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ORIGINAL ARTICLE

Limited transpiration under high vapor pressure deficits of creeping bentgrass by application of Daconil-Action®

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Abstract

Main conclusion **First observation that chemical spray can induce limited-transpiration rate under high vapor pressure deficit. It appears that acibenzolar may be key in inducing this water conservation trait.**

Irrigation and water use have become major issues in management of turfgrasses. Plant health products that have been introduced into the turfgrass market have been observed to improve plant performance in water stress conditions. In this study, we evaluated whether a selection of common plant health products alter the ability of creeping bentgrass (*Agrostis stolonifera* L.) to control transpiration under high vapor pressure deficit (VPD). The plant health treatments—Daconil Action®, Insignia®, and Signature®—were applied to plots on golf course putting greens located in Raleigh NC and in Scottsdale, AZ. Using intact cores removed from the putting greens, transpiration rates were measured over a range of VPDs in controlled conditions. In all cases stretching over a 3-year period, bentgrass cores from field plots treated with Daconil-Action limited transpiration under high VPD conditions, while check treatments with water, and others treated with Insignia® or Signature® did not. Transpiration control became engaged when VPDs reached values ranging from 1.39 to 2.50 kPa, and was not strongly influenced by the field temperature at which the bentgrass was growing. Because all plots in NC had been treated with chlorothalonil—the key ingredient in Daconil Action to control diseases—it was concluded that the likely chemical ingredient in Daconil Action triggering the transpiration control response was acibenzolar. This is the first evidence that the limited-transpiration trait can be induced by a chemical application, and it implies significant potential for ameliorating drought vulnerability in cool-season turfgrasses, and likely other plant species.

Keywords Daconil action · Creeping bentgrass · Transpiration rate · Vapor pressure deficit · Water conservation

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Introduction

Water shortages are becoming prevalent in all plant production systems. With increases in population, availability of water for irrigation of landscapes, and turfgrasses in particular, is increasingly under pressure, dictating a need for water conservation strategies (Beard and Kenna 2008; GCSAA 2009; Cathey et al. 2011).

The driving gradient of water loss from plants is the vapor pressure difference between leaves and the surrounding atmosphere, referred to as vapor pressure deficit (VPD; Kramer 1983). High atmospheric VPD most commonly occurs at high temperatures, when saturated vapor pressure of the atmosphere is elevated. Transpiration rates can be modulated by decreasing stomatal conductance. Indeed, an effective physiological strategy to avoid drought stress would be for plants to limit stomatal conductance and transpiration only under high atmospheric VPD. This allows CO_2 exchange and photosynthesis to proceed unimpeded until elevated water loss occurs under high VPD. Such a limiting transpiration mechanism at high VPD has been found to be a key genetic component of drought tolerance with several crop species, including

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soybean (*Glycine max* (L.) Merr.) (Sadok and Sinclair 2009; Seversike et al. 2013), maize (*Zea mays* L.) (Yang et al. 2012; Gholipoor et al. 2013), and sorghum (*Sorghum bicolor* L.) (Shekoofa et al. 2014). In these cases, transpiration rate increases linearly with increasing VPD until a breakpoint VPD is reached above which there is little or no further increase in transpiration rate with increasing VPD.

Recent studies with tall fescue (*Festuca arundinacea* Schreb.) have revealed the presence of the limited-transpiration trait under high VPD similar to those with the crop species (Sermons et al. 2012). In experiments where VPD was increased step-wise at an optimal temperature of 21 °C, expression of a limited-transpiration response occurred at 1.45 kPa and little additional transpiration rate increase occurred at higher VPD, indicating stomatal conductance control. After a shift to a temperature of 29 °C, well above fescue's optimal growth range, transpiration control was lost. Although some transpiration control was present after an extended acclimation period (7 days), the degree of recovery was limited. A similar heat sensitivity of the transpiration control mechanism is being addressed in plant breeding programs developing drought-tolerant crop species (Yang et al. 2012; Seversike et al. 2013).

