**Morphological and Physiological Growth Responses of Kentucky Bluegrass to Foliar Applications of Iron, a Cytokinin, and Growth Regulator-like Chemicals**

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(ABSTRACT)

A series of studies were conducted to examine morphological and physiological responses of Kentucky bluegrass (*Poa pratensis L.*) following foliar applications of chelated iron phosphate citrate (Fe), the synthetic cytokinin benzyladenine (BA), the systemic triazole fungicides propiconazole and triadimefon, and MZ63 cold water seaweed extract. Applications of Fe at 112 mg $m^{-2}$, BA at 6 mg $m^{-2}$, propiconazole and triadimefon at 42 and 150 mg $m^{-2}$, respectively, and MZ63 seaweed extract at 0.32 ml $m^{-2}$ enhanced root and shoot growth and development of seedling Kentucky bluegrass.

Repeated applications of BA, the triazoles, or MZ63 in late summer or fall and spring tended to slightly increase post-transplant rooting and sod strength of Kentucky bluegrass as compared to single applications. Repeated applications of Fe applied alone in late summer or fall and spring increased Kentucky bluegrass rooting as compared to single applications of Fe. However, the potential for reduced sod strength and post-transplant rooting was also indicated following single summer applications of chelated Fe at 112 mg $m^{-2}$.

Kentucky bluegrass growth from various combinations of BA, the triazoles, MZ63 seaweed extract and Fe were highly variable. The nature of the responses indicated the

possibility of an adverse interaction between the growth promoting activities of chelated Fe and the other materials.

Kentucky bluegrass seedlings treated with Fe, BA, the triazoles, or MZ63 seaweed extract had increased photosynthetic rates on a land area basis, but not on a per gram shoot dry weight basis. These results suggested the larger photosynthetic rates were probably in response to an increased leaf area resulting from stimulation of leaf and lateral bud initiation.

Benzyladenine was the most active material in delaying the senescence-like response of excised Kentucky bluegrass leaves as measured by carbon dioxide exchange, percent chlorophyll fluorescence decay, and leaf color ratings. Applications of Fe or propiconazole also delayed excision-induced senescence of Kentucky bluegrass leaves, while the anti-senescence activity of triadimefon was highly variable. Combinations of Fe with BA or the triazoles did not further promote a delay in excision-induced senescence.

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# Chapter 1

# Introduction

The rate of germination and establishment of Kentucky bluegrass (*Poa pratensis L.*) is relatively slow as compared to many cool-season turfgrass species. Enhancing root and shoot development of seeded Kentucky bluegrass will increase the rate of establishment and provide a mature, high-quality turf. Over time, enhancement of seedling development will provide ground cover that will reduce water runoff and erosion.

Immediate benefits of enhancing sod establishment will be realized by sod producers. Sod production is a rapidly expanding industry in Virginia. The 1982 Virginia Turfgrass Survey estimated the total value of sod sold in the state at more than $1.8 million. Producers strive to establish and harvest a sod crop as rapidly as possible, while maintaining an acceptable level of quality and turf vigor. Treatments that would reduce the period of establishment to harvest and simultaneously improve sod quality would be of immense value to producers. Profits in sod production are inversely proportional to the interval between establishment and harvest.

Increasing sod strength and post-harvest rooting of Kentucky bluegrass turf is also desirable. Improved strength will result in sod that can be handled more readily at both harvest and transplanting. Enhanced transplant rooting of the mature sod will result in rapid turf establishment and reduce the potential for moisture stress. Therefore, less irrigation would be necessary for transplanted sod and the potential for nutrient uptake would be promoted.

Delayed leaf senescence of Kentucky bluegrass turf would promote an increase in leaf area that could provide for a larger photosynthetic capacity. Materials with cytokinin-like properties have been linked to anti-senescence responses in many plants (Thimann, 1980), as well as the promotion of lateral bud initiation (Ali and Fletcher, 1971). Hence, photosynthetic (Ps) activity of the plant could be increased either by the anti-senescence effects of the chemicals or leaf and lateral bud initiation and development. Larger leaf areas for Kentucky bluegrass would be particularly beneficial as winter approaches. Larger leaf areas might result in increased Ps and higher levels of carbohydrates could be available for maintenance of the plant throughout the winter. Increased levels of carbohydrate reserves would improve shoot regrowth the following spring without depleting reserves needed for continuing root initiation and development.

Research reported here was conducted to study the morphological and physiological responses of Kentucky bluegrass to treatments with iron, a synthetic cytokinin, and other chemicals (systemic fungicides and seaweed extracts) with plant growth regulator activities that have sometimes been labeled as cytokinin-like (CK-like). Benzyladenine (BA, 6-benzylaminopurine), a synthetic cytokinin, has been reported to increase Ps and delay leaf senescence of many plants (Adedipe et al., 1971; Varga and Bruinsma, 1973; Tao et al., 1983). Benzyladenine will be referred to as a synthetic cytokinin throughout these studies.

The systemic fungicides, triadimefon (1-(4-chlorophenoxy)-3, 3-dimethyl-1-(1H-1, 2, 4-triazol-1-yl)-2-butanone) and propiconazole (1-(2-(2,4-dichlorophenyl)-4-propyl-1, 3-dioxolan-2-ylmethyl)-1H-1, 2, 4-triazole) have been reported to cause CK-like growth responses as observed by measurement of increased plant growth and delayed leaf senescence in many crops (Buchenauer et al., 1981; Kettlewell et al., 1982; Ballard et al., 1984; Gautam et al., 1984). However, researchers no longer strictly classify the plant growth regulator activities of triadimefon and propiconazole as CK-like, since the chemicals have also been reported to act as either gibberellic acid biosynthesis inhibitors or abscisic acid-like compounds (Kane and Smiley, 1983; Fletcher and Arnold, 1986; Wulster et al., 1987). Although most of the plant growth regulator activities observed in our studies with Kentucky bluegrass could be classified as CK-like, it appears triadimefon and propiconazole would be better described as plant growth regulator-like (PGR-like) chemicals. The growth-promoting responses apparently are non-target effects that supplement the fungicidal activity of the systemic triazoles.

Many seaweed extracts contain plant growth substances (Hussain and Boney, 1969; Finnie and Van Staden, 1985). The seaweed extracts used in the studies contained natural cytokinins as well as auxins, gibberellic acids, and micronutrients. Many of the growth responses of Kentucky bluegrass treated with the seaweed extracts were possibly stimulated by CK-like activity of the extracts, but the varied composition of these compounds prompted them to be labeled PGR-like materials as well.

The objectives of these studies were to:

i) determine the effectiveness of iron (Fe), BA, and other materials for enhancement of seedling Kentucky bluegrass development.

ii) evaluate the influence of Fe, BA, and other materials for improvement of sod strength and post-harvest sod rooting of Kentucky bluegrass.

iii) examine potential anti-senescence activity of various combinations of Fe, BA, and triazoles applied to Kentucky bluegrass.

# Chapter 2

# Literature Review

## *Cytokinins and Cytokinin-like Growth Regulators*

Cytokinins were first identified in 1955 (Richmond and Lang, 1957) and have since been isolated from over 40 plant species (Weaver, 1972). As a plant hormone, they were first identified as control mechanisms in cell division and differentiation, but since have been implicated in the processes of senescence and apical dominance (Jones, 1973; Wareing and Philips, 1981). Cytokinin materials commonly applied to plants are either naturally occurring or synthetic materials. One of the most biologically active synthetic cytokinins is an adenine derivative known as benzylaminopurine (BA) (Skoog et al., 1967). A cytokinin derivative known as kinetin (6-furfurylaminopurine) was isolated in

1955 from herring sperm DNA (Moore, 1979), but has not been identified in plants. The first naturally occurring cytokinin isolated was zeatin (Letham, 1963). As its name implies, zeatin was isolated from corn. Natural cytokinins used in exogenous applications have also been extracted from seaweed (Hussain and Boney, 1969). Systemic fungicides such as benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) and carbendazim (2-(methoxycarbonylamino)-benzimidazole) have also been documented as having cytokinin-like properties (Skene, 1972; Thomas, 1974; Buchenauer and Rohner, 1981).

Fletcher and Arnold (1986) reported no cytokinin-like (CK-like) activity for triadimefon in the cucumber (*Cucumis sativus*) cotyledon bioassay, but indicated the growth regulating response observed was a secondary response due to a stimulation of root growth. They reported increased cytokinin, chlorophyll (Chl), and potassium levels in the cotyledons of treated plants. Fletcher and Arnold (1986) proposed the increase in Chl concentration was due to greater cytokinin and potassium levels, as had previously been reported (Green and Muir, 1978; Fletcher et al., 1982). The mode of action of the systemic triazole fungicides triadimefon and propiconazole has been reported to be a blockage of C-14 demethylation in the sterol biosynthetic pathway (Buchenauer and Rohner, 1981, Henry and Sisler, 1981). This is an important step in the terpenoid biosynthetic pathway and is a precursor to the biosynthesis of cytokinins, abscisic acid, and gibberellic acid. Plant growth regulator effects of triadimefon have been reversed by gibberellic acid treatments (Buchenauer and Rohner, 1981; Kane and Smiley, 1983).

