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THE EFFECT OF SEA WEED (Ascophyllum nodosum) EXTRACT ON ANTIOXIDANT ACTIVITIES AND DROUGHT TOLERANCE OF TALL FESCUE (Festuca arundinacea Schreb.)

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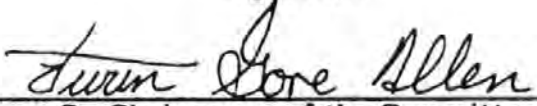JAMAL YOUSEF AYAD, B.S., M.S.

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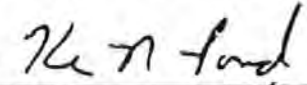
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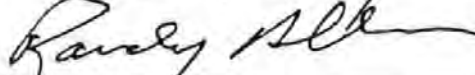
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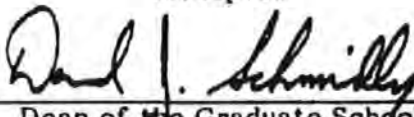
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THE EFFECT OF SEAWEED (ASCOPHYLLUM NODOSUM) EXTRACT ON ANTIOXIDANT ACTIVITIES AND DROUGHT TOLERANCE OF TALL FESCUE (FESTUCA ARUNDINACEA SCHREB.)
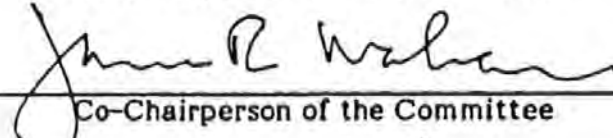
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ABSTRACT

Plants have developed enzymatic and nonenzymatic antioxidant mechanisms to prevent oxidation of cellular compartments. Enhancing these mechanisms might help plants cope with encountered stresses. Greenhouse and field studies were conducted to examine the influence of seaweed (*Ascophyllum nodosum*) extract on antioxidant enzymes activities, forage growth, and persistence of tall fescue (*Festuca arundinacea* Schreb.). Furthermore, effects of soil moisture, plant genotype, and infection with the endophyte *Neotyphodium coenophialum* ([Morgan-Jones and Gams] Glenn, Bacon and Hanlin) were investigated. In a greenhouse experiment, seaweed extract was applied to 'Martin' tall fescue at 0, 2, 4, 6, 8, and 10 kg ha^{-1} in a randomized block design with four replicates. Seaweed extract linearly increased ($P < 0.05$) glutathione reductase activity. Superoxide dismutase and ascorbate peroxidase were also increased but responses differed by time and treatment rates. In a second greenhouse experiment, seaweed extract was applied at 4 kg ha^{-1} to endophyte-infected and non-infected 'Georgia Jessup' and 'KY-31' tall fescue grown with 50-100% and 30-100% field capacity soil moisture in a completely randomized design with four replications. Glutathione reductase activity increased ($P < 0.05$) in both genotypes in response to seaweed extract and moisture stress and tended to increase ($P < 0.07$) in response to the endophyte. Seaweed extract increased ($P < 0.05$) superoxide

LIST OF TABLES

endophyte-free fescue was found to eliminate animal problems, but endophyte-free genotypes are generally less persistent than infected genotypes particularly in drought and heat stressed environments.

Drought stress is a major limiting factor for crop production in many regions of the world. Water deficit stress results in reduction of plant growth that is correlated to the diminished rate of CO_2 assimilation. Excessive levels of the active oxygen species are usually produced under stress conditions. These active species can cause deleterious injuries to plant cellular structure and metabolism. Plant cells are usually protected against such effects by a complex of antioxidant systems. The endogenous protective mechanisms include carotenoid, glutathione, ascorbic acid, alpha-tocopherol, and several enzymes, including superoxide dismutase, ascorbate peroxidase, and glutathione reductase. These components scavenge the active oxygen species in the cell compartments. However, the observed reductions in plant performance under different stresses indicate that plants cannot compensate for the effects of all stresses.

There is evidence that plant growth regulators could be used to partially counteract environmental constraints. Natural growth substances contained in various seaweeds and their extract have been shown to produce changes in plant growth and stress tolerance. The most widely investigated seaweed is *Ascophyllum nodosum*. The mode of action of seaweed extract on plant growth and stress tolerance is not well understood. First, it was thought that effects

2

CHAPTER II

LITERATURE REVIEW

Plant Growth Regulators and Seaweed Extract

Plant growth and development is influenced by various endogenous and exogenous variables. The resulting physiological processes regulate the rhythm of growth and development. Plant growth regulators have been used over the past decades for several purposes. "They are compounds, either naturally occurring or chemically synthesized, which regulate biosynthesis and /or metabolic systems and, thus, affect the expression of biological responses in living tissues" (Yokoyama and Keithly 1991; p. 19).

Plant hormones have been described as chemical messengers regulating responses to environmental signals as well as regulating normal plant developmental changes (Morgan 1990). Abscisic acid (ABA) has been reported as a signal inducing stomatal closure (Davies and Zhang 1991). Small changes in ABA distribution were reported to trigger stomatal closure (Morgan 1990). Cytokinins play a role in stomatal regulation under water stress. However, ABA and cytokinin have an opposite role in drought stress. Increased levels of ABA and decreased cytokinin activity under water stress favor stomatal closure and reduce water transpiration (Morgan 1990). Levels of other plant hormones like

4

different plant types, stage of growth and season of the year at harvest, and the different methods of seaweed processing (Featnoby-Smith and Van Staden 1984: Hofman et al. 1986; Mairah et al. 1989). Methods of application, frequency, rate and timing of the application account for additional variables in seaweed extract responses (Nelson and Van Staden 1984: Crouch. 1990).

Foliar application of seaweed concentrate to seedlings of *Pinus pinea* L. increased shoot length and weight and decreased in the root to shoot ratio (Atzmon and Van Staden 1994). They also found that root drenches accelerated root growth and increased lateral root dry weight. This indicated that root application of seaweed concentrate improved seedling quality and increased the ability of seedlings to survive transplanting into pots. Seaweed extract as a foliar fertilizer has been noted to have various beneficial effects on many crops (Button and Noyes 1964).

Although seaweed products have been utilized in agricultural practices for many years. the precise mechanism by which they elicit beneficial growth responses is still not fully understood (Crouch and Van Staden 1993). Seaweed is known to contain a wide range of minerals but does not satisfactorily account for the total changes in mineral content of plants treated with seaweed extract (Blunden 1977). Seaweed contains both carbohydrates which stimulate plant growth and those which might reduce growth (Blunden and Woods 1969). Moreover. some of these effects have been attributed to the presence of growth substances such as cytokinins. which are known to occur at relatively high levels

6

1986). On the other hand, the results of field experiments indicated no significant increase in wheat yield in response to seaweed extract from *Durvillaea potatorum* or *Ecklonia maxima* (Myers and Perry 1986). Similar responses to seaweed extract application on barley (*Hordeum vulgare*) plants where no effect on the yield was observed (Taylor et al. 1990).

In seed germination studies, seaweed rates of 0.5 and 1.0% (v/v) improved germination of creeping red fescue (*Festuca rubra*) seeds (Button and Noyes 1964). They found that high rates, above 5% (v/v), retarded seedling emergence. Seaweed extract has been also tested on beet (*Beta vulgaris*) seeds (Wilczek and Ng 1982). They found that treated seed had better germination rates than the control at temperatures of 10, 15, 20, and 30°C but not at 25°C.

The effect of seaweed on plant growth under stress conditions has been studied. Higher grain production of wheat has been reported in response to seaweed application on wheat grown under water stress (Mooney and Van Staden 1985). Under K deficiency, Becket and Van Staden (1989) found that application of seaweed concentrate improved both grain number and grain weight. However, no significant increase in yield was observed under sufficient supply of potassium. The effect of seaweed concentrate on cucumber (*Cucumis sativus*) root growth has been studied (Nelson and Van Staden 1984). They reported an increased root mass and root to shoot ratios after five weeks of treatment. Seaweed extracts have been reported to increase chlorophyll

8

inability of plants to withstand environmental stress is the primary source of agronomic loss in agriculture. Water stress influences plant growth at various levels of plant development (Smith and Griffith 1993). As soil water declines, wilting occurs when water movement in dry soil toward the root becomes slow and unable to replace water loss by plants (Hsiao 1973). Higher plants respond to water deficit in several ways. Stomatal closure, leaf rolling, and osmotic adjustments are well known morphological and physiological adaptations to water stress (Zhang and Kirkham 1994). As a result of that, the flux of CO_2 is lowered at the same time resulting in a decreased rate of CO_2 fixation (Kaiser 1987; Smirnof and Colombe 1988). Several reports suggested that water stress reduced photosynthetic rate through limiting CO_2 diffusion due to reduced stomatal conductivity, which reduces intercellular CO_2 concentration(Lawlor 1995; Turner et al. 1978).

Plant growth is the result of cell division, enlargement and differentiation. All these factors are affected by water status of the plants (Mckersie and Leshem 1994). Water stress inhibits growth through inhibition of various physiological and biochemical processes including photosynthesis, respiration, hormones, and nutrient uptake and metabolism (Kramer and Boyer 1995). Volaire and Thomas (1995) investigated the role of physiological responses in survival of prolonged soil moisture deficit in vegetative plants of two *Dactylis glomerata* populations. As drought progressed, leaf extension decreased to zero, water status declined, and water soluble-carbohydrates at first increased then decreased. They also

10

implicated in many degradative processes of plants, including aging, wounding, pathogen attack, and exposure of plants to "xenobiotics" or to some stress situations (Elstner 1987; Leshem 1988).

The limitation of carbon dioxide exchange and metabolic fixation during water stress results in exposure of chloroplasts to excess excitation energy. Although there are many photoprotective mechanisms through which plants can dissipate light energy (Smirnoff 1993). some of the excitation energy may be diverted to activate molecular oxygen (Bailey and Walker 1992).

Photosynthetic electron transport is maintained at a relatively higher rate in the stressed leaves as compared to the large decrease in the rate of CO_2 fixation (Kaiser 1987). This imbalance may result in the over-reduction of the electron transport chain components and facilitate the transfer of electrons to O_2 (Baisak et al. 1994). This univalent reduction of O_2 gives rise to the formation of toxic O_2 species which are highly reactive and in the absence of any protective mechanism can damage various aspects of cell structure and function (Elstner 1982; Asada and Takahashi 1987; Smirnoff 1993). Other environmental stresses such as excessive light. temperature extremes, air pollution. wounding or herbicides can disturb normal cellular metabolism and upset the balance of oxygen free radical production (Elstner et al. 1987).

The antioxidative defense enzymes, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase showed increased activity in Ni-treated drought sensitive seedling of wheat (Pandolfini et al. 1996). Antioxidant enzymes have also been found in higher levels in maize (*Zea mays*) plants with greater drought resistance (Del Longo et al. 1993), and in cotton (*Gossypium hirsutum* L.) plants exposed to drought (Burke et al. 1985).

Superoxide dismutase

Superoxide dismutase is the most efficient scavenger of the superoxide anions and is an essential part of the ascorbate-glutathione cycle (Beyer et al. 1991). It is an important agent for protecting leaves from harmful effects of membrane lipid destruction (Dhindsa et al. 1981). Plants have different forms of SOD containing Cu and Zn, Fe, or Mn as prosthetic metals. Copper-SOD and Zn-SOD are 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). Recent research results indicated that plants that over-express SOD, such as alfalfa (*Medicago sativa*) and tobacco (*Nicotiana tabaci*), are more tolerant to paraquat, and freezing and more resistant to photoinhibition (Bowler et al. 1991: Gupta et al. 1993). Drought has been found to enhance cytosolic Cu/ZnSOD of tomato plants while chloroplastic Cu/ZnSOD remained unaffected (Bowler et al.

14

inactivation (Burke et al. 1985). The potential role of glutathione reductase and glutathione in tolerance mechanisms to oxidative and water stresses has been examined in several plants, but results are ambiguous (Smith et al. 1989). Drought influences the amount of glutathione reductase activity present in leaves, but the means whereby the enzyme is elevated relative to controls depends on the plant (Smith et al. 1989). Withholding water for 5 d from barley plants grown in the greenhouse caused increased levels of glutathione reductase (Smirnoff and Colombe 1988). On the other hand, field grown cotton and wheat plants showed an increase in enzyme activity through an inhibition of the decline in activity that normally occurs during the growing season of irrigated crops (Burke et al. 1985). Other studies showed that leaf position, planting density, and canopy temperature are important variables that affect levels of glutathione reductase in response to water stress (Burke and Hatfield 1987: Smith et al. 1989).

Ascorbate peroxidase

This enzyme catalyzes the peroxidation of ascorbate to dehydroascorbate, and dehydroascorbate reductase utilizes reduced glutathione to maintain ascorbate pool in a reduced form (Jablonski and Anderson 1978). High levels of ascorbate reductase have been detected in chloroplasts (Gillham and Doge 1986). Bender et al. (1994) determined the enzyme activities of ascorbate peroxidase and glutathione reductase in wheat flag leaves at weekly

16

were found to inhibit the activity of toxic oxygen species, hydrogen peroxide and superoxide which are the major elements for chlorophyll degradation during senescence (Sexton and Woulhouse 1984). Moreover, cytokinins might be used as signals for triggering of other antioxidant systems (Schmidt and Zhang 1997). Increased concentrations of SOD, photosynthetic capacity, chlorophyll content and α-tocopherol (vitamin E precursor) in turfgrass have been observed in response to seaweed extract (Schmidt and Zhang 1997). They also indicated that application of seaweed extracts and humic acids enhanced antioxidant activities under both favorable moisture and drought stress conditions. More reports indicated increased activity of superoxide dismutase as a result of seaweed application on tall fescue (Coelho et al. 1997). Moreover, in lambs grazing seaweed-treated tall fescue, a linear increase ($P < 0.13$) in serum vitamin A and serum Se ($P < 0.10$) were observed (Fike 1995). Similar trends were observed in cattle (Allen et al. 1997). This suggests that it may be possible to reduce or prevent oxidative damage by strengthening the defense mechanisms through enhancing the function of antioxidants with exogenous application of certain plant growth regulators.

Tall Fescue

Tall fescue is a cool-season grass that is found growing in wet places throughout Europe and North Africa (Borrill 1976). Meadow fescue has been described by Linnaeus in 1753 as *Festuca elatior*. A few years later, tall fescue

18

presence and concentration all suggested that well managed tall fescue had high quality and should result in good animal performance. This poor performance of animals attracted the attention of investigators into tall fescue after its rapid acreage expansion (Fribourg et al. 1991).

