Methiozolin Effects on Annual Bluegrass Control at Various Seasonal Timings and Creeping Bentgrass Injury in Shade

by

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Abstract

Methiozolin is a new herbicide with efficacy against annual bluegrass (*Poa annua* L.; ABG) and is being investigated as a selective herbicide in creeping bentgrass (*Agrostis stolonifera* L.; CBG) golf course greens, tees and fairways. However, the ideal seasonal timing for methiozolin remains unclear, wide ranges of ABG control have been reported and not all seasonal timings have been tested. Additionally, golf course superintendents often manage shaded tees and fairways, yet the influence of methiozolin on CBG grown in reduced light is unknown. Field studies were conducted from 2015-2017 in New Jersey on mixed CBG and ABG soil-based putting greens to investigate a wide range of spring and fall timings of methiozolin. Sequential applications totaling 1.44 kg ai ha^{-1} controlled ABG 72% to 87% (study 1) and 77% to 93% (study 2) at 12 WAIT and best control resulted from Sep 1 and Oct 1 seasonal timings. CBG injury remained at acceptable levels throughout both studies at all seasonal timings. Oct 1 (Study 2) and Nov 1 seasonal timings reduced total green cover up to 20% and voids in coverage persisted until the following spring. Greenhouse experiments (repeated in time) were conducted in New Jersey testing sequential methiozolin applications totaling 2.88 kg ai ha^{-1} on 6 cultivars of CBG maintained at fairway height in 3 shade treatments. Methiozolin reduced CBG clipping dry weights $\leq$ 50% at 21 and 42 DAIT among all cultivars in 40% shade. The cultivars Princeville and 007 were significantly less tolerant to methiozolin in all shade treatments. Results suggest that methiozolin Sep 1 seasonal timing is optimal for achieving ABG control while providing good CBG safety, late fall timings should be avoided and moderate (40%) shade reduces CBG vigor enhancing injury following applications of methiozolin.

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Abbreviations

ai	active ingredient
ANOVA	analysis of variance
C	degrees Celsius
cm	centimeters
DAIT	days after initial treatment
g	grams
h	hours
ha	hectare
kg	kilograms
NJ	New Jersey
N	nitrogen
m	meters
mm	millimeters
PAR	photosynthetically active radiation
PGRs	plant growth regulators
ppm	parts per million
PRE	preemergent
TE	trinexapac-ethyl
WAIT	weeks after initial treatment

I. Literature Review

Annual Bluegrass

Annual bluegrass (*Poa Annua* L.) is one of the most adapted and widespread plants in the world, found in arid deserts of Africa, mountainsides in Europe, islands in the sub-Antarctic and on all seven continents (Warwick, 1979; Holm, 1997; Frenot et al., 1999; Huff, 2003). Annual bluegrass can produce an exceptional golf course putting surface when grown in favorable conditions without stress, however it is considered a weed when inhabiting creeping bentgrass (*Agrostis stolonifera* L.) golf putting greens, since maintaining favorable growing conditions for annual bluegrass can be problematic in summer, particularly in the northeast United States (Beard et al., 1978; Huff, 2003; Turgeon and Vargas Jr, 2003; McElroy et al., 2011).

Annual bluegrass is a bunch type, cool-season, annual grass in the Poaceae family and is believed to have originated in Europe (Beard and Beard, 2005). Annual bluegrass is an allotetraploid with 28 chromosomes (2n = 4x = 28) originating from two diploid species, *Poa supina* Schrad. and *Poa infirma* H.B.K. (Tutin, 1957; Turgeon, 2012). *P. supina* has a prostrate growth habit and is a perennial species, while *P. infirma* has an upright growth habit and is an annual species; both species are native to Europe (Darmency and Gasquez, 1997). Annual bluegrass has a folded vernation, membranous pointed ligule, no auricles, broad collar and 2-3 mm wide leaves that are smooth on both sides and slightly taper to a boat-shaped tip. Annual bluegrass turf has a light green color and the plant produces a light-brown, terminal open-panicle inflorescence.

Annual bluegrass produces most seed during the spring, dies with the summer heat followed by germination in late summer or early fall and dormancy or semi-dormancy in the winter (Lush, 1988). Although the majority of annual bluegrass germinates in the early fall, it has been observed germinating year-round, with seedling emergence anytime between August and May (Kaminski and Dernoeden, 2007). Prolific seed production in the spring is characteristic of annual bluegrass, yet can occur year round, with a single plant producing as many as 2,250 seeds year^{-1} (Holm, 1997). Vast seed production is one of many traits which incite golf course superintendents and turfgrass researchers to classify annual bluegrass as a weed in creeping bentgrass putting greens.

Annual bluegrass is generally categorized by growing type as either the annual biotype *Poa annua* L. var. *annua* Timm or the perennial biotype *Poa annua* L. f. *reptans* (Hausskn.) T. Koyama. The annual biotype has a bunch type growth habit and the perennial biotype has a stoloniferous, prostrate growth habit. The perennial biotype is sometimes referred to as the greens biotype of *Poa annua* and persists under close mowing and frequent irrigation (Lush, 1988, 1989; Vargas and Turgeon, 2004; Turgeon, 2012). The perennial biotype demonstrates the adaptability of *Poa annua* ecotypes to various environmental conditions (McElroy et al., 2002). La Mantia and Huff (2011) characterized the perennial biotype as the greens phenotype and reported that advanced generations of progeny resulting from perennial biotype parents do not follow conventional single-gene inheritance models. Subsequent generations were found to revert back to annual biotypes, leading them to suggest that the perennial greens biotype is unstable and regulated by an epigenetic mechanism. The development of either the perennial and annual biotype is influenced by cultural practices and environmental conditions (Gaussoin and Branham, 1989; Turgeon and Vargas Jr, 2003; Carson et al., 2005; La Mantia and Huff, 2011).

The genetic diversity of annual bluegrass enables the plant to adapt to a wide variety of climatic and cultural conditions (Huff, 2003). The adaptation of the perennial or greens type annual bluegrass to golf course putting greens is aided by the plants wide range of genotypic and phenotypic variability (Lush, 1989; Dionne et al., 2010). Lush (1989) found that annual bluegrass morphology on putting greens significantly varied from populations in the rough and fairway areas by morphology, verdure and seed qualities. The perennial biotype favors cultural and maintenance practices employed on greens, including higher nitrogen fertility, lower mowing heights and frequent irrigation (Carson et al., 2005; Slavens et al., 2011).

Creeping bentgrass is well-suited for golf course putting greens due to its prostrate and stoloniferous growth habit, fine leaf texture, relative heat tolerance, tolerance for low mowing heights and ability to form a high quality, uniform stand with superior ball roll and recuperative capability (Warnke, 2003; Beard and Beard, 2005; Turgeon, 2012). Creeping bentgrass greens are susceptible to diseases such as dollar spot (*Sclerotinia homeocarpa*), brown patch (*Rhizoctonia solani*) and gray snow mold (*Typhula incarnata*) and to damage from insect and weed pests (Turgeon, 2012) as well as summer stress and decline due to various biotic and abiotic factors (Dernoeden, 2012).

Annual bluegrass greens are sometimes intentionally established and maintained providing an excellent putting surface when intensively managed, specifically in regions of cool to mild summers, including the coastal temperate climates found in the Pacific Northwest United States (Huff, 2003). However annual bluegrass is poorly adapted for use on putting greens in many geographical regions of the United States where golf courses are located, such as the humid continental climate of portions of the Northeast, the desert Southwest and the humid sub-tropical climate of the Southeast (Turgeon and Vargas Jr, 2003). Annual bluegrass is less heat and drought tolerant than creeping bentgrass and frequently will not persist in weather-induced summer stress periods common to these areas (Lush, 1988). Most newly established putting greens in the Northern United States are a monostand of one or more cultivars (variety) of creeping bentgrass, yet within a few years they are invaded by annual bluegrass (Watschke et al., 1979). In the southeast United States, annual bluegrass germination occurs simultaneous to bermudagrass (*Cynodon dactylon* (L.) Pers.) dormancy resulting in dramatic visual weed infestations and providing the annual bluegrass with a competitive advantage (Johnson and Murphy, 1995).

Annual bluegrass is problematic as a putting surface due to poor heat tolerance, shallow root system and broad susceptibility to numerous pests (Vargas and Turgeon, 2004). Annual bluegrass is generally more susceptible to diseases than creeping bentgrass, with major diseases including: anthracnose (*Colletotrichum cereale*), brown patch, dollar spot, summer patch (*magnaporthe poae*), waitea patch (*Rhizoctonia zeae*), pink snow mold (*Michrodochium nivale*) and gray snow mold (*Typhula incarnate*) (Huff, 2003).

Anthracnose and anthracnose basal rot are particularly destructive diseases of annual bluegrass in the Northeast United States, yet can be found on all cool-season species and many warm-season species (Smiley et al., 2005). Anthracnose is closely tied to cultural practices and applying more frequent, lighter rates of nitrogen, rolling, mowing at appropriate heights and well timed fungicide applications have reduced anthracnose severity and limited disease occurrence (Inguagiato et al., 2009; Turgeon, 2012). Susceptibility to anthracnose has been found to vary among biotypes of annual bluegrass (Murphy et al., 2008).

In and around the major centers for golf in the Northeast United States the annual bluegrass weevil (*Listronotus maculicollis* Dietz) is a major insect pest which feeds preferentially on *Poa annua* and has a substantial economic impact on the golf course industry in the region (Vittum and Tashiro, 1987; McGraw et al., 2007; Held and Potter, 2012). Annual bluegrass weevils use the inside of the leaf sheath to deposit their eggs so that newly emerged immature stages (instars) can feed on the plant (Vittum, 2006). A minimum of two complete generations occur per year in the Northeast US with the first generation reaching maturity in early summer (Vittum and Tashiro, 1987). The adults will feed on closely mown annual bluegrass then overwinter in plant debris at sites up to 60m away from a golf course fairway (McGraw and Koppenhöfer, 2010). The damage caused by the annual bluegrass weevil is extremely severe (Cowles et al., 2008) and difficult to manage due to pyrethroid insecticide resistance and varying susceptibility of the different life stages of instars and adults to insecticides (McGraw et al., 2007). Preventative insecticide applications are the convention at Northeast United States golf courses for annual bluegrass weevil control and typically applied at least twice in the spring and summer with accurate seasonal timing essential to affect the susceptible instar stages of the pest (Vittum, 2006; Cowles et al., 2008).

Cultural Methods of Annual Bluegrass Control

Professional turfgrass managers maintaining creeping bentgrass greens employ numerous *Poa annua* control strategies with general goals being either total eradication of annual bluegrass, suppressing the population or keeping it as healthy as possible. Various cultural (maintenance) control techniques can be utilized in order to control annual bluegrass populations, however they produce inconsistent results by location (Reicher et al., 2012).

Maintaining appropriate height of cut, irrigation frequency and duration, fertility levels, soil pH and 100% cover of creeping bentgrass are considered sound cultural practices to control annual bluegrass (Busey, 2003; Turgeon and Vargas Jr, 2003). In an experiment with two creeping perennial ryegrasses (‘CSI' and ‘PPG-PR171’) and two traditional cultivars (‘Azimuth' and ‘Presidio') it was found creeping perennial cultivars significantly reduced annual bluegrass seedling emergence and establishment (Masin and Macolino, 2016), indicating that dense, vigorous, lateral turfgrass growth inhibits weed establishment.

Manipulating nitrogen (N) phosphorous (P) and potassium (K) fertility is essential for annual bluegrass growth or control. Application of N results in increased annual bluegrass vigor and proliferation of the greens ecotype of *Poa annua* (Beard et al., 1978). Optimal nitrogen fertility for annual bluegrass putting greens is applied sequentially throughout the growing season and was found to be between 147 and 220 kg N ha $^{-1}$ annually (Vargas and Turgeon, 2004). Creeping bentgrass has a lower annual N fertility requirement (Turgeon, 2012) and a trend among turfgrass managers is to limit N fertility to a minimum required amount in order to control annual bluegrass, however the long term impacts of this strategy on the sustainability of soil and creeping bentgrass health are unknown (McElroy and Bhowmik, 2013). Micronutrients may also play a role in annual bluegrass establishment (Varco and Sartain, 1986; Xu and Mancino, 2001). The addition of zinc up to 320 ppm reduced average *Poa annua* germination rates, with zinc sulfate being more effective than zinc oxide (Guertal et al., 2006).

Phosphorous applications aided germination, establishment and clipping yield of annual bluegrass (Goss, 1974; Varco and Sartain, 1986). P and K play an important role in annual bluegrass plant health and impact the bentgrass to annual bluegrass population dynamic (Goss et al., 1976). Additionally, annual bluegrass does not tolerate acidic soils as well as creeping bentgrass (Beard et al., 1978; Turgeon and Vargas Jr, 2003) and applications of sulfur or acidifying nitrogen fertilizer can inhibit annual bluegrass seed germination (Varco and Sartain, 1986).

McElroy et al. (2004) evaluated 8 ecotypes of *Poa annua* and found optimal temperature for seed germination among all ecotypes to be 19 C (day) - 10 C (night) and that high temperatures 39 C (day) to 29 C (night) significantly reduced germination. Frénot et al. (1999) reported the majority of annual bluegrass ecotypes collected germinated at temperature between 5 C and 20 C. Wu et al. (1987) suggested mowing height, fertility and irrigation practices affected germination rates of *Poa annua* and found a 60% mean index of germination on greens compared to 5% mean index of germination in rough. Annual bluegrass seeds can germinate under various light conditions, including total darkness, and germination varies by ecotype (Itoh et al., 1997; McElroy et al., 2004).

Chemical Methods of Annual Bluegrass Control

Mitigating the consequences from the vast seed production of annual bluegrass is important considering that seeds can remain viable in the soil for over 6 years (Bogart, 1972; Huff, 2003). Applications of plant growth regulators (PGRs), specifically Proxy® (Ethephon, Bayer Environmental Science, NC, USA) applied at 3.8 kg ai ha^{-1} in a tank mix with Primo Maxx® (Trinexapac-ethyl; Syngenta Corp., NC, USA) at 0.1 kg ai ha^{-1} reduced *Poa annua* seedhead production from 56% to over 80% (Watschke et al., 1979; McCullough et al., 2006; Borger, 2008). A novel concept of ethephon applications in winter has been reported to suppress annual bluegrass seedhead production 5-7 times greater than spring applications (Askew, 2017). Alternatively, Gaussoin and Branham (1989) reported that clipping removal reduced the *Poa* *annua* seedbank in the soil by 60% and when followed by overseeding with creeping bentgrass the annual bluegrass population decreased 28%.

PGRs are frequently utilized as a method of chemical control of annual bluegrass on creeping bentgrass putting greens in the Northern United States. PGRs reduce *Poa annua* populations on greens by inhibiting the growth of annual bluegrass to a greater extent than creeping bentgrass, thus giving the desirable species a competitive advantage (McCullough et al., 2005). Flurprimidol (SePRO Corp., IN, USA) and Paclobutrazol (Syngenta Corp., NC, USA) are two common PGRs used to control annual bluegrass (Vargas and Turgeon, 2004; Danneberger, 2006). Both are Type II PGRs and inhibit the synthesis of gibberellic acid early in the pathway (Watschke et al., 1979; McCullough et al., 2006). Johnson and Murphy (1996) found that 3 spring and fall applications of paclobutrazol reduced perennial biotypes of *Poa annua* by at least 57%, however fluprimidol applied at the same timings resulted in no greater than a 47% reduction in annual bluegrass. Flurprimidol controls annual bluegrass more slowly than paclobutrazol (Christians, 2001). Flurprimidol applied monthly from May to October at 0.28 and 0.56 kg ai ha^{-1} reduced annual bluegrass populations in a creeping bentgrass fairway 78% and 74% respectively (Bigelow et al., 2007).

In addition to selective regulation of annual bluegrass growth in creeping bentgrass golf course putting greens and fairways, certain PGRs improve quality of turfgrass grown in reduced light conditions. Applications of trinexapac-ethyl (TE) in combination with various nitrogen rates improves shaded turfgrass color, texture and quality (Qian and Engelke, 1999; Goss et al., 2002; Steinke and Stier, 2003; Ervin et al., 2017). Steinke and Stier (2003) reported that monthly or bimonthly applications of TE at rates of 0.05 kg ai ha^{-1} significantly improved turfgrass quality, density and increased chlorophyll levels in creeping bentgrass, Kentucky bluegrass and supina bluegrass. Qian and Engelke (1999) found monthly or bimonthly repeated applications of TE at 0.05 or 0.10 kg ha^{-1} vastly improved the shade tolerance of Diamond zoysiagrass. However, Gardner and Wherley (2005) studied 3 cool-season turfgrass species treated with TE totaling 0.29 kg ai ha^{-1} and grown under deciduous tree shade equaling 9% of full irradiance and reported no difference in color or quality compared to the non-treated control. The authors suggest neutral density shade fabric used in previous research does not accurately reproduce the shade resulting from a true deciduous tree canopy.

PGRs used in turfgrass management are conventionally classified by the mode of growth inhibition within the plant. All PGR types and classes, I-II and A-E respectively, are used in some way to control annual bluegrass. Additionally type II class A PGRs are reported to improve creeping bentgrass turf quality grown in reduced light environments (Christians, 2001).

There are limited herbicide options in creeping bentgrass putting greens and those that are labeled for greens can cause injury if misapplied (Dernoeden, 2012). There are even fewer options when considering herbicides for *Poa annua* control. There is currently one selective pre-emergent herbicide (PRE) for *Poa annua* used on creeping bentgrass putting greens, bensulide (S-(O,O-diisopropyl phosphorodithioate) ester of N-(2-mercapto) benzenesulfonamide; Bensumec®, PBI Gordon Corp., MO, USA) although damage to root systems of non-target species has been observed with repeated use (Callahan, 1972; Landschoot et al., 1993). The new herbicides amicarbazone (Amicarbazone: 4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide; Xonerate®, Arysta LifeScience, NC, USA) and bispyribac-sodium (Sodium 2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy]benzoate; Velocity®, Valent USA Corp., CA, USA) are labeled for post-emergent (POST) annual bluegrass control in creeping bentgrass turf (Anonymous, 2017a; b). However, they are not labeled for use on greens and both are injurious to desirable turf thus requiring precise seasonal timing with amicarbazone (McCullough et al., 2010). Reduction in annual bluegrass has not been consistent with bispyribac-sodium by location (McCullough et al., 2009). On warm-season putting greens, such as bermudagrass, there are relatively more herbicide options for annual bluegrass control (Yelverton and McCarty, 2001).

