudagrass, Kentucky bluegrass, perennial ryegrass, and zoysiagrass equal to trinexapac-ethyl at 0.70, 0.22, 0.60, and 0.27 kg a.i. ha^{-1}, respectively. Collectively, these data suggest prohexadione Ca suppreses growth of common turfgrass species. Aggressive growing grasses such as bermudagrass may require higher rates and/or multiple applications to effectively manage height and reduce mowing. If registered for use in turfgrass at a competitive price, prohexadione Ca could reduce mowing expense to turfgrass professionals. Future studies should evaluate different application intervals to maximize product effectiveness.

ACKNOWLEGEMENTS

Appreciation is expressed to the personnel at the Virginia Tech Turfgrass Research Center.

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Table 1. Analysis of variance of main effects and interactions of prohexadione calcium (PC) percent reduction of clipping biomass for each species in field experiments.

Species	Parameter	df	F value	P value
Bermudagrass	Experiment	2	55	0.0001
	Rep (experiment)	6	21	0.0001
	PC rate	4	11	0.0022
	Time after treatment	5	4	0.0449
	Experiment*time after treatment	10	14	0.0001
	Experiment*PC rate	8	3	0.0089
	PC rate*time after treatment	20	3	0.0042
	Experiment*PC rate*time after treatment	40	1	0.5319
Kentucky bluegrass	Experiment	2	3	0.0373
	Rep (experiment)	6	18	0.0001
	PC rate	4	3	0.0762
	Time after treatment	5	17	0.0001
	Experiment*time after treatment	10	4	0.0001
	Experiment*PC rate	8	9	0.0001
	PC rate*time after treatment	20	1	0.5397
	Experiment*PC rate*time after treatment	40	1	0.9851
Perennial ryegrass	Experiment	2	69	0.0001
	Rep (experiment)	6	10	0.0001
	PC rate	4	4	0.0500
	Time after treatment	5	1	0.3460
	Experiment*time after treatment	10	24	0.001
	Experiment*PC rate	8	4	0.0003
	PC rate*time after treatment	20	1	0.2197
	Experiment*PC rate*time after treatment	40	1	0.9972

Zoysiagrass	Experiment	2	161	0.0001
	Rep (experiment)	6	9	0.0001
	PC rate	4	3	0.1158
	Time after treatment	5	2	0.1482
	Experiment*time after treatment	10	25	0.0001
	Experiment*PC rate	8	2.12	0.0360
	PC rate*time after treatment	20	1.14	0.3538
	Experiment*PC rate*time after treatment	40	0.68	0.9235

Fig. 1. Effect of time after treatment on prohexadione Ca percent reduction of perennial ryegrass clipping biomass. Pooled over prohexadione Ca rate. Bars represent LSD for each experiment. Prohexadione Ca was applied at 0 and 3 wk.

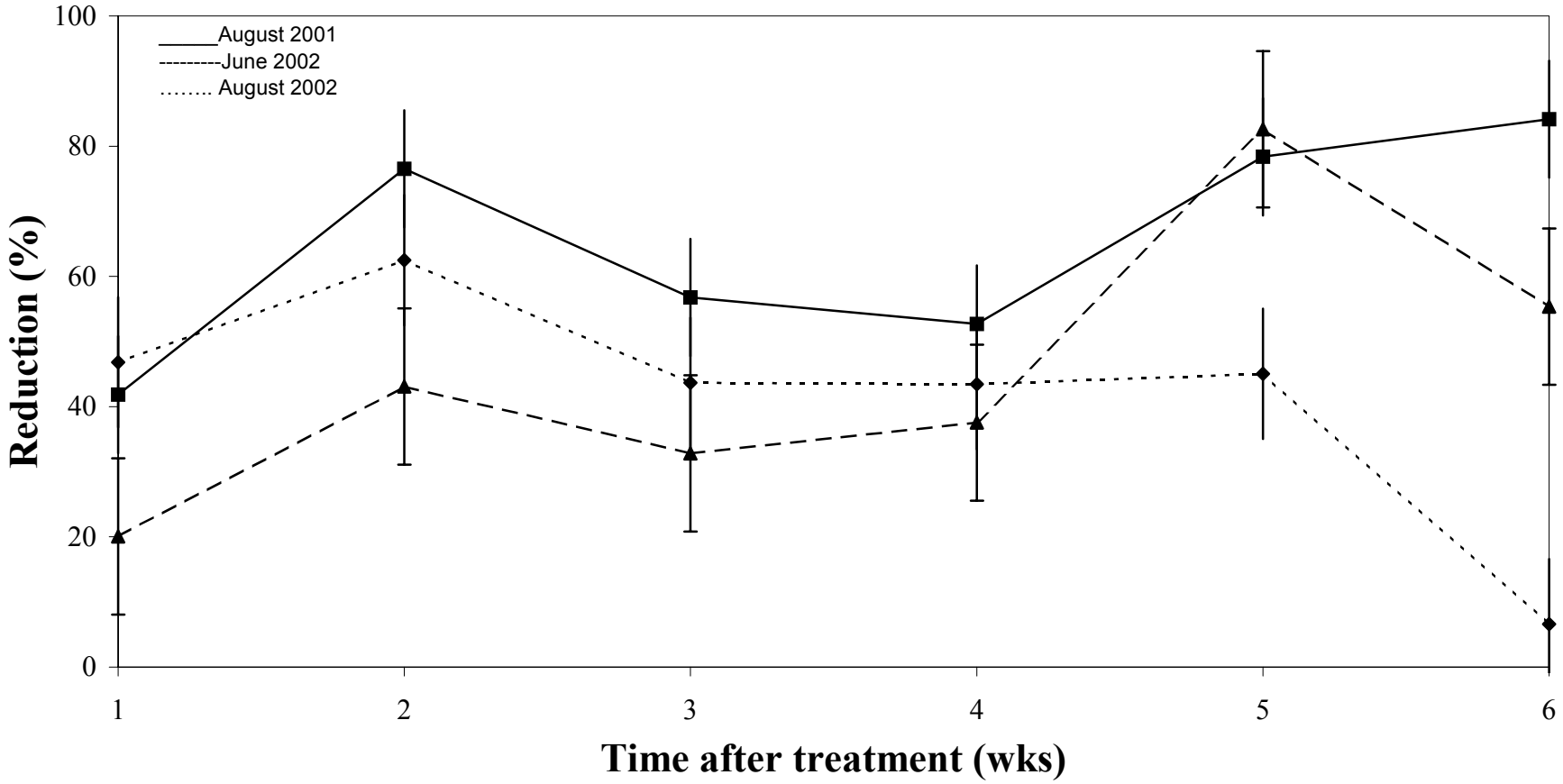

Fig. 2. Effect of prohexadione Ca (PC) rate on percent reduction of perennial ryegrass clipping biomass over a six-week period. Pooled over time after treatment. Bars represent SE for each mean. Trinexapac-ethyl equivalent rate denoted with a line and a (x).

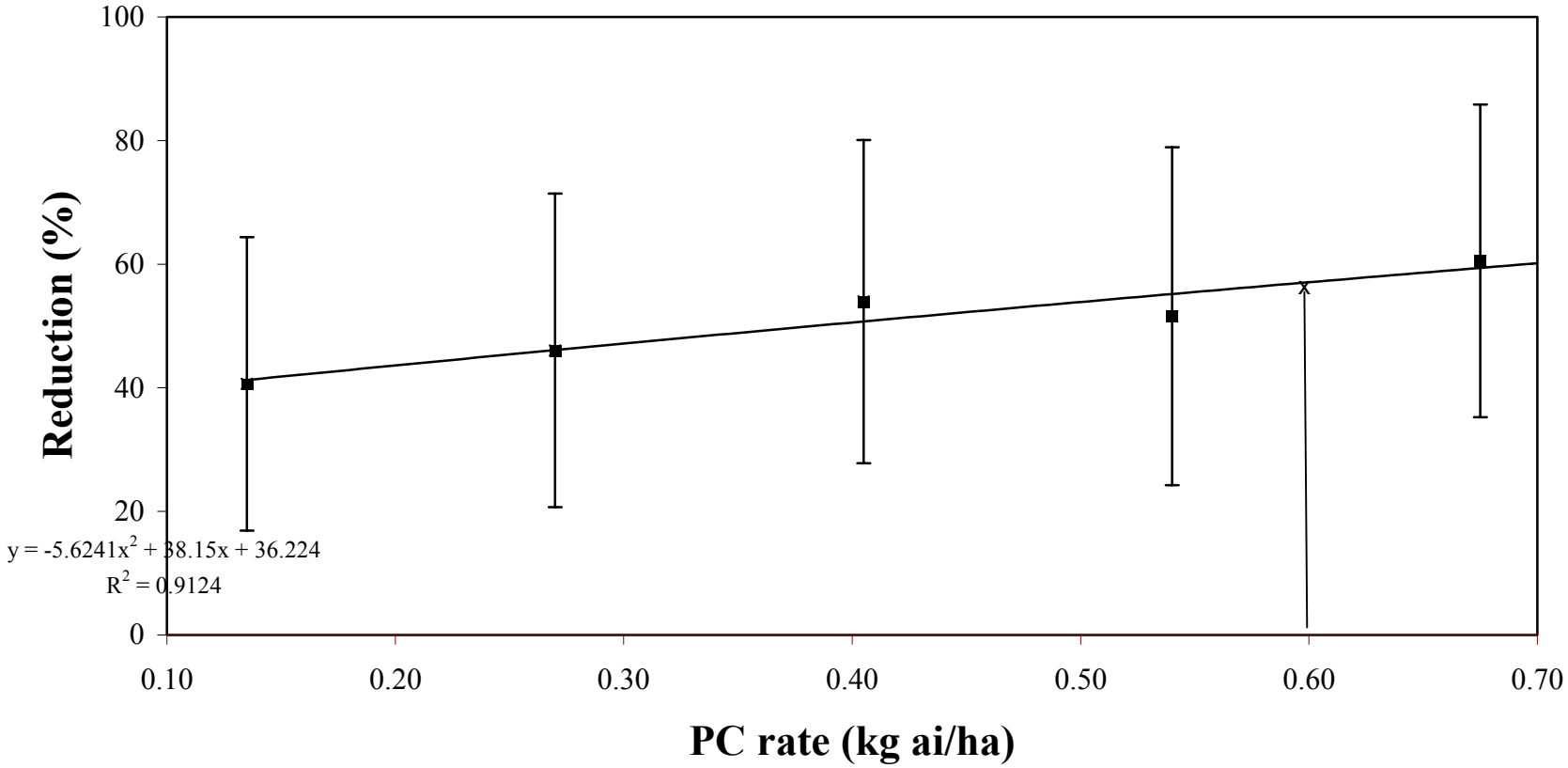

Fig. 3. Effect of time after treatment on prohexadione Ca percent reduction of Kentucky bluegrass clipping biomass. Pooled over trial and PC rate. Bars represent LSD for the experiment. Prohexadione Ca was applied at 0 and 3 wk.

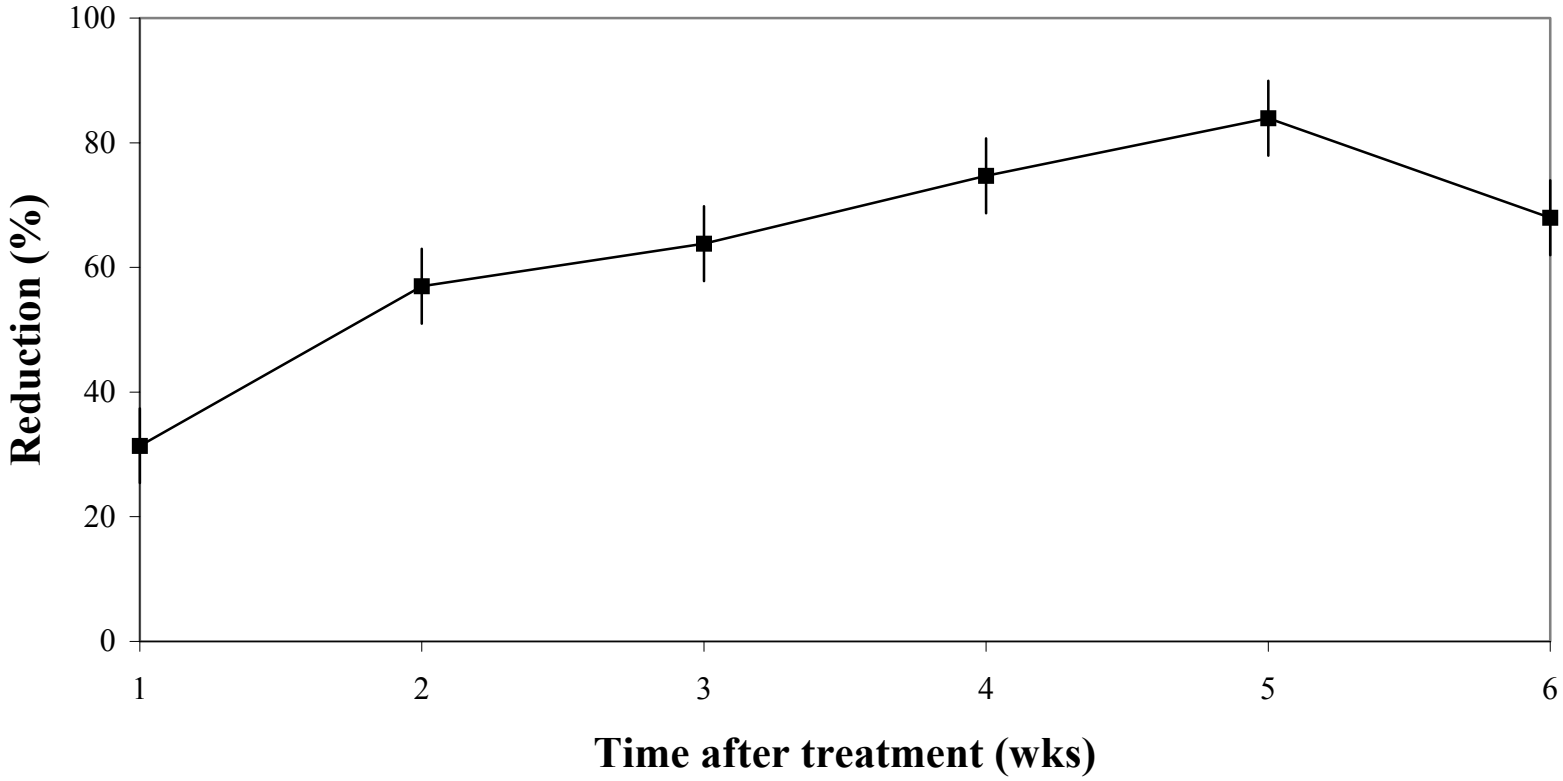

Fig. 4. Effect of prohexadione Ca (PC) rate on percent reduction of Kentucky bluegrass clipping biomass over a six-week period. Pooled over time after treatment. Bars are SE of each mean. Trinexapac-ethyl equivalent rate denoted with a line and a (x).

