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Research Article

In vitro and preventative field evaluations of potential biological control agents and synthetic fungicides for control of *Clarireedia jacksonii* sp. nov.

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Abbreviations: AUDPC: Area Under Disease Progress Curve; BCA: Biological Control Agents; BL: *Bacillus Licheniformis* SB 3086; BS: *Bacillus Subtilis* QST 713; DDi: Distilled De-ionized; DS: Dollar Spot; DSC: Dose Response Curve; EO: Essential oils; ETP: Epipolythiodioxopiperazine; LSD: Least Significant Difference; PDA: Potato Dextrose Agar; SG: *Streptomyces Griseoviridis*; TH: *Trichoderma Harzianum*

Abstract

Clarireedia jacksonii sp. nov. Formerly *Sclerotinia homoeocarpa* F.T. Bennett, the causal agent of dollar spot (DS), is the most destructive pathogen in turfgrass. Symptoms appear as circular patches 10-40 mm in diameter with small tan lesions surrounded by a darker band, sometimes presenting an hour glass appearance. A multi-year study was initiated with the objective of determining the efficacy of biological control agents (BCA) and tank mixes of BCA's and synthetic fungicides on DS control. Nutrient source was also evaluated to determine any interaction with the BCA's and tank mixes. *In vitro* studies evaluated the efficacy of synthetic and BCA's for *C. jacksonii* control. Quarter strength potato dextrose agar was amended with ¼, ½ and full labeled rates of various products. Chlorothalonil at all rates provided greatest (> 90%) control of *C. jacksonii* for study duration. Biological control agents provided best efficacy at ¼ and ½ label rates. *Streptomyces griseoviridis* provided least efficacy and may have exacerbated formation of *C. jacksonii.* Preventative field evaluations for synthetic and BCA's provided different results between two study years. In Year 1, all treatments had < 15% disease severity for the duration of the study. In year 2, disease pressure was extremely elevated. Synthetic program 1, centered on azoxystrobin + propiconazole applications and conventional fertility sources, provided best results with < 5% disease severity for the duration of the study. Reduced synthetic program 1, and synthetic program 2 followed closely with < 10% disease severity. Reduced synthetic programs were based on monthly applications of either chlorothalonil or pyraclostrobin every 30 day, alternated with biofungicide applications. Synthetic program 2 utilized rotation applications of pyraclostrobin and chlorothalonil every 14 days. Organic programs, utilizing only biofungicides and organic fertility sources, provided the least amount of control and exceeded the 15% threshold by the second month of the evaluation period.

Introduction

Clarireedia jacksonii sp. nov. formerly *Sclerotinia homoeocarpa* F.T. Bennett, the causal agent of dollar spot (DS), is the most destructive pathogen in turfgrass [1,2]. Dollar spot affects both cool- and warm-season grasses, during a wide range of growing conditions. Symptoms include circular patches 10-40 mm in diameter with small tan lesions surrounded by a darker band, sometimes presenting an hour glass appearance. During favorable conditions, spots may coalesce to form larger irregular shaped patches [2,3].

Biological controls have been used since the late 1980's, with the production of *Bacillus thuringiensis* Berliner [4]. Interest in biological controls has increased with growing public concerns about synthetic chemical applications.

Increased use of synthetic fungicides has resulted in fungicide tolerant strains and increased fungicide residues in the food chain, possibly exceeding safe limits [5,6].

Biological control agents (BCA) offer multiple modes of control, including antagonism, antibiosis or mycoparasitism. Antagonism is an interaction between the biocontrol product and the pathogen. Competition for nutrients exceeds the supply by the two organisms, resulting in decreased population sizes. One example is the saprophytic *Fusarium oxysporum* Schlecht, which competes with pathogenic *F. oxysporum*. Similarly, *Psuedomonas fluorescens* Flugge has a high affinity for ferric iron causing shortages for other organisms [6-8].

Pathogen control can also be achieved by metabolite production. Certain BCA's manufacture lipopeptides or lytic enzymes that are able to degrade pathogen cell walls. Typically, the *Bacilli* based BCA's achieve control by this method [9]. *Bacillus licheniformis* strain SB3086 is a soil-borne bacterium that produces antifungal metabolites [10]. *Bacillus licheniformis* is registered as Ecoguard by Novozymes. *Bacillus subtilis*, formulated as Rhapsody by AgraQuest, has a similar mode of action.

Lipopeptides are divided into three families; fengycin, surfactin and iturin A., and are amphiphilic compounds comprised of a lipid tail plus a cyclic oligopeptide. Control of pathogens has been attributed to fengycin and iturin A families of *Bacilli*. Lipopeptides interact with pathogen membranes creating pores, causing membrane instability, resulting in desiccation of the mycelium. Studies have also indicated that surfactin and fengycin can promote induced systemic resistance [11]. Aiding the control achieved by *Bacilli* products are their long-lived spores, which are resistant to environmental conditions such as heat and desiccation that normally limit BCA control [12].