Soil fumigation is a chemical method of *Poa annua* control on golf courses. Methyl bromide (CH_3Br) was a highly effective and widely used fumigant for sterilizing *Poa annua* seeds prior to greens renovations and grow-ins until a complete ban on production and import took effect January 1, 2005, resulting from its classification as a Class I ozone-depleting substance (US EPA). An alternative and effective fumigant is dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) which completely controlled annual bluegrass emergence on a putting green when applied at rates greater than 291 kg ai ha^{-1} in summer and covered with plastic for 1 week (Landschoot et al., 2004).

Herbicide resistance is an important consideration when developing chemical control regimes for annual bluegrass. Resistance to simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) has been documented for over two decades (Darmency and Gasquez, 1981; Hutto et al., 2004). In the United States, annual bluegrass has been found resistant to mitotic inhibiting herbicides, inhibitors of acetolactate synthase, photosystem II inhibitors, and inhibitors of enolpyruvylshikimate-3-phosphate (EPSP synthase) (Holt and Lebaron, 1990; Binkholder et al., 2011; Cross et al., 2015). Cross et al., (2015) simulated 6 different herbicide regimens to determine time to selection for resistant *Poa annua* and estimated glyphosate (Isopropylamine salt of N-(phosphonomethyl) glycine; Roundup®, Monsanto, MO, USA) alone, applied annually would result in resistant *Poa annua* within 10 years and that summer PRE + fall POST and alternating glyphosate each year would delay development of resistance for up to 25 years. McElroy (2012) suggests two effective strategies for managing herbicide resistance in turf are rotating herbicide mode of action and improving the overall health of the desired turfgrass, thereby limiting opportunities for weed establishment.

Methiozolin

A new herbicide, methiozolin (5{2, 6-difluorobenzyl}oxymethyl-5-methyl-3, 3{3-methylthiophen-2-yl}-1, 2-isoxazoline; PoaCure®), has been developed and manufactured by Moghu Research Center in Daejeon, South Korea for selective control of grassy weeds. Methiozolin is a member of the isoxazoline class of chemical compounds, (Koo et al., 2010; McCullough et al., 2013a) a class that has many uses in medicine and corrosion inhibition (Bhatnagar et al., 1989; Yıldırım and Cetin, 2008). Methiozolin was discovered evaluating derivatives of 5-benzyloxymethyl-1, 2-isoxazoline for herbicidal activity and safety in rice (*Oryza sativa* L.) production (Ryu et al., 2002). When synthesized, methiozolin exists as two isomers, (S)- and (R)-, as well as a racemic mixture of the two, however the (S)-isomer and racemic mixture demonstrated herbicidal activity, but the (R)-isomer did not (Nam et al., 2012).

Methiozolin has been found to control annual bluegrass, barnyardgrass [*Echinochloa crus-galli*(L.) P. Beauv.] and goosegrass [*Eleusine indica* (L) Gaertn.], among others (Koo et al., 2008, 2010, 2014; Nam et al., 2012; Brosnan et al., 2013; McCullough et al., 2013a; Xiong et al., 2015). Methiozolin is reported as safe on creeping bentgrass, as well as on established zoysiagrass (*Zoysia japonica* Steud.), Kentucky bluegrass (*Poa pratensis* L.), and perennial ryegrass (*Lolium perenne* L.) when applied at 0.5 - 1.0 kg ai ha^{-1} (Koo et al., 2008, 2010; Yu and McCullough, 2014). Higher rates of methiozolin resulted in zoysia grass injury 6 weeks after treatment with 13% to 27% injury corresponding to 2 and 4 kg ai ha^{-1} methiozolin. Methiozolin injured 50% of annual bluegrass at 1.67 kg ai ha^{-1}, however required 6.72 kg ai ha^{-1} to cause the same injury to creeping bentgrass (Yu and McCullough, 2014). Hoisington et al. (2014) found that 25% total injury occurred at 1.1 kg ai ha^{-1} for creeping bentgrass, 0.2 kg ai ha^{-1} for velvet bentgrass (*agrostis canina* L.) and 0.3 kg ai ha^{-1} for colonial bentgrass, (*agrostis capillaris* L) respectively. Similarly rates required for 50% clipping dry weight reduction were 1.4 kg ai ha^{-1}, 0.4 kg ai ha^{-1} and 0.4 kg ai ha^{-1} on creeping, colonial and velvet bentgrass, respectively. Among 9 cultivars of creeping bentgrass, "Penn A-4" demonstrated the highest tolerance to methiozolin, requiring 4.5 kg ai ha^{-1} for 25% total injury and all cultivars tolerated 0.5 kg ai ha^{-1} (Hoisington et al., 2014).

The mode of action of methiozolin is unknown, however 2 have been suggested: cell wall biosynthesis inhibition (CBI) and inhibition of tyrosine aminotransferase (TAT) (Lee et al., 2007; Grossmann et al., 2012; Venner et al., 2012). Lee et al (2007) reported that methiozolin affected cell wall biosynthesis. However, CBI may be a secondary mode of action based on comparison to other CBI herbicide effects on cellulose production (Sabba and Vaughn, 1999; Brabham et al., 2014). Yu and Mcullough (2014) found that differential target site binding or fate in the roots may play a role in methiozolin selectivity for annual bluegrass.

Grossman et al. (2012) further investigated the possibility that methiozolin blocks the conversion of L-tyrosine to 4-HPP, catalyzed by tyrosine aminotransferase (TAT) using the model species duckweed (*Lemna paucicostata* L.). Upon L-tyrosine conversion to 4-HPP, 4-HPP dioxegenase (4-HPPD) catalyzes the creation of homogentisate (Moran, 2005). This is a vital first step of the prenylquinone pathway and inhibition disrupts the eventual synthesis of plastoquinones, tocopherols and other compounds (Lopukhina et al., 2001). When plants are exposed to stress, more protectant compounds, including tocopherols are produced to mitigate injury. Higher levels of precursor, homogenticisic acid via transamination of 4-HPP by TAT is required to elevate protectant compound levels (Lee and Facchini, 2011; Riewe et al., 2012). In the model species thale-cress (*Arabidopsis thaliana* L. Heynh.) there are 44 genes which encode for aminotransferases, seven of those being TAT genes (Prabhu and Hudson, 2010; Grossmann et al., 2012). Inhibition of the isoenzyme TAT7 in thale-cress occurred following applications of methiozolin and compounds structurally similar to methiozolin (Grossmann et al., 2012). Based on the high rates of methiozolin needed to inhibit TAT7, Grossmann et al. (2012) proposed that TAT isoenzymes in more sensitive species could be inhibited at much lower rates.

Methiozolin has been found to be absorbed by both the leaf and roots (Koo et al., 2014). Yu and McCullough (2014) had similar findings and reported primarily acropetal movement of methiozolin. Flessner et.al (2013) reported little translocation of methiozolin when applied to the leaf or via hydroponic solution. Out of the total foliar applied, labeled methiozolin, 10% was recovered above the treated leaf, 1.4% below and 1.3% in all other parts evaluated, yet when root applied, the majority of the herbicide translocation was to the crown (30 μg) not the leaf (8 μg) (Flessner et al., 2013). Methiozolin has a relatively high log K_{ow} (octanol-water partitioning coeffecient) of 3.9 which is a factor in limiting translocation and plays a role in persistence in the soil (Koo et al., 2010; Flessner et al., 2015). Temperature affects the absorption of methiozolin, with 15% absorption at 10-15 C compared to 35% at 25-30 C and growing chamber temperatures of 10 C required higher methiozolin rates than 20 C and 30 C to achieve 50% injury on annual bluegrass (McCullough et al., 2013a).

Studies using non-radio labeled methiozolin resulted in better control of annual bluegrass with soil-plus-foliar treatments compared to soil-alone or foliar-alone single application treatments (Brosnan et al., 2013). Flessner et al. (2013) reported similar findings with foliar-plus-soil treatments with best control at 1.68 kg ai ha^{-1} methiozolin. In contrast, Koo et al. (2014) found that applications to the leaf had no herbicidal activity and applications of soil-plus-foliar were equivalent to soil alone.

Methiozolin has been investigated as a POST applied selective herbicide (Brosnan et al., 2013), although PRE activity has been documented. PRE treatment resulted in annual bluegrass seed germination control at methiozolin applications of 1.50 kg ai ha^{-1} and above (Nam et al., 2012). Flessner et al. (2013) supported these findings, reporting inhibition of annual bluegrass seed germination at 1.68 kg ai ha^{-1}. Establishment of creeping bentgrass from seed following methiozolin treatments of 0.56 kg ai ha^{-1} resulted in 18% total creeping bentgrass green cover and 1.12 kg ai ha^{-1} and 2.24 kg ai ha^{-1} yielded 6% green cover, at 8 weeks after seeding (McCullough et al., 2013a). Creeping bentgrass cover increased to 44%, 58% and 64% for treatments of 0.56 kg ai ha^{-1} , 1.12 kg ai ha^{-1} and 2.24 kg ai ha^{-1} , respectively, when seeding was delayed until 2 weeks after methiozolin application. PRE activity of methiozolin is supported by Askew and McNulty (2014) who found that existing annual bluegrass control was not achieved with fall treatments, yet annual bluegrass population was reduced 25% 3 months after fall treatment. Field studies were conducted on creeping bentgrass putting greens in Missouri and Texas investigating sequential PRE-only methiozolin applications at 1.0 kg ai ha^{-1} in the fall prior to *poa annua* emergence and the authors found annual bluegrass < 5% the following May compared to 30% cover in the non-treated check (Haguewood et al., 2012)

Field studies have been conducted with methiozolin to evaluate annual bluegrass control and creeping bentgrass injury. McCullough et al. (2013) noted two applications of methiozolin at 0.84 kg ai ha^{-1} and 1.68 kg ai ha^{-1} resulted in 58% and 93% annual bluegrass control, respectively, with treatments initiated in February/March or May. However, lower methiozolin rates of 0.42 kg ai ha^{-1}, yielded 23% annual bluegrass control at 8 weeks after treatment when initiated in February/Match and 50% control at 8 weeks after treatments when initiated in May. The field study discussed earlier in Virginia also investigated spring application of methiozolin at rates of 0.5 kg a.i. ha^{-1} and 0.75 kg a.i. ha^{-1} and found no significant injury or discoloration on creeping bentgrass (Askew and McNulty, 2014). Brosnan et al., (2013) evaluated methiozolin control of annual bluegrass on golf putting greens in Tennessee and Texas reporting that control following applications in the fall was significantly affected by the methiozolin rate, application time and sequential applications. Annual bluegrass control was greater at 1.0 kg ai ha^{-1} than 0.5 kg ai ha^{-1} methiozolin treatment and single applications in November and December resulted in better control than those applied in October. The researchers also found sequential applications of methiozolin made October-November, November-December or three applications (October-November-December), had greater annual bluegrass control than a single application on a sand-based putting green. Xiong et al. (2015) carried out field studies evaluating sequential methiozolin application safety on creeping bentgrass greens. Treatments were methiozolin at 0.25, 0.50, 0.75, or 1.00 kg a.i. ha^{-1} applied 1, 2, or 4 weeks apart, totaling 2.00 to 2.25 kg a.i. ha^{-1} per season. Methiozolin applications resulted in consistent annual bluegrass control up to 95%, compared with the non-treated control. Phytotoxicity was observed at times, but was at acceptable levels at all times, suggesting that methiozolin sequential treatments applied beginning in early fall provide effective control of annual bluegrass with acceptable safety to creeping bentgrass greens (Xiong et al., 2015). Findings by Koo et al. (2014) further supported early fall timing of sequential methiozolin applications rather than in the spring. Annual bluegrass control was 80 to 100% following sequential fall applications and approximately 60%

following spring applications of 0.5 and 1.0 kg a.i. ha^{-1} with only temporary and acceptable injury to creeping bentgrass reported. Han and Kaminski (2012) reported similar findings with fall application timings of methiozolin providing greatest annual bluegrass control and that multiple, sequential applications resulted in both Pre and Post activity. Sequential or single spring applications of methiozolin at 0.50 kg ai ha^{-1} did not control annual bluegrass on a putting green in Indiana, yet annual bluegrass was controlled 44% with sequential March + April + May treatments of 1.0 kg ai ha^{-1} (Trappe et al., 2013).

Rootzone material, organic matter and soil moisture can influence the behavior of herbicides and can affect annual bluegrass control (McCullough and Hart, 2006; McCurdy et al., 2008; Hoyle et al., 2012). Brosnan et al. (2013) stated that annual bluegrass control was greater on soil-based greens than sand-based greens following a single, fall applications of 0.05 and 1.00 kg a.i. ha^{-1} methiozolin. In an experiment investigating soil mobility of methiozolin, Flessner et al. (2015) found the mean Kd (sorption coefficient) value to be 13.8 mL g^{-1} and that out of the total applied, 24% remained plant available. Soil organic matter is reported to be a significant factor in sorption of methiozolin in sand-based rootzones (Brosnan et al., 2013; Flessner et al., 2015). Hwang et al. (2013) investigated the herbicide in aerobic and anaerobic soils and reported the soil half life of methiozolin was approximately 49 days and that of the total applied, 16-18% was found in the soil 120 days after treatment in aerobic soils. However in anaerobic soils no detectable methiozolin degradation occurred, indicating aerobic soil microbes are most responsible for biotic degradation of methiozolin in soil.

Annual bluegrass control and creeping bentgrass injury was not influenced when methiozolin was applied simultaneously with ammonium sulfate or iron sulfate as nutrient tank mix partners (Flessner et al., 2017). Including chelated iron in herbicide treatments for annual bluegrass control has been shown to reduce discoloration of creeping bentgrass without affecting herbicide efficacy (McCullough et al., 2009).

II. Influence of Methiozolin on Annual Bluegrass Control and Creeping Bentgrass Injury on Soil-Based Putting Greens at Various Seasonal Timings

Introduction

Annual bluegrass (*Poa annua* L.) is often considered a weed in creeping bentgrass (*Agrostis stolonifera* L.) golf course putting greens. Creeping bentgrass is a preferred turfgrass species for golf course putting greens in suitable climates due to its fine leaf texture, color, relative heat and drought tolerance, recuperative capacity and ball roll (Turgeon, 2012). Annual bluegrass exhibits many undesirable characteristics which negatively affect overall quality of a golf course putting green including light green color, poor tolerance to heat and drought stress, high susceptibility to pests and prolific seedhead production (Vargas and Turgeon, 2004; Beard and Beard, 2005).

The most common strategies currently used for annual bluegrass control in golf course putting greens are cultural practices which create conditions favorable to creeping bentgrass and unfavorable to annual bluegrass and the use of plant growth regulators (PGRs) or a combination of the two strategies (Bogart, 1972; Gaussoin and Branham, 1989; Huff, 2003). Cultural practices which encourage creeping bentgrass growth over annual bluegrass include limiting irrigation, low nitrogen and phosphorous fertility, soil pH < 6.0 and limiting the seed bank in the soil. PGRs which inhibit gibberellic acid synthesis early in the pathway (type II class B) have been found to regulate annual bluegrass growth more than creeping bentgrass growth (Bigelow et al., 2007). Paclobutrazol, Trimmit® (Syngenta Corp., NC, USA) is used to selectively regulate annual bluegrass growth in creeping bentgrass golf course greens. However no combination of PGRs and cultural practices completely controls annual bluegrass and constant applications are required to suppress annual bluegrass populations (McCullough et al., 2005).

Chemical control options on golf course greens are limited. Currently the PRE bensulide is the only herbicide labeled for use on creeping bentgrass putting greens. However annual bluegrass control is poor and issues with root damage to creeping bentgrass plants has been reported (Callahan, 1972; Landschoot et al., 1993). The two relatively new herbicides amicarbazone and bispyribac-sodium have labels for POST annual bluegrass control in creeping bentgrass turf (Anonymous, 2017a; b). However, they are not labeled for putting greens and precise application timing is necessary with amicarbazone and control has been inconsistent with bispyribac-sodium (McCullough et al., 2009). The soil fumigant dazomet provides very good control of annual bluegrass emergence but is only practical during construction or renovation of putting greens (Landschoot et al., 2004). There are currently no POST herbicides labeled for annual bluegrass control on creeping bentgrass putting greens. Generally, there are more herbicide options for annual bluegrass control in bermudagrass putting greens (Yelverton and McCarty, 2001).

Herbicide resistance is an increasingly important issue regarding chemical control regimes for annual bluegrass. Resistant annual bluegrass populations have been reported to nearly every herbicide mode of action including mitotic inhibiting herbicides, inhibitors of acetolactate synthase, inhibitors of very long chain FAS, photosystem II inhibitors, photosystem I inhibitors and inhibitors of EPSP synthase (Darmency and Gasquez, 1981; Holt and Lebaron, 1990; Hutto et al., 2004; Binkholder et al., 2011; McElroy, 2012; Cross et al., 2015).

Methiozolin is an experimental herbicide in the isoxazoline class of chemical compounds shown to selectively control numerous species of grass weeds including annual bluegrass (McNulty et al., 2011; Han and Kaminski, 2012; Nam et al., 2012; McCullough et al., 2013a; Koo et al., 2014; Brosnan et al., 2017). One reported mode of action of methiozolin is the inhibition of tyrosine amino transferase (TAT) an enzyme required for plastoquinone and tocopherol synthesis, which thereby interferes with electron transport in photosystem II and carotenoid pigment production (Grossmann et al., 2012). Cell wall biosynthesis has also been reported as a mode of action (Lee et al., 2007).

