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THE EFFECT OF TURFGRASS GROWTH RETARDANTS ON  
PHOTOSYNTHESIS, PIGMENT CONTENT, AND DISCOLORATION  
OF KENTUCKY BLUEGRASS (*Poa pratensis* L.)

A Thesis Presented

by

LESLEY A. SPOKAS

Submitted to the Graduate School of the  
University of Massachusetts in partial fulfillment  
of the requirements for the degree of

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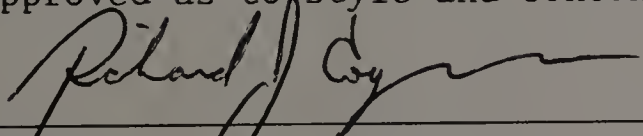
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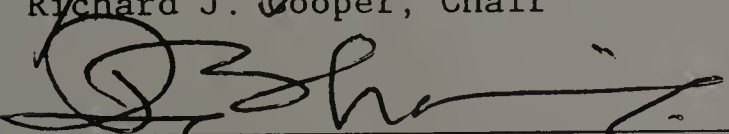
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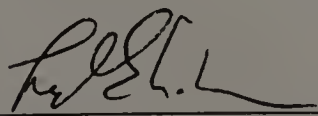
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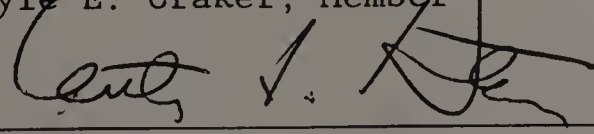
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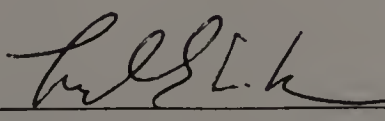
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## DEDICATION

I dedicate this manuscript, and the accomplishment that it represents to the three most important people in my life.

To Bo, friend and partner, strongest supporter, closest confidant;

To Eric and Melissa, my hopes for the future;

for your love, understanding, and faith in me,

thank you.

## ACKNOWLEDGEMENTS

I would like to extend my sincere appreciation to the following people without whose encouragement and support this would never have come to pass.

Dr. Richard Cooper, friend and major professor, for the support and encouragement from the beginning; for always being there when I needed to discuss a problem, and for giving me the freedom to accomplish the task.

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## CHAPTER 1

### INTRODUCTION

Reduction of turfgrass growth using plant growth regulating (PGR) compounds has been the subject of research for more than 40 years (14). The majority of these studies have been efficacy trials, which have demonstrated the amount and duration of growth suppression (6,8,32). Many studies have noted an objectionable level of turf discoloration associated with PGR use (6,19,32). This discoloration has relegated the use of these potentially valuable chemicals to low quality turf areas, where high visual quality is not essential.

The discoloration associated with PGR treated turf may be due to increased disease incidence (20,36). Although there does appear to be a correlation between increased disease activity and the use of certain PGRs, other studies have shown discoloration to exist in the absence of disease activity (6,8). Additionally, Cooper et al. (9) reported that discoloration occurred on PGR treated annual bluegrass (*Poa annua* L.) even when disease was prevented by use of fungicides.

Another explanation offered for the discoloration associated with PGR use is that injured and otherwise non-green plant material is visible due to the inability of treated turf to produce sufficient new growth to replace naturally senescing leaves (1). If this were the sole cause of discoloration, discoloration would be expected to last for the duration of growth suppression and would probably become more severe with time. To the contrary, discoloration tends to subside after several weeks, while growth suppression continues (7).

The use of PGRs has many effects on the development and architecture of a turfgrass canopy. The stand remains unmown, therefore increasing the quantity of both living and dead tissue present. Additional stand changes which have been reported in response to various compounds include: reduced rhizome growth (41), thinning (11), reduced tillering (13), and increased turf density and stimulated tillering (7). Changes in the physical structure of the canopy affect the microenvironment of individual leaves. Light quantity, spectral distribution, and relative humidity levels of an unmown sward, such as a PGR treated turf, are different than those of a mown stand. PGR treatment also results in a greater portion of the stand being comprised of mature and senescing leaves (37). Independent of PGR treatment, these types of changes in canopy architecture, microenvironment, and mean leaf age, have been shown to affect the apparent photosynthetic rate of grass swards (21,28,30,38,39). There is, however, limited research investigating photosynthetic changes resulting from PGR treatment.

If PGRs are to be accepted for use on medium to high quality turf, discoloration must be reduced to an acceptable level. The first step in reducing discoloration is to determine how PGR's induce discoloration. These studies were undertaken to trace the developmental sequence of discoloration and growth suppression in PGR treated Kentucky bluegrass (*Poa pratensis* L.) and to determine whether a relationship exists between PGR induced discoloration and either photosynthetic rate or pigment content.

## CHAPTER 2

### LITERATURE REVIEW

Mowing of turf is necessary to maintain utility for most applications. Mowing frequencies range from once or twice per year for low quality areas such as roadside turf to daily for high quality low cut areas such as golf greens. Mowing, however, is both expensive and physiologically detrimental to the grass plant. On average, the expense of mowing has been estimated to be approximately \$12.00 per ha per mowing (1). The reduction of leaf area by mowing reduces the photosynthetic capacity of the plant, leading to a reduction in carbohydrate production (35) and storage, sometimes leading to a temporary stoppage of root growth. Reduced root growth is accompanied by a decrease in water uptake, while cut blade ends increase transpiration (35).

Efforts to overcome the expense and detrimental effects of mowing have led to the development of various plant growth regulating (PGR) compounds capable of reducing mowing requirements. Numerous compounds have been evaluated for efficacy during the past 40 years (8,11,12,19,32). The growth regulating effects of maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) (MH) were first described in 1949 by Schoene (33) who reported a total cessation of growth lasting up to six weeks. MH applied to Kentucky bluegrass, colonial bentgrass (*Agrostis tenuis* Sibth.), perennial ryegrass (*Lolium perenne* L.) and redtop (*Agrostis alba* L.) does, however, cause discoloration and reduction in turf density (14). Similar growth regulation and discoloration has been

noted with use of chloroflurenol (2,7-dichloro-9-hydroxyfluorene-9-carboxylic acid) (14).

The potential benefits of these early PGRs led to the development and screening of many compounds for growth regulating activity during the 1970's. Elkins et al. (12) reported a series of experiments using MH, chloroflurenol, and several experimental compounds concluding that these PGRs exhibited greater discoloration on intensively managed 'Merion' Kentucky bluegrass than on low to moderate quality turf of the same cultivar. Jagschitz (19) tested several other experimental compounds as well as MH, reporting that those compounds demonstrated to be most effective at suppressing growth also caused unacceptable discoloration of the turf. Turf quality is rated on a scale of 1 to 9 with 1 = brown turf, 6 = average turf, and 9 = ideal turf. Quality components are color, uniformity, density, texture, growth habit and smoothness. Unacceptable discoloration would result in turf quality of less than 6.

The search for growth regulators which provide effective growth suppression while maintaining acceptable visual quality has produced relatively few marketable compounds. Amidochlor, (N-[(acetylamino)methyl]-2-chloro-N-2,6(diethylphenyl)acetamide), and mefluidide, [N-(2,4-dimethyl-5-[(trifluoromethyl)sulfonyl]amino)phenyl)acetamide], are two PGRs commercially available for use on turf for several years. Both of these compounds are mitotic inhibitors, retarding plant growth by interrupting cell division in the apical meristem (37). Amidochlor is absorbed primarily by the roots of mature grass plants (2), suppressing growth of Kentucky bluegrass, creeping red fescue

(*Festuca rubra* L. ssp. *rubra*), perennial ryegrass and tall fescue (*Festuca arundinacea* Shreb.) for approximately 6 weeks when applied at rates ranging from 1.68 to 3.36 kg a.i. ha<sup>-1</sup> (5,32). Mefluidide, in contrast, is foliarly absorbed and remains primarily within the leaf onto which it is applied (1,26). Secondary apical meristem activity is inhibited as the compound does not appear to translocate basipetally. Duration of growth suppression of Kentucky bluegrass treated with mefluidide at 0.14 or 0.28 kg a.i. ha<sup>-1</sup> ranges from 6 to 12 weeks (14,32).

Research has consistently demonstrated significant discoloration following mefluidide application to commonly used turfgrass species. Cooper et al. (10) reported that discoloration of annual bluegrass treated with 0.07 to 0.28 kg a.i. ha<sup>-1</sup> became visible 7 to 8 days after treatment (DAT) for all rates and lasted 2 to 3 weeks. Bhowmik (6) reported 24% injury (based on 0 = no injury; 100 = dead turf) in a Kentucky bluegrass (cv Baron) - red fescue (cv Pennfine) stand, accompanied by a drop in turf quality from 9 to 7.5. Christians (8) reported that although mefluidide applied at 0.28 and 0.56 kg a.i. ha<sup>-1</sup> reduced the quality of treated Kentucky bluegrass compared to nontreated plots overall turf quality was commercially acceptable. Pennucci and Jagschitz (32), applied mefluidide at 0.14 and 0.28 kg a.i. ha<sup>-1</sup> to Kentucky bluegrass, red fescue, tall fescue, and perennial ryegrass and reported no discoloration to any species during the first 28 DAT. Injury for all species was less than 2.0 (based on a scale of 1 - 10 where 10 = brown turf) 4-8 weeks after treatment. Mefluidide treated

turf has been reported to be darker green, seedhead free, and of better overall quality than nontreated turf once discoloration has dissipated (9,34,37).

