Elucidating Influence of Temperature and Environmental Stress on Turfgrass Response to Mesotrione and Evaluation of Potential Synergistic Admixtures to Improve Mesotrione Efficacy

Daniel B. Ricker

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

Masters of Science

In

PLANT PATHOLOGY, PHYSIOLOGY, AND WEED SCIENCE

Shawn Askew, Chair

Erik Ervin

Lloyd Hipkins

December 14, 2006

Blacksburg, Virginia

Keywords: Carotenoids, chlorophyll, mesotrione, environmental stress, and smooth crabgrass.

ABSTRACT

Smooth crabgrass (*Digitaria ischaemum* Schreb. *ex* Muhl.) is a problematic annual weed in managed coolseason turfgrass. Currently, turfgrass managers can control smooth crabgrass preemergence and postemergence with only a few selective herbicides. Mesotrione is a new chemical compound that has proven effective in controlling mature crabgrass with minimal coolseason turfgrass injury. Mesotrione controls weeds by blocking an enzyme called *p*hydroxyphenylpyruvate dioxygenase (HPPD). HPPD inhibition leads to a block in the biosynthesis of plastoquinone (PQ). PQ is an essential cofactor for phytoene desaturase, thus HPPD inhibitors disrupt carotenoid biosynthesis and cause tissue bleaching. Bleaching in a golf course management system is undesirable. Mesotrione was evaluated with other postemergent admixtures to alleviate bleaching, while providing acceptable smooth crabgrass control. Also, environmental conditions were evaluated to test potential mesotrione bleaching on tolerant perennial ryegrass.

AUTHOR’S ACKNOWLEDGEMENTS

Appreciation is expressed to Shawn Askew for granting me the opprotunity for this degree and working with me to help it be a success. To Erik Ervin and Lloyd Hipkins , thank you for being part of my committee and offering advice on how to improve my project. To everyone else working in Plant Pathology and Weed Science at Virginia Tech, all the people at Glade Road Research Facility and the Turfgrass Research Fields, I thank you for making this project a sucess. To my friends ands family thanks for sticking with me through the ups and downs, God bless you all.

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OBJECTIVES

(1) Evaluate mesotrione applied alone and in combination with potentially synergistic herbicides to improve late-season weed control and possibly reduce the number of mesotrione treatments.

(2) Determine perennial ryegrass response to mesotrione during high air temperature and other environmental stresses, including: leaf wetness, photosynthetically active radiation (PAR), soil moisture, solar radiation (SR) and soil temperature.

Introduction

Overview of Mesotrione. The discovery of mesotrione began in 1977 when a U.S. scientist observed few weeds growing underneath a bottlebrush (*Callistemon citrinus*) plant. A detailed soil analysis revealed an allopathic chemical was being released. The compound released was leptospermore, a chemical previously isolated from the oil of an Australian myrtaceous plant (Mitchell et al. 2001). Further evaluation of leptospermore showed it produced bleaching symptoms on an array of broadleaf and grassy weeds at 1.0 kg ha^{-1}. Subsequent to the work with leptospermore, triketones were discovered in 1982. These compounds became broad-spectrum preemergent and postemergent herbicides on grasses and broadleaves, with excellent corn tolerance. Mesotrione can effectively control weeds at 100-225 g ha^{-1} as a preemergent or 70-150 g ha^{-1} as a postemergent application (Lee et al. 1997).

Mesotrione has a dissociation constant (pKa) of 3.12 at 20 C making it a weak acid. Because of mesotrione's weak acidity the degree of ionization is dependent on pH, resulting in pH dependency for water solubility. Mesotrione water solubility ranges from 2.2 g $litre^{-1}$ at pH 4.8 (20 C) to 22 g $litre^{-1}$ at pH 9 (20 C). High water solubility at neutral pH results in ideal plant uptake and translocation.

Plant uptake of radio labeled mesotrione applied to corn, common lambquarters, giant foxtail (*Setaria faberi* Herrm), and ivyleaf morningglory (*Ipomoea hederacea* (L.) Jacq.*)* was evaluated 1, 6, and 24 hours after application. Uptake in each weed species was rapid, with 55 to 90% of the applied compound absorbed after 24 hours (Mitchell et al. 2001). Following a foliar application, [^{14}C] mesotrione is acropetally and basipetally translocated throughout the plant. When applied to common lambquarters, 48% of applied radioactivity moved out of the treated leaf seven days after treatment and analysis of the total extract showed that 42% was still parent mesotrione. In contrast, 14% of applied radioactivity moved out of the treated corn leaf seven days after treatment but analysis did not yielded any parent mesotrione. Apparently, rapid metabolism of mesotrione is the primary mechanism of tolerance in corn compared to other species (Mitchell et al. 2001).

Mitchell et al. (2001) evaluated mesotrione metabolism in whole shoots of corn, barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.*)*, common lambquarters, giant foxtail, ivyleaf morningglory, large crabgrass (*Digitaria sanguinalis* (L.) Scop.*)*, and redroot pigweed (*Amaranthus retroflexus* L.*)* treated with [^{14}C] mesotrione at 72 g ha^{-1} and evaluated seven days after treatment for the presence of unchanged parent compounds. Results found that the metabolism of mesotrione was slower in the three broadleaf weeds, while corn, large crabgrass, giant foxtail, and barnyardgrass all metabolized mesotrione rapidly. Large crabgrass metabolized mesotrione the fastest of the grassy weeds tested. Corn metabolized mesotrione the fastest out of all plants tested indicating that the selectivity of mesotrione is based on plant metabolism. It also may explain the need for multiple applications to the same weed species, such as crabgrass with, faster mesotrione metabolism (Mitchell et al. 2001).

Inhibition of phytoene desaturase leads to the disruption of carotenoid biosynthesis, a mechanism previously believed to be the only way of causing bleaching symptoms in target plants. After the discovery of leptospermore from the bottlebrush plant, researchers began to examine how other mechanisms in the plant may affect carotenoid biosynthesis by indirectly disrupting phytoene desaturase. Lee et al. (1997) discovered that members of the triketone herbicide family target the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD). HPPD inhibition leads to a block in the biosynthesis of plastoquinone (PQ). PQ is an essential cofactor for phytoene desaturase, thus HPPD inhibitors disrupt carotenoid biosynthesis and cause tissue bleaching (Hess 2000; Lee et al. 1997).

Literature evaluating mesotrione on cool-season turf has increased over the years. Beam et al. (2006) studied mesotrione for creeping bentgrass control in cool-season turf. Askew et al. (2003), Beam et al (2006) and Bhowmik and Drohen (2001) reported control of difficult to control perennial weeds and other grassy and broadleaf weeds using HPPD inhibiting herbicides. They also noted discoloration of desired turf, as treatment rates increased and under certain environmental conditions. Bhowmik and Drohen (2001) noted that tolerant cool-season turfgrass species may metabolize mesotrione faster than susceptible species and some cultivars of the same species may be more susceptible to HPPD inhibiting herbicides. Similar with Askew et al. (2003), Bhowmik and Drohen (2001) observed injury to tolerant species under summer stress. In order to safely recommend HPPD inhibiting herbicides to control difficult weeds in turfgrass, environmental conditions such as moisture stress or high air temperature must be evaluated to ensure applications do not cause undesired cool-season turfgrass injury.

Moisture stress symptoms in plants occur when the transpiration rates exceed water uptake. Plant roots trigger a reduction in stomatal conductance, abscisic acid (ABA) is produced, and cytosolic Ca^{2+} concentrations are elevated, forcing stomatal closure. As the stomates begin to close, photosynthesis and plant growth rate decline, due to alterations in nitrogen metabolism and reduced carbon availability. Other mechanisms to combat moisture stress include the production of osmolyte and the activation of genes to produce new proteins available for plant tissue survival (Mata and Lamattina 2001).

Herbicide efficacy and safety is affected by environmental conditions (Hinz and Owen 1994; Reynolds et al. 1988). Factors affecting herbicide fate include plant morphology which may disrupt herbicide retention and absorption and plant physiology which may affect translocation and metabolism (Petersen and Hurle 2001; Reynolds et al. 1988). Research has demonstrated that broadleaf postemergence herbicides applied to plants under water stress show differential herbicide retention, absorption, translocation, and metabolism. However, results from research assessing similar conditions for postemergence annual grass herbicides were inconclusive (Kidder and Behrens 1988).

Environmental effects on Mesotrione activity. In the transition zone, cool-season turf is subjected to an assortment of summer stresses, including: air temperature, relative humidity, soil moisture, and light intensity. Moisture stress can last for prolonged periods in non-irrigated turf and cause severe reduction in turfgrass quality. Research has show a reduction in Kentucky bluegrass, perennial ryegrass, and tall fescue dry root weight, leaf water potential, and photochemical efficiency under water stress (Jiang and Haung 2000). Research has also reported cool-season turf tolerance to moisture stress by preconditioning. Purposely exposing plants to moisture stress prior to stressful conditions enables the plant to more effectively protect itself. This methods essentially trains the plant to reduce osmotic potential and enhance stomatal opening which allows for a higher photosynthetic rate than a typical plant under moisture stress. Moisture stress preconditioning effectively prepares the plant to maintain normal functions longer, reducing turfgrass injury and allowing for rapid recovery (Bennett and Sullivan 1981; Thomas et al. 1976).

Previous research has shown that turfgrass species, tolerant to herbicides under normal growing conditions, may become injured when applications are made during periods of moisture stress (Dernoeden 2002). Mesotrione safety on Kentucky bluegrass, tall fescue, and perennial ryegrass has been demonstrated under normal growing conditions but evaluations during periods of environmental stress have not been conducted (Askew et al. 2003). Research using acifluorfen, fluazifop, and glyphosate have shown that herbicidal activity is influenced by air temperature, relative humidity, and moisture availability (Alscher and Hess 1993; Kells et al. 1984; McWhorter 1981; McWhorter and Azlin 1978; Wichert et al. 1992; Wills 1984; Wills and McWhorter 1981).

Air temperature can affect postemergence herbicide efficacy and herbicide absorption (Fausey and Renner 2001; Hammerton 1967; Johnson and Young 2002; Watlz et al. 2004). Nimbal (1996) reported MSMA controlled common cocklebur (*Xanthium strumarium* L.) but efficacy depended on climatic conditions during and after treatment. MSMA control of wild mustard (*Brassica kaber* (D.C.) L.C. Wheeler) and wild oat (*Avena fatura* L.) increased when temperature increased from 10 C to 30 C (Miller et al. 1981). Glyphosate provided better johnsongrass (*Sorghum halepense* (L.) Pers.) contol at 35 C than at 24 C or 29 C (Jordan 1977; McWhorter 1981; McWhorter and Azlin 1978). Research indicates that glyphosate applied at 32 C controlled bermudagrass more effectively than a similar application made at 24 C (Jordan 1977). Other research showed bentazon controlled redroot pigweed (Wichert et al. 1992) and common ragweed (*Ambrosia artemisiifolia* L.) more at increased temperature and humidity (Ritter and Coble 1981).

Isolating temperature as a primary factor related to herbicide efficacy is a difficult process because temperature influences plant germination, growth rate, and metabolic activity, all of which impacting herbicide efficacy (Bayer 1987). Bayer (1987) divided temperature influence into three categories: pre-spray, at time of application, and post-spraying

<u>Pre-spray:</u> Prior to herbicide treatment plants are developing and undergoing changes anatomically and physiologically. Biochemical processes are directly affected

by temperature changes in the environment. Higher temperatures tend to produce thicker cuticles, reduce stomatal openings, and change gas exchange. These morphological processes facilitate plant survival during high temperatures.

Cuticle enlargement is directly related to higher temperatures and prevents water loss. However, a thicker cuticle efficiently prevents foliar applied herbicide from entering the leaf, limiting herbicide absorption. Temperatures prior to spraying have a direct effect on metabolic activity in the plant.

<u>Time of Application</u>: Air temperatures at the time of treatment can also influence herbicide efficacy. Herbicide efficacy is related spray droplet retention on the leaf surface. Air temperature that causes rapid dehydration of spray droplets reduces herbicide concentration on the leaf and plant uptake. Herbicide applied to plants must remain in a position that allows for leaf absorption. Therefore, treatments designed to avoid the hottest part of the day are more effective (Bayer 1987).

<u>Post-application</u>: Air temperature after a treatment influences physiological processes in the plant that may impact herbicide activity and efficacy. Generally, an increase in air temperature increases herbicide activity inside the plant. Uptake and penetration increase with an increase in air temperature to a certain degree. Once a threshold is reached, the plant reduces uptake and focuses on survival mechanisms (Bayer 1987; Price 1983).

Air temperatures are believed to affect translocation of the herbicide through the xylem and phloem. Non-published field observations indicate that fluctuating temperatures may cause more toxicity than constant temperature (Cudney 1987). McWhorter (1981) reported an increase in air temperature from 18 to 35 C resulted in a four fold increase of [^{14}C] metriflefen translocation in johnsongrass. Jordan (1977) and

Wills and McWhorter (1981) reported more [^{14}C] glyphosate translocation and absorption at 32 C than 22 C in bermudagrass and cotton (*Gossypium hirsutum* L.).