Antibiosis is achieved by species that produce microbial toxins detrimental to the target pathogen [13]. Branching bacteria, such as *Streptomyces*, and *Penicillium* fungi, are mostly responsible for the production of antibiotics. Control can be achieved by direct contact between the antibiotic and pathogen, as well as by systemic translocation through the host plant. Plant pathogen antibiotics include streptomycin, tetracylines and cyclohexamide. Streptomycin has been shown effective on oomycetes. The same antibiotics gave rise to strobilurin class fungicides, originally synthesized from a fungus [7]. *Streptomyces griseochromogenes* Fukunaga produces blasticidin, used to control rice blast (*Magnaporthe grisea* (T. T. Heber) M. E. Barr). *Rhizoctonia solani* J. G. Kuhn and *Pythium ultimum* Trow can also be controlled by gliotoxin and gliovirin produced by *Gliocladium virens* R. D. Stipanovic and C. R. Howell [7]. Gliotoxin and gliovirin are secondary metabolites produced by fungi grouped in the epipolythiodioxopiperazine (ETP) class of peptides. Known antagonism toward soilborne pathogens such as *Rhizoctonia solani* date back to the 1930's [14].

Mycoparasitism involves direct interaction between pathogen and BCA [7,15]. Four primary groups are considered for mycoparasitism including hypoviruses, facultative parasites, obligate bacterial pathogens and predators. Plant Shield HC, which contains *Trichoderma harzianum* Rifai, has been shown to wrap around the hyphae of *Rhizoctonia* and produces chitenases and glucanases that break down cell walls [7]. This parasitism process typically occurs when cellulose from organic matter is low. Levels of control with products containing *T. harzianum* or similar compounds, therefore, may be dependent upon the available nutrient sources [8]. *Trichoderma harzianum* tends to perform better when nutrients are available and initial pathogen levels are low, indicating the most suitable application timing as a preventative application [16]. *Trichoderma atroviride* suppressed *Clarireedia spp* on creeping bentgrass by 67.5% [17].

Essential oils (EO) such as clove oil, wintergreen oil and thyme oil, are derived from plants. Essential oils limit hyphal growth and induce lysis and cytoplasmic evacuation in fungi. Changes in cell walls, including plasma membrane mitochondrial structure, along with enzymatic reactions cause inhibition of growth [14]. Similar to most fungicides, dose response curves (DRC) indicate a rate response with the use of BCA's. Research indicates a decrease in disease control often occurs with study duration [18]. Poacic acid suppressed *C. jacksonii* growth by 93% *in vitro*, in field studies disease severity was suppressed in one of two trial years [19].

A multi-year study was initiated with the objective of determining the efficacy of BCA's and tank mixes of BCA's and synthetic fungicides on DS control. Nutrient source was also evaluated to determine any interaction with the BCA's and tank mixes.

Materials and methods

In vitro studies

Samples of *C. jacksonii* were collected from a creeping bentgrass [*Agrostis stolonifera* L. var. *palustris* (Huds) cv. L-93] putting green at Clemson University, Clemson, SC (34.67°N, 82.84°W). Blades of blighted turf were surface sterilized by soaking leaf blades in 5% bleach and 95% distilled de-ionized (ddi) water for 30 seconds. Blades were then rinsed in ddi water and patted dry on a sterile paper towel. Blighted leaf blades were initially plated on potato dextrose agar (PDA) amended with streptomycin and ampicillin. Samples were then transferred to PDA using sterile techniques. Cultured plates were allowed to grow until mycelium reached the outer edge of the petri dishes.

Products screened for efficacy included: chlorothalonil, *Bacillus licheniformis* (BL), *Streptomyces griseoviridis* (SG), *Trichoderma harzianum* (TH), *Bacillus subtilis* QST 713 (BS), and a combination of rosemary oil, clove oil, plus thyme oil (EO). Rates screened were 100, 50 and 25% of the label recommended field rates (Table 1). Products were added to ¼ strength PDA after it had cooled to 60°C. Agar was stirred using an autoclaved magnetic stir. Twenty ml aliquots were added to 100 × 15 mm petri dishes. Five mm discs from the leading edge of *C. jacksonii* were taken and placed into the middle of the amended medium. Discs were placed so that mycelia growth came in direct contact with amended media. Discs from the same isolate of *C. jacksonii* were placed on each concentration of fungicide-amended media. Plates were incubated at a constant 23°C.

Growth prior to 24 hours was excluded to avoid any differences in establishment. Mycelia growth was recorded daily along 4 axes, and 4 daily diameters were averaged to obtain average daily growth. Inhibition was calculated as [1 – (average mean colony diameter on amended medium divided by average mean colony diameter on unamended medium)] × 100%. Areas under disease progress curve (AUDPC) were calculated for each fungicide and rate using the following equation:

$$AUDPC = \sum [(Y_1 + Y_2)/2] (T_2 - T_1) \quad (1)$$

where Y equals rating date and T equals days between rating date [20]. Study design was a 6 by 4 factorial, with 6 fungicide programs and 4 fungicide rates. Mean separations were performed using Fisher's least significant difference (LSD) with an alpha of 0.05.

Field studies

Studies were conducted at Clemson University, Clemson, SC (34.67°N, 82.84°W) on a creeping bentgrass putting green from May 2008 to December 2009. The site was an 11-year old 85:15 USGA sand:peat greens construction. Mowing height was ~4 mm, and greens were mowed 5-7 times weekly. Irrigation was applied on a 3-day interval to prevent moisture stress.

