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# Changes in Endogenous Carotenoid Pools of Turf and Weed Species as Affected by Mesotrione and Environmental Conditions

James D. McCurdy
*University of Tennesee - Knoxville*

## Recommended Citation

To the Graduate Council:

I am submitting herewith a thesis written by James D. McCurdy entitled "Changes in Endogenous Carotenoid Pools of Turf and Weed Species as Affected by Mesotrione and Environmental Conditions." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

J. Scott McElroy, Major Professor

We have read this thesis and recommend its acceptance:

Carl E. Sams, John C. Sorochan, Dean A. Kopsell

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Carolyn R. Hodges, Vice Provost
and Dean of the Graduate School

(Original signatures are on file with official student records)

**CHANGES IN ENDOGENOUS CAROTENOID POOLS OF TURF AND WEED**

**SPECIES AS AFFECTED BY MESOTRIONE AND ENVIRONMENTAL**

**CONDITIONS**

A Thesis Presented for the Master of Science Degree.

The University of Tennessee, Knoxville.

James D. McCurdy

May 2008

**THESIS ABSTRACT**

Mesotrione, a carotenoid biosynthesis inhibiting herbicide, was evaluated for its use in turfgrass systems. Experiments were conducted to evaluate smooth crabgrass (*Digitaria ischaemum*) control with preemergence applications of mesotrione plus prodiamine. Experiments evaluated the influence of application timing on the efficacy of mesotrione plus prodiamine combinations and compared mesotrione plus prodiamine to current preemergence and early-postemergence herbicide treatments used for control of crabgrass. Greenhouse studies were conducted to compare the effects of foliar, soil, and soil plus foliar application of mesotrione on yellow nutsedge (*Cyperus esculentus*) and large crabgrass (*Digitaria sanguinalis*). Research was conducted in environmental growth rooms to investigate the effects of light intensity and temperature on perennial ryegrass (*Lolium perenne*) and large crabgrass carotenoid composition following mesotrione application.

# ACKNOWLEDGEMENTS

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# I. LITERATURE REVIEW

# Introduction

Mesotrione, 2-(4-(methylsulfonyl)-2-nitrobenzoyl)cyclohexane-1,3 cyclohexanedione, is a member of the triketone family of herbicides, which are chemically derived from leptospermone, a natural phytotoxin obtained from the Californian bottlebrush plant (*Callistemon citrinus* Stapf.;Mitchell et al., 2001;Vencil et al., 2002). Mesotrione is a carotenoid biosynthesis inhibitor which is currently labeled for use in European and U.S. maize (*Zea mayes* L.) production (Mitchell et al. 2001). Carotenoid biosynthesis inhibitors are often referred to as "bleachers" because of the characteristic white growth resulting after treatment. Tissue whitening is a result of the inhibition of carotenoid biosynthesis and the destruction of existing chlorophyll. Mesotrione is a selective preemergence and postemergence herbicide which has been developed for control of annual broadleaf and certain grass weeds. Mesotrione is currently being considered for use in many turfgrass species; however, little is known about the impact of environmental conditions on mesotrione physiology.

# Carotenoids

Carotenoids are $C_{40}$ isoprenoid compounds which form lipid soluble red, orange, and yellow pigments. Carotenoids are associated with photosynthetic light harvesting complexes (LHCs) where they transfer light energy to the photosynthetic reaction center and act in photoprotection by quenching free radicals, singlet oxygen, and other reactive oxygen species (Havaux 1998; Sandmann and Boger 1997). If carotenoids are not present in the photosystem or they are incapable of quenching excess energy,

considerable damage and degradation of thylakoid membranes may occur (Siefermann-Harms 1987).

Carotenoid formation begins with the dimerization of the $C_{20}$ isoprenoid compound geranyl-geranyl pyrophosphate to produce phytoene, a precursor to other carotenoids (Norris et al. 1995). Phytoene desaturase catalyzes the desaturation of phytoene to produce ζ-carotene, which then undergoes two further desaturation reactions to yield lycopene (Norris et al. 1995). Branching of the pathway occurs when lycopene is cyclized to form either α-carotene or β-carotene. Lutein and lutein 5, 6-epoxide (epoxylutein) are derived from α-carotene; while neoxanthin and the xanthophyll cycle pigments zeaxanthin, antheraxanthin, and violaxanthin, are formed from β-carotene (Demmig-Adams et al. 1996).

### Mode of Action

Mesotrione competitively inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) which subsequently inhibits the conversion of tyrosine to plastoquinone and α-tocopherol (Prysbilla et al., 1993). Plastoquinone is a critical cofactor for phytoene desaturase as well as an intermediate electron carrier between the carotenoid desaturase enzyme and the photosynthetic electron transport chain (Mayer et al. 1990; Norris et al. 1995; Prysbilla et al. 1993).

Personal accounts indicate that in 1977, Zeneca scientists at the Western Research Center (California) observed that relatively few weeds grew under Californian bottlebrush plant (*C. citrinus*). The allelopathic compound excreted by the plant was found to be leptospermone (Mitchell et al. 2001). HPPD was found to be a viable

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herbicide target in 1982 while Zeneca Agrochemical scientist investigated the herbicidal 2-benzoyl-1,3-cyclohexanediones, commonly referred to as the triketones or benzoylcyclohexanediones (Ellis et al. 1995; Lee et al. 1997, 1998).  The first benzoylcyclohexanedione herbicide to be commercialized was sulcotrione or SC-0051, first registered for use in 1993 (Beraud et al. 1993).  Sulcotrione, a post emergent broad-leaf herbicide used in European maize, resulted from these original efforts, as did mesotrione, which was developed under the name ZA-1296 for use in US maize (Lee et al. 1998).

## Crop Resistance and Weed Control

Mesotrione is a selective soil and foliar applied herbicide for control of annual broadleaf and certain grass weeds in corn (Sprague et al. 1999; Young et al. 1999). Symptoms in plants which are sensitive to mesotrione are bleaching followed by necrosis (Vencil et al. 2002).  Mesotrione, a weak acid, has a dissociation constant (pKa) of 3.12 at 20°C (Lee et al. 1997).   This weak acidity means that the degree of ionization is dependent on pH (Lee et al. 1997).  Lee et al. (1997) suggested that the acidity of HPPD inhibitors might affect transport and uptake in plants.  Mesotrione is rapidly taken up by weed species following foliar application and is distributed within the plants by both acropetal and basipetal movement (Mitchell et al. 2001).  Mitchell et al. (2001) reported the uptake of [$^{14}$C] mesotrione in several weed species and maize to be 55 to 90% within 24 hours, with the amount of absorbed [$^{14}$C] mesotrione in maize being slightly less than for the weed species.  This slower absorbance may contribute to maize's resistance to mesotrione (Mitchell et al. 2001).  Mitchell et al. (2001) also found that *Zea mays* has the

ability to rapidly metabolize mesotrione. Barta and Boger (1995) demonstrated that the tolerance of maize to SC-0051, sulcotrione, was due to its metabolism rather than enzyme insensitivity. The slower uptake of mesotrione, relative to susceptible weed species, may also contribute to its utility as a selective herbicide for use in maize (Mitchell et al. 2001).

Young et al. (1999) demonstrated that foliar application of mesotrione improved the spectrum of weeds controlled; however, the activity of many foliar applied herbicides may be influenced by environmental factors, such as temperature and relative humidity (Bayer 1987; Cudney 1987). Johnson and Young (2002) demonstrated that influence of relative humidity and temperature on mesotrione efficacy is species dependent and should be considered during field applications.

Johnson and Young (2002) found that common water hemp (*Amaranthus rudis* Sauer) and large crabgrass (*Digitaria sanguinalis* L.), both $C_4$ plants, are more susceptible to mesotrione at 18°C than 32°C. In the same study, cocklebur (*Xanthium strumarium* L.) and velvetleaf (*Abutilon theophrasti* (L.) Medic.), $C_3$ plants, are more susceptible to mesotrione efficacy at 32°C rather than 18°C (Johnson and Young 2002). Johnson and Young (2002) speculate $C_4$ plants' decreased metabolism compared to that of $C_3$ plants' in response to lower temperatures may decrease the metabolism of mesotrione. However, the role of photosynthetic pathways in herbicide efficacy is not supported consistently by the literature. High temperatures may increase fluidity of the cuticle and plasma membrane resulting in greater uptake of foliar applied herbicides; however, as temperature increases, the metabolic activity of the plant may increase and be of greater importance for some species (Johnson and Young 2002).

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It has been demonstrated that foliar applications of mesotrione are more effective than soil applications (Young et al. 1999), but it is necessary to evaluate the soil activity of mesotrione in turfgrass systems. Understanding the effects of environmental conditions upon mesotrione efficacy may help explain differences in activity of foliar applications of mesotrione under different environmental conditions.

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## II. MESOTRIONE PLUS PRODIAMINE FOR SMOOTH CRABGRASS (*DIGITARIA ISCHAEMUM*) CONTROL IN ESTABLISHED BERMUDAGRASS TURF

## Abstract

Crabgrass species (*Digitaria* spp.) are problematic weeds in bermudagrass (*Cynodon dactylon*) turf which can be controlled by preemergence herbicide applications. Due to the difficulty in predicting crabgrass emergence and other prevailing management constraints, preemergence herbicide applications are not always properly timed. Mesotrione controls crabgrass both preemergence and postemergence; however, relatively short soil-residual activity limits its use as a preemergence herbicide. Two experiments were conducted to evaluate smooth crabgrass (*Digitaria ischaemum*) control with preemergence applications of mesotrione plus prodiamine. The first experiment evaluated the influence of application timing on the efficacy of mesotrione plus prodiamine combinations. Applications were made every two weeks from 15 Mar to 24 May. Mesotrione plus prodiamine controlled smooth crabgrass more consistently across all application dates than either mesotrione or prodiamine applied alone. The second experiment evaluated mesotrione along with current preemergence and early-postemergence herbicide treatments used for control of crabgrass. When applied at the 1 to 2 tiller growth stage, mesotrione plus prodiamine controlled smooth crabgrass 99% when rated 31 Aug. Bermudagrass injury from mesotrione ranged from 9 to 44%, but did not result in any reduction in turf plant density. Mesotrione plus prodiamine is an effective tank-mixture when prodiamine alone is not applied in a timely fashion; however, variable and excessive turf injury is a potential impediment to mesotrione use on bermudagrass turf.

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## Introduction

Crabgrass species (*Digitaria* spp.) are problematic weeds in bermudagrass (*Cynodon dactylon* L.) turf (Dernoeden and Grande 1983; Webster 2004). *Digitaria* spp. include smooth crabgrass [*D. ischaemum* (Schreb) Schreb. ex Muhl Schreb.], large crabgrass [*D. sanguinalis* (L.) Scop.], southern crabgrass [*D. ciliaris* (Retz.) Koel.], and tropical crabgrass [*D. bicornis* (Lam.) Roemer & J.A. Schultes ex Loud]. Crabgrass can be selectively controlled in turf by both preemergence and postemergence herbicide applications (Bhowmik and Bingham 1990; Dernoeden and Krouse 1991; Johnson 1975). Effective preemergence crabgrass control depends upon preemergence herbicide application prior to crabgrass emergence (Watschke et al. 1976). Crabgrass emergence can be difficult to predict because weather conditions vary from year to year; therefore, preemergence herbicide applications are not always properly timed (Masin et al. 2005). Turfgrass managers would benefit from the ability to control crabgrass both preemergence and postemergence with a single application. Preemergence and postemergence herbicides applied in a single application would enable a turf manager to control emerged crabgrass plants and provide residual control of crabgrass. Johnson (1996) found that a tank-mixture of dithiopyr, a preemergence herbicide, and MSMA, a postemergence herbicide, controlled large crabgrass longer than when herbicides were applied alone. Another possible solution may be to tank-mix a preemergence herbicide with a postemergence herbicide such as mesotrione.

Mesotrione is a carotenoid biosynthesis inhibitor currently being evaluated for use in turfgrass. Symptoms in plants which are sensitive to mesotrione are bleaching

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followed by necrosis within 3 to 5 days after treatment (Mitchell et al. 2001). Tissue whitening is a result of the inhibition of carotenoid biosynthesis and the destruction of existing chlorophyll (Hess 2000; Mitchell et al. 2001). Typical application rates range from 100 to 230 g ai/ha when applied as a preemergence and 70 to 150 g/ha as a postemergence application (Mitchell et al. 2001). Previous research has shown that mesotrione is a viable herbicide for the preemergence and postemergence control of crabgrass in corn (*Zea mays* L.) (Mitchell et al. 2001; Ohmes et al. 2000). Mesotrione is safe on many turfgrass species, including Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* L.), and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] (Askew et al. 2004; McElroy 2005; McElroy et al. 2005). However, mesotrione has been reported to cause minor injury to bermudagrass (Willis et al. 2007).

Mesotrione controls a variety of common turf weeds postemergence, such as large crabgrass, goosegrass [*Eleusine indica* (L.) Gaertn.], nimblewill (*Muhlenbergia schreberi* J.F. Gmel.), ground ivy (*Glechoma hederacea* L.), common purslane (*Portulaca oleracea* L.), black medic (*Medicago lupulina* L.), dallisgrass (*Paspalum dilatatum* Poir.), creeping bentgrass (*Agrostis stolonifera* L.), yellow nutsedge (*Cyperus esculentus* L.), and dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers) (Beam et al. 2006; Giese et al. 2005; Johnson et al. 2002; Reicher et al. 2006; Reicher and Weisenberger 2006). Variable preemergence control of *Ipomoea* spp. and common cocklebur (*Xanthium strumarium* L.) has been reported; however, control was higher with postemergence application (Johnson et al. 1999; Young et al. 1999). Mesotrione has been reported to

lack preemergence activity due to a relatively short soil half-life, 4.5 to 32 days depending upon soil pH (Dyson et al. 2002).

