Evaluation of Crown Membrane Health and Gas Accumulation in Response to Ice Stress and Management Practices of Creeping Bentgrass and Poa Annua

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Objectives:
1. *Investigate whether crown membrane fatty acid ratios and composition may correlate to toxic gas accumulation and are they differentially accumulated between creeping bentgrass and poa annua under ice cover stress*
2. *Evaluate how lipid profiles and FFA change over a time course of ice cover*
3. *Use a simulated ice cover experiment to determine whether membrane health changes due to incubation of turf with specific ice cover*
4. *Evaluate whether chemical treatments commonly used in the turf industry reduce turf loss due to ice cover, particularly related to membrane disruption or FFA accumulation*

Creeping bentgrass and annual bluegrass are two important putting green species that are sensitive to ice cover damage. Creeping bentgrass is typically more tolerant to ice stress than annual bluegrass. A major cause of damage under prolonged ice cover is the accumulation of toxic gases and damage to grass crown tissue. Several management practices have been reported to improve turf survival of winter, but have not been investigated in controlled studies. This project aimed to determine whether commonly used plant growth regulators (PGRs) and an oil based product, Civitas, have an effect on turf survival of ice cover and is that survival related to membrane or crown health.

Separate creeping bentgrass and annual bluegrass fields were maintained at the Hancock Turfgrass Research Center at Michigan State University. Plots of both species were treated in late summer through fall of 2014 and 2015 every two weeks with: Civitas, mefluidide, propiconazole, or trinexapac ethyl at label recommended rates. Turfgrass plots underwent natural acclimation to cold conditions in fall of 2014 and 2015. Turfgrass plugs were then taken on 11 Nov. 2014 and 25 Nov. 2015 from each plot, planted in 4 inch plastic pots in native soil, and then transferred to an environmentally controlled low temperature growth chamber (-4^{o}C) where they underwent 1) no ice or 2) ice cover (0.5” thick) treatments. Turfgrass plugs were taken out of the low temperature growth chamber at 0, 20, 40, and 60 days after temperature treatments. Plants were then destructively sampled by cutting the plants in half. Half of the plant went to gas chromatography mass spectroscopy (GC/MS) for analysis of free fatty acids while the other half went towards a percent regrowth assay in a greenhouse.

In 2016, ice covered plugs treated with Civitas had the greatest regrowth after 40 days in the low temperature growth chamber. Plugs that were treated with mefluidide, propiconazole, or not treated had the same amount of regrowth after 40 days in the low temperature growth chamber. Trinexapac-ethyl treated plugs had less regrowth than untreated plugs. After 60 days in the growth chamber, civitas and mefluidide treated plugs had greater regrowth than the untreated plugs, while trinexapac-ethyl treated plugs had less regrowth than the untreated control (Figure 2). Similar results were found in 2015 on all sampling dates. Civitas, mefulidide, and propiconazole treated plugs had greater regrowth than trinexapac-ethyl and untreated plugs.

After 60 days in the low temperature growth chamber under non-ice covered conditions, plugs treated with mefluidide or propiconazole had greater regrowth when compared to the untreated plugs. No differences were detected between non-ice covered treatments after the 20 and 40 day sampling times. In 2015, Civitas had greater regrowth than untreated plugs on all sampling days while mefluidide had more regrowth than the control after 20 and 60 days and propiconazole had greater regrowth after 20 and 40 days in the low temperature growth chamber (Figure 3).

For both years, at 20 (Table 1), 40 (Table 2) and 60 days (Table 3) under ice cover, the majority of the fatty acids detected within annual bluegrass were linolenic acid, linoleic acid, oleic acid, stearic acid, palmitoleic acid, and palmitic acid. Plants that were treated with mefluidide, propiconazole, and Civitas have a greater percentage of polyunsaturated fatty acids, with linoleic acid being the most increased due to these chemical treatments, compared with trinexapac-ethyl and untreated samples (Table 1). The unsaturated fatty acid linoleic acid is a precursor to the plant hormone jasmonic acid, a hormone involved in stress responses and the induced systemic resistance pathway. Further evaluation of these profiles is needed and is being performed.