Table 1: Treatments applied during *in vitro* evaluation of growth suppression on *Clarireedia jacksonii* sp.

Treatment	Rate (ppm)
Bacillus subtilis ½ label rate	7.85
Bacillus subtilis ½ label rate	15.70
Bacillus subtilis full label rate	31.40
Bacillus licheniformis ¼ label rate	1.63
Bacillus licheniformis ½ label rate	3.25
Bacillus licheniformis full label rate	6.50
Essential oils ¼ label rate	1600
Essential oils ½ label rate	3200
Essential oils full label rate	6400
Trichoderma harzianum ¼ label rate	5.40
Trichoderma harzianum ½ label rate	10.80
Trichoderma harzianum full label rate	21.50
Streptomyces griseoviridis ¼ label rate	0.01
Streptomyces griseoviridis ½ label rate	0.02
Streptomyces griseoviridis full label rate	0.04
Chlorothalonil ¼ label rate	117.5
Chlorothalonil ½ label rate	235.0
Chlorothalonil full label rate	470.0

Nitrogen treatments were applied via organic or synthetic sources, a total of 1.81 kg ha^{-1} N was applied annually by all products (Table 2). Additional potash (K_2O) and phosphorous (P_2O_5) was applied through separate products to provide a 1-1-2 ratio of N-P-K. Granular products included EndoRoots 3-3-5 (LebanonTurf, Lebanon, PA) and 13-0-26 (Andersons, Sussex, WI). Granular products were applied at 0.11 kg ha^{-1} N, with the exception of May and July which was at 0.23 kg ha^{-1} N. Novozymes Turf Vigor 9-3-6 (LebanonTurf, Lebanon, PA) was applied at 0.11 kg ha^{-1} N. Inorganic liquid nitrogen was applied at 0.11 kg ha^{-1} N via 7-0-14 (Harrell's Turf Specialty, Inc., Lakeland, FL). Treatments with "organic" as the first word such as organic reduced 1 (OR1), indicates fertility was derived from organic sources.

In addition to the nutrients, treatments were applied with the goal to increase rootzone beneficial bacteria and fungi. Treatments included: a BCA based program, reduced synthetic fungicide program and a conventional fungicide program. All products evaluated during the studies were registered for use in South Carolina at the time of application (Table 3). Experimental design was a split plot with two different fertility treatments as main effects and five fungicide programs as split plots. Four replications of 1.5 × 1.5 m plots were used in the experimental design. Spray applications were made using a pressurized CO_2 backpack boom sprayer, through 8003 flat-fan nozzles (Tee jet, Spraying Systems CO., Roswell, GA) with a water carrier volume of 374 L ha^{-1}.

Synthetic products were conventional programs and considered industry standards (Table 4). Synthetic program 1 (SS1) and synthetic program 2 (SS2) were based on synthetic nitrogen source + synthetic fungicides applied every 14 days. Programs were selected from previous research conducted at Pee Dee research center, Florence, SC by S. B. Martin (personal communication). Synthetic fungicide programs 1 and 2 were duplicated using an organic nitrogen source and are referred to as OS1 and OS2, hereafter.

Reduced synthetic

Two fungicide programs were based on applications of chlorothalonil or pyraclostrobin on a 30-day interval, instead of the traditional 14-day interval (Table 4). Chlorothalonil and pyraclostrobin applications were followed by BCA's on an alternate 30-day interval. Fungicide programs were duplicated

Table 2: Nitrogen fertility applied to L-93 creeping bentgrass during a two-year study initiated in June 2008 to provide a total of 1.81 kg N ha^{-1} in Clemson, SC.

Fertility	Mar†	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Organic	kg N ha^{-1}								
Turf Vigor 9-3-6			0.11	0.11	0.11	0.11	0.11	0.11	0.11
EndoRoots 8-3-5	0.11	0.11	0.11	0.23			0.11	0.23	0.11
Inorganic									
Liquid 7-0-14			0.11	0.11	0.11	0.11	0.11	0.11	0.11
Granular 13-0-26	0.11	0.11	0.11	0.23			0.11	0.23	0.11

†No applications made during months of January and February.

Table 3: Fungicides trade name, common name and manufacturer of products used during a two-year study on L-93 creeping bentgrass for the control and suppression of dollar spot (*Clarireedia jacksonii* sp. nov).

Common Name	Trade Name	Manufacturer
Chlorothalonil	Daconil Ultrex WP	Syngenta, Greensboro NC 27419
Azoxystrobin + propiconazole	Headway SC	Syngenta, Greensboro NC 27419
Azoxystrobin	Heritage WG	Syngenta, Greensboro NC 27419
Fludioxonil	Medallion WP	Syngenta, Greensboro NC 27419
Pyraclostrobin	Subdue MAXX SC	Syngenta, Greensboro NC 27419
Trifloxystrobin + triadimefon	Insignia WG	BASF, Research Triangle Park NC 27709
Iprodione	Tartan SC	Bayer, Research Triangle Park NC 27709
Aluminum tris	26 GT SC	Bayer, Research Triangle Park NC 27709
Mancozeb	Chipco Signature WP	Bayer, Research Triangle Park NC 27709
Chlorothalonil + thiophante methyl	Fore Rainshield SC	Dow AgroScience, Indianapolis IN 46268
Mancozeb	Spectro 90 SC	Cleary Chemical Corp. Dayton NJ 08810
Bacillus subtilis QST 713	Rhapsody SC	AgraQuest, Davis CA 95916
Bacillus licheniformis SB3086	EcoGuard SC	Novozymes, Salem VA 24153
Trichoderma harzianum strain KRL-AG2	Plant Shield HC WP	BioWorks, Geneva NY 14456
Streptomyces griseoviridis strain K61	Mycostop WP	Verdera, Espoo Finland
Clove, thyme rosemary oils	Paradigm L	Parkway Research, Pleasant Plains IL 62677
Endomycorrhiza	EndoRoots SC	Novozymes, Salem VA 24153
Bacillus licheniformis SB3086	Turf Vigor SC	Novozymes, Salem VA 24153

