Influence of Plant Growth Regulators on Turfgrass Growth, Antioxidant Status, and Drought Tolerance

by

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(ABSTRACT)

A series of studies were conducted to examine the antioxidant status, drought and disease tolerance, and growth response to foliar application of soluble seaweed (*Ascophyllum nodosum*) extracts (SE) and humic acid (HA; 25% active HA or 2.9% active HA) in tall fescue (*Festuca arundinacea* Schreb), Kentucky bluegrass (*Poa pratensis* L.) and creeping bentgrass (*Agrostis palusttis* Huds.) grown under low (-0.5 MPa) and high (-0.03 MPa) soil moisture environments.

Foliar application of humic acid (2.9 % active HA) at 23.7 and 47.4 l ha^{-1} improved leaf water status, shoot and root development in tall fescue, Kentucky bluegrass and creeping bentgrass grown under drought. Humic acid (2.9% active HA) at 15.5 l ha^{-1} or SE at 326 g ha^{-1} significantly reduced dollarspot incidence and improved turf quality in creeping bentgrass.

Drought stress induced an increase of antioxidants α-tocopherol and ascorbic acid concentrations in the three turfgrass species. In the experiment with Kentucky bluegrass, drought stress increased β-carotene concentration, but did not significantly influence superoxide dismutase (SOD) activity.

Foliar application of humic acid (25% active HA) at 5 l ha^{-1} and/or SE at 326 g ha^{-1} consistently enhanced α-tocopherol and ascorbic acid concentrations, leaf water status, and growth in the three cool-season turfgrass species grown under low and high soil moisture environments. In the experiment with Kentucky bluegrass, application of HA at 5 l ha^{-1} plus SE at 326 g ha^{-1} also increased β-carotene content and SOD activity under low and high soil moisture environments. There were close positive correlations between the antioxidant status and shoot or root growth in the three turfgrass species regardless of soil moisture levels.

The antioxidant SOD activity, photosynthetic capacity in terms of F_{vm690}, and chlorophyll content in terms of F_{m730}/F_{m690} exhibited a seasonal fluctuation in endophyte [*Neotiphodium coenophialum* (Morgan Jones and Gams) Glenn, Bacon, Price and Hanlin] -free and endophyte-infected tall fescue. Application of SE enhanced SOD activity, photosynthetic capacity, and chlorophyll content in tall fescue, especially at 4 weeks after SE treatment. The SOD activity, photosynthetic capacity and chlorophyll content were not significantly influenced by the endophyte infection. A close positive correlation between SOD and photosynthetic capacity during the summer was found in endophyte-free and endophyte-infected tall fescue.

DEDICATION

To my wife, Jianfeng, and to my son, Jiwei

To the loving memory of my parents

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Influence of Plant Growth Regulators on

Turfgrass Growth, Antioxidant Status, and Drought Tolerance

List of Tables

List of Figures

Chapter 1
Introduction and objectives

Plant growth regulators (PGRs) play an important role in modern agriculture and turf management. Proper application of certain PGRs may improve turf quality and thereby benefit environments (Schmidt, 1990). Recently, increasing interest has been developed in utilizing PGRs with multiple functions in turf management. Natural products, seaweed extract (SE) and humic acid (HA) are common sources of PGRs that exhibit multiple functions. These sources of PGRs could not only regulate plant growth and development but also increase plant resistance to various environmental stresses, such as drought, salinity, and low temperature. Seaweed extracts, which possess cytokinin-like and auxin-like properties, can stimulate endogenous cytokinin activities of plants (Crouch, 1990). When applied to turfgrasses, SE enhances root growth, delays senescence, improves turf quality (Schmidt, 1990; Crouch, 1990), increases tolerance to drought (Nabati et al., 1991), and regulates cell membrane components under drought stress (Yan and Schmidt, 1993). Humic acid, which has auxin-like activity, not only enhances plant growth and nutrient uptake but also improves stress resistance (Senn, 1991). However, physiological mechanisms of the PGRs' effects on drought tolerance are still not clear.

Plant antioxidants and their role in the plant defense system have received increasing attention within the last decade (Larson, 1988; Alscher and Hess, 1993; Smirnoff,1995). Research results suggest that many environmental stresses such as drought, salinity, low temperature, and herbicide pollution damage plants directly or indirectly through the endogenous reactive oxygen species (Larson, 1988; Price and Hendry, 1989; Sminoff, 1993; Thompson et al., 1987). Water stress causes various physiological and biological changes in plants, one of which is the accumulation of reactive oxygen species in the cell. The reactive oxygen radicals are toxic and may result in a series of injuries to plant metabolism. It damages photosynthetic components, inactivates protein and enzymes, destroys cell membrane structure and permeability by causing lipid peroxidation (Price and Hendry, 1987, 1989, 1991; Winston, 1990).

Plants possess various antioxidants to cope with the oxidative stress. These include lipid soluble antioxidants (e.g. α-tocopherol, β-carotene), water soluble reductants(e.g. ascorbic acid,

glutathione), and enzymatic antioxidants (e.g.superoxide dismutase, catalase, and enzymes of the ascorbate/glutathione cycle). Alpha-tocopherol, ascorbic acid, β-carotene, and superoxide dismutase (SOD) are important antioxidants which protect plants by suppressing oxidative injury. Alpha-tocopherol, which is located within biological membranes, can quench oxygen radicals, stabilize cell membranes by influencing lipid organization, and protect chlorophyll (Hess, 1993). Ascorbic acid scavenges many types of free radicals affecting many enzymes activities and also is required for regeneration of α-tocopherol (Smirnoff, 1995). β-carotene is not only an accessory pigment in the photosynthetic apparatus but also quenches singlet oxygen efficiently (Mckersie and Leshem, 1994). Superoxide dismutase is the most efficient scavenger of the superoxide anion and an essential component of the ascorbate-glutathione cycle for the detoxification of toxic oxygen species (Nakano et al., 1981).

The close relationship between antioxidant activity (such as SOD) and stress tolerance has been identified in several crops such as maize (Malan et al.,1990) and tobacco (Perl et al., 1991). These antioxidant activities change rapidly when a plant suffers from biotic or abiotic stresses (Price and Hendry, 1989; Smirnoff, 1993, 1995; Bowler, 1992;). Meanwhile, chlorophyll fluorescence also increased significantly under drought. However, little is known about the mechanism involved. It is unknown whether exogenous PGR (such as SE) affect stress tolerance via their influence on antioxidant activities or whether it is possible to manipulate the antioxidant level to increase drought stress tolerance. Information on these relationships is important to understand the mechanism of PGRs' functions and to improve drought stress tolerance, and turfgrass quality.

The objectives of this study were:

i) to evaluate the effect of seaweed extracts (SE) and humic acids (HA) on turfgrass tolerance to drought stress.

ii) to determine the influence of exogenous SE and HA on the status of plant antioxidants (α-tocopherol, ascorbic acid, SOD and β-carotene) and growth in different turfgrass species grown under high and low soil moisture regimes.

iii) to examine the relationship among PGR, antioxidant status, and function of photosynthetic apparatus in turfgrass.

Chapter 2
Literature Review

Water stress and plant growth

Water is imperative for plant growth and development. Water deficit stress, permanent or temporary, limits the growth and distribution of natural vegetation and the performance of cultivated plants more than any other environmental factor (Kramer, 1983). Although research and practices aimed at improving water stress resistance and water use efficiency have been carried out for many years, the mechanism involved is still not clear (Smith et al., 1993). Futher understanding and manipulating plant water relations and water stress tolerance can significantly improve plant productivity, turfgrass management, and environmental quality.

Water deficit stress can be defined as situations in which plant water potential and turgor are reduced enough to interfere with normal functions (Hsiao, 1973). Smirnoff (1993) separates water deficit from desiccation. Water stress is considerred to be a moderate loss of water which leads to stomatal closure and limitation of gas exchange. Desiccation is a much more extensive loss of water which can potentially lead to gross disruption of metabolism and cell structure and eventually to the cessation of enzyme-catalyzed reactions (Smirnoff, 1993). Water deficit stress occurs when transpiration exceeds the absorption of water from soil (Kozlowski, 1968). Deficits may happen under high irrigation when soil water is available because of higher transpiration rate or poor water quality (Smith and Griffiths, 1993). As soil water potential declines, daytime wilting occurs when the movement of water toward the roots in the drying soil becomes too slow to replace daytime water loss (Hsiao, 1973). Water stress is characterized by reduction of water content, turgor, total water potential, wilting, closure of stomata, and decrease in cell enlargement and growth. Servere water stress may result in arrest of photosynthesis, disturbance of metabolism, and finally dying (Mckersie and Leshem, 1994).

Plant growth is defined as irreversible increase of size and is accomplished through cell devision, cell enlargement, and differentiation (Lyndon, 1990). It involves genetic, physiological,

ecological, and morphological events and their complex interactions. Since plants are rooted in one place, they encounter frequent environmental stresses including water stress.

Water stress influences plant growth at various levels from cell to community (Smith and Griffiths, 1993). The quantity and quality of plant growth depend on cell devision, enlargement, and differentiation, and all of these events are affected by water stress (Mckersie et al., 1994). Hsiao (1973) concluded that water stress inhibits cell enlargement more than cell devision. It reduces plant growth through inhibition of various physiological and biochemical precesses, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and hormones (Kramer,1983). As a result, plant size, leaf area, and productivity are reduced.

Initially, reduced water potential decreases the chemical activity of water and thereby modifies the structure of water in the cell. A low chemical activity of water can cause a change in the structure of the sphere of hydration around proteins and thereby reduce their efficacy. A loss of turgor may cause a change in the spatial position of transport channels, membrane enzymes, and decrease membrane thickness (Nelson and Orcutt, 1996).

Cell growth (expansion) is one of the most drought sensitive physiological processes due to the reduction of turgor pressure. Cell expansion can only occur when turgor pressure is greater than the cell wall yield threshold. Water stress greatly suppresses cell expansion and plant growth due to the low turgor pressure (Mckersie and Leshem, 1994).

Water stress reduces the rate of photosynthesis (Bjorkman and Powles, 1984; Chaves, 1991; Cornic and Briantais, 1991; Daraux, 1992; Lawlor, 1995). Lawlor (1995) pointed out that water stress reduces photosynthetic rate through limiting CO_2 diffusion due to the reduced stomatal conductivity, which reduces intercellular CO_2 concentration (Turner et al., 1978). Mckersie et al. (1994) indicated that water stress influences the plants by disruption of membrane structure and concomitant organelle disarray, impairment of stomatal function, and disturbance of CO_2 diffusion. The limitations of carbon dioxide exchange and metabolic fixation result in exposure of chloroplasts to excess excitation energy. Much of this can be dissipated by various photoprotective mechanisms, including dissipation as heat via carotenoids, photorespiration, crassulacean acid metabolism (CAM) idling, leaf movements and other morphological features which minimize light absorption (Smirnoff, 1993). However, some of excitation energy may be diverted to activate molecular

oxygen, and excess reactive oxygen species, will be produced and accumulated in the cell (Walker, 1992). All these species (e.g. superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen) are reactive and may damage cells by causing lipid peroxidation and inactivation of enzymes (Larson, 1988; Thompson, 1987; Winston, 1990).

Plant growth regulators and water stress tolerance

Plant growth regulators are substances that influence physiological processes of plants at very low concentrations (Frankenberger, Jr. and Arshad, 1995). When produced endogenously by plants, they are often referred to as phytohormones (plant hormones). Plant hormones have been viewed as chemical messengers regulating the normal progression of developmental changes as well as responses to environmental signals (Morgan, 1990). The term PGR includes a large number of synthetic and naturally occurring compounds. Nickell (1982) defined PGRs as either naturally or synthetic compounds that are applied directly to a target plant to alter its life precesses or its structure to improve quality, increase yields, or facilitate harvesting. Both the term PGR and phytohormones has been used interchangeably, particularlly when referring to auxins, gibberellins, cytokinins, ethylene and abcissic acid (Frankenberger Jr. And Arshad, 1995).

Abscissic acid (ABA) has been recognized as a signal inducing stomata closure (Davies and Zhang, 1991). Small changes in ABA distribution may trigger initial stomata closure, and the closure is maintained by rise in bulk ABA (Morgan, 1990). Gollan et al. (1986) indicated that water relations of leaves had not altered when leaf conductance decreased as the soil dried out. This suggested that elevated ABA concentration in the roots may serve as a signal for stomata closing. Zhang and Davies (1987) showed that dehydration of root tips increased their ABA production, and application of ABA to roots increased ABA content of leaves.

Cytokinin plays a role in stomatal regulation under water stress. It has been reported that water stress lowered cytokinin activity, and rewatering of drought-stressed plants restored the activity (Reid and Wample, 1985). A hypothesis has been proposed that ABA and cytokinin have opposite roles in drought stress. The increase of ABA and decrease of cytokinin under water stress favor stomatal closure and reduce water loss through transpiration (Morgan, 1990). Although

stomatal closure was viewed as an adaptive strategy of plants to water stress, it may interfere with gas exchange and result in oxidative stress in plant cells, especially under high light (Demmig et al., 1988; Smirnoff, 1995).

Other hormones (auxin, gibberellins, and ethylene) are also responsive to water stress. Auxin and gibberellins have been shown to decline under water stress in some plants (Aharoni et al.,1977; Guinn and Brummet, 1988). In contrast, rapid increase of ethylene has been observed in the plants grown under water deficit stress. It was noted that water stress enhances respiration, senescence and ripening which are all related to increased ethylene levels (Morgan, 1990). Lipid peroxidation due to activated oxygen under water stress may be related to the increase of ethylene level (Hildebrand and Grayburn, 1991; Leshem, 1981; Smirnoff, 1993).

Plant hormones may serve as a signal for plant adaptation to the stressful environments. Increased ABA level may enhance stomata closure and reduced water loss through transpiration (Davies and Zhang, 1991). Accumulation of metabolites (proline, polyols, quaternary amines, sugars, ions, polyamines, etc.) and osmotic adjustment under water stress may be related to regulatory functions of the phytohormones (Drolet et al., 1986; Smirnoff and Cumbes, 1989; Mckersie et al., 1994). Recently, these metabolites have been reviewed as antioxidants.

Despite the fact that plants are capable of synthesizing phytohormones and absorbing phytohormones generated by soil microorganisms, they may also respond to exogenous application of hormones during certain growth stage and under specific conditions (Frankenberger and Arshed, 1995). Plant may not synthesize enough endogenous phytohormones for optimal growth and development under suboptimal climatic and environmental conditions. Proper exogenous application of PGRs may enhance plant growth and tolerance to water stress (Arteca, 1996; Hall et al., 1993).

Plant growth regulators play an accepted role in some turfgrass management system (Chalmer, 1990). Traditionally, PGRs are used as growth retardents to reduce mowing frequency (Schott and Walter, 1991). Most of these PGRs are synthesized chemicals, and some of them are derived from herbicides. Excessive use of these PGRs has a risk of polluting environments.

In recent years, some natural products have received increasing attentions (Crouch, 1990; Schmidt, 1990; 1993; Senn, 1987;1991). These naturally derived, and hormone-containing

products, can not only stimulate plant growth and development, but also improve plant resistance to environmental stresses. Additionally, these organic substances have no harmful threat to the environment quality. These products, a group of plant growth regulators, are also called biostimulants and include seaweed extracts, humic acids, and vitamins (Schmidt, 1993).

Two plant growth regulators: seaweed extracts and humic acid

Seaweed extracts

Seaweed extracts (SE) and seaweed have been used as fertilizers and soil conditioners for centuries (Aitken and Senn, 1965; Crouch, 1990). Seaweed extracts contain not only most of the major and minor nutrients, amino acids, and vitamins B_1, B_2, C, E, but also cytokinins, auxin, GAs, and ABA-like growth substances (Abetz, 1980; Finnie and Van Staden, 1985; Munda and Gubensek, 1975; Moor and Van Staden, 1986; Stephenson, 1968).

Initially, enhanced plant growth with seaweed application was attributed to its soil conditioning properties. Later, it was showed that increased trace element supply could explain only some of the beneficial effects of seaweed (Francki, 1960). Low rates of seaweed extract could also promote plant growth significantly (Crouch, 1992, 1993). Thereafter, it was suggested that organic compounds, rather than mineral elements, were responsible for improved growth (Brain et al., 1973; Blunden, 1977; Crouch, 1993; Finnie et al., 1985). In recent years, it has been demonstrated that seaweed products contain phytohormones, and the stimulating effects of seaweed extracts may be attributed to these components, especially cytokinins.

Seaweed extracts exhibit various benificial effects on plant growth and development (Crouch, 1990; Crouch et al., 1990). They may enhance nutrient uptake, regulate plant growth substances, increase chlorophyll content, protein synthesis and cell division, promote root and shoot growth and improve seed gemination (Beckett et al., 1990; Button and Noyes, 1964; Van Staden et al., 1994; Yan, 1993).

Seaweed extracts have been used as a cytokinin-like growth regulator and exhibit multiple functions in turf management (Schmidt, 1990). When applied to plants, SE stimulates shoot growth

and branching (Temple and Bomke, 1989), increase root growth and lateral root development (Metting et al., 1990), improve nutrient uptake (Yan , 1993), enhance resistance to diseases (Featan by-Smith et al., 1983) and environmental stresses such as drought and salinity (Nabati, 1991; Nabati et al., 1994). It has been observed that cytokinin or cytokinin-like growth regulators can inhibit the activity of free radical groups, hydrogen peroxide, and superoxide, which are the major elements for chlorophyll degradation during senescence (Sexton and Woulhouse, 1984). Under drought stress, they may replace endogenous cytokinin whose production is reduced by stress conditions (Fletcher et al, 1988). It has been indicated that a range of naturally occurring and synthetic cytokinins can enhance stomatal aperture, transpiration, and the protein (polysomes/ribosomes) associated with activity of guard cells(Jewer and Incoll, 1980).

Humic acid

Humic acid is one of the major components of humus. Humates are natural organic substances, high in humic acid and containing most of known trace minerals necessary to the development of plant life (Senn, 1991).

Humates have long been used as a soil conditioner, fertilizer, soil supplement. Humus holds not just micronutrient metal ions, but also the essential macronutrients: nitrate, phosphate, and potassium. The significance of humic acids is not just limited to its function as a reservoir of mineral plant nutrients and regulator of their liberation (Senn, 1991). Recent research showed that humic acid can be used as a growth regulator to regulate hormone level, improve plant growth and enhance stress tolerance (Piccolo, 1992).

Tan et al. (1979) indicated that humic acid (HA) was in general beneficial to shoot and root growth of corn plants. Dry matter yield in corn shoots was stimulated by HA, especially when the plants were treated with HA at a concentration of 640 ppm. Fagbenro and Agboola (1993) noted that HA was not only beneficial to the growth but also nutrient uptake of teak seedlings. The uptake of N, P, K, Mg, Ca, Zn, Fe, and Cu by the seedlings was increased with application of HA. Humic acid produced a proliferation of roots at optimum concentration of 0.05%. These results showed that HA can stimulate plant growth and nutrients uptake at relatively low concentration. The stimulating effects of HA may be related to its hormone activity.

O'Donnell (1973) found that HA from leonardite exhibits auxin-like effects. It greatly enhanced root initiation of *Pelargonium hortorum* cuttings. Piccolo et al. (1992) indicated that humic extract with the highest acidity and smallest molecular size is the only material showing auxin-like activity.

Several hypotheses have been proposed to explain the effect of HA. These include the formation of complex between HA and mineral ions, catalysis of HA to enzymes in plant, influence of HA on respiration and photosynthesis, stimulation of nucleic acid metabolism (Schnitzer and Khan, 1972), and hormone activity of HA (O'Donnell, 1973).