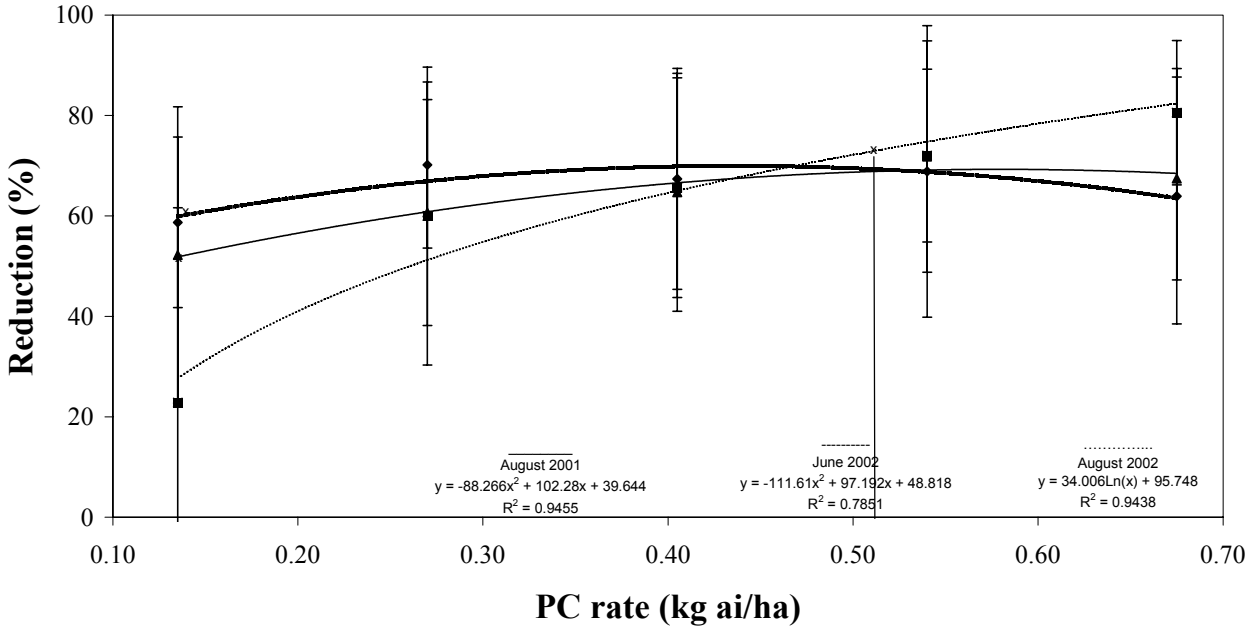

Fig. 5. Effect of time after treatment on prohexadione Ca percent reduction of zoysiagrass clipping biomass. Pooled over prohexadione Ca rate. Bars represent the LSD for each experiment. Prohexadione Ca applications were made at 0 and 3 wk.

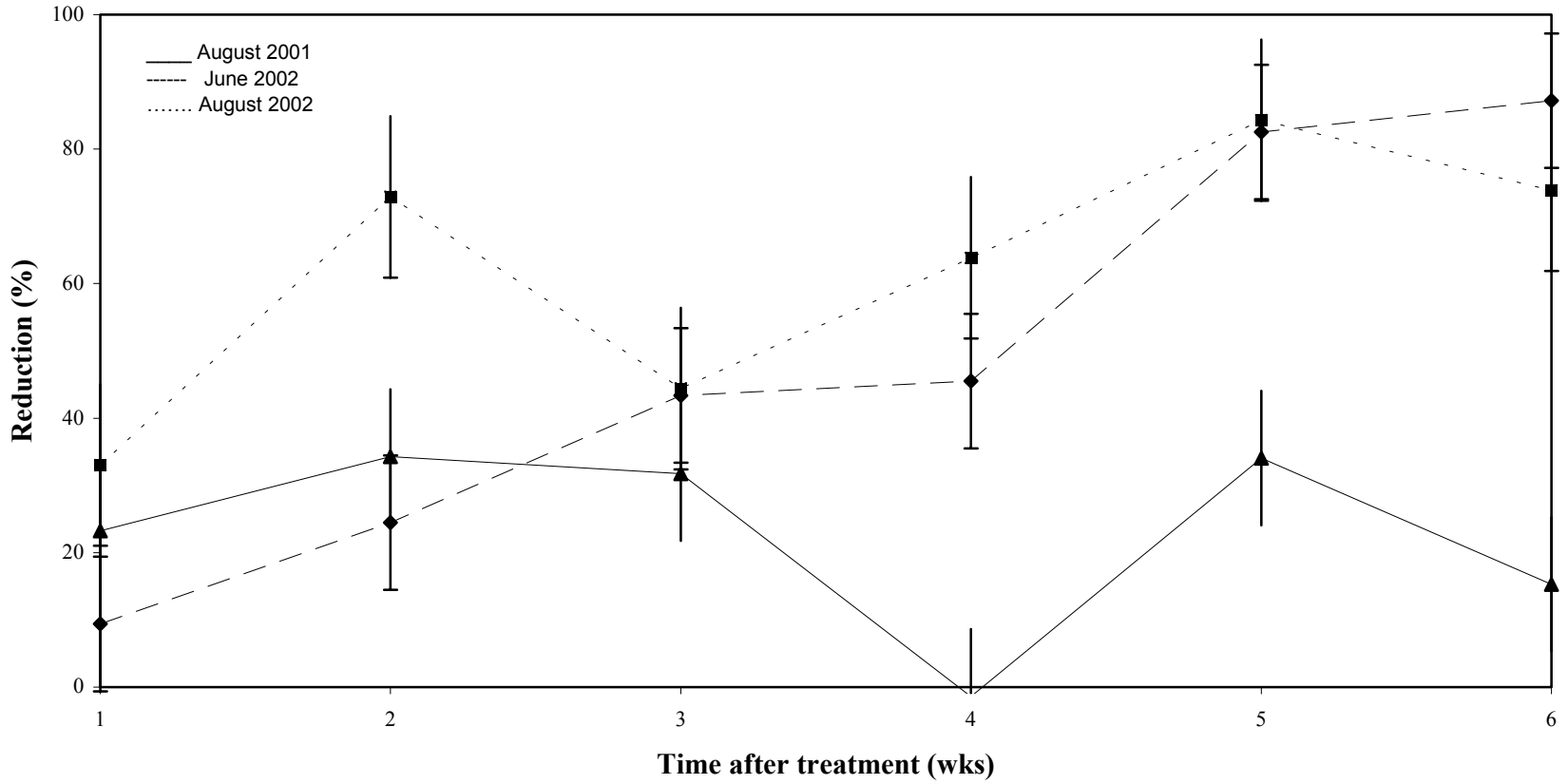

Fig. 6. Effect of prohexadione Ca (PC) rate on percent reduction of zoysiagrass clipping biomass over a six-week period. Pooled over time after treatment. Bars represent SE for each mean. Trinexapac ethyl equivalent rate denoted with a line and a (x).

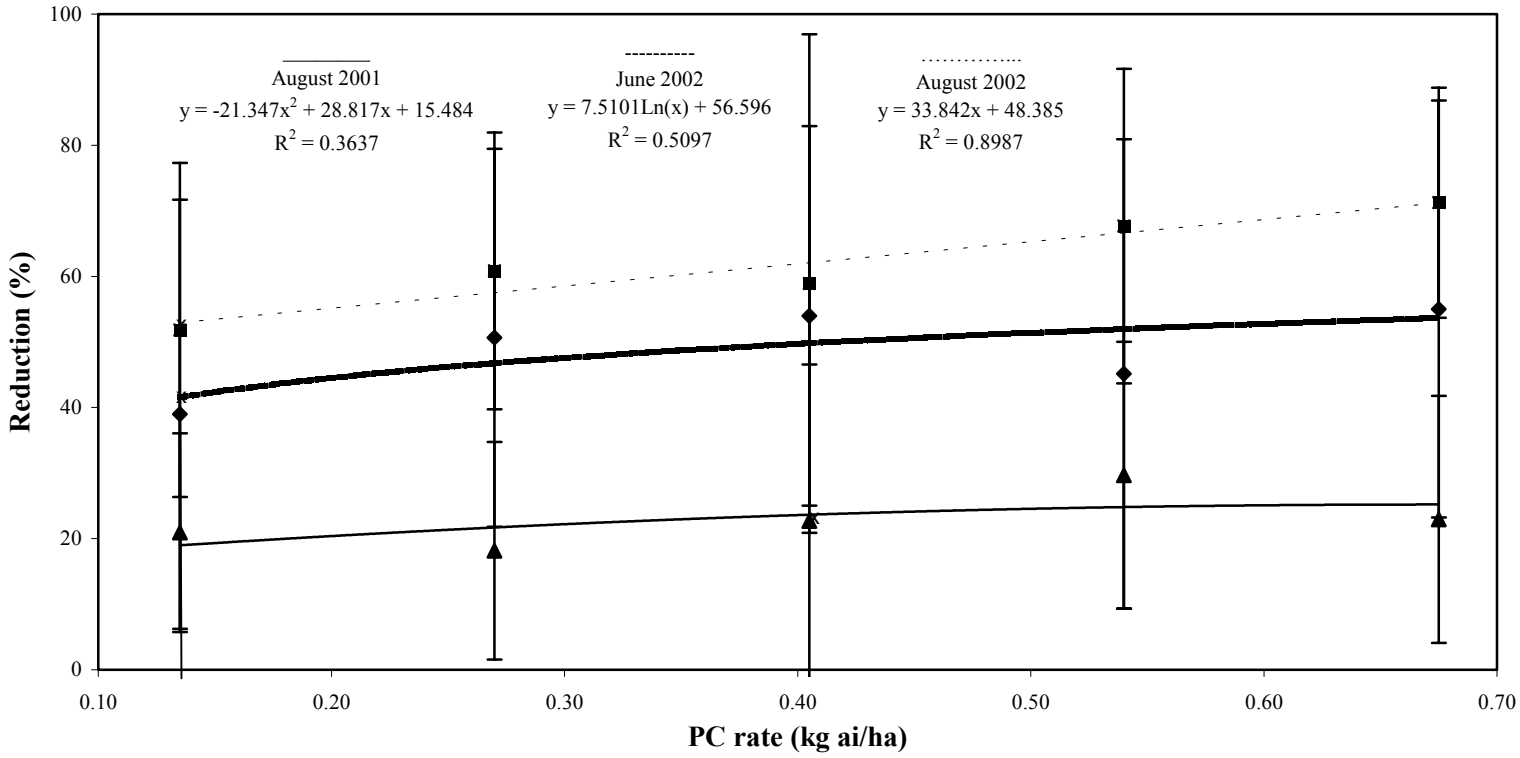

Fig. 7. Effect of time after treatment on prohexadione Ca percent reduction of bermudagrass clipping biomass. Pooled over prohexadione Ca rate. Bars represent LSD for each experiment. Prohexadione Ca applications were made at 0 and 3 wk.

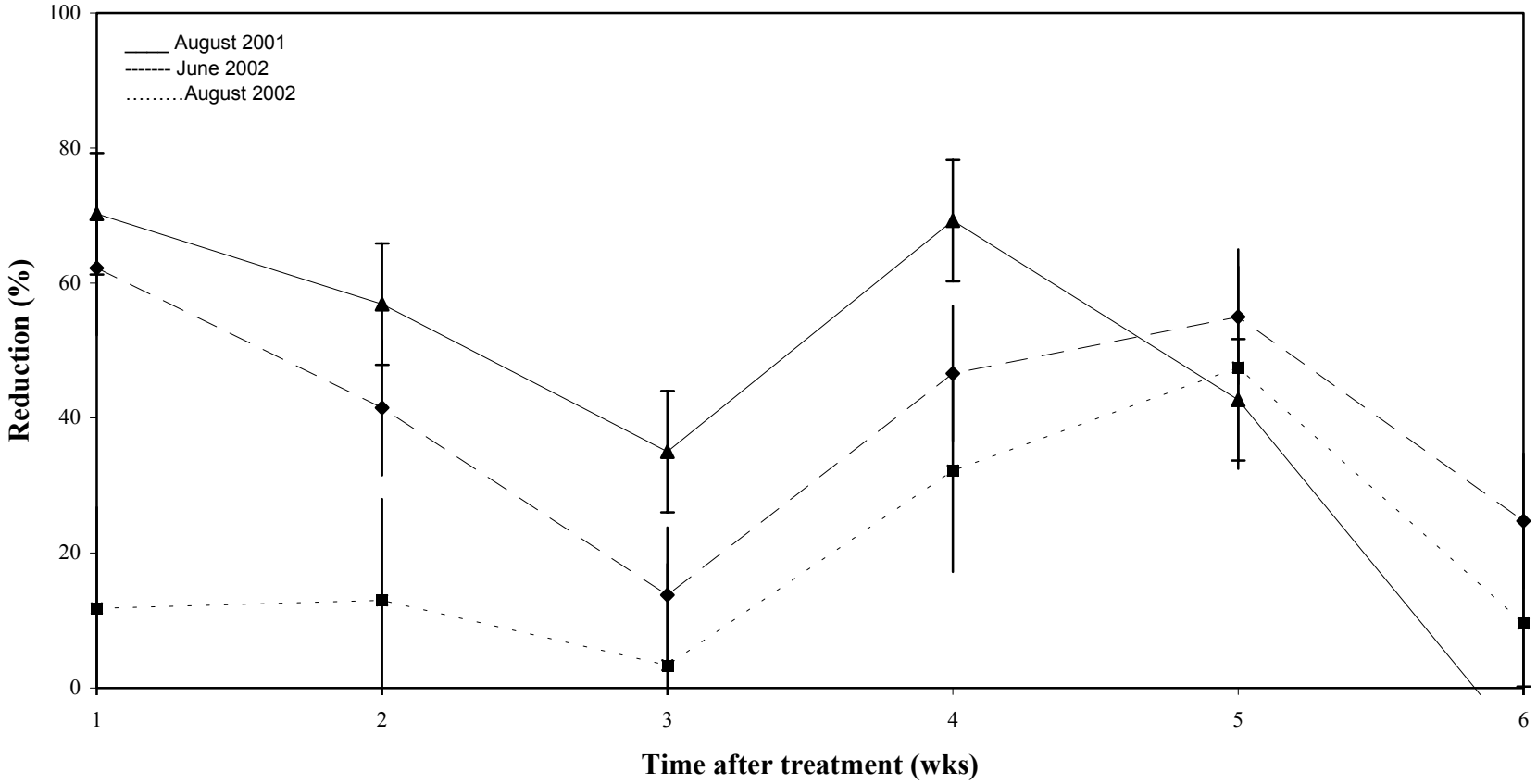

Fig. 8. Effect of prohexadione Ca (PC) rate on percent reduction of bermudagrass clipping biomass over a six-week period. Pooled over time after treatment. Bars represent SE of each mean. Trinexapac-ethyl equivalent rate denoted a line and a (x).