with either an organic or synthetic nitrogen source. Programs applied for preventative control of DS were synthetic nitrogen + chlorothalonil + BCA (SR1), synthetic nitrogen + pyraclostrobin + BCA (SR2), organic nitrogen + chlorothalonil + BCA (OR1) and organic nitrogen + pyraclostrobin + BCA (OR2).

Organic products were applied on a 14-day interval in an attempt to maintain similar control as the synthetic programs (Table 4). Four products were split into two separate applications, assuring plots received two fungicides on a weekly basis. Organic organic (OO) was a combination of an organic nitrogen source and BL + EO + SG + BS.

Turfgrass plots were rated weekly for disease severity and turfgrass quality. Disease ratings were collected using a line intersect grid rating method. Each plot was divided into 289 subplots, considered grids, measuring 100 × 100 mm each. Disease symptoms in any portion of a grid was considered a "hit". Percentage disease was calculated by taking the number of "hits" and dividing by the total number of grids, then multiplying by 100. An acceptable percent disease was arbitrarily set at ≤ 15 percent. Areas under disease progress curve (AUDPC) was calculated as shown earlier.

Turfgrass quality was rated on a scale of 1-9, 1 = brown dead turf, 7 = minimum accepted and 9= dark green dense turf [21]. Weekly readings were averaged each month for statistical analysis.

Analysis of variance (ANOVA) and means separation were performed on all data sets using the SAS statistical software package JMP Pro 9.1 (SAS Institute Inc., SAS Campus Drive, Cary NC 27513, USA). Data was analyzed individually for each evaluation date. Significant means were separated using Fisher's LSD test ($p = 0.05$).

Results

In vitro studies

Experimental runs were not significantly different, thus, were pooled. After day 2, Chlorothalonil at ¼, ½ and full rates provided highest control, > 95% for the study duration (Table 5). In the first 6 days of the evaluation period, BL ¼, BS ¼, TH ½, TH full and EO full provided > 80% DS control. *Bacillus licheniformis* ½ and full rate; and BS ½ rate provided moderate, ~60% DS control. Biological control agents applied at less than the label rate provided increased activity. Quarter rates of BS provided 85% control by day 3 compared to < 75% for ½ and full rates. Similar trends were observed with BL ¼ rate providing > 90% control at day 3 compared to < 85% control for ½ and full rates. *Trichoderma harzianum* provided the quickest activity at ½ and full rates, > 85% control by day 3. However, full rate failed to maintain efficacy. Control gradually dropped to < 50% control for full rate, where ½ rate maintained 85% control for study duration (Table 5).

All products, with the exception of SG, provided a DRC. DRC's were used to calculate EC_{50}, EC_{85} and EC_{90} rates (Table 6). EC_{50} rates are a standard for evaluation of potential fungicides [1]. It was pre-determined 15% disease severity would be acceptable for a reduced or fully organic fungicide program, resulting in the calculation of EC_{85} rates. EC_{90} rates were calculated to closer match accepted current commercial fungicide efficacy. Chlorothalonil DRC's revealed excellent control at all rates evaluated (Table 6).

Field studies

In the first year, acceptable turf quality (≥ 7) was maintained by all plots in June through August. By September, OO and SR2 treatments resulted in below acceptable quality ratings (< 7), and these along with SS1 in October, remained unacceptable for the duration of the study (Table 7). At the

Table 4: Synthetic, reduced synthetic and organic fungicide programs, products, formulation, rates and application dates for control of dollar spot (*Clarireedia jacksonii* sp. nov) on L-93 creeping bentgrass. Synthetic programs solely used fungicides synthetically manufactured for turfgrass, reduced programs used a combination of synthetically manufactured and organic products and organic programs contained no synthetic fungicides.