Recent research showed that HA, when applied to turfgrass, can stimulate shoot and root growth, and improve resistance to environmental stress in turfgrass (Schmidt, 1990; Goatley and Schmidt, 1990). This observation is consistent with the results of Piccolo (1992), who showed that HA exhibits an auxin-like activity. However, the mechanism of these two PGRs' stimulating effects on plants has not been well established.

Water stress, reactive oxygen species, and plant antioxidant systems

Increasing evidence suggests that many damaging environmental stresses, such as drought, salinity, temperature, herbicides, etc. have their effects, directly or indirectly, through the formation of reactive oxygen species following impairment of the electron transport system (Ahuja and Kaur, 1985; Alscher and Hess, 1993; Badiani et al., 1990; Barden and Bramlage, 1994; Bowler, 1992; Buckland et al., 1991; Gallerani et al., 1990; Gillham and Dodge, 1987; Hess, 1992; Law et al., 1983; Moran et al. 1994; Moran et al., 1994; Price and Hendry, 1987; Quartacci et al., 1992; Smirnoff, 1993; Spychalla et al., 1990). Water stress is known to cause various physiological and biological effects on plants. The decrease in photosynthesis (Kaiser, 1987; Lawlor, 1995; Ludlow and Powles, 1988), the closure of stomata (Chaves, 1991), and osmotic adjustment (Nelson and Orcutt, 1996; Sen Gupta, 1988) appear to be typical plant responses to water deficit at the first stage. Closure of stomata and reduction of photosynthesis in plant leaves disturb the supply of CO_2 for photosynthesis and expose chloroplasts to excess excitation energy (Tanaka et al., 1990). When water-stressed leaves were illuminated, the reducing power from the photosystem was not consumed

for CO_2 fixation but for the activation of O_2 (Asada et al., 1983). Excess reactive oxygen species are accumulated in the cell under drought (Moran et al., 1994).

Reactive oxygen species are produced by excess transfer energy from triplet excited chlorophyll to oxygen (singlet oxygen formation) or photoreduction of oxygen (formation of superoxide, hydrogen peroxide, and hydroxyl radical) (Mckersie and Leshem, 1994). Excess accumulation of reactive oxygen species results in a series of oxidative injuries to plants (Elstner 1982; 1991; Hess, 1993; Winston, 1990; Wolff, 1986).

Oxidative injury involves the initial formation of reactive oxygen species and its subsequent reaction with macromolecules. Proteins, lipids, polysaccharides and nucleic acids can be damaged (Davies, 1987; Elstner, 1991; Mckersie and Leshem, 1994; Thompson et al., 1987). As a result, normal cell metabolism can be seriously disturbed.

Plants possess intrinsic antioxidant defense mechanisms for coping with reactive oxygen species (Salin, 1987). Essentially, antioxidant defensive systems fall into three general classes: 1) the lipid- soluble, membrane-associated antioxidants (e.g.α-tocopherol, β-carotene); 2) the water-soluble reductants (e.g. glutathione, ascorbate); and 3) enzymatic antioxidants (e.g. superoxide dismutase, catalase and enzymes of the ascorbate/glutathione cycle (Smirnoff, 1995; Zhang and Kirkman, 1994). It has been suggested that the formation of reactive oxygen species is an inherent consequence of metabolism and that control of their levels is essential for normal function. The toxicity of an externally imposed biotic or abiotic oxidative stress can be partly attributed to the overriding of existing resistance mechanisms. Only when those mechanisms are overwhelmed would injury occur (Doulis, 1994). This suggests that the strengthening of the defense mechanisms, through enhancing the functions of their components (such as ascobic acid, α-tocopherol, β-carotene and superoxide dismutase) may reduce or prevent oxidative injury and improve water stress resistance of plants.

Alpha-tocopherol. Alpha-tocopherol (Vitamin E or 5,7,8 trimethyl-tocol) plays a unique role as an antioxidant and a stabilizer for biological membranes. It is synthesized in chloroplasts and proplastids and located in membranes of the cell, especially the thylakoid membranes of the chloroplasts. The structure and location of α-tocopherol determine its function as a membrane

stablizer (Hess, 1993; Smirnoff, 1995). Development of HPLC technology facilitates the separation and determination of α-tocopherol accurately (Hakansson et al., 1987; Lumley, 1993; Pocklington and Dieflenbacher, 1988; Weber, 1984; Yao, 1990).

Alpha-tocopherol, concentrated in chloroplasts and present in high concentrations both in chloroplast envelope and in the thylakoid membranes, traps lipid peroxy radicals in the deep inner regions of membranes. It stabilizes the bilayer by affecting lipid organization (Kagan, 1989), and protects chlorophyll against red light-induced degradation (Van Hasselt et al., 1979). Alpha-tocopherol is also shown to protect the plant against various environmental stresses such as drought (Hess, 1993; Price and Hendry, 1987; Smirnoff, 1993; Winston, 1990).

Ascorbic acid (AA). Ascorbate (vitamin C) occurs in all plant tissues, usually being higher in photosynthetic cells and meristems (and some fruits). About 30 to 40% of the total ascorbate is in the chloroplast, and stromal concentrations as high as 50 mM have been reported (Foyer, 1993). It is highest in the mature leaf, where the chloroplasts are fully developed and the chlorophyll levels are highest. Although it has been determined that D-glucose is the precursor of L-ascorbic acid, the synthetic pathway has not been totally understood (Smirnoff, 1995).

Ascorbic acid has effects on many physiological processes including the regulation of growth, differentiation and metabolism of plants (Foyer, 1993). A fundamental role of AA in the plant defense system is to protect metabolic processes against H_2O_2 and other toxic derivatives of oxygen. Acting essentially as a reductant and reacting with and scavenging many types of free radicals**,** AA reacts non-enzymatically with superoxide, hydrogen peroxide, and singlet oxygen . It can react indirectly by regenerating α-tocopherol or in the synthesis of zeaxanthin in the xanthophyll cycle. Therefore, AA influences many enzyme activities, and minimizes the damage caused by oxidative process through synergic function with other antioxidants(Asada et al., 1992; Chinoy, 1969; Foyer, 1993; Kuner and Ederer, 1985).

Beta-carotene. Beta-carotene is present in the chloroplasts of all green plants. It is exclusively bound to the core complexes of PSI and PSII. Protection against the damaging effects of oxygen at this site is essential for chloroplast functioning. β-carotene, as an accesory pigment and

an effctive antioxidant, plays a unique role in protecting photochemical processes (Burton and Ingold, 1984).

The carotenoids can exist in a ground state or in one of two excited states after the absorption of light energy. In terms of their antioxidant properties, carotenoids can protect the photosystems in one of four ways: a) by reacting with lipid peroxidation products to terminate chain reactions; b) by scavenging singlet oxygen and dissipating the energy as heat; c) by reacting with triplet or excited chlorophyll molecules to prevent formation of singlet oxygen; d) by the dissipating the excess excitation energy through the xanthophyll cycle (Demmig et al., 1988). The main protective role of β-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and therefore avoids oxidative stress completely.

Superoxide dismutase. Superoxide dismutases (SODs), discovered by McCord and Fridovich in 1969, are ubiquitous metalloenzymes that catalyze the disproportionation of superoxide radical anions to dioxygen and hydrogen peroxide. Superoxide dismutases are the most efficient scavengers of the superoxide anion and an essential component of the ascorbate-glutathione cycle (Bannister et al., 1987; Foster and Hess, 1982; Beyer et al., 1991; Halliwell, 1974; Harper and Harvey, 1978; Monk et al., 1989; Nakano et al., 1980) for the detoxification of toxic oxygen species. Higher plants have SODs containing Cu and Zn, Fe, or Mn as prosthetic metals. Copper/ Zn-SOD is found in both chloroplasts and cytosols, whereas Mn-SOD is found in the matrix of mitochondria. Iron-SOD has been reported in chloroplasts, mitochondria, and peroxisomes of petals in a few plants (Bowler et al.,1992).

Superoxide dismutase has been one of the most widely studied enzymes in the antioxidant system (Bowler, 1991; 1994). The importance of SOD for aerobic growth has been established by demonstration that the SOD-deficient mutant of *E. coli* (Carlioz and Tonati, 1986) are hypersensitive to oxygen. Recent research indicates that tobacco and alfalfa expressing high levels of Mn-SOD targeted to chloroplasts or mitochondria are more tolerant to paraquat and freezing, respectively (Bowler et al., 1991; Mckersie et al., 1994). Copper/Zn-SOD over-expression in tobacco (Sen Gupta et al., 1993; Perl et al., 1993) increases tolerance to oxidative treatments. In the case of tobacco over-expressing Cu/Zn-SOD in the cytoplasm and chloroplasts, the plants are

markedly more resistant to loss of photosynthetic capacity (photoinhibition) when exposed to high light intensity at low temperature (Sen Gupta et al.,1993). Several researchers have indicated that SODs play an important role in water stress tolerance of plants (Bowler, 1992).

Plant antioxidants and water stress tolerance

Oxidative stress, characterized by excess accumulation of reactive oxygen radicals, could result from various environmental stresses such as water stress (Dhindsa, 1991; Gamble and Burke, 1984; Seel et al., 1992; Senaratna et al., 1984; Smirnoff, 1988; 1993), chilling stress (Wise and Naylor, 1987), air pollution (Fryer et al., 1992), and herbicides (Doulis, 1994). It was noted that antioxidant activity is significantly different between stress-sensitive and stress - tolerant cultivars of the same species when they are grown under stress. In other words, plant antioxidants are sensitive to stress, and their activity may be related to the capacity of plant stress tolerance (Bowler, 1992; Stuhlfanth et al., 1990).

Alpha-tocopherol. Photosynthetic apparatus and membrane could be affected by water stress. Alpha-tocopherol is a lipid-soluble antioxidant associated with biological membrane of cells, especially the membrane of photosynthetic apparatus (Lawlor, 1995). Research has shown that water-deficiency may result in an increase of tocopherol concentration in plant tissues (Hashim et al., 1993; Price and Hendry, 1989, 1991; Moran et al., 1994). Burger et al. (1951) noted that tocopherol content of soybean leaves was increased as the amount of rainfall decreased. This is consistent with the reports of Tanaka et al. (1990), who showed that subjecting spinach to water deficit increased the content of α-tocopherol in the leaves. Based on the studies of 10 different grass species under water stress, Price and Hendry (1989) found that drought stress led to an increase of 1 to 3-fold of α-tocopherol concentration in 9 out of 10 species. They pointed out that the species with a high tolerance of stress are defended through tocopherol. Moreover, highly significant correlations were observed between stress tolerance and γ-tocopherol concentration (the precursor of α-tocopherol; Spearmans rank correlation coefficient $r = 0.731$).

Ascorbic acid. Ascorbic acid is distributed in the cytosol as a water-soluble antioxidant. Dry (and thus sunnier and warmer) conditions have been noted to increase the AA content in turnip greens (Reder et al., 1943), onions, and black currant (Shirochenkova et al., 1985). Black currant grown in hot, dry years were found to contain more than twice the AA of those grown in wet years. Stocker (1960) has reported an enhancement in AA content of plants which were subjected to severe water stress. Research results indicate that, under moderately dry conditions, a slight increase in the total AA concentration occurred. There was an extraordinary upsurge in the total AA content along with abnormally high oxidation-reduction ratio and very high level of respiration under extreme drought conditions, suggesting a complete breakdown of the metabolism (Chinoy et al., 1969). Mukherjee and Choudhuri (1983) noted that AA increased significantly under water stress. In contrast, Price and Hendry (1989) found a decrease of ascorbate content of drought-stressed treatment in 9 out of 10 grass species. It is obvious that the relationship between antioxidant activity and drought stress tolerance is still not clear.

Beta-carotene. Beta-carotene can protect chlorophyll and prevent photoinhibition. It is closely associated with photosynthetic apparatus and quenches singlet oxygen efficiently. Stuhlfauth et al. (1990) found that acclimation of *Digitalis lannta* to low water potential results in a 25% increase of β-carotene.

Superoxide dismutase. Superoxide dismutases are closely related to the capability of plant tolerance to various stresses, such as water stress, chilling stress, herbicides, and pathogens (Ahuja and Kaur, 1985; Badiani et al., 1990; Bowler et al., 1992). It was reported that SOD enhances water stress tolerance of plants. In tomato, cytosolic Cu/Zn-SOD was induced strongly by drought, while chloroplastic Cu/Zn-SOD remained largely unaffected (Bowler et al.,1992). In two mosses, the drought-tolerant *Tortula ruralis* and drought-sensitive *Cratoneuron filicinum* were compared (Dhindsa et al., 1981). The drought-tolerant moss showed lower levels of lipid peroxidation, together with increased levels of SOD; the opposite occurred in the sensitive moss. Drought tolerant and intolerant maize inbreds were analyzed by Malan et al. (1990), and resistance was found to correlate with Cu/Zn-SOD. Drought stressed tomato showed increases both in

transcript levels of cytosolic Cu/Zn-SOD gene (eight-fold) and enzyme activity (five-fold), whereas an increase in enzyme activity was observed in chloroplastic Cu/Zn-SOD (2.4-fold) without a corresponding increase in transcript levels (Perl et al., 1993).

Plant growth regulators, antioxidant activity, and water stress tolerance

Both plant hormones and antioxidants are endogenous organic substances that regulate plant growth and development. They play an important role in plant tolerance to water stress. As water potential decreases, increased ABA and reduced cytokinin result in stomatal closure. GA and IAA are reduced due to the water stress. Ethylene increases rapidly as a response to water stress. The alteration of these hormones may facilitate plants to adapt to stressful environment and improve water stress tolerance by retaining moisture in the plant tissues (Saab et al., 1990).

It has been noted that various antioxidants change rapidly as water potential decreases. In most cases, α-tocopherol, β-carotene, and SODs are increased significantly under water stress (Bowler, 1992; Smirnoff 1993). The change of the antioxidants may enhance water stress resistance of plants through detoxifying reactive oxygen species.

The biochemical relationship between plant hormones and antioxidant responses to water stress is being revealed (Hildebrand and Grayburn, 1991). It was noted that cytokinin can not only prevent the formation of reactive oxygen species, but also scavenge the free radicals (Heshem, 1981). Therefore, cytokinin may play a dual role in suppressing free radical damage and water stress injury. Seaweed extracts, as a cytokinin - containing material, may increase antioxidant activity and improve water stress resistance through suppressing reactive oxygen species.

Water stress induces ethylence production (Apelbaum and Yang, 1981; Kacperska and Kuback-Zebalska, 1989). Hildebrand and Grayburn (1991) reported that the increase of ethylene under oxidative stress is associated with lipid peroxidation caused by activated oxygen radicals. Lipoxygenases (linoleate: oxygen oxidoreductase, EC 1.13.11.12) are a class of dioxygenases that catalyze the peroxidation of polyunsaturated fatty acids and other molecules containing *cis,cis*-1,4-pentadiene moieties. Kacperska and Kukacka-Zebalska (1985) noted the similarities between lipoxygenase activity and ethylene production and proposed lipoxygenase as a possible in vivo ethylene - forming enzyme. A recent study showed that this enzyme is also involved in oxidative

stress response (Hildebrand and Grayburn, 1991). It may be concluded that possible linkage exists between antioxidant defense system and hormones action under stress.

There are a few reports on influences of exogenous PGRs on antioxidant status. Yokoyama et al. (1991) showed that the synthesized PGR enhanced β-carotene concentration substantially. GA and IAA were found to increase AA content in potato and soybean sprouts (Kiryukhin, 1969; Kim, 1988). Pauls and Thompson (1982) found that cytokinin and antioxidants influenced cell membrane tolerance to ozone damage. Ethephon increased AA content in 7 out of 21 cases, and deceased AA content in 8 out of 21 cases (Mozafar, 1994). Almost all these experiments were conducted to evaluate effects of PGRs on vitamin contents of vegetable crops in normal growing conditions. No drought stress treatment was involved in these studies. Little is known about influences of PGRs on antioxidant activities and then drought tolerance in turfgrasses under drought stress conditions. However, these studies do suggest that it is possible to regulate antioxidant activities with exogenous PGRs.

There is a need to examine the influence of PGRs on antioxidants activities under drought stress and well-watered condition, and investigate the relationship among PGR application, antioxidant status, and drought stress tolerance in different kinds of turfgrasses. Information from this study is important to understand functions of PGRs, reveal the physiological mechanism of drought stress, and improve turfgrass quality.

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Chapter 3

Influence of plant growth regulators on turfgrass growth and disease resistance

Introduction

Turfgrass growth and quality are significantly impacted by various biotic and abiotic stresses. Traditionally, a high quality turf is maintained through proper fertilization, irrigation, mowing, disease control and other cultivation practices (Watschke and Schmidt, 1992). However, as water resources decline, drought stress becomes a major limiting factor in turf management in many parts of the world (Kenna and Horst, 1993). Water stress causes stomatal closure, reduces photosynthesis, and suppresses growth (Bjorkman and Powles, 1984; Gollan et al., 1986; Smith and Griffiths, 1993). Additionally, improper management of turfgrass, such as excess nitrogen application, results in increased disease occurrence and thus poor turf quality.

Utilization of plant growth regulators has become an accepted practice in some turfgrass management systems (Yan et al. 1993). Traditionally, PGRs are used as growth retardants to suppress shoot growth and thus reduce mowing frequency (Schott and Walter, 1991). Most of these PGRs are synthesized inorganic or organic chemicals, and some of them are herbicides. The large amounts of these chemicals used during the growing season has increased environmental concerns (Beard, 1982). In the past decade, hormone-containing natural products, a group of PGRs, have received increasing interest (Crouch, 1990; Schmidt, 1990; Senn, 1991). The PGSs not only improve turfgrass growth and stress resistance but also avoid environmental pollution (Beckett et al., 1990; Crouch, 1990; Yan et al.1993).

Humic acid (HA) and seaweed extract (SE) are two plant growth regulators. Seaweed extract contains not only various mineral nutrients but also phytohormones and vitamins (Blunden, 1977; Brain et al., 1973; Senn, 1987). When applied to plants, SE not only increases endogenous cytokinin concentration but also enhances nutrient uptake, increase shoot and root growth, increases chlorophyll content, and improves seed gemination (Finnie and Van-Staden, 1985; Van-Staden et al.,1994; Yan, 1993). Seaweed extract has been shown to improve turfgrass growth and increase

tolerance to environmental stresses (Nabati, 1991; Nabati et al. 1994). Humic acid contains valuable plant nutritional substances not found in chemical fertilizers (Senn,1991). Research showed that HA, which possess auxin-like activity, can be used as a growth regulator to regulate endogenous hormone levels, improve plant growth, and facilitate stress tolerance (Frgbenro and Agboola, 1993; O'Donnell, 1973; Piccolo, 1992).

Dollarspot, caused by *Sclerotinia homoeocarpa* Bennett, is a serious disease of turfgrass on golf course putting greens, closely mown fairways, bowling greens, and home lawns (Goodman and Burpee, 1991). Creeping bentgrass and annual bluegrass are highly susceptible to ***S***. *homoeocarpa*. Dollarspot has traditionally been reduced with high rates of N fertilization, adequate water supply, avoidance or removal of dew and guttation fluid from turf, and the application of fungicides (Vargas, 1981). No dollarspot-resistant cultivars of creeping bentgrass or annual bluegrass are available (Goodman and Burpee, 1991).

Since disease-free golf greens and fairways are highly valued, frequent applications of inorganic or synthetic-organic fungicides are necessary for controlling this disease (Smiley, 1983; Vargas, 1981). However, large amounts of fungicides used each year could result in environmental pollution. Therefore, development and implementation of an alternative control for dollarspot may reduce fungicide (such as Banner) use and minimize the risk of the pollution of water resources. Little research has been reported on the effects of the PGR, such as SE and HA, in controlling this disease.