Since mesotrione has postemergence activity and limited preemergence activity, research was conducted to determine if the addition of mesotrione to prodiamine, a commonly used crabgrass preemergence herbicide, could potentially provide postemergence and extended preemergence control of smooth crabgrass in established bermudagrass turf. The objectives of the two experiments were to evaluate turf injury and smooth crabgrass control for 1) mesotrione and prodiamine applied alone and in combination when applied at different spring application timings and 2) mesotrione plus prodiamine compared with other standard herbicide treatments used for control of crabgrass.

**Materials and Methods**

Two experiments were conducted at three locations to evaluate the utility of mesotrione in combination with prodiamine for control of smooth crabgrass. The first experiment evaluated the efficacy of mesotrione, prodiamine, and mesotrione plus prodiamine as influenced by application timing (referred to hereafter as the Timing Experiment). The second experiment evaluated mesotrione plus prodiamine against traditional preemergence crabgrass control scenarios (referred to hereafter as the Comparison Experiment). Each experiment was conducted three times as follows: at Milt Dickens Park in Oak Ridge, TN, in 2005; at the University of Tennessee West Tennessee Research and Education Center in Jackson, TN, in 2006; and at the University

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of Tennessee East Tennessee Research and Education Center - Plant Sciences Unit in Knoxville, TN, in 2006.

All locations were mowed between one and three times per week at approximately 2.5 cm. Experiments at Oak Ridge and Jackson were not provided with supplemental fertilizer or irrigation water for the duration of the study. Experiments at Knoxville received 24.5 kg N/ha with a 24-6-12 fertilizer[1] per month and were irrigated as needed to supplement rainfall. At Oak Ridge the soil type was Greendale silt loam [Fine-loamy, siliceous, semiactive, mesic Fluventic Dystrudept] with pH 5.9 and 4.0% organic matter. At Jackson the soil type was Lexington silt loam [Fine-silty, mixed, active, thermic Ultic Hapludalf] with pH 6.6 and 1.0% organic matter. At Knoxville the soil type was Sequatichie loam soil [Fine-loamy, siliceous, semiactive, thermic Humic Hapludult] with pH 6.2 and 2.1% organic matter. All areas were naturally infested with smooth crabgrass.

For both studies and at all locations, research was conducted in a randomized complete block design with four replicates and the size of experimental units were 4.5 $m^2$. Herbicides were applied in a water carrier volume of 280 L/ha with a $CO_2$-pressurized backpack sprayer equipped with 11002 XR flat fan nozzles[2] at 276 kPa. Soil temperature at a 2 cm depth was recorded using a hand held soil thermometer. Mesotrione was always applied with a nonionic surfactant[3] at 0.25% v/v. In order to illustrate season-long control, smooth crabgrass control was visually rated 31 Aug relative to the non-treated check on a 0 to 100% scale where 0 constituted no population reduction and 100 constituted complete elimination of all smooth crabgrass plants.

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Control estimates focused on the presence or absence of crabgrass plants and did not include crabgrass injury symptoms. Bermudagrass injury was rated visually on a 0 to 100% scale where 0 constituted no injury relative to the non-treated check and 100 constituted complete bermudagrass death. Crabgrass growth stage was recorded at each application date as reported in Table 1A. (All tables and figures are in the appendices.)

**Timing Experiment.** Research was conducted to evaluate mesotrione, prodiamine, and mesotrione plus prodiamine applied every two weeks from 15 Mar to 24 May (6 application timings). Herbicide rates were mesotrione at 280 g/ha, prodiamine at 1.12 kg/ha, or a combined treatment of both mesotrione and prodiamine at the previously mentioned rates. Bermudagrass injury was visually rated every two weeks beginning 29 Mar and concluding 6 weeks after 24 May. Smooth crabgrass control was rated as a final end of season rating on August 31. All data were subject to ANOVA (P = 0.05) and analyzed as a factorial (three herbicide treatments by six application timings) with the three separate environments considered fixed effects in the model. The herbicide treatment by application timing by environment interaction was evaluated to determine if there was an interaction over location or years. Means separation for treatment comparisons was accomplished using Fisher's protected LSD (P ≤ 0.05).

**Comparison Experiment.** Research was conducted to compare mesotrione plus prodiamine to traditional preemergence crabgrass control scenarios. Herbicide treatments are listed in Table 2A and include a non-treated control. All preemergence treatments were applied on 15 Mar; 8 weeks after initial treatment (WAIT) applications were applied on 9 May; and smooth crabgrass at 1 to 2 tillers of growth was treated on 15 Jun

in 2005, and 1 Jun in 2006.  Bermudagrass injury was rated biweekly beginning two weeks after study initiation and concluding six weeks after final herbicide applications. Data analysis and means separation were performed similar to that described in the Timing Experiment.

## Results and Discussion

**Timing Experiment**.  Due to a herbicide treatment by application timing by environment interaction, data were not pooled over environments (data not shown).  A herbicide treatment by application timing interaction was observed within each environment; therefore, the interaction is reported rather than the main effects.  In Oak Ridge, prodiamine applied 15 Mar to 26 Apr controlled smooth crabgrass greater than 90% (Figure 1A).  However, control decreased to 73% when prodiamine was applied 10 May and 55% when applied 24 May.  Mesotrione controlled smooth crabgrass less than 20% when applied 15 Mar to 26 Apr.  Smooth crabgrass control increased to 39% when mesotrione was applied 10 May and 60% applied 24 May.  Smooth crabgrass control with mesotrione plus prodiamine was more consistent than either herbicide applied alone, as control with the combination was 79% or greater for all application timings.

The trends in the herbicide treatments for control of smooth crabgrass at Jackson were similar to those observed at Oak Ridge.  Prodiamine, applied 15 Mar to 26 Apr, controlled smooth crabgrass greater than 95%.  However, control of smooth crabgrass with prodiamine decreased to 87% when applied 10 May and 75% when applied 24 May. Although control increased across some application dates, mesotrione controlled smooth

crabgrass less than 73% regardless of application date. Mesotrione plus prodiamine controlled smooth crabgrass greater than 90% across all application dates.

Results observed at Knoxville differed slightly from those of the Oak Ridge and Jackson locations. Smooth crabgrass control with prodiamine decreased with earlier application dates than at the two previous locations. Prodiamine controlled smooth crabgrass 96% when applied 15 and 29 Mar, 70% when applied 12 Apr, but less than 20% when applied after 26 Apr. Because prodiamine lacks postemergence activity, this decrease in control could be attributed to an earlier smooth crabgrass emergence. However, no observations of smooth crabgrass abundance were taken, only the average growth stage of emerged plants (Table 1A). Smooth crabgrass plants were at a more advanced growth stage at the Knoxville location than at either Oak Ridge or Jackson locations. Smooth crabgrass control with mesotrione was less than 20% when applied 15 Mar to 12 Apr. When applied 26 Apr, mesotrione control was 97%. However, mesotrione control was less than 45% when applied 10 and 24 May. Mesotrione plus prodiamine controlled smooth crabgrass more effectively than either prodiamine or mesotrione, with greater than 95% control when applied 15 Mar through 26 Apr, but only 66% control when applied 10 May, and 41% control when applied 24 May. The observed decrease in control provided by mesotrione and mesotrione plus prodiamine applied after 26 Apr could be due to the timing of smooth crabgrass emergence and the resulting plant size.

Timing of emergence is affected by soil temperature and water potential (Forcella et al. 2000). Several studies have predicted crabgrass emergence using temperature.

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King and Oliver (1994) reported that crabgrass germination occurred between 10 to 15°C soil temperature. Moreno and McCarty (1994) reported 60% emergence of smooth crabgrass occurring at 15°C soil temperature. Soil temperatures were only recorded at application dates and not continuously across each experiment (data not shown). Thus, they fail to explain observed location interaction with control of smooth crabgrass. Soil fertility is also thought to influence seedling emergence (Forcella et al. 2000). Unlike the Jackson and Oak Ridge locations, the Knoxville location was irrigated and fertilized which may have influenced the timing of smooth crabgrass emergence as well as growth stage. On 12 Apr, crabgrass growth stage was 1- to 2-leaf at Knoxville, whereas it was not emerged at Oak Ridge and at the 1-leaf growth stage at Jackson. Previous research indicates that mesotrione controls crabgrass less with increasing plant growth stage (Whaley et al. 2006).

At all locations, due to a lack of postemergence activity, control of smooth crabgrass with prodiamine decreased after the 26 Apr application date. Due to a lack of preemergence activity, smooth crabgrass control with mesotrione was 0% when applied 15 Mar and 29 Mar. Prodiamine plus mesotrione provided a more consistent means of controlling smooth crabgrass over application dates of 15 Mar through 24 May than either mesotrione or prodiamine. Tank-mixing mesotrione with prodiamine could provide acceptable smooth crabgrass control with a single herbicide application within a larger application timing window. However, it is necessary to compare prodiamine plus mesotrione to traditional crabgrass preemergence control scenarios.

**Comparison Study.** Quinclorac and dithiopyr treatments, applied at 1 to 2 tillers growth stage, controlled smooth crabgrass 73 and 62%, respectively. All other treatments, including mesotrione plus prodiamine, controlled smooth crabgrass greater than 90%. Thus, the combination of prodiamine and mesotrione was the only treatment that resulted in excellent smooth crabgrass control after emergence was observed. As stated earlier, this can be critical if an intended preemergence herbicide application is not timed correctly. Quinclorac resistance has been reported in certain biotypes of smooth crabgrass (Koo et al. 1997). However, in this case, lack of control was likely due to the limited residual preemergence activity of quinclorac. Reicher et al. (1999) reported that quinclorac, when applied pre-plant incorporated and at emergence in spring-seeded Kentucky bluegrass or perennial ryegrass, failed to provide season-long large crabgrass control. Previous research indicates that dithiopyr controls crabgrass postemergence only if applied prior to tillering (Enache and Ilnicki 1991; Reicher et al. 1999). Therefore, dithiopyr applied at 1 to 2 tillers failed to adequately control smooth crabgrass.

**Bermudagrass Injury.** Bermudagrass injury from mesotrione containing treatments was sporadic, ranging from 9 to 44% across both studies (data not shown). In the timing study, no consistency in bermudagrass injury was observed across timings or locations. Even in comparisons of the Knoxville and Jackson locations in 2006, injury exceeded 30% at Knoxville for 24 Apr application, but was less than 15% at Jackson. Conversely, injury exceeded 30% at Jackson but was less than 20% at Knoxville for applications performed on 24 May. Willis et al. (2007) reported similar injury (12 to 18%) when mesotrione was applied to bermudagrass. External factors such as humidity

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and air temperature have been reported to influence mesotrione activity (Johnson and Young 2002).  Therefore, attempts were made to correlate the observed injury to external factors.  Bermudagrass injury could not be correlated with humidity, air temperature, green-up, or mixture with prodiamine.  While the potential for turf injury greatly limits the adoption of mesotrione for use in bermudagrass turf, bermudagrass injury was limited to tissue whitening, with no tissue necrosis or decrease in turf stand observed at any time.  Therefore, when no other weed control option is available, and if the user can tolerate injury, mesotrione may be an effective tool for weed management in bermudagrass.

Due to the many constraints that turfgrass managers encounter, such as those due to weather and budgeting, timely application of preemergence herbicides can be a difficult task.  Mesotrione has utility for turf weed management for control of annual grasses such as smooth crabgrass without reducing bermudagrass turf stand density.  However, even with a tank-mixture of prodiamine plus mesotrione, control of smooth crabgrass may be dependent upon emergence and plant growth stage at the time of application.  Mesotrione plus prodiamine gives turfgrass managers the option of a single late-preemergence or early-postemergence application while providing the same smooth crabgrass control as an effectively timed traditional preemergence herbicide application.

Little is known about tank-mixing other preemergence herbicides with mesotrione; therefore, future research should include a thorough evaluation of other preemergence herbicide combinations with mesotrione.  Future research should also include an evaluation of mesotrione plus prodiamine control of other *Digitaria* spp.

**Sources of Materials**

[1] Harrells, Inc., PO Box 807, Lakeland, FL 33802 (http://www.harrells.com).

[2] TeeJet Extended Range spray tips.  Spraying Systems Co., North Avenue and Shmale Road, Wheaton, IL 60189.

[3] Non-ionic surfactant; X-77® Spreader (Alkylphenol ethoxylate, alcohol ethoxylate, tall oil fatty acid, 2,2' dihydroxydithyl ether and dimethylpolysiloxane), Loveland Products, Inc., PO Box 1286, Greeley, CO 80632.

[4] MSO® Concentrate (Methylated vegetable oil, alcohol ethoxylate, tall oil fatty acid), Loveland Products, Inc., PO Box 1286, Greeley, CO 80632.

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**Appendix A.**

**Table 1A.** Growth stage of smooth crabgrass on herbicide application dates at Oak Ridge, TN, in 2005, at Jackson, TN, in 2006, and at Knoxville, TN, in 2006.

| | Application Timing | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Timing Experiment | | | | | | Comparison Experiment [a] | |
| | 15 Mar | 29 Mar | 12 Apr | 26 Apr | 10 May | 24 May | PRE | 8 WAIT |
| Oak Ridge | none | none | 1 leaf | 1 to 2 leaf | 2 to 3 leaf | 1 to 2 tillers | none | 2 to 3 leaf |
| Jackson | none | none | none | 1 to 2 leaf | 2 to 3 leaf | 1 to 2 tillers | none | 2 to 3 leaf |
| Knoxville | none | none | 1 to 2 leaf | 1 to 2 leaf | 2 to 3 leaf | 1 to 2 tillers | none | 2 to 3 leaf |

[a] PRE applications were applied 14 Mar; 8 WAIT, applied 9 May. Abbreviations: PRE, preemergence; WAIT, weeks after initial treatment.