Environmental conditions may be responsible for variable weed control observed with foliar applied mesotrione (Johnson and Young. 2002). C_4 plants, common waterhemp *(Amaranthus rudis* Sauer*)* and large crabgrass were more susceptible to mesotrione at 18 C compared to 32 C. This is contrary to C_3 plants in previous research involving temperature and herbicide efficacy (Kells et al. 1984; McWhorter and Azlin 1978; Wills and McWhorter 1981). Physiological difference in plant metabolism is believed to be the reason why mesotrione was less effective at higher temperatures. C_4 plants have increased growth and metabolic activity compared to C_3 plants at higher temperatures (Wills 1984). At low air temperatures, mesotrione applied to C_4 plants metabolizes slower, increasing herbicide efficacy (Johnson and Young 2002). Mesotrione is less effective on C_4 plants than C_3 plants at higher temperatures due to increased C_4 metabolism. However, C_3 plants may be more injured by mesotrione at high air temperatures due to temperature stress and reduced metabolic activity (Johnson and Young 2002). Mesotrione acts selectively to control a variety of annual broadleaves, grasses, and perennial weeds in Kentucky bluegrass, perennial ryegrass, and tall fescue, all C_3 grass species. Thus, previous work suggests that high temperature may lead to increased injury on C_3 turfgrasses treated with mesotrione but this hypothesis has not been tested.

Improving smooth crabgrass control with mesotrione admixtures. Smooth crabgrass (*Digitaria ischaemum* Schreb. *ex* Muhl.) is a problematic annual weed in managed cool-season turfgrass. Currently, turfgrass managers can control smooth crabgrass preemergence and postemergence. Preemergence herbicides include bensulide, dithiopyr, oryzalin, oxadiazon, pendimethalin, prodiamine, and trifluralin (Askew and

Hipkins 2005). Treatments must be applied prior to climatic conditions favorable for smooth crabgrass germination (Beard 2002; Christians 2004; Monaco 2002; Reicher 1999). If treatments are applied too late, preemergence herbicides will be ineffective in controlling established smooth crabgrass. Likewise, smooth crabgrass may escape control sooner than expected because environmental conditions influence residual time for preemergence treatments and smooth crabgrass may escape treatment sooner than labeled predictions (Beard 2002; Christians 2004). In such instances, smooth crabgrass that escapes preemergence treatments must be controlled with postemergence herbicides. Currently, DSMA, fenoxaprop-ethyl, MSMA, and quinclorac are registered to control emerged smooth crabgrass in cool-season turf (Askew and Hipkins 2005).

A common management practice is to delay preemergence herbicide treatments until seedlings are observed and tank-mix postemergence and preemergence herbicides to control smooth crabgrass seedlings and extend residual control (Beard 2002; Christians 2004; Monaco 2002). Tank-mixing minimizes environmental impact by reducing the unnecessary application of preemergence herbicide on non-infested areas (Johnson 1994; 1996). Green (1991) reported that the best method of reducing herbicide rates, while still providing satisfactory control, was to tank-mix herbicides. Researchers at The Pennsylvania State University reported excellent control of smooth crabgrass 12 weeks after treatment with a postemergence and preemergence tank-mix (Watschke et al. 2005). Tank-mixing two or more herbicides in a turfgrass management system also controlled broadleaf and grassy weeds, reduced application time, lowered cost, and delayed weed resistance from developing (Johnson 1996). However, tank-mixed herbicides have shown mixed results in different locations around the United States. In Maryland, tank-mix combinations demonstrated less control than non tank-mixed treatments (Dernoeden et al. 2003). Herbicide and plant physiology can impact tank-mixtures (Zhang et al. 1995).

Zhang (1995) concluded that herbicides from the same chemical family had a higher frequency of synergism than herbicides from different chemical families.

The cool-season turf growth cycle consists of rapid growth in the spring, slowed growth in the summer, and rapid growth in the fall. Using preemergence or postemergence herbicides to control smooth crabgrass competition early in the season allows cool-season turfgrasses to more efficiently utilize nutrients, water, space, and light to develop healthy foliage and roots prior to summer stress (Beard 2002; Christians 2004; Turgeon 1991). In situations where preemergence treatments breakdown rapidly or early postemergence treatments do not completely control smooth crabgrass, late season infestations are likely. Smooth crabgrass controlled late in the season provides cool-season turf uninterrupted and accelerated growth during a peak growth period. Fall growth of cool-season turf provides the plant roots with carbohydrates to store during winter dormancy (Beard 2002; Christians 2004; Turgeon 1991). Hence, late season smooth crabgrass is beneficial to cool-season turfgrass health, quality and rapid spring greenup (Beard 2002; Christians 2004). Research has also shown that early summer postemergence treatments at the 1 to 3 leaf stage provide better smooth crabgrass control than the same application made at the 2 to 3 tiller stage (Chism and Bingham 1991; Dernoeden et al. 1992; Johnson 1996; 1997).

Mature smooth crabgrass is difficult to control and repeated postemergence herbicide treatments often do not provide adequate control (Dernoeden et al. 2003). In corn (*Zea mays* L.*) r*esearch has shown that mesotrione combined with atrazine and applied postemergence controlled larger or more difficult to control weeds than either herbicide alone (Armel et al. 2005; Armel et al. 2003; Whaley et al. 2006). Postemergence crabgrass herbicides effectively control smooth crabgrass populations but often control few other weeds. Mesotrione effectively controls a wide variety of broadleaf, grassy, and difficult to control perennial weeds while affording safety on Kentucky bluegrass (*Poa pratensis* L.*)*, perennial ryegrass (*Lolium perenne* L.*)*, and tall fescue (*Festuca arundinacea* Schreb.) (Askew et al. 2003). Mesotrione controls annual sowthistle (*Sonchus cleraceus* L.*)*, black medic (*Medicago lupulina* L.*)*, seedling broadleaf plantain (*Plantago major* L.*)*, crabgrass (*Digitaria spp.*), common lambquarters (*Chenopodium album* L.*)*, common purslane (*Portulaca oleracea* L.), Virginia buttonweed, (*Diodia virginiana* L.*)*, white clover (*Trifolium repens* L.*)*, and yellow woodsorrel (*Oxalis stricta* L.) (Askew et al. 2003; Keese et al. 2005). It also has demonstrated selective control of creeping bentgrass (*Agrostis stolonifera* L.*)* and nimblewill (*Muhlenbergia schreberi* J.F. Gmel.*)* in cool-season golf course roughs and home lawns, while sequential applications of mesotrione has suppressed or controlled common bermudagrass (*Cynodon dactylon* (L.) Pers.) (Beam et al. 2006). However, Askew et al. (2003) observed mesotrione treatments occasionally caused unacceptable and unexplained cool-season turfgrass injury. Furthermore, mesotrione seldom controls weeds with one application, but controls several troublesome species when applied two or more times in sequence (Askew et al. 2003).

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Evaluation of Potential Admixtures to Improve Postemergence Smooth Crabgrass (*Digitaria ischaemum* Schreb. *ex* Muhl.) Control in Cool-season Turfgrass with Mesotrione.

DANIEL B. RICKER AND SHAWN D. ASKEW[1]

Abstract: Mesotrione is under evaluation for registration in turfgrass for weed control, but often requires repeat treatments. Previous research in agricultural crops indicates tank mixtures with mesotrione may improve weed control. Three field trials were conducted in 2005 and 2006 in Blacksburg, VA on smooth crabgrass in perennial ryegrass and tall fescue. Data indicate mesotrione applied in combination with bentazon, bromoxynil, or carfentrazone, controlled smooth crabgrass better than any of these herbicides applied alone at all sites. Adding mesotrione to MSMA and quinclorac improved smooth crabgrass on of three sites. Sequential mesotrione applications improved long term weed control.

Nomenclature: Basagran; bromoxynil; carfentrazone; mesotrione; MSMA; quinclorac; smooth crabgrass, *Digitaria ischaemum* Schreb. *ex* Muhl.#[2]DIGSA; Kentucky bluegrass, *Poa pratensis* L. 'Kelly' #POAPR Perennial ryegrass, *Lolium perenne* L. 'Unknown' #LOLPE.

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[1] Graduate Research Assistant and Assistant Professor respectively, Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, 435 Old Glade Road, Virginia Tech Box 0330, Blacksburg, VA 24060-0330, Corresponding author's E-mail: saskew@vt.edu.

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

Additional index words: Postemergence herbicides, synergism, turfgrass injury,

Abbreviations: fb, followed by; POST, postemergence; DAT, days after treatment**.**

INTRODUCTION

Smooth crabgrass (*Digitaria ischaemum* Schreb. *ex* Muhl.) is an annual problematic weed on managed cool-season turf throughout the country. Currently, turfgrass managers can control smooth crabgrass preemergence or postemergence. Preemergence herbicides include bensulide, dithiopyr, oryzalin, oxadiazon, pendimethalin, prodiamine, and trifluralin (Askew and Hipkins 2005). Treatments must be applied at the appropriate time preceding climatic conditions favorable for smooth crabgrass germination (Beard 2002; Christians 2004; Reicher et al. 1999). If treatments are applied too late, preemergence herbicides will be ineffective in controlling established smooth crabgrass. Likewise, smooth crabgrass may escape control sooner than expected because environmental conditions influence residual time for preemergence treatments and smooth crabgrass may escape treatment sooner than labeled predictions (Beard 2002; Christians 2004). Therefore, smooth crabgrass that escapes preemergence treatments must be controlled with postemergence herbicides. Currently, DSMA, fenoxaprop-ethyl, MSMA, and quinclorac are registered to control emerged smooth crabgrass in cool-season turf (Askew and Hipkins 2005).

A common turfgrass management practice is to delay preemergence herbicide treatments until seedlings are observed and tank-mix postemergence and preemergence herbicides to control smooth crabgrass seedlings and extend residual control (Beard 2002; Christians 2004). Tank-mixing minimizes environmental impact and may reduce wasted preemergence herbicide on non-infested areas (Johnson 1994; 1996). Green (1991) reported that the best method of minimizing effective herbicide rates, yet still providing control, was to tank-mix herbicides. Researchers at The Pennsylvania State University reported excellent control of smooth crabgrass 12 weeks after treatment with a postemergence and preemergence tank-mix (Watschke et al. 2005). Tank-mixing two or more herbicides in a turfgrass management system also controlled broadleaf and grassy weeds, reduced application time, lowered cost, and delayed weed resistance from developing (Johnson 1996). However, tank-mixed herbicides have shown diverse results in different locations around the United States. In Maryland, tank-mix combinations demonstrated less control than non tank-mixed treatments (Dernoeden et al. 1992). Herbicide and plant physiology can impact tank-mixtures (Zhang et al. 1995). Zhang (1995) concluded that herbicides from the same chemical family had a higher frequency of synergism than herbicides from different chemical families.

Mature smooth crabgrass is difficult to control and repeated postemergence herbicide treatments often do not provide adequate control (Dernoeden et al. 2003). In corn (*Zea mays* L.*) r*esearch has shown that mesotrione combined with atrazine and applied postemergence controlled larger or more difficult weeds than either herbicide alone (Armel et al. 2005; Armel et al. 2003; Whaley et al. 2006). Postemergence crabgrass herbicides effectively control smooth crabgrass populations but often control few other weeds. Mesotrione effectively controls a wide variety of broadleaf, grassy, and difficult to control perennial weeds while maintaining safety on Kentucky bluegrass (*Poa pratensis* L.*),* perennial ryegrass (*Lolium perenne* L.*),* and tall fescue (*Festuca arundinacea* Schreb.) (Askew et al. 2003). Mesotrione controls annual sowthistle (*Sonchus cleraceus* L.*),* black medic (*Medicago lupulina* L.*)*, seedling broadleaf plantain (*Plantago major* L.*),* crabgrass (*Digitaria spp.*), common lambquarters (*Chenopodium album* L.*),* common purslane (*Portulaca oleracea* L.), Virginia buttonweed, (*Diodia virginiana* L.*),* white clover (*Trifolium repens* L.*)*, and yellow woodsorrel (*Oxalis stricta*

L.) (Askew et al. 2003; Keese et al. 2005). Research demonstrated selective control of creeping bentgrass (*Agrostis stolonifera* L.*)* and nimblewill (*Muhlenbergia schreberi* J.F. Gmel.*)* from cool-season golf course rough and home lawns, while sequential applications of mesotrione has suppressed or controlled common bermudagrass (*Cynodon dactylon* (L.) Pers.) (Beam et al. 2006). Furthermore, mesotrione seldom controls weeds when applied once, but controls several troublesome species when applied two or more times in sequence (Askew et al. 2003).