Most tall fescue pastures established prior to 1987 are endophyte infected (Shelby and Dalrymple 1987). The source of endophyte infection for these pastures was probably the original collection site of Kentucky-31, since seed collected from that site were highly endophyte-infected (Pedersen et al 1991).

The high productivity of livestock grazing endophyte-free tall fescue compared to endophyte-infected tall fescue is well documented (Steudeman and Hoveland 1988). However, the agronomic differences between endophyte-infected and endophyte-free tall fescue are not completely understood (Pedesen et al., 1991).

Tall Fescue and Growth Regulators

The effects of growth regulators on the physiological and biochemical mechanisms of plants is complex (Schmidt and Zhang 1997). Plants may acclimate to stress conditions by changing their membrane composition (Hale and Orcutt 1987). Yan (1993) reported increased membrane fluidity and salt stress tolerance of ryegrass (*Lolium perenne*) in response to the application of cytokinin-like materials. This might indicate that cytokinin acts as a signal for

(Clay, 1989). Presence of the fungi is often associated with enhanced fitness of their host grass (Siegel et al. 1987). Since some fungi coevolved with their grass hosts and are non-parasitic, the endophyte-plant relationship is truly symbiotic (Fibroug et al. 1991).

Bacon et al. (1977) found intrecellular hyphae growing within tall fescue. They referred to the fescue endophyte as a biotype of *Epichloe typhina*. Based on *in vitro* similarities, Morgan-Jones and Gams (1982) placed the endophyte of fescue in the genus *Acremonium coenophialum*. In 1996, Glenn et al. reclassified tall fescue endophyte into *Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon and Hanlin.

The infection of tall fescue by a consistently symptomless, nonsporulating endophytic fungus was reported in 1941 from New Zealand (Fribourg et al. 1991). However, the significance of the impact of the endophyte's presence did not attract general attention until several decades later when the economic impact resulting from association of a fungal endophyte infected tall fescue with animal problems was recognized (Fribourg et al. 1991).

The fungus grows as the seed germinates, invading the seedling shortly after germination. Infection of the first and subsequent leaves does not occur until sheath differentiation occurs. Herd et al. (1977) found that the concentration of endophyte's metabolic activity in plant tissues decreased with increasing plant size. They reported that approximately 70% of endophyte metabolic activity present in a plant is located in the leaf sheath. This suggested

22

Endophyte infection status has been found to improve seedling performance (Pedersen and Burrus 1989). Endophyte status may also affect seedling survivals. Bouton and Burton (1988) reported that endophyte-infected tall fescue had twice the number of surviving plants, compared to endophyte-free plants of the same genetic lines, four months after seeding. In North and Central Texas, stand maintenance and forage availability were improved in pastures showing high levels of infection with endophytic fungi compared to pastures with low infection levels (Read and Camp 1986).

The influence of ecological and environmental factors on the response of the endophyte and tall fescue has received substantial attention. Endophyte infection influenced leaf mass depending upon genotype while the relative benefit of endophyte on "pseudostem mass" was affected by defoliation (Belesky and Fedders 1996). In some cases endophyte gave growth and size advantage to the host and did not in others.

The mechanism involved in this mutualistic symbiosis relative to plant-fungus compatibility, increased plant growth and morphological alterations are poorly understood. Plant growth regulators produced by fungi have been associated with growth and morphological alterations of similar mutualism (Meyer 1974). It was observed that the endophyte of tall fescue produced indole acetic acid but not detectable levels of cytokinins and gibberellins (De Battista et

24

Bush and Burrus, 1988). However, environmental factors such as heat, moisture, and soil fertility are known to alter alkaloid production in the plant. Belesky et al. (1989) reported that decreased moisture increased loline alkaloid concentration. Also, ergot alkaloid production is influenced by both drought and soil fertility (Arachvaleta et al. 1992). Moreover, Hill et al. (1991b) and Agee and Hill (1994) indicated that production of ergovaline in tall fescue is influenced not only by the endophyte, but, also by moisture, temperature, and plant genotype.

Several attempts have been made to solve the problems of fescue toxicosis. Negative animal effects of tall fescue infected with the endophyte are reduced by substituting endophyte free tall fescue (Collins 1991). Utilization of low endophyte cultivars of tall fescue has been shown to improve animal performance; however, insufficient information is available on the direct effects of endophyte infection on the productivity and composition of tall fescue herbage (Fritz and Collins 1991). Studies have shown improved animal performance compared to animals consuming endophyte-infected tall fescue (Hoveland et al. 1983; Neal and Schmidt 1985; Read and Camp 1986). However, endophyte-free tall fescue is less tolerant to drought and heat stressed environments, overgrazing and insect damage than endophyte-infected fescue (West et al. 1988; Johnson et al. 1985). This resulted in stand loss and lower forage production of endophyte-free fescue, especially under drought conditions (Read and Camp 1986; Gates and Wyatt 1989; West et al. 1988).

Nitrogen assimilation has been demonstrated to be more efficient in endophyte infected plants (Bacon et al. 1986; Lyons et al., 1986). Increased response to nitrogen fertilization associated with the endophyte has been observed in a single clone in the greenhouse (Archevaleta et al. 1989).

Endophyte had no effect on leaf osmotic potential and minimal effect on water soluble mineral and sugar concentrations, and endophyte-mediated adaptation to drought stress was an avoidance mechanism. Because enhanced drought tolerance is a result of specific interactions between plant genotypes and endophyte isolates, selection of endophyte specifically for enhancing drought tolerance in tall fescue is not likely to have a general effect on the plant population (Hill et al. 1996).

Insect resistance due to endophyte infection in tall fescue has not yet been well documented in the field. However, the lack of insect damage in endophyte-infected tall fescue in the field suggests such a relationship (Bacon and Siegel 1988). Endophyte-induced insect resistance has been documented in the laboratory and the greenhouse (Clay et al. 1985; Johnson et al. 1985). Roberts et al. (1992) reported that endophyte infected KY 31 tall fescue expressed more chitinase than endophyte free KY 31 tall fescue which emphasis that chitinase is related to tall fescue persistence. Chitinase is an antifungal hydrolase associated with disease resistance (Joose 1995).

Even though the cellular protection system will allow plants to tolerate some level of stress, the observed reductions in plant performance indicate that plants cannot fully compensate for the effects of all stresses (Burke and Mahan 1991).

Seaweed extract has been used in agriculture for centuries (Crouch 1990). Since these extracts have been derived from natural materials, they have no known harmful effect on the environment. Their application can stimulate plant growth and development and improve resistance to environmental stresses (Schmidt 1993). Improved root and shoot growth of turfgrass (Zhang 1997) and membrane permeability in ryegrass (*Lolium perenne*; Yan 1993) are means of enhancing plant stress tolerance.

Enhancement of antioxidant metabolism might improve plant growth performance under stress. The application of seaweed extract increased SOD, chlorophyl content, and α-tocopherol under both favorable moisture and drought stress conditions (Schmidt and Zhang 1997). Increased activity of SOD, in tall fescue (*Festuca arundinacea* Schreb.) sprayed with seaweed extract was reported by Allen et al. (1997) and Coelho et al. (1997).

Seaweed extracts have been shown to have cytokinin activity that is capable of producing physiological changes at low application rates (Brain et al. 1973). Naturally occurring and synthetic cytokinins are known to promote growth and development under adverse environmental conditions (Nabati et al. 1991).

were kept under frequent irrigation and fertilization using Hoagland solution (Hoagland and Arnon 1938). Plant shoots were cut to 2 cm above the soil surface 1-wk before the time of seaweed extract application.

Six seaweed (*Ascophylum nodosum*; Acadian Seaplants Limited, Dartmouth, Nova Scotia, Canada) extract levels [0 (Control), 2, 4, 6, 8, and 10 kg ha '] were applied in water solution to the surface of the sand on 15 Jan. 1996. Seaweed extract treatments were arranged in a completely randomized block design with four replicates. Blocking was on the original plant material in order to minimize potential effects of plant genotype variation. Leaf samples were taken at day 1, 7, 14, 21, 28, and 42 after treatments were applied to monitor effects on SOD, glutathione reductase and ascorbate peroxidase activities in plant tissues. Fully expanded leaf blades were harvested, frozen in liquid N, and stored in a freezer at -90°C until they were extracted and analyzed.

Enzyme extraction

For assays of glutathione reductase and superoxide dismutase, 0.5 g of fresh leaf segments were thoroughly ground and homogenized with a cold mortar and pestle using 5-ml of 50-mM K-phosphate buffer (pH 7.0) containing 0.1-mM EDTA and 1% polyvinylpolypyrrolidone (Madamanchi and Alscher 1991). The homogenate was centrifuged at 14,000 g for 15 min at 4°C and the

$$\text{Enzyme (Units/ml)} = (\frac{V}{v} - 1)(\text{dilution factor})$$

where V and v represent the rate of the assay reaction in absence and in presence of the superoxide dismutase respectively (Giannopoltis and Ries 1977).

Glutathione reductase assay. Glutathione reductase activity was determined from the rate of NADPH oxidation measured by the decrease in absorbance at 340 nm (E=6.2 mM cm⁻¹) following the procedure of Foyer and Halliwell (1976). The 1-ml assay mixture contained 0.1-M Tris-HCl buffer (pH 7.8), 2 mM-EDTA, 50-µM NADPH, 0.5-mM GSSG, and 30 µl of the crude enzyme extract. The assay was initiated by the addition of NADPH and was monitored at 25°C using a spectrophotometer (DU-100, Beckman Instrument Inc., Fullerto, CA). The initial velocity of the reaction was determined, and activity was expressed as µmole of NADPH oxidized hr⁻¹ mg⁻¹ fresh weight.

Ascorbate peroxidase assay. Ascorbate peroxidase activity was measured according to (Allen 1995) by monitoring the rate of ascorbate oxidation at 290nm (E=2.8 mM cm⁻¹). The reaction mixture contained 50mM HEPES buffer (pH 7.0), 1mM EDTA, 1.0mM H_2O_2, 0.5 mM ascorbate, and 30µl enzyme extract. The H_2O_2 was added last. Tubes were stirred and the absorbance was read at 290nm using a spectrophotometer (DU-100, Beckman Instrument Inc., Fullerton CA). Ascorbate peroxidase activity is expressed as µmole of ascorbate oxidized h⁻¹g⁻¹ of fresh weight.

yielded a dark green powder with a strong amine odor. Few peaks were detected by GC indicating the presence of proteins and/or carbohydrates that are not resolvable by GC. The water extract resulted in a dark powder with little discernible odor. The water extract was further resolved with a Sep-Pak C_{18} solid phase extraction column. After loading, the column was washed 3X with water and 3 separate fractions (F1, F2, F3) were collected and freeze-dried.

Bioassay of seaweed fractions

Endophyte-free 'Kentucky-31' tall fescue, obtained from Dr. Henry Fribourg, Univ. of Tennessee, was established in the greenhouse in 100 x 41 x 23 cm plastic flats on 3 Oct. 1995. Plants were kept under uniform conditions and water was supplied to be non-limiting. Nutrients were supplied through applying Hoaglands' solution (Hoagland and Arnon 1938) once a week. Single plants were transplanted into plastic pots, containing 3.5 kg sand, on 7 Feb. 1997. Plant shoots and roots were cut to 7 and 3 cm, respectively, at the time of transplanting.

The following treatments were applied to tall fescue on 7 Feb. 1997, at the time of transplanting: (1) Control (water); (2) Intact seaweed extract (SWE); organic solvent extracts (3) CH_2Cl_2 and (4) MeOH; (5) Water soluble extract and its soluble fractions (6) Fraction 1 (F1), (7) Fraction 2 (F2), and (8) Fraction 3 (F3).

Results

Experiment 1

An interaction between time and treatment was present ($P \le 0.05$) for SOD. therefore, treatments were tested further by time. By day 1, seaweed extract appeared to increase SOD activity at application rates of up to 6 kg ha^{-1} but resulted in lower SOD activity at higher treatment rates (quadratic effect. $P \le 0.06$: Table 3.1). By day 7. this quadratic response ($P \le 0.05$) was also observed. Effect of seaweed on SOD tended ($P \le 0.08$) to be quadratic on d 14 but by day 21. SOD activity was increased in response to all increasing rates of seaweed application (linear response, $P \le 0.05$). Superoxide dismutase activity of tall fescue was also higher ($P \le 0.01$) for the mean of seaweed extract treated plants. compared to the control at 21-d. No effect of seaweed on SOD was observed at either day 28 or day 42.

Effects of seaweed extract on glutathione reductase were consistent across sampling dates and no interaction was observed. Thus, effects of seaweed extract were tested over time for this antioxidant (Table 3.2). Averaged over all sampling dates, glutathione reductase activity increased linearly ($P \le 0.05$) with increasing seaweed extract rates. Moreover, the mean glutathione reductase activity of seaweed extract treated plants tended ($P \le 0.06$) to be higher than the control. When the data were examined by time, the response

38

Table 3.2. The effect of seaweed extract rates on glutathione reductase activity (μmole NADPH h^{-1} g^{-1} fresh weight) of 'Martin' tall fescue at 1, 7, 21 and 42 days following application.

Seaweed rate kg.ha^{-1}	Days after seaweed application				
	Mean[†‡]	1[§]	7	21	42[¶]
0	3298	3024	3250	2978	3940
2	3853	3673	3949	3903	3886
4	3298	3350	3150	2551	4141
6	3986	4566	2762	3324	5292
8	4204	4774	3410	3652	4981
10	4041	4146	3815	3287	4915
SE	270	324	591	472	531

[†] Linear effect of seaweed ($P \leq 0.05$).
[‡] the control differed from the mean of the seaweed treatments ($P \leq 0.06$).
[§] Linear effect of seaweed ($P \leq 0.01$).
[¶] Linear effect of seaweed ($P \leq 0.06$).

Table 3.3. The effect of seaweed extract rates on ascorbate peroxidase activity
(μmole ascorbate h^{-1} g^{-1} fresh weight) of 'Martin' tall fescue at 1, 7, 21
and 42 days following application.[†]

Seaweed rate kg.ha^{-1}	Days after seaweed application			
	1[‡]	7[§]	21[¶#]	42[#††]
0	746	470	909	1076
2	725	625	3380	1677
4	1726	704	1324	2007
6	834	404	1707	2315
8	803	931	1792	2943
10	923	1052	1646	2539
SE	145	149	403	413

[†]   Time x treatment interaction ($P \leq 0.05$).
[‡]   Quadratic effect of seaweed ($P \leq 0.05$).
[§]   Linear effect of seaweed ($P \leq 0.05$).
[¶]   Cubic effect of seaweed ($P \leq 0.05$).
[#]   Control vs. seaweed ($P \leq 0.05$).
[††]   Linear effect of seaweed ($P \leq 0.01$).

compared to the mean of the fractioned parts. Ascorbate peroxidase activity was higher ($P \leq 0.01$) in tall fescue treated with crude seaweed extract compared to the mean of the seaweed fractions.