In addition to genetic sources of transpiration regulation under high VPD, another option might be to have a chemically induced transpiration regulation by spraying foliage. Such an option would be useful for use with plant species in which a genetic resource for the trait has not be identified, or it is not expressed at high temperatures such as in tall fescue. In addition, a chemical spray may allow more controlled management of water such that the chemical is applied only when more controlled water use is required. Otherwise, stomata can be allowed to remain fully open to maximize growth. While there is currently no direct evidence that an external chemical spray can induce the limited-transpiration response, numerous 'plant health' chemical treatments appear to protect turfgrasses from severe drought stress in high temperature summer months, which is when high VPD conditions commonly occur. One possibility is that the physiological benefits of the plant health treatments include transpiration control and avoidance of drought-stress damage.

The objective of the experiments described in this manuscript was to evaluate whether a select group of 'plant health' chemicals led to or enhanced transpiration control at high VPD. The approach involved treatment applications to plots of creeping bentgrass (*Agrostis stolonifera* L.) on golf course putting greens, periodic removal of intact plant/soil cores, and then moving the cores into a controlled environment for analyses of their VPD response. The chemical groups were selected from those commonly proposed to have plant heath advantages—Daconil-Action® (Syngenta Corp.), Insignia® (BASF Corp.), and Signature® (Bayer Corp.). The treatment applications in the field were done at two locations and the samplings of cores were done over a range of field temperatures. Measurement of VPD responses was conducted at a relatively high temperature for this 'cool-season' grass species to determine whether effects were expressed under high temperature conditions.

Materials and methods

Treatment plots were established on plots of creeping bentgrass putting greens at the N.C. State University Turfgrass Research Laboratory in Raleigh, NC, and also at Desert Mountain Golf Club in Scottsdale, AZ. The greens were built to USGA specifications, with soils 85–90 % sand and 10–15 % peat. Four spray treatments were applied at both locations: (1) a water-only check; (2) Daconil Action (Chlorothalonil, Acibenzolar-*S*-methyl); (3) Insignia (Pyraclostrobin); and (4) Signature (Fosetyl-Al). At the NC site, Primo Maxx® (Trinexapac-ethyl) was included with all treatments; Appear® was included in the Daconil Action treatment, and Daconil Ultrex (Chlorothalonil) and Subdue (Mefenoxam) were applied every other week to suppress fungal diseases. None of the additions were included at AZ. Details regarding each of the chemicals and application amounts are given in Table 1. The spray volume for all treatments was 81 mL m^{-2}. Plots were maintained free of water-deficit stress and weeds using best management practices, and all plots were judged to be of excellent quality.

The experimental design was four 0.9 m × 1.8 m plots for each chemical treatment. The chemical treatments were applied at two-week intervals using a backpack sprayer system at a pressure of 40 psi. Applications were done in the morning before 09:00 am (EST) on various dates in 2012 and 2013 in NC and 2013 and 2014 in AZ (refer to Tables 2, 3). Treatments were repeatedly applied to the same plots during each year, and the sampling of turf cores began only after two treatments had been applied to the plots. Cores (10-cm diameter and approximately 12-cm deep) were collected using a putting green cup cutter on the morning following chemical spray applications with no intervening irrigation of the plots. At NC, one core was harvested from three replicate plots of each of the four treatments. The cores were immediately placed into specially constructed 10-cm diameter polyvinyl chloride pots. The pots were transported to the North Carolina State University Phytotron and put into a walk-in growth chamber with light provided at 600 µm m^{-2} h^{-1} during a 12-h light period and day/night temperatures controlled at 32/26 °C.

Table 1 Chemicals applied to creeping bentgrass in field experiments

Product	Application	Active ingredient	% Active ingredient	Intended use	Plant health properties[a]
Daconil Action	1.27 μL m^{-2}	Chlorothalonil	53.9	Fungicide/plant health	Stimulates production of pathogenesis-related proteins and activates systemic acquired resistance and reduces environmental stresses
Appear	2.18 μL m^{-2}	Potassium phosphite	53.3	Plant health	Improved disease control (like Daconil action, this enhances SAR), even in extreme temperatures, and improves turf quality and appearance (with the pigment)
Insignia SC	0.25 μL m^{-2}	Pyraclostrobin	23.3	Fungicide/plant health	Controls diseases, helps with stress management including: drought/moisture, mechanical, and temperature extremes
Signature 80 WDG	1.22 g m^{-2}	Fosetyl-al	80.0	Fungicide/plant health	Directly controls diseases, induces resistance, manages radiation and improves carbohydrate accumulation
Primo Maxx	0.05 μL m^{-2}	Trinexapac-ethyl	11.3	Growth regulator	(Not a "plant health" product)—reduces gibberellin production resulting in less leaf extension, denser turf canopy, and improved green color
Daconil Ultrex	1.07 g m^{-2}	Chlorothalonil	82.5	Fungicide	(Not a "plant health" product) fungicide: activates systemic acquired resistance (as with Daconil Action)
Subdue Maxx	0.36 μL m^{-2}	Mefenoxam	22.0	Fungicide	(Not a "plant health" product)—used for pythium control

