NET PHOTOSYNTHESIS AND CHLOROPHYLL CONTENT AS AFFECTED BY SYNTHETIC PLANT GROWTH REGULATORS

ABSTRACT

Research was conducted to determine the effects of the synthetic plant growth regulators mefluidide and EL-500 on net photosynthesis, total chlorophyll, and dry weight/unit leaf area of annual bluegrass (Poa annua var. reptans (Hausskn.) Timm.) and creeping bentgrass (Agrostis palustris Huds cv. 'Penncross'). Plants were treated with mefluidide at 0.00, 0.14, 0.28, and 0.56 kg ha\(^{-1}\) or EL-500 at 0.00, 1.12, 2.24, and 4.48 kg ha\(^{-1}\) and data was collected 9 days after treatment (DAT) for mefluidide treatments and 10 DAT for EL-500 treatments. EL-500 rates decreased carbon dioxide exchange rate (CER) of annual bluegrass 38% compared to untreated plants, while creeping bentgrass CER was unaffected by EL-500 rates. EL-500 increased the total chlorophyll of creeping bentgrass 20% over untreated plants. Increasing rates of mefluidide had no effect on photosynthesis of either species but did increase total chlorophyll 83% and dry weight/unit area 37% over untreated plants. In a separate study, both species were treated with
mefluidide at 0.00 and 0.28 kg ha⁻¹ or EL-500 at 0.00 and 2.24 kg ha⁻¹ and data were collected 4, 8, 16, 32, and 64 days after treatment (DAT). Mefluidide inhibited CER of both species 4 and 8 DAT after treatment. Inhibition 4 DAT, averaged across species, was 44% and at 8 DAT, 55% compared to untreated plants. El-500 inhibited both species 58% 16 DAT. Both compounds increased total chlorophyll 16 DAT. Mefluidide increased chlorophyll 80%, while the EL-500 increase was 51%. Both compounds inhibit photosynthesis and increase total chlorophyll under qualifying conditions. Further research into specifics of these alterations and possible reversal of treatment effects with exogenous gibberellic acid applications warrants attention.

INTRODUCTION

Plant growth regulators (PGR's) are currently receiving a great deal of attention in turfgrass industry and research. The uses for PGR's in turf range from vegetative control of highway roadside grasses to seedhead suppression and species conversion on intensively managed golf course turf. However, inconsistencies in efficacy and lack of information on physiological effects imposed by PGR application has prompted research interest on PGR effects on turfgrass physiological properties.

Mefluidide (N-[2,4-dimethyl-5 ([trifluoromethyl] sulfonyl) amino] phenyl] acetamide) and EL-500 (α-(1-methylethyl)- α-[4-trifluoro methoxy)phenyl] 5-pyrimidine
methanol) are synthetic PGR's currently utilized in turfgrass management. Physiologically, mefluidide and EL-500 are known to inhibit gibberellin biosynthesis by blocking ent-kaurene oxidation (34) which ultimately inhibits cell elongation. Further, mefluidide has been shown to influence the polar transport of IAA (16). Specifically on turfgrass species mefluidide is known to inhibit seedhead production of annual bluegrass (*Poa annua* L.) (7,28) and increase root elongation and depth of rooting of annual bluegrass (7). In Kentucky bluegrass (*Poa pratensis* L.) mefluidide has been found to reduce root and tiller production but increase rhizome length (5,12,14,31), while EL-500 increases tiller production (10). EL-500 is reported to exhibit selective growth suppression of annual bluegrass in mixed stands with perennial ryegrass (*Lolium perenne* L.) (3) or creeping bentgrass (*Agrostis palustris* Huds.) (32). Additionally, both PGR's have been shown to alter photosynthate partitioning in Kentucky bluegrass (18). Visual effects of turfgrasses treated with mefluidide or EL-500 range from an initial loss in quality due partially to phytotoxicity to an eventual increase in quality due in part to enhancement of green color (4,7,15,22,28). The reported turf quality and morphological effects and differences in photosynthate partitioning indicates the possibility of an alteration in a physiological process such as carbon dioxide assimilation and/or chlorophyll synthesis. The objectives of this
research were to evaluate the effect of mefluidide and EL-500 applications on the net photosynthesis, total chlorophyll and leaf dry weight/unit area of annual bluegrass and creeping bentgrass.

MATERIALS AND METHODS

Plant Material. Tillers were obtained from mature stands of annual bluegrass and creeping bentgrass located at the Hancock Turfgrass Research Center, East Lansing, MI and transplanted into styrofoam cups. The growth medium was 5 sandy loam : 3 sand : 1 peat moss (by volume). Plants were grown in the greenhouse where temperatures fluctuated between 16 and 24 °C. Plants were watered twice daily with an automatic misting system and fertilized weekly with 55 mg urea in 50 ml tap water (0.28 kg ha⁻¹).