MATERIAL AND METHODS

Three field trials were conducted at the Virginia Tech Turfgrass Research Center in Blacksburg, VA during the summers of 2005 and 2006. Site one (S1) and site three (S3) contained an unknown variety of turf type tall fescue (*Festuca arundinacea* Schreb.*)* and 'Kelly' Kentucky bluegrass (*Poa pratensis* L.*)*. Site two (S2) contained a variety of cool-season and warm-season grasses, mainly fescue (*Festuca* spp) and perennial ryegrass (*Lolium perenne* L.*)*, with areas of common bermudagrass (*Cynodon dactylon* (L.) Pers.) and creeping bentgrass (*Agrostis stolonifera* L.*)*, all varieties unknown. Each site was treated with Trimec Classic™ at 9.6 L/ ha to control broadleaf weeds 4 weeks prior to trial initiation. S1 and S2 were severely infested (>80% cover) with smooth crabgrass (*Digitaria ischaemum* Schreb. *ex* Muhl.) while S3 was moderately infested (40% to 60% cover). Prior to initiation in 2005, the growth stage of smooth crabgrass was the 3 to 4 tiller stage while in 2006, the growth stage was 1 to 2 tillers. Each site was mowed twice per week at 6.35 cm with clippings returned and fertilized on a monthly basis with 10-10-10 at 243.9 kg/ha to deliver 24.39 kg/ha of nitrogen [N], phosphorus [P], and potassium [K] per application. Supplemental irrigation was implemented on all research sites as needed to prevent turf wilt. The soil at site one and two was Groseclose loam (clayey, mixed, mesic, Typic Hapludalfs) with pH 6.0 and 1.8% organic matter. S3 was also Groseclose soil type but with a pH of 5.5 and organic matter 2.3%.

For all trials, the experimental design was a randomized complete block with treatments arranged in a five-by-two-by-two factorial and replicated four times. Plots were 1.82 m by 1.82 m plot at S1 and S2 and 1.21 m by 2.45 m at S3. The first factor consists of five post emergence herbicides; bentazon[3] at 0.28 kg ai/ha, bromoxynil[4] at 0.28 kg ai/ha, carfentrazone-ethyl[5] at 0.035 kg ai/ha, MSMA[6] at 1.68 kg ai/ha, and quinclorac[7] at 0.84 kg ai/ha. The second factor consists of mesotrione[8] at 0.14 kg ai/ha as a mixture with the above herbicides and each herbicide applied alone. Finally, all of the aforementioned treatment options were either followed by a sequential mesotrione treatment at 0.14 kg ai/ha three weeks later or not. Comparison treatments included a non treated control for a total of 23 treatments. Adjuvants were added accordingly to label instructions and include 1% v/v of crop oil concentrate[9] for quinclorac and 0.25%

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[3] Basagran® Herbicide, BASF, 100 Campus Drive, Florham Park, New Jersey 07932.

[4] Buctril® Herbicide, Bayer CropScience LP, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

[5] QuickSilver™ T&O Herbicide, FMC Corporation, Agricultural Products Group, Philadelphia, PA 19103.

[6] MSMA Herbicide, PBI Gordon. 1217 West 12th Street, Kansas City MO, 64101

[7] Drive® Herbicide. BASF, 100 Campus Drive, Florham Park, New Jersey 07932.

[8] Callisto® Herbicide, Syngenta Crop Protection, Inc., Greensboro, NC 27409.

[9] Crop Oil Concentrate wetter/spreader/penatrant adjuvant consisting of a 83% paraffin base petroleum oil and 17% constituents ineffective as spray adjuvant. Loveland Products Inc, PO Box 1286 Greeley, CO 80632-1286.

v/v nonionic surfactant[10] used for mesotrione, bromoxynil and bentazon. Initial treatments were applied on July 15, 2005, July 16, 2005 and August 08, 2006 for S1, S2 and S3, respectively. Sequential applications occurred 3 weeks after trial initiation. Application equipment consisted of a CO_2 backpack sprayer, calibrated to deliver 287 L/ha using TeeJet™ VS11004 flat-fan nozzles[11].

Data was collected at trial initiation and 3, 14, 24, 35 and 42 days after initial treatment (DAT). Data included initial percentage of crabgrass cover, turfgrass quality, turfgrass color, turfgrass injury, and smooth crabgrass control. Turfgrass color was visually estimated on a 1 to 9 scale where 1 = brown turf; 5 = minimal acceptable turfgrass color; and 9 = optimal greenness. Turfgrass injury was visually rated on a 0% to 100% scale where 0% = no injury and 100% = dead turf. Turfgrass quality was visually assessed on a 1 to 9 scale. 1 = dead turf; 5 = minimal acceptable level; and 9 = uniform, optimum green color, density and acceptable rough conditions. Smooth crabgrass control was visually estimated on a 0% to 100% scale where 0% = no control and 100% = compete control.

Data were analyzed using methods described in Colby (1967) and similar assumptions outlined in (Abendroth et al. 2006).

$$\text{Expected value} = [(\text{herb1} + \text{herb2}) - (\text{herb1} \times \text{herb2})/100]$$

Herb1 is the observed smooth crabgrass control of [mesotrione at 0.14 kg ai/ha] applied alone. Herb2 is observed smooth crabgrass control of [bentazon at 0.28 kg ai/ha,

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[10] Kinetic®, a nonionic wetter/spreader/penatrant adjuvant consisting of a 99% proprietary blend of polyalkyleneoxide, modified polydimethylsiloxane, and polyoxypropylene-polyoxyethylene copolymers. Helena Chemical Company, 225 Schilling Blvd., Suite 300, Collierville, TN 38017.

[11] VS1104 Teejet® spray nozzles. Spraying Systems Co., North Avenue, Wheaton, IL 60189.

bromoxynil at 0.28 kg ai/ha, carfentrazone-ethyl at 0.035 kg ai/ha, MSMA at 1.68 kg ai/ha, and quinclorac at 0.84 kg ai/ha] applied alone.

The assumption being that the biologically accepted reference model used is a Multiplicative Survival Model (MSM) instead of the Additive Dose Model (ADM). In general, ADM is most effective when the herbicides being combined have similar modes of action. In this case, MSM is a more effective model because the mode of action of mesotrione and the additives are dissimilar Herbicide Handbook 2003; Mitchell et al. 2001) The results using the MSM model were analyzed in SAS to dictate differences in experimental combinations from the predicted response yielding a positive (synergistic), neutral (additive) or negative (antagonistic) value (WSSA 1985). Smooth crabgrass control means from admixtures and sequential applications were compared using Fisher's protected LSD at P = 0.05.

RESULTS AND DISCUSSION

Herbicide applications are most effective when weed species are young, actively growing and environmental conditions are favorable for herbicide penetration and absorption to target weeds. Herbicide efficacy is greatly reduced when applied to mature weeds, dense infestations or during the summer, when cool-season turfgrass is not actively growing (figure 1). Herbicide admixtures or sequential applications are often needed for effective weed control in these situations.

Of the five herbicides used in this study, quinclorac and MSMA are the only two labeled for smooth crabgrass control in cool-season turf (Anonymous 2005d; 2005e). Bentazon is labeled for broadleaf weeds and sedge control (Anonymous 2005a). Bromoxynil and carfentrazone-ethyl are labeled for a wide range of broadleaf weed control (Anonymous 2005b; 2005f). Mesotrione, not currently labeled for cool-season turf use, also controls a wide variety of broadleaf weeds and annual grasses (Anonymous 2005c).

Only smooth crabgrass control and cool-season turfgrass injury are reported. The visually observed control in the field did not outweigh the expected control of quinclorac at 0.84 kg ai/ha plus mesotrione at 0.14 kg ai/ha indicating these admixtures as additives at S1 and S3, 24 and 35 DAT. But at S2 these same admixtures were antagonistic (table 3). MSMA at 1.68 kg ai/ha plus mesotrione at 0.14 kg ai/ha was an additive at S2 and S3, 24 DAT. MSMA at 1.68 kg ai/ha plus mesotrione at 0.14 kg ai/ha was antagonistic at S1, 24 DAT and at all three locations 35 DAT. Bentazon at 0.28 kg ai/ha, bromoxynil at 0.28 kg ai/ha, carfentrazone-ethyl at 0.035 kg ai/ha did not control smooth crabgrass [control = 0%] applied alone at any time. Bentazon at 0.28 kg ai/ha plus mesotrione at 0.14 kg ai/ha, bromoxynil at 0.28 kg ai/ha plus mesotrione at 0.14 kg ai/ha, and carfentrazone-ethyl at 0.035 kg ai/ha plus mesotrione at 0.14 kg ai/ha were synergistic 24 DAT for S2 and S3 (table 1). However, at S1 bromoxynil at 0.28 kg ai/ha plus mesotrione at 0.14 kg ai/ha admixture were antagonistic at 24 DAT (table 2). At S1 only bentazon at 0.28 kg ai/ha plus mesotrione at 0.14 kg ai/ha was synergistic 35 DAT (table 2). At S2, bentazon at 0.28 kg ai/ha plus mesotrione at 0.14 kg ai/ha, bromoxynil at 0.28 kg ai/ha plus mesotrione at 0.14 kg ai/ha, and carfentrazone-ethyl at 0.035 kg ai/ha plus mesotrione at 0.14 kg ai/ha were synergistic 35 DAT. At S3 no synergism occurred for any treatment 35 DAT (table 2).

A sequential application of mesotrione at 0.14 kg ai/ha three weeks after initiation increased smooth crabgrass control for bentazon at 0.28 kg ai/ha, bromoxynil at 0.28 kg ai/ha, carfentrazone-ethyl at 0.035 kg ai/ha, MSMA at 1.68 kg ai/ha, and quinclorac at 0.84 kg ai/ha at S1 and S3, 14 days after sequential (DAS) (table 1). At S2, quinclorac at

0.84 kg ai/ha followed by (fb) mesotrione at 0.14 kg ai/ha compared to quinclorac did not improve smooth crabgrass control 14 DAS (table 1).

The most injury occurred S3. Quinclorac at 0.84 kg ai/ha plus mesotrione at 0.14 kg ai/ha fb mesotrione at 0.14 kg ai/ha and quinclorac alone at 0.84 kg ai/ha fb mesotrione at 0.14 kg ai/ha injured cool-season turf 34% and 25%, respectively 14 DAS. No other treatment at S3 injured the turf. At S1 and S2 minor injury (<10%) occurred from treatments that contained mesotrione, 7 DAT. In both situations the turf quickly recovered within one week and no other injury was observed for the duration of the trial.

The most effective herbicide treatments occur when applications are made prior to smooth crabgrass maturity (Dernoeden et al. 2003). In situations when weed pressure was extensive [> 80% cover], and smooth crabgrass had exceeded 3 to 4 tillers, as in S1 and S2 herbicide efficacy decreased. The two trials conducted in 2005 were initiated on dense (>80% cover) stands of mature smooth crabgrass (3 to 4 tillers) and the treatments were applied during July when cool-season turfgrass aren't actively growing. The single treatments of MSMA at 1.68 kg ai/ha and quinclorac at 0.84 kg ai/ha only controlled smooth crabgrass 29% at S1 and 24% and 53%, respectively at S2, 35 DAT. Smooth crabgrass that was not initially controlled outperformed the cool-season turfgrass during the summer months.

In 2006, the trial area was moderately infested with 1 to 2 tillering, seedling smooth crabgrass. (40% to 60% cover) and the growth stage was 1 to 2 tillers, drastically younger growth stage than 2005. The 2006 trial demonstrated that a single application of MSMA and quinclorac, and combinations of bentazon, bromoxynil a, carfentrazone-ethyl with mesotrione controlled smooth crabgrass effectively (table 1). In 2006, all treatments with mesotrione improved smooth crabgrass control at S3, 14 DAS. Therefore, when smooth crabgrass was mature or infestations were severe, a single application of MSMA or quinclorac did not effectively control smooth crabgrass. Adding mesotrione at 0.14 kg ai/ha did improve weed control, however were not biologically significant. Adding mesotrione at 0.14 kg ai/ha to bentazon at 0.28 kg ai/ha, bromoxynil at 0.28 kg ai/ha, or carfentrazone-ethyl at 0.035 kg ai/ha plus mesotrione at 0.14 kg ai/ha fb mesotrione at 0.14 kg ai/ha extended smooth crabgrass control longer than without a sequential application.

Table 2.1: Effects of herbicides applied alone or as admixtures, with or without a sequential mesotrione application on visual smooth crabgrass control 24 days after treatment (DAT).

		Smooth Crabgrass Control (%)					
		without Mesotrione			with Mesotrione		
Herbicide	Rate (kg ai/ha)	Site[b] 1	Site 2	Site 3	Site 1	Site 2	Site 3
Bentazon	0.28	0 c[c]*	0 b*	0 c*	58 b*	60 a*	76 bc*
Bromoxynil	0.28	0 c*[d]	0 b*	0 c*	34 c*	70 a*	70 c*
Carfentrazone-ethyl	0.035	0 c*	0 b*	0 c*	35 c*	45 b*	71 c*
Mesotrione[a]	0.14	—	—	—	25 d*	30 c*	58 d*
MSMA	1.68	23 b*	33 a	53 b*	36 c*	36 bc	81 b*
Quinclorac	0.84	65 a	30 a*	84 a	68 a	45 b*	90 a
LSD		1.8	4.9	6.2	6.3	11.9	8.1

[a] Mesotrione without mesotrione = non treated plots. Mesotrione with mesotrione = a single applications of mesotrione at 0.14 kg ai/ha.

[b] Site 1 and site 2 corresponds to 2005, and site 3 corrresponds to 2006.

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

[d] Means followed by the symbol * are significantly difference according to Fisher's protected LSD at P= 0.05 for the comparison of a sequential mesotrione application

[e] A sequential mesotrione application was applied 3 weeks after trial initiation.

