THE EFFECT OF SEAWEED CONCENTRATE ON TURFGRASS GROWTH, NEMATODE TOLERANCE AND PROTEIN SYNTHESIS UNDER MOISTURE STRESS CONDITIONS

BY

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(ABSTRACT)

A preliminary experiment was conducted to determine the effects of salinity and moisture stress on the pathogenicity of root-knot nematodes (RKN) in turfgrass plants. The results indicated that RKN infection adversely affected both visual and functional parameters of bentgrass. Salinity and moisture stress further exaggerated the damage caused by RKN.

Under well-watered conditions, the effects of SWC and RKN infection on bentgrass plants were studied. Applications of SWC at 0.5 liter ha^{-1} and 1.0 liter ha^{-1} effectively enhanced bentgrass growth under both RKN-free and RKN-infected condition. It was shown that RKN caused less damage to SWC-treated plants than to non-treated plants. In addition, a soil drench of 0.5 liter ha^{-1} and 1.0 liter ha^{-1} at 10 day intervals was required to enhance bentgrass growth under RKN-free and RKN-infected conditions, respectively.

The effects of seven SWC treatments on the growth of nematode-free and RKN infected bentgrass plants were tested under three irrigation regimes. Rooting and leaf moisture

parameters, quality and clipping yield were all improved to some degree by SWC applications. High dosage SWC treatments, applied as a soil drench at one liter ha^{-1} every 10 days, were most effective in improving plant growth. Application of SWC was more beneficial to RKN-infected plants than RKN-free plants, and to abiotically stressed plants than to abiotic stress-free plants.

In a separate study, seaweed application was also shown to enhance both top growth and root growth of lance nematode (Hoplolaimus galeatus) or RKN infected bentgrass grown under drought or salinity stress condition. With SWC application, almost all of the symptoms caused by nematode infection and the abiotic stress were partially overcome. In addition, root development, leaf water status and clipping yield were all improved. It was apparent that soil drench SWC treatments were more effective in enhancing bentgrass growth than foliar SWC treatments. Application of SWC slightly reduced the number of nematodes per unit of fresh root (for RKN) and per unit weight of soil (for lance nematodes).

Protein extracted from SWC-treated or non-treated ryegrass plants under different stress conditions indicated that SWC altered plant protein synthesis, possibly by inducing selective gene expressions.

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CHAPTER 1

INTRODUCTION AND OBJECTIVES

Moisture and salinity stress are often major obstacles to maintain high quality turfgrass. Although very little research has been conducted to evaluate the effects of nematode infection on turfgrass, nematode damage has been an increasing concern in turfgrass management. The common methods used so far to improve turf quality under moisture and salinity stress, and nematode infection include increasing amount of irrigation and application of synthetic fertilizers and pesticides. In some areas, limited water resources, however, make it impossible to irrigate turfgrass extensively and frequently. In addition, increased utilization of agricultural chemicals, especially fertilizers and pesticides has had a negative impact on the environment. As a result, development of natural biodegradable products which control nematode activities on turfgrass, and/or increase plant tolerances to moisture and salinity stress is of both economical and environmental importance.

Seaweed is a natural biodegradable product that is in plentiful supply on the earth. If seaweed products increase turfgrass tolerance to moisture stress and/or nematode damage, they may play an important role in reducing the amount of agricultural chemicals used in turf management.

Seaweed or seaweed products have been used as food, fodder and soil conditioners for many centuries. Recently, seaweed concentrate (SWC) has been shown to enhance plant root growth, nutrient uptake, quality and resistance to pest and diseases, in more than 80 plant species. A few studies have reported that SWC possesses nematicidal activity. Research on the effect of SWC on turfgrass is very limited.

The reason SWC induces these beneficial effects is uncertain. The hypothesis that improved plant growth due to seaweed application was attributed to improved soil structure was refuted because SWC was applied in very small dosages. A possible additive effect of both nutrient uptake and plant growth regulatory action has been proposed.

Growth regulators in seaweed were thought to be mainly responsible for the beneficial effects of SWC on plants. Seaweed contains many kinds of plant growth regulators, such as auxins, gibberellin, abscisic acid and cytokinin-like substances. Among these plant growth regulators, cytokinin has been singled out as having particular significance.

However, the effect of plant growth regulators in seaweed only explains some of the benefits of SWC, but not all. Therefore, additional research is needed to exploit the mechanisms that SWC induces in these beneficial responses from plants.

The objectives of this project were:

1). To determine the effects of soil salinity and moisture stress on the pathogenicity of root-knot nematodes (RKN) in bentgrass plants, and on the development of RKN population.

2). To ascertain the beneficial effects of SWC on RKN infected or nematode-free bentgrass under different stress conditions.

3). To detect and characterize novel proteins of perennial ryegrass induced by SWC application under various stress conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 BENEFICIAL EFFECTS OF SEAWEED CONCENTRATE ON PLANTS

Seaweeds have been used as fertilizers and soil conditioners for many years. Fertilization with marine algae dates back to the ancient Greeks and Vikings. According to Booth (1965), brown algae was used for manuring on the coastal lands of France, Ireland, Scotland and Normandy by the 12th Century. However, land application of seaweeds was limited to coastal areas, since they were not easily processed and transported. Therefore, it became necessary to process the seaweeds in some way before they became economic manures for inland farmers.

As a result, seaweed processing has become a world-wide business today, with a huge amount of seaweed products sold each year. The seaweed products developed so far can be placed into three groups: (1) meals to apply to soil in large volumes or to blend into defined rooting media for greenhouse crops, (2) powdered or liquid extracts, and (3) concentrates used as root dips, soil drenches and as foliar sprays (Booth, 1969; Senn, 1987; Crouch, 1990).

Many researchers have evaluated plant responses to seaweed application, and the conclusions are many and varied.

2.1.1 Improved Root Growth and Functions

Effects of seaweed concentrates (SWC) on plant roots are similar to those induced by cytokinins, and both are well documented (Blunden et al., 1979; Featonby-Smith and Van Staden, 1983a). Although the mechanisms in which SWC affects plant roots have not been positively established, there are several explanations about how SWC improves root growth and function. Booth (1969) reported that commonly used SWC products contain considerable quantities of phenolic compounds and mannitol which stimulate root development and exhibit growth promotive properties (Poapst and Dunkee, 1967). Root extension and lateral root development have been enhanced by using low concentrations of SWC while they are inhibited by high concentrations of SWC (Finnie and Van Staden, 1985). Some very strong polar molecules, such as fucoidin and alginates, formed in the process of seaweed degradation, improve soil aggregation and crumb structure. Of course, better soil structure is more suitable for root growth and function.

In addition to the enhancement of root growth, SWC application can also increase nutrient uptake (Francki, 1960; Aitken and Senn, 1965; Offermans, 1968). Research reports that SWC can increase the availability of nitrogen (Booth, 1966; Caiozzi et al., 1968; Senn and Kingman, 1978; Beckett and Van Staden, 1989 and 1990b), phosphorus (Booth, 1966; Caiozzi et al., 1968), potassium (Booth, 1966; Becket and Van Staden, 1989), calcium (Senn and Kingman, 1978), manganese (Blunden, 1972), magnesium (Senn and Kingman, 1978), iron (Booth, 1966), and zinc (Becket and Van Staden, 1990a) in soils. There are at least two ways that SWC may increase nutrient uptake. It can stimulate an increased root system, which has a larger absorption surface area that may absorb more mineral nutrients. Furthermore, certain elements in seaweed may be important in the chelation of metals to yield soluble complexes which may promote mineral absorption (Senn and Kingman, 1978).

2.1.2 Increased Vegetative and Economic Yields

In field studies Blunden et al.(1979) demonstrated close correlations between SWC applications and increased crop yields. Under water stress, dry mass of roots, straw and grain of wheat were increased by applying SWC (Mooney and Van Staden, 1985). Nelson and Van Staden (1986) also showed enhancements of vegetative production, fertile spikelets, number of kernels, culm diameter, culm length, culm length : culm diameter ratio, and total kernel nitrogen in wheat plants treated with SWC. Nelson and Van Staden (1986) and others (Featonby-Smith and Van Staden, 1983a; Featonby-Smith and Van Staden, 1983b) stated that SWC application at a suitable level affected vegetative growth more than economic yield. Such beneficial effects are partly due to improved growth parameters during early stages of plant development, and partly due to a delaying of senescence and better redistribution of assimilates during the reproductive stages.

Other studies, however, have not demonstrated increase in plant yield resulting from the use of SWC. Gupta and MacLeod (1982), in a field and greenhouse experiment, found no significant difference between the yields of SWC-treated and non-treated peas or wheat. Bleyaert (1984) found that SWC had no effect on the growth, development or production of greenhouse tomato. Similar results were reported by Miers and Perry (1986). In 1990, Taylor et al. reported that in their four year study, the effect of SWC on the growth and yield of barley was not significant.

Contradictions in the conclusions from these experiments may be attributed to experimental design, number of replications, complexity of SWC composition, and different commercial SWC products.

2.1.3 Increased Tolerance to Pathogen Attacks and Abiotic Stresses

It has been reported that SWC application increases plant tolerance to fungal, bacterial and insect attacks (Senn et al., 1961; Booth, 1964; Driggers and Marucci, 1964; Aitken and Senn, 1965; Stephensen, 1966; Booth, 1966 and 1969). Application of SWC to plants has suppressed fungal (Khaleafa et al., 1975), bacterial (Booth, 1969), and virus (Pozsar et al., 1967) infections. Crouch (1990) suggested that healthier plants induced by SWC application are better able to withstand pest attacks. Laminarin, a component of many brown seaweeds (Mitchell, 1963), and cytokinin (Featonby-smith and Van Staden, 1983a) in SWC may be responsible for the increased plant tolerance to these pathogens.

The effect of SWC on plant tolerance to abiotic stresses has not been well researched. Senn et al.(1961) showed that treated lettuce plants were more tolerant to frost (-2 °C). Information on the influence of SWC on plant tolerance to heat, cold, salt and drought is not available. More research needs to be done in these areas. There are some indications that SWC-treated plants show increased tolerance to these stress conditions, considering that SWC can induce healthier and more vigorous plants with deeper rooting systems.

2.1.4 Increased Tolerance to Nematode Infection

Numerous reports indicated that SWCs are responsible for increased plant tolerance to nematode infection (Tarjan, 1977; Tarjan and Frederich, 1983). Tomato plants grown in nematode-infested soil, after treatment with SWC, generated a larger root system, which resulted in higher economic and biologic yields (Featonby-Smith and Van Staden, 1983a). A reduction in root-knot nematode infection as reported by Crouch (1990) indicated that application of SWC improved all of the measured parameters (plant weight, leaf surface area, number of flowers, root length and etc.) of tomato plants infected with nematodes. In addition, the degree of nematode infection in the SWC-treated plants was significantly lower than the control. Hormones, especially cytokinins, play an important role in nematode infection and development in the roots of susceptible hosts (Dropkin et al., 1969; Sawhney and Webster, 1975). It was reported that higher concentrations of kinetin, not only decreased larval penetration into plant roots, but also inhibited the development of those that entered (Dropkin et al., 1969). After nematode infection, cytokinin levels in the host root exudates decreased (Bergeson, 1972). It was thought that reduced shoot growth associated with nematode infection may result from a decrease in cytokinin levels (Featonby-Smith and Van Staden, 1983b). Addition of cytokinin to plants with SWC application may compensate the decline in cytokinin caused by nematode infection.

Cytokinin may also suppress the reproduction and development of invading nematodes (Hussey, 1985). Featonby-Smith and Van Staden (1983b) detected smaller numbers of RKN in roots after SWC application, compared with the control. In vitro experiments, DeWaele et al.(1988) showed that application of SWC at various concentrations suppressed nematode reproduction. It was inferred that SWC acted indirectly on the nematodes through the influence on the host plants.

The beneficial effects obtained with SWC applications were usually attributed to the presence of cytokinin in SWC (Abetz, 1980). Later, however, 1-aminocyclopropane-1-carboxylic acid (ACC, a precursor of ethylene) was detected in seaweed concentrate by Van Staden (1985), which indicated that ACC may be the causative factor. Both cytokinin and ACC have been implicated in host resistance to nematode infections (Dropkin et al., 1969; Sawhney and Webster, 1975). High concentrations of cytokinin or ethylene favor susceptibility (Veech, 1981). Symptoms of plants with nematode infection were at least partially due to the increased ethylene production in the plants (Glazer et al., 1984).

2.1.5 Other Effects of SWC

Possibly because of hormones in SWC, such as cytokinins, improved seed germination and the breaking of dormancy were recorded after SWC treatment in table beet (Wilczek and Timothy, 1982) and creeping red fescue grass (Button and Noyes, 1964).

Cytokinin, the main proposed active component of SWC that induces beneficial plant responses, regulates a number of plant functions including cell division, protein and CO_2 metabolism, enzyme formation and leaf senescence, shoot elongation and fruit set. Moreover, it is reported that cytokinin treatment augmented the ratio of RNA to DNA, suggesting that a critical effect of cytokinin in senescence might be the maintenance of protein synthesizing machinery, perhaps by regulating RNA (Letham et al., 1977). Therefore, cytokinin-containing SWC may exhibit similar functions.

Povolny (1969a, 1969b and 1972) found the hardness of peach increased when sprayed with SWC, which delayed the ripening of apple, peach and apricot fruits on the trees and prolonged the storage period. The financial losses in treated fruit were suppressed by 35% in comparison with the untreated control. Controlling ripening makes it possible to prevent the losses due to overripening and to prolong the period of fruit consumption.

2.2. NEMATODES AND PLANTS

2.2.1 Roles of Nematodes in Crop Losses

Nematodes are nonsegmented worms that possess a body cavity and a complete digestive tract (Poinar, 1983). It has been estimated that at least 21,000 nematode species have been described, and 500,000 species exist in the world (Decker Heinz, 1989). In 100 cm^3 of arable land or garden soil, there are 4000 to 5000 or more nematode individuals (Decker Heinz, 1989), although not all of them are plant parasitic. In spite of the huge number of nematodes, their damage is often overlooked due to the following reasons: 1) their microscopic size makes them difficult to detect; 2) they are soilborne parasites; 3) the damage they cause is reductive or suppressive, rather than destructive; 4) the above ground symptoms of nematode infestation are similar to other diseases; and, 5) their obligate parasitic characteristic makes them difficult to work with.

It was estimated that the losses caused by nematodes in 1957 constituted 10% of the USA total harvest (Elmer, 1958). The annual damage caused by nematodes in California alone, during the same year was between $90 to 140 million (Allen and Maggenti, 1959). Poinar (1983) reported that a 10% crop yield reduction in USA was caused by these worms in 1967, even though more than 100 million pounds of nematicide was applied. In 1970, he stated that $60 million was spent on treating 1.7 million acres of nematode infested land in USA, and still, the annual crop loss was more than $1.5 billion. The loss reached $4 billion in 1976, according to his report. LeClerq (1964) estimated the losses of individual crops in terms of the percentage of the total annual production:

Tomato.. 8%

Common bean, Lima bean and Melon................. 5%

Sugar beet, Potato, Lemon, Orange, Peach and
Raspberry................................. 4%

Lucerne, Maize, Arachis, Tobacco and Lespedeza....3%

Cotton and Soybean..............................2%

In developing countries, crop rotation is limited because of high population pressure and limited arable land; therefore, the losses caused by nematodes could be higher than in developed countries. In those countries, nematodes damage crops each year, but few are aware of the causal agent because nematologists are rare. In addition, because most undeveloped countries are located in tropical or subtropical regions, nematodes, which are warm temperature loving animals, are more active and cause more damage than under temperate climates.

According to the report by Dowler and Van Gundy (1984), the average losses caused by plant-parasitic nematodes on all investigated major crops were about 10%. Losses in individual incidents may be as high as 100%. In fact, the real losses may be much higher than this due to the difficulty to eliminate the effect of other factors, and the tendency to attribute nematode damage to other factors. Besides, the above financial estimates do not include the indirect monetary losses of increased inputs to crop production, such as water and fertilizer.

2.2.2 Interactions of Nematodes With Other Factors

Thousands of plant diseases are induced by fungi, bacteria and viruses, but most involve synergism with other pathogens. However, most phytopathological literature deals with diseases caused by one pathogen.

Atkinson (1891) was among the first to report an interaction between nematodes and fungi. He observed that interaction by root-knot nematodes (Meloidogyne spp.) always increased the severity of Fusarium wilt of cotton. In fact, nematode influence on Fusarium wilt expression is so profound that wilt control in a number of crops is based upon concomitant root-knot nematode control (Powell et al., 1971). This seems true even if the cultivar involved is Fusarium wilt-resistant. Since Atkinson's early observation, the root-knot-Fusarium interaction has been reported on tomato (Jenkins and Coursen, 1957; Goode and McGuire, 1967), tobacco (Porter and Powell, 1967), peas (Davis, 1963; Davis and Jenkins, 1963), cotton (Minton and Minton, 1963), soybean (Ross, 1965), sugar beet (Jorgenson, 1970), eggplant and pepper (Mountain and McKeen, 1962; Olthof and Reynes, 1969), potato (Mountain and McKeen, 1962), elm and maple (Dwinell and Sinclair, 1967), alfalfa (Kushner and Crittenden, 1967), peanuts (Minton and Jackson, 1967) and winter wheat (Benedict and Mountain, 1956).

Much of the research on nematode-fungi disease complexes has shown that the fungus component of an interaction often has an effect on nematode population. Infection by fungi may reduce (Ryder and Crittenden, 1965; Littrell and Johnson, 1969; James, 1966; Jorgenson, 1970) or increase (Ross, 1965; Mountain and McKeen, 1962; Faulkner and Skotland, 1965; Dwinell and Sinclair, 1967) the populations of nematodes, or increase the ratio of males to female nematodes due to an unfavorable environment for nematode development (Ketudat, 1969).

2.2.3 Nematodes and Turfgrass

Nematode damage is generally a much greater problem in the southern than in northern United States. Many kinds of nematodes are found in most, if not all, turf fields in the world. Damage to turf caused by nematodes have become more and more of a concern in turfgrass management. Prior to 1900, a scientist in Holland recorded the occurrence of nematode from meadow land. However, people did not recognize nematodes as plant pathogens of turfgrass until the 1950's. Introduction of nematicides provided a chemical tool to demonstrate the effects of nematode activities through control. Extensive research, concerning the type and extent of nematode damage to turfgrasses, was done between the 1950's and 1960's. General associations between turfgrass and nematode genera were established (Table 2-1).

Table 2.1 A list of nematode spp. found on turfgrasses

	Common Name	Scientific Name
Endoparasitic	Burrowing nematode	Radopholus smilus
	Cyst nematode	Heterodera spp.
	Cystoid nematode	Meloidodera App.
	Lesion nematode	Pratylenchus spp.
	Root-knot nematode	Meloidogyne spp.
Ectoparasitic	Awl nematode	Dolichodorus spp.
	Dagger nematode	Xiphinema spp.
	Lance nematode	Hoplolaimus spp.
	Needle nematode	Longidorus spp.
	Pin nematode	Paratylenchus spp.
	Ring nematode	Criconemells spp.
	Sheath nematode	Hemicycliophora spp.
	Spiral nematode	Helicotylenchus spp.
	Sting nematode	Belonolaimus spp.
	Stubby-root nematode	Trichodorus spp
	Stunt nematode	Tylenchorhynchus spp

In 1976, two out of the six nematode species of Criconemoides found by Safford and Riedel in an Ohio golf course turf were dominant (73% and 22%), and each of the other nematode species made up less than 3% of the nematode composition. The results showed that geographical site location and turfgrass species did not correlate with the nematode species found. It was believed that the cutting height of grass, soil moisture and soil texture may be more important to the infection by specific nematode species (Safford and Riedel, 1976). Four nematode species were identified on turf in Hawaii (Tashiro and Murdoch, 1977). The nematode counts of the species from chlorotic spots and from normal turf on the same greens revealed no correlation between population density of plant-parasitic nematodes in or outside the affected areas.

Five of the ten species identified in 16 golf courses throughout New York state were the most abundant and reported for the first time in New York (Murdoch, Tashiro and Harrison, 1978). No turf injury was associated with nematode infection on these courses. Eisenback (unpublished) conducted a preliminary survey of golf courses in the mid-Atlantic region of the US to determine the genera and species that commonly occur on turf, and detected nematodes from 14 genera.

In a study on the effects of bermudagrass N treatments and cultural practices on the plant-parasitic nematode populations, White and Dickens (1984) found the sources of N had a limited effect, and topdressing, vertical mowing, or core aerification treatments had no consistent effect on nematode population. As to nematode control in turfgrass, Radewald (1973) recommended starting with nematode-free materials, preplant chemical treatments, and postplant chemical treatments.

A survey of the literature of the past 20 years revealed few publications on turfgrass parasitic nematodes (Murdoch and et al., 1978; Safford and Riedel, 1976; Swier, 1978; Tashiro and et al., 1977). The works that have been reported are general associations between turf and nematode genera. It is believed that nematodes injure plant roots by feeding on root cells. Damaged roots may have necrotic lesions or swellings and knots. Wilting is often the first visual symptom of nematode infection on turfgrass because of the reduced size of the root system and the inability to take up water. Other symptoms include yellowing of the leaves, slow growth, and a gradual thinning of the turf. Although nematodes usually do not directly kill host plants, they can seriously weaken them and predispose the turf to other root pathogens. After feeding on diseased tissue, nematodes can spread diseases by attacking healthy roots. Therefore, the interaction between nematodes and other disease agents (e.g. fungi, viruses and bacteria) combined with stress factors, especially drought and high temperature, could result in severe damage to the turfgrass.

