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Assessment of Drought Stress on Physiology Growth of *Agrostis palustris* Huds. as Affected by Plant Bioregulators and Nutrients

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Abstract: This study examines the effect of two materials with Plant Growth Regulator (PGR) properties that might affect drought stress tolerance of creeping bentgrass (*Agrostis palustris* Huds.). The materials were applied with or without Fe 3 weeks before creeping bentgrass was transplanted and subsequently subjected to drought and recovery. Low and high N fertility (LNF and HNF) were also variables in this study. Creeping bentgrass grown on a Groseclose silt loam received 25 (LNF) or 50 (HNF) kg ha^{-1} of N from urea. Ten days after fertilization, plots were treated with either a Fortified Seaweed (*Ascophyllum nodosum*) Extract (FSE), at 9.3 L ha^{-1} or propiconazole (PPC), at 0.93 L active ingredient (a.i) ha^{-1}. Each PGR was applied alone or in conjunction, with chelated Fe at 1.1 kg Fe ha^{-1}. Two weeks after the application of PGRs treatments, plugs were transplanted into a container and after 2 additional weeks, were placed under a rain shelter. After 3 weeks of withholding water, the plants were irrigated to field capacity three times per week. Foliar application of FSE or PPC either alone or in combination with Fe caused an increased tolerance to drought regardless of N fertility levels. Cumulative Evapotranspiration (CET) of treated plants was greater under the HNF, resulting in wilt after 3 weeks of withholding water. After 2 weeks of withholding water at the both N fertility levels and 3 weeks at the LNF, FSE and Fe, resulted in significantly less wilting than in the control plants. Foliage recovery of treated plants following a drought was significantly greater at the LNF than HNF. Both PGRs with or without Fe, significantly improved root growth regardless of N fertility. When measured at the termination of the study.

Key words: Cumulative evapotranspiration, propiconazole, low nitrogen fertility, high nitrogen fertility, fortified seaweed (*Ascophyllum nodosum*) extract, plant growth regulator, green leaf density, leaf water status

INTRODUCTION

Drought is a major limiting factor of crop development in many parts of the world and it suppresses growth and causes a loss of crop quality (White, 1996; Carrow and Duncan, 2003; Zhang and Ervin, 2004). Drought typically results in substantial water deficits in the soil and consequently in plant tissues. If drought persists, significant injuries to plants result (Ribaut and Pilet, 1991; White *et al*., 1993; Richter, 1997; DaCosta *et al*., 2004). Water deficits accompanying rapid wilting in the above ground parts of plant cause a change in the protoplasm of leaf tissues and impair permeability of the cell membrane to water (Chaves, 1991; Pattanagul and Madore, 1999). Low soil water potential can limit the allocation of carbon resources to the root system and reduce intermediary metabolism, water absorption and mineral uptake (Keller and Ludlow, 1993; Passioura, 1994; Volaire and Thomas, 1995; Richter, 1997; Wanek and Richter, 1997; Schmidhalter *et al*., 1998; Pattanagul and Madore, 1999).

Application of heavy rates of N to plants grown in dry soil have a negative impact on plant water use efficiency and dry matter distribution (Heitholt, 1990). Reduction of root growth, increase of certain diseases and high water use have been associated with excessive use of N fertilizer (Yust *et al*., 1984; Heitholt, 1990).

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Growth quality and yield of grass species have been improved under stressful environments by supplying mineral nutrients and Plant Growth Regulators (PGR) (Schmidt and Synder, 1984; Crouch *et al*., 1990; Nabati *et al*., 1991; Glinski *et al*., 1992; Zhang and Schmidt, 2000; Zhang *et al*., 2002, 2003a, b; Zhang and Ervin, 2004; Nabati *et al*., 2008). Compounds with PGR activity not only can increase growth but also can protect plants against various environmental stresses (Nabati *et al*., 1994; Zhang and Schmidt, 2000; Zhang *et al*., 2002, 2003a, b; Zhang and Ervinl, 2004; Nabati *et al*., 2008). Research indicated that plants receiving cytokinin-like materials appeared to survive longer than untreated plants during drought stress (Davies *et al*., 1988; Adami *et al*., 1998; Ervin *et al*., 2003; Nabati *et al*., 2008). Plant morphological and chemical changes caused by environmental stresses include decreased root growth, epicuticular wax, chloroplast and chlorophyll content. Such changes may be mediated by an enhanced activity of free-radical systems (Fletcher and Hofstra, 1988; Nilsen and Orcutt, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997; Pinhero and Fletcher, 1994; Jiang and Huang, 2001; DaCosta *et al*., 2004).