Summary Points

- Annual bluegrass regrowth after simulated ice cover in a growth chamber was significantly affected by plant growth regulator or Civitas treatments.
- Enhanced survival of annual bluegrass after treatment with plant growth regulators or Civitas could be related to shifts in fatty acid accumulation.
- Trinexapac ethyl treated plugs had less survival than the untreated plugs under ice cover on two sampling dates.

Figure 1 – Annual bluegrass plants treated with Civitas, mefluidide, propiconazole, trinexapac-ethyl, or untreated under ice (0.5" thick) in a low temperature growth chamber (-4°C)

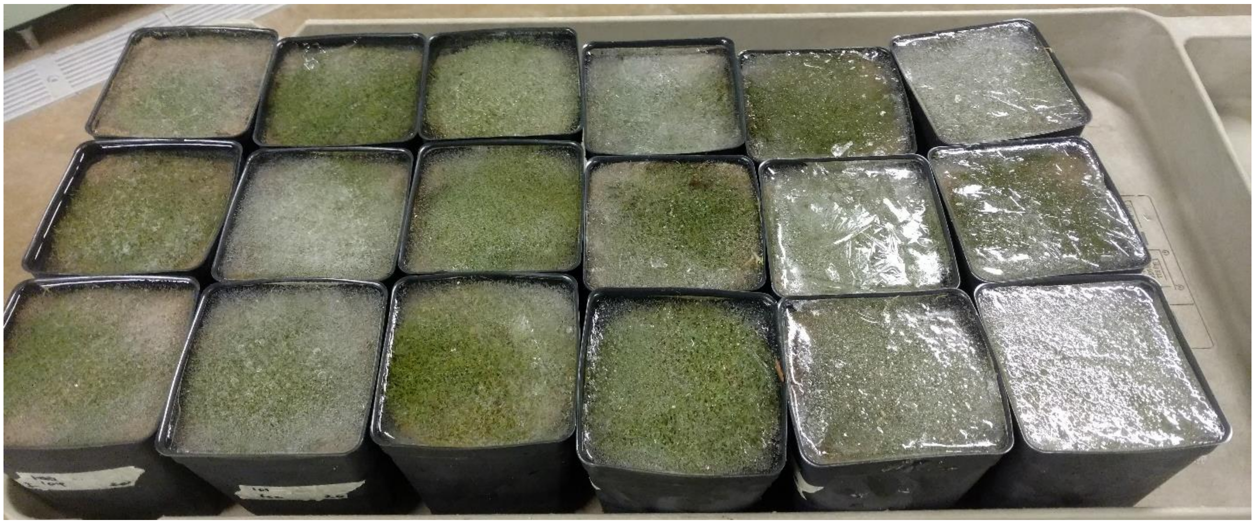

Figure 2 – Regrowth (%) of annual bluegrass plugs maintained under ice cover in 2016 (A) and 2015 (B) a low temperature growth chamber (-4 °C) that were treated with different plant growth regulating compounds. Different letters indicate statistically significant differences within a sampling day ($P \leq 0.05$). NS represents sampling days with no significance ($P \leq 0.05$).