Dernoden (11) evaluated April and June applications of mefluidide at 0.28 and 0.56 kg a.i. ha<sup>-1</sup> to Kentucky bluegrass for four successive years. Mefluidide treated plots consistently exhibited fair (0.28 kg a.i. ha<sup>-1</sup>) to poor (0.56 kg a.i. ha<sup>-1</sup>) color, with some loss of density at the higher rate (11). While acceptable color normally returned by August, overall quality continued to be unacceptable until October due to severe infestation by crabgrass (*Digitaria* ssp.) (11). Although mefluidide treated turf visually appeared less dense during the growing season, no differences in tiller number or leaf number existed between mefluidide treated and nontreated turf when samples were harvested during the early spring of the fifth year (11).

Dernoden's (11) observations on the effect of mefluidide on tillers are consistent with those of other researchers. Christians (8) determined that mefluidide applications of 0.28 and 0.56 kg a.i. ha<sup>-1</sup> to Kentucky bluegrass neither stimulated nor reduced rhizome development. Freeborg and Daniel (14) reported no difference in tiller number caused by mefluidide application at 0.28 kg a.i. ha<sup>-1</sup> to greenhouse grown 'Wabash' Kentucky bluegrass during the first year of their study. An unexplained significant reduction in the number of tillers, however, did occur during the second year of the study (14).

Amidochlor application to fine turf has been studied less than mefluidide. Pennucci and Jagshitz (32) reported that amidochlor applied at rates of 1.68 to 3.36 kg a.i. ha<sup>-1</sup> reduced growth for 5 to 6 weeks on

the four cool-season grasses tested regardless of application rate, with little to no discoloration during the first 4 weeks after treatment. Amidochlor caused significant injury to red fescue, perennial ryegrass and tall fescue 4 to 8 weeks after application. Amidochlor induced injury to Kentucky bluegrass was moderate (25 to 30%) and not evident until 8 to 12 weeks after application. Bhowmik (6) evaluated amidochlor applications of 2.2, 2.8, and 3.4 kg a.i. ha<sup>-1</sup> to a Kentucky bluegrass (cv Baron) - red fescue (cv Pennfine) sod at 100% green-up during three successive years, reporting that average turf quality was excellent for 14 days after treatment (DAT), but declined significantly by 21 DAT. Additional studies by Bhowmik (5), revealed that turf height was reduced up to 28 DAT with amidochlor application, but vertical growth rate was accelerated 42 DAT.

While PGR induced growth suppression and discoloration have been investigated fairly thoroughly, effects on physiological processes including photosynthesis, are not extensive. One of the first papers to document physiological changes in grass plants associated with PGR use was that of Nelson et al. (30). These researchers reported that ancymidol (α-Cyclopropyl-α-[p-methoxyphenyl]-5-pyrimidine methanol) reduced the carbon dioxide exchange rate (CER) of both bermudagrass (*Cynodon dactylon* [L.] Pers.) and tall fescue when expressed on a surface area basis. When CER for these two species was expressed on a leaf area basis, however, no difference between treated and nontreated plants was observed. Decreased CER was associated with decreased top

weight, indicating that a reduction in quantity of photosynthetic tissue was responsible for the apparent decrease in photosynthesis.

In recent studies, Breuniger (7) demonstrated that flurprimidol (a-[1-methyl]-a-[4-(trifluoromethoxy)phenyl]-5-pyrimidine methanol) did not effect photosynthesis of greenhouse grown Kentucky bluegrass eight weeks after treatment. These plants had not developed any discoloration from the PGR application. Gaussoin et al. (15) reported that application of fluriprimidol at 2.24 kg a.i. ha<sup>-1</sup> to a mixed stand of annual bluegrass and creeping bentgrass (*Agrostis plaustris* Huds.) resulted in a 52% reduction in net photosynthesis for annual bluegrass and a 29% reduction in net photosynthesis for creeping bentgrass.

In addition to a possible direct effect on photosynthesis, PGRs induce a number of changes in canopy architecture which might indirectly affect photosynthesis. Studies of various grass species under normal growing conditions have been conducted. Jewiss and Woledge (21) noted that tall fescue photosynthesis increased slightly for approximately 10 days after full expansion of the leaf blade and then decreased rapidly until the leaf had senesced (21). Woledge (39) working with perennial ryegrass observed that the photosynthetic capacity or maximum APR (Apparent Photosynthetic Rate) for any leaf decreased as the canopy developed. Additionally, Ollerenshaw and Incoll (31) determined that secondary tillers developing in unmown swards had lower leaf APRs than established tillers in the same sward.

Woledge and Leafé (40) measured the photosynthetic rate of field grown perennial ryegrass plants during three growing periods: at the beginning of the growing season, following a mid-season harvest, and



following a late-season harvest. Canopy APR at the beginning of any of these growth periods was ascertained to equal individual leaf APR (40) because leaves were small and well illuminated. As total leaf area increased, individual leaf APR decreased, and canopy APR decreased. The decreased APR of both individual leaves and the canopy with increasing leaf growth was believed to be due to decreased light penetration and greater shading within the canopy. Woledge (38) reported that when photosynthetic measurements were made in bright light (2500 ft. candles), plants which had been grown in full sunlight had a greater APR than plants which had been grown in the shade. Conversely, when photosynthetic measurements were made at low light intensities (250 or 500 ft. candles) plants grown in the shade were determined to have greater APR than those grown in full sun. Morgan and Brown (29) suggested that shading of lower leaves by upper leaves in the canopy increased the effects of aging on photosynthetic rate.

Both Morgan and Brown (28) and Krans and Beard (23) suggested that mowing affected canopy photosynthesis. Morgan and Brown (28) worked with bermudagrass mown to a height of 6 cm weekly or monthly, measuring carbon dioxide exchange rate weekly. These researchers ascertained that swards with equal leaf area produced lower carbon dioxide exchange rates when mown weekly than when mown on a monthly basis. In contrast, Krans and Beard (23) observed that apparent photosynthesis per unit leaf area was greater in Kentucky bluegrass mowed every 3 to 4 days than in plants mowed every 7 or 14 days. Apparent photosynthesis per unit leaf area was also greater in plants mown to 2.5 cm than in plants mown to 6.2 cm (23). Turf mown either more frequently or to a lower height, such as

those in Krans and Beard's study (23), may have had a greater percentage of younger leaves and thus higher CER than infrequently mown turf. Morgan and Brown (28) attributed the lower CER of weekly mown swards to a lower percentage of live phytomass (77%), compared to the monthly mown sward (87%). An additional possibility for the difference in CER between the two swards may be the relative age of the leaves comprising the phytomass. In the weekly mown sward the leaves grew 'relatively prostrate' (28), thus most of the leaf tissue was below the mowing height and was not removed in mowing. Conversely, the monthly mown sward grew upright and 'virtually all' (28) of the leaves in the canopy were removed by mowing. When CER measurements were taken on equal leaf area for these two mowing regimes leaf tissue for the weekly mown sward was perhaps several weeks old while the monthly mown sward was comprised of relatively young leaves when CER measurements were taken.

## CHAPTER 3

### MATERIALS AND METHODS

#### Plant Material

Studies were conducted on a mature stand of 'Baron' Kentucky bluegrass growing on a Hadley silt loam soil (coarse silty, mixed, nonacid, mesic typic Udifluvent) at the University of Massachusetts Turfgrass Research Facility, South Deerfield, MA. Prior to use in these experiments the turf was maintained at a height of 5 cm with twice weekly mowings with a reel mower and fertilized at a rate of 2.24 kg N ha<sup>-1</sup> year<sup>-1</sup>. Pesticide applications were made as needed to maintain healthy turf. No supplemental irrigation was used. Following initiation of experiments, mown treatments were maintained at a height of 5 cm by mowing weekly with a reel mower. Turf in greenhouse experiments was watered daily, as needed to prevent drought stress. Turf transplanted to sand was fertilized weekly with half strength Hoagland's solution (17) to provide 0.56 kg N ha<sup>-1</sup>. Field experiments and greenhouse turf growing in silt loam received no fertilization during the course of the experiments. Field experiments were irrigated as needed to prevent wilt. No pesticides were used.

For initial greenhouse studies sod was harvested in the field, with soil removed as close to the crown/root interface as possible. The sod was then transplanted into 13 cm square plastic pots filled with quartz sand (50:50 [v:v] coarse to fine). Sod was grown in this manner to facilitate measurement of photosynthesis without interference from soil microbial respiration. Development of a steel box with dimensions comparable to the plastic pots allowed measurements to be made on sod

grown under conditions which more closely duplicated field conditions. Sod for subsequent greenhouse experiments was, therefore, harvested to a depth of 7.6 cm and transplanted into 30 x 40 x 7.6 cm wooden flats. Sod for all experiments was allowed to acclimate to greenhouse conditions for 4 weeks before experiments were initiated. Temperatures in the greenhouse during all experiments averaged  $\approx 21^{\circ}$  C, with daily fluctuations between 20 and  $32^{\circ}$  C. Daily humidity levels fluctuated between 30 and 70%. No supplemental lighting was used for growth.