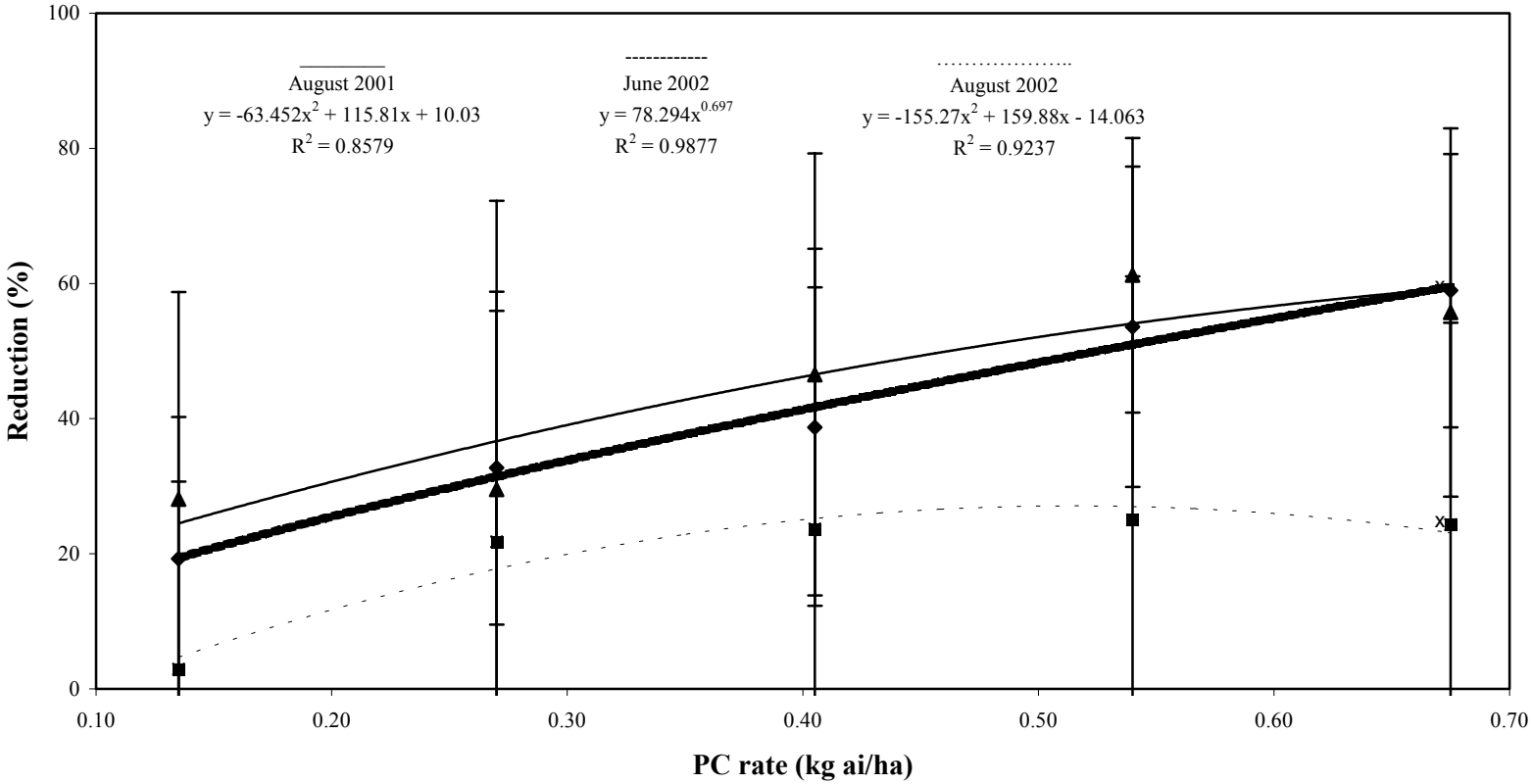

Fig. 9. Effect of time after treatment on prohexadione Ca percent reduction of perennial ryegrass clipping biomass in the greenhouse. Pooled over prohexadione Ca rate. Bars represent SE for each mean. Prohexadione Ca applications were made at 0 and 3 wk.

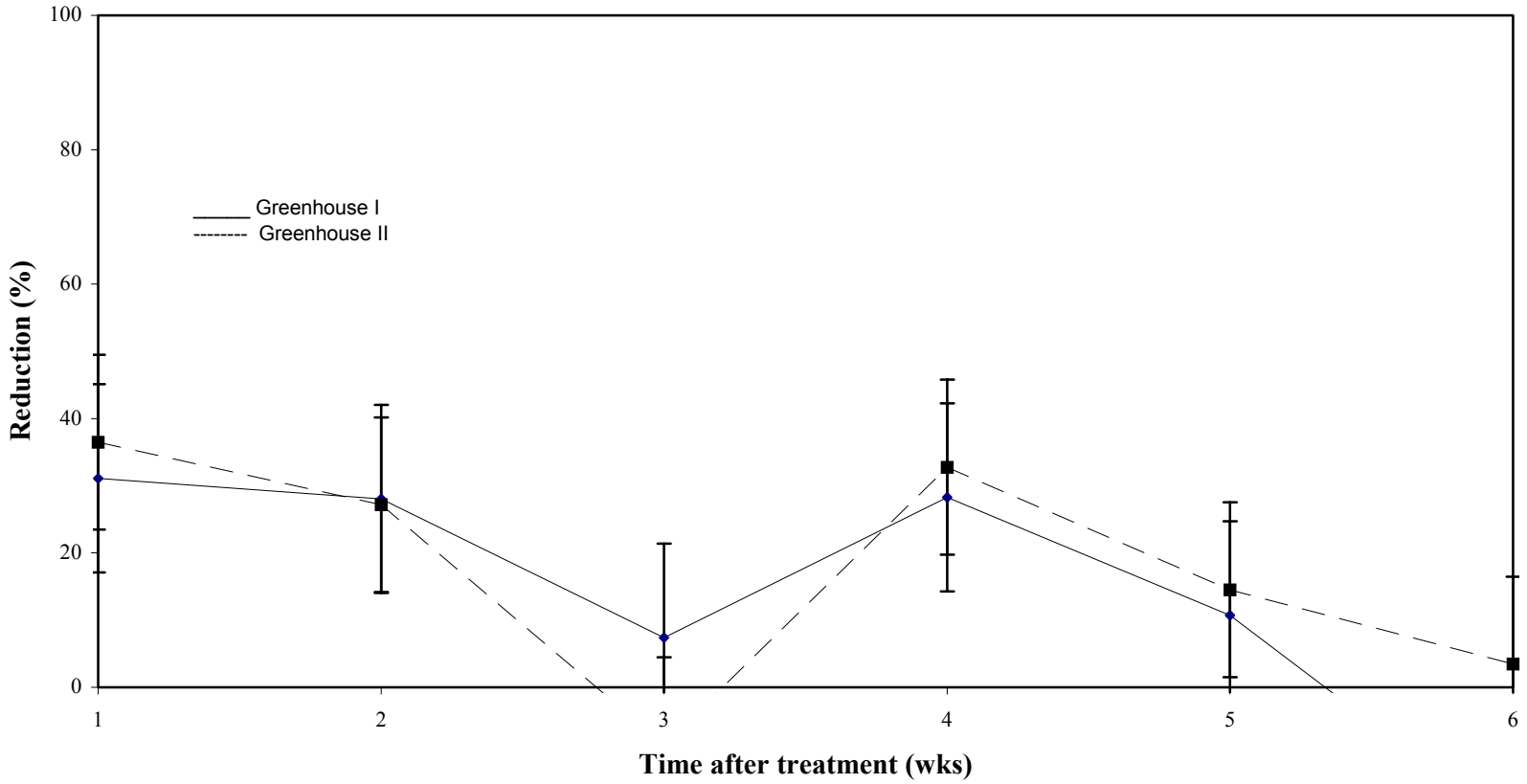

Fig. 10. Effect of time after treatment on prohexadione Ca percent reduction of Kentucky bluegrass clipping biomass in the greenhouse. Pooled over greenhouses and prohexadione Ca rate. Bars represent SE for each mean. Prohexadione Ca applications were made at 0 and 3 wk.

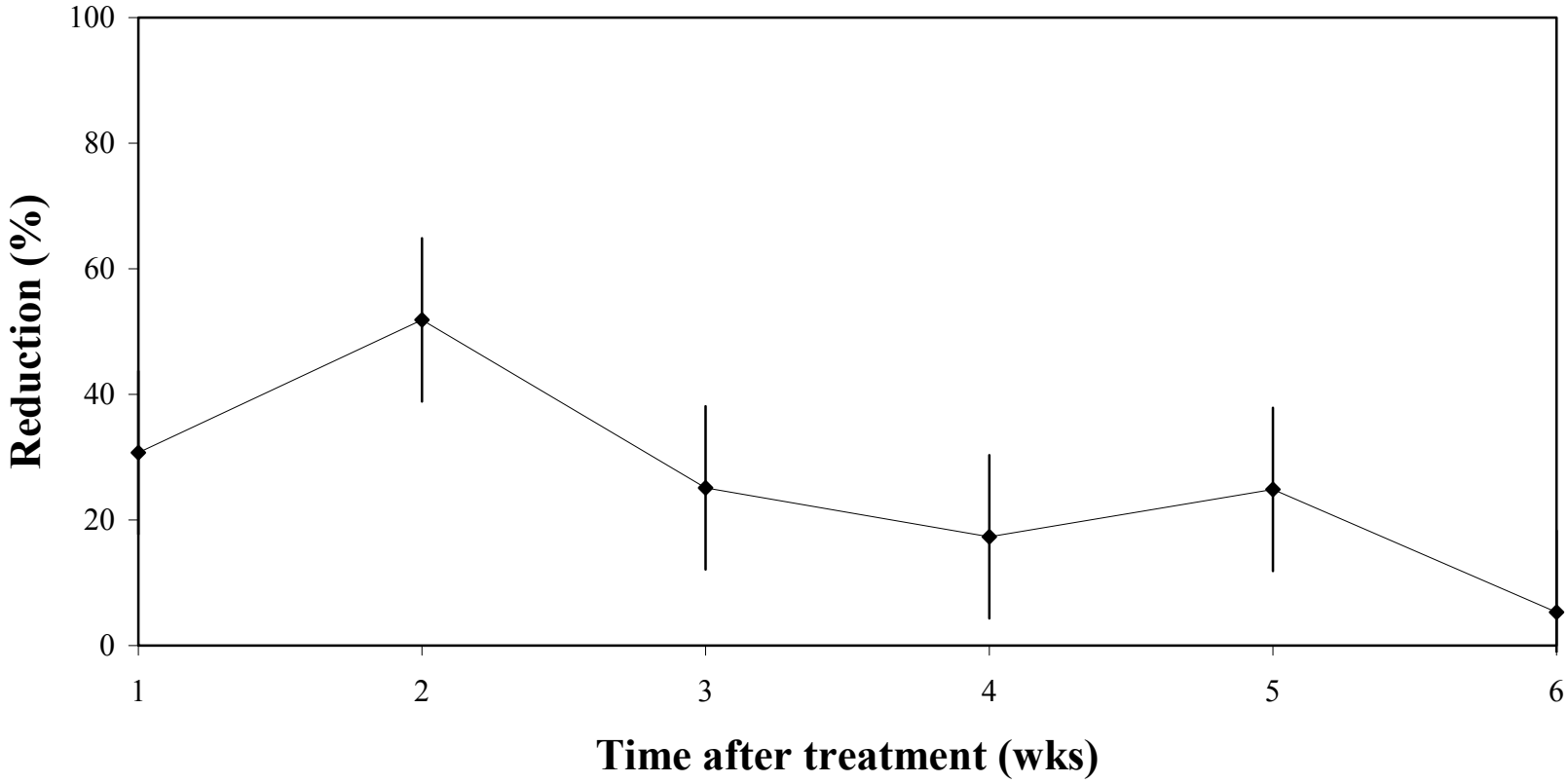

Fig. 11. Effect of prohexadione Ca rate on percent reduction of Kentucky bluegrass clipping biomass in the greenhouse over a six-week period. Pooled over time after treatment and greenhouses. Bars represent SE of each mean. Trinexapac ethyl equivalent rate denoted with a line and a (x).

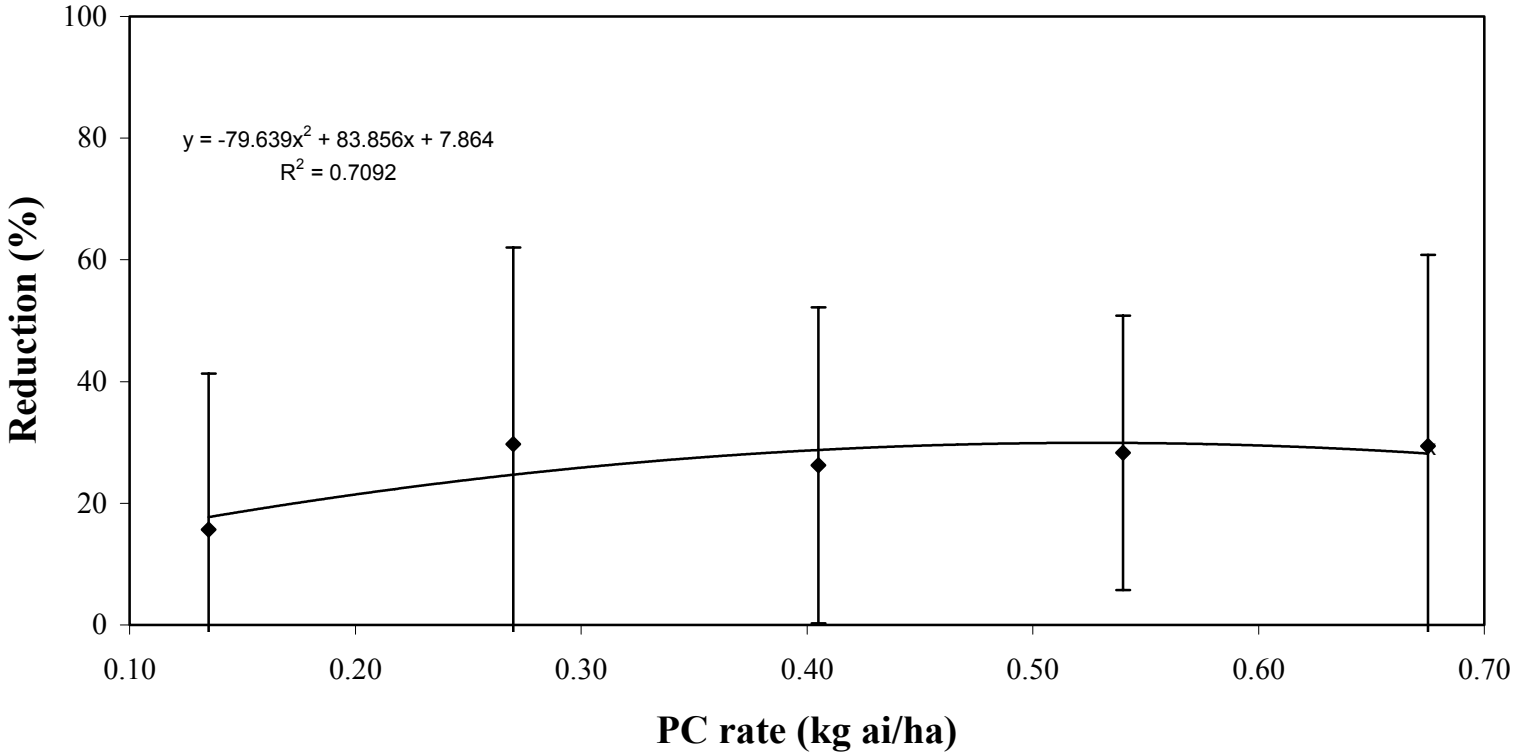

Table 2. Analysis of variance of main effects and interactions of prohexadione calcium (PC) percent reduction of clipping biomass in greenhouse experiments.