Discussion

Experiment 1

Application of seaweed extract increased activities of the antioxidant enzymes: superoxide dismutase, glutathione reductase, and ascorbate peroxidase. An increase in SOD in response to seaweed extract has been shown previously (Allen et al. 1997; Coelho et al. 1997; Zhang 1997). As far as we are aware, the effects of seaweed extract on glutathione reductase and ascorbate peroxidase have not been previously demonstrated.

The enhancement of antioxidant enzyme activity varied over seaweed extract rate and time after the application. The activity of SOD appeared to increase in response to seaweed extract rate up to 6 kg ha^{-1} but no advantage to higher rates was evident. The activity of glutathione reductase and ascorbate peroxidase appeared to respond to rates at least through 8 kg ha^{-1}.

The finding of enhanced α-tocopherol, ascorbic acid, β-carotene and SOD activity of tall fescue, Kentucky bluegrass (*Poa pratensis*), and creeping bent grass (*Agrostis palustris* Huds *A. Stolonifera* L. cv. 'Penncross) activity (Zhang 1997) at 0.32 kg seaweed extract ha^{-1} suggests that activity may be increased even at lower rates.

44

differences in forage mass of tall fescue grown in the greenhouse or in the field due to seaweed extract treatment at a rate of 4 kg ha^{-1}. On the other hand, Nabati et al. (1994) indicated an improvement in shoot growth of Kentucky bluegrass in response to seaweed extract fortified with peat, humic acid, and thiamine at a rate of 38 L ha^{-1}. Zhang (1997) also reported an increased dry clipping weight of Kentucky bluegrass in response to seaweed extract at a rate of 0.3 kg ha^{-1}. Thus, the effect of seaweed extract on plant growth may be both rate and species dependent.

The enhancement of the antioxidant enzyme activity by seaweed extract application suggests its use as a bioassay for seaweed fractions. In our experiment, the increased antioxidant activities in response to seaweed extract was 30%, 56% and 82% higher than the control for SOD, glutathione reductase, and ascorbate peroxidase. The higher enzyme activities in response to the intact seaweed extract compared to the fractions appeared to indicate that fractioning seaweed extract especially through organic solvents resulted in at least some loss of this effect. The 36%, 50%, and 19% increase in SOD, glutathione reductase, and ascorbate peroxidase activities, respectively, in response to water soluble Fraction 3 compared to the control might indicate that water soluble factors could be responsible for the observed seaweed responses.

Moreover, the application rate for the different fractions of seaweed extract was based on the application rate of intact seaweed extract. In the previous experiment, the increased rates of seaweed showed a quadratic

46

Except for the reduction in shoot weight in response to organic solvent fraction (methanol), plant biomass was not affected by seaweed extract or its water soluble fractions. Therefore, antioxidant enzyme activities and not biomass could be used to assay for seaweed extract activity. The use of water soluble Fraction 3 resulted in similar responses to the use of intact seaweed extract at least for SOD and glutathione reductase. This suggests that the active component of seaweed extract appeared to be water soluble. However, further investigation is needed to identify the active component or components in seaweed extract and the optimum rates at which they induce their responses.

Tall fescue (*Festuca arundinacea* Schreb.) is a widely distributed cool-season forage in the south-eastern United States. Water availability and high summer temperatures appeared to limit tall fescue growth and persistence (Sleper and Buckner 1995). However, tall fescue plants that are infected with the endophyte fungus *Neotyphodium coenophialum* ([Morgan-Jones and Gams] Glenn, Bacon, and Hanlin; Glenn et al. 1996) often have increased drought tolerance, increased insect and nematode resistance, and improved N metabolism and assimilation (Pedersen et al. 1990). Clay (1990) related these characteristics in part to the presence of fungal alkaloids (Clay 1990). More over, the massive rooting of tall fescue is usually considered a major factor in its wide adaptation.

Numerous studies have reported beneficial symbiotic relationships among the grass endophytes *N. coenophialum* and *N. lolli* and their respective hosts of tall fescue and perennial ryegrass (*Lolium perenne*; Latch et al. 1985; Hill et al. 1991; West et al. 1993). Endophyte infected plants had a greater tillering rate and tiller survival as well as enhanced root and shoot dry matter accumulation (reviewed by Malinowski et al. 1997).

Malinowski et al. (1997) determined the influence of endophyte on selected growth aspects and plant water status of meadow fescue (*Festuca pratensis* Huds). They found that plants associated with the endophyte have increased root and shoot growth and were better adjusted to water depletion. Under water stress, stomatal activity was affected by the endophyte which

50

Goatley and Schmidt (1990b) conducted field and greenhouse experiments to measure seedling Kentucky bluegrass (*Poa pratensis* L.) growth responses to foliar applications of benzyladenine or a fortified seaweed extract (containing 500 mg L^{-1} of glycol kinetin and 500 μg L^{-1} gibberellins) applied alone or in combination with chelated Fe. The seaweed extract significantly increased root and shoot growth in both field and greenhouse experiments. The seaweed extract also increased the gross CO_2 exchange rate on a land area basis 4 and 6 wk after treatment in a winter experiment.

Development and the use of cultivars and management strategies resulting in improved drought resistance continues to be one of the most important needs in crop production due to the limited water resources available for agriculture. New management strategies to improve drought tolerance may include the use of growth regulators (Marcum and Jiang 1997).

Antioxidant systems within plants, including superoxide dismutase, glutathione reductase and ascorbate peroxidase, help to protect cells from damages due to active oxygen species produced under water stress. Moreover, *N. coenophialum* has provided tall fescue plants with more stress tolerance compared with non-infected plants, but the mechanisms involved are not clear. The suggested relationship to plant and fungal alkaloids may be due at least in part to the antioxidant activity of the alkaloid (Larson 1988). Seaweed extracts have been noted recently to improve plant growth and antioxidant activities in turfgrasses (Zhang at al. 1997) and in tall fescue (Coelho et al. 1997). To our

52

Six seaweed extract rates of 0 (Control), 2, 4, 6, 8, and 10 kg ha^{-1} were supplied in water solution to the sand on the day of transplanting. Water was applied to the tubes to maintain moisture level at field capacity and 30-50% of field capacity gravimetricly. Water treatments began at the time of transplanting and continued until the experiment was terminated on 19 April 1996.

At the end of the experiment, roots were washed from sand. Shoot and root biomass was measured after drying in an oven at 55°C for 48 hr. Root to shoot ratio was calculated. Expanded plant canopy height from the root-shoot junction to the tip of the longest leaf was measured on the fresh sample prior to drying.

Data were analyzed as a completely randomized design with a 6 x 2 factorial arrangement of treatments with four replicates of each treatment (Steel and Torrie 1981). Effects of water, seaweed extract, and their interactions were tested. Linear, quadratic, and cubic effects of seaweed extract rates were tested by orthogonal contrasts. The control was also compared with the mean of the seaweed extract treatments.

Experiment 2

Seeds of both endophyte-infected and -free Kentucky 31 obtained from Dr. Henry Fribourg, Univ. Of Tennessee and of Georgia Jessup tall fescue from Dr. Joe Bouton, University of Georgia, were established in the greenhouse as previously described. These seed sources provided genetically similar plants,

54

measure SOD, glutathione reductase, and ascorbate peroxidase activity. On 16 Feb. 1997 all plants were harvested at 2 cm, weighed, dried at 55°C and reweighed to determine total dry weight of aerial plant parts.

Data were analyzed as a completely randomized design with a factorial arrangement of treatments (Steel and Torrie 1981). Effects of treatments and their interactions were tested.

<div align="center">

Results
</div>

Experiment 1.

Total dry plant weight (roots plus shoots) of tall fescue was reduced ($P \leq$ 0.06) in response to the low moisture level (Figure 4.1). However, there was an interaction ($P \leq 0.05$) between moisture and seaweed extract rate treatments. Thus, the data were analyzed by moisture level.

Seaweed extract reduced total dry plant weight under field capacity moisture level (quadratic response; $P \leq 0.01$). Additionally, the control differed ($P \leq 0.01$) from the mean of the seaweed treatment. No effect of seaweed treatments was observed at the low moisture level on total plant weight.

Shoot dry weight was reduced ($P \leq 0.01$) in response to moisture level (Figure 4.2). No seaweed effect was observed on shoot dry weight. For root dry weight, an interaction ($P \leq 0.05$) between moisture and seaweed extract rate treatments were observed (Figure 4.3) and, therefore, data were analyzed by

<div align="center">

56
</div>
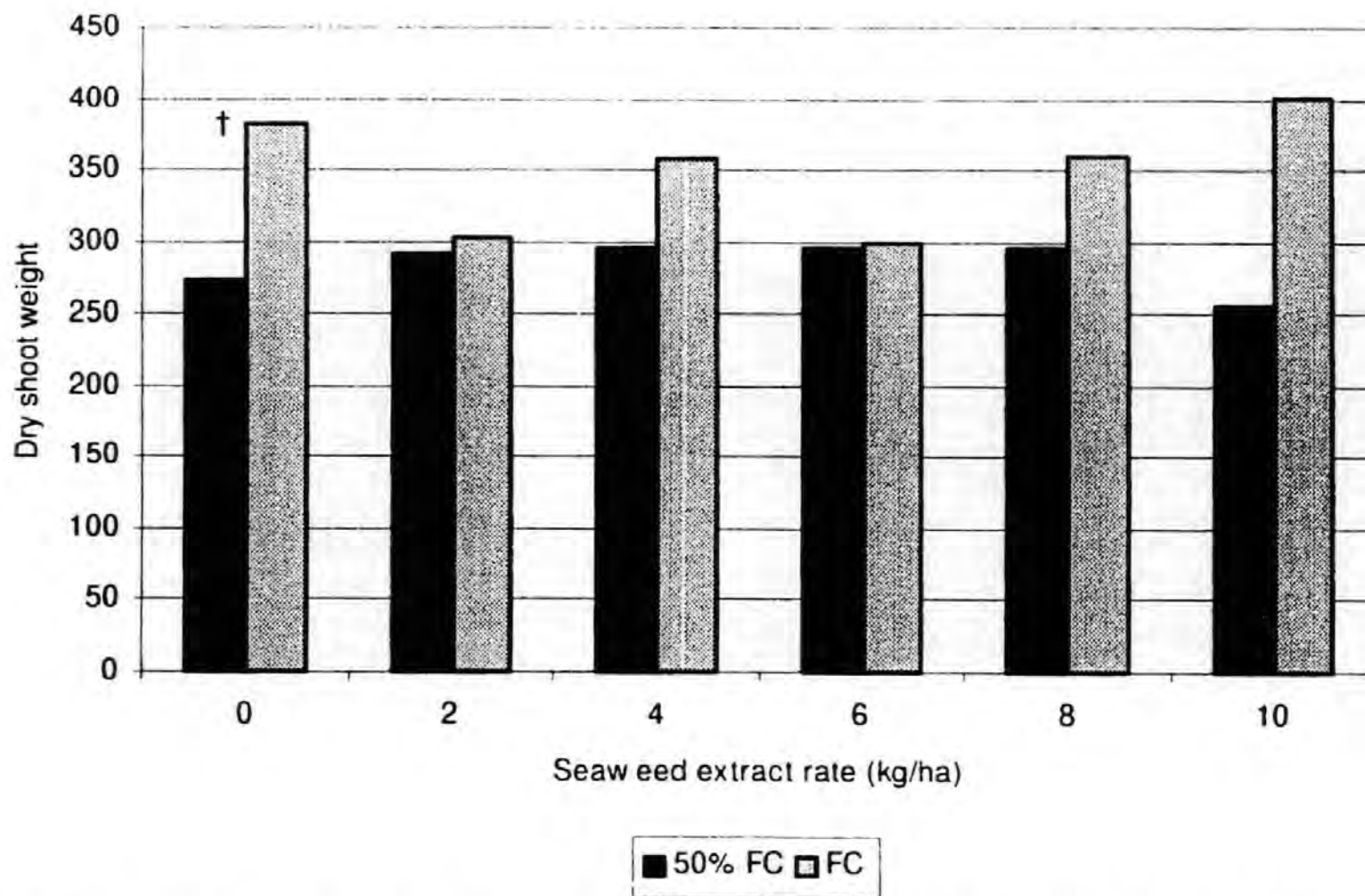
Figure 4.2. Effect of moisture level and seaweed extract rate on dry plant shoot weight of tall fescue. [† Indicated difference due to moisture level ($P \leq 0.01$)].

moisture. Root weight was reduced by seaweed extract under the field capacity moisture treatment (quadratic response; $P \leq 0.01$). Moreover, the control differed ($P \leq 0.01$) from the mean of seaweed extract treatments. No effect of seaweed extract was observed under the lower moisture level treatment.

Root to shoot ratio was affected by both moisture and seaweed rate (Figure 4.4). Seaweed extract reduced root to shoot ratio linearly ($P \leq 0.01$) in response to increasing seaweed extract rate under field capacity moisture condition. The control differed ($P \leq 0.01$) from the mean of seaweed extract treatments. No changes in root to shoot ratio in response to seaweed extract was observed under higher moisture stress.

Experiment 2

Water stress reduced ($P \leq 0.01$) shoot growth of both KY-31 and Georgia Jessup tall fescue (1.2 vs. 1.6 mg plant[-1]: SE 0.06; Table 4.1). Endophyte-infected fescue had higher ($P \leq 0.05$) shoot dry weight than endophyte-free tall fescue (1.5 vs. 1.3 mg plant[-1]; SE 0.06) but the effect was modified by moisture levels (Interaction $P \leq 0.05$). Interactions ($P \leq 0.01$) among moisture, genotype, and endophyte treatments were found, thus, the data were further analyzed by moisture treatments. Georgia Jessup tall fescue plants had greater ($P \leq 0.05$) plant shoot weight compared to KY-31 plants (1.5 vs. 1.3 mg plant[-1]: SE 0.06).

Table 4.1. Effect of moisture level, genotype, endophyte status (E+, E-), and seaweed extract (E+, E-) on dry plant shoot weight (mg) of tall fescue.