#### Chemical Application

The growth regulators amidochlor and mefluidide were chosen for this research because they have similar modes of action (37), have been reported to be effective in suppressing growth (32), and characteristically produce different degrees of discoloration (32). Both materials are commercially available and are widely used. Amidochlor and mefluidide were applied at  $2.8 \text{ kg ai ha}^{-1}$  and  $0.56 \text{ kg ai ha}^{-1}$  respectively, the manufacturers' highest recommended rate. Applications were made using a  $\text{CO}_2$  powered backpack sprayer equipped with flat fan nozzles at a pressure of 207 kPa for greenhouse studies and at 152 kPa for field applications. Applications for all experiments were made in a carrier volume of  $600 \text{ L ha}^{-1}$ . Amidochlor applications were watered with 1.2 cm of water within 24 hours of application to ensure root absorption.

#### Developmental Sequence of Discoloration and Growth Suppression

Individual plant studies were conducted in the field during 1989 to document the developmental sequence of discoloration and duration of growth suppression. Four days after treatment application plugs (5 cm

in diameter and 8 cm in depth) were removed at random from each plot. Plugs were thinned to 2 - 3 tillers each. Primary tillers were chosen at random from the center of the plug. Plugs were then transplanted into 5 cm square plastic pots filled with soil, placed in a flat and left in the field. Blade length (collar to tip) and relative tiller position of each leaf were determined for all leaves on all tillers. The oldest green leaf of each tiller was designated leaf number 1, the next oldest leaf 2, and continuing in this manner for all visible leaves. Leaf length, number of lesions, and coloration were characterized weekly for blades present at time of treatment as well as leaves which developed after treatment.

Measurements of turf quality (color and density) were made weekly following treatment application. Turf quality ratings were based on a scale of 1 to 9 with 1 representing brown turf, 6 average turf, and 9 ideal turf. Ideal turf is deep green in color and has uniform texture and density with no weeds present. Time until onset of discoloration was noted and discoloration characterized for each experiment. Growth suppression was determined by weekly measurements of mean turf height prior to mowing. Changes in stand density were quantified by removing four plugs (5 cm in diameter) from each plot and counting the number of tillers per plug, as well as the number of green leaves per tiller. Leaves were considered green if more than 50% of the existing blade surface was green.

#### Measurement of Physiological Parameters

Pigments were assessed daily using mature blade tissue harvested at random within each treatment. Fresh samples were weighed immediately

and macerated with a mortar and pestle. Pigments were extracted with 80% (v/v) acetone to which a 'pinch' of magnesium carbonate ( $\text{MgCO}_3$ ) was added. Samples were maintained in darkness at  $5^\circ\text{C}$  until analysis. Pigment content was determined using a Coleman Model 124D double beam spectrophotometer (Coleman Instruments, Maywood, IL). Total chlorophyll content was determined using equation 1 (4) while total carotenoid content was determined using equation 2 (24).

$$\text{Total Chlorophyll } (\mu\text{g ml}^{-1}) = \text{Abs}_{663} \times 8.02 + \text{Abs}_{645} \times 20.2 \quad (1)$$

$$\text{Total Carotenoid } (\mu\text{g ml}^{-1}) = \text{Abs}_{475} \times 4.0 \quad (2)$$

Preliminary studies in the greenhouse determined that maximum photosynthetic capacity was reached at 0900 h and continued until 1500 h. Photosynthetic measurements were taken during this time period. Field measurements were taken only during the morning hours, however, usually between (0930 and 1100 h) before temperatures reached  $30^\circ\text{C}$ , at which point the rate of photorespiration interfered with measurement of photosynthesis. Photosynthetic measurements were made using a LI-COR portable photosynthesis system (Model LI-6000, LI-COR<sup>®</sup>, Inc., Lincoln, NE). For greenhouse studies, treated pots were placed 15 cm beneath a light bank consisting of four Sylvania 120 WER 40 light bulbs suspended above a 12 cm deep water bath (Fig. 3.1). Irradiance at canopy height averaged  $800 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$  during photosynthetic measurements. If ambient light levels in the greenhouse were lower than  $800 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ , samples were allowed to acclimate for five minutes under the lights to assure light saturation before photosynthetic rate was determined.

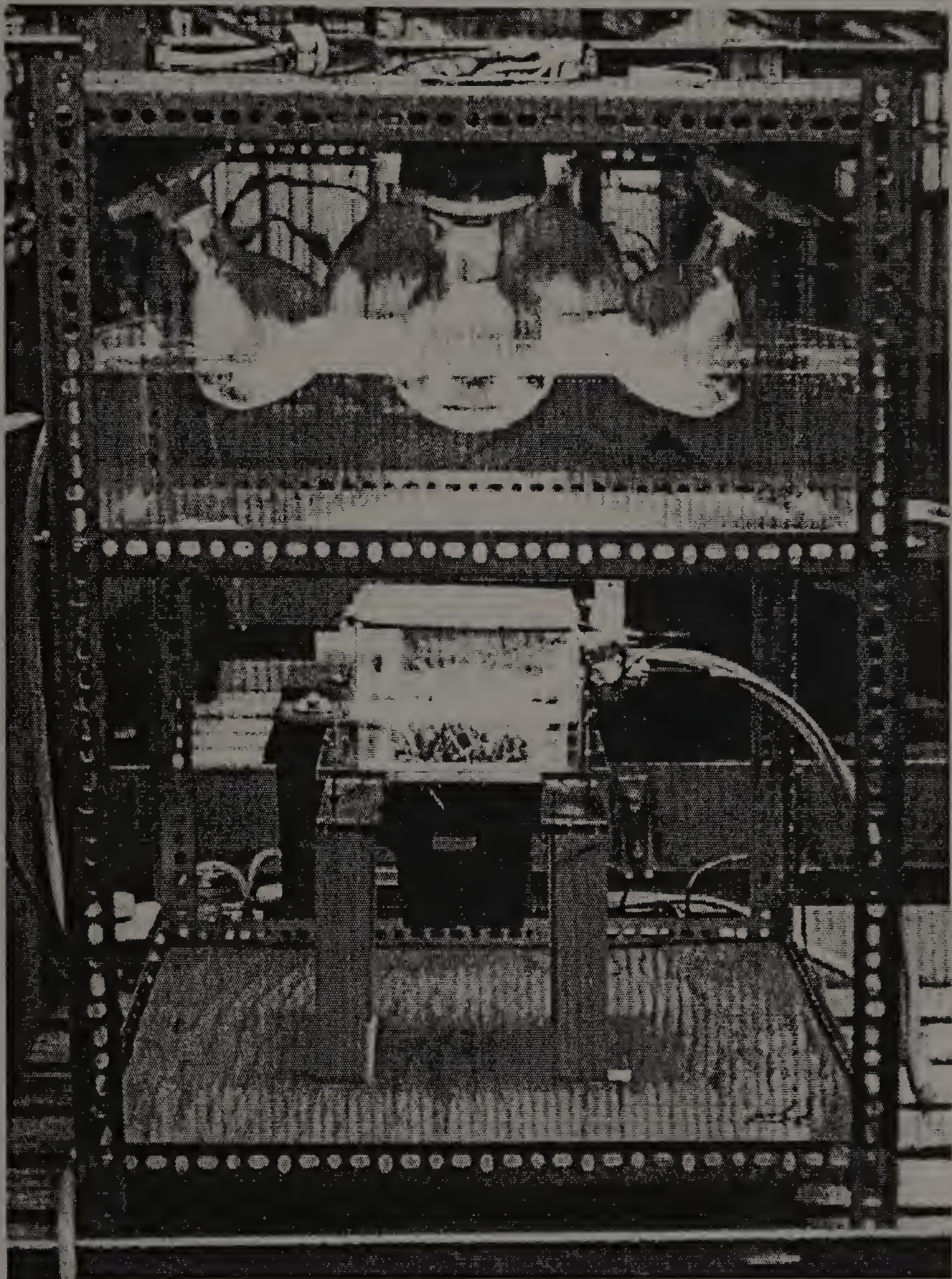


Figure 3.1. Light stand used in photosynthetic measurements; modified LI-COR<sup>®</sup> LI-6000 four liter chamber in use in the greenhouse.

Preliminary studies indicated that at  $800 \text{ } \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ , light was not a limiting factor in determining photosynthetic rate. Modification of the LI-6000 4 L chamber (Fig. 3.2) allowed measurement of  $1.7 \text{ dm}^2$  of turf canopy. The modification consisted of removal of the bottom half of the 4 L chamber and replacement with an open ended chamber which formed an airtight seal with the plastic pot. The new chamber bottom was constructed from MARGARD<sup>®</sup> (General Electric Co., Speciality Plastics Division, Pittsfield, MA.) and coated with teflon to prevent water adsorption. For all photosynthetic measurements of turf growing in soil, interference from soil and thatch respiration was eliminated by flooding. The bottom of the chamber was filled to a minimum depth of 2.5 cm standing water.