Species	Parameter	df	F value	P value
Kentucky bluegrass	Experiment	2	3	0.0811
	Rep (experiment)	6	5	0.0006
	PC rate	4	29	0.0032
	Time after treatment	5	9	0.0146
	Experiment*time after treatment	10	1	0.2812
	Experiment*PC rate	8	0	0.9912
	PC rate*time after treatment	20	2	0.1545
	Experiment*PC rate*time after treatment	40	1	0.9984
Perennial ryegrass	Experiment	1	2	0.1999
	Rep (experiment)	4	1	0.4432
	PC rate	4	2	0.1999
	Time after treatment	5	7	0.0234
	Experiment*time after treatment	5	4	0.0041
	Experiment*PC rate	4	2.12	0.0832
	PC rate*time after treatment	20	2	0.1205
	Experiment*PC rate*time after treatment	20	1	0.7732

Table 3. Effect of prohexadione Ca and trinexapac-ethyl on turfgrass color five weeks after initial treatment. †

Treatment	Rate	Turfgrass color			
		Bermudagrass	Kentucky bluegrass	Perennial ryegrass	Zoysiagrass
	kg a.i. ha^{-1}	1-9			
Nontreated		6.0a‡	6.8a	7.0	6.5a
Prohexadione Ca§	0.14	6.0a	6.4b	6.7	5.9b
	0.27	5.9ab	6.4b	6.5	5.5bcd
	0.41	6.0a	6.4b	6.5	5.6bc
	0.54	6.0a	6.1bc	6.6	5.2cd
	0.67	5.6b	6.0c	6.5	5.1d
Trinexapac-ethyl¶	0.29	4.7c			
	0.22		6.3bc		
	0.38			6.5	
	0.10				5.5bcd

† Data pooled over three experiment timings.

‡ Means within a column followed by the same letter are not significantly different based on Fisher's Protected LSD at P≤0.05.

§. A second prohexadione Ca at identical rates was applied three weeks after initial treatment.

¶ A second trinexapac-ethyl at identical rates was applied four weeks after initial treatment.

Chapter III. Effect of Prohexadione Calcium on Annual Bluegrass (*Poa annua*) Control and Turfgrass Seedling Development.[1]

Abstract: Annual bluegrass is difficult to control in Kentucky bluegrass because only a few selective herbicides are available. Paclobutrazol is a growth regulator that selectively suppresses annual bluegrass in Kentucky bluegrass. Prohexadione Ca regulates turfgrass growth similar to paclobutrazol and may be useful for selective annual bluegrass suppression. Tests were conducted at three locations in Virginia in the fall of 2001 and 2002 to determine effects of prohexadione Ca rate on annual bluegrass control and Kentucky bluegrass injury. Prohexadione Ca was applied twice at two locations and three times at one location, three wk apart, at 0.14, 0.27, 0.41, 0.54, and 0.68 kg ai/ha. A nonionic surfactant was included with each treatment at 0.25% v/v. Two applications three weeks apart of paclobutazol at 0.56 kg ai/ha and prohexadione Ca at 0.41 kg ai/ha with crop oil concentrate or methylated seed oil were included as comparisons. Increasing prohexadione Ca rate increased annual bluegrass control, but did not affect Kentucky bluegrass injury. Highest annual bluegrass control was noted at 8 WAIT. Prohexadione Ca at 0.65, 0.48, and 0.53 kg a.i. ha^{-1} controlled annual bluegrass equivalent to paclobutrazol at 3, 6, and 8 WAIT, respectively. Annual bluegrass control was similar with prohexadione Ca at 0.41 kg ai/ha mixed with all adjuvants tested. Greenhouse tests were also conducted to determine effects of prohexadione Ca on seedling development. Prohexadione Ca at 0.41 kg ai/ha and paclobutrazol at 0.56 kg

[1] Received for publication Date and in revised form Date.

ai/ha were applied to bare soil immediately prior to seeding perennial ryegrasss. Seedling height and color were rated at 2 and 5 WAIT. Prohexadione Ca did not affect height or color of perennial ryegrass seedlings when applied prior to seeding. Results indicate prohexadione Ca suppresses annual bluegrass and does not affect seedling development.

Nomenclature: Paclobutrazol; prohexadione calcium; annual bluegrass, *Poa annua* L. #[2] POAAN; Kentucky bluegrass, *Poa pratensis* L; perennial ryegrass, *Lolium perenne* L. 'Prosport'.

Additional index words: Interseeding, plant growth regulator, turfgrass injury, weed control.

Abbreviations: WAIT, weeks after initial treatment; WAT, weeks after treatment.

This chapter is formatted for publication in Weed Technology.

INTRODUCTION

Annual bluegrass is a troublesome weed in turf that produces abundant seeds and adapts to close mowing and compacted soils (Watschke et al. 1979; Wu et al. 1992). Annual bluegrass is difficult to control in Kentucky bluegrass due to lack of available selective herbicides. Herbicide options for postemergence annual bluegrass control are limited in Kentucky bluegrass. Plant growth regulators such as flurprimidol and paclobutrazol affect annual bluegrass more than other turfgrass species, thus enabling the desired turf to compete with annual bluegrass (Baldwin 1993; Gibson et al. 1998; Isgrigg

[2] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

et al. 1998; Johnson and Murphy 1995, 1996; Woosley et al. 2003). Isgrigg and Yelverton (1999) reported paclobutrazol applied twice in the fall and twice in the spring reduced stands of annual bluegrass at least 80% by the end of year two. Yelverton et al. (1999) reported paclobutrazol at 0.28 and 0.56 kg ai/ha applied twice in fall and twice in spring controlled annual bluegrass 50 and 85%, respectively. Woosley et al. (2003) reported paclobutrazol at 0.28 and 0.14 kg ai/ha controlled annual bluegrass 85%.

Miyazawa et al. (1991) suggested that prohexadione Ca could be used to manage grass height and preliminary research supports this assumption (Beam, unpublished data). Effects of prohexadione Ca on annual bluegrass have not been documented. Prohexadione Ca works similarly to trinexapac-ethyl, paclobutrazol, and flurprimidol to inhibit gibberellin production in turfgrass (Rademacher 2000). Trinexapac-ethyl is the most commonly used turfgrass growth regulator and depends on foliar absorption for effectiveness (Fagerness and Penner 1998). Lack of soil residual activity allows trinexapac-ethyl to suppress existing turfgrass while not affecting interseeded turfgrass (Waltz et al. 1997).

Paclobutrazol and flurprimidol cannot be used during turfgrass seeding due to residual effects on germinating seedlings (Anonymous 2002; Gaussoin and Branham 1987; Haley and Fermanian 1989; Murphy 2001; Yelverton et al. 1999). Although useful at seeding, trinexapac-ethyl does not control annual bluegrass like paclobutrazol or flurprimidol (Gibson et al. 1998). A plant growth regulator that selectively suppresses or controls annual bluegrass without harming seeded turfgrass would allow interseeding concurrent with treatment and improve annual bluegrass control efforts. Effects of prohexadione Ca on annual bluegrass or turfgrass seedling establishment have not been

documented. If, like trinexapac-ethyl, prohexadione Ca depends predominately on foliar absorption, using a proper adjuvant such as crop oil concentrate or methylated seed oil could increase annual bluegrass control compared to nonionic surfactant.

The objectives of these experiments were to determine the effect of prohexadione Ca at rates between 0.14 and 0.67 kg ai/ha on annual bluegrass and Kentucky bluegrass compared to paclobutrazol, evaluate the influence of nonionic surfactant, crop oil concentrate, and methylated seed oil adjuvants on efficacy of prohexadione calcium for annual bluegrass control, and to compare prohexadione Ca to paclobutrazol for effects on perennial ryegrass seedling establishment.

MATERIALS AND METHODS

Three greenhouse studies were conducted at the Glade Road Research Facility, in Blacksburg, VA in February 2002 and June 2003. Prohexadione Ca at 0.41 kg ai/ha and paclobutrazol 0.56 kg ai/ha were applied to bare soil with an air-driven, single-nozzle greenhouse track sprayer[3] calibrated to deliver 281 L/ha. Soil was a Groseclose loam (clayey, mixed, mesic, Typic Hapludalfs) with 1.8% organic matter and pH 6.6. Pots (103-cm^2) were watered to field capacity then 'Prosport' perennial ryegrass was seeded at 242 kg/ha. Pots were watered daily to field capacity, and greenhouse average temperature was 20 C. Seedling height (average of each pot measured from the soil surface to top of plant) and color (on 1-9 scale where 1=dead turf and 9=darkest green turf) were rated at 2 and 5 weeks after treatment (WAT). Number of perennial ryegrass seedlings was also counted 2 WAT.

[3] Allen Track Sprayer, Allen Machine Works, 607 East Miller Road, Midland, MI 48640.

Field studies for annual bluegrass control were conducted at the Virginia Tech Golf Course in Blacksburg, VA on an established Kentucky bluegrass fairway in October 2001 and 2002, and at Stoney Creek Golf Course in Wintergreen, VA in November 2001. Soil at the Virginia Tech Golf Course was a Groseclose loam (clayey, mixed, mesic, Typic Hapludalfs) pH 6.5 with 2% organic matter. Soil at the Wintergreen location was a Wintergreen loam (Fine, mixed, subactive, mesic Typic Paleudults) pH 5.0 with 4% organic matter. Plot size was 1.83 m^2 and irrigation and fertilizer were applied as needed by golf course personnel. Prohexadione calcium was applied at 0, 0.14, 0.27, 0.41, 0.54, and 0.68 kg ai/ha. The treatment was delivered with a CO_2-pressurized sprayer calibrated to deliver 281 L/ha at 221 kPa and contained a non-ionic surfactant[4] at 0.25 % v/v. In addition, prohexadione Ca at 0.41 kg ai/ha was also applied with crop oil concentrate[5] or methylated seed oil[6] each at 1% v/v. At the Virginia Tech Course in 2001 all treatments were applied three times, three wk apart. At all other locations treatments were consistent except paclobutrazol at 0.56 kg ai/ha was used as a comparison treatment in these studies, and only two treatments of each growth regulator were made rather than three. All studies were visually rated at 3, 6, 8, and 17 weeks after initial treatment (WAIT) for turf injury (0 to 100 with 0 = no injury and 100 = dead turf) and annual

[4] Promate Kinetic™, 99% nonionic oganosilicate surfactant. Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

[5] Agridex® paraffin-based petroleum oil concentrate with 83% heavy range, paraffinic petroleum hydrocarbons and 17% surfactant emulsifiers (polyoxyethylene sorbitan fatty acid esters). Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

[6] Meth Oil™ methylated seed oil. Methyl soyate blend. Riverside/Terra Corp., Terra Centre, 600 4th Street, Sioux City, IA 51101.

bluegrass control (0 to 100 with 0 = no difference from control plots and 100 = completely dead).

All experiments were conducted as randomized complete block designs with treatments replicated three times. Accounting for study repetition, field and greenhouse treatments were replicated nine times. Data variance was evaluated by plotting residuals. When analyzing percentage data, nontreated controls were deleted to stabilize variance. If needed, either arcsine square-root or log transformation was applied prior to ANOVA to improve variance homogeneity. A combined ANOVA was conducted with sums of squares partitioned to evaluate linear, quadratic, and higher-order effects of prohexadione Ca rate and trial effects. Trial effects were considered random and the mean squares of these effects were used to test treatment effects as is appropriate for a combined analysis (McIntosh 1983). Polynomial regressions were used to describe the effect of prohexadione Ca rate on measured responses as indicated by the ANOVA. If second-order effects were more significant, the quadratic curve was applied; otherwise the linear equation was applied. Other factors were separated using Fisher's protected LSD at P = 0.05.

RESULTS AND DISCUSSION

Greenhouse experiments. Prohexadione Ca and paclobutrazol did not alter number of perennial ryegrass seedlings per pot 2 WAT when compared to the nontreated (Data not presented). The interaction of location by treatment was significant for perennial ryegrass color two and five WAT. Paclobutrazol increased turfgrass color in all experiments two WAT and in two experiments five WAT (Table 1). Prohexadione Ca

did not affect perennial ryegrass color at two or five WAT in any experiment, when compared to the nontreated (Table 1).

The main effect of growth regulator treatment was significant for height of perennial ryegrass 2 and 5 WAT. Height of perennial ryegrass was stunted by paclobutrazol but not by prohexadione calcium at 2 and 5 WAT, when compared to the nontreated (Table 2). Our results are consistent with previous research concerning the effects of paclobutrazol on turfgrass seedling development. Yelverton et al. (1999) reported paclobutrazol inhibited bentgrass seedling growth when applied 2 wk or less prior to seeding of bentgrass. Murphy (2001) reported paclobutrazol decreased perennial ryegrass establishment for up to four months after overseeding. We could find no previous research that utilized prohexadione Ca prior to turfgrass seeding. In a lab experiment root uptake of ^{14}C prohexadione Ca was minimal (Beam, unpublished data). Prohexadione Ca has a half-life in the range of hours in the soil and may be the reason for lack of soil activity (Rademacher 2000).

Annual bluegrass control. The main effect of prohexadione Ca rate was significant for annual bluegrass control 3, 6, and 8 WAIT. As prohexadione Ca rate increased, annual bluegrass control increased linearly 3, 6, and 8 WAIT (Figure 1). Each 0.1 kg ai/ha increase in prohexadione Ca resulted in a 3.7, 5.1, and 6.0% increase in annual bluegrasss control at 3, 6, and 8 WAIT, respectively (Figure 1). Maximum annual bluegrass control was 71% at 8 WAIT when prohexadione Ca was applied at 0.68 kg ai/ha. Prohexadione Ca at 0.65, 0.48, and 0.53 kg a.i. ha^{-1} controlled annual bluegrass equivalent to paclobutrazol at 3, 6, and 8 WAIT, respectively (Figure 1). While effects of prohexadione Ca on annual bluegrass have not been acknowledged, annual bluegrass

control with paclobutrazol is well documented (Isgrigg et al. 1998; Johnson and Murphy 1995, 1996; Woosley et al. 2003).

The interaction of year by prohexadione Ca rate was significant for annual bluegrass control 17 WAIT. Annual bluegrass control was not rated at Stoney Creek Golf Course 17 WAIT. At the Virginia Tech Golf Course in 2001, increasing prohexadione Ca rate up to 0.41 kg ai/ha increased annual bluegrass control to 50% 17 WAIT, while rates above 0.41 kg ai/ha did not improve control (Figure 2). At the Virginia Tech Golf Course in 2002, prohexadione Ca, regardless of rate, controlled annual bluegrass less than 10% 17 WAIT (Figure 2). Prohexadione Ca at an estimated 1.3 kg ai/ha was equivalent to paclobutrazol (22%) for annual bluegrass control at the Virginia Tech Golf Course in 2002, 17 WAT. In 2001, paclobutrazol was not applied and prohexadione Ca controlled annual bluegrass between 30 and 50% 17 WAIT. In 2001, prohexadione Ca was applied three times and increased annual bluegrass control 17 WAIT compared to 2002 when it was only applied twice. Annual bluegrass control with paclobutrazol has been reported in other studies, and multiple applications were needed to effectively control annual bluegrass (Baldwin 1993; Gibson et al. 1998; Woosley et al. 2003). Yelverton et al. (1999) reported paclobutrazol at 0.56 kg ai/ha applied twice in fall and twice in spring controlled annual bluegrass 85%.

