Plant Growth Regulator Effects on Bacterial Etiolation of Creeping Bentgrass Putting Green Turf Caused by *Acidovorax avenae*

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Abstract

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Bacterial etiolation, caused by *Acidovorax avenae*, is a widespread problem in creeping bentgrass putting green turf. The symptoms normally appear as abnormally elongated turfgrass stems and leaves. Observations at multiple field sites suggest the involvement of plant growth regulators (i.e., GA-biosynthesis inhibitors) commonly applied to turf, alluding to a phytohormone imbalance caused by the bacterium. A 2-year field study examined the effects of trinexapac-ethyl, flurprimidol, and paclobutrazol on bacterial etiolation severity caused by *A. avenae*. Trinexapac-ethyl applied at 0.05 kg a.i. ha⁻¹ every 7 days and 0.10 kg ha⁻¹ every 14 days

increased etiolation compared with all other treatments in both years. Flurprimidol and paclobutrazol were not different from the control but high-rate applications caused phytotoxicity that lowered turf quality early in 2014. When the etiolated turfgrass was removed with mowing, turfgrass treated with trinexapac-ethyl exhibited the highest turfgrass quality on most rating dates. Results from this work illustrate that using plant growth regulator materials with different modes of action is a solution to managing creeping bentgrass growth while limiting the potential for bacterial etiolation outbreaks.

Creeping bentgrass (*Agrostis stolonifera* L. (CBG)) is a popular cool-season species that is utilized in northern and transition climate zones for golf course putting greens. Abiotic and biotic stresses are common during the summer months and, thus, intense management is required to prevent disease and maintain turf quality throughout the growing season. In recent years, bacterial etiolation (caused by *Acidovorax avenae* and *Xanthomonas translucens*) has emerged as a growing concern for many turfgrass managers (Giordano et al. 2012; Roberts et al. 2014). Symptoms are often described as abnormal elongation of turfgrass stems and leaves that can occur rapidly overnight. Rapid growth of turfgrass plants is often associated with yellowing or chlorosis, which also detracts from the visual aesthetics of the putting green. Bacterial etiolation normally begins in the outer areas of the putting green where equipment traffic is concentrated and can continue to the interior of the green during stressful environmental conditions. Individual etiolated plants are often unsightly and can impede playability if not removed through frequent mowing. In rare cases, etiolation can progress into a bacterial decline that results in necrosis and significant turf loss (Latin 2012). Due to the rarity of bacterial diseases in turf, management practices are not well established and the lack of labeled antibiotic materials has caused turf managers to apply fungicides that rarely reduce symptoms. Many turf practitioners have questioned whether management practices employed to improve playability may be increasing plant stress and exacerbating symptoms but research is necessary to understand the etiology of this disease and to develop effective management options.

Putting green turf must endure multiple stress factors, including constantly low mowing heights (i.e., <2.6 mm), low fertilization, and regular traffic from equipment and golfers (Zontek 2004). In an effort to maintain playability, plant growth regulators (PGR) are

often utilized to reduce clipping yields and improve surface uniformity. In the past two decades, the use of PGRs has become a fundamental practice in putting green management, mainly through the adoption of application strategies established with the release of trinexapac-ethyl (TE), one of the most utilized PGRs in the turfgrass industry (Bigelow et al. 2007; Reicher et al. 2013). PGRs are categorized into two main groups: type I regulators that inhibit cell division and type II regulators that inhibit processes in the gibberellin biosynthesis pathway and, thus, limit cell elongation. Gibberellins are considered a major class of plant hormones, which are compounds produced by plants that influence metabolic processes at very low concentrations (Raskin 1992). Gibberellins induce cell elongation and are designated as GA_(x), where *x* corresponds to the order of discovery (MacMillan 2001; Rodrigues et al. 2012). TE is a type II PGR that inhibits the 3β-hydroxylase conversion of GA₂₀ to GA₁ and is absorbed through the foliage (Ervin and Zhang 2007b). In addition to gibberellin inhibition, regular TE applications may improve heat stress tolerance, shade tolerance, drought tolerance, and turf density, and reduce anthracnose severity caused by *Colletotrichum cereale* Manns sensu lato Crouch, Clarke, & Hillman (Ervin and Koski 1998; Ervin and Zhang 2007a; Ervin et al. 2004; Inguagiato et al. 2008; McCann and Huang 2008). Other GA-biosynthesis inhibitors include paclobutrazol and flurprimidol, which are root absorbed and inhibit *ent*-kaurene oxidase that converts *ent*-kaurene to *ent*-kaurenoic acid via *ent*-kaurenol and *ent*-kaurenal early in the GA-biosynthesis pathway (Fidanza et al. 2006; Lenton et al. 1994; Rademacher et al. 1987). Although these products are not as popular as TE, they are used for the suppression of *Poa annua* var. *annua* and are sometimes applied in a mixture with TE (Reicher et al. 2013).