Treatment Synthetic 1 (S.1)	Formulation	Rate per ha^{-1}	App. Date
Azoxystrobin + propiconazole + mefenoxam	15.27 SC + 2 ME	4.77 L + 03.18 L	7-May
Chlorothalonil + pyraclostrobin	82.5 WDG + 80 DG	15.8 kg + 12.20 g	21-May
Azoxystrobin + propiconazole + chlorothalonil	15.27 SC + 82.5 WDG	4.77 L + 11.45 L	4-Jun
Chlorothalonil + pyraclostrobin	82.5 WDG + 80 DG	15.8 kg + 12.20 g	18-Jun
Mefenoxam + azoxystrobin	2 ME + 50 WDG	3.18 L + 03.18 L	2-Jul
Azoxystrobin + propiconazole +mancozeb	15.27 SC +75 WP	4.77 L + 24.4 g	16-Jul
Chlorthalonil + fludioxonil	82.5 WDG + 50 WP	15.8 kg + 00.76 g	30-Jul
Chlorthalonil + pyraclostrobin	82.5 WDG + 80 DG	15.8 kg + 12.2 g	13-Aug
Azoxystrobin + propiconazole +chlorothalonil	15.27 SC + 82.5 WDG	4.77 L + 15.8 kg	27-Aug
Mefenoxam + azoxystrobin	2 ME + 50 WDG	3.18 L + 03.18 L	10-Oct
Treatment Synthetic 2 (S.2)			
Trifloxystrobin + triadimefon	4.17 & 20.86 SC	6.36 L	7-May
Pyraclostrobin	20 WDG	2.75 g	21-May
Mancozeb	90 WDG	17.57 g	4-Jun
Pyraclostrobin + chlorothalonil	80 DG + 82.5 WDG	12.2g + 15.8k g	18-Jun
Pyraclostrobin	20 WDG	2.75 g	2-Jul
Pyraclostrobin + chlorothalonil	80 DG + 82.5 WDG	12.2g + 15.8k g	16-Jul
Iprodione	2 F	12.72 L	30-Jul
Trifloxystrobin + triadimefon	4.17 & 20.86 SC	6.36 L	13-Aug
Pyraclostrobin + chlorothalonil	80 DG + 82.5 WDG	12.2g + 15.8 kg	27-Aug
Pyraclostrobin	20 WDG	2.75 g	10-Oct
Treatment Reduced 1 (R.1)			
Pyraclostrobin	20 WDG	2.75 g	7-May fb every 30 days
Bacillus licheniformis	0.14 SC	63.6 L	7-may fb every 14 days
Clove, thyme and rosemary oils	38 L	9.54 L	7-May fb every 14 days
Trichoderma harzianum strain KRL-AG2	1.15 WP	0.5 g	7-May fb every 14days
Treatment Reduced 2 (R.2)			
chlorothalonil	82.5 WDG	15.8 kg	7-May fb every 30 days
Streptomyces griseoviridis strain K61	4.0 WDG	0.21 g	7-May fb every 14 days
Bacillus subtilis	1.34 SC	19.1 L	7-May fb every 14 days
Treatment Organic 1 (O.1)			
Bacillus licheniformis	0.14 SC	63.6 L	7-May fb every 14 days
Clove, thyme and rosemary oils	38 L	9.54 L	7-May fb every 14 days
Streptomyces griseoviridis strain K61	4.0 WP	0.21 g	14-May fb every 14 days
Bacillus subtilis	1.34 SC	19.1 L	14-May fb every 14 days

Table 5: *Clarireedia jacksonii* sp. nov control during *in vitro* evaluation of *Bacillus subtilis, Bacillus licheniformis, Trichoderma harzianum, Streptomyces griseoviridis*, essential oils and chlorothalonil. Treatments were added to ¼ strength potato dextrose agar plates. Five mm inoculation discs were inserted in the middle of all plates; growth was evaluated after a 24-hour incubation period.

		Control					
		Day					
Treatment	Rate†	1	2	3	4	5	6
		%					
Chlorothalonil	1/4	$88.9^{a\text{-}d\ddagger}$	95.9^{a}	97.9^{a}	97.9^{a}	97.9^{ab}	97.9^{a}
Chlorothalonil	1/2	89.5^{abc}	98.1^{a}	98.1^{a}	98.1^{ab}	98.1^{a}	98.1^{a}
Chlorothalonil	full	93.5^{ab}	97.6^{a}	98.8^{a}	98.8^{a}	98.3^{a}	98.8^{a}
B. subtilis	1/4	80.9^{c}	77.3^{b}	85.0^{cd}	85.0^{d}	85.0^{e}	85.0^{cd}
B. subtilis	1/2	67.8^{d}	57.0^{c}	71.2^{e}	71.2^{f}	71.2^{f}	61.2^{e}
B. subtilis	full	100^{a}	57.3^{c}	67.8^{e}	67.8^{f}	67.8^{f}	60.3^{e}
B. licheniformis	1/4	83.7^{bc}	82.0^{b}	91.3^{b}	91.3^{b}	91.3^{c}	91.3^{b}
B. licheniformis	1/2	77.2^{cd}	64.2^{c}	82.2^{d}	82.2^{d}	82.2^{e}	82.2^{d}
B. licheniformis	full	77.1^{cd}	83.5^{b}	82.3^{d}	82.3^{d}	82.3^{e}	82.3^{cd}
T. harzianum	1/4	35.4^{e}	41.5^{d}	31.5^{f}	30.0^{g}	10.4^{h}	6.9^{g}
T. harzianum	1/2	100^{a}	76.6^{b}	85.8^{bcd}	85.8^{cd}	85.8^{de}	82.4^{cd}
T. harzianum	full	87.6^{abc}	85.6^{b}	89.7^{bc}	89.7^{bc}	89.7^{cd}	81.5^{d}
S. griseoviridis	1/4	0^{g}	0^{g}	0^{h}	0^{h}	0^{i}	0^{h}
S. griseoviridis	1/2	0^{g}	0^{g}	0^{h}	0^{h}	0^{i}	0^{h}
S. griseoviridis	full	0^{g}	0^{g}	0^{h}	0^{h}	0^{i}	0^{h}
Essential oils	1/4	100^{a}	20.4^{e}	18.6^{g}	0^{h}	0^{i}	0^{h}
Essential oils	1/2	100^{a}	85.3^{b}	84.9^{cd}	76.1^{e}	0^{i}	34.1^{f}
Essential oils	full	100^{a}	97.3^{a}	97.8^{a}	97.5^{a}	0^{i}	87.4^{bc}
Untreated		0^{g}	0^{g}	0^{h}	0^{h}	0^{i}	0^{h}