2.2.4 Nematode Control

Nematodes must be considered in any pest management program, because they can cause problems in almost any cropping situation. The large number of nematode species and their feeding behaviors, necessitate the development of different methods and measures to control them. All the following methods have been employed in nematode control.

Physical Methods: High temperature, steaming soil and treating plants with hot water. these methods are expensive and can not be used extensively.

Chemical Methods: Chemical nematicides are either fumigants or non-fumigants. Both are effective in destroying nematodes in the soil environment, but they must be applied when soil conditions are suitable. Some of these chemicals may affect beneficial organisms in the soil and most of them could cause environmental pollution and human hazards (Crouch, 1990).

Host-plant Resistance: Developing and using resistant plant varieties is the most efficient and economical way to control nematode damage. It is known that resistant genetic resources are available in many crops, and resistant varieties could be successfully planted to avoid nematode damage. However, variability of nematode races and difficulty of inserting nematode resistant genes into a desired genetic background, make this a continuing battle. Plant breeders face an even greater challenge with perennial crops.

Cultural Practices: Cultural practices, such as growing cover crops, crop rotation, and use of trap crops, have been used to reduce nematode damage. The main problem of this method is that farmers can not keep their land occupied with most profitable crops, so that there is little chance or desire to fallow or even to conduct crop rotation, especially in highly populated countries.

Biological Control: Using the nematodes' natural enemies, such as fungi, protozoans and other soil-dwelling organisms could decrease the population of nematodes. Although this method has great potential for controlling nematodes, it is still in the early stage of experimention, and much research is necessary before it becomes a practical solution.

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CHAPTER 3

RESPONSE OF BENTGRASS TO THE INTERACTION OF ABIOTIC STRESSES AND NEMATODE INFECTION

INTRODUCTION

Nematodes are important plant pathogens that limit world agricultural production (Taylor, Sasser and Nelson, 1982). They occur all over the world (Sasser and Carter, 1985) and parasitize most agricultural crops (Taylor and Sasser, 1978; Sasser, Carter and Taylor, 1982; Taylor, Sasser and Nelson, 1982). Many of the nematode species attack plant roots. Responses of plants to nematode infection include alteration in respiration, water relationships, photosynthesis, and nutritional deficiencies (Hussey, 1985). Furthermore, plant growth and yield are suppressed because of removal of nutrients (Dropkin, 1969; Sasser, 1980), decrease in nutrient absorption and translocation (Jones and Dropkin, 1975), as well as retardation of root growth (Lewis and McClure, 1975).

Under favorable plant growth conditions, nematode influences on plant development may be difficult to detect, because of the high compensation ability of host plants. Powell (1971) believed that nematodes damage host plants mainly through interactions with other stress factors. It is likely that different factors co-influencing the same host plants will influence each other. For example, soil salinity and moisture stress not only affect plant growth, but also influence the reproduction and development of nematodes existing in the soil. Studies concerning the roles of salinity in nematode-host interactions have shown that as salinity increases, nematode damage to cotton, tomato and sweet orange increase (Van Gundy and Martin, 1961; Heald and Heilman, 1971; Maggenti and Hardan, 1973). However, studies on the interaction of nematodes and salinity have not been conducted on turfgrass. In order to better manage turfgrass and reduce nematode damage, it is important to understand the relationships among nematodes, stress factors and host-plants. The objective of this study was to determine the effects of soil salinity and moisture stress on the pathogenicity of nematodes in bentgrass plants, and on the development of the nematode population.

MATERIALS AND METHODS

These experiments were conducted under greenhouse conditions, with an average daily temperature range of 22 °C to 40 °C, from January 29 to June 18, 1993. A 2.5 cm thick mature sod of bentgrass (Agrostis palustris Huds, cv. Penncross) was placed on each of 48 nineteen liter cylindrical plastic containers (27 cm in diameter), which had been filled with methyl bromide-fumigated sandy loam soil (pH=6.8) and topped with an expanded metal screen. Just before transplanting, soils in 24 of the containers were inoculated with root-knot nematodes (RKN) (Meloidogyne naasi) (7800 eggs/container) by injecting 10 ml water-RKN suspension 2.5 cm deep into 5 locations in each container. The inoculum of the RKN was obtained from previously inoculated bentgrass. The soils in the rest of the containers (24) were RKN-free and used as controls.

From transplanting on January 29 to May 4, maximum plant vigor was maintained with watering one liter of tap water per container every other day, and fertilizing with 58 kg N ha^{-1}, 25 kg P ha^{-1} and 48 kg K ha^{-1} each month for all containers. The height of the turf was maintained at 0.6 cm at seven-day intervals, with a mower adapted for microplots. On May 4, all containers were watered gravimetrically to provide 13% moisture (field capacity) of soil dry weight. Then, the containers were arranged into two experiments, each consisted of 24 containers (12 with RKN and 12 without RKN). The containers in one experiment were exposed to adequate irrigation or salinity watering while the containers in a second experiment received either adequate irrigation or drought stress. In the adequate irrigation and the salinity treatment, one liter of tap water or 0.2% NaCl-water solution was added to separate containers, every other day. For all the treatments, soil water content in each container was balanced to field capacity at 14 day interval. For the drought treatment, no water was added to the designated containers for 14 days intervals. Then, each container was irrigated to field capacity again. At the termination of the experiment, the following parameters were measured: 1) visual quality with 9 = best, 6 = acceptable, and 1 = all dead plants; 2) plant shoot density with 9 = most and 1 = poorest; 3) leaf color with 1 = lightest green and 9 = darkest green; 4) wilting rating with 9 = lowest and 1 = highest; 5) leaf water potential, ascertained with leaf hydraulic pressure (LHP) (Cox and Hugher, 1982); 6) clipping yield; 7) root mass as determined by the energy required for vertically lifting roots from soil (VLR) (Schmidt et al., 1986); 8) root length as expressed by the longest root measured after pulling the roots out from each container; and 9) nematode density determined as the number of RKN eggs per gram root and as per container by the Clorox extraction (Hussey and Barker, 1973).

Root sample was collected from 5 to 15 cm below soil surface in each container for RKN extraction. Total number of RKN eggs in each container was determined by multiplying eggs/g. fresh root and VLR. Soil salinity stress level was measured and expressed as electrical conductivity (Greenberg et al., 1981).

A 2 x 2 factorial design with 6 replications was used for each experiment. Analysis of variance (ANOVA) with SAS was applied to the data collected from each experiment, and means were separated by Duncan's multiple range test at 5% probability when the ANOVA indicated significant treatment effects.

RESULTS

Effects of RKN infection, stress factors or their combinations on the bentgrass plants.

Salt, drought stress and nematode infection adversely affected growth and development of the bentgrass plants. Figures 3-1 and 3-2 show the effect of salinity and drought on the foliage of bentgrass with and without RKN infection. Almost every plant aspect was influenced by salt, drought stress, nematode infection, or their treatment combinations. Under salt or drought stress, RKN infection altered plant appearances significantly. However, RKN did not affect appearances of the bentgrass under adequate irrigation. Similar results were observed with root development as affected by RKN, abiotic stress or their combinations (Figures 3-3 and 3-4). Under adequate irrigation, RKN infection affected VLR and root length less than under either salinity or drought stress conditions.

When bentgrass was infected with RKN and subjected to drought or saline irrigation, turf quality decreased significantly (Figure 3-5). The effects of RKN infection on turf density, leaf color and wilting rate were not significant under adequate irrigation, but when subjected to the abiotic-stresses these parameters were adversely affected whether RKN was present or not (Figure 3-6, 3-7, and 3-8). Salinity irrigation or drought stress alone caused a significant reduction in these parameters, compared with the non-stress treatment. With the addition of RKN infection the salt treatment decreased density and color rating further. The combination of RKN and drought stress significantly affected all the three parameters negatively, compared with drought stress alone. Leaf hydraulic pressure was the only parameter which significantly increased with RKN inoculation under adequate irrigation, but not under abiotic stress conditions (Figure 3-9). As compared with RKN inoculated turf, LHP was significantly higher with saline irrigation, but not drought. The combination of nematode inoculation and saline irrigation or drought did not significantly increase LHP.

Clipping yield is a function of many plant parameters. Nematode infection alone reduced fresh clipping weight sharply (Figure 3-10). Moreover, fresh clippings from bentgrass grown under salt stress or the combination of RKN and salt stress were significantly lower than bentgrass grown under no stress condition. However, there was no significant difference in clipping yield between salt, RKN or their combined treatments. Drought stress alone had no significant effect on clipping yield. However, fresh clipping weight from grass subjected to drought stress in combination with nematode infection was significantly lower than the non-stressed control.

It was found that VLR was not affected by RKN infection alone (Fig.3-11). However, under drought or salinity conditions, VLR significantly decreased. The combination of drought and nematode infection reduced VLR to a greater extent than either parameter alone, whereas, the combination of salt and nematode infection did not further affect VLR when compared with the grass treated with salt irrigation alone.

Similar results could be seen on the root length (Fig. 3-12).

Effects of salt and drought stress on the reproduction of the nematodes.

The number of RKN eggs in 5 grams of fresh root (egg density) from bentgrass grown under salinity, drought or no stress condition are listed in Table 3-1 and Table 3-2. The RKN egg densities were significantly higher under drought than under adequate irrigation. The egg density obtained from grass subjected to salinity treatment was not significantly different from that of adequate irrigated plants.

Table 3-1. Numbers of RKN eggs in bentgrass fresh root as influenced by salt

Stress Treatment	Number of RKN Eggs: Per 5 gm Root	Per Container**
Normal Irrigation	2,833a*	110,740a
Saline Irrigation	3,034a	105,636a

*. Means with a different letter in each column were significantly different at 0.05 probability level.
**. Total number of RKN eggs was estimated by the product between eggs/g. fresh root and VLR.

Table 3-2. Numbers of RKN eggs in bentgrass fresh root as influenced by soil moisture

Stress Treatment	Number of RKN Eggs	
	Per 5 gm Root	Per Container**
Normal Irrigation	2,833b*	110,740a
Saline Irrigation	3,456a	90,047b

*. Means with a different letter in each column were significantly different at 0.05 probability level.
**. Total number of RKN eggs was estimated by the product between eggs/g. fresh root and VLR.

Although eggs/g root from drought-stressed plants was higher, the total number/container was lower compared with that of the adequate irrigation. A similar trend could be found by comparing salinity and adequate irrigation, i.e. under salt stress condition, egg density was 7% higher, but total egg number in each container was 5% lower than that of Adequate irrigation. The lower egg number but high egg density in the stressed containers was a reflection of the salt and the drought treatment reduced root mass (lower VLR).

DISCUSSION AND CONCLUSION

RKN infection adversely affected turf quality, leaf color, LHP, and fresh clipping yield. Drought and salinity stress negatively influenced turf quality, color, density, wilting, LHP and root development. Salinity irrigation also reduced clipping yields. It has been shown that RKN infection affects water and nutrient absorption and upward translocation by the root system (Hussey, 1985). Juvenile RKN primarily penetrate roots directly behind the root cap, then they migrate intercellularly in the cortex to the region of cell differentiation (Starr, 1981). Therefore, RKN essentially affect the whole plant by influencing root functions (Hussey, 1985). Above-ground symptoms exhibited by RKN-infected plants are similar to those produced on any plants with a damaged and/or malfunctioning root system. These symptoms include suppressed shoot growth, nutritional deficiencies exhibited by leaves and temporary wilting (Hussey, 1985). All of the alterations detected in the current study are coincident with or derived from the above symptoms.

Drought influence on bentgrass infected with RKN exacerbated adverse effects on all parameters measured except for LHP and clipping yield. Salinity irrigation caused a further reduction only in turf quality, color and density where the bentgrass was infected with nematodes. Evidently, the salinity irrigation did not influence the bentgrass as much as the drought treatment. Others (Powell, 1971; Hussey, 1985) also found abiotic factors which induce plant stress, such as drought, salinity and temperature extremes, increase nematode damage to plants. Under these stresses, water is a key for plant survival. As stated above, nematode infections have been shown to reduce water absorption and translocation, therefore, nematode infection in conjunction with certain abiotic stresses may create a severe water deficiency as was reflected with the water potential measurements (Fig.3-9).

The population increase of RKN is related to food supply, and adaptation to the physical and biological environment. Soil moisture and salinity are the two most important physical factors that affect nematode activities. If soil moisture fails to fall into the range from 40 to 60% of field capacity, nematode activities decrease (Hussey, 1985). Guiran (1978) reported that dry soil inhibit both juvenile emergence and egg hatch.

The osmotic potential of soil solution can not only affect plant growth, but also alter RKN behavior. Wallace (1969) found that hatching of RKN eggs was reduced by low osmotic potential. Reversat (1981) reported that RKN consumed food reserves 7.5 times less under salinity conditions than under normal conditions. Our results agree that the total number of RKN in each container of stress treatments was lower than in the stress-free treatment. The lower total egg numbers/container in drought stress treatments in the current study resulted from the drought inhibition of RKN development. The lower RKN eggs/g root under normal irrigation, on the other hand, was due to the fact that egg density was diluted by the larger root system.

Fig.3-1 Effect of drought and RKN infection on bentgrass foliar growth. 19 weeks after RKN inoculation, with D_0=adequate irrigation, D_1=drought, N_0=RKN free and N_1=RKN infected.

Fig.3-2. Effect of Salinity and RKN infection on bentgrass foliar growth. 19 weeks after RKN inoculation; with D_0=adequate irrigation, D_2=salinity, N_0=RKN free and N_1=RKN infected.

Fig.3-3 Effect of drought and RKN infection on bentgrass roots. 19 weeks after RKN inoculation; with D_0=adequate irrigation, D_1=drought, N_0=RKN free and N_1=RKN infected.

Fig.3-4. Effect of Salinity and RKN infection on tbentgrass root. 19 weeks after RKN inoculation; with D_0=adequate irrigation, D_2=salinity, N_0=RKN free and N_1=RKN infected.

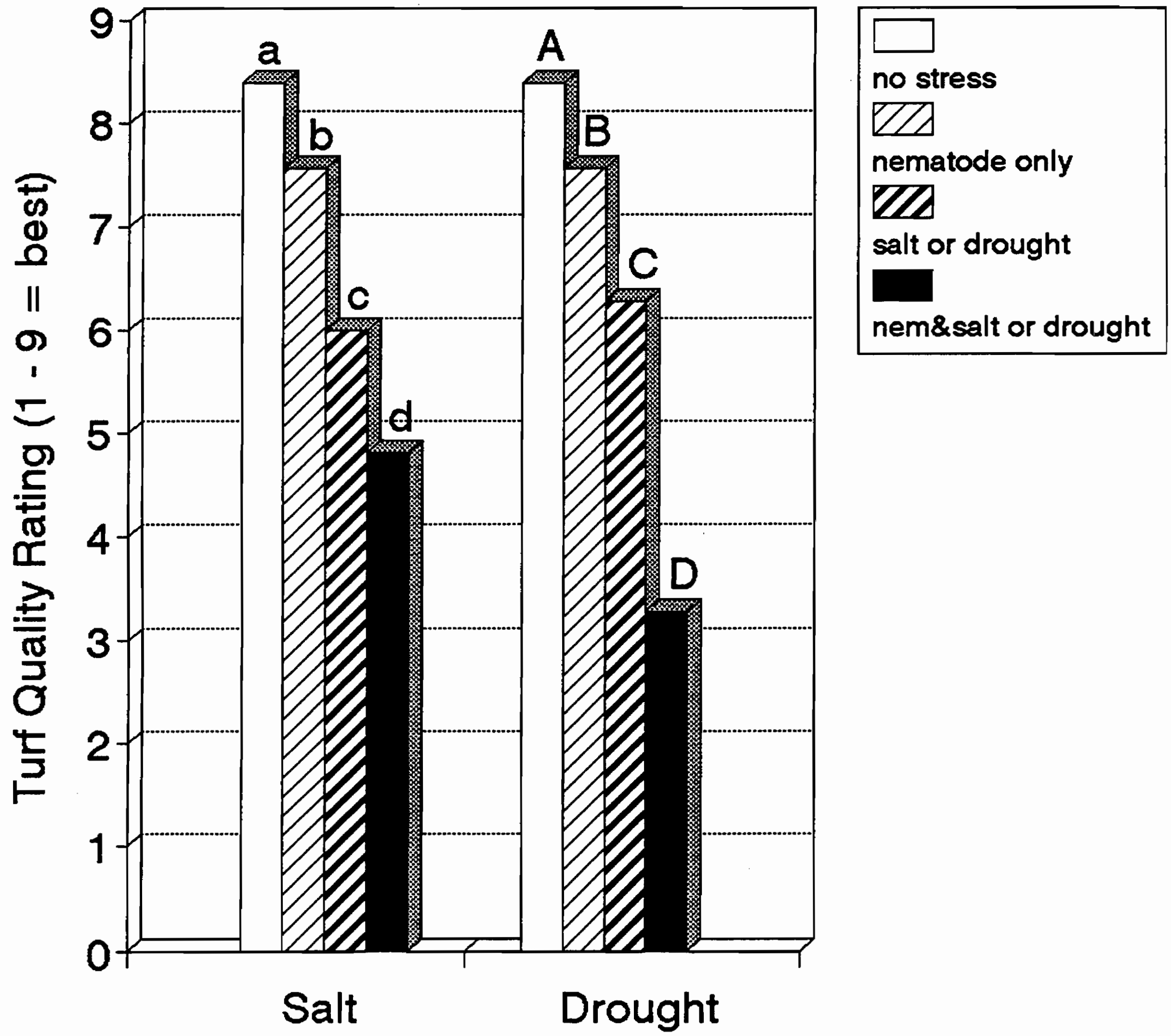

Figure 3-5 The effect of RKN infection and salt or drought stress on bentgrass quality. The data used are the means from two visual ratings recorded 17 and 19 weeks after RKN inoculation. The letter a, b, c and d differentiate the effects of the treatments in salinity group, while A, B, C and D indicate the differences among the treatments in drought group. The bars in each group indicated by the same letter are not significantly different at 0.05 probability level.

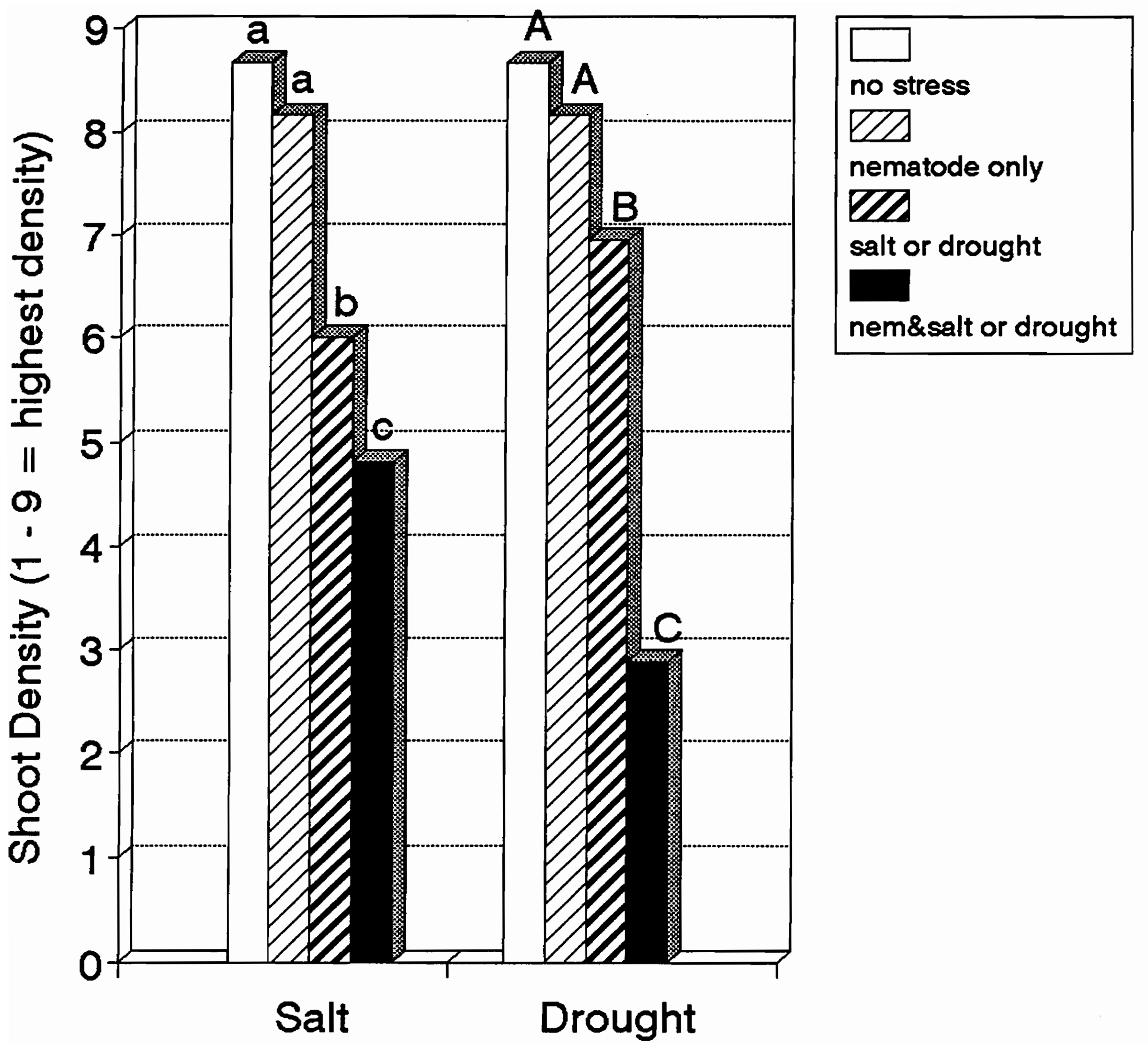

Figure 3-6. The effect of RKN infection and salt or drought stress on bentgrass density (9 to 1 with 9 = most). The data are the means from two visual ratings recorded 17 and 19 weeks after RKN inoculation. The letter a, b and c differentiate the effects of the treatments in salinity group, while A, B and C indicate the differences among the treatments in drought group. The bars in each group indicated by the same letter are not significantly different at 0.05 probability level.