Net Photosynthesis Determination. Net photosynthesis was measured using a open gas analysis system previously described by Augustine et al. (2) as modified by Sams and Flore (30). Measurements were made on 5 to 15 leaves placed into the assimilation chamber such that leaf to leaf shading was minimal. Immature and senescing leaves were excluded from the chamber to ensure maximum gas exchange. Assimilation chamber conditions were; ambient temperature of 21 °C, ambient CO₂ between 320 and 345 μL L⁻¹, light intensity of > 1000 μmol m⁻² s⁻¹ and leaf to air vapor pressure deficits < 1.5 KPa. Net photosynthesis was calculated as a molar flux, using the mole fraction of CO₂ as suggested by Cowen (8).
calculated on a leaf area basis using basic computer programs designed by Moon and Flore (23).

**Chlorophyll Determination.** Chlorophyll was extracted from fresh leaf material using N,N-dimethylformamide as described by Moran and Porath (24). Samples were maintained in the dark at 4-5 °C for 48 hrs prior to analysis on a Beckman spectrophotometer. Total chlorophyll was calculated based on extinction coefficients determined by Inskeep and Bloom (19). Chlorophyll was expressed on a leaf area basis.

**Leaf Dry Weight/Unit Area.** One day prior to determination of net photosynthesis 5 to 10 leaves were clipped from each plant sample and the leaf area determined on a Li-Cor leaf area meter. Leaves were then dried in a forced air dryer at 65 °C for 48 hrs and dry weight recorded. The leaf area and dry weight data were used to determine mg dry weight per cm² leaf area. Additionally this data was used to calculate total chlorophyll on a dry weight basis.

**Rate Study**

Plants were treated with mefluidide at 0.00, 0.14, 0.28, and 0.56 kg ha⁻¹ or EL-500 at 0.00, 1.12, 2.24, and 4.48 kg ha⁻¹. Treatments were applied via a conveyor belt sprayer equipped with an 8002E nozzle calibrated to deliver 770 L ha⁻¹ at 0.21 MPa. Net photosynthesis, total chlorophyll and dry weight/unit area were determined as previously described. Data were collected for mefluidide treatments 10 days after treatment (DAT) and EL-500 treatments nine DAT. Data collected were expressed as a
percent of the control rate and the two compounds were analyzed separately. Treatment design was a 2 (species) X 4 (rate) factorial. The mefluidide rate study utilized four replications and the EL-500 rate study, five replications. An analysis of variance (AOV) was calculated for each variable and means separated with the least significant difference (LSD) multiple comparison technique.

**Time Course Study**

Plants were treated with mefluidide at 0.00 and 0.28 kg ha\(^{-1}\) or EL-500 at 0.00 and 2.24 kg ha\(^{-1}\). Treatments were applied as described in the rate study. Data were collected on net photosynthesis and total chlorophyll 4, 8, 16, 32 and 64 DAT. Data collected were expressed as a percent of the control rate and the two compounds were analyzed separately. Treatment design was a 2 (species) X 2 (rate) factorial with 4 replications. An AOV was calculated for each evaluation date and means separated by the LSD multiple comparison technique.

**RESULTS**

**Rate Study**

**EL-500.** Results of the AOV found species and EL-500 rates to be significant sources of variability. As rate increased net photosynthesis of both species declined (Figure 4). However, the net photosynthesis of creeping bentgrass was never significantly affected by EL-500 rates when compared to the control while rates of 2.24 and 4.56 kg ha\(^{-1}\) significantly inhibited photosynthesis of annual bluegrass.
When expressed on a dry rate basis EL-500 rates significantly affected total chlorophyll, but when expressed on a leaf area basis total chlorophyll was not affected. Species did not differ in their response to rates of EL-500, when chlorophyll was expressed on a dry weight basis (Figure 4). However, species were significantly different when chlorophyll was expressed on a leaf area basis. When the data for net photosynthesis and total chlorophyll (leaf area) are averaged across EL-500 rates photosynthesis of annual bluegrass was significantly reduced and total chlorophyll of creeping bentgrass increased when compared to the control (Figure 5).

**Mefluidide.** Total chlorophyll was significantly affected by mefluidide rate but not by species when expressed on a leaf area or dry weight basis. The trend was for increased total chlorophyll from 0.00 to 0.28 kg ha\(^{-1}\) with significant differences for both parameters at 0.28 kg ha\(^{-1}\). Total chlorophyll appeared to decline at 0.56 kg ha\(^{-1}\) but did not differ from the control (Figure 6). Mefluidide rates significantly affected dry weight/unit area equally for both species with an increase in dry weight/unit area as rate increased (Figure 6).