[f] 24 Days after treatment is 3 days after the sequential mesotrione application.

Table 2.2: Effects of herbicides applied alone or as admixtures, with or without a sequential mesotrione application on visual smooth crabgrass control 35 days after treatment (DAT).

		Smooth Crabgrass Control (%)					
		without Mesotrione			with Mesotrione		
Herbicide	Rate (kg ai/ha)	Site[b] 1	Site 2	Site 3	Site 1	Site 2	Site 3
Bentazon	0.28	0 b[c]*	0 c*	0 c*	73 a*	66 ab*	76 b*
Bromoxynil	0.28	0 b*[d]	0 c*	0 c*	48 c*	80 a*	78 b*
Carfentrazone-ethyl	0.035	0 b*	0 c*	0 c*	23 c*	25 c*	78 b*
Mesotrione[a]	0.14	—	—	—	55 b*	63 b*	73 b*
MSMA	1.68	29 a*	24 b*	53 b*	53 b*	53 c*	70 b*
Quinclorac	0.84	29 a*	52 a	78 a*	60 b*	35 c	90 a*
LSD		6.6	7.2	5.9	8.2	15.2	8.1

[a] Mesotrione without mesotrione = non treated plots. Mesotrione with mesotrione = a single applications of mesotrione at 0.14 kg ai/ha.

[b] Site 1 and site 2 corresponds to 2005, and site 3 corrresponds to 2006.

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

[d] Means followed by the symbol * are significantly difference according to Fisher's protected LSD at P= 0.05 for the comparison of a sequential mesotrione application

[e] A sequential mesotrione application was applied 3 weeks after trial initiation.

[f] 35 Days after treatment is 14 days after the sequential mesotrione application.

Table 2.3: Synergistic admixtures 24 and 35 days after treatment (DAT) for visual smooth crabgrass control.

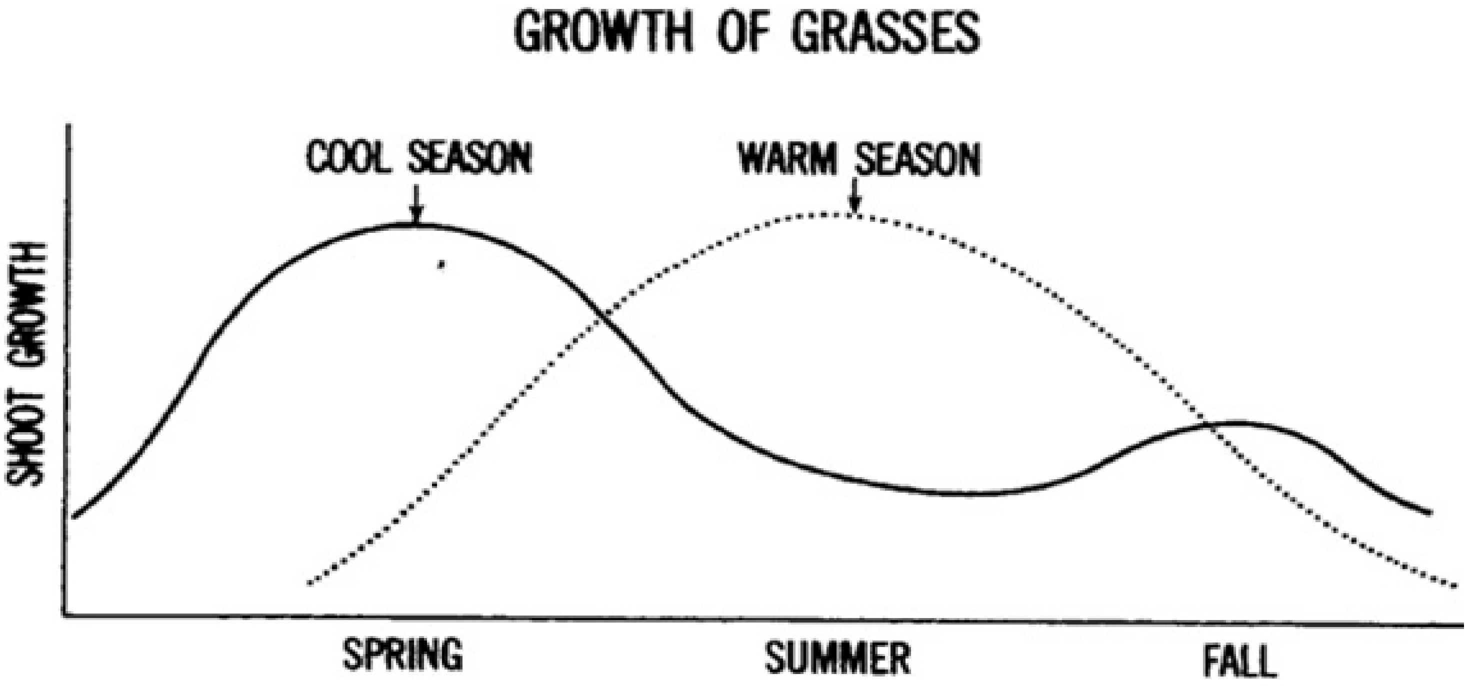
		Smooth Crabgrass Control (%)					
		24 DAT			35 DAT		
Herbicide	Rate (kg ai/ha)	Site[b] 1	Site 2	Site 3	Site 1	Site 2	Site 3
Bentazon	0.28	58 (23)+	60 (36)+	76 (58)+	73 (40)+	66 (49)+	76 (68)±
Bromoxynil	0.28	34 (23)±	70 (36)+	70 (58)+	48 (40)±	80 (49)+	78 (68)±
Carfentrazone-ethyl	0.035	35 (23)+	45 (36)+	71 (58)+	23 (40)–	25 (49)–	78 (68)±
MSMA	1.68	36 (51)–	36 (69)–	81 (79)±	53 (57)±	53 (75)–	70 (84)–
Quinclorac	0.84	68 (73)±	50 (62)–	90 (93)±	60 (58)±	35 (60)–	90 (92)±
LSD							

[a] Values in parentheses are the expected values from the modified Colby (1967) equation.

[b] Site 1 and site 2 corresponds to 2005, and site 3 corrresponds to 2006.

[c] Observed and expected means were compared using Fisher's protected LSD at P =0.05. Synergistic treatments are indicated by [+], additive treatments are indicated by [±] and antagonistic treatments are indicated by [–].

Figure 2.1: Turfgrass growth cycle (Christians 2002)



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Ricker and Askew: Mesotrione and temperature

Air Temperature Effects on Perennial Ryegrass (*Lolium perenne* L.) Response to Foliar Applied Mesotrione

Daniel B. Ricker

Plant Pathology and Weed Science, Virginia Tech, Blacksburg, Virginia, 24060

Shawn D. Askew

Corresponding author. Plant Pathology and Weed Science, Virginia Tech, Blacksburg, Virginia, 24060: saskew@vt.edu.

Abstract

Currently, mesotrione is not labeled for use on perennial ryegrass turf, however mesotrione at 0.14 kg ai/ha plus 0.25% v/v NIS applied sequentially three weeks apart controls many annual grassy and broadleaf weeds as well as suppress difficult to control perennial weeds in perennial ryegrass (Askew et al. 2005; Keese et al 2005). Growth chambers were used to evaluate mesotrione toxicity on *Lolium perenne* L. (perennial ryegrass) at a range of temperatures between 12.7 and 23.8 C.

Mesotrione visual injury was not correlated with chlorophyll content, total carotenoids and PE values. Carotenoids and PE exhibited curvilinear response to temperature with a peak of 82 μg g^{-1} fresh weight and 0.703 Fv/Fm, respectively at 23.8 C. Visually estimated perennial ryegrass injury was dependent on both temperature and mesotrione rate. Mesotrione applied at 0.14 kg ai/ha caused the highest visual injury response at 23.8 C with less injury occurring at higher or lower temperatures. Mesotrione rates of 0.21 and 0.28 kg ai/ha injured perennial ryegrass 15% to 20% and 18% to 25%, respectively regardless of temperature.

Nomenclature: Mesotrione, 2-[4-(methlysulfonyl)-2-nitrobenzoyl]-1,3-cyclohexane, *Lolium perenne* L. perennial ryegrass 'field general'; days after treatment, DAT.

Key words: Carotenoids, chlorophyll, photochemical efficiency.

Introduction

In the transition zone, cool-season turf is subjected to an assortment of summer stresses, including: air temperature, relative humidity, moisture, and light intensity. Previous research has shown that turfgrass species, tolerant to herbicides under normal growing conditions, may become injured when applied during periods of moisture stress (Dernoeden 2002). Mesotrione safety on Kentucky bluegrass, tall fescue, and perennial ryegrass has been observed under normal growing conditions but temperature stress evaluation has not been conducted (Askew et al. 2003). Research using acifluorfen, fluazifop, and glyphosate indicate that herbicidal activity is influenced by high air temperatures, relative humidity, and moisture availability (Alscher and Hess 1993; Kells et al. 1984; McWhorter 1981; McWhorter and Azlin 1978; Wichert et al. 1992; Wills 1984; Wills and McWhorter 1981).

Air temperature can affect postemergence herbicide efficacy and herbicide absorption (Fausey and Renner 2001; Hammerton 1967; Johnson and Young 2002; Watlz et al. 2004). Nimbal (1996) reported MSMA controls common cocklebur (*Xanthium strumarium* L.) but efficacy depends on climatic conditions during and after treatment. MSMA control of wild mustard (*Brassica kaber* (D.C.) L.C. Wheeler) and wild oat (*Avena fatura* L.) increased when temperature increased from 10 C to 30 C (Miller et al. 1981). Glyphosate controlled johnsongrass (*Sorghum halepense* (L.) Pers.) more at 35 C than at 24 C or 29 C (Jordan 1977; McWhorter 1981; McWhorter and Azlin 1978). Research indicates that glyphosate applied at 32 C controlled bermudagrass more than a similar application made at 24 C (Jordan 1977). Other research showed bentazon controlled redroot pigweed (Wichert et al. 1992) and common ragweed (*Ambrosia artemisiifolia* L.) more at increased temperature and humidity (Ritter and Coble 1981).

Air temperatures are believed to affect translocation of the herbicide through the xylem and phloem. Non-published field observations indicate that fluctuating temperatures may cause more toxicity than constant temperature (Cudney 1987). McWhorter (1981) reported an increase in air temperature from 18 to 35 C resulted in a four fold increase of ^{14}C-metriflefen translocation in johnsongrass. Jordan (1977) and Wills and McWhorter (1981) reported more ^{14}C-glyphosate translocation and absorption at 32 C than 22 C in bermudagrass and cotton (*Gossypium hirsutum* L.).

The objective of this research is to assess which air temperatures cause the most perennial ryegrass injury from mesotrione.

Material and Methods

A randomized complete block design experiment was conducted in growth chambers at Blacksburg, VA. Treatments were arranged in a split plot design with four day/night temperature regimes as main plots (12.7 C/7.2 C, 18.3 C/12.7 C, 23.8 C /18.3 C, and 29.4 C/23.8 C, three mesotrione rates (0.14, 0.21 and 0.28 kg ai/ha) as sub plots, and four 10 cm by 10cm plastic pots as subsamples. Treatments were replicated in time with temperatures established in four growth chambers changed randomly each time a new replication is started. The study was conducted three times with a total of three replications each time.

Four growth chambers were manufactured from household chest freezers[1]. Temperatures were regulated with two thermostats[2] in each chamber to control the freezer compressor at preset day and night temperatures. A Clear Polyester Mylar® cover[3] was placed on the opening of each chamber and a small fan[4] provided air circulation. Hobo® probes[5] were used in each growth chamber to record air temperature for each replication.

Soil was a 75%/25% sand/top soil mixture with Milorganite® (6-2-0) fertilizer at 829.3 kg/ha. And had 3.4% organic matter, 6.3 pH and 12.0 meq/100g cation exchange capacity. Overhead lights were mounted 1.2 m above plants to provide light exposure for 14 hours per day at 480 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR). Plants were watered every other day using unfiltered greenhouse tap water.

Two-year-old ‘Field General’ perennial ryegrass plants were harvested from a research fairway in Blacksburg, VA in 10 cm by 10 cm squares and transplanted into plastic pots. All leaves on harvested plants were clipped to 1.5 cm. Plants were selected and pruned to ensure visual uniformity. Plants were then allowed to acclimate at 18.3 C (65 F) for three days prior to treatment and being placed in desired temperature regimes.

Mesotrione plus a nonionic surfactant [NIS][6] at 0.25% v/v was applied at the indicated rates using an enclosed spray chamber. The spray chamber is equipped with a TeeJet®[7] 8001E nozzle and calibrated to deliver 287 L/ha at 275 kPa. Trays containing plant material were placed 30 cm below the nozzle to ensure full plant coverage.

