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Chemical plant protectants and plant growth regulator effects on annual bluegrass survival of ice cover

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Abstract

Annual bluegrass (*Poa annua* L; ABG) is susceptible to damage from ice cover. Effects of chemical treatments on ice survival under low temperature growth chamber conditions were studied. Field putting green plots of ABG were treated in the fall of 2014 and 2015 with Civitas, mefluidide, propiconazole or trinexapac-ethyl (TE) at label rates. After acclimation, turfgrass cores from each plot were transferred to a low temperature growth chamber, received ice or no ice treatment and were sampled at 0, 20, 40 and 60 days. Ice was applied by misting plants at low temperature. In 2014, Civitas-, mefluidide- and propiconazole-treated plugs had the greatest amount of regrowth after ice and no ice treatments on most days; comparable results were found in 2015 but fewer statistical differences were detected. TE-treated plants were no different from the untreated controls on most days in either year. Plants that were treated with mefluidide, propiconazole and Civitas had more polyunsaturated fatty acids (FA) in crown tissue, with linoleic acid approximately 50% greater compared to TE and untreated samples. Civitas, mefluidide or propiconazole treatments improved ABG survival of simulated ice conditions, which could be associated with the changes to saturated and unsaturated FA ratios that were observed in this study.

1 | INTRODUCTION

Turfgrasses or other perennial crops can be subjected to prolonged periods of ice cover in many northern or temperate regions of the world. Freezing rains, ice storms, poor soil drainage and refreezing of snow melt can cause ice accumulation on top of plant canopies (Mckersie & Leshem, 1994). Ice cover can cause anoxic conditions, crown hydration and reduced freeze tolerance in ABG (Olien & Smith, 1981; Rochette, Dionne, & Desjardins, 2000; Tompkins, Ross, & Moroz, 2004). The primary cause of death to turfgrass under ice sheets is most likely from oxygen depletion and toxic gas accumulation (Pessarakli, 2008). Annual bluegrass (*Poa annua*; ABG) is a turfgrass putting green species that is susceptible to ice damage, which has been reported to survive an average of 60 days of ice cover (Beard, 1964; Tompkins et al., 2004; Vargas & Turgeon, 2004). Past research performed at Michigan State University in a freezing chamber has shown that ABG necrosis can occur in 45 days at −4°C under a 1.27-cm ice layer (Beard, 1964). For comparison, creeping bentgrass (*Agrostis stolonifera*), another common putting green species, can survive under ice cover for up to 120 days without significant injury. Ice damage is a significant issue for golf courses since ABG areas frequently endure prolonged ice cover in northern regions. This damage is costly as it causes turfgrass managers to repair or completely renovate ice damaged putting greens.

There are positives and negatives of current methods of ice management or ice damage prevention used on golf courses. Ice removal can be an effective way to prevent ice damage; however, it is costly, laborious and can cause mechanical damage to putting greens. Another difficulty with ice removal is removal is highly time-sensitive. Tompkins et al. (2004) showed there was little to no benefit of removing ice from ABG after 45 days of ice cover while removing ice prior to 45 days may be beneficial depending on environmental conditions. Another winter turf management strategy is to cover greens with an impermeable or permeable cover or mulch material. These cover materials can raise crown level

soil temperatures to increase overwintering survival; however, inconsistent results in winter protection often occur due to unpredictable winter temperature fluctuations and conditions (Dionne, Dube, Laganiere, & Desjardins, 1999). Better, more reliable, and reduced risk strategies are needed to protect turfgrasses from winter damage such as ice cover. Therefore, this research aims to evaluate simple chemical management strategies that could promote ABG survival of ice cover.

In addition to new management practices, a better understanding of the physiological mechanisms of ABG that result in ice sensitivity and those that could promote tolerance is needed. Therefore, another goal of the research was to evaluate changes in physiology that occur during treatments and during recovery. Winter preparatory management practices are performed in the fall, when turfgrasses undergo the process of acclimation. Acclimation is induced by a reduction in photoperiod and reduced daytime/night-time temperatures (Guy, 2003; Limin & Fowler, 1985). This change in environmental conditions can change physiological characteristics in a plant such as increasing antioxidant scavenging, accumulation of antifreeze proteins and alteration of cell membrane composition (Munshaw et al., 2006). During acclimation, cell membrane fatty acid (FA) profiles can be altered from a saturated FA profile to a more unsaturated FA profile (Baird et al., 1998; Shang, Zhang, Munshaw, & Ervin, 2006). Shifts in FA profiles have been observed in multiple plant species in response to cold acclimation or cold temperature treatment (Cyril, Powell, Duncan, & Baird, 2002; Hoffman, DaCosta, Ebdon, & Watkins, 2010; Samala, Yan, & Baird, 1998) but have yet to be investigated in ABG. A shift in fatty acid composition towards more unsaturated FAs than saturated FAs is associated with ice encasement tolerance (Dalmannsdóttir, Helgadóttir, & Gudleifsson, 2001; Hetherington, McKersie, & Borochov, 1987). Whether chemical treatment of ABG can alter FA profile shifts during acclimation and following ice stress deserves investigation.

The chemical treatments used in this study include plant growth regulators (PGRs; mefluidide, trinexapac-ethyl, TE), a fungicide (propiconazole), and a plant protective treatment registered as a fungicide-containing mineral oil (a mixture of food-grade isoparaffins) as the active ingredient. Many turfgrass managers already use these compounds during the growing seasons to alter the growth habits of ABG or to prevent disease. It is not known whether any of these treatments could play a negative or positive role in winter survival and regrowth following ice stress of ABG. Therefore, we hypothesize that altering plant growth and other physiological changes associated with applications of PGRs or fungicides during acclimation could alter turfgrass regrowth following winter conditions. Our objectives were to evaluate how per cent regrowth and physiological attributes associated with acclimation, such as changes in FA profiles, may be affected by chemical treatments and duration of ice cover of ABG under both ice and no ice in a low temperature growth chamber.

