Dollar Spot and Brown Patch Incidence in Creeping Bentgrass as Affected by Acibenzolar-S-Methyl and Biostimulants

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Abstract. There is interest in identifying cultural practices that may reduce fungicide requirements of creeping bentgrass (Agrostis palustris Huds.) putting greens. Our objective was to evaluate the plant defense activator ASM in combination with 12 biostimulants for the potential to reduce dollar spot (Sclerotinia homoeocarpa F.T. Bennett) and brown patch (Rhizoctonia solani Kuhn) in a blend of ‘Cato’: ‘Crenshaw’ creeping bentgrass during 2000 and 2001. The experimental design was a split-plot with ASM as the whole plot, and biostimulants as the subplots. ASM was applied biweekly as a.i. at 35 g·ha−1 and biostimulants were applied according to manufacturers’ recommendations. Sclerotinia homoeocarpa in some subplots was reduced by 38% with ASM, but levels were ≥1500/ m2 in Aug. 2000, and turf quality was unacceptable through most of the study period. None of the biostimulants reduced dollar spot or brown patch with ASM. None of the biostimulants reduced dollar spot or brown patch in creeping bentgrass when compared to biweekly applications of soluble N at 4.9 kg·ha−1. Dollar spot suppression achieved with ASM warrants additional studies to determine how it might be used to reduce fungicide inputs on creeping bentgrass putting greens. Chemical name used: acibenzolar-S-methyl (ASM).

Fungicides are used routinely, often on a preventive schedule, to suppress dollar spot and brown patch in creeping bentgrass putting greens. As environmental stewards, golf course superintendents are interested in identifying ways to reduce fungicide use.

Plants have evolved numerous and complex defense mechanisms to survive attacks by fungal pathogens, most of which are triggered once the pathogen enters the plant. However, there are nonfungal organic chemicals that can be applied to plant leaves to stimulate defense responses. Research indicates that this induced resistance is likely associated with production of pathogenesis-related proteins (Hammerschmidt, 1999).

Acibenzolar-S-methyl [(ASM), Actigard, Syngenta Corp., Basel, Switzerland] is a plant defense activator that induces systemic acquired resistance in plants, and has antifungal, antibacterial, and antiviral activity (Cole, 1999). The induced resistance is mediated via a salicylic acid-dependent process, and can be triggered by applying salicylic acid, or ASM, which triggers a similar response (Hammerschmidt, 1999). Fungal diseases in tobacco (Nicotiana tabacum Linn.) (Cole, 1999) and cabbage (Brassica spp.) (Cubeta et al., 1999) were suppressed following ASM application. There is no published information on ASM effects on diseases of creeping bentgrass.

Some biostimulant manufacturers have marketed products to enhance turf quality, improve fungicidal chemicals that may serve to suppress turfgrass diseases, primarily by creating a healthier, more vigorous plant. However, there has been no published research to verify this. The term “biostimulant” refers to products that contain one or more of a broad range of ingredients, including nutrients, organic acids, hormones, vitamins, microbial inoculants, plant extracts and others (Karnok, 2000a). Some ingredients contained in biostimulants are produced by turfgrasses and other plants. For example, cytokinins, including adenine and zeatin, are commonly extracted from seaweed (kelp) and incorporated into biostimulant formulations. Plant responses to cytokinin may include: improved wound healing; delay of senescence and chlorosis; enhanced chloroplast development; promotion of cell division and elongation; and enhanced root elongation and root hair development (Horgan, 1984). Under good growing conditions, the turfgrass plant may have adequate levels of all hormones to ensure normal growth, and little benefit will occur from application of additional hormones. However, when the plant is exposed to certain environmental and cultural stresses, levels of some hormones, such as cytokinins, may drop (Liu et al., 2002). Under these conditions, application of cytokinins or other plant hormones could help ameliorate the stress (Liu et al., 2002).

Humic acids may also be found in some biostimulants, and have been shown to increase cell membrane permeability (Hewitt, 1952; Visser, 1985); increase oxygen uptake, respiration and photosynthesis (Aili et al., 1964); increase root and cell elongation (Vaugham, 1974); increase ion transport; and produce cytokinin-like growth responses (Cacco and Civelli, 1973). Humate materials and beneficial microorganisms including bacteria, yeasts, and actinomycetes, increased quality, and root and shoot mass of a 6-month old ‘Tifway’ bermudagrass [Cynodon dactylon (L.) Pers. × C. transvaalensis Burt-Davy] turf (Kuti, 1998).