There were no significant differences noted for annual bluegrass control between prohexadione Ca at 0.41 kg ai/ha mixed with nonionic surfactant, crop oil concentrate, or methylated seed 3, 6, 8, and 17 WAIT (Data not presented).

Prohexadione Ca inhibits gibberellin production similar to trinexapac-ethyl by blocking the 3ß-hydroxylase enzyme which converts GA_{20} to the growth active GA_1.

Annual bluegrass control by prohexadione Ca may be from a build of gibberellin precursors that negatively affect annual bluegrass more than Kentucky bluegrass. Paclobutrazol and prohexadione Ca increase cytokinin and abscisic acid, and decrease ethylene concentration in responsive tissues and paclobutrazol also affects sterol formation (Rademacher 2000), which may led to annual bluegrass control.

Kentucky bluegrass injury. There were no significant differences for Kentucky bluegrass injury 3 and 8 WAIT. The interaction of location by prohexadione Ca rate was significant for Kentucky bluegrass injury 6 and 17 WAIT. At the Virginia Tech Golf Course in 2002 6 WAIT, prohexadione Ca at 0.14 to 0.67 kg ai/ha injured Kentucky bluegrass between 10 and 30% 6 WAIT (Figure 3). Colder weather in 2002 compared to 2001 may have increased Kentucky bluegrass injury from growth regulator treatment. Prohexadione Ca at 0.27 kg ai/ha was equivalent to paclobutrazol for Kentucky bluegrass injury 6 WAIT. At 17 WAIT prohexadione Ca injured Kentucky bluegrass less than 10% at all locations and paclobutrazol injured Kentucky bluegrass 17% 17 WAIT (Data not presented).

There were no significant differences noted for Kentucky bluegrass injury between prohexadione Ca at 0.41 kg ai/ha mixed with nonionic surfactant, crop oil concentrate, or methylated seed 3, 6, 8, and 17 WAIT (Data not presented).

Prohexadione Ca at 0.41 kg ai/ha suppressed annual bluegrass comparable to paclobutrazol without affecting overseeding. Isgrigg et al. (1998) reported bentgrass growth increased 2 wk sooner than annual bluegrass when paclobutrazol was applied, which enabled creeping bentgrass to compete successfully with annual bluegrass. Annual bluegrass control programs with prohexadione calcium would inhibit annual bluegrass

similar to paclobutrazol, but could also utilize overseeding to move closer to a desirable turfgrass monoculture. Future studies should look at spring and fall application timings of prohexadione calcium, as well as effects on bentgrass putting greens, to maximize this active ingredient in turfgrass.

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Table 1. Effect of prohexadione Ca and paclobutrazol on perennial ryegrass color 2 and 5 WAT.[a]

		2 WAT			5 WAT		
Treatment	Rate	2002	2003-1	2003-2	2002[c]	2003-1	2003-2
	kg ai /ha	1-9[b]					
Nontreated		6 b[c]	7 b	7 b	6.3	7 b	7 b
Paclobutrazol	0.56	7 a	8 a	8 a	7	8 a	8 a
Prohexadione Ca	0.41	6 b	7 b	7 b	7	7 b	7 b

[a] Abbreviations: 2002, 2003-1, and 2003-2 represent greenhouse trials conducted at the Glade Road Research Facility, Blacksburg, VA in greenhouse #1 in February 2002, greenhouse #1 in June 2003, and greenhouse #2 in June 2003, respectively; WAT, weeks after treatment.

[b] Color rated on 1-9 scale where 1 is dead turf and 9 is darkest green color.

[c] Means followed by the same letter are not significantly different according to Fisher's protected LSD at P = 0.05.

Table 2. Effect of prohexadione Ca and paclobutrazol on perennial ryegrass height 2 and 5 WAT averaged over three greenhouse trials between 2002 and 2003.[a]

Treatment	Rate	Height	
		2 WAIT	5 WAIT
	kg ai/ha	cm	
Nontreated		6.9 a[b]	9.1 a
Paclobutrazol	0.56	1.9 b	2.1 b
Prohexadione Ca	0.41	6.0 a	8.1 a

[a] Abbreviations: WAT, weeks after treatment.

[b] Means followed by the same letter are not significantly different according to Fisher's protected LSD at P = 0.05.

***Figure 1*. Effect of prohexadione Ca rate on annual bluegrass control 3, 6, and 8 WAIT, pooled over three locations. Equivalent paclobutrazol rate denoted with a line and a (x).**

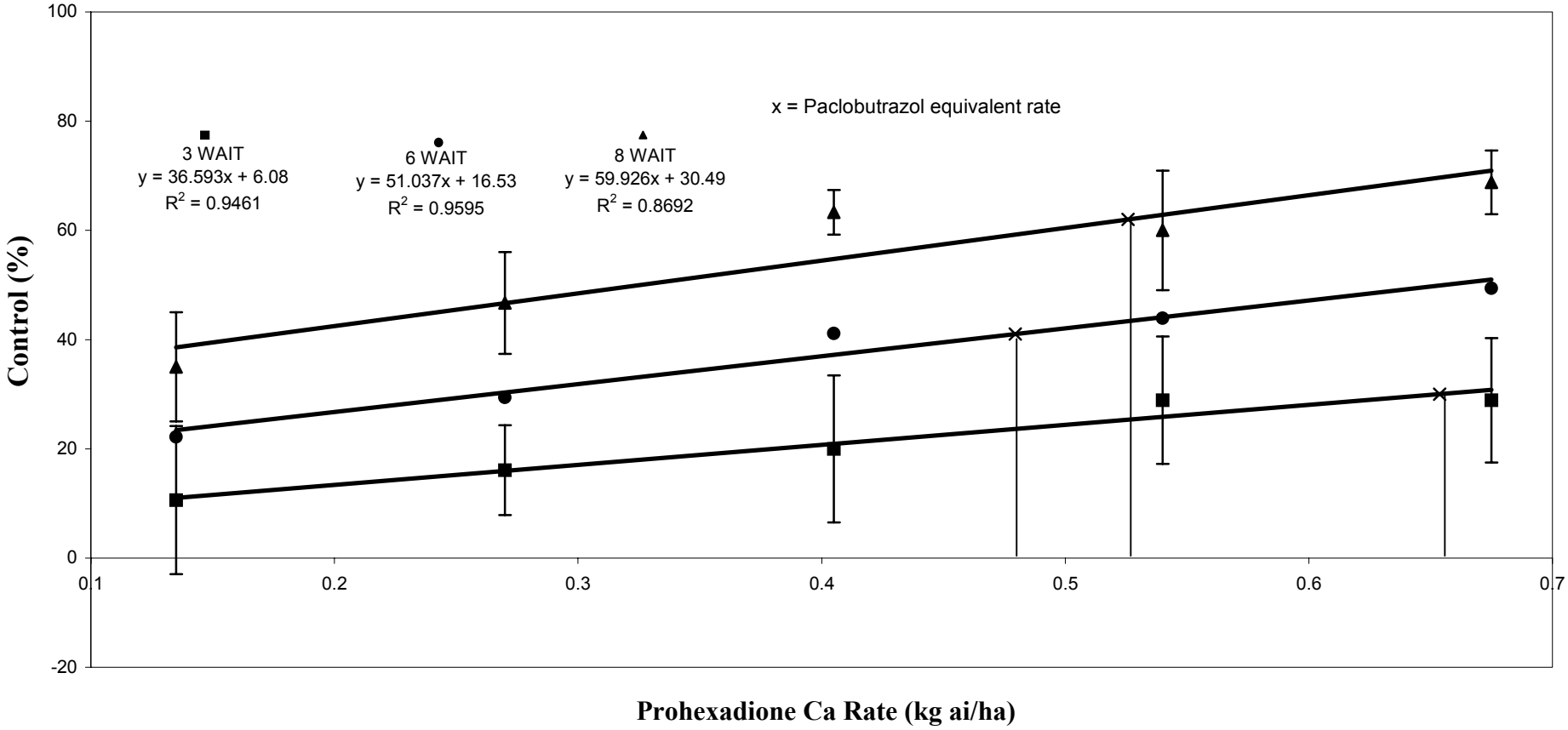


***Figure 2*. Effects of prohexadione Ca rate on annual bluegrass control 17 WAIT, by location. Equivalent paclobutrazol rate denoted with a line and a (x).**

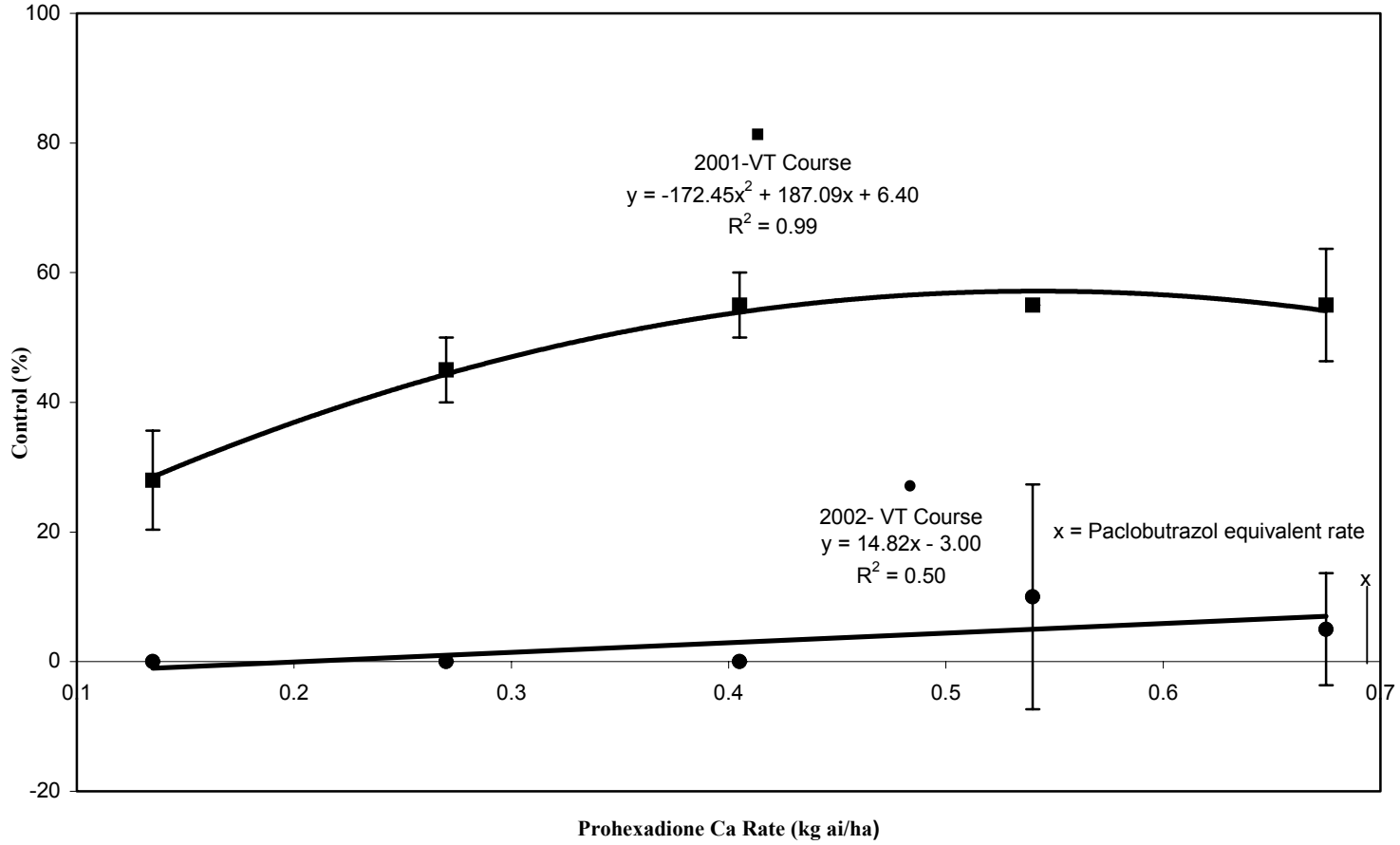
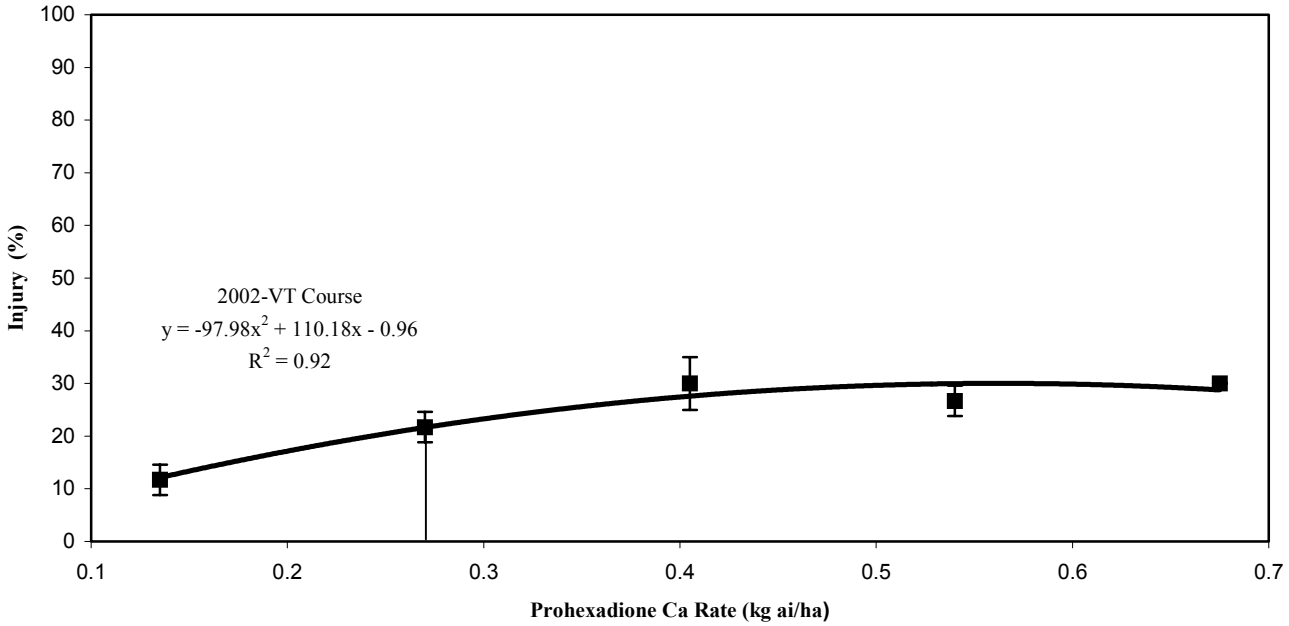


Figure 3. **Effects of prohexadione Ca rate on Kentucky bluegrass injury 6 WAIT at Virginia Tech Golf Course 2002. Equivalent paclobutrazol rate denoted with a line and a (x).**



Chapter IV. Absorption, Translocation, and Metabolism of Prohexadione Calcium in Annual Bluegrass (*Poa annua*), Creeping Bentgrass (*Agrostis stolonifera*), Kentucky Bluegrass (*Poa pratensis*), and Perennial Ryegrass (*Lolium perenne*)[1]

JOSH B. BEAM and SHAWN D. ASKEW[2]

Abstract. Prohexadione calcium is an experimental turfgrass growth regulator that selectively controls or suppresses annual bluegrass in desirable turfgrass such as creeping bentgrass, Kentucky bluegrass, and perennial ryegrass. To help explain interspecific differences in turfgrass and weed response to prohexadione Ca, two laboratory trials were conducted to measure ^{14}C-prohexadione Ca absorption, translocation, and metabolism in these four species. Annual and Kentucky bluegrass absorbed more prohexadione Ca than creeping bentgrass and perennial ryegrass when averaged over harvest timing and trial. Neither translocation out of the treated leaf or metabolism of prohexadione Ca differed between species. When averaged over species and trial, 22% of recovered prohexadione Ca was metabolized within 1 HAT, and plants metabolized an additional 0.7% each additional hour for a period of 48 hours. Previous research indicates that annual and Kentucky bluegrass growth is suppressed more by prohexadione Ca than creeping

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bentgrass and perennial ryegrass. Increased prohexadione Ca absorption partially explained this trend in the current study.