2 | MATERIALS AND METHODS

2.1 | Plant materials and growing conditions

This study was conducted from July to November in 2014 and 2015 on the same ABG field each year with the same plots used for each treatment at the Michigan State University Hancock Turfgrass Research Center in East Lansing, Michigan. Field plots consisted of mature ABG grown on a top-dressed Colwood brookston loam soil. A light sand topdressing was brushed into the canopy weekly from May through September at a depth of 2.0 mm. The site was maintained at a 3.3 mm mowing height and was mown three times weekly. The plots were irrigated nightly to replace approximately 100% potential evapotranspiration to avoid drought stress. Irrigation was withheld when rainfall events exceeded 100% potential evapotranspiration. Potential evapotranspiration was retrieved from the weather station located at the research facility based on the FAO Penman–Monteith Equation (Allen, Pereira, Raes, & Smith, 1998). The study area was fertilized with 127.2 kg N/ha each year including foliar feeding 4.9 kg N/ha weekly June through September of 2014 and 2015. Plots were fertilized with 24.4 kg N/ha on 2 June and 1 September, using a 18-9-18 (N-P-K) granular fertilizer (Andersons Golf Products, Maumee, OH). Fungicides and insecticides were applied preventatively and curatively to avoid turfgrass loss due to disease or insect damage, respectively, to all plots equally and should not have had an influence on our results.

2.2 | Chemical treatments

All chemical treatments began on 31 July 2014 and on 4 August 2015 and were applied at label recommended rates every two weeks (Figure 1). All treatments were applied with a pressure-calibrated backpack sprayer (591 L/ha at 275 kPa) equipped with four flat fan nozzles (DG8002 DS, Teejet Technologies, Wheaton, IL). The foliar chemical treatments included (a), a fungicide product containing a mineral oil [CIVITAS TURF DEFENSE™ (Civitas), Petro-Canada, Mississauga, Ontario] at a rate of 40.6 L/ha, (b), mefluidide (Embark T&O, PBI-Gordon Corp., Kansas City, MO) at a rate of 1.6 L/ha, (c), propiconazole (Banner Maxx, Syngenta Crop Protection, Greensboro, NC) at a rate of 6.4 L/ha and (d), TE (Primo Maxx, Syngenta Crop Protection, Greensboro, NC) at a rate of 0.4 L/ha. Untreated plots were sprayed with water and were utilized as the control plots.

Turfgrass plugs (10-cm-diameter) were taken by hand with a turf corer from each plot on 11 November 2014 and 25 November 2015 to a depth of 10 cm, after adequate natural plant acclimation. Temperatures optimum for cold hardening were based on Dionne, Castonguay, Nadeau, and Desjardins (2001) where plants experienced approximately two weeks of temperatures at or below 2°C. This met our requirement for cold hardening. Eight turfgrass plugs were taken from each plot of the five chemical treatments (four sampling time points, two ice cover treatments, with four replications) with a total of 160

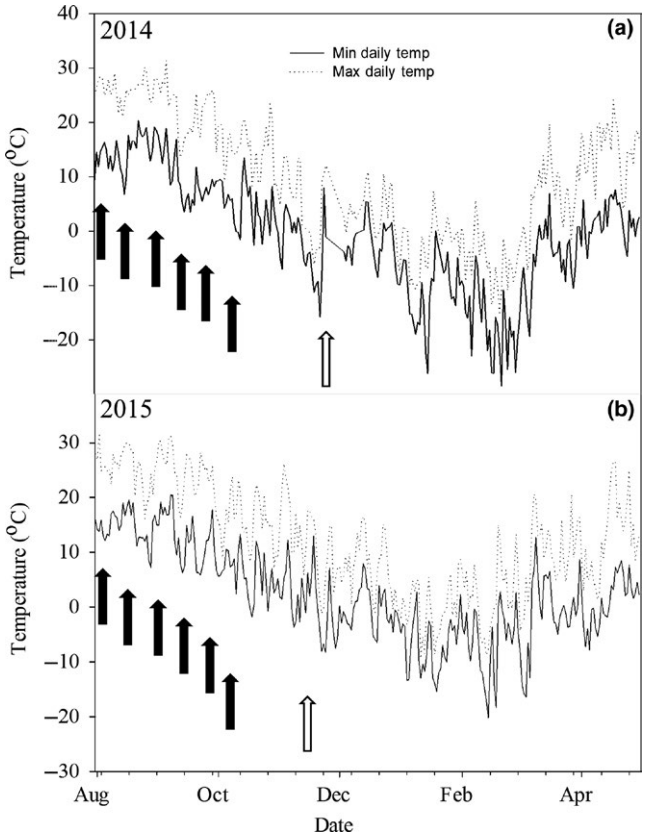

FIGURE 1 Daily minimum and maximum temperature for the Hancock Turfgrass Research Center in East Lansing, MI, from (a) 31 July 2014 to 3 April 2015 and (b) 31 July 2015 to 30 April 2016. Black filled in arrows indicate when field treatment applications were applied in each year. Open arrows indicate when annual bluegrass plugs were removed from the field

turfgrass plugs being used for the experiment. Once all plugs were removed from plots, they were immediately planted in 10.2-cm-diameter plastic pots with a depth of 15.2 cm in sandy loam soil and transferred to a low temperature growth chamber and allowed to acclimate at −2°C for two weeks with a light level of 200 μmol m^{-2} s^{-1} with a 10-hr photoperiod prior to low temperature treatment. Growth chamber conditions included a light level of 200 μmol m^{-2} s^{-1} with a 10-hr photoperiod and a temperature of −4°C. Treatments inside the growth chamber consisted of (a) no ice or (b) ice cover treatments. Ice cover treatment involved misting deionized water over pots to form an ice layer (1.3-cm-thick). Ice-covered and non-ice-covered plugs were separated to one half of the growth chamber for ease of misting. Ice layers were monitored for depth throughout the duration of the study, and water was added by misting of ice layers showed any loss of depth. Four turfgrass plugs were taken out from the low temperature growth chamber from each chemical treatment, at 0, 20, 40 and 60 days from both ice- and non-ice-covered treatments.