Field studies have investigated methiozolin at both PRE and POST application timings on putting greens. Xiong et al. (2015) reported up to 95% control of annual bluegrass with fall timings of sequential treatments of 0.3 to 1.0 kg ai ha^{-1} applied 2 weeks apart. Similar findings were reported by Koo et al. (2014) with methiozolin at 0.5 – 1.0 kg ai ha^{-1} resulting in 80-100% annual bluegrass control at fall application timings compared to < 60% control at spring timings. Annual bluegrass control at February/March and May timings with sequential applications of methiozolin totaling 1.68 kg ai ha^{-1} resulted in < 70% control compared to > 90% control at 3.36 kg ai ha^{-1} (McCullough et al., 2013a). Sequential applications of the same total rate result in better annual bluegrass control than single applications and seasonal timing may influence annual bluegrass control (Han and Kaminski, 2012; Brosnan et al., 2013; McCullough et al., 2013a).

Creeping bentgrass injury has been reported following methiozolin applications at rates required for effective annual bluegrass control. McNulty et al. (2011) reported creeping bentgrass injury at 25 and 40% with methiozolin at rates of 2.0 and 4.0 kg ai ha^{-1}, respectively, in Virginia. McCullough et al. (2013) found creeping bentgrass injury in growth chamber experiments to be 2 to 4 times greater at 10 C compared to 20 C and 30 C, respectively. Venner et al. (2012) reported creeping bentgrass injury of 30-80 % from late fall applications of methiozolin at 2.0 kg ai ha^{-1} and that recovery was not achieved until the following May.

Numerous studies have been conducted on paclobutrazol investigating annual bluegrass control and creeping bentgrass injury. Ervin et al. (2017) reported paclobutrazol applied alone over 2 years at 0.20 to 0.40 kg ai ha^{-1} decreased annual bluegrass coverage to 5% on creeping bentgrass greens. In experiments conducted in NJ, paclobutrazol at 0.20 kg ai ha^{-1} applied repeatedly resulted in acceptable creeping bentgrass quality across numerous seasonal timings (McCullough et al., 2005).

Wide ranges of annual bluegrass control have been reported in field studies investigating sequential fall and spring application timings of methiozolin. Additionally it remains unclear how seasonal timing affects overall annual bluegrass control in situ on soil-based creeping bentgrass putting greens. Little research-based information exists on the effect of various seasonal timings on creeping bentgrass injury in situ, specifically on very late or early season applications. Further data related to timing methiozolin applications would be useful in improving methiozolin efficacy for annual bluegrass control programs and minimizing creeping bentgrass injury.

The objectives of this study were to 1) determine the influence of various seasonal timings of methiozolin on annual bluegrass control on soil-based putting greens and 2) investigate the effect of a wide range of methiozolin seasonal timings on creeping bentgrass injury.

Materials and Methods

Field studies were conducted between September 2015 and May 2017 at Greenbriar Woodlands Golf Course in Toms River, Ocean County, NJ (40.03°N, 74.20° W). Research plots were established on two separate putting greens, the 4th green in 2015-2016 (Field Study 1) and the 3rd green in 2016-2017 (Field Study 2). Greens were mixed creeping bentgrass and annual bluegrass and established in 1986 with Penncross creeping bentgrass (Tee-2-Green Inc., Hubbard, OR). Putting greens remained open for golf play for the duration of the studies and were mowed 6 times a week during the growing season and rolled 3 times a week with mowing at heights between 3.2 mm and 4.0 mm using walk-behind greens mowers (Flex 21, The Toro Company, Bloomington, MN). Irrigation was applied as needed only when wilt occurred. Pesticides were applied routinely during the studies (Appendix). No significant pest occurrences were noted during the study. Granular fertilizer was applied both study years in April and September at the rate of 0.45 kg Nitrogen ha^{-1} (Contec DG, 19-0-15, The Andersons Inc, Maumee, OH) and foliar applications of nitrogen, including various micronutrients, were applied regularly throughout the growing season totaling 3.36 kg Nitrogen ha^{-1}. No applications of PGRs or herbicides were made. Core cultivation was completed 1 month prior to trial initiation Study 1 and Study 2. No type of cultivation took place during the study period.

Rootzone material composition was native soil, “push-up greens” and both greens were equal soil texture of 83 % sand, 9 % silt and 8% clay (CLC Labs, Westerville, OH). The soil series present at the site is Evesboro Sand according to NRCS Web Soil Survey results and the soil is classified as mesic, coated Lamellic Quartzipsamments. Average organic matter was 2.8% in Study 1 and 3.3 % in Study 2 determined using the United States Golf Association (USGA) method ASTM D 2974. Soil testing during the studies indicated adequate yet not excessive plant available nutrients (Table 12). Soil pH was 6.0 in Study 1 and 5.9 in Study 2 and cation exchange capacity (CEC) was 4.1 in Study 1 and 4.4 in Study 2 (CLC Labs, Westerville, OH). The golf course superintendent approximated the composition of the greens to be 50% annual bluegrass and 50% creeping bentgrass, on average across the entirety of the putting green, at trial initiation dates of September 1 for both locations.

The field study was arranged as a randomized complete block design with 3 replications. A running check of non-treated control 0.2 m wide was maintained between individual replication to assist in visual ratings. Actual treated area for each replication was 1.1 m x 1.3 m.

Main factors for the study included methiozolin totaling 1.44 kg ai ha^{-1}, a treated control of paclobutrazol totaling 0.28 kg ai ha^{-1} and 5 seasonal timings. Sequential methiozolin applications of 0.72 kg ai ha^{-1} and paclobutrazol applications of 0.14 kg ai kg ha^{-1} were applied 14 days apart. Fall seasonal timings were September 1, October 1 and November 1. Spring initial application timings were April 1 and May 1.

Treatments were applied using a CO_2 powered backpack sprayer (BellSpray Inc.) at 172 kPa calibrated to deliver 281 L ha^{-1} spray volume equipped with 8002XR flat fan nozzles (Tee Jet Technologies, Wheaton, IL) on a 0.76m, 4 nozzle boom. Overhead irrigation was activated immediately following all applications to supply 3.5 mm of water. A physical rain gauge (Model 6330, Stratus Precision) was positioned adjacent to the small plot research both years and all precipitation event totals and monthly averages were recorded (Figure 2). Precipitation, chemical applications and treatment details were recorded in ARM 2016-2017 (Gylling Data Management).

Plots were evaluated at 0, 12 and 24 WAIT for percent annual bluegrass cover. Percent annual bluegrass cover was recorded thrice during the study and was objectively determined using a 1.1 m x 1.3 m grid of 50 intersecting lines. Annual bluegrass plants at each intersection were scored as present or absent and percent area containing ABG determined by dividing the number of intersections in which ABG was present by the total number of intersection within the rating grid. Percent reduction in annual bluegrass cover was then calculated and is referred to as percent control of annual bluegrass from henceforth.

Creeping bentgrass injury was recorded at 4, 12, 24 and 52 WAIT and rated on a 0-5 scale where 0 = no injury, 5 = all creeping bentgrass plants in treatment area dead and 1 = maximum acceptable injury. Creeping bentgrass injury was a visual comparison of replications to the non-treated control. No creeping bentgrass injury was recorded in either study at 52 WAIT therefore this rating date was excluded from data analysis.

Percent reduction in total green cover was calculated from percent total green cover in the non-treated control and rated on a 0-100% scale where 0% = no voids in turf or reduction in green cover, 100% = entire plot dead/brown or no green cover and 10% reduction in total green cover = maximum acceptable reduction in green cover. Percent total green cover was evaluated at 4, 12, 24 and 52 WAIT. No reductions in percent green cover were recorded in either study at 52 WAIT therefore this rating date was excluded from data analysis.

Turfgrass quality was evaluated at 4, 12, 24 and 52 WAIT and was rated on 1 to 9 scale, where 1 = entire plot brown or dead; 7 = minimum acceptable quality for a golf course putting green; and 9 = optimum turfgrass quality. Turfgrass color was rated on 1 to 9 scale, where 1 = entire plot brown or dead; 7 = minimum acceptable color for a golf course putting green; and 9 = optimum dark green color. Ratings were a visual comparison of treatments to the non-treated control. No difference in visual turf quality or color between treatments and replications were recorded in either study at 52 WAIT therefore this rating date was excluded from data analysis.

All data were subjected to ANOVA in IBM SPSS Statistics V23.0 (2015 IBM Corp. Armonk, NY). When appropriate means were compared using Fisher's Least Significant Difference (LSD) test conducted at the .05 significance level. ANOVA revealed that repetition in time and replications were significant between each field study experiment. Therefore data from each run of the field study were analyzed separately.

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Results and Discussion

Annual Bluegrass Control. Annual bluegrass percent cover was reduced in all methiozolin treatments at all seasonal timings across both runs of the field study (Table 3). In the first year of the field study sequential methiozolin applications totaling 1.44 kg ai ha^{-1} initiated on Sep 1 and Oct 1 resulted in the lowest percent annual bluegrass cover 7% and 11%, respectively, at 12 WAIT and the Nov 1 timing resulted in the highest percent annual bluegrass cover at 16% (Table 3). At 24 WAIT percent annual bluegrass cover was similar for all seasonal timings ranging from 9% to 17% in study 1. In study 2 percent annual bluegrass cover at 24 WAIT ranged from 7% to 17% and Nov 1 and May 1 timings resulted in significantly more annual bluegrass cover than the nontreated control. These results are consistent with responses reported by Xiong et al. (2015) and Han and Kaminski (2012) where late summer or early fall application timings provided best annual bluegrass control.

In both runs of the study and at all seasonal timings methiozolin resulted in greater annual bluegrass control than the treated control paclobutrazol at 0.28 kg ai ha^{-1} (Table 2). Across all paclobutrazol seasonal timings mean control levels were 57 % at 12 WAIT in study 1 and 54% in study 2, however, percent annual bluegrass cover increased by 24 WAIT dissimilarly to methiozolin (Table 5).

The rate of methiozolin (1.44 kg ai ha^{-1}) was equivalent to 1.5 times the experimental label rate for golf course putting greens and was selected to elicit plant response since creeping bentgrass injury at putting green height was being investigated (Anonymous, 2016). Annual bluegrass control of 90 to 95% using methiozolin has been reported by previous researchers when using rates from 2.0 to 3.3 kg ai ha^{-1} (McCullough et al., 2013a; Xiong et al., 2015). Herbicide rates were selected considering the small plot research was conducted on two putting greens which remained in use throughout both runs of the study. Koo et al. (2014) found similar levels of annual bluegrass control when using 0.5 to 1.0 lb kg ai ha^{-1} of methiozolin at limited timings.

Creeping Bentgrass Injury. In field study 1 methiozolin applications totaling 1.44 lb kg ai ha^{-1} caused creeping bentgrass injury at Sep 1, Nov 1 and Apr 1 seasonal timings at 4 WAIT and Nov 1 at 12 WAIT (Table 6). In field study 1 injury to creeping bentgrass across all treatments and timings in the study was acceptable at all evaluation dates. Nov 1 treatment timings resulted in significantly more creeping bentgrass injury at 4 and 12 WAIT than all other seasonal timings, however the injury rating (0.7) was within acceptable range for creeping bentgrass putting greens (injury < 1.0 = acceptable). No significant injury was recorded after 24 WAIT.

In field study 2 creeping bentgrass visual injury was recorded at all seasonal timings at 4 WAIT and 12 WAIT, however was within acceptable range for creeping bentgrass putting greens (injury < 1.0 = acceptable) with the exception of Nov 1 timing at 4 and 12 WAIT (Table 7). November 1 seasonal timing of methiozolin resulted in unacceptable visual injury which persisted up to 12 WAIT. These findings are consistent with previous research indicating creeping bentgrass may be sensitive to methiozolin at lower temperatures (McCullough et al., 2013a; Xiong et al., 2015). The mean daily temperature recorded at Greenbriar Woodlands Golf Course in November was 12 degrees C in field study 1 and 9 degrees C in field study 2 (Figure 1). Creeping bentgrass recovered from both acceptable and unacceptable visual injury by 24 WAIT for all seasonal timings in both runs of the field study.

Percent Reduction in Total Green Cover. Percent reduction in total green cover treatment effect varied among treatments and across runs of the field study and was greatest at 4 WAIT (Figure 2). In the field study only Oct 1 (study 2 only) and Nov 1 seasonal timings of methiozolin at 1.44 kg ai ha^{-1} significantly reduced total green cover. Oct 1 seasonal timing reduced green cover 8.0% and 18.7% at 4 WAIT in study 1 and study 2, respectively. The 8.0% reduction in total green cover observed following Oct 1 timing decreased to 0.0 % at 12 WAIT in study 1 however in study 2 the putting green only recovered to the level of 12.0% reduction in green cover. The Nov 1timing reduced green cover 13.3% and 17.0% at 4 WAIT in study 1 and study 2 respectively. Reductions in total green cover observed following Nov 1 timings decreased to 8.7% at 12 WAIT in study 1 however in study 2 the reduction in green cover increased to 19.0%. However, annual bluegrass injury and plant death constituted > 90% of the total reduction in percent green cover. Creeping bentgrass injury accounted for ≤ 3.5% and 5.0% of the reduction in total green cover in study 1 and study 2, respectively. No unacceptable reductions in total green cover were observed at 24 WAIT of methiozolin for any seasonal timing. Reductions in total green cover were significantly greater among all seasonal timings of the treated control paclobutrazol at 0.28 kg ai ha^{-1} and the voids in green cover persisted after 12 WAIT (data not shown). Reductions ≥ 40% were observed at 4 WAIT of Nov 1 timings of paclobutrazol across both runs of the field study. This supports previous studies reporting methiozolin herbicidal activity occurs less rapidly following treatments to annual bluegrass than with select PGR regimes (Venner et al., 2012).

Turfgrass Color and Quality. Turfgrass color following methiozolin totaling 1.44 kg ai ha^{-1} remained acceptable throughout study 1 with the exception of 4 WAIT at the Nov 1 timing (Table 10). In the second field study there was a reduction in turfgrass color among the 3 fall methiozolin seasonal timings that persisted to 12 WAIT. There was no effect of color enhancement compared to the non-treated control following any methiozolin seasonal timings in either study. The treated control paclobutrazol resulted in the both the lowest color ratings (4 WAIT) and the highest color ratings (24 WAIT) for individual rating dates across both runs of the study which supports earlier research reporting color enhancement following initial injury from paclobutrazol (Johnson and Murphy, 1995; McCullough et al., 2005; Danneberger, 2006).

Turfgrass quality was acceptable among all seasonal timings of methiozolin in both field studies with the exception of the Nov 1 timing (Table 11). At 4 WAIT turfgrass quality was reduced slightly below acceptable levels (6.8 in study 1 and 6.7 in study 2). At 12 WAIT methiozolin Nov 1 replications in study 1 recovered to acceptable quality, however in study 2 the unacceptable reduction in quality was observed until 24 WAIT. At 24 WAIT turf quality was acceptable and similar to the non-treated control for all seasonal timings of methiozolin. There was no effect of turf quality enhancement observed following methiozolin treatments. Although Nov 1 timings led to the greatest reduction in turf quality there was no significant difference in turf quality between the other 2 fall seasonal timings and the 2 spring seasonal timings within each run of the field study. A greater reduction in turf quality was observed with the treated control paclobutrazol at 0.28 kg ai ha^{-1} among all 3 fall seasonal timings and the reduced putting green quality persisted through the winter.

Conclusions. Methiozolin applied at 1.44 kg ai ha^{-1} was not injurious to creeping bentgrass at September/October fall seasonal timings or April/May spring timings. This is consistent with findings in previous experiments testing timings individually using rates ≤ 1.44 kg ai ha^{-1}. However levels of annual bluegrass control observed in our field studies were slightly less than reported in previous research when using rates > 1.44 kg ai ha^{-1} methiozolin (Han and Kaminski, 2012; Hoyle et al., 2012; Trappe et al., 2013; Askew and McNulty, 2014). Our field study suggests that sequential methiozolin timings with excellent creeping bentgrass safety do not consistently provide very good annual bluegrass control.

Late fall applications of methiozolin to soil-based putting greens composed of mixed annual bluegrass and creeping bentgrass where annual bluegrass is > 50% may result in unacceptable reductions in total green cover which persist through the winter and recovery in the following spring. McCullough et al (2013) reported increased creeping bentgrass injury following methiozolin applied in low temperatures similar to the injury observed in our field study at the November seasonal timings. The reduction observed in total green cover at 12 and 24 WAIT with late fall seasonal timings in both studies was attributed to annual bluegrass plant death and reduced creeping bentgrass growth vigor. Methiozolin applications in the spring and late summer/early fall coincide with more vigorous creeping bentgrass growth which subsequently fills the voids in green cover from annual bluegrass necrosis.

Enhanced herbicide activity was observed in field study 2 among the fall seasonal timings compared to study 1. In the fall of study 2 mean monthly precipitation was significantly greater than in the fall of study 1 (Figure 1). Flessner et al. (2015) found the methiozolin Kd (sorption coefficient) value to be 13.8 mL g^{-1} and that organic matter and soil moisture influence the behavior of the herbicide and can affect annual bluegrass control (Hoyle et al., 2012; Hwang et al., 2013). The two research plots were established on adjacent putting greens of equal soil texture (loamy sand) however in field study 2 the putting green % organic matter was slightly higher than in study 1 (Table 12). Soil organic matter is reported to be a significant factor in sorption of methiozolin in rootzones (Brosnan et al., 2013; Flessner et al., 2015)

Future research may be warranted to investigate the effect of soil texture, organic matter and soil moisture in situ on methiozolin herbicidal activity in soil-based putting greens. Quantifying the effects of specific environmental variables could provide further insight into more clearly defining the optimal timing of methiozolin for annual bluegrass control on creeping bentgrass golf course putting greens. On creeping bentgrass putting greens with low percentages of annual bluegrass (< 10%) very late or early seasonal timings may be acceptable since the source of reduction in green cover is annual bluegrass plant necrosis not creeping bentgrass injury. These seasonal timings may yet be useful in settings where any voids in green cover are not tolerated during the golf season. The development of resistant populations is an important consideration with any herbicide for annual bluegrass control. Field studies demonstrate that repeated use of methiozolin is required to maintain good to very good control, therefore investigating selection pressure associated with long-term methiozolin use for annual bluegrass control would be appropriate.