**Time Course Study**

**EL-500.** Results of the AOV found no significant differences between species for net photosynthesis on any of the measurement dates. Photosynthesis, averaged across species, gradually declined from 4 to 16 DAT with a significant
Figure 4. Effect of EL-500 rates on net photosynthesis of annual bluegrass and creeping bentgrass (vertical bar represents LSD at P=0.05) and chlorophyll content (mean of both species). Rate effect on chlorophyll content was non-significant (NS) on a leaf area basis and significant (P=0.05) on a dry weight basis.
Figure 5. Effect of EL-500 on net photosynthesis and chlorophyll content (leaf area basis). Vertical bars represent LSD (P=0.05). Data for each species averaged across 3 rates of EL-500.
Figure 6. Effect of mefluidide rates on chlorophyll content and dry weight/unit leaf area of annual bluegrass and creeping bentgrass. Data points represent mean of both species.
difference between the EL-500 treated plants and the control on the 16 DAT measurement date. On the 32 and 64 DAT measurement photosynthesis was higher in the treated plants but not significantly different from the control (Figure 7). The total chlorophyll of treated plants was significantly affected at 16 DAT (Figure 7). A difference between species was also noted on this date with annual bluegrass having a total chlorophyll 133% and creeping bentgrass 167% of their respective control plants.

**Mefluidide.** Results of the AOV found no significant differences between species for net photosynthesis on any of the measurement dates. On the 4 and 8 DAT measurement dates photosynthesis, when averaged across species, was significantly reduced by mefluidide treatment (Figure 7). As was seen with EL-500 treated plants at 16 DAT mefluidide treated plants had a significantly higher total chlorophyll than control plants (Figure 7).

**DISCUSSION**

Both EL-500 and mefluidide were found to inhibit photosynthesis. As EL-500 rate increased net photosynthesis decreased. No rate effect was shown for mefluidide when measured at 9 DAT. However in the time course study, mefluidide at 0.28 kg ha⁻¹ significantly inhibited photosynthesis 4 and 8 DAT, while EL-500 treated plants were significantly inhibited 16 DAT. It appears that the window of inhibition is extremely narrow for both compounds and the lack of a significant rate effect for mefluidide might be
Figure 7. Effect of EL-500 and mefluidide on net photosynthesis and chlorophyll content (leaf area basis) of annual bluegrass and creeping bentgrass. Data points represent mean of both species. Asterisks adjacent to data point indicate significant difference between control and PGR treatment on respective evaluation date. * and ** indicate significance at P=0.05 or P=0.01 respectively.
explained by data being collected after the inhibition window. The early inhibition of photosynthesis by mefluidide versus the relatively late inhibition by EL-500 is probably due to contrasting modes of uptake for these two compounds. Mefluidide is primarily foliar absorbed (13) whereas EL-500 is primarily root absorbed (ELANCO Products Co., 1983, personal communication). The foliar absorbed mefluidide would be absorbed faster than the EL-500 hence the inhibition is exhibited earlier.

Mefluidide and EL-500 are known to inhibit gibberellic acid (GA) biosynthesis (6,16). Studies have indicated that GA's might be considered photosynthesis promoters (9). Exogenous applications of GA3 to the roots of tomato (Lycopersicon spp.) plants has been shown to increase leaf photosynthesis (1). Application of GA3 to foliage of 'Ormond' and 'Pee Dee' bermudagrass (Cynodon dactylon [L.] Pers.) (21) has been shown to increase net photosynthesis, after these two cultivars were grown at chilling temperatures. However, it does not appear that GA directly affects electron transport because studies have shown no increase in photosynthetic activity of isolated chloroplasts treated with GA (29). Additionally GA's have been shown to alter sink strength in sunflower (Helianthus annuus L.) (33) and alter sink strength and increase acid invertase activity in bean (Phaseolus vulgaris L.) (25). Altered sink strength or assimilate accumulation has been shown to affect
photosynthesis in plants (27) and specifically in grasses (17, 26). Although studies are limited it does appear that GA's act as photosynthesis promoters. Conversely a compound which inhibits GA biosynthesis, such as mefluidide or EL-500, may inhibit photosynthesis by reducing endogenous levels of GA.

El-500 exhibited selectivity in inhibition of photosynthesis and effect on chlorophyll concentration in annual bluegrass and creeping bentgrass. Net photosynthesis and total chlorophyll of annual bluegrass were decreased by EL-500 treatment while creeping bentgrass was unaffected. Field applications of EL-500 have been reported to selectively suppress growth of annual bluegrass in mixed stands with perennial ryegrass (3) or creeping bentgrass (32).

Based on results of dry weight/unit area data increasing rates of mefluidide increased leaf thickness of both species. This increase in leaf thickness was reflected in the chlorophyll data. Whether expressed on a leaf area or dry weight basis mefluidide increased total chlorophyll, however the response was more pronounced on a leaf area basis. In mefluidide treated plants it appears that the increase in total chlorophyll is primarily an increase reflected in an increased leaf width. The same response was not shown for EL-500. However, Devlin and Kosanski (11) reported a increase in leaf thickness in corn leaves following EL-500 treatment. Examination of the AOV finds
the level of significance for this attribute to be $P = 0.09$. If the arbitrary level for significance is increased to 0.1 then the increase in total chlorophyll in EL-500 treated plants is not a reflection of the increase in leaf width.

Both compounds investigated altered the physiological and morphological attributes evaluated. Further research is necessary to investigate the extent of these alterations. Possible avenues of investigation might include direct effects of PGR's on $O_2$ evolution in isolated chloroplasts and reversal of treatment effects by exogenous application of GA.
REFERENCES