The response of plants to exogenous cytokinin application is highly dependent on whether the material is naturally occurring or synthetic. Zeatin applications to oat (*Avena sativa L.*) leaf segments were much more active in promoting plant growth than BA (Varga and Bruinsma, 1973; Tao et al., 1983). However, BA was markedly more effective in delaying leaf senescence. Letham (1967) proposed the response differences

were due to differing modes of action of the materials. Kaminek and Lustinec (1978) suggested the difference in response was due to more rapid enzymatic degradation of zeatin into metabolites that had little or no cytokinin-like properties. Research by Tao et al. (1983) supported this hypothesis. They reported many metabolic products of BA had cytokinin-like properties while those of zeatin did not.

There has been no direct demonstration of cytokinin synthesis within the root, but indirect measurements support this idea (Torrey, 1966). Senescence of plants experimentally placed under moisture stress was hastened and was found to correlate negatively with the concentration of cytokinins in the xylem exudate (Torrey, 1966; Itai and Vaadia, 1971; Sitton et al., 1973). Xylem exudates from decapitated apple (*Malus spp.*) roots had similar cytokinin contents to those of xylem sap of the shoot (Jones, 1973). Environmental stresses due to factors such as soil moisture, light intensity, soil salinity, and temperature extremes accelerate senescence (Torrey, 1966; Fletcher and North, 1984).

## Cytokinins, Growth Regulators with Cytokinin-like Activity and Plant Senescence

Cell metabolism is greatly altered at the onset of senescence in the leaf. Proteolysis and RNA hydrolysis are the first events identified in senescing oat leaves (Thimann, 1985). This is followed by the subsequent loss of Chl (Phillips et al., 1969; Maunders et al., 1983). An accumulation of free amino acids and reduced sugars also has been observed and these compounds were mobilized for transport out of the leaf (Thimann et al., 1974). Makino et al. (1983) reported decreased ribulose bisphosphate carboxylase

(RuBPCase) activity. The eventual disruption of membrane integrity has also been observed in leaves after a number of days (Tripathi et al., 1982; Trippi and Thimann, 1983). Tetley and Thimann (1974) reported a large increase in respiration occurred in oat leaves during the senescence process.

Exogenous applications of cytokinins have been shown to prevent or delay leaf senescence. Benzyladenine has been determined to be a very active anti-senescence compound when applied to plants (Varga and Bruinsma, 1973; Tao et al., 1983). Mothes and Engelbrecht (1961) observed an accumulation of amino acids and an increase in protein synthesis in selected areas of tobacco leaves previously treated with kinetin. Tetley and Thimann (1974) found kinetin prevented a rise in respiratory $O_2$ evolution of detached leaves. Applications of the fungicide carbendazim to detached wheat (*Triticum aestivum L.*) leaves have decreased senescence and protected cellular organelles from disorganization that was observed in untreated control leaves.

Addition of kinetin to detached *Xanthium* leaves temporarily arrested the senescence process and maintained Chl, DNA, RNA, and protein levels (Osborne, 1962). Kettlewell and Davies (1982) reported propiconazole treatments on winter wheat retarded visible leaf yellowing and maintained photosynthetic rates per unit leaf area at higher levels than the control. Propiconazole and triadimefon treatments maintained green leaf color of winter wheat and retarded Chl degradation in cereal leaves (Buchenauer et al., 1981, Ballard et al., 1984). Triadimefon and etaconazole (1-((2-(2, 4-dichlorophenyl)-4-ethyl-1, 3-dioxolan-2-yl)methyl)-1*H*-1, 2, 4-triazole) treatments increased total non-structural carbohydrate levels and Chl retention of Kentucky bluegrass (Kane and Smiley, 1983).

## Cytokinin and Growth Regulator-like Chemical Effects on Photosynthesis

Photosynthetic activity has been shown to increase from cytokinin applications. Low concentrations of BA increased the photosynthetic (Ps) rate and activity of RuBPCase and glyceraldehyde 3-phosphate dehydrogenase of seven agricultural plants (Chernyad'ev et al., 1984; Chernyad'ev et al., 1986). Gautam et al. (1984) reported increased Ps rates and Chl concentrations in soybeans (*Glycine max*) treated with the systemic fungicide triadimefon. Propiconazole maintained Ps rates and delayed the onset of leaf senescence of winter wheat (Kettlewell et al., 1982 Davies, et al., 1984). Adedipe et al. (1971) found BA treated beans (*Phaseolus vulgaris L.*) had increased Chl and carotenoid levels and suggested the retardation of leaf senescence was due to a maintenance of Ps activity. Chlorophyll formation in etiolated cucumber plants and in tobacco cell cultures was enhanced by treatments of zeatin, kinetin, and BA (Fletcher and McCullagh, 1971; Dei, 1983; Axelos et al., 1984). Kinetin stimulated grana production and Chl production in wheat coleoptiles (Volfova et al., 1978). Benzyladenine treated leaves of big bluestem (*Andropogon gerardi Vitman*) contained higher Chl contents than untreated plants (Towne and Owensby, 1983).

## Proposed Mechanisms for Cytokinin Response

Light, increased stomatal aperture, and $CO_2$ and $O_2$ concentrations appear to be important regulators of senescence and are somehow interrelated with cytokinin levels in the leaf (Thimann et al., 1977; Thimann and Satler, 1979a; Satler and Thimann, 1983; Trippi and De Luca d'Oro, 1985). The degree of stomatal opening and its relationship

to cytokinin applications has been studied. Stomatal opening in the epidermis of detached *Kalanchoe daigremontiana Hamet et. Parr.* leaves was promoted by zeatin and kinetin (Jewer and Incoll, 1981). Cooper and Digby (1972) observed an increase in stomatal aperture of barley (*Hordeum vulgare L.*) following kinetin treatment. Transpiration rates in a large number of plants have been shown to increase following cytokinin applications (Luke and Freeman, 1968; Pallas and Box, 1970). However, Thimann (1985) reported stomatal opening of the dicot *Tropaeolum* was not directly linked to the prevention of leaf senescence and concluded that while stomatal closure definitely seemed to accelerate senescence, stomatal opening did not necessarily decrease the senescence rate.

The effect of light on senescence delay has been negated by floating hypostomatous leaves with stomatal surface downward or by floating the leaves on a strong osmoticum (Kuraishi, 1976; Tetley and Satler, 1979a). Therefore, the effect of light appears to be primarily related to its promotion of stomatal opening (Thimann, 1980). Thimann and Satler (1979 b) reported that senescence in darkness could be strongly delayed by treatments with kinetin and BA.

The concentrations of $CO_2$ and $O_2$ have been reported to be very important in the leaf senescence process. Oat seedling leaves placed in air largely free of $CO_2$ senesced even in the presence of white light (Satler and Thimann, 1983). Stomates were open and senescence still occurred. Senescence in the light was accelerated by pure $O_2$, but greatly delayed in both light and dark by pure $N_2$. Satler and Thimann (1983) proposed these phenomena could be explained by a combination of oxidation and photooxidation processes. Trippi and De Luca d'Oro (1985) reported senescence of oat leaves increased as $O_2$ levels in the light were increased. They also determined irradiance over 40 W $m^{-1}$ accelerated senescence and proposed this could be due to photooxidation. They theo-

rized an excess of $O_2$ over the requirement for respiratory $O_2$ levels could stimulate secondary oxidations leading to hydroperoxide formation.

The precise mechanism of senescence delay is further complicated by the presence (or absence) of abscisic acid and ethylene. Thimann and Satler (1979a) reported abscisic acid induced stomatal closure and increased the senescence rate of detached oat leaves, but could be reversed by BA treatment. Abscisic acid promotes stomatal closure and has been shown to increase rapidly in leaves that are water-stressed (Wright and Hiron, 1969). In effect, abscisic acid acts as an antagonist to the metabolic activities of cytokinins (Thimann, 1985). Even less is known about the mode of action of ethylene and its contribution to leaf senescence. Aharoni (1979) showed ethylene enhanced leaf senescence of several plant species. Sisler and Pian (1973) reported ethylene increased the rate of senescence of detached tobacco leaves and promoted leaf curing. Though cytokinins have been shown to reverse the senescence process, they also have been shown to promote ethylene production (Gepstein and Thimann, 1981). Thimann (1985) concludes the senescence process most likely is controlled by a complex interaction of all the major plant hormones.

## Cytokinins, Growth Regulators with Cytokinin-like Activity, and Plant Growth

Cytokinins and kinin-like materials increase plant growth and yield of some agronomic crops. Mishra and Gaur (1985) reported applications of kinetin to barley in growth stage III promoted marginal tiller formation and increased ear number. Davies et al. (1984) indicated treatments with propiconazole were effective in increasing grain

yield of wheat if applied early in the growing season. Applications of BA to spring barley at pre-heading stage resulted in a 57% increase in grain yield (Williams and Cartwright, 1980).