Propicanozole, a triazole compound with both fungicidal and PGR properties, is capable of protecting plants under harsh environments, including low and high temperature and water deficit (Fletcher and Hofstra, 1988; Nabati *et al*., 1991, 1994, 2008; Zhang and Schmidt, 2000; Zhang *et al*., 2003a). The mode by which traizoles protects plants from stresses is thought to be associated with phytohormonal modifications, such as increases in cytokinins.

Emerson *et al*. (1986) noted that winter wheat (*Triticum aestivum* L.) treated with the PGRs ethephon, mefluidide and cycocel produced higher yield under a low rate of N as compared to a higher rate of N. Cytokinins added to growth medium without additional N enhanced birch (*Acer pseudoplatanus* L.) seedling growth (Horgan and Wareing, 1990).

The objectives of this study were to evaluate responses of creeping bentgrass under drought stress and two fertility regimes when treated with PGR and/or Fe and to evaluate the effects of these treatments on recovery from drought stress of creeping bentgrass.

MATERIALS AND METHODS

The study was conducted at the Virginia Tech Turfgrass Research Center, Blacksburg, Virginia, USA. Field plots of creeping bentgrass (*Agrostis palustris* Huds.) were established on a Groseclose silt loam (clayey, Kaolinitic, mesic typic Hapludult) with a pH 6.2. Soil P and K were 21 and 37 μg g^{-1}, respectively. The plots received 25 or 50 kg N ha^{-1} of as urea for low- or high- N regimes, respectively. Nitrogen and subsequent PGR treatments were applied to 0.9×1.8 m plots arranged in a randomized complete block design with four replications. Following ANOVA analysis means of treatment effects were separated according to Duncan's multiple rang test.

Ten days after N fertilization, plots were treated with a Fortified Seaweed (*Ascophyllum nodosum*) Extract (FSE) at 9.3 L ha^{-1} or propiconazole (PPC) at 0.93 L active ingredient (a.i) ha^{-1}. Each PGR was applied alone or in conjunction with Fe (Na-diethylene-triamine-pentaacetate chelated) at 1.1 kg (a.i) ha^{-1}. The FSE was an extract of seaweed (3%) and peat humus (7%) fortified with ascorbic acid, α-tocopherol, glycine and thiamine (FSE) supplied by Roots Inc. New Haven, CT. The PPC [1-{2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2yl}methyl-1 H-1,2,4-triazole] was supplied (as "Banner") by Novartis, Greensboro, NC and is a triazole fungicide with known PGR properties. All chemicals were applied with a compressed-air sprayer that delivered 784 L ha^{-1} of solution at a pressure of 290 kPa.

Two weeks after PGR treatments, 14×2 cm diameter deep plugs were taken from each field plot and transplanted into rings made from 14 cm diameter plastic PVC pipe cut to a 5 cm depth. Two steel wires with 3 mm diameter were inserted at right angles through holes drilled 6.2 mm from the rings. Hardware cloth with 6 mm mesh, had been cut to fit the inside of the ring and rested on the steel wires to allow the roots grow through uniformly, (Schmidt and Synder, 1986). The transplanted plugs were permitted to grow in the rings for additional 2 weeks with daily watering and then the rings were placed on surface of the same Groseclose soil in drained 15×15 m metal containers. Approximate field capacity of each container has been estimated by thoroughly watering the container, allowing it to drain for 24 h and then weighing.

Two weeks after transplanting into the metal containers (during which time the plants were watered every other day or as needed), the containers were placed under a clear plastic rain shelter for 3 weeks during which time water was withheld and Cumulative Evapotranspiration (CET), wilting, Leaf Water Status (LWS) and Foliage Dry Weight (FDW) were monitored. After 3 weeks of withholding water, the plants were removed from the rain shelter and irrigated to field capacity three times per week. Green-Leaf Density (GLD), leaf color, LWS, FDW and Root-Pull Strength (RPS) were measured over 3 weeks of post-drought watering.

Measurements during drought: Cumulative evapotranspiration was determined after 1 and 3 weeks of withholding water by weighing the metal containers and subtracting those values from weights of the containers when at the original approximate field capacity. (Fresh weight of biomass was deemed negligible). Percent wilting was determined by placing a 4 cm diameter ring on the plugs and determining the number of non-wilted leaves, using indicators such as bluish color and leaf rolling inside the ring. The mean of two ratings per plug was recorded for percent wilting.

