The Horticultural Spray Oil, Civitas™, Causes Chronic Phytotoxicity on Cool-season Golf Turf

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Abstract. Petroleum-derived spray oils (PDSOs) have been used for pest management in horticulture and agronomy for over a century. Civitas™ is a new PDSO designed for use in the turfgrass industry. It is commonly mixed with low rates of pesticides to reduce the environmental impact and improve plant stress tolerance. Civitas can cause phytotoxicity, which has limited its acceptance by the turfgrass industry. Civitas is mixed with a green pigment called Harmonizer™ to sustain acceptable turfgrass color. A field study and a growth chamber study were designed to quantify phytotoxicity, understand the role of Harmonizer, and isolate the cause of Civitas-induced phytotoxicity. Civitas, Harmonizer, their combination (Two-Pack), and a water-only control were applied to a research putting surface in Ithaca, NY, during 2012 and 2013. Civitas and Harmonizer were applied every 2 weeks at the rates of 5.0 and 0.3 mL·m⁻², respectively. Visual turfgrass quality rating and canopy temperature were quantified several times weekly. Civitas caused chlorosis and decline in visual quality during both years. Harmonizer masked chlorosis but did not prevent a drop in stand density during the second field season. Treatments were replicated on annual bluegrass (*Poa annua* L.) in a growth chamber experiment. Civitas did not increase electrolyte leakage or alter the composition of cuticle; however, there were signs of oil persistence on the leaves and stomata and evidence of reduced gas exchange. Chlorosis resulting from oil persistence and reduced gas exchange is consistent with chronic PDSO phytotoxicity. This research demonstrated the potential for phytotoxicity with high rates of Civitas. Lower application rates likely reduce the potential for phytotoxicity but may also minimize the pest control benefits associated with the product.

Petroleum-derived spray oils, commonly referred to as horticultural spray oils, have been used to control insect and fungal pests in horticultural crops since the 1800s (Agnello, 2002). There has been renewed interest in PDSO because these products, when properly refined, have minimal human toxicity and environmental impact (Ebbon, 2002). Although there is debate as to how PDSOs affect plant pests, they can be lethal to plant pests and have the potential to stimulate innate plant defenses (Cortes-Barco et al., 2010a; Northover and Timmer, 2002; Traverner, 2002).

Civitas™ (Suncor Energy, Mississauga, Ontario, Canada) is a novel PDSO that contains a mixture of food-grade isoparaffins (alkanes)

ranging in size from 16 to 33 carbons in length and an emulsifier (Hsiang et al., 2013). It is currently produced and marketed by Petro-Canada for disease and insect control in golf turf management. Civitas represents a novel approach to turf pest management because it primes plant defense yet has no fungicidal and limited fungistatic properties (Cortes-Barco et al., 2010a). Specifically, Cortes-Barco et al. (2010a, 2010b) demonstrated that Civitas primed or activated genes involved in induced systemic resistance, including genes involved in the jasmonic acid pathway when applied to both *Nicotiana bethamiana* and creeping bentgrass (*Agrostis stolonifera* L.). Consequently, several studies have shown reduced pesticide requirements when using Civitas in a disease management program (Aynardi et al., 2011; Aynardi and Uddin, 2013a, 2013b; McCall and Focht, 2010; Popko et al., 2010; Popko and Jung, 2013).

Acute and chronic phytotoxicity is a known limitation of PDSOs (Hodgkinson et al., 2002). Acute phytotoxicity results from direct damage to plant tissue including leaf lesions, fruit sunburn, and cell membrane disruption (Hodgkinson et al., 2002). Chronic

phytotoxicity arises from inhibition of stomatal conductance (g_s) causing reductions in transpiration, photosynthesis, and respiration and an increase in photorespiration, photo-oxidation, and oxidative stress (Hodgkinson et al., 2002). For example, a paraffinic PDSO was shown to reduce g_s in grapes and altered carbon partitioning (Finger et al., 2002). Similar results have been demonstrated in other horticultural crops (Rethwisch et al., 1992).