Warren et al. (1974) indicated applications of benomyl and thiabendazole increased foliar dry weights of Kentucky bluegrass and creeping bentgrass (*Agrostis palustris Huds.*). Smiley et al. (1985) reported benomyl increased root and rhizome production and sod strength of Kentucky bluegrass. Kane and Smiley (1983) reported both benomyl and BA increased leaf extension rates and chl retention of Merion Kentucky bluegrass. Kane and Smiley (1983) also determined foliar applications of triadimefon or etaconazole at levels of 300 to 600 mg a.i. $m^{-2}$ suppressed Kentucky bluegrass root and shoot growth and suggested the growth regulating activity was caused by an anti-gibberellin mode of action. Triadimefon reduced drought stress and increased yield in soybeans, peas (*Pisum spp.*), and annual bluegrass (*Poa annua*) (Fletcher and Nath, 1984; Pennypacker et al., 1982).

Fletcher and Hofstra (1985) reported growth regulator activity from triadimefon applied as a soil drench to beans paralleled that of abscisic acid. Triadimefon increased the stomatal resistance of treated tomato (*Lycopersicon esculentum Mill.*) and reduced injury of bean plants exposed to ozone. Fletcher and Hofstra further determined triadimefon treatment improved the cold tolerance of cabbage (*Brassica oleracea*) and barley (*Hordeum vulgare*). The researchers suggested the growth regulating properties of triadimefon were mediated by interfering with the isoprenoid pathway and thus altering the balance of plant hormones synthesized in the pathway. Fletcher and Hofstra (1985) proposed the ultimate triadimefon plant growth regulator-like (PGR-like) effect could be dependent on the dynamic equilibrium of cytokinins, gibberellic acids, and abscisic acid at specific stages of plant growth and develpment.

Propiconazole applied at a level of 20 mg $L^{-1}$ water as a soil drench and triadimefon applied at a level of 100 mg $L^{-1}$ as a bulb soak significantly increased heights of lily (*Lilium longiflorum Thunb.*) plants as compared to untreated controls (Wulster et al., 1987). However, propiconazole and triadimefon applied at levels of 500 mg $L^{-1}$ as a bulb soak significantly reduced lily height and the PGR-like activity was proposed to be caused by a blockage of gibberellic acid biosynthesis.

## Cytokinin Effect on Initiation of Lateral Buds and Lateral Root Primordia

Ali and Fletcher (1971) reported BA applied to inhibited buds of soybean initiated new lateral bud growth. Lee et al. (1974) concluded that addition of the synthetic cytokinin reversed lateral bud inhibition in peas that had been treated with the antibiotic hadacidin. Lateral bud inhibition of *Begonia* caused by high temperature and long days was completely overcome by BA treatments (Heide, 1965).

Root treatments with cytokinin materials had differing effects on root initiation and elongation. Heide (1965) reported cytokinins inhibited lateral root formation of *Begonia* cuttings. Goodwin and Morris (1979) showed that cytokinins inhibited lateral root production, but promoted lateral stem outgrowth. However, other researchers have found exogenous cytokinin effects to be concentration dependent. Cytokinin concentrations greater than $10^{-6}$ M showed marked inhibition of root primordia initiation and emergence in peas, but concentrations less than $10^{-6}$ M exhibited small but real promotions in the number of lateral root primordia (Wightman et al., 1980). They proposed the lower concentration of cytokinins were interacting with endogenous auxins and stimulating lateral root primordia initiation. Smith and Thorpe (1975) reported the ef-

fect of exogenous kinetin applications to *Pinus radiata* roots to be highly dependent on the time of the application. The earliest stages of root development were the most sensitive and roots were inhibited. Eriksen (1974) confirmed these results using BA and peas. He further showed low concentrations of BA appeared to act synergistically with some other growth substance (not identified) and promote root development. Skoog et al., (1973) observed a cytokinin antagonist reduced initiation and development of roots in wheat and radish seedlings, and *Coleus* spp. cuttings. The inhibition could be completely reversed by application of BA.

Hussain and Boney (1969) detected CK-like substances in the extracts of the sea algae *Laminaria digitata* and confirmed their cytokinin properties with the radish cotyledon bioassay. Brain et al. (1973) reported 0.1 and 0.01% concentrations of seaweed extracts produced increases in growth of tissue cultured cells of a cytokinin-requiring strain of *Atropa belladonna.* An extract from another brown alga (*Ecklonia maxima*) increased shoot and root growth of *Beta vulgaris* and increased the endogenous concentration of cytokinins in the root as well (Featonby-Smith and Van Staden, 1983). Working with nutrient stressed cucumbers, Nelson and Van Staden (1984) observed an increase in root mass and root/shoot ratio from seaweed extract at a 1:500 dilution. Finnie and Van Staden (1985) reported increased root extension and elongation of *in vivo* cultured tomato roots when seaweed concentrate was applied at concentrations of $10^{-7}$ M and less. They further showed the response mimicked that of cytokinins and not indoleacetic acid, gibberellic acid, or abscisic acid.

Applications of BA at a concentration of $10^{-4}$ M to pea seedlings with the root tips removed inhibited lateral root initiation, but applications of $10^{-6}$ to $10^{-5}$ M BA as a cotyledon substitute promoted lateral root initiation (Hinchee and Rost, 1986). Hinchee and Rost determined shoot or cotyledon derived cytokinins could be transported to the

primary root and act as inhibitors or promoters of lateral root initiation depending on cytokinin concentrations relative to that of endogenous auxin concentrations.

## *Chlorophyll Fluorescence*

Pigment systems of green plants trap light energy in the form of photons and pass the energy on to other molecules. When a photon is absorbed, an electron in the conjugated double bond system is promoted from a bonding orbital to a non-bonding orbital. The pigment molecule is then said to be in an excited state. The electron of the excited molecule is promoted to a higher vibrational level of the non-bonding orbital. The promoted electron then drops from the highest vibrational level to the lowest one of the non-bonding orbital in a series of steps known as the vibrational cascade. The cascade is a non-radiative process by which quanta of energy equal to the difference in energy of the successive vibrational levels are dissipated and absorbed by other molecules in the photosystem (Goodwin and Mercer, 1983).

The pigment molecule remains in an excited state after completion of the vibrational cascade. Promoted electrons in the non-bonding orbital and electrons in the bonding orbitals of the pigment molecule have opposite spins and are said to be paired. In this state the molecule is called a singlet. The electron in the non-bonding molecular orbital is quickly dropped back to the lowest vibrational level of the bonding orbital, and the energy of this drop of electrons from an excited state to a ground state is utilized in photochemical reactions or dissipated in several other ways. One way energy is dissi-

pated is by fluorescence. As excited electrons are passed down lower energy levels within the pigment systems, a photon can be emitted as the electron moves back to the lowest energy level. The energy of the photon emitted during fluorescence is less than that of the photon originally absorbed by the pigment system and the emitted photon will have a longer wavelength (Goodwin and Mercer, 1983).

A correlation between Chl fluorescence and carbon assimilation was recognized as early as 1874 (Muller, 1874). In the 1930's, Kautsky and co-workers developed light sensitive devices and signal recorders to monitor Chl fluorescence. Several systems have been developed to simultaneously measure Chl fluorescence, $O_2$ evolution, and $CO_2$ uptake (Delieu and Walker, 1983, Ogren and Baker, 1985).

There is general agreement that fluorescence of plant tissue measured by optical devices at room temperature is largely emitted by photosystem 2 (PS II) and the associated light harvesting complex (Vredenberg and Sloota, 1967). The fluorescence undergoes characteristic variations following a dark to light transition. Traces of fluorescence following the induction period are known as the Kautsky effect (Schreiber, 1983). A characteristic trace is given in Fig. 1. Four points on the curve are commonly reported and have been interpreted. Level O denotes constant fluorescence seen after a dark period when all reaction centers of PS II are open. Any fluorescence above this initial level is termed variable fluorescence. The rise to a peak (P) is often characterized by an inflection (I) point. The fluorescence rise from level O is considered to reflect the reduction of electron acceptor Q (Krause and Weis, 1984).