**Table 2A.** Effect of mesotrione plus prodiamine tank-mixture and traditional preemergence crabgrass control scenarios on smooth crabgrass (Digitaria ischaemum) control in bermudagrass (Cynodon dactylon L.) turf.[a]

| Herbicide | Rate | Application | Smooth Crabgrass Control [c] |
|---|---|---|---|
| | kg ai/ha | | —— % —— |
| Dithiopyr | 0.56 | PRE | 92 |
| Prodiamine | 1.12 | PRE | 95 |
| Pendimethalin | 3.36 | PRE | 91 |
| Oxadiazon | 4.48 | PRE | 92 |
| Dithiopyr | 0.43 | PRE and 8 WAIT | 93 |
| Prodiamine | 0.43 | PRE and 8 WAIT | 92 |
| Prodiamine plus | 1.12 plus | 1-2 Tiller | 99 |
| Quinclorac [d] | 0.84 | 1-2 Tiller | 73 |
| Dithiopyr | 0.56 | 1-2 Tiller | 62 |
| LSD (*P*=0.05) | | | 10 |

[a] Visual estimates of control were taken on 31 Aug at Oak Ridge, TN, in 2005, at Jackson, TN, in 2006, and at Knoxville, TN, in 2006.

[b] PRE applications were applied 14 Mar; 8 WAIT, applied 9 May; and 1-2 Tiller, applied 15 Jun in 2005, and 1 Jun in 2006. Abbreviations: PRE, preemergence; WAIT, weeks after initial treatment; LSD, least significant difference.

[c] Treatment by location interaction was not significant (P = 0.05). Therefore, smooth crabgrass control was combined across all locations.

[d] All quinclorac applications included 1.75 L/ha methylated seed oil[4]

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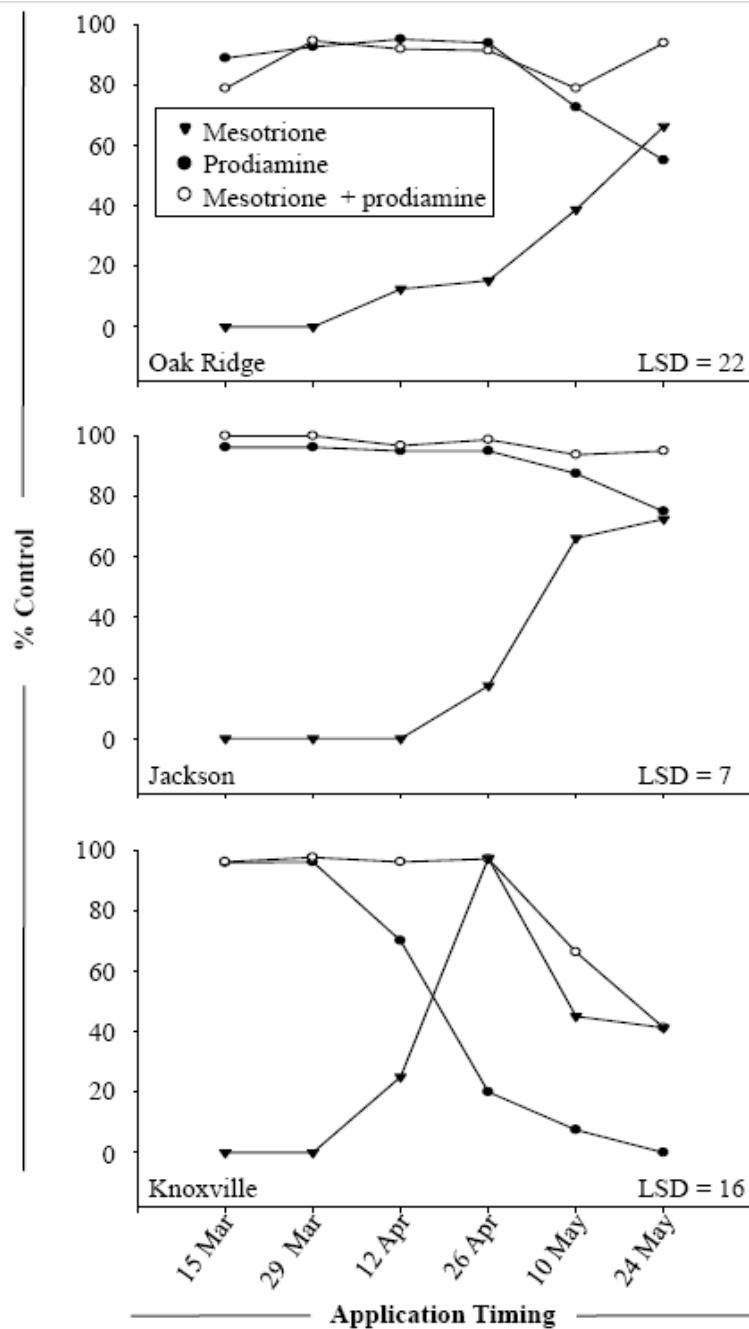
**Figure 1A.** Smooth crabgrass (*Digitaria ischaemum*) control in bermudagrass (*Cynodon dactylon* L.) turf visually rated 31 Aug at Oak Ridge, TN, in 2005, at Jackson, TN, in 2006, and at Knoxville, TN, in 2006. The LSD (P=0.05) value is provided for comparison of any treatment mean.

# III. YELLOW NUTSEDGE (*CYPERUS ESCULENTUS*) AND LARGE CRABGRASS (*DIGITARIA SANGUINALIS*) RESPONSE TO SOIL VS. FOLIAR APPLIED MESOTRIONE

**Abstract**

Mesotrione, a carotenoid biosynthesis inhibitor, is being evaluated for use in turfgrass systems. It has been hypothesized that root absorption of soil applied mesotrione is necessary for effective weed control. Greenhouse studies were conducted to compare the effects of foliar, soil, and soil plus foliar applied mesotrione at 0.14 and 0.28 kg ai/ha on yellow nutsedge (*Cyperus esculentus*) and large crabgrass (*Digitaria sanguinalis*). Mesotrione applied at 0.28 kg/ha controlled both yellow nutsedge and large crabgrass more effectively than mesotrione applied at 0.14 kg/ha. Soil and soil plus foliar applied mesotrione at 0.28 kg/ha controlled yellow nutsedge greater than foliar applied mesotrione 56 days after treatment (DAT). Soil plus foliar applied mesotrione at 0.28 kg/ha controlled large crabgrass 83% 28 DAT, which was greater than any other treatment. Soil and soil plus foliar applied mesotrione at both rates reduced large crabgrass foliar dry weight more effectively than foliar applied mesotrione. Results indicate that root absorption of mesotrione from soil is beneficial for the effective control of both yellow nutsedge and large crabgrass.

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**Introduction**

*Cyperus* spp. and *Digitaria* spp. are genre comprising many common turfgrass weeds. Yellow nutsedge (*Cyperus esculentus* L.) is a perennial weed which is difficult to control in turfgrass due to its ability to propagate via rhizomes and underground tubers (Tumbleson and Kommedahl 1961; Nelson and Renner 2002). Yellow nutsedge tubers may remain viable following herbicide application; therefore, adequate control depends upon reducing the tuber population (Tumbleson and Kommedahl 1961). Large crabgrass (*Digitaria sanguinalis* (L.) Scop) is an annual, grass weed which is selectively controlled in turfgrass by both preemergence and postemergence herbicide applications (Bhowmik and Bingham 1990; Dernoeden and Krouse 1991; Johnson 1975).

Mesotrione is a carotenoid biosynthesis inhibitor which is being evaluated for use in turfgrass systems. Mesotrione is safe on many turfgrass species including Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* L.), and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] (Askew et al. 2004; McElroy 2005; McElroy et al. 2005; Willis et al. 2007). Mesotrione has been reported to cause minor injury to bermudagrass in the form of bleaching but does not reduce turfgrass stand density (Willis et al. 2007; McCurdy et al. 2008). Mesotrione has been reported to control creeping bentgrass (*Agrostis stolonifera* L.) as well as other common turfgrass weeds postemergence, including Digitaria spp., goosegrass [*Eleusine indica* (L.) Gaertn.], nimblewill (*Muhlenbergia schreberi* J.F. Gmel.), ground ivy (*Glechoma hederacea* L.), common purslane (*Portulaca oleracea* L.), black medic (*Medicago lupulina* L.), dallisgrass (*Paspalum dilatatum* Poir.), and dandelion

(*Taraxacum officinale* G.H. Weber ex Wiggers) (Beam et al. 2006; Giese et al. 2005; Johnson et al. 2002; Reicher et al. 2006)  Reicher and Weisenberger (2006) reported that mesotrione controls yellow nutsedge; however, previous research has shown yellow nutsedge control with mesotrione to be inconsistent (Johnson et al. 2002).

Young et al. (1999) demonstrated that foliar applications of mesotrione were more effective than soil applications at controlling certain weed species.  However, un-published observations led us to hypothesize that root absorption of soil applied mesotrione is necessary for effective weed control.  In turfgrass, postemergence herbicides that can be either root or foliar absorbed allow greater flexibility for turfgrass managers than foliar-alone absorbed herbicides.  Herbicides capable of root absorption allow for granular or liquid application.  Additionally, they can be dislodged from foliage by post-application irrigation to prevent off target herbicide movement from traffic or surface water movement, often without sacrificing weed control.  Research was initiated to evaluate effectiveness of soil, foliar, and soil plus foliar applied mesotrione for control of yellow nutsedge and large crabgrass.

**Materials and Methods**

Research was conducted at the University of Tennessee, Knoxville, TN in an environmentally controlled glasshouse.  Trials were conducted in a randomized complete block design with a 2 by 3 factorial treatment arrangement.  Factorial levels were two mesotrione rates (0.14 or 0.28 kg ai/ha) by three application methods (soil, foliar, or soil plus foliar).  Run one was conducted fall 2006, and run two was conducted spring 2007.

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A nontreated check was included for both yellow nutsedge and large crabgrass experiments.

Three yellow nutsedge tubers were sown 2 cm deep and seed of large crabgrass were sown 0.5 cm deep in 12 cm diameter plastic pots (500 ml volume and 95 cm$^2$ surface area) containing Sequatichie loam soil [Fine-loamy, siliceous, semiactive, thermic Humic Hapludult] with pH 6.2 and 2.1% organic matter. Pots were overhead irrigated 3 minutes twice daily utilizing a mist irrigation system. Pots were fertilized with a complete fertilizer[1] (5.0 g/m$^2$) on a biweekly basis prior to treatment and resuming 7 days after treatment (DAT). Plants were grown under natural lighting. Pots were randomized every 2 days to account for potential environmental variation within greenhouse conditions.

Prior to herbicide treatment, all pots were thinned to two yellow nutsedge plants (20 cm ± 5 cm tall) or two large crabgrass plants (2-3 tillers each). Mesotrione was soil, foliar, or soil plus foliar applied at 0.14 or 0.28 kg/ha. All treatments included 0.25% v/v non-ionic surfactant[2]. Mesotrione was soil applied by diluting in 10 ml of tap water the amount of mesotrione that would contact the surface area of the pot had it been treated soil plus foliar. Using a syringe, the dilute mesotrione solution was applied evenly on the soil surface without any foliar contact at the base of the plant. Foliar and soil plus foliar applied mesotrione was applied in a water carrier volume of 280 L/ha with a $CO_2$-pressurized backpack sprayer equipped with 11002 XR flat fan nozzles[3] at 276 kPa. The soil of foliar applied pots was covered with approximately 2 cm peat moss to intercept mesotrione before contacting soil surface. Peat moss from foliar applied pots was

removed immediately following herbicide application. All plants were allowed to dry for 15 minutes in full sun prior to being returned to the greenhouse. To insure foliar applied mesotrione did not contact soil, all pots were sub-irrigated until 7 DAT; after which they were overhead-irrigated as described previously.

Plant injury was evaluated visually for phytotoxicity on a 0 (no phytotoxic response) to 100 (complete plant death) % scale 28 and 56 DAT. Plant foliage was harvested by clipping plants 1.5 cm above the soil surface 28 DAT. Subsequently, plants were allowed to regrow. Plant injury was assessed 56 DAT; foliage was harvested at the soil level; and roots were washed free of soil using forced water. All plant biomass was oven dried at 60ºC for 72 hours and weighed.

Data were subjected to ANOVA (P = 0.05). ANOVA was conducted as a randomized complete block with factorial arrangement (Steele et al. 1997). ANOVA results were used to select main effects and interactions were separated by Fisher's protected LSD (P = 0.05). A run by mesotrione rate by application method interaction was observed for all dry weights. Therefore, dry weights for fall and spring runs are discussed separately. Main effects and interactions are presented according to ANOVA, with precedence given to higher-order interactions within the factorial arrangement (Steele et al. 1997).