2.3 | Fatty acid analysis

Extraction of FAs was performed with approximately 200 mg crown tissue (fresh weight) from ice-covered and non-ice-covered samples at 0, 20, 40 and 60 days according to the method of Welti et al. (2002) with modifications. Crown tissue was harvested after turf crowns were thawed. Leaf and root material were removed by cutting materials away from individual tillers leaving only crown tissue. Frozen crown material was transferred into test tubes containing 3 ml of preheated isopropanol (75°C) with 0.01% butylated hydroxytoluene (BHT). Samples were placed in a 75°C water bath for 15 min. After the samples had cooled, 1.5 ml chloroform and 0.6 ml distilled water were added and the samples were capped and shaken at room temperature for 5 hr. After 5 hr, the lower layer containing chloroform and the lipids were transferred into new test tubes. An additional 4 ml of chloroform/methanol (2:1) with 0.01% BHT was added to the tubes containing the crown material. The tubes were recapped and placed on a shaker for 15 hr. After extraction, 1 ml KCL was added to the tubes containing the extracted lipids and chloroform and centrifuged at 5,000 *g* for 10 min. After 10 min, the top thin layer was removed, 2 ml of distilled water was added and the tubes were centrifuged for an additional 10 min at 5,000 *g*. The top thin layer was removed, and the remaining sample was evaporated using vacuum centrifugation. Samples were preserved in 1 ml chloroform and stored at −80°C until analysis. Remaining crown material was dried in an oven 70°C for determination of dry weight. Gas chromatography–mass spectroscopy was utilized to quantify FAs. As a measure of fatty acid unsaturation (Cyril et al., 2002), the double bond index (DBI) for each chemical treatment was then calculated as DBI = 0 × ([16:0] + [18:0]) + 1 × 1 × ([16:1] + [18:1]) + 2 × ([16:2] + [18:2]) + 3 × [18:3].

2.4 | Per cent regrowth

After either 0, 20, 40 or 60 days in the low temperature growth chamber, a set of turfgrass plugs were removed from the low temperature chamber and transferred to a 4°C chamber for 7 days, which served as a de-acclimation period. Plants were then transferred to a greenhouse. Greenhouse conditions were maintained at average day/night temperatures of 23/16°C and an average 12-hr photoperiod at 400 μmol m^{-2} s^{-1} photosynthetically active radiation. Per cent regrowth was observed for turfgrass plugs taken from the low temperature growth chamber after a 20-day regrowth period in the greenhouse. Regrowth was determined by visual inspection and manual counting of the number of living and dead tillers or leaves arising from crowns. Living plants were divided by the number of total plants multiplied by 100 achieved the per cent regrowth.

2.5 | Experimental design and statistical analysis

Field plots were arranged as a completely randomized design with four replications. The growth chamber experiment was conducted as a split plot design with ice treatment as the whole plot with replications split within each whole plot. Chemical treatment and sampling time were completely randomized within the whole plot.

Effect	Per cent regrowth		Fatty acids		Double bond index	
	2014	2015	2014	2015	2014	2015
Rep						
Ice treatment (I)	*	**	ns	ns	ns	ns
Chemical treatment (T)	***	***	***	***	***	***
T × I	ns†	ns	ns	ns	ns	ns
Days (D)	***	***	***	***	**	***
T × D	***	**	***	***	***	**
I × D	ns	ns	ns	ns	ns	ns
T × I × D	ns	ns	ns	ns	ns	ns

TABLE 1 Analysis of variance for main treatment factors and interactions of per cent regrowth, fatty acid analysis, double bond index and ratio of 18:3/18:2 fatty acids under chemical and ice cover treatments of annual bluegrass in East Lansing, MI during 2014 and 2015

*p values ≤0.05. **p value ≤0.01. ***p value ≤0.001. †ns: not significant with p value >0.05.