#### Experimental Design and Statistical Analysis

All experiments were carried out utilizing a randomized complete block design consisting of four replicates of each treatment. Field plots measured 1.0 by 3.0 meters. Greenhouse treatments were blocked on the greenhouse bench. All data were subjected to either an analysis of variance (ANOVA) or an analysis of covariance using PROC GLM of the SAS System (SAS Institute, Inc., 1988). Analysis of covariance was used when the continuous variable time was included in the analysis (appendix). When indicated by the ANOVA F test, means were separated by use of a pair-wise t test or Duncan's New Multiple Range test, as warranted by the comparisons being made.



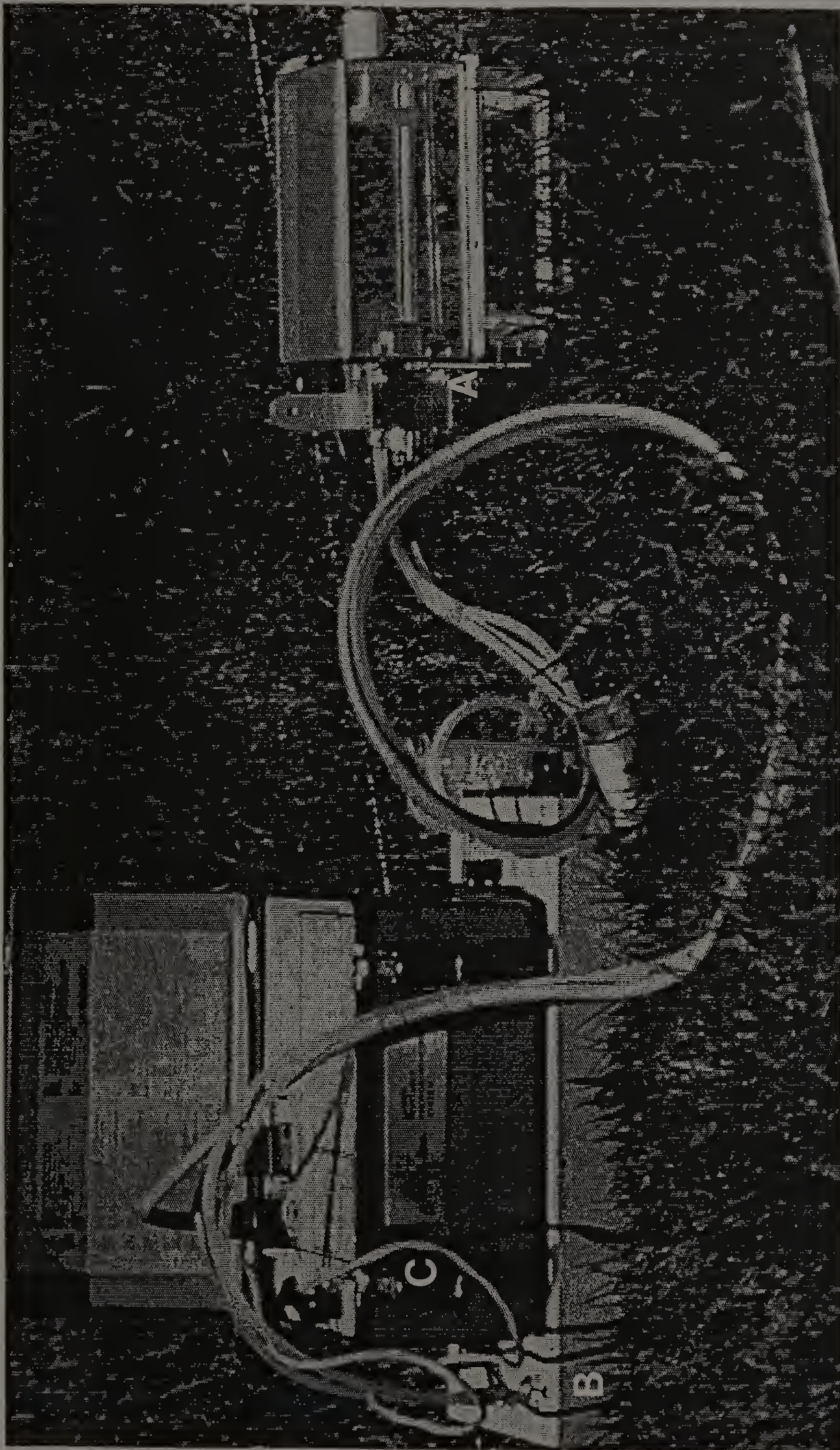


Figure 3.2. The modified LI-COR® LI-6000 portable photosynthesis system in use in the field, showing A) modified four liter chamber, B) CO<sub>2</sub> analyzer, and C) computer console.

## CHAPTER 4

### RESULTS AND DISCUSSION

#### Developmental Sequence of Discoloration

PGRs were applied to turf growing in the greenhouse on 28 May 1987. Discoloration became noticeable on mefluidide treated turf 9 days after treatment (DAT). Although no specific lesions or bleaching were noticed at this time, the turf appeared slightly chlorotic and lacking in vigor. By 14 DAT, discoloration had evolved into distinct lesions yellow-orange or reddish-brown in color, beginning at the leaf tip and progressing toward the base. Blades with cut ends were no more severely injured than whole blades. Discolored blades appeared withered. Approximately 50% of the blades of mefluidide treated plants were affected. Blades lacking distinct lesions appeared lighter green than nontreated blades. Many of the blades showing no discoloration appeared flaccid. New growth first became evident in mefluidide treated plants 35 DAT, being yellow-green in color and making the darker green of the existing blades more noticeable (Plate 1). Mefluidide treated plants had new growth visible in three of four pots at 41 DAT and all four had at least 50% new growth by 60 DAT.

Amidochlor treated plants exhibited a slight tip bleaching 14 DAT, with injury affecting from 10 to 30% of the leaf blades. Amidochlor is root absorbed (2), rather than foliarly absorbed as is mefluidide (1), and normally produces little discoloration. Tip bleaching was more severe on blades with cut ends than on blades with intact leaf tips. This higher incidence of discoloration on leaves with cut ends may have



Plate 1. Variation in color of 'Baron' Kentucky bluegrass 37 days after 28 April mefluidide application in the greenhouse. Arrows indicate (A) pale green new growth and (B) dark green treated blades.

been due to increased absorption of the compound directly into the leaf tissue through the cut blade end. Although two days had elapsed between the last mowing and amidochlor application the healed leaf tip remained a means of entry not available on intact blades. Amidochlor induced injury was less distinct at 21 DAT with approximately 5 - 10% of the blades still showing injury. Amidochlor treated turf had recovered acceptable turf quality by 29 DAT.

Field applications of mefluidide on 11 June 1987 failed to produce any discoloration (data not presented). Similar results were observed during a fall 1987 greenhouse experiment. A review of the conditions of both applications and consultations with others (18,27) suggested that mefluidide induced discoloration was related to flower initiation.

To investigate a possible relationship between stage of plant development and discoloration, an application of PGRs in the spring of 1988 was timed to coincide with 100% green-up (12 May), early inflorescence development (27 May), or emergence of inflorescences (18 June) of Kentucky bluegrass. Discoloration was most evident on the oldest blades of tillers, with early discoloration appearing as a yellowish cast in the understory. Nondiscolored blades of mefluidide treated plants were a darker green 21 DAT following the 12 May application, making discolored and diseased blades more noticeable. Mefluidide treated turf appeared less dense than nontreated or amidochlor treated turf regardless of application date.

Turf quality was significantly reduced by mefluidide application beginning 14 DAT when applied at greenup and 6 DAT when applied at the later developmental stages (Table 4.1). Following application at green-

Table 4.1. Quality of field grown 'Baron' Kentucky bluegrass in response to PGR application at varying developmental stages.

Treatment	Turf quality <sup>†</sup>						
	12 May (green-up)						
	Days after treatment						
	14	21	29	37	43	58	63
Mefluidide	3.5 c <sup>‡</sup>	2.5 c	2.5 c	4.0 b	5.0	5.6	6.0
Amidochlor	7.0 b	4.3 b	5.0 b	5.1 a	5.3	5.4	5.6
Nontreated (mown)	8.0 a	5.0 a	6.0 a	5.8 a	5.4	5.5	5.8
	**	**	**	**	NS	NS	NS
	27 May (early inflorescence development)						
	Days after treatment						
	6	14	22	28	36	43	52
Mefluidide	4.0 b	4.0 b	3.0 b	3.0 b	3.0 c	4.8 c	4.9 c
Amidochlor	4.3 a	6.0 a	6.1 a	5.9 a	5.4 b	5.4 b	5.4 b
Nontreated (mown)	4.9 a	6.0 a	6.0 a	6.0 a	6.0 a	6.0 a	6.0 a
	**	**	**	**	**	**	*
	18 June (inflorescences emerged)						
	Days after treatment						
	6	14	21	30	41		
Mefluidide	5.5 b	4.8 b	4.0 b	4.0 b	5.2		
Amidochlor	6.0 a	5.6 a	5.9 a	5.1 a	5.6		
Nontreated (mown)	6.1 a	5.6 a	6.0 a	5.8 a	5.8		
	**	*	**	**	**		NS

† Turf quality based on a rating of 1 to 9 (1 = brown or dead turf 9 = dark green, ideal turf).