Schmidt and Chalmers (1993) found that N applied in conjunction with fortified seaweed extract, benzyladenine, or chelated iron improved ‘Tifgreen’ bermudagrass [Cynodon dactylon (L.) Pers. × C. transvaalensis Burt-Davy] color and enhanced postdormancy turf coverage. Fall color and growth of bermudagrass has also been improved with applications of gibberellic acid (Karnok, 2000b).

More information is needed regarding fungicide alternatives for disease suppression in creeping bentgrass. Hence, our objective was to evaluate dollar spot and brown patch incidence in creeping bentgrass in response to application of ASM and 12 biostimulants.

Materials and Methods

This study was initiated in May 2000 on a sand-based golf green at the Rocky Ford Turfgrass Research Center in Manhattan, Kans. The rootzone mix was composed of 84% sand, 14% silt, and 2% clay (v/v/v). A soil test at the initiation of the study indicated a pH of 7.7, 112 mg·kg−1 P; 32 mg·kg−1 K; and 1438 mg·kg−1 Ca. The plot area was composed of a blend of ‘Cato’: ‘Crenshaw’ creeping bentgrass that had been seeded in 1998. Turf was mowed 6 d weekly at 4 mm and irrigated to prevent stress with 5 mm of water daily. A 20N–0P–4K fertilizer containing ≥25% soluble N, and 75% N from methylene ureas, was applied the entire study to provide N at 49 kg·ha−1 in April, May, September, and October, and N at 24.5 kg·ha−1 in June, July, and August.

Plots were arranged in a split-plot randomized complete-block design with three replications. Whole plots measured 4.2 × 6.3 m and consisted of ASM (treated or nontreated) applied as a.i. at 35 g·ha−1. This rate was recommended by the manufacturer based upon their preliminary research. Acibenzolar-S-methyl was applied on 14-d intervals beginning on 5 May and ending on 15 Sept., 2000. In 2001, the initial treatment was on 20 Apr., and the last application was on 19 Sept. Applications were made using a CO2 sprayer pressured at 138 kPa to deliver water at 815 L·ha−1.

Sub-plots measured 0.9 × 2.1 m and were biostimulant treatments (Table 1) and a urea-treated control. All products were applied according to label recommendations. Application rates are presented as the amount
of product applied. Liquid formulations were applied as described for ASM. Granular formulations were applied using a hand-held shaker bottle. Initial application dates each year were the same as indicated above for ASM. Biostimulants that contained N, and the total N applied per year from each: Aminoplex (2.85 kg·ha⁻¹), Flexx (2.4 kg·ha⁻¹), Turf Vigor (51.6 kg·ha⁻¹), and Focus 15G (42 kg·ha⁻¹). A biweekly application of N at 4.9 kg·ha⁻¹ from urea (46N–0P–0K) was included as the control, and provided a total annual N level of 49 kg·ha⁻¹. Acibenzolar-S-methyl, biostimulants, and N were applied at 0900 h on respective treatment days, and to the same plots in each year.

Data were collected on dollar spot and brown patch incidence, and turf quality. *Sclerotinia homoeocarpa* infection centers were counted every two weeks between 7 June and 14 Sept. 2000 and 23 May and 20 Sept. 2001 using a 27-cm-diameter template that was randomly tossed two times per plot. Values were then converted to number of infection centers per square meter. Percentage of each plot infested with brown patch was rated visually using a 0% to 100% scale in 2000 on 19, 26, and 31 July; 10, 16, 23, and 30 Aug.; and 6 and 14 Sept. In 2001, brown patch ratings were done on 12, 27, and 31 July; 22 Aug.; and 6 Sep. Dollar spot and brown patch data were analyzed using Area Under the Disease Progress Curve (AUDPC) (Campbell and Madden, 1991). Data were subjected to analysis of variance (ANOVA), and means separated using Fisher’s protected least significant difference (LSD) (P < 0.05). Turf quality was rated visually once weekly on a 0 to 9 scale, where 0 = dead turf; 7 = acceptable quality for a putting green; and 9 = optimum color, density, and uniformity. Disease and turf quality responses of all biostimulant-treated turf was compared to turf treated with the biweekly application of urea (4.9 kg·ha⁻¹) for purposes of discussion.