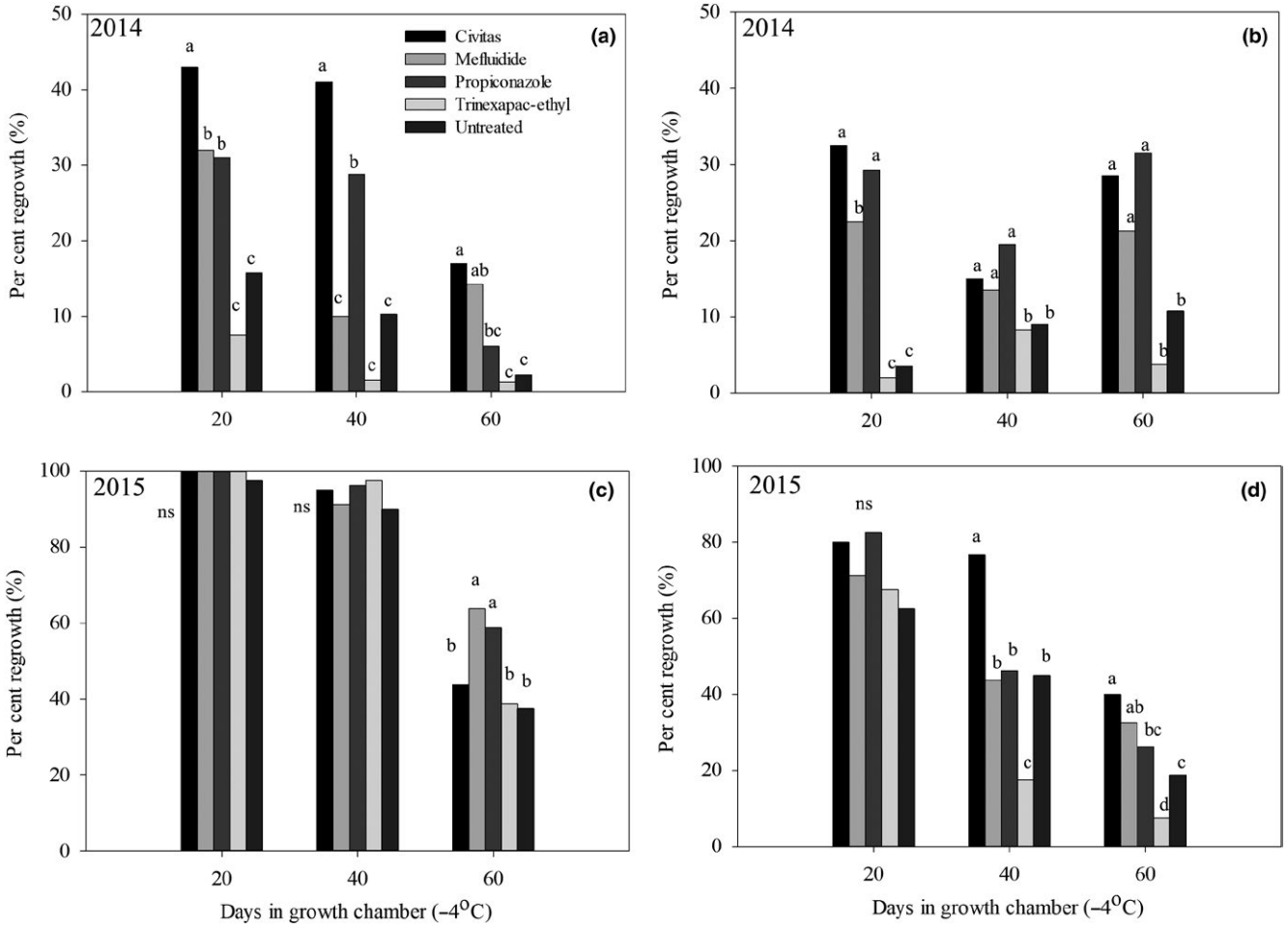

FIGURE 2 Per cent regrowth of annual bluegrass treated with a given chemical treatment after 20, 40 and 60 days in the low temperature growth chamber (−4°C) under (a) non-ice cover conditions in 2014, (b) ice-covered conditions in 2014, (c) non-ice cover conditions in 2015 and (d) ice-covered conditions in 2015. Means with the same letter on a given rating days are not significantly different based on least significant difference values ($p \leq 0.05$)

The experiment was repeated using the same growth chamber. All data were subjected to analysis of variance (ANOVA) using SAS 9.4 (SAS institute Inc., Cary, NC) mixed model procedure. For analyzing the data, time (year) was a fixed factor in the model. When ANOVA indicated a significant year effect, results are presented separately by year. Mean separations were performed by using Fischer's Protected Least Significant Differences (LSD) at the $p \leq 0.05$ level.

3 | RESULTS

Main effects and the interactions of chemical treatment and day were significant for per cent regrowth, fatty acid analysis and DBI in 2014 and 2015 (Table 1). Ice cover main effects were only significant in 2014 and 2015 for per cent regrowth of ABG; therefore, fatty acid analysis and DBI were pooled over the ice treatment data (ice or no ice). Significant time effects were observed within per cent regrowth, fatty acid analysis and DBI data collections resulting in data being presented separately by year.

Typical fall weather conditions were recorded during the chemical treatments in the field, which indicates plants acclimated naturally during treatments (Figure 1). Chemical treatments had a significant effect on non-ice-covered regrowth after 20 days at −4°C in 2014 with Civitas treatment having greater regrowth than all other treatments while mefluidide and propiconazole treatments had greater regrowth than TE and untreated controls (Figure 2a). After 40 days of incubation at −4°C, Civitas-treated plants again had the greatest regrowth when compared to other treatments while propiconazole treatment had greater regrowth than mefluidide, TE and untreated plants (Figure 2a). After 60 days in the low temperature growth chamber, TE and the untreated had the least amount of regrowth when compared to other treatments while the mefluidide treatment had the greatest regrowth during both years of the experiment (Figure 2a).

Chemical treatments had a significant effect on regrowth of ice-covered ABG at 20, 40 and 60 days in the low temperature growth chamber. In general, during both years of the experiment under ice cover, TE and untreated control plants had the lowest regrowth on most days compared to the other PGR treatments (Figure 2b). Specifically, after 20 days at −4°C under ice cover, Civitas-, mefluidide- and propiconazole-treated plants had the greatest regrowth in 2014 while TE and the untreated plants had the least amount of regrowth. In 2015, there was no significant treatment effect after 20 days in the low temperature growth chamber; however, after 40 days under ice cover, Civitas had the greatest regrowth while TE had the lowest per cent regrowth compared to mefluidide, propiconazole and the untreated, which were not significantly different from each other. Comparable results were observed after 60 days under ice cover in 2015 with Civitas having the greatest per cent regrowth and TE having the least amount of regrowth compared to the other treatments.