	Moisture level (% of field capacity) [t]							
	30 - 100 [‡]				50 - 100			
	Genotype				Genotype			
	GA [§]		KY-31		GA		KY-31	
	Endophyte		Endophyte		Endophyte		Endophyte	
Seaweed	E+	E-	E+	E-	E+	E-	E+	E-
S+	1.5	1.0	1.2	1.1	1.9	1.8	1.5	1.5
S-	1.8	0.8	1.1	1.0	1.6	1.8	1.7	1.4

[t] Moisture x genotype x endophyte interaction ($P \leq 0.01$, SE=0.11).

[‡] Genotype x endophyte interaction ($P \leq 0.01$, SE=0.08).

[§] E+ differed from E-, averaged over seaweed (1.65 vs. 0.9; $P \leq 0.01$, SE=0.09)

Table 4.2. Effect of moisture level, genotype, endophyte status (E+, E-), and seaweed extract (E+, E-) on extended plant height (cm) of tall fescue.

	Moisture level (% of field capacity) [†]							
	30 - 100 [‡]				50 - 100			
	Genotype				Genotype			
	GA		KY-31		GA		KY-31	
	Endophyte		Endophyte		Endophyte		Endophyte	
Seaweed	E+	E- [§]	E+	E- [§]	E+	E-	E+	E-
S+	28.3	25.8	28.6	30.5	31.9	32.3	29.6	34.3
S-	30.5	21.9	32.9	22.5	29.0	31.0	30.3	36.5

[†] Moisture x endophyte interaction ($P \leq 0.05$, SE=1.68).

[‡] Endophyte x seaweed interaction ($P \leq 0.05$, SE=1.89).

[§] S+ differed from S- across endophyte free of both genotypes (28.1 vs. 2.2; $P \leq 0.05$, SE=1.70).

Table 4.3. Effect of moisture level, genotype, endophyte status (E+, E-), and seaweed extract (E+, E-) on number of tillers per plant of tall fescue.

	Moisture level (% of field capacity) [†]							
	30 - 100 [‡]				50 - 100			
	Genotype				Genotype			
	GA		KY-31[§]		GA		KY-31	
	Endophyte		Endophyte		Endophyte		Endophyte	
Seaweed	E+	E-	E+	E- [¶]	E+	E-	E+	E-
S+	26	23	21	22	19	19	15	20
S-	25	21	15	16	15	19	19	16

[†] Moisture x genotype x endophyte x seaweed interaction ($P \leq 0.05$, SE=2.4).

[‡] Genotype x endophyte x seaweed interaction ($P \leq 0.05$, SE=2.2).

[§] Endophyte x seaweed interaction ($P \leq 0.05$, SE=2.5).

[¶] S+ differed from S- ($P \leq 0.05$, SE=1.7).

over both endophyte-infected and non-infected plants. In Georgia Jessup tall fescue, only the endophyte-infected plants had higher ($P \leq 0.05$) enzyme activities in response to seaweed extract.

Effect of moisture, genotype, endophyte, and seaweed on glutathione reductase were consistent and no interaction were observed. Glutathione reductase activity increased ($P \leq 0.05$) under water stress (Table 4.5). Endophyte-infected tall fescue of both genotypes tended to be higher ($P \leq 0.07$) than the non-infected plants. The application of seaweed extract enhanced ($P \leq 0.05$) the activity of the enzyme.

Moisture stress increased ($P \leq 0.01$) ascorbate peroxidase activity (1374 vs. 1043; SE 74; Table 4.6). Seaweed extract also increased ($P \leq 0.01$) ascorbate peroxidase activity (1354 vs. 1063; SE 74). However, interaction ($P \leq 0.01$) among moisture, genotype, and seaweed were observed. Under low moisture stress, there was a genotype by seaweed interaction where the application of seaweed extract increased ($P \leq 0.05$) ascorbate peroxidase activity only in KY-31 and not in Georgia Jessup plants. Under the high moisture stress. Georgia Jessup plants had higher enzyme activities ($P \leq 0.05$) than KY-31. Ascorbate peroxidase activity was increased ($P \leq 0.05$) by seaweed extract and tended ($P \leq 0.07$) to be increased by the endophyte.

68

Table 4.6: Effect of moisture level, genotype, endophyte status (E+, E-), and seaweed extract (E+, E-) on ascorbate peroxidase activity (µmole ascorbate h^{-1} g^{-1} fresh weight) of tall fescue.

	Moisture level (% of field capacity) [1]							
	30 - 100 [‡§]				50 - 100 [¶]			
	Genotype				Genotype			
	GA		KY-31		GA		KY-31[#]	
	Endophyte		Endophyte		Endophyte		Endophyte	
Seaweed	E+	E-	E+	E-	E+	E-	E+	E-
S+	2184	1742	1472	952	1004	907	1220	1350
S-	1328	1007	1224	1079	1007	1242	672	944

[1]   Moisture x genotype x seaweed interaction ($P \leq 0.01$, SE=12.2).

[‡]   GA differed from KY-31, averaged over endophyte and seaweed extract (1565 vs. 1182, $P \leq 0.05$, SE=129).

[§]   S+ differed from SW-, Averaged over genotype and endophyte (1589 vs. 1160, $P \leq 0.05$ SE=129).

[¶]   Genotype x seaweed interaction ($P \leq 0.01$, SE=11.3).

[#]   S+ differed from SW-, Averaged over endophyte ($P \leq 0.05$, SE=74).

to be cytokinin (Brain 1973; Blunden and Wildgoose 1977). Finnie and van Staden (1985) reported a stimulation in *in vitro* cultured tomato roots in response to seaweed extract diluted by a factor of 400-600. Similar effect were observed when they used low concentration of the cytokinin zeatin (10 and 100 nmol l^{-1}). But when Finnie and van Staden (1985) used a dilution factor of 100, root growth was inhibited. It is also possible that under drought stress, lack of a dequate moisture was the overriding factor and obscured any effect of seaweed extract. It is also possible that tall fescue reacts differently than the other plant species recited in the literature.

Experiment 2

Results of this experiment further demonstrated the lack of response of aerial plant growth to seaweed application at 4 kg ha^{-1} that was observed in Experiment 1. The increased shoot growth of the endophyte infected Georgia Jessup compared to non-infected plants under moisture stress is in agreement with previous studies. It is not clear why endophyte-infected KY-31 did not out yield the non-infected KY-31 in the presence of moisture stress. While many studies have shown that the endophyte presence tends to increase shoot mass and tillering (Arachevaleta et al. 1989; De Battista et al. 1990) in tall fescue, the effects were not consistent across host genotypes (Belesky et al. 1987; Rice et al. 1990). The genotype by moisture interaction observed in our research supports this finding that genotype modifies the effect of endophyte. Hill et al.

72

this response. To our knowledge, no previous studies have been conducted to evaluate the effect of endophyte presence on antioxidant activities other than the results reported from field studies in Virginia and Mississippi for SOD (Allen et al. 1997; Coelho et al. 1997).

The effect of moisture stress on antioxidant activity observed in our experiment is consistent with previously reported data. However, SOD and ascorbate peroxidase responses were modified by genotype and seaweed extract. Baisak et al. (1994) found that water stress caused an increase in SOD and GR activity in wheat under water stress. Ascorbate peroxidase activity increased under mild water stress but declined during severe water stress. They suggested that the different components of the active oxygen scavenging system appear to be modulated differentially under water stress.

Zhang and Kirkham (1994) reported an early increased SOD activity in most of seven wheat species, used in their study, but SOD decreased with further increase in magnitude of water stress. They suggested that hexaploid wheat had less efficient antioxidant systems than tetraploid and diploid wheats. Moreover, exposing barley (*Hordeum vulgare*) and tef (*Eragrostis tef*) to severe water deficit (< -3.0 MPa) resulted in increased activity (leaf dry weight basis) of glutathione reductase and monodehydroascorbate reductase in barley and ascorbate peroxidase and monodehydroascorpate reductase in tef (Smirnoff and

74

Water stress reduced root and shoot growth and root to shoot ratio. Application of seaweed extract had little effect on growth of either endophyte-infected or non-infected tall fescue under stress but may reduce root growth in non-water stressed plants. The endophyte association with Georgia Jessup tall fescue resulted in higher shoot weight only under stress. No such differences were observed with KY-31 fescue. Georgia Jessup produced more biomass than KY-31.

water deficit stresses (Bacon 1993). Several reports indicated that endophyte-infected plants often showed greater growth rate and tillering than non-infected plants (Arachevaleta et al. 1989; Hill et al. 1991a). This trend is affected by the genotype (Belesky et al. 1989). Stomatal conductance and transpiration rate declined faster in infected plants compared to the non-infected (Elmi et al. 1990). Elbersen et al. (1991) reported an enhancement in osmotic adjustment by endophyte infection after prolonged drought.

Several animal disorders have been associated with the endophyte collectively known as 'fescue toxicity' (Schmidt and Osborn 1993). Ergovaline and peramine alkaloids have been associated with fescue toxicosis (Garner et al. 1993; Siegel et al. 1990). Two main approaches are used to reduce the impact of fescue toxicosis; eradication of infected fescue and re-establishing new endophyte-free cultivars (Fribourg et al. 1988) and management of infected fescue to minimize the toxic effects on animals (Schmidt and Osborn 1993). Removal of the endophyte leaves the plant less able to stand environmental stresses, overgrazing, and insect pressure (Deflice and Henning 1990; West et al. 1988).

Seaweed extract has been reported to have beneficial effects on plants (Metting et al. 1990). Commercial seaweed extracts that are mostly applied as a foliar spray or as a flush to the soil are processed primarily from brown algae (*Phaeochyceae*; Verkleij 1992). The timing and frequency of application appears crop dependent. Verkleij (1992) suggested that seaweed extract should

78

the control. Effects of seaweed application became apparent in plants stressed during the vegetative phase of growth where straw and grain yield were higher in sprayed plants (Mooney and van Staden 1985).

Seaweed extract treatments tended to increase P concentration in cucumber (*Cucumis sativa*) leaves and to decrease N concentration (Nelson and van Staden 1984b). Application of kinetin at the same rate as in seaweed extract gave similar results on potato (*Solanum tuberosum*) yield (Blunden and Wildgoose 1977).

Foliar application of seaweed concentrate (Kelpack 66) on growth and cytokinin content of bean (*Phaseolus vulgaris*) plants was studied by Featonby-Smith and van Staden (1984). Seaweed concentrate at a dilution of 1:500 improved growth of bean plants. Moreover, endogenous cytokinin activity was increased in the treated bean plants compared to the control. Concentration of cytokinin in seaweed extracts ranged from 20 to 30 μg kg^{-1} kinetin equivalent in fresh matter (Featonby-Smith and van Staden 1983).

The results of seaweed extract experiments indicate that the most beneficial results were obtained when plants were under stresses (Verkleij 1992). This could explain partially the poor performance of seaweed extract on crops under ideal conditions such as found by Kuisma (1989) on potatoes. Seaweed quality, extraction method, storage condition, soil type, crop type, and growth stage are variables that play a role in determining the kind and magnitude of response to seaweed extract application (Verklij 1992).

The South Plains of West Texas is a semiarid environment well suited to warm-season forages. However, a cool-season grass could be useful in extending grazing season and add flexibility to livestock systems. Tall fescue is a cool-season grass that can be grown under irrigation but is not well adapted to this environment where summer temperatures often exceed 38°C and annual precipitation averages 45 cm per year. The presence of the endophyte and the use of plant growth regulators may be useful to improve tall fescue tolerance to the prevailing environmental stresses. From this stand point and depending on the previous preliminary greenhouse experiments, the following study was designed to explore the effect of seaweed extract and the endophyte on the growth, yield, quality, and antioxidant activities of tall fescue plants grown under different levels of moisture stress in the field.

Materials and Methods

Effects of seaweed extract, endophyte fungus, and irrigation level were investigated during two successive growing seasons (1996 and 1997) at the Texas Tech University Erskine Street Field Experimental Station, Lubbock, TX (33° 45' north latitude, 101° 45' west longitude, and 1133 m altitude) The experimental site was located on an Amarillo fine sandy loam (fine mixed thermic typic paleustalf) with a pH of 7.9. Effects of seaweed extract, endophyte status of 'Kentucky 31' tall fescue, and irrigation level on plant growth and persistence, antioxidant activities, and forage quality were tested. Genetically similar

82

In 1996, seaweed extract was applied in water solution using pressurized sprayers on 12 Mar., 1996 and moisture treatments were begun on 29 Apr., 1996. Seaweed extract was reapplied to the plots on 25 July, 1996. Fescue was harvested at a height of 7 cm each time a growth stage was reached that would be appropriate for cutting hay. Four harvests were obtained from the first growing season on 10 May, 1996, 20 June, 1996, 25 July, 1996, 18 Sep., 1996. Forage mass was determined by mowing a strip 0.6 x 2.0 m and 0.6 x 0.4 m from the center of each plot.

For 1997, seaweed extract was applied to both sets of 48 plots on 11 Mar. 1997. Thus, during 1997, 96 plots were included in the study. The three irrigation treatments for plots used during 1996 were continued without interruption. Irrigation treatments for plots initiated in 1997 began on the day that the seaweed treatments were first applied (11 Mar.). Plots of both sets were harvested at a height of 7 cm before seaweed extract was applied. Plots were harvested and forage mass was determined by clipping an area of 0.6 x 0.4 m on 13 May, 1997, 9 June, 1997, and 10 Sep., 1997.

Forage was dried in a forced air oven (55°C) to a constant weight and weighed to determine forage mass. Total seasonal yield was cultivated as the sum of the forage mass at each harvest within a growing season.

In Year 1 plots, green leaves of tall fescue (~ 2 gm) were collected from within each plot at 7 and 21 d after seaweed extract application and then at 30-d intervals until 11 Mar. 1997 for the determination of superoxide dismutase,

84

Data was analyzed following the general linear model procedure (GLM) of SAS (1982) for a randomized complete block design with a 2 x 2 x 3 factorial arrangement of treatments using a model that tested effects of treatments, block, time, and all interactions. For Year 1, the data for sampling dates that occurred 7 and 21 d after the initial seaweed application were analyzed separately because the dates occurred prior to initiation of irrigation treatments. Data for these two dates were analyzed as 2 x 2 factorial arrangement of treatments with 12 replications of each treatment. For Year 2 data, effect of year was included in the model. Orthogonal contrasts were used to test linear and quadratic effects of irrigation treatments.