The sporadic growth associated with bacterial etiolation in CBG suggests a phytohormone imbalance, possibly gibberellin, within etiolated plants. The symptoms appear similar to bakanae, or foolish seedling of rice (*Oryza sativa* L.), where the fungus *Gibberella fujikuroi* (Sawada) Ito produces GA and causes infected plants to grow taller than surrounding, noninfected plants (Kurosawa 1926). Previous research has shown weekly applications of TE (active ingredient [a.i.] at 0.05 kg a.i. ha⁻¹) were associated with significantly more bacterial etiolation in CBG, caused by *A. avenae*, than plots not receiving TE (Roberts et al. 2015). Other PGR products are available in the turfgrass market and, due to the specific nature of TE applications on GA-biosynthesis, many have questioned whether other PGRs such as flurprimidol and paclobutrazol may also influence bacterial

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*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table is published online.

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etiolation development. The objective of this research was to examine the impact of TE, flurprimidol, and paclobutrazol applications on bacterial etiolation and turf quality of CBG putting green turf. Results build on previous research by comparing multiple PGR for their impact on bacterial etiolation. The research helps to improve our understanding of how etiolation develops and aids in developing management strategies for this increasing phenomenon.

Materials and Methods

Field evaluation of PGRs on bacterial etiolation. A 2-year field experiment was initiated on 5 April 2013 to evaluate the impact of TE, flurprimidol, and paclobutrazol on bacterial etiolation of CBG ('Penn-A1') putting green turf, caused by *A. avenae*. The plot area was mowed five to six times per week at a bench height setting of 3.2 to 4.1 mm; the height of cut was increased during periods of high temperatures and frequent rainfall to avoid scalping. Prior to application of treatments, N was applied at 20 kg ha⁻¹ from March to April 2013 and 38 kg ha⁻¹ from February to April 2014. During treatment application periods, N was applied at 5 to 10 kg ha⁻¹ every 10 to 14 days as needed. Fertilization was applied as urea and ammoniacal sources (i.e., 46-0-0 or 12-0-0) or using complete sources (i.e., 25-5-18, 18-3-4, and 25-6-12) that also delivered P₂O₅ and K₂O when applied; elemental P and K were applied at 6 and 37 kg ha⁻¹ in 2013 and 44 and 75 kg ha⁻¹ in 2014, respectively.

Insect pests were suppressed with chlorantranilipole (Acelepryn; Syngenta Crop Protection, Greensboro, NC) while fungal diseases like dollar spot (*Sclerotinia homoeocarpa* F. T. Benn.), brown patch (*Rhizoctonia solani* Kühn), and Pythium diseases were suppressed with fungicides, including boscalid (Emerald; BASF Corp., Research Triangle Park, NC), vinclozolin (Curalan EG; BASF Corp.), iprodione (26GT; Bayer Environmental Science, Research Triangle Park, NC), azoxystrobin (Heritage TL; Syngenta Crop Protection), flutalonil (ProStar; Bayer Environmental Science), and mefenoxam (SubdueMaxx; Syngenta Crop Protection). The site was irrigated to prevent drought stress and support turfgrass growth throughout each year. Modified alkylated polyol (Revolution; Aquatrols, Paulsboro, NJ) and alcohol ethoxates + polyethylene + polypropylene glycols (Cascade Plus; Precision Laboratories LLC, Waukegan, IL) were applied every 21 to 28 days throughout each growing season to maintain water infiltration. All maintenance was performed on the entire plot area to maintain uniformity across treatment blocks.

The study was arranged in a randomized complete block design with four replications. Treatments consisted of TE (PrimoMaxx; Syngenta Crop Protection) applied at 0.05 kg a.i. ha⁻¹ every 7 days, 0.05 kg a.i. ha⁻¹ applied every 14 days, and 0.10 kg a.i. ha⁻¹ applied every 14 days; flurprimidol (Cutless; SePRO Corp., Carmel, IN) applied at 0.11 kg a.i. ha⁻¹ every 14 days and 0.21 kg a.i. ha⁻¹ every 14 days; paclobutrazol (Trimmit; Syngenta Crop Protection) applied at 0.07 kg a.i. ha⁻¹ every 14 days and 0.14 kg a.i. ha⁻¹ every 14 days; and a nontreated control. Treatments were applied using a CO₂-pressurized system equipped with an air-induction even nozzle (Model AI9504E; Spraying Systems Co., Carol Stream, IL) calibrated to deliver each treatment rate in H₂O at 407 liters ha⁻¹. Treatments were applied from 5 April to 11 October 2013 and 11 April to 15 August 2014. The plot area was irrigated 4 to 6 h post application per label recommendation to water in flurprimidol and paclobutrazol treatments. Delayed irrigation allowed foliar absorption of TE treatments before watering in flurprimidol and paclobutrazol, which are root absorbed.