Six FAs, either unsaturated or saturated, were detected in crown tissue of ABG plants. The saturated FAs included palmitic acid (16:0) and stearic acid (18:0). The monounsaturated FAs were palmitoleic acid (16:1) and oleic acid (18:1), and the polyunsaturated FAs were linoleic acid (18:2) and α-linolenic acid (18:3). The fatty acid hexadecadienoic acid (16:2) was not observed in measurable quantities. Significant effects from PGR treatments were observed on FA composition of ABG crown membrane profiles and were consistent for each time point of sampling (0, 20, 40 or 60 days of ice or non-ice cover treatment). Ice treatment duration and ice treatment did not change FA composition within a given treatment.

Concentrations of the saturated FA palmitic acid (16:0) were significantly lower in the Civitas, propiconazole and mefluidide treatments when compared to the untreated control and TE-treated plants after 20 and 40 days of ice or no ice treated plants in 2014

TABLE 2 Saturated fatty acid contents of crown tissue of annual bluegrass treated in the field with foliar chemical applications and then exposed to 0, 20, 40 and 60 days in a low temperature growth chamber (−4°C) in 2014 and 2015. Within each column, means followed by the same letter are not significantly different ($p \leq 0.05$)

	2014				2015			
	Stress treatment period (day)				Stress treatment period (day)			
	0	20	40	60	0	20	40	60
16:0[†]	Molar percentage (mol %)							
Civitas	29.50 a[‡]	30.50 bc	29.25 bc	26.95 c	29.2 b	29.27 bc	27.63 b	26.63 b
Propiconazole	29.20 a	26.90 c	27.38 bc	26.95 c	30.07 b	28.87 bc	25.68 b	28.50 b
Mefluidide	23.75 b	29.30 c	24.25 c	29.58 bc	26.37 b	25.32 c	26.05 b	27.75 b
Trinexapac-ethyl	33.75 a	33.40 ab	31.98 ab	34.18 a	34.37 a	33.25 ab	33.35 a	35.03 a
Untreated	31.75 a	35.00 a	34.88 a	32.63 ab	34.77 a	34.52 a	33.15 a	34.30 a
18:0								
Civitas	28.27 bc	29.10 b	28.83 b	29.03 b	30.17 b	28.40 b	26.70 b	27.55 b
Propiconazole	25.60 c	26.20 b	25.90 b	26.25 b	25.22 c	26.00 b	26.48 b	25.18 b
Mefluidide	27.57 c	26.20 b	26.18 b	25.88 b	28.77 b	26.20 b	26.80 b	26.48 b
Trinexapac-ethyl	32.22 b	35.40 a	36.43 a	35.43 a	30.57 ab	35.50 a	36.98 a	36.80 a
Untreated	38.07 a	37.00 a	36.90 a	36.38 a	32.97 a	37.47 a	29.05 b	35.38 a

[†]The fatty acid ratios are (C, number of carbon atoms)/(D, number of double bonds) and are composed of palmitic (16:0) and stearic (18:0) acids. [‡]Means followed by the same letter within each column for each fatty acid are not significantly different based on Fisher's protected LSD ($\alpha = 0.05$).

TABLE 3 Changes in the unsaturated fatty acid contents of crown tissue of annual bluegrass treated in the field with foliar chemical applications and then exposed to 0, 20, 40 and 60 days of ice cover in a low temperature growth chamber (−4°C) in 2014 and 2015. Within each column for each fatty acid, means followed by the same letter are not significantly different ($p \leq 0.05$). Columns with no letters indicate no significant differences among chemical treatments

	2014 Stress treatment period (day)				2015 Stress treatment period (day)			
	0	20	40	60	0	20	40	60
16:1[†]	Molar percentage (mol %)							
Civitas	7.80	6.60 ab	6.28 a	6.08 a	8.08	6.65 a	6.60	6.10
Propiconazole	6.05	4.70 c	6.65 a	4.00 b	5.90	6.30 a	4.98	6.50
Mefluidide	4.78	6.30 ab	4.15 b	6.60 a	4.50	4.70 c	4.78	5.20
Trinexapac-ethyl	5.85	7.20 a	7.05 a	6.33 a	7.10	6.20 ab	5.83	4.93
Untreated	5.93	5.50 bc	4.78 b	4.33 b	5.28	4.97 bc	5.28	4.95
18:1								
Civitas	9.37 a[‡]	6.70	7.08	6.93 a	8.80 a	8.45 a	7.85 a	6.75
Propiconazole	7.55 ab	5.30	6.33	4.40 b	7.52 ab	6.77 ab	5.13 b	6.20
Mefluidide	5.67 b	7.10	5.35	6.60 ab	4.85 c	6.57 bc	5.85 b	4.78
Trinexapac-ethyl	5.72 b	5.80	6.08	5.90 ab	5.72 bc	4.85 c	5.43 b	5.65
Untreated	6.42 b	5.60	5.73	5.73 ab	7.32 ab	6.50 bc	5.90 b	7.08
18:2								
Civitas	13.55 b	13.50 b	12.93 bc	13.53 bc	12.47 b	14.05 b	12.90 b	17.00 b
Propiconazole	20.57 a	21.60 a	19.30 a	17.25 ab	20.07 a	18.60 a	23.53 a	24.03 ab
Mefluidide	22.42 a	19.00 a	22.03 a	22.00 a	19.92 a	20.42 a	24.28 a	21.20 a
Trinexapac-ethyl	8.15 c	9.00 bc	9.33 bc	9.73 cd	8.37 c	8.65 c	8.40 c	8.80 c
Untreated	8.17 c	8.00 c	7.63 c	7.13 d	8.90 c	7.95 c	15.50 b	9.13 c
18:3								
Civitas	9.95	12.90 ab	13.43 ab	13.38 a	8.78	12.35 ab	16.10 a	13.85 a
Propiconazole	10.93	15.30 a	10.33 ab	10.60 ab	10.60	11.25 abc	10.45 b	10.53 ab
Mefluidide	14.58	11.70 ab	15.93 a	10.25 ab	14.43	15.17 a	9.78 b	12.35 bc
Trinexapac-ethyl	13.40	9.30 b	9.65 b	9.18 b	12.83	8.70 bc	9.43 b	6.30 d
Untreated	9.30	8.10 b	8.15 b	7.08 b	8.28	6.87 c	9.63 b	8.65 cd

[†]The fatty acid ratios are (C, number of carbon atoms)/(D, number of double bonds) and are composed of palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids. [‡]Means followed by the same letter within each column for each fatty acid are not significantly different based on Fisher's protected LSD ($\alpha = 0.05$).