Krause and Weis (1984) proposed a transient block of photosystem 1 (PS I) electron transport leads to the fluorescence rise to P and is due to a lack of efficiency of electron acceptors of PS I and a reduced pool of oxidized NADP. Duysens and Sweers (1963) reported maximum fluorescence levels were obtained upon addition of the electron transport inhibitor DCMU (3-(3,4-dichlorophenyl) -1,1-dimethylurea). Typical Kautsky

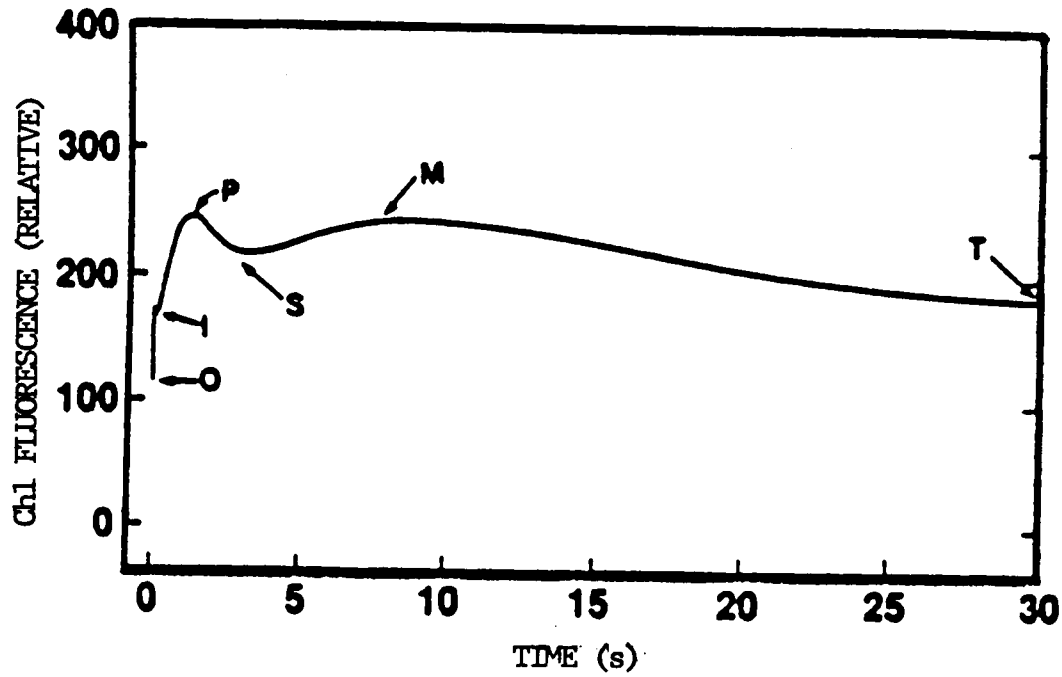


**Figure 1.** A typical chlorophyll fluorescence induction curve following a dark-light transition (after Shaw et al., 1986).

effect plots showed fluorescence declines from a peak to a terminal (T) state. The decline in fluorescence is a result of fluorescence quenching. Duysens and Sweers (1963) proposed Q quenched fluorescence in the oxidized state and labeled this mechanism Qq. Krause (1973) showed the establishment of a proton gradient across the thylakoid membrane provided a potent fluorescence quenching mechanism (Qe) when the transthylakoid pH gradient was high. The O to P fluorescence transient has been linked to the oxidation status of Q (Duysens and Sweers, 1963, Papageorgiou, 1975). Q was rapidly reduced upon illumination, Qq quenching was suppressed, and there was an immediate rise in fluorescence. Qq quenching slowly increased (fluorescence decreased) as Q reoxidized to a steady state value. Simultaneously, Qe quenching rose as a proton gradient was established (Krause, 1973). Qe quenching decreased as ATP was consumed during the reductive pentose phosphate cycle and this resulted in point M on the Kautsky plot. Qe gradually increased as electron transport occurred and a proton gradient was established. However, Ireland et al. (1984) showed the onset of carbon assimilation increased Qq as NADP was reduced and Qe quenching was decreased as ATP was utilized. The terminal (T) fluorescence plot was reached when Qe and Qq reached steady-state conditions (Papageorgiou, 1975).

Kautsky and Hirsch (1931) reported Chl fluorescence had the potential to be utilized as a tool for measuring photosynthetic productivity. Fluorescence monitoring of intact tissue provided a measure of photosynthesis without damage or disturbance to the plant (Baker and Bradbury, 1981). Simultaneous measurements of $CO_2$ fixation and Chl fluorescence indicated $CO_2$ fixation did not begin until after point P of the fluorescence curve and was correlated with the S-M transient on the curve. Ireland et al. (1984) further showed an increase in $CO_2$ fixation coincided with fluorescence quenching and both curves oscillated in an anti-parallel manner until steady-state levels were reached.

Walker et al. (1983) reported anti-parallel but phase shifted relationships between the curves of $O_2$ evolution and $CO_2$ uptake, and Chl fluorescence. Rises in fluorescence anticipated declines in carbon assimilation and $O_2$ evolution. However, Ogawa (1982) indicated the curve relationships between $O_2$ evolution and Chl fluorescence were complementary instead of anti-parallel. These results indicated a high relative yield of fluorescence did not correlate with a low photosynthetic rate. Ireland et al. (1984) reported changes in $CO_2$ concentrations resulted in fluorescence and $CO_2$ fixation curves that did not strongly correlate.

## *Iron*

Iron (Fe) is the micronutrient most commonly deficient in turf. Iron deficiency is most common in soils that are: i) alkaline, ii) high in phosphate, manganese, or zinc, iii) high in organic matter, iv) waterlogged, or v) have excessively thatched turf (Beard, 1973). Plants suffering from Fe deficiency are characterized by having leaves with various degrees of interveinal chlorosis (DeKock, 1955). Iron deficiency symptoms are similar to those of nitrogen deficiency except chlorosis appears first on younger leaves (Oertli, 1963). This chlorotic pattern occurs because iron does not readily move within the plant from older to younger leaf tissue (Brown; 1978, Kannan and Pandey, 1982). Plants fed labelled $^{59}Fe$ had increased amounts of Fe in veinal areas, but under Fe stress conditions, Fe decreases disproportionately faster in veinal areas as compared to interveinal areas (DeKock, 1955).

Foliar sprays of Fe solutions have been successfully used to correct deficiency problems. Withee and Carlson (1959) reported a 4% solution of $FeSO_4$ applied as a foliar application to sorghum (*Sorghum halapense L.*) was more practical and more beneficial in reducing chlorosis than a soil treatment with Fe.

## Iron Availability and Plant Absorption

Iron exists in the soil in the forms of $Fe^{3+}$, $Fe(OH)^{2+}$, $Fe(OH_4)^{+}$, and $Fe^{2+}$, but the amount of iron in solution is very small compared to the total iron content in the soil (Marschner, 1979). Soil pH is a very important factor determining the solubility of $Fe^{3+}$ since the solubility of the ferric ion is reduced by 1000 fold for each pH unit above four (Wallace and Lunt, 1960).

Iron must be reduced from the $Fe^{3+}$ state to $Fe^{2+}$ prior to absorption by the root (Ambler et al., 1970; Chaney et al., 1972). Plants differ in their ability to carry out this reduction process and are classed as being *efficient* or *inefficient* with respect to Fe uptake (Christ, 1974; Brown, 1978). Efficient plants respond to Fe stress by i) releasing $H^{+}$ from their roots, ii)releasing reducing compounds from roots, iii) reducing $Fe^{3+}$ to $Fe^{2+}$ in or on their roots, iv) increasing organic acid contents in their roots (Brown, 1961). Efficient plants apparently have a mechanism by which they can secrete a reductant to convert $Fe^{3+}$ to $Fe^{2+}$. Wallace (1981) proposed monocots did not excrete $H^{+}$ under Fe deficiency conditions, but dicots did. The excretion of $H^{+}$ lowered the pH around the roots and resulted in less stable Fe chelates in the soil and greater Fe uptake in the dicots. Olsen et al. (1982) proposed caffeic acid, a component of the shikimic acid

pathway, was a primary reductant released by the roots of some plants to facilitate Fe uptake.

Romheld and Marschner (1986) reported evidence of two different strategies of Fe uptake by Fe deficient plants depending on whether the species was graminaceous or non-graminaceous. They reported non-graminaceous species were characterized by having an inducible plasma membrane-bound reductase that enhanced $H^+$ release from the roots. Romheld and Marschner (1986) further determined graminaceous species under Fe stress were characterized by enhanced release of phytosiderophores (non-proteinogenic amino acids such as mugineic and avenic acid) that are released by the roots to form stable chelates with $Fe^{3+}$ The graminaceous species further appeared to have highly specific uptake systems for the phytosiderophores. These stress mechanisms appear to be active only when Fe deficiency occurs.

The uptake of Fe by roots is primarily by active absorption. Branton and Jacobson (1962) indicated the addition of the uncoupler 2,4-dinitrophenol (DNP) to the nutrient solution of peas significantly reduced Fe uptake. Decorticated pea plants showed increased Fe absorption due to transpiration, but they concluded passive uptake was not nearly as important as active transport. Iron absorption by tobacco (*Nicotiana tabacum L.*) leaf cells was increased in the presence of light and succinate (Kannan and Wittwer, 1967). Leaf cell absorption was also sensitive to temperature change and addition of DNP. These factors further suggested Fe uptake was an active process.

Brown (1978) proposed Fe is taken up in $Fe^{2+}$ form, oxidized back to $Fe^{3+}$ form, is chelated by organic acids, and is transported in the xylem throughout the plant. The uptake of Fe from the chelated form also is dependent on getting the Fe reduced to the ferrous form, but the process is more complex. One group of researchers proposed the first step in chelated $Fe^{3+}$ uptake is binding of the chelated Fe to a site on the plasmalemma, followed by reduction of $Fe^{3+}$ to $Fe^{2+}$. Then the $Fe^{3+}$ is split from the

chelate and possible, but not obligatory, expulsion of the chelate from the root can follow (Wallace, 1983; Wallace and Wallace, 1983; Wallace et al., 1984). Other scientists have reported the same $Fe^{3+}$ binding mechanism, but do not believe the chelate is taken up by the roots (Bienfait, et al., 1982; Romheld and Marschner, 1983). Tiffin (1966, 1970) reported an Fe citrate compound was the primary Fe species transported in sunflower (*Helianthus spp.*) and soybean. The citric acid form appeared to be the one that moved within the plant.