### Results

There was no interaction between ASM and biostimulants for dollar spot or for brown patch (Table 2). Creeping bentgrass response to ASM concerning AUDPC values for dollar spot and brown patch was similar across years and biostimulant treatments; therefore, ASM data are presented as means over years and biostimulant treatments; therefore, ASM data are presented as means over years and biostimulant treatments. Biostimulants affected dollar spot levels differently between years; hence, biostimulant effects are presented separately for each year.

**Dollar spot.** By 21 June, 2000, there was an average of 500 *S. homoeocarpa* infection centers/m², and numbers peaked on 16 Aug. at 150/m² (data not shown). In 2001, *S. homoeocarpa* infection centers numbered < 50/m² on all dates except 12 June (average of 80/m²) and 20 Sept. (average of 150/m²).

When averaged across years and biostimulant treatments, creeping bentgrass treated with ASM had an AUDPC value for dollar spot (AUDPC = 4899) was 38% lower (P < 0.05) than nontreated turf (AUDPC = 7843).

Creeping bentgrass treated with biostimulants had equivalent, or more, dollar spot than urea-treated turf in 2000 (Table 3). Biostimulants that resulted in a statistically similar level of dollar spot in creeping bentgrass when compared to biweekly urea applications were Roots 1>2>3 with *bacillus* complex, Turf Vigor, and 710–132 Microbial Fungicide. Turf treated with other biostimulants had higher AUDPC values for dollar spot than that receiving biweekly applications of urea.

In 2001, none of the biostimulants suppressed dollar spot compared to urea (Table 3). Creeping bentgrass treated with Roots 1>2>3 with *bacillus* complex, Turf Vigor, and 710–132 Microbial Fungicide had higher dollar spot numbers in 2001 compared to the same plot in 2000. Silica, alfalfa meal, and molasses were added to each biostimulant at the rate of 2.5 kg·ha⁻¹. Creeping bentgrass treated with biostimulants had equivalent, or more, dollar spot than nontreated turf (AUDPC = 7843).

### Table 1. Description of biostimulants evaluated.

| Biostimulant trade name | Rate of product | Application interval | Formulation and contents |
|-------------------------|----------------|---------------------|--------------------------|
| Roots 1>2>3 premix with *bacillus* complex | 19. L·ha⁻¹ | 2 weeks | Liquid; soil enhancing microbes with 1.2% K, 1.0% P; 2.2% chelated iron |
| Roots 1>2>3 premix with *standup* (Roots, Inc., Independence, Mo.) | 7.7 L·ha⁻¹ | 2 weeks | Liquid; soil enhancing microbes with 1.2% K, 1.0% P; 2.2% chelated iron |
| Aminoplex (Grigg Bros., Albion, Idaho) | 9.5 L·ha⁻¹ | 2 weeks | Liquid; N–3%; fermentation products–45%; natural plant and organic extracts–21%; and inert material–31% |
| Colonize T&O (Plant Health Care, Inc., Pittsburgh) | 0.5 kg·ha⁻¹ | 2 weeks | Granular; isolavone; soluble humic acids; seaweed extract derived from Ascophyllum nodosum; soluble extract from Yucca schidigera; vitamins, sugars, and amino acids; beneficial rhizosphere bacteria |
| Compete Plus (Plant Health Care, Inc., Pittsburgh) | 2 kg·ha⁻¹ | 2 weeks | Granular; water-dispersible soil inoculant that contains numerous strains of beneficial rhizosphere bacteria and fungi including selected species of *Bacillus*, *Streptomyces* and *Pseudomonas* bacteria, and *Trichoderma* fungi |
| Flexx (Plant Health Care, Inc., Pittsburgh) | 8 kg·ha⁻¹ | 2 weeks | Granular; 3N–0P–17K; humic and fulvic acids derived from solubilized leonardite; cold water sea kelp extract; Ascophyllum nodosum; amino acids; 18 vitamins; and soluble Yucca plant extract |
| Turf Vigor (Sybron Chemicals, Inc., Salem, Va.) | 57 L·ha⁻¹ | 2 weeks | Liquid; 9N–1P–5K; composed of Kelp, 6 specific bacilli strains with unique phytohormone production capabilities and micronutrient package (including 0.1% Fe) |
| 710–132 Microbial Fungicide (Sybron Chemicals, Salem, Va.) | 16 L·ha⁻¹ | 2 weeks | Liquid; composed of Kelp, small NPK, 1 bacilli strain and micronutrient package (including 0.1% Fe). |
| Focus Liquid (PBI Gordon, Kansas City, Mo.) | 26 L·ha⁻¹ | April, June, Aug, Oct | Liquid; 4.8% Kelp extract, 35.4% humic/fulvic acid, 1.4% chelated iron and 58.4% inert |
| Focus 15G (PBI Gordon, Kansas City, Mo.) | 200 kg·ha⁻¹ | Spring, Summer, Fall | 7% N; 5.2% humic/fulvic acid; 0.72% kelp extract, 0.04% iron |
| Launch (PBI Gordon, Kansas City, Mo.) | 102 L·ha⁻¹ | Monthly | 73% manure extract, 1.2% kelp, 0.35% chelated iron, 9% humic acid |
| Bolster (Natural Fertilizer of America, Cannon Falls, Minn.) | 9.5 L·ha⁻¹ | 2 weeks | Liquid; 2.0% sulfur, 5.0% iron; plant food sources derived from ferrous sulfate; 2.0% solubilized seaweed; and 4.0% humic acids from leonardite. |