(Table 2). For instance, in 2014 after 40 days, Civitas-, mefluidide- and propiconazole-treated ABG had 16.1, 30.5 and 21.5% lower palmitic acid content when compared to the untreated control, respectively (Table 2). Civitas, propiconazole and mefluidide had lower concentrations of the saturated FA stearic acid (18:0) when compared to the untreated controls except after 40 days in 2015; TE-treated ABG had the same stearic acid content as the untreated control except 0 day in 2014 (Table 2). Civitas had increased concentrations of the monounsaturated FA palmitoleic (16:1) at 20, 40 and 60 days in 2014 when compared to the untreated control (Table 3). Oleic acid (18:1) concentration was only increased by Civitas treatments after 0 day in 2014 and 20 and 40 days in 2015 when compared to the untreated control (Table 3). Civitas, propiconazole and mefluidide increased concentrations of the polyunsaturated FA linoleic acid (18:2) in both 2014 and 2015 and at 0, 20, 40 and 60 days compared to the untreated control, except for Civitas after 40 days while TE-treated ABG was never different from the untreated control (Table 3). For instance, Civitas, propiconazole and mefluidide had 39.7, 60.3 and 63.6% greater linoleic acid concentrations, respectively, when compared to the untreated control after 0 day in 2014. The polyunsaturated FA linolenic acid (18:3) was increased by Civitas treatment after 60 days in 2014 and 20, 40 and 60 days of ice cover in 2015. For example, Civitas had 47.1% and 37.5% higher amounts of linolenic acid when compared to the untreated control after 60 days of ice cover in 2014 and 2015, respectively (Table 3).

Double bond index indicates a degree to which chemical treatments altered unsaturated fatty acids within ABG. In both 2014 and 2015, Civitas-, propiconazole- and mefluidide-treated

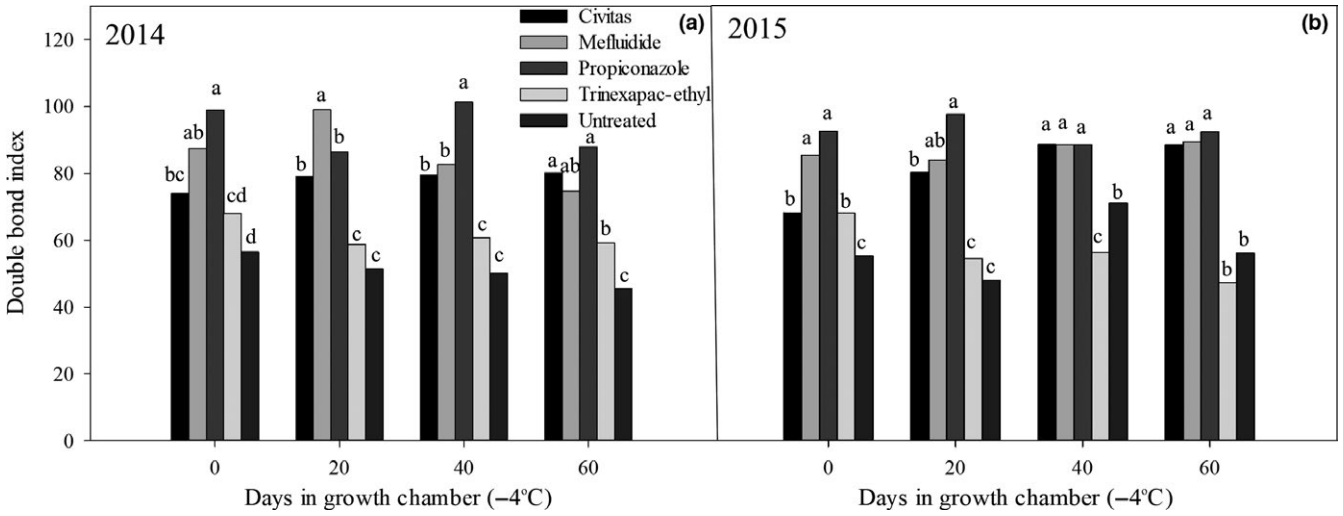

FIGURE 3 Double bond index for fatty acids of annual bluegrass treated with foliar applications of a given chemical treatment after 0, 20, 40 and 60 days in the low temperature growth chamber (−4°C) in (a) 2014 and (b) 2015. Means with the same letter on a given rating day are not significantly different based on least significant difference values ($p \leq 0.05$)

ABG had a greater DBI than the untreated control on all sampling days except 0 days in 2014 (Figure 3). For example, in 2015 after 40 days in the growth chamber, Civitas, propiconazole and mefluidide each had 19.7, 19.6 and 19.6% greater DBI than the untreated control, respectively. The DBIs of TE-treated plants were generally not significantly different than the DBI of untreated control plants.