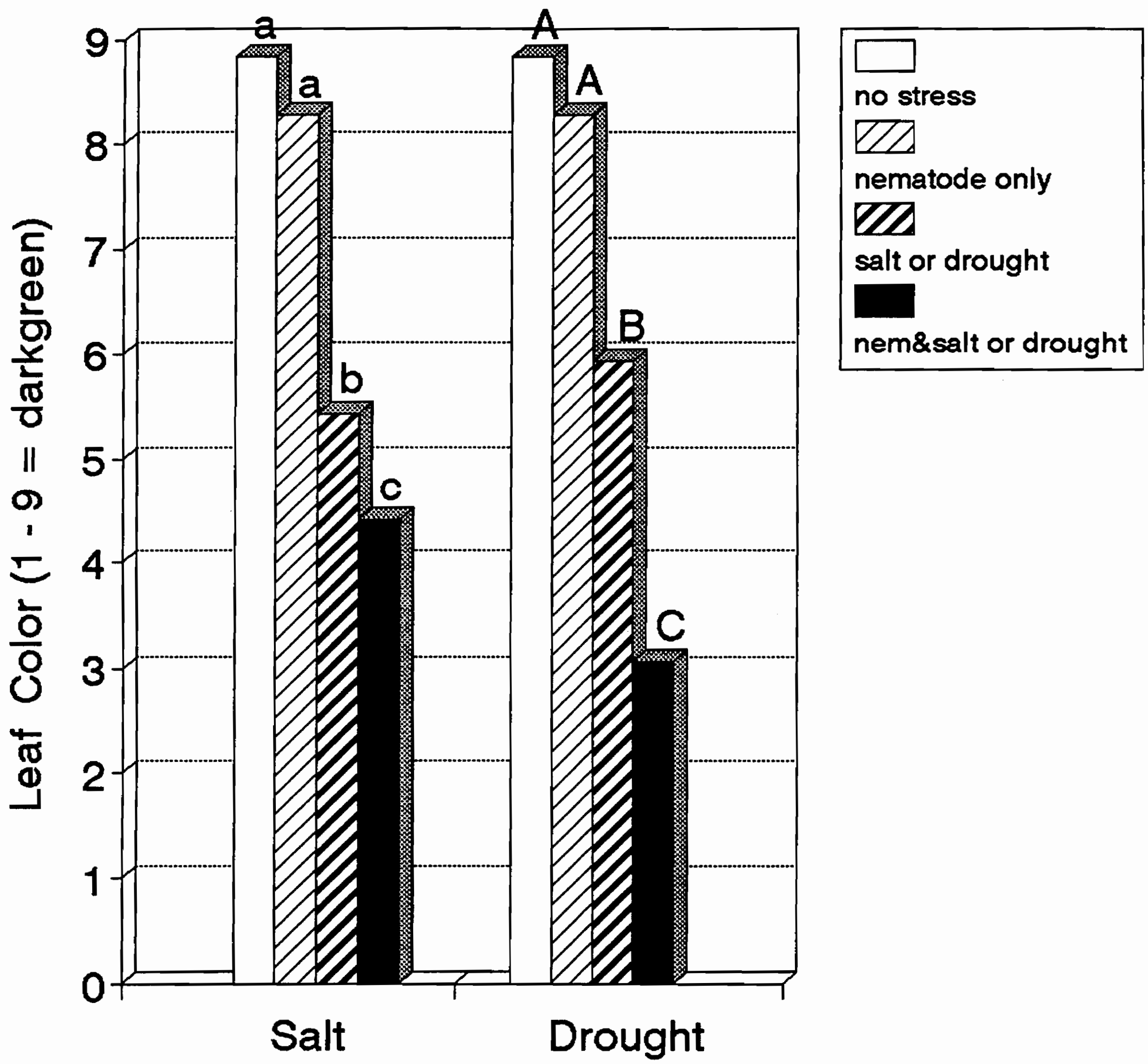

Figure 3-7 The effect of RKN infection and salt or drought stress on bentgrass leaf color (1 to 9 with 9 = darkest green). The data are the means from two visual ratings recorded 17 and 19 weeks RKN inoculation. The letter a, b and c differentiate the effects of the treatments in salinity group, while A, B and C indicate the differences among the treatments in drought group. The bars in each group indicated by the same letter are not significantly different at 0.05 probability level.

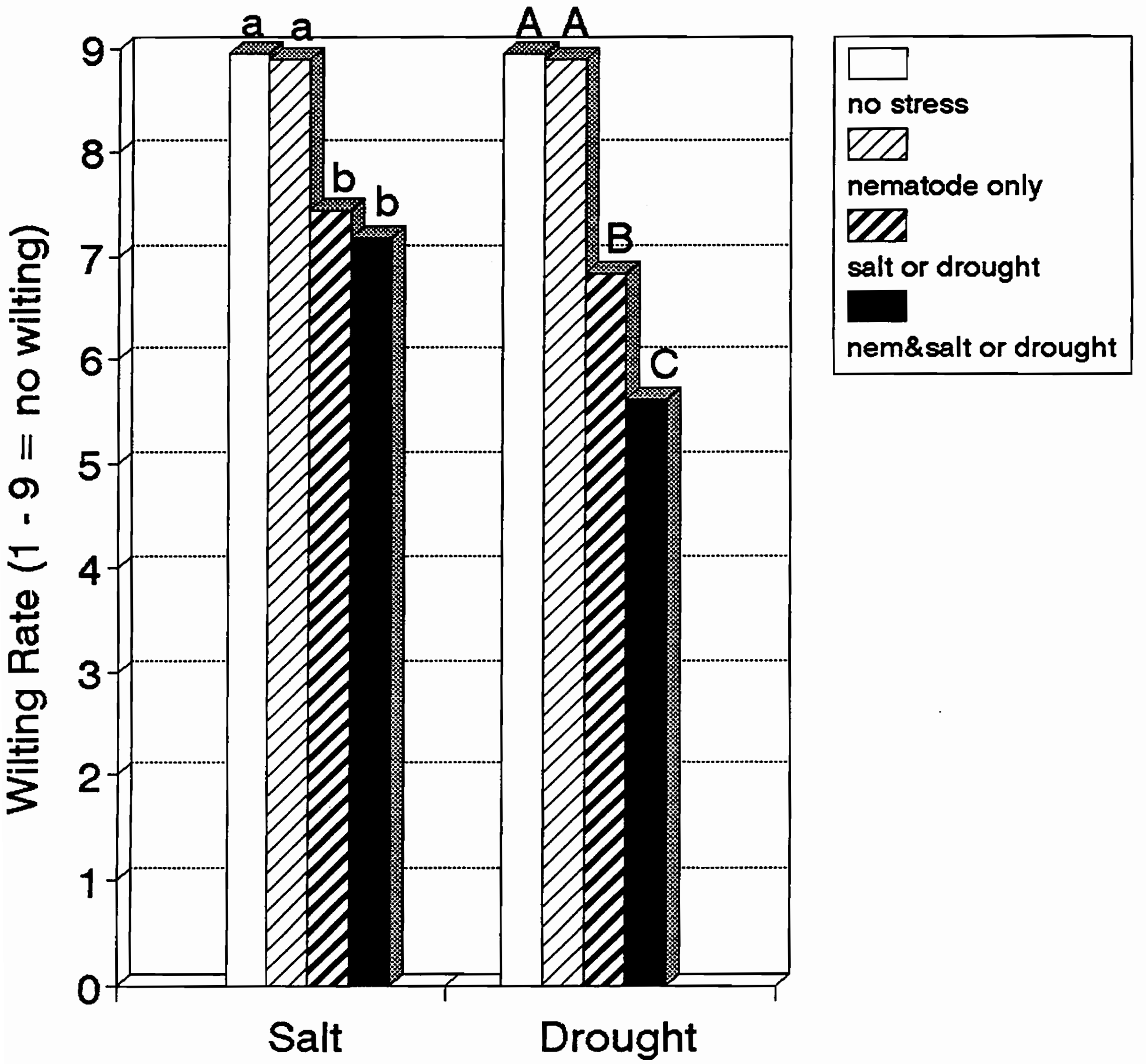

Figure 3-8 The effect of RKN infection and salt or drought stress on bentgrass wilting (1 to 9 with 9 = least). The data are the means from two visual ratings recorded 17 and 19 weeks after RKN inoculation. The letter a and b differentiate the effects of the treatments in salinity group, while A, B and C indicate the differences among the treatments in drought group. The bars in each group indicated by the same letter are not significantly different at 0.05 probability level.

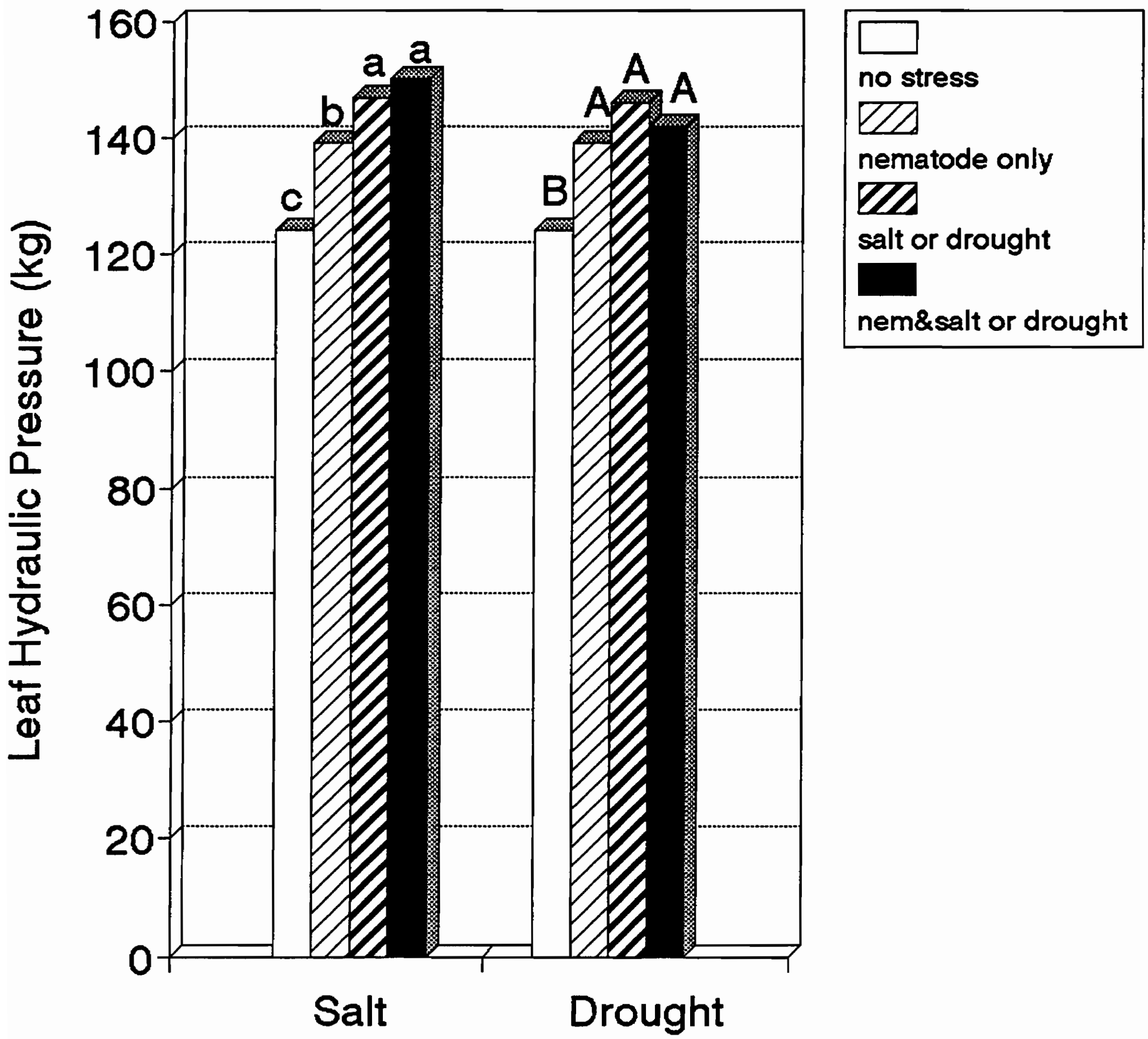

Figure 3-9 The effect of RKN infection and salt or drought stress on bentgrass leaf hydraulic pressure. The data are the means from two measurements 17 and 19 weeks after RKN inoculation. The letter a, b and c differentiate the effects of the treatments in salinity group, while A and B indicate the differences among the treatments in drought group. The bars in each group indicated by the same letter are not significantly different at 0.05 probability level.

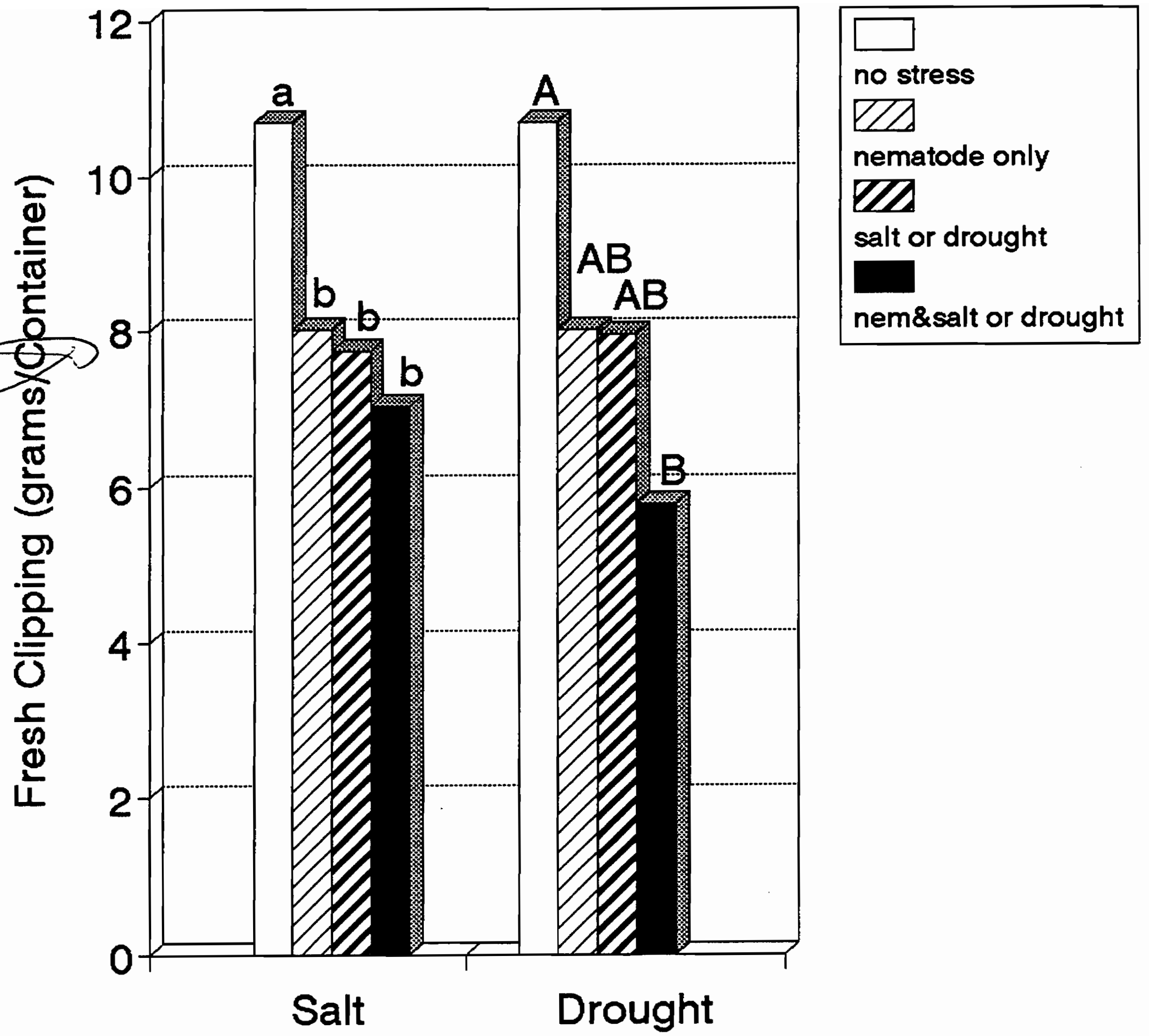

Figure 3-10 The effect of RKN infection and salt or drought stress on bentgrass fresh clipping weight. The data are the means from two collections conducted 17 and 19 weeks after RKN inoculation. The letter a and b differentiate the effects of the treatments in salinity group, while A and B indicate the differences among the treatments in drought group. The bars in each group indicated by a same letter are not significantly different at 0.05 probability level.

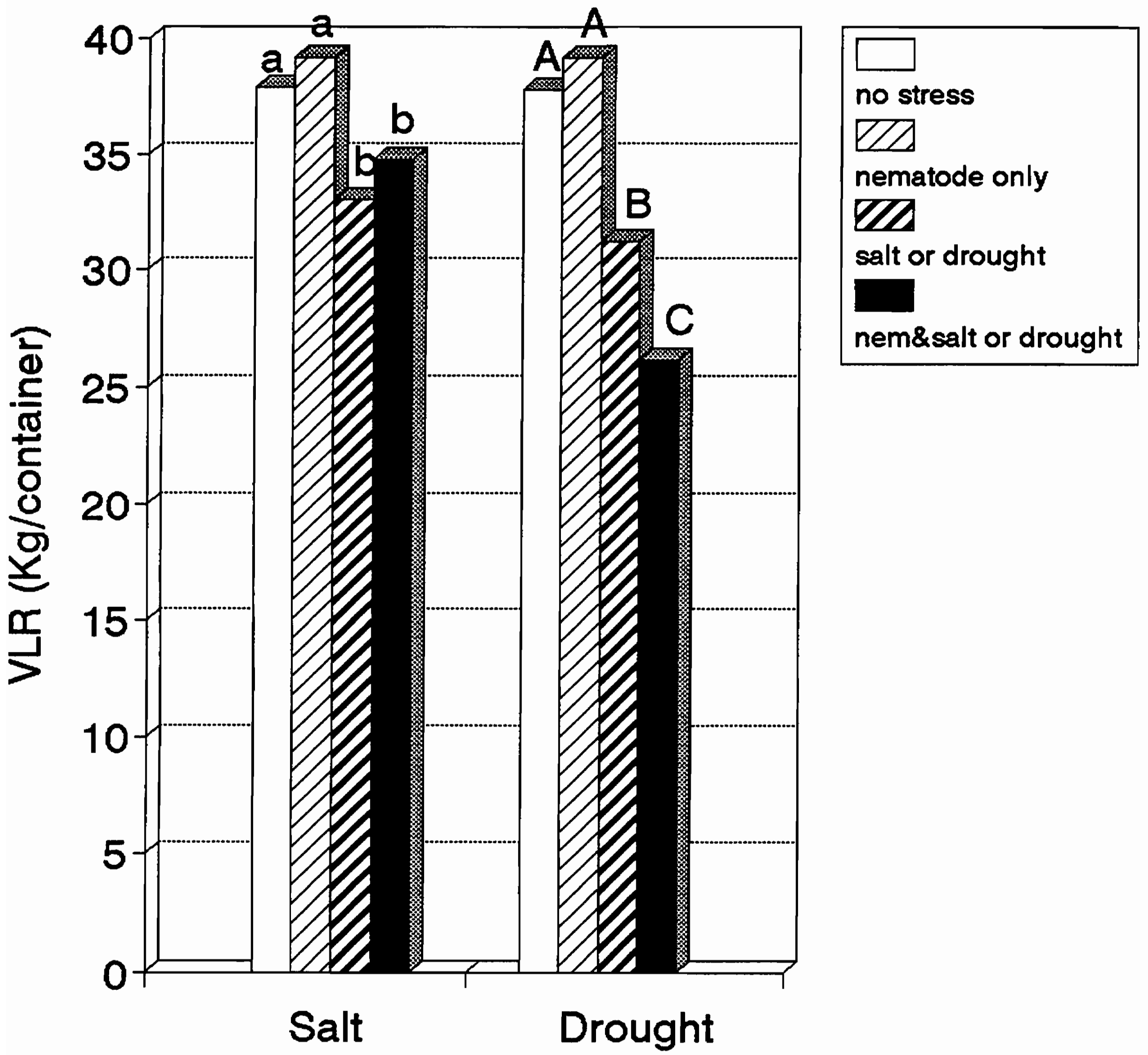

Figure 3-11 The effect of RKN infection and salt or drought stress on the energy required for vertically lifting bentgrass roots from soils (VLR). The data were collected 19 weeks after RKN inoculation. The letter a and b differentiate the effects of the treatments in salinity group, while A, B and C indicate the differences among the treatments in drought group. The bars in each group indicated by the same letter are not significantly different at 0.05 probability level.