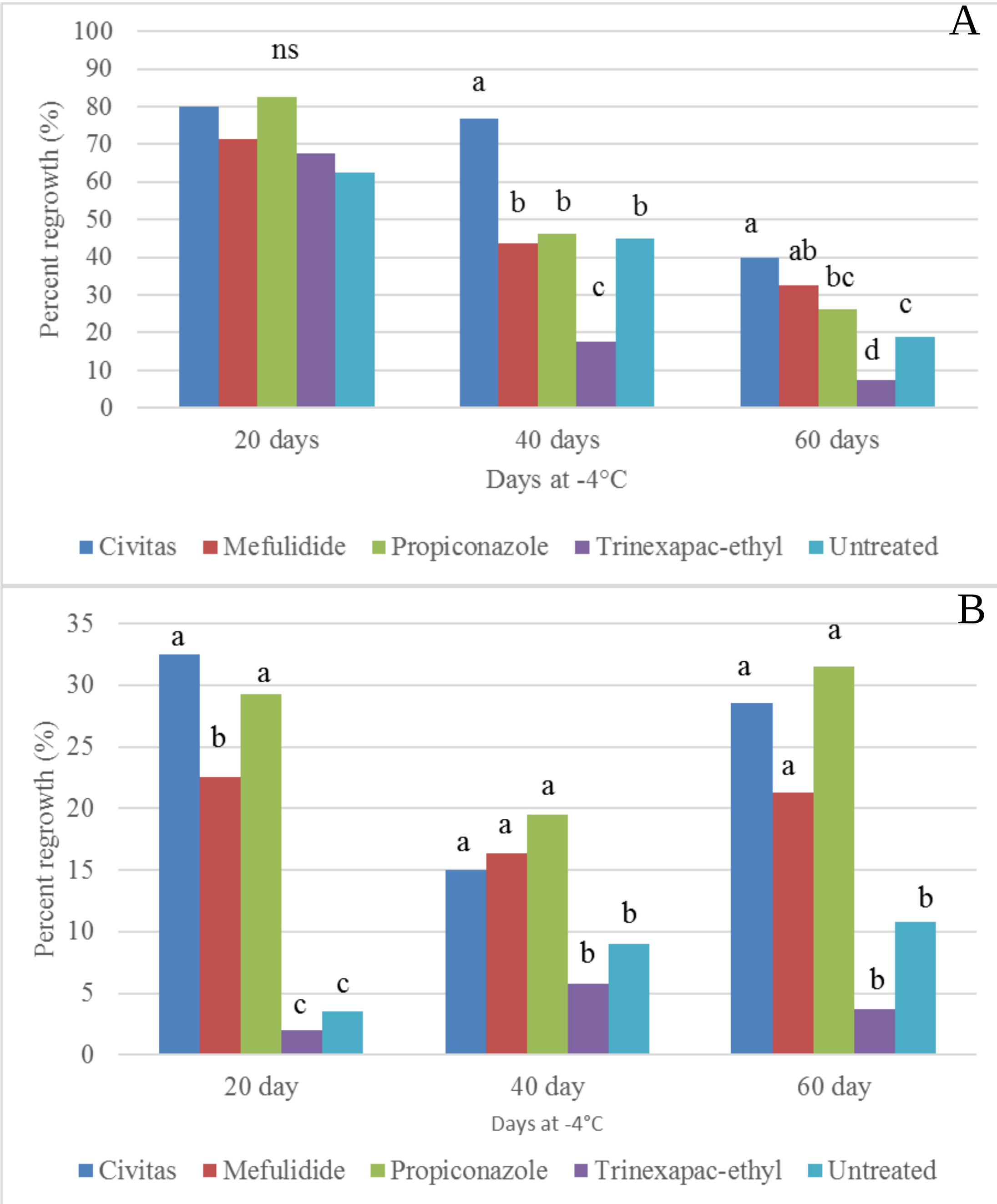

Figure 3. – Regrowth (%) of annual bluegrass plugs maintained with no ice cover in 2016 (A) and 2015 (B) in a low temperature growth chamber (-4 °C) that were treated with different plant growth regulating compounds. Different letters indicate statistically significant differences within a sampling day (P ≤ 0.05). NS represents sampling days with no significance (P≤ 0.05).