Inoculation procedures. The plot area was inoculated with *A. avenae* isolate PT-1 on 10, 11, and 12 June 2013; and 4, 5, and 6 June; 1 and 2 July; and 6 and 7 August 2014. Additional inoculations were required in the 2014 season because symptoms were slow to develop and did not persist as in 2013. To prepare for inoculation, bacteria were grown on nutrient agar for 24 to 48 h, plates were flooded with 100 mM potassium phosphate buffer containing 1% peptone, cells were scraped from the media surface, and the resulting suspension was collected for inoculation. The cell suspension was diluted to approximately 10⁹ CFU ml⁻¹ using a previously calculated calibration curve based on the optical density at 600 nm. Dilution

plating was also used to confirm CFU concentration. The bacterial suspension was diluted 10-fold at the experimental site in distilled H₂O. The final suspension (approximately 10⁸ CFU ml⁻¹) was applied to freshly cut, wet foliage (i.e., light irrigation) using a motorized backpack sprayer (SHR-210; Echo Inc., Lake Zurich, IL) equipped with a three-nozzle boom (Model 8004VS; Spraying Systems Co.) calibrated to deliver 816 liters ha⁻¹. After inoculation, the plot area was covered with a permeable cover (Evergreen, Ontario, Canada) to maintain leaf wetness through the nighttime hours. The cover was removed the following day between 0900 and 1000 h in order to prevent turf desiccation and allow for daily maintenance practices. Turf exhibiting etiolation was sampled multiple times throughout each season and *A. avenae* isolation was attempted at multiple time points across the 2-year study.

Data collection and analysis. Etiolation severity within each plot was quantified every 7 to 14 days using a grid count method to calculate the percentage of symptomatic turf. The method utilized a grid containing 171 (5 × 5 cm) square observations that was overlaid on each plot, and positive etiolation observation counts (i.e., the presence of abnormally elongated, chlorotic stems, or leaves) were transformed to a percentage using the formula $(n/171) \times 100$, where n was the number of squares observed with positive etiolation symptoms. Turf quality was rated using a 1-to-9 scale, where 1 = completely dead and 9 = healthy. Turfgrass color, density, and uniformity were all components of turf quality. Turfgrass quality was rated after morning mowing; thus, in the absence of etiolation. Phytotoxicity was also rated when visible using a 0-to-5 scale, where 0 = no phytotoxicity, 1 = some discoloration, 2 = extensive discoloration, 3 = discoloration and thinning, 4 = severe discoloration and thinning, and 5 = completely dead. In addition to individual rating dates for each of the measured components, area under progress curve (AUPC) values were also calculated to obtain a season-long measure of etiolation and turfgrass quality, which were termed AUEPC and AUQPC, respectively. AUPC was calculated using the trapezoidal method with the formula $A = \sum_{i=1}^{N-1} ((y_i + y_{i+1})/2) \times (t_{i+1} - t_i)$, where A = AUEPC or AUQPC, i = interval point for rating, N = number of ratings, y = rating value, and t = time point of rating (Madden et al. 2007). All data were subjected to analysis of variance using the MIXED procedure in SAS 9.4 for Windows and means were separated using Tukey's honestly significant difference.

Results

Field evaluation of PGRs on bacterial etiolation. Bacterial etiolation developed each year following inoculation with *A. avenae*; however, year and year-PGR effects were significant in the model. Therefore, data are presented as individual years. In 2013, etiolation symptoms developed 8 days postinoculation and increased rapidly, with a peak of 66% observed on 17 June (Fig. 1). Symptoms declined as the season progressed into early-July but increased again in late-July before declining once again as the season ended. Etiolation was consistently high among plots treated with TE, especially with TE applied at 0.10 kg a.i. ha⁻¹ every 14 days and 0.05 kg a.i. ha⁻¹ every 7 days; these two treatments exhibited more etiolation than all other treatments and the nontreated control on six of eight rating dates and when comparing AUEPC (Table 1). Reducing the TE rate to 0.05 kg a.i. ha⁻¹ every 14 days reduced etiolation compared with the higher rates on four of eight rating dates and when comparing AUEPC. Among the other PGRs tested, both flurprimidol and paclobutrazol applied at both rates were always similar to the nontreated control; results for AUEPC were also statistically similar when compared with the nontreated control.