[a] Properties based on claims made by marketing company

Core sampling from the plots in AZ was the same as in NC except once the cores were harvested they were each placed in heavy plastic sleeves slightly larger than the cores. The sleeves maintained the integrity of the cores and prevented water loss. The cores were tightly packed in a luggage container and on the same day taken as carry-on luggage on a commercial airliner to NC. Once the cores arrived at the Phytotron at NCSU, the cores were installed in the 10-cm polyvinyl chloride pots used in the transpiration measurements.

Once in the growth chamber, all cores were watered and allowed to acclimate for 1 day. Then, a 6-L clear chamber was attached to the top of each pot so that VPD could be controlled and transpiration rate measured. The protocol for measuring the transpiration response to VPD was similar to that described by Fletcher et al. (2007). Transpiration responses were measured on two consecutive days using step-wise increases in VPD, starting from low (0.5–1.5 kPa) then to medium (1.5–2.5 kPa), and next a high VPD range (2.5–3.5 kPa). At each step, the VPD in the chambers was allowed to stabilize for 30 min and then water loss was measured gravimetrically over a 1-h period. Transpiration rate for each pot was expressed as g m^{-2} min^{-1}. The cores were watered after the first day of measurement to ensure well-watered conditions. Environmental sensors were used to measure temperature and humidity in each chamber, allowing calculation and monitoring of VPD.

Statistical analysis

Transpiration rate data from the 2 days of measurement for a set of cores were combined and initially subjected to a two-segment linear regression analysis (Prism 6.0, GraphPad, Software Inc.). The regression generated slopes for each of the two segments and the VPD at which the two segments intersect. The intersection of the two linear segments is defined as the breakpoint in the transpiration response to increasing VPD. If the slopes of the two linear segments were not significantly different ($p < 0.05$), then all data for that treatment were re-analyzed using a single linear regression. This approach allowed clear differentiation between the expression of the limited-transpiration trait with a VPD breakpoint, or the lack of expression of the trait.

Results

Bentgrass core samples treated with the plant health chemicals were collected for measurement of transpiration rate response to VPD on seven dates in NC and four dates in AZ (Tables 2, 3). A clear segregation was observed in all cases between the two-segment transpiration response to increasing VPD obtained from the Daconil-Action spray and the linear response of the other treatments, as

Table 2 Results from single- and two-segment linear regressions of transpiration responses of creeping Bentgrass cores exposed to step-wise increases in VPD