### Results and Discussion

**Yellow Nutsedge** control did not differ due to a run by mesotrione rate by application method interaction (P > 0.05); therefore, data were pooled over runs (Table 1B). Foliar and soil plus foliar applied mesotrione at 0.14 kg/ha controlled yellow

nutsedge less than all other treatments 28 DAT (41 and 39%, respectively). Soil applied mesotrione at 0.14 kg/ha controlled yellow nutsedge greater than all other 0.14 kg/ha applications 28 DAT (57%). Due to a lack of foliar interception, soil applied treatments may have had greater amounts of herbicide for root absorption than soil plus foliar applied treatments. The 10 ml carrier volume of the soil applied treatment may also have aided in herbicide movement into the root zone as well as uptake and translocation. However, we utilized previously published methodology for conducting soil compared to foliar greenhouse research trials (McElroy et al. 2004; Wilcut 1998). Mesotrione applied at 0.28 kg/ha controlled yellow nutsedge 57 to 65% 28 DAT; however, no differences in control were observed due to application methods. Soil applied mesotrione at 0.14 kg/ha controlled yellow nutsedge greater than all other 0.14 kg/ha applications 56 DAT (81%). Foliar applied mesotrione at 0.28 kg/ha controlled yellow nutsedge less than other 0.28 kg/ha applications (64%). Soil and soil plus foliar applied mesotrione at 0.28 kg/ha controlled yellow nutsedge 96 and 84%, respectively, 56 DAT. Research has shown that foliar applied mesotrione may control weeds greater than soil applied (Young et al. 1999). However our results indicate that mesotrione control of yellow nutsedge is enhanced by soil application.

Yellow nutsedge foliar- and root-dry weights are separated by experimental run due to a run by mesotrione rate by application method interaction (Table 2B). Run one foliar and soil plus foliar applied mesotrione at 0.14 kg/ha did not reduce yellow nutsedge foliar-dry weights 56 DAT. All other applications, regardless of application method, reduced foliar-dry weights greater than 90% 56 DAT. Soil applied mesotrione,

regardless of rate, reduced yellow nutsedge foliar-dry weights to zero, a 100% reduction. Effects upon yellow nutsedge root-dry weights were similar 56 DAT. Reduction in yellow nutsedge root-dry weights due to soil plus foliar applied mesotrione at 0.14 kg/ha did not differ from the non-treated check or any other treatment. All other application methods, regardless of rate, reduced yellow nutsedge root-dry weights 76 to 85%.

Run two yellow nutsedge foliar- and root-dry weight reductions were similar to those observed fall 2006. Soil applied mesotrione at 0.14 kg/ha reduced yellow nutsedge foliar-dry weights less than all other treatments 28 DAT (39%). Soil plus foliar applied mesotrione at 0.28 kg/ha reduced yellow nutsedge foliar-dry weights greater than all application methods of mesotrione at 0.14 kg/ha (84%). Foliar and soil applied mesotrione at 0.14 kg/ha did not reduce foliar- or root-dry weights 56 DAT. However, reductions in root-dry weights due to foliar and soil applied mesotrione at 0.14 kg/ha did not differ from reductions due to all other treatments. Soil plus foliar applied mesotrione at 0.14 kg/ha and mesotrione applied at 0.28 kg/ha, regardless of application method, reduced yellow nutsedge root-dry weights 79 to 98% 56 DAT.

**Large crabgrass** control did not differ due to a run by mesotrione rate by application method interaction ($P > 0.05$); therefore, data were pooled over run (Table 1B). Foliar applied mesotrione at 0.14 kg/ha controlled large crabgrass less than all other treatments 28 DAT (19%). Soil plus foliar applied mesotrione at 0.14 kg/ha and foliar applied mesotrione at 0.28 kg/ha controlled large crabgrass greater than foliar applied mesotrione at 0.14 kg/ha but less than all other treatments 28 DAT. Soil applied mesotrione at 0.14 kg/ha controlled large crabgrass greater than other 0.14 kg/ha

applications 28 DAT (60%). Soil applied mesotrione at 0.28 kg/ha controlled large crabgrass greater than soil applied mesotrione at 0.14 kg/ha (71%). Soil plus foliar applied mesotrione at 0.28 kg/ha controlled large crabgrass greater than all other treatments 28 DAT (83%). Foliar applied mesotrione at 0.14 kg/ha did not control large crabgrass 56 DAT. Soil applied mesotrione at 0.14 kg/ha controlled large crabgrass greater than soil plus foliar applied mesotrione at 0.14 kg/ha 56 DAT (91%). Soil and soil plus foliar applied mesotrione at 0.28 kg/ha controlled large crabgrass equally as well as soil applied mesotrione at 0.14 kg/ha (90 and 98%, respectively). These results indicate that mesotrione control of large crabgrass is enhanced by soil application. Selectivity of crop and weed species is due to differential absorption and metabolism of mesotrione (Witchert et al. 1999). In order to affect plant growth, root absorbed mesotrione must translocate to the actively growing shoot meristems via the xylem. Previous research demonstrates mesotrione translocation both acropetally and basipetally through xylem and phloem of plant tissue (Witchert et al. 1999).

Large crabgrass foliar- and root-dry weights are separated by experimental run due to a run by mesotrione rate by application method interaction (Table 3B). During run one, foliar applied mesotrione at 0.14 kg/ha reduced foliar-dry weights less than all other treatments 28 DAT (63%). Soil and soil plus foliar applied mesotrione, regardless of rate, and foliar applied mesotrione at 0.28 kg/ha reduced large crabgrass foliar-dry weights equally as well 28 DAT (87 to 98%). Foliar applied mesotrione at 0.14 kg/ha did not reduce large crabgrass foliar-dry weights 56 DAT. Soil and soil plus foliar applied mesotrione, regardless of rate, and foliar applied mesotrione at 0.28 kg/ha reduced large

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crabgrass foliar-dry weights equally as well  56 DAT (73 to 100%).  Foliar applied mesotrione at 0.14 kg/ha reduced large crabgrass root-dry weights less than all other mesotrione treatments 56 DAT (39%).  All other applications reduced large crabgrass root-dry weights greater than 85%.

Run two large crabgrass foliar- and root-dry weight reductions were similar to those observed fall 2006; however, it appears that almost all applications were less effective.  Foliar applied mesotrione at 0.14 kg/ha mesotrione reduced large crabgrass foliar-dry weights as well as foliar applied mesotrione at 0.28 kg/ha but less than all other treatments.  Both rates of soil applied mesotrione and soil plus foliar applied mesotrione at 0.14 kg/ha reduced foliar-dry weights as well as soil plus foliar applied mesotrione at 0.28 kg/ha and greater than all other treatments 28 DAT.  Reductions in large crabgrass foliar-dry weights following 56 DAT were slightly different than those discussed for fall 2006.  Foliar applied mesotrione, regardless of rate, and soil applied mesotrione at 0.28 kg/ha did not reduce large crabgrass foliar-dry weights 56 DAT.  Soil applied mesotrione at 0.14 kg/ha and soil plus foliar applied mesotrione, regardless of rate, reduced foliar-dry weights greater than foliar applied mesotrione at 0.14 kg/ha and equal to foliar and soil applied mesotrione at 0.28 kg/ha 56 DAT.  Despite application method and rate, large crabgrass root-dry weight reductions did not differ from those observed in the non-treated check.  Well established root mass and the lack of root degradation may have caused similarity between treated and nontreated root-dry weight.  Large crabgrass plants were 2-3 tillers in growth stage; therefore, root mass was well established prior to mesotrione treatment.

Differences in yellow nutsedge and large crabgrass foliar- and root-dry weight reductions due to experimental run may be due to the effect of temperature on mesotrione efficacy. Previous research has reported that mesotrione efficacy is affected by environmental conditions; and that plants with a $C_4$ metabolism, such as large crabgrass and yellow nutsedge, are more susceptible to injury at temperatures below optimal growing conditions (Johnson and Young 2002). Run one was conducted fall 2006 when greenhouse temperatures were cooler (average: 22 ºC); whereas, run two greenhouse temperatures were warmer (average: 29 ºC) in spring 2007. In general, foliar- and root-dry weights of both yellow nutsedge and large crabgrass were less in the fall than in the spring.

**Research Implications.** These data indicate that mesotrione absorbed by roots from soil is required for complete control of both yellow nutsedge and large crabgrass. Due to a lack of turf canopy, applications performed in this study may not fully mimic field applications, as a healthy turf canopy may impede the penetration of mesotrione into the soil. However, the role of soil applied mesotrione demonstrated by this research justifies the use of application methods that increase potential for root absorption, such as post application irrigation or granular mechanisms.

Mesotrione injures creeping bentgrass which is a desirable turfgrass species for golf course putting greens. Due to mesotrione control of creeping bentgrass and the potential for root absorption, mesotrione applications near putting greens may be limited. Previous research demonstrates the potential for herbicide movement onto non-target surfaces, both by mechanical and hydraulic movement (Barker et al. 2005; Starrett et al.

1996).  Utilization of herbicides in close proximity to creeping bentgrass is a problem previously reported, specifically in the case of sulfonylurea herbicides (Barker et al. 2005).  Barker et al. (2005) report post application tracking of rimsulfuron onto creeping bentgrass putting greens may be reduced by using the lowest effective rate of herbicide and applying irrigation prior to traffic.  Similarly, in order to prevent mesotrione injury to non-target surfaces, either through mechanical or hydraulic movement of the herbicide, mesotrione applications may require reduced rates or even a buffer zone near susceptible turf species such as creeping bentgrass.

Due to the effectiveness of soil applied mesotrione, future research should include an evaluation of post-application irrigation techniques and of granular applied mesotrione near susceptible turf.  Future research should also focus upon mesotrione efficacy in other turfgrass weed species, the effects of soil moisture on the root absorption of mesotrione, and the effects of post-application precipitation on mesotrione efficacy.

## Sources of Materials

[1] Harrells, Inc., PO Box 807, Lakeland, FL 33802 (http://www.harrells.com).

[2] Non-ionic surfactant; X-77® Spreader (Alkylphenol ethoxylate, alcohol ethoxylate, tall oil fatty acid, 2,2' dihydroxydithyl ether and dimethylpolysiloxane), Loveland Products, Inc., PO Box 1286, Greeley, CO 80632.

[3] TeeJet Extended Range spray tips. Spraying Systems Co., North Avenue and Shmale Road, Wheaton, IL 60189.

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**Appendix B.**

**Table 1B.** Effects of mesotrione rate and application method on control of yellow nutsedge and large crabgrass [a,b].

| Mesotrione rate kg ai/ha [c] | Application | Yellow Nutsedge Control | | Large Crabgrass Control | |
|---|---|---|---|---|---|
| | | 28 DAT | 56 DAT | 28 DAT | 56 DAT |
| | | ———————————% Control——————————— | | | |
| | Foliar | 39 | 28 | 19 | 13 |
| 0.14 | Soil | 57 | 81 | 60 | 91 |
| | Soil plus Foliar | 41 | 54 | 38 | 62 |
| | Foliar | 57 | 64 | 38 | 71 |
| 0.28 | Soil | 58 | 96 | 71 | 90 |
| | Soil plus Foliar | 65 | 84 | 83 | 98 |
| | LSD (P = 0.05) | 11 | 15 | 9 | 20 |

[a] Abbreviations:  DAT, days after initial treatment; LSD, least significant difference.

[b] Yellow nutsedge and large crabgrass control was visually rated 28 and 56 DAT on a 0 (no phytotoxic response) to 100 (complete plant death) scale.  Data pooled over two experimental

[c] All mesotrione applications included a 0.25% v/v non ionic surfactant.

**Table 2B.** Effects of mesotrione rate and application method on yellow nutsedge foliar- and root-dry weight reduction [a,b].

| Mesotrione rate | | Foliar-Dry Weight | | | | Root-Dry Weight | |
| | | 28 DAT | | 56 DAT | | | |
| kg ai/ha [c] | Application | Fall | Spring | Fall | Spring | Fall | Spring |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | % Change compared to nontreated control | | | | | |
| | Foliar | 80 | 51 | 37 | 46 | 78 | 59 |
| 0.14 | Soil | 81 | 39 | 100 | 55 | 85 | 35 |
| | Soil plus foliar | 62 | 51 | 0 | 65 | 41 | 79 |
| | Foliar | 84 | 80 | 91 | 71 | 76 | 79 |
| 0.28 | Soil | 85 | 80 | 100 | 87 | 82 | 98 |
| | Soil plus foliar | 82 | 84 | 94 | 90 | 81 | 91 |
| | LSD (P=0.05) | 25 | 30 | 52 | 62 | 53 | 35 |

[a] Abbreviations:  DAT, days after initial treatment; LSD, least significant difference.

[b] A significant (P = 0.05) run by treatment interaction prevented pooling over experimental run.

[c] All mesotrione applications included a 0.25% v/v non ionic surfactant.

**Table 3B.** Effects of mesotrione rate and application method on large crabgrass foliar- and root-dry weight reduction [a,b].

| Mesotrione rate kg ai/ha [c] | Application | Foliar-Dry Weight | | | | Root-Dry Weight | |
|---|---|---|---|---|---|---|---|
| | | 28 DAT | | 56 DAT | | | |
| | | Fall | Spring | Fall | Spring | Fall | Spring |
| | | % Change compared to nontreated control | | | | | |
| 0.14 | Foliar | 63 | 29 | 14 | 5 | 39 | (-71) [d] |
| | Soil | 98 | 74 | 100 | 82 | 99 | 80 |
| | Soil plus foliar | 87 | 86 | 73 | 77 | 87 | 87 |
| 0.28 | Foliar | 94 | 54 | 75 | 69 | 86 | 16 |
| | Soil | 97 | 76 | 100 | 49 | 97 | 70 |
| | Soil plus foliar | 97 | 69 | 100 | 100 | 98 | 97 |
| | LSD (P=0.05) | 21 | 29 | 32 | 71 | 27 | 72 |

[a] Abbreviations: DAT, days after initial treatment; LSD, least significant difference.

[b] A significant (P = 0.05) run by treatment interaction prevented pooling over experimental run.

[c] All mesotrione applications included a 0.25% v/v non ionic surfactant.

[d] Indicates an increase in root-dry weight relative to the untreated check.