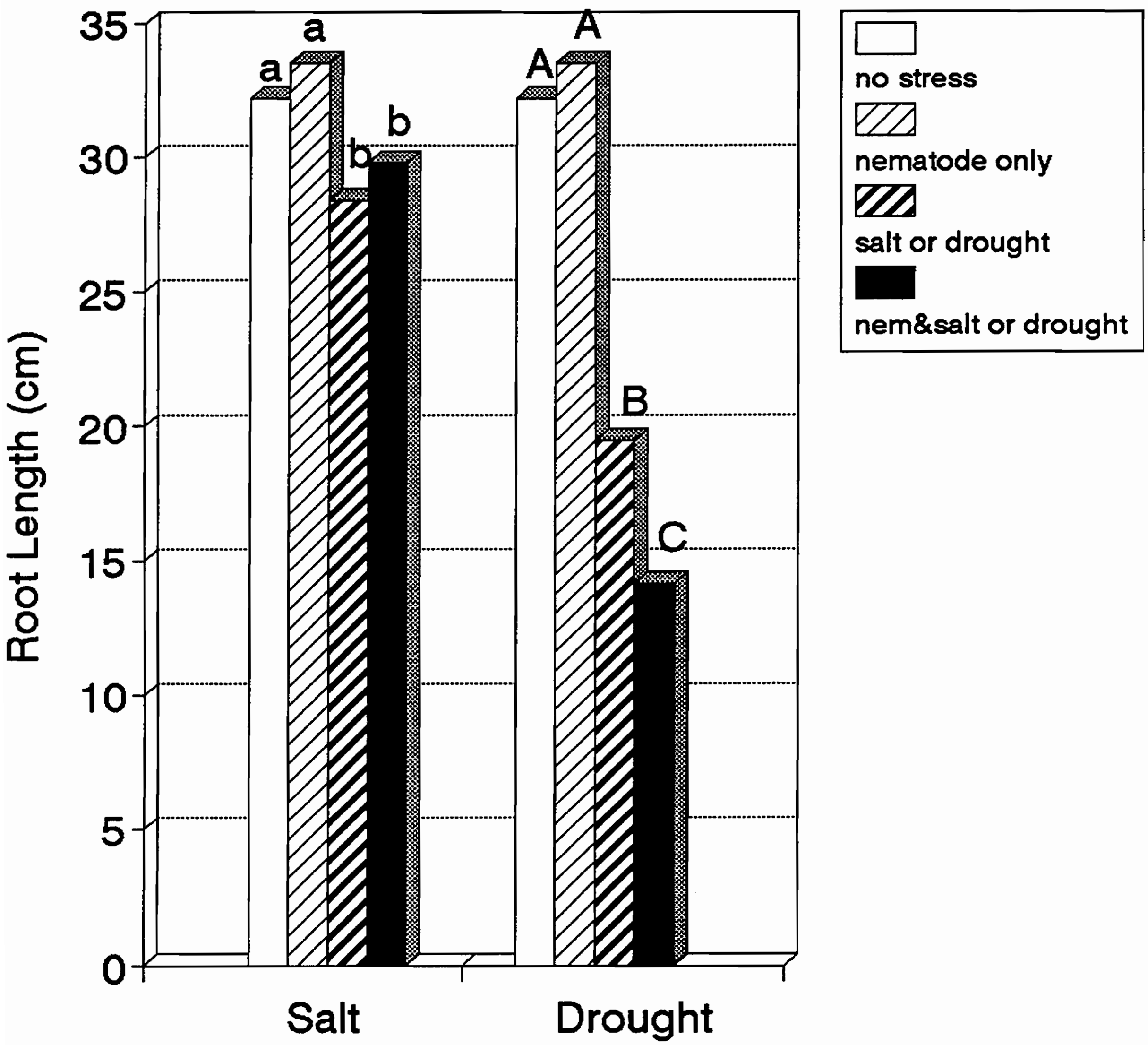

Figure 3-12 The effect of RKN infection and salt or drought stress on bentgrass root length. The data were collected 19 weeks after RKN inoculation. The letter a and b differentiate the effects of the treatments in salinity group, while A, B and C indicate the differences among the treatments in drought group. The bars in each group indicated by the same letter are not significantly different at 0.05 probability level.

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CHAPTER 4

THE EFFECT OF SEAWEED CONCENTRATE AND ROOT-KNOT NEMATODE ON THE GROWTH OF BENTGRASS UNDER WELL-WATERED CONDITIONS

INTRODUCTION

Seaweed concentrates (SWC) are made from storm-cast or freshly cut seaweed, belonging to the brown algae (Phaeophyceae), and sold commercially throughout the world as fertilizer supplements. It is believed that SWC contains all major and minor plant nutrients (Stephenson, 1968; Lobban et al., 1985), trace elements (Booth, 1964; Lobban et al., 1985), and most importantly, plant hormones, such as cytokinins (Brain et al., 1973; Blunden, 1977; Featonby-Smith and Van Staden, 1983), gibberellins (Taylor et al., 1977; Wildgoose et al., 1978), and auxins (Sumera and Cajipe, 1981). When applied to plants, SWC enhanced root growth, nutrient uptake, quality and resistance to pest and diseases (Crouch, 1990; Verkleiji, 1992). A few studies have reported that SWC possesses nematicidal activity. Featonby-Smith and Van Staden (1983) reported a 40% reduction in root-knot nematode (RKN) infection in tomato plants after SWC-soil drench. Extracts from 4 of 18 marine algae species, collected from Florida, inhibited the development of RKN in soil, and enhanced the growth of the tomato plants (Paracer et al., 1987).

In turfgrass management, nematode damage is often a problem (Chapter 3; Rafford et al., 1976; Murdoch et al., 1978), resulting in poor turfgrass quality and poorly developed root systems. Turfgrass managers have a special interest in naturally occurring products that improve turfgrass quality and growth, and soil fertility, and that enhance pest and disease resistance. This is becoming more important, since the use of chemical fertilizers and pesticides is often environmentally undesirable. Therefore, if the enhancements to root growth, nutrient uptake and disease resistance by SWC application could be proved in turfgrass, chemical usage in turfgrass management will be greatly reduced. This study is designed to ascertain the effects of SWC on growth of creeping bentgrass under RKN-free and infested conditions.

MATERIALS AND METHODS

This experiment was carried out under natural light and temperature under a clear plastic shelter from September 7, to October 23, 1992, at the Virginia Tech Turfgrass Research Center, Blacksburg, VA. and in a greenhouse from October 24, 1992 to January 15, 1993. A 2.5 cm thick bentgrass (Agrostis palustris Huds, cv. Penncross) sod , which was established in methyl-bromide fumigated soil, was placed on the top of each 19-liter cylindrical plastic container (27 cm in diameter) filled with fumigated sandy-loam soil and topped with an expanded metal screen. One week later, RKN (Meloidogyne naasi) was introduced to 5 locations in each container by injecting 10 ml inoculum solution 5 cm deep at two concentrations, 0 and 5800 eggs/container. The sod was then treated with three concentrations of SWC (Aqua-10 Laboratories) of 0, 0.5 and 1.0 liter/hectare, two weeks after the transplanting. The SWC used for each treatment was applied with one liter of tap water per container as a soil drench every 10 days for a total of 10 applications. The sod in each container was irrigated with one liter of water every other day, and fertilized with 58 kg N ha^{-1}, 25 kg P ha^{-1} and 48 kg K ha^{-1} each month. When SWC was applied in the same day, irrigation was omitted. The height of the turfgrass was maintained at 0.6 cm at seven day intervals, with a mower adapted for micro-plots.

Fifteen and seventeen weeks after RKN inoculation, the following parameters were recorded from the plants in each container. The fresh clipping yield was the average of the last two mowings, and dry clipping yield was determined in the same way as well. Leaf moisture content was calculated as (fresh clipping yield - dry clipping yield)/fresh clipping yield. Turf quality was visually rated with 1 to 9 scale (9 = best). Leaf water potential was estimated as leaf hydraulic pressure (LHP) with a leaf pressure press (Cox and Hugher, 1982). Root mass was evaluated 17 weeks after inoculation by measuring the energy required for vertically lifting roots from soils (VLR) (Schmidt et al.,1986).

The experiment was designed as a 2 (RKN treatments) x 3 (SWC treatments) factorial with three replications. Analyses of variance (ANOVA) with SAS were applied to the means of different measurements. Means for each treatment were separated by Duncan's multiple range test at 5% probability when the ANOVA indicated significant treatment effects.

RESULTS

ANOVA indicated the effects of RKN infection, SWC treatments and the interactions between RKN and SWC for all the measured parameters were significant at 0.05 probability level, except for leaf moisture content and dry clipping yield. Mean separations were conducted under the same RKN levels or the same SWC dosages for all of the parameters for convenience in discussion.

The overall appearance of the bentgrass plants, which continually received SWC every 10 days during the sixteen week period, whether RKN-free or RKN-infected, was better than that of the control. The quality of RKN-free turf was improved by SWC applications at both the 0.5 and 1.0 liter/ha dosage, although only the quality of the turf treated with 0.5 liter SWC/ha was significantly higher than the control (Fig. 4-1). Under RKN-infection, turfgrass quality, treated with 1.0 liter/ha SWC dosage was significantly higher than that of its non-treated counterpart. Generally, there was little effect of RKN-infection on turfgrass quality.

Application of SWC slightly but non-significantly increased leaf moisture content of RKN-free or RKN-infected plants (Fig.4-2). RKN infection had little effect on leaf moisture content. However, LHP was greatly reduced by SWC applications, regardless of RKN infection (Fig.4-3). Both SWC applications at 0.5 and 1.0 liter/ha similarly decreased LHP of RKN-free or RKN-infected bentgrass plants. Under both SWC application rates, the RKN infection increased LHP significantly possibly by affecting root functions.

Clipping fresh weight was increased 50% and 60% in RKN-free bentgrass treated with 0.5 and 1.0 liter SWC/ha, respectively, when compared to control plants (Fig.4-4). Under RKN-infestation, only the plants treated with 1.0 liter SWC/ha generated significantly more fresh clipping weight. Infection with RKN significantly reduced the fresh clipping yields from the control plants and the plants treated with 0.5 liter SWC/ha. Clipping yield from bentgrass treated with 1 liter SWC/ha did not significantly decrease when infected with RKN. Dry clipping weight increase was not significant with SWC treatments under either RKN-free or RKN-infection (Fig.4-5).

Application of SWC significantly increased VLR of RKN-free or RKN-infected plants (Fig.4-6). In addition, SWC applied at 1.0 liter/ha was more effective at improving root growth of RKN-infected plants than at 0.5 liter/ha. Bentgrass VLR did not differ between the two concentrations of SWC under the RKN-free condition (Fig.4-6).

CONCLUSIONS

Infection by root-knot nematode reduces turf quality and fresh clipping yield while increasing LHP of bentgrass. More parameters are expected to be influenced by RKN under stress conditions because the additive effects between RKN and stress conditions on bentgrass (Chapter 3).

Application of SWC at 0.5 liter/ha to RKN-free plants significantly improved turf quality, reduced LHP, increased fresh clipping yield and VLR. Applied at 1.0 liter/ha, SWC induced the same results except with a slightly improved quality that was non-significant. It is suggested that SWC at 0.5 liter/ha applied as soil drench at 10 day intervals is the maximum that can be used under RKN-free condition.

Under RKN-infection, applying SWC at 0.5 liter/ha decreased LHP and increased VLR. In addition to these two parameters, 1.0 liter/ha of SWC application improved turf quality and increased fresh clipping. One liter SWC/ha, applied as a soil drench at 10 day intervals, is effective in enhancing bentgrass growth under RKN-infected conditions.

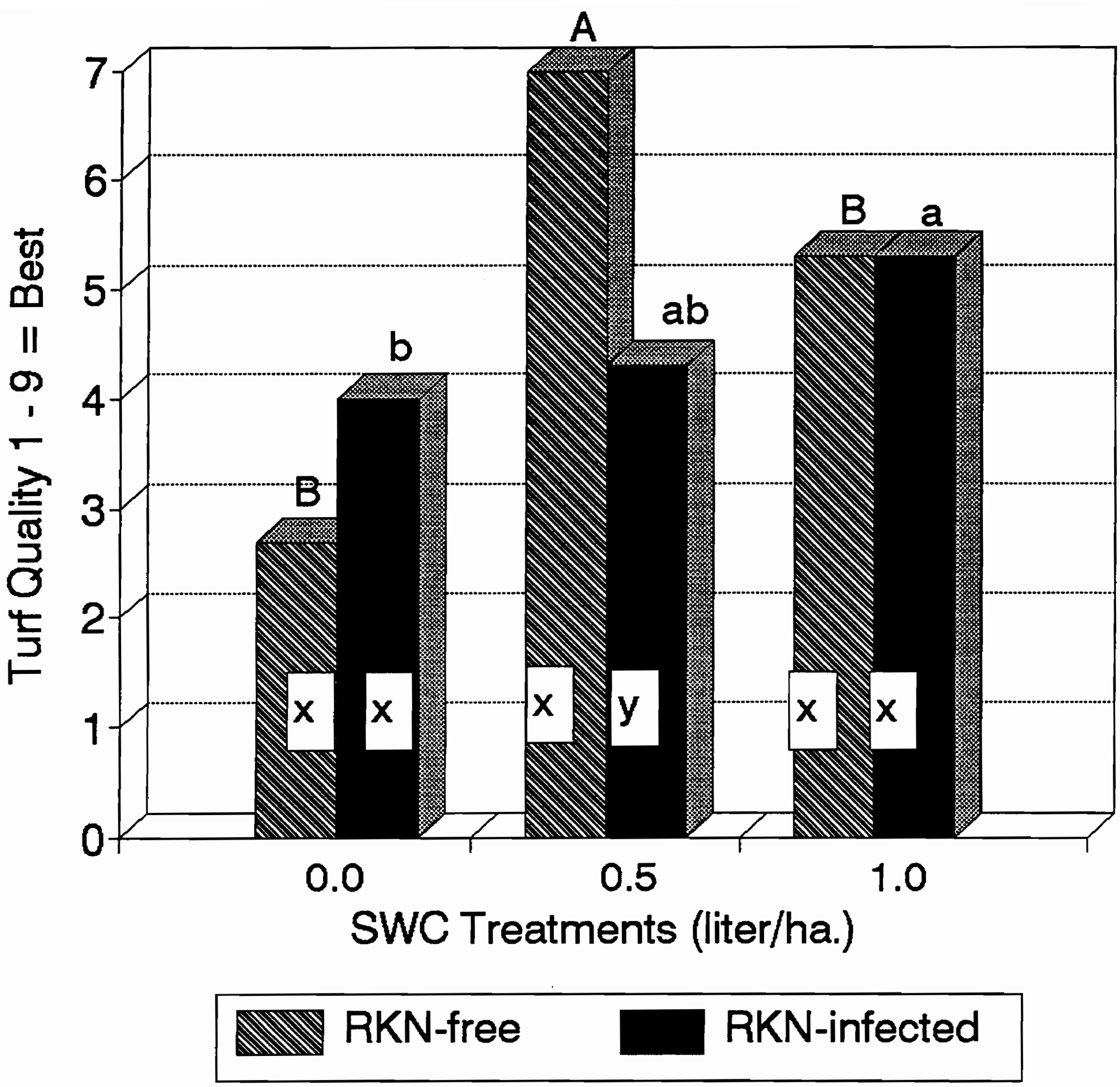

Figure 4-1. The effect of seaweed concentrate application and Root-Knot nematode on bentgrass quality, fifteen weeks after RKN inoculation. The letters A and B, and a and b differentiate the differences of turf quality ratings of the bentgrass treated with three SWC solutions under RKN-free and RKN-infection respectively. The letters of x and y indicate the difference between RKN-free and infected treatment under same SWC concentration. The means within each group indicated by the same letter are not significantly different at 0.05.

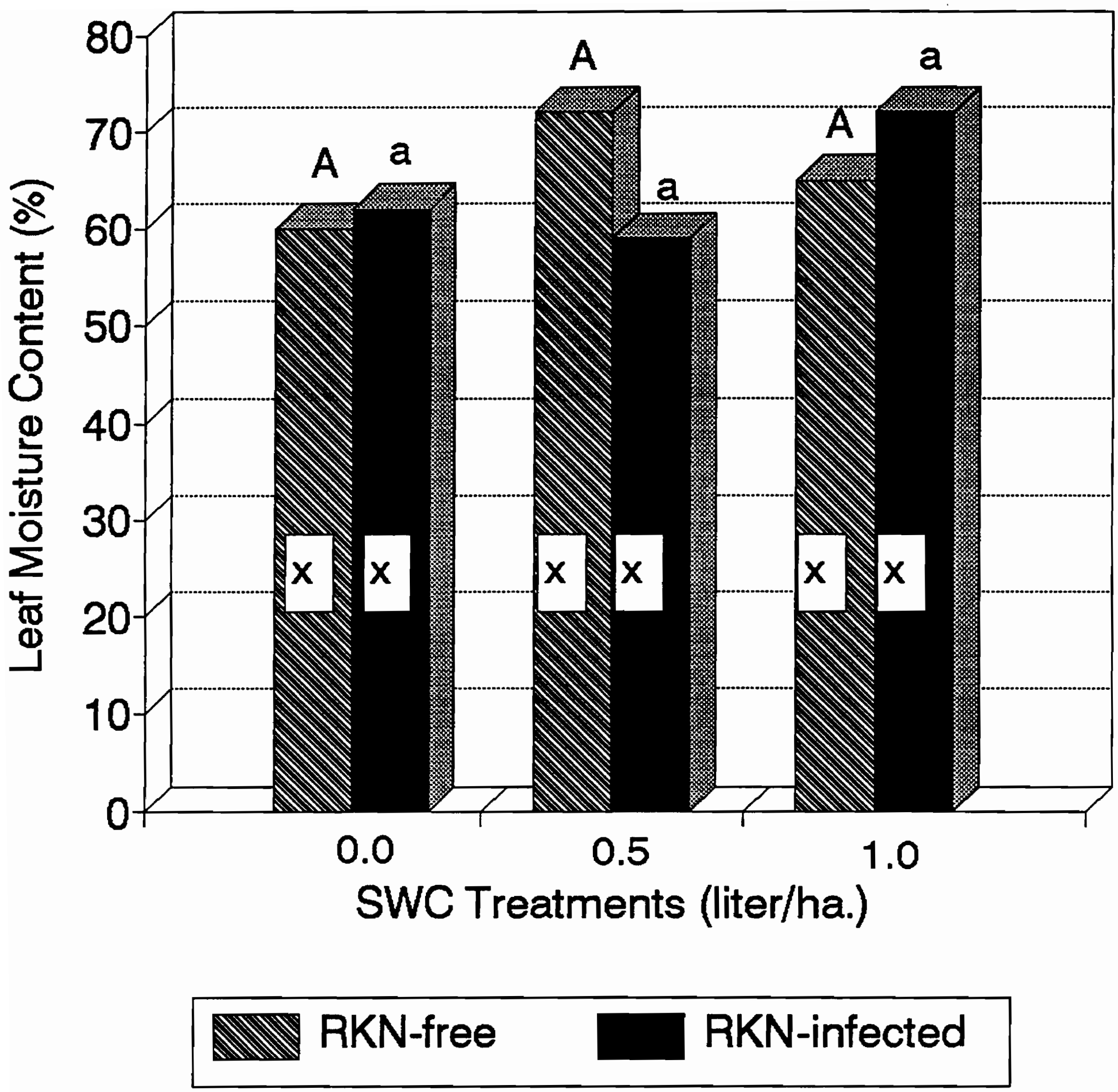

Figure 4-2. The effect of seaweed concentrate and RKN on leaf moisture content of bentgrass, fifteen weeks after RKN inoculation. The letters A and B, and a and b differentiate the differences of turf quality ratings of the bentgrass treated with three SWC solutions under RKN-free and RKN-infection respectively. The letter of x indicates the difference between RKN-free and infected treatment under same SWC concentration. The means within each group indicated by the same letter are not significantly different at 0.05.