†Rates were either ¼, ½ or full labeled rates for dollar spot control.
‡Means with the same letter are not significantly different based on Fisher's least significant difference test (α = 0.05).

Table 6: Effective concentration for 50% control (EC_{50}), 85% control (EC_{85}) and 90% control (EC_{90}) in parts per million (ppm) for synthetic and biological control agents on *Clarireedia jacksonii* sp. nov plated on ¼ strength potato dextrose agar (PDA).

Treatment	EC_{50}†	EC_{85}	EC_{90}
		ppm	
T. harzianum	8.3	10.6	ND‡
S. griseoviridis	ND	ND	ND
B. subtilis	4.7	ND	ND
B. licheniformis	0.9	1.6	1.6
Clove, thyme, rosemary oil	4815	ND	ND
Chlorothalonil	60.7	103.5	110.1

†Rates were calculated by using linear regression and visually calculating EC_{50}, EC_{85}, EC_{90} rates using JMP 9.1.
‡ND = Not Detectable.

Table 7: Turf quality ratings for L-93 creeping bentgrass during 2008 and 2009 for a preventative dollar spot study using various organic and synthetic control agents listed in table 4.

	Turfgrass Quality†												
	2008							2009					
Treatment	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.		Jun.	Jul.	Aug.	Sep.	Oct.	Nov.
Organic organic	$7.3^{ab\ddagger}$	7.0b	7.0^{b}	6.8^{c}	6.3^{c}	6.0^{b}		7.3^{ab}	6.0^{a}	6.6^{cd}	6.3^{cd}	6.5^{b}	6.5^{b}
Organic reduced 1	7.0^{ab}	7.3^{ab}	7.3^{ab}	7.0^{bc}	6.8^{abc}	6.1^{ab}		7.0^{ab}	6.3^{a}	7.0^{bcd}	6.6^{cd}	6.6^{b}	6.6^{b}
Organic reduced 2	7.6^{ab}	7.3^{ab}	7.3^{ab}	7.3^{abc}	7.3^{ab}	7.0^{ab}		7.6^{ab}	6.5^{a}	7.1^{abc}	6.6^{cd}	6.8^{ab}	6.8^{ab}
Organic synthetic 1	7.8^{ab}	7.3^{ab}	7.3^{ab}	7.0^{bc}	7.1^{ab}	7.3^{ab}		7.8^{ab}	6.3^{a}	7.5^{ab}	7.6^{ab}	7.6^{a}	7.6^{a}
Organic synthetic 2	7.5^{ab}	7.3^{ab}	7.3^{ab}	7.5^{ab}	7.0^{abc}	6.3^{ab}		7.5^{ab}	6.3^{a}	7.0^{bcd}	6.8^{cd}	6.6^{b}	6.6^{b}
Synthetic organic	7.3^{ab}	7.3^{ab}	7.3^{ab}	7.6^{a}	6.6^{bc}	6.0^{b}		7.3^{ab}	6.0^{a}	6.5^{d}	6.1^{d}	6.3^{b}	6.3^{b}
Synthetic reduced 1	8.0^{a}	7.3^{ab}	7.3^{ab}	7.0^{bc}	7.1^{ab}	7.5^{a}		8.0^{a}	6.5^{a}	7.1^{abc}	7.0^{bc}	7.0^{ab}	7.0^{ab}
Synthetic reduced 2	7.5^{ab}	7.3^{ab}	7.3^{ab}	7.5^{ab}	7.5^{a}	7.1^{ab}		7.5^{ab}	6.0^{a}	7.1^{abc}	6.6^{cd}	6.8^{ab}	6.8^{ab}
Synthetic synthetic 1	7.5^{ab}	7.5^{a}	7.5^{a}	7.5^{ab}	6.6^{bc}	6.3^{ab}		7.5^{ab}	6.5^{a}	7.6^{a}	8.0^{a}	7.6^{a}	7.6^{a}
Synthetic synthetic 2	7.5^{ab}	7.0^{b}	7.0^{b}	6.8^{c}	7.1^{ab}	6.5^{ab}		7.5^{ab}	6.0^{a}	7.1^{abc}	6.5^{cd}	6.5^{b}	6.5^{b}

†Turfgrass quality was rated on a scale of (1 – 9), 1 = dead turf and 9 = ideal turf, 7 was set as the minimum acceptable turfgrass quality.
‡Means with the same letter are not significantly different based on Fisher's least significant difference test (α = 0.05).

initiation of the second year, all plots had ≥ 7 turf quality (Table 7). However, by July, a significant increase in disease pressure occurred, resulting in all plots falling below acceptable visual quality (Table 7). By 15 August, all programs had acceptable quality with the exception of OO and SO (Table 7). From August to November, programs OS1, SR1 and SS1 were the only treatments to maintain acceptable quality (≥ 7) (Table 7).