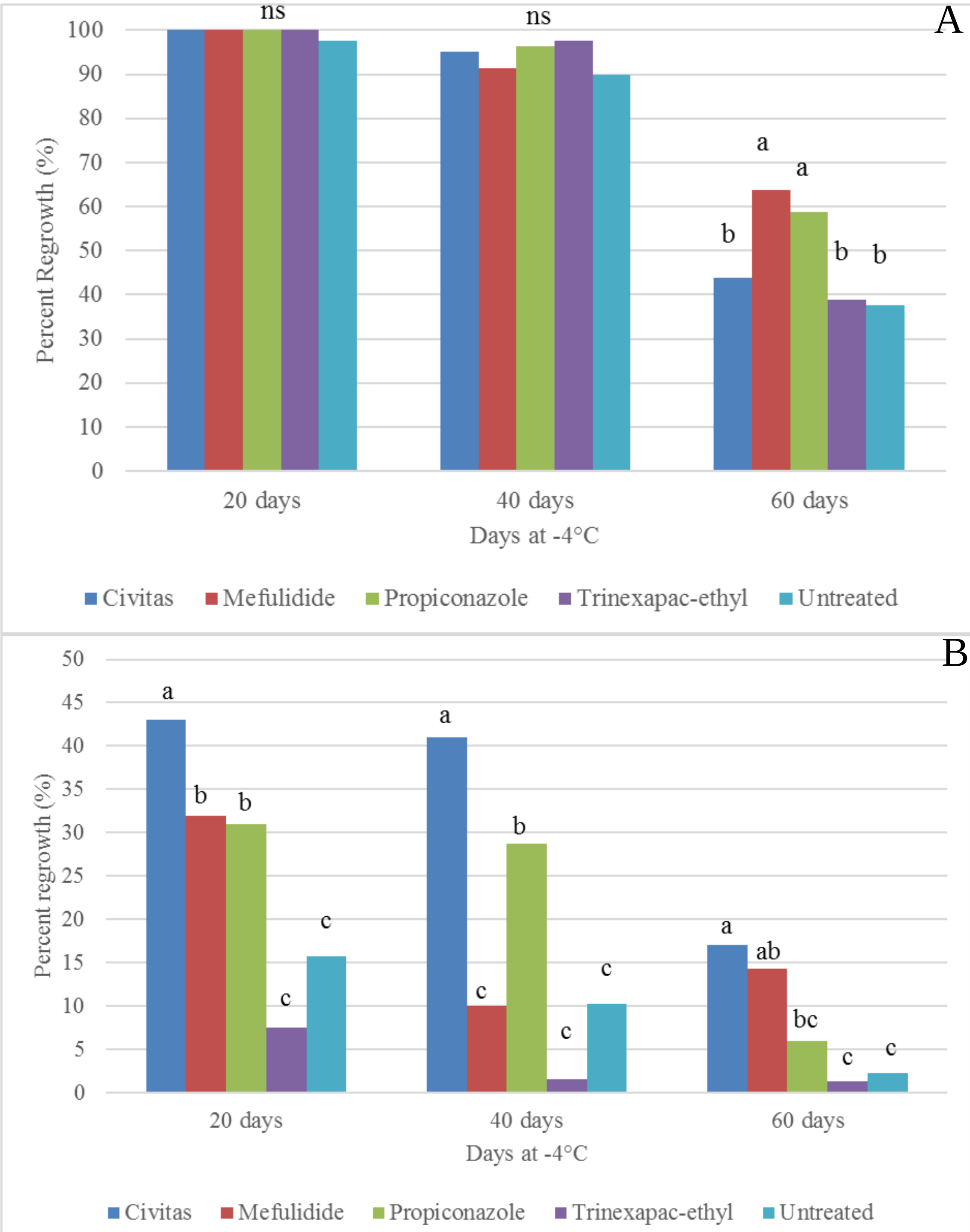

Table 1. List of free fatty acids found in annual bluegrass crown tissue exposed to different chemical treatments after 20 days of ice coverin 2015 and 2016. The fatty acid designation ratios are (C, number of carbon atoms)/(D, number of double bonds). Different letters indicate statistically significant differences within each column ($P \leq 0.05$).

	2015						2016					
	Fatty Acids						Fatty Acids					
	Molar percentage (mol %)						Molar percentage (mol %)					
	saturated		unsaturated				saturated		unsaturated			
	16:0	18:0	16:1	18:1	18:2	18:3	16:0	18:0	16:1	18:1	18:2	18:3
	Palmitic acid	Stearic acid	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid	Palmitic acid	Stearic acid	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid
Civitas	30.5 bc	29.1 b	6.6 ab	6.7a	13.5 b	12.9 ab	29.27 bc	28.4 b	6.65 a	8.45 a	14.05 b	12.35 ab
Propiconazole	26.9 c	26.2 b	4.7 c	5.3a	21.6 a	15.3 a	28.87 bc	26 b	6.3 a	6.77 ab	18.6 a	11.25 abc
Mefluidide	29.3 c	26.2 b	6.3 ab	7.1a	19.0 a	11.7 ab	25.32 c	26.2 b	4.7 c	6.57 bc	20.42 a	15.17 a
Trinexapac-ethyl	33.4 ab	35.4 a	7.2 a	5.8a	9.0 bc	9.3 b	33.25 ab	35.5 a	6.2 ab	4.85 c	8.65 c	8.7 bc
Untreated	35.0 a	37.0 a	5.5 bc	5.6a	8.0 c	8.1 b	34.52 a	37.47 a	4.97 bc	6.5 bc	7.95 c	6.87 c
LSD	3.83	4.73	1.33	2.22	4.83	5.73	4.49	4.01	1.25	1.84	3.44	5.02

Table 2. List of free fatty acids found in annual bluegrass crown tissue exposed to different chemical treatments after 40 days of ice cover in 2015 and 2016. The fatty acid designation ratios are (C, number of carbon atoms)/(D, number of double bonds). Different letters indicate statistically significant differences within each column (P ≤ 0.05).