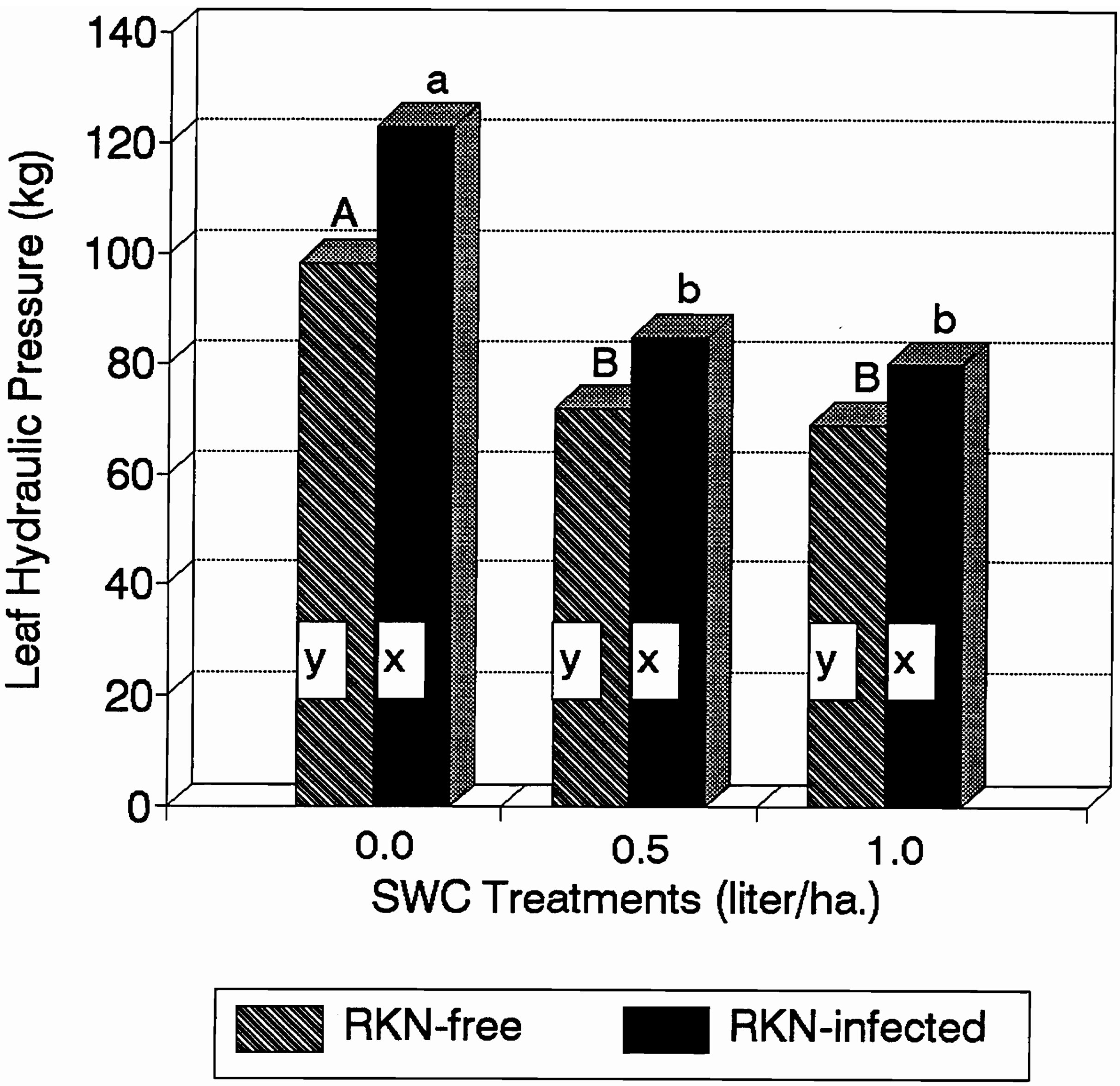

Figure 4-3. The effect of seaweed concentrate and RKN on leaf hydraulic pressure (LHP) of bentgrass, fifteen weeks after RKN inoculation. The letters A and B, and a and b differentiate the differences of turf quality ratings of the bentgrass treated with three SWC solutions under RKN-free and RKN-infection respectively. The letters of x and y indicate the difference between RKN-free and infected treatment under same SWC concentration. The means within each group indicated by the same letter are not significantly different at 0.05.

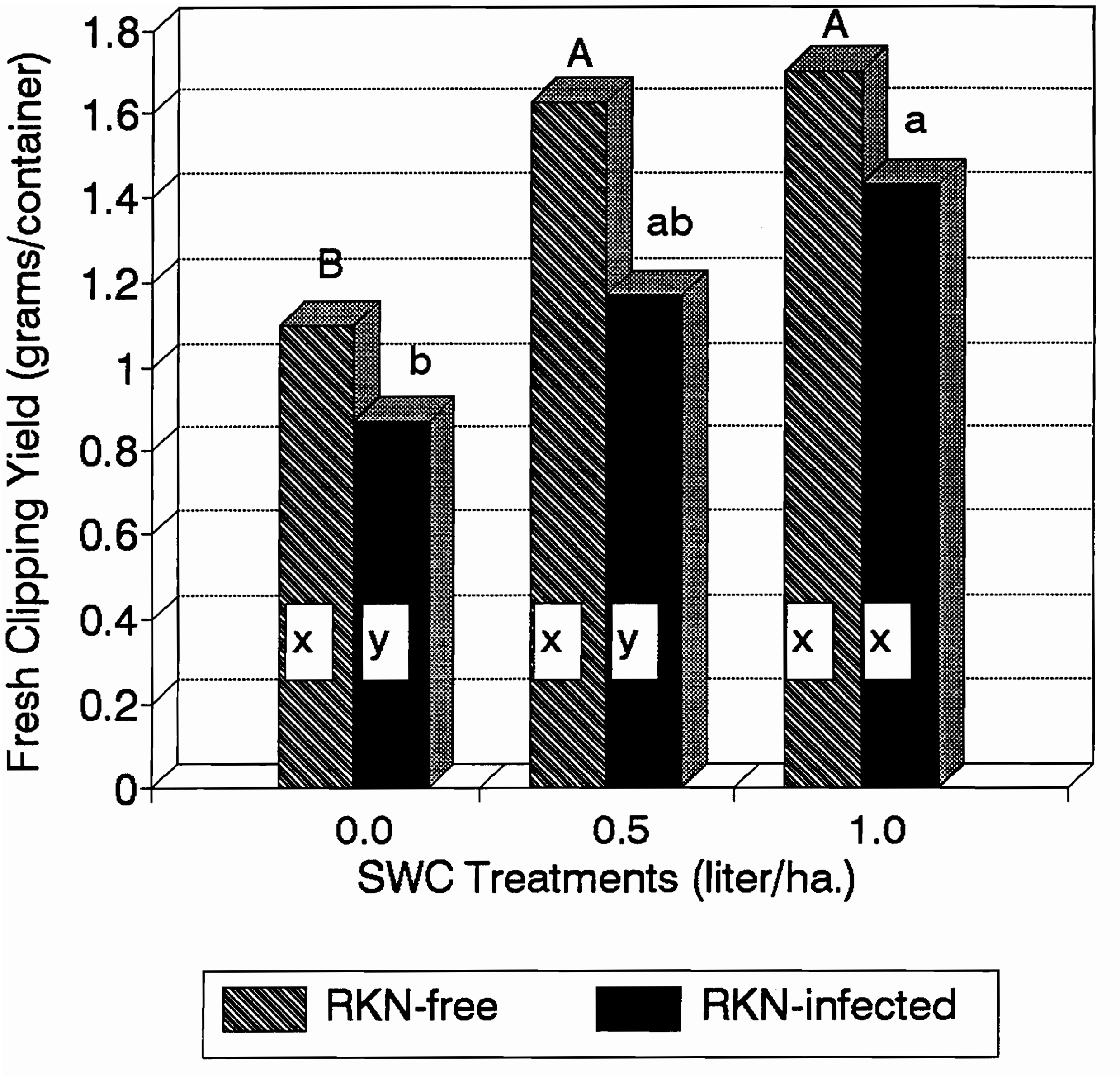

Figure 4-4. The effect of seaweed concentrate and RKN on fresh clipping yield of bentgrass, fifteen weeks after RKN inoculation. The letters A and B, and a and b differentiate the differences of turf quality ratings of the bentgrass treated with three SWC solutions under RKN-free and RKN-infection respectively. The letters of x and y indicate the difference between RKN-free and infected treatment under same SWC concentration. The means within each group indicated by the same letter are not significantly different at 0.05.

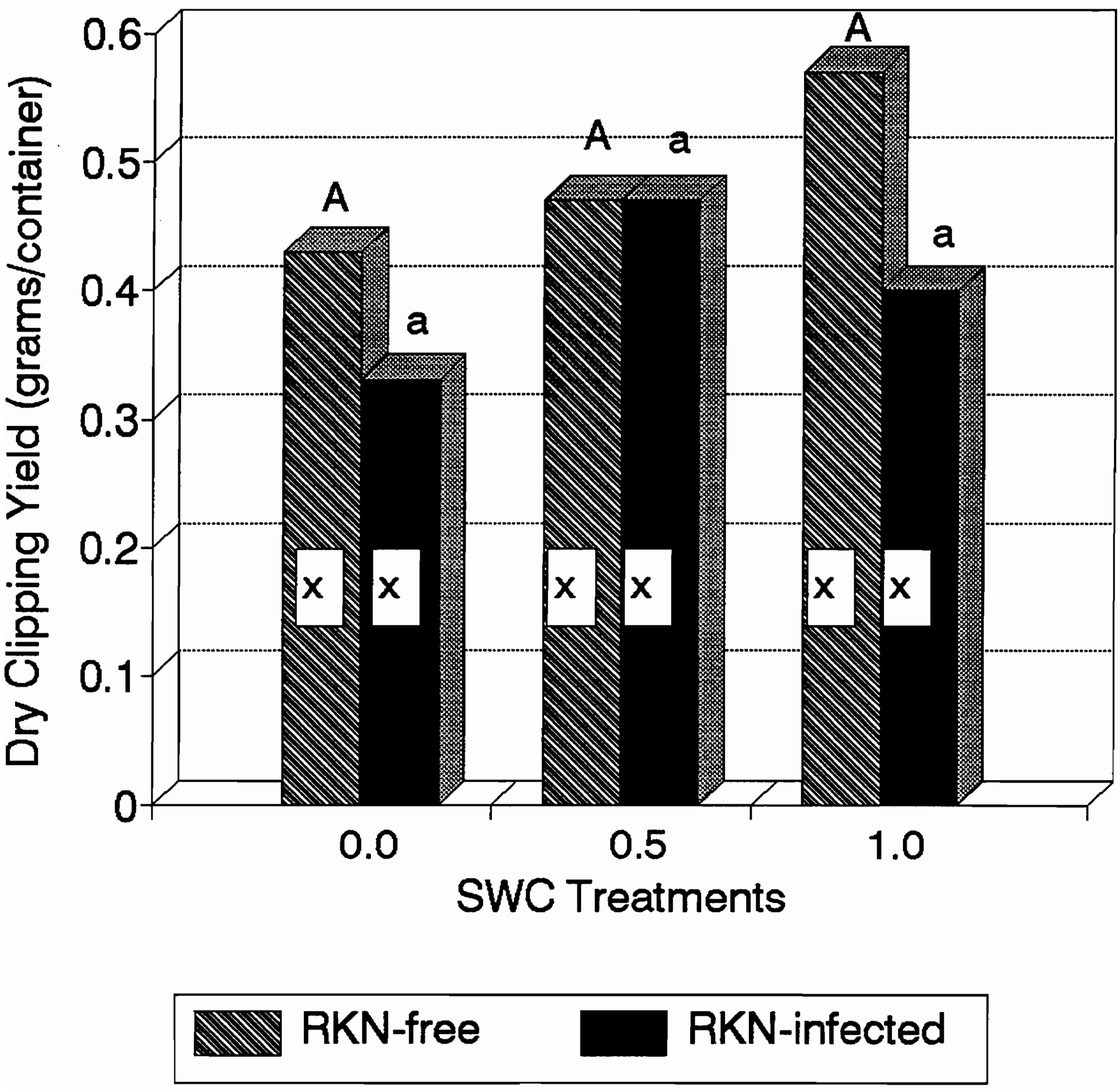

Figure 4-5. The effect of seaweed concentrate and RKN on dry clipping yield of bentgrass, fifteen weeks after RKN inoculation. The letters A and a differentiate the differences of turf quality ratings of the bentgrass treated with three SWC solutions under RKN-free and RKN-infection respectively. The letter of x indicates the difference between RKN-free and infected treatment under same SWC concentration. The means within each group indicated by the same letter are not significantly different at 0.05.

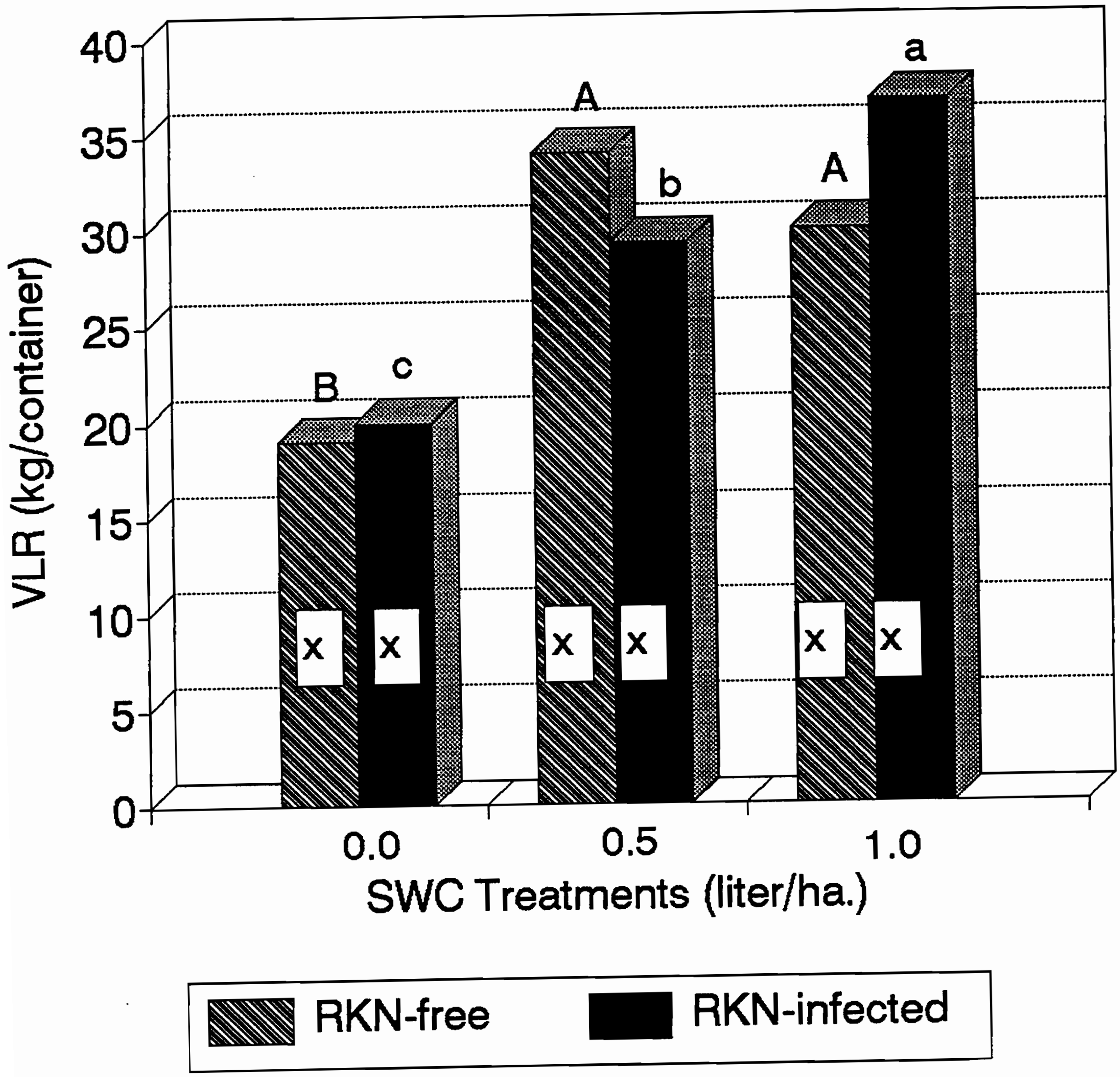

Figure 4-6. The effect of seaweed concentrate and RKN on the energy required for vertically lifting roots of bentgrass, seventeen weeks after RKN inoculation. The letters A and B, and a b and c differentiate the differences of turf quality ratings of the bentgrass treated with three SWC solutions under RKN-free and RKN-infection respectively. The letter of x indicates the difference between RKN-free and infected treatment under same SWC concentration. The means within each group indicated by the same letter are not significantly different at 0.05.

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CHAPTER 5

THE EFFECT OF SEAWEED CONCENTRATE ON THE GROWTH OF NEMATODE-FREE AND NEMATODE-INFECTED BENTGRASS PLANTS UNDER THREE WATERING REGIMES

INTRODUCTION

Bentgrass (Agrostis palustris Huds) is one of the most favorable hosts of plant-parasitic nematodes, considering it is not crop rotated and the type of management it normally receives. While it is difficult to estimate the economic losses in turfgrass caused by nematodes, 10% was thought to be an average loss for major agricultural crops (Dowler and Van Gundy, 1984). At times, an individual incident may be as high as 50% (Mai, 1985). In most parts of the world, huge amounts of money are spent to reduce the damages caused by plant-parasitic nematodes each year (Spears, 1964; LeClerq, 1964; Poinar, 1983).

Since nematodes often attack plant roots, nematode-infected turfgrass plants usually exhibit similar symptoms to those having damaged and/or malfunctioning root systems, such as wilting, yellow leaves, slow growth, and a gradual thinning of the turf (Heald and Perry, 1969). Therefore, nematode infections greatly reduce aesthetic and recreation value of turfgrass. Moreover, nematodes can damage turfgrass through the interactions with other pathogens, e.g. fungi (Jenkins et al., 1957; Powell, 1971), virus (Taylor and Brown, 1981; Lamberti and Roco, 1987), and abiotic factors (Chapter 3; Senn and Kingman, 1978). These interactions may result in severe damage to the host turfgrass plants.

In any turfgrass culture, nematode controls should be considered because almost all turf is infected by plant-parasitic nematodes. Most of the efficient nematicides however have been restricted in many locations due to their environmental and health hazards (Crouch, 1990). In fact, it is often impractical to use nematicides on large scale turfgrass management systems.

Exogenous application of seaweed concentrate (SWC) has been shown to improve growth and development of various agricultural and horticultural crops under either nematode-free or nematode infection, and to suppress the nematode populations (Crouch, 1990; Verkleij, 1992). The overall appearance of nematode-infected tomato plants was improved with SWC application (Featonby-Smith and Van Staden, 1983a; Crouch, 1990). Under nematode infection, roots, shoots and fruit weight of tomato were increased after SWC applications (Featonby-Smith and Van Staden, 1983a). Plant weight of citrus seedlings was also increased with both foliar and soil drench treatments with SWC (Tarjan, 1977).

In addition, application of SWC reduced nematode infection in maize (De Waele et al., 1988). If application of SWC improves the growth and development of nematode-infected turfgrass, and suppresses nematode populations, environmental impact from nematicides could be greatly reduced.

The objective of these studies was to ascertain the effect of SWC application on the growth of nematode-free and nematode-infected bentgrass under three irrigation regimes.

MATERIALS AND METHODS

These experiments were carried out under natural light and temperature from September 7, to October 23, 1992, under a clear plastic shelter at the Virginia Tech Turfgrass

Research Center, Blacksburg, VA. and in a greenhouse from October 24, 1992 to January 15, 1993.

A 2.5 cm thick mature creeping bentgrass (Agrostis palustris Huds, cv. Penncross) sod was placed on 19 liter cylindrical plastic containers (27 cm in diameter), each of which had been filled with methyl bromide-fumigated sandy loam soil and topped with an expanded metal screen. One week after transplanting, root-knot-nematodes (RKN, Meloidogyne naasi) extracted from greenhouse cultures (Hussey and Barker, 1973) were introduced into each container by injecting the inoculum solution 5 cm deep into five locations to provide two population levels, 0 and 5800 eggs per container. The sod was then treated with SWC (Aqua-10 Laboratories) at seven different seaweed concentrations as indicated in Table 5-1. Seaweed treatments S_0, S_1, S_2, S_3, and S_6 were applied only one week later after transplanting while S_4 and S_5 were applied at 10 day intervals, a total of 10 times. For the control and soil drench treatments (S_0 through S_5), the required SWC was diluted with the appropriate volume of water which was consistent with the water regime, then the solution was applied as a soil drench to each container. For the foliar treatment (S_6), the SWC was sprayed on the shoots on each container, after the required amount of water was irrigated.

Table 5-1. Description of the SWC treatments

SWC	SWC rate (liter/ha)	application method	frequency
S_0	0.0	soil drench	10 times
S_1	0.5	soil drench	once
S_2	1.0	soil drench	once
S_3	2.0	soil drench	once
S_4	0.5	soil drench	every 10 days,10 times
S_5	1.0	soil drench	every 10 days,10 times
S_6	1.0	foliar spray	once

*. Whenever SWC application was unscheduled, the same amount of water was used as soil drench.

From transplanting to November 1, 1992, nutrient levels and soil moisture in all the containers were maintained for maximum plant vigor by irrigating with one liter of water every other day and fertilizing with 58 kg N ha^{-1}, 25 kg P ha^{-1} and 48 kg K ha^{-1} every four weeks to each container after transplanting. From November 1 to the end of the experiment (January 15, 1993), the containers were arranged into six groups for six trials: trial 1: high water regime, one liter water $container^{-1}$ 5 $days^{-1}$, nematode free; trial 2: high water regime, one liter water $container^{-1}$ 5 $days^{-1}$, RKN infected; trial 3: medium water regime, one liter water $container^{-1}$ 10 $days^{-1}$, nematode free; trial 4: medium water regime, one liter water $container^{-1}$ 10 $days^{-1}$, RKN infected; trial 5: low water regime, one liter water $container^{-1}$ 15 $days^{-1}$, nematode free; trial 6: low water regime, one liter water $container^{-1}$ 15 $days^{-1}$, RKN infected. The height of the turf was maintained at 0.6 cm at seven day intervals, with a mower adapted for the containers.