Data were collected at 0, 7, and 14 days after treatment (DAT), as 7 and 14 DAT represent the most common range of time that turfgrass injury due to mesotrione treatment had been observed in previous field research (Askew et al. 2005; Beam et al. 2006) . Data consisted of visually estimated turfgrass injury and turfgrass color. The rating scale for perennial ryegrass injury is 0 to 100%, where 0% is no injury and 100% is dead grass. Turfgrass color was rated on a 1.0 to 9.0 scale where 1.0 = brown turf, 6.0 = minimally acceptable, and 9.0 = dark green. Additionally, each pot was measured for photochemical efficiency (PE) using a chlorophyll fluorometer[8]. Three reading were taken on the leaf re-growth in each subsample. Finally, leaf blades were harvested from each subsample and analyzed separately for chlorophyll content, and total carotenoids. Chlorophyll a, chlorophyll b and carotenoid content was extracted and measured by cross referencing a calibration curve with spectrophotometer readings outlined in (Lichtenthaler 1987).

Data were analyzed statistically and results were summarized in charts and tables. Analysis of variance was conducted with PC SAS. Trials were considered random and treatment effects were tested with the mean square associated with the random variable. Quantitative temperature series were explained with regression analysis where possible. Data variance were tested by plotting residuals and stabilized, where needed, with arcsin square root or log transformation. Data were averaged over the three trials no treatment by replication interactions occurred.

Results and Discussion

This trial was conducted in growth chambers to limit abiotic and biotic environmental factors. Previous research has shown that increased mesotrione rates injure normally tolerant perennial ryegrass (Askew et al. 2005; Bhowmik and Drohen 2001). As expected, increasing the rate of mesotrione increased perennial ryegrass injury. Regardless of temperature, increasing mesotrione rate decreased chlorophyll a, chlorophyll b, chlorophyll a + b, total carotenoids and PE (data not shown) while perennial ryegrass injury increased. (figure 1).

Temperature data collected from the Hobo® probes were (+/-) 0.5 degrees from the desired growth chamber temperature for the duration of each replication (figure 2). Optimum perennial ryegrass growth occurs when air temperatures are between 18.3 C and 23.8 C (Christians 2004). Perennial ryegrass total carotenoid levels were dependent on temperature. As the air temperature reached optimum growing conditions carotenoid production and PE increased with a peak of 82 $\mu g\ g^{-1}$ fresh weight and 0.703 Fv/Fm, respectively at 23.8 C before declining (figure 3).

(Askew et al. 2005; Beam et al. 2006; Bhowmik and Drohen 2001) reported that mesotrione at 0.14 kg ai/ha plus 0.25% v/v NIS is the safest herbicide rate to use on perennial ryegrass. Increasing temperatures, increased perennial ryegrass injury when treated with mesotrione at 0.14 kg ai/ha plus 0.25% v/v NIS. Perennial ryegrass was injured 2.7% at 12.7 C day / 7.2 C night, while perennial ryegrass was injured 15% at 23.8 C day / 18.3 C night. Mesotrione at 0.21 ai kg/ha and 0.28 ai kg/ha injured perennial ryegrass between 15 to 20% and 18 to 25%, respectively regardless of temperature (figure 4).

Similar with glyphosate, mesotrione applied postemergence showed more activity at higher air temperatures. Also, translocation and absorption of mesotrione occur more rapidly at high air temperatures instead of low temperatures (Johnson and Young 2002). Even though most studies show that increasing air temperatures increases weed control, injury to normally tolerant species can occur in a similar manor.

Visually estimated perennial ryegrass injury was the strongest effect measured 7 and 14 DAT. Unfortunately, many of the photosynthetic pigments measured were not significantly correlated to visually estimated injury. Previous research has shown that carotenoid biosynthesis inhibiting herbicides significantly reduce photosynthetic processes in susceptible weed species, thus reducing biomass (Creech et al. 2004). However, no previous research has demonstrated total carotenoids as a degree of severity indicator of bleaching symptoms on susceptible plant material (Creech et al. 2004; Kana et al. 2004).

Kim et al. (2004) reported that the death mechanism of plants treated with carotenoid biosynthesis inhibiting herbicides is different depending on the developmental stage of the plant. Mature, green tissue bleaching occurs from oxidative stresses through the photosynthetic electron transport chain. While underdeveloped, younger leaf blades lose photosystem II functions, reducing carbohydrate production and eventually tissue death. Growth after carotenoid biosynthesis inhibiting herbicide application does occur, but do not effect existing carotenoids. Similar to Kim et al. (2004), Hess (2000) also reported that mature plant tissues formed prior to application do not typically show bleaching symptoms, however new leaves formed after application typically do. Harvesting leaves prior to application, before new leaves were produced, could explain the lack of significant correlation between visual injury and measured chlorophyll a, chlorophyll b, chlorophyll a + b, total carotenoids and PE values.

Currently, mesotrione is not labeled for use on perennial ryegrass turf, however, mesotrione at 0.14 kg ai/ha plus 0.25% v/v NIS applied sequentially three weeks apart controls many annual grassy and broadleaf weeds, additionally, it suppresses many a difficult to control perennial weeds in perennial ryegrass (Askew et al. 2005; Keese et al 2005). The data suggests that mesotrione applied at 0.14 kg ai/ha is affected by temperature. Perennial ryegrass injury was more severe at optimum growth temperatures. Increasing mesotrione rates to 0.21 and 0.28 kg ai/ha injured perennial ryegrass regardless of temperature.

SOURCES OF MATERIALS

[1]Kenmore Elite 24.9 cu. ft. Chest Freezer model #16592. Manufactured by Sears, Roebuck and Co. 3333 Beverly Road, Hoffman Estates, IL 60179.

[2] Temperature thermostat model# A19ARC. Manufactured by Johnson Controls Inc. 5757 N. Green Bay Ave. Milwaukee, WI 53209.

[3] Clear Polyester Mylar®. Manufactured by United States Plastic Corp., 1390 Neubrecht Rd. Lima, Ohio 45801-3196.

[4] Holmes box fan model# 3733. Manufactured by Sunbeam Products Inc., 4101 Howard Bush Drive, Neosho, MO 64850

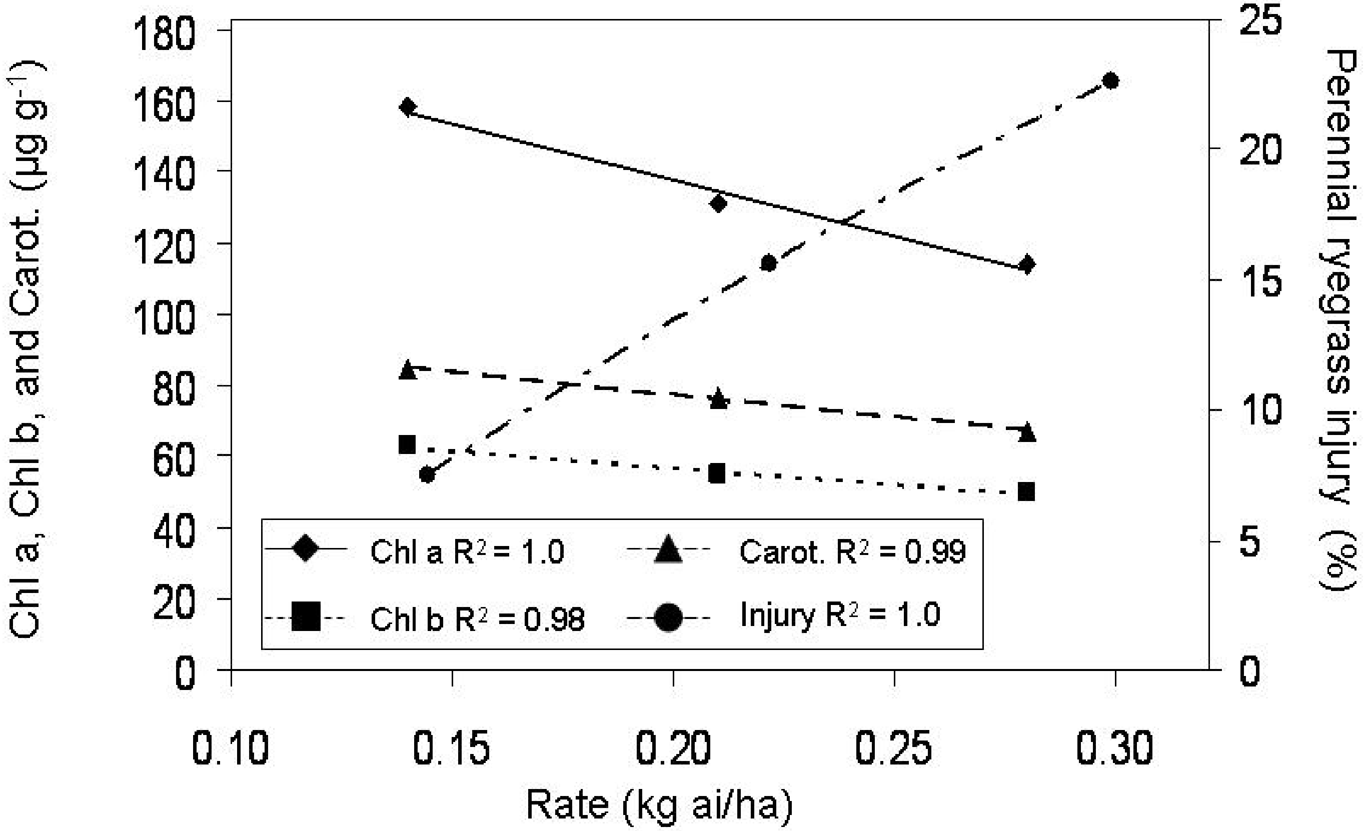
[5] Hobo® probes model H8. Manufactured by Onset Computers Corp., PO Box 3450 Pocasset, MA 02559-3450.

[6] Induce® nonionic low foam wetter/spreader adjuvant contains 90% nonionic surfactant (alkylarylpolyoxyalkane ether and isopropanol), free fatty acids, and 10% water. Manufactured by Helena Chemical Company, Suite 500, 6075 Poplar Avenue, Memphis, TN 38137.

[7] TeeJet® nozzle 8001E. Manufactured by TeeJet Agricultural Spray Products, PO Box 7900, Wheaton, IL 60189-7900.

[8] Chlorophyll fluorometer model OS1-FL. Manufactured by Opti-Sciences, 8 Winn Avenue, Hudson, NH 03051.

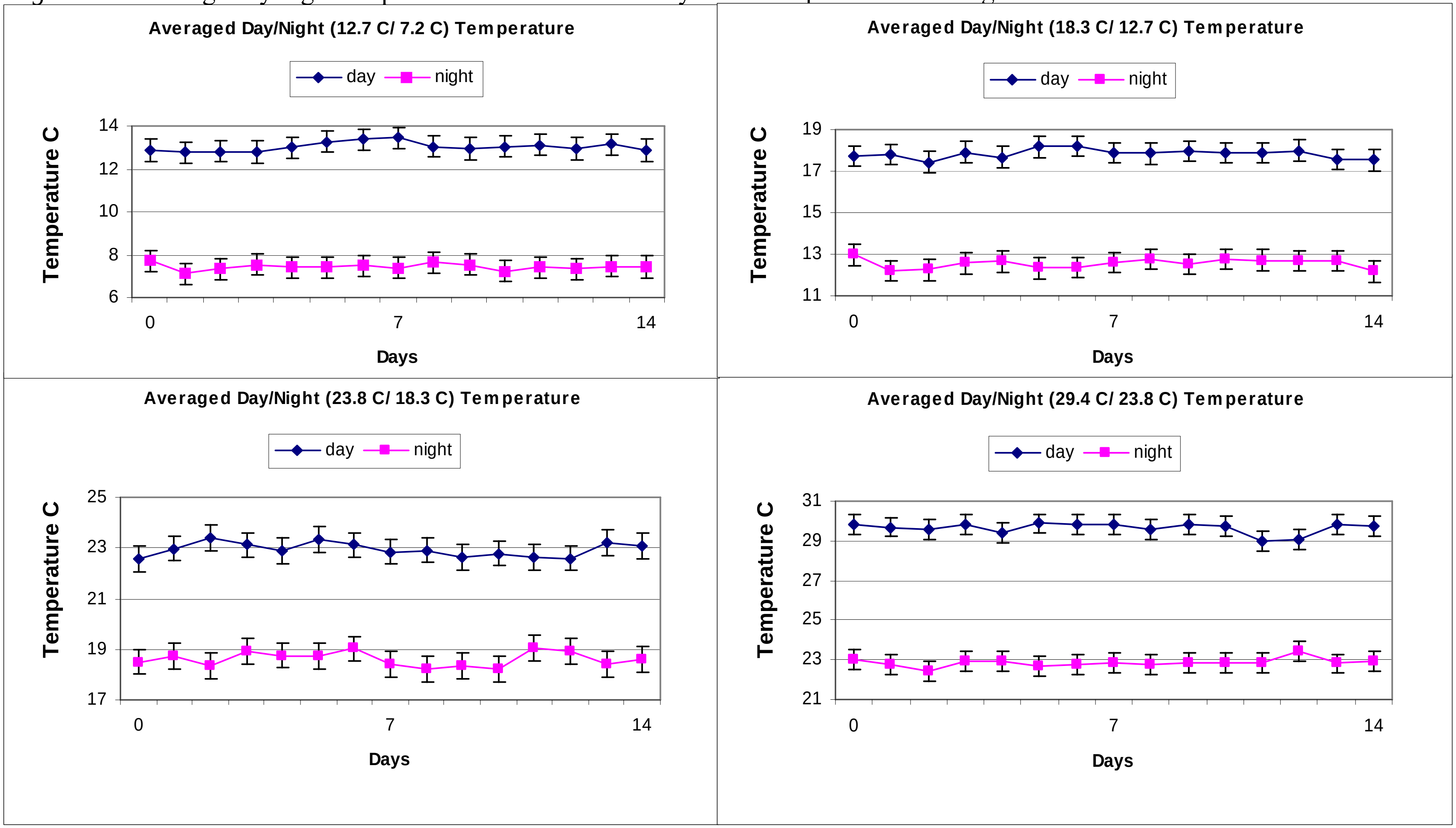
Figure 3.1: Chlorophyll a, chlorophyll b, carotenoids and perennial ryegrass injury at increasing mesotrione rates.