Date	Temp. (°C)	Treatment	TR VPD breakpoint ± SE	Slope 1 ± SE	Slope 2 ± SE	R^2
26 Sep, 2012	27	Water	–	4.99 ± 0.59	–	0.81
		Daconil Action	2.50 ± 0.14	8.00 ± 1.51	1.08 ± 0.57	0.94
		Insignia	–	4.10 ± 0.53	–	0.78
		Signature	–	3.99 ± 0.66	–	0.69
2 Oct, 2012	30	Water	–	5.58 ± 0.53	–	0.87
		Daconil Action	2.44 ± 0.25	6.54 ± 1.42	0.66 ± 1.49	0.80
		Insignia	–	4.71 ± 0.53	–	0.82
		Signature	–	5.14 ± 0.55	–	0.84
10 Oct, 2012	18	Water	–	9.38 ± 0.81		0.88
		Daconil Action	2.10 ± 0.29	12.23 ± 4.84	0.85 ± 1.36	0.89
		Insignia	–	4.99 ± 0.57	–	0.83
		Signature	–	4.46 ± 0.49	–	0.83
5 June, 2013	26	Water	–	4.69 ± 0.40	–	0.88
		Daconil Action	1.50 ± 0.29	8.05 ± 1.03	2.36 ± 1.15	0.91
		Insignia	–	3.84 ± 0.55	–	0.78
		Signature	–	3.50 ± 0.57	–	0.68
9 June, 2013	30	Water	–	4.46 ± 0.72	–	0.69
		Daconil Action	1.39 ± 0.27	10.53 ± 3.04	2.97 ± 1.15	0.90
		Insignia	–	5.80 ± 0.49	–	0.90
		Signature	–	3.38 ± 0.40	–	0.81
1 July, 2013	26	Water	–	3.57 ± 0.21	–	0.93
		Daconil Action	1.90 ± 0.79	5.05 ± 3.69	1.21 ± 1.00	0.75
		Insignia	–	3.65 ± 0.42	–	0.82
		Signature	–	3.21 ± 0.36	–	0.82
29 July, 2013	27	Water	–	3.46 ± 0.49	–	0.75
		Daconil Action	1.82 ± 0.51	5.56 ± 1.72	2.57 ± 0.81	0.86
		Insignia	–	3.21 ± 0.13	–	0.97
		Signature	–	3.31 ± 0.42	–	0.78

The cores were taken from replicated plots treated with various plant health chemicals on putting greens at NC State University in 2012 and 2013. Values for the regression slopes are expressed as g m^{-2} min^{-1} kPa^{-1}. All of the data are means of analyses with three cores, and all transpiration responses to VPD were measured at 32 °C. Dashes for breakpoint and slope 2 reflect the fact that there was a single linear response and no breakpoint was detected. Dates and temperatures are the time of core removal

illustrated in Fig. 1. In all tests, there was a clear two-segment response in the results from the Daconil-Action treatment while the data for the water spray and the other two chemical treatments had a linear response over the entire range of tested VPD (Tables 2, 3).

The VPD breakpoint value observed as a result of the Daconil-Action spray of the bentgrass plots varied among tests. In NC, the VPD breakpoint ranged from 1.39 kPa to a high of 2.50 kPa (Table 2). In AZ, the VPD breakpoint for Daconil-Action ranged from 1.80 to 2.50 kPa. The values of the individual VPD breakpoints were not found to correspond to the time of year or temperature conditions when the treatments were applied and samples collected.

The initial slope of the two-segment response exhibited by the Daconil-Action treatment was somewhat greater than the slope of the single linear regression of the other treatments. On average, the increase in slope was 1.5 times greater than the water spray treatment. Above the breakpoint VPD, the regression slope was substantially decreased below the initial slope and the slope of the other treatments. This indicates a substantial conservation of water by creeping bentgrass under high VPD following Daconil-Action treatment.

Discussion

The current research explored the possibility that a selected group of 'plant health' chemicals might trigger the ability of creeping bentgrass to limit transpiration rate at high VPD. The results from two very different geographic locations with different climates consistently

Table 3 Results from single- and two-segment linear regressions of transpiration responses of creeping bentgrass cores exposed to step-wise increases in VPD

Date	Temp. (°C)	Treatment	TR VPD breakpoint ± SE	Slope 1 ± SE	Slope 2 ± SE	R^2
10 Aug, 2013	34	Water	–	3.08 ± 0.45	–	0.75
	34	Daconil Action	2.30 ± 0.47	4.65 ± 0.45	2.42 ± 0.91	0.96
16 Sept, 2013	37	Water	–	3.80 ± 0.34	–	0.88
	37	Daconil Action	2.50 ± 1.85	4.56 ± 0.81	2.76 ± 2.93	0.88
26 July, 2014	35	Water	–	2.34 ± 0.37	–	0.83
9 Aug, 2014	33	Daconil Action	2.38 ± 0.60	3.35 ± 1.14	0.81 ± 0.88	0.90
31 Aug, 2014	36	Daconil Action	1.80 ± 0.39	4.09 ± 1.12	1.02 ± 0.53	0.96
27 Sept, 2014	26	Water	–	3.17 ± 0.66	–	0.74