Results

Weather

Total seasonal precipitation during the two growing seasons was 80.84 cm from April 29, 1996 through Sept. 10, 1997 (Figure 5.1). The monthly distribution of rainfall varied but followed a distribution pattern typical of the South Plains region. The long-term mean precipitation for this region is 45 cm. The highest monthly precipitation during the experimental period was in June, 1996 and Apr., 1997. Effective rainfall started in May of 1996 while it started earlier in April the next year. However, the rain came through July and August of 1996 was higher than the same period in 1997 season.

Total amount of irrigation water added reflected the water lost by evaporation (Figure 5.1). During the 18 months that spanned two growing seasons, 342.7 cm of water were added to replace 100% PET. The evaporation measured in this experiment was less than the 100 cm average of the region. Half this amount was added to replace 50% PET. The monthly-minimum and maximum temperatures are shown in Figure 5.2 and were close to long-term average for this region.

Forage Yield

For forage mass, interactions were present among irrigation level, endophyte. seaweed extract, and harvest date (Appendix, Tables A.1-A.3). Because some effects were cumulative and total seasonal yield was of interest, effects of treatments were examined for total seasonal yield (Appendix, Table A.4). Further interactions of endophyte status and seaweed extract with irrigation level were present, thus, the results were tested by irrigation level. Within irrigation level, the effects of endophyte and seaweed extract were consistent over the three total seasonal yields.

When plants were irrigated to replace 100% PET, endophyte-infected fescue tended ($P \leq 0.09$) to produce more total seasonal dry matter yield than endophyte-free plants (Figure 5.3). No effect of seaweed extract was observed at this irrigation level. At 50% PET replacement, effects of endophyte were not
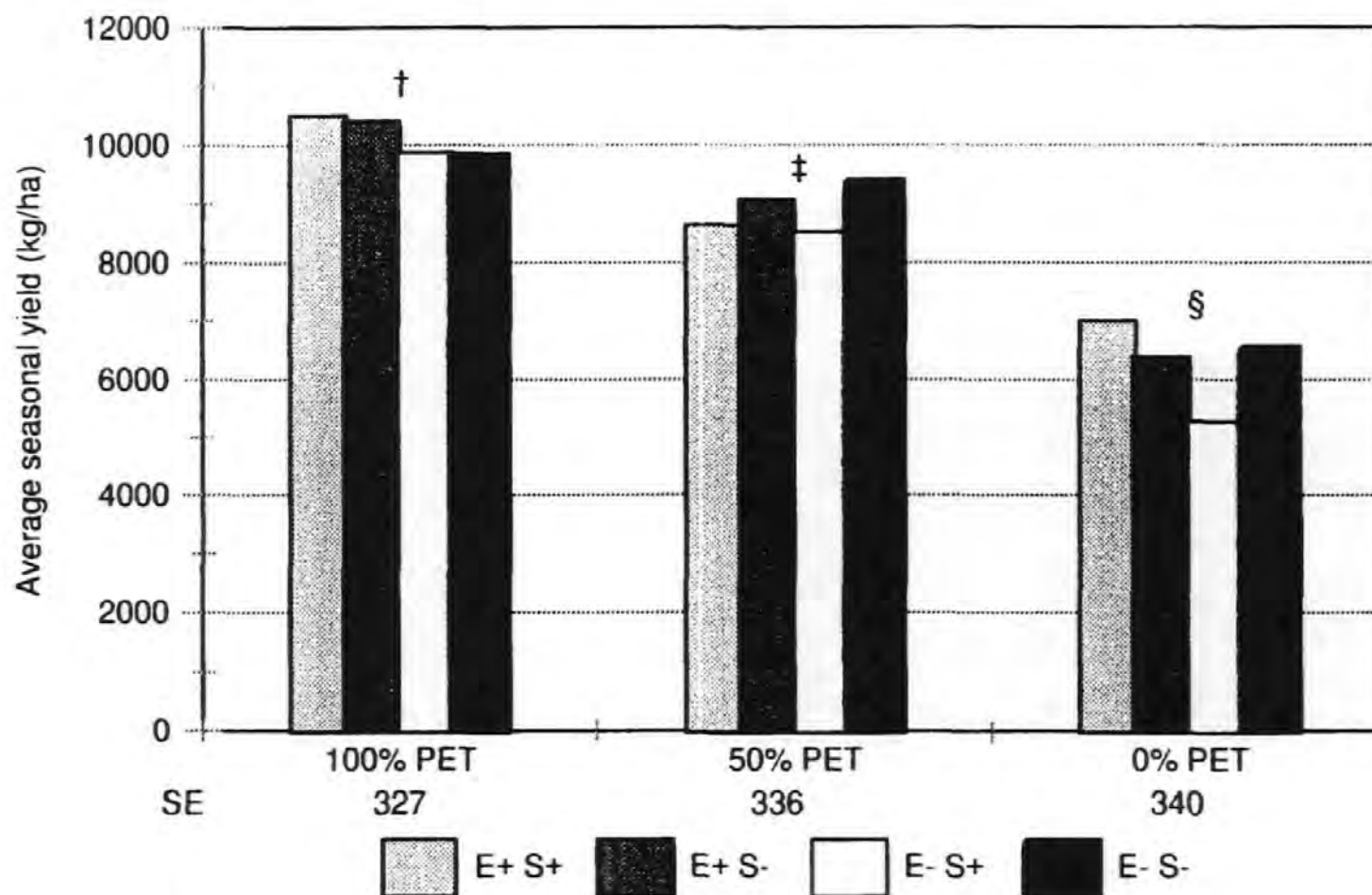
Figure 5.3. Effect of endophyte (E+, E-) and seaweed extract (S+, S-) on average total seasonal yield of tall fescue grown under different irrigation levels during 1996 and 1997 growing seasons. [[1] Effect of the endophyte ($P \leq 0.09$); [2] Effect of seaweed ($P \leq 0.06$); [§] Endophyte x seaweed interaction ($P \leq 0.05$), and effect of endophyte ($P \leq 0.05$)]
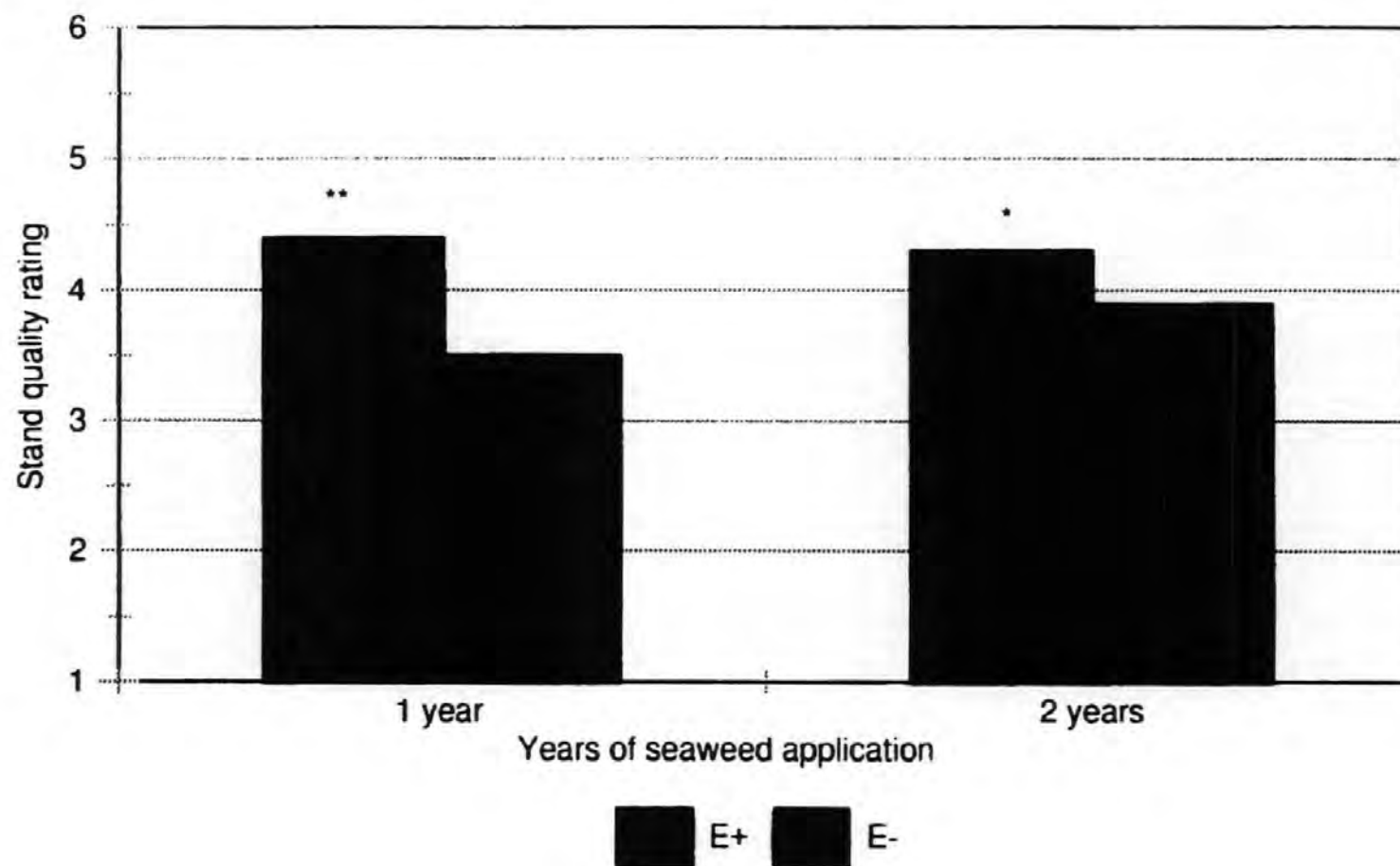
Figure 5.4. Effect of endophyte status (E+, E-) on stand quality ratings of tall fescue grown in plots treated for one year (1997) and for two years (1996, 1997), respectively. [*, ** indicted difference between E+ and E- ($P \leq$ 0.05, 0.01, respectively)].
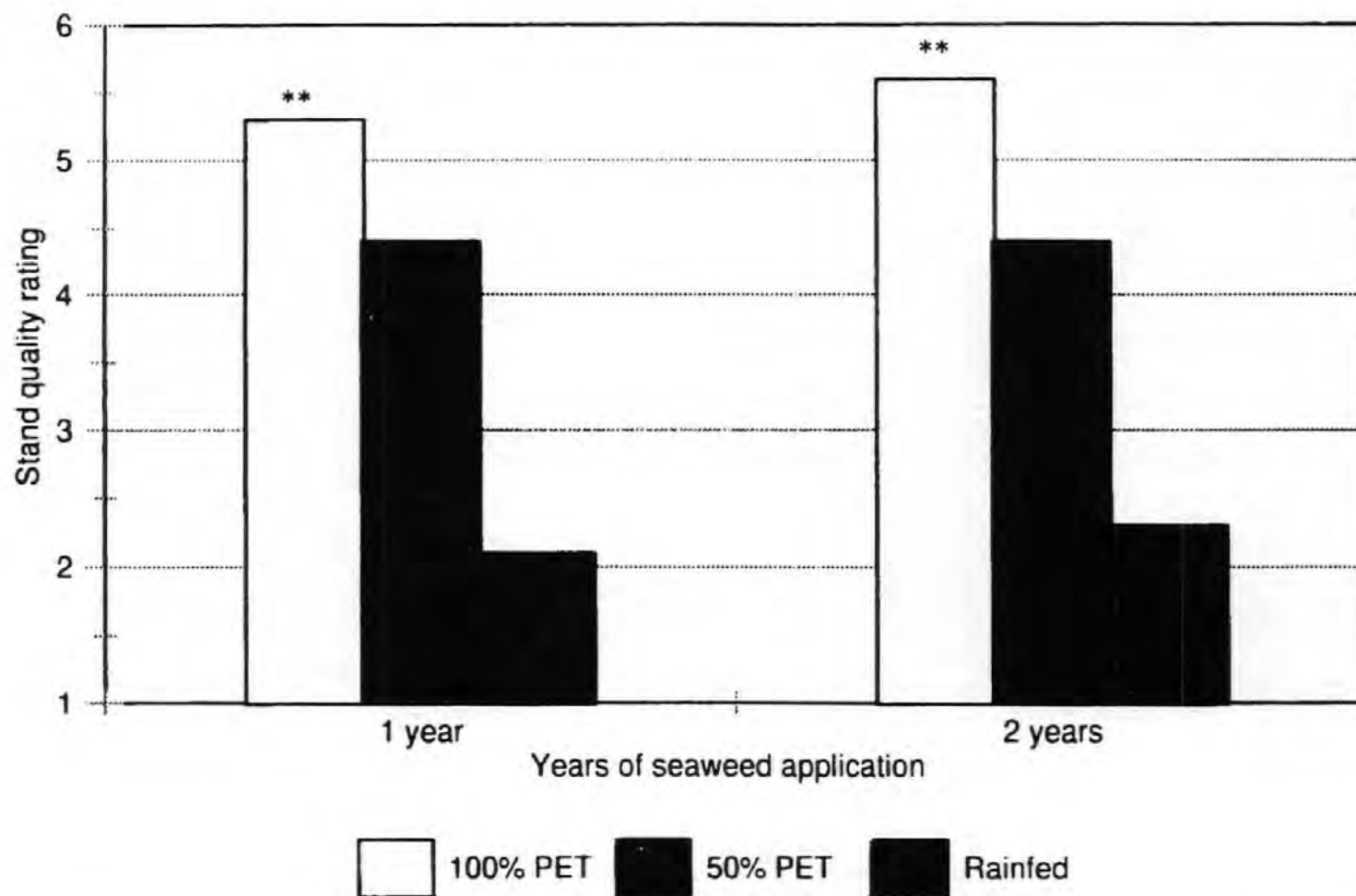
Figure 5.6. Effect of irrigation on stand quality ratings of tall fescue grown in plots treated for one year (1997) and for two years (1996, 1997), respectively. [** indicated difference due to irrigation ($P \leq 0.01$)].

Table 5.1. Effect of endophyte (E+, E-) and seaweed extract (S+, S-) on superoxide dismutase (SOD), glutathione reductase (GR), and ascorbate peroxidase (ASP) activity of tall fescue grown in the field.