Bacterial etiolation symptoms were slow to develop in 2014 and multiple inoculations were performed in an effort to increase severity across the plot area. Additional inoculations were successful in increasing etiolation, with a maximum of 25% observed in TE-treated plots on 17 July (Fig. 2). Etiolation was observed throughout July and into mid-August before symptoms decreased at the end of the season. Similar to 2013, TE applied at 0.05 kg ha⁻¹ every 7 days and 0.10 kg ha⁻¹ every 14 days resulted in the highest level of

etiolation compared with all other treatments, including the non-treated control, on multiple rating dates. Applications of TE at 0.05 kg ha⁻¹ every 14 days generally had less etiolation than the same product at higher rates and was only significantly higher than the nontreated control on 2 of 11 rating dates. Both rates of flurprimidol and paclobutrazol were similar to the nontreated control, resulting in significantly less etiolation than TE applied at higher rates.

Turf quality did not diminish as a result of bacterial decline in either year of study; however, quality differences among treatments were observed. In 2013, PGR applications influenced turf quality on 11 of 14 rating dates and when comparing AUQPC (Tables 1 and 2). TE applied at 0.05 kg a.i. ha⁻¹ every 7 days and 0.10 kg ha⁻¹ every 14 days typically exhibited the best turf quality, as shown on individual rating dates and in AUQPC. It should also be noted that TE treatments often resulted in the only plots with acceptable (i.e., >5) turf quality. Turf quality in flurprimidol- and paclobutrazol-treated plots was similar to the nontreated control on most rating dates; however, turf quality of these plots was lower than TE-treated plots on six rating dates later in the season.

Fig. 1. Plant growth regulator effects on bacterial etiolation, caused by *Acidovorax avenae*, of 'Penn-A1' creeping bentgrass putting green turf in 2013. Error bars represent Tukey's honestly significant difference ($P < 0.05$ probability, MIXED analysis of variance). The entire plot area was inoculated on 10, 11, and 12 June 2013. Symptoms were rated using a grid-count method that was transformed into percent etiolation.

Phytotoxicity associated with PGR applications was not observed in 2013.

Trends were similar in 2014, with both high-rate TE applications exhibiting the best turf quality during the year. However, these treatments were frequently similar to the nontreated control, and significant differences occurred at fewer instances compared with 2013 (Table 3). Differences in AUQPC were not observed (Table 1). Flurprimidol and paclobutrazol reduced turf quality compared with TE, which was a direct result of phytotoxicity. Phytotoxicity was observed with flurprimidol and paclobutrazol applications in 2014; both rates had significant phytotoxicity effects in mid-May, with the higher rates causing more phytotoxicity that extended longer into the season (Table 4).

Discussion

PGRs were evaluated for their effect on bacterial etiolation caused by *A. avenae*. Etiolation developed after inoculation each year. The severity of etiolation was less in 2014 than in 2013 but an application of propiconazole in early-season field maintenance may have

Fig. 2. Plant growth regulator effects on bacterial etiolation, caused by *Acidovorax avenae*, of 'Penn-A1' creeping bentgrass putting green turf in 2014. Error bars represent Tukey's honestly significant difference ($P < 0.05$ probability, MIXED analysis of variance). The entire plot area was inoculated on 4, 5, and 6 June; 1 and 2 July; and 6 and 7 August 2014. Symptoms were rated using a grid-count method that was transformed into percent etiolation.

Table 1. Plant growth regulator effects on area under progress curves for etiolation (AUEPC) and turf quality (AUQPC) of 'Penn-A1' creeping bentgrass putting green turf in summer 2013 and 2014^w

Treatment (kg a.i. ha ⁻¹) ^x	Int. (days) ^y	AUEPC		AUQPC	
		2013	2014	2013	2014
Trinexapac-ethyl (0.05)	7	1045 a	805 a	541 a	573
Trinexapac-ethyl (0.05)	14	433 a	702 ab	508 ab	550
Trinexapac-ethyl (0.10)	14	874 a	543 b	552 a	590
Flurprimidol (0.11)	14	123 c	196 c	487 bc	554
Flurprimidol (0.21)	14	150 c	116 c	471 bc	539
Paclobutrazol (0.07)	14	146 c	115 c	484 bc	576
Paclobutrazol (0.14)	14	170 c	159 c	469 bc	520
Nontreated	...	166 c	226 c	454 c	550
Tukey's HSD	...	245	219	48	84
Source of variation ($Pr < 0.05$) ^z	...	***	***	***	NS