## Biochemical Activity of Iron in Plants

The most obvious symptom of Fe deficiency is chlorosis, but no conclusive relationship between leaf tissue Fe concentration and degree of chlorosis has been found. Terry (1980) reported Chl content of Fe-stressed bean leaves correlated positively with leaf Fe concentration. Terry and Low (1982) also reported Chl content was quantitatively related to the bound Fe content of the lamellae. They further proposed Fe deficiency reduced Chl and lamellar Fe contents by retarding the formation of new thylakoids rather than by diminishing Chl synthesis *per se*. Jacobson (1945) found a definite relationship between Fe and Chl content of leaves and indicated that only a certain fraction of Fe was *active* in Chl formation. However, O'Toole (1966) reported chlorotic leaves of grasses had higher Fe content than those plants not under Fe stress. O'Toole believed there was an active Fe concentration in the leaves that was used for the biosynthesis of enzymes and Chl precursors, but reported a large percentage of Fe was bound in an *inactive* form as well.

# Chapter 3

# Immature Kentucky Bluegrass Sod Development as Influenced by Applications of Iron, Benzyladenine, and Other Materials

## *Introduction*

Numerous field trials were performed on Kentucky bluegrass sod in the fall and spring of 1986 and 1987. These trials were performed to determine:

i) if Fe, benzyladenine (BA), or other materials with plant growth regulator-like (PGR-like) activity might increase Kentucky bluegrass rooting and sod strength.

ii) optimal levels and timing of Fe, BA, or PGR-like material application.

iii) the requirement for single or repeated Fe, BA, or PGR-like material applications.

# *Materials and Methods*

## Summer applications (Trial 1)

Treatments for two field experiments were applied in July 1986 at the Turfgrass Research Center in Blacksburg, VA on similar areas of 'Plush' Kentucky bluegrass. The sod was established in the fall of 1985 on a Groseclose silt loam (a clayey, kaolinitic, mesic Typic Hapludult) with a pH of 6.2. Nitrogen, $P_2O_5$, and $K_2O$ were applied at 49 kg $ha^{-1}$ each at seeding with a 10-10-10 fertilizer. Additional N (49 kg $ha^{-1}$) was applied in February 1986. The turf was mowed at 3.8 cm and irrigated as needed to avoid wilt. Treatments were applied to 0.9-m by 1.8-m plots arranged in randomized complete block designs replicated four times. Treatments (Table 1) were various combinations of Fe as a chelated Fe phosphate citrate (RGB Laboratories, Inc., Kansas City, MO), triadimefon, propiconazole, benzyladenine (BA), MZ63 cold water seaweed extract (Agrimar Corp., Atlanta, GA), Panasea hot water seaweed extract (Emerald Isle, Ltd., Ann Arbor, MI), and Aqua Gro soil wetting agent (a combination of polyoxyethylene esters of cyclic acids and polyoxyethylene ethers of alkylated phenols, Aquatrols Corp., Pennsauken, NJ). One experiment received a single application on 11 September 1986 and the other trial received repeated applications at the full levels on 9 July and 11 September 1986. Repeated applications resulted in the turf receiving twice as much material as single applications. Commercially available or experimental formulations of compounds were used. A compressed-air boom sprayer that delivered 748 L $ha^{-1}$ of chemical solution at a pressure of 276 kPa was used for foliar applications.

Sod from each plot was cut 1 week following the final chemical application with a sod harvester set at a cutting depth of 2.2 cm. Harvest dates were 18 September for both experiments. Sod was further cut into 900 $cm^2$ pieces and the sod squares were transplanted on 900 $cm^2$ pieces of expanded metal that had been lightly pressed into a prepared soil area. Transplanted sod was irrigated as needed to prevent the sod from drying.

Measurements of post-transplant rooting were made 4 to 6 weeks after sod was transplanted. Measurement dates were 20 October 1986 for both experiments. Rooting of the sod was measured by vertically lifting the metal frames from the soil. A hand-held push/pull gauge scale (model DPPH-100, John Chatillon & Sons, Inc., 83-30 Kew Gardens Road, Kew Gardens, NY) with four metal hooks extending from the base was used to determine the vertical lift of the transplanted frames in a method similar to that described by Schmidt et al. (1986). The hooks were attached to the metal squares in the soil and the amount of force required to pull the roots free from the soil was recorded. Schmidt et al. (1986) reported a significant correlation between vertical root lift force and root mass.

Post-transplant rooting data were analyzed by an analysis of variance (ANOVA); when significance ($p < 0.05$) was indicated, Hsu's procedure and Dunnett's test were performed. Hsu's analysis was used to gather information on a large number of treatments (Lentner and Bishop, 1986). Hsu's procedure groups treatments in classifications of the "highest or best" categories. Therefore, treatments grouped according to Hsu's procedure were those that increased post-transplant rooting and sod strength the most. Hsu's procedure is useful in large scale studies designed to select the group of most promising treatments. Dunnett's test is specific for comparisons with a control treatment (Lentner and Bishop, 1986). Dunnett's test does not provide provide information

about differences among chemical treatments, but does indicate the significance of response of a chemical treatment over the untreated control.

## Fall and spring applications (Trial 2)

A 'Plush' Kentucky bluegrass sod seeded in fall 1986 was used in this trial. Three similarly maintained areas of turf received chemical applications on either 12 November 1986, 12 November 1986 plus 8 April 1987, or only on 8 April 1987. Treatments are listed in Table 2. Plot maintenance and experimental methods were as previously described. Sod was harvested for root lift studies on 10 May 1987, and frames were set in a prepared soil area on this date. Vertical root lift measurements to measure root growth were determined by lifting the frames from the soil on 13 June 1987. In addition to measuring rooting, sod strength data were collected from two areas in each plot. Sod strength measurements were made on 15 June 1987 by removing two 0.3-m by 0.3-m pieces of sod from the field and averaging the force required to break the sod by stretching. Sod strength was determined by securing the piece of sod on a sod stretching machine consisting of one metal unit mounted on a track and driven by an electric powered gear activator and a second, immobile frame. The Chatillon push/pull gauge scale was attached to the mobile unit to measure the force required to break the sod when the unit was placed in motion. Force was recorded in kilograms at the point of sod break. All data were subjected to ANOVA, and when a significant ANOVA test was indicated, Hsu's procedure and Dunnett's test were performed.

# *Results*

## Repeated summer applications (Trial 1)

***Repeat applications of Fe, a BA, and other materials to Kentucky bluegrass sod on 9 July 1986 and 11 September 1986. Vertical root lifts made 20 October 1986.***

The triazole fungicides, propiconazole and triadimefon, were the most active materials in promotion of root development of transplanted sod (Table 1). Benzyladenine applied alone also significantly improved root growth and was placed in the highest rooting group. Untreated controls had the lowest vertical root lift values, and every treatment placed in the best rooting group also had significantly greater root lift values than the control. Propiconazole at 21 mg $m^{-2}$ or 42 mg $m^{-2}$ and the combination of triadimefon plus Fe provided significantly greater Kentucky bluegrass root enhancement than the control for the repeated application experiment (Table 1).

## Single summer applications (Trial 1)

***Applications of Fe, BA, and other materials to Kentucky bluegrass sod on 11 September 1986. Vertical root lifts made 20 October 1986.***

Table 1. Post-transplant rooting of Kentucky bluegrass following applications of Fe, benzyladenine (BA) and other materials from single applications on 11 Sept. and repeated applications on 9 July and 11 September 1986. Vertical root lifts measured 20 October 1986.

| Substance | | Iron | Application | |
|---|---|---|---|---|
| Material applied | Level | level | Single | Repeated |
| | —— mg a.i. $m^{-2}$– | | —— kg $900^{-1}$ $cm^{-2}$– | |
| BA | 6 | 0 | 70.8* a | 70.0* |
| | | 112 | 68.3 | 68.8* |
| Propiconazole | 21 | 0 | 84.8* a | 87.8* a |
| | 42 | 0 | 88.5* a | 78.3* a |
| | 84 | 0 | 70.8* a | 69.3* |
| Triadimefon | 75 | 0 | 72.3* a | 69.5* |
| | | 112 | 68.8 | 69.5* |
| | 150 | 0 | 68.0 | 84.3* a |
| | | 112 | 80.5* a | 67.3* |
| | | 112 (Urea)† | 71.0 | 74.5* a |
| MZ63 seaweed extract‡ | 0.08 ml | 0 | 67.5 | 65.5 |
| | | 112 | 69.5 | 71.8* |
| | 0.16 ml | 0 | 67.5 | 58.8 |
| | | 112 | 67.3 | 68.5* |
| | 0.32 ml | 0 | 59.8 | 72.8* |
| Panasea seaweed extract‡ | 2.5 ml | 0 | 65.5 | 62.3 |
| | | 112 | 68.8 | 70.8* |
| None | 0 | 112 | 53.8 | 66.0* |
| Control | 0 | 0 | 51.8 | 51.8 |
| LSD (Dunnett's, 0.05) | | | 18.6 | 22.1 |
| Grand mean | | | 69.0 | 69.3 |

* indicates means within the same column are placed in the "significantly higher" group at the 0.05 probability level according to Hsu's procedure. Means within the same column followed by the letter "a" are significantly different from the control at the 0.05 probability level according to Dunnett's test.