Most modern PDSOs are highly paraffinic and are considered safe when mixed with water at concentrations less than 2% by weight (Beattie, 1990). Currently, Civitas is applied at 2.5% to 5% concentrations and can result in turf chlorosis within hours of application. To mask the chlorosis, Civitas is applied with Harmonizer™ (Suncor Energy), which is a green phthalocyanine pigment (Nash, 2011). This type of pigment has been shown to mask injury and mitigate ultraviolet stress (Ervin et al., 2004). The original commercial formulation, Two-Pack, consisted of the Civitas and Harmonizer packaged separately to be mixed on-site. A new pre-mixed single-product formulation, Civitas One™, was introduced in 2013.

The objectives of this project were to characterize the phytotoxic response of a mixed cool-season golf putting surface treated with Civitas, Harmonizer, or their combination in the field and determine how phytotoxicity is elicited in a growth chamber. Our hypothesis was that Civitas causes chronic phytotoxicity as a result of oil persistence and reduced g_s .

Materials and Methods

Field assessment of phytotoxicity. This study was conducted during two field seasons on a mature mixed stand of ≈30% annual bluegrass and 70% creeping bentgrass (*Agrostis stolonifera* L. cv. L-93) managed as a putting surface at the Cornell University Turf and Landscape Research Center in Ithaca, NY. The green was constructed from the on-site Arkport fine sandy loam soil (originally mixed, active, mesic lamellic hapludalf) and topdressed regularly with sand meeting U.S. Golf Association specifications (USGA Green Section Staff, 2004) that resulted in a 7.5-cm layer at the beginning of the study.

The putting surface was mowed 6 d per week at 3 mm with a fixed-head walking greensmower (Toro Greenmaster 1000; Toro Co., Bloomington, MN). Plots were irrigated to prevent drought stress and trafficked daily with a modified traffic device fitted with golf spikes designed to simulate 30,000 rounds of golf/year (Cockerham and Brinkman, 1989). Liquid ammonium sulfate fertilizer was applied weekly at 10 kg nitrogen/ha throughout the growing season. Topdressing was applied monthly to an approximate depth of 1 mm. Fungal disease outbreaks were controlled with chlorothalonil as needed.

Treatments included Civitas, Harmonizer, their combination hereafter called the

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Two-Pack, and a non-treated control. Treatments were arranged in a randomized complete block design with four replicates. Civitas and Harmonizer were applied every 2 weeks at rates of 5.0 mL·m⁻² and 0.3 mL·m⁻², respectively, with a CO₂-powered backpack sprayer equipped with two TeeJet AI 8004 nozzles (TeeJet Technologies, Wheaton, IL) calibrated to deliver 810 L·ha⁻¹ at 275 kPa. Applications began on 22 Aug. 2012 and 31 May 2013 and continued until final measurements were taken on 30 Sept. 2012 and 30 Aug. 2013.

Visual turfgrass quality rating and canopy temperature were recorded several times per week during each year. Visual turfgrass quality rating is a composite rating accounting for factors such as color, canopy density, and surface uniformity. It was rated on a 1 to 9 scale where 1 represents completely dead turf, 6 was considered acceptable, and 9 represents perfect putting surface quality (Skogley and Sawyer, 1992).

Surface canopy temperature was measured with a FLIR i7 IR camera (FLIR Systems, Inc., Portland, OR). The camera was positioned ≈7 m above the putting surface and ≈10 m south of the first block. The FLIR allowed for all 20 plots to be measured simultaneously for reduced variability associated with changing sky conditions. FLIR images were imported into the FLIR Tools software package (FLIR Systems, Inc.) to determine the average temperature within each plot.

Visual turfgrass quality and surface temperature data were subjected to repeated measures analysis in JMP 10 (Version 10.0.1; SAS Institute, Cary, NC). The random term in the model was Civitas × Harmonizer nested within the experimental plot. The main effects of the Civitas, Harmonizer, date, and block as well as all interactions were examined. Means were separated with Fisher's least significant difference (LSD) ($\alpha = 0.05$) when appropriate.