^w Values within a treatment grouping followed by the same letter do not significantly differ by Tukey's honestly significant difference (HSD) test, $\alpha = 0.05$. AUEPC and AUQPC served as an experiment-long measure of etiolation calculated using the trapezoidal method with the formula $A = \sum_{i=1}^{N-1} ((y_i + y_{i+1})/2) \times (t_{i+1} - t_i)$. The entire plot area was inoculated with *Acidovorax avenae* on 10, 11, and 12 June 2013; and 4, 5, and 6 June; 1 and 2 July; and 6 and 7 August 2014.

^x Treatments were applied using a CO₂ pressurized sprayer calibrated to deliver the appropriate product rate in 407 liters ha⁻¹ from 5 April to 11 October 2013 and 11 April to 15 August 2014.

^y Treatment interval.

^z Significance of treatments was determined using the MIXED procedure in SAS, with *** corresponding to significance level 0.001; NS = nonsignificant.

influenced symptom development, requiring multiple inoculations throughout the 2014 season. It is also important to note that etiolation fluctuated greatly across each season, which often coincided with the prevailing weather patterns (data not shown). Heavy rain events followed by days of low humidity and high temperature often resulted in the most severe etiolation, and this coincided with many outbreaks observed in previous years. Additional research to determine how weather impacts etiolation development will not only improve our knowledge of how this condition develops but also aid in recreating symptoms for research trials.

Similar results were observed with respective PGR treatments across both years of study. Higher TE application rates (i.e., 0.05 kg a.i. ha⁻¹ every 7 days and 0.10 kg a.i. ha⁻¹ every 14 days) consistently exhibited the most etiolation across both years. Decreasing the frequency of TE application to 0.05 kg a.i. ha⁻¹ every 14 days often reduced etiolation. These results were expected because previous research showed that TE applied at 0.05 kg a.i. ha⁻¹ every 7 days increased bacterial etiolation caused by *A. avenae* when compared with the same rate every 14 days and a nontreated control (Roberts et al. 2015). Conversely, applications of flurprimidol and paclobutrazol did not influence etiolation development. The differences observed between the PGR evaluated in this research could be related to the different modes of action associated with each compound, because TE inhibits a later step in GA biosynthesis while flurprimidol and paclobutrazol inhibit early GA biosynthesis (Ervin and Zhang 2007b; Lenton et al. 1994; Rademacher et al. 1987). Before initiating research on *A. avenae* in CBG, some practitioners speculated that PGR applications were a possible cause of etiolation symptoms (Bigelow 2007). Although our research illustrates that increased etiolation symptoms occur with frequent TE applications, this relationship was only present in *A. avenae*-inoculated turf, whereas TE applications on noninoculated turf maintained in the growth chamber did not etiolate (Supplementary Table S1). Hence, both *A. avenae* and frequent TE applications were necessary for the severe etiolation observed in our study.

Although previous reports of turf disease caused by *A. avenae* may refer to a bacterial decline, a decline of turf was not observed in this study (Giordano et al. 2012; Latin 2012). In fact, despite having the most etiolation symptoms across all treatments in both years of study, TE treatments always exhibited the best turf quality or were no different from the nontreated control. Multiple factors may have contributed to the lack of turf quality differences within our trial, because improved turf quality within heavily etiolated plots was not expected. First, mowing heights on the research green were always 3.2 mm or greater, whereas some golf courses experiencing etiolation and bacterial decline mow below 2.5 mm. Although these