†Urea applied at the rate of 980 mg $m^{-2}$.

‡Levels of MZ63 cold water seaweed extract (Agrimar Corp., Atlanta, GA) and Panasea hot water seaweed extract (Emerald Isle, Ltd., Ann Arbor, MI) are expressed in ml product $m^{-2}$.

The top root enhancement treatments placed in Hsu's best rooting category were those including applications of propiconazole or triadimefon (Table 1). Many of the combinations of growth regulating chemicals plus Fe were placed in the best root strength grouping as well. The four treatments with the largest root lift data were significantly greater than the control (Table 1). Propiconazole applied at 21 mg $m^{-2}$ or 42 mg $m^{-2}$, and triadimefon at 150 mg $m^{-2}$ applied alone and with Fe and urea had significantly larger root lift values than the control.

## Single fall application (Trial 2)

***Application of Fe, BA, and other materials to Kentucky bluegrass sod on 12 November 1986. Vertical root lifts made 13 June 1987 and sod strength measured 15 June 1987.***

This experiment examined the long-term response to materials applied in November and transplanted in May. The ANOVA for post-transplant root growth and sod strength data revealed no significant differences. Therefore, only the treatment means for rooting and sod strength data are presented (Tables 2 and 3). Although not significantly different, both sets of data indicated many of the treatments tended to increase root initiation, root development, and sod strength as compared to the control.

There appeared to be little long-term rooting response evident from applications of Aqua Gro. Other treatments were quite variable in their performance. Fe applied at 112 mg $m^{-2}$ caused greater root growth than the Fe treatment of 56 mg $m^{-2}$ (Table 2).

Sod strength for a mature Kentucky bluegrass sod suitable for harvest was determined to be approximately 22 kg for a 0.3-m by 0.3-m piece of sod in independent field studies. No treated sod in the single fall application experiment attained this level of sod

Table 2. Post-transplant rooting of Kentucky bluegrass following single applications of Fe, benzyladenine (BA) and other materials on 12 November 1986 or 8 April 1987, or repeated applications on both dates. Vertical root lifts measured 13 June 1987.

| Substance | | Iron | | Application | | |
|---|---|---|---|---|---|---|
| | | | | Single | Fall + | Single |
| Material applied | Level | level | | fall | spring | spring |
| | — mg a.i. $m^{-2}$ — | | | — kg $900^{-1}$ $cm^{-2}$ — | | |
| BA | 6 | 0 | | 31.3 | 21.0 | 32.5 |
| | | 112 | | 19.8 | 29.0* | 36.3 |
| Propiconazole | 21 | 0 | | 30.8 | 21.3 | 35.3 |
| | 42 | 0 | | 30.0 | 33.8* | 24.8 |
| | | 112 | | -- | -- | 30.0 |
| | 84 | 0 | | 28.8 | 42.0* a | 33.5 |
| Triadimefon | 150 | 0 | | 26.5 | 41.3* a | 34.5 |
| | | 0 | (Aqua Gro)† | 22.8 | 24.5 | 41.3 |
| | | 112 | | 32.0 | 28.8* | 32.3 |
| | | 112 | (Urea)† | 30.5 | 33.5* | 33.0 |
| MZ63 seaweed extract‡ | 0.08 ml | 0 | | 25.3 | 29.0* | 34.5 |
| | | 112 | | 35.8 | 38.3* | 39.8 |
| | 0.16 ml | 0 | | 31.3 | 29.3* | 23.8 |
| | | 112 | | 27.5 | 32.0* | 26.0 |
| | 0.32 ml | 0 | | 29.8 | 21.8 | 33.3 |
| | | 112 | | 28.5 | 27.5* | 30.0 |
| Panasea seaweed extract‡ | 2.5 ml | 0 | | 28.5 | 28.8* | 33.5 |
| | | 112 | | 29.0 | 25.5 | 36.5 |
| Aqua Gro | 2.5 ml | 0 | | 23.3 | 21.0 | 30.8 |
| None | 0 | 56 | | 26.5 | 23.8 | 29.5 |
| | 0 | 112 | | 30.5 | 39.0* | 28.0 |
| | 0 | 112 | (Urea)† | 26.3 | 24.3 | 37.5 |
| Control | 0 | 0 | | 21.8 | 25.7 | 26.8 |
| LSD (Dunnett's, 0.05) | | | | NS | 15.0 | NS |
| Grand mean | | | | 26.6 | 28.2 | 32.3 |

* indicates means within the same column are placed in the "significantly higher" group at the 0.05 probability level according to Hsu's procedure. Means within the same column followed by the letter "a" are significantly different from the control at the 0.05 probability level according to Dunnett's test.

†Additions were urea applied at 980 mg $m^{-2}$ and soil wetting agent Aqua Gro (Aquatrols Corp., Pennsauken, NJ) at 2.5 ml product $m^{-2}$.

‡Levels of MZ63 cold water seaweed extract (Agrimar Corp., Atlanta, GA) and Panasea hot water seaweed extract (Emerald Isle, Ltd., Ann Arbor, MI) expressed in ml product $m^{-2}$.

strength, although BA plus Fe, propiconazole at 84 mg $m^{-2}$ or 42 mg $m^{-2}$, and the combination of triadimefon plus Fe plus urea were near the 22 kg value (Table 3). These data indicated the triazoles, propiconazole and triadimefon, tended to have better long-term response than treatments of MZ63 (seaweed extract) and Aqua Gro (soil wetting agent). Iron applied at 112 mg $m^{-2}$ caused a larger sod strength increase than Fe applied at 56 mg $m^{-2}$. Again, this suggested the higher rate of Fe was required for long term response.

## Repeated fall and spring applications (Trial 2)

***Repeated applications of Fe, BA, and other materials to Kentucky bluegrass sod on 12 November 1986 and 8 April 1987. Root lifts and sod strength measured 13 June and 15 June 1987.***

The ANOVA of root growth and sod strength data in the repeated application experiment was significant. As in previous studies, treatment with the triazoles significantly increased rooting. Increased rooting appeared to be a non-target effect of the treatment and apparently was not due to fungicidal activity. Sod treated with propiconazole at 84 mg $m^{-2}$ or triadimefon at 150 mg $m^{-2}$ had the largest vertical root lift measurements of any treatments and were the only treatments significantly different from the control (Table 2). Vertical root lift data again indicated the application of Fe at 112 mg $m^{-2}$ to bluegrass in both fall and spring resulted in more root growth than Fe applied at 56 mg $m^{-2}$.

Hsu's procedure placed the control treatment in the grouping of best sod strength treatments. Therefore, Dunnett's test comparing all other treatments with the control

Table 3. Sod strength of Kentucky bluegrass following single applications of Fe, benzyladenine (BA), and other materials on 12 November 1986 or 8 April 1987, or repeated applications on both dates. Sod strength measured 15 June 1987.

| Substance | | Iron | | Application | | |
|---|---|---|---|---|---|---|
| | | | | Single | Fall + | Single |
| Material applied | Level | level | | fall | spring | spring |
| | mg a.i. $m^{-2}$ | | | kg 30 $cm^{-1}$ | | |
| BA | 6 | 0 | | 16.5 | 19.1* | 17.8* |
| | | 112 | | 20.9 | 13.9 | 17.3* |
| Propiconazole | 21 | 0 | | 16.6 | 16.9* | 19.1* |
| | 42 | 0 | | 19.4 | 21.3* | 19.0* |
| | | 112 | | -- | -- | 15.3* |
| | 84 | 0 | | 19.8 | 22.4* | 16.4* |
| Triadimefon | 150 | 0 | | 16.4 | 14.9* | 13.8 |
| | | 0 | (Aqua Gro)† | 14.9 | 16.9* | 11.0 |
| | | 112 | | 16.4 | 17.3* | 21.5* |
| | | 112 | (Urea)† | 19.1 | 18.8* | 15.0* |
| MZ63 seaweed extract‡ | 0.08 ml | 0 | | 14.4 | 15.1 | 16.0* |
| | | 112 | | 16.0 | 18.3* | 16.3* |
| | 0.16 ml | 0 | | 17.8 | 15.4 | 13.1 |
| | | 112 | | 14.0 | 17.0* | 13.9* |
| | 0.32 ml | 0 | | 18.4 | 18.5* | 15.8* |
| | | 112 | | 14.3 | 16.6* | 14.9* |
| Panasea seaweed extract‡ | 2.5 ml | 0 | | 15.4 | 19.1* | 14.5* |
| | | 112 | | 17.1 | 19.5* | 15.6* |
| Aqua Gro | 2.5 ml | 0 | | 13.6 | 18.4* | 12.4 |
| None | 0 | 56 | | 13.4 | 17.5* | 17.3* |
| | 0 | 112 | | 18.3 | 18.3* | 12.3 |
| | 0 | 112 | (Urea)† | 14.5 | 16.1* | 14.0* |
| Control | 0 | 0 | | 15.4 | 16.4* | 15.0* |
| Grand mean | | | | 16.5 | 17.6 | 15.5 |

* indicates means within the same column are placed in the "significantly higher" group at the 0.05 probability level according to Hsu's procedure.