Growth chamber assessment of phytotoxicity. A growth chamber study was conducted to assess the phytotoxic response of Civitas and Harmonizer on membrane integrity, gas exchange, and cuticle composition and structure. Annual bluegrass (*Poa annua* L. cv. DW-184) was established in a Percival WE-1012 growth chamber fitted with a mixed incandescent and fluorescent light source (Percival Scientific, Perry, IA) at Cornell University in Ithaca, NY. Plants received ≈450 μmol·m⁻²·s⁻¹ photosynthetically active radiation (PAR) for 16 h at 30 °C and 50% relative humidity and 8 h of darkness at 20 °C and 70% relative humidity.

Ray Leach SC10 3.8 × 210-cm cone-tainers (Stuewe & Sons, Tangent, OR) were filled and packed with non-amended sand meeting USGA specifications for putting green construction (USGA Green Section Staff, 2004). Approximately 10 seeds of annual bluegrass were placed in cone-tainers that were filled with sand to 15 mm below the top of the cone-tainer to allow for uniform clipping height. Cone-tainers were

fertilized weekly for the first 10 weeks of establishment with 10 mL of a fertilizer solution containing 200, 50, 200, and 1 mg·L⁻¹ nitrogen, phosphorus, potassium, and chelated iron, respectively. After establishment, cone-tainers received 8 mL of the previously mentioned fertilizer mix every 2 weeks. Cone-tainers were clipped several times per week and were watered daily with tap water to prevent drought stress. Treatments were initiated 12 weeks after germination at which point multiple tillers had completely filled the cone-tainer.

The study was conducted twice as a randomized complete block design with four replicates. Treatments included Civitas, Harmonizer, Two-Pack, Civitas One, and a non-treated control. The Civitas and the Harmonizer were applied alone at the rates of 5.0 and 0.3 mL·m⁻², respectively, or combined on-site to create the Two-Pack. Civitas One was applied at the rate 5.5 mL·m⁻², which is equivalent to the Two-Pack.

Applications were made with a CO₂-powered backpack sprayer equipped with a TeeJet AI 8004 nozzle (TeeJet Technologies) calibrated to deliver 81 mL·m⁻² at 275 kPa. All cone-tainers were arranged into a single line to ensure each cone-tainer was 50 cm beneath the nozzle tip. In an effort to determine changes in the cuticle morphology and to visualize changes in cuticle structure after successive applications, an additional application of Two-Pack and Civitas One was made to additional cone-tainers 10 d after the first application.

All measured observations occurred 48 h after treatment applications except for the scanning electron micrographs that occurred 48 h after the first and second application. Membrane integrity was determined by electrolyte leakage (EL) described by Liu and Huang (2000). Briefly, 50 mg of wet green leaf tissue was rinsed with Milli-Q ultrapure H₂O (EMD Millipore, Billerica, MA), dried with a Kimwipe (Kimberly-Clark, Irving, TX), and placed in a 50-mL volumetric flask, which contained 40 mL of Milli-Q H₂O. Tissue was gently shaken in darkness for 24 h before initial electrical conductivity (EC) measurement with an Orion Star conductivity meter (Thermo Fisher, Waltham, MA). Samples were then autoclaved for 20 min to release total plant electrolytes, allowed to cool to ≈20 °C, and conductivity was re-measured. Electrolyte leakage was expressed as the percent of initial conductivity divided by the total conductivity.

Hexane extraction and gas chromatography-mass spectrometry (GC-MS) was used to quantify epicuticular wax content. The method was based on that described by Bethea (2012) and Jenks et al. (2001). Approximately 500 mg of fresh green tissue was weighed and submerged in 25 mL of hexane for 50 s to remove the cuticle. Ten micrograms of tetracosane (C₂₄ alkane; Sigma Aldrich, St. Louis, MO) was added to each extraction and served as the internal standard. The extract was poured into a 50-mL beaker, evaporated to less than 4 mL, and stored in

4-mL glass vials with TFE-lined caps (Wheaton Science Products, Millville, NJ) at -23 °C. Before analysis, samples were transferred into 5-mL Weaton™ V Vials™ (Wheaton Science Products, Millville, NJ) and evaporated to dryness under a N₂ stream in a fume hood. Dried samples were derivatized with 350 μL of bis(trimethylsilyl)-acetamide (Sigma-Aldrich) at 85 °C for 25 min.