‡ Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range test (0.05).

\* \*\* Significant at the 0.05 or 0.01 probability level, respectively. NS Nonsignificant (0.05).

up (12 May), discoloration persisted for 28 days with turf recovering acceptable quality by 43 DAT. Extended cool wet weather during this period resulted in severe red thread (*Laetisaria fuciformis* McAlp.) disease incidence on the experimental area. Disease injury observed on plants treated with mefluidide at 100% green-up is shown in Plate 2. The increased activity of the disease on PGR treated turf may have been due to a decreased recuperative potential induced by the growth regulator. Turf treated with mefluidide during early inflorescence development (27 May) did not recover acceptable quality for the duration of the study (Plate 3). Turf receiving mefluidide application following inflorescence emergence was discolored for 36 days, but, by 41 DAT had quality comparable to nontreated turf.

Amidochlor treated Kentucky bluegrass exhibited little to no discoloration regardless of developmental stage at time of application. Quality of amidochlor treated turf was significantly lower than nontreated turf 14 to 29 DAT following 12 May application (Table 4.1). The presence of seedheads detracted from overall turf quality following amidochlor application during early inflorescence development (27 May). Quality of amidochlor treated turf was comparable to nontreated turf following 18 June application. Amidochlor treated plants exhibited quality equal to or superior to mefluidide treated plants throughout the evaluation.

Mefluidide application prior to inflorescence development either in the greenhouse or at 100% green-up in the field resulted in significant discoloration lasting for approximately 5 weeks. Discoloration resulting from mefluidide application during early



Plate 2. Incidence of red thread on 'Baron' Kentucky bluegrass 19 days after 12 May mefluidide application. Arrow indicates disease.



Plate 3. Developmental sequence of discoloration on treated 'Baron' Kentucky bluegrass following 27 May mefluidide application [A) 6 DAT, B) 14 DAT, and C) 45 DAT].



inflorescence development was not as severe, but of greater duration. Persistence of discoloration might be expected as a result of inflorescence initiation diverting photosynthate away from the developing leaves. Once flower initiation occurs, leaf primordia growth is inhibited while axillary bud development is enhanced (25). This reversal of growth pattern persists until removal of the apical dome, either by inflorescence maturation and seed set, or by mowing. Application of mefluidide to turf once this reversal of growth pattern has occurred would effectively eliminate the plant's ability to produce new leaves, thus reducing the turf's ability to recover from discoloration. Mefluidide application after seedhead emergence resulted in little or no discoloration. Following seed set the growth pattern reverses again, the primary sink for photosynthate would revert to vegetative growth, such as development of new leaves and secondary tillers. Discoloration occurring following mefluidide application at this stage of development would be short lived, due to the renewed development of leaf primordia and the availability of sufficient carbohydrates to ensure recuperative potential.

Apparent loss of density was observed following mefluidide application both in the greenhouse and in the field. Other researchers (11) have reported a visible loss of density, with conflicting results as to effect on tiller number (7,11,13,14). In order to assess PGR effects on turf density, four plugs (5 cm in diameter) were removed from each plot 24 DAT following 18 June application. The number of tillers per plug and the number of green leaves per tiller were counted. Neither mefluidide nor amidochlor application affected the number of tillers per

plug (Table 4.2). Mefluidide application did, however, result in fewer green leaves per tiller.

Table 4.2. Effect of PGRs on field grown 'Baron' Kentucky bluegrass density.

Treatment <sup>†</sup>	Tillers/plug	Green leaves /tiller
Mefluidide	43.0	1.4
Amidochlor	44.3	2.6
Nontreated, mown	42.8	2.5
LSD <sub>0.05</sub>	NS	0.2

† Treatments applied 18 June 1988, samples were taken 13 July 1988, 25 DAT.

NS Nonsignificant (0.05).

#### Individual Plant Study

In order to document the development and duration of PGR induced discoloration, individual tillers were removed from each plot four days after treatment following 27 May application. Eight tillers from each treatment were characterized for length and number of green leaves present. Changes in tillers were measured and described weekly.

Half of the mefluidide treated tillers developed new growth during the 7 week study period. New leaves developed on three (tiller # 1, 5 & 8 in Table 4.3), and one developed a new tiller (# 3). Tiller number eight started to develop new growth 31 DAT which shriveled soon after becoming visible, followed by death of the tiller. Death of this tiller

Table 4.3 Effect of mefluidide on leaf growth and emergence of new leaves and tillers in field grown 'Baron' Kentucky bluegrass.

Tiller	Leaf	Days after treatment							
		4	10	17	24	31	39	45	52
		Length of green tissue (cm)							
1	1	2.8	†						
	2	5.0	5.3	5.5	5.5	†			
	3	---	---	---	---	1.4	1.8	1.8	†
	4	---	---	---	---	3.1	3.1	3.1	†
	5	---	---	---	---	2.0	5.5	5.5	5.5
	6	---	---	---	---	---	§	4.3	6.1
	7	---	---	---	---	---	---	---	4.0
2	1	2.0	†						
	2	4.5	4.5	4.5	4.5	4.8	5.0	5.4	5.1
3	1	3.5	†						
	2	5.0	5.3	5.6	5.9	5.8	5.8	7.1	7.1 ¶0.2 2.4
4	1	3.0	†						
	2	5.7	5.7	6.1	6.3	6.3	#		
5	1	3.2	3.2	3.2	3.2	3.2	3.2	†	
	2	2.5	2.7	2.9	3.0	2.7	2.7	†	
	3	---	---	---	---	---	3.1	3.1	3.1
	4	---	---	---	---	---	---	5.0	5.0
	5	---	---	---	---	---	---	3.8	3.8
6	1	3.3	†						
	2	2.2	†						
	3	2.0	2.5	2.6	2.7	2.5	#		
7	1	2.5	†						
	2	4.0	†						
	3	4.0	4.0	4.0	4.0	#			
8	1	2.2	†						
	2	1.5	1.5	1.5	1.5	1.5	1.5	#	
	3	2.5	2.5	2.5	2.5	2.6	2.6		
	4	---	---	---	---	§	†		
	5	---	---	---	---	§	†		

† Leaf no longer green.  
 ‡ Leaf not visible at this time.  
 § Leaf visible, but not long enough to measure.  
 ¶ New tiller.  
 # Tiller dead.

was attributed to growing conditions, most likely drought stress. None of the other tillers which died had exhibited any sign of new growth prior to senescence.

New leaves which were long enough to measure were produced by five of eight amidochlor treated tillers by 31 DAT (# 3,4,5,6,and 8 in Table 4.4). One tiller (# 8) produced both new leaves and new tillers. Tiller # 2 produced a new tiller just prior to its own death. Tillers 1 and 7 died during the 4th and 6th week, respectively.

All of the nontreated tillers produced either new leaves or new tillers during the observation period (Table 4.5). Tillers 2,4,7 and 8 died during the 5th week, but all four had produced new tillers prior to senescence.

Individual plant studies corroborated the conclusion that apparent loss of density associated with mefluidide application was due to visual perception. The number of treated tillers which died was no greater than the number of nontreated tillers that died. Nontreated tillers were, however, on crowns which produced new tillers, continuing the life of the plant. No evidence of new tillers was observed on most of the treated tillers. Individual plants were studied following PGR application during early inflorescence development 27 May. PGR application at this time may possibly suppress tillering because those tillers which would normally flower and die, stimulating the crown to produce new tillers (25) are left in a 'state of readiness' to flower and therefore, produce neither inflorescences nor tillers.

Hanson and Branham's study of photosynthate distribution following PGR application (16) corroborates the results of the individual plant

Table 4.4 Effect of amidochlor on leaf growth and emergence of new leaves and tillers in field grown 'Baron' Kentucky bluegrass.