The objectives of this research were to evaluate the influences of HA on growth in three turfgrass species (experiment I), and examine the effects of HA and SE in controlling dollarspot in creeping bentgrass (experiment II).

Materials and methods

Experiment I

This greenhouse study used Kentucky bluegrass (*Poa Pratensis* L. cv. 'Plush'), tall fescue (*Festuca arundinacea* Schreb. cv.' Rebel Jr.') and creeping bentgrass (*Agrostis palustris* Huds. cv. 'Penncross') which were established at the Turfgrass Research Center at Virginia Tech, Blacksburg, Va in September 30, 1992 on a Groseclose silt loam (a clayey, kaolinitic, mesic Typic Hapludult)

with pH of 6.2. Soil P and K content at the time of this experiment were 21 and 34 ug g^{-1}, respectively. Urea was applied at 50 kg N ha^{-1} in May, 1993.

Plugs (10 cm in diameter) were taken from the field on 2 March 1993 and placed in 10 cm x 5-cm plastic rings with a hardware cloth bottom. On the next day, the plugs were treated with HA (2.9% active humic acid) (Plant Wise Biostimulant Co., Louisville, KY) at the rates of 0, 11.8, 23.7, and 47.4 l ha^{-1}. The HA was diluted with water and spread evenly on foliage with a 40 cc syringe. The needle of the syringe was modified to generate the mist of the liquid. The plugs which were to be treated were laid out within an area of 4 $foot^2$. The diluted humic acid (30 ml) was applied to this area. The treated plugs were permitted to grow under a rain shelter.

Fifteen kilograms of dry Groseclose silt loam soil adjusted to 11.6 % moisture level (which was equivalent to a water potential of -0.5 MPa) (Nabati, 1991) were placed into terrarium-like boxes (30 x 40 x 75 cm) constructed with 0.15 mm clear plastic sides and tops. Each terrarium received 3 g of 20-20-20 fertilizer.

One week after treatment with HA, the bottom of the rings was removed and the plugs were transplanted into the terrariums. Each terrarium received eight plugs. The terrariums were sealed and placed in an air-conditioned greenhouse in which the temperature was maintained at 21^{o}C in the day and 18^{o} C at night. The average daily air temperatures inside the terrariums obtained with thermocouples were 22^{o} C and 24 ^{o}C at 8 am and 2 pm, respectively, during the growing season. The tops of the terraria were removed each week to check for disease or wilting and then sealed. No additional HA, fertilizer, or water were added for the period of the experiment.

The water stress level of leaf (WSL) was measured with a hydraulic leaf press (Campbell Scientific Inc, Logan, Utah) according to the procedures described by Nabati (1991) and Yan (1993) 2 and 4 weeks after transplanting. The last fully emerged leaf from a tiller were removed and placed in a Champbell-brewster leaf press chamber. The chamber was activated by a hydraulic pump that was capable of compressing the leaf. The relative pressure that caused water to be exudated uniformly from the tip, bottom, and edges of the leaf was recorded (Nabati, 1991). The greater the relative pressure required to force water from the leaf cells, the less the leaf moisture content. The mean of three measurements from each treated plug were taken for leaf water status.

Four weeks after transplanting, the plant height was measured and the shoots were collected

and weighed. Thereafter, the plugs were gently removed from the terrariums and the soil was washed from the roots. The root length was measured, and root dry weights were determined after drying in an oven at 60°C for 24 hours.

A randomized complete block design was used, and treatments were replicated four times. The means of treatment effects were compared based on a least significant difference at $\alpha = 0.1$.

Experiment II

This field study used creeping bentgrass (*Agrostis palustris* Huds A. *stolonifera* L. cv. "Penncross") which was established in the Turfgrass Research Center at Virginia Tech, Blacksburg, Virginia in September 1992 on a Groseclose silt loam (a clayey, kaolinitic, mesic Typic Hapludult) with a pH of 6.2. Soil P and K content at the time of this experiment were 21 and 34 *u*g g^{-1}, respectively. Urea was applied at 50 kg N ha^{-1} in May 1993.

Plots, 1.5 m x 1.5 m, were treated with HA and seaweed (*Ascophyllum nodosum*) extract (SE) on July and August 1993 when dollarspot disease was prevalent. The rate of HA (2.9% active humic acid) was 1.7, 5.2, 15.5 and 47.4 l ha^{-1}. The soluble SE was dissolved in water and applied at rates of 163 and 326 g ha^{-1}. The SE solution was sprayed at a rate of 35 ml m^2. A compressed-air boom sprayer that delivered 123 l ha^{-1} of solution at a pressure of 276 KPa was used for foliage application. Both SE and HA were supplied from Plant Wise Biostimulant Co. (Louisville, KY). All treatments were arranged in a randomized complete experimental block design with four replications. Dollarspot incidence was evaluated based on a visual scale of 1 to 9 with 9 indicating most disease incidence from one week after treatment. Meanwhile, the leaf color was also evaluated based on a visual scale of 1 to 9 with 9 indicating the greenest color or the best turf quality.

The means of treatment effects on dollarspot and leaf color were compared based on a least significant difference (LSD) at $\alpha = 0.1$.

Results

Experiment I.

Water status in terms of water stress level (WSL) of Kentucky bluegrass, tall fescue and creeping bentgrass was significantly improved with foliar application of HA (Table 3.1). As measured with a hydraulic press, all turfgrasses treated with various levels of HA showed higher leaf

moisture content as compared with the non-treated control at 3 and 5 weeks after treatment.

The leaf water status was improved as the rate of HA increased beginning at the third week after treatment. The high rate of HA exhibited greater effect on tall fescue and Kentucky bluegrass leaf moisture content at the third week after HA application. However, leaf water status became similar among the three HA treatments on creeping bentgrass at three weeks and all grasses at five weeks after treatment, which was significantly higher when compared with the control (Table 3.1).

There was substantial difference in leaf water status among the three grass species. Tall fescue generally exhibited better water status as compared with Kentucky bluegrass and creeping bentgrass (Table 3.1).

Table 3.2 shows that root length and root dry weight were increased in the three grass species treated with high doses of HA. However, the effects of HA on root length and root dry weight differed with grass species.

The greater mass and deeper root systems of all grass species stimulated with exogenous application of HA may be one aspect in improving drought tolerance because of greater efficiency in water absorption from the soil low in moisture.

Foliar application of HA enhanced plant height of all three grass species studied (Table 3.3). The clipping dry weight was increased significantly in tall fescue treated with HA applied at 23.7 l ha^{-1}. All doses of HA significantly enhanced clipping yields of Kentucky bluegrass. However, the enhancement of creeping bentgrass clipping yields associated with HA treatments did not differ significantly from the non-treated bentgrass. It appears that effects of HA doses differs with grass species.

Experiment II.

Visual evaluation for dollarspot incidence in creeping bentgrass treated with different rates of HA and SE indicates that in early August, lower dollarspot incidence was present in response to either the higher rates (326 g ha^{-1}) of SE or medium rates (15.5 l ha^{-1}) of HA (Table 3.4). The HA did not impact dollarspot incidence when applied at the highest (46 l ha^{-1}) or lowest rate (1.72 l ha^{-1}). In addition, the low doses of SE did not reduce dollarspot incidence during July through September.

The leaf color of dollarspot-infected creeping bentgrass was improved with application of HA or SE (Table 3.5). During August and September, the leaf color of PGR-treated turf was

significantly greener than that of non-treated turf. This color improvement can be attributed to less dollarspot incidence and/or delayed senescence.

The correlation between dollarspot incidence and leaf color was significant ($R^2 = 0.7163$; $P < 0.05$) (Fig.3.1). This indicated that turf quality in terms of leaf color can be improved by controlling dollarspot disease with SE or HA.

Table 3.1. Water stress level as influenced by humic acid (HA) in the three turfgrass species grown under low (-0.5 MPa) soil moisture.

HA Amount	Water Stress Level (Relative pressure)		
(l ha^{-1})	tall fescue	Kentucky bluegrass	creeping bentgrass
	---------- 24 March ----------		
0	117.6 a	172.0 a	180.0 a
11.8	108.0 ab	156.8 ab	171.2 a
23.7	92.3 ab	149.0 ab	135.0 b
47.4	90.6 b	135.0 b	135.5 b
	----------11 April ----------		
0	175.5 a	182.9 a	168.8 a
11.8	117.5 b	137.1 b	146.8 bc
23.7	125.4 b	136.7 b	133.8 c
47.4	129.1b	144.5 b	130.2 c

The plugs (10-cm diameter) were taken from the field and transplanted into the terrariums on 2 March 1993. The plugs were grown under the low soil moisture (-0.5 MPa) for 6 weeks.
The humic acid product contains 2.9 % active humic acid.
Within columns, means followed by same letters are not significantly different based on LSD at $\alpha = 0.1$

Table 3.2. Root growth as influenced by humic acid (HA) in the three turfgrass species grown under low (-0.5 MPa) soil moisture.

HA Amount ($l\ ha^{-1}$)	tall fescue	Kentucky bluegrass	creeping bentgrass
	---------- Root Length (cm) ----------		
0	10.3 b	9.0 b	3.7 b
11.8	13.8 ab	13.3 ab	5.5 a
23.7	13.6 ab	13.6 ab	5.6 a
47.4	16.9 a	17.8 a	4.3 b
	----------Root Dry Weight (mg $plug^{-1}$) ----------		
0	26.0 b	21.3 b	68.7 c
11.8	38.0 a	23.0 b	81.5 bc
23.7	33.8 ab	30.0 ab	100.0 ab
47.4	41.0 a	35.0 a	121.0 a

The plugs (10-cm diameter) were taken from the field and transplanted into the terrariums on 2 March 1993. The plugs were grown under the low soil moisture (-0.5 MPa) for 6 weeks.
The humic acid product contains 2.9 % active humic acid.
Within columns, means followed by same letters are not significantly different based on LSD at $\alpha = 0.1$.

Table 3.3. Shoot growth as influenced by humic acid (HA) in the three turfgrass species grown under low (-0.5 MPa) soil moisture.

HA Amount ($l\ ha^{-1}$)	tall fescue	Kentucky bluegrass	creeping bentgrass
	----------Plant Height (cm) ----------		
0	14.5 b	11.3 b	10.5 b
11.8	19.0 a	12.4 b	12.4 ab
23.7	20.4 a	15.9 a	12.3 ab
47.4	18.5 a	15.8 a	14.4 a
	---------- Shoot Dry Weight (mg $plug^{-1}$) ----------		
0	159.7 c	75.5 c	110.5 a
11.8	215.8 b	111.0 b	122.8 a
23.7	268.0 a	101.8 b	120.0 a
47.4	153.7 c	133.38 a	124.5 a

The plugs (10-cm diameter) were taken from the field and transplanted into the terrariums on 2 March 1993. The plugs were grown under the low soil moisture (-0.5 MPa) for 6 weeks.
The humic acid product contains 2.9 % active humic acid.
Within columns, means followed by same letters are not significantly different based on LSD at $\alpha = 0.1$.

Table 3.4. Dollarspot incidence of creeping bentgrass as influenced by plant growth regulator (humic acid or seaweed extracts)

	Amount[a]		Dollarspot Incidence[b]		
PGRs	HA (l ha^{-1})	SE (g ha^{-1})	21 July	3 Aug.	23 Sept.
HA	46.7	0	4.0 a	3.5 ab	3.0 b
HA	15.5	0	3.0 ab	2.5 b	2.0 c
HA	5.2	0	4.0 a	3.3 b	1.8 c
HA	1.7	0	4.0 a	4.5 a	3.8 a
SE	0	326	2.8 b	2.5 b	4.0 a
SE	0	163	4.0 a	4.5 a	4.0 a
Control	0	0	3.8 a	4.5 a	3.3 ab

[a] The humic acid product contain 2.9% active humic acid.
[b] The dollarspot incidence was visually rated based on a scale of 1 to 9 with 9 = the severest dollarspot incidence.
Within same columns, means followed by same letters are not significantly different based on LSD at $\alpha = 0.1$.

Table 3.5. The leaf color of creeping bentgrass as influenced by plant growth regulator (humic acid or seaweed extracts)

PGRs	Amount		Leaf Color[a]		
	HA (l ha^{-1})	SE (g ha^{-1})	21 July	3 Aug.	23 Sept.
HA	46.7	0	6.8 a	7.0 ab	5.5 b
HA	15.5	0	7.5 a	7.5 a	5.8 ab
HA	5.2	0	7.0 a	7.3 a	6.5 a
HA	1.7	0	6.8 a	6.8 b	5.3 b
SE	0	326	7.3 a	7.4 a	5.5 b
SE	0	163	6.9 a	7.1 ab	5.0 bc
Control	0	0	7.0 a	6.5 b	4.3 c

[a]The leaf color was visually rated based a scale of 1 to 9 (9 = greenest).
Means followed by same letters within same column are not significantly different based on LSD at $\alpha = 0.1$.

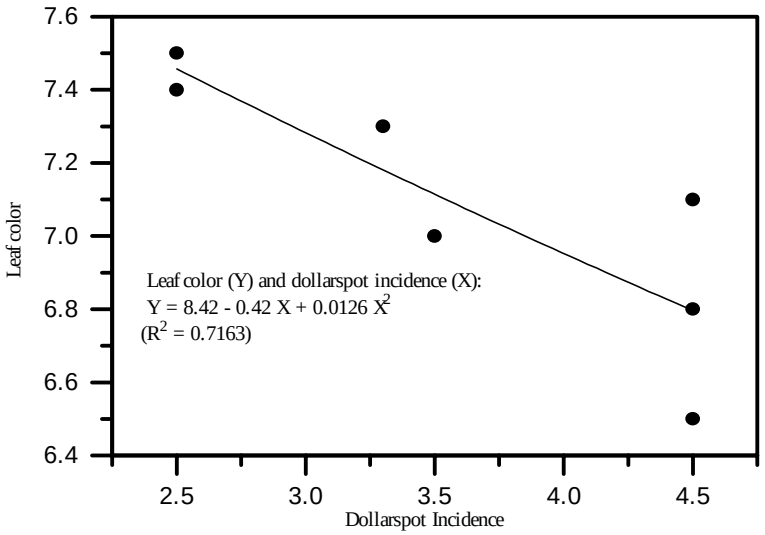

Fig.3.1. Relationship between leaf color and dollarspot incidence in creeping bentgrass (Aug. 1993)

Discussion

Leaf water status was improved with foliar application of proper rates of HA in all three turfgrass species (Table 3.1). Since the turfgrasses were grown in low soil moisture conditions in this study, increased water content in the leaf tissues may indicate better drought tolerance. In other words, HA may influence plant growth through impacting plant water relations.

Root development was enhanced with application of higher rates of HA in the three turfgrass species (Table 3.2). This is consistent with the results by Tan et al. (1979), and Fagbenro and Agboola (1993) who indicated a beneficial effect of HA on root growth in several plant species. O'Donnell (1973) noted that HA from leonardite exhibits auxin-like effects and greatly enhanced root initiation of *Pelargonium hortorun* cuttings. The results of this study showed that HA may enhance both root initiation (numbers) and individual root length. The hormones contained in the PGR may enhance the capacity of the plants to retain moisture, and the greater root system may contribute to the better water status of the leaves.

Schnitzer and Khan (1972) noted that humic substances may indirectly impact nutrient uptake through the function of HA and fulvic acid (FA), and directly impact the plants when they were absorbed by roots. They indicated that HA promoted the accumulation of reducible sugar, which increased wilting resistance through enhancing the osmotic pressure inside plants. Enhancement by HA of peroxidase activity, seed germination, nutrient uptake, and root growth were also observed by several researchers. Schnitzer and Poapst (1967) indicated that application of FA (3000-6000 ppm), extracts from a Podzol Bh horizon, enhanced root formation by 300% in bean stem segments. These results suggest that HA may promote root growth and moisture status of leaves by enhancing osmotic adjustment and root initiation.

Humic acid increased clipping production, although not statistically significant in creeping bentgrass. This supports the results with corn by Tan et al. (1979). Fagbenro and Agboola (1993) found HA enhanced not only plant growth but also nutrient uptake of teak seedlings. Humic acid influences respiration and photosynthesis (Schnitzer and Khan, 1972) and hormone activity (O'Donnell, 1973). This study showed that HA exhibited an effect on root development which may

be responsible for the better water relations and shoot growth of turfgrasses under stress.

Humic acid or SE suppressed dollarspot incidence of creeping bentgrass when they were applied at a proper rate. However, the pathological mechanism of these hormone-containing materials' impact on this disease has not been established.

A significant negative correlation between leaf color and dollarspot incidence suggests that turfgrass leaf color and thus quality can be improved by controlling dollarspot incidence through application of plant growth regulators in creeping bentgrass. The incidence of dollarspot, a serious fungal disease (Fenstermacher, 1980), is increased when turfgrass suffers from various environmental stresses, such as drought and high temperature (Hall et al., 1993; Vargas, 1981). Seaweed extract and HA may suppress dollarspot incidence and improve leaf color by improving plant water relations and growth (Table 3.1 and Table 3.2).

Nabati (1991) reported that HA and SE enhanced drought tolerance of Kentucky bluegrass. Exogenous cytokinin has been shown to reduce the stomatal resistance and increase photosynthesis (Arteca, 1996). Yokoyama et al. (1991) and Kim (1988) found that the growth regulators enhanced antioxidant concentration, such as β-carotene and ascorbic acid, in several plant species. Since HA or SE, the two PGRs, contain various micronutrients, vitamins, and hormones, the enhanced disease resistance of creeping bentgrass with application of these PGRs may be related to the hormonal activity and antioxidants (vitamins) status. Further research is needed to examine the impacts of these PGRs on stress tolerance and antioxidants status of turfgrass.

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Chapter 4

Alpha-tocopherol status and growth responses of Kentucky bluegrass to plant growth regulators and drought

Introduction

Water deficits limit the growth and distribution of natural vegetation and the performance of cultivated plants more than any other environmental factors (Kramer, 1983). Water stress causes various physiological and biological changes of plants. The decrease in photosynthesis, the closure of stomata, and osmotic adjustment are considered to be typical plant responses to the first stage of water stress (Lawlor, 1995). Closure of stomata by a decrease of water content in plant leaves disturbs the supply of CO_2 for photosynthesis (Tanaka et al., 1990). Reduction of stomatal conductance and photosynthesis in water-stressed plants results in exposure of cells to excess excitation energy, especially under high-light conditions. The excess excitation energy may be diverted to activate molecular oxygen and stimulate excess production of active oxygen species which are toxic to cells. These active oxygen species such as superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen may damage proteins, lipids and nucleic acids and cause dysfunction of plant metabolism (Thompson et al., 1987; Winston,1990; Smirnoff, 1993).

Increasing evidence shows that many damaging environmental stresses, such as drought, directly or indirectly, occur through the formation of reactive oxygen species following impairment of the electron transport system (Price and Hendry, 1989). Plants possess intrinsic antioxidant defense systems to detoxify reactive oxygen species and minimize oxidative injury. These include lipid soluble, membrane-associated antioxidants (e.g. α-tocopherol, ß-carotene), water soluble reductants (e.g. ascorbic acid, glutathione), and enzymatic antioxidants (e.g. superoxide dismutase, catalase and the enzymes of the ascorbate/ glutathione cycle). It has been suggested that the formation of reactive oxygen species is an inherent consequence of metabolism and that control of their levels is essential for normal function of cells (Doulis, 1994).