Leaf water status was determined by removing that last fully emerged leaf from a tiller and placing it in a Campbell-Brewster leaf press. The press was activated by a hydraulic pump that was capable of compressing the leaf as described by Nabati *et al*. (1994). The pressure that caused water to be exuded uniformly from tips, bottom and edges of the leaves was recorded. The greater the pressure required to express water, the poorer the LWS, i.e., less water was contained in the leaf tissues. Mean of three LWS measurements from each plug were recorded. Shoot dry weight accumulation was approximated by weighing harvested foliage clipped at 5 cm from soil surface (after withholding water for 3 week) and re-weighing after drying at 60°C for 24 h.

Post-drought measurements: Green leaf density was evaluated by placing a 4 cm diameter ring over the transplanted plugs and counting the number of leaves that were green. The measurements per plug were recorded at 2 and 3 week after re-watering. Overall color of plants was rated based upon a visual score of 9 for dark green, 6 for acceptable color and 1 for no green color. Leaf water status was determined at 2 and 3 weeks after re-watering as described earlier.

Root development was determined by using a modified vertical root lift method described by Schmidt and Synder (1986). A hand-held pulling gauge scale (Model DPPH-100, John Chatillion and Sons, Inc., Kew Garden, NY) with two metal hooks was attached to the plug rings and lifted vertically. The amount of force required to pull the ring free from the soils is correlated to root mass. Shoot dry weight accumulation was determined 3 weeks after re-watering by harvesting at a height of 5 cm and weighing after oven drying at 60°C for 24 h.

RESULTS

Cumulative evapotranspiration averaged 32% greater with the HNF than LNF (Table 1). However, none of the PGR or Fe treatment significantly affected CET during the first week water was withheld (Table 1). After 3 week, no differences in CET occurred between any PGR treatment and the control under LNF. Under HNF, the FSE treatment lowered CET, while PPC+Fe-treated plants had significantly higher CET compared to the control (Table 1). Two weeks after the water was withheld, regardless of N fertility, grasses treated with PGR or without Fe, except for the PPC alone treatment wilted less. The PPC plus Fe treatment was the most protective against wilting (Table 1). After 3 week of drought, all treated plants, except the PPC-treated plants, grown under LNF had a lower percentage of wilted leaves than the non-treated control. Again after 3 week the PPC plus chelated Fe was most effective at reducing wilt at both levels of N regimes.

The LWS was more favorable in plants treated with PPC, FSE and chelated Fe after 1 week without watering (Table 2). Prior treatment with PPC alone or in combination with Fe significantly stimulated biomass production (FDW) as compared to the control plants (Table 2). Nitrogen treatments were without effect on FDW and there was no N and PGR treatments interaction.

Table 1: Cumulative Evaporatranspiration (CET) 1 and 3 and wilting 2 and 3 weeks after withholding water of creeping bentgrass starting 3 weeks after chemical treatment as affected by propiconazole (PPC), Fortified Seaweed Extract (FSE), Fe and two nitrogen fertility regimes

			Nitrogen rates (kg ha^{-1})								
			CET					Wilt			
	Amount		1 week			3 weeks		2 weeks		3 weeks	
	PGR	Fe	25	50	Mean	25	50	25	50	25	50
Treatments	(L ha^{-1})	(kg ha^{-1})	kg/m^2/plant					Wilting (%)			
Control	0.00	0.0	18.5	24.0	20.3a†	27.1ab	33.0b	30b†	33b	56a	65a
Fe	0.00	1.1	19.7	21.5	20.6a	28.0a	33.0b	20d	26c	39b	48c
FSE	9.30	0.0	19.7	23.4	25.5a	25.5ab	25.5c	22c	24cd	44b	49c
PPC	0.93	0.0	14.8	23.5	19.1a	22.0b	33.0b	41a	39a	57a	57b
FSE+Fe	9.30	1.1	17.3	21.7	19.5a	28.0a	29.8b	25c	27c	44b	53c
PPC+Fe	0.93	1.1	14.8	23.7	16.2a	26.3ab	38.3a	17e	21d	27c	35d
Source of variation											
Nitrogen Fertility (NF)			*			*		NS		*	
Plant Growth Regulator (PGR)			NS			*		*		*	
NF×PGR			NS			*		*		*	

*Significant at the 10% probability level; NS: Non-Significant at 10% probability level. †Within a column, means followed by the same letter(s) are not significantly different at p = 0.1% according to Duncan's multiple range test

Table 2: Leaf Water Status (LWS) 1, Foliage Dry Weight (FDW) 3 and Green-Leaf Density (GLD) 2 and 3 weeks after withholding water of creeping bentgrass starting 3, 5 and 6 weeks after chemical treatment as affected by propiconazole (PPC), Fortified Seaweed Extract (FSE), Fe and two nitrogen fertility regimes.