The cores were taken from replicated plots treated with various plant health chemicals on putting greens at Desert Mountain, Scottsdale, AZ in 2013 and 2014. Values for the regression slopes are expressed as g m^{-2} min^{-1} kPa^{-1}. All of the data are means of analyses with four cores, and all transpiration responses to VPD were measured at 32 °C. Dashes for breakpoint and slope 2 reflect the fact that there was a single linear response and no breakpoint was detected. Dates and temperatures are the time of core removal

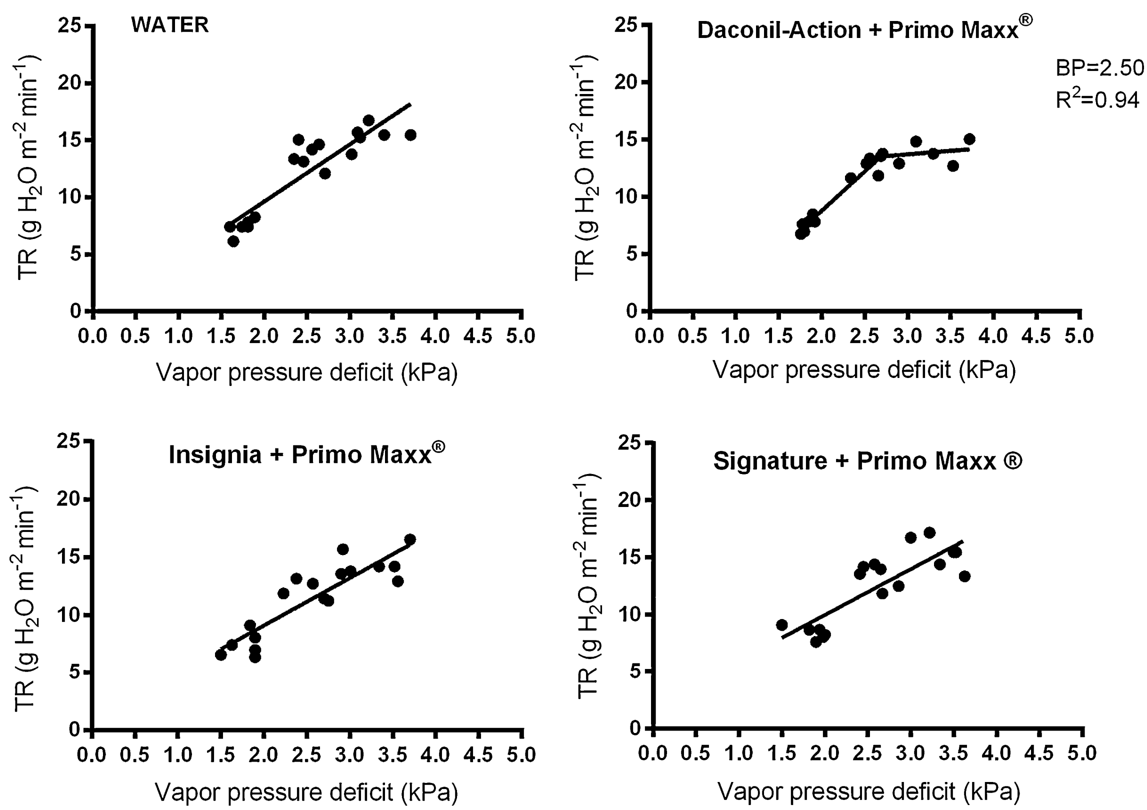

Fig. 1 Plots of transpiration rate versus atmospheric vapor pressure deficit for cores collected at Raleigh, NC, on 26 September 2012 from creeping bentgrass plots sprayed with three chemical treatments and water

showed that foliar spraying with Daconil-Action triggered the limited-transpiration response at high VPD, in contrast to the other treatments. The limited-transpiration response became engaged when VPD increased to the range of 1.4–2.5 kPa. This type of response to high VPD has now been identified as a key genetic trait in the development of important drought-tolerant field crops, soybean, corn, and sorghum (Sadok and Sinclair 2009; Yang et al. 2012; Gholipoor et al. 2013; Seversike et al. 2013; Shekoofa et al. 2014).