Alpha-tocopherol (vitamin E), which is hydrophobic and associated with membranes, is one of the most important components of cellular defense against oxidative injury. It reacts with

superoxide, lipid peroxy radical, and singlet oxygen and terminates the chain reactions of lipid peroxidation (Fryer, 1992; Hess, 1993). A significant increase of α-tocopherol concentration is observed in several plant species when they are subjected to drought (Price and Hendry, 1989; Hashim et al., 1993). Burger et al.(1983) noted that α-tocopherol content of soybean leaves is increased as the amount of rainfall decreased. Tanaka et al. (1990) showed that subjecting spinach to water deficits increased the content of α-tocopherol in the leaves. Price and Hendry (1989) found that drought led to the production of superoxide radical anions in the plants, and, as the drought continued, there was a rise in the peroxidation of membranes and loss of control over the uptake of transition metals, especially that of Fe, into the cells. Drought stress was also noted to cause an increase in the radical scavenging vitamin E (α-tocopherol) and an increase in glutathione in the plant tissues. Highly significant correlations were observed between stress tolerance and tocopherol concentration (Spearmans rank correlation coefficient r =0.731) (Price and Hendry, 1989). Moran et al.(1994) noted that drought (leaf water potential = 1.3 MPa) induced a 67% increase of α-tocopherol.

Proper application of plant growth regulators to turfgrasses can increase plant vigor and growth (Schmidt, 1990), stimulate root growth, and improve the resistance to various environmental stresses (drought, salinity, temperature, etc.) (Schmidt, 1990; Nabati et al., 1994).

Recent studies indicate that seaweed extracts (SE) improved cell membrane fluidity and permeability, resulting in better tolerance to drought in ryegrass (Yan and Schmidt, 1993). However, it is not clear whether this improvement is due to the effects of plant growth regulators on the status of reactive oxygen species scavengers (such as α-tocopherol). Little research has been done on the biochemical mechanisms of exogenous PGRs' effects on the antioxidant and water stress tolerance.

The objectives of this study were to determine the effects of exogenous PGRs and water stress on α-tocopherol concentration of Kentucky bluegrass; ascertain the relationship among PGRs, antioxidant activity, and water stress tolerance; and provide information for manipulating turfgrass performance with PGRs.

Materials and methods

PGRs application and plant culture. Kentucky bluegrass (*Poa praetensis* L. cv. 'Plush') seeds were sown at 0.12 g in a 5 x 10-cm diam. plastic ring. Each ring contained the same amount

of Groseclose silt loam soil (a clayey, kaolinitic, mesic Typic Hapludult) with the ring bottom covered with plastic film. When the seedlings were 2-wk old, seaweed (*Ascophyllum nodosum*) (SE) and/or HA were applied on the seedlings according to the rates listed in Table 4.1. The dry SE was dissolved in water, and the SE solution was sprayed evenly on foliage in a rate of 80 ml m^2. The HA was diluted before application at a rate of 80 ml m^2. Both HA and SE were supplied by Plant Wise Biostimulant Co., Louisville, KY.

Drought stress treatment. Drought stress treatment began on 3 - wk old seedlings. Silt loam top soil was air-dried and then sieved through a 2-mm screen. This soil was mixed with appropriate water to obtain two moisture levels: 11.6% and 20.1% (which are equivalent to a water potential of - 0.5 and - 0.03 MPa, respectively) (Nabati, 1991) . A 20-20-20 soluble fertilizer was mixed with water to provide 34 kg N ha^{-1}. After thoroughly mixing the soil with water, the soil was placed in a plastic bag, sealed and allowed to equilibrate for 72 hr.

Terrarium-like boxes (30 x 40 x 75 cm) were constructed with 0.15 mm clear plastic sides and tops. Into each box was placed 15 kg of soil adjusted to one of the two moisture levels. The plastic bottom of the rings were removed and the seedling in each ring was transplanted to the terrariums. Each of the terrariums received seven plugs. The seven treated grass plugs within each moisture level were arranged randomly within each terrarium and replicated three times. The terrariums tops were sealed with clear plastic film and the terrariums were randomly placed in an air-conditioned greenhouse at The Turfgrass Research Center of Virginia Tech, Blacksburg, Va, and rotated 1/4 a turn three times a week. Thermocouples were used to record air temperature inside each terrarium. Average air temperature was maintained at 23 C and 25 C at 8 am and 2 pm, respectively, during the experiment. Water stress level (WSL) for all treatments was measured with a hydraulic press (Campbell Scientific Inc, Logan, Utah) at 6 weeks after germination according to the procedure described in Chapter 3.

Fresh leaves of the 6-wk old seedlings were sampled from each treated ring. The leaves were frozen with a small amount of liquid N, and then stored in -20°C before being homogenized. Plant height was measured and the plugs were removed from the growing medium. The soil was washed away from the roots. The root length was measured, and the root mass was dried in an oven at 60°C for 24 hours before being weighed.

Extraction of α- tocopherol from leaves. Alpha-tocopherol analysis was based on the methods of Tanaka et al. (1990). Ethanol (80% aqueous solution) was used as the extraction medium for α-tocopherol. Fresh leaf segments (500 mg) of Kentucky bluegrass were homogenized with a Polytron PT 3000 (Kinematia AG) in 10 ml of 80% ethanol with 0.01% of butylated hydroxytoluence (BHT) at a speed of 29,200 rpm for 2 minutes. The homogenate was then centrifuged in the presence of Argon at 2,800 rpm for 30 min at 16 ^{o}C. To 5 ml of the clear supernatant, 1.0 ml of n-hexane (85%) was added, and the mixture was fully agitated with S/P Vortex Mixer (Baxter Scientific products) for 2 minutes.

Separation and determination of α-tocopherol. Two hours after agitation of the mixture, the upper layer of hexane in the mixture was collected, filtered with a Nylon Acrodisc 13 mm x 0.2 µm filter and applied to a High-Performance Liquid Chromatography (HPLC). The HPLC system consisted of a HPLC pump (Shimadzu LC 600), with a 20 µl injection loop, a variable wavelength fluorescence detector (Shimadzu RF 535 fluorescence HPLC monitor), a HPLC system controller (Shimadzu SCL-6B), and a CR 501 chromatopac (Shimadzu, Japan). Separations were performed on an analytical column of Supelcosil LC-Si (4.6 X 250 mm) from Supelco and filtered with a guard column (50 x 4.6 mm) at room temperature. The mobile phase was a mixture of hexane-isopropanol (99:1). Alpha-tocopherol was detected by measuring the fluorescence intensity at 325 nm while exciting at 292 nm. Alpha-tocopherol from Sigma was used to develop a standard curve for the calculation of α-tocopherol concentration in the samples. The α-tocopherol concentration was adjusted based on the recovery percentage (82%).

The data were subjected to ANOVA analysis, and treatments were compared with Duncan's multiple range test.

Results

Responses of plant growth to water stress and PGRs

The average leaf water status, in terms of water stress level (WSL) determined with a Campbell hydraulic leaf press, was decreased by 16.7% due to the drought stress (Table 4.1). This showed that the water stress applied in this study was severe enough to significantly reduce leaf

water content.

Exogenous application of SE and/or HA improved the leaf water status significantly, especially under the water stress conditions (Table 4.1). The highest leaf moisture content occurred when the seedlings were treated with HA and /or SE at the higher rates.

The shoot growth was suppressed substantially due to the water stress. On average, plant height (Table 4.2) and clipping dry weight (Table 4.3) of the grass grown under low soil moisture were only 38.7% and 21.5%, respectively, of those in the high soil moisture. However, exogenous PGR enhanced the shoot growth of Kentucky bluegrass under both low and high soil moisture conditions (Table 4.3). The plant height and fresh clipping weight were significantly increased with application of the high doses of SE, HA, or their combinations.

Root length was not significantly influenced by soil moisture level or PGR treatments (Table 4.4). The high dosage of HA, SE alone and the combination tended to have longer roots.

The root dry weight was not significantly reduced under low soil moisture. Application of the high doses SE and/or HA increased root mass significantly in both low and high soil moisture environments (Table 4.5). The PGRs appeared to have better effects on root development under water stress than under the high soil moisture conditions.

Response of α-tocopherol to water stress and PGRs

Alpha-tocopherol concentration increased from 15.0 μg g^{-1} dry leaves in the high soil moisture regime to 45.3 μg g^{-1} dry leaves under water stress (Table 4.6).

Foliar application of the PGR further increased α-tocopherol content of Kentucky bluegrass significantly under both high and low soil moisture levels (Table 4.6). The highest α-tocopherol concentration occurred when the seedlings were treated with HA at 5 l ha^{-1} or HA plus SE at 2.5 l + 326 g ha^{-1}. The results of this study indicated that exogenous SE or HA, two PGRs, enhanced endogenous concentration of α-tocopherol.

Antioxidant α-tocopherol and turfgrass growth

A positive correlation between α-tocopherol concentration and root or shoot growth was observed in Kentucky bluegrass under both high and low soil moisture (Fig.4.1; Fig.4.2). The correlation coefficients (r) were statistically significant ($p<0.1$) between α-tocopherol concentration and root dry weight or shoot dry weight. This indicated that plants endogenous concentration of

antioxidant α-tocopherol is related to the plant growth.

TABLE 4.1. Water stress level (WSL) of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- Relative Pressure ----------		
HA	2.5	0	187.7	216.7	202.2b
HA	5.0	0	171.0	211.7	191.3bcd
SE	0	163	184.7	215.0	199.8bc
SE	0	326	153.0	190.0	171.5cd
HA+SE	2.5	163	175.5	191.7	183.7bcd
HA+SE	2.5	326	154.0	171.7	162.8d
Control	0	0	219.6	256.7	238.2a
Mean			177.9 y	207.6 x	

The seeds were sown in the ring (10 cm diameter) and germinated under high soil moisture. Two weeks after germination, the seedlings were treated with the PGRs and transplanted into the terrarium under low (-0.5 MPa) soil moisture. The seedlings were grown for 4 weeks before being analyzed (see Materials and Methods).

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.1$.

Moisture: $p = 0.0015$; PGR: $p = 0.0015$; Moisture*PGR: $p = 0.9774$.

TABLE 4.2. Plant height of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	----------Plant Height (cm)----------		
HA	2.5	0	16.8	5.8	11.3a
HA	5.0	0	18.3	5.7	12.0a
SE	0	163	14.8	7.1	10.9a
SE	0	326	17.2	7.1	12.1a
HA+SE	2.5	163	16.7	6.0	11.4a
HA+SE	2.5	326	17.7	7.1	12.4a
Control	0.0	0.0	14.8	6.3	10.5a
Mean			16.6x	6.4y	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.1$.
Moisture: p = 0.0001; PGR: p = 0.9843; Moisture*PGR: p = 0.7090.

TABLE 4.3. Clipping dry weight of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	------ Clipping dry Weight (g $plug^{-1}$)------		
HA	2.5	0	1.817	0.330	1.073bc
HA	5.0	0	2.010	0.407	1.208ab
SE	0	163	1.833	0.380	1.107bc
SE	0	326	2.243	0.603	1.423a
HA+SE	2.5	163	1.783	0.287	1.035bc
HA+SE	2.5	326	2.047	0.513	1.280ab
Control	0	0	1.377	0.293	0.835c
Mean			1.873 x	0.402 y	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.1$.
Moisture: p = 0.0001; PGR: p = 0.0152; Moisture*PGR: p = 0.6061.

TABLE 4.4. Root length of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	ml ha^{-1}	g ha^{-1}	---------- Root Length (cm) ----------		
HA	2,500	0	10.3	7.5	8.9a
HA	5,000	0	12.0	8.6	10.3a
SE	0	163	10.7	8.3	9.5a
SE	0	326	12.2	8.0	10.1a
HA+SE	2,500	163	8.9	7.9	8.4a
HA+SE	2,500	326	11.8	9.4	10.6a
Control	0.0	0.0	8.2	5.5	6.8a
Mean			10.6 x	7.9 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.1$.
Moisture: p = 0.0631; PGR: p = 0.8369; Moisture*PGR: p = 0.9180.

TABLE 4.5. Root dry weight of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	-------- Root Dry Weight (g $plug^{-1}$) -------		
HA	2.5	0	0.129	0.187	0.158bc
HA	5.0	0	0.190	0.228	0.209ab
SE	0	163	0.164	0.198	0.181abc
SE	0	326	0.179	0.271	0.230ab
HA+SE	2.5	163	0.189	0.160	0.163bc
HA+SE	2.5	326	0.228	0.268	0.248a
Control	0	0	0.123	0.122	0.122c
Mean			0.172 x	0.205 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.1$.
Moisture: p = 0.1492; PGR: p = 0.0983; Moisture*PGR: p = 0.9545.

TABLE 4.6. Alpha-tocopherol of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	ml ha^{-1}	g ha^{-1}	--------α- Tocopherol (ug g^{-1} DM) -------		
HA	2.5	0	19.72	86.19	52.96ab
HA	5.0	0	29.15	118.09	73.62a
SE	0	163	18.17	46.15	32.16b
SE	0	326	30.95	79.32	55.14ab
HA+SE	2.5	163	24.70	75.92	50.31ab
HA+SE	2.5	326	30.61	109.60	70.11a
Control	0	0	15.04	45.26	30.15b
Mean			24.05y	80.08x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.1$.
Moisture: p = 0.0001; PGR: p = 0.0435; Moisture*PGR: p = 0.3220.

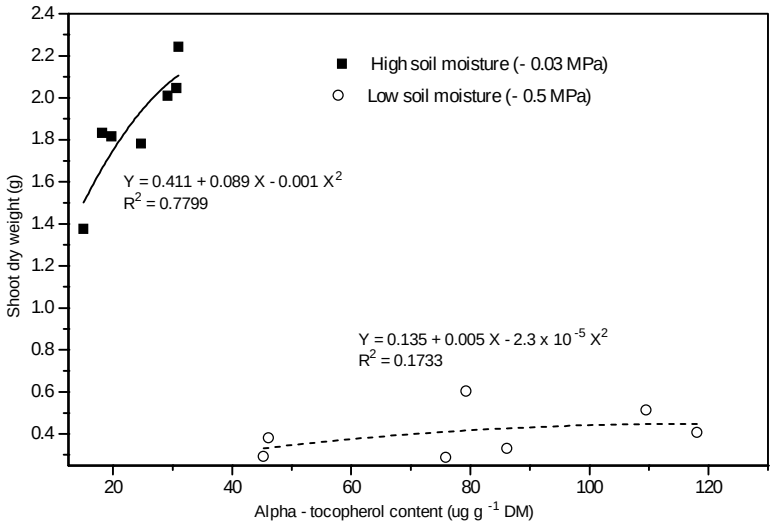

Fig.4.1. Relationship between α -tocopherol and shoot growth in Kentucky bluegrass grown under low and high soil moisture.

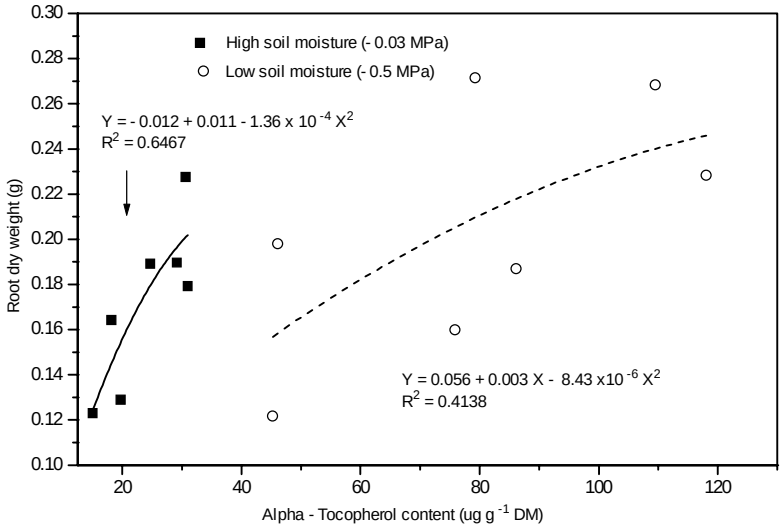

Fig.4.2. Relationship between α-tocopherol and root development in kentucky bluegrass grown under low and high soil moisture.

Discussion

The shoot and root growth of Kentucky bluegrass were significantly suppressed due to the water stress applied in this study (Table 4.4 and Table 4.5) and was consistent with the results obtained by Nabati (1991). The impacts of water stress on plant growth have been well documented (Kramer, 1983). Suppression of plant growth has been attributed to the reduced cell expansion by low turgor pressure under drought. The Kentucky bluegrass grew slowly and had no obvious guttation on the leaves produced under the drought imposed in this study. This suggests that the drought treatment in this study was severe enough for interfering cell functions.

Water stress resulted in an significant increase of antioxidant α-tocopherol endogenous concentration of Kentucky bluegrass seedlings (Table 4.6) as previously reported by Price and Hendry (1989), Tanaka et al.(1990), and Moran et al. (1994). Alpha-tocopherol is synthesized in the chloroplasts and closely associated with the thylakoid membrane of the chloroplasts. The thylakoid membrane, which contains substantial unsaturated lipids, is one of the major sites of oxidative damage through lipid peroxidation. Lawlor (1995) noted that water stress causes accumulation of reactive oxygen species in the chloroplasts. This may result in an increase of α-tocopherol which quenches oxygen radicals within the membrane and terminates chain reactions that cause oxidative damage.

Closure of stomata appears to be a typical response to water stress. Rapid rise of ABA level has been attributed to the factor signaling this response (Davies and Zhang, 1991). Closure of stomata and reduction of photosynthesis interfere with gas exchange and a higher O_2/CO_2 ratio is realized. As a result, excess excitation energy may be diverted to activate molecular oxygen, and excess reactive oxygen species are produced and may damage proteins, lipids, and DNA.

The rapid increase of α-tocopherol under the conditions described above indicated that plant the antioxidant systems exhibit a similar response to water stress as to other environmental stresses, such as salinity (Gossett et al., 1994), chilling (Fryer, 1992), and herbicides (Doulis 1994). However, the signal triggering this response is still not clear. It has been suggested that the increase of α-tocopherol under stress may be related to the degradation of chlorophyll, because phytol from chlorophyll degradation may be used for the synthesis of α-tocopherol (Hess, 1993).

This study showed that the two PGRs, SE and HA, enhanced the antioxidant α-tocopherol content under both high soil moisture and water stress. Humic acid and SE have been shown to

improve turfgrass growth and resistance to environmental stresses (Nabati et al. 1994). These stimulating effects have been attributed to the hormone activity of PGRs (Crouch, 1990). The result of this study suggested that the phytohormone cytokinin may serve as a signal to trigger antioxidant system responses. The support evidence for this explanation is also from the study by Kim (1988) and Yokoyama et al.(1991), who showed that exogenous hormones impacted antioxidant (ascorbic acid and β-carotene) status.

This study indicated that the concentration of α-tocopherol is positively correlated to the growth of Kentucky bluegrass under low or high soil moisture conditions (Fig.4.1). Positive correlations were also found among PGRs, growth and α-tocopherol level. Increased antioxidant activity may condition plants to improve turfgrass quality under various environmental stresses including water stress. Manipulation of turfgrass with environmentally compatible PGRs, such as SE and HA, can improve water stress tolerance and turf quality without sacrificing the environment.

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Chapter 5

Influence of plant growth regulators on hydrophobic and hydrophilic antioxidant status and growth of three turfgrass species subjected to drought

Introduction

Water deficits are a major limiting factor in turfgrass management. Avoidance of water stress, tolerance of water stress, and water use efficiency are major mechanisms for plants to tolerate water deficit environments (Kenna and Horst, 1993). Since turfgrass is grown under varying environmental situations, tolerance mechanisms of the plant (e.g. osmotic adjustment and accumulation of protective solutes) are major strategies for turfgrass to survive water stress environments. There is wide variation among turfgrass species in terms of drought tolerance. It has been well documented that tall fescue shows better drought tolerance than Kentucky bluegrass or creeping bentgrass (Turgeon, 1991). Although the deep root system of tall fescue has been attributed to its good drought tolerance, the physiological basis of drought tolerance still remains unclear.