Nomenclature: Prohexadione calcium; annual bluegrass, *Poa annua* L. #[3] POAAN; creeping bentgrass, *Agrostis stolonifera* L., 'Penncross'; Kentucky bluegrass, *Poa pratensis* L. 'Kelly'; perennial ryegrass, *Lolium perenne* L. 'Prosport'.

Additional index words: Growth suppression, weed control.

Abbreviations: HAT**,** hours after initial treatment; PGR, plant growth regulator.

This chapter is formatted for publication in Weed Technology.

INTRODUCTION

Miyazawa et al. (1991) suggested that prohexadione Ca could be used to manage turfgrass height and research supports this assumption (Beam et al. 2002, 2003). Prohexadione Ca inhibits gibberellin production in turfgrass similar to trinexapac-ethyl (Rademacher, 2000). Trinexapac-ethyl is the most commonly used turfgrass growth regulator because it does not discolor desirable turfgrass or annual bluegrass and has no soil residual activity (Fagerness and Penner 1998a). Since trinexapac-ethyl does not control annual bluegrass, turfgrass managers use paclobutrazol or flurprimidol when faced with the need to suppress annual bluegrass infestations. However, these chemicals can be more injurious to desirable turfgrass and preclude seeding thin areas due to soil residual activity (Haley and Fermanian 1989; Isgrigg 1998; Yelverton et al. 1999).

[3] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

Studies in Virginia indicate that prohexadione Ca selectively controls or suppresses annual bluegrass in Kentucky bluegrass (Beam et al. 2002, 2003) and creeping bentgrass (unpublished data), equivalent to paclobutrazol. Other Virginia field trials indicate that prohexadione Ca at rates between 0.14 and 0.67 kg ai/ha affects percent reduction in turfgrass clipping biomass differently between Kentucky bluegrass and perennial ryegrass (Beam et al. 2002, 2003).

Although several studies have evaluated turfgrass response to plant growth regulators (PGRs), few studies have examined absorption, translocation, and metabolism of PGRs in turf species. Fagerness and Penner (1998b) examined absorption and translocation of ^{14}C-trinexapac-ethyl in Kentucky bluegrass and found that the crown region absorbed more radioactivity than leaf blades or roots. Several studies have reported differences in species response to PGRs based on visual assessment or clipping biomass. The objective of this experiment was to determine the rates of absorption, translocation, and metabolism of prohexadione Ca in annual bluegrass, creeping bentgrass, perennial ryegrass, and Kentucky bluegrass in an attempt to elucidate reasons for differential plant response to prohexadione Ca.

MATERIALS AND METHODS

Annual bluegrass, 'Penncross' creeping bentgrass, 'Prosport' perennial ryegrass, and 'Kelly' Kentucky bluegrass were transplanted from field grown plants thinned to one tiller and one fully expanded leaf and placed in 0.25% Hoagland solution (Menn and McBee 1970) in the greenhouse with average nighttime and daytime temperatures of 20 and 30 C, respectively. Supplemental lighting was provided and average irradiance was

17.2 W/m^2. Plants acclimated for 1 week before treatment. Foliar absorption and translocation were determined by applying three 1-µl droplets of treatment solution to the newest fully expanded leaf blade on each plant. The solution consisted of 3,5- ^{14}C-labeled prohexadione Ca (Figure 1) with 91% purity and 2.1 kBq, in 88% 1M HCL, 12% Acetonitrile, and 0.25% v/v nonionic surfactant[4]. Plants were harvested at 1, 12, 24, and 48 hours after treatment (HAT). Each treatment was replicated three times and the study was repeated in time. Preliminary results indicated prohexadione Ca was being lost via CO_2 from treated leaves; therefore air derived from each plant was vacuumed through a 50 ml glass burette containing 20 g of glass beads and 20 ml of 3M NaOH. At each harvest, each NaOH container was emptied and rinsed with 20 ml of acetonitrile: water (3:7) and a 1 ml aliquot of the solution was added to 19 ml scintillation cocktail[5] and analyzed with a liquid scintillation spectrometer (LSS)[6].

Treated leaves were washed with 10 ml of 1:1 methanol: water. Rinsates were collected in 20-ml liquid scintillation vials and a 1 ml aliquot of each was added to 19 ml scintillation cocktail and analyzed with a LSS.

The remaining plant parts were separated into treated leaf, other foliage, and roots and dried and stored in an oven at 50 C until oxidized. Samples were then oxidized in a sample oxidizer[7] and released $^{14}CO_2$ was captured in scintillation cocktail[8] and quantified

[4] Promate Kinetic™, 99% nonionic oganosilicate surfactant. Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

[5] Scintiverse BD. Fisher Scientific, Fair Lawn, NJ 07410.

[6] LS 6500 Multipurpose Scintillation Counter. Beckman Coulter, Inc, 4300 N. Harbor Blvd., PO Box 3100 Fullerton, CA 92834-3100.

[7] Packard Model 307, Packard Instrument Company, 2200 Warrenville rd.; Downers Grove, IL 60515.

by LSS. The Hoagland solution was also measured and a 1-ml sample was analyzed with 19 ml scintillation cocktail to quantify root-exuded ^{14}C.

Total percentage recovery of ^{14}C-prohexadione Ca applied was calculated by combining the ^{14}C activity recovered in the CO_2, washes, and plant parts, and dividing by the total ^{14}C activity applied. Percent translocation was determined by dividing total activity from plant parts by the total ^{14}C-prohexadione Ca activity absorbed.

To evaluate metabolism, plants were treated, harvested, as previously described, and stored in freezer at -4 C. Plant tissues were ground with tissue grinders[9] in 15 ml acetonitrile: water (3:7). Extract and plant parts were vacuum filtered and washed with solvent. The extract was dried completely with a stream of nitrogen gas and resuspended in 0.2 ml acetonitrile: water (3:7). A 100 µl subsample of extract was spotted on silica gel thin layer chromatography plates and developed in a 90:7:3 solution of isopropyl ether, formic acid, and water. Plates were air-dried and radioactive proportions were determined by scanning with a radiochromatogram scanner[10]. Radioactive trace peaks were integrated with Win-Scan® and parent herbicide was identified by comparing values from the corresponding standard spotted on each plate. Data consisted of the percentage parent herbicide and sum percentage of all metabolites.

Data were subjected to ANOVA with sums of squares partitioned to reflect a split-split-plot treatment structure and trial effects. The four harvest timings were considered main plots, the four plant species were considered subplots, and the five portions of

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[8] Carbosorb E and Permaflour E+. PerkinElmer Life and Analytical Sciences 549 Albany St., Boston, MA 02198-2512.

[9] Pyrex®. Corning One Riverfront Plaza, Corning, NY 14831.

[10] Bioscan 200. Bioscan, Inc., Washington, DC.

quantified radioactivity (CO_2, foliage, roots (including root exudates from Hoagland solution), treated leaf, and rinse) were considered subsubplots. Homogeneity of variance was tested based on visual inspection of plotted residuals and appropriate transformations were performed to satisfy assumptions of ANOVA. Trial effects were considered random and mean squares were tested appropriately based on the treatment design (McIntosh 1983). Where main plot effects were significant, regressions were used to explain the relationship of measured responses over time. Effects of species and plant parts were separated via Fisher's Protected LSD test at P=0.05.

RESULTS AND DISCUSSION

Foliar absorption and translocation. Recovery of applied ^{14}C averaged 95%. The interaction of plant species by plant part was significant for percent-recovered radioactivity. Annual and Kentucky bluegrass had less in rinse and therefore absorbed more radioactivity than creeping bentgrass and perennial ryegrass when averaged over harvest timing and trial (Table 1). Creeping bentgrass absorbed less radioactivity than perennial ryegrass (Table 1). Decreased absorption by perennial ryegrass compared to annual and Kentucky bluegrass is consistent with field applications of prohexadione Ca. Beam et al. (2002, 2003) reported prohexadione Ca suppressed annual and Kentucky bluegrass at 0.4 kg ai/ha and perennial ryegrass at 0.7 kg ai/ha. Perennial ryegrass and creeping bentgrass cuticles may have been thicker when compared to annual and Kentucky bluegrass leading to decreased absorption in these species.

Percent $^{14}CO_2$ captured in vacuumed air was less than 4% of recovered radioactivity and varied by species (Table 1). When averaged over harvest timing and trial annual

bluegrass released more $^{14}CO_2$ than creeping bentgrass and perennial ryegrass (Table 1). Increased $^{14}CO_2$ evolution from annual and Kentucky bluegrass may be partially due to higher ^{14}C prohexadione Ca absorption rates in these two species.

Between 11 and 15% of ^{14}C was recovered from foliage above and below the treated leaf regardless of species (Table 1). Basipetal translocation in annual bluegrass resulted in more ^{14}C recovered from root tissue of this species than from creeping bentgrass or perennial ryegrass (Table 1). However, not more than 11% of recovered ^{14}C was found in roots of any species.

The majority of recovered ^{14}C remained in the treated leaves of all species. Kentucky bluegrass maintained more ^{14}C in treated leaves than annual bluegrass and creeping bentgrass and the amount was not different from perennial ryegrass (Table 1). Although annual and Kentucky bluegrass absorbed ^{14}C prohexadione Ca equivalently, Kentucky bluegrass retained more ^{14}C in the treated leaf compared to annual bluegrass. Although this difference in prohexadione Ca translocation out of the treated leaf supports observed annual bluegrass control in Kentucky bluegrass by prohexadione Ca, any difference in prohexadione Ca translocation is minimal and probably does not account for selective annual bluegrass suppression observed in field trials (Beam et al. 2002, 2003), since there was no difference between annual and Kentucky bluegrass in amount of radioactivity in foliage and roots (Table 1).

The interaction of harvest timing by plant part was significant for percent-recovered radioactivity. Radioactivity recovered in the leaf wash decreased over time as more ^{14}C prohexadione Ca was absorbed (Figure 1). Fagerness and Penner (1998b) reported trinexapac-ethyl increased in absorption over time in Kentucky bluegrass leaf blades. In

our study, percent radioactivity increased in CO2, other foliage, root, and treated leaf over time (Figure 1). While radioactive CO_2 increased over time, amount recovered was less than 10% at any harvest timing (Figure 1). Percent radioactivity in treated leaves did not fluctuate substantially over time (Figure 1), 47% of recovered radioactivity was in treated leaves 1-hr after treatment and 50% was still in treated leaf 48 HAT. Percent radioactivity in other foliage and roots increased over time, percent recovered was less than 20% at any harvest timing for both plant parts. Apparently, highest prohexadione Ca absorption occurs within 1 HAT and most remains in treated leaf.

Metabolism. Prohexadione calcium was metabolized in the treated leaf into two polar metabolites, for clarity these were combined in the figure and discussion. The interaction of timing by metabolite was significant for percent metabolite in the treated leaf. Since the interaction of species by metabolite was not significant, the metabolism differences between species are most likely not the reason for the differences in growth suppression seen under field conditions. Percent prohexadione Ca decreased and polar metabolites increased linearly over time (Figure 2). Only 22% of recovered prohexadione Ca was metabolized within 1 HAT, but plants metabolized an average of 0.7% of prohexadione Ca each additional hour for a period of 48 hours. Percent metabolites increased 0.7% every hour, as well. At 48 HAT only 43% of applied radioactivity remaining in treated leaf was prohexadione Ca.

Annual and Kentucky bluegrass absorbed more prohexadione Ca than creeping bentgrass and perennial ryegrass and this likely lead to the differential rate response seen in field applied prohexadione Ca. Beam et al. (2002, 2003) reported prohexadione Ca suppressed annual and Kentucky bluegrass at lower rates than perennial ryegrass. At 48

HAT 50% of recovered radioactivity remained in treated leaf, of this only 43% was prohexadione Ca. Therefore, metabolism of the active ingredient is the reason multiple applications are required for continued growth suppression in field conditions. Also since prohexadione Ca translocation to other foliage was minimum, thorough coverage of prohexadione Ca is essential for complete growth suppression of the target plant species. Differences in annual and Kentucky bluegrass reported from field-applied prohexadione Ca are only partially explained in this research. Future research should examine affinity of the 3ß hydroxylase enzyme in each species for prohexadione Ca.

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Table 1. Percent radioactivity in each plant part separated by species based on percent recovery data pooled over harvest timing and trial.[a]

	Radioactivity in each part				
Species	CO_2	Rinse	Treated leaf	Foliage	Roots
	%				
Annual bluegrass	4 a[b]	17 c	53 b	15	11 a
Creeping bentgrass	2 b	31 a	46 b	11	9 bc
Kentucky bluegrass	3 ab	15 c	60 a	12	10 ab
Perennial ryegrass	2 b	24 b	54 ab	13	7 c

[a] Abbreviations: CO_2, percent radioactive carbon dioxide exuded from each plant; Foliage, percent radioactivity that moved out of treated leaf into other vegetative portions of the plant (a combination of above and below treated leaf); Rinse, percent radioactivity that was removed from the treated leaf with methanol and water; Roots, percent radioactivity that moved to the roots; Treated leaf, percent radioactivity that did not move out of the treated leaf.

[b] Means followed by the same letter are not significantly different according to Fisher's protected LSD at P = 0.05.