# IV. EFFECTS OF MESOTRIONE ON PERENNIAL RYEGRASS CAROTENOID CONCENTRATIONS UNDER VARYING ENVIRONMENTAL CONDITIONS

**Abstract**

Mesotrione, a carotenoid biosynthesis inhibitor, is currently being evaluated for use in turfgrass. Mesotrione has been reported to injure perennial ryegrass (*Lolium perenne* L.). Research was conducted to investigate the effects of mesotrione on perennial ryegrass carotenoid concentrations under varying environmental conditions. Perennial ryegrass was treated with mesotrione (0.28 kg ai/ha) and subsequently placed in an environmental growth chamber at 600, 1100, or 1600 $\mu mol/m^2/s$ irradiance and 18, 26, or 34ºC. Leaf tissue was harvested 3, 7, and 21 days after treatment (DAT). Percent bleaching, percent necrosis, foliar weight, and photochemical efficiency were recorded as an indication of mesotrione efficacy. Temperature and irradiance levels did not affect mesotrione efficacy in perennial ryegrass. The highest amount of bleaching (8%) was observed 7 DAT in treated plants. Treatment with mesotrione did not result in decreased perennial ryegrass foliar weights. Treated plants displayed a lower photochemical efficiency 3 and 7 DAT than nontreated plants; although, plants recovered to the level of the nontreated by 21 DAT. Carotenoids were quantified using HPLC analysis. Carotenoid levels were similar to those reported in creeping bentgrass and many green leafy vegetable and herbal crops. Chlorophyll, β-carotene, lutein, and violaxanthin decreased due to treatment with mesotrione while phytoene, zeaxanthin, and antheraxanthin increased. Phytoene was undetectable in nontreated plants, but was 1.9 mg/100g fresh weight in treated plants. Despite carotenoid biosynthesis inhibition by mesotrione, the photoprotecting carotenoids zeaxanthin and antheraxanthin increased. These data indicate that mesotrione efficacy does not vary between the tested irradiance

and temperature levels.  However injury is a potential concern when applying mesotrione to perennial ryegrass.

## Introduction

Mesotrione is a carotenoid biosynthesis inhibitor which is currently being evaluated for use in turfgrass. Mesotrione is a selective preemergence and postemergence herbicide which controls a variety of common turfgrass weeds (Beam et al. 2006; Giese et al. 2005; Johnson and Young 2002). Symptoms in plants which are sensitive to mesotrione are bleaching followed by necrosis within 3-5 days after treatment (Vencil et al. 2002). Tissue whitening is a result of the inhibition of carotenoid biosynthesis and the destruction of existing chlorophyll (Mayonado et al. 1989; Mitchell et al. 2001). Tolerance to mesotrione has been reported in some turfgrass species, including perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.), tall fescue (*Festuca arundinacea* L.), and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] (Askew et al. 2004; McElroy 2005; McElroy et al. 2005). However, injury can occur in perennial ryegrass (Beam et al. 2006).

Mesotrione competitively inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), the enzyme responsible for the conversion of tyrosine to plastoquinone and α-tocopherol (Prysbilla et al. 1993). Plastoquinone is a cofactor for phytoene desaturase, a crucial enzyme of the carotenoid biosynthesis pathway (Norris et al. 1995). Carotenoids are $C_{40}$ isoprenoid compounds which form lipid soluble red, orange, and yellow pigments. Carotenoids are associated with photosynthetic light harvesting complexes (LHCs) where they transfer light energy to the photosynthetic reaction center and act in photoprotection by quenching free radicals, singlet oxygen, and other reactive oxygen species (Havaux 1998; Sandmann and Boger 1997). If carotenoids

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are not present in the photosystem or they are incapable of quenching excess energy, considerable damage and degradation of thylakoid membranes may occur (Siefermann-Harms 1987).

Carotenoid formation begins with the dimerization of the $C_{20}$ isoprenoid compound geranyl-geranyl pyrophosphate to produce phytoene, a precursor to other carotenoids. Phytoene desaturase catalyzes the desaturation of phytoene to produce $\zeta$-carotene, which then undergoes two further desaturation reactions to yield lycopene. Branching of the pathway occurs when lycopene is cyclized to form either α-carotene or β-carotene. Lutein and lutein 5,6-epoxide (epoxylutein) are derived from α-carotene; while neoxanthin and the xanthophyll cycle pigments zeaxanthin, antheraxanthin, and violaxanthin, are formed from β-carotene (Demmig-Adams et al. 1996; Norris et al. 1995).

The activity of many foliar applied herbicides may be influenced by environmental factors, such as temperature and relative humidity (Bayer 1987; Cudney 1987). Previous research demonstrates the influence of relative humidity and temperature on mesotrione efficacy is species dependent. Johnson and Young (2002) reported common water hemp (*Amaranthus rudis* Sauer) and large crabgrass (*Digitaria sanguinalis* L.), both $C_4$ plants, were more susceptible to mesotrione at 18°C than 32°C. In the same study, cocklebur (*Xanthium strumarium* L.) and velvetleaf (*Abutilon theophrasti* (L.) Medic.), both $C_3$ plants, were more susceptible to mesotrione efficacy at 32°C rather than 18°C (Johnson and Young 2002). Johnson and Young (2002) speculate $C_4$ plants' decreased metabolism compared to that of $C_3$ plants', in response to lower

temperatures may decrease the metabolism of mesotrione. However, the role of photosynthetic pathways in herbicide efficacy is not supported consistently by the literature. High temperatures may increase fluidity of the cuticle and plasma membrane resulting in greater uptake of foliar applied herbicides; however, as temperature increases, plant metabolic activity may increase, and be of greater importance for some species (Johnson and Young 2002). The objective of this research was to investigate the effects of mesotrione on perennial ryegrass carotenoid concentrations under varying environmental conditions. Understanding the effects of environmental conditions upon mesotrione efficacy may allow turfgrass managers to more effectively control weeds while minimizing injury to perennial ryegrass turf.

**Materials and Method**

Research was conducted at the University of Tennessee, Knoxville. Seed of 'Palmer IV' perennial ryegrass[1] (*L. perenne* L.) were planted approximately 0.5 cm deep in 12 cm diameter plastic pots (500 ml volume and 95 cm$^2$ surface area) containing silt-loam soil [Sequatichie loam soil (Fine-loamy, siliceous, semiactive, thermic Humic Hapludult) with pH 6.2 and 2.1% organic matter]. Seeds were germinated at 26ºC and 50% relative humidity in an environmental growth room[2] at 1100 μmol/m$^2$/s irradiance with a 16 h photoperiod. Irradiance was provided by a mixture of metal halide and high pressure sodium lamps. Throughout the experiment, pots were overhead irrigated twice daily to maintain adequate soil moisture, fertilized with a complete fertilizer[3] (5.0 g N/m$^2$) on a weekly basis prior to treatment and resuming 7 days after treatment (DAT),

and randomized every two days to account for potential variation within the environmental growth room.

Pots were thinned to 5 plants per pot one week after planting. Pots were treated 14 days after emergence with mesotrione at 0.28 kg ai/ha plus 0.25% v/v non-ionic surfactant[4] applied in a water carrier volume of 280 L/ha with a $CO_2$-pressurized backpack sprayer equipped with 11002 XR flat fan nozzles[5] at 276 kPa. Plants were subsequently placed within an environmental growth room at 18, 26, or 34°C. Irradiance levels were achieved by manipulating the proximity of the plants to the overhead light source. Due to the limited availability of environmental growth rooms, the three temperature regimes could not be conducted simultaneously. For this reason, great care was taken to ensure plants were of identical growth stage and size prior to treatment, and that they were fertilized and irrigated identically.

Four treated pots and four nontreated pots were randomly selected from each irradiance level 3, 7, and 21 DAT. Two photochemical efficiency ($F_v/F_m$) ratings per pot were taken mid canopy as an indication of photoinhibition and overall plant health using a modulated fluorometer[6]. Percent bleached tissue and percent necrotic tissue were recorded visually as an indication of mesotrione efficacy. All plants were harvested at soil level and immediately placed on ice for transfer to storage at -80°C. Prior to carotenoid extraction, plant material was weighed to obtain sample fresh weights (FW). Carotenoids were extracted and quantified according to previously published methods (Emenhiser et al. 1996; Kopsell et al. 2003; McElroy et al. 2006). Plant material was first homogenized in liquid N using a mortar and pestle. A subsample weighing

approximately 0.5 g was placed into a Potter-Elvehjem tissue grinder tube[7] with 0.8 mL of ethyl-β-apo-8-carotenoate, as an internal standard, and 2.5 mL of tetrahydrofuran (THF) stabilized with 2,6-Di-*tert*-butyl-4-methoxyphenol (BHT). The sample was homogenized using 25 insertions with a Potter-Elvehjem tissue grinder pestle attached to a drill press[8] set at 540 rpm while the tube was immersed in ice to dissipate heat. The tube was then centrifuged for 3 minutes at 500 $g_n$. Using a Pasteur pipette, supernatant was placed into a conical 15-mL test tube, capped, and held on ice during the remainder of the extraction. The sample pellet was resuspended in 2.0 mL THF, and the extraction procedure was repeated 5 times until the supernatant was clear. The combined supernatants were reduced to 1.0 mL under N stream[9]. To each 1.0 mL sample, 4.0 mL MeOH was added and vortexed. Samples were filtered through a 0.2 μm polytetrafluoroethylene filter[10] prior to analysis by high performance liquid chromatography (HPLC).

An Agilent 1100 series HPLC unit with a photodiode array detector[11] was used for sample separation. Samples were analyzed for carotenoids using a ProntoSIL $C_{30}$ RP column (4.6 by 250 mm)[12] with a 5.0-mm particle size and 200-A° pore size fitted with a guard column (4 by 23 mm, 7.0 mm; S-5) (Nesterenko and Sink 2003). The column was maintained at 30ºC by a thermostatted column comparment. Pigment separation was performed using an isocratic mixture of methanol/methyl-*tert*-butyl-ether 89:10.9% (v/v) plus 0.1% triethylamine. Eluted compounds from a 20-mL injection were detected. Data were collected, recorded, and integrated using 1100 HPLC Chem-Station Software[13]. Carotenoids were selected based upon their active roles in photoprotection and light

harvesting. Phytoene was detected at 290 nm. Carotenoids detected at 453 nm include lutein, epoxylutein, violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, and β-carotene. α-Carotene was undetectable and therefore not quantified. Chlorophyll *a* and chlorophyll *b* were detected at 652 nm. Peak assignment was performed by comparing retention times and line spectra (250-650 nm) obtained from photodiode array detection with authentic standards purchased from a commercial vender[14]. Concentrations of the authentic standards were determined spectrophotometrically using quantitative spectroscopic data (Davies and Köst 1988). HPLC recovery rates of ethyl-β-apo-8-carotenoate (53 to 91%) were used to estimate extraction efficacy. All carotenoid concentrations were calculated on a mg/100g FW leaf tissue basis. Ratios of zeaxanthin plus anteraxanthin to zeaxanthin plus antheraxanthin plus violaxanthin (Z+A / Z+A+V ratio), and epoxylutein to lutein (ELU/LU ratio) were calculated for comparison.

The experimental design was completely random with a two by three factorial treatment arrangement (two mesotrione treatments by three irradiance levels). Within each treatment scheme, harvest intervals (0, 7, and 21 DAT) were analyzed as samples. The experiment was conducted at three temperatures (18, 26, or 34ºC) with four replicates within each temperature. A model with equal variance was fit to data and a likelihood ratio test was used to test if variance were unequal between temperatures. Independent analysis of temperatures was conducted, and visual verification confirmed that results were similar for each temperature. Equal variance among runs allowed for data pooling over temperatures. The Lavene test was used to test for equal variance

among treatments. All data were subjected to ANOVA (P = 0.05). ANOVA results were used to select main effects. Means were seperated by Fisher's protected LSD.

## Results and Discussion

Although temperature is known to influence mesotrione efficacy as well as carotenoid concentration, effects did not differ due to temperature in this experiment. Therefore, all data were pooled across temperature. A treatment by harvest interval interaction was observed for percent bleaching and percent necrosis (Table 3C). When evaluating perennial ryegrass response to mesotrione, at no time was bleaching of nontreated plants observed. Treated plant bleaching 3 and 7 DAT was 7 to 8% but less than 1% 21 DAT. Treated plant necrosis was 2 to 3% 7 and 21 DAT while nontreated plant necrosis was less than 1% 21 DAT. When evaluating perennial ryegrass foliar weights, there was no significant effects due to treatment with mesotrione or irradiance level.

**Carotenoid Quantification.** Treated plant chlorophyll *a* concentrations (177.4 mg/100g FW) were less than those of nontreated plants (191.0 mg/100g FW) (Table 3C). Chlorophyll *a* concentrations 3 DAT (204.4 mg/100g FW) were greater than concentrations observed 7 and 21 DAT (182.2 and 166.3 mg/100g FW, respectively; Table 2C). Although chlorophyll *b* did not vary due to treatment with mesotrione, concentrations 3 DAT (70.8 mg/100g FW) were greater than 7 and 21 DAT (62.0 mg/100g FW). Chlorophyll *a* to *b* ratios were approximately 3 to 1 throughout the experiment and did not differ due to irradiance level, harvest interval, or treatment with mesotrione. Total quantified carotenoids did not vary due to treatment with mesotrione

or irradiance level; however, total concentrations were greater 3 DAT (46.0 mg/100g FW) than 7 and 21 DAT (41.4 and 40.7 mg/100g FW, respectively; Table 2C). These results are comparable to previous studies in which isoxaflutole, an HPPD inhibitor, was applied to 'Prelude' perennial ryegrass. Bhowmik and Drohen (2001) reported decreases in chlorophyll *a* concentrations due to treatment with isoxaflutole; however, total carotenoids were unaffected.