III. Evaluation of the Tolerance of Creeping Bentgrass Cultivars to Methiozolin in Shade

Introduction

Annual bluegrass is a problematic winter annual, grassy weed in creeping bentgrass that often invades newly constructed or renovated fairways and tees within a few years. Intensive cultural practices and chemical treatments targeting the weed result in variable control (Beard et al., 1978; Huff, 2003). Conditions maintained on fairways can favor establishment of annual bluegrass, however the shallow rooting habit and sensitivity to various pests and abiotic stress factors often results in annual bluegrass death during periodic summer stress causing voids in green cover affecting ball role and visual quality (Watschke et al., 1979; Turgeon and Vargas Jr, 2003; Beard and Beard, 2005; Smiley et al., 2005).

There are few herbicides effective in controlling annual bluegrass POST in cool-season golf course fairways and tees. Amicarbazone (Amicarbazone: 4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide; Xonerate®, Arysta LifeScience, NC, USA) and bispyribac-sodium (Sodium 2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy]benzoate; Velocity®, Valent USA Corp., CA, USA) are labeled for annual bluegrass control in cool-season turfgrass, however turfgrass sensitivity is problematic and applications require careful planning due to temperature restrictions (McCullough et al., 2010; Anonymous, 2017b).

Methiozolin is a new herbicide produced by Moghu Research Center, LLC, in Daejeon, South Korea for annual bluegrass control in cool-season turfgrass. Methiozolin reportedly affects cell wall biosynthesis, however, CBI may be a secondary mode of action based on comparison to other CBI herbicide effects on cellulose production (Sabba and Vaughn, 1999; Brabham et al., 2014). Grossman et al. (2012) found methiozolin blocks the conversion of L-tyrosine to 4-HPP, catalyzed by tyrosine aminotransferase (TAT) using the model species duckweed (*Lemna paucicostata* L.).

Methiozolin is absorbed by both the leaf and roots (Koo et al., 2014). Yu and McCullough (2014) reported similar findings and primarily acropetal movement of methiozolin. There is little translocation of methiozolin when applied to the leaf or via hydroponic solution and when root applied the majority of the herbicide translocation was to the crown not the leaf (Flessner et al., 2013). Methiozolin has a relatively high log K_{ow} (octanol-water partitioning coeffecient) of 3.9 which is a factor in limiting translocation and plays a role in persistence in the soil (Koo et al., 2010; Flessner et al., 2015).

Previous studies have reported mixed results using methiozolin rates > 1.0 kg ai ha^{-1} with wide ranges of annual bluegrass control across spring and fall seasonal timings and suggesting creeping bentgrass injury may depend on environmental conditions. Control of >80% was reported at methiozolin rates of 0.5 to 1.0 kg ai ha^{-1} with acceptable injury to creeping bentgrass (Koo et al., 2014). McCullough (2013) found methiozolin rates as high as 3.36 kg ai ha^{-1} did not injure creeping bentgrass and provided >90% annual bluegrass control. Creeping bentgrass is reported to have greater tolerance to methiozolin compared to colonial and velvet bentgrass species, < 25% total injury at rates of 2.4 kg ai ha^{-1} (Hoisington et al., 2014). Limited research based data is available on the effect of methiozolin on creeping bentgrass injury by cultivar and methiozolin effect on creeping bentgrass in reduced light growing conditions is unknown.

Numerous studies have reported plant growth regulators (PGRs) effectiveness in controlling annual bluegrass. PGRs are widely used for control of annual bluegrass on creeping bentgrass fairways and tees in the Northern United States. PGRs reduce *Poa annua* populations in creeping bentgrass turf by selectively inhibiting the growth of annual bluegrass to a greater extent than creeping bentgrass. (McCullough et al., 2005). Flurprimidol (alpha-(1-methylethyl)-alpha-[4-(trifluoromethyoxy)phenyl]-5-pyrimidinemethanol); Cutless® (SePRO Corp., IN, USA) is a common PGRs used to control annual bluegrass (Vargas and Turgeon, 2004; Danneberger, 2006). It is a class B, type II PGR and inhibits the synthesis of gibberellic acid early in the pathway (McCullough et al., 2006). Flurprimidol reduces annual bluegrass more slowly than the other widely used Class B, Type II PGR paclobutrazol (Christians, 2001). Flurprimidol applied monthly from May to October at 0.28 and 0.56 kg ai ha^{-1} reduced annual bluegrass populations in a creeping bentgrass fairway 78% and 74% respectively (Bigelow et al., 2007).

In addition to selective regulation of annual bluegrass growth in creeping bentgrass, class A, type II PGRs are found to improve quality of turfgrass grown in reduced light conditions. trinexapac-ethyl (TE) in combination with nitrogen improved shaded turfgrass color, texture and quality (Qian and Engelke, 1999; Goss et al., 2002; Steinke and Stier, 2003; Ervin et al., 2017). Turfgrass grown in reduced light conditions has reduced recuperative capacity, elongated shoot growth and reduced non-structural carbohydrate compared to plants in full-irradiance (Qian and Engelke, 1999).

The use directions for methiozolin require that no PGRs are used until annual bluegrass control is completed (Anonymous, 2016). PGRs remain a widely used chemical control method for annual bluegrass in creeping bentgrass fairways and are also used to improve the quality of turfgrass grown in reduced light environments. Deciduous tree shade frequently impacts creeping bentgrass golf course tees and fairways and it is estimated that 20 to 25% of all turfgrass is impacted in some way by shade (Beard, 1973). The effect of methiozolin on creeping bentgrass grown in a shade environment is unknown. Evaluation of methiozolin safety on creeping bentgrass grown in shade environments would add to our knowledge of the risk for potential injury from the herbicide. The objectives of the greenhouse experiments were 1) to investigate the tolerance of fairway height creeping bentgrass to methiozolin when grown in reduced light conditions and 2) evaluate the response of various creeping bentgrass cultivars to methiozolin in shade environments.

Materials and Methods

A pot study was established to investigate potential injury from methiozolin application to creeping bentgrass cultivars grown in shade environments. Greenhouses experiments were conducted in 2017 in Jackson, NJ (Greenhouse Study 1) from March 13 to June 5 and in Toms River, NJ (Greenhouse Study 2) from August 1 to October 31. Creeping bentgrass cultivars were Penn A-1, Penn A-4, 007, L93, Princeville and Declaration (Table 14). In each study 2 additional cultivars (AU Victory and Penncross) were eliminated from the study due to poor seed establishment, reducing the number of cultivars from 8 to 6. Cultivars were selected based on previous herbicide research, observations in the field and availability.

Pots used for the study measured 103.2 cm^2 and had a depth of 10.2 cm (McConkey & Co., Sumner, WA). Pots were filled with heat-treated top-dressing composed of 50% reed-sedge peat and 50% sand on volume:volume basis (ParTac Peat Corp., Great Meadows, NJ). Soil pH was 7.2, soil organic matter was 10.0% and infiltration rate was 16.5 cm $hour^{-1}$. All cultivars were seeded at the rate of 49 kg seed ha^{-1}. Soil was kept moist and pots covered with wet landscape fabric until germination occurred. Pots were mowed bi weekly at 1.9 cm using hand held shears (Gardena 8893-U 3 inch). Mean greenhouse temperatures were 19°C (night) and 25°C (day) during each study.

Pots were fertilized at 0 and 10 days using 12-24-18 (N-P-K) liquid fertilizer (Plant food Co, Cranbury, NJ) at the rate of 36.6 kg P_2O_5 ha^{-1} and thereafter every 14 days with 20-3-3 fertilizer at the rate of 12.2 kg Nitrogen ha^{-1}. Two preventative fungicide applications were made, 0.6 kg a.i. ha^{-1} azoxystrobin (Methyl (*E*)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) (Heritage, Syngenta, Greensboro, NC) and 7.6 kg a.i. ha^{-1} chlorothalonil (Tetrachloroisophtalonitrile) (Daconil Ultrex, Syngenta, Greensboro, NC). Acelepryn (Chlorantraniliprole; 3-Bromo-N-[4-chloro-2methyl[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide) was applied preventatively for insect pests at the rate of 0.11 kg ai ha^{-1}.

Plants were acclimated to designated reductions of full irradiance for 48 h prior to treatment initiation. Study treatments were initiated 7 weeks after seeding. Shade treatments included reductions of full irradiance of 0% (full sun), 40% (moderate shade) and 80% (severe shade). Shade environments were established using 40% and 80% woven light reducing polyethylene neutral density shade fabric (Sunblocker™).

Photosynthetic photon flux density (LiCor LI250, Lincoln, NE) was measured on each response variable rating date in each shade treatment to objectively evaluate actual percent reduction of full-irradience. In run 1 of the experiment full sun measurements were 32 mol m^{-2} d^{-1} (14 d), 39 mol m^{-2} d^{-1} (28 d) and 44 mol m^{-2} d^{-1} (42 d). In run 2 measurements were 36 mol

m^{-2} d^{-1}(14 d), 29 mol m^{-2} d^{-1} (28 d) and 25 mol m^{-2} d^{-1} (42 d). Mean reduction of full irradiance recorded across all treatment and evaluation dates in both studies was 39% and 77% for moderate and severe shade treatments, respectively, relative to the full sun measurements. The DLI requirement for acceptable quality of creeping bentgrass has been reported as 30 mol m^{-2} d^{-1} (Russell, 2018). Very low irradiance, 4.7 mol m^{-2} d^{-1} PAR, was reported to influence carotenoid levels in 'Crenshaw' creeping bentgrass, with lutein content variations most common (McElroy et al., 2006).

Herbicide treatments included methiozolin 1.44 kg ai ha^{-1} $application^{-1}$ and a treated control flurprimidol at 0.28 kg ai ha^{-1} $application^{-1}$. Sequential applications were applied 10 days after initial treatment (DAIT). Total methiozolin dose was 2.88 kg ai ha^{-1} and flurprimidol was 0.56 kg ai ha^{-1}.

Experimental design was randomized complete block design with 3 replications plus a non-treated check for each shade treatment. Treatments were applied using a CO2 powered backpack sprayer (BellSpray Inc.) at 172.4 kPa calibrated to deliver 281.0 L ha^{-1} spray volume equipped with 8002XR flat fan nozzles (Tee Jet Technologies, Wheaton, IL) on a .76m, 4 nozzle boom. Irrigation was applied immediately following all applications to supply 3.5 mm of water.

Clippings were harvested at 21 and 42 DAIT in accordance with the routine mowing schedule. Pots were mown to a height of 1.9 cm and clippings collected by individual pot. Clipping dry weights (CDW) were recorded (Model EJ 120, A&D Weighing, San Jose, CA) after drying clippings in a forced-air oven at 80ºC for 72 hours (Quincy Lab, model 10GC).

Creeping bentgrass visual injury was evaluated at 14, 28 and 42 DAIT and rated on a scale from 0-5 where 0 = no injury, 1= maximum acceptable injury and 5 = entire pot dead/brown. Creeping bentgrass injury was a visual comparison of treated pots to the non-treated control.

Percent creeping bentgrass total green cover was evaluated at 14, 28 and 42 DAIT and then converted to percent reduction in green cover and rated on a scale from 0-100% where 0% = no reduction or voids in total green cover, 10% = maximum acceptable reduction in green cover and 100% = entire pot brown or dead.

Creeping bentgrass turfgrass quality and color were evaluated at 14, 28 and 42 DAIT and was rated on 1 to 9 scale, where 1 = entire plot brown or dead; 7 = minimum acceptable quality for a golf course putting green; and 9 = optimum turfgrass quality. Turfgrass color was rated on 1 to 9 scale, where 1 = entire plot brown or dead; 7 = minimum acceptable color for a golf course putting green; and 9 = optimum dark green color. Ratings were a visual comparison of treatments to the non-treated control in each shade treatment.

All data were subjected to factorial ANOVA in JMP 14.1 (SAS, Inc., Cary, NC, 2018). When appropriate means were compared using the least square means comparison test at the 0.05 significance level and means grouped according to Tukey's HSD (honestly significant difference) test. Statistical analysis revealed there were no significant interactions in time or replication between greenhouse studies so data were pooled from each run of the experiment.

Results and Discussion

Creeping Bentgrass Clipping Dry Weights (CDW). As expected CDW were reduced within cultivar and treatment in accordance with % reduction of full irradiance (table 15). Methiozolin at 2.88 kg ai ha^{-1} reduced CDW of all cultivars at all evaluation dates relative to the non-treated control of each cultivar in each respective shade treatment with the exception of the severe shade treatment at 42 DAIT (Figure 4). All cultivars in the non-treated control at 42 DAIT in severe shade had CDW ≤ compared to the same cultivar treated with methiozolin, except L93. At 21 DAIT methiozolin treated pots of cultivars L93, Declaration and A-1 had significantly greater mean CDW at both moderate (0.35-0.38 g) and severe (0.16-0.25 g) reductions of full irradiance. At 42 DAIT, although creeping bentgrass growth was reduced, this pattern continued with CDW at 0.24 – 0.29 g in moderate shade and 0.23- 0.30 g in severe shade. The cultivar A-4 had a mixed response to methiozolin across shade treatments similar to that described by Hoisington et al. (2014). The cultivars Princeville and 007 had the most pronounced and consistent CDW reduction to shade and chemical treatments (Figure 3). At 42 DAIT in severe shade no measurable clippings (0.0 g) were harvested from Princeville and 007 pots (Table 16).

In general, methiozolin treatments had similar reductions in CDW as the treated control flurprimidol at 0.56 kg ai ha^{-1} in the moderate shade treatments, however in full sun and severe shade flurprimidol reduced CDW more than methiozolin. The effect of herbicide treatment on CDW in full sun and severe shade was greater at 42 DAIT compared to 21 DAIT. This observation in our study is consistent with the product experimental label suggesting no PGRs are needed while in methiozolin protocol due to reduced creeping bentgrass growth following herbicide treatments (Anonymous, 2016). Both methiozolin and flurprimidol resulted in greater CDW reductions within individual shade treatments compared to the non-treated control, with the exception of severe shade at 42 DAIT in which cultivar and shade were the main effect. In general creeping bentgrass growth and vigor was greatly reduced in severe shade across all chemical treatments and cultivars.

Creeping Bentgrass Injury. Visual injury ratings were consistent with reductions in CDW recorded at 21 and 42 DAIT in describing treatment effects. Methiozolin totaling 2.88 kg ai ha^{-1} was slightly injurious to creeping bentgrass at 14 DAIT in severe shade however in moderate shade and full sun injury was acceptable for all cultivars (Figure 5). At 28 and 42 DAIT only the cultivars A-4, Princeville and 007 were injured in full sun and moderate shade, yet in severe shade all cultivars had unacceptable injury (Figure 6 & 7). The cultivars L93, Declaration and A-1 had acceptable levels of injury in full sun and moderate shade and were only slightly injured in severe shade with the exception of A-1. Results suggest these 3 cultivars are more tolerant to methiozolin and flurprimidol in moderate shade environments. Creeping bentgrass injury was unacceptable at many evaluations and higher than most previous research, however the effects of the rate of methiozolin 2.88 kg ai ha^{-1} and shade accounted for the enhanced injury (Haguewood et al., 2012; Hoyle et al., 2012; Xiong et al., 2015). Visual injury evaluations provided a more consistent response by cultivar to shade and chemical treatment compared to CDW. The treated control flurprimidol at 0.56 kg ai ha^{-1} did not consistently affect creeping bentgrass injury in moderate or severe shade.

Percent Reduction in Creeping Bentgrass Cover. Methiozolin significantly reduced creeping bentgrass cover among all cultivars in all shade treatments relative to the non-treated control at 14 DAIT (Table 19). Similar to observations with CDW and injury, the cultivars 007 and

Princeville had the greatest reduction in green cover relative to the non-treated control at 14 DAIT. Mean percent green cover in these pots was reduced 13 and 19% with Princeville and 16 and 17% with 007 for moderate and severe shade, respectively. Reductions in green cover were not as great among pots in full sun following sequential methiozolin applications. However at 28 and 42 DAIT the effect of methiozolin on percent green cover by cultivar was not significant except for A-4 at 42 DAIT in severe shade. At 42 DAIT all non-treated control pots in severe shade, except those containing cultivars 007 and A-4, had similar reductions in bentgrass green cover as methiozolin and flurprimidol, suggesting the level of reduction in full irradiance set as the severe shade treatment had the greatest effect on creeping bentgrass response variables and obscured cultivar x chemical treatment effects. Although a reduction in total green cover was observed in severe shade, all surviving creeping bentgrass plants were not necessarily injured or chlorotic. The treated control flurprimidol at 0.56 kg ai ha^{-1} did not prevent reductions in green cover among shade treatments (Table 20). No reductions in percent green cover were caused by disease as reported in previous shade experiments (Koh et al., 2003).

Creeping Bentgrass Quality and Color. Methiozolin influenced visual quality ratings for 2 of the 6 cultivars. Visual quality ratings were significantly affected by methiozolin treatment within full sun and moderate shade treatments for the cultivars 007 and Princeville (Table 21). In severe shade methiozolin treated Princeville and 007 pots had similar quality as pots in the non-treated control. Among the cultivars L93, Declaration, A-1 and A-4 methiozolin did not influence turf quality and observed reductions in quality over time (28 and 42 DAIT) were attributed to increasing shade. The treated control flurprimidol at 0.56 kg ai ha^{-1} did not influence turf quality (data not shown) however slightly enhanced turf color only at 28 DAIT, which is consistent with previous research indicating color enhancement following flurprimidol (Johnson and Murphy, 1995; Danneberger, 2006; Bigelow et al., 2007). Methiozolin reduced visual color ratings of Princeville and 007 in full sun and moderate shade at all evaluation dates and severe shade at 14 DAIT. No effect was observed among other methiozolin treated cultivar pots when compared to the non-treated control. The effect of severe shade on quality and color was comparable to observations on reduction in green cover, where after 28 DAIT decreased turf quality and color were similar for all treatments.