In regards to AUDPC duration throughout year 1, program SR1 had the lowest disease accumulation at 6 and OR1 had the highest AUDPC at 51 (Table 8). When evaluating monthly disease occurrence, severity did not differ among treatments from June through August in the first year (Table 9). Significant treatment differences initially occurred in September and continued through November as disease pressure increased. Disease severity was low and acceptable in all plots, except OR1 in November (~16%) (Table 9). Overall, in year 1, least disease was associated with SR1, OS1, and OS2.

Monthly disease severity in year 2 was elevated in comparison to year 1, most likely due to weather conditions (Table 9). In June year 2, differences were not observed in disease severity, but by July, OO and SO exceeded 15% disease incidence followed by OR1, OS2 and SS2 in August (Table 9). Programs OS1 and SS1 were the only treatments in September, October, and November with disease severity of ≤ 15% (Table 9). Program SS1 had lowest AUDPC at 9.33, while SO had highest disease severity ~13% and AUDPC at 231.00 (Table 8). Overall, in 2009, SR1, SS1 and OS1 provided greatest control (≤ 77) based on AUDPC (Table 8).

Discussion

In vitro studies

A unique trend was noticed with BCA's during the evaluation period. Lower rates of BCA's had increased efficacy over higher rates, the exception was EO's and TH. Essential oils provided increased efficacy as rate increased. Similar trends, with decreased spore germination as rates of EO's increased. During the evaluation period, ¼ rates of BL and BS appeared to establish a containment zone around the inoculation plug quicker than the ½ and full-strength treatments [5]. Fungistatic activity was evident when BL and BS were viewed with light microscopy. Hyphae of *C. jacksonii* on agar amended with BL and BS had much shorter, stubby mycelium than the untreated. Mycelium looked similar to a rootzone parasitized by nematodes, exhibiting short, stubby roots.

Streptomyces hygroscopicus, *S. diastaticus* and *S. galbus* for control of dollar spot on *Poa pratensis* L. *Streptomyces hygroscopicus* was the only treatment to decrease disease infection [22]. The authors also indicate *Streptomyces* spp. have traditionally been used to control soil-borne pathogens. Results from the current study indicate that *Streptomyces griseoviridis* failed to provide suppression or control of the pathogen, *Clarireedia jacksonii*, similar to 3 of 4 *Streptomyces* species evaluated by [22].

In conclusion, half and full rates of BCA's exhibited a clear reduction in residual control compared to all rates

Table 8: Dollar spot (*Clarireedia jacksonii* sp. nov) area under disease progress curve (AUDPC) values for year 1 and year 2 during a preventative fungicide study evaluating various organic and synthetic control agents listed in table 4.

	AUDPC†	
Treatment	**2008**	**2009**
Organic organic	41.66^{ab‡}	184.66^{ab}
Organic reduced 1	51.00^{a}	94.00^{bcd}
Organic reduced 2	12.66^{ab}	99.00^{bcd}
Organic synthetic 1	10.00^{ab}	10.66^{cd}
Organic synthetic 2	23.00^{ab}	89.33^{bcd}
Synthetic organic	35.33^{ab}	231.00^{a}
Synthetic reduced 1	6.00^{b}	77.66^{cd}
Synthetic reduced 2	12.33^{ab}	93.00^{bcd}
Synthetic synthetic 1	27.66^{ab}	9.33^{d}
Synthetic synthetic 2	18.00^{ab}	114.33^{bc}

†AUDPC was calculated using $\sum [(Y_1 + Y_2)/2]\ (T_2 - T_1)$. Lower values = better control.
‡Means with the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$).

Table 9: Disease severity ratings for L-93 creeping bentgrass during 2008 and 2009 for a preventative dollar spot study using various organic and synthetic control agents listed in table 4.