	2015						**2016**					
	Fatty Acids						**Fatty Acids**					
	Molar percentage (mol %)						**Molar percentage (mol %)**					
	saturated		**unsaturated**				**saturated**		**unsaturated**			
	16:0	**18:0**	**16:1**	**18:1**	**18:2**	**18:3**	**16:0**	**18:0**	**16:1**	**18:1**	**18:2**	**18:3**
	Palmitic acid	**Stearic acid**	**Palmitoleic acid**	**Oleic acid**	**Linoleic acid**	**Linolenic acid**	**Palmitic acid**	**Stearic acid**	**Palmitoleic acid**	**Oleic acid**	**Linoleic acid**	**Linolenic acid**
Civitas	29.25 bc	28.83 b	6.28 a	7.08	12.93 bc	13.43 ab	27.63 b	26.7 b	6.60	7.85 a	12.9 b	16.1 a
Propiconazole	27.38 bc	25.9 b	6.65 a	6.33	19.30 a	10.33 ab	25.68 b	26.48 b	4.98	5.13 b	23.53 a	10.45 b
Mefluidide	24.25 c	26.18 b	4.15 b	5.35	22.03 a	15.93 a	26.05 b	26.8 b	4.78	5.85 b	24.28 a	9.78 b
Trinexapac-ethyl	31.98 ab	36.43 a	7.05 a	6.08	9.33 bc	9.65 b	33.35 a	36.98 a	5.83	5.43 b	8.4 c	9.43 b
Untreated	34.88 a	36.90 a	4.78 b	5.73	7.63 c	8.15 b	33.15 a	29.05 b	5.28	5.9 b	15.5 b	9.63 b
LSD	5.53	4.43	1.14	NS	4.79	6.00	3.35	3.02	NS	1.62	3.35	2.39

Table 3. List of free fatty acids found in annual bluegrass crown tissue exposed to different chemical treatments after 60 days of ice cover in 2015 and 2016. The fatty acid designation ratios are (C, number of carbon atoms)/(D, number of double bonds). Different letters indicate statistically significant differences within each column (P ≤ 0.05).

	2015						**2016**					
	Fatty Acids						**Fatty Acids**					
	Molar percentage (mol %)						**Molar percentage (mol %)**					
	saturated		**unsaturated**				**saturated**		**unsaturated**			
	16:0	**18:0**	**16:1**	**18:1**	**18:2**	**18:3**	**16:0**	**18:0**	**16:1**	**18:1**	**18:2**	**18:3**
	Palmitic acid	**Stearic acid**	**Palmitoleic acid**	**Oleic acid**	**Linoleic acid**	**Linolenic acid**	**Palmitic acid**	**Stearic acid**	**Palmitoleic acid**	**Oleic acid**	**Linoleic acid**	**Linolenic acid**
Civitas	26.95 c	29.03 b	6.08 a	6.93 a	13.53 bc	13.38 a	26.63 b	27.55 b	6.10	6.75	17 b	13.85 a
Propiconazole	26.95 c	26.25 b	4.00 b	4.40 b	17.25 ab	10.60 ab	28.5 b	25.18 b	6.50	6.20	24.03 ab	10.53 ab
Mefluidide	29.58 bc	25.88 b	6.60 a	6.60 ab	22.00 a	10.25 ab	27.75 b	26.48 b	5.20	4.78	21.2 a	12.35 bc
Trinexapac-ethyl	34.18 a	35.43 a	6.33 a	5.90 ab	9.73 cd	9.18 b	35.03 a	36.8 a	4.93	5.65	8.8 c	6.3 d
Untreated	32.63 ab	36.38 a	4.33 b	5.73 ab	7.13 d	7.08 b	34.3 a	35.38 a	4.95	7.08	9.13 c	8.65 cd
LSD	4.22	5.28	1.26	2.25	5.79	3.90	3.49	1.38	NS	NS	4.61	3.18