### Table 2. Analysis of variance for acibenzolar-S-methyl (ASM) and biostimulant treatments on the development of dollar spot and brown patch in a Crenshaw-Cato creeping bentgrass blend at Manhattan, Kans., in 2000 and 2001.

| Source of variation | df | Dollar spot | Brown patch |
|---------------------|----|-------------|-------------|
| Year (YR)           | 1  | **         | NS          |
| Acibenzolar-S-methyl (ASM) | 1  | *          | NS          |
| Biostimulant (BIO)  | 12 | **         | NS          |
| YR × ASM            | 1  | NS         | NS          |
| ASM × BIO           | 12 | NS         | NS          |
| YR × BIO            | 12 | NS         | NS          |
| YR × ASM × BIO      | 12 | NS         | NS          |

*Area under the disease progression curve (AUDPC) represents counts of *S. homoeocarpa* infection centers on eight dates between 7 June and 14 Sept. 2000, and nine dates between 23 May and 20 Sept. 2001. Brown spot AUDPC values represent data from nine dates between 19 July and 14 Sept. 2000 and five dates between 12 July and 6 Sept. 2001. "*" Non-significant or significant at P < 0.05 or 0.01, respectively.

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than turf treated biweekly with urea.

Brown patch. In 2000, brown patch was first observed on 19 July, peaked on 10 Aug. with >16% of the plot area affected, and declined to <5% of plot area affected by 23 Aug. (data not shown). In 2001, two brown patch outbreaks occurred: 31 July (≥25% of plot area affected) and 10 Sept. (=5% of plot area affected) (data not shown). Neither ASM nor biostimulants had any effect on brown patch. (Table 2).

Turf quality. The ASM provided a higher (P < 0.05) turf quality rating (quality = 3.7) than nontreated turf (quality = 3.4) in only Aug. 2000.

In 2000, mean monthly quality ratings for turf in all biostimulant treatments and the urea control were less than acceptable (i.e., ratings ≤7) in all months (Table 4). Considering all treatments across all months, none of the biostimulants provided higher quality ratings than turf treated biweekly with urea.

After May 2001, turf in all biostimulant treatments had quality ratings that were less than acceptable (Table 4). Turf treated biweekly with urea had statistically similar quality to all biostimulant-treated turf.

**Discussion**

Creeping bentgrass visual quality was relatively low throughout both years of the study as a result of nonuniformity resulting from the presence of *S. homeocarpa* infection centers. When averaged over all biostimulant treatments and the urea control, ASM reduced dollar spot AUDPC by 38%. Despite the moderate reduction in dollar spot provided by ASM, improved quality in ASM vs. nontreated turf occurred in only Aug. 2000 when disease pressure was higher than at any time during study. Hence, although ASM provided a moderate reduction in dollar spot, it did not suppress the disease to a level that resulted in a significant increase in quality under the disease pressure observed in this test.

The moderate effectiveness of ASM in our test warrants that it be investigated further as a tool for reducing fungicide inputs on creeping bentgrass greens. For example, it is possible that reduced fungicide application levels or frequencies could be employed if ASM is included as part of the dollar spot management protocol. Brown patch was not affected by ASM application. Hence, any fungicide reduction strategy in creeping bentgrass using ASM would have to account for lack of brown patch suppression.