4 | DISCUSSION

A reduction in ABG regrowth occurred in 20 days at −4°C under either ice cover or non-ice-covered conditions in 2014. While a reduction in ABG regrowth occurred in 40 days under ice cover in 2015. Previous research has found a survival range of 45–90 days for ABG (Beard, 1964; Tompkins et al., 2004; Vargas & Turgeon, 2004). The differences between studies in the number of days under ice that causes damage are likely due to differences in experimental factors, different turf management strategies prior to turf plugs being put into low temperature conditions (management practices have changed significantly in the past 50 years), or since ABG can exhibit a large degree of ecotype variability (Dionne et al., 2010). Compared to 50 years ago, mowing heights are lower, new chemistries are applied, and enhanced cultural practices that can add traffic stress of turf are commonly employed (Beard, Beard, & Beard, 2014). It is important to note that the cold and ice treatments in the low temperature growth chamber may not be representative of field conditions. This growth chamber condition causes freezing of the entire canopy and root zone (−4°C) and would be a more extreme form of stress than in the field. Under field conditions, soil temperatures at crown level (0.5 cm depth) typically remain just below 0°C while soil temperatures at 10 and 20 cm depths are typically warmer than at the crown level during the winter months (Dionne et al., 1999).

Shifts in membrane FA compositions can play a significant role in plant acclimation to low temperatures, particularly in perennial turfgrass species. Un-acclimated cell membranes can become rigid under low temperatures, and increases in unsaturated FAs can help plant membranes retain some degree of fluidity (Iba, 2002). Cell membrane FA composition that is common in most plants includes saturated FAs [palmitic acid (16:0) and stearic acid (18:0)], monounsaturated FAs [palmitoleic acid (16:1) and oleic acid (18:1)], and the polyunsaturated FAs [linoleic acid (18:2) and α-linolenic acid (18:3)] (Millar, Smith, & Kunst, 2000). In perennial ryegrass (*Lolium perenne*), saturated FAs (stearic and palmitic acid) were found to decrease in concentration while polyunsaturated FAs (linoleic and α-linolenic) were found to increase in concentration due to cold acclimation in crown tissue after 21 days of acclimation (Hoffman et al., 2010). ABG crown FAs did not change significantly between each sampling day or between ice cover treatments. This suggests that ABG crown FAs were not altered during ice cover treatment and that any FA changes that occurred happened during chemical treatments during the acclimation period. Thus, future work could more specifically evaluate ABG FA changes during the acclimation or chemical treatment period. Some chemical treatments evaluated in this study did significantly alter FA composition in ABG crown tissue, which could have promoted ice survival and regrowth during recover from ice conditions. Whether any FA changes were a direct or indirect effect of chemical treatment cannot be determined by the results of the study and could be investigated in the future.

Plant growth regulators are used primarily in turfgrass management to reduce above ground growth, thereby reducing mowing requirements, or to inhibit seed head production. Mefluidide inhibits cell elongation and division and is applied to limit ABG flowering and seed head production in golf course putting greens in the spring (Haguewood, Song, Smeda, Moss, & Xiong, 2013; Tautvydas, 1984). The mode of action of mefluidide was only recently determined to

be associated with inhibition of 3-ketoacyl-CoA synthase, which is involved in very long chain fatty acid synthesis (Tresch, Heilmann, Christiansen, Looser, & Grossmann, 2012). Inhibition of long chain FAs could be related to our results regarding mefluidide effects on saturated and unsaturated FA found here; however, more detailed biochemical analysis would be needed to determine this. Mefluidide, a mitosis inhibitor, had similar FA contents to Civitas and propiconazole treatments. Previous research with mefluidide shows that mefluidide-treated maize had increased abscisic acid content when compared to untreated maize prior and during cold (26°C) treatment (Zhang, Li, & Brenner, 1986). Increases in abscisic acid contents have been shown to increase cold hardiness in plants (Li, Ryu, Tseng, & Chen, 1989). Mefluidide treatment of corn seedling leaves increased linoleic acid concentrations and decreased palmitic acid concentrations when compared to untreated corn leaves (Zhang & Chen, 1991).

Gibberellic acid (GA) biosynthesis is inhibited by TE, which reduces cell elongation. It has been shown to reduce grass clipping yield in multiple grass species including Kentucky bluegrass (*Poa pratensis*), perennial ryegrass, creeping bentgrass and ABG (Ervin & Koski, 1998, 2001; Kreuser & Soldat, 2012; Landry & Murphy, 2000). The chemical TE has been shown to decrease tolerance to heat stress in Kentucky bluegrass (Heckman, Horst, Gaussoin, & Young, 2001). However, TE has also been shown to play a role in improving heat tolerance when combined with drought stress in creeping bentgrass (McCann & Huang, 2006). Heckman et al. (2001) concluded that it could have been due to TE being in the same class as cyclohexanedione, which interrupts lipid synthesis. Effects on lipid synthesis are relevant to the FA results found here. In TE-treated plants, palmitoleic acid, an unsaturated fatty acid, was higher on some days in 2014 in crown tissue than in untreated control plants. We did not see any effects of TE treatment of plants on FA profiles compared to untreated plants. Therefore, it is not yet fully clear how TE may alter lipid or FA synthesis and how it may impact abiotic stress tolerance.

TE-treated ABG had the same regrowth as the untreated controls on most days measured; except for after ice cover at 40 and 60 days in 2015, it had significantly less regrowth than the controls. Murata, Sato, Takahashi, and Hamazaki (1982) found that there was no relationship between freeze-sensitive plants and freeze-tolerant plants and their palmitoleic acid content. Thus, it is not yet clear why TE treatment may have reduced regrowth following prolonged low temperature or ice cover conditions. It is possible that TE repression of GA lasted into the spring or that other factors such as carbohydrate or hormonal responses to TE treatment could play a role in this reduction of regrowth. In Kentucky bluegrass, TE application inhibited growth by reducing GA and reducing the amount of labelled carbon in the form of photosynthates in the crown of the plant when compared to plants not treated with TE (Hanson & Branham, 1987). Rossi and Buelow (1997) showed that applying recommended label rates of TE in the fall on fairway height ABG caused increased necrosis when compared to untreated areas the following spring when ratings occurred. However, GA accumulation in plants during acclimation is thought to be negatively associated with cold tolerance (Shan et al., 2007). Future research is needed to determine if carbohydrates or GA may play a role in ABG sensitivity or tolerance to winter conditions. Based on our results, applying TE to ABG in the late summer into fall during acclimation could cause no change or could decrease ABG survival.