The antioxidant content of a plant is closely related to its stress tolerance (Alscher and Hess, 1993; Smirnoff, 1995). It has been established that environmental stresses may damage the plants through accumulation of toxic reactive oxygen species such as peroxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen. Drought-induced oxidative damage has been observed in higher plants (Moran et al., 1994; Price and Hendry, 1991; Smirnoff et al., 1993; Smith and Griffiths, 1993). The severity of this damage largely depends on the status of antioxidant systems, since plants develop antioxidants to remove toxic reactive oxygen species and protect the plant cells from lipid peroxidation and inactivation of enzymes that occur under stress (Smirnoff, 1993; Van Hasselt et al., 1979; Winston, 1990).

Plant antioxidant status exhibits a wide variation from species to species (Hess, 1995), with a close relationship between antioxidant activity and water stress tolerance (Smirnoff, 1993). Price and Hendry (1989) indicated that the antioxidant α-tocopherol content increased significantly as a response to water stress in 9 out of 10 grass species examined. Tanaka et al. (1990) showed that

drought stress enhanced antioxidant activity in spinach. Several researchers reported that drought induced a significant increase in the antioxidant status in plants (Moran et al., 1994; Mukherjee and Choudhuri, 1983; Smirnoff et al., 1988). Analysis of antioxidant status of grasses with different water stress tolerance may lead to a better understanding of the mechanisms involved.

Alpha-tocopherol and ascorbic acid are two important antioxidants in higher plants (Alscher and Hess, 1993; Foyer, 1993; Larson, 1988; Price and Hendry, 1987). They are concentrated in the chloroplasts and the cytosol (ascorbic acid), and protect the photosynthetic apparatus under stress by scavenging excess reactive oxygen species (Smirnoff, 1995). Although some research on the α-tocopherol and ascorbic acid responses to drought has been reported (Kuner and Ederer, 1985), the manipulation of endogenous α-tocopherol and ascorbic acid in drought stressed turfgrass with exogenous growth regulators has not been well established.

Regulation of turfgrass performance with plant growth regulators has been practiced for many years (Arteca, 1996). Proper application of certain PGRs can not only improve turfgrass growth but also enhance stress tolerance (Schmidt, 1990). Seaweed and HA are two environmentally compatible natural products. Containing phytohormones and showing auxin, or cytokinin-like activity (Crouch, 1990), when these materials are applied to turfgrass, they enhance the tolerance to salinity, drought, chilling and other environmental stresses (Piccolo, et al., 1992; Senn, 1991; Van Staden et al., 1994). Examination of different stress tolerant grasses treated with these plant growth regulators may provide information for managing turfgrass for better tolerance to water stress.

The objectives of this study were to examine the influence of exogenous plant growth regulators on endogenous hydrophobic and hydrophilic antioxidant concentrations, and their impact on drought tolerance of three turfgrass species and to provide information for turfgrass cultural practices to enhance stress resistance of turfgrass.

Materials and methods

PGRs application and drought stress treatment. creeping bentgrass (*Agrostis palustris* Huds. cv. "Penncross"), Kentucky bluegrass (*Poa praetensis* L. cv. 'Plush'), and tall fescue (*Festuca arundinacea* Schreb. cv. "Rebel Jr.") seeds were sown at rates 0.06, 0.12, and 0.25 g ring^{-1}, respectively, in a 10-cm diameter ring. Each ring had the bottom covered with plastic film and

contained the same amount of Groseclose silt loam soil (a clayey, Kaolinitic, mesic Typic hapludult). When the seedlings were 2- wk old, soluble seaweed (*Ascophyllum nodosum*) extracts (SE) at a rate of 326 g ha^{-1}, humic acid (HA; 25% active humic acid) at a rate of 5 l ha^{-1} or the combination were sprayed evenly on the foliage according to the procedure described in Chapter 4. Both HA and SE were supplied by Plant Wise Biostimulant Co., Louisville, Kentucky.

Silt loam top soil was air-dried for 2 weeks and then sieved through a 2-mm screen. This soil was mixed with appropriate water to obtain two moisture levels: 11.6% and 20.1% (which are equivalent to a water potential of - 0.5 and - 0.03 MPa). A 20-20-20 soluble fertilizer was mixed with water to provide 34 kg N ha^{-1} (3 g N box^{-1}). After thoroughly mixing the soil with water, the soil was placed in a plastic bag, sealed, and allowed to equilibrate for 72 hr.

Terrarium-like boxes (30 x 40 x 75 cm) were constructed with 0.15 mm clear plastic sides and tops. Fifteen kg of soil adjusted to one of the two moisture levels was placed into each terrarium. One week after treatment of the seedlings, the plastic bottom of the rings were removed and the seedling in each ring was transplanted to the terrarium. Each of the terrariums received 8 plugs. The terrarium tops were sealed with clear plastic, and the terrariums were placed in an air-conditioned greenhouse. Thermocouples were used to record air temperature inside each terrarium twice daily. The average temperature was 23 ^{o}C at 8 am and 26 ^{o}C at 2 pm, respectively, during the period of the experiment.

Water stress level (WSL) for all treatments were measured with a hydraulic leaf press (Campbell Scientific Inc, Logan, Utah) 7 wk. after germination according to the procedure described in Chapter 3. In addition, plant height was measured and fresh leaves of the 7-wk old seedlings were sampled from each treatment at this time. The leaves were frozen with a small amount of liquid N, and then stored at -20 ^{o}C before being homogenized. Following the WSL measurement, the soil was washed from the roots which were dried in an oven at 60 ^{o}C for 24 hrs before being weighed.

The four PGR treatments within each moisture level were arranged randomly within each terrarium and replicated four times. The terrariums were randomly placed in an air-conditioned greenhouse at the Turfgrass Research Center of Virginia Tech, Blacksburg, Va, and rotated 1/4 turn three times a week.

Extraction of α- tocopherol from leaves. (as described in Chapter 4).

Extraction of ascorbic acid from leaves. The fresh leaves were sampled and frozen with liquid N and stored at -20 ºC. The frozen leaves segments (1,000 mg) were homogenized in 7 ml of 5% metaphosphoric acid with a Polytron (PT3000, Kinematia, AG) at a speed of 29200 rpm for 3 minutes. Ice water was used to maintain the samples at a low temperature during the homogenization. The homogenate was then centrifuged with a refrigerated centrifuge at 4000 rpm for 40 minutes. Clear supernatant (4 ml) was analyzed by a High-performance Liquid Chromatography (HPLC).

Determination of α-tocopherol concentration. The fully agitated mixture stood for 2-hr before further analysis (see details in Chapter 4). The α-tocopherol concentration were adjusted based on the recovery percentage (82%).

Determination of ascorbic acid concentration. The clear supernatant was passed through a Nylon Acrodisc 13 mm x 0.2 *u*m filter before being analyzed by a HPLC. The HPLC system consisted of a HPLC pump [8]SPD-6A[9], with a 20 *u*l injection loop, a variable wavelength UV detector [8]Shimadzu RF 535 fluorescence HPLC monitor[9], a HPLC system controller [8]Shimadzu SCL-6B[9], and a CR 501 chromatopac [8]Shimadzu, Japan[9]. Separations were performed on an analytical column of Supelcosil LC-8-BD [8]4.6 X 150 mm [9] from Supelco and filtered with a guard column [8]50 x 4.6 mm[9]. A mixture of methanol and 4.3 mM hexane sulfornate w/0.1 triethylamine [8]PH 2.8 w/phosphoric acid·15˘85[9] was used as the mobile phase. Ascorbic acid was detected by measuring the absorbance intensity at 245 nm. Ascorbic acid from Aldrich was used to develop the standard curve. The AA concentration was adjusted based on the recovery percentage [8]62[59] for AA.

A two factor analysis of variance (ANOVA) and a least significant difference (LSD) test were performed on each data set.

Results

Responses of plant growth to water stress and PGRs

The average leaf water status, in terms of water stress level (WSL) determined with the hydraulic press, was decreased due to the water stress in the three cool-season turfgrass species

(Tables 5.1, 5.2, and 5.3.). Among the three grasses, Kentucky bluegrass appeared to have the best water status (the lowest WSL).

The leaf water status was improved significantly with application of the PGRs under both high and low soil moisture environments (Tables 5.1, 5.2, and 5.3.). In other words, SE and/or HA enhanced water stress tolerance. It appeared that the combination of SE and HA had better effects on plant water status than when either material was applied alone.

Water stress reduced shoot growth in the three turfgrasses examined. The plant height and clipping dry weight were significantly reduced under drought stress in the three grass species (Tables 5.4 through 5.9). On average, shoot dry weight was reduced by 25.7% in creeping bentgrass, 38.6% in tall fescue, and 21.4% in Kentucky bluegrass, respectively, under drought as compared with those in high soil moisture levels.

Application of the PGRs enhanced plant height of the three grasses under both high and low soil moisture (Tables 5.4, 5.5, and 5.6).

The clipping dry weights were increased with application of PGRs to the three grasses grown under the high and low soil moisture environments (Tables 5.7, 5.8, and 5.9). It appears that SE and/or HA have greater stimulating effects under water stress than under high soil moisture. Among the three grasses, creeping bentgrass showed the greatest response to the PGRs.

Application of the PGRs improved root growth as determined by root length measurements in three grasses grown under both the high and low soil moisture (Tables 5.10, 5.11, and 5.12). However, only the combination of HA and SE enhanced root length significantly in the three grasses.

The root dry weight of tall fescue and Kentucky bluegrass was significantly enhanced with all PGR treatments (Table 5.13, Table 5.14). Although application of HA or SE alone did not increase root mass of creeping bentgrass, the combination of HA and SE caused a significant increase of root mass of creeping bentgrass (Table 5.15).

The interaction between growth regulators and moisture levels differed with grass species. The moisture and PGR interaction for shoot weight was significant in creeping bentgrass, but not in the other two species (Table 5.7, Table 5.8, Table 5.9). In contrast, the moisture and PGR interaction for root length was significantly in tall fescue and Kentucky bluegrass but not in

creeping bentgrass (Tables 5.10, 5.11 and 5.12). The SE and HA showed greater stimulating effects on root growth under drought than in the high moisture conditions.

Response of α-tocopherol and ascorbic acid to water stress and PGRs

Water stress resulted in a significant increase of exogenous α-tocopherol concentrations in the three turfgrass species (Tables 5.16, 5.17, and 5.18).

The combination of SE with HA enhanced α-tocopherol content in the three grasses under high and low moisture (Tables 5.16, 5.17, and 5.18). Among the three grasses, tall fescue and Kentucky bluegrass contained a higher level of α-tocopherol and a lower level of ascorbic acid as compared with creeping bentgrass.

water deficit did not influence ascorbic acid content in all three grass species. Application of the PGRs significantly enhanced ascorbic acid content in the three grass species (Tables 5.19, 5.20, and 5.21). This showed that a lipid-soluble, membrane- associated antioxidant (α-tocopherol) may be more sensitive to water deficit than a water- soluble reductant (ascorbic acid). However, the ascorbic acid concentration on dry weight basis was enhanced with application of the PGRs (Tables 5.19, 5.20, and 5.21).

Since the experimental design did not include the grass species, no statistical comparison can be done on the ascorbic acid content among the three grasses. However, because the three grasses were treated on the same date and grown under the same experimental conditions and antioxidant analysis procedures were the same, the ascorbic acid content of three grasses was compared based on the average of different PGR treatments (Tables 5.19, 5.20, and 5.21). The data showed that the ascorbic acid status of tall fescue and Kentucky bluegrass exhibited greater response to the PGR treatments than did that of creeping bentgrass.

Antioxidant status and turfgrass growth

Positive correlations between antioxidant (α-tocopherol and ascorbic acid) concentrations and root or shoot growth were observed in the three grasses grown under both high and low soil moisture levels (Fig.5.1 through 5.4). Since the relationships between the antioxidants and plant growth were similar in both tall fescue and Kentucky bluegrass, the comparisons were made only for tall fescue and creeping bentgrass. Fig.5.1 and 5.2 show that turfgrass with high level of α-tocopherol produced better shoot and root growth under high and low soil moisture environment as

compared with the turfgrass containing low level of this antioxidant. However, the correlation between α-tocopherol and shoot or root weight differed with grass species. Tall fescue contained a higher level of α-tocopherol and showed a closer relationship between the antioxidant and growth (the r value ranged from 0.8936 to 0.9637) as compared with creeping bentgrass (the r value ranged from 0.6496 to 9479) (Fig.5.1 and 5.2). Similarly, there was a positive correlation between ascorbic acid and shoot or root growth in tall fescue and creeping bentgrass grown under drought and high soil moisture environments (Fig.5.3 and 5.4).

A significant correlation between α-tocopherol and ascorbic acid content was found in tall fescue and creeping bentgrass grown under low soil moisture condition, but not in creeping bentgrass under high soil moisture (Fig. 5.5). This result indicated that the turfgrass with high level of α-tocopherol generally contained more ascorbic acid.

TABLE 5.1. Water stress level of tall fescue as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- Relative Pressure ----------		
HA	5	0	186.3	232.5	209.4 b
SE	0	326	177.5	220.0	198.8 bc
HA+SE	5	326	161.3	220.0	190.6 c
Control	0	0	207.5	260.1	233.8 a
Mean			183.1 y	233.1 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
HA = Humic acid (25% active humic acid).
SE = Soluble seaweed extracts from *ASCOPHYLLUM nodosum*.
Moisture: p = 0.0001; PGR: p = 0.0001; Moisture * PGR: p = 0.7262.

TABLE 5.2. Water stress level of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- Relative Pressure ----------		
HA	5.0	0	168.1	200.0	184.0 b
SE	0	326	161.3	185.0	183.3 b
HA+SE	5.0	326	160.0	206.7	173.2 c
Control	0	0	197.3	228.3	212.8 a
Mean			171.7 y	205.0 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.0001; PGR: p = 0.0001; Moisture * PGR: p = 0.2800.

TABLE 5.3. Water stress level of creeping bentgrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- Relative Pressure ----------		
HA	5	0	243.3	284.0	263.7 b
SE	0	326	253.3	313.3	283.3 ab
HA+SE	5	326	226.7	243.3	235.0 c
Control	0	0	273.3	330.0	301.7 a
Mean			249.2 y	289.3 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.0011; PGR: p = 0.0035; Moisture * PGR: p = 0.2061.

TABLE 5.4. Plant height of tall fescue as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- cm ----------		
HA	5	0	9.7	5.9	7.8 b
SE	0	326	10.8	6.5	8.6 a
HA+SE	5	326	10.9	7.7	9.3 a
Control	0	0	8.8	5.5	7.1 b
Mean			10.0 x	6.4 y	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: $p = 0.0001$; PGR: $p = 0.0013$; Moisture * PGR: $p = 0.7288$.

TABLE 5.5. Plant height of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- cm ----------		
HA	5	0	9.7	6.7	8.2 b
SE	0	326	8.8	6.3	7.6 b
HA+SE	5	326	10.5	7.7	9.1 a
Control	0	0	7.8	5.5	6.7 c
Mean			9.2 x	6.6 y	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.0001; PGR: p = 0.0003; Moisture * PGR: p = 0.8655.

TABLE 5.6. Plant height of creeping bentgrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- cm ----------		
HA	5	0	10.7	7.0	8.8 bc
SE	0	326	11.2	7.1	9.2 b
HA+SE	5	326	12.8	7.7	10.3 a
Control	0	0	9.2	5.6	7.4 c
Mean			11.0 x	7.0 y	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.0001; PGR: p = 0.0126; Moisture * PGR: p = 0.1139.

TABLE 5.7. Shoot dry weight of tall fescue as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- mg $plug^{-1}$ ----------		
HA	5	0	616.8	385.8	500.8 bc
SE	0	326	668.2	425.5	546.9 ab
HA+SE	5	326	695.8	480.6	588.2 a
Control	0	0	589.2	285.7	437.5 c
Mean			642.3 x	394.4 y	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.0001; PGR: p = 0.0063; Moisture * PGR: p = 0.6644.

TABLE 5.8. Shoot dry weight of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- mg $plug^{-1}$ ----------		
HA	5	0	708.4	542.2	625.3 b
SE	0	326	713.2	557.7	635.5 b
HA+SE	5	326	901.1	769.5	835.3 a
Control	0	0	623.1	444.8	534.0 b
Mean			736.4 x	578.6 y	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.0341; PGR: p = 0.0409; Moisture * PGR: p = 0.9956.

TABLE 5.9. Shoot dry weight of creeping bentgrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	----------mg $plug^{-1}$ ----------		
HA	5	0	617.5 b	491.3 b	554.4
SE	0	326	724.1 a	472.0 bc	601.9
HA+SE	5	326	722.7 a	542.4 a	632.6
Control	0	0	551.8 b	415.0 c	483.4
Mean			654.0 x	486.1 y	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.0001; PGR: p = 0.0025; Moisture * PGR: p = 0.0300.

TABLE 5.10. Root length of tall fescue as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- cm ----------		
HA	5.0	0	8.9 ab	7.8 c	8.4
SE	0	326	10.1 a	11.0 b	10.6
HA+SE	5.0	326	10.3 a	15.8 a	13.0
Control	0	0	7.3 b	5.1 d	6.2
Mean			9.1 x	9.9 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.2513; PGR: p = 0.0001; Moisture * PGR: p = 0.0016.

TABLE 5.11. Root length of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- cm ----------		
HA	5	0	11.0	8.1	9.5 a
SE	0	326	11.4	8.9	10.2 a
HA+SE	5	326	11.8	9.5	10.6 a
Control	0	0.0	8.1	6.1	7.1 a
Mean			10.6 x	8.1 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.0001; PGR: p = 0.0005; Moisture * PGR: p = 0.9326.

TABLE 5.12. Root length of creeping bentgrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- cm ----------		
HA	5	0	5.5	4.9	5.2 b
SE	0	326	6.5	4.2	5.3 b
HA+SE	5	326	9.4	7.0	8.2 a
Control	0	0	4.3	3.7	4.0 b
Mean			6.4 x	5.1 y	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.0216; PGR: p = 0.0005; Moisture * PGR: p = 0.2831.

TABLE 5.13. Root dry weight of tall fescue as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- mg $plug^{-1}$----------		
HA	5	0	55.5	63.5	59.5 b
SE	0	326	70.5	71.5	71.0 ab
HA+SE	5	326	78.8	94.6	86.7 a
Control	0	0	37.2	38.5	37.8 c
Mean			60.5 x	67.0 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.4636; PGR: p = 0.0049; Moisture * PGR: p = 0.9225.

TABLE 5.14. Root dry weight of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- mg plug-1----------		
HA	5	0	90.3	97.1	93.7 b
SE	0	326	96.6	104.0	100.3 b
HA+SE	5	326	153.2	159.5	156.4 a
Control	0	0	52.8	64.0	58.4 c
Mean			98.2 x	106.2 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.5244; PGR: p = 0.0004; Moisture * PGR: p = 0.9989.

TABLE 5.15. Root dry weight of creeping bentgrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	---------- mg $plug^{-1}$ ----------		
HA	5	0	84.1	65.4	74.2 b
SE	0	326	96.4	94.6	95.5 b
HA+SE	5	326	124.1	156.8	140.5 a
Control	0	0	50.6	55.5	53.1 b
Mean			88.8 y	96.5 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.3659; PGR: p = 0.0001; Moisture*PGR: p = 0.2402.

TABLE 5.16. Alpha-tocopherol concentration of tall fescue as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	----------ug g^{-1} dw ----------		
HA	5	0	328.2	546.4	437.3 b
SE	0	326	345.3	435.2	390.3 b
HA+SE	5	326	474.9	644.4	559.3 a
Control	0	0	223.6	268.6	246.1 c
Mean			343.0 y	473.7 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD atα = 0.1.
Moisture: p = 0.0001; PGR: p = 0.0001; Moisture*PGR: p = 0.1247.