Tiller	Leaf	Days after treatment							
		4	10	17	24	31	39	45	52
		Length of green tissue (cm)							
1	1	3.0	†						
	2	4.7	5.6	5.9	#				
2	1	1.2	†						
	2	2.4	3.2	4.9	4.2	#	¶2.6	2.6	2.6
							1.2	2.9	2.9
							--	0.2	2.5
3	1	2.5	2.5	3.0	3.7	†			
	2	7.5	†						
	3	--†	--	--	§	2.8	4.5	4.6	4.6
	4	--	--	--	--	--	§	2.2	4.0
4	1	2.2	†						
	2	2.3	†						
	3	4.5	5.0	5.5	6.5	8.0	8.0	8.1	8.1
	4	--	--	--	--	3.2	3.3	3.3	3.3
	5	--	--	--	--	1.0	3.5	5.7	5.7
	6	--	--	--	--	--	--	1.1	6.0
5	1	4.0	†						
	2	1.0	†						
	3	4.5	4.5	5.4	5.8	8.5	8.5	8.5	8.5
	4	--	--	--	--	2.2	2.2	2.2	2.2
	5	--	--	--	--	2.4	3.6	3.8	1.0
	6	--	--	--	--	--	1.7	3.9	4.0
	7	--	--	--	--	--	--	0.3	4.3
6	1	3.0	†						
	2	5.8	6.0	†					
	3	0.9	1.5	1.7	3.0	5.5	5.5	3.8	†
	4	--	--	--	--	--	2.5	2.5	†
	5	--	--	--	--	--	2.0	6.0	6.2
	6	--	--	--	--	--	--	0.5	6.4
7	1	2.3	†						
	2	7.7	†						
	3	2.8	4.1	5.0	5.7	†			
	4	--	--	--	0.5	0.9	#		
8	1	2.1	†						
	2	2.0	2.0	2.0	2.0	2.0	2.3	2.3	†
	3	1.0	2.1	3.1	4.7	5.8	6.2	6.2	†
	4	--	--	--	--	2.6	2.6	2.6	2.6
	5	--	--	--	--	§	3.3††	4.6	2.0
	6	--	--	--	--	--	--	1.6	5.9
	7	--	--	--	--	--	--	--	0.7
¶	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	§	0.5	2.0	§	0.5	1.7	§	1.8
	3	--	§	3.7	--	0.5	3.1	--	2.8
	4	--	--	0.5	--	--	0.8	--	0.5.

† Leaf no longer green.  
 ‡ Leaf not visible at this time.  
 § Leaf visible, but not long enough to measure.  
 ¶ New tiller.  
 # Tiller dead.  
 †† Three new tillers visible, growth listed below

Table 4.5 Leaf growth and emergence of new tillers in untreated field grown 'Baron' Kentucky bluegrass.

Tiller	Leaf	Days after treatment								
		4	10	17	24	31	39	45	52	
		Length of green tissue (cm)								
1	1	4.2	†							
	2	3.6	†							
	3	2.1	3.5	4.5	5.2	†				
	4	--†	--	--	--	2.8	3.0	3.0	†	
	5	--	--	--	--	2.1	4.7	4.7	5.5	
	6	--	--	--	--	--	3.1	5.0	5.7	
	7	--	--	--	--	--	--	1.6	3.0	
2	1	3.0	†				¶0.4	1.5	1.5	1.5
	2	2.5	†				1.8	2.8	2.8	†
	3	5.6	6.4	6.4	6.4	#	0.6	1.1	4.0	4.7
							--	--	--	4.3
3	1	2.2	†							
	2	2.7	†							
	3	1.8	2.5	2.7	2.7	3.0 <sup>††</sup>	3.2	3.2	1.0	
	4	--	--	--	--	2.3	2.3	2.9	2.9	
		¶	1	1.3	1.3	†	0.8	0.8	†	
			2	0.6	2.2	2.2	2.0	2.7	2.8	
			3	--	4.0	4.0	2.6	4.0	4.0	
			4	--	3.0	8.0	--	--	3.6	
			5	--	--	1.1				
4	1	2.5	†				¶1.8	1.8	4.0	†
	2	3.3	3.5	†	--			3.1	6.6	
	3	0.6	1.5	1.8	1.8	#	§	6.3	6.4	
5	1	3.5	3.5	3.5	3.5	3.5	2.7	†		
	2	3.4	3.4	3.4	3.4	3.4	3.0	3.0	3.0	
	3	1.0	1.3	1.5	2.0	2.7	3.5	4.2	4.2	
	4	--	--	--	--	--	--	2.5	3.5	
6	1	1.8	†							
	2	2.5	2.5	2.5	2.5	2.5	2.5	†		
	3	2.5	2.8	3.2	4.9	6.7	6.8	6.8	†	
	4	--	--	--	--	3.2	4.0	4.0	4.5	
	5	--	--	--	--	--	§	2.0	4.6	
7	1	3.5	†							
	2	3.0	†							
	3	3.4	4.7	5.2	5.3	#††				
		¶	1	1.1	#		1.0	1.0	#	
			2	3.1			0.5	1.3		
			3	3.4			--	0.8		
8	1	3.2	†				¶1.3	1.3	1.3	†
	2	3.0	†				1.8	1.8	3.0	3.0
	3	2.5	2.5	2.7	3.0	#	--	--	1.0	4.0
							--	--	--	0.8

† Leaf no longer green.  
 ‡ Leaf not visible at this time.  
 § Leaf visible, but not long enough to measure.  
 ¶ New tiller.  
 # Tiller dead.  
 †† New tillers visible, growth listed below.

study. These researchers report that only 8% of labeled carbon recovered from mefluidide treated plants 4 weeks after treatment was in immature leaves and only 4% in auxillary shoots. Amidochlor treated plants had 29% in immature leaves and 8% in auxillary shoots. Nontreated plants had 12% labeled carbon in immature leaves and 49% in auxillary shoots (16). This distribution of labeled photosynthate indicates PGR treated plants would have little reserves to use to produce new tillers, as was evidenced in the individual plant study. Only 3 of 16 treated crowns produced new tillers as compared to 5 of 8 nontreated crowns which produced new tillers. The greater percentage of photosynthate observed in immature leaves of amidochlor treated plants parallels the increased number of new leaves that developed on amidochlor treated tillers as compared with either mefluidide or nontreated tillers.

Results of individual plant studies contributed to a better understanding of the developmental sequence of discoloration. Distinct lesions occur on the blade tips at the onset of discoloration and appear to precede total bleaching of the blade. Discoloration begins on the oldest leaf of the tiller, and may in fact, be early senescence since discolored blades do not recover. If discolored blades are replaced by growth of new blades, as with amidochlor treated turf, then recovery of turf quality occurs. In contrast, if discolored blades are not replaced with new growth, as happened with mefluidide treated tillers, then discoloration continues. Reduction in visual density as reported (11) did occur in mefluidide treated plots, although no reduction in tiller number was observed. The claim of the manufacturer that the reduction in turf quality associated with mefluidide use is "due to visibility of

blades damaged in mowing, thatch, old clippings, and otherwise nongreen parts of the understory" (1) is partly corroborated by the reduction in the number of green leaves per tiller. This reduction in blade number, however, does not appear to be the primary cause for decreased turf quality as implied by the manufacturer.

#### Growth Suppression

Growth suppression of PGR treated turf was determined by direct measurement of mean turf height within each plot. During initial greenhouse experiments nontreated plants were both mown and nonmown. Mown treatments were mown after measurement of mean turf height. For example turf measured on 21 DAT was last mown on 14 DAT. Measurements of nontreated mown turf in Table 4.6 represent the growth of 7, 8, 12 and 19 days, respectively. Mefluidide treated turf was significantly shorter than nonmown turf when rated 21, 29, and 41 DAT (Table 4.6). Amidochlor treated turf was longer than mefluidide treated turf 21 to 41 DAT and longer than mown turf from 21 DAT through 60 DAT. Amidochlor treated turf was of comparable height to nonmown turf 29 and 60 DAT indicating that growth suppression was short-lived. Growth suppression in this experiment was similar to that reported by Bhowmik (5), although he noted a flush of growth 42 DAT which did not occur in this experiment.

In field experiments, amidochlor treated plants were taller than nontreated, mown plants beginning 29 DAT following 12 May application and taller than all other plants beginning 14 DAT following 27 May application (Table 4.7). This growth differential lasted throughout the study, indicating that amidochlor is more of a growth suppressor causing



Table 4.6. Growth suppression of greenhouse grown 'Baron' Kentucky bluegrass treated with PGRs.

Treatment <sup>†</sup>	Turf height (cm)				
	Days after treatment				
	14	21	29	41	60
Mefluidide	7.6	7.0 c <sup>‡</sup>	6.9 b	7.1 c	10.8 a
Amidochlor	8.3	8.0 b	9.8 a	10.4 b	10.6 a
Mown, nontreated <sup>§</sup>	7.3	7.3 bc	8.1 b	6.9 c	7.6 b
Nonmown, nontreated	8.4	9.3 a	10.3 a	11.6 a	12.0 a
	NS	**	**	**	*

† Treatments applied 28 April, 1987.

‡ Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range test (0.05).

§ Treatments were mown immediately after measurement. Mean height represents regrowth since the previous measurement.

\* \*\* Significant at the 0.05 or 0.01 probability levels, respectively.

NS Nonsignificant (0.05)

a reduction of growth rate rather than a total cessation of growth as with a growth inhibitor. Mefluidide application to turf at green-up (12 May) or during inflorescence development (27 May) resulted in turf which was not significantly longer than nontreated mown turf for 29 and 22, DAT respectively. Once inflorescences had become visible ( $\approx$  15 June) neither PGR was effective in suppressing growth.

#### Measurement of Physiological Parameters

##### Pigment Content

In order to determine if a decline in pigment content preceded the onset of discoloration, pigment analysis was conducted in the greenhouse

Table 4.7. Growth suppression of field grown 'Baron' Kentucky bluegrass in response to PGR application at varying developmental stages.