		Days after seaweed application					
		7-d			21-d		
Endophyte	Seaweed	SOD[†]	GR[‡§]	ASP[‡]	SOD	GR[¶]	ASP
		10^3		10^2	10^3		10^2
E+	S+	2.4	95	5.2	1.5	83	4.0
	S-	2.0	23	2.7	1.2	62	3.4
E-	S+	2.0	25	3.2	1.2	40	4.3
	S-	1.7	23	2.1	1.0	24	2.1

[†] Effect of endophyte and seaweed extract ($P \leq 0.05$, SE=0.10).
[‡] Endophyte x seaweed interaction ($P \leq 0.05$, SE=10, 28).
[§] For endophyte-infected tall fescue, S+ differed from S- ($P \leq 0.05$, SE=14).
[¶] Effect of endophyte ($P \leq 0.01$, SE=7).

96
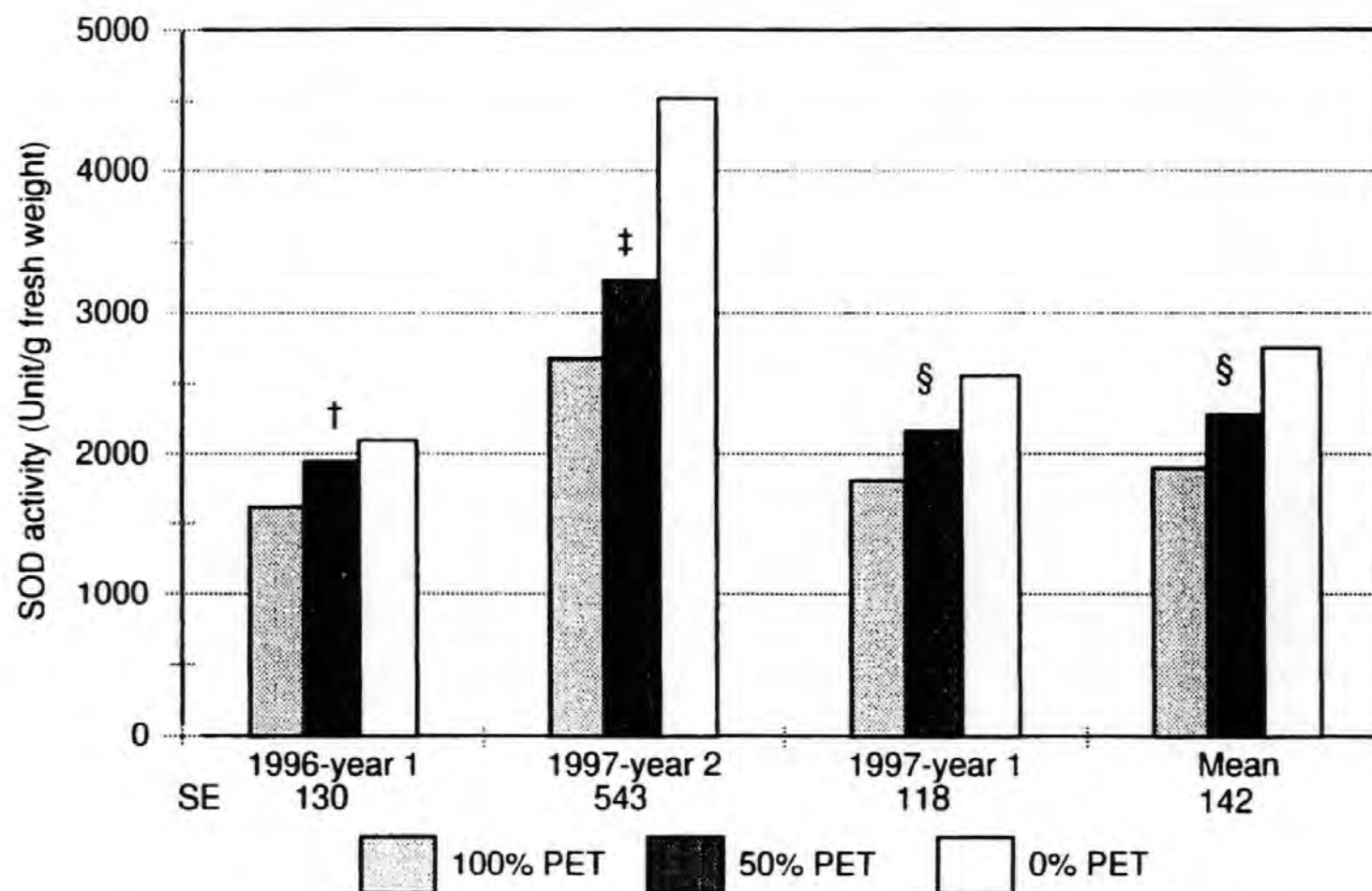
Figure 5.7. Effect of irrigation on superoxide dismutase (SOD) activity of tall fescue grown in the field during 1996 and 1997 growing seasons. [†‡§ Indicates linear effect of irrigation on SOD; $P \leq$ 0.01, 0.05, and 0.001, respectively]
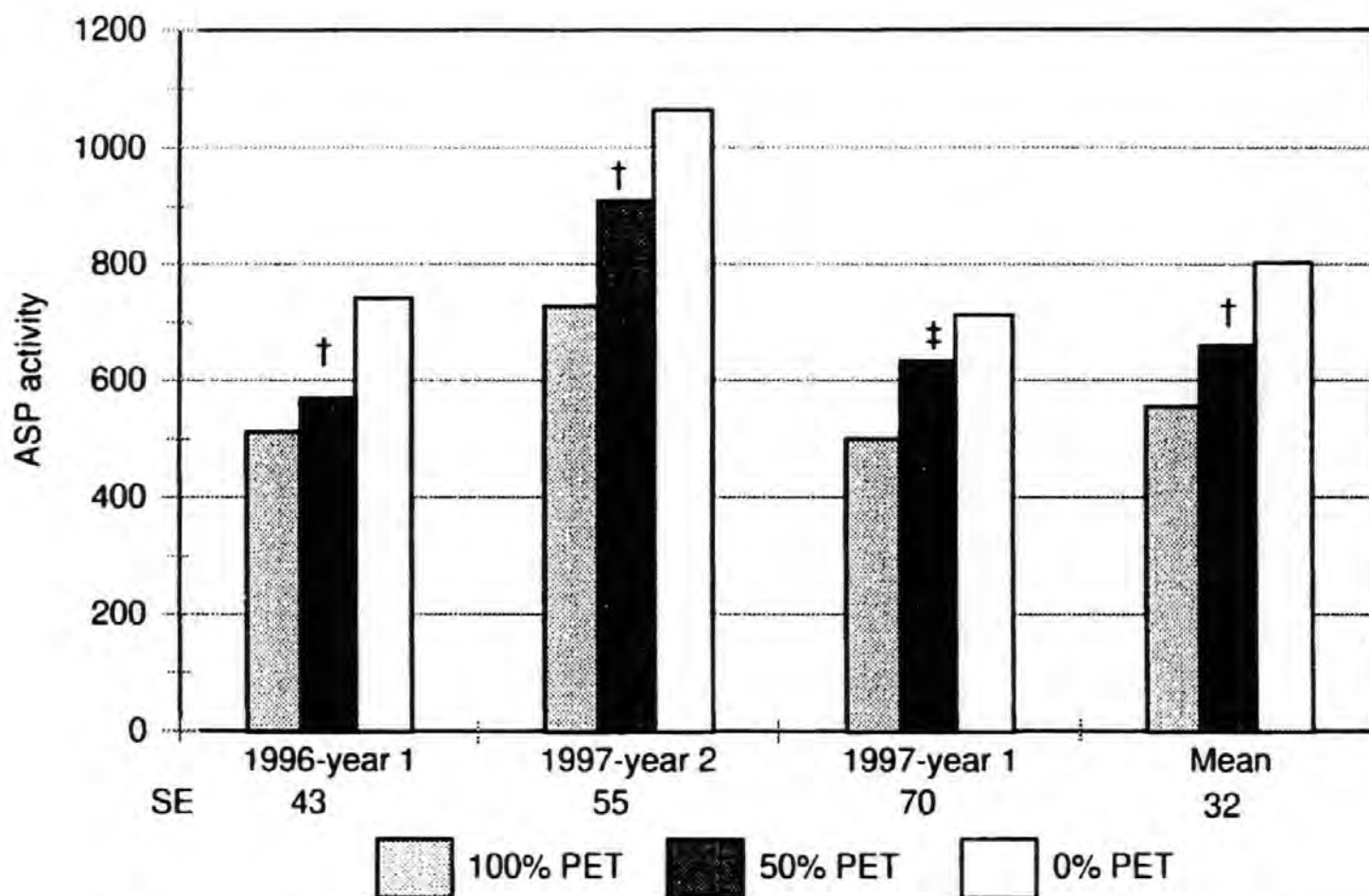
Figure 5.9. Effect of irrigation on ascorbate peroxidase (ASP) activity of tall fescue grown in the field during 1996 and 1997 growing seasons. [†‡ Indicates linear effect of irrigation on ASP; $P \leq 0.001, 0.05$, respectively]

did increase ($P \leq 0.05$) in the two sets of plots during 1997 (Figure 5.11). When

effect of endophyte was averaged over all sampling dates, the increase ($P \leq$

0.001) in SOD and glutathione reductase activity in response to the endophyte

was clearly evident. The enhancement ($P \leq 0.05$) of ascorbate peroxidase

activity in response to the endophyte presence was only significant when results

were analyzed across all sampling dates (Figure 5.12).

Effect of seaweed extract

Seaweed extract application tended ($P \leq 0.10$) to increase SOD activity in

1996-year 1 plots (Figure 5.13). However, in 1997-year 2 plots, SOD activity

was higher ($P \leq 0.05$) in seaweed treated plants compared to non-treated tall

fescue. Moreover, the mean SOD activity averaged across growing seasons

was increased ($P \leq 0.05$) in response to seaweed extract.