	Amount		Nitrogen rates (kg ha^{-1}): LWS at 1 week			FDW at 3 weeks			GLD at 2 weeks			GLD at 3 weeks		
	PGR	Fe	25	50	Mean	25	50	Mean	25	50	Mean	25	50	Mean
Treatments	(L ha^{-1})	(kg ha^{-1})	Relative pressure			g plug^{-1}			Leaf No. (cm^{-2})					
Control	0.00	0.0	195	225	210a†	0.80	0.87	0.83c†	3.96	3.73	3.80c	5.55	5.95	5.71b†
Fe	0.00	1.1	175	180	175cd	0.97	0.98	0.97ab	4.36	4.04	4.20b	6.34	6.50	6.26a
FSE	9.30	0.0	165	185	175cd	0.90	0.83	0.87bc	4.20	3.09	3.65c	6.25	5.63	6.03b
PPC	0.93	0.0	150	180	165d	1.00	0.87	0.90b	4.12	3.17	3.65c	5.55	4.68	5.07c
FSE+Fe	9.30	1.1	180	195	187b	0.87	0.90	0.91bc	4.20	3.73	3.96c	5.87	5.55	5.71b
PPC+Fe	0.93	1.1	175	190	180bc	1.07	1.00	1.02a	4.36	4.12	4.6a	6.82	5.87	6.34a
Source of variation														
Nitrogen Fertility (NF)			*			NS			*			*		
Plant Growth Regulator (PGR)			*			*			*			*		
NF×PGR			NS			NS			NS			NS		

*Significant at the 10% probability level; NS: Non-Significant at 10% probability level. †Within a column, means followed by the same letter(s) are not significantly different at p = 0.1% according to Duncan's multiple range test

Table 3: Leaf color, Leaf Water Status (LWS), Root-Pull Strength (RPS), Foliage Dry Weight (FDW) 3 weeks after re-watering of creeping bentgrass starting at 6 weeks after chemical treatment as affected by propiconazole (PPC), Fortified Seaweed Extract (FSE), Fe and two nitrogen fertility regimes

	Amount		Nitrogen rates (kg ha^{-1}): Leaf color			LWS		RPS		FDW		
	PGR	Fe	25	50	Mean	25	50	25	50	25	50	Mean
Treatments	(L ha^{-1})	(kg ha^{-1})	Rating¶			Relative pressure		g m^{-2}		g plug^{-1}		
Control	0.00	0.0	7.2	8.0	7.6c†	195a†	220a	20c†	24d	0.67	0.86	0.77b†
Fe	0.00	1.1	8.2	8.8	8.5b	175bc	190c	24b	34a	0.86	1.00	0.93ab
FSE	9.30	0.0	8.2	8.7	8.4b	170c	197bc	29a	30b	0.90	0.80	0.86ab
PPC	0.93	0.0	8.7	8.7	8.7a	150d	194bc	29a	30b	0.93	0.73	0.83ab
FSE+Fe	9.30	1.1	8.4	8.2	8.3b	190a	202b	29a	27c	0.93	0.93	0.93ab
PPC+Fe	0.93	1.1	8.6	8.6	8.6a	180b	186c	27ab	34a	1.13	1.06	1.10a
Source of variation												
Nitrogen Fertility (NF)			NS			*		*		NS		
Plant Growth Regulator (PGR)			*			*		*		*		
NF×PGR			NS			*		*		NS		

*Significant at the 10% probability level; NS: Non-Significant at 10% probability level. †Within a column, means followed by the same letter(s) are not significantly different at p = 0.1% according to Duncan's multiple range test, ¶Leaf color was ranked from 1 to 9, where, 1 is no green color, 6 is acceptable and 9 is the best

The GLD after re-watering following drought was significantly greater for plants grown under LNF than HNF (Table 2). Propiconazole plus Fe and Fe alone were the only treatments that increased bentgrass leaf density 2 and 3 weeks after irrigation. The grass color was not significantly influenced by N fertility 3 weeks after recovering from drought; however, foliar application of PGR with or without Fe produced a darker green leaf than in the control plants regardless of N fertility (Table 3). Plants that received PPC with or without Fe had the darkest green color 3 weeks after watering was resumed.

Plants that received the LNF had a lower LWS (higher leaf moisture content) than HNF 3 weeks after termination of the drought (Table 3). All treatments, expect FSE plus Fe under LNF significantly caused an increase in leaf water content 3 weeks after the drought.