At the end of the experiment, the following parameters were measured:

1) leaf moisture content: (a) expressed as leaf hydraulic pressure (LHP) (Cox and Hugher, 1982), (b) calculated from

fresh and dry clipping yield; 2) visual quality: 9 = best and 1 = worst; 3) fresh clipping yield; 4) dry clipping yield; 5) root mass: expressed as the energy required for vertically lifting roots from soil (VLR) (Schmidt, et al.,1986); 6) root length: expressed by measurement of the longest root. 7) nematode density determined as the number of RKN eggs per gram root and as per container by the Clorox extraction (Hussey and Barker. 1973). Root sample was collected from 5 to 15 cm below soil surface in each container for RKN extraction. Total number of RKN eggs in each container was determined by multiplying eggs/g. fresh root and VLR.

Randomized complete block experimental design was used for each trial, with 7 treatments (SWC) and 3 replications. Analyses of variance (ANOVA) were applied to the data of each trial, and means were separated by Duncan's multiple range test at 5% probability when ANOVA indicated significant treatment effects.

RESULTS

Rooting Parameters: Application of SWC nonsignificantly increased VLR of RKN-free plants under high water regime

(Table 5-2). Under the same water regime, however, RKN infected plants treated with frequent-soil-drench SWC (S_4 and S_5) and high-dosage-single application of SWC (S_3) possessed significantly larger VLR than the control. Under the other two water regimes (medium and low), SWC application significantly increased VLR of RKN-free and RKN-infected plants, i.e. treatments S_5 and S_6 significantly increased VLR of RKN free plants grown under medium and low water regimes, while RKN-infected plants treated with S_2 and S_6, and S_4, S_5 and S_6 generated significantly heavier root system under medium and low water regime, respectively.

Application of the SWC treatments enhanced root elongation at various degree (Table 5-3). Compared with the control (S_0), SWC application increased the root length of RKN-infected plants under high and low water regime. Under the high water regime, S_4 and S_5 significantly increased root length. However, only S_5 elongated the roots of RKN-infected and noninfected plants under the low water regime. Under the medium water regime no significant difference was detected in root length regardless of RKN-free or infected plants.

Leaf Moisture Parameters: Application of SWC significantly decreased LHP of both RKN-free and RKN-infected plants under each water regime (Table 5-4). Treatments S_4 and S_5 provided significantly better leaf moisture (Lower LHP) regardless of water regime or nematode infection. The S_2 and S_3 also effectively decreased LHP of RKN-infected bentgrass under various conditions. At the lower dosages (S_1 and S_6), leaf moisture enhancement was variable.

Wilting was evident only in the plants subjected to the medium and the low irrigation (Table 5-5). All SWC treatments significantly alleviated wilting symptoms of bentgrass grown under medium irrigation, except for the low dosage foliar SWC treatment (S_6). The 0.5 liter SWC per ha treatment (S_1) was the only treatment that did not reduce wilting symptoms of RKN-free plants under low water regime. Under the same water regime, only the 0.5 liter SWC per ha frequently applied SWC treatment (S_4) significantly reduced wilting of the bentgrass grown in RKN infected soil.

Leaf water content was affected relatively little by SWC applications (Table 5-6). Under high and medium irrigation, SWC-treated plants did not have a higher leaf moisture content than the non-treated bentgrass. The water content of RKN-free plants grown under low irrigation, however, was significantly increased in S_4, S_5 and S_6. Under the same irrigation, the leaf water content of RKN-infected plants was not different significantly.

Quality Parameters: Applications of all the soil-drench SWC treatments improved the turf quality of RKN-free bentgrass under high irrigation (Table 5-7). Under identical irrigation, all the SWC treatments significantly increased the quality of RKN-infected bentgrass except for S_1. Applications of one liter SWC per hectare or higher caused substantial enhancement in turfgrass quality regardless of RKN-infection. Bentgrass that received least water was improved in quality , compared with the control in the water regimes, only when SWC was applied at 0.5 or 1.0 liter per ha. every 10 days (S_4 and S_5).

Under each irrigation regime, leaf color of RKN-infected bentgrass treated with SWC was more acceptable than their non-treated counterpart (Table 5-8). Compared with the control, significant improvements in leaf color of RKN-infected plants were detected in the plants treated with S_1, S_3, S_4 and S_5 under high water regime, S_4 and S_5 under medium regime water, and S_2, S_3, S_4 and S_5 under low water regime. Application of SWC caused less improvements in leaf color on RKN-free plants than in RKN-infected plants. Only under medium irrigation regime, the effect of SWC on leaf color of RKN-free plants was significant (Table 5-8). The S_2, S_3, S_4 and S_5 treatments significantly improved turf color.

Clipping yield: Seaweed application, however, did not significantly increase fresh clipping of RKN-free plants regardless of water regime (Table 5-9), except that treatment S_5 significantly increased fresh clipping under medium water regime with or without RKN. The application of S_4 on turfgrass grown under all water regimes caused a significant increase in clipping yield of RKN-infected bentgrass. Under high and medium water regime, fresh clippings from RKN-infected bentgrass increased significantly in the S_2, S_4, S_5 and S_6 applications. On the average, high-concentrate SWC treatment (S_3) and high frequency SWC treatments (S_4 and S_5) were most effective in increasing dry clipping yield than low concentrate and single treatments (S_1, S_2 and S_6). Only the bentgrass that was irrigated least frequently and drenched with the highest dosage of SWC (S_5) resulted in a significant increase in dry clipping weight regardless of RKN-infection (Table 5-10). In addition, application of S_2 significantly increased the dry clipping yield of RKN-infected bentgrass grown under low water regime.

DISCUSSION

Root growth of RKN-free bentgrass plants was enhanced with SWC application under various moisture conditions (Table 5-2 and 5-3). High concentrate and frequent SWC drench application (S_5) was the most effective SWC treatment in improving root mass (VLR) and root length. The effect of SWC on root growth has been well documented (Nelson and Van Staden, 1984a; Crouch, 1990).

Although seaweed products contain all of the required elements needed for plant growth (Stephenson, 1968), addition of these elements are not adequate to explain the effects of SWC on plant growth (Blunden 1977). The occurrence of cytokinin-like substances in commercial seaweed extracts have been reported by many people (Blunden and Wildgoose, 1979; Featonby-Smith and Van Staden, 1983b and 1984; Finnie and Van Staden 1985). Plants response similarly to SWC and cytokinin treatments (Finnie and Van Staden, 1985), but not the same (Nelson and Van Staden, 1985). Plant growth regulatory activity of seaweeds may be responsible for SWC effect on root growth improvement (Finnie and Van Staden, 1985), and may be related to increased nutrient uptake (Senn and Kingman, 1978).

It is believed that considerable quantities of phenolic compounds in seaweed (Booth, 1969), stimulate root development and exhibit growth promoter properties (Jackson, 1965; Poapst and Dunkee, 1967). Root extension and lateral root development were found to be enhanced with SWC applications (Finne and Van Staten, 1985). In addition, some very strong polar molecules, formed in the process of seaweed degradation, may improve soil structure, which was more suitable for root growth and function. Application of SWC can also increase root nutrient uptake (Francki, 1960; Aitken and Senn, 1965) and the availability of nitrogen (Beckett and Van Staden, 1989), phosphorus (Booth, 1966), potassium (Booth, 1966), calcium (Senn and Kingman, 1978), manganese (Blunden, 1972), magnesium (Senn and Kingman, 1978), iron (Booth, 1966) and zinc (Becket and Van Staden, 1990a) in soil. The enhanced root growth and functions could be the primary reason for the improvements in other parameters detected in this study; higher quality, more acceptable color, higher clipping yield and better leaf moisture status. Exogenous hormones in seaweed may also play an important role in the improvements in these parameters.

Under RKN infestation, SWC applications were effective in improving the growth and quality parameters of bentgrass plants measured in this study. Application of SWC was more effective, in this regard, on RKN-infected turfgrass than on RKN-free grass. The main explanation for this is that the damages caused by RKN infection and moisture stress on most of these parameters were additive (Chapter 3), and created a more severe stress. The effect of SWC application is more obvious when plants are under a severe stress.

Numerous studies have shown SWC to beneficially affect the growth of nematode-infected plants, and to suppress development of nematodes (Stephenson, 1968; Tarjan, 1977; Tarjan & Frederich, 1983). Under nematode infection, Featonby-Smith and Van Staden (1983a) reported that the overall appearance of tomato plants treated with SWC was better than the untreated control. Both fresh and dry mass of root, shoot and fruits were increased in their experiment. In particular, they found that the number of RKN inside the roots was reduced, although the number in the soil was increased after SWC application compared to the controls.

In a study conducted to test the influence of SWC application on the production of <u>Pratylenchus</u> <u>zeae</u> (Nematoda) on maize, DeWaele et al.(1988) concluded that all SWC treatments suppressed the reproduction of <u>P. zeae</u> by 47-63% compared with the untreated control, but plant growth was not significantly stimulated by any of the SWC treatments.

However, in the greenhouse experiment, neither the reproduction of P. zeae or plant growth was affected by any SWC treatment.

An aspect worth considering is why SWC application has the ability to make plants more tolerant to nematode damage. The cytokinin content in SWC, was thought to be responsible for this. High concentrations of kinetin were shown not only to decrease nematode juvenile penetration, but also inhibit the development of nematodes which did enter roots (Dropkin et al, 1969).

In other reports, however, it is believed that giant cells and galls usually develop on growth regulator-treated plants (Hussey, 1985). Cytokinin levels may increase in root-knot nematode infected plants (Owens and Bottio, 1966), and have been reported to be higher in noninfected root-knot susceptible plants than in resistant plants (Van Staden and Dimalla, 1977). Cytokinin exogenously supplied to nematode resistant plants, reverse the resistance response and made plants susceptible to nematode infection.

What causes this contradiction is unclear. RKN species penetrate and damage root tips, which are sources of cytokinin, therefore, RKN infection may interrupt the synthesis of cytokinin by temporarily inhibiting root growth. Therefore, it may be predicted that cytokinin concentration decreases in host plants after nematode invasion.

Although the effect of cytokinin in SWC on nematode development is unclear, the reasons for SWC to improve plant growth under nematode infection can be hypothesized as follows: (a) the decreased nutrient absorption and translocation caused by nematode infection may be compensated with increases in root mass induced with SWC application; (b) retarded root growth by nematode infection may also be compensated with the stimulatory effect of SWC; (c) SWC cytokinins may suppress the growth and development of nematodes (Dropkin et al., 1969).

Table 5-2. Effect of SWC on the energy required for vertically lifting roots of RKN-free or RKN-infected bentgrass grown under different watering regimes.

	Irrigation Regime					
	High		Medium		Low	
SWC[a] (l/ha)	RKN -[b]	RKN +	RKN -	RKN +	RKN -	RKN +
S0 (0.0)	[c]20.0a	17.3c	22.2c	20.0b	16.0b	16.0c
S1 (0.5)	23.5a[d]	21.0abc	21.8c	24.0ab	22.5ab	19.0bc
S2 (1.0)	26.3a	21.0abc	22.0bc	26.3a	20.3b	16.3c
S3 (2.0)	24.0a	23.3ab	25.0abc	24.0ab	22.3ab	19.0bc
S4 (0.5)	24.5a	25.3a	24.0bc	24.5ab	19.8b	25.8a
S5 (1.0)	25.3a	24.3a	30.0ab	25.3ab	24.0a	25.0a
S6 (1.0)	20.5a	18.8bc	31.8a	20.5b	24.0a	22.5ab

[a]: S0, S4 and S5 were applied 10 times, while rest of the SWC treatments were applied once. All the SWC treatments were soil drenched except for S6 which was foliarly sprayed.
[b]: RKN-: RKN free; RKN+: initially inoculated with 5800 RKN eggs.
[c]: The data were collected 17 weeks after RKN inoculation.
[d]: The letters within each column differentiate the effects of the SWC treatments under same water regime and same RKN inoculation. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 5-3. Effect of SWC on root length (cm) of RKN-free or RKN-infected bentgrass grown under different watering regimes.

	Irrigation Regime					
	High		Medium		Low	
SWC[a] (l/ha)	RKN -[b]	RKN +	RKN -	RKN +	RKN -	RKN +
S0 (0.0)	[c]21.3a	20.0b	22.8a	20.8a	23.5bc	17.8b
S1 (0.5)	27.3a[d]	25.0ab	24.5a	21.0a	23.8bc	23.0ab
S2 (1.0)	25.0a	26.0ab	30.3a	21.8a	20.0c	18.5ab
S3 (2.0)	31.5a	24.0ab	28.3a	19.0a	22.8bc	18.3b
S4 (0.5)	27.5a	30.0a	27.5a	20.8a	23.5bc	23.0ab
S5 (1.0)	29.5a	30.0a	34.8a	20.3a	30.8a	25.3a
S6 (1.0)	25.8a	22.0b	30.0a	20.5a	24.0bc	19.0ab

[a]: S0, S4 and S5 were applied 10 times, while rest of the SWC treatments were applied once. All the SWC treatments were soil drenched except for S6 which was foliarly sprayed.
[b]: RKN-: RKN free; RKN+: initially inoculated with 5800 RKN eggs.
[c]: The data were collected 17 weeks after RKN inoculation.
[d]: The letters within each column differentiate the effects of the SWC treatments under same water regime and same RKN inoculation. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 5-4. Effect of SWC on leaf hydraulic pressure (kg) of RKN-free or RKN-infected bentgrass grown under different watering regimes.

	Irrigation Regime					
	High		Medium		Low	
SWC[a] (l/ha)	RKN -[b]	RKN +	RKN -	RKN +	RKN -	RKN +
S0 (0.0)	[c]99a	109a	124a	138a	130a	137a
S1 (0.5)	89ab[d]	93b	125a	129ab	111bc	124ab
S2 (1.0)	81bc	88bc	113ab	111bcd	114ab	119bc
S3 (2.0)	80bc	84cd	113ab	106cd	110bc	112bc
S4 (0.5)	76cd	77de	98b	109bcd	110bc	107c
S5 (1.0)	65d	73e	98b	98d	94c	105c
S6 (1.0)	95a	86bc	115ab	120abc	99bc	111bc

[a]: S0, S4 and S5 were applied 10 times, while rest of the SWC treatments were applied once. All the SWC treatments were soil drenched except for S6 which was foliarly sprayed.
[b]: RKN-: RKN free; RKN+: initially inoculated with 5800 RKN eggs.
[c]: The data were collected 17 weeks after RKN inoculation.
[d]: The letters within each column differentiate the effects of the SWC treatments under same water regime and same RKN inoculation. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 5-5. Effect of SWC on wilting (1-9 with 9=least wilting) of RKN-free or RKN-infected bentgrass grown under different watering regimes.

	Irrigation Regime					
	High		Medium		Low	
SWC[a] (l/ha)	RKN -[b]	RKN +	RKN -	RKN +	RKN -	RKN +
S0 (0.0)	[c]9.0a	9.0a	2.8b	1.5b	2.2c	3.5b
S1 (0.5)	9.0a[d]	9.0a	5.8a	5.0a	3.8bc	2.5b
S2 (1.0)	9.0a	9.0a	6.5a	4.8a	6.3a	4.5b
S3 (2.0)	9.0a	9.0a	6.8a	5.8a	7.0a	4.8ab
S4 (0.5)	9.0a	9.0a	6.8a	5.0a	6.8a	5.3a
S5 (1.0)	9.0a	9.0a	6.5a	6.5a	6.5a	4.8ab
S6 (1.0)	9.0a	9.0a	1.0b	1.5b	5.3ab	5.0ab

[a]: S0, S4 and S5 were applied 10 times, while rest of the SWC treatments were applied once. All the SWC treatments were soil drenched except for S6 which was foliarly sprayed.
[b]: RKN-: RKN free; RKN+: initially inoculated with 5800 RKN eggs.
[c]: The data were collected 17 weeks after RKN inoculation.
[d]: The letters within each column differentiate the effects of the SWC treatments under same water regime and same RKN inoculation. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 5-6. Effect of SWC on leaf water content (%) of RKN-free or RKN-infected bentgrass grown under different watering regimes.

	Irrigation Regime					
	High		Medium		Low	
SWC[a] (l/ha)	[b]RKN -	RKN +	RKN -	RKN +	RKN -	RKN +
S0 (0.0)	[c]82a	74a	73a	62a	69b	65a
S1 (0.5)	78a[d]	73a	75a	64a	69b	78a
S2 (1.0)	75a	77a	68a	73a	75ab	68a
S3 (2.0)	77a	75a	73a	64a	76ab	68a
S4 (0.5)	76a	70a	74a	75a	80a	79a
S5 (1.0)	78a	79a	76a	79a	84a	75a
S6 (1.0)	74a	74a	70a	72a	76a	74a

[a]: S0, S4 and S5 were applied 10 times, while rest of the SWC treatments were applied once. All the SWC treatments were soil drenched except for S6 which was foliarly sprayed.
[b]: RKN-: RKN free; RKN+: initially inoculated with 5800 RKN eggs.
[c]: The data were collected 17 weeks after RKN inoculation.
[d]: The letters within each column differentiate the effects of the SWC treatments under same water regime and same RKN inoculation. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 5-7. Effect of SWC on Quality (1-9 with 9=best) of RKN-free or RKN-infected bentgrass grown under different watering regimes.

	Irrigation Regime					
	High		Medium		Low	
SWC[a] (l/ha)	[b]RKN -	RKN +	RKN -	RKN +	RKN -	RKN +
S0 (0.0)	[c]6.5d	3.5d	4.8ab	2.7b	3.75b	5.0ab
S1 (0.5)	7.3bc[d]	4.8cd	4.3ab	5.0ab	3.75b	2.75b
S2 (1.0)	7.3bc	5.3bc	4.8ab	4.7ab	4.0b	3.5ab
S3 (2.0)	7.3bc	6.5ab	5.8ab	3.5ab	4.3ab	4.5ab
S4 (0.5)	7.8ab	5.3bc	6.0a	5.2ab	5.0ab	5.8a
S5 (1.0)	8.0a	6.8a	3.0bc	6.0a	6.5a	5.5a
S6 (1.0)	7.0cd	6.0abc	4.0ab	2.2b	5.3ab	4.0ab

[a]: S0, S4 and S5 were applied 10 times, while rest of the SWC treatments were applied once. All the SWC treatments were soil drenched except for S6 which was foliarly sprayed.
[b]: RKN-: RKN free; RKN+: initially inoculated with 5800 RKN eggs.
[c]: The data were collected 17 weeks after RKN inoculation.
[d]: The letters within each column differentiate the effects of the SWC treatments under same water regime and same RKN inoculation. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 5-8. Effect of SWC on leaf color (1-9 with 9=darkest green) of RKN-free or RKN-infected bentgrass grown under different watering regimes.

SWC[a] (l/ha)	Irrigation Regime: High [b]RKN -	High RKN +	Medium RKN -	Medium RKN +	Low RKN -	Low RKN +
S0 (0.0)	[c]6.3a	4.5c	2.3c	2.5b	4.8a	2.5c
S1 (0.5)	6.3a[d]	6.3ab	4.5abc	4.5ab	5.3a	3.0c
S2 (1.0)	6.8a	4.8bc	5.0ab	4.3ab	6.5a	5.0ab
S3 (2.0)	7.5a	6.3ab	6.0ab	5.3ab	6.3a	6.0a
S4 (0.5)	7.5a	6.8a	5.8ab	5.5a	6.0a	5.0ab
S5 (1.0)	7.5a	6.5a	6.3a	6.3a	5.5a	5.8a
S6 (1.0)	6.3a	6.0abc	3.8bc	3.5ab	5.5a	3.5bc

[a]: S0, S4 and S5 were applied 10 times, while rest of the SWC treatments were applied once. All the SWC treatments were soil drenched except for S6 which was foliarly sprayed.
[b]: RKN-: RKN free; RKN+: initially inoculated with 5800 RKN eggs.
[c]: The data were collected 17 weeks after RKN inoculation.
[d]: The letters within each column differentiate the effects of the SWC treatments under same water regime and same RKN inoculation. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 5-9. Effect of SWC on fresh clipping yield (g) of RKN-free or RKN-infected bentgrass grown under different watering regimes.

	Irrigation Regime					
	High		Medium		Low	
SWC[a] (l/ha)[b]	RKN -	RKN +	RKN -	RKN +	RKN -	RKN +
S0 (0.0)	[c]3.2a	1.0c	1.3b	1.0d	1.75a	1.0b
S1 (0.5)	3.3a[d]	2.1ab	1.5ab	1.5cd	1.23a	0.98b
S2 (1.0)	3.3a	2.7a	1.6ab	3.0ab	1.30a	1.5ab
S3 (2.0)	3.5a	1.8bc	1.7ab	1.7bcd	1.53a	1.2ab
S4 (0.5)	2.7a	2.2ab	1.7ab	2.5abc	1.60a	1.63a
S5 (1.0)	3.3a	2.8a	2.4a	3.3a	1.38a	1.7ab
S6 (1.0)	3.0a	2.6ab	1.2b	3.0ab	1.78a	1.5ab

[a]: S0, S4 and S5 were applied 10 times, while rest of the treatments were applied once. All the SWC treatments were drenched except for S6 which was foliarly sprayed.
[b]: RKN-: RKN free; RKN+: initially inoculated with 5800 eggs.
[c]: The data were collected 17 weeks after RKN inoculation.17
[d]: The letters within each column differentiate the effects the SWC treatments under same water regime and same RKN inoculation. Within each column, means followed by the letter were not significantly different at 0.05 probability.