Conclusions. The cultivars Princeville and 007 maintained at fairway height are sensitive to methiozolin applied at 2.88 kg ai ha^{-1} when grown in moderate shade (40%). The cultivars A-1 and A-4 are not consistently affected by methiozolin treatments in full sun or shade. Methiozolin has good safety on the cultivars L93 and Declaration in full sun and shade up to 40% reduction of full irradiance.

Severe shade appears to affect the cultivars Princeville and 007 regardless of chemical treatment. Differences in cultivar response may be caused by creeping bentgrass density type, 007 is classified as high density and Princeville as moderately-high improved density (Brilman, 2008). Further research investigating differences in tolerance of creeping bentgrass cultivars to methiozolin at the genetic level would be useful in understanding cultivar sensitivity to the new herbicide.

Effect on CDW was similar for methiozolin and flurprimidol and consistent with previously studies suggesting the herbicide activity of methiozolin regulates growth of creeping bentgrass to an extent which PGRs would not be required during treatment protocol (Koo et al., 2008). Although chemical treatment effects were detected at 14 DAIT, severe shade treatment of

80% reduction of full irradiance over time suppressed creeping bentgrass growth excessively. Experimental design considered previous studies using 80% shade fabric in which this effect was not observed (Koh et al., 2003). It is estimated the day light integral (DLI) was ≤ 5 mol m^{-2} d^{-1} for extended periods in the severe shade treatment. Future research may be more effective at elucidating effects of methiozloin by limiting shade reductions to ≤ 60% of full irradiance. Future research may be warranted to investigate the tolerance of various density types of creeping bentgrass cultivars to methiozolin since numerous cultivars are used on tees and fairways and potential injury from methiozolin is a concern of many golf course superintendents.

Table 1. Herbicide materials used in field study

Commercial Trade Name	Manufacturer	Active Ingredient	Formulation	Source	Application Rate
Poacure	Moghu Research Center, Daejeon, South Korea	Methiozolin	25 % EC 0.25 kg ai L^{-1}	Moghu Research Center Ltd	10.21 ml in 1.0 L of water
Trimmit	Syngenta Corp., NC, USA	Paclobutrazol	22 % SC 0.24 kg ai L^{-1}	Grassroots Turf Products Inc. Randolph, NJ	4.17 ml in 1.0 L of water

Table 2. Percent control of annual bluegrass in response to various seasonal timings of sequential methiozolin applications across both runs of field study

	Percent annual bluegrass control †			
	Study 1		Study 2	
	12 WAIT§	24 WAIT	12 WAIT	24 WAIT
Methiozolin Timing†	------------ 0-100% ------------		------------ 0-100% -------------	
September	87 a‡	77 ab	93 a	86 a
October	79 ab	77 ab	92 a	84 a
November	72 b	70 b	77 b	70 b
April	76 b	78 ab	83 ab	79 ab
May	77 ab	82 a	82 ab	69 b

† Two sequential applications, 2 WAIT. Total methiozolin 1.44 kg ai ha^{-1}

‡Within evaluation dates means followed by the same letter are not significantly different via means separation at the α= 0.05 level

§ WAIT = weeks after initial treatment

Table 3. Percent annual bluegrass cover on a mixed creeping bentgrass and annual bluegrass putting green as influenced by various seasonal timings of methiozolin and paclobutrazol applications,
Field Study 1.

	Percent annual bluegrass †		
	0 WAIT§	12 WAIT	24 WAIT
Treatment Timing	------------------------ 0-100%		------------------------
Methiozolin 1.44 kg ai ha $^{-1}$			
01 Sep	55	7 e	12 c
01 Oct	53	10 de	12 c
01 Nov	57	16 bcd	17 c
01 Apr	52	13 cde	11 c
01 May	51	12 cde	9 c
Paclobutrazol 0.28 kg ai ha $^{-1}$			
01 Sep	51	22 ab	37 b
01 Oct	51	19 bc	33 b
01 Nov	49	29 b	39 b
01 Apr	53	19 bcd	31 b
01 May	54	21 b	31 b
Non-treated Control	53	49 a	51 a

† Percent annual bluegrass determined using 1.1 m x 1.3 m grid of 50 intersections, annual bluegrass plants were counted as present or absent at each intersection and converted to percentage.
‡ Within evaluation dates means followed by the same letter are not significantly different via means separation at the $\alpha = 0.05$ level
§ WAIT = weeks after initial treatment

Treatment date is initial application. Second application 2 WAIT. At each application methiozolin was 0.72 kg ai ha $^{-1}$ and paclobutrazol was 0.14 kg ai ha $^{-1}$.

Table 4. Percent annual bluegrass cover on a mixed creeping bentgrass and annual bluegrass putting green as influenced by various seasonal timings of methiozolin and paclobutrazol applications, Field Study 2.

	Percent annual bluegrass †		
	0 WAIT§	12 WAIT	24 WAIT
Treatment Timing	------------------------------	0-100%	------------------------------
Methiozolin 1.44 kg ai ha $^{-1}$			
01 Sep	51	3 d	7 c
01 Oct	52	4 d	8 c
01 Nov	53	12 cd	16 c
01 Apr	52	9 cd	11 c
01 May	55	10 cd	17 c
Paclobutrazol 0.28 kg ai ha $^{-1}$			
01 Sep	53	23 b	29 b
01 Oct	53	25 b	33 b
01 Nov	51	23 b	35 b
01 Apr	51	28 b	25 b
01 May	55	21 b	37 b
Non-treated Control	53	49 a	51 a

† Percent annual bluegrass determined using 1.1 m x 1.3 m grid of 50 intersecting lines, annual bluegrass plants were counted as present or absent at each intersection and converted to percentage.
‡Within evaluation dates means followed by the same letter are not significantly different via means separation at the $\alpha = 0.05$ level
§ WAIT = weeks after initial treatment

Treatment date is initial application. Second application 2 WAIT. At each application methiozolin was 0.72 kg ai ha $^{-1}$ and paclobutrazol was 0.14 kg ai ha $^{-1}$.

Table 5. Percent control of annual bluegrass in response to various seasonal timings of sequential paclobutrazol applications across both runs of field study

	Percent annual bluegrass control †			
	Study 1		Study 2	
	12 WAIT§	24 WAIT	12 WAIT	24 WAIT
Paclobutrazol Timing	------------ 0-100% ------------		------------ 0-100% -------------	
September	56 ab‡	28 ab	57 ab	45 ab
October	62 a	34 ab	54 ab	38 b
November	41 b	21 b	55 ab	32 b
April	65 a	42 a	44 b	50 a
May	60 a	41 a	61 a	33 b

†Percent control based on percent annual bluegrass coverage at 0 WAIT

‡ Within evaluation dates means followed by the same letter are not significantly different via means separation at the α= 0.05 level

§ WAIT = weeks after initial treatment

Treatment date is initial application. Second applications 2 WAIT. Total paclobutrazol applied was 0.28 kg ai ha^{-1}

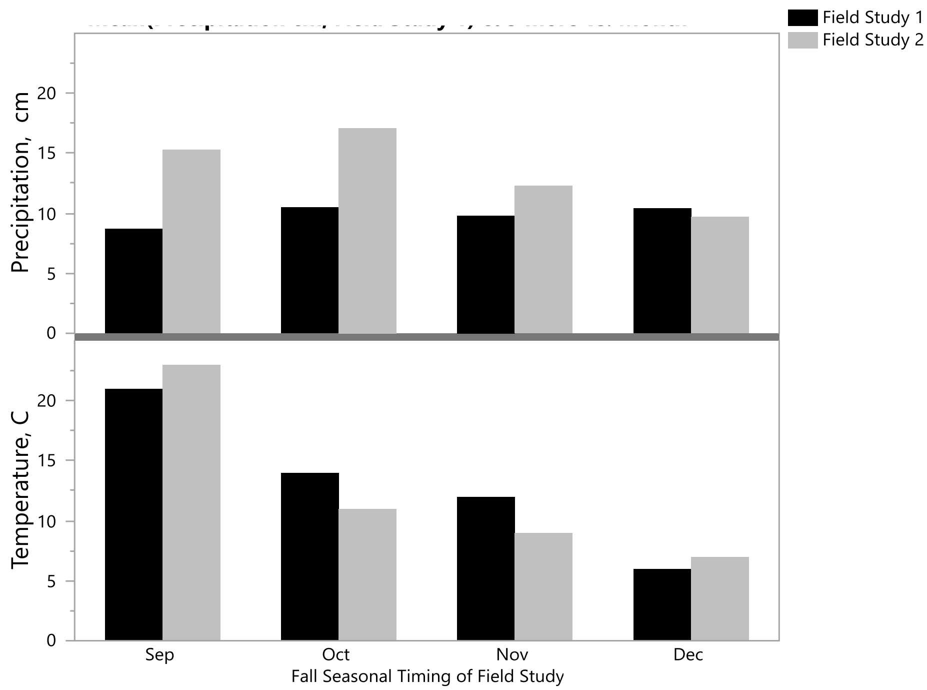

Figure 1. Monthly precipitation and temperature at fall seasonal timings of field study across both runs of experiment. Data recorded by golf course staff at site of small plot research using a physical rain gauge (Model 6330, Stratus Inc) and thermometer (Model 115-01, Fischer Instruments) Each bar represents total monthly precipitation and average temperature from first to last day of month.

Table 6. Effect of methiozolin and paclobutrazol on creeping bentgrass injury by seasonal timing, Field Study 1

	Creeping bentgrass injury†		
Treatment and Timing	4 WAIT§	12 WAIT	24 WAIT
	------------------------- 0-5 -------------------------		
Methiozolin 1.44 kg ai ha $^{-1}$			
Sep 1	0.3 cd‡	0.0 b	0.0 b
Oct 1	0.0 d	0.0 b	0.0 b
Nov 1	0.7 bcd	0.7 ab	0.3 b
Apr 1	0.3 cd	0.3 b	0.3 b
May 1	0.0 d	0.0 b	0.0 b
Paclobutrazol 0.28 kg ai ha $^{-1}$			
Sep 1	1.0 abc	0.7 ab	0.7 b
Oct 1	0.7 bcd	1.0 ab	1.0 ab
Nov 1	1.7 a	2.0 a	2.0 a
Apr 1	1.3 ab	1.0 ab	0.7 b
May 1	0.7 bcd	1.3 ab	0.7 b
Non-treated Control	0.0 d	0.0 b	0.0 b

† Creeping bentgrass injury was visually assessed using a 0 to 5 scale where 0 = no injury and 5 = all plants dead. Maximum acceptable injury = 1.0
‡ Within evaluation dates means followed by the same letter are not significantly different via means separation at the α= 0.05 level
§ WAIT= weeks after initial treatment

Treatment date is initial application. Second application 2 WAIT. At each application methiozolin was 0.72 kg ai ha $^{-1}$ and paclobutrazol was 0.14 kg ai ha $^{-1}$.

Table 7. Effect of methiozolin and paclobutrazol on creeping bentgrass injury by seasonal timing, Field Study 2

Treatment and Timing	Creeping bentgrass injury[†]		
	4 WAIT[§]	12 WAIT	24 WAIT
	------------------------- 0-5 --------------------------		
Methiozolin 1.44 kg ai ha $^{-1}$			
Sep 1	0.7 b[‡]	0.3 bc	0.0 b
Oct 1	0.3 b	0.7 b	0.0 b
Nov 1	1.0 ab	1.0 ab	0.3 b
Apr 1	0.3 b	0.0 b	0.3 b
May 1	0.3 b	0.3 bc	0.0 b
Paclobutrazol 0.28 kg ai ha $^{-1}$			
Sep 1	1.0 ab	0.7 b	0.7 b
Oct 1	1.3 ab	1.0 ab	1.0 ab
Nov 1	2.3 a	2.3 a	1.3 a
Apr 1	0.7 b	1.0 ab	0.0 b
May 1	0.3 b	0.7 b	0.0 b
Non-treated Control	0.0 b	0.0 b	0.0 b

[†] Creeping bentgrass injury was visually assessed using a 0 to 5 scale where 0 = no injury and 5 = all plants dead. Maximum acceptable injury = 1.0
[‡] Within evaluation dates means followed by the same letter are not significantly different via means separation at the $\alpha = 0.05$ level
[§] WAIT= weeks after initial treatment

Treatment date is initial application. Second application 2 WAIT. At each application methiozolin was 0.72 kg ai ha $^{-1}$ and paclobutrazol was 0.14 kg ai ha $^{-1}$.

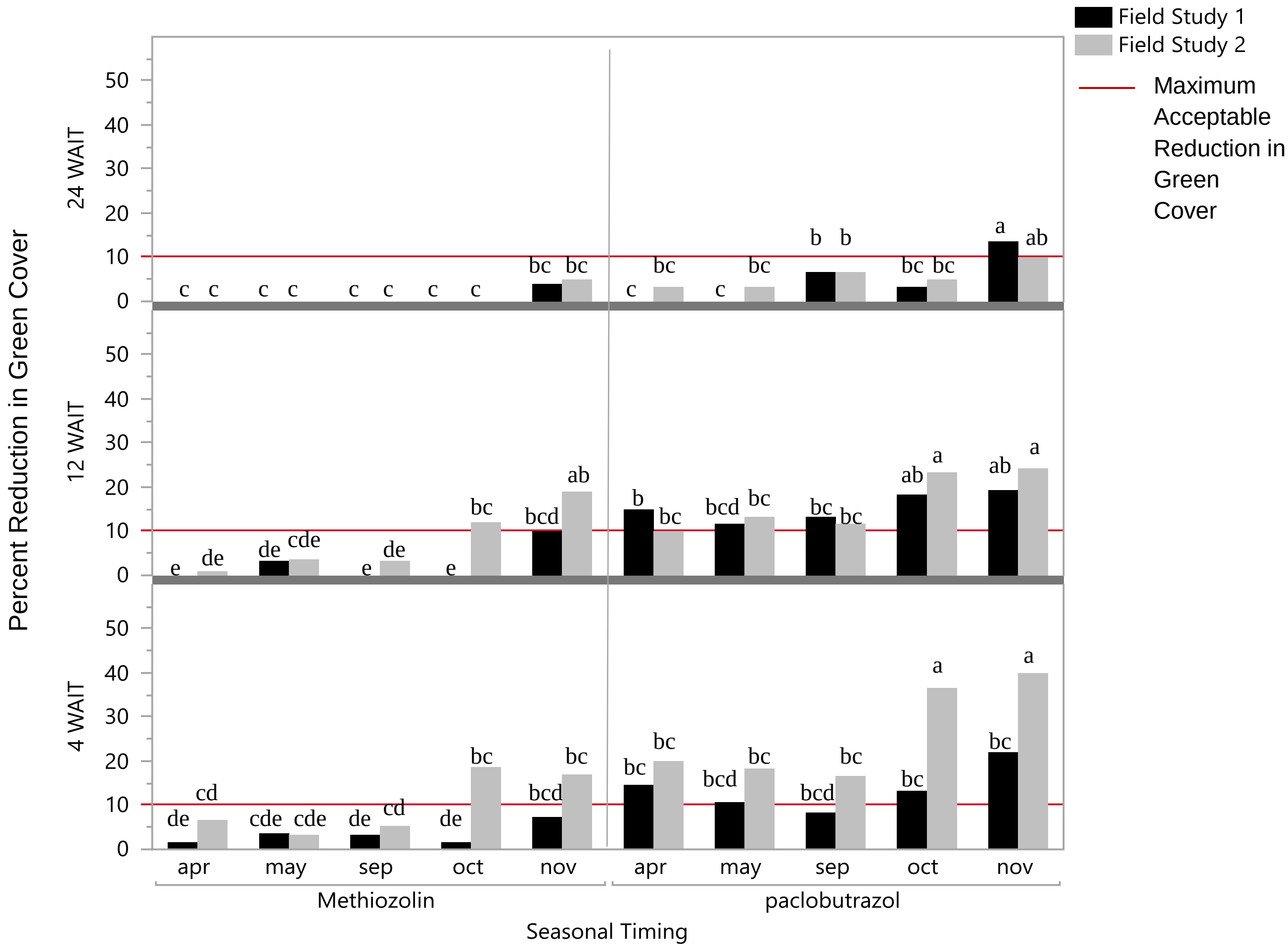

Figure 2. Percent reduction in total green cover on a mixed creeping bentgrass and annual bluegrass putting green as influenced by various seasonal timings of methiozolin relative to the treated control, Field Study 1 and Field Study 2. Data presented on a 0 to 100 % scale where 0% reduction = entire plot green/covered and 100% reduction = entire plot brown/dead scale. Treatment timings with the same letter above the bar are not significantly different at α 0.05 within the WAIT.
WAIT = Weeks after initial treatment

Table 8. Effect of methiozolin & paclobutrazol on percent reduction in total green cover by seasonal timing, Field Study 1

	Percent reduction in total green cover[†]		
	4 WAIT[§]	12 WAIT	24 WAIT
Treatment and Timing	---------------- 0-100% Reduction ----------------		
Methiozolin 1.44 kg ai ha $^{-1}$			
Sep 1	3.3 c[‡]	0.0 d	0.0 b
Oct 1	8.0 bc	0.0 d	0.3 b
Nov 1	13.3 bc	8.7 bc	0.0 b
Apr 1	3.0 c	0.0 d	0.0 b
May 1	1.0 c	3.3 cd	1.3 b
Paclobutrazol 0.28 kg ai ha $^{-1}$			
Sep 1	18.3 b	13.3 b	6.7 ab
Oct 1	20.0 b	18.3 ab	3.3 b
Nov 1	41.3 a	26.7 a	16.7 a
Apr 1	17.0 b	15.0 b	0.7 b
May 1	13.3 bc	11.7 bc	0.0 b
Non-treated Control	0.0 d	0.0 d	0.0 b

[†] Percent reduction in total green cover on a 0 to 100 % scale where 0% reduction = entire plot green and 100% reduction = entire plot brown/dead scale. Maximum acceptable reduction in percent green cover = 10%.
[‡] Within evaluation dates means followed by the same letter are not significantly different via means separation at the α= 0.05 level
[§] WAIT= weeks after initial treatment

Treatment date is initial application. Second application 2 WAIT. At each application methiozolin was 0.72 kg ai ha $^{-1}$ and paclobutrazol was 0.14 kg ai ha $^{-1}$.