[a] Data averaged over 14 days after treatment as no replication by treatment interactions occurred.

[b] Chlorophyll a = -317.86x + 201.35; chlorophyll b = -92.857x + 75.3; carotenoids = -125.71x + 102.53; injury = -115.31x2 + 156.5x - 12.18.

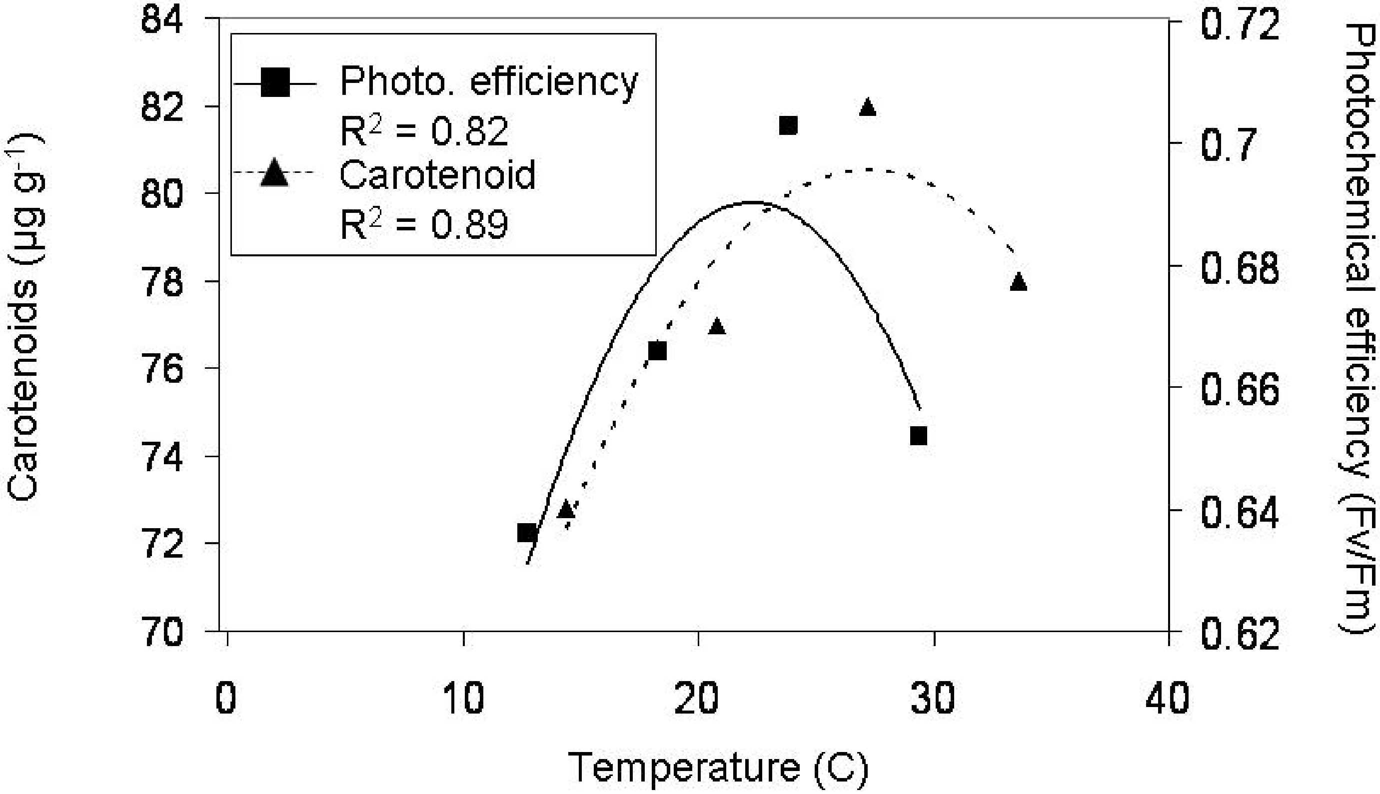
Figure 3.2: Average day/night temperature data over the 14 day treatment period for each growth chamber.



[a] Temperature data averaged over all three studies.

[b] Actual temperature was (+/-) 0.5 C from desire temperature.

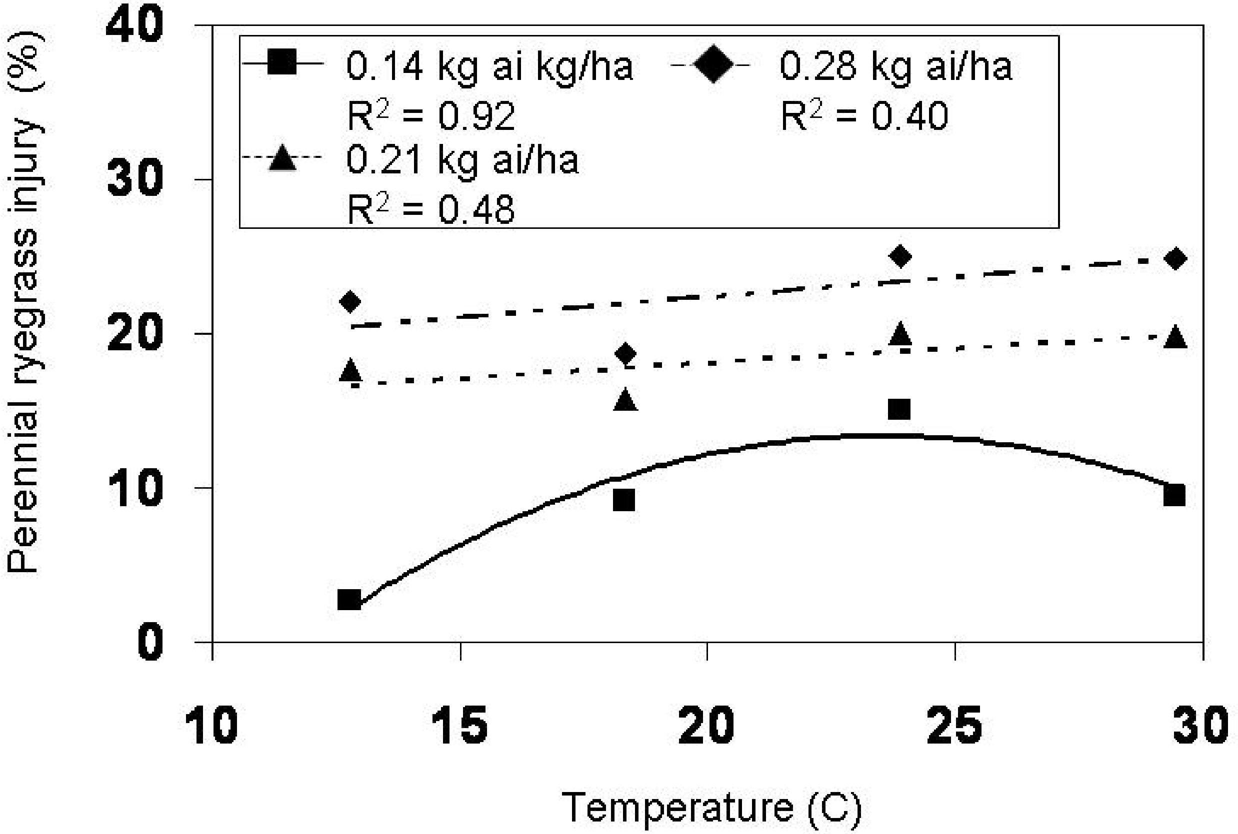
Figure 3.3: Total carotenoids and photochemical efficiency at each temperature regime.



[a] Data averaged over 14 days after treatment as no replication by treatment interactions occurred.

[b] Carotenoid = $-0.0664x^2 + 3.1752x + 42.582$; Photochemical efficiency = $-0.0007x^2 + 0.0292x + 0.3649$.

Figure 3.4: Perennial ryegrass injury at mesotrione rates across different temperatures.



[a] Data averaged over 14 days after treatment as no replication by treatment interactions occurred.

[b] 0.14 kg ai/ha = $-0.0969x^2 + 4.5605x - 40.304$; 0.21 kg ai/ha = $0.1962x + 14.133$; 0.28 kg ai/ha = $0.2592x + 17.178$

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Environmental Effects on Perennial Ryegrass (*Lolium perenne* L.) Response to Foliar Applied Mesotrione

Daniel B. Ricker

Plant Pathology and Weed Science, Virginia Tech, Blacksburg, Virginia, 24060

Shawn D. Askew

Corresponding author. Plant Pathology and Weed Science, Virginia Tech, Blacksburg, Virginia, 24060: saskew@vt.edu.

Abstract

Several cool-season turfgrasses tolerate mesotrione, prompting a request to label its use in turfgrass in the fall of 2006. Turfgrass species that are considered tolerant, however often succumb to discoloration. Perennial ryegrass and fine fescue are especially vulnerable to this unpredicted discoloration. In an effort to determine environmental conditions that might contribute to turfgrass discoloration by mesotrione an experiment was conducted in Blacksburg, VA staring on March 08, 2006. Mesotrione was applied at 0.14 kg ai/ha plus NIS at 0.25% v/v on a weekly basis to evaluate turfgrass response a range of environmental conditions of 17 weeks.

Only two mesotrione treatments, May 30th and June 28th, resulted in unacceptable turfgrass injury. At these two treatment dates, mesotrione injured perennial ryegrass 35% while the other treatment dates resulted in no more than 12% injury. Canonical discriminate analysis indicated a correlation between some measured responses, such as chlorophyll and carotenoids, but was not useful for determining environmental conditions that lead to turfgrass injury. Linear regressions indicated that turfgrass injury, assessed visually and with digital photography, was positively correlated with soil moisture but not with solar radiation, photosynthetically active radiation, dew period or temperature. By examining the two treatments dates that resulted in turfgrass discoloration, it was noted that air temperatures 10 days prior to treatment (DPT) and 10 days after treatment (DAT) were consistent with optimum growth temperatures for perennial ryegrass. Percent soil volumetric water content (%VWC) also increase 10 DPT then declined after treatment.

Nomenclature: Mesotrione, 2-[4-(methlysulfonyl)-2-nitrobenzoyl]-1,3-cyclohexane, *Lolium perenne* L., perennial ryegrass 'Field General'; *Festuca longifolia* L. hard fescue 'Aurora Gold'; days after treatment, DAT.

Key words: Digital image analysis, carotenoids, chlorophyll.

Introduction

Mesotrione is part of the triketone herbicide family targeting the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD) (Lee et al. 1997). HPPD inhibition leads to a block in the biosynthesis of plastoquinone (PQ). PQ is an essential cofactor for phytoene desaturase, thus HPPD inhibitors disrupt carotenoid biosynthesis and cause tissue bleaching (Hess 2000; Lee et al. 1997). Currently, mesotrione is registered for use in corn (*Zea mays* L.) however recent work suggests that mesotrione has excellent uses in turfgrass weed control (Askew et al. 2003; Beam et al. 2006; Bhowmik and Drohen 2001; Keese et al. 2005).

In the transition zone, perennial ryegrass turfgrass is subjected to an assortment of summer stresses, including: air temperature, relative humidity, moisture, and light intensity. Moisture stress can last for prolonged periods in non-irrigated turf and cause severe reduction in turfgrass quality. Research showed a reduction in Kentucky bluegrass, perennial ryegrass, and tall fescue dry root weight, leaf water potential and photochemical efficiency under water stress (Jiang and Haung 2000).

Previous research has shown that turfgrass species, tolerant to herbicides under normal growing conditions, may become injured when applied during periods of moisture stress (Dernoeden 2002). Mesotrione safety on Kentucky bluegrass, tall fescue, and perennial ryegrass has been observed under normal growing conditions but environmental stress evaluation has not been conducted (Askew et al. 2003). Research using acifluorfen, fluazifop, and glyphosate indicate that herbicidal activity is influenced by high air temperatures, relative humidity, and moisture availability (Alscher and Hess 1993; Kells et al. 1984; McWhorter 1981; McWhorter and Azlin 1978; Wichert et al. 1992; Wills 1984; Wills and McWhorter 1981).