TABLE 5.17. Alpha-tocopherol concentration of Kentucky bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	----------ug g^{-1} dw ----------		
HA	5	0	285.2	425.6	355.4 b
SE	0	326	324.9	406.7	365.8 b
HA+SE	5	326	470.4	573.0	521.7 a
Control	0	0	201.3	312.5	256.9 c
Mean			320.4 y	429.4 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD atα = 0.1.
Moisture: p = 0.0007; PGR: p = 0.0001; Moisture*PGR: p = 0.8823.

TABLE 5.18. Alpha-tocopherol concentration of creeping bentgrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	----------ug g^{-1}dw ----------		
HA	5	0	103.9	191.0	138.7 ab
SE	0	326	223.4	134.3	117.4 b
HA+SE	5	326	106.1	205.3	216.1 a
Control	0	0	51.6	83.6	64.4 b
Mean			121.3 y	153.6 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.5440; PGR: p = 0.2549; Moisture*PGR: p = 0.9125.

TABLE 5.19. Ascorbic acid concentration of tall fescue as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	----------mg g^{-1} dw ----------		
HA	5	0	1.533	1.447	1.491 b
SE	0	326	1.626	1.380	1.503 b
HA+SE	5	326	1.875	2.221	2.048 a
Control	0	0	1129	1.017	1.073 c
Mean			1.541 x	1.517 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD at $\alpha = 0.1$.
Moisture: p = 0.8263; PGR: p = 0.0001; Moisture*PGR: p = 0.2879.

TABLE 5.20. Ascorbic acid concentration of bluegrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	----------mg g^{-1} dw ----------		
HA	5	0	0.826	0.708	0.767 b
SE	0	326	0.683	0.700	0.691 bc
HA+SE	5	326	0.940	1.047	0.994 a
Control	0	0	0.733	0.497	0.615 c
Mean			0.796 x	0.738 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD atα = 0.1.
Moisture: p = 0.1001; PGR: p = 0.0001; Moisture*PGR: p = 0.2402.

TABLE 5.21. Ascorbic acid concentration of creeping bentgrass as influenced by plant growth regulators (humic acid and/or seaweed extract) and water deficit

	Amount		Soil Moisture Level (MPa)		
Treatment	HA	SE	-0.03	-0.5	Mean
	l ha^{-1}	g ha^{-1}	----------mg g^{-1} dw ----------		
HA	5	0	2.630	3.078	2.854 ab
SE	0	326	2.806	3.111	2.958 ab
HA+SE	5	326	3.320	3.799	3.560 a
Control	0	0	1.809	2.371	2.090 b
Mean			2.641 x	3.090 x	

Means within a column (a, b) or row (x, y) followed by the same letter are not significantly different based on LSD atα = 0.1.
Moisture: p = 0.3363; PGR: p = 0.2089; Moisture*PGR: p = 0.9973.

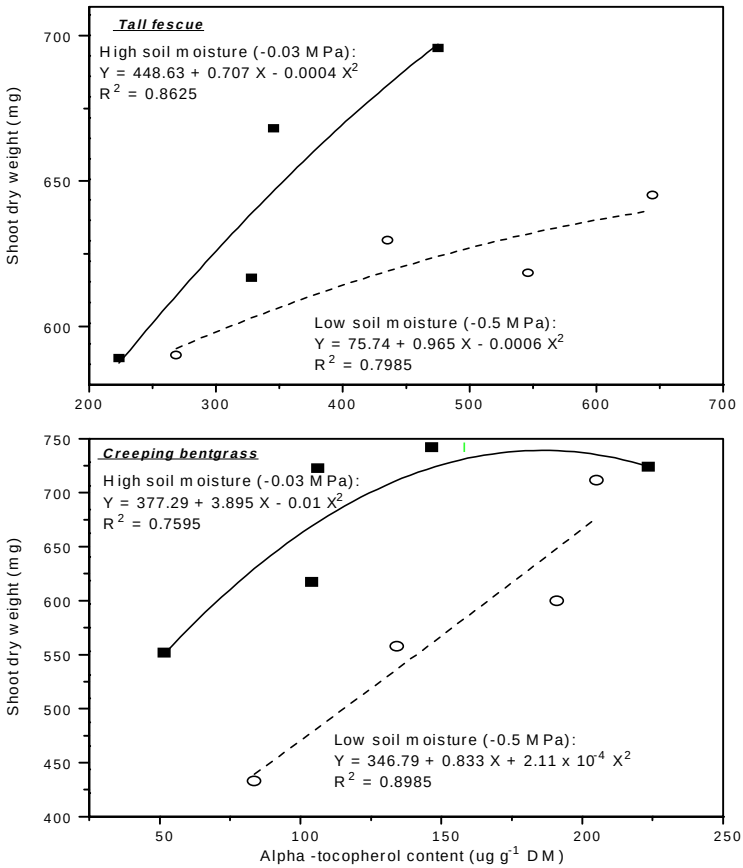

Fig.5.1. Relationship between α-tocopherol content and shoot weight in tall fescue and creeping bentgrass grown in high and low soil moisture.

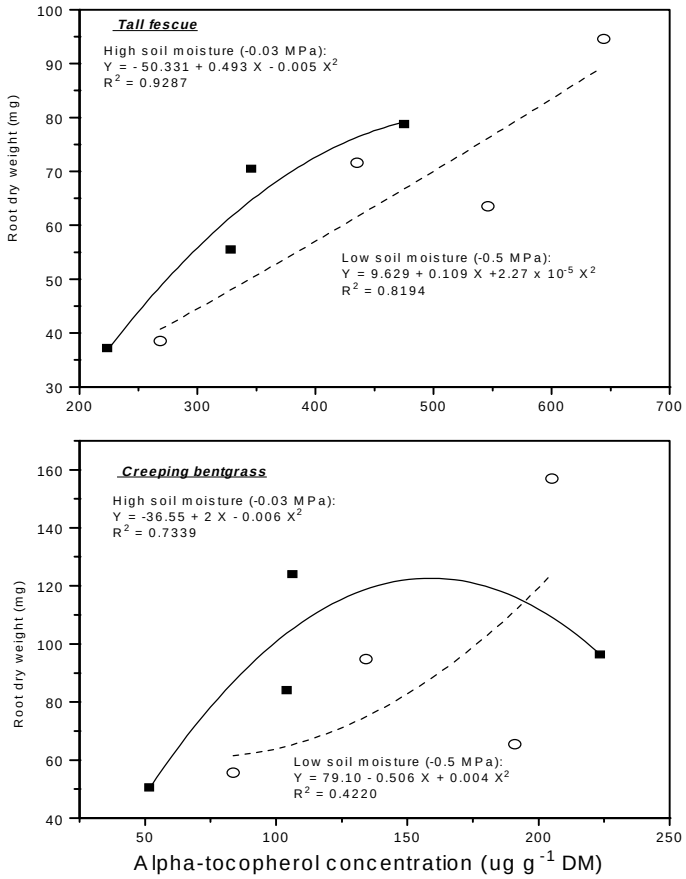

Fig.5.2. Relationship between shoot α - tocopherol content and root weight in tall fescue and creeping bentgrass grown under low and high soil moisture environments .

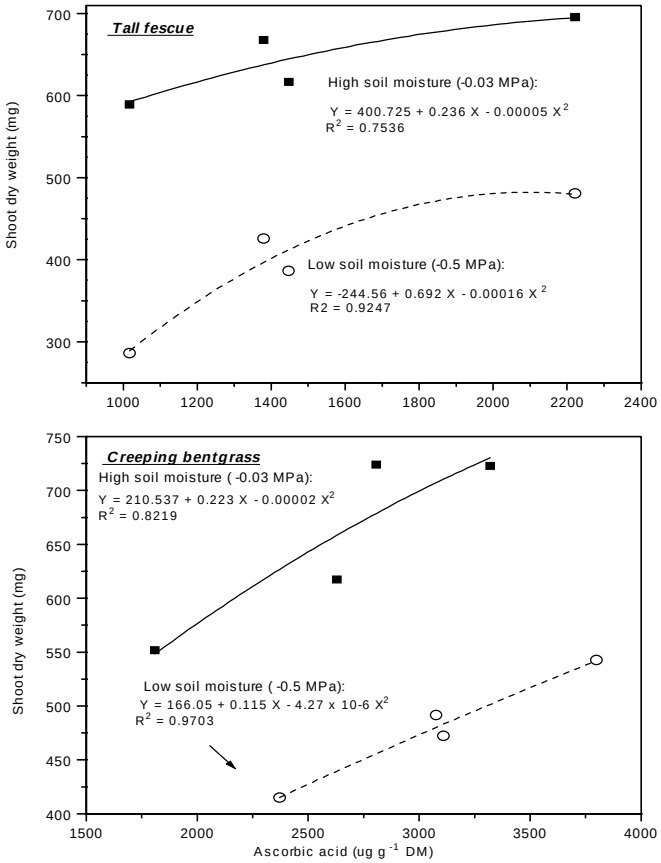

Fig.5.3. Relationship between shoot weight and ascorbic acid content in tall fescue and creeping bentgrass grown under the two soil moisture levels.

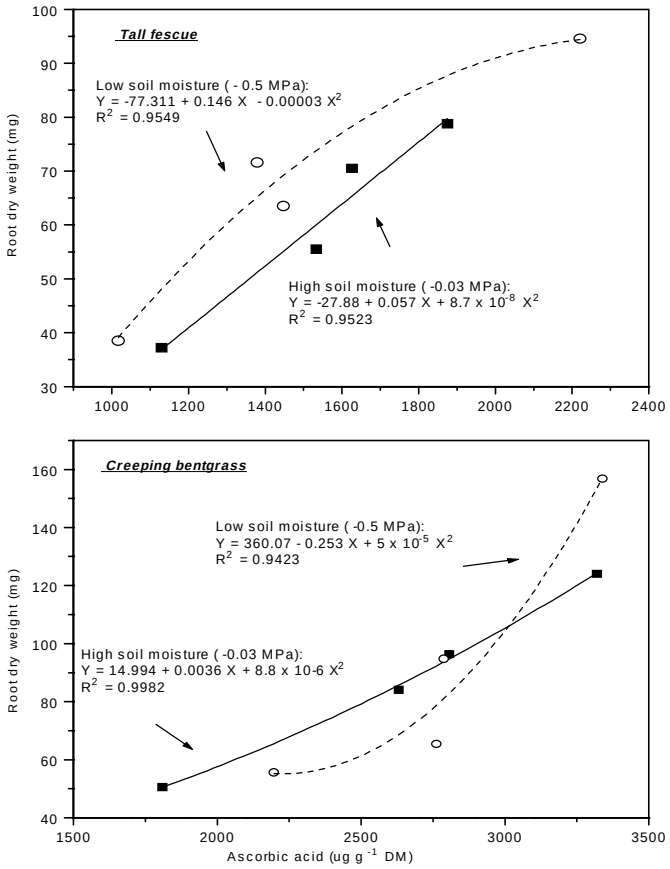

Fig.5.4. Relationship between root weight and shoot ascorbic acid content in tall fescue and creeping bentgrass grown under the two soil moisture envieonments.

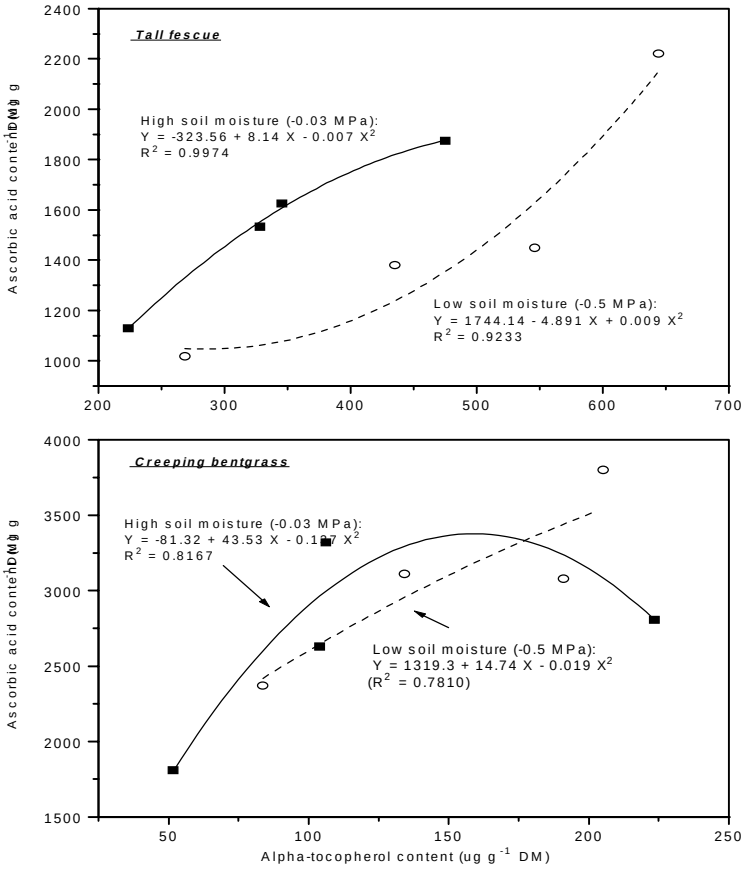

Fig. 5.5. Relationship between a-tocopherol and ascorbic acid content in tall fescue and creeping bentgrass grown under two soil moisture levels.

Discussion

Foliar application of SE and HA improved leaf water status of the three turfgrass species. Seaweed extracts enable the roots to access moisture normally not available to plants (Senn, 1987). The improvement of SE on water relation in this study may result from greater root activity, which may be more important than root mass in enhancing leaf moisture content.

Kononova (1966) reported that humic substances (60 ppm) enhanced root development and plant growth. It was noted that humic substances altered the carbohydrate metabolism of plants and promoted the accumulation of reducible sugars. The plant cells with a high level of these sugars retain more moisture. Low-molecular-weight humic substances, such as fulvic acid, enhance ion transport (such as iron) which may regulate transpiration rate and reduce water loss. In addition, the interactions between HA and hormones were observed in most cases (Schnitzer and Khan, 1972). Humic acid may impact the leaf water status through influencing osmotic adjustment and transpiration rate.

Water stress reduced the shoot and root growth significantly in the three grass species. This result supports previous studies by White et al. (1993), who showed that water stress suppressed growth of Kentucky bluegrass. It has been well documented that water stress results in stomatal closure, reduction of photosynthesis, and oxidative stress (Lawlor, 1995). These factors may contribute to the reduction of the plant growth.

Water stress resulted in a significant increases in antioxidant α-tocopherol endogenous concentration in the three turfgrass species. This result is consistent with previous research conducted by Price and Hendry (1989), Moran et al. (1994), and Tanaka et al.(1990). Alpha-tocopherol is a lipid-soluble antioxidant and concentrated in the chloroplasts, especially the thylakoid

membranes. The increase of this antioxidant may be triggered by excess production of reactive oxygen species in the photosynthetic apparatus under water stress. Increased α-tocopherol levels may serve as an acclimation strategy of plants to tolerate water deficits.

Water stress did not induce an increase of ascorbic acid on dry weight basis. Ascorbic acid not only quenches reactive oxygen species but also regenerates α-tocopherol (Alscher and Hess, 1993). The actual concentration of ascorbic acid results from the balance between synthesis and breakdown. Smirnoff(1993) noted that reduction of ascorbic acid levels under water stress may be due to the depletion by excess reactive oxygen species or utilization for cycling α-tocopherol.

The photosynthetic apparatus is sensitive to water deficit. Closure of stomata and direct inhibition of Calvin cycle enzymes result in exposure of cells to excess excitation energy (Smirnoff, 1995). This excess energy may be diverted to activate molecular oxygen. Excess accumulation of reactive oxygen species damages various macromolecules, resulting in lipid peroxidation and enzyme inactivation (Elstner, 1982). The active oxygen-induced damage to the cell may be minimized or prevented by increased antioxidant activity (Doulis, 1994).

Exogenous application of SE and HA enhanced the antioxidant (α-tocopherol and ascorbic acid) status in all three turfgrass species. Seaweed extracts contain not only cytokinin, gibberellin, and other phytohormones but also micronutrients and vitamins. Crouch (1993) noted that growth improvement by application of SE may result from the activity of cytokinin. Cytokinin can act as a hormone as well as a antioxidant to influence plant metabolism. Cytokinin contained in SE may be responsible for the enhancement of antioxidant status.

The results of this study also showed that turfgrass with higher antioxidant levels performed better under water stress. A closer correlation between antioxidant and growth in tall fescue (and Kentucky bluegrass) as compared with that in creeping bentgrass suggests that drought-tolerant tall fescue and Kentucky bluegrass may adapt to drought through an increase of their antioxidant status. Seaweed extract and HA may enhance hydrophobic and hydrophilic antioxidant activity and thus promote growth and leaf water status. It may be concluded that antioxidant status could be manipulated with exogenous application of plant growth regulators.

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Chapter 6

Low-molecular weight and enzymatic antioxidant response to plant growth regulators and drought in Kentucky bluegrass

Introduction

Water deficit stress affects plants at various levels. Stomatal closure, reduction of photosynthesis and osmotic adjustment are typical responses of plants to water stress at the first stage (Tanaka et al., 1990). Water stress-induced stomatal closure interferes with exchange of CO_2 and O_2 in the cell. It is well documented that a large proportion of the electron flux is diverted from CO_2 assimilation to O_2 reduction under stressed conditions as a consequence of the decrease in C_i (intercellular CO_2 concentration) and g_s (stomatal conductance) (Cornic and Briantais, 1991). Reduction of O_2 in the Mehler reaction results in formation of reactive oxygen species such as superoxide and hydrogen peroxide. In addition, absorption of excess light by chlorophyll leads to the formation of triplet excited chlorophyll which can then pass excitation energy to oxygen resulting in formation of singlet oxygen (Lawlor, 1995).

Research results suggest that many environmental stresses, such as drought, salinity, low temperature and herbicide pollution, damage plants through the reactive oxygen species (Quartacci et al., 1992; Larson, 1988; Monk et al., 1989; Price and Hendry, 1989, 1991; Smirnoff, 1993). Plants develop various antioxidants to cope with oxidative stress. Alpha-tocopherol, β-carotene, and superoxide dismutase (SOD) are important antioxidants which protect plants by suppressing oxidative injury. Alpha-tocopherol, which is located within

biological membranes, can quench oxygen radicals, stabilize cell membranes by influencing lipid organization, and protect chlorophyll (Hess, 1993). Water deficit may result in an increase of tocopherol concentration in plant tissues (Price and Hendry, 1989; Hashim et al., 1993; Tanaka et al., 1990). Based on the studies of 10 different grass species under water stress, Price and Hendry (1989) found that drought stress led to an increase of one to three fold of α-tocopherol concentration in 9 out of 10 species. They pointed out that species with a high tolerance of stress are defended through tocopherol.

Beta-carotene can protect chlorophyll and prevent the plants from photoinhibition. It is closely associated with the photosynthetic apparatus and quenches singlet oxygen efficiently. Stuhlfauth et al. (1990) found that acclimation of *Digitalis lannta* to low water potential resulted in a 25% increase of β-carotene.

Superoxide dismutase is the most efficient scavengers of the superoxide anion and an essential component of the ascorbate-glutathione cycle for the detoxification of toxic reactive oxygen species (Nakano et al., 1980). The close relationship between SOD activity and drought stress tolerance has been observed in several crops, such as maize (Malan et al.,1990) and tobacco (Perl et al., 1993). In tomato, cytosolic Cu/ZnSOD was induced strongly by drought while chloroplastic Cu/ZnSOD remained largely unaffected (Bowler et al., 1992). Drought stressed tomato showed increases both in transcript levels of cytosolic Cu/Zn SOD gene (eight fold) and enzyme activity (five fold); whereas, an increase in enzyme activity was observed in chloroplastic Cu/Zn-SOD (2.4-fold) without a corresponding increase in transcript levels (Perl et al., 1993). Although it was reported that major antioxidants showed differential responses to environmental stress, few studies have been done on the relationship among different antioxidants under drought stress.