Table 9. Effect of methiozolin & paclobutrazol on percent reduction in total green cover by seasonal timing, Field Study 2

	Percent reduction in total green cover†		
	4 WAIT§	12 WAIT	24 WAIT
Treatment and Timing‡	---------------- 0-100% reduction ----------------		
Methiozolin 1.44 kg ai ha $^{-1}$			
Sep 1	5.3 c	3.3 de	0.0 c
Oct 1	18.7 bc	12.0 b-e	0.0 c
Nov 1	17.0 bc	19.0 abc	5.0 abc
Apr 1	6.7 c	1.0 e	0.0 c
May 1	3.3 c	3.7 de	0.0 c
Paclobutrazol 0.28 kg ai ha $^{-1}$			
Sep 1	16.7 bc	11.7 bcd	6.7 ab
Oct 1	36.7 ab	23.3 ab	5.0 abc
Nov 1	40.0 a	24.3 a	10.0 a
Apr 1	20.0 abc	10.0 cde	3.3 bc
May 1	18.3 abc	13.3 a-d	3.3 bc
Non-treated Control	0.3 c	0.0 e	0.0 b

† Percent reduction in total green cover on a 0 to 100 % scale where 0% reduction = entire plot green and 100% reduction = entire plot brown/dead scale. Maximum acceptable reduction in percent green cover = 10%.
‡ Within evaluation dates means followed by the same letter are not significantly different via means separation at the α= 0.05 level
§ WAIT= weeks after initial treatment

Treatment date is initial application. Second application 2 WAIT. At each application methiozolin was 0.72 kg ai ha $^{-1}$ and paclobutrazol was 0.14 kg ai ha $^{-1}$.

Table 10. Creeping bentgrass color in a mixed creeping bentgrass and annual bluegrass putting green as influenced by various seasonal timings of methiozolin applications. Field Study 1 and Field Study 2

	Turfgrass Color†		
	4 WAIT§	12 WAIT	24 WAIT
Treatment and Timing	1-9		
	Field Study 1		
Methiozolin 1.44 kg ai ha $^{-1}$			
Sep 1	7.7 a‡	7.3 ab	7.5 ab
Oct 1	7.9 a	7.3 ab	7.3 b
Nov 1	6.8 ab	7.1 ab	7.3 b
Apr 1	7.2 ab	7.3 ab	7.2 b
May 1	7.6 a	7.8 ab	7.8 ab
Paclobutrazol .28 kg ai ha $^{-1}$			
Sep 1	6.8 ab	7.6 ab	8.0 ab
Oct 1	6.3 b	6.8 b	7.8 ab
Nov 1	7.2 ab	6.3 b	8.3 a
Apr 1	7.2 ab	7.8 ab	7.7 ab
May 1	7.7 a	8.3 a	7.4 ab
Non-treated Control	7.9 a	7.5 ab	7.7 ab
	Field Study 2		
Methiozolin 1.44 kg ai ha $^{-1}$			
Sep 1	6.3 bc	6.8 bcd	7.4 ab
Oct 1	6.7 ab	6.3 cd	7.3 ab
Nov 1	6.4 ab	6.0 cde	7.0 b
Apr 1	7.3 ab	7.0 bc	7.4 ab
May 1	7.7 a	7.4 ab	8.0 ab
Paclobutrazol .28 kg ai ha $^{-1}$			
Sep 1	6.0 bc	6.5 cd	7.8 ab
Oct 1	5.5 c	5.7 de	7.8 ab
Nov 1	5.5 c	5.5 e	8.2 a
Apr 1	6.8 ab	7.8 ab	8.0 ab
May 1	6.8 ab	8.3 a	7.8 ab
Non-treated Control	7.6 a	7.7 ab	7.6 ab

† Color assessed using a 1- 9 scale, where 1 = brown or dead plot, 9 = dark green or ideal turf color and minimum acceptable color for putting green = 7

‡ Within evaluation dates means followed by the same letter are not significantly different via means separation at the α= 0.05 level

§ WAIT= weeks after initial treatment

Treatment date is initial application. Second application 2 WAIT. Each application was methiozolin 0.72 kg ai ha $^{-1}$ and paclobutrazol 0.28 kg ai ha $^{-1}$

Table 11. Creeping bentgrass quality in a mixed creeping bentgrass and annual bluegrass putting green as influenced by various seasonal timings of methiozolin applications. Field Study 1 and Field Study 2

	Turfgrass Quality†		
	4 WAIT§	12 WAIT	24 WAIT
Treatment and Timing		1-9	
		Field Study 1	
Methiozolin 1.44 kg ai ha $^{-1}$			
Sep 1	7.3 ab‡	7.8 ab	7.6 b
Oct 1	7.8 a	7.0 b	7.1 c
Nov 1	6.8 ab	7.2 b	7.4 bc
Apr 1	7.3 ab	8.0 a	7.2 c
May 1	7.5 a	7.8 ab	7.8 ab
Paclobutrazol .28 kg ai ha $^{-1}$			
Sep 1	7.0 ab	7.8 ab	8.1 ab
Oct 1	6.3 b	6.8 bc	7.7 b
Nov 1	6.8 ab	6.3 c	8.3 a
Apr 1	7.3ab	7.7 ab	7.8 ab
May 1	7.6 a	8.0 a	7.5 b
Non-treated Control	7.9 a	7.7 ab	7.7 b
		Field Study 2	
Methiozolin 1.44 kg ai ha $^{-1}$			
Sep 1	7.4 ab	7.1 bc	7.7 ab
Oct 1	7.6 ab	7.1 bc	7.3 ab
Nov 1	6.7 cd	6.7 cd	7.3 ab
Apr 1	7.1 bc	7.8 ab	8.0 a
May 1	7.3 bc	7.7 ab	7.3 ab
Paclobutrazol .28 kg ai ha $^{-1}$			
Sep 1	7.0 bc	6.9 bc	7.7 ab
Oct 1	6.8 cd	6.6 cd	7.5 ab
Nov 1	6.3 d	6.0 d	7.8 ab
Apr 1	7.4 ab	7.3 abc	7.0 b
May 1	8.0 a	8.2 a	7.6 ab
Non-treated Control	7.6 ab	7.7 ab	7.4 ab

† Quality assessed using a 1- 9 scale, where 1 = brown or dead plot, 9 = optimal turf quality and minimum acceptable quality for putting green = 7

‡ Within evaluation dates means followed by the same letter are not significantly different via means separation at the α= 0.05 level

§ WAIT= weeks after initial treatment

2 sequential applications, methiozolin 0.72 kg ai ha $^{-1}$ and paclobutrazol 0.28 kg ai ha $^{-1}$

Table 12. Soil nutrient[†], texture and organic matter analysis at field study initiation.

Field Study 1 (2015-2016)

Soil pH	Cation Exchange Capacity	% Base Saturation				Percent Organic Matter[‡]	USDA Textural Class[§]
		K	Ca	Mg	H		Sand-Silt-Clay
5.9	4.4	3.3	64	16	16	2.8	83-09-08 (Evesboro Loamy Sand)

Field Study 2 (2016-2017)

Soil pH	Cation Exchange Capacity	% Base Saturation				Percent Organic Matter	USDA Textural Class
		K	Ca	Mg	H		Sand-Silt-Clay
6.0	4.1	9.5	70	20	16	3.3	83-09-08 (Evesboro Loamy Sand)

[†] Mehlich-I soil test extraction
[‡] Loss on ignition
[§] Method conforms to ASTM D 422-63

Table 13. Herbicide materials used in greenhouse experiments.

Commercial Trade Name	Manufacturer	Active Ingredient	Formulation	Source	Individual Application Rate
Poacure	Moghu Research Center, Daejeon, South Korea	Methiozolin	25 % EC 0.25 kg ai L^{-1}	Moghu Research Center Ltd	20.42 ml in 1.0 L of water
Cutless	SePro Corp., IN, USA	Flurprimidol	16 % MEC 0.16 kg ai L^{-1}	Grassroots Turf Products Inc. Randolph, NJ	6.5 ml in 1.0 L of water

Table 14. Creeping bentgrass cultivars established in greenhouse experiments.

Cultivar Name	Producer	Source	Bentgrass Density Type
A 1	Tee 2 Green Corp., Hubbard, OR	All certified seed supplied by Grassroots Turf Products Inc. Randolph, NJ	Ultra-Very High Density
A 4	Tee 2 Green Corp., Hubbard, OR		Ultra-Very High Density
007	Seed Research of Oregon. Halsey, OR		High Density
Declaration	Lebanon Seaboard Corp., Lebanon, PA		Ultra-Very High Density
L 93	Jacklin Seed Co., Liberty Lake, WA		Improved-Moderately High Density
Princeville	LESCO Inc., Cleveland, OH		Improved-Moderately High Density

Table 15. Clipping dry weight of 6 creeping bentgrass cultivars as influenced by level of shade and treatment, Greenhouse Experiments, 21 days after initial treatment.

	Clipping Dry Weight† - 21 DAIT§								
	Full Sun (0% shade)			Moderate Shade (40%)			Severe Shade (80%)		
	Methiozolin	Flurprimidol	UC	Methiozolin	Flurprimidol	UC	Methiozolin	Flurprimidol	UC
CULTIVAR		--------------- g ---------------			--------------- g ---------------			--------------- g ---------------	
L93	0.61 abc‡	0.49 bcd	0.72 ab	0.37 bcd	0.31 def	0.44 abc	0.20 bc	0.16 bcd	0.29 ab
Declaration	0.49 bcd	0.47 cd	0.78 a	0.35 cde	0.28 def	0.50 a	0.16 bcd	0.16 bcd	0.34 a
A-1	0.54 bc	0.50 bc	0.72 ab	0.38 bcd	0.27 d-g	0.40 abc	0.24 abc	0.13 cd	0.27 ab
A-4	0.55 bc	0.23 e	0.64 abc	0.27 efg	0.29 d-f	0.48 ab	0.09 cd	0.12 cd	0.21 bc
Princeville	0.29 de	0.26 de	0.54 abc	0.17 gh	0.25 fg	0.31 def	0.09 cd	0.11 cd	0.19 bc
007	0.18 e	0.22 e	0.53 abc	0.11 h	0.22 fg	0.22 fgh	0.05 d	0.11 cd	0.16 bcd

† Clippings collected from individual pots at 21 DAIT at time of routine pot mowing, oven dried for 72 h and weight recorded in grams.

‡CDW means within shade treatments followed by the same letter are not significantly different at the α 0.05 level according to the least square means comparison test.

§DAIT = days after initial treatment.

Methiozolin applied at 0 and 10 DAIT totaling 2.88 kg ai ha^{-1}, total flurprimidol applied 0.56 kg ai ha^{-1}.

Table 16. Clipping dry weight by creeping bentgrass cultivar as influenced by level of shade, cultivar and treatment, Greenhouse Experiments, 42 days after initial treatment.

	Clipping Dry Weight[†] - 42 DAIT[§]								
	Full Sun (0% shade)			Moderate Shade (40%)			Severe Shade (80%)		
	Methiozolin	Flurprimidol	UC	Methiozolin	Flurprimidol	UC	Methiozolin	Flurprimidol	UC
CULTIVAR	--------------- g ---------------			--------------- g ---------------			--------------- g ---------------		
L93	0.58 abc[‡]	0.47 bcd	0.79 a	0.29 ab	0.28 ab	0.40 a	0.30 a	0.24 bc	0.32 a
Declaration	0.50 bcd	0.54 bcd	0.64 ab	0.27 ab	0.28 ab	0.41 a	0.26 ab	0.07 de	0.19 bcd
A-1	0.54 bcd	0.45 bcd	0.66 ab	0.24 b	0.23 b	0.29 ab	0.23 bc	0.11 cde	0.19 bcd
A-4	0.37 cd	0.26 ef	0.53 bcd	0.12 bc	0.15 bc	0.23 b	0.16 cd	0.02 de	0.12 cde
Princeville	0.30 def	0.20 f	0.35 cd	0.17 bc	0.12 bc	0.12 bc	0.00 e	0.12 cde	0.06 de
007	0.32 cd	0.29 def	0.64 ab	0.05 c	0.10 bc	0.10 bc	0.00 e	0.00 e	0.00 e

[†] Clippings collected from individual pots at 21 DAIT at time of routine turf mowing, oven dried for 72 h and weight recorded in grams.

[‡]CDW means within shade treatments followed by the same letter are not significantly different at the α 0.05 level according to the least square means comparison test.

[§]DAIT = days after initial treatment.

Methiozolin applied at 0 and 10 DAIT totaling 2.88 kg ai ha^{-1}, total flurprimidol applied 0.56 kg ai ha^{-1}.

Table 17. Analysis of variance for methiozolin treated creeping bentgrass clipping dry weight as affected by amount of shade, cultivar and their interaction

	---------------------- P > F ----------------------	
	21 DAIT	42 DAIT
	Run 1	
Shade	0.0003	< .0001
Cultivar	< .0001	< .0001
Shade x Cultivar	0.0091	0.5440
	Run 2	
Shade	< .0001	< .0001
Cultivar	< .0001	< .0001
Shade x Cultivar	0.0046	0.4290

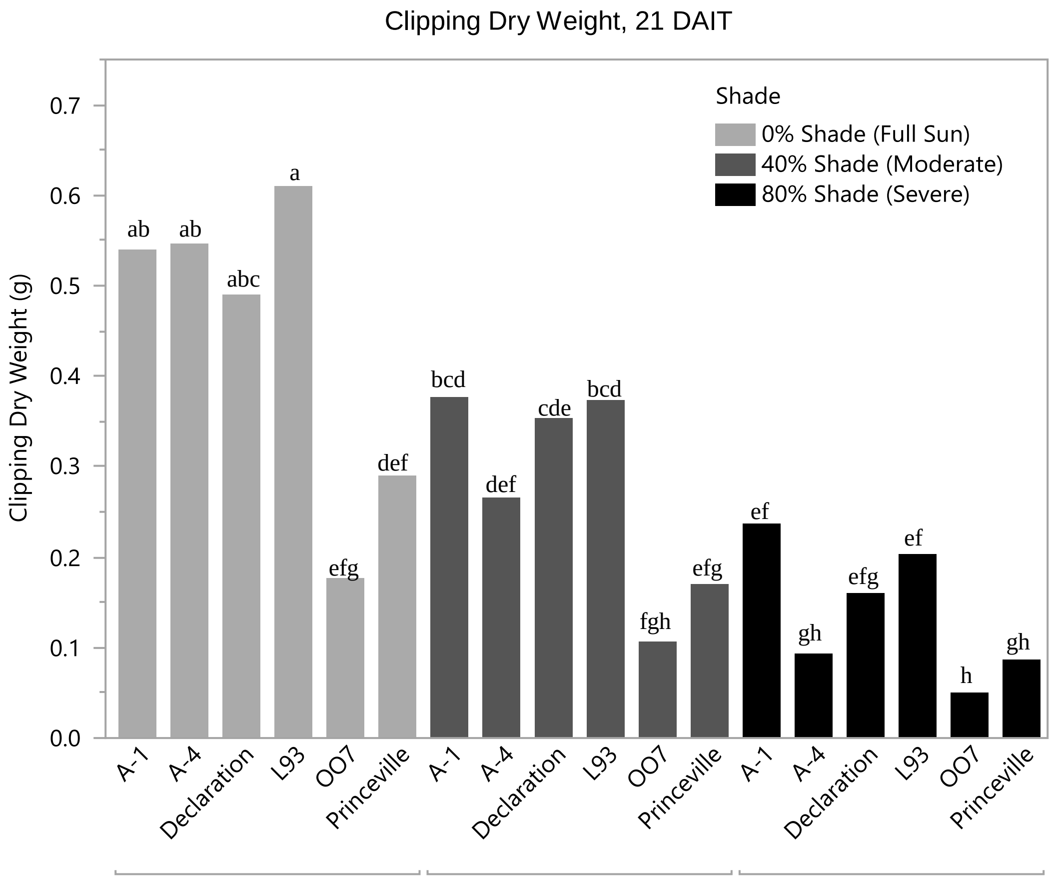

Figure 3. Effect of increasing shade on clipping dry weight of 6 creeping bentgrass cultivars at 21 Days after initial treatment of methiozolin. Cultivars with the same letter above the bar are not significantly different at the α 0.05 level across experiments. Sequential methiozolin applications totaling 2.88 kg ai ha^{-1}.
DAIT= days after initial treatment