The fungicide products used in this study, propiconazole and Civitas, are both thought to have PGR-like effects on turfgrasses (Elliott, 1999). Propiconazole is a demethylation inhibitor fungicide with a triazole chemistry. Literature regarding the effects of fungicide treatments on abiotic stresses is lacking, particularly for those associated with winter conditions. Paclobutrazol, a PGR with a similar chemistry to propiconazole, increased protection to chilling injury of bell pepper fruits (*Capsicum annuum*; Lurie, Ronen, Lipsker, & Aloni, 1994) The researchers were not able to determine the mechanism. For instance, paclobutrazol treatment did not alter phospholipid content of bell pepper fruits when compared to untreated plants (Lurie et al., 1994). Civitas can cause PGR-like effects such as altering carbon assimilation and carbon partitioning in the plant (Kreuser, 2014). Civitas is also known to be an induced systemic resistance (ISR) activator. The ISR pathway is a plant defence response pathway that can be induced by chemicals, pathogens or other abiotic stresses (Cortes-Barco, Hsiang, & Goodwin, 2010; Hsiang, Goodwin, & Cortes, 2011). Despite their differences in chemistry or mechanism of action, Civitas, propiconazole or mefluidide each may be effective treatments to increase ABG survival of winter or ice conditions. However, the mechanism associated with these treatments is not yet clear.

The major FA that was up-regulated by Civitas, mefluidide and propiconazole during acclimation was linolenic acid, which is an 18-carbon omega-3 trienoic polyunsaturated FA found in membrane lipids of plants (Upchurch, 2008). For Civitas, effects on linolenic acid could be through the promotion of ISR, for which the primary up-regulated signalling hormone is jasmonic acid. Linolenic acid is a precursor to jasmonic acid (Farmer & Ryan, 1992); however, whether ISR and jasmonic acid played a role in effecting linolenic during winter stresses cannot be directly concluded from the results of our study. Linolenic acid is known to play a role in cold tolerance in some species. For instance, a cold tolerant variety of seashore paspalum (*Paspalum vaginatum* Sw) was found to have higher concentrations of linolenic acid when compared to cultivars that were not cold tolerant (Cyril et al., 2002). However, past research on wheat varieties reveals that increases in linolenic acid synthesis do not necessarily indicate that tolerance to freezing will occur (de la Roche, 1979). Our research suggests that applications of Civitas, mefluidide or propiconazole may cause an increase in linolenic acid of ABG crown membrane tissue, which could be associated with increased tolerance to low temperature and ice stress.

It is possible that regulation of FAs by Civitas, mefluidide and propiconazole could have improved ice and low temperature tolerance. These three treatments generally caused greater levels of monounsaturated and polyunsaturated accumulation in crown tissues, which may have played a role in maintaining membrane fluidity

during acclimation to maintain membrane integrity during low temperature and ice conditions. Future research could investigate these treatments specifically for membrane integrity maintenance, as this was not directly measured in the current study.

It is important to note that ABG is notorious for being a turfgrass species that is highly plastic and variable in response to spring freeze–thaw cycles. ABG frequently can undergo premature de-acclimation (Espevig, Höglind, & Aamlid, 2014; Hoffman, DaCosta, & Ebdon, 2014). De-acclimation is a change in physiological attributes within the plant that lead to increased plant growth and a decrease in freeze tolerance. Optimally, de-acclimation happens gradually in response to an increase in environmental temperatures during the spring (Arora, Rowland, Odgen, & Dhanaraj, 2004; Kalberer, Wisniewski, & Arora, 2006). Applying the PGR mefluidide in the field to winter wheat varieties delayed the loss of cold hardiness during the winter in Saskatchewan (Gusta, O'Connor, & Reaney, 1990). It is not yet clear if increases in spring regrowth following winter would be a benefit or detriment to ABG if unseasonable winter or spring conditions existed. Evaluating the treatments used in our study under ice in the field and under experimental de-acclimation cycles would help to better understand potential ABG responses to PGR treatment. Additionally, Civitas and demethylation inhibitor fungicides can cause phytotoxicity to some turfgrasses at certain rates and times of year (Elliott, 1995; Kreuser & Rossi, 2014). Therefore, it is important to note that the treatments proposed here could slightly reduce fall turf quality.

5 | CONCLUSIONS

Civitas, mefluidide and propiconazole treatments increased unsaturated FA ratios and per cent regrowth when compared to untreated controls on most days measured. Enhanced crown survival due to the treatments could have played a role in increased regrowth after ice or no ice treatments. A shift in crown tissue FAs from saturated to unsaturated FAs in response to chemical treatments could have played a role in enhanced survival of annual bluegrass plants following simulated winter conditions. It is not clear whether TE treatment may decrease or cause no change in low temperature or ice survival of ABG. Future work should evaluate these treatments under ice in field conditions, determine potential effects on de-acclimation and tested on other turfgrass species that are sensitive to cold or winterkill stresses. Additionally, investigating the direct mechanism of these treatments on enhanced regrowth may be warranted such as whether other physiological factors, like shifts in carbohydrate storage, could have played a role in ABG acclimation. Our results indicate that some of the chemical treatments described here could be feasible treatment methods to enhance ABG survival of winter conditions.

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