Air temperature can affect postemergence herbicide efficacy and herbicide absorption (Fausey and Renner 2001; Hammerton 1967; Johnson and Young 2002; Watlz et al. 2004). Nimbal (1996) reported MSMA controls common cocklebur (*Xanthium strumarium* L.) but efficacy depends on climatic conditions during and after treatment. MSMA control of wild mustard (*Brassica kaber* (D.C.) L.C. Wheeler) and wild oat (*Avena fatura* L.) increased when temperature increased from 10 C to 30 C (Miller et al. 1981). Glyphosate controlled johnsongrass (*Sorghum halepense* (L.) Pers.) more at 35 C than at 24 C or 29 C (Jordan 1977; McWhorter 1981; McWhorter and Azlin 1978). Research indicates that glyphosate applied at 32 C controlled bermudagrass more than a similar application made at 24 C (Jordan 1977).

Air temperatures are believed to affect translocation of the herbicide through the xylem and phloem. Non-published field observations indicate that fluctuating temperatures may cause more toxicity than constant temperature (Cudney 1987). McWhorter (1981) reported an increase in air temperature from 18 to 35 C resulted in a four fold increase of ^{14}C-metriflefen translocation in johnsongrass. Jordan (1977) and Wills and McWhorter (1981) reported more ^{14}C-glyphosate translocation and absorption at 32 C than 22 C in bermudagrass and cotton (*Gossypium hirsutum* L.).

Environmental conditions may be responsible for variable weed control observed with foliar applied mesotrione (Johnson and Young. 2002). C_4 plants, common waterhemp *(Amaranthus rudis* Sauer*)* and large crabgrass were more susceptible to mesotrione at 18 C compared to 32 C contrary to C_3 plants in previous research involving temperature and herbicide efficacy (Kells et al. 1984; McWhorter and Azlin 1978; Wills and McWhorter 1981). Physiological difference in plant metabolism is believed to be the reason why mesotrione was less effective at higher temperatures. C_4 plants have improved growth and metabolic activity compared to C_3 plants at higher temperatures (Wills 1984). At low air temperatures, mesotrione applied to C_4 plants metabolizes slower, increasing herbicide efficacy (Johnson and Young 2002). Mesotrione is less effective on C_4 plants than C_3 plants at higher temperatures due to increased C_4 metabolism. However, C_3 plants may be more injured by mesotrione at high air temperatures due to temperature stress (Johnson and Young 2002). Mesotrione acts selectively to control a variety of annual broadleaves, grasses, and perennial weeds in Kentucky bluegrass, perennial ryegrass, and tall fescue, all C_3 grass species. Thus, previous work suggests that high temperature may lead to increased injury on C_3 turfgrasses treated with mesotrione but this hypothesis has not been tested.

The objectives of this research is to evaluate air temperature, leaf wetness, photosynthetically active radiation (PAR), soil moisture, solar radiation (SR) and soil temperature on perennial ryegrass injury to foliar applied mesotrione.

Material and Methods

A randomized complete block design experiment was conducted in Blacksburg, VA starting on March 08, 2006. Mesotrione was applied at 0.14 kg ai/ha plus NIS at 0.25% v/v on a weekly basis to random plots evaluating mesotrione against environmental conditions over 17 weeks. Treatments were replicated four times and the experiment was conducted in two locations on “Field General” perennial ryegrass and a third location on shaded hard fescue “Aurora Gold”. A modified ConeJet®[1] full cone spray nozzle was calibrated to deliver 861 L/ha at 103.4 kPa and used in conjunction with a 27 cm diameter plastic shield to create a 0.32 m^2 mesotrione treated circle. Soil organic material was 3.3, 2.0, and 3.5%, pH was 5.5, 5.1, and 5.9 and cation exchange capacity was 5.9, 6.8 and 4.0 meq/100g for sites 1,2, and 3, respectively. All sites were fertilized once per month with 10-10-10 at 243.9 kg/ha to deliver 0.22 kg/ha of nitrogen, potassium, and phosphorus per application. Chlorothalonil[2] was applied at 12.66 kg ai/ha on July 19, 2006 and August 14, 2006 to control dollar spot (*Sclerotinia homeocarpa)* and brown patch (*Rhizoctonia solani*). Supplemental irrigation was applied when plant wilt was detected. Site one mowed 3 times per week at 1.27 cm while site two and three were mowed twice per week at a height of 7.65 cm.

Data were collected at 0, 5, and 10 days after herbicide treatment, as these timings represent the most common range of time that turfgrass injury due to mesotrione treatment has been observed in recent field research (Askew et al. 2005; Beam et al. 2006). Visually estimated turfgrass injury and turfgrass color were assessed on a rating scale of 0 to 100%, where 0% is no injury and 100% is dead grass and 1 to 9 scale where 1 = brown turf, 6 = acceptable color, and 9 = dark green color.

A 1.2 m by 1.2 m by 1.3 m wooden phytotoxicity sensitivity meter was constructed and designed using digital photography to capture changes in plant phytotoxicity after mesotrione applications. A nautical alkaline battery[3], 1200 watt power inverter and two 45 cm ballasts holding four incandescent light bulbs supplied supplemental lighting to the phytotoxicity sensitivity meter, 1.3 m from the turf canopy. The phytotoxicity sensitivity meter was completely seal not allowing any natural light to enter. Using a digital Rebel™ ET[4] camera, photographs of each plot were taken during data collection times. The manual settings were used and the aperture and F-stop were recorded. The photographs were digitally analyzed using SigmaScan Pro 5.0[5] computer program and methods outlined in Karcher and Richardson (2005). Additionally, plants within each plot were harvested and analyzed separately for chlorophyll content.

Chlorophyll a, chlorophyll b and carotenoids were extracted and measured by cross referencing a calibration curve with spectrophotometer readings (Lichtenthaler 1987).

Environmental data was collected using a Watchdog® series data logger[6]. Hourly readings were logged for 17 weeks. Readings included air temperature [C], leaf wetness [LW (0 = dry and 15 = wet)], photosynthetically active radiation (PAR) [uM/m^{2}s], soil moisture measured as volumetric water content [%VWC], solar radiation (SR) [wat/m^2] and soil temperature [C].

Injury to perennial ryegrass greater than 35%, 10 days after treatment (DAT) was statistical analyzed. Environmental data collected 10 and 5 days before treatment (DBT) and after treatment were analyzed statistically from the date of treatment that caused the most perennial ryegrass injury and the results were summarized in charts and tables.

Analysis of variance was conducted with PC SAS. Trials were considered random and treatment effects were tested with the mean square associated with the random variable. Quantitative temperature series were explained with regression analysis where possible. Data variance were tested by plotting residuals and stabilized, where needed, with arcsin square root or log transformation.

Digital photography and chlorophyll content were analyzed separately from the environmental data and results were summarized in tables. Analysis of variance was conducted with PC SAS. Trials were considered random and treatment effects were tested with the mean square associated with the random variable (McIntosh 1983).

Results and Discussion

Environmental

May 30, 2006: At site one, LW ranged from 4.55 10 DBT to 6.27 at initiation and finally declining to 5.27 10 DAT. However, at site two LW was lowest at 10 DPT however the highest LW occurred at 5 DAT, with LW returning near the 10 DPT level, 5 days later. At site one PAR and SR ranged from 795.7 to 834.2 and 429.3 to 461.4, respectively 10 DPT. PAR and SR peaked at treatment then declined 5 and 10 DAT. Site two followed the same trend where PAR and SR ranged from 775.3 to 826.3 and 418.2 to 457.8, respectively 10 DPT then declining 5 and 10 DAT. At both sites, air temperature and soil temperature increased from 10 DPT until trial initiation then continued to decline 5 and 10 DAT. At site one VWC levels ranged between 15.09 to 15.47% 10 and 5 DPT. VWC levels increased to 18.89% 5 DAT, and a slight decline to 18.41% 5 days later. Site two VWC levels ranged between 15.09 to 14.53% 10 and 5 DPT. 5 DAT VWC levels dropped nearly 4% then increased to 17.9% 5 days later (table 1).

June 28, 2006: LW at both sites were highest 5 DPT. At site one, LW declined 5 and 10 DAT, while at site two the lowest LW occurred at trial initiation and increased 5 and 10 DAT. At both sites PAR and SR increased from 10 DPT until peaking at trial initiation. Then at both sites, PAR and SR declined 5 and 10 DAT. At site one air temperature increased from 22.6 C at 10 DPT to 22.8 C at trial initiation then decreased to 22.8 C, 10 DAT. Soil temperature at site one continued to decline from 22.2 C at 10 DPT to 20.2 C at 10 DAT. At site two, air temperature continued to decline from 22.3 C at 10 DPT until 21.2 at 10 DAT while soil temperature remained constant. VWC increased from 15.7% 10 DPT to 21.5% at 5 DPT, then peaked at 27.1% at site one. At site VWC increased from 17.6% 10 DPT to 22.6% at 5 DPT, then peaked at 22.8%. After trial initiation VWC at both sites steadily declined 5 and 10 DAT (table2).

Prior to herbicide treatment plants are developing and undergoing changes anatomically and physiologically. Biochemical processes are directly affected by temperature changes in the environment. Higher temperatures tend to produce thicker cuticles, reduce stomatal openings, and change gas exchange. These morphological processes facilitate plant survival during high temperatures (Bayer 1987).

Temperatures prior to spraying have a direct effect on metabolic activity in the plant. Coast fiddleneck *(Amsinckia spectabilis* L.) control with 2,4-D increased as air temperatures increased from 10 C to 20 C (Bayer 1987). Dicamba and DSMA were more effective in controlling redroot pigweed at higher air temperatures than at lower temperatures (Nalewaja et al. 1975).

Air temperatures at the time of treatment can also influence herbicide efficacy. As the air temperature rises, spray droplets dry rapidly on the leaf surface. Rapid dehydration of spray droplets reduces herbicide concentration and plant uptake. Herbicide applied to plants must remain in a position that allows for leaf absorption. Therefore, treatments designed to avoid the hottest part of the day are more effective (Bayer 1987).

Air temperature after a treatment manipulates physiological processes in the plant that impacts herbicide activity and efficacy. Generally, an increase in air temperature increases herbicide activity inside the plant. Uptake and penetration increase with an increase in air temperature to a certain degree. Once a threshold is reached, the plant reduces uptake and focuses on survival mechanisms (Bayer 1987; Price 1983).

Air temperatures are believed to affect translocation of the herbicide through the xylem and phloem. Non-published field observations indicate that fluctuating temperatures may cause more toxicity than constant temperature (Cudney 1987). Greater absorption and translocation of perennial ryegrass occurs when air temperatures are between 18.3 C and 23.8 C (Christians 2004), coinciding with the temperature ranges recorded at both dates that mesotrione caused unacceptable injury.

Perennial ryegrass was not injured greater than 12% at any other time. The injury to perennial ryegrass occurred at two specific dates at two different locations yet, the weather data that was collected was difficult to interpret. On May 30, 2006, at both sites the only similarities are that air temperature and soil temperature increased until treatment then declined. With the exception of site 2 on June 28, 2006 air temperatures air and soil temperatures followed a similar pattern at the first date.

With the exception of site 1 on May 30, 2006, percent VWC increased until treatment then declined. Under dryer conditions transpiration exceeds water uptake. Roots then signal a systemic response to reduce stomatal conductance by producing abscisic acid (ABA) that elevates guard cells cytosolic Ca^2 concentrations closing the stomates. Herbicide absorption is reduced under dryer conditions. As more water becomes available stomates will remain open longer to reestablish cellular water levels. Herbicide absorption is easier and quicker when stomates are open than when the stomates are closed. Increasing VWC allows mesotrione to enter the plants through the stomates, dyer conditions that occurred after slows metabolism and translocation and the herbicide injures normally tolerant perennial ryegrass.

Severe perennial ryegrass injury occurred at two specific treatment dates. The most influential environmental responses were air and soil temperature and soil moisture. The injury occurred during perennial ryegrass optimum temperatures and when soil moisture was low, increased near application, and then declined. Mesotrione toxicity on perennial ryegrass is unknown, yet air and soil temperature and soil moisture play a role in predicting perennial ryegrass injury. More research needs to be conducted to determine other factors that may be involving perennial ryegrass injury phenomenon.

Phototoxic Sensitivity

At two specific treatment dates mesotrione applied at 0.14 kg ai/ha plus NIS at 0.25% v/v perennial ryegrass injury exceeded 35%, while the rest of the treatment dates resulted in injury that did not exceed 12%. Using methods outlined in Karcher and Richardson (2005) the percentage of green cover declined as perennial ryegrass injury increased for treatments made on May 30th and June 28, 2006 (table 2). Visually observed perennial ryegrass injury and reduced perennial ryegrass green could not be made with injury less than 12%. Digital analysis of the photographs was a difficult process. The most effective results were photographs taken of lush, dark green, immaculately maintained perennial ryegrass. Unfortunately, abiotic and biotic factors affect perennial ryegrass quality in the field. The photograph taken of less optimal perennial ryegrass plots often distinguished cosmetic blemishes, mower clippings, and thatch as perennial ryegrass injury however, visually estimated injury ratings could distinguish between herbicide toxicity and environmental error.

Karcher and Richardson (2005) have developed an excellent way to evaluate turfgrass injury. However, the most effective results come from initial photographs taken on lush, dark green turf. Any blemishes or debris distorts the ability of the computer program to assess the photograph, resulting in unusable data.

SOURCES OF MATERIAL

[1] ConeJet® nozzle. Manufactured by TeeJet Agricultural Spray Products, PO Box 7900, Wheaton, IL 60189-7900.