It has been proposed that α-tocopherol is maintained until ascorbic acid has been completely oxidized, then α-tocopherol depletion occurs. Kunert and Ederer (1985) examined the relationship between vitamin E (α-tocopherol) and vitamin C (ascorbic acid) and found that the vitamin C to vitamin E ratio changes during the life cycle of the beech leaf. Finckh and Kunert (1985) noted that both the amount and ratio of vitamin E and C contributed to the

sensitivity of a seedling to the herbicide diphenylether. A few researchers examined the responses of α-tocopherol, SOD, and β-carotene to water stress but did not report the correlations among these antioxidants.

Proper application of certain plant growth regulators may improve turf quality and enhance stress tolerance (Schmidt, 1990; Schott and Walter, 1991; Van Staden, 1994). Both SE and HA, which possess cytokinin-like and auxin-like properties, can enhance plant growth, delay senescence and improve turf quality (Crouch, 1992; Fagbenro and Agboola, 1993; Finnie and Van Staden, 1985; Schmidt, 1990), increase tolerance to drought (Nabati, 1991), and improve nutrient uptake (Senn, 1991). However, the physiological mechanisms of the PGRs' effects on drought tolerance is still not fully understood.

The objectives of this study were to examine the interactive responses of various antioxidants to water stress and exogenous application of PGR, and to provide information for manipulation of water stress tolerance through alteration of antioxidant activity with growth regulators.

Materials and Methods

Plant culture, PGRs application, and drought stress treatment. Seeds of Kentucky bluegrass (*Poa pratensis* L. cv. 'Plush') were sown in a 10-cm diameter and 5 cm deep ring containing the same amount of Groseclose silt loam soil at 0.12 g ring $^{-1}$ in the greenhouse on 11 November 1995 (see Chapter 4 for details).

The combination of HA (25% active humic acid) and SE (dry formula) at rate of 5 l ha^{-1} and 326 g ha^{-1} was applied evenly on foliage of 2-wk old Kentucky bluegrass seedlings according to the procedures described in previous chapters. The two PGRs were supplied by Plant wise Biostimulant Co. (Louisville, KY).

Water stress treatments began when the seedlings were 3-wk old. The terrarium-like boxes (30 x 40 x 75 cm) were used, and two moisture levels (11.6 % and 20.1% which were equivalent to a water potentials of -0.5 and -0.03 MPa, respectively) were obtained by adding appropriate water (see Chapter 4 for details).

The treated seedlings of Kentucky bluegrass were permitted to grow in the terrariums under one of the two moisture regimes for 5 weeks. The fresh leaves from each treatment were sampled and frozen immediately with liquid N and then stored at - 20 ^{o}C.

Antioxidants determination.

Alpha-tocopherol. Alpha-tocopherol was extracted and determined according to the procedures described in Chapter 5.

Beta-carotene. Beta-carotene was extracted with 40 ml of HPLC-grade hexane containing 0.01% butylated hydroxytoluene (BHT). Frozen leaf segments (500 mg) were homogenized with a Polytron PT3000 (Kinematia AG) in the extraction medium for 2 minutes. The crude extract was washed with a saturated NaCl solution. Trace water was removed from hexane by filtering through anhydrous powdered sodium sulfate (5 g). The hexane phase was then evaporated to dryness and redissolved in a mobile phase containing MeOH/Acetonitrile/DCM (40:48:12, v/v/v).

The sample in the mobile phase was filtered with a Nylon acrodisc 13 mm x 0.2 µm filter and analyzed by a HPLC. The HPLC system consisted of a HPLC pump (Shimadzu LC 600), with a 20 µl injection loop, a UV detector (SPD-6A), a HPLC system controller (Shimadzu SCL-6B), and a CR501 chromatopac (Shimadzu, Japan).

Separations were performed on an analytical column of Pinnacle 3 µm ODS (100 x 4.6 mm) from Restek Corporation with a C-18 guard column (50 x 2.0 mm) (Whatman 30-38 µm mesh). The mobile phase was a mixture of methanol/acetonitrile/dichloromethane (40:48:12, v/v/v). Beta-carotene was detected with the UV detector set at 450 nm, and the concentration of β-carotene in the plant extracts was determined using peak height against the standard curve. Light was eliminated with aluminum foil, and dilutions were prepared when necessary to keep the absorption in the linear range of the standard curve. The β-carotene concentrations were calculated based on the standard curve.

Superoxide dismutase. Superoxide dismutase was analyzed according to the methods of Giannopolitis and Ries (1977) with the following modifications.

The frozen samples (1.0 g fresh weight) were homogenized with a Polytron in 10 ml

of 0.05 M Na_2HPO_4/NaH_2PO_4 (pH 7.0) buffer. The homogenates were filtered through four layers of cheesecloth and then centrifuged at 4 C for 20 min at 15,000 x g. The supernatants were collected and used for SOD assay. The crude SOD extracts were diluted ten fold with the extraction medium.

The reaction mixture was composed of 1.3 µM riboflavin, 13 mM methionine, 63 µM nitro blue tetrazolium (NBT), 0.05 M sodium carbonate (pH 10.2), and appropriate volume (50 µl) of the diluted extract. Deionized distilled H_2O was added to bring to the final volume of 3 ml. The riboflavin was added last. The mixture was illuminated in glass test tubes selected for uniform thickness and color. Identical solutions that were not illuminated served as blanks.

The reaction device consisted of a glass test tube holder (two beakers) immersed in water maintained at 25° C. A circular fluorescent lamp (GE, FC 8T9-WW-RS) was attached on the outside of the beaker, and the whole device was set in an insulated chest. The reaction was initiated and terminated by turning the light on and off. The light was on for 10 min.

Appropriate dilution of the supernatant was performed when necessary to ensure that the absorbance value of the sample fell within the linear section of the standard curve.

One enzyme unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of nitro blue tetrazolium (NBT) reduction measured at 560 nm on a spectrophotometer. The SOD activity was determined according to the equation:

SOD units g^{-1} fw = [(V/v) -1] (dilution factor)

Where $V = V_2 - V_1$, V_1 and V_2 are absorbance in absence of SOD without and with light, respectively. $V_1 = 0$. $v = v_2 - v_1$. v_1 and v_2 are absorbance in presence of SOD without and with light, respectively.

The standard SOD (Sigma)(1 mg of the product contains 4400 units SOD) was used to develop standard curve to verify the relationship between SOD activity and V/v ratio at 560 nm.

Results

Alpha-tocopherol response to water stress and PGRs

Water stress did not significantly influence α-tocopherol content (dry weight basis) in Kentucky bluegrass. However, application of SE and HA significantly enhanced α-tocopherol content under both low and high soil moisture (Table 6.1).

Beta-carotene response to water stress and PGRs

Water stress resulted in an increase of β-carotene in Kentucky bluegrass (Table 6.1). Beta-carotene concentration was 272.9 and 420.3 μg g^{-1} DM in the grass grown in high and low soil moisture, respectively.

Application of HA plus SE enhanced β-carotene status under both low and high water stress conditions (Table 6.1). Beta-carotene and α-tocopherol exhibited a similar response to exogenous application of the growth regulators.

Superoxide dismutase response to water stress and PGRs

The enzymatic antioxidant SOD activity of Kentucky bluegrass was not significantly influenced by water stress (Table 6.1). However, exogenous application of SE and HA promoted SOD activity, especially under drought stress. Table 6.1 shows that SOD activity in turfgrass subjected to drought was higher when the turf was treated with the PGRs as compared with non-treated control.

Relationship among the antioxidants.

Positive correlations among the three antioxidants were observed in Kentucky bluegrass grown under low and high soil moisture (Table 6.2). The correlation coefficients among the three antioxidants were statistically significant ($p < 0.1$).

Table 6.1. Alpha-tocopherol, β-carotene concentration and superoxide dismutase activity of Kentucky bluegrass as influenced by plant growth regulators and water deficit

		Soil Moisture Level (MPa)		
Treatment	Amount (ha^{-1})	-0.03	-0.5	Mean
		---------- α-tocopherol (μg g^{-1} DM) ----------		
HA+SE	5 l +326 g	554.5	534.3	544.4 a
Control	0	267.2	301.8	284.5 b
Mean		410.8 x	418.0 x	
		---------- SOD activity (unit g^{-1} DM) ----------		
HA + SE	5 l +326 g	7737.4	7132.2	7435.0 a
Control	0	6671.5	4198.6	5435.0 b
Mean		7204.0 x	5665.0 x	
		----------β-carotene content (μg g^{-1} DM) --------		
HA + SE	5 l +326 g	348.8	530.4	452.6 a
Control	0	196.9	310.2	261.6 b
Mean		272.9 y	420.3 x	

Means within each column (a, b) or row (x, y) followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.1$.
Alpha-tocopherol:
Moisture: p = 0.8898; PGR: p = 0.0003; Moisture * PGR: p = 0.5999.
Superoxide dismutase:
Moisture: p = 0.1206; PGR: p = 0.0925; Moisture*PGR: p = 0.5428. .
Beta-carotene:
Moisture: p = 0.0404; PGR: p = 0.0117; Moisture*PGR: p = 0.5971.

Table 6.2. Relationship among superoxide dismutase (SOD) activity (unit g^{-1} DM), α-tocopherol and β-carotene concentration (µg g^{-1} DM) of Kentucky bluegrass grown under high(-0.03 MPa) and low (0.5MPa) soil moisture

Soil Moisture	Antioxidant	Equation	Correlation Coefficient	P value
- 0.03 MPa	α-Tocopherol (Y) and SOD (X)	Y = 37.31+0.0518 X	0.6100	0.1083
	β-carotene(Y) and SOD(X)	Y = -43.06 + 0.0439 X	0.6297	0.0943
	β-carotene (Y) and α-Tocopherol (X)	Y = 55.34 + 0.5295 X	0.6461	0.0835
- 0.5 MPa	α-Tocopherol (Y) and SOD (X)	Y = 316.61 + 0.0179 X	0.2723	0.5141
	β-carotene (Y) and SOD (X)	Y = 146.58+0.0483X	0.8534	0.0070
	β-carotene (Y) and α-Tocopherol (X)	Y=180.47+0.5737X	0.6663	0.0712

Discussion

This study showed that α-tocopherol, β-carotene, and SOD status exhibited a parallel response to water stress. Alpha-tocopherol and β-carotene, two lipid-soluble antioxidants, are concentrated in the chloroplast. They not only protect chlorophyll by dissipating excess excitation energy but also scavenge reactive oxygen species. Van Hasselt et al. (1979) noted that both α-tocopherol and β-carotene effectively protected chlorophyll against red light-induced degradation. Alpha-tocopherol is distributed and performs its functions near the surface of membranes, while β-carotene is a component of photosynthetic light harvesting complex. Therefore, both antioxidants are closely related to the photosynthetic apparatus.

Superoxide dismutase has a central role in the defense against oxidative stress (Bowler et al., 1992). Of the two isozymes of SODs found in higher plants, Cu/Zn-SOD is located in both chloroplast and cytosol. The SOD can efficiently convert toxic superoxide anion into hydrogen peroxide, which further changes to water. This process occurs in the photosynthetic apparatus of chloroplasts.

It has been well documented that the photosynthetic apparatus, which is rich in unsaturated fatty acids, is a major target of reactive oxygen species (Lawlor, 1995). Under drought stress, excess reactive oxygen species attack lipids, proteins, and nucleic acids. This results in lipid peroxidation, protein inactivation, and DNA alteration (Mckersie et al., 1994). An high status of the antioxidants may be considered to be an adaptive mechanism of plants to drought stress.

Results of this study show that α-tocopherol, β-carotene, and SOD were enhanced with exogenous application of SE and HA. This is consistent with the results by Yokoyama et al. (1991) and Kim (1988), who showed that the antioxidants (ascorbic acid and β-carotene) increased substantially when plants were treated with plant growth regulators. Exogenous application of a synthesized PGR promoted β-carotene significantly (Yokoyama et al., 1991).

Seaweed extracts contain substantial amounts of hormones, especially cytokinin (Crouch, 1990). It has been reported that exogenous cytokinin enhances endogenous cytokinin

activity (Yan, 1993). Foliar spray with kinetin can stimulate photosynthetic rates (Treharne et al., 1970). Meidner (1967) noted that kinetin promoted stomatal openings by increasing endogenous cytokinin levels. Increase in net photosynthetic rate was shown to be primarily due to a decrease in stomatal resistance. Cytokinin also stimulates chloroplast development (Parthier, 1979), enhances phloem loading (Daie, 1986), and delays senescence. The effects of SE well match these functions of cytokinin. In addition, auxin-like activity of HA has also been established (Piccolo, 1990). Seaweed extract and HA may impact plant photosynthesis and growth by enhancing antioxidant systems through their hormonal activity.

Positive correlations among α-tocopherol, β-carotene and SOD activity were observed in both high and low soil moisture, and the correlation coefficients were statistically significant in almost all cases (Table 6.2). This suggests that the three antioxidants work cooperatively in suppressing oxidative injury induced by water stress. The responsive mechanisms of these antioxidants may be similar. This indicated that the two lipid-soluble antioxidants and enzymatic antioxidant (SOD) exhibit a similar response to water stress and exogenous growth regulators, and function elaborately in protecting plant under drought.

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Chapter 7

Influence of seaweed extract and endophyte infection on seasonal fluctuation of superoxide dismutase activity and photosynthetic function in tall fescue

Introduction

Tall fescue (*Festuce arundinacea* Schreb.) is not only a common cool season turfgrass species but also an important forage in many parts of the world. The growth and quality of tall fescue are frequently suppressed by various environmental stresses such as drought, salinity, and nutrient deficit (Watschke and Schmidt, 1992), and biotic stresses such as endophyte infection.

Tall fescue commonly acts as host for the endophyte-fungus [*Neotiphodium coenophialum* (Morgan-Jones and Games) Glenn, Bacon, Price, and Hanlin] in a relationship which is generally considered mutualistically symbiotic. Endophyte infected plants show better tolerance to various abiotic and biotic stresses. However, endophyte infected forage grasses are toxic to grazing animals (Bacon et al., 1986). The biological mechanisms of improvement of stress tolerance of plants infected by endophyte have not been understood.

Some environmental stresses impact plants through the reactive oxygen species (Smirnoff, 1995). Although reactive oxygen species are normal metabolic products in plant cells, their excess accumulation will cause damage to the cell components (proteins, lipids, nucleic acids, etc.) and result in a dysfunctions of normal metabolism (Doulis et al., 1994).

Superoxide dismutase (SOD), which catalyzes peroxide radical anions to dioxygen and hydrogen peroxide, is the most efficient antioxidant enzyme in a plant's defensive system (Smirnoff, 1995). The status of this enzyme is closely related to the plant stress tolerance (Bowler et al. 1992). Malan et al. (1990) noted that water stress resistance of maize inbreds was correlated to the activity of Cu/Zn-SOD. Drought stressed tomato showed increases both in transcript levels of cytosolic Cu/Zn-SOD gene (eight-fold) and enzyme activity (five-fold) (Perl et al. 1993). This suggests that plants with high SOD activity possess better tolerance to stressful environments.

Lawlor (1995) noted that the photosynthetic apparatus is a major target of toxic reactive

oxygen species. The photosynthetic efficiency largely depends on the balance between antioxidant status and active oxygen production. Inefficiency of antioxidant scavenging systems result in excess accumulation of reactive oxygen species, which damages photosynthetic electron transport components (Smirnoff et al., 1995). Stress conditions may block the light-driven photosynthetic electron transport and result in an increased loss of absorbed light energy via chlorophyll fluorescence (Miles, 1990; Lichtenthaler, 1988). Somersalo and Krause (1988) indicated that changes of chlorophyll fluorescence were related to photoinhibition of photosynthesis and cold acclimation of plants.

The chlorophyll fluorescence can be considered an intrinsic fluorescent probe of photosynthetic system in the chloroplasts (Lichtenthaler, 1988). Fluorescent emission by the chlorophyll of the photosystems makes it possible to carry out non-destructive assays to examine photochemical events of the photosynthetic process and monitor the function of photosynthetic apparatus and even antioxidant status, especially when plants are grown under stress.

Seasonal variation of SOD activity has been observed in white pine needles and the SOD activity was increased from the spring to the winter (Anderson et al., 1992). Bolhar-Nordenkampf and Lechner (1988) indicated that the ratio of variable fluorescence (F_v) to maximum fluorescence (F_m) (F_{vm}) declined from December through March, then increased from March through June. However, no study has been done on the antioxidant status of tall fescue in different seasons and its relation to the chlorophyll fluorescence emission.

Hormone action was involved during the acclimation of plants to stress (Morgan, 1990). Seaweed extract, a hormone-containing materials, has been shown to impact plant growth (Crouch, 1990). It delayed senescence, enhanced photosynthetic rate, and improved stress resistance when applied to turfgrass (Goatley and Schmidt, 1990; Nabati et al. 1994). However, the mechanism of SE'effect on photosynthesis remains unclear. There are no reports on the impact of seaweed on antioxidant status and chlorophyll fluorescence emissions in higher plants.

The objective of this study was to examine the influence of SE and endophyte infection on SOD activity and chlorophyll fluorescence of the tall fescue in different seasons and to determine the possibility of monitoring the antioxidant activity through the change of chlorophyll fluorescence.

Materials and methods

This experiment was carried out at the Southwest Virginia Research and Extension Center at Glade Spring, Virginia (81 40' west longitude; 30 47' north latitude). The tall fescue '(*Festuca arundinacea* Schreb, cv 'Kentucky-31') was established in 1985 in 1.5 ha plot. Forty-eight yearling angus and angus x hereford steers were randomized to four paddocks of endophyte infected (E+; 80%) and four paddocks of endophyte-free (E-; < 5%) tall fescue. Steers grazed from April through October. A complete randomized block design was used and each plot replicated four times.

Nitrogen was applied at 60 kg ha^{-1} in early April 1995. The grass was treated with soluble seaweed extract from *ASCOPHYLLUM nodosum* at a rate of 3.5 kg ha^{-1} in April and July 1996. The dry seaweed extract was dissolved in tap water and the solution was sprayed evenly on the foliage at a volume of 35 ml m^{-2}.

Fresh leaves from each plot were sampled at 28-d intervals from April through November. The samples were frozen immediately with liquid N and maintained at -20 ^{o}C before analysis. The SOD activity was determined according the procedure described in Chapter 6.

At the same time of sampling for SOD analysis, chlorophyll fluorescence was detected with a dual wavelength fluorometer (Model *OS-50*) (Optic- Science, Tyngsboro, MA). The canopy of the tall fescue was covered for 15 minutes with a black ring (10 cm diameter) to exclude light. Chlorophyll fluorescence was excited by a 635 µm source and detected at 690 µm and 730 µm wavelength for each plot. The intensity of excitation source was 400 µE. The various parameters of chlorophyll fluorescence (F_o, F_m, F_{vm}, etc.) were recorded automatically. Three measurements were taken from each plot. The data were downloaded to a computer and subjected to ANOVA analysis with SAS.

The shape of the chlorophyll fluorescence emission spectra is determined mainly by two parameters, the chlorophyll content and the photosynthetic capacity of the leaf. The ratio of the two fluorescence maxima F_{m690}/F_{m730} is a suitable stress indicator of plants. The F_{m690}/F_{m730} increases not only with decreasing chlorophyll content but also when the process of photosynthesis declines (Rinderle and Lichtenthaler , 1988). Therefore, the higher the ratio F_{m730}/F_{m690} (a reciprocal of ratio F_{m690}/F_{m730}), the higher the chlorophyll content.