†Additions were urea applied at 980 mg $m^{-2}$ and soil wetting agent Aqua Gro (Aquatrols Corp., Pennsauken, NJ) at 2.5 ml product $m^{-2}$.

‡Levels of MZ63 cold water seaweed extract (Agrimar Corp., Atlanta, GA) and Panasea hot water seaweed extract (Emerald Isle, Ltd., Ann Arbor, MI) expressed in ml product $m^{-2}$.

was not performed. Although not significant, trends indicated greater sod strength for the applications of propiconazole at 42 or 84 mg $m^{-2}$ (Table 3).

## Single spring application (Trial 2)

***Application of Fe, BA, and other materials to Kentucky bluegrass sod on 8 April 1987. Vertical root lifts made 13 June 1987 and sod strength measured 15 June 1987.***

Root growth data was not significantly different for the single April application according to ANOVA. Therefore, only treatment means are presented for this data (Table 2). Significant differences in sod strength data were indicated by ANOVA and Dunnett's test and Hsu's procedure were performed for this data (Table 3).

Sod strength data for the single April application in trial 1 showed sod treated with the triazoles, triadimefon and propiconazole, and the synthetic cytokinin, BA, had the highest sod strength values. However, Hsu's procedure once again included the control in the grouping of best treatments. For this reason, Dunnett's procedure was not performed. Unlike previous tests, Fe applied at 56 mg $m^{-2}$ resulted in larger sod strength values than Fe applied at 112 mg $m^{-2}$ (Table 3).

# *Discussion*

Hsu's procedure grouped treatments that had the largest post-transplant root growth and sod strength. However, the placement of treatments in the groups was not consistent throughout the studies. Certain treatments and levels of Fe, BA, and other materials were selected for further field, greenhouse, and laboratory studies to examine the morphological and physiological responses of treated Kentucky bluegrass (Table 4). These materials provided the most consistent improvement of root and shoot growth. Consideration was also given to the necessity of selecting levels of Fe, BA, and other materials that would be cost-effective and still provide enhanced Kentucky bluegrass growth response.

Foliar applications of propiconazole at 42 mg $m^{-2}$ or triadimefon at 150 mg $m^{-2}$ consistently stimulated post-transplant root growth and sod strength in both trials. The stimulation appeared to be a non-target growth regulating effect, since no signs of disease were observed in the field plots. Applications of propiconazole at 0.42 mg $m^{-2}$ or triadimefon at 150 mg $m^{-2}$ have been reported to increase photosynthetic (Ps) rates and chlorophyll (Chl) concentrations of soybeans and wheat (Kettlewell et al., 1982; Davies et al., 1984; Gautam et al., 1984). Propiconazole and triadimefon were also reported to increase yields of wheat, soybeans, and peas by stimulating plant growth (Davies et al., 1984; Fletcher and Nath, 1984). Levels of propiconazole and triadimefon selected for further study are recommended use levels for disease control. The non-target growth regulator-like activity of triazoles on Kentucky bluegrass turf was previously reported by Kane and Smiley (1983). The promotion of Kentucky bluegrass rooting and sod strength with these materials could be a result of increased Ps rates. Growth stimulation

Table 4. Various combinations of Fe, BA, and other materials selected for further field, greenhouse, and laboratory studies on Kentucky bluegrass.

| Material | Level | Iron level |
|---|---|---|
| | mg a.i. $m^{-2}$ | |
| BA | 6 | 0 |
| | | 112 |
| Propiconazole | 42 | 0 |
| | | 112 |
| Triadimefon | 150 | 0 |
| | | 112 |
| MZ63 seaweed extract† | 0.32 ml | 0 |
| | | 112 |
| None | 0 | 0 |
| | | 112 |

†The level of MZ63 cold water seaweed extract (Agrimar Corp., Atlanta, GA) is expressed in ml product $m^{-2}$.

also could be due to increased cell division and elongation resulting from cytokinin-like activities of the chemicals.

Single and repeated applications of BA tended to stimulate Kentucky bluegrass rooting and sod strength. Still, the rooting and sod strength responses were generally less than those obtained with applications of propiconazole or triadimefon. Kane and Smiley (1983) reported BA increased leaf extension rates and Chl retention of 'Merion' Kentucky bluegrass. However, other researchers reported synthetic cytokinins such as BA were markedly more effective in delaying leaf senescence than promoting plant growth (Varga and Bruinsma, 1973; Tao et al., 1983). Benzyladenine has been shown to increase Ps rates and Chl concentrations of many plants (Adedipe et al., 1971; Chernyad'ev et al., 1984; Gautam et al., 1984). Again, increased rooting and sod strength by BA could have been caused by stimulated cell division and elongation or increased Ps.

Foliar applications of MZ63 cold water seaweed extract generally tended to increase post-transplant rooting and sod strength. Selection of the optimum level was difficult. Although not always significant, MZ63 applied at 0.08 ml $m^{-2}$ or 0.32 ml $m^{-2}$ increased rooting and sod strength. The 0.08 ml level of MZ63 would be less costly for producers, but other research (data not shown) has indicated very little growth promotion from the 0.08 ml level of MZ63. Rooting and sod strength values following treatment with 0.32 ml $m^{-2}$ were not always the largest, but the 0.32 ml level did consistently promote the two vigor indices measured in the experiments.

The combination of triadimefon plus Fe plus urea was very effective for the enhancement of post-transplant sod rooting and sod strength. The combinations of urea with Fe, BA, and the other materials are worthy of further research. The soil wetting agent, Aqua Gro, and the hot water seaweed extract, Panasea, caused some increases in

Kentucky bluegrass rooting and sod strength, but generally did not stimulate growth as much as the other materials. Prior research has shown that non-ionic surfactants like Aqua-Gro influence plant growth in addition to improving soil wetting characteristics (Westwood and Batjer, 1958; Stowe, 1960; Parr and Norman, 1964). The high application levels of Panasea as compared to the much lower levels of MZ63 suggest MZ63 probably has more PGR-like activity for promoting Kentucky bluegrass growth.

Chelated iron phosphate citrate applied on Kentucky bluegrass alone at 112 mg $m^{-2}$ generally increased Kentucky bluegrass rooting and sod strength as compared to Fe at 56 mg $m^{-2}$ or the untreated control. Kentucky bluegrass treated with Fe at 112 mg $m^{-2}$ in July and September in trial 1 had greater post-transplant root growth as compared to the single application of Fe at 112 mg $m^{-2}$ in September (Table 1). These data indicate an increase in rooting from repeated Fe applications made approximately 8 weeks apart. The increased rooting responses could have been due to the growth promotion from the foliar Fe treatment or possibly the extended growing period of the sod receiving repeat Fe applications.

General trends indicated Fe applied at 112 mg $m^{-2}$ in the fall and spring in trial 2 increased rooting as compared to single Fe applications in either the fall or spring, although significant differences were not always observed (Table 2). Post-transplant root growth (as indicated by vertical lifts) following a single application of Fe at 112 mg $m^{-2}$ in the spring was similar to root growth measurements recorded for bluegrass treated with the same level of Fe in the fall. This suggests that fall Fe fertilization could stimulate Kentucky bluegrass rooting the following spring. The addition of urea to Fe applied at either 56 or 112 mg $m^{-2}$ did not increase rooting for either fall or repeated fall and spring applications (Table 2). Although not significantly different, the addition of

urea to Fe at 112 mg $m^{-2}$ improved rooting in the single spring application as compared to Fe alone at 112 mg $m^{-2}$.

Sod strength data following single and repeated applications of chelated iron in trial 2 were variable (Table 3). The single application of Fe at 112 mg $m^{-2}$ in April caused lower sod strength than the untreated control. In contrast, sod strength for the repeated applications of Fe in the fall and spring were higher than sod strength values of the single spring application.

The combinations of Fe plus BA and the other materials provided mixed results for post-transplant sod rooting and sod strength. The addition of Fe at 112 mg $m^{-2}$ to triadimefon applied at 150 mg $m^{-2}$ generally increased rooting and sod strength as compared to triadimefon applied alone, although many of the differences were not significant. The addition of Fe to MZ63 at all levels generally stimulated root growth and sod strength. The combination of Fe plus propiconazole at 42 mg $m^{-2}$ was included in trial 2 for the April application only. Trends indicated post-transplant root growth for the Fe plus propiconazole combination was enhanced as compared to propiconazole applied alone, but sod strength for the combination of Fe plus propiconazole was less than sod strength of Kentucky bluegrass treated with propiconazole alone (Tables 2 and 3).

