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The Growth of Kentucky Bluegrass (*Poa pratensis* cv. Plush) as Affected by Plant Growth Regulators and Iron (Fe), Grown under Limited Soil Moisture Regimes

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Abstract: This research was conducted to determine if exogenous plant growth regulators (PGR) or Fe could alleviate some of the effect of low soil moisture content on Kentucky blue grass (*Poa pratensis* cv. Plush). Two week old seedlings were treated with seaweed (*Ascophyllum nodosum*) extract (SWE) at 9.3 L ha^{-1} or Propiconalzole (PPC) alone at 0.93 L active ingredient (a.i) ha^{-1} alone or in combination with chelated Fe at 1.1 kg ha^{-1}. Transplanted seedlings were grown under three soil moisture regimes (-0.5, -0.3 and -0.03 MPa) for 6 weeks. The experiment was repeated once. Foliage growth of the treated plants increased as soil water potential increased. Application of Fe provided the most consistent enhancement of foliage growth regardless of the soil moisture regimes. All PGR treatments improved leaf water content compared with control plants. Root length and root mass measurements made in the difference experiments were positively correlated. However, root length decreased significantly with soil moisture level; while root mass did not. The results showed that exogenous application of SWE or PPC, alone or with Fe reduced the influence of low soil moisture content. Seedlings did not differ in P and K contents in leaf tissues regardless of the soil moisture regimes or PGR treatments. The results indicate that the main effects of the PGR on growth of Kentucky bluegrass under low soil moisture content were associated with aspects other than P and K nutrition.

Key words: Kentucky bluegrass (*Poa pratensis* cv. Plush), propiconalzole, seaweed extract, plant growth regulator, root growth, leaf water content, shoot growth

INTRODUCTION

Growth and quality of grass species stand are significantly affected by various abiotic and biotic stresses. Research studies have showed that the quality of plant growth performances can be maintained under proper fertilization, irrigation, insect, disease control and other cultivation practices (Watschke and Schmidt, 1992). However, as water resources and moisture availability in soil decline, drought stress becomes a major limiting factor in growth and development of the plant species (Kenna and Horst, 1993). Plants subjected to soil moisture stress grow more slowly; the growth rate of various organs is suppressed (White *et al*., 1993; DaCosta *et al*., 2004). Lateral bud initiation and rhizome formation in many grass species are reduced drastically during periods of soil water deficits (DaCosta *et al*., 2004). Water stress is also characterized by the reduction of plant water content, wilting, closure of stomata, reduction of hormonal activities, lowered availability of nutrients (especially P and K). These reduced photosynthesis and decreased cell enlargement (McKersie and Leshem, 1994; Lawlor, 1995; Nilsen and Orcutt, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997; DaCosta *et al*., 2004). Research has been shown that some of plant growth regulators could play an acceptable role in some turfgrass managements (Chalmers, 1990). Application of proper exogenous of plant growth regulators to an appropriate growth stage of plant may help to enhance growth and development and tolerance to water stress (Arteca, 1996; Hall and Bingham, 1993). Application of plant growth regulators in conjunction with mineral nutrients has drawn considerable attention for crop production in adverse environments (Crouch, 1990). When chelated iron applied as iron fertilization has been shown to improve root growth and water relationships of creeping Bentgrass plants (*Agrostis palustris Hud*) (Glinski *et al*., 1992). The study has shown that iron plays an important role in chlorophyll formation and is used on fine turf to provide a greening response without increasing shoot growth (Glinski *et al*., 1992; Zhang *et al*., 2002). Two natural occurring chemicals, i.e., Humic Acid (HA) and Seaweed

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Extract (SWE) and a synthetic Propiconazole (PPC) are three plant growth regulators which have been used recently to stimulate plant growth under harsh environments (Zhang and Schmidt, 1997, 1999, 2000a, b; Allen *et al*., 2001; Fike *et al*., 2001; Zhang *et al*., 2003a). Several studies indicated that application of humic acid to grass species could promote shoot and root growth and improve plant resistance to adverse environments (Goatley and Schmidt, 1990a; Nabati *et al*., 1994; Schmidt and Zhang, 1997, 1999; Ervin *et al*., 2003).

Propiconazole, a triazole compound which has both fungicidal and growth bio-regulatory properties, is capable of protecting plants under harsh environments, including low and high temperatures and drought conditions. The mode by which traizole protects plants from stresses is associated with plant hormonal modification, such as an increase in cytokinins (Fletcher and Hofstra, 1988; Zhang and Schmidt, 2000b). Aqueous SWE and PPC products exhibit various beneficial effects on plant growth regulation. Application of these substances could cause an increase in shoot and root growth, improve nutrient uptake, increase photosynthesis and enhance its resistance to disease and environmental stresses such as drought and salinity in various species (Crouch, 1990; Meeting *et al*., 1990; Nabati *et al*., 1991, 1994; Yan, 1993; Schmidt and Zhang, 1997; Zhang and Schmidt, 1999; Zhang *et al*., 2003a, c; Zhang and Ervin, 2004). The objective of this study was to determine response of the transplanted seedlings of Kentucky bluegrass grown under different soil moisture regimes and foliar application of PGR materials and chelated Fe.