Compounds were separated and identified by GC-MS using an Agilent 6890N GC (Santa Clara, CA) equipped with split/splitless injector and a J&W Scientific DuraGuard DB-5ms (30 m × 0.25 mm × 0.25-μm film with a 10-m guard column, Folsom, CA) coupled to a JEOL-GCMate II mass spectrometer (Akishima-Shi, Tokyo, Japan). One-microliter injection volumes were used with both split mode (1:150 split ratio) to quantify large components and splitless mode with an inlet purge wait time of 1 min to quantify minor components. Gas chromatographic separation was performed with helium as the carrier gas at a constant flow rate of 1.0 mL·min⁻¹. The initial oven temperature of 50 °C was held for 2 min followed by heating to 200 °C at a rate of 40 °C·min⁻¹ and to 300 °C at a rate of 4 °C·min⁻¹. The final temperature was held for 14.25 min for a total analysis time of 45 min. Injector, interface, and ion-source temperatures were kept at 250, 280, and 250 °C, respectively. Mass spectrums were acquired in positive-ion mode using electron-impact ionization with electron energy of 70 eV and filament current of 0.3 mA. Preamp gain was set to X1 and detector voltage to 350 V. Masses were measured with linear magnetic-field scans from m/z 35 to 600 with 0.2 scan/s scan speed and 0.1-s interscan delay using external calibration. Nominal mass resolution (10% valley) was 500 and actual resolution on PFK calibrant before acquisition was ≈650.

Major peaks were identified from m/z peak data described in the Wiley NIST database summarized by Bethea (2012). Area under each peak was integrated in TSS Pro 3.0 (Shader Analytical and Consulting Laboratories, Inc., Detroit, MI). Electron impact ionization allowed for peak area of each compound to be compared with the internal standard by molar ratio and calculate the quantity in μg·g⁻¹ fresh weight.

Epicuticular wax morphological structure and stomata were examined with a Hitachi SU-70 Ultra-High Resolution Analytical Field Emission Scanning Electron Microscope (Hitachi, Chiyoda, Tokyo, Japan) at Suncor Petro-Canada Research Laboratory in Mississauga, Ontario, Canada. Treatments examined included a non-treated control, Civitas and, and Civitas One 24 and 48 h after the first and second applications. One-millimeter sections of leaves were fixed to the aluminum stage with carbon tape. The microscope was set at 1 kV and 9.5-mm working distance. Micrographs were taken at 500×, 2500×, and 5000× magnification.

Carbon exchange rate (CER) and transpiration were measured with a LI-COR 6400XT photosynthesis meter with 6400-17L Lighted

Fig. 1. Turfgrass visual quality and surface temperature as affected by Civitas and Harmonizer and environmental conditions during the field experiment in 2012 and 2013.

Table 1. Summary of repeated-measures analysis of variance table of main effects and significant interactions from the field experiment.

Source	Visual quality rating		Surface temperature	
	df	F-ratio	df	F-ratio
Civitas (C)	1	69.16***	1	155.44***
Harmonizer (H)	1	143.98***	1	127.45***
Date	47	8.81***	35	11,648.38***
C × H	1	35.74***	1	1.85
C × date	47	11.88***	35	8.65***
H × date	47	5.80***	35	6.65***
C × H × date	47	6.12***	35	1.47*

*Significant at the 0.05 *P* level.

**Significant at the 0.01 *P* level.

***Significant at the 0.001 *P* level.

Whole Plant *Arabidopsis* Chamber (LI-COR, Lincoln, NE). Negative CER indicates net carbon (C) loss, whereas positive CER indicates C assimilation. The chamber was configured to provide constant flow rate at

500 $\mu\text{mol}/\text{air}/\text{s}$ and a 400 $\mu\text{L}\cdot\text{L}^{-1}$ CO_2 concentration. Light response curves were created with an AutoProgram that adjusted photosynthetic photon flux density (PPFD) from 2000 to 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of PAR. Carbon

exchange rate and transpiration were measured automatically after CO_2 and H_2O concentrations of the sample had stabilized when cv values were less than 5%. The stability values were optimized for time efficiency and measurement precision. PPFD was then automatically reduced to the next lowest intensity. Light response curves were created 48 h after application at PPFD values of 2000, 1500, 1000, 750, 400, 200, and 0 PPFD.