Glutathione reductase activity was consistently increased ($P \leq 0.001$) in

response to seaweed extract for 1997-year 1 and year 2 plots as well as for the

mean activity (Figure 5.14). For plots only during Year 2, the effect of seaweed

on increasing glutathione reductase was most evident in rainfed plots (Seaweed

x moisture interaction: $P \leq 0.05$). No significant differences due to seaweed
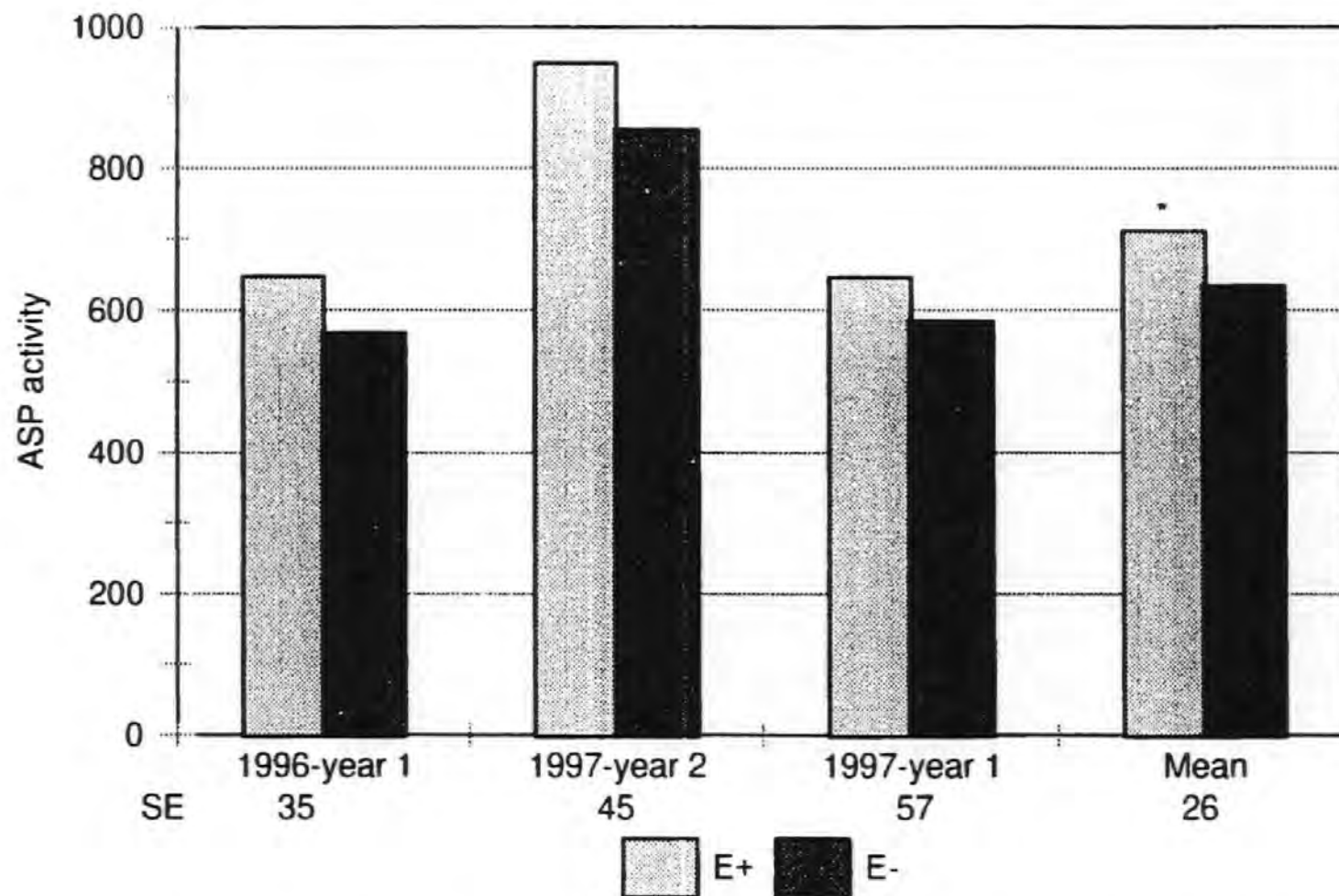
Figure 5.12. Effect of Endophyte status (E+, E-) on ascorbate peroxidase (ASP) activity of tall fescue grown in the field during 1996 and 1997 growing seasons. [* Indicates that E+ differed from E-; $P \le 0.05$]
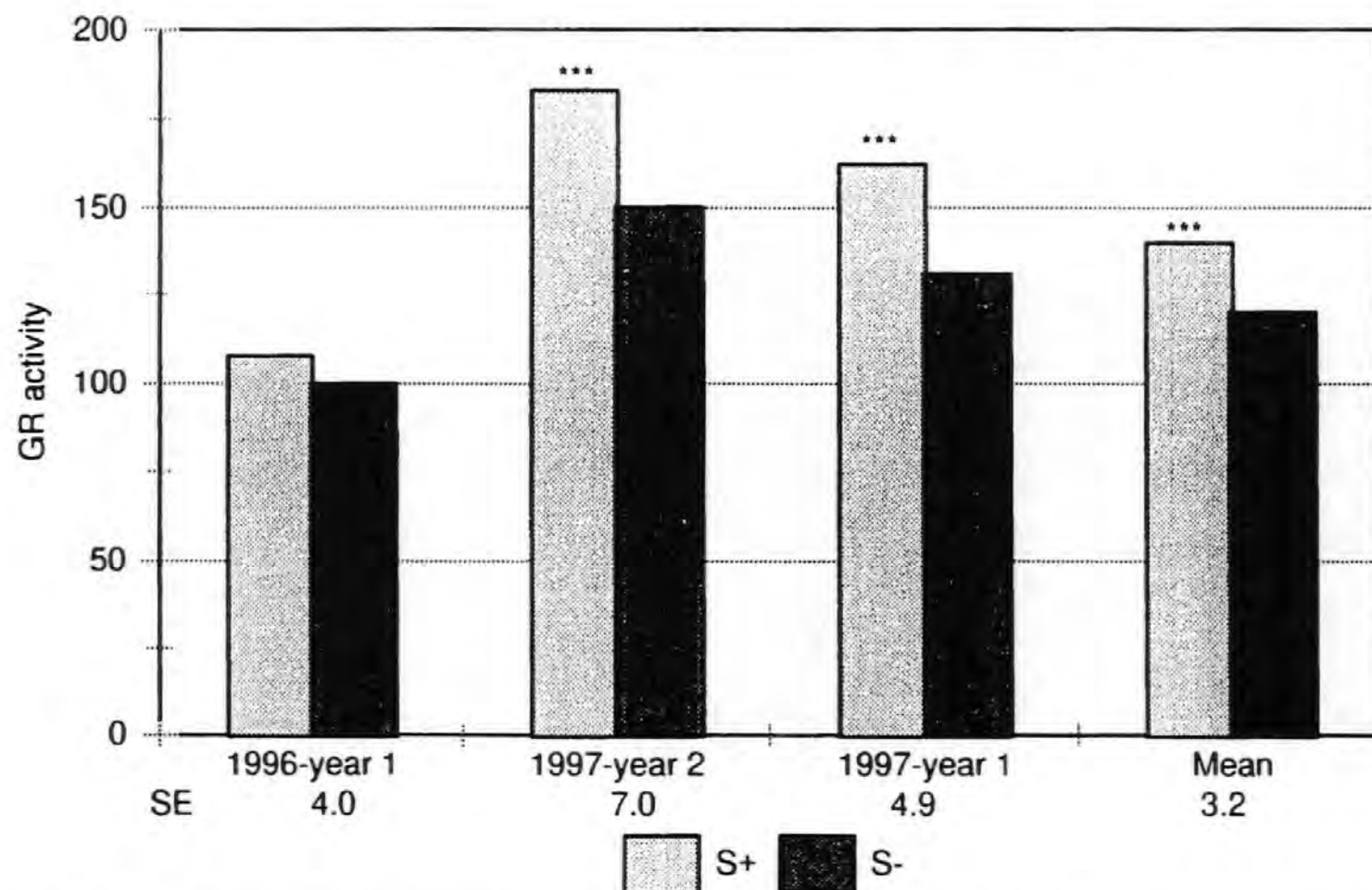
Figure 5.14. Effect of 1year and 2 years of seaweed extract (S+, S-) application on glutathione reductase (GR) activity of tall fescue grown in the field during 1996 and 1997 growing seasons. [*** Indicates that S+ differed from S-; $P \leq 0.001$]
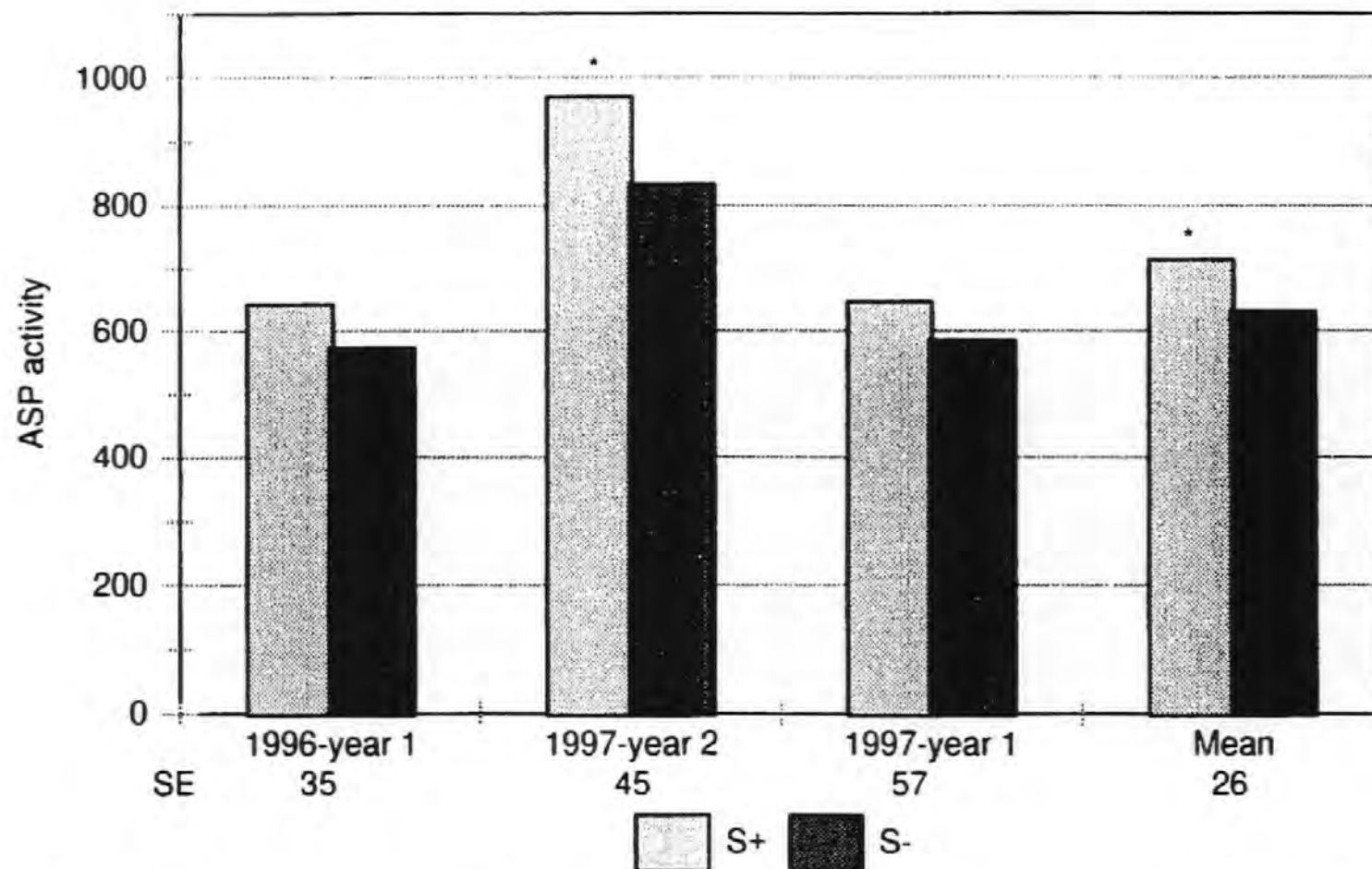
Figure 5.15. Effect of 1 year and 2 years of seaweed extract (S+, S-) application on ascorbate peroxidase (ASP) activity of tall fescue grown in the field during 1996 and 1997 growing seasons. [* Indicates that S+ differed from S-; $P \leq 0.05$]

seaweed extract at Virginia. In fact, Coelho et al. (1997) observed a reduction in forage mass in response to seaweed extract application on endophyte-free tall fescue. Myers and Perry (1986) reported no significant yield increase in wheat in response to seaweed extract from *Durvillaea potatorum* or *Ecklonia maxima*. No effect of foliar seaweed application on barley yield was found in a 2-yr Canadian experiment (Taylor et al. 1990). In contrast, many beneficial effects on crop yield have been reported for seaweed extract on turfgrass (Schmidt and Zhang 1997), wheat (Nelson and Van Staden 1986), sugar beat (Fetonby-Smith and Van Staden 1983) and potato (Blunden and Wildgoose 1977).

The endophyte presence appeared to increase forage yield, however, this response was dependent on water status and seaweed extract. Our results indicated that the increase in yield in response to the endophyte was observed only under full watering. This was perhaps related to the further stress of high temperatures or high light intensity to which the plants were subjected. The South High Plains is a very stressful environment for a cool season grasses and irrigation alleviates only one of these stresses. When irrigation was reduced and the stress level increased, no beneficial effect of the endophyte was observed.

Previous research has indicated that presence of the endophyte generally increase growth of tall fescue under stress. Read and Walker (1990) reported that forage dry matter yields averaged 55% higher in high-endophyte pastures of 'AU Triumph', 'Kenhy', and 'KY-31' than in low-endophyte pastures of the same

Although drought influences the amount of glutathione reductase activity present in leaves, the means whereby the enzyme is elevated relative to controls depends on the plant (Smith et al. 1989). Other studies have showed that leaf position, planting density, and canopy temperature are important variables that affect levels of glutathione reductase in response to water stress (Burke and Hatfield 1987; Smith eta al. 1989). Withholding water for 5 d from barley plants grown in the greenhouse caused increased levels of the glutathione reductase (Smirnoff and Colombe 1988). On the other hand, field grown cotton and wheat plants showed an increase in enzyme activity through an inhibition of the decline in activity that normally occurs during the growing season of irrigated crops (Burke et al. 1985).

The endophyte presence also increased SOD, glutathione reductase, and ascorbate peroxidase activities in tall fescue. However, the increase was more consistent for SOD and glutathione reductase than for ascorbate peroxidase which was significant only when the results were analyzed across all sampling dates and seasons. To our knowledge, no previous studies have been undertaken to determine the relationship between the endophyte and these antioxidant components. This increase in antioxidant activity may account at least in part for the ecological advantage of the endophyte-infected plants.

Our field plot studies verified results from the greenhouse experiments (Chapters III and IV) that seaweed extract application increased activity of SOD, glutathione reductase, and ascorbate peroxidase.

112

spring and summer time appeared to overcome the advantage of the endophyte presence. However, the endophyte-infected plots had a higher stand quality ratings than the endophyte free at the end of the experiment.

No consistent responses of seasonal forage yield to seaweed extract were observed. On the other hand a reduction of yield was obtained when seaweed extract was applied to the non-infected tall fescue under lower irrigation levels. The stand quality ratings indicated improvement with the application of seaweed extract which might be reflected into better survivability. Results of our experiment do not generally support the hypothesis that seaweed extract improves plant stress tolerance but these results may be dependent on the application rate and time.

Superoxide dismutase, glutathione reductase, and ascorbate peroxidase activities were linearly increased with decreasing irrigation levels, but level of increase in these antioxidants was not enough to protect plants from growth reduction under high water deficit and heat stress.

The endophyte presence stimulated the antioxidant enzyme metabolism. But was more consistent for SOD and glutathione reductase than for ascorbate peroxidase. This suggests that the reported advantages of the endophyte may be related at least in part to the enhancement of these antioxidants.

Seaweed extract application enhanced activity of SOD, glutathione, and ascorbate peroxidase across all seasons. The effect of seaweed on SOD and glutathione reductase activity was more consistent than ascorbate peroxidase

114

CHAPTER VI

OVERALL DISCUSSION AND CONCLUSIONS

Seaweed extract applied to tall fescue at rates in our studies either had no effect on plant growth or reduced plant growth. This was particularly evident in the endophyte-free tall fescue under water stress. The observed reduction in plant growth was probably due to a reduction in root growth as suggested by effects observed on plants in sand cultures. Although a range of treatment levels were tested in our research. all levels may have been too high to stimulate an increase in growth. Fike et al. (1997) observed a greater forage mass in tall fescue treated with 1.5 kg ha^{-1} as compared with that treated with 3 kg ha^{-1}. Coelho et al. (1997) suggested that 3.4 kg ha^{-1} of seaweed extract may depress top growth in tall fescue and that the effect appeared greater in endophyte-free plants. Finnie and Van Staden (1985) found that applying seaweed extract diluted by a factor 400-600 to *in vitro* cultured tomato roots stimulated root growth. But when they increased the concentration by using a dilution factor of 100. root growth was inhibited. Zhang (1997) found 70% increase in clipping dry weight of Kentucky bluegrass grown in the greenhouse in response to foliar application of 0.32 kg ha^{-1} of seaweed extract compared to the control. Comparing these results with our findings suggested that the rates used in our studies could have been too high. even at the lowest rates.

The endophyte-infected plants in our greenhouse study had a greater shoot weight than the non-infected plants especially under stress. On the other hand, under field conditions, the endophyte presence did not increase dry seasonal forage yield under the lower irrigation levels but did increase plant growth at the highest irrigation level. However, higher stand quality ratings averaged over other treatments, were observed in the endophyte infected plots compared with non-infected plants at the end of the 2-yr experiment. This suggests that some stress tolerance was provided by the endophyte. Several studies have focused on the importance of the endophyte on tall fescue growth and persistence under environmental stresses (Funk et al. 1984; Read and Camp 1986).

Although the presence of endophyte fungus in tall fescue has been considered as an ecological advantage to the grass, especially in stressful environments (Bacon and Siegle 1988), the high heat and drought stress encountered in the High Plains of West Texas could be beyond the tolerance of such symbiosis. The mechanisms by which the endophyte enhances host survival during drought is incompletely understood. However, several factors have been considered including leaf rolling (Arechavaleta et al. 1989), improved root growth (Belesky et al. 1989), and enhanced osmotic adjustment (West et al. 1989). Moreover, the endophyte is thought to induce a sort of incipient stress that somehow preconditions the host to drought (West et al. 1994). The

fully irrigated fescue over the partially irrigated may not justify the use of such amount of irrigation water. In this field study, irrigation was continuous throughout the 18 months of the experiment which included late autumn and winter time where no significant plant growth is usually obtained. From a production stand point, water use could be minimized during winter and mid-summer when plants are dormant or growth is slow.