Table 5-10. Effect of SWC on dry clipping (g) of RKN-free or RKN-infected bentgrass grown under different watering regimes.

	Irrigation Regime					
	High		Medium		Low	
SWC[a] (l/ha)	RKN -[b]	RKN +	RKN -	RKN +	RKN -	RKN +
S0 (0.0)	[c]0.55a	0.63a	0.35a	0.33a	0.23b	0.33b
S1 (0.5)	0.70a[d]	0.55a	0.35a	0.55a	0.38ab	0.23b
S2 (1.0)	0.83a	0.63a	0.48a	0.80a	0.33ab	0.45a
S3 (2.0)	0.78a	0.45a	0.50a	0.58a	0.35ab	0.35ab
S4 (0.5)	0.68a	0.63a	0.38a	0.68a	0.33ab	0.35ab
S5 (1.0)	0.68a	0.60a	0.55a	0.70a	0.55a	0.40a
S6 (1.0)	0.75a	0.65a	0.35a	0.80a	0.28ab	0.38ab

[a]: S0, S4 and S5 were applied 10 times, while rest of the SWC treatments were applied once. All the SWC treatments were soil drenched except for S6 which was foliarly sprayed.
[b]: RKN-: RKN free; RKN+: initially inoculated with 5800 RKN eggs.
[c]: The data were collected 17 weeks after RKN inoculation.
[d]: The letters within each column differentiate the effects of the SWC treatments under same water regime and same RKN inoculation. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

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CHAPTER 6

THE EFFECT OF SEAWEED CONCENTRATE ON THE GROWTH OF NEMATODE-INFESTED BENTGRASS PLANTS UNDER DROUGHT OR SALINITY CONDITION

INTRODUCTION

Since 1743 when the wheat gall nematode was first detected, nematodes have been recognized as plant pathogens. Nematodes can be found all over the world. They attack plants by sucking host cell contents, dissolving intercellular cement, breaking cell walls, suppressing host cell division, inducing cell proliferation and gall formation, digesting plant tissues (Christie, 1960), and changing hormone balance inside host plants (Hussey, 1985). Nematode infections may also make the host plants more susceptible to the damages caused by other pathogens, such as fungi (Powell, 1971; Bergeson, 1972), bacteria (Pitcher, 1963), and viruses (Taylor and Brown, 1981), and abiotic stresses (Heald and Heilman, 1971; Maggenti and Hardan, 1973).

Nematode injury results in plant stunting, nutrition deficiencies, and uneven or patchy growth in fields. Specific symptoms vary with host plant species and nematode species. However, root gall formation is a typical symptom with root-knot-nematode (Meloidogyne) infection. Above-ground symptoms shown by nematode infected plants are similar to those produced on the plants having a damaged and/or malfunctioning root system.

The limited nematode research on turfgrass systems has concentrated on the associations between nematode species and turfgrass species (Safford and Riedel, 1976, Tashiro and Murdoch, 1977, Murdoch, Tashiro and Harrison, 1978). At least 16 nematode species have been associated with turfgrass (Turgeon, 1980 and Heald and Perry, 1969). Although we know very little about the relationship between nematodes and their turfgrass hosts, nematode damage is a greater problem in turfgrass than in agricultural crops, because it is not rotated or cultivated as most other crops. Wilting, yellow leaves, slow growth and a gradual thinning are most common symptoms of nematode infection in turfgrass (Heald and Perry, 1969). In previous studies concerning nematode infection on bentgrass (Chapter 3 and 4), it was shown that the affected parameters include quality, leaf moisture, clipping yield, root length and root mass. In addition, an additive effect between nematode infection and drought or salinity stress on bentgrass plants was detected (Chapter 3). Therefore, control of nematode infection on turfgrass reduces some of the stresses caused by drought or salinity.

Procedures used to control nematode infection of turfgrass include physical, chemical and biological methods, as well as host-plant resistance and cultural practices. Regardless of the procedures, it is difficult to control nematodes in turfgrass systems. Physical methods (e.g. treating nematode vectors with steam or hot water) are expensive and can not be used extensively; biological methods have not been developed sufficiently to be effective; host-plant resistances are available in some plant species, but not in all turfgrass species. Cultural practices can be conducted extensively but they are not very effective (White and Dickens, 1984). On the other hand, chemical methods are relatively effective, but, most cause environmental pollution and hazards to humans (Crouch, 1990). In fact, some chemical nematicides have already been banned because of their negative ecological impacts.

The development of a natural bio-degradable product that reduces nematode damage to plants would be of both economic and environmental significance. Seaweed concentrate (SWC) has been known to improve growth and development of plants, and to enhance plant resistances to their pathogens (Crouch, 1990). Besides, many beneficial responses were observed on the bentgrass plants treated with SWC (Chapter 4 and 5).

The objective of this study was to ascertain the effect of seaweed concentrate on the growth of nematode-infested bentgrass plants, and on the reproduction of nematodes under salinity or drought stress conditions.

MATERIALS AND METHODS

This experiment was carried out under greenhouse conditions with a daily average temperature range from 22 °C to 40 °C, at Virginia Tech, Blacksburg, Virginia, from January 29 to June 18, 1993.

Plant Materials: A 2.5 cm thick mature sod of bentgrass (Agrostis palustris Huds, cv. Penncross) was placed on each of nineteen liter cylindrical plastic containers (27 cm in diameter), which had been filled with methyl bromide-fumigated sandy loam soil (pH=6.8) and topped with an expanded metal screen. Nutrient levels and soil moisture were maintained for maximum plant vigor by fertilizing with 58 kg N ha^{-1}, 25 kg P

ha^{-1} and 48 kg K ha^{-1} each month and watering one liter of tap water every other day. The height of the turf was maintained at 0.6 cm at seven day intervals, with a mower adapted for microplots.

Inoculum: Two nematode genera, root-knot (Meloidogyne naasi) and lance (Hoplolaimus galeatus) nematodes were involved in this study. Root-knot nematode (RKN) were obtained from Dr. Eisenback (Nematologist, Virginia Tech). Lance nematodes were originally extracted from a bentgrass sample collected from a golf course in south eastern Pennsylvania. Pure inoculum from the two nematode genera, RKN and Lance, were increased on barley and bentgrass plants, respectively. Soils in the containers used in this study were inoculated as follows: 10 ml tap water, 10 ml lance solution(7800 juveniles), and 10 ml RKN inoculum solution (7080 eggs). The inoculum was introduced by injecting the solutions 2.5 cm deep in 5 locations before placing the expanded metal pieces on the soil surface.

SWC Treatments: One week after transplanting, sod was treated with SWC (Aqua-10 Laboratory) at six concentrations (S_0, S_1, S_2, S_3, F_1 and F_2) as indicated in Table 6-1. An appropriate amount of SWC was diluted to one liter with water for the soil drench treatments, while the SWC was sprayed on the shoots. In each application, the same amount of water was used as a soil drench for the untreated and prior to foliar treatments.

Table 6-1. SWC treatments applied to the bentgrass inoculated with RKN or Lance nematodes

SWC treatment	SWC Rate (liter/ha)	application procedure	frequency	number of applicat.
S_0	0.0	soil drench	twice a month	10 times
S_1	2.0	soil drench	once a month	5 times
S_2	2.0	soil drench	twice a month	10 times
S_3	4.0	soil drench	twice a month	10 times
F_1	2.0	foliar spray	once a month	5 times
F_2	2.0	foliar spray	twice a month	10 times

*: When scheduled irrigation coincided with SWC treatment schedule, the seaweed was applied with the irrigation water

Stress Treatment: Plants were all well watered with tap water uniformly to maintain maximum vigor from transplanting to April 15. On April 15, sufficient water was added to each container gravimetrically to bring the soil to field capacity (13%). The containers were arranged into four groups, with each for one trial: trial 1: drought stress and lance nematode infection; trial 2: drought stress and RKN infection; trial 3: salinity stress and lance nematode infection; trial 4: salinity stress and RKN infection. For the drought stress treatment, water was added to field capacity in 14 day intervals. On April 20, each container in the salt stress treatment started to receive one liter of 0.2% NaCl solution every other day until the end of the experiment. Water content in each container was balanced by weight with tap water at 14-day intervals.

Data Collection: At the end of the last two water-wilting cycles, the following parameters were measured: 1) leaf moisture content: (a) measured as leaf hydraulic pressure (LHP) (Cox and Hugher, 1982), (b) calculated from fresh and dry clipping yield; 2) visual wilting rating (9 = no wilting and 1 = most severe wilting); 3) visual quality: 9 = best and 1 = worst; 4) leaf color with 1-9=darkest green; 5) shoot density with 1-9=most; 6) clipping yield (fresh weight); 7) clipping yield (dry weight); 8) root mass: estimated by the energy required for vertically lifting roots from soil (VLR) (Schmidt et al., 1986); 9) nematode concentration: equal amount of soil sample (Lance) or root sample (RKN) was taken from each container. Lance nematodes were extracted with the sugar flotation procedure (Jenkins, 1964) while RKN were with Clorox methods (Hussey and Barker, 1973). Nematode concentration was expressed as the number of adult nematodes in 10 grams of soil (for the Lance) or the eggs in one gram of fresh roots for the RKN.

Each trial was designed as a randomized complete block design, with treatment = 6 (SWC treatments) and replications = 3. Analyses of variance (ANOVA) were applied to the data of each trial, and means were separated by Duncan's multiple range test at 5% probability when ANOVA indicated significant treatment effect.

RESULTS AND DISCUSSION

Overall Appearance

Under salinity and drought stress conditions, Lance or RKN infected-bentgrass exhibited a series of symptoms which included yellow leaves, thinning, lower clipping yield, and poor turf quality. However, with SWC applications, almost all of the symptoms were overcome to some degree (Figure 6-1 and 6-2). It appeared that the soil drench SWC treatments (S_1 and S_2) improved bentgrass more than foliar treatments (F_1 and F_2). It was previously reported that the overall appearance of nematode-infected tomato plants was improved by SWC application (Featonby-Smith and Van Staden, 1983). The improvement could be attributed to SWC's beneficial effect on plant functions, such as rooting (Booth, 1969; Bucket and Van Staden, 1989), and to the SWC suppression of nematode reproduction (Stephenson, 1968; Tarjan, 1977; Tarjan and Frederich, 1983; Featonby-Smith and Van Staden, 1983; De Waele et al., 1988)

Rooting Parameters

Bentgrass VLR was improved to various degrees with all SWC treatments regardless of nematode infection or drought or salt stress (Table 6-2). Under drought stress, the effect of SWC on VLR of lance infected bentgrass plants was not significant ($p=0.05$), although VLR was increased by 50% with soil drench SWC applications (Table 6-2). However, S_1 and S_2 treatments significantly increased VLR of RKN infected plants grown under drought stress. One possible explanation could be that the amount of SWC in the S_3 treatment was too high causing the associated concentration of cytokinin to stimulate the top growth and to inhibit the root growth. In the salt stress treatments, SWC treatments did not cause any significant difference in VLR of RKN-infected plants. In contrast, the S_3 treatment induced significantly higher VLR of the bentgrass infected with Lance nematode (Table 6-2). The foliar SWC treatments (F_1 and F_2) caused a nonsignificant increase in VLR regardless of nematode infection or stress treatments.

Stimulatory effects of SWC on root systems were well documented (Featonby-Smith and Van Staden, 1983; Nelson and Van Staden,1984). It was found that both root extension and lateral root development were enhanced by a suitable concentration of SWC (Finne and Van Staden,1985). Besides, SWC may improve soil structure, which further improves the suitability for root growth (Crouch, 1990).

Leaf Water Status

Three leaf water parameters, leaf-water content, LHP and leaf wilting were measured in this study (Table 6-3, 6-4 and 6-5). Under drought stress, the leaf water content of Lance-infected bentgrass was increased by all SWC treatments except for the low-concentrate foliar treatment (F_1), while RKN-infected plants showed little influence (Table 6-3). Applications of SWC on lance or RKN-infected plants did not cause significant leaf water content change under salinity condition.

Only S_3 treatment caused a significant decrease in LHP under drought and Lance infected condition (Table 6-4). However, all SWC treatments significantly improved leaf-water-status (lower LHP) of the plants grown under drought and RKN infected condition. Once again, SWC applications did not affect LHP significantly under salinity condition, possible because salinity treatments were not strong enough.

Most the SWC treatments significantly reduced wilting of the bentgrass under drought condition (Table 6-5). Because wilting did not occur under salinity condition, no wilting difference was found among the SWC treatments.

It is obvious that large, vigorous and well-functioning root systems, induced by SWC applications, were more efficient in water absorption and translocation. In addition, SWC could suppress nematode activities and reduce the damage to roots (Stephenson, 1968; Tarjan, 1977; Tarjan and Frederich, 1983; Featonby-Smith and Van Staden, 1983; De Waele et al., 1988).

Turf Quality Parameters

Quality parameters of turfgrass are affected by many factors, and usually sensitive to environment stresses. In this study, quality (Table 6-6), color (Table 6-7) and shoot density (Table 6-8) were all improved by SWC applications. Under either drought or salinity stress, nematode-infected bentgrass show improved quality, more acceptable color and increased shoot density after SWC applications. Soil-drench SWC treatments (S_1, S_2 and S_3) were more effective than foliar SWC treatments (F_1 and F_2), and higher concentrate treatments were more effective than low concentrate treatments (S_2 vs S_1 and F_2 vs F_1).

Improved turf quality by SWC may be attributed to the cytokinin components of SWC. Cytokinin increases cell divisions, retards leaf aging and senescence. Of course, more developed and better functioning root systems caused by SWC treatments may also reduce the stress on the plants, delay leaf aging and senescence.

Clipping Yields

Although excessive clipping yield is undesirable in turf management, clipping yield is a general indicator of the plant growth and development. Under stressed condition, higher clipping yield usually means the plants are more adapted to environmental conditions. In this study, both fresh and dry clippings from nematode-infected and drought-stressed plants were significantly increased by S_3 only (Table 6-9 and 6-10). Although not significant, clipping yield increases were associated with SWC application under salinity stress.

The effects of SWC on plant yields are well documented (Blunden, 1977; Mooney and Van Staden, 1985; Nelson and Van Staden, 1986). In fact, SWC applications affect plant vegetative yield more than economic yield (Nelson and Van Staden, 1986; Featonby-Smith and Van Staden, 1983a, 1983b). Increased yields were partly attributed to the improved growth during early stage of plant development, and partly to a delayed senescence and better translocation.

Nematode Density

Application of SWC (S_2 and S_3) slightly reduced the number of RKN eggs per unit of fresh root weight and Lance nematodes per unit weight of soil, but the SWC treatments did not differ significantly from the control(Table 6-11). Previous studies have shown that SWC application suppressed nematode development on tomato (Featonby-Smith and Van Staden, 1983) and maize (De Waele et al., 1988). The lower density of nematodes could be caused by the suppression activity of SWC (Paracer, et al., 1987).

Table 6-2. Effect of Seaweed Concentrate on VLR of bentgrass inoculated with RKN or Lance nematodes and grown under drought or saline stress.

SWC Treatment[a] (liter/ha)		Stress Treatment			
		Drought Stress		Salt Stress	
		Lance	RKN	Lance	RKN
S0	(0.0)	[b]17.0a	26.3c	19.3b	22.7a
S1	(2.0)	21.3a[c]	31.0b	26.7ab	26.7a
S2	(2.0)	26.0a	38.0a	25.7ab	31.0a
F1	(2.0)	19.0a	26.7c	26.3ab	29.0a
F2	(2.0)	25.0a	29.3bc	26.3ab	30.3a
S3	(4.0)	23.0a	30.0bc	29.0a	26.3a

[a]: S0, S2 and F2 were applied for 10 times while S1 and F1 for 5 times only.
[b]: Data were collected 17 weeks after nematode inoculation.
[c]: The letter a, b and c within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 6-3. Effect of Seaweed Concentrate on leaf water content (%) of Bentgrass inoculated with RKN or Lance nematodes and grown under drought or saline stress.

SWC Treatment[a] (liter/ha)	Stress Treatment			
	Drought Stress		Salt Stress	
	Lance	RKN	Lance	RKN
S0 (0.0)	[b]70c	71ab	75a	58a
S1 (2.0)	74a[c]	66b	69a	64a
S2 (2.0)	75a	73a	71a	70a
F1 (2.0)	70c	70ab	71a	65a
F2 (2.0)	72b	74a	69a	67a
S3 (4.0)	74a	72a	67a	69a

[a]: S0, S2 and F2 were applied for 10 times while S1 and F1 for 5 times only.
[b]: Data were collected 15 and 17 weeks after nematode inoculation.
[c]: The letter a, b and c within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 6-4. Effect of Seaweed Concentrate on LHP of Bentgrass inoculated with RKN or Lance nematodes and grown under drought or saline stress.

SWC Treatment[a] (liter/ha)		Stress Treatment			
		Drought Stress		Salt Stress	
		Lance	RKN	Lance	RKN
S0	(0.0)	[b]161a	177a	131a	140a
S1	(2.0)	137ab[c]	147b	120a	132a
S2	(2.0)	133ab	123c	117a	128a
F1	(2.0)	148ab	148b	127a	140a
F2	(2.0)	154ab	147b	123a	137a
S3	(4.0)	118b	132bc	115a	122a

[a]: S0, S2 and F2 were applied for 10 times while S1 and F1 for 5 times only.
[b]: Data were collected 15 and 17 weeks after nematode inoculation.
[c]: The letter a, b and c within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 6-5. Effect of Seaweed Concentrate on Wilting of Bentgrass inoculated with RKN or Lance nematodes and grown under drought or saline stress. Rating 1 to 9 = no wilt.

SWC Treatment[a] (liter/ha)	Stress Treatment			
	Drought Stress		Salt Stress	
	Lance	RKN	Lance	RKN
S0 (0.0)	[b]2.7c	3.7c	9.0a	9.0a
S1 (2.0)	6.3ab[c]	4.7bc	9.0a	9.0a
S2 (2.0)	7.7a	7.00a	9.0a	9.0a
F1 (2.0)	5.3ab	4.7bc	9.0a	9.0a
F2 (2.0)	4.0bc	6.3ab	9.0a	9.0a
S3 (4.0)	6.0ab	7.7a	9.0a	9.0a

[a]: S0, S2 and F2 were applied for 10 times while S1 and F1 for 5 times only.
[b]: Data were collected 15 and 17 weeks after nematode inoculation.
[c]: The letter a, b and c within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 6-6. Effect of Seaweed Concentrate on turfgrass quality of Bentgrass inoculated with RKN or Lance nematodes and grown under drought or saline stress. Rating 1 to 9 = best.

SWC Treatment[a] (liter/ha)	Stress Treatment			
	Drought Stress		Salt Stress	
	Lance	RKN	Lance	RKN
S0 (0.0)	[b]3.0c	4.0c	3.7c	3.3b
S1 (2.0)	6.3abc[c]	6.7ab	5.0bc	5.0ab
S2 (2.0)	8.3a	5.7abc	8.3a	6.7a
F1 (2.0)	4.3bc	5.0bc	7.3ab	5.0ab
F2 (2.0)	6.0abc	5.0bc	7.3ab	5.0ab
S3 (4.0)	7.7ab	7.3a	7.0ab	6.3a

[a]: S0, S2 and F2 were applied for 10 times while S1 and F1 for 5 times only.
[b]: Data were collected 15 and 17 weeks after nematode inoculation.
[c]: The letter a, b and c within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 6-7. Effect of Seaweed Concentrate on turfgrass leaf color of Bentgrass inoculated with RKN or Lance nematodes and grown under drought or saline stress. Rating 9 = best and 1=worst.

SWC Treatment[a] (liter/ha)	Stress Treatment			
	Drought Stress		Salt Stress	
	Lance	RKN	Lance	RKN
S0 (0.0)	[b]3.0c	3.0b	7.0c	7.7b
S1 (2.0)	5.3abc[c]	5.7a	8.0b	9.0a
S2 (2.0)	6.3ab	5.7a	9.0a	9.0a
F1 (2.0)	4.0bc	3.7b	7.3c	8.0b
F2 (2.0)	5.3abc	5.7a	8.0b	9.0a
S3 (4.0)	7.7a	7.3a	9.0a	9.0a

[a]: S0, S2 and F2 were applied for 10 times while S1 and F1 for 5 times only.
[b]: Data were collected 15 and 17 weeks after nematode inoculation.
[c]: The letter a, b and c within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 6-8. Effect of Seaweed Concentrate on turfgrass shoot density of Bentgrass inoculated with RKN or Lance nematodes and grown under drought or saline stress. Rating 1 to 9 = best.

SWC Treatment[a] (liter/ha)		Stress Treatment			
		Drought Stress		Salt Stress	
		Lance	RKN	Lance	RKN
S0	(0.0)	[b]3.3b	4.0b	5.0b	2.0b
S1	(2.0)	5.3ab[c]	6.3a	5.3ab	3.7ab
S2	(2.0)	7.7a	5.3ab	8.7a	4.3a
F1	(2.0)	3.0b	5.3ab	7.7ab	3.7ab
F2	(2.0)	3.3b	4.3b	6.7ab	3.7ab
S3	(4.0)	7.3a	6.3a	6.0ab	5.0a

[a]: S0, S2 and F2 were applied for 10 times while S1 and F1 for 5 times only.
[b]: Data were collected 15 and 17 weeks after nematode inoculation.
[c]: The letter a, b and c within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 6-9. Effect of Seaweed Concentrate on fresh clipping yield (g) of Bentgrass inoculated with RKN or Lance nematodes and grown under drought or saline stress.