Treatment	Turf height (cm)						
	12 May (green-up)						
	Days after treatment						
	14	21	29	37	43	58	63
Mefluidide	7.8 c <sup>†</sup>	8.5 b	9.2 b	10.0 b	12.3 a	13.5 a	14.8 a
Amidochlor	9.6 b	11.3 a	13.0 a	13.3 a	13.9 a	14.1 a	16.1 a
Nontreated <sup>‡</sup>	11.9 a	9.6 ab	7.8 b	7.5 c	7.0 b	7.0 b	5.4 b
	**	**	**	**	**	**	**
	27 May (early inflorescence development)						
	Days after treatment						
	6	14	22	28	36	43	52
Mefluidide	7.8 b	8.3 b	8.3 b	8.4 b	8.4 b	9.1 b	10.9 b
Amidochlor	9.3 a	10.9 a	11.1 a	11.3 a	11.8 a	11.8 a	11.8 a
Nontreated	9.3 a	7.5 b	7.8 b	7.0 c	7.0 c	6.3 c	6.0 c
	**	*	**	**	**	**	**
	18 June (inflorescences emerged)						
	Days after treatment						
	6	14	21	30	41		
Mefluidide	8.3	8.3	8.3	8.3 a	11.9		
Amidochlor	8.5	9.3	9.3	9.0 a	12.5		
Nontreated	8.6	7.3	7.5	6.8 b	11.8		
	NS	NS	NS	**	NS		

† Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range test.

‡ Nontreated plants were mown to a height of 6 cm immediately following measurement. Mean turf height represents regrowth since the previous mowing.

\* \*\* Significant at the 0.05 or 0.01 probability level, respectively. NS Nonsignificant (0.05).

(Fall 1987) and in the field following June 1987 and 27 May 1988 application. In all cases, evaluation of pigment content began 3 DAT and lasted until no perceptible change in leaf appearance and pigment content remained (21 DAT). No differences in pigment content were found in either greenhouse or field experiments during 1987 (data not presented). Following 27 May 1988 application, total chlorophyll content of mefluidide treated turf began to increase 5 DAT (Table 4.8). This trend

Table 4.8. Effect of mefluidide on 'Baron' Kentucky bluegrass pigment content.

Mefluidide <sup>†</sup> kg a.i. ha <sup>-1</sup>	DAT											
	3	4	5	6	7	8	10	12	14	18	21	
	Total chlorophyll ( $\mu\text{g g}^{-1}$ ) <sup>‡</sup>											
0.56	87	87	109	95	79	83	77	116	104	91	126	
0	106	107	112	85	59	61	61	100	96	83	117	
	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	
	Total carotenoids ( $\mu\text{g g}^{-1}$ ) <sup>‡</sup>											
0.56	18	19	27	23	18	21	19	28	25	23	29	
0	22	26	26	21	15	15	17	24	23	21	27	
	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	

† Mefluidide applied in the field 27 May 1988.

‡ Fresh weight.

\* Significant at the 0.05 probability level.

NS Nonsignificant (0.05).

was found to be significant 7 and 8 DAT. Total carotenoid content of mefluidide treated turf was significantly higher than nontreated turf 8 DAT. Amidochlor application had no effect on either total chlorophyll or total carotenoid content of leaf tissue.

While one might expect reduced pigment content associated with discoloration, pigment content of mefluidide treated turf was found to be significantly greater than nontreated turf 7 and 8 DAT. A possible explanation for this phenomenon is that treated tillers may continue to produce the same amount of pigment as untreated tillers, thus concentrating pigments within treated blades. This might explain, in part, the darker green color often associated with mefluidide application and seen in this study.

At the conclusion of the spring 1987 greenhouse study (60 DAT) pigment content of the 'dark green' leaves of mefluidide treated plants was compared to the 'pale green' of the new growth. Although the concentration of pigment was higher in the 'dark green' tissue, there were no statistical differences (Table 4.9). Variability within groups and insufficient sample size may have contributed to the lack of statistical significance.

Table 4.9. Pigment content of mefluidide treated 'Baron' Kentucky bluegrass 60 days after 28 April 1988 application in the greenhouse.

	Pigment content †	
	Total chlorophyll $\mu\text{g g}^{-1}$	Total carotenoids $\mu\text{g g}^{-1}$
Dark green‡	265	32.4
Pale green	182	29.9
	NS	NS

† Fresh weight.

‡ Leaves were selected for groups based on visual observation.

NS Non significant (0.05).

## Photosynthesis

Preliminary experiments were conducted in the greenhouse to determine the parameters for measurement of canopy photosynthesis of Kentucky bluegrass. Factors which were determined from these experiments were light saturation for maximum APR (Apparent Photosynthetic Rate); time of day for photosynthetic measurements, and methods of eliminating interference from root and soil microbial respiration. Light saturation for Kentucky bluegrass was determined to be  $800 \mu\text{mol PAR m}^{-2}$ . In the greenhouse, photosynthetic measurements could be taken any time after 0900 h when maximum APR was reached and before 1500 h when APR began to decline. In the field, however, it was necessary to take APR measurements in the morning hours before the temperature in the photosynthetic chamber reached a level which stimulated photorespiration. Measurement of APR in the field, therefore, was usually done before 1100 h. For all experiments conducted on turf growing in soil, it was necessary to flood the canopy at the time of measurement to avoid interference from root and soil microbial respiration. For greenhouse experiments conducted on sod transplanted into quartz sand measurement of APR could be taken with or without flooding the sand. Magnesium Perchlorate [ $\text{Mg}(\text{ClO}_4)_2$ ] was used as a desiccant in the air circulation system prior to the sample entering the analyzer so little or no water vapor was present in the sample at the time of analysis. The presence of the water used to flood the turf had the additional benefit of providing a source of moisture to the leaves, so that stomatal resistance was not increased due to removal of moisture by the desiccant.

In a greenhouse experiment conducted during spring 1987, APR of mefluidide treated turf was lower than nontreated mown turf 13, 19 and 39 DAT and lower than nonmown nontreated turf on all dates for which a significant difference in APR existed (Table 4.10). Mefluidide treatment resulted in a lower APR than amidochlor treated turf on 33 and 39 DAT. Amidochlor treated turf had a lower APR than nonmown nontreated

Table 4.10. Photosynthetic rate of PGR treated 'Baron' Kentucky bluegrass.

Treatment <sup>†</sup>	Apparent photosynthetic rate									
	(mg CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )									
	Days after treatment									
	13	16	19	21	23	25	29	31	33	39
Mefluidide	.30 c <sup>‡</sup>	.25	.31 c	.37	.36 b	.28 b	.39	.26 b	.32 b	.27 b
Amidochlor	.32 bc	.32	.34 bc	.38	.42 ab	.35 ab	.38	.35 ab	.42 a	.36 a
Nontreated (mown)	.36 ab	.30	.37 ab	.44	.35 ab	.32 ab	.43	.32 ab	.36 b	.32 a
Nontreated (nonmown)	.38 a	.35	.40 a	.47	.48 a	.40 a	.48	.40 a	.46 a	.34 a
	*	NS	**	NS	**	*	NS	*	**	**

† Treatments applied in the greenhouse 28 April 1987.

‡ Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range test (0.05).

\* \*\* Significant at the 0.05 or 0.01 probability level, respectively.  
NS Nonsignificant (0.05).

turf only 13 and 19 DAT. The APR of amidochlor treated turf which was comparable to that of nonmown nontreated turf 21 through 39 DAT was due in part, to the lack of growth suppression exhibited by this compound. No difference in APR was observed between mown and nonmown nontreated

turf. Under normal maintenance practices turf would be mown regularly. Thus, for subsequent studies nontreated turf was mown in order to duplicate normal turf conditions.

Mefluidide reduced APR briefly following 12 May application (Table 4.11), after which it was not significantly lower than nontreated turf. Amidochlor treated turf had a higher APR than both nontreated and mefluidide treated turf beginning 21 DAT and lasting until 46 DAT.

Table 4.11. Photosynthetic rate of PGR treated 'Baron' Kentucky bluegrass in the field.

Treatment	Apparent photosynthetic rate (mg CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )						
	12 May (green-up)						
	Days after treatment						
	14	21	29	39	46	63	
Mefluidide	.16 b <sup>†</sup>	.18 b	.17	.21 b	.23 b	.19	
Amidochlor	.18 ab	.27 a	.20	.28 a	.29 a	.24	
Nontreated (mown)	.26 a	.26 b	.21	.20 b	.22 b	.22	
	*	*	NS	*	*	NS	
	18 June (inflorescence visible)						
	Days after treatment						
	9	11	17	19	23	25	41
Mefluidide	.20	.21	.21	.21	.15	.13	.22
Amidochlor	.25	.24	.33	.30	.26	.19	.18
Nontreated (mown)	.20	.19	.19	.28	.22	.18	.22
	NS	NS	NS	NS	NS	NS	NS

† Means within a column followed by the same letter are not significantly different according to Duncan's New Multiple Range test (0.05).

\* \*\* Significant at the 0.05 or 0.01 probability level, respectively.