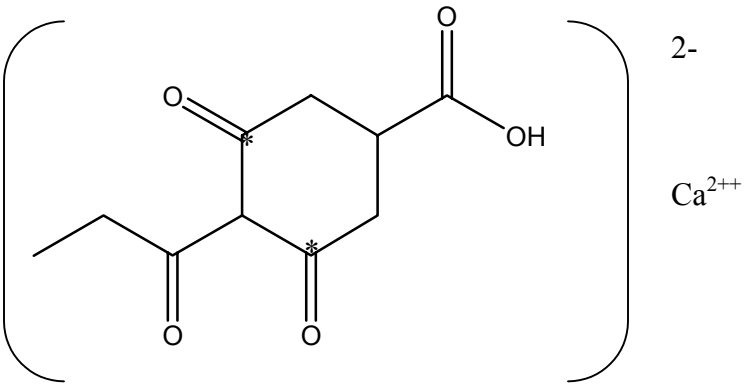


Figure 1. Chemical structure of the calcium salt of 3,5-dioxo-4 propionylcyclohexane-carboxylic acid (prohexadione calcium), ^{14}C-labeled carbons denoted with an asterick (*).

Figure 2. Effect of time after treatment on radioactivity distribution averaged over plant species and trial.

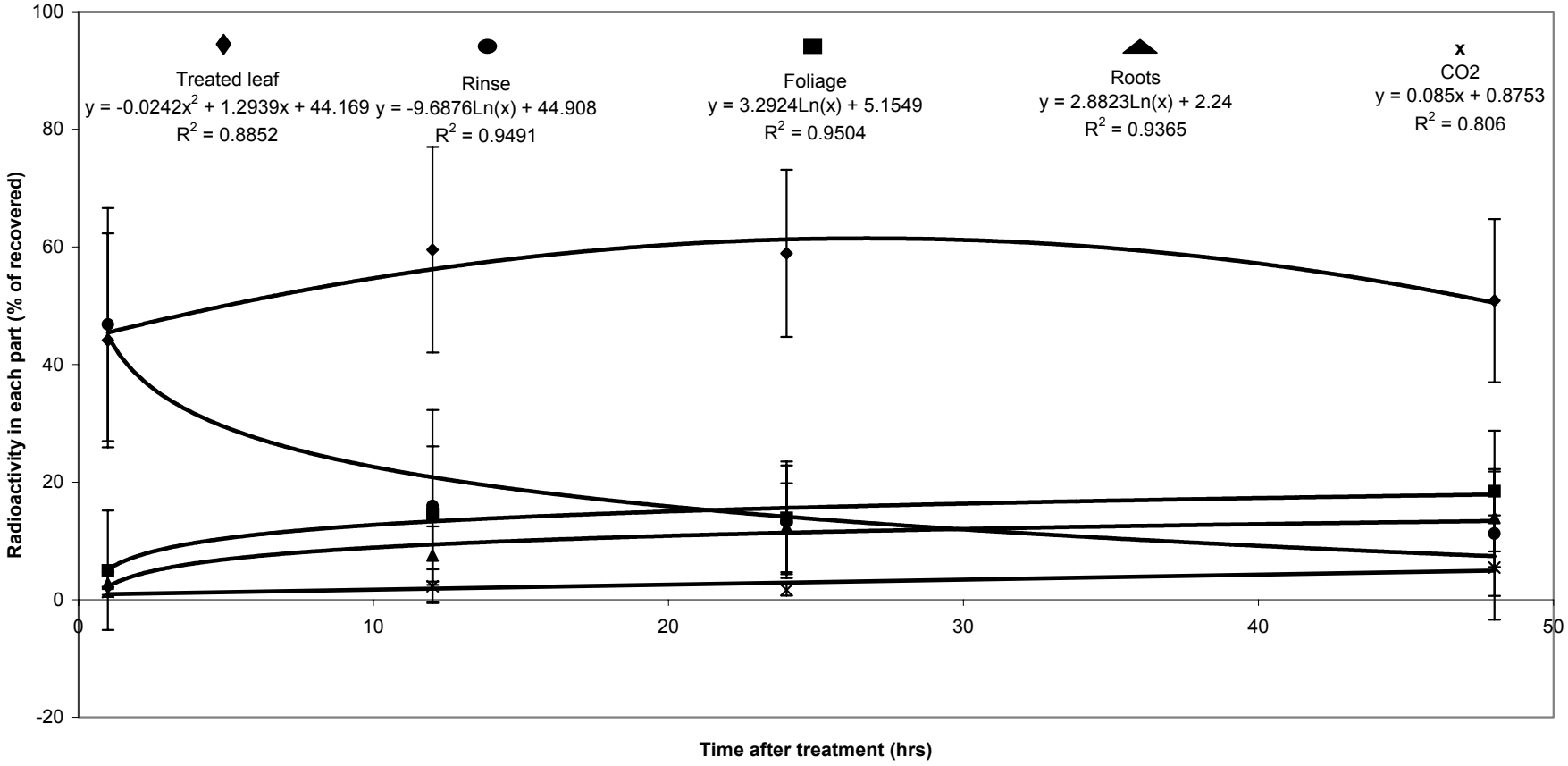
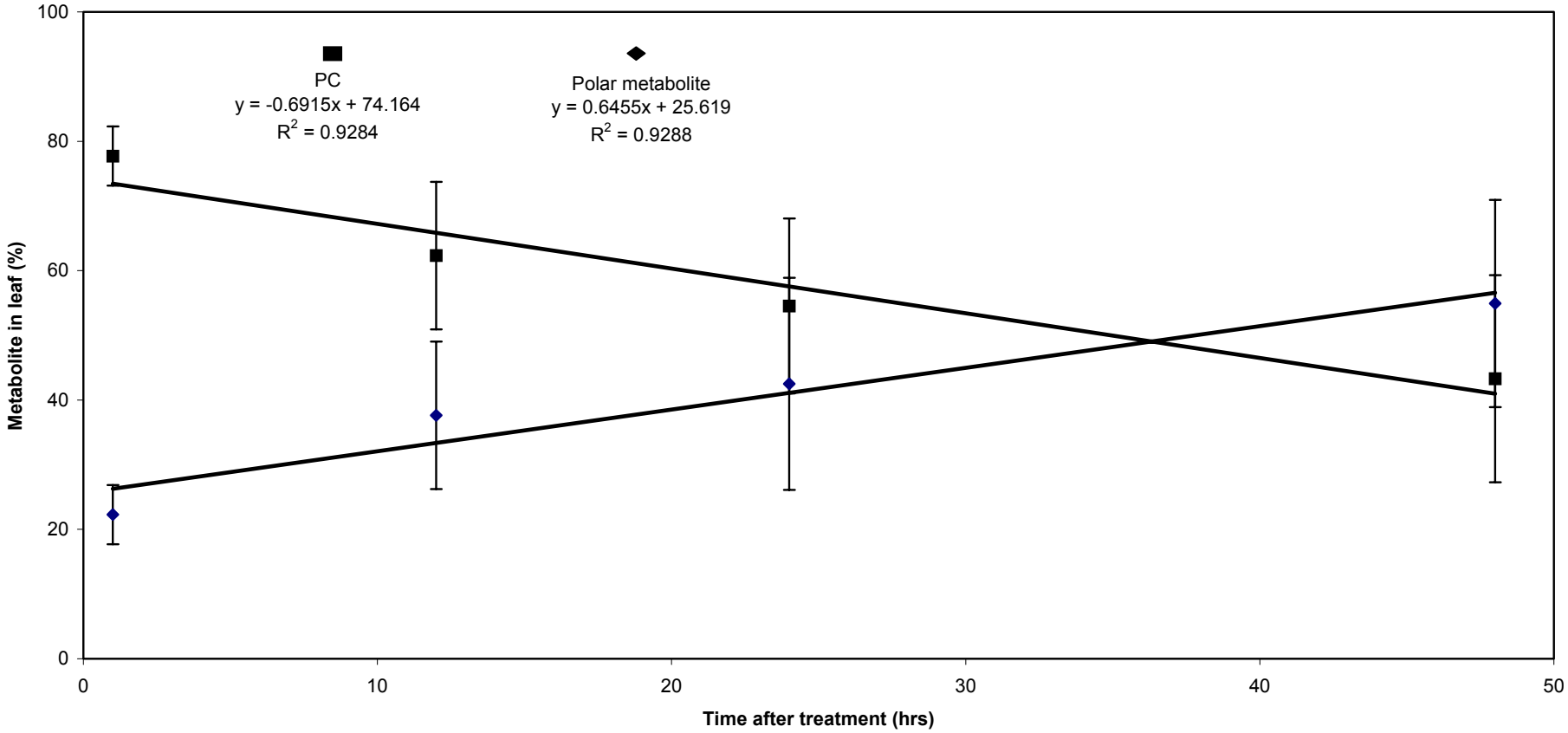


Figure 3. Effect of time after treatment on percent prohexadione calcium (PC) and polar metabolites in treated leaves, averaged over plant species and trials**.**



Chapter V. Derivation of the Nucleotide Sequence Coding for the Acetolactate Synthase Enzyme in Annual Bluegrass (*Poa annua*)

Abstract: While chlorsulfuron, halosulfuron, imazaquin, and metsulfuron have been registered for use in turfgrass for many years, their primary use was not for annual bluegrass control. Bispyribac sodium, foramsulfuron, rimsulfuron, sulfosulfuron, and trifloxysulfuron inhibit the acetolactate synthase (ALS) enzyme and are labeled to control or suppress annual bluegrass in turfgrass. Annual bluegrass populations resistant to other classes of herbicide chemistry have been reported under selection pressure. Sequencing the ALS gene in annual bluegrass gives a backbone to future studies examining resistant populations that may develop. To accomplish this, genomic DNA was extracted from annual bluegrass and then amplified using polymerase chain reaction (PCR). Custom primers were designed based on sequence information from barley, corn, downy brome, Italian ryegrass, and rice. The PCR amplicons were cloned into an *E. coli* vector, and subsequently extracted and sequenced. Amino acid sequences derived from DNA sequence data were greater than 87% similar to species used as primers.

Nomenclature: Bispyribac sodium, chlorsulfuron, foramsulfuron, halosulfuron, imazaquin, metsulfuron, rimsulfuron, sulfosulfuron, and trifloxysulfuron; annual bluegrass, *Poa annua* L. #[1] POAAN; downy brome, *Bromus tectorum* L. # BROTE; barley, *Hordeum vulgare* L; corn, *Zea mays* L.; Italian ryegrass, *Lolium multiflorum* LAM.; rice, *Oryza sativa* L..

Additional index words: Herbicide resistance, weed control.

[1] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

Abbreviations: ALS, acetolactate synthase; BLAST basic local alignment sequencing tool; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA.

This chapter is formatted for publication in Weed Technology.

INTRODUCTION

Herbicides that inhibit acetolactate synthase (ALS) have been registered for use in turfgrass since 1988 (Gaul and Christians 1988; Larocque and Christians 1985; US EPA Pesticide Product Label System). Acetolactate synthase-inhibiting herbicides will selectively control annual bluegrass in warm-season turfgrass, such as bermudagrass. These herbicides were first used in wheat (*Triticum aestivum* L.), corn, and soybean (*Glycine max* (L.) Merr.), and resistant weeds were discovered in these crops within five years of herbicide use (Primiani et al. 1990). Currently, 83 weed species are resistant to ALS-inhibiting herbicides (Heap 1997, 2004).

Herbicide resistance can develop from a mutation in gene sequence that codes for a different amino acid in the target enzyme (Devine et al. 1993). This change leads to reduced or no herbicide binding in resistant plants. Ultimately, resistant plant populations will replace susceptible populations after continued use of the herbicide.

Phenological and morphological variation abounds in annual bluegrass (Wu et al. 1992). Currently, annual bluegrass populations resistant to ALS-inhibiting herbicides have not been reported. Annual bluegrass populations resistant to triazine, bipyridilium, thiocarbamate, urea, ethofumesate, and dinitroaniline herbicides have been reported (Bulcke and Callens

1998; Dyer and Bowman 1986; Heap 1997, 2004; Himme et al. 1984; Kelly et al. 1999; Mengistu et al. 2000; Struve et al. 1987; Vaughn and Gasquez 1987).

As of 2004, nine ALS-inhibiting herbicides were marketed for use in turf in the United States. While chlorsulfuron, halosulfuron, imazaquin, and metsulfuron have been registered for use in turfgrass for many years, their primary use was not for annual bluegrass control. Foramsulfuron, trifloxysulfuron, rimsulfuron, bispyribac sodium, and sulfosulfuron were first used in turfgrass in the past three years, and are labeled for annual bluegrass control or suppression. As such, the selection pressure for developing annual bluegrass populations resistant to ALS-inhibiting herbicides has increased.

Heap (1997) reported that new cases of weeds resistant to ALS-inhibiting herbicides have increased faster than any other herbicide group. Sequencing the ALS gene in annual bluegrass is the first step in studies to determine the likelihood and consequences of resistant annual bluegrass populations in turfgrass areas. To date annual bluegrass populations resistant to ALS-inhibiting herbicides have not been discovered and the gene coding for ALS production in annual bluegrass has not been sequenced. The objective of this study was to determine the nucleotide and derived amino acid sequence of the ALS gene in annual bluegrass.

MATERIALS AND METHODS

Genomic DNA was extracted from one annual bluegrass plant that was collected from an area with no known history of ALS-herbicide use following the DNeasy Plant Mini Kit

Handbook[2]. Samples were then frozen at –20 C until used in polymerase chain reaction (PCR). A search was conducted on GenBank[3] to find ALS sequences of rice (GI:12082314), corn (GI:22138; GI:22140), barley (GI:3075507), annual ryegrass (GI:11120575), and downy brome (GI:19698559). Conserved regions between species were identified using Sequencher[4] alignment software. Custom primers were designed and synthesized based on the consensus sequence of related species. Primers used were (5` GTCATCACCAACCACCTCTTC 3`) and (5` AAAATCTGGATATATCTCACTCTCA 3`). The PCR was preformed in 25-µl reaction volume with 1.5 mM 10x buffer, 200 µM dNTPs, 0.1µM forward primer, 0.1 µM reverse primer, 0.063 µl Taq polymerase, 0.5 µg template DNA. The reaction was set up for 95 C for two minutes, followed by 40 cycles of 95 C for one minute, 50 C for one minute, and 72 C for one minute. The final extension was 72 C for two minutes, thirty seconds. All plant ALS genes sequenced to date have lacked introns, and therefore sequencing of cDNA will not be necessary since DNA sequences will contain needed information to create amino acid sequence (Mazur et al. 1987; Fang et al. 1992).

The ALS gene was amplified from annual bluegrass genomic DNA isolates using polymerase chain reaction (PCR). The PCR product was then cloned into an *E. coli* DNA topoisomerase cloning vector; *E.Coli* was subsequently transformed and cultured on petri plates containing LB agar with 50 µg/ml of ampicillin. Plasmid DNA from the plates was

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[2] DNeasy Plant Mini Kit Handbook™. 2000. Qiagen Inc. 28159 Avenue Statford; Valencia, CA 91355-1106.

[3] NCBI website. 2004. Available at (http://www.ncbi.nlm.nih.gov/). National Center for Biotechnology Information, National Library of Medicine Building 38A, Bethesda, MD 20894.

[4] Sequencher™. 2003. Available at (http://www.genecodes.com/) Gene Codes Corporation, 775 Technology Drive, Suite 100A, Ann Arbor, Michigan 48108 USA.

extracted via the Qiagen Spin Miniprep Kit[5], and the ALS DNA insert was sequenced with T7 and T3 primers using sequencing chemistry[6]. Nucleotide sequences were obtained using on a genetic analyzer[7] at the Core Laboratory Facility at Virginia Bioinformatics Institute at Virginia Tech.