Electrolyte leakage, cuticle components, and CER at each light level were subject to analysis of variance in JMP 10 (Version 10.0.1; SAS Institute). Analysis of covariance was used for the transpiration data resulting from the linear response to PPFD. The main effects of Civitas component, block, run, and all interactions were examined and nonsignificant effects and interactions were removed from the final statistical

Table 2. The effect of Civitas and Harmonizer on putting green tiller density at the end of the 2013 growing season.

Treatment	df	Tiller density no./cm ²
Harmonizer		14.2 a
Control		13.3 ab
Civitas		12.4 bc
Two-Pack		11.6 c
Analysis of variance		
Civitas	1	***
Harmonizer	1	***
Civitas × Harmonizer	1	***

***Significant at the 0.001 *P* level.

Means followed by the same letter are not significantly different according to least significant difference (0.05).

Table 3. Electrolyte leakage of turfgrass leaves treated with different Civitas formulations during the growth chamber experiment.

Treatment	df	Electrolyte leakage (%)
Civitas		13.5 a
Control		10.5 ab
Two-Pack		9.4 b
Harmonizer		8.6 b
Civitas One		8.5 b
Analysis of variance		
Treatment (T)	4	*
Run (R)	1	NS
T × R	4	NS

*Significant at the 0.05 *P* level.

Means followed by the same letter are not significantly different according to least significant difference (0.05).

NS = nonsignificant.

model. Means were separated with Fisher's LSD ($\alpha = 0.05$) when appropriate.

Results and Discussion

Civitas phytotoxicity field assessment. The Ithaca, NY, air temperatures were within the daily air temperature by 1 °C during the 2012 and 2013 growing seasons. Maximum daily air temperatures were greater during 2013 than 2012 (Fig. 1). The total daily light integral was more variable in 2013 than 2012 because there were more clear days in 2013 and the study started later in 2012 than 2013 when daylengths were shorter. The weather station was struck by lightning on 8 Aug. 2013 and stopped logging data after the event.

The Civitas and the Harmonizer had a significant effect on visual quality and canopy temperature (Table 1). Civitas applied by itself reduced visual quality rating below acceptable limits (less than 6) because of chlorosis on 44 of the 47 rating dates (Fig. 1). Comparatively, the control plot had acceptable quality during all of the rating dates during both years. Addition of Harmonizer increased visual quality ratings an average of 0.68 units compared with the control over 2 years of observations.

There was a significant Civitas × Harmonizer × date interaction (Table 1). In 2012, plots that received Harmonizer, the

Two-Pack, and the control had similar turfgrass quality ratings throughout the season (Fig. 1). Turfgrass visual quality declined with Civitas treatment as the season progressed in 2012. In 2013, plots that received only Harmonizer or the control consistently had acceptable visual quality rating. In contrast, Civitas produced unacceptable quality ratings on all rating days in 2013. Combination of Harmonizer with Civitas masked the phytotoxicity from May until mid-July; however, visual quality rapidly declined below acceptable levels beginning 24 July 2013. The decline in quality resulted from thinning of the turfgrass canopy. The decline in Two-Pack quality rating during 2013 likely occurred because the experimental run started earlier in 2013 and ran a month longer than 2012. Air temperatures and solar radiation were also greater during 2013 than 2012. At the end of the 2013 growing season, six 20-mm diameter plugs were taken from each plot and tillers were counted. Canopy tiller density declined with Civitas despite application of Harmonizer (Table 2). Tiller density results were specific to 2013 treatment application because the entire plot area was moved to a different location on the putting surface between 2012 and 2013. The decline in turfgrass visual quality rating and stand density is most likely the result of the high labeled application rate of 5.0 mL·m⁻². Since the initiation of this study, turfgrass managers have reduced application rates by 50% to 75% to minimize the potential of phytotoxicity. More research is required to determine how much Civitas needs to be applied to elicit induced systemic resistance and minimize the potential for phytotoxicity.