differences may seem minuscule, incremental decreases in mowing height increase the likelihood of scalping, which could contribute to thinning and necrosis commonly observed with bacterial decline. Therefore, future research evaluating the effect of mowing height on bacterial etiolation and decline would be beneficial. Second, it is important to note that turf quality was evaluated following morning mowing; thus, etiolation was removed. Our study intended to capture quality differences outside of the abnormal elongation and chlorosis (i.e., thinning from decline), which was simply removed through mowing. Even though etiolation was not observed in their study, McCann and Huang (2007) previously showed that TE applications improved turf quality of CBG during heat and drought stress. Environmental stress factors are even more common when managing CBG during the summer in the transition zone, which is one of the reasons turf managers have adopted frequent use of TE to manage putting greens through the summer months (Reicher et al. 2013). Although not always significant, flurprimidol and paclobutrazol applications at low and high rates typically exhibited lower turf quality than TE; however, both were often similar to the nontreated control. This was not expected because similar treatments applied to CBG in a growth chamber experiment showed that all PGR improved turf quality relative to the nontreated control after three applications. Differences in turf quality among treatments applied in the field were more apparent in 2013, when individual rating dates and AUQPC values showed that TE applied at high rates (0.05 kg a.i. ha⁻¹ every 7 days and 0.10 kg a.i. ha⁻¹ every 14 days) had the best turf quality. Flurprimidol and paclobutrazol at both rates were no different than TE applied at 0.05 kg ha⁻¹ every 14 days or the nontreated control. Reductions in turf quality from flurprimidol and paclobutrazol applications were also observed on a few individual rating dates in 2014, when high rates of each compound caused significant phytotoxicity (i.e., leaf purpling and discoloration). These reductions in turf quality could be related to overregulation of GA, or phytotoxicity may have been worse due to low nighttime temperatures occurring around the same time period (i.e., 7 to 9°C nightly minimum), which can worsen phytotoxic effects associated with high-rate applications of early-GA-biosynthesis inhibitors (Bigelow et al. 2007). Regardless, CBG normally recovers, as it did in our study (Bigelow 2012). In some cases, discoloration associated with PGR applications can also be corrected with soluble nitrogen or chelated iron. Additional research has shown that shoot reductions in wheat (*Triticum aestivum* L.) resulting from paclobutrazol applications can be corrected with GA₃ applications (Lenton et al. 1994). Although turf quality may benefit more from regular TE applications, both flurprimidol and

Table 2. Plant growth regulator effects on turf quality of ‘Penn-A1’ creeping bentgrass putting green turf in summer 2013

Treatment (kg a.i. ha ⁻¹) ^x	Days ^y	Turf quality (1 to 9, where 9 = best) ^w													
		13 May	21 May	28 May	14 June	18 June	24 June	2 July	7 July	16 July	22 July	29 July	8 August	16 August	23 August
Trinexapac-ethyl (0.05)	7	5.3	5.8	4.5	4.9 a	5.1 a	5.1 a	5.4 a	5.0 a	5.3 ab	4.8 ab	5.7 a	6.6 a	6.1 a	4.5 ab
Trinexapac-ethyl (0.05)	14	5.3	6.0	4.5	5.0 a	4.5 ab	4.5 ab	5.1 a	4.5 ab	4.9 abc	4.7 ab	5.0 abc	6.0 a	5.2 abc	4.1 abc
Trinexapac-ethyl (0.10)	14	6.0	6.3	4.8	4.8 ab	5.1 a	4.8 ab	5.2 a	5.1 a	5.7 a	5.2 a	5.8 a	6.8 a	5.9 ab	4.6 a
Flurprimidol (0.11)	14	5.3	6.1	4.6	4.5 ab	4.1 b	4.3 ab	4.5 b	4.1 b	4.3 bc	4.8 ab	4.8 bc	6.0 a	4.9 abcd	4.1 abc
Flurprimidol (0.21)	14	5.8	5.5	4	4.5 ab	4.3 ab	4.3 ab	4.3 b	4.1 b	4.0 c	4.8 ab	4.7 bc	6.1 a	4.5 cd	3.9 bcd
Paclobutrazol (0.07)	14	5.5	6.0	4.3	4.5 ab	4.3 ab	4.6 ab	4.5 b	4.3 b	4.0 c	4.9 ab	4.8 bc	6.1 a	4.7 bcd	4.0 abcd
Paclobutrazol (0.14)	14	4.3	4.9	4.1	3.9 b	4.6 ab	4.5 ab	4.5 b	4.3 b	4.3 bc	5.1 ab	5.2 ab	6.0 a	4.8 abcd	3.8 cd
Nontreated	...	5.3	6.3	4.6	5.2 a	4.0 b	4.1 b	4.5 b	4.3 b	3.9 c	4.1 b	4.4 c	4.0 b	3.6 d	3.4 d
Tukey's HSD	...	2.0	1.8	0.9	0.9	0.9	0.9	0.6	0.6	1.2	1.0	0.8	1.7	1.4	0.7
Variation (<i>Pr</i> < 0.05) ^z	...	NS	NS	NS	**	***	**	***	***	***	0.08	***	***	***	***

^w Turf quality was rated using a scale of 1 to 9, where 1 = completely dead and 9 = healthy. Turf density, color, and uniformity were all components of turf quality.

Values within a treatment grouping followed by the same letter do not significantly differ by Tukey's honestly significant difference (HSD) test, $\alpha = 0.05$.