Basic local alignment sequencing tool (BLAST)[8] searches were conducted using the sequence data, identifying strong similarity (E-score 0.0) between annual bluegrass ALS sequences and known ALS data from other plant species. Biology Workbench[9], Chromas[10], Lasergene[11], and Sequencher software were utilized for sequence analysis. Biology Workbench was also utilized to deduce the amino acid translation from DNA sequence data.

RESULTS AND DISCUSSION

The first 1460 bases and corresponding amino acid sequences (486) of the annual bluegrass ALS gene are listed in Figure 1. The known start codons and known domains of

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[5] Qiagen Spin Miniprep Kit™. Qiagen Inc. 28159 Avenue Statford; Valencia, CA 91355-1106.

[6] Applied Biosystems BigDye 3.1. Applied Biosystems. 850 Lincoln Centre Drive, Foster City, CA 94404.

[7] Applied Biosystems 3700 DNA Analyzer. Applied Biosystems. 850 Lincoln Centre Drive, Foster City, CA 94404.

[8] NCBI website. 2004. Available at (http://www.ncbi.nlm.nih.gov/BLAST/). National Center for Biotechnology Information, National Library of Medicine Building 38A, Bethesda, MD 20894.

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[10] Chromas 1.45. 1996. Griffith University, Queensland, Australia. Latest version can be found on http://www.technelysium.com.au/chromas.html.

[11] Lasergene. 2004. Available at (http://www.dnastar.com/). DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715.

resistance to ALS inhibiting herbicides are marked in Figure 1 as well (Wright et al. 1998). Our sequence did not extend to the stop codon nor include the fifth domain of resistance. Annual bluegrass ALS amino acid sequence was 89, 89, 89, 88, 88, and 87% similar to Italian ryegrass, wheat, barley, downy brome, rice, and corn, respectively (Figure 2). The aforementioned species are the only grasses listed on the NCBI database with known ALS nucleotide sequences. By comparison, annual bluegrass was 81% similar to redrood pigweed (*Amaranthus retroflexus* L.), mouse-ear cress (*Arabidopsis thaliana* (L.) Heynh.), and wild mustard (*Raphanus raphanistrum* L.). In addition to the six grasses and three broadleaf species mentioned above, thirteen other broadleaf plants and five primitive organisms were also discovered on an NCBI search of ALS genomes.

As of 2004, ten grass species were reported to be resistant to ALS-inhibiting herbicides (Heap 2004). Saari et al (1992) reported perennial ryegrass biotypes were less sensitive to inhibition by ALS-inhibiting herbicides. Preston and Powles (2002) reported that the frequency of resistant Wimmera ryegrass *(Lolium rigidum* Gaudin) individuals to sulfometuron-methyl varied from 2.2×10^{-5} to 1.2×10^{-4}. Sweeney and Danneberger (1995) using RAPD markers, discovered differences in annual bluegrass populations on greens and fairways. With the morphological differences in annual bluegrass (Wu et al. 1992) and increasing selection pressure through use of ALS-inhibiting herbicides, annual bluegrass resistant to these herbicides will likely occur within the next few years. The information attained in this study will be used in future studies to predict the likelihood of developing annual bluegrass populations resistant to ALS-inhibiting herbicides and methods to prolong or prevent resistance development.

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Figure 1. Annual bluegrass ALS genomic DNA (B.) and corresponding amino acid sequence (A.). Start codons are in italics and domains of known amino acid changes are listed in bold.

A. M I S G R E **F A L** V I T N H L F R H E Q

B. *atg*attagcggccgcga**attcgccct**tgtcatcaccaaccacctcttccggcacgagcag 61

A. G E A F A A S G Y A R A S G R V G V C V

B. ggggaggcgttcgccgcgtccgggtacgcccgcgcctccggccgcgtcggggtctgcgtc 121

A. A T S G P G A T N L V S A L A D A L L D

B. gccacctccggccccggcgccaccaacctcgtctccgcgctcgccgacgctctgctcgac 181

A. S I P M V **A I T G Q V *P* R R M I G** T D ***A***

B. tccatcccgatggt**cgccatcacggggcaggtcccgcgccgcatgatcggcac**gga**cgcc** 241

A. **F Q E T P** I V E V T R S I T K H N Y L V

B. **ttccaggagacgcc**gatcgtggaggtcacccgttccatcaccaagcacaattacctggtc 301

A. L D V E D I P R V I Q E A F F L A S S G

B. cttgacgtggaggacatcccccgcgtcattcaggaagccttcttcctcgcctcctccggc 361

A. R P G P V L V D I P K D I Q Q Q M A V P

B. cggccggggccggtgctggtcgacatccccaaggacatccagcagcagatggccgtgcct 421

A. V W D A P M S L P G Y I A R L P K P P A

B. gtctgggacgcgccaatgagtctgccagggtacattgctcgcctccctaagccgccggct 481

A. T E L L E Q V L R L V G E A R R P I L Y

B. accgaattgcttgagcaggtcctgcgtctggttggtgaggctcggcgcccaattctgtat 541

A. V G G G C S A S G E E L R R F V E L T G

B. gttggtggtggctgctctgcgtccggcgaggagttgcgccgctttgttgagctcactggg 601

A. I P V T T T L M G L G N F P S D D P L S

B. atcccagtgacaactaccctcatgggtcttggcaacttccccagcgatgacccactgtct 661

A. L R M L G M H G T V Y A N Y X V D K A D

B. ctgcgtatgcttgggatgcatggtacagtctacgccaattacgcvgtagataaggctgac 721

A. L L L A F G V R F D D R V T G K I E A F

B. ctgctgcttgcatttggtgtgcggtttgatgaccgtgtgactggaaaaatagaggctttt 781

A. A S R S K I V H I D I D P A E I G K N K

B. gcaagcaggtccaagattgtgcacattgacattgatccagctgagattggcaagaacaag 841

A. Q P H V S I C A D V K I A L E G L N S L

B. cagccacacgtctccatttgtgcagatgtcaagatcgctttggagggcttgaattctctt 901

A. L L N G S K T H K S L D F S S W H E E L

B. ctgctaaatgggagcaaaacacacaagagtttagattttagttcgtggcatgaggagttg 961

A. D Q Q K R E F P L G F K T F G E A I P P

B. gaccagcagaagagggagtttcctctgggattcaaaacttttggtgaggcgatcccacca 1021

A. Q Y A I Q V L D E L T K G E A I I A T G

B. caatatgctatccaggtactggatgagctgaccaaaggggaggcgatcattgccactggt 1081

A. V G Q H Q M W A A Q Y Y T Y K R P R Q W

B. gttgggcagcaccagatgtgggcggctcagtattacacgtacaagcggccacgtcagtgg 1141

A. L S S A G L G A M G F G L P A A A G A A

B. ctgtcttcggctggtcttggagcaatggggtttgggttgccagctgcagctggtgctgct 1201

A. V A N P G V T V V D I D G D G S F L M N

B. gtggccaacccaggtgtcacagttgttgacattgatggagatggtagcttcctcatgaat 1261

A. I Q E L A L I R I E N L P V K V M I L N

B. attcaggagttggcactgattcgtattgagaacctccctgttaaggtgatgatactgaac 1321

A. N Q H L G M V V **Q *W* E D** R F Y K A N R A

B. aaccaacatctgggaatggtggt**gcagtgggagga**caggttttacaaggccaatcgggcg 1381

A. H T Y L G N P E N E S E I Y P D F K G E

B. cacacttaccttgggaacccagaaaatgagagtgagatatatccagattttaagggcgaa 1441

A. F V K P A D

B. ttcgttaaacctgcagatg 1460

Figure 2. Comparison of ALS amino acid sequences of annual bluegrass, corn, Italian ryegrass, rice, wheat, downy brome, and barley. Conserved sequences across species are indicated with a space, sequences that do not match annual bluegrass are listed in bold.

```
Annual bluegrass      ---------------------------------------------
Ryegrass            MATATSTAVAFSGATATLPKPRTLPRHLLPSSRRALAAPIRCSAVSPS
Downy brome ----------------------------------------------------
Barley        ------------------------------------------------
Wheat          -  ----------------------------------------AASPAA
Rice             MATTAAAAAAALSAAATAKTGRKNHQRHHVLPARGRVGAAAVRCSAVSPV
Corn 108           MATAAAASTALTGATTAAPKAR--RRAHLLATRRALAAPIRCSAASPA
Corn 109         --MATAATAAAALTGATTATPKSR--RRAHHLATRRALAAPIRCSALSRA


Annual bluegrass-----  ----------------------------------------M
Ryegrass         -  -SPAPPATALRPWGPSEPRKGADILVEALERCGISDVFAYPGGASMEI
Downy brome     ---  -------LRPWGPSEPRKGADILVEALERCGIVDVFAYPGGASMEI
Barley              ---------------------------------------------
Wheat             T--SVAPPATALRPWGPSEPRKGADILVEALERCGIVDVFAYPGGASMEI
Rice              TPPSPAPPATPLRPWGPAEPRKGADILVEALERCGVSDVFAYPGGASMEI
Corn 108          MP--MAPPATPLRPWGPTDPRKGADILVESLERCGVRDVFAYPGGASMEI
Corn 109          TP--TAPPATPLRPWGPNEPRKGSDILVEALERCGVRDVFAYPGGASMEI


Annual bluegrass ISGREFALVITNHLFRHEQGEAFAASGYARASGRVGVCVATSGPGATNLV
Ryegrass         HQALTSSPL
Downy brome      HQALTRSP           V
Barley             -LTRSP
Wheat            HQALTRSP
Rice             HQALTRSP
Corn 108         HQALTRSP  A                  S       I
Corn 109         HQALTRSP  A                  S       I


Annual bluegrass SALADALLDSIPMVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVL
Ryegrass                           Q
Downy brome
Barley
Wheat
Rice                     V
Corn 108                 V
Corn 109                 V


Annual bluegrass DVEDIPRVIQEAFFLASSGRPGPVLVDIPKDIQQQMAVPVWDAPMSLPGY
Ryegrass
Downy brome                                              A
Barley                                                     T
Wheat                                                      T
Rice                                                       TS N
Corn 108           D     V                                 K
Corn 109           D     V                               A  T
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Annual bluegrass IARLPKPPATELLEQVLRLVGEARRPILYVGGGCSASGEELRRFVELTGI
Ryegrass                                E              DV
Downy brome                              K          A
Barley                                              A
Wheat                                   S           A
Rice                                    S               D   W
Corn 108                                S   V       A
Corn 109                    F           S   V       A       C

Annual bluegrass PVTTTLMGLGNFPSDDPLSLRMLGMHGTVYANYXVDKADLLLAFGVRFDD
Ryegrass                                            A
Downy brome                                         A
Barley                                              A
Wheat                                               A
Rice                                                A
Corn 108                                            A           L
Corn 109                                            A

Annual bluegrass RVTGKIEAFASRSKIVHIDIDPAEIGKNKQPHVSICADVKIALEGLNSLL
Ryegrass                                                    L      -AV
Downy brome                                                 L      -D
Barley                                                      L      -G
Wheat                                                       L      -A
Rice                        A                               L      -A
Corn 108                    A    V                          L      -A
Corn 109                  G A                               L      -T

Annual bluegrass LNGSKTHKSLDFSSWHEELDQQKREFPLGFKTFGEAIPPQYAIQVLDELT
Ryegrass          T   CD  F      D  E         Y
Downy brome           AQ     GP Q   E    T    Y
Barley            S   AQQG   GP  K            Y
Wheat                 AQQG   GP  K
Rice              QQ T KT S   A  N            Y     E
Corn 108          E  TSK  F  G                 Y  SNEE Q
Corn 109          E  TSK  F  G   D             Y IFNEE

Annual bluegrass KGEAIIATGVGQHQMWAAQYYTYKRPRQWLSSAGLGAMGFGLPAAAGAAV
Ryegrass                                    V                   T
Downy brome                          S                          S
Barley                                          S               S
Wheat                                           S
Rice                                                            S
Corn 108              G                                         S
Corn 109

Annual bluegrass ANPGVTVVDIDGDGSFLMNIQELALIRIENLPVKVMILNNQHLGMVVQWE
Ryegrass
Downy brome
Barley
Wheat
Rice                                                 V          L
Corn 108                          V    M           FV
Corn 109                          I    M           FV
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Annual bluegrass DRFYKANRAHTYLGNPENESEIYPDFK---GEFVKPAD------------
Ryegrass                                    VTIAKG NV  VRVTKRSEVRAAI
Downy brome                                 VTIAKG NV  VRVTKKSEVRAAI
Barley                                      VTIAKG NV  VRVTKKSEVSAAI
Wheat                                       VTIAKG NV  VRVTKKSEVTAAI
Rice                                        VTIAKG NI  VRVTKKSEVRAAI
Corn 108                                    VTIAKG NI  VRVTKKNEVRAAI
Corn 109                  F                 VAIAKG NI  VRVTKKSEVHAAI

Annual bluegrass      -------------------------------------------
Ryegrass              KKMLETPGPYLLDIIVPHQEHVLPMIPSGGAFKDIIMEGDGRISY
Downy brome           QKMLDTPGPYLLDIIVPHQEHVLPMIPSGGAFKDIIMEGDGRIEY
Barley                KKMLETPGPYLLDIIVPHQEHVLPMIPSGGAFKDMIMEGDGRTSY
Wheat                 KKMLETPGPYLLDIIVPHQEHVLPMIPNGGAFKDMIMEGDGRTSY
Rice                  KKMLETPGPYLLDIIVPHQEHVLPMIPIGGAFKDMILDGDGRTVY
Corn 108              KKMLETPGPYLLDIIVPHQEHVLPMIPSGGAFKDMILDGDGRTVY
Corn 109              KKMLEAPGPYLLDIIVPHQEHVLPMIPSGGAFKDMILDGDGRTVY
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Vita

Joshua Bart Beam was born on April 25, 1977 to Charles and Jackie Beam. He grew up and worked on a small family farm in Delight, NC. He accepted Jesus Christ as his Lord and savior at age 8. He graduated from Burns High School in 1995 and received numerous scholarships to attend North Carolina State University (NCSU) in Raleigh, NC. He was very active in intramural football and basketball while at NCSU. He worked for the USDA-Natural Resources Conservation Service during the summers of 1997 and 1998, while continuing his work on the family farm. He graduated from NCSU in 1999 with a B.S. degree in Agronomy Soil Science Concentration. He continued his education at NCSU, and received a Master of Science degree in the Department of Crop Science under the direction of Drs. David Jordan and Alan York in May 2001. He received the Outstanding Graduate Master's Student award in the Crop Science Department at NCSU in 2001. After leaving N. C. State, he then worked at BASF Corporation for the summer of 2001. In August 2001, he continued his education at Virginia Polytechnic Institute and State University and is pursing a Doctor of Philosophy degree under the direction of Dr. Shawn Askew. While at Virginia Tech he has worked in the Weed Clinic and received the Virginia Crop Protection Association scholarship. He was married to Gay Cashwell on November 22, 2003. They have been happily married ever since.