It was anecdotally observed that turf treated with Harmonizer was slow to recover from traffic and pest damage. Because this site contained a significant amount of annual bluegrass, recovery likely would be through germination from the soil seed bank. Harmonizer contains a chelated copper molecule. It is possible that routine application of copper-based pigments may inhibit germination in voids. Bristow et al. (2013) showed application of the heavy metal zinc (Zn) to newly established cool-season turfgrass resulted in decline and death. However, mature turfgrass stands were less affected by Zn application.

There was a significant Civitas × Harmonizer × date interaction for putting surface canopy temperature (Table 1). On days with high solar radiation and low cloud cover, Harmonizer and Civitas applied alone increased canopy temperatures between 0 and 1.6 °C. A combination of Civitas and Harmonizer had an additive effect on surface canopy temperature often increasing temperatures by 0 to 2 °C (Fig. 1). McCarty et al. (2013) found that application of phthalocyanine pigments to heat-stressed turfgrass did not affect transpiration rate. Therefore, Harmonizer seemed to increase canopy temperature because of its ability to absorb light energy. The increase in canopy temperature did not have an obvious effect on turfgrass health because the Harmonizer treatment

consistently had the best quality rating, whereas Civitas had the worst quality despite similar canopy temperatures. The increase in canopy temperature with Civitas suggests gs and transpiration may be inhibited. Inhibition of transpiration with other PDSOs has been well described in other plant species (Hodgkinson et al., 2002).

Civitas phytotoxicity growth chamber assessment. There was no evidence of membrane damage resulting from application of Civitas to annual bluegrass because EL of Civitas-treated plants was similar to control plants (Table 3). Electrolyte leakage did not exceed 20%, a level typical of non-stressed turfgrass plants, whereas EL values of heat- or drought-stressed can reach 40% to 85% (Liu and Huang, 2000; Yang et al., 2013). Plants treated with only Civitas had EL values between 8% and 14%. Plants that received Harmonizer, the Two-Pack, or the Civitas One had lower levels of EL compared with Civitas but were the statistically the same as the non-treated control.

Harmonizer is composed of a polychlorinated copper (Cu) II phthalocyanine pigment (Nash, 2011). Phthalocyanines chelate Cu²⁺ and result in a stable blue-green-colored pigment. Although it is possible that Harmonizer stabilized plant membranes, reduced EL with Harmonizer is likely an artifact of autoclaving Harmonizer which causes damage to the chelating agent. This could release Cu²⁺ into the solution and increase the EC after autoclaving during sample preparation. In turn, the denominator in the EL calculation would increase and reduce % EL. Alternatively, the phthalocyanine may have chelated electrolytes that were liberated before the sample is autoclaved, which would reduce the solution EC and % EL. Despite the uncertainty as to why Harmonizer reduced EL, it is clear that the Civitas does not damage membranes and rules out acute phytotoxicity (Hodgkinson et al., 2002).

Treatments did not alter quantity or composition of the plant's natural epicuticular wax. There were seven principle components of annual bluegrass epicuticular wax identified through GS-MS: C₁₆ and C₁₈ fatty acids and C₁₈, C₂₂, C₂₄, C₂₆, and C₂₈ primary alcohols (Fig. 2). Other minor peaks representing less than 1% of the total epicuticular wax were ignored. Total epicuticular wax load averaged 59.4 µg·g⁻¹ fresh weight. Primary alcohols comprised 96.1% of the epicuticular wax, whereas primary C₁₆ and C₁₈ saturated fatty acids comprised 2.4% and 1.5%, respectively. The primary alcohol 1-hexacosanol was the predominant species at 86% of the total epicuticular wax or 51.1 µg·g⁻¹ fresh weight. There was a significant run effect for four of the seven principle components (Table 4). Total C₁₈, C₂₂, C₂₄, and C₂₈ primary alcohol species increased from run one to run two. Although there was not a clear explanation, factors such as irrigation frequency and amount are known to change epicuticular wax load (Kim et al., 2007a, 2007b; Kosma et al., 2009).