MATERIALS AND METHODS

The study was conducted at the Virginia Tech Turfgrass Research Center, Blacksburg, VA, USA. Kentucky bluegrass (*Poa pratensis* L.) seeds were sown at a rate of 16 kg ha^{-1} in 26×52 cm flats filled with Groseclose silt loam (Typic Hapludult, pH 6.4). The soil P and K contents were 30 and 57 μg g^{-1}, respectively. Seeded flats received 50 to 60 mL of water twice a day. Two weeks after germination, when the grass plants had formed two-leaf, seedlings were treated with one of two PGR materials and Fe alone or in combination. One PGR was carried out at a low-temperature-water extract of seaweed (*Ascophyllum nodosum*) (3%) and peat humus (7%) fortified with ascorbic acid, alpha tocopherol, glycine and thiamine (SWE) marketed by Root Inc., New heaven, CT. The SWE was applied at 9.3 L ha^{-1}. The second PGR, propiconazole [1-{2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2yl}methyl-1-H-1,2,4-triazol] (PPC) was applied at 0.93 L a.i. ha^{-1}. The Fe source was Na-diethylene-triamine-pentaacetate chelated iron applied at 1.1 kg a.i. ha^{-1}. All treatments were applied with a compressed-air sprayer with 784 L water ha^{-1} at 290 KPa. The treated seedlings were irrigated daily and grown in a greenhouse with a mean temperature of approximately 24°C for daytime and 20°C during night.

Groseclose soil was air-dried in a greenhouse for two weeks and then screened through a 2 mm mesh sieve. Moisture release curves, according to the methods of Richards (1965), showed the soil required 11.6, 14.4 and 20.1 g of water per 100 g of soil to develop soil water potentials of -0.5, -0.3 and -0.03 MPa, respectively. Aliquots of 22 kg of this soil with appropriate water and 20-20-20 (N: P: K) soluble chemical fertilizers (to supply 50 kg N ha^{-1}) were mixed thoroughly before being sealed into plastic containers and allowed to equilibrate for 72 h.

Terrarium-like boxes (30 cm high ×40 cm wide ×75 cm long) were constructed with 0.15 cm clear plastic sides and tops. Each of these boxes was placed at 22 kg of soil adjusted to one of the three moisture regimes. This provided a 7.5 cm soil depth. Plugs, 3.8 cm in diameter by 2 cm deep, of the treated Kentucky bluegrass seedlings were removed from the flats 2 weeks after PGR treatments. The soil was gently washed away from the roots and plugs were transplanted to a terrarium containing one of the three moisture regimes. Each terrarium received 24 plugs.

A tape was used to seal each terrarium. Straws were placed in two, 0.5 cm diameter holes in each top to partially ventilate the terrarium while minimizing moisture losses and condensation. The terraria were placed in a greenhouse in which temperature was maintained at 21°C during the day and 18°C at night. Thermocouples were used to record soil and air temperatures inside the terraria twice a day, at 9 am and at 7 pm. Mean air temperatures within the terraria were 24, 22 and 21 °C in the morning and 21, 20 and 22°C in the evening for -0.5, -0.3 and -0.03 MPa soil water potentials, respectively. Mean soil temperatures were 18, 20 and 22°C at 9 am and 20, 23 and 22°C at 7 pm for -0.5, -0.3 and -0.03 MPa soil water potentials, respectively.

The tops of the terraria were removed weekly at late afternoon and replaced after checking for diseases or wilting. The seedlings received no additional PGR, fertilizer, or water treatments for 6 weeks after transplanting. Three weeks after transplanting, the seedlings were clipped at 5 cm. Plant height, percent protein of whole plants, root length and P and K concentration of tissues were determined at 6 weeks after transplanting. The experiment was repeated for the determinations of root dry weights, as well as Leaf Water Status (LWS).

For the second experiment, leaf water status was determined by placing the last fully emerge leaf from a tiller in a Campbell Scientific Model J-14 leaf press. The leaf press was activated by a hydraulic pump that was capable of compressing the leaf till it exuded moisture as described by Nabati *et al.* (1994). The pressure that caused water to be exuded uniformly from tip, bottom and the edges of the leaf was recorded. The greater the pressure required to exude water, the higher the LWS of grass grown under different soil moisture regimes indicated the lower the leaf water content. The means for LWS were determined from three leaf measurements of each treatment.