When evaluating individual carotenoid concentrations as affected by mesotrione treatment, changes occurred in phytoene, β-carotene, zeaxanthin, antheraxanthin, violaxanthin, lutein, epoxy-lutein, but not in neoxanthin (Table 3C). Phytoene concentrations were 1.9 mg/100g FW in treated plants and undetectable in nontreated plants. A treatment by harvest interval interaction was observed for phytoene concentrations (Table 4C). Phytoene concentrations of treated plants 3 DAT were 2.7 mg/100g FW and subsequently decreased 7 and 21 DAT (1.7 and 1.3 mg/100g FW, respectively). Phytoene concentrations were also affected by irradiance levels. Phytoene concentrations of plants grown at 600 μmol/m$^2$/s (1.1 mg/100g FW) were greater than those of plants grown at higher irradiance levels (0.9 mg/100g FW). Previous research reports HPPD-inhibition increases phytoene concentrations due to the indirect inhibition of phytoene desaturase (Mayonado et al. 1989; Soeda and Uchida 1987). Phytoene is a precursor to lycopene, which is cyclized to form either α-carotene, precursor to lutein, or β-carotene, precursor to the xanthophyll cycle. α-carotene was undetectable and therefore not quantified; however, β-carotene concentrations of treated plants (7.8

mg/100g FW) were less than those of nontreated plants (9.1 mg/100g FW), presumably due to the decrease in the precursor phytoene.

The xanthophyll cycle pigments (zeaxanthin, antheraxanthin, and violaxanthin) are antioxidants and components of light harvesting complexes (Demmig-Adams et al. 1996; Niyogi et al. 1997). The ratio of photoprotecting pigments zeaxanthin and antheraxanthin to total xanthophyll pigments (Z+A / Z+A+V) varied due to both treatment and irradiance level while total xanthophylls (Z+A+V) were similar in both treated and nontreated plants (8.5 to 8.8 mg/100g FW; Table 5C). Z+A / Z+A+V was greater in treated plants (0.5:1) than nontreated plants (0.4:1). Z+A / Z+A+V was lower (0.4:1) in plants grown at 600 $\mu mol/m^2/s$ than in plants grown at higher irradiance levels (0.5:1). A treatment by harvest interval interaction was also observed for Z+A / Z+A+V ratios. Z+A / Z+A+V increased in nontreated plants from 0.4:1 3 and 7 DAT to 0.5:1 21 DAT while remaining steady at 0.5:1 in treated plants. Within the xanthophyll cycle, zeaxanthin is the primary carotenoid responsible for preventing photoinhibition (Demmig-Adams et al. 1999). Increases in photoprotection have been linked to zeaxanthin quenching of singlet oxygen and free radicals in chloroplast membranes (Baroli et al. 2003). Zeaxanthin concentrations of treated plants were greater (2.0 mg/100g FW) than those of nontreated plants (1.4 mg/100g FW). Antheraxanthin concentrations did not differ due to treatment; however, concentrations 3 DAT (2.6 mg/100g FW) were greater than those 7 and 21 DAT (2.2 mg/100g FW). A treatment by harvest interval interaction was observed for zeaxanthin. Zeaxanthin concentrations of treated plants decreased from 2.3 mg/100g FW 3 DAT to 1.7 mg/100g FW 21 DAT

which was identical to concentrations of nontreated plants. Unlike other xanthophyll cycle pigments, violaxanthin concentrations of treated plants (4.4 mg/100g FW) were less than those of nontreated plants (4.9 mg/100g FW). Results indicate that zeaxanthin was preferentially accumulated in treated plants. Xanthophyll cycle pigments zeaxanthin and antheraxanthin have previously been shown to accumulate in high irradiance conditions due to the increased activity of the pH dependent enzyme violaxanthin deepoxidase (Demmig-Adams et al. 1996; Niyogi et al. 1997). However, increases due to irradiance were not observed in perennial ryegrass. Increased binding of zeaxanthin to photosystem II proteins allows for more efficient quenching of excess energy, a process known as non-photochemical quenching (NPQ) (Horton et al. 1996; Li et al. 2000).

Similar to violaxanthin, lutein concentrations of treated plants (19.4 mg/100g FW) were less than those of untreated plants (20.8 mg/100g FW). Lutein functions as an integral subunit of LHCs. In plant mutants void of the xanthophyll cycle carotenoids, lutein functions in NPQ as a photoprotectant against oxidative damage (Niyogi et al. 1997). Epoxy-lutein can also function as a light-harvesting pigment under low irradiance conditions with shifts from lutein to epoxy-lutein occurring in low irradiance (Bungard et al. 1999; Niyogi et al. 1997). Although epoxy-lutein did not differ due to treatment; concentrations did vary due to harvest date. Epoxy-lutein decreased from 1.1 mg/100g FW 3 DAT to less than 1.0 mg/100g FW 7 and 21 DAT. No differences in epoxy-lutein to lutein ratios (ELU/LU) were observed.

Palmer IV perennial ryegrass produced carotenoid concentrations comparable to 'Crenshaw' creeping bentgrass (*Agrostis stolonifera* L.) grown at 554 μmol/m$^2$/s.

McElroy et al. (2006) reported concentrations of β-carotene, violaxanthin, lutein, epoxy-lutein, and neoxanthin in creeping bentgrass as 5.8, 3.2, 19.1, 0.9, and 3.9 mg/100g FW. Perennial ryegrass in the current study produced higher concentrations of β-carotene, lower concentrations of violaxanthin, and similar concentrations of neoxanthin, lutein, and epoxy-lutein.  Concentrations were also similar to many green leafy vegetable and herbal crops.  Khachick et al. (1992) reported levels of β-carotene, violaxanthin, lutein, epoxy-lutein, and neoxanthin in raw unprocessed spinach (*Spinacia oleracea* L.) as 8.9, 7.4, 9.5, 0.5, and 2.4 mg/100 g FW, respectively.  Perennial ryegrass produced similar levels of β-carotene, violaxanthin, epoxy-lutein, and neoxanthin as those reported for spinach but higher levels of lutein.  Kopsell et al. (2007) reported levels of β-carotene, zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, lutein, and epoxy-lutein in various *Brassica* sp. as 3.5, 0.4, 0.6, 1.6, 1.8, 8.0, and 0.4 mg/100g FW.  Perennial ryegrass produced higher levels of all carotenoids.

**Photochemical Efficiency.**   Photochemical efficiency, measured as the chlorophyll fluorescence parameter $F_v/F_m$, varied due to treatment (Table 3C).  Treated plant photochemical efficiency (0.6490) was less than that of nontreated plants (0.7180). A treatment by harvest interval interaction was also observed.   Treated plant photochemical efficiency increased from 0.6334 3 DAT to 0.6738 21 DAT; whereas, nontreated plant photochemical efficiency decreased from 0.7218 7 DAT to 0.6991 21 DAT.  Previous research has shown that decreases in photochemical efficiency may occur due to increases in zeaxanthin concentration.  Zeaxanthin concentrations of treated perennial ryegrass plants 3 and 7 DAT were greater than those of nontreated plants.  It is

thought that zeaxanthin accumulation decreases photochemical efficiency by decreasing the size of light-harvesting antenna, and that zeaxanthin directly quenches singlet oxygen and free radicals of the chloroplast membrane (Baroli et al. 2003; Croce et al. 1999).

**Conclusions.** In summary, perennial ryegrass injury did not vary due to temperature or irradiance levels. Carotenoid levels of perennial ryegrass were similar to those reported in creeping bentgrass and many green leafy vegetable and herbal crops. Total carotenoid concentrations did not vary due to treatment with mesotrione or light intensity. In treated plants, HPPD inhibition indirectly inhibited phytoene desaturase which increased phytoene concentrations and decreased β-carotene concentrations. Total xanthophyll concentrations did not differ due to treatment with mesotrione; however, photoprotecting pigments zeaxanthin and antheraxanthin increased while violaxanthin decreased. The observed accumulation of zeaxanthin and accompanying drop in perennial ryegrass photochemical efficiency is consistent with previous studies and is thought to be, in part, due to a decrease in the size of light-harvesting antenna (Baroli et al. 2003; Croce et al. 1999). Clearly bleaching of perennial ryegrass turf is a concern to turfgrass managers; however, injury was limited to bleaching with no reduction in foliar weight and plants had recovered to an acceptable level 21 DAT. Temperature and irradiance levels did not influence visual mesotrione injury; however, turfgrass managers should use precaution when applying any herbicide to turf undergoing summer heat-stress.

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**Sources of Materials**

[1] Palmer IV perennial ryegrass. Proseeds Marketing Jefferson, OR.

[2] Environmental Growth Chambers, Chargrin Falls, OH.

[3] Harrells, Inc., PO Box 807, Lakeland, FL.

[4] Non-ionic surfactant; X-77® Spreader (Alkylphenol ethoxylate, alcohol ethoxylate, tall oil fatty acid, 2,2' dihydroxydithyl ether and dimethylpolysiloxane), Loveland Products, Inc, Greeley, CO.

[5] TeeJet Extended Range spray tips. Spraying Systems Co.,Wheaton, IL.

[6] OS1-F1 Modulated Fluorometer. Opti Sciences, Hudson NH.

[7] Potter-Elvehjem tissue grinder tube; Kontes, Vineland, NJ.

[8] Craftsman 15-inch drill press; Sears, Roebuck, and Co., Hoffman Estates, IL.

[9] N-EVAP 111; Orgnomation Inc., Berlin, MA.

[10] Econofilter PTFE 25/20; Agilent Technologies, Wilmington, DE.

[11, 13] Agilent Technologies, Palo Alto, CA.

[12] MAC-MOD Analytical Inc., Chadds Ford, PA.

[14] CaroteNature GmbH, Lupsingen, Switzerland, http://www.carotenature.com

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**Appendix C.**

**Table 1C.** Percent bleaching, percent necrosis, and photochemical efficiency ($F_v/F_m$) pooled over treatments as affected by harvest interval. All data were subjected to ANOVA (P = 0.05). Means of significant effects are seperated by Fisher's Protected LSD (P = 0.05).

| DAT | Chl *a*/*b* | Elu/Lu | Z+A+V | Z+A / Z+A+V | % Bleaching | % Necrosis | $F_v/F_m$ |
|---|---|---|---|---|---|---|---|
| 3 | 2.9 | 0.1 a | 9.5 a | 0.5 | 3.4 a | 0.0 b | 0.678 |
| 7 | 3.0 | 0.5 b | 8.5 b | 0.4 | 4.1 a | 0.8 a | 0.686 |
| 21 | 2.9 | 0.1 b | 7.9 b | 0.5 | 0.0 b | 1.4 a | 0.686 |
| P -value | ns [a] | 0.0001 | 0.0001 | ns | < 0.0001 | 0.002 | ns |

Abbreviations: DAT, days after treatment.

[a] Significance of DAT reaction terms when data were subjected to ANOVA (P = 0.05).

**Table 2C.** Carotenoid concentrations (mg/100g FW) pooled over treatments as affected by harvest interval.   All data were subjected to ANOVA (P = 0.05).   Means of significant effects are seperated by Fisher's Protected LSD (P = 0.05).

| DAT | Totals | Phytoene | β-Carotene | Zea-xanthin | Antera-xanthin | Viola-xanthin | Neo-xanthin | Lutein | Epoxy-lutein | Chl $a$ | Chl $b$ |
|-----|--------|----------|-----------|-------------|----------------|---------------|-------------|--------|--------------|--------|--------|
| | | | | | | mg/100g FW | | | | | |
| 3 | 46.0 a | 1.4 a | 9.2 a | 1.8 | 2.6 a | 5.1 a | 3.8 | 21.2 | 1.1 a | 204.4 a | 70.8 a |
| 7 | 41.4 b | 0.8 b | 8.5 ab | 1.6 | 2.2 b | 4.8 a | 3.4 | 19.4 | 0.9 b | 182.2 b | 61.9 b |
| 21 | 40.7 b | 0.7 b | 7.8 b | 1.7 | 2.2 b | 4.1 b | 3.4 | 19.6 | 0.9 b | 166.3 c | 57.1 b |
| P -value | 0.002 [a] | < 0.0001 | 0.007 | ns | 0.0004 | < 0.0001 | ns | ns | < 0.0001 | < 0.0001 | < 0.0001 |

Abbreviations:  DAT, days after treatment; FW, fresh weight.

[a] Significance of DAT reaction terms when data were subjected to ANOVA (P = 0.05).

**Table 3C.** Pigment concentrations, composition ratios, percent bleaching and necrosis, and $F_v/F_m$ of 'Palmer IV' perennial ryegrass due to mesotrione treatment by day after treatment interaction[a] and mesotrione treatment.

| | DAT | Treated | Nontreated | P -value |
|---|---|---|---|---|
| | | mg/100g fresh weight | | |
| Chlorophyll *a* | 3 | 196.0 | 212.3 | |
| | 7 | 171.0 | 193.3 | ns [b] |
| | 21 | 165.3 | 167.4 | |
| | Mean | 177.4 | 191.0 | 0.034 [c] |
| Chlorophyll *b* | 3 | 69.5 | 72.0 | |
| | 7 | 60.7 | 63.0 | ns |
| | 21 | 57.3 | 56.9 | |
| | Mean | 62.5 | 64 | ns |
| Chlorphyll *a/b* | 3 | 2.8 | 3.0 | |
| | 7 | 2.9 | 3.1 | ns |
| | 21 | 2.0 | 2.9 | |
| | Mean | 2.9 | 3 | ns |
| % Bleaching | 3 | 6.7 a | 0.0 b | |
| | 7 | 8.2 a | 0.0 b | < 0.0001 |
| | 21 | 0.4 b | 0.0 b | |
| | Mean | 5.1 | 0.0 | < 0.0001 |
| % Necrosis | 3 | 0.0 b | 0.0 b | |
| | 7 | 1.7 a | 0.0 b | 0.01 |
| | 21 | 2.5 a | 0.3 b | |
| | Mean | 1.4 | 0.1 | < 0.0001 |
| Fv/Fm | 3 | 0.633 d | 0.723 ab | |
| | 7 | 0.640 d | 0.732 a | 0.0003 |
| | 21 | 0.674 c | 0.699 bc | |
| | Mean | 0.649 | 0.718 | < 0.0001 |

[a] Means of significant effects are seperated by Fisher's Protected LSD (P = 0.05).
[b] Significance of treatment by DAT interaction term.
[c] Significance of treatment main effect pooled across DAT.