Application of Civitas, Civitas One, or Two-Pack resulted in a broad peak on the

Fig. 2. A screenshot of two 150-split total ion current chromatographs from (A) epicuticular waxes extracted from a non-treated control and (B) Civitas One-treated turf sample. Major peaks were a C₁₆ and C₁₈ (13.4 and 15.08 min) fatty acid; a C₂₄ alkane internal standard (19.27 min); and a C₁₈, C₂₂, C₂₄, C₂₆, and C₂₈ primary alcohol (16.51, 21.81, 25.20, 28.52, and 31.59 min).

total ion current chromatogram from 9 to 24 min into the recording (Fig. 2). The Civitas signature was absent from the Harmonizer and control plants. Electron-impact ionization allowed for integration of the entire Civitas peak. On molar average, there was 12.2 times more Civitas on treated leaves than C₂₆ 1-hexacosanol, the dominant natural component of annual bluegrass

epicuticular wax. There was no statistical difference among the Civitas, Civitas One, and Two-Pack treatments.

Leaves of the non-treated control had a regular and uniform epicuticle structure with dense primary alcohol platelets (Barthlott et al., 1998; Jeffree, 2006). These crystal structures are known to be very regular and dependent on their chemical composition.

Platelet crystal structure is common when epicuticular wax is composed mainly of primary alcohols (Barthlott et al., 1998; Jeffree, 2006). Scanning electron microscopy clearly shows that both the Civitas One and Two-Pack alter the morphology of the epicuticular wax (Fig. 3) most notably 48 h after the second application of Civitas.

Table 4. Differences in epicuticular wax composition between the two experimental runs.

Run	df	C ₁₆ FA ^z	C ₁₈ FA	C ₁₈ PA	C ₂₂ PA	C ₂₄ PA	C ₂₆ PA	C ₂₈ PA
$\mu\text{g}\cdot\text{g}^{-1}$ fresh weight								
1		1.26 a ^y	0.97 a	0.59 b	1.36 b	0.88 b	46.33 a	1.69 b
2		1.59 a	0.78 a	0.96 a	2.39 a	1.37 a	55.37 a	2.33 a
Analysis of variance								
Treatment (T)	4	NS	NS	NS	NS	NS	NS	NS
Run (R)	1	NS	NS	*	**	*	NS	*
T × R	4	NS	NS	NS	NS	NS	NS	NS

^zThe size of the fatty acid (FA) or primary alcohol (PA) in number of C atoms.

^yMeans followed by the same letter are not significantly different according to least significant difference (0.05).

NS = nonsignificant.

Fig. 3. Scanning electron micrograph of leaf epicuticular wax morphology and turfgrass stomata. (A, C, E) Turfgrass leaves treated with water, 48 h after Two-Pack application, and 48 h after the second Two-Pack application, respectively, at 500× magnification. (B, D, F) Turfgrass leaves treated with water, 48 h after Two-Pack application, and 48 h after the second Two-Pack application, respectively, at 2500× magnification. Images were representative of the entire leaf surface.

Dissolved epicuticular waxes are known to recrystallize after the solvent is allowed to evaporate (Jetter and Reiderer, 1994; Koch et al., 2006; Neinhuis et al., 2001). However, recrystallization did not occur on turfgrass leaves treated with Civitas and it is plausible that Civitas disrupted the normal crystal structure and inhibited recrystallization. The altered cuticle morphology could influence leaf boundary layer and leaf

surface wettability by altering surface tension. This may have significant implications for irrigation management and pesticide absorption (Fernandez and Eichert, 2009; Yoshimitsu et al., 2002).

In addition to the altered crystalline structure of the cuticle, the stomata appear occluded (Fig. 3). It is unclear if the substance is the Civitas, redistributed plant epicuticular wax, or both. In any event, the level of

additional wax on the leaves resulting from Civitas was significant. It is likely that both redistribution of natural epicuticular waxes and the addition of Civitas reduced g_s and transpiration, which caused increased canopy temperature in the field study. These findings have been observed in other species and have been implicated to cause chronic phytotoxicity resulting from oil persistence and stomatal impedance (Hodgkinson et al., 2002).

Transpiration rates increased linearly as light level increased from 0 to 2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR (data not shown). The Two-Pack and Civitas One treatments in Run 1 and Civitas alone treatment in Run 2 had a reduced transpiration rate compared with the non-treated control (Table 5). There was also a significant run and treatment × run interaction. The LI-COR 6400 was configured to have a constant air flow rate through the chamber. This led to small differences in relative humidity and vapor pressure deficit between the two runs, which can explain the run and treatment × run interaction.