Oxygen is an essential component of life. However, under environmental stresses, highly reactive oxygen species such as singlet oxygen and superoxide are generated (Scandalios 1997). These oxygen species are highly reactive and cytotoxic. They react with unsaturated fatty acids to cause peroxidation of essential lipids in cell membrane and cellular organelles which leads to leakage ended by cellular death (Scandalios 1997). The plants possess both enzymatic and non-enzymatic cellular protection against oxidation. However, individual plant stress responses do not occur in isolation, but they are often insufficient by themselves to provide comprehensive protection. Therefore, plants with improved scavenging mechanisms for active oxygen species would be of great agricultural value since environmental stresses overwhelm the normal protection capacity of plants.

Water stress in our greenhouse and field studies increased activities of SOD, glutathione reductase, and ascorbate peroxidase. Drought was reported to influence glutathione reductase activity in plant leaves, but the enhancement level depends on the plant (Smith et al. 1989). Field studies have showed that

120

Zn, Fe, Mn, and S into the synthesis of these enzymes which might be reflected into nutrient deficiency that limit plant growth. Missaoui (1998) found that growth of bromegrass (*Bromus wildonowii*) was increased by application of seaweed extract plus added S and that the response was greater than either S or seaweed extract applied alone.

The mechanisms by which seaweed extracts influence plant growth and antioxidant activities is not clear. However, the additive effects of enhanced nutrient uptake and regulatory action of plant growth substances contained in the seaweed extract are possible factors in the obtained responses (Crouch and Van Staden 1993). In 1973, Brain et al. reported high cytokinin-like activity of one commercial seaweed extract product. Since the identification of cytokinins and auxins in several commercial seaweed extracts, they were implied in their possible involvement in regulation of plant growth (Williams et al. 1981). The concentration of cytokinin in seaweed extracts ranged from 20 to 30 µg kg^{-1} kinetin equivalent in fresh matter (Featonby-Smith and Van Staden 1984).

Few direct attempts have been made to evaluate the possibility of improving crop productivity through the application of cytokinins (Frankenberger and Arshad 1995). Cytokinins play a vital role in nutrient mobilization (Kuiper and Stall 1987), stimulation of protein synthesis, and promotion of chloroplast development and chlorophyll II synthesis (Salisbury et al. 1992). Exogenous application of cytokinins was found to stimulate protein synthesis (McGaw 1987). Their effect on protein synthesis is though to be at the translational or post

122

(Saker et al. 1997). The enhancement of SOD in the forage in response to seaweed application may have influenced the immune system directly through an increase in antioxidant activity in both the plant and the animal.

Results of these studies indicate that potential benefits of seaweed extract are in the area of antioxidant activities. Practical uses for this tool now need investigation. The increased antioxidant activity may be greater in endophyte-infected tall fescue than in non-infected fescue plants. The role of the endophyte on increasing antioxidant levels needs further investigation. The reduced plant growth in response to seaweed extract application or the lack of response might be related to the rates of application and lower rates should be investigated. Further research to study the effect of lower seaweed extract application rates on plant growth and immune responses in grazing animals should be conducted. Also, more research in the area of timing and frequency of seaweed extract application on tall fescue should be studied.

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Table A.1. Effect of irrigation, endophyte and seaweed extract application on dry forage mass (kg ha⁻¹) of tall fescue grown in the field during yr 1 of 1996 growing season.

Irrigation	Endophyte	Seaweed	10 May [tt]	20 Jun. [ts¶#]	25 July [t]	18 Sep. [tt]
100% PET						
	E+	S+	3492	1596	2418	2587
		S-	4127	2253	2747	2260
	E-	S+	3803	1913	2614	2226
		S-	3596	1574	2445	1930
50% PET	E+	S+	3462	1611	2321	2216
		S-	3420	1349	2317	2161
	E-	S+	4288	1367	1823	1871
		S-	4633	1462	2494	2334
Rainfed	E+	S+	3948	1027	1657	1388
		S-	3163	899	1247	1279
	E-	S+	3307	560	588	742
		S-	3517	687	1214	960

[t] Irrigation x endophyte x seaweed interaction ($P \leq 0.05$, SE= 254, 194, 213).

[‡] For 50% PET, E+ differed from E- ($P \leq 0.05$, SE=191).

[§] For 100% PET, there was endophyte x seaweed interaction ($P \leq 0.05$, SE=156).

[¶] For 100% PET, S+ differed from S- for E- tall fescue ($P \leq 0.05$, SE=65).

[#] For rainfed treatment, E+ differed from E- ($P \leq 0.05$, SE=98).

[tt] Effect of endophyte ($P \leq 0.05$, SE=88), and effect of irrigation ($P \leq 0.01$, SE=107)

Table A.3. Effect of irrigation, endophyte and seaweed extract application on dry forage mass (kg ha⁻¹) of tall fescue grown in the field during yr 1 of 1997 growing season.

Irrigation	Endophyte	Seaweed	13 May[†‡§]	9 Jul.[¶#††]	10 Sep.[‡‡§§¶¶##]
100% PET					
	E+	S+	5219	2954	2109
		S-	5565	2914	1323
	E-	S+	4811	2180	1479
		S-	5029	2857	1467
50% PET	E+	S+	5151	2002	1036
		S-	4571	2805	854
	E-	S+	4016	2089	807
		S-	4858	2460	803
Rainfed	E+	S+	4950	2074	322
		S-	3638	1770	177
	E-	S+	3632	1522	115
		S-	4868	2300	401

[†] Irrigation x endophte x seaweed interaction ($P \leq 0.05$, SE=297, 210).

[‡] For rainfed treatment, there was endophyte x seaweed interaction ($P \leq 0.01$, SE=245).

[§] S+ differed from S- for both E+ and E- tall fescue under rainfed treatment ($P \leq 0.05$, SE=142, 243).

[¶] For 50% PET, S+ differed from S- ($P \leq 0.05$, SE=162).

[#] For rainfed treatments, there was endophyte x seaweed ($P \leq 0.05$, SE=185).

[††] For rainfed treatments, S+ differed from S- of E- tall fescue ($P \leq 0.05$, SE=145).

[‡‡] Irrigation x seaweed ($P \leq 0.05$, SE=75) and endophyte x seaweed interaction ($P \leq 0.01$, SE=61).

[§§] For 100% PET and rainfed plots, seaweed x endophyte interaction ($P \leq 0.05$, SE=138).

[¶¶] For 100% PET, S+ differed from S- of E+ tall fescue ($P \leq 0.05$, SE=175).

[##] For rainfed plots, S+ differed from S- of E- tall fescue ($P \leq 0.01$, SE=32).

Table A.5. Effect of irrigation and seaweed extract on superoxide dismutase activity (10^3 unit.g^{-1} fresh weight) of endophyte-infected and -free tall fescue grown during 1996 and 1997 growing season.

	Irrigation												
	100% PET				50% PET				Rainfed				
	Endophyte				Endophyte				Endophyte				
Days after	E+		E-		E+		E-		E+		E-		
application	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	SE
12 Mar., 96													
51	0.7	0.7	0.7	0.7	0.7	0.8	0.8	0.8	0.9	0.9	0.9	0.8	0.06
83 [†‡§¶#]	1.7	1.5	1.1	1.2	2.7	1.4	0.9	0.7	1.7	1.1	2.0	1.0	0.23
113d [††‡‡]	1.1	0.6	1.0	0.6	1.2	1.2	1.1	1.0	1.3	1.7	1.2	1.4	0.08
25 July, 97													
8 [§§]	0.9	0.9	0.7	0.8	1.0	1.0	1.0	0.8	1.3	1.2	1.3	0.9	0.10
38 [¶¶##]	2.1	2.2	2.0	1.8	2.6	2.5	2.5	1.9	3.4	2.4	2.6	2.9	0.24
98 [†‡‡‡]	3.8	4.2	3.8	3.0	4.2	4.6	5.6	3.5	6.4	5.2	3.0	2.4	0.61
189 [‡‡‡]	2.1	1.8	1.4	2.3	3.0	2.4	2.2	2.2	4.1	2.1	3.0	1.8	0.58
11 Mar., 1997													
28 [§§§¶¶¶]	8.9	6.6	5.4	2.7	8.6	7.0	7.5	5.7	15.3	12.2	7.9	6.0	1.13
63 [###]	1.3	1.1	1.3	1.0	1.4	1.3	1.3	1.2	1.8	1.5	1.8	1.4	0.13
120 [§§]	1.0	0.9	1.0	0.8	1.2	1.2	1.1	1.5	1.5	1.7	1.8	1.3	0.11

Table A.6. Effect of irrigation and seaweed extract on superoxide dismutase activity (10^3 unit.g[1] fresh weight) of endophyte-infected and -free tall fescue grown during yr 1 of 1997 growing season.

	Irrigation												
	100% PET				50% PET				Rainfed				
	Endophyte				Endophyte				Endophyte				
Days after	E+		E-		E+		E-		E+		E-		
application	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	SE
11 Mar., 97													
7 [1]	3.0	2.7	2.7	2.4	3.5	3.3	3.3	2.8	4.9	3.7	4.0	3.2	0.26
14 [‡]	2.5	2.1	2.1	2.2	2.8	2.4	2.9	2.2	3.2	2.5	2.7	2.3	0.22
63 [1]	1.4	1.2	1.0	0.9	1.9	1.1	1.5	1.3	2.1	1.8	1.8	1.6	0.19
120 [§]	1.3	1.5	0.9	1.0	1.4	1.7	1.4	1.1	1.8	1.5	2.0	1.6	0.25

[1] Effect of irrigation ($P \le 0.01$, SE=0.13, 0.93), endophyte, and seaweed ($P \le 0.01$, SE=0.10, 0.08).

[‡] Effect of irrigation ($P \le 0.05$, SE=0.11) and seaweed ($P \le 0.01$, SE=0.09).

[§] Effect of irrigation ($P \le 0.05$, SE=0.13).

Table A.7, Continued

†   The effect of irrigation ($P \le 0.05$, SE=5.5, 10.7) and seaweed , ($P \le 0.01$, SE=4.5, 8.8).

‡   Irrigation x endophyte interaction ($P \le 0.01$, SE=4.4).

§   for 50% PET, effect of endophyte and seaweed ($P \le 0.05$, SE=5.3).

¶   Effect of seaweed ($P \le 0.01$, SE=2.5).

Effect of irrigation ($P \le 0.05$, 0.01, 0.01, SE=3.3, 10.1, 8.4).

††   Irrigation x endophyte x seaweed interaction ($P \le 0.05$, SE=4.8).

‡‡   For 50% PET, endophyte x seaweed interaction ($P \le 0.05$, SE=3.7).

§§   For E+ tall fescue, S+ differed from S- across 50% PET ($P \le 0.05$, SE=2.9).

¶¶   For rainfed plots, effect of endophte ($P \le 0.01$, SE=4.5).

Effect of irrigation ($P \le 0.01$, SE=9.5) and endophyte ($P \le 0.05$, SE=7.8).

†††   Endophyte x seaweed interaction ($P \le 0.05$, SE=9.7).

‡‡‡   S+ differed from S- across all E+ tall fescue ($P \le 0.01$, SE=10.2).

§§§   Effect of irrigation ($P \le 0.01$, SE=6.8), endophyte ($P \le 0.01$, SE=5.6), and seaweed ($P \le 0.05$, SE=5.6).

Table A.9. Effect of irrigation and seaweed extract on ascorbate peroxidase activity (10^2 μmole ascorbate h^{-1}.g^{-1} fresh weight) of endophyte-infected and -free tall fescue grown during 1996 and 1997 growing season.

Days after application	Irrigation												
	100% PET				50% PET				Rainfed				
	Endophyte				Endophyte				Endophyte				
	E+		E-		E+		E-		E+		E-		
	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	SE
12 Mar., 1996													
51[†]	2.8	2.5	2.5	2.6	2.9	2.8	2.7	2.7	3.3	2.9	3.5	2.9	0.17
83[‡]	1.5	1.8	2.4	2.0	4.4	3.4	4.0	1.1	9.9	5.5	5.3	2.3	1.83
113[§¶#]	3.7	2.8	4.0	3.3	4.6	4.2	2.7	3.4	6.4	5.7	5.1	5.0	0.39
25 July, 1996													
8[†]	1.9	1.5	1.6	1.3	2.1	1.9	2.1	1.6	2.6	2.5	2.5	2.5	0.22
38[‡]	6.6	5.4	5.5	4.3	6.7	6.2	5.5	5.6	8.6	7.6	7.6	6.8	0.94
98	13.9	11.6	13.5	12.1	11.6	11.9	12.0	12.4	12.6	16.3	14.0	10.0	1.52
189[††]	9.1	8.6	8.0	6.6	12.5	8.9	9.0	9.8	15.0	14.9	13.5	13.2	1.16
11 Mar., 1997													
28[‡‡§§¶¶##†††‡‡‡]	4.7	4.1	3.5	3.1	5.0	4.3	5.9	3.2	9.1	5.4	7.1	5.7	0.40
63[†]	10.5	9.9	10.4	9.3	14.1	11.3	11.8	12.6	15.2	12.5	14.4	12.1	0.87
120[‡]	8.7	7.4	9.0	6.8	14.0	9.1	9.6	8.2	11.0	14.3	10.6	10.5	1.61

Table A.10. Effect of irrigation and seaweed extract on ascorbate peroxidase activity (10^2 µmole ascorbate $h^{-1}.g^{-1}$ fresh weight) of endophyte-infected and -free tall fescue grown during yr 1 of 1997 growing season.

	Irrigation												
	100% PET				50% PET				Rainfed				
	Endophyte				Endophyte				Endophyte				
Days after	E+		E-		E+		E-		E+		E-		
application	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	SE
11 Mar., 1997													
7 [†]	1.7	0.9	1.1	1.0	2.0	1.4	1.7	1.4	2.1	1.7	2.0	1.6	0.13
14 [‡]	1.4	1.2	1.3	0.9	1.5	1.2	1.5	1.3	2.1	1.7	2.1	1.6	0.17
63 [§]	12.2	12.0	12.1	11.0	13.5	13.7	14.0	12.3	15.7	14.5	15.4	13.7	1.38
120 [§]	6.4	6.4	5.3	5.1	11.7	7.2	8.3	8.7	11.8	11.1	8.2	8.7	1.49

[†] Effect of irrigation ($P \le 0.01$, SE=0.06), endophyte ($P \le 0.05$, SE=0.05), and seaweed ($P \le 0.01$, SE=0.5).

[‡] Effect of irrigation ($P \le 0.01$, SE=0.08) and seaweed ($P \le 0.01$, SE=0.07).

[§] Effect of irrigation ($P \le 0.05, 0.01$, SE=0.69, 0.75).