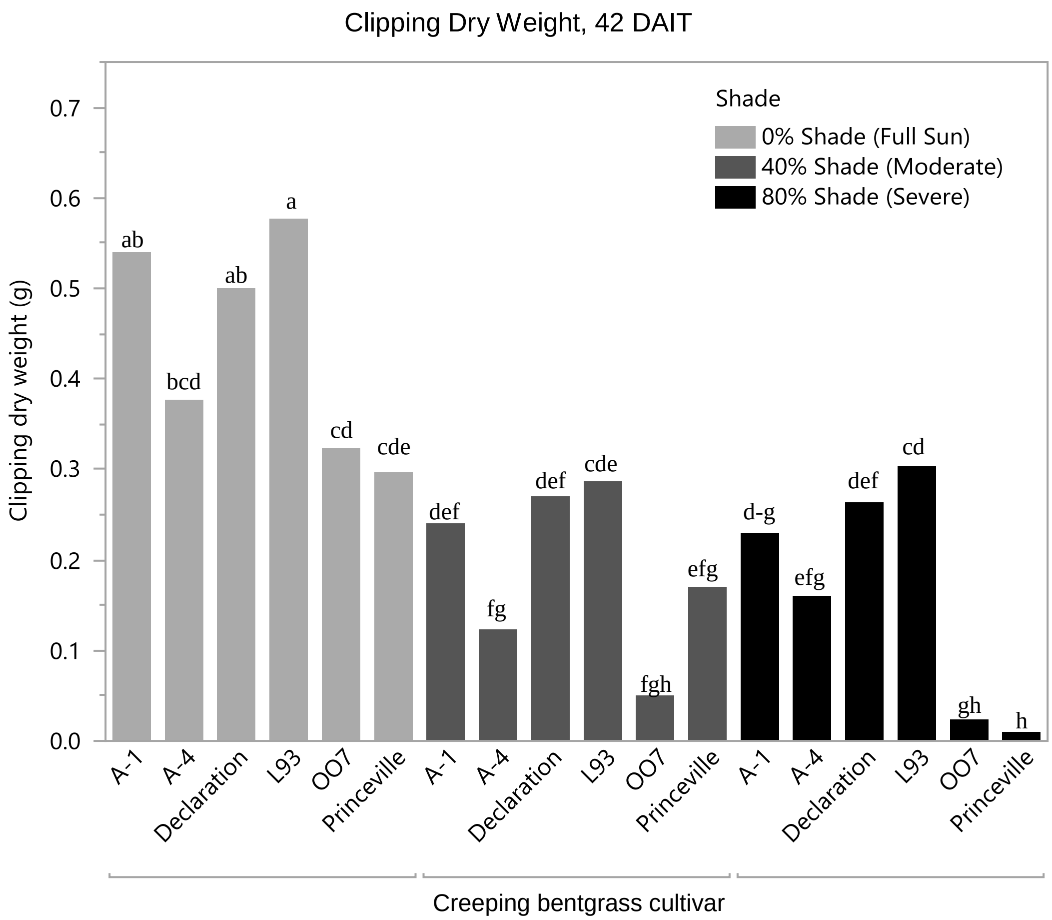

Figure 4. Effect of increasing shade on clipping dry weight of 6 creeping bentgrass cultivars at 42 Days after initial treatment of methiozolin. Cultivars with the same letter above the bar are not significantly different at the α 0.05 level across experiments. Sequential methiozolin applications totaled 2.88 kg ai ha^{-1}.
DAIT= days after initial treatment

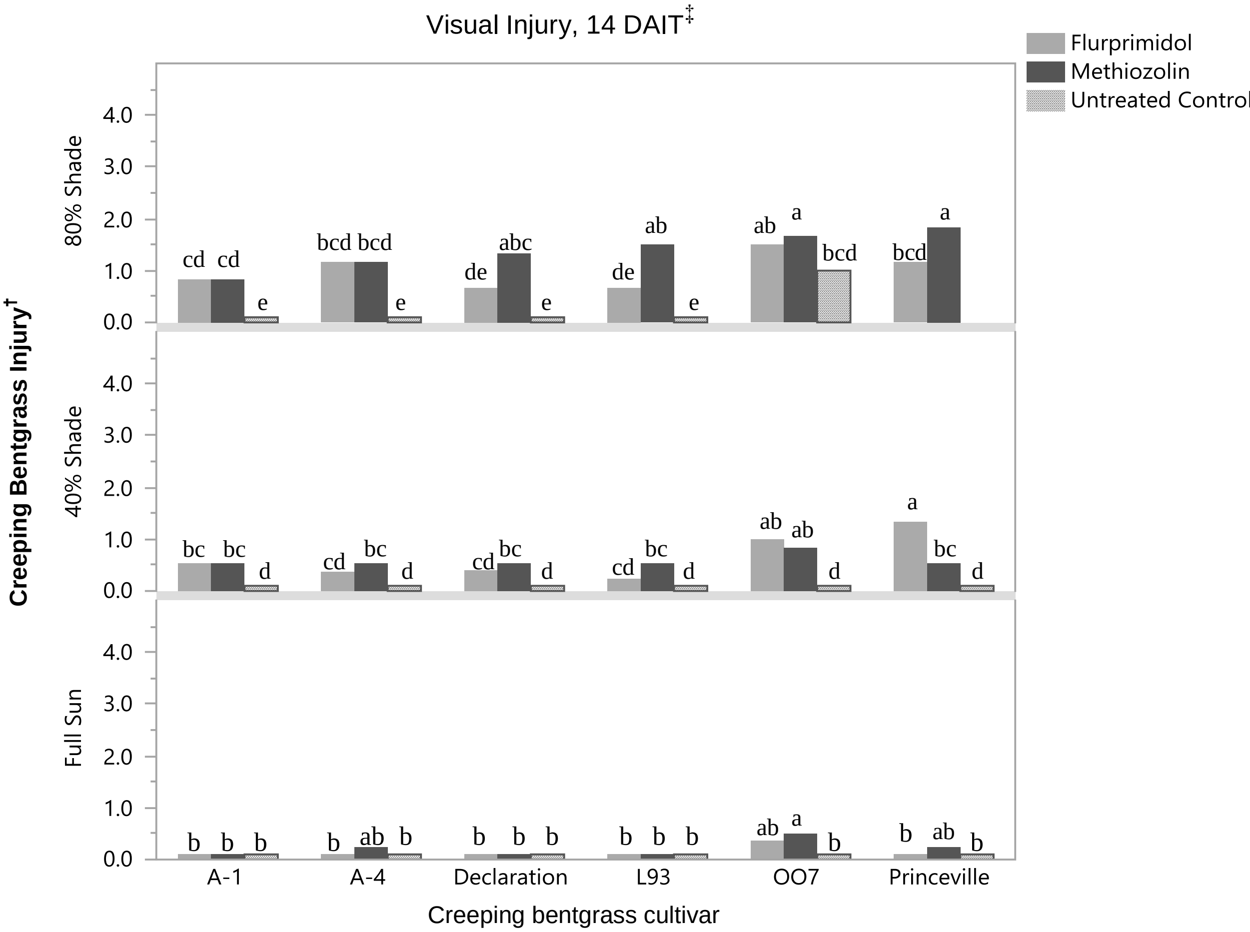

Figure 5. Effect of increasing shade on visual injury of 6 creeping bentgrass cultivars at 14 days after initial treatment of methiozolin.

Cultivars with the same letter above the bar are not significantly different at the α 0.05 level across experiments.

† Creeping bentgrass visual injury assessed using a 0 to 5 scale where

0 = no injury and 5 = all plants dead. Maximum acceptable injury = 1.0.

Sequential methiozolin totaled 2.88 kg ai ha^{-1}

Sequential flurprimidol totaled 0.56 kg ai ha^{-1}

‡ DAIT= days after initial treatment

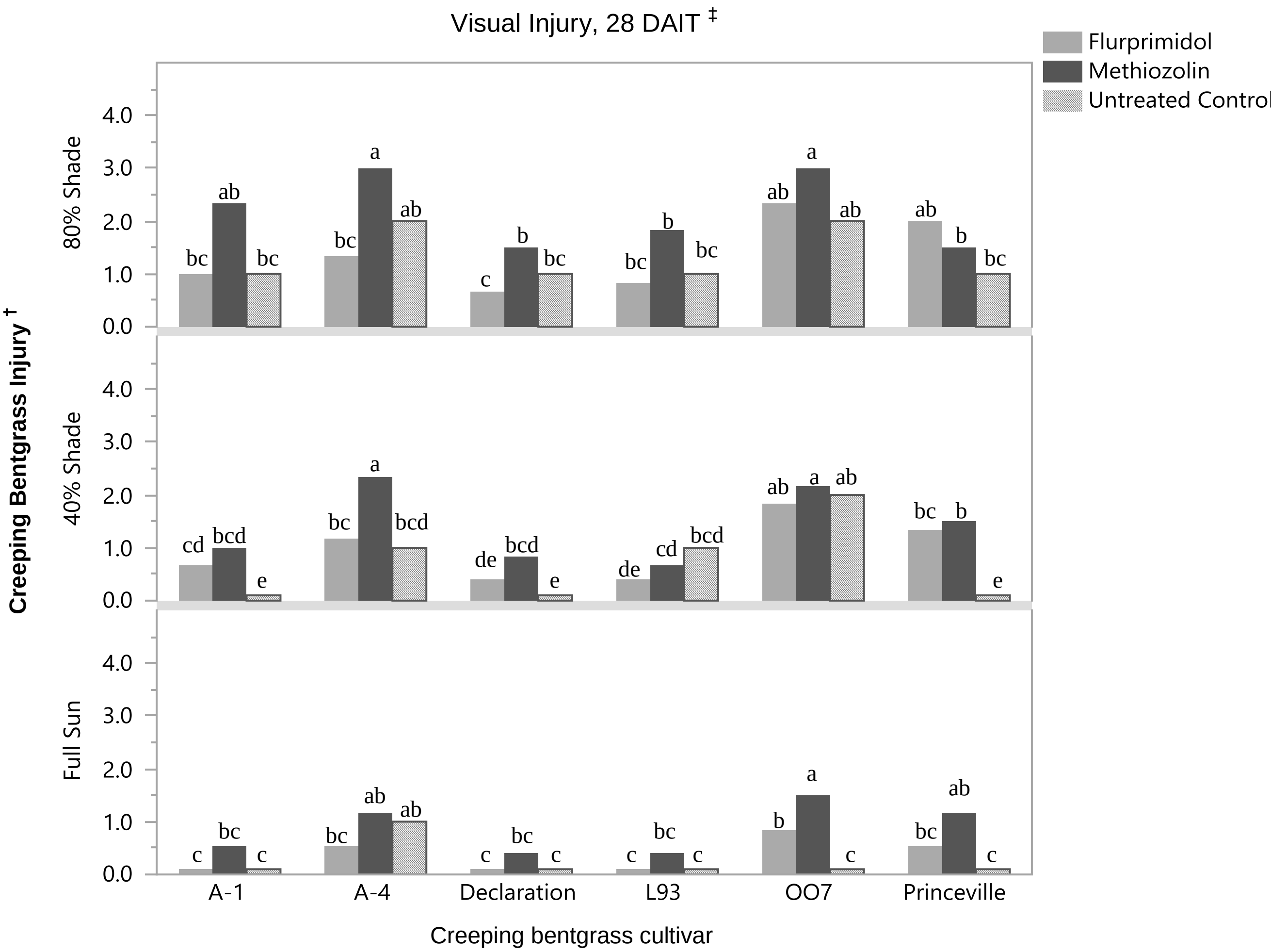

Figure 6. Effect of increasing shade on visual injury of 6 creeping bentgrass cultivars at 28 days after initial treatment of methiozolin. Cultivars with the same letter above the bar are not significantly different at the α 0.05 level across experiments.

† Creeping bentgrass visual injury assessed using a 0 to 5 scale where 0 = no injury and 5 = all plants dead. Maximum acceptable injury = 1.0.

Sequential methiozolin applications totaled 2.88 kg ai ha^{-1}
Sequential flurprimidol applications totaled 0.56 kg ai ha^{-1}

‡ DAIT= days after initial treatment

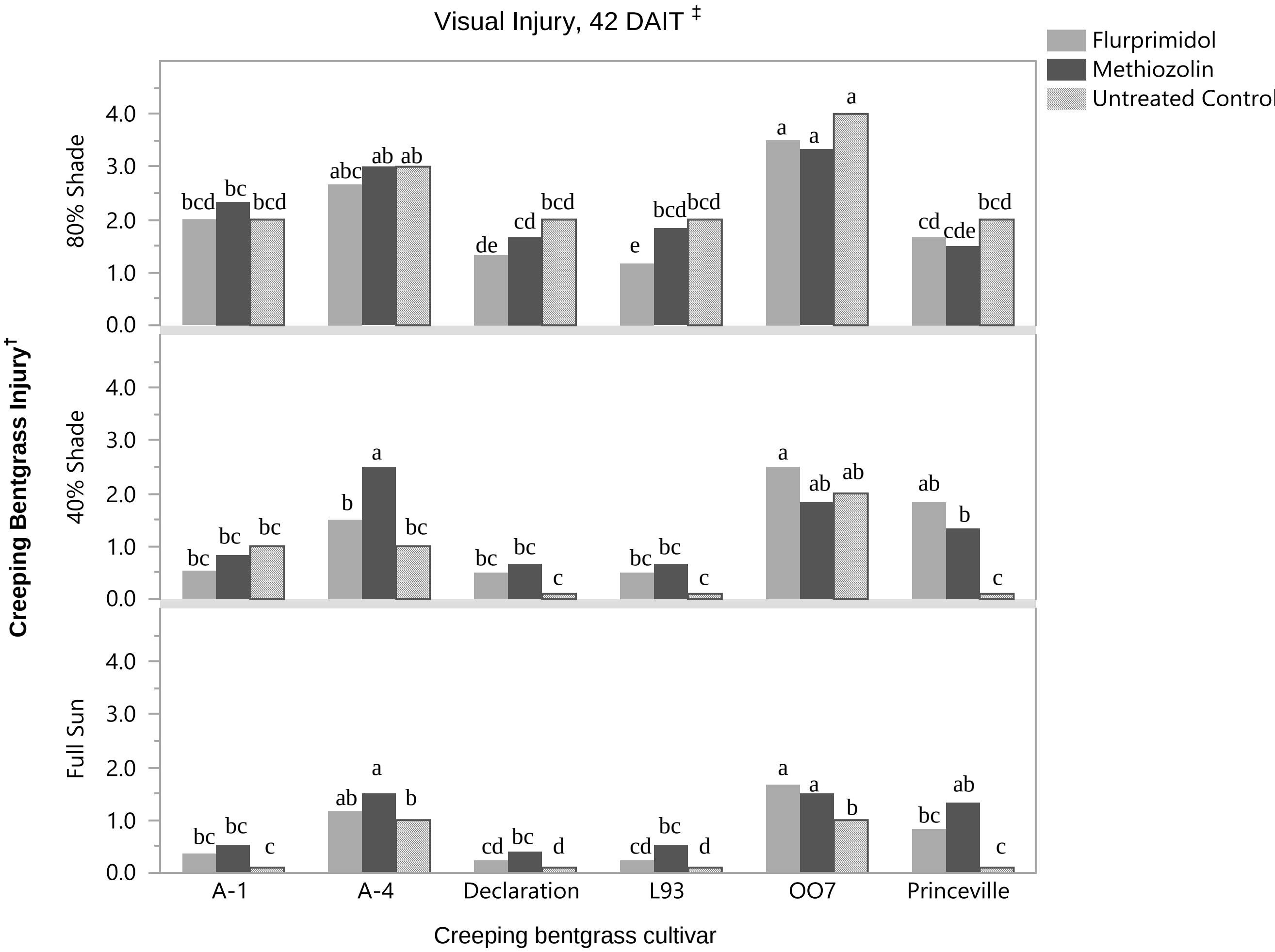

Figure 7. Effect of increasing shade on visual injury of 6 creeping bentgrass cultivars at 42 days after initial treatment of methiozolin. Cultivars with the same letter above the bar are not significantly different at the α 0.05 level across experiments.

† Creeping bentgrass visual injury assessed using a 0 to 5 scale where 0 = no injury and 5 = all plants dead. Maximum acceptable injury = 1.0.

Sequential methiozolin totaled 2.88 kg ai ha^{-1}
Sequential flurprimidol totaled 0.56 kg ai ha^{-1}

‡ DAIT= days after initial treatment

Table 18. Source of variation for creeping bentgrass visual response variables as affected by treatment, shade, cultivar and their interaction

	-------------------- P > F ---------------------		
	14 DAIT	28 DAIT	42 DAIT
	Percent Reduction in Total Green Cover		
Treatment	< .0001	< .0001	0.0003
Cultivar	< .0001	< .0001	< .0001
Shade	< .0001	< .0001	< .0001
Treatment x Cultivar	< .0002	0.1889	0.1259
Treatment x Shade	0.3764	0.0019	0.6944
Cultivar x Shade	0.0011	< .0001	0.3068
Treatment x Cultivar x Shade	0.0053	0.4032	0.0895
	Turfgrass Visual Quality		
Treatment	< .0001	< .0001	0.1262
Cultivar	< .0001	< .0001	< .0001
Shade	< .0001	< .0001	< .0001
Treatment x Cultivar	< .0001	0.2597	0.0464
Treatment x Shade	0.4499	0.0788	0.3005
Cultivar x Shade	0.1607	< .0001	0.4364
Treatment x Cultivar x Shade	0.0799	0.9828	0.1955
	Turfgrass Visual Color		
Treatment	0.0004	0.0003	0.0622
Cultivar	< .0001	< .0001	< .0001
Shade	< .0001	< .0001	< .0001
Treatment x Cultivar	< .0001	0.5727	0.0017
Treatment x Shade	< .0001	0.0604	< .0001
Cultivar x Shade	0.5021	0.7331	0.6870
Treatment x Cultivar x Shade	0.4804	0.4430	0.2143

Table 19. Effect of methiozolin on percent reduction in total green cover of 6 creeping bentgrass cultivars as influenced by level of shade, Greenhouse Experiments.