Environmental stresses induce loss of photosynthetic capacity. The $F_{vm}690$, a ratio of variable

fluorescence (F_{v690}) to maximum fluorescence (F_{m690}) of PS II, provides adequate means to assess the photosynthetic capacity of plants (Bolhar-nordenkampf and Lechner, 1988). Photoinhibition of photosynthesis is defined as a decrease in efficiency and capacity of photosynthetic O_2 evolution or CO_2 assimilation, caused by excessive light energy. The higher the F_{vm690}, the less photoinhibition of photosynthesis, and the higher the photosynthetic capacity.

Results

The F_{vm690} showed a seasonal fluctuation in all the treatments. It remained the same from April through June, then increased gradually from July through September, and thereafter gradually dropped from September through November (Fig 7.1).

Seaweed extract increased F_{vm690} from April through November. The differences of F_{vm690} between SE and non-SE treatments were significant ($p < 0.1$) in May, July, September, and November (data not shown). Seaweed extract significantly improved photosynthetic capacity during summer and late fall when photosynthetic capacity was relative low.

Endophyte infection influenced photosynthetic capacity in tall fescue. Endophyte infection caused a decrease of photosynthetic capacity of tall fescue, especially during early growing season (from May through August).

The F_{m730}/F_{m690} reached the first peak in May, then decreased from June to July, and reached second peak in September, then reduced from October through November (Fig.7.2).

Seaweed extract enhanced chlorophyll content, in terms of F_{m730}/F_{m690}, during the growth season, especially in May and July. The F_{m730}/F_{m690} in the non-treated tall fescue decreased significantly , while The F_{m730}/F_{m690} of SE-treated tall fescue remained high in July (Fig.7.2). On average, SE increased the F_{m730}/F_{m690} significantly in May, July, and September (data not shown).

Endophyte infection influenced chlorophyll content, in terms of F_{m730}/F_{m690}, in tall fescue (Fig.7.2). The tall fescue with endophyte infection exhibited a lower chlorophyll content (F_{m730}/F_{m690}) as compared with the tall fescue without endophyte infection, especially in the early growing seasons.

Superoxide dismutase activity decreased from April through July and then increased gradually from July through November. Seaweed extract enhanced the SOD activity significantly in all months (Fig.7.3). However, the SOD activity was not affected or slightly increased by endophyte infection

in tall fescue.

There was close correlations of SOD activity with F_{vm690} and F_{m730}/F_{m690} during the active growing period (from July through September) (Fig 7.4), although not in early or late seasons.

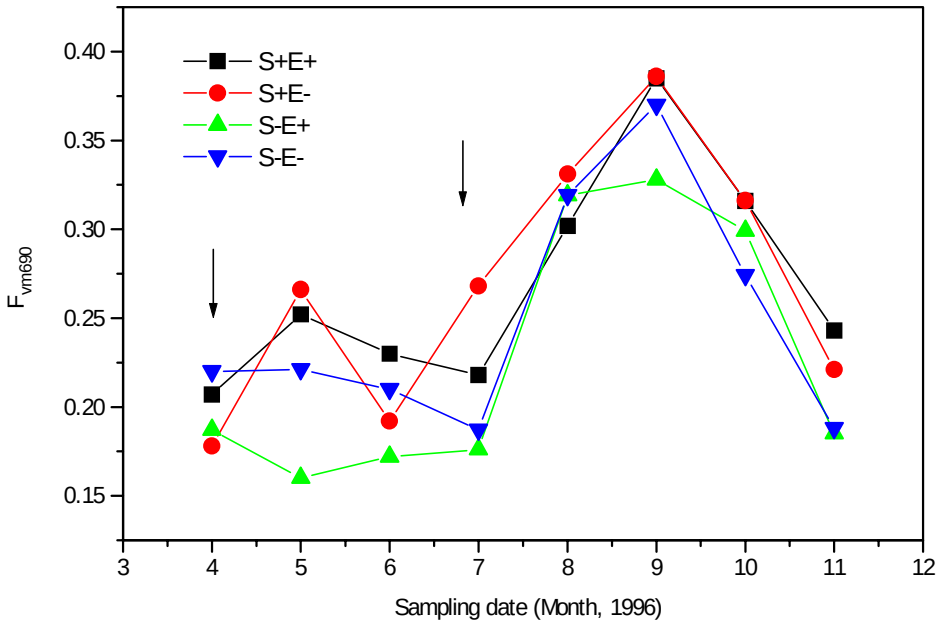

Fig.7.1. Effects of seaweed extract and endophyte infectione on F_{vm690} in tall fescue (Glade Spring, Virginia) (The arrow indicates the SE application date)

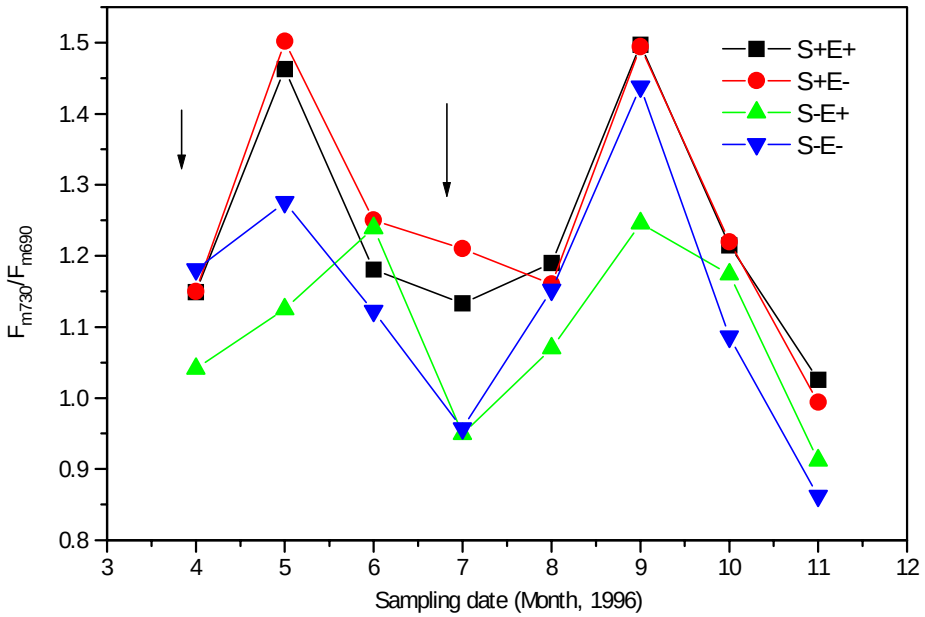

Fig.7.2. Effects of seaweed extract and endophyte infection on F_{m730}/F_{m690} (chlorophyll content) in tall fescue (Glade Spring, Virginia) (The arrow indicates the SE application date).

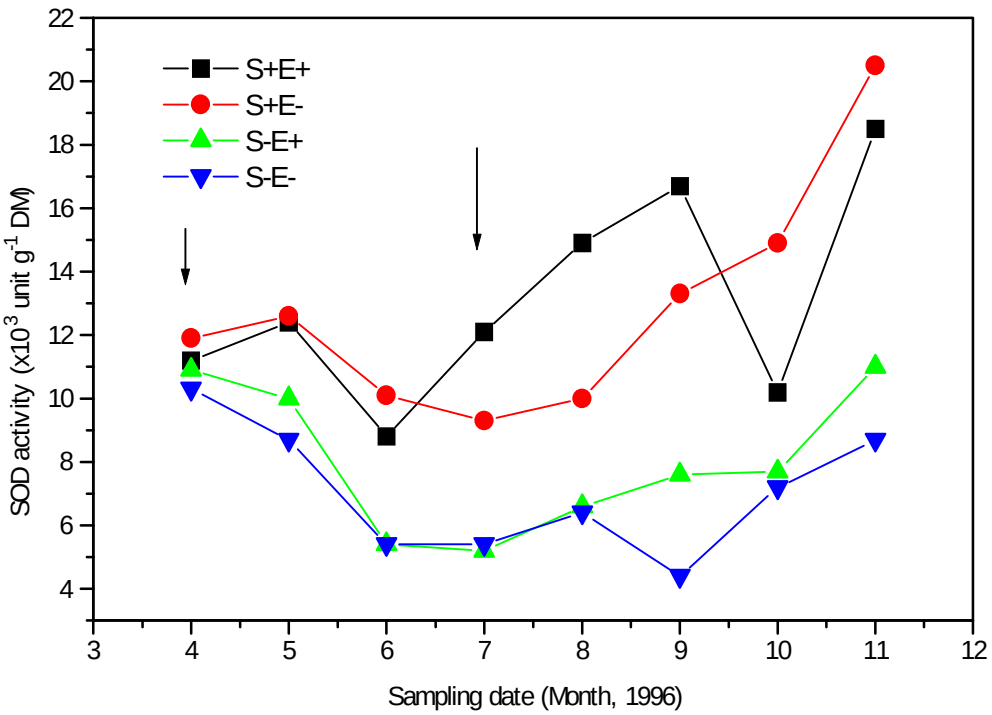

Fig.7.3. Effects of seaweed extract and endophyte infection on SOD activity in tall fescue (Glade Spring, Virginia) (The arrow indicates the date of SE application)

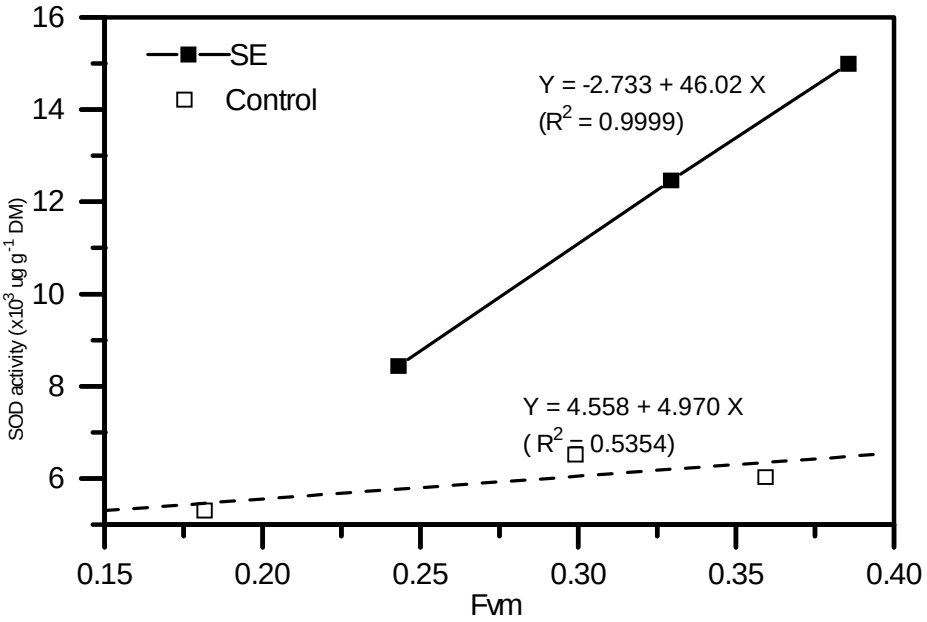

Fig.7.4. Relationship between SOD activity and photosynthetic capacity (Fvm) in tall fescue from July through September.

Discussions

The photosynthetic capacity in terms of F_{vm690} of tall fescue did not show substantial change from April through July, and then increased rapidly from July through October (Fig.7.1). This seasonal change may resulted from the interactive effects of leaves growth stage with environmental factors. The canopy consisted of fully developed leaves and high light intensity contributed to the high photosynthetic capacity. This result is consistent with the study reported by Bolhar-Nordenkampf et al. (1988). The decrease of F_{vm690} in late fall may be related to the leaf senescence , because low chlorophyll content in the leaves was observed during this growing period (Fig.7.2).

The chlorophyll content of tall fescue in terms of F_{m730}/F_{m690} showed a similar seasonal change as F_{vm690} did from July through November. The tall fescue with a high chlorophyll content exhibited a high photosynthetic capacity in terms of F_{vm690}. The Fvm and F_{m730}/F_{m690} are stress indicators which monitor the functions of photosynthetic apparatus. Environmental stresses such as drought and high-light or biotic stresses such as endophyte infection may cause a significant photoinhibition or even photooxidation, under which low Fvm and F_{m730}/F_{m690} occurs.

The seasonal change of SOD activity matched well with chlorophyll content (F_{m730}/F_{m690}) in most period of the growing season (Fig.7.2 and Fig.7.3). The change of SOD activity found in this study was similar to the results reported by Doulis et al. (1993) with red spruce and Anderson et al. (1992) with white pine. It appeared that tall fescue with high SOD activity showed high chlorophyll content and photosynthetic capacity. However, SOD activity increased while photosynthetic capacity decreased in October and November. This different response may be resulted from leaf senescence process in which excess free radicals damaged photosynthetic components and triggered an increase of SOD activity.

Endophyte infection reduced photosynthetic capacity in terms of F_{vm690} and chlorophyll content in terms of F_{m730}/F_{m690} in the early growing season (fig.7.1. and 7.2), but did not significantly impact SOD activity in tall fescue. This result is inconsistent with study of Richardson et al. (1993), who noted that endophyte infection enhances photosynthesis at low water potential Endophyte is known to play a key role in improving both tall fescue ‘s drought tolerance and pest resistance. The effects of endophyte infection on plant stress resistance and photosynthesis may be related to the environmental conditions in which the plants grow. In this study, both the endophyte-free and the

endophyte-infected tall fescue treatments grew well because no severe drought stress occurred during the growing season. This may partly explain why the endophyte-infected tall fescue did not increase photosynthetic capacity of tall fescue.

Foliar application of SE enhanced SOD activity and photosynthetic capacity in terms of F_{vm690}, delayed senescence (high chlorophyll content in terms of F_{m730}/F_{m690}) in the tall fescue, specially during the summer. The high F_{vm690} and F_{m730}/F_{m690} in the tall fescue treated with SE suggest that SE may improve the function of photosynthetic apparatus via reducing photoinhibition. Goatley (1988) used chlorophyll fluorescence to monitor leaf senescence and showed that SE significantly reduced the leaf senescence and increased photosynthetic rate on a land area basis. The findings in this study are supported by the study by Goatley (1988).

Senn (1987) noted that SE, which contain hormones, vitamins, micronutrients and other bioactive compounds, impact plant metabolism. Cytokinin, a component contained in SE, has been showed to act as an antioxidant in suppressing the toxicity of reactive oxygen species (Leshem, 1981). This current research leads to the conclusion that SE influences photosynthetic activity possibly through antioxidant activity. The growth and quality of tall fescue could be improved with application of PGRs , especially during the growing season with high environmental stress.

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Chapter 8

Summary

Field, greenhouse, and laboratory studies were carried out to investigate the influence of plant growth regulators (PGRs), seaweed extract (SE) from *Ascophyllum nodosum* and humic acid (HA), on the antioxidant status, photosynthetic activity, drought tolerance and growth of three cool- season turfgrass species grown under low and high soil moisture. The two PGRs were supplied by Plant Wise Biostimulants Co. (Louisville, KY, USA). .

Foliar application of the HA product at 23.7 and 47.4 l ha^{-1} improved leaf water status and growth of tall fescue, Kentucky bluegrass and creeping bentgrass grown under drought 6 weeks after HA treatment. The leaf water status of the three grass species was improved with application of HA at rates of 11.8, 23.7, and 47.4 l ha^{-1}. However, the root weight and shoot weight were significantly increased only with application of high rates of HA (23.7 and 47.4 l ha^{-1}). The root length and plant height responses to HA differed with turfgrass species. The result of this study is consistent with the results reported by Tan et al. (1979), Fagbenro and Agboola (1993), and O'Donnell (1973).

Humic acid, which posses auxin-like activity, may improve leaf water status by increasing reducible sugar accumulation, osmotic pressure of the cells, and regulation of stomatal behavior under water stress. Since the plants were grown in the terrariums with a limited soil moisture, increases of shoot and root growth can be only accomplished with improvement of photosynthetic function. A high level of leaf water status is a vital factor to the normal photosynthetic function. The HA induced better leaf water relations may contributed to the improvement of plant growth.

Humic acid (2.9% active HA) at 15.5 l ha^{-1} or SE at 326 g ha^{-1} significantly reduced dollarspot incidence and improved turf quality in creeping bentgrass. The incidence of dollarspot, a serious fungus-infected disease, could be increased by various environmental stresses including drought. Although a significant suppression of this disease with application of the PGRs was identified in this study, it is not known what compound or compounds present in the PGRs that brings about the observed effects. The PGRs may reduce the dollarspot incidence through improving leaf water status

and growth vigor.

Although shoot growth was suppressed by drought stress, the root length and root weight were not obviously reduced in all three grasses grown under drought environments. The leaf water status was higher in tall fescue and Kentucky bluegrass as compared with the creeping bentgrass under drought. However, there was no significant moisture and PGRs interaction on shoot and root growth.

Drought stress induced an increase of the antioxidants α-tocopherol and ascorbic acid concentrations in the three turfgrass species. In the experiment with Kentucky bluegrass, drought stress enhanced β-carotene concentration, but did not significantly influence superoxide dismutase activity. This result is consistent with the results obtained by Moran et al. (1994), Price and Hendry (1989), Spyropoulos and Mavrommatis (1978), and Tanaka et al. (1990). Stomatal closure and reduction of photosynthesis under water deficit cause excess production of reactive oxygen species because excitation energy is diverted to activate O_2, not for CO_2 fixation. High levels of antioxidants can efficiently scavenge reactive oxygen species and prevent photosynthetic apparatus from oxidative injury.

Foliar application of humic acid (25% active HA) at 5 l ha^{-1} and/or SE at 326 g ha^{-1} consistently enhanced the α-tocopherol and ascorbic acid content, leaf water status, and growth in the three grass species grown under low or high soil moisture environments. The tall fescue and Kentucky bluegrass contained higher level of α-tocopherol and lower level of ascorbic acid as compared with the creeping bentgrass, a species with poor drought tolerance. A significant increase of the antioxidants in the turfgrasses treated with the small amount of the PGRs can not be explained solely by their nutritional value. Homonal activity of the PGRs may contribute to these effects.

In the separate experiment with Kentucky bluegrass, β-carotene and superoxide dismutase status were increased with application of SE and HA under low (-0.5 MPa) and high (-0.03 MPa) soil moistures. It is possible that the antioxidant systems are triggered by a common signal from the PGRs.

There were close correlations between antioxidants and shoot or root growth in the three grass species under low and high soil moisture. High levels of antioxidants were coincidence with better plant growth regardless of soil moisture levels. Since antioxidants protect photosynthetic function through reducing chlorophyll degradation and membrane lipid peroxidation under oxidative stress,

turfgrass with high concentration of antioxidants may produce better photosynthetic function and thus growth.

The enzymatic antioxidant superoxide dismutase activity, photosynthetic capacity in terms of F_{vm690} and chlorophyll content in terms of F_{m730}/F_{m690} exhibited a seasonal fluctuation in endophyte-infected and endophyte-free tall fescue. Superoxide dismutase activity decreased from April through July and then increased from July through November. This results is consistent with the result reported by Anderson et al. (1992) with red pine. High level of SOD activity during winter may be induced by low temperature stress.

Application of SE to the grass enhanced SOD activity, photosynthetic capacity, and chlorophyll content, especially at one month after SE treatment. However, endophyte infection did not significantly influence the SOD activity, although it reduced photosynthetic capacity and chlorophyll content of tall fescue during the summer.

Positive correlations between SOD activity and photosynthetic capacity during the summer were found in both SE treated and the control. This suggests that antioxidant status may be related to the photosynthetic function of tall fescue. However, the photosynthetic capacity and the chlorophyll content decreased from September through November, while SOD activity increased during the same period. The reduction of photosynthetic capacity and chlorophyll content during the late growing season may result from leaves senescence and low temperature.

Vita

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