Six weeks after transplanting, the plants as plugs were removed; the soil was washed away from the roots and root length was recorded. Tillers were separated from roots and dry matter accumulation was determined by weighting after drying the plant tissues in an oven at 60°C for 24 h total protein of the whole plants was determined on a dry weight basis by Kjeldahl Auto 1030 Analyzer (Prabin and Co AB). Mineral composition (P and K) of leaf tissues was determined on a dry weight basis following perchloric acid digestion according to the method of Jones and Steyn (1973). Phosphorus and potassium contents were analyzed by inductively coupled plasma spectrogra.

Experimental design being used was a factorial arranged in a randomized complete block design with four replications. One factor was soil moisture regimes and the other factor was chemical treatments. Data were statistically analyzed with the use of analysis of variance and the separation of means were carried out by Duncan's Multiple Range Test (DMRT) at 5% probability.

RESULTS AND DISCUSSION

Applications of SWE and PPC either alone or in combination with Fe caused different growth responses under different soil moisture conditions, as has been observed by other workers (Schmidt and Snyder, 1984; Walster *et al.*, 1987; Pinhero and Fletcher, 1994; Zhang and Schmidt, 1999; Zhang *et al.*, 2003b; Zhang and Ervin, 2004). The smallest plants were attained under -0.5 MPa soil moisture (Table 1). Several studies have suggested that limited soil moisture content tends to reduce lateral bud initiation, rhizome production and growth rate of various organs, resulting in the reduction of plant size (Mckersie and Leshem, 1994; Lawlor, 1995; DaCosta *et al.*, 2004). Foliar growth of PGR treated plants generally increased when compared with untreated plants.

Shoot dry weight obtained in the second experiment correlated (r = 0.6520) with the shoot heights measured in

Table 1: Plant height and root length of Kentucky bluegrass at 8 weeks after chemical treatment and 6 weeks after transplanting into various soil moisture regimes as affected by propiconazol (PPC), seaweed extract (SWE) and Fe

Soil moisture MPa	SWE ($L\ ha^{-1}$)	PPC ($L\ ha^{-1}$)	Fe ($kg\ ha^{-1}$)	Plant height (cm)	Root length (cm)
-0.03	0.0	0.00	0.0	20.7^{c}†	10.2^{c}
	0.0	0.00	1.1	26.2^{a}	13.5^{a}
	9.3	0.00	0.0	23.0^{b}	12.2^{ab}
	0.0	0.93	0.0	25.2^{a}	12.7^{a}
	9.3	0.00	1.1	23.0^{b}	13.0^{a}
	0.0	0.93	1.1	19.5^{c}	10.7^{bc}
-0.3	0.0	0.00	0.0	16.7^{b}	11.0^{e}
	0.0	0.00	1.1	19.5^{a}	15.0^{bc}
	9.3	0.00	0.0	19.2^{a}	13.7^{cd}
	0.0	0.93	0.0	19.2^{a}	15.5^{b}
	9.3	0.00	1.1	14.0^{c}	17.7^{a}
	0.0	0.93	1.1	18.2^{a}	12.5^{d}
-0.5	0.0	0.00	0.0	13.5^{bc}	11.7^{c}
	0.0	0.00	1.1	18.0^{a}	15.5^{b}
	9.3	0.00	0.0	15.0^{b}	16.2^{ab}
	0.0	0.93	0.0	15.5^{b}	15.0^{ab}
	9.3	0.00	1.1	12.2^{c}	17.2^{a}
	0.0	0.93	1.1	14.7^{b}	15.2^{b}
Source of variation					
Soil moisture (SM)				**	**
Plant growth regulator (PGR)				**	**
SM×PGR				**	**

**: Significant at 1% probability level. †: Within a column and moisture levels means followed by the same superscript letter are not significantly different at p = 5% according to Duncan's Multiple Range Test

the first experiment (Table 1, 2). Although all PGR and Fe treatments caused an increase in the shoot development of the grasses treated-seedling with Fe more consistently and it produced a significant increase in shoot growth regardless of the moisture regimes.

Root length being measured during the first experiment and the root dry weight obtained in the second experiment were relatively correlated (r = 0.7092). In the first experiment, root length increased as soil moisture decreased (Table 1). However, soil moisture did not significantly affect root weight in the second experiment (Table 2). All PGR or Fe treatments increased root development, especially when the grass was grown under moisture stresses. Zhang *et al.* (2003a, b) reported that foliar application of SWE + HA and PPC alone, or in combination with SWE + HA improve rooting and quality of Tall Fescue and Kentucky Bluegrass plants. The studies also indicated some PGRs such as SWE, HA and PPC may enhance root growth of grass subjected to stress by increasing the antioxidant defending system and increasing endogenous cytokinin and auxin levels. These results support previous findings that cytokinin-like and triazole materials are capable of stimulating root growth and development under adverse conditions (Nelson and Van Staden, 1984; Fletcher and Hofstra, 1988; Goatley and Schmidt, 1990b; Nabati *et al.*, 1991; Zhang and Ervin, 2004).