We hypothesized that a moderate dollar spot reduction achieved with ASM may be enhanced if used in combination with one or more biostimulants. However, biostimulants had no effect on dollar spot, or actually increased AUDPC values compared to a biweekly application of N at 4.9 kg ha⁻¹. Hence, although others have observed positive effects of biostimulants as they relate to turfgrass stress tolerance (Schmidt, 1991), sod rooting (Schmidt, 1991), and post-dormancy turf coverage (Schmidt and Chalmers, 1993), dollar spot suppression in creeping bentgrass does not appear to be a benefit.

An increase in dollar spot following application of some biostimulants relative to biweekly N applications may have been due, in part, to the fact that some products contained no, or very low levels of N, and N deficiencies are commonly associated with greater dollar spot severity (Christians, 1998). This was particularly the case in 2001 when only non-N containing biostimulants (Roots 1>2>3 premix with Standup, Compete Plus, or Focus Liquid) exhibited higher numbers of *S. homeocarpa* infection centers compared to urea-treated turf. Alternatively, some fertilizer sources have been shown to increase brown patch severity in perennial ryegrass, possibly by acting as a nutrient source for the fungus (Fidanza and Dernoeden, 1996). Some biostimulants may serve a similar dollar spot-enhancing role in creeping bentgrass.

Biostimulants had no effect on brown patch in creeping bentgrass. Nitrogen fertilization can be particularly effective in decreasing dollar spot severity when used in combination with a fungicide. 

| Treatment¹ | May | June | July | Aug. | Sept. |
|------------|-----|------|------|------|-------|
| Urea control | 5.2 | 5.2 | 5.3 | 4.0 ab | 5.5 a |
| Roots 1>2>3 premix with bacillus complex | 5.2 | 5.1 | 4.8 d | 3.4 cd | 4.0 c |
| Roots 1>2>3 premix with standup | 4.9 | 5.4 | 4.9 cd | 3.4 cd | 4.0 c |
| Aminoplex | 4.8 | 5.0 | 4.9 bd | 3.4 cd | 4.1 bc |
| Colonize T&O | 5.0 | 4.7 | 4.7 d | 3.3 cd | 4.0 c |
| Compete Plus | 4.7 | 4.5 | 4.7 d | 3.3 cd | 4.0 c |
| Flex® | 5.0 | 4.9 | 4.9 | 3.4 cd | 4.1 c |
| Turf Vigor | 4.7 | 5.1 | 5.2 ab | 4.1 a | 5.3 a |
| 710-132 Microbial Fungicide | 5.3 | 5.4 | 5.2 a-c | 3.8 a-c | 4.6 b |
| Focus Liquid | 5.3 | 5.0 | 4.9 d | 3.3 cd | 3.9 c |
| Focus 15G | 5.1 | 4.9 | 5.2 ab | 3.4 cd | 4.0 c |
| Launch | 5.1 | 4.9 | 4.9 cd | 3.2 d | 4.1 bc |
| Bolster | 5.1 | 4.7 | 5.0 b | 3.3 cd | 4.0 c |

¹Turf quality was rated visually on a 0 to 9 scale, where 0 = dead turf, 7 = acceptable putting green quality, and 9 = optimum color and density.
²Means in 2000 are from three dates in May, two dates in June, five dates in July, four dates in August, and four dates in September. Means in 2001 are from five dates in May, three dates in June, four dates in July and August, and two dates in September.
³All treatments were applied according to label instructions. Urea was applied to provide N at 4.9 kg ha⁻¹ in addition to routine fertilizer described in methods.
⁴Numbers followed by the same letter in a column are not significantly different (P > 0.05) according to an F-least significant difference mean separation test.
promote brown patch, but the low levels of N contained in some biostimulants did not enhance the disease when compared to biweekly applications of N at 4.9 kg·ha⁻¹.

Although a few manufacturers implied that application of their particular biostimulant may enhance turf vigor and suppress diseases, not all products evaluated were marketed with disease suppression as a primary benefit of using the product. Clearly, some of these products may offer turfgrass growth benefits that we did not measure. As they relate to disease incidence in creeping bentgrass, however, avenues other than the use of biostimulants should be sought to reduce fungicide requirements on creeping bentgrass managed under the conditions described herein.

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