SWC Treatment[a] (liter/ha)	Stress Treatment			
	Drought Stress		Salt Stress	
	Lance	RKN	Lance	RKN
S0 (0.0)	[b]6.00b	6.92b	4.43a	7.07a
S1 (2.0)	7.86ab[c]	7.88b	7.17a	6.33a
S2 (2.0)	7.84ab	8.12ab	8.50a	7.37a
F1 (2.0)	7.48ab	5.05b	6.17a	5.17a
F2 (2.0)	6.77b	8.74ab	7.80a	6.10a
S3 (4.0)	12.0a	12.03a	7.67a	7.93a

[a]: S0, S2 and F2 were applied for 10 times while S1 and F1 for 5 times only.
[b]: Data were collected 15 and 17 weeks after nematode inoculation.
[c]: The letter a, b and c within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 6-10. Effect of Seaweed Concentrate on dry clipping yield (g) of Bentgrass inoculated with RKN or Lance nematodes and grown under drought or saline stress.

SWC Treatment[a] (liter/ha)	Stress Treatment			
	Drought Stress		Salt Stress	
	Lance	RKN	Lance	RKN
S0 (0.0)	[b]1.73b	1.93bc	1.33a	2.10a
S1 (2.0)	2.00ab[c]	2.68ab	2.23a	2.20a
S2 (2.0)	1.93ab	2.17bc	2.50a	2.23a
F1 (2.0)	2.20ab	1.49c	1.80a	1.80a
F2 (2.0)	1.88ab	2.30bc	2.40a	2.00a
S3 (4.0)	3.09a	3.34a	2.46a	2.47a

[a]: S0, S2 and F2 were applied for 10 times while S1 and F1 for 5 times only.
[b]: Data were collected 15 and 17 weeks after nematode inoculation.
[c]: The letter a, b and c within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Table 6-11. Comparison of the nematode numbers among the selected SWC treatments.

SWC Treatment[a] (liter/ha)		Stress Treatment			
		Drought Stress		Salt Stress	
		Lance	RKN	Lance	RKN
S0	(0.0)	488a[b]	711a	506a	644a
S2	(2.0)	470a	652a	440a	590a
S3	(4.0)	488a	658a	488a	572a

[a]: Data were collected 17 weeks after nematode inoculation.
[b]: The letters within each column indicated the differences among the SWC treatments under same water regime and same nematode infection. Within each column, means followed by the same letter were not significantly different at 0.05 probability.

Fig.6-1a

Fig.6-1b

Figure 6-1. The effect of swc on the general appearance of bentgrass under drought and lance nematode infection. a: SWC soil drench treatments (S1 and S2) and control (S0). b: SWC foliar treatments (F1 and F2) and control (S0)

Fig.6-2a

Fig.6-2b

Figure 6-2. The effect of swc on the general appearance of bentgrass under drought and RKN nematode infection. a: SWC soil drench treatments (S1 and S2) and control (S0). b: SWC foliar treatments (F1 and F2) and control (S0)

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CHAPTER 7

NOVEL PROTEINS INDUCED BY SEAWEED CONCENTRATE IN RYEGRASS UNDER VARIOUS STRESS CONDITIONS

INTRODUCTION

The applications of seaweed concentrate (SWC) induced a series of beneficial responses by plants, such as higher yields (Featonby-Smith and Van Staden, 1987); increased nutrient uptake (Bokil et al., 1972) and root growth (Crouch and Van Staden, 1990), reduced incidence of pathogen and insect attack (Stephenson, 1966; Booth, 1964; Senn et al., 1961), increased tolerance to frost (Senn et al., 1961), salinity stress (Chapter 6), moisture stress (Chapter 5 and 6) and nematode infection (Tarjan, 1977; De Waele et al., 1988).

The reason SWC induces these beneficial effects is uncertain. The hypothesis that improved plant growth with seaweed application was attributed to its soil conditioning properties was refuted because SWC was applied in very small dosages (Crouch, 1990). A possible additive effect of both nutrient uptake and of plant growth regulatory action has been proposed (Bokil et al., 1972; Aitken and Senn, 1965).

Many kinds of plant growth regulatory substances were detected, such as auxins or auxin-like compounds (Kingman and Moore, 1982), gibberellin-like substances (Taylor and Wilkinson, 1977; Crouch, 1990), components with similar activities to abscisic acid (Hussian and Boney, 1973; Kingman and Moore, 1982) and cytokinin-like substances (Van Staden and Breen, 1973; Mooney and Van Staden, 1986). Among these plant growth regulators, cytokinins have been singled out as of particular significance. It was shown that the responses of plants to SWC and cytokinins were closely correlated to each other (Featonby-Smith and Van Staden, 1983; Naito, Tsuji and Hatakeyama, 1978). The beneficial influences of SWC on plants may mainly be induced by its cytokinin compounds, which enhance protein synthesis, cell division (Pozsar, EL Hammady and Kiraly, 1967, Miller, 1963), nutrient mobilization (Letham, 1978), and retard senescence (Shaw, Bhattacharya and Quick, 1965), as well as inhibit pathogen infection (Dekker, 1963).

Protein synthesis of plants may be altered by cytokinin application (Pozsar, EL Hammady and Kiraly, 1967; Miller, 1963). Therefore, cytokinin-containing SWC may improve plant growth through its effects on protein synthesis and cell division. Although many studies have been conducted to characterize the novel proteins induced by ABA applications

(Lobroeaux et al., 1993; Anderberg et al., 1992; Chang and Walling, 1991), similar research with cytokinins is very limited (Dominov et al., 1992).

The objective of this study was to detect and characterize novel proteins of ryegrass induced by SWC application under various stress conditions.

MATERIALS AND METHODS

Plant Materials

Perennial ryegrass (Lolium perenne L.) seeds (0.1 g) were placed between two pieces of Whatman #1 filter papers, soaked with distilled water or SWC solution (1 ml SWC solution supplied by Aqua-10 Laboratory), was diluted to one liter with tap water). Then the papers were rolled to form a cylinder. Five cylinders were placed into each of 12 one-liter beakers, six of which contained the water-treated seeds and another six contained SWC-treated seeds. The beakers were transferred into a dark growth chamber set at 22 °C. Fifteen ml of distilled water was injected daily into the bottom of each beaker of non-SWC treated cylinders. The same amount of SWC solution was injected into the bottom of each beaker containing the SWC treatment.

After seven days, plants in the four beakers (two treated with SWC and two not) were subjected to one of three different stress treatments: 1) non-stress: the daily water-regime was continued, and plants were harvested when 10 days old; 2) drought-treatment: watering was withheld until the plants show wilting symptoms, and 3) salinity-treatment: 15 ml of 0.5% NaCl solution was added daily to each beaker until the seedlings were 14 days old at which time they were harvested. Shoot samples for protein analysis were collected from each treatment, and fixed with liquid nitrogen and immediately stored at -70 °C.

Protein Sample Preparation

The protein extraction procedure of Sarhan and Perras (1987) were used in this study. Shoots were cut into one cm pieces and placed in a mortar with dry ice, ground into powder and homogenized at 4 °C in proper amount of 100 mM Tris-Hcl (pH 8.0) with 1 mM PMSF (Phenylmethylsulfonyl fluoride). The homogenate was filtered and centrifuged at 165,000 Xg for 90 minutes in a Beckman 50.1 rotor. Soluble proteins were precipitated with an equal volume of 20% trichloroacetic acid for 30 minutes at 4 °C, collected by centrifugation, and dissolved in 0.1 N NaOH for protein assay. Lysis buffer was added to the protein sample according to the concentration of protein in the solution measured by Bradford procedure (1976). Then the protein sample was stored at -70 °C for two-dimensional gel analysis.

Two-dimensional Gel Analysis

Two-dimensional PAGE was conducted by following the procedure described by Anderson (1988) with some modifications.

ISO:the first dimension:

A 20 milligram protein sample was used in this study. The electrofocusing gel solution for ISO was prepared as follows:

Tube Gel		
Urea	9 M	1.9000 g
Ampholyte (40%)	2%	1.75 ml (pH 3-10)
Acrylamide Stock	3.3%T	0.365 ml
DD water		1.13 ml
Degas		
NP-40 Stock (20%)	2%	0.35 ml
Ammonium persulfate	10%	25 ul
TEMED	10%	25 ul

The gel solution was pressed into glass tubes (1.5 mm in diameter) and allowed to polymerize for one hour.

The high tank of the system was filled with 20 mM NaoH and the low tank with 0.085% H_3PO_4. The ISO set-up was prefocused for 30 minutes at 250 volts. The tank solutions were replaced with fresh ones for focusing. A 20 milligram protein sample was loaded to each glass tube. The gels were focused for 2.5 hours under 500 volts. At the end of focusing, the tube gels were extruded into an equilibration buffer for 5 to 10 minutes, and used immediately or stored at -70 °C.

Slab Gel Electrophoresis:

An 8 cm x 10 cm running gel was made by pouring the gel solution indicated below into slab gel cassettes. The running

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Running Gel:

6.25 ml	30% acrylamide/0.8% bisacrylamide
9.03 ml	L10 buffer
Degas	
156 ul	10% SDS
2.8 ul	TEMED
188 ul	10% ammonium persulfate

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gel was overlaid with water saturated n-butanol and allowed to polymerize for two hours. One and half hours after starting the polymerization of the running gel, a stacking gel solution (see below) was poured into the cassette. A 1.5 mm comb was inserted into the cassettes immediately. Polymerization occurred for 30 minutes.

Stacking Gel:

2.4 ml	Tris/SDS buffer
1.9 ml	DD water
0.7 ml	30% Acrylamide/0.8% bisacrylamide

Degas	
100 ul	10% ammonium persulfate
3.75 ul	TEMED

Slab gels, loaded with the first dimensional gel and a standard protein, were transferred into the DALT tank with the cassettes. The tank was filled with DALT tank buffer. Each gel was run at 25 Ampere current until the dye was just out of the edge of the gel. At the end of the electrophoresis, the DALT cassettes were unloaded and the gel was prepared for staining.

Detection of Proteins

Proteins in the gel were detected with silver staining described by Bradford (1976).

Silver Staining: All of the following operations were done on an orbital shaker.

1). The gel was placed into a plastic box containing fixing solution for 30 minutes

2). The gel was transferred into destaining solution for at least 60 minutes

3). The gel was placed in 10% glutaraldehyde for 30 minutes

4). The gel was washed 4 times with DD water for 30 minutes each wash

5). The gel was stained with silver nitrate solution for 15 minutes with vigorous shaking

6). The gel was transferred to another box and washed 5 times with DD water

7). The gel was transferred to another box containing developer and vigorously shaken until the bands appeared as intense as desired

8). The gel was transferred to Kodak Rapid Fix for 5 minutes

9). The gel was washed exhaustively in water to remove the Rapid Fix

10). The picture of the gel was taken and/or a dry gel was made to maintain a permanent gel record.

To make a dry gel, the gel was placed between two cellophane membrane backing sheets; this Sandwich was placed between two sheets of Whitman 3MM filter paper and dried at room temperature for two days.

The molecular weights (mw) of the detected polypeptides were estimated by checking a curve plotted with the relative mobility of the molecule (rf) against the log mw of the standard protein. The rf here was calculated from (distance migrated by protein)/distance migrated by the dye.

RESULTS

A 2-D-PAGE of soluble proteins extracted from 10-day old ryegrass seedlings grown under well-watered condition, are shown in Figure 7-1a. About 13 spots were visible on the gels. Few differences in the intensities of individual spots were found between the SWC-treated (Fig.7-1b) and non-treated seedlings (Fig.7-1a). A novel polypeptide (number 2), however, appeared on the 2-D-PAGE of SWC-treated seedlings (Figure 7-1b), with a molecular weight 52.1 kD. In addition, spot number 1 shows a polypeptide (mw=27.5 kD), which was intensified after SWC application (Figure 7-1b), compared with its non-treated counterpart (Figure 7-1a).

Under drought-stress conditions, about 17 reproducible polypeptide spots were detected in the 2-D-PAGE of ryegrass seedlings (Fig.7-2). After SWC application, novel polypeptide spots detected by silver staining included spot 1, 2, 3 and 4, with 22.9, 28.2, 52.5 and 81.3 kD mw, respectively. Contrasted to the well-watered seedlings (spot 1 in Figure 7-2a and 7-2b), the intensity of spot 5 (27.5 kD) of drought-stressed seedlings was reduced.

Few variations were detected in the 2-D-PAGE of salinity-stressed seedlings after SWC application (Figure 7-3a and 7-3b). The only difference caused by SWC application was that a new polypeptide (spot 1, 52.1 kD) was added.

Table 7-1. The most prominent spots and their molecular weight

Spot Number	molecular weight (kD)	no stress		Drought		Salinity	
		S0	S1	S0	S1	S0	S1
1	22.9				+		
2	28.2				+		
3	52.5		+		+		+
4	81.3	+	+		+	+	+
5	27.5	+	++	++	+		
6	53.5	+	+	+	+	+	+
7	64.6	+	+	+	+	+	+
8	30.2	+	+	+	+		
9	31.6	+	+	+	+		
10	33.1	+	+	+	+		
11	32.4	+	+	+	+		

+: spot present; ++: spot intensified; blank: spot absent.

S_0=no seaweed treatment and S_1=seaweed treatment.

DISCUSSION

Application of SWC altered protein synthesis and induced a series of modifications on the 2-D-PAGEs of ryegrass seedlings. Under well-watered condition, SWC-treated seedlings had at least one more polypeptide (spot 2 on Figure 7-1b), while synthesis of another polypeptide decreased (spot 1 on Figure 7-1). More novel polypeptide (spot 1, 2, 3 and 4) were detected in drought stress seedlings after SWC application. Under salinity-stress condition, very little variation was found on the PAGEs of SWC-treated and non-treated seedlings.

Spot 2, 3 and 1 on Figure 7-1, 7-2 and 7-3 respectively with a similar molecular weight (about 52.5 kD), may be the same polypeptide induced by SWC under three different stress conditions. More work, such as protein sequencing, needs be done to confirm this. The resolutions of the 2-D-PAGE of drought-stressed seedlings were higher than those of well-watered and salinity-stressed seedlings. As one of the results, more variations among the protein spots could be expected after resolutions of the gels are further improved. Under salinity-stressed condition, polypeptide with low molecular weights disappeared. The reasons for this is unknown.

Plants change their protein metabolism in response to various stress conditions. Novel proteins were synthesized by plants under salinity, water-stress, heat stress, chilling, anaerobic conditions, pathogenesis and wounding (Reviewed by Dubey, 1993). The production of such stress-proteins possibly provides evolutionary value to the plants for survival under adverse environmental situation, (Dubey, 1993). Any factors which increase or decrease such proteins may change the survival fitness of the plants under certain environmental conditions. The application of SWC to plants induces many beneficial responses (Crouch, 1990; Verkleij, 1992). The current study indicated that SWC altered plant protein synthesis, which possibly means that SWC modifies gene expression in plants. Such modification may further cause accumulation or depletion of certain proteins. Further work needs to be done to associate such protein alterations with the beneficial responses.

Fig.7-1a

Fig.7-1b

Figure 7-1. Silver-Stained 2-D protein patterns of the shoots from well-watered 10-d-old ryegrass plants. A. control plants; B. SWC-treated plants. The number 1 points to polypeptide whose amounts is increased. Number 2 to the newly induced polypeptide by SWC.

Fig.7-2a

Fig.7-2b

Figure 7-2. Silver-Stained 2-D protein patterns of the shoots from drought-stressed 10-d-old ryegrass plants. A. control plants; B. SWC-treated plants. The number 1 points to polypeptide whose amounts is decreased. Number 2,3 and 4 to the newly induced polypeptide by SWC. Number 5 to the polypeptide whose amounts is increased.

Fig.7-3a

Fig.7-3b

Figure 7-3. Silver-Stained 2-D protein patterns of the shoots from salinity-stressed 10-d-old ryegrass plants. A. control plants; B. SWC-treated plants. The number 1 points to the newly induced polypeptide by SWC.

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CHAPTER 8

SUMMARY

As the negative effects of synthetic fertilizers and pesticides become apparent, considerable attention has been focused on the development and use of natural biodegradable materials that enhance plant growth and increase plant resistances to biotic and/or abiotic stress conditions. These materials are specially important to turfgrass management considering the extensive locations and the special values of turfgrass. Seaweed is one of the natural products which has not been extensively exploited. Therefore, there is a need to investigate the effects of seaweed products on turfgrass. The objective of this project is to exploit the possibilities to reduce the amount of applied agricultural chemicals by using SWC in turfgrass managements. Apart from examining the effects of SWC on bentgrass under favorable growing conditions, influences of SWC on bentgrass plants under nematode infection, moisture stress, salinity stress and their combinations were studied. Lastly, a preliminary research was conducted to test the effects of SWC on protein synthesis of plants under various stress conditions.

Study of the literature shows that man started to use seaweeds as fertilizers 1,000 years ago, and seaweed products have been applied to over 80 plant species. The beneficial responses of plants, induced by seaweed products include increased yield, shoot weight and root weight, delayed senescence, improved frost resistance, increased nutrient uptake and germination rate, enhanced root growth and reduced incidence of pathogen infections. Information on the effects of seaweed products on turfgrass is limited.

The mechanism for SWC to induce such beneficial responses from plants are uncertain although early researchers attributed the beneficial activities of SWC to its soil conditioning properties and the nutrient constituents. This inference was excluded since there is too little dry seaweed product applied in the SWC treatments. Plant growth regulatory activities of SWC have been recently believed to be mainly responsible to the beneficial effects, but plant growth regulators in SWC can only explain some of the SWC effects. Therefore, there may be other active components in seaweed products which have not been detected.

Infection of RKN negatively influenced both visual and functional characteristics of bentgrass plants. The affected parameters included quality, color, water potential and clipping yields. Under drought or salinity stress conditions, more parameters of bentgrass were affected negatively by RKN infection, such as shoot density, wilting rates and root development. Therefore, nematode damage on turfgrass may be a severe problem, especially under abiotic stresses.

In the absence of abiotic stress conditions, application of SWC at 0.5 liter/ha. to RKN-free plants significantly improve turf quality, reduced leaf water potential, increased fresh clipping yield and root mass. When applied at 1.0 liter/ha., SWC induced the same responses of plants except for a nonsignificant quality-improvement. It is suggested that SWC be applied at 0.5 liter/ha. as a soil drench at 10 day intervals for 10 times is the maximum to be applied under RKN-free condition. Under RKN-infestation, applying SWC at 0.5 liter/ha. as a soil drench for 10 times improved leaf moisture status, and increased root mass. In addition to these two parameters, 1.0 liter/ha. SWC treatment improved turf quality and fresh clipping. One liter SWC/ha. is recommended to apply as a soil drench at 10 day intervals under RKN-infected conditions.

In a separate study, the effects of seven SWC treatments on the growth of nematode-free and infected bentgrass plants were tested under three water regimes. Eight parameters were studied and all of them were improved to some degree by all

SWC applications. High concentration, frequent and soil-drench SWC treatments were most effective to improve plant growth. Application of SWC was more effective to RKN-infected plants than nematode-free plants, and to abiotically stressed plants than abiotic stress free plants.

Seaweed application was also shown to enhance the growth of bentgrass grown under drought or salinity stress and in soil infested with lance nematodes or RKN. With SWC applications, almost all of the symptoms caused by nematode infection and abiotic stress were partially overcome. In addition, rooting, leaf water status and clipping yield were all improved. It was apparent that soil drench SWC treatments were more effective than foliar SWC treatments. Application of SWC reduced the number of nematodes per unit of fresh root (for RKN) and per unit weight of soil (for lance).

In order to explore the reason SWC induced such beneficial responses from plants, protein was extracted from SWC-treated or nontreated ryegrass plants under well-watered conditions, moisture or salinity stress condition. Variations on the 2-D-gels of the proteins indicated that SWC altered plant protein synthesis, possibly by inducing selective gene expressions.

Most of this project was devoted to exploit the effects of SWC on bentgrass grown under various stress conditions, but left the reasons why SWC caused these effects unanswered. Various active components have been determined in seaweed products, and none is responsible for all the claimed SWC effects on plants. Total effects of these separate components on plants are not equivalent to these of SWC products. There are at least two explanations for this. One is that effects of the separate components in SWC are additive to plant growth. The other is that some active components in SWC have not been detected. If the former is true, the influences of summations applications of the concentrated elements could be of great importance to crop production. If the latter could be proved, a new plant growth regulator may be isolated from SWC.

The effects of SWC on plant protein synthesis obtained in this project is tentative, though we feel comfortable to state that SWC alters plant protein synthesis. More efforts are needed before the kinds and sequences of the induced proteins are ascertained. After these proteins are sequenced, DNA sequences conferring these proteins could be discovered, which makes it possible to obtain transgenic plants.

As we learn more about the positive influences of SWC on plants and the reasons for SWC to induce such beneficial responses, applications of seaweed products could become more standard in plant production, because of their relatively low price and biodegradable characteristic.

VITA

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