Table 2: Shoot, root dry weight and leaf water status of Kentucky bluegrass at 8 weeks after chemical treatment and 6 weeks after transplanting into various soil moisture regimes as affected by propiconazol (PPC), seaweed extract(SWE) and Fe

Soil moisture MPa	SWE ($L\ ha^{-1}$)	PPC ($L\ ha^{-1}$)	Fe ($kg\ ha^{-1}$)	Shoot dry weight ($mg\ cm^{-2}$)	Root dry weight ($mg\ cm^{-2}$)	Leaf water status (MPa)
-0.03	0.0	0.00	0.0	40^{b}†	23^{a}	2.3^{a}
	0.0	0.00	1.1	51^{ab}	33^{a}	1.8^{bc}
	9.3	0.00	0.0	51^{ab}	28^{a}	1.6^{c}
	0.0	0.93	0.0	62^{a}	27^{a}	2.0^{b}
	9.3	0.00	1.1	50^{ab}	31^{a}	1.8^{bc}
	0.0	0.93	1.1	48^{ab}	24^{a}	1.9^{bc}
-0.3	0.0	0.00	0.0	36^{b}	21^{a}	2.4^{a}
	0.0	0.00	1.1	55^{a}	32^{a}	2.0^{b}
	9.3	0.00	0.0	37^{b}	25^{a}	2.0^{b}
	0.0	0.93	0.0	40^{b}	35^{a}	2.1^{b}
	9.3	0.00	1.1	39^{b}	37^{a}	2.0^{b}
	0.0	0.93	1.1	21^{c}	28^{a}	2.1^{b}
-0.5	0.0	0.00	0.0	31^{b}	16^{d}	2.6^{a}
	0.0	0.00	1.1	50^{a}	44^{a}	2.2^{b}
	9.3	0.00	0.0	32^{b}	31^{bc}	2.2^{b}
	0.0	0.93	0.0	35^{b}	28^{c}	1.9^{c}
	9.3	0.00	1.1	32^{b}	33^{b}	2.2^{b}
	0.0	0.93	1.1	50^{a}	36^{b}	2.3^{b}
Source of variation						
Soil moisture (SM)				**	NS	**
Plant growth regulator (PGR)				**	*	**
SM×PGR				**	NS	NS

**,*: Significant at the 1 and 5% probability level, respectively; NS: Non-significant at 5% probability level. †: Within a column and moisture levels means followed by the same superscript letter are not significantly different at p = 5% according to Duncan's multiple range test

As soil water potential decreased, leaf water content of treated plants increased (Table 2). Higher Leaf Water Stress (LWS) values indicate greater likelihood of leaf moisture stress. The highest LWS values were developed under plants grown at the lowest soil moisture level. All PGR treated grasses had lower LWS values and improved leaf water content when compared with control plants grown under the three soil moisture regimes. Leaf water status measured in the second experiment was correlated (r = -0.6327) with the root length measurements in the first experiment. This indicated that the results of both experiments revealed that root growth increased with an increase in the leaf water content and is associated with PGR treatments.

The results in this study indicated that the application of PGRs with or without Fe could be helpful for leaf water content improvement when grass species is grown under low soil moisture regime. Zhang and Schmidt (2000b) 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 positively correlated between antioxidants and shoot or root growth where the result was found in the two grass species (*Festuca arundinacea* and *Agrostis palustris*). Improvement of growth and LWS of turfgrass treated with SWE and HA may be related to its high antioxidant concentration. The leaf concentration of either P or K did not differ significantly between the Kentucky bluegrass seedlings those subjected to PGR or moisture treatments (data not shown). Neither did protein content of whole plants differ between treatments (data not shown). This confirms the results of other workers that mineral nutrients did not appear to be responsible for stimulating the plant growth activity during periods when soil water was limited (Crouch, 1990; Schmidt and Zhang, 1997; DaCosta *et al*., 2004). The findings of this study indicate that application of SWE or PPC either alone or in conjunction with Fe aided Kentucky bluegrass to tolerate against moisture stresses possibly by impacting the organic metabolism of the grass.

In addition of a statement above our results show that application of PGR significantly affected a positive growth with low energy consumption, when plant is confronted with the limited water availability. In such condition, morphological growth, in particular root growth development enable to expand and grow deep into the soil profiles in order to absorb and conserve water in the leaf tissues. This growth phenomenon which is stimulated by PGR treatment is a unique factor for grass survival under limited water in dry area.

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