[2] Chlorothalonil trade name Daconil. Manufactured by Syngenta Professional Products P.O. Box 18300, Greensboro, NC 27419.

[3] Lifeline alkaline marine battery. Manufactured by Lifeline Marine & Rv Batteries, 955 Todd Avenue, Azusa, CA 91702

[4] Rebel digital camera model# EOS Rebel K2. Manufactured by U.S.A. Canon Inc, One Canon Plaza, Lake Success, NY 11042.

[5] SigmaScan Pro. Manufactured by Systat Software, Inc.,1735, Technology Drive, Ste 430 San Jose, CA 95110.

[6] Watchdog™ data logger station model# 800. Manufactured by Spectrum Technologies, Inc.,12360 South Industrial Dr., East Plainfield, IL 60585.

Table 4.1: Environmental data on May 30, 2006.

Site 1						
Days	Dew Period	PAR (uM/m^2s)	SRD (wat/m^2)	Soil Temp (C)	Air Temp (C)	%VWC[d]
-10[a]	4.55a[b]	834.2ab	461.4ab	14.9d	16.2c	15.09b
-5	5.58a	795.7ab	429.3ab	20.6b	20.1b	15.44b
0	6.27a	957.2a	516.9a	23.5a	22.8a	15.47b
5	5.65a	715.5ab	389.3ab	19.7bc	21.8b	18.89a
10	5.27a	692.4b	378.6b	18.5c	20.4c	18.41a
LSD	4.22	138.6	107.4	3.79	1.73	0.78
Site 2						
-10	4.4b	826.3a	457.8a	18.6d	18.2d	15.09c
-5	5.4ab	775.3a	418.2a	20.5c	20.8b	15.44c
0	5.2ab	895.2a	487.4a	23.4a	24.5a	14.53c
5	5.8a	708.1a	384.7a	22.5a	20.1ab	10.32a
10	4.8b	667.3a	367.1a	21.3b	18.8cd	17.9b
LSD	1.49	437.9	242.3	1.39	3.59	0.791

[a] A minus sign before the number of days indicates the days before treatment. No sign before the number of days indicated days after treatment. Mesotrione at 0.14 kg ai/ha applied at day 0.

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

[c] Hourly data averaged for each day.

[d] %VWC = percent volumetric water content.

Table 4.2: Environmental data on June 28, 2006.

<table>
<tr><th colspan="7">Site 1</th></tr>
<tr><th>Days</th><th>Dew Period</th><th>PAR (uM/m^2s)</th><th>SRD (wat/m^2)</th><th>Soil Temp (C)</th><th>Air Temp (C)</th><th>%VWC[d]</th></tr>
<tr><td>-10[a]</td><td>6.83b[b]</td><td>666.1b</td><td>364.1ab</td><td>22.2a</td><td>22.6b</td><td>15.7d</td></tr>
<tr><td>-5</td><td>8.3a</td><td>529.6b</td><td>285.3a</td><td>21.9a</td><td>23.0ab</td><td>21.4c</td></tr>
<tr><td>0</td><td>6.96ab</td><td>815.3ab</td><td>451.7ab</td><td>21.9a</td><td>23.9a</td><td>27.1a</td></tr>
<tr><td>5</td><td>6.61b</td><td>786.8b</td><td>433.1a</td><td>21.6ab</td><td>23.1b</td><td>24.6b</td></tr>
<tr><td>10</td><td>6.49b</td><td>706.3a</td><td>393.4a</td><td>20.2b</td><td>22.8b</td><td>25.1ab</td></tr>
<tr><td>LSD</td><td>1.96</td><td>184.2</td><td>102.61</td><td>1.04</td><td>1.09</td><td>3.1</td></tr>
<tr><th colspan="7">Site 2</th></tr>
<tr><td>-10</td><td>7.68a</td><td>644.3bc</td><td>348.6a</td><td>23.4a</td><td>22.3a</td><td>17.6c</td></tr>
<tr><td>-5</td><td>8.11a</td><td>564.7c</td><td>301.3a</td><td>23.6a</td><td>21.9ab</td><td>22.6a</td></tr>
<tr><td>0</td><td>5.02b</td><td>881.9ab</td><td>467.2a</td><td>23.6a</td><td>22.4ab</td><td>22.8a</td></tr>
<tr><td>5</td><td>6.23b</td><td>818.9c</td><td>447.4a</td><td>23.6a</td><td>21.9ab</td><td>18.9b</td></tr>
<tr><td>10</td><td>6.37b</td><td>768.2ab</td><td>423.8a</td><td>23.3a</td><td>21.1b</td><td>18.9b</td></tr>
<tr><td>LSD</td><td>1.59</td><td>190.1</td><td>193.3</td><td>0.464</td><td>1.01</td><td>2.11</td></tr>
</table>

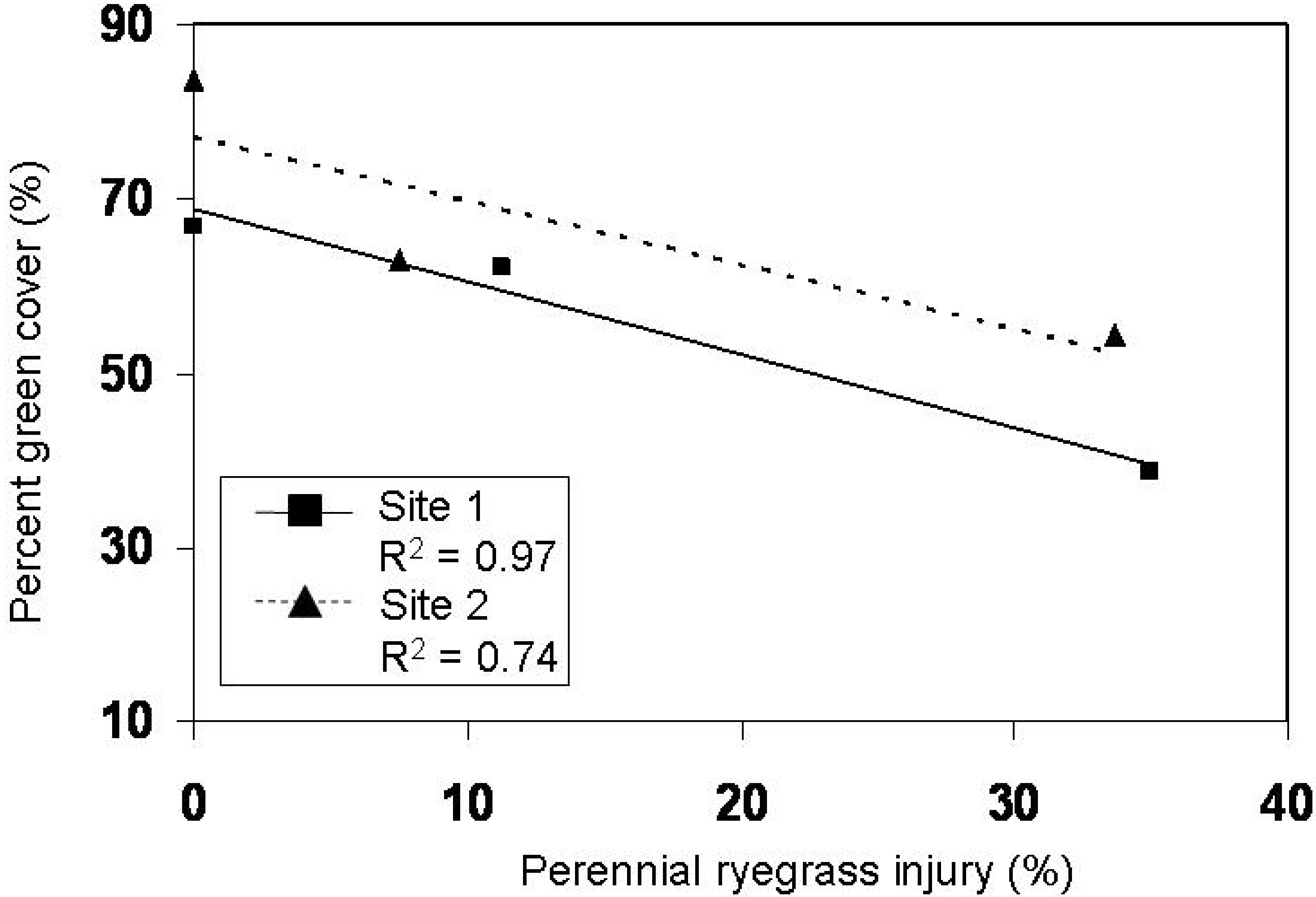
[a] A minus sign before the number of days indicates the days before treatment. No sign before the number of days indicated days after treatment. Mesotrione at 0.14 kg ai/ha applied at day 0.

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

[c] Hourly data averaged for each day.

[d] %VVC = percent volumetric water content.

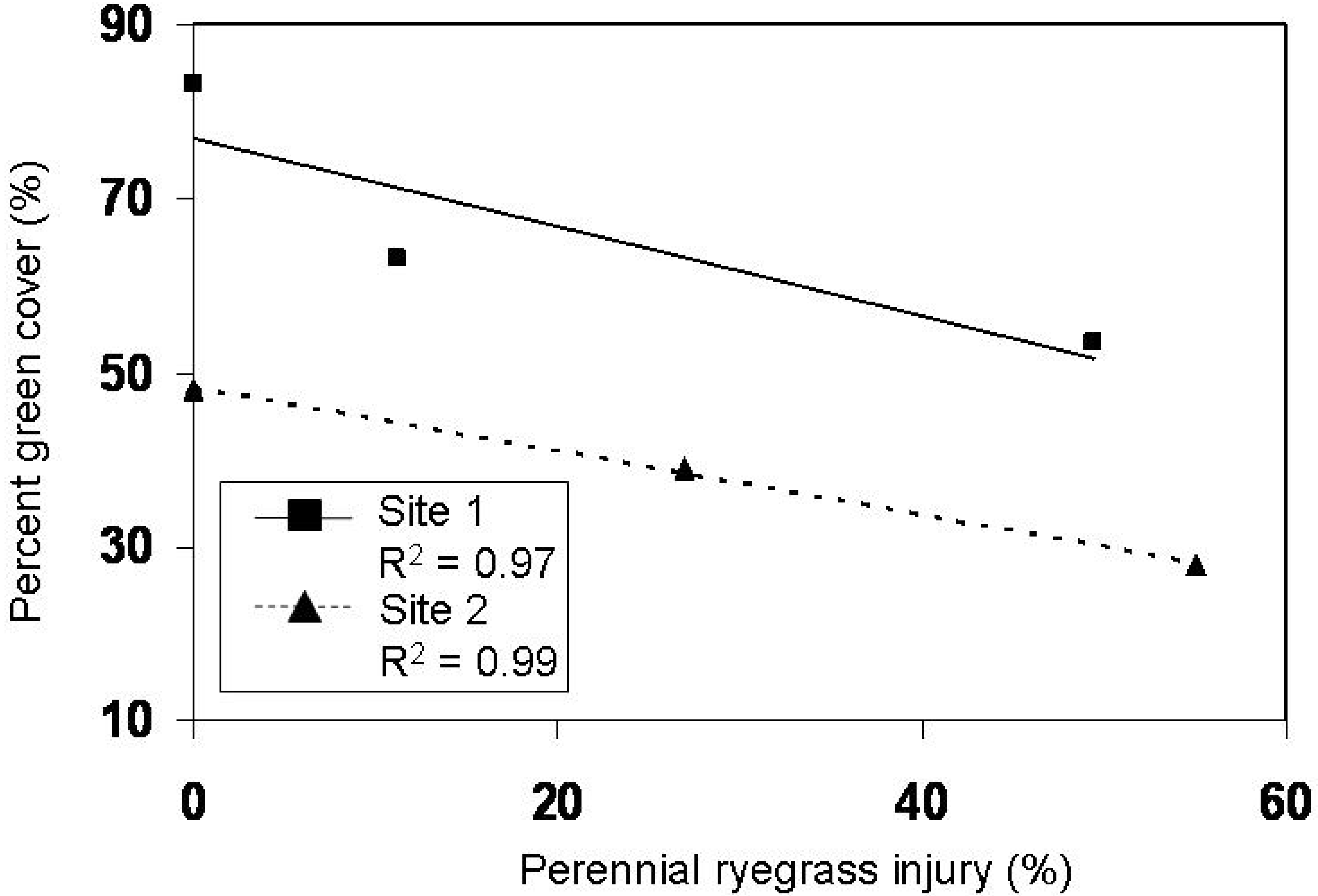
Figure 4.1: Digital analysis of perennial ryegrass green cover versus perennial ryegrass injury from May 30, 2006 treatment.



[a] Data averaged over 0, 5 and 10 days after treatment for each site.

[b] Site 1 y = -0.8311x + 68.58; Site 2 y = -0.7325x + 77.092.

Figure 4.2: Digital analysis of perennial ryegrass green cover versus perennial ryegrass injury from May 30, 2006 treatment.



[a] Data averaged over 0, 5 and 10 days after treatment for each site.

[b] Site 1 y = -0.3638x + 48.278; Site 2 y = -0.3638x + 48.278.

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