**Table 4C.** Pigment concentrations and composition ratios of 'Palmer IV' perennial ryegrass due to mesotrione treatment by day after treatment interaction[a] and mesotrione treatment.

| | DAT | Treated | Nontreated | P-value |
|---|---|---|---|---|
| | | mg/100g fresh weight | | |
| Total Carotenoids | 3 | 45.7 | 46.3 | |
| | 7 | 40.0 | 42.7 | ns [b] |
| | 21 | 41.3 | 40.2 | |
| | Mean | 42.3 | 43.5 | ns [c] |
| Phytoene | 3 | 2.7 a | 0.0 d | |
| | 7 | 1.7 b | 0.0 d | < 0.0001 |
| | 21 | 1.3 c | 0.0 d | |
| | Mean | 1.9 | 0 | < 0.0001 |
| β-Carotene | 3 | 8.4 | 9.9 | |
| | 7 | 7.6 | 9.5 | ns |
| | 21 | 7.5 | 8.1 | |
| | Mean | 7.8 | 9.1 | 0.0002 |
| Lutein | 3 | 20.2 | 22.1 | |
| | 7 | 18.4 | 20.5 | ns |
| | 21 | 19.4 | 19.7 | |
| | Mean | 19.4 | 20.8 | 0.02 |
| Epoxy-Lutein | 3 | 1.1 | 1.1 | |
| | 7 | 0.8 | 0.9 | ns |
| | 21 | 0.9 | 0.9 | |
| | Mean | 0.9 | 1.0 | ns |
| E-lutein/Lutein | 3 | 0.06 | 0.05 | |
| | 7 | 0.05 | 0.05 | ns |
| | 21 | 0.05 | 0.05 | |
| | Mean | 0.05 | 0.05 | ns |

[a] Means of significant effects are seperated by Fisher's Protected LSD (P = 0.05).
[b] Significance of treatment by DAT interaction term.
[c] Significance of treatment main effect pooled across DAT.

**Table 5C.** Pigment concentrations and composition ratios of 'Palmer IV' perennial ryegrass due to mesotrione treatment by day after treatment interaction[a] and mesotrione treatment.

| | DAT | Treated | Nontreated | P-value |
|---|---|---|---|---|
| | | mg/100g fresh weight | | |
| Zeaxanthin | 3 | 2.3 a | 1.4 cd | 0.02 [b] |
| | 7 | 1.9 ab | 1.2 d | |
| | 21 | 1.7 bcd | 1.7 bc | |
| | Mean | 2.0 | 1.4 | 0.0007 [c] |
| Antheraxanthin | 3 | 2.6 | 2.5 | |
| | 7 | 2.3 | 2.0 | ns |
| | 21 | 2.2 | 2.3 | |
| | Mean | 2.4 | 2.3 | ns |
| Violaxanthin | 3 | 4.7 | 5.4 | |
| | 7 | 4.4 | 5.1 | ns |
| | 21 | 4.1 | 4.1 | |
| | Mean | 4.4 | 4.9 | 0.0019 |
| Neoxanthin | 3 | 3.6 | 4.0 | |
| | 7 | 3.3 | 3.6 | ns |
| | 21 | 3.4 | 3.4 | |
| | Mean | 3.4 | 3.7 | ns |
| Z+A+V | 3 | 9.7 | 9.3 | |
| | 7 | 8.8 | 8.2 | ns |
| | 21 | 7.8 | 8.0 | |
| | Mean | 8.8 | 8.5 | ns |
| Z+A / Z+A+V | 3 | 0.5 a | 0.4 b | |
| | 7 | 0.5 a | 0.4 b | 0.002 |
| | 21 | 0.5 a | 0.5 a | |
| | Mean | 0.5 | 0.4 | < 0.0001 |

[a] Means of significant effects are seperated by Fisher's Protected LSD (P = 0.05).
[b] Significance of treatment by DAT interaction term.
[c] Significance of treatment main effect pooled across DAT.

# V. EFFECTS OF MESOTRIONE ON LARGE CRABGRASS CONTROL AND CAROTENOID CONCENTRATIONS UNDER VARYING ENVIRONMENTAL CONDITIONS

**Abstract**

Research was conducted to investigate the effects of mesotrione on large crabgrass (*Digitaria sanguinalis*) under varying environmental conditions. Large crabgrass was treated with mesotrione (0.28 kg ai/ha) and subsequently placed in an environmental growth chamber at 600, 1100, or 1600 $\mu$mol/m$^2$/s irradiance and 18, 26, or 34ºC. Leaf tissue was harvested 3, 7, and 21 days after treatment (DAT). Chlorophyll *a*, chlorophyll *b*, and total carotenoids were quantified spectrophotometrically. Percent bleaching, percent necrosis, foliar weight, and photochemical efficiency were recorded as an indication of mesotrione efficacy. Mesotrione control of large crabgrass was similar at temperature levels between 18 and 32ºC. Likewise, irradiance did not affect large crabgrass control as foliar weights were similar between 600 and 1600 $\mu$mol/m$^2$/s irradiance. However, treated and nontreated chlorophyll *a* concentrations were reduced at 1600 $\mu$mol/m$^2$/s irradiance. Mesotrione reduced large crabgrass chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations. Similarly, chlorophyll *a* to chlorophyll *b* ratios were decreased in treated plants. Mesotrione treated large crabgrass bleaching was highest 7 DAT and decreased 21 DAT. Treated plants were less than 10% necrotic 3 and 7 DAT but nearly 35% necrotic 21 DAT. Photochemical efficiency was reduced by mesotrione; however, trended towards recovery 21 DAT. For these reasons, secondary applications of mesotrione or other post-emergence herbicides may more effectively control large crabgrass when applied prior to 21 DAT.

Mesotrione is a carotenoid biosynthesis inhibitor which is currently labeled for use in turfgrass systems and maize (*Zea mayes* L.) production (Mitchell et al. 2001). Large crabgrass (*Digitaria sanguinalis* L. Scop) is an annual, grass weed which is selectively controlled by both preemergence and postemergence herbicide applications (Bhowmik and Bingham 1990; Dernoeden and Krouse 1991; Johnson 1975). Mesotrione control of large crabgrass has been reported to vary due to temperature and relative humidity. Johnson and Young (2002) reported that the influence of relative humidity and temperature on mesotrione efficacy is species dependent and that large crabgrass was more susceptible to mesotrione at 18°C than 32°C. Johnson and Young (2002) speculate that the decreased metabolism of $C_4$ plants in response to lower temperatures may decrease their metabolism of mesotrione. However, the role of temperature on herbicide efficacy is not supported consistently by the literature. High temperatures may increase fluidity of the cuticle and plasma membrane resulting in greater uptake of foliar applied herbicides; however, as temperature increases, the metabolic activity of the plant may increase and be of greater importance for some species (Johnson and Young 2002).

Symptoms in plants which are sensitive to mesotrione are bleaching followed by necrosis within 3-5 days after treatment (Vencil et al. 2002). Tissue whitening is a result of the inhibition of carotenoid biosynthesis and the destruction of existing chlorophyll (Mayonado et al. 1989; Mitchell et al. 2001). Mesotrione competitively inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) which prevents the biosynthesis of α-tocopherol and plastoquinone (Prysbilla et al. 1993). α-tocopherol is a known scavenger of reactive oxygen species (Trebst et al. 2002). Plastoquinone is responsible

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for electron transfer in the light dependent reactions of photosynthesis and is a cofactor for phytoene desaturase, a crucial enzyme of the carotenoid biosynthesis pathway (Norris et al. 1995). Carotenoids are $C_{40}$ isoprenoid compounds which form lipid soluble red, orange, and yellow pigments. Carotenoids are associated with photosynthetic light harvesting complexes (LHCs) where they transfer light energy to the photosynthetic reaction center and act in photoprotection by quenching free radicals, singlet oxygen, and other reactive oxygen species (Havaux 1998; Sandmann and Boger 1997). If carotenoids are not present in the photosystem or they are incapable of quenching excess energy, considerable damage and degradation of membranes may occur (Siefermann-Harms 1987).

Research was conducted to investigate the effects of mesotrione on large crabgrass control and pigment concentrations under varying environmental conditions. Understanding the effects of environmental conditions upon mesotrione efficacy may allow turfgrass managers to more effectively control large crabgrass.

## Materials and Method

Research was conducted at the University of Tennessee, Knoxville. Seeds of large crabgrass were planted approximately 0.5 cm deep in 12 cm diameter plastic pots (500 ml volume and 95 $cm^2$ surface area) containing silt-loam soil [Sequatichie loam soil (Fine-loamy, siliceous, semiactive, thermic Humic Hapludult) with pH 6.2 and 2.1% organic matter]. Seeds were germinated at 26ºC and 50% relative humidity in an environmental growth room[1] at 1100 μmol/m$^2$/s irradiance with a 16-hour photoperiod. Irradiance was provided by a mixture of metal halide and high pressure sodium lamps.

Throughout the experiment, pots were overhead irrigated twice daily to maintain adequate soil moisture, fertilized with a complete fertilizer[2] (5.0 g $N/m^2$) on a weekly basis prior to treatment and resuming 7 days after treatment (DAT), and randomized every two days to account for potential variation within the environmental growth room.

Pots were thinned to 2 plants per pot one week after planting. Pots were treated 16 days after emergence when plants were at the 1- to 2-tillers of growth stage with mesotrione at 0.28 kg ai/ha plus 0.25% v/v non-ionic surfactant[3] applied in a water carrier volume of 280 L/ha with a CO2-pressurized backpack sprayer equipped with 11002 XR flat fan nozzles[4] at 276 kPa. Plants were subsequently placed within an environmental growth room at 18, 26, or 34ºC. Irradiance levels (600, 1100, or 1600 $\mu mol/m^2/s$ irradiance) were achieved by manipulating the proximity of plants to the overhead light source. Due to the limited availability of environmental growth rooms, the three temperature regimes could not be conducted simultaneously. For this reason, great care was taken to ensure plants were of identical growth stage and size prior to treatment, and that they were fertilized and irrigated identically.

Four treated pots and four nontreated pots were randomly selected from each irradiance level 3, 7, and 21 days after treatment (DAT). Photochemical efficiency ($F_v/F_m$) ratings were taken mid-leaf on the second fully extended leaf of each plant as an indication of photoinhibition and overall plant health using a modulated fluorometer[5]. Percent bleached tissue and percent necrotic tissue were recorded visually as an indication of mesotrione efficacy. All plants were harvested at soil level and immediately frozen in liquid N then placed on ice for transfer to storage at -80ºC.

Prior to carotenoid extraction, plant material was weighed to obtain sample fresh-weights (FW). Large crabgrass stems were removed and samples were homogenized in liquid N using a mortar and pestle. A subsample weighing approximately 0.1 g was placed into a test tube (16 by 150 mm) with 10 mL of acetone. The sample was further homogenized for 30 seconds at 20,000 rpm with a Power Gen Model 125 Homogenizer[5]. The tube was then placed into a centrifuge for 10 minutes at 500 $g_n$. Supernatants were spectrophotometrically[6] analyzed for chlorophyll *a*, chlorophyll *b*, and total carotenoids (β-carotene plus xanthophylls) at absorbance spectra 661.6, 644.8, and 470.0 nm, respectively. Pigment concentrations were calculated from absorbance results according to previously published models (Table 1D) (Lichtenthaler 1987).

The experimental design was completely random with a two by three factorial treatment arrangement (two mesotrione treatments by three irradiance levels). Within each treatment scheme, harvest intervals (0, 7, and 21 DAT) were analyzed as samples. The experiment was conducted at three temperatures (18, 26, and 34ºC) with four replicates. A model with equal variance was fit to data and a likelihood ratio test was used to test if variance were unequal between temperatures. Independent analysis of temperatures was conducted, and visual verification confirmed that results were similar for each temperature. Equal variance among runs allowed for data pooling over temperatures. The Lavene test was used to test for equal variance among treatments. All data were subjected to ANOVA (P = 0.05). ANOVA results were used to select main effects. Means were seperated by LSD.

## Results and Discussion

Although temperature is known to influence mesotrione efficacy as well as carotenoid concentration, mesotrione control of large crabgrass did not differ due to temperature levels between 18 and 32ºC; therefore, all data were pooled across temperature. A treatment by harvest interval interaction was observed for percent bleaching, percent necrosis, foliar weight, and photochemical efficiency ($F_v/F_m$) (Table 2D). While nontreated large crabgrass bleaching was negligible ($\leq$ 1%), treated plant bleaching increased from 27% 3 DAT to 44% 7 DAT then decreased to 31% 21 DAT. Both treated and nontreated large crabgrass necrosis was minor 3 DAT (< 2%) and remained minor in nontreated plants. However, bleached tissue of treated plants quickly became necrotic. Treated plant necrosis increased to 8% 7 DAT and 35% 21 DAT. Large crabgrass FW of treated and nontreated plants were similar 3 DAT (5.7 and 7.3 g, respectively). As expected treated and nontreated large crabgrass fresh weights increased 7 and 21 DAT; however, nontreated plant fresh weights (11.2 and 24.2 g, respectively) were greater than those of treated plants (8.1 and 10.4 g, respectively).