From the two chemical components of Daconil-Action, it seems evident that acibenzolar was responsible for activation of the transpiration-limiting response. This conclusion is based on the fact that the other active ingredient in Daconil-Action is chlorothalonil, and chlorothalonil is also a component of the other two fungicide treatments that did not induce the limited-transpiration response. In addition, there was no indication that the pigment Appear was a controlling factor. It was absent from the Daconil-Action treatment in AZ, and Appear is structurally similar to the pigment in Signature, which did not trigger the limited-transpiration response to high VPD. We are aware of observations that pigment covers leaf tissues and the suggestion that it could limit gas exchange through stomata (McCarty et al. 2013). If that had occurred in our VPD analyses, however, it would most likely lead to uniform lowering of a linear transpiration slope as VPD increased. However, that did not happen in the Signature treatment, nor was there any indication of a VPD breakpoint.

These experiments have generated the first evidence that the limited-transpiration control at high VPD can be chemically induced. The importance cannot be over-stated. It opens the potential for moderating water-deficit stress in bentgrass through a management approach. Water-deficit stress at high temperature is typically the main abiotic stress problem for bentgrass management. The impact on water conservation of the limited-transpiration response at high VPD would be important in many areas where bentgrass is grown. The benefit on water savings would be especially great in AZ. Our preliminary analyses of environmental conditions in AZ indicate that VPD exceeds 2.5 kPa by 09:00 am throughout much of the daylight hours from late May until early October. The maximum VPD can reach values from 4.0 to 5.0 kPa. Even in the humid Southeast, VPD can exceed the 1.5–2.5 breakpoint range in a typical afternoon throughout the summer which coincides with the highest probability of water-deficit stress and wilting. For example, in Raleigh in 2013, which was a relatively rainy year, the VPD exceeded the average measured 1.65 kPa breakpoint in the afternoons of 70 out of 122 days from May until the end of August (Lake Wheeler Rd Field Lab, climate.ncsu.edu/cronos). Consequently, spraying bentgrass with Daconil-Action would be expected to decrease afternoon water loss and the likelihood of wilting. In drier years, one might expect that water use would decline and extend the intervals between irrigation applications.

Functionally, a critical aspect of a limited-transpiration response is that it is present in high temperature ranges when VPD is highest. Finding high temperature stability in the response was one of the more challenging hurdles in the development of drought-tolerant soybean (Carter and Rufty 1993). While the basis for the variation in the VPD breakpoints with the Daconil-Action treatments is not known, there was no obvious destabilization of the response at high temperature as the breakpoint was always present. Furthermore, statistical analyses failed to find a clear relationship with field temperatures, and all transpiration measurements were made at 32 °C, a temperature much higher than the optimal growth range for this cool-season turfgrass. It is conceivable that breakpoints were being influenced by other components in the management programs (e.g., nutrition) or, perhaps, plant acclimation to other environmental signals (Sermons et al. 2012). We also cannot know how long the transpiration control persists after Daconil-Action is applied. In this study, cores were collected and VPD measurements made within a day after applications; so it is unclear how much longer the effects would persist. Current management strategies in the Southeast generally involve Daconil-Action applications at 14-day intervals during summer months.

The increase in transpiration rate with Daconil-Action in the low VPD range was surprising. At low VPD, the slope of the transpiration rate increase with VPD averaged 1.5 times greater than for the water spray. In the natural environment, the effect of this greater transpiration rate would be experienced most often in mornings when VPD is still low, and absolute transpiration rate also is low. Therefore, the potential impact of this increase in transpiration would be relatively small compared to total daily water loss. On the other hand, increased morning transpiration rates might allow increased photosynthetic activity as a result of increased stomatal conductance (Kramer 1983).

Our results should not be interpreted to mean that Insignia and Signature do not have a beneficial impact during drought stress. Our experiments only tested changes in transpiration with varying VPD. Drought stress can involve a number of negative physiological responses, which end up impacting turfgrass health and quality. The often observed positive impacts of Insignia and Signature during stresses like drought, for example, could reflect positive influences on physiological stability that minimize quality decline (Huang and Xiaozhong 2009).

Author contribution statement A. Shekoofa performed the transpiration measurements and did the initial analysis of results. D. Carley oversaw the field experiments. All authors contributed to examining the results and to preparing the manuscript.

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