At the termination of the study (after 3 weeks recovery from drought), Root Pull Strength (RPS) was greater with HNF than LNF. Root development of PGR-treated plants was significantly enhanced at both N levels (Table 3). Iron had less effect on root pull strength under LNF as compared to other treatments, while addition of Fe increased the effect of PPC and reduced the effect of FSW at the HNF regimes. Foliage dry weights were not significantly different between N fertility regimes and only the PPC plus Fe stimulated foliar growth than untreated plants after watering was resumed (Table 3). The HNF caused increased vegetative growth and that resulted in higher CET than the LNF. Higher CET, more wilted leaves and poorer LWS in the plants grown at the HNF was followed by slower growth recovery from drought. Lower CET, low to moderate LWS and less wilting of leaves associated with the LNF accounted for better growth recovery from drought stress.

DISCUSSION

Foliar application of the PGRs with or without Fe to bentgrass under different N fertility regimes caused differences in water use by bentgrass during and following drought. Apparently drought conditions produced several effects, especially at the HNF, which caused a decrease in the re-growth capacity of plants. Individual Plants which wilted the most and had poorer LWS were the slowest to recover from drought which indicated a positive correlation (r = 0.842). These findings also suggest that plant re-growth following drought is promoted by foliar application of PGR with or without Fe.

Plants transpirated relatively large amounts of water during the first week, but as the drought progressed, the plants lost less water regardless of N fertility. Plants with lower LWS produced more leaf dry matter under both N fertility regimes following a drought. These findings support earlier study by Crouch *et al*. (1990) and Zhang and Schmidt (2000), who noted that foliar applications of PGR, in particular cytokinins-like material, might increase the activity of enzymes of endogenous hormones in leaves that help plants tolerate drought. Zhang and Schmidt (2000) also reported that the leaf water content of tall fescue was improved with application of the Hormone-Containing Products such as seaweed extract or humic acid. A study by Nabati *et al*. (2008) reported that the application of PGRs with or without Fe on Kentucky bluegrass grown under limited soil moisture regime, enhanced leaf water content in plant. Compounds containing cytokinins-like material, is acting as an antioxidant which capable of maintaining the integrity of the tonoplast membrane and repressing lipoxygenase: an enzyme responsible for leaf senescence in plant species (Thimann, 1987; Musgrave 1994).

Exogenous PGR plus Fe and chelated Fe treatments played a significant role in minimizing the drought effects on growth and physiology of bentgrass. Foliar applications of PGR with or without Fe improved LWS when water became limited to plants and subsequently caused an increase after water was again made available. This improvement may correspond to activation of endogenous hormones that protect cell membranes of leaf tissue during drought (Crouch *et al*., 1990). Zhang and Schmidt (2000) reported that Hormone-Containing Products such as seaweed extract or humic acid treatments significantly improved leaf water status and shoot and root growth of the grasses grown under high and low soil moisture. The appearance of a dark green color is presumably an indication of chlorophyll development following the stressful condition. Other research indicates that PPC stimulate antioxidant development which deactivate free radical groups, such as superoxide and hydrogen peroxide which accelerate leaf senescence and chlorophyll break down during water deficiency (Upadhyaya *et al*., 1985; Zhang and Schmidt 1999). Several other workers have reported that PPC has been shown to develop other antioxidants that function better; which it not only increases antioxidant levels, it also promotes photochemical activities, resistance to senescence and root mass (Zhang *et al*., 2003a, b; Zhang and Schmidt, 2000; Dexter, 1973) reported that high rate of N fertilization reduced drought tolerance of temperate grass species.

The present study showed that HNF stimulated CET, wilting and caused poorer LWS, whereas low CET, low to moderate LWS and less wilting of leaves associated with LNF. Emerson *et al*. (1986) found similar response for winter wheat under low irrigation and different rates of N fertility following foliar applications of three PGRs, ethephon, mefluidide and cycocel. Even with higher CET and moderate LWS, PGR and Fe treatments lowered leaf wilting and improved of root development. Zhang and Schmidt (2000) reported that seaweed extract and humic acid treatments significantly increased α-tocopherol and ascorbic acid concentration of grasses grown under high and low soil moisture contents and this event positively correlated between antioxidants and shoot or root growth in *Festuca arundinacea*. Improved root development would allow the plants to obtain deeper moisture when water was limited near the soil surface.

From study presented here with PGRs in conjunctions with Fe and N the findings have revealed that growth stimulus of the bentgrass has impacted by certain of biochemical changes within plant other than mineral nutrition that promoted physiological and morphological growth activities under harsh environment

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