Large crabgrass pigment concentrations have not previously been reported; however, our analysis shows that concentrations are comparable to many green leafy vegetable and herbal crops. Large crabgrass produced pigment concentrations comparable to many green leafy vegetable and herbal crops. Kopsell et al. (2007) reported levels of chlorophyll *a* and chlorophyll *b* in various *Brassica* sp. as 83.5 and 24.3 mg/100g FW. Similarly, nontreated large crabgrass chlorophyll *a* and chlorophyll *b* concentrations were 86.6 and 28.4 mg/100g FW. Chlorophyll *a* to chlorophyll *b* ratios

did not differ due to treatment with mesotrione; however, ratios pooled over treatment decreased with increasing harvest date (Table 3D). Ratios also varied due to irradiance level (Table 4D). Chlorophyll *a* to chlorophyll *b* was greatest in plants grown at 600 $\mu$mol/m$^2$/s irradiance (3.2:1). This difference is attributed to Chlorophyll *a* concentrations which differed due to irradiance level rather than chlorophyll *b* concentrations which did not. Chlorophyll *a* concentrations of plants grown at 600 $\mu$mol/m2/s irradiance (70.0 mg/100g FW) were greater than those of plants grown at 1600 $\mu$mol/m$^2$/s (62.0 mg/100g FW).

When evaluating large crabgrass pigment concentrations, a treatment by harvest interval interaction was observed for chlorophyll *a*, chlorophyll *b*, and total carotenoids (Table 2D). Treated plant chlorophyll *a* concentrations (45.4 mg/100g FW) were less than those of nontreated plants (86.6 mg/100g FW). Treated plant chlorophyll *a* concentrations were greater 3 DAT (59.0 mg/100g FW) than 7 and 21 DAT (38.5 and 38.7 mg/100g FW, respectively). Nontreated plant chlorophyll *a* concentrations were similar 3 and 7 DAT (94.6 and 92.7, respectively) and decreased 21 DAT (72.5 mg/100g FW). Treated plant chlorophyll *b* concentrations (14.5 mg/100g FW) were approximately half of concentrations observed in nontreated plants (28.4 mg/100g FW). Treated plant chlorophyll *b* concentrations were greater 3 DAT (17.7 mg/100g FW) than 7 and 21 DAT (12.5 and 13.3 mg/100g FW). Results are comparable to previous studies in which isoxaflutole, an HPPD inhibitor, was applied to 'Prelude' perennial ryegrass. Bhowmik and Drohen (2001) reported perennial ryegrass chlorophyll *a* concentrations decreased due to treatment with isoxaflutole. Chlorophyll degradation may be linked to

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PS II associated D1 protein destruction by reactive oxygen species. Reactive oxygen species are normally quenched by α-tocopherol and carotenoids; however, HPPD inhibition prevents the production of these photoprotecting compounds (Havaux 1998; Sandmann and Boger 1997; Trebst et al 2002 ).

Treated plant carotenoid concentrations (24.8 mg/100g FW) were less than those of nontreated plants (44.4 mg/100g FW) and varied due to DAT. Treated plant concentrations 3 DAT (29.8 mg/100g FW) were greater than 7 and 21 DAT (20.8 and 23.8 mg/100g FW). Nontreated plant concentrations were greater 3 and 7 DAT (46.4 and 46.8 mg/100g FW) than 21 DAT (39.8 mg/100g FW). Decreased carotenoid concentrations are attributed to HPPD-inhibition which increases phytoene concentrations due to the indirect inhibition of phytoene desaturase (Mayonado et al. 1989; Soeda and Uchida 1987). Phytoene is a precursor to lycopene, which is cyclized to form either α-carotene, precursor to lutein, or β-carotene, precursor to the xanthophyll cycle pigments zeaxanthin, antheraxanthin, and violaxanthin. Previous research has implicated zeaxanthin accumulation in decreasing the size of light-harvesting antenna. Carotenoids dissipate excitation energy from triplet chlorophyll and prevent the formation of singlet oxygen and other reactive oxygen species. Together these mechanisms operate in photoprotection of light harvesting complexes; at the same time, however, they lower photochemical efficiency (Baroli et al. 2003; Croce et al. 1999).

Large crabgrass photochemical efficiency, measured as the chlorophyll fluorescence parameter $F_v/F_m$, decreased due to treatment with mesotrione (Table 2D). Treated plant photochemical efficiency (0.235) was less than that of nontreated plants

(0.671) which remained similar 3, 7, and 21 DAT. Treated plant photochemical efficiency ratings decreased from 0.183 3 DAT to 0.053 7 DAT and then recovered to 0.470 21 DAT. Recovered photochemical efficiency occurred between 7 and 21 DAT. Previous research demonstrates secondary applications of mesotrione may be required for complete weed control. Large crabgrass may be more susceptible to secondary mesotrione applications prior to 21 DAT. Additionally mesotrione control of large crabgrass may decrease with increasing plant size (McCurdy et al. 2008). Frequent mesotrione applications may negatively affect sensitive crops. Crop and weed selectivity are due to differential absorption and metabolism of mesotrione and may differ for individual crop and weed sensitivity (Mitchell et al. 2001). Therefore, optimal application intervals may depend upon individual crop and weed sensitivity.

Conclusions

Our analysis shows that large crabgrass pigment concentrations are comparable to many green leafy vegetable and herbal crops. Mesotrione efficacy depends upon degradation of light harvesting and photosynthetic complexes. Limited or excessive light and temperature upon these complexes may affect mesotrione control of large crabgrass; however, our research could not confirm mesotrione control of large crabgrass differs due to temperature levels between 18 and 32ºC as FW responded similarly to mesotrione at all temperatures. Likewise, FW responded similarly to mesotrione at irradiance levels between 600 and 1600 $\mu$mol/m$^2$/s. Chlorophyll *a* concentration, which was highest in plants grown at 600 $\mu$mol/m$^2$/s, was the only pigment to vary due to irradiance level. Treated large crabgrass bleaching was highest and photochemical efficiency was lowest 7

DAT, which indicates some plant recovery occurs prior to 21 DAT.  For these reasons, secondary applications of mesotrione or other post-emergence herbicides may be more effective prior to 21 DAT.

## Sources of Materials

[1] Environmental Growth Chambers, Chargrin Falls, OH

[2] Harrells, Inc., PO Box 807, Lakeland, FL.

[3] Non-ionic surfactant; X-77® Spreader (Alkylphenol ethoxylate, alcohol ethoxylate, tall oil fatty acid, 2,2' dihydroxydithyl ether and dimethylpolysiloxane), Loveland Products, Inc, Greeley, CO.

[4] TeeJet Extended Range spray tips. Spraying Systems Co.,Wheaton, IL .

[5] Power Gen 125. Fisher Scientific, www.fishersci.com.

[6] OS1-F1 Modulated Fluorometer. Opti Sciences, Hudson NH.

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**Appendix D.**

**Table 1D.** Equations for determination of chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations. [a,b]

| Carotenoid | Equation |
|---|---|
| Chl *a* | $11.24\ A_{661.6} - 2.04\ A_{644.8}$ |
| Chl *b* | $20.13\ A_{646.8} - 5.10\ A_{663.2}$ |
| Carotenoids | $\dfrac{1000\ A_{470.0} - 1.90(\text{Chl } a) - 63.14(\text{Chl } b)}{214}$ |

[a] Pigment concentrations obtained by inserting the measured absorbance values are μg/mL plant extract solution which were converted to mg/100g large crabgrass fresh weight.

[b] Equations are based upon previously published research (Lictenthaler, 1987).

**Table 2D.** Percent bleaching, percent necrosis, fresh weight, and photochemcial efficiency (Fv/Fm) due to mesotrione treatment by day after treatment interaction[a] and mesotrione treatment.

|  | DAT | Treated | Nontreated | P-value |
|---|---|---|---|---|
|  |  | mg/100g fresh weight | | |
| % Bleaching | 3 | 26.5 b | 0.8 c |  |
|  | 7 | 43.9 a | 1.0 c | 0.0009[b] |
|  | 21 | 31.4 b | 0.7 c |  |
|  | Mean | 34 | 0.8 | < 0.0001[c] |
| % Necrosis | 3 | 1.9 c | 0.0 c |  |
|  | 7 | 8.2 b | 0.0 c | < 0.0001 |
|  | 21 | 34.9 a | 1.7 c |  |
|  | Mean | 15 | 0.6 | < 0.0001 |
| FW (g) | 3 | 5.7 d | 7.3 d |  |
|  | 7 | 8.1 cd | 11.2 b | < 0.0001 |
|  | 21 | 10.4 bc | 24.2 a |  |
|  | Mean | 8.1 | 14.2 | < 0.0001 |
| Fv/Fm | 3 | 0.1826 c | 0.6557 a |  |
|  | 7 | 0.0533 d | 0.6759 a | < 0.0001 |
|  | 21 | 0.4704 b | 0.6816 a |  |
|  | Mean | 0.2354 | 0.671 | < 0.0001 |

[a] Means of significant effects are seperated by Fisher's Protected LSD (P = 0.05).
[b] Significance of treatment by DAT interaction term.
[c] Significance of treatment main effect pooled across DAT.

**Tabel 3D.** Pigment concentrations (mg/100g FW) due to mesotrione treatment by day after treatment interaction[a] and mesotrione treatment..

|  | DAT | Treated | Nontreated | P-value |
|---|---|---|---|---|
|  |  | mg/100g fresh weight | | |
| Chl *a* / *b* | 3 | 3.3 | 3.3 | |
| | 7 | 3.1 | 3.1 | ns[a] |
| | 21 | 2.9 | 2.9 | |
| | Mean | 3.1 | 3.1 | ns[b] |
| Chl *a* | 3 | 59.0 c | 94.6 a | |
| | 7 | 38.5 d | 92.7 a | 0.0012 |
| | 21 | 38.7 d | 72.5 b | |
| | Mean | 45.4 | 86.6 | < 0.0001 |
| Chl *b* | 3 | 17.7 c | 28.7 a | |
| | 7 | 12.5 d | 30.9 a | 0.0015 |
| | 21 | 13.3 d | 25.5 b | |
| | Mean | 14.5 | 28.4 | < 0.0001 |
| Total Carotenoids | 3 | 29.8 c | 46.4 a | |
| | 7 | 20.8 d | 46.8 a | 0.0017 |
| | 21 | 23.8 d | 39.8 b | |
| | Mean | 24.8 | 44.4 | < 0.0001 |

[a] Means of significant effects are seperated by Fisher's Protected LSD (P = 0.05).
[b] Significance of treatment by DAT interaction term.
[c] Significance of treatment main effect pooled across DAT.

**Table 4D.** Percent bleaching, percent necrosis, photochemcial efficiency ($F_v/F_m$), and pigment concentrations (mg/100g FW) pooled over treatments as affected by harvest interval. All data were subjected to ANOVA (P = 0.05). Means of significant effects are seperated by Fisher's Protected LSD (P = 0.05).

| DAT | % Bleaching | % Necrosis | FW (g) | $F_v/F_m$ | Chl $a$/$b$ | Chl $a$ | Chl $b$ | Total Carotenoids |
|---|---|---|---|---|---|---|---|---|
| 3 | 13.7 b | 1.0 c | 6.5 c | 0.419 b | 3.3 a | 76.8 a | 23.2 a | 38.1 a |
| 7 | 22.4 a | 4.1 b | 9.7 b | 0.365 c | 3.1 b | 65.6 b | 21.7 a | 33.8 b |
| 21 | 16.0 b | 18.3 a | 17.3 a | 0.576 a | 2.9 c | 55.6 c | 19.4 b | 31.8 b |
| P-value | 0.0006[a] | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.0021 | 0.0002 |

Abbreviations: fresh weight, FW; days after treatment, DAT.

[a] Significance of DAT reaction terms when data were subjected to ANOVA (P = 0.05).

**Table 5D.** Percent bleaching, percent necrosis, photochemcial efficiency ($F_v/F_m$), and pigment concentrations (mg/100g FW) pooled over treatments as affected by irradiance level. All data were subjected to ANOVA (P = 0.05). Means of significant effects are seperated by Fisher's Protected LSD (P = 0.05).

| $\mu mol/m^2/s$ | % Bleaching | % Necrosis | FW (g) | $F_v/F_m$ | Chl $a$ /$b$ | Chl $a$ | Chl $b$ | Total Carotenoids |
|---|---|---|---|---|---|---|---|---|
| 600 | 17.6 | 7.6 | 12.4 | 0.465 | 3.2 a | 70.0 a | 22.0 | 33.8 |
| 1100 | 17.1 | 8.1 | 10.7 | 0.451 | 3.0 b | 66.0 ab | 22.0 | 36.1 |
| 1600 | 17.3 | 7.6 | 10.4 | 0.444 | 2.8 b | 62.0 b | 22.0 | 33.9 |
| P-value | ns[a] | ns | ns | ns | 0.0056 | 0.0292 | ns | ns |

Abbreviations: fresh weight, FW; days after treatment, DAT.

[a] Significance of DAT reaction terms when data were subjected to ANOVA (P = 0.05).

# VITA

James Dewey McCurdy, son of Bob and Suzanne McCurdy, was born the 21$^{st}$ of July, 1984. He grew up on a small farm in North West Tennessee, where his family grew row-crops, livestock, and turfgrass. James graduated from Gibson County High School in May, 2002 and entered the University of Tennessee, Martin, where in 2006 he earned a Bachelor of Agriculture degree in Plant Sciences. James was appointed a Graduate Research Assistantship August 2006 at the University of Tennessee, Knoxville. He received a Masters of Science degree in Plant Sciences in May 2008.