	Percent Reduction in Total Green Cover†								
	Full Sun (0% shade)			Moderate Shade (40%)			Severe Shade (80%)		
	14 DAIT§	28 DAIT	42 DAIT	14 DAIT	28 DAIT	42 DAIT	14 DAIT	28 DAIT	42 DAIT
Methiozolin 2.88 kg ai ha $^{-1}$	------- 0-100% Reduction -------			------- 0-100% Reduction -------			------- 0-100% Reduction -------		
L93	3.3 c‡	6.7 c	5.8 bc	8.3 de	3.3 d	8.3 b	12.5 c	18.3 bc	25.0 bc
Declaration	11.7 b	6.7 c	5.0 bc	7.5 e	5.8 cd	8.3 b	20.8 b	13.0 c	19.3 c
A-1	6.7 bc	9.2 bc	1.7 c	10.0 cde	10.0 c	11.7 ab	18.3 bc	25.8 ab	31.7 ab
A-4	8.3 bc	6.7 c	4.2 bc	16.7 bcd	11.7 c	13.3 ab	26.7 ab	19.2 bc	36.7 a
Princeville	20.8 a	11.7 ab	16.7 a	26.7 a	18.3 bc	16.7 ab	36.7 a	27.5 ab	32.3 ab
007	21.7 a	14.2 a	18.3 a	23.3 ab	25.0 a	22.5 a	31.7 ab	31.7 a	33.6 ab
Non-treated Control									
L93	0.1 d	3.8 cd	2.5 bc	8.8 cde	6.3 cd	12.5 ab	15.0 bc	17.5 bc	22.5 bc
Declaration	6.0 bc	0.1 d	4.0 bc	8.8 cde	11.3 c	10.0 ab	21.3 b	13.8 c	21.2 bc
A-1	1.0 cd	3.8 cd	5.0 bc	8.8 cde	11.3 c	10.0 ab	20.0 b	21.2 b	30.0 ab
A-4	2.5 cd	0.1 d	5.0 bc	10.0 cd	11.5 c	8.0 b	18.8 bc	21.2 b	23.8 bc
Princeville	5.0 c	1.3 d	7.5 bc	13.8 bcd	18.8 bc	15.0 ab	17.5 bc	30.8 a	35.3 a
007	5.0c	2.5 cd	3.8 bc	18.8 bc	25.0 a	16.3 ab	15.0 bc	30.5 a	21.3 bc

† Percent reduction in total creeping bentgrass green cover rated on a 0 to 100 % scale where 0% reduction = entire plot green and 100% reduction = entire plot brown/dead scale.

‡Column means followed by same letter are not significantly different via the least square means comparison test at α= 0.05 level

§ DAIT = days after initial treatment

Table 20. Effect of flurprimidol on percent reduction in creeping bentgrass total green cover as influenced by shade treatment, Greenhouse Experiments

	Percent Reduction in Total Green Cover†		
	14 DAIT§	28 DAIT	42 DAIT
Flurprimidol 0.56 kg ai ha^{-1}	---------------- 0-100% Reduction ----------------		
Full Sun (0% Shade)			
L93	6.7 e‡	8.3 fg	9.2 fg
Declaration	8.3 e	5.0 g	4.0 g
A-1	8.3 e	6.7 fg	5.8 g
A-4	27.7 cd	14.2 d-g	15.0 c-f
Princeville	20.0 cd	15.9 d-g	17.5 c-f
007	13.3 de	6.3 fg	11.7 def
Moderate Shade (40%)			
L93	12.5 de	13.3 d-g	10.0 ef
Declaration	12.5 de	9.7 efg	10.8 ef
A-1	10.8 de	10.9 efg	15.0 c-f
A-4	12.5 de	16.7 c-f	10.8 ef
Princeville	28.3 bcd	22.5 bcd	30.0 ab
007	33.3 bc	31.7 ab	27.5 bcd
Severe Shade (80%)			
L93	18.3 cd	18.2 c-f	22.5 b-e
Declaration	37.5 ab	20.0 cde	25.0 bcd
A-1	17.5 cd	20.9 cde	29.2 bc
A-4	34.2 bc	27.5 abc	28.3 bcd
Princeville	50.0 a	28.3 abc	30.8 ab
007	19.2 cd	35.5 a	43.0 a

† Percent reduction in total green cover visually assessed on a 0 to 100 % scale where 0% reduction = entire plot green and 100% reduction = entire plot brown/dead scale. Maximum acceptable reduction in creeping bentgrass cover = 10%.
‡Within evaluation dates means followed by the same letter are not significantly different at the α= 0.05 level
§ DAIT= days after initial treatment

Table 21. Visual quality ratings for 6 creeping bentgrass cultivars as influenced by level of shade and methiozolin treatment, Greenhouse Experiments.

	Turfgrass Quality†								
	Full Sun (0% shade)			Moderate Shade (40%)			Severe Shade (80%)		
	14 DAIT§	28 DAIT	42 DAIT	14 DAIT	28 DAIT	42 DAIT	14 DAIT	28 DAIT	42 DAIT
Methiozolin 2.88 kg ai ha^{-1}	------- 1-9 -------			------- 1-9 -------			------- 1-9 -------		
L93	7.3 ab‡	6.2 b	6.3 ab	7.0 a	6.5 a	5.9 ab	6.3 a	5.6 a	6.0 a
Declaration	6.3 bcd	6.0 bc	6.4 ab	6.3 ab	6.4 ab	6.3 a	6.0 ab	5.5 a	5.4 ab
A-1	7.4 ab	6.8 ab	6.3 ab	6.6 ab	6.2 ab	6.0 ab	6.1 a	5.0 ab	4.3 bc
A-4	7.2 bc	6.5 ab	6.8 a	6.4 ab	6.0 bc	5.3 bc	5.6 ab	5.7 a	4.7 b
Princeville	5.3 e	4.7 c	4.4 c	5.8 ab	3.9 d	3.3 d	4.9 bc	3.7 bc	3.3 cd
007	5.8 de	5.3 bc	4.9 c	5.1 b	4.7 cd	5.3 bc	5.4 b	3.0 c	3.9 bcd
Non-treated Control									
L93	7.6 a	6.8 ab	6.5 ab	6.6 ab	6.8 a	5.5 b	5.5 b	5.5 a	4.8 b
Declaration	7.4 ab	6.8 ab	6.9 a	6.8 ab	6.5 ab	6.1 ab	5.5 b	5.0 ab	4.6 b
A-1	7.0 bc	7.4 a	7.1 a	7.8 ab	6.1 bc	5.5 b	5.2 bc	5.5 a	4.0 bcd
A-4	7.0 bc	7.5 a	7.0 a	6.5 ab	6.6 a	5.5 b	5.6 ab	5.0 ab	5.5 ab
Princeville	6.0 cde	5.9 bc	6.1 bc	5.9 ab	4.5 cd	5.5 b	3.9 c	3.5 bc	2.4 d
007	7.1 bc	6.3 b	6.6 ab	5.8 ab	4.9 bcd	4.6 c	3.9 c	4.4 b	3.8 bcd

† Quality assessed using a 1- 9 scale, where 1 = brown or dead plot, 9 = optimal turf quality and minimum quality for putting green = 7

‡Means in column followed by the same letter are not significantly different at the α 0.05 level according to the least square means comparison test.

§DAIT = days after initial treatment.

Table 22. Visual color ratings for 6 creeping bentgrass cultivars as influenced by level of shade and methiozolin treatment, Greenhouse Experiments.

	Turfgrass Color†								
	Full Sun (0% shade)			Moderate Shade (40%)			Severe Shade (80%)		
	14 DAIT	28 DAIT	42 DAIT	14 DAIT	28 DAIT	42 DAIT	14 DAIT	28 DAIT	42 DAIT
Methiozolin 2.88 kg ai ha^{-1}	------- 1-9 -------			------- 1-9 -------			------- 1-9 -------		
L93	7.0 ab	5.9 cd	6.2 b	7.1 a	6.3 ab	5.7 ab	6.1 ab	5.3 ab	6.2 a
Declaration	6.7 bc	5.7 cde	6.6 ab	6.4 ab	6.4 ab	6.3 a	6.3 a	5.7 a	5.2 ab
A-1	7.3 ab	6.6 bc	6.5 ab	6.5 ab	6.3 ab	5.9 ab	5.8 ab	4.8 bc	4.7 bc
A-4	7.0 ab	6.7 ab	6.4 ab	6.3 bc	6.2 ab	5.4 b	5.8 ab	5.8 a	4.8 bc
Princeville	5.1 d	5.0 e	4.8 c	5.9 bc	3.8 d	3.1 c	5.0 cd	3.3 cd	3.3 cd
007	6.3 c	5.6 de	4.7 c	5.4 c	5.0 bcd	5.1 b	5.3 bcd	3.0 d	3.8 cd
Non-treated Control									
L93	7.4 a	6.5 bc	6.8 ab	6.5 ab	6.5 ab	5.3 b	5.4 bcd	5.5 ab	4.6 bc
Declaration	7.4 a	6.8 ab	6.8 ab	6.5 ab	6.3 ab	5.8 ab	5.0 cd	4.4 bcd	4.5 bc
A-1	7.0 ab	7.4 a	7.2 a	6.9 ab	6.0 ab	5.8 ab	4.5 cde	5.5 ab	4.0 bcd
A-4	7.5 a	6.9 ab	6.5 ab	6.3 bc	6.8 a	5.9 ab	5.4 bcd	5.0 bc	4.8 bc
Princeville	6.3 c	6.1 bcd	6.0 b	6.1 bc	4.4 cd	5.3 b	3.8 e	3.9 bcd	3.0 d
007	7.3 ab	6.3 bcd	6.5 ab	6 bc	5.1 cd	5.0 b	4.3 de	4.0 cd	3.8 cd

† Color assessed using a 1- 9 scale, where 1 = brown or dead plot, 9 = optimal dark green color and min. quality for putting green = 7

‡Means in column followed by the same letter are not significantly different at the α 0.05 level according to the least square means comparison test.

§DAIT = days after initial treatment.

Field Study 1 Field Study 2

Figure 8. Image of enhanced methiozolin activity and subsequent annual bluegrass control with significantly greater reduction in percent green cover observed in October and November seasonal timings during Field Study 2, relative to Field Study 1.

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Appendix

Weed Science Society of America, Approved Common and Chemical Nomenclature

Amicarbazone	4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-trizole-1-carboxamide
Bensulide	O,Obis(1methylethyl)S[2[(phenylsulfonyl)amino]ethyl] phosphorodithioate
Bispyribac	2,6-bis[(4,6-dimethoxy-2-pyrimidinyl)oxy]benzoic acid
Dazomet	tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione
Dithiopyr	S,S-dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarbothioate
Ethofumesate	(6)-2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate
Flurprimidol	((alpha-(1-methylethyl)-alpha-[4-(trifluoromethyoxy)phenyl]-5-pyrimidinemethanol)
Glyphosate	N-(phosphonomethyl)glycine
Mesotrione	2-(4-mesyl-2-nitrobenzoyl)-3-hydroxycylohex-2-enone
Methiozolin	(5{2, 6-difluorobenzyl}oxymethyl-5-methyl-3, 3{3-methylthiophen-2-yl}-1, 2-isoxazoline
Methyl bromide	bromomethane
Paclobutrazol	((±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol
Simazine	(2-chloro-4,6-bis(ethylamino)-s-triazine)
Trinexapac-ethyl	(RS)-4-cyclopropyl(hydroxy)methylene-3,5 dioxocyclohexanecarboxylate

Appendix, Table 1. Pesticide and chemical applications to putting green #4 for the duration of field study 1, 2015-2016.

Date (Mo/d/yr)	Commercial Trade Name	Manufacturer	Active Ingredient (Common Name)	Formulation	Application Rate (In United States Customary Units)
08/25/15	Spectator Ultra	LESCO Corp., Cleveland, OH	Propiaconazole	Liquid, EC	44.0 fl oz $acre^{-1}$
09/03/15	Triple crown	FMC Corp., Philadelphia, PA	Bifenthrin, Imidacloprid, Zeta-cypermethrin	Liquid, F	10.0 fl oz $acre^{-1}$
09/09/15	Dithane 75	Dow Agroscience, Indianapolis, IN	Mancozeb	Dry, DF	22.0 lbs $acre^{-1}$
09/24/15	LESCO 18 Plus	LESCO Inc., Cleveland, OH	Iprodione	Liquid, F	2.5 gal $acre^{-1}$
09/24/15	LESCO Wet	LESCO Inc., Cleveland, OH	Ethylene Glycol Butyl Ether	Liquid	1.25 gal $acre^{-1}$
10/07/15	Mainsail	PROKoZ Inc., Apharetta, GA	Chlorothalonil	Dry, WDG	9.0 lbs $acre^{-1}$
10/07/15	Emerald	BASF Corp., Florham Park, NJ	Boscalid	Dry, WDG	8.0 oz $acre^{-1}$
11/03/15	Dithane 75	Dow Agroscience, Indianapolis, IN	Mancozeb	Dry, DF	16.5 lbs $acre^{-1}$
12/28/15	Turfcide 400	AMVAC Corp., Los Angeles, CA	Pentachloro-nitrobenzene	Liquid, F	5.0 gal $acre^{-1}$
04/12/16	Chlopyrifos	Makhteshim Agan Inc., Raleigh, NC	Chlorpyrifos	Liquid, SC	0.25 gal $acre^{-1}$
04/14/16	Daconil	Syngenta Crop Protection LLC., Greensboro, NC	Chlorothalonil	Liquid, F	1.25 gal $acre^{-1}$
04/14/16	Clearys 3336	Cleary Chemical Corp., Dayton, NJ	Thiophanate-methyl	Liquid, F	88.0 fl oz $acre^{-1}$

(Appendix, Table 1. Cont.)

05/09/16	Emerald	BASF Corp., Florham Park, NJ	Boscalid	Dry, WDG	8.0 oz $acre^{-1}$
05/09/16	Docket	Syngenta Crop Protection LLC., Greensboro, NC	Chlorothalonil	Dry, WS	5.5 lbs $acre^{-1}$
05/09/16	K-Phyte	Plantfood Co., Cranbury, NJ	Phosphite	Liquid	1.25 gal $acre^{-1}$
05/23/16	Banol	Bayer Crop Science, Research Triangle Pk, NC	Propamocarb-hydrochloride	Liquid, SC	84.0 fl oz $acre^{-1}$
05/23/16	Acelepryn	Syngenta Crop Protection LLC., Greensboro, NC	Chlorantraniliprole	Liquid, SC	20.0 fl oz $acre^{-1}$

Appendix, Table 2. Pesticide and chemical applications to putting green #3 for the duration of field study 2, 2016-2017.

Date (Mo/d/yr)	Commercial Trade Name	Manufacturer	Active Ingredient (Common Name)	Formulation	Application Rate (US Customary Units)
08/30/16	Chipco 26019	Bayer Crop Science, Research Triangle Pk, NC	Iprodione	Liquid, F	44.0 fl oz acre^{-1}
08/30/16	Clearys 3336	Cleary Chemical Corp., Dayton, NJ	Thiophanate-methyl	Liquid, F	88.0 fl oz acre^{-1}
08/30/16	Revolution	Aquatrols Corp., Paulsboro, NJ	Oxirane, methyl-, polymer with oxirane, dimethyl ether	Liquid	2.5 gal acre^{-1}
09/13/16	Daconil	Syngenta Crop Protection LLC., Greensboro, NC	Chlorothalonil	Liquid, F	1.25 gal acre^{-1}
09/21/16	Heritage 50W	Syngenta Crop Protection LLC., Greensboro, NC	Azoxystrobin	Dry, WP	17.0 oz acre^{-1}
09/21/16	Lesco Wet	LESCO Inc., Cleveland, OH	Ethylene Glycol Butyl Ether	Liquid	1.25 gal acre^{-1}
09/21/16	Imidacloprid	Makhteshim Agan Inc., Raleigh, NC	Imidacloprid	Liquid, WSP	6.4 oz acre^{-1}
10/05/16	Spectator Ultra	LESCO Corp., Cleveland, OH	Propiaconazole	Liquid, EC	44.0 fl oz acre^{-1}
10/05/16	K-Phyte	Plantfood Co., Cranbury, NJ	Phosphite	Liquid	1.25 gal acre^{-1}
10/07/15	Emerald	BASF Corp., Florham Park, NJ	Boscalid	Powder, WDG	8.0 oz acre^{-1}
10/19/16	Docket	Syngenta Crop Protection LLC., Greensboro, NC	Chlorothalonil	Dry, WS	5.5 lbs acre^{-1}

(Appendix, Table 2. Cont.)

10/19/16	Emerald	BASF Corp., Florham Park, NJ	Boscalid	Dry, WDG	8.0 oz $acre^{-1}$
10/19/16	K-Phyte	Plantfood Co., Cranbury, NJ	Phosphite	Liquid	1.0 gal $acre^{-1}$
11/02/16	Dithane 75	Dow Agroscience, Indianapolis, IN	Mancozeb	Powder, DF	16.5 lbs $acre^{-1}$
01/08/17	Turfcide 400	AMVAC Corp., Los Angeles, CA	Pentachloro-nitrobenzene	Liquid, F	5.0 gal $acre^{-1}$
04/12/17	Chlopyrifos	Makhteshim Agan Inc., Raleigh, NC	Chlorpyrifos	Liquid, SC	0.25 gal $acre^{-1}$
05/01/17	Daconil	Syngenta Crop Protection LLC., Greensboro, NC	Chlorothalonil	Liquid, F	1.5 gal $acre^{-1}$
05/01/17	Clearys 3336	Cleary Chemical Corp., Dayton, NJ	Thiophanate-methyl	Liquid, F	88.0 fl oz $acre^{-1}$
05/12/17	LESCO 18 Plus	LESCO Inc., Cleveland, OH	Iprodione	Liquid, F	2.5 gal $acre^{-1}$
05/12/17	Triple crown	FMC Corp., Philadelphia, PA	Bifenthrin, Imidacloprid, Zeta-cypermethrin	Liquid, F	10.0 fl oz $acre^{-1}$
05/12/17	Revolution	Aquatrols Corp., Paulsboro, NJ	Oxirane, methyl-, polymer with oxirane, dimethyl ether	Liquid	2.5 gal $acre^{-1}$
05/24/17	Bayleton 50	Bayer Crop Science, Research Triangle Pk, NC	Triadimefon	Dry, WSP	2.8 lbs $acre^{-1}$
05/24/17	Provaunt	EI du Pont de Nemours and Co., Wilmington, DE	Indoxacarb	Dry, WDG	12.0 oz $acre^{-1}$
05/24/17	K-Phyte	Plantfood Co., Cranbury, NJ	Phosphite	Liquid	1.25 gal $acre^{-1}$
05/24/17	ProStar 70	Bayer Crop Science, Research Triangle Pk, NC	Flutolanil	Dry, WG	6.0 lbs $acre^{-1}$