In contrast to Harmonizer, Civitas significantly reduced CER and radiation use efficiency (RUE) as PPFD intensity increased (Table 6; Fig. 4). A formulation effect was noted because the Civitas One reduced CER and RUE more than Civitas and Two-Pack. Treatment differences were not statistically different below a PPFD of 750 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR. There were no differences in CER in darkness, which represents maximum dark respiration rate (Hay and Porter, 2006). This suggests the Civitas treatments did not affect respiration rate. Like with the transpiration data, the significant run effect was most likely the result of a small difference in chamber vapor deficit gradient between runs.

Reduction in maximum photosynthetic rate at higher PPFD is commonly the result of substrate (CO_2) limitation and can occur when stomata are closed or obstructed (Hay and Porter, 2006). Future study is needed to determine the duration of reduced CER/RUE and transpiration. Long-term reduction in RUE has the potential to reduce nonstructural carbohydrates and increase photo-oxidation as photosynthesis becomes substrate-limited. Harmonizer and the Two-Pack could safely be eliminated from future experiments because they did not alter CER and transpiration rate compared with the control or Civitas treatments, respectively. This change would offer substantial time savings in the future.

Conclusion

Application of the PDSO, Civitas, to a mixed annual bluegrass and creeping bentgrass golf putting surface resulted in chlorosis, canopy thinning, and elevated surface temperatures. Addition of the phthalocyanine pigment, Harmonizer, with Civitas masked the chlorosis in both years; however, Harmonizer was unable to mitigate the decline in turfgrass visual quality rating because stand density declined was observed during the second year. Civitas was applied at the high labeled rate (5% w/v). Beattie (1990) stated

Table 5. Transpiration rate as affected by treatment, photon flux density, and run during the growth chamber experiment.

Run	Treatment	df	Transpiration rate
			unit
1	Control	4	13.62 cd ²
	Harmonizer	4	13.57 cd
	Civitas	4	13.3 cd
	Two-Pack	4	12.98 f
	Civitas One	4	13.09 ef
2	Control	4	14.18 a
	Harmonizer	4	14.08 ab
	Civitas	4	13.78 bc
	Two-Pack	4	14.12 a
	Civitas One	4	13.95 ab

Analysis of covariance

Source of variation	df	F-ratio
Treatment (T)	4	***
Photon flux density (PPFD)	1	***
Run (R)	1	***
T × PPFD	4	NS
T × R	4	*
PPFD × R	1	NS
T × PPFD × R	4	NS

*Means followed by the same letter are not significantly different according to least significant difference (0.05).

*Significant at the 0.05 *P* level.

***Significant at the 0.001 *P* level.

NS = nonsignificant.

Table 6. Carbon exchange rate analysis of variance from the growth chamber experiment.

Source	df	F-ratio
Treatment (T)	4	28.9***
Photon flux density (PPFD)	6	1,184.1***
Run (R)	1	8.6**
T × PPFD	24	2.4***
T × R	4	2.13
PPFD × R	6	0.02
T × PPFD × R	24	0.25

*Significant at the 0.05 *P* level.

**Significant at the 0.01 *P* level.

***Significant at the 0.001 *P* level.

PDSO applied at concentrations greater than 2% w/v had increased potential to cause phytotoxicity. Decreasing Civitas application rate has the potential to decrease the risk of phytotoxicity.

The chlorosis associated with Civitas can be described as chronic phytotoxicity because there was significant evidence of oil persistence, stomatal occlusion on micrographs, and reduced gas exchange at high PPFD. This is known to increase oxidative stress under conditions of high light intensity or temperature. There was no evidence of membrane disruption or alteration of quantity or composition of epicuticular wax to support acute phytotoxicity, although wax morphology was altered. Further research is required to understand how Civitas affects C partitioning, photoinhibition, water use efficiency, and pesticide absorption. Additionally, research needs to be conducted to optimize application rate. Lower application rates may reduce the potential for phytotoxicity but may also lessen other Civitas benefits such as induced systemic resistance and increased pesticide efficacy.

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Fig. 4. The effect of photosynthetic flux density and Civitas component or formulation on carbon exchange rate.

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