NS Nonsignificant (0.05).

An analysis of covariance was done on data gathered following 27 May 1988 PGR application and revealed that turf treated with mefluidide had a significantly lower APR than nontreated turf 14 - 39 DAT (Fig. 4.1). Amidochlor treated turf was not significantly different from nontreated turf following 27 May application. PGR treatment had no effect on APR when turf was treated following inflorescence emergence (18 June).

A large degree of variation occurred in the measured photosynthetic rate for all experiments regardless of the treatment. Archbold and Houtz (3) report similar difficulties using the LI-6000 to measure photosynthetic rate in PGR treated strawberry (*Fragaria spp*) plants. The effect of mefluidide application on APR was variable. While decreased APR occurred at some point during all experiments, the relationship was obscured by extreme variability. Greater accuracy in measurements or a considerably greater number of replications are probably necessary to reduce variability and enhance accuracy.

Hanson and Branham's (16) findings that significantly less photosynthate was transported to immature leaves of mefluidide treated plants may explain the lack of new leaf development found in mefluidide treated turf. Diversion of photosynthate to roots (16) during the period of growth suppression corroborates the research of Cooper et al. (9) and may also explain the 'flush' of growth which follows growth suppression of mefluidide treated turf.

The APR of nontreated turf was not effected by mowing. This would indicate that although there was more leaf tissue contributing to APR in the nonmown sward, the contribution of each leaf was lower. Decreased



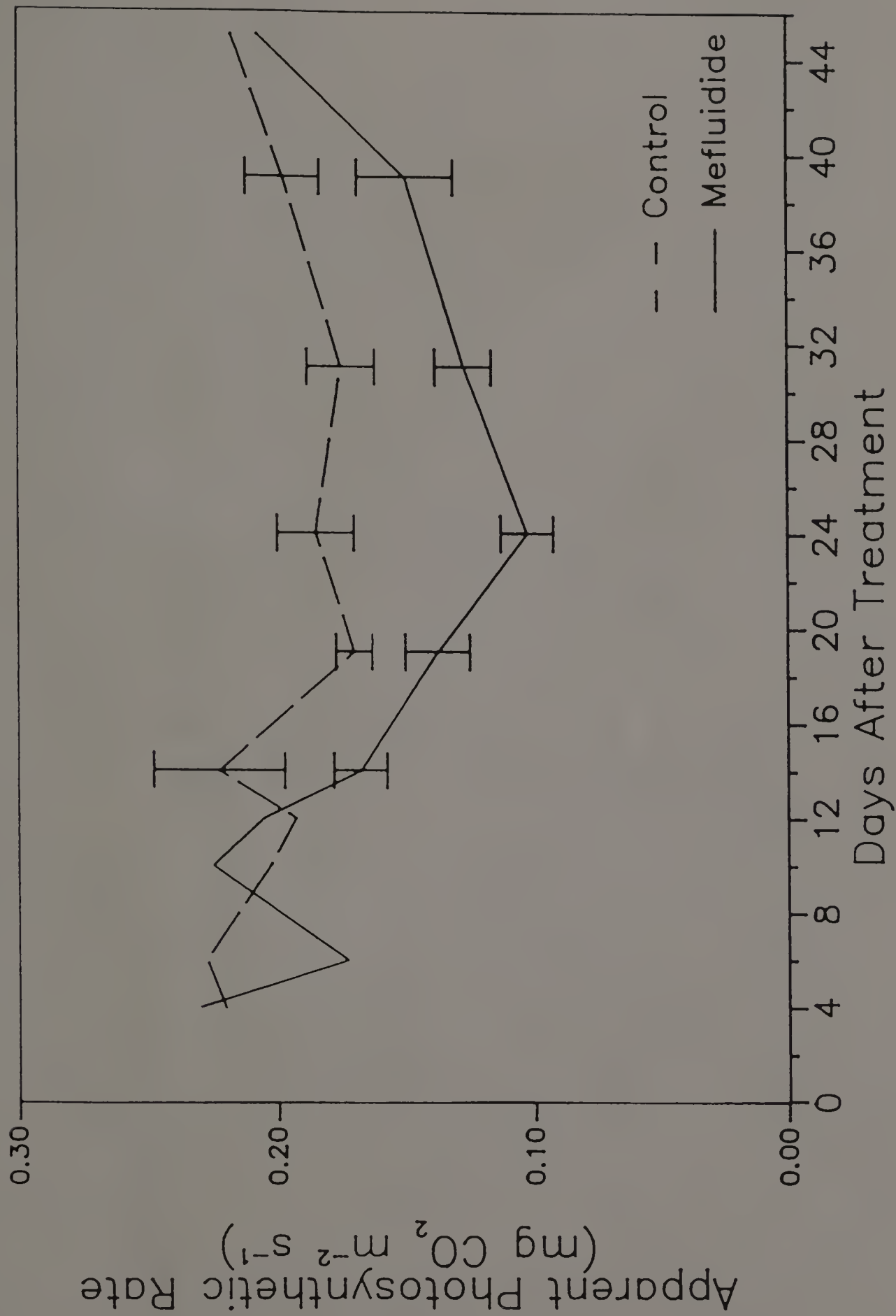


Figure 4.1. Apparent photosynthetic rate of field grown 'Baron' Kentucky bluegrass turf following 27 May mefluidide application. Vertical bars indicate  $\pm$  one standard error of the mean.

photosynthesis per leaf may have been related to increased mean leaf age and canopy development, both of which have been demonstrated to reduce individual leaf photosynthetic rate (21,29,39,40). The mown sward was comprised of younger blades, which although shorter than nonmown blades, were presumably contributing more per leaf to canopy APR, as was demonstrated by Krans and Beard (23) and Morgan and Brown (28). The APR of mefluidide treated turf was lower than nontreated turf whenever a difference occurred. This reduction was probably due to the decreased number and increased age of the leaf blades comprising the canopy. Amidochlor treated turf exhibited little growth suppression, resulting in a canopy comparable to nonmown nontreated turf. The similar canopy composition of these two swards would be expected to produce comparable photosynthetic rates.

## CHAPTER 5

### SUMMARY AND CONCLUSIONS

Application of the growth regulator amidochlor caused discernable discoloration to the turf in only one of the experiments conducted for these studies. Discoloration was slight ( $\approx 30\%$ ) and short lived ( $\leq 2$  weeks). These results are similar to those reported by Bhowmik (5) who noted that discoloration affected 32% of amidochlor treated blades, but that quality remained above 6 on a scale of 1 to 9. In all other experiments no discoloration was associated with this compound. A decrease in turf quality resulted from all mefluidide applications, with quality decline being greatest following application at green-up (12 May).

Distinct lesions occur on the blade tips at the onset of discoloration and appear to precede total bleaching of the blade. Discoloration begins on the oldest leaf of the tiller, and may in fact, be early senescence since discolored blades do not recover. If discolored blades are replaced by growth of new blades, as with amidochlor treated turf, then recovery of turf quality occurs. If, on the other hand, discolored blades are not replaced with new growth, as happened with mefluidide treated tillers, then discoloration continues.

Reduction in visual density as reported (11) did occur in mefluidide treated plots. Mefluidide treatment resulted in fewer green leaves per tiller although no reduction in tiller number was observed. This reduction in the number of green leaves increased the amount of the understory which was visible, but was incidental to the discoloration of green leaf tissue directly attributable to mefluidide application.

Growth suppression is the objective of a PGR. In these experiments mefluidide provided acceptable growth suppression lasting 4 to 5 weeks on average. While several studies have reported greater duration of growth suppression (6,14), these experiments are in agreement with most other reports (9,10,32,34). Amidochlor induced growth suppression was brief (2 to 3) weeks, and occurred only when application was made prior to inflorescence initiation (28 April 1987, 12 May 1988). While these results initially appear to conflict with most published studies (5,6,32), they in fact do not, since the published studies were all conducted in early spring. There is insufficient published data available on applications made after initiation of inflorescences.

Pigment content of PGR treated turf was assessed to determine if a reduction in pigments was the cause of discoloration associated with PGR application. Amidochlor had no effect on pigment content, while mefluidide application appeared to increase pigment content in treated leaves. Although differences were evident results were inconclusive. It is, however, not likely that a PGR induced decrease in pigment content is the cause of discoloration associated with these compounds.

The time differential between onset of discoloration and decreased APR indicates a lack of cause/effect relationship. Furthermore, the decreased APR of mefluidide treated turf cannot be solely attributed to the effects of discoloration. Changes in sward composition caused by growth suppression contributed to decreased APR.

APPENDIX

ANALYSIS OF COVARIANCE AND PARAMETER ESTIMATES

Source	DF	Sum of squares	Mean square	F value
Single model	3	2.839	0.9464	421**
Unexplained	77	0.173	0.0022	
Seperate models	6	2.862	0.9541	477**
Error	74	0.1502	0.002	
Unexplained by 2 lines	3	0.0229	0.0076	3.76*

Treatment	Intercept	Linear coefficient	Quadratic coefficient
Nontreated	0.2484	- 0.005241	0.0001002
Mefluidide	0.2754	- 0.01104	0.0002058

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