^x Treatments were applied using a CO₂ pressurized sprayer calibrated to deliver the appropriate product rate in 407 liters ha⁻¹ from 5 April to 11 October 2013.

^y Treatment interval.

^z Source of variation. Significance of treatments was determined using the MIXED procedure in SAS, with *** and ** corresponding to significance levels 0.001 and 0.01, respectively; NS = nonsignificant.

paclobutrazol can be applied to reduce clipping yields while also limiting bacterial etiolation compared with TE, making each of these compounds a potential alternative for plant growth regulation in putting greens suffering from bacterial etiolation.

Additional products exist with a mixture of flurprimidol and TE and a mixture of paclobutrazol, flurprimidol, and TE. Bigelow et al. (2007) also showed that mixing TE with flurprimidol can reduce annual bluegrass populations in CBG fairway turf while providing an enhanced green color. Although both flurprimidol and paclobutrazol are more commonly used for annual bluegrass suppression, it may be beneficial to utilize these early-GA-biosynthesis inhibitors to limit bacterial etiolation. Future research to evaluate mixtures of PGR may show that increases in etiolation from TE applications can be counteracted with paclobutrazol and flurprimidol while still achieving the improved turf quality. It may also be possible to utilize flurprimidol and paclobutrazol as curative applications for bacterial etiolation while still utilizing TE in a season-long PGR schedule; however, additional research is needed for confirmation.

Due to the differences observed with PGR applications and bacterial etiolation caused by *A. avenae*, it is possible that *A. avenae* might be altering GA metabolism in infected plants. Several bacteria can synthesize usable forms of GA, including *Acetobacter diazotrophicus*, *Azospirillum lipoferum*, *A. brasilense*, *Bacillus licheniformis*,

B. pumilus, *Herbospirillum seropedicae*, and *Rhizobium phaseoli* (MacMillan 2001). Research presented here illustrates that altering PGR mode of action to inhibit early GA biosynthesis can significantly reduce bacterial etiolation, suggesting that GA production may be involved in symptom expression. Additional research is needed to evaluate *Acidovorax avenae* phytohormone production and survival. It is possible that the bacterium is producing multiple gibberellins that may stimulate etiolation. *A. avenae* could also produce effectors, which are small-secreted microbial proteins that can bind to plant metabolic sites and aid in infection. These effectors may alter plant gibberellin production and metabolism (Galán and Collmer 1999). Multiple effectors have been described in other species of *Acidovorax*, including those that influence cell mobility and biofilm formation, while many other bacterial effectors are widely known to influence phytohormone production (Bahar et al. 2009; Kazan and Lyons 2014). Future research incorporating improved sequencing technologies will likely improve our ability to describe these effectors and, ultimately, improve our understanding of the bacterial etiolation development.

Results presented here build on previous recommendations for reducing bacterial etiolation in CBG putting greens. Although TE applied at 0.05 kg a.i. ha⁻¹ every 7 days and 0.10 kg ha⁻¹ every 14 days improved turf quality, an increase in bacterial etiolation caused by

Table 3. Plant growth regulator effects on turf quality of ‘Penn-A1’ creeping bentgrass putting green turf in summer 2014

Treatment (kg a.i. ha ⁻¹) ^x	Days ^y	Turf quality (1 to 9, where 9 = best) ^w															
		18 April	2 May	7 May	14 May	21 May	27 May	3 June	12 June	18 June	24 June	3 July	17 July	21 July	29 July	4 August	11 August
Trinexapac-ethyl (0.05)	7	4.0	4.4	4.4	4.3 ab	5.4 a	5.3	6.3	5.6 a	5.4 a	4.5 a	3.5	3.8	3.9	4.4	4.8 a	5.6 ab
Trinexapac-ethyl (0.05)	14	4.5	4.4	4.4	4.3 ab	4.8 ab	4.8	5.9	5.1 ab	4.5 ab	4.1 ab	3.7	3.5	3.6	4.3	4.6 ab	4.9 ab
Trinexapac-ethyl (0.10)	14	4.5	4.5	4.5	4.6 a	5.1 a	4.9	6.0	5.9 a	5.4 a	4.6 a	4.0	3.9	4.0	4.6	4.7 ab	5.8 a
Flurprimidol (0.11)	14	4.0	4.3	4.5	4.3 ab	5.0 ab	5.0	6.0	5.4 a	5.1 ab	4.1 ab	3.5	3.6	3.7	4.4	4.5 ab	5.1 ab
Flurprimidol (0.21)	14	4.5	4.9	4.8	3.9 ab	3.9 b	4.3	5.5	4.1 b	4.4 ab	4.1 ab	3.8	3.8	3.8	4.1	4.4 b	4.9 b
Paclobutrazol (0.07)	14	4.8	4.9	4.9	4.0 ab	4.5 b	4.5	6.1	4.8 ab	4.5 ab	4.6 a	4.1	3.9	4.1	4.6	4.7 ab	5.0 ab
Paclobutrazol (0.14)	14	4.5	4.6	4.3	3.4 b	3.9 b	4.0	5.6	4.0 b	4.0 b	3.6 b	3.5	3.8	3.8	4.1	4.6 ab	5.0 ab
Nontreated	...	4.3	4.3	4.9	4.6 a	5.0 ab	5.3	6.0	5.1 ab	4.5 ab	4.1 ab	3.4	3.4	3.5	4.0	4.6 ab	4.9 b
Tukey's HSD	...	1.3	1.4	1.2	1.1	1.1	1.5	1.7	1.2	1.2	0.8	0.8	0.5	0.9	0.7	0.5	0.9
Variation (<i>Pr</i> < 0.05) ^z	...	NS	NS	NS	*	**	NS	NS	***	**	*	NS	NS	NS	NS	*	*