	Disease Severity†											
	2008						**2009**					
	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.
Organic organic	0.0^{a‡}	1.0^{ab}	1.0^{ab}	5.6^{ab}	11.6^{ab}	13.0^{ab}	0.0^{a}	33.0^{a}	43.0^{a}	35.6^{ab}	35.0^{ab}	35.0^{ab}
Organic reduced 1	0.0^{a}	4.3^{a}	4.3^{a}	9.0^{a}	14.3^{a}	16.0^{a}	0.0^{a}	12.3^{cd}	16.0^{bc}	19.3^{bc}	21.6^{bc}	21.6^{bc}
Organic reduced 2	0.0^{a}	0.6^{b}	0.6^{b}	1.6^{b}	3.3^{ab}	3.3^{ab}	0.0^{a}	12.3^{cd}	7.6^{bc}	16.6^{cd}	29.6^{ab}	29.6^{ab}
Organic synthetic 1	0.0^{a}	1.0^{b}	1.0^{b}	0.6^{b}	2.3^{ab}	2.0^{b}	0.0^{a}	2.3^{d}	2.0^{cd}	0.6^{d}	1.3^{c}	1.3^{c}
Organic synthetic 2	0.0^{a}	0.3^{b}	0.3^{b}	2.6^{ab}	7.3^{ab}	9.3^{ab}	0.0^{a}	5.0^{cd}	16.3^{bc}	15.6^{cd}	24.6^{bc}	24.6^{bc}
Synthetic organic	0.0^{a}	1.0^{b}	1.0^{b}	5.6^{ab}	11.6^{ab}	13.0^{ab}	0.0^{a}	28.6^{ab}	46.6^{a}	44.6^{a}	54.0^{a}	54.0^{a}
Synthetic reduced 1	0.0^{a}	0.0^{b}	0.0^{b}	0.0^{b}	1.6^{b}	1.3^{b}	0.0^{a}	11.3^{cd}	12.3^{bcd}	15.6^{cd}	17.6^{bc}	17.6^{bc}
Synthetic reduced 2	0.0^{a}	0.3^{b}	0.3^{b}	2.6^{ab}	2.3^{ab}	3.6^{ab}	0.0^{a}	19.3^{abc}	9.3^{bcd}	16.0^{cd}	22.6^{bc}	22.6^{bc}
Synthetic synthetic 1	0.0^{a}	1.3^{b}	1.3^{b}	3.0^{ab}	9.0^{ab}	10.0^{ab}	0.0^{a}	1.3^{d}	1.3^{d}	1.0^{d}	1.3^{c}	1.3^{c}
Synthetic synthetic 2	0.0^{a}	1.6^{b}	1.6^{ab}	3.0^{ab}	7.6^{ab}	7.6^{ab}	0.0^{a}	14.6^{bcd}	17.0b	22.3^{bc}	28.6^{bcd}	28.6^{bcd}

of chlorothalonil and TH 10.6 ppm and BL 1.6 ppm (Table 6). *B. licheniformis* at 1.6 ppm was successful in providing > 90% control, which would be acceptable to most turfgrass managers. All other BCA products evaluated might provide some additional efficacy in tank mixtures during periods of reduced disease pressure. Since BCA's evaluated provided less efficacy and residual activity, their use would probably be restricted over that of conventional fungicides such as chlorothalonil.

Field studies

One plausible explanation for yearly control differences was varying rainfall for the two years. During year 1, South Carolina experienced one of the worst droughts on record, averaging 96 cm of rain statewide, while during year 2, total rainfall was 147 cm. Data indicated a significant increase in disease following months with high rainfall totals. Similar trends were noticed with plant quality over the two years. This reduction in control between years is a particular concern. Biological control agents need to perform acceptably when disease pressures are elevated to be considered as a viable alternative to synthetic fungicides. One theory at study initiation was an accumulation or loading period would be required to realize any benefit to the organic fertility. Microorganism thresholds would have to be reached in order to compete with pathogen causal agents, providing a reduction in disease pressure. Lack of long-term residual control for the BCA's became evident during the second year of the study. Additional studies need to evaluate rootzone augmentation with these species to determine the potential to produce a suppressive soil.

Previous research indicated spray applications of *T. harzianum* provided both rhizosphere and foliar antagonist populations at effective levels [23]. Weekly applications were able to provide similar control to that provided by synthetic fungicides. Monthly spray applications of *T. harzianum* were able to reduce DS, but did not achieve similar control as weekly applications [24]. Similar trends were noticed with SR1 and OR1 where DS was reduced in comparison to programs not containing *T. harzianum*. Jacobson, et al. [25], indicated programs combining azoxystrobin and *Bacillus* based BCA's provided the best control of *Rhizoctonia* crown rot. Reduced programs received a spray application on a weekly basis, where synthetic programs received applications on a bi-weekly basis. However, this increase in applications and man-hours may be offset by the decrease in environmental impact of these reduced synthetic programs. Previous research evaluating environmental impact quotient revealed less impact to the environment than conventional programs [26].

In our research, ¼ label rate for synthetic programs showed the ability to maintain similar disease control as label rate synthetic programs when bentgrass is grown under lower rainfall conditions. During periods of increased disease pressure, BCA's lost some residual efficacy. Turf managers attempting to use ¼ label rate for synthetic programs must remain diligent with applications and monitor weather patterns to be successful. Additional research is required to understand the interaction between fertility formulation and how fungicide efficacy is impacted. Continuation of this study evaluating timing, rates and the accumulation of bio-control organisms and disease severity, may reveal if a loading period is required for successful bio-based programs.

In conclusion, all treatments provided acceptable DS control for the study duration during the drier year 1, with the exception of OR1, which fell below the acceptable threshold of 15% DS severity during November. During the wetter second study year, DS control was noticeably decreased. Programs OS1, SS1 and SR1 provided best overall quality, but fell below acceptable quality (≤ 7) during July (Table 7).

Visual Quality ratings were highest during year one, with OS1, SR1 and SR2 providing ≥ 7 for the duration of the study (Table 7). Programs SS1 and OS1 were the only treatments to provide acceptable visual quality during year two, with the exception of July when all treatments fell below an acceptable quality rating of 7 (Table 7).

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