^w Turf quality was rated using a scale of 1 to 9, where 1 = completely dead and 9 = healthy. Turf density, color, and uniformity were all components of turf quality.

Values within a treatment grouping followed by the same letter do not significantly differ by Tukey's honestly significant difference (HSD) test, $\alpha = 0.05$.

^x Treatments were applied using a CO₂ pressurized sprayer calibrated to deliver the appropriate product rate in 407 liters ha⁻¹ from 11 April to 15 August 2014.

^y Treatment interval.

^z Source of variation. Significance of treatments was determined using the MIXED procedure in SAS, with ***, **, and * corresponding to significance levels 0.001, 0.01, and 0.05, respectively; NS = nonsignificant.

Table 4. Phytotoxicity effects associated with plant growth regulator applications on ‘Penn-A1’ creeping bentgrass putting green turf in summer 2014

Treatment (kg a.i. ha ⁻¹) ^x	Days ^y	Phytotoxicity ^w							
		14 May	21 May	27 May	3 June	12 June	18 June	24 June	3 July
Trinexapac-ethyl (0.05)	7	0.0 d	0.0 c	0.1 b	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b
Trinexapac-ethyl (0.05)	14	0.0 d	0.0 c	0.0 b	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b
Trinexapac-ethyl (0.10)	14	0.0 d	0.0 c	0.0 b	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b
Flurprimidol (0.11)	14	0.5 cd	0.3 c	0.0 b	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b
Flurprimidol (0.21)	14	1.5 ab	1.5 ab	0.6 a	0.5 b	0.9 ab	0.5 ab	0.4 a	0.3 b
Paclobutrazol (0.07)	14	1.0 bc	1.0 b	0.1 b	0.0 c	0.5 bc	0.0 b	0.0 b	0.0 b
Paclobutrazol (0.14)	14	2.0 a	2.0 a	1.0 a	1.0 a	1.5 a	0.6 a	0.4 a	0.8 a
Nontreated	...	0.0 d	0.0 c	0.0 b	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b
Tukey's HSD	...	0.7	0.6	0.5	0.5	0.9	0.5	0.4	0.5
Variation (<i>Pr</i> < 0.05) ^z	...	***	***	***	***	***	***	*	***

^w Phytotoxicity was rated using a scale of 0 to 5, where 0 = no effects, 1 to 3 = discoloration, and 4 to 5 = thinning. Values within a treatment grouping followed by the same letter do not significantly differ by Tukey's honestly significant difference (HSD) test, $\alpha = 0.05$.

^x Treatments were applied using a CO₂ pressurized sprayer calibrated to deliver the appropriate product rate in 407 liters ha⁻¹ from 11 April to 15 August 2014.

^y Treatment interval.

^z Source of variation. Significance of treatments was determined using the MIXED procedure in SAS, with *** and * corresponding to significance levels 0.001 and 0.05, respectively.

A. avenae was observed. Previous research suggested reducing or eliminating PGR applications altogether; however, our research provides evidence that altering applications to include early-GA-biosynthesis inhibitors can reduce the potential for etiolation development. Moreover, these effects were observed regardless of application rate when compared with TE. Most turfgrass managers would likely be apprehensive about applying flurprimidol and paclobutrazol during the summer months but our study showed that only high application rates reduced turf quality. Although more questions are raised regarding the etiology of this disease, information provided here improves our understanding of how PGR applications influence symptom development. Additional research will ultimately improve our knowledge of bacterial etiolation and provide new methods for control.

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