The treatment of BA plus Fe tended to decrease root growth as compared to BA applied alone in the single summer application in trial 1 (Table 1). Post-transplant rooting and sod strength data comparing BA alone and the combination of BA plus Fe in trial 2 were highly variable (Tables 2 and 3). The single November application of Fe plus BA tended to decrease rooting as compared to BA applied alone (Table 2), but enhanced sod strength (Table 3). While not always statistically significant, the combination of BA plus Fe applied either in the fall and spring or the spring alone appeared to

increase Kentucky bluegrass rooting and decrease sod strength as compared to BA applied alone. The variable rooting and sod strength responses following treatment with BA plus Fe suggested the possibility of an interaction between the synthetic cytokinin and the chelated Fe. Research has indicated some chelates have auxin-like activity when applied at low concentrations (Heath and Clark, 1956a, 1956b; Thimann and Takahashi, 1958; Wallace et al., 1962). No information was available concerning the likelihood of an adverse interaction between BA and the chelated Fe phosphate citrate used in this study.

Previous reports indicated field applications of BA or materials with PGR-like activity on Kentucky bluegrass provided mixed results. Kane and Smiley (1983) proposed inconsistent field responses to applications of triadimefon and etaconazole could have been caused by chemical binding to organic matter, photoinstability, volatilization from leaf surfaces, and microbial breakdown. The same factors could have caused the variable Kentucky bluegrass responses observed in our studies. However, the levels of triadimefon and etaconazole applied by Kane and Smiley (1983) were two to four times larger than the levels applied in these trials, and the possibility existed that plant growth was decreased in their studies due to phytotoxic effects of supraoptimal application levels.

# Chapter 4

# Rooting and Sod Strength of a Mature Kentucky Bluegrass Sod Following Applications of Iron, Benzyladenine, and Other Materials

## *Introduction*

Field studies initiated in the summer of 1987 were performed to determine:

i) whether benzyladenine (BA), two triazoles, propiconazole and triadimefon, or MZ63 seaweed extract could improve the sod strength and subsequent rooting of transplanted Kentucky bluegrass sod.

ii) if applications of a chelated Fe compound either alone or in combination with BA, the triazoles, or MZ63 seaweed extract could stimulate Kentucky bluegrass rooting and sod strength.

## *Materials and Methods*

The experiments were performed in the summer of 1987 on a September 1986 seeded 'Plush' Kentucky bluegrass sod established on a Lodi silt loam (pH 6.4) at the Turfgrass Research Center, Blacksburg, VA. At the time of harvest, this area was considered to be a mature sod acceptable for harvest. Nitrogen, $P_2O_5$, and $K_2O$ were applied at seeding at 49 kg $ha^{-1}$ each, with a 10-10-10 fertilizer. Additional N was applied at 49 kg $ha^{-1}$ in March 1987. The turf was maintained at a mowing height of 3.8 cm and was irrigated as needed to promote turf establishment. Foliar treatments of various combinations of Fe, BA, triazoles, and MZ63 seaweed extract were applied to 0.9-m by 1.8-m plots randomized in a complete block. A compressed-air boom sprayer that delivered 748 L $ha^{-1}$ at 276 kPa was used for foliar applications.

Treatments for experiment 1 were applied on 7 July 1987 and replicated four times (Table 5). Kentucky bluegrass sod pieces 30-cm by 30-cm were harvested on 14 July with a sod harvester cutting at a depth of 2.2 cm and sod was placed on 900 $cm^2$ expanded metal squares set in a newly prepared soil area as described in Chapter 3. Roots of the sod were permitted to grow through the metal squares into the soil. Irrigation was applied as needed at establishment to prevent the sod from drying. On 11 August, the metal squares were vertically lifted by the technique described in Chapter 3. A sec-

ond Kentucky bluegrass sod harvest was made on 4 August 1987. The sod was cut at a depth of 1.3 cm instead of 2.2 cm due to extremely dry soil conditions. Transplanted sod squares were grown until 16 September when vertical root lifts were made. Sod strength of the 7 July treated sod was measured on 4 August and again on 31 August by the methods described in Chapter 3.

Treatments applied in experiment 2 on 24 August were replicated three times (Table 5). Sod was harvested on 1 September and transplanted on metal squares in a newly prepared planting area as previously described. Vertical lift force to determine post-transplant root growth was measured on 29 September. Sod strength was measured on 21 September and again on 19 October by the method described in Chapter 3.

The treatments for experiment 3 were also applied on 24 August. Treatments and levels listed in Table 5 were replicated eight times. Sod was harvested 31 August and sod squares transplanted and established as previously described. Vertical lift force was measured on 29 September. Sod strength was measured 24 September and again on 19 October. Sod was harvested at a depth of 1.3 cm on 19 October due to dry soil conditions.

The design in each experiment was a randomized complete block, two-factor factorial with BA, the triazoles, or MZ63, and the other factor being the Fe chelate. The ANOVA were performed for each parameter of each experiment and treatment means were separated by calculated LSD values.

Table 5. Various combinations of Fe, BA, triazoles and MZ63 seaweed extract applied to Kentucky bluegrass to determine post-transplant rooting and sod strength responses in experiments 1, 2, and 3.

| Material | Level | Iron level | Experiment† |
|---|---|---|---|
| | —— mg a.i. $m^{-2}$— | | |
| BA | 6 | 0 | 1, 2 |
| | | 112 | 1, 2 |
| Propiconazole | 42 | 0 | 1, 2, 3 |
| | | 112 | 1, 2, 3 |
| Triadimefon | 150 | 0 | 1, 2, 3 |
| | | 112 | 1, 2, 3 |
| MZ63‡ | 0.32 ml | 0 | 1 |
| | | 112 | 1 |
| None | 0 | 0 | 1, 2 ,3 |
| | | 112 | 1, 2, 3 |

†The numbers 1, 2, and 3 indicate the experiments in which the treatments were included.

‡The level of MZ63 cold water seaweed extract (Agrimar Corp., Atlanta, GA) is expressed in ml product $m^{-2}$.

# *Results*

## July application of Fe, BA, triazoles, and MZ63 (Experiment 1)

***Kentucky bluegrass growth responses from combinations of Fe, BA, triazoles, and MZ63 seaweed extract on 7 July 1987. Root lifts made on 11 August and 16 September and sod strength measured 4 August and 31 August 1987.***

The 11 August vertical root lift of sod squares transplanted on 14 July indicated treatments of propiconazole, triadimefon, and MZ63 seaweed extract significantly increased root growth of Kentucky bluegrass treated 1 week before transplanting (Table 6). Root lift values of sod squares treated with these materials were approximately twice as great as sod squares not treated. Benzyladenine treatment did not significantly increase post-harvest rooting of Kentucky bluegrass sod. The mean values for BA treated sod were only slightly higher than those for sod squares receiving no materials. Combinations of Fe plus BA, triazoles, or MZ63 did not increase rooting (Table 6). The mean root lift values of sod receiving Fe treatment were actually lower than the mean values for untreated sod.

The 16 September vertical root lift indicated propiconazole caused a more significant increase in Kentucky bluegrass rooting than any other treatment (Table 6). Triadimefon-treated had better root growth than sod receiving no material with plant growth regulator-like (PGR-like) activity. Mean root lift values of sod treated with MZ63 or BA did not differ significantly from the untreated sod. Again, the Fe applica-

Table 6. Post-transplant rooting and sod strength following foliar applications of combinations of Fe, BA, triazoles and MZ63 seaweed extract on 7 July 1987 in experiment 1.

| Material applied | Date of observation: 11 Aug. Fe (mg $m^{-2}$) 0 | 112 | avg. | 16 Sept. Fe (mg $m^{-2}$) 0 | 112 | avg. | 4 Aug. Fe (mg $m^{-2}$) 0 | 112 | avg. | 31 Aug. Fe (mg $m^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | kg 900 $^{-1}$ $cm^{-2}$ Root lift | | | | | | kg 30 $cm^{-1}$ Sod strength | | | | | |
| BA | 19.3 | 17.3 | 18.3B‡ | 28.5 | 30.3 | 29.4BC | 12.4 | 12.0 | 12.2A | 10.4 | 11.8 | 11.1B |
| Propiconazole | 31.5 | 31.8 | 31.6A | 38.3 | 44.0 | 41.2A | 10.0 | 10.0 | 10.0B | 15.1 | 15.1 | 15.1A |
| Triadimefon | 39.8 | 33.0 | 36.4A | 38.0 | 27.5 | 32.8B | 8.8 | 9.3 | 9.1B | 15.3 | 15.8 | 15.6A |
| MZ63 seaweed extract | 32.5 | 30.3 | 31.4A | 30.8 | 29.5 | 30.2BC | 9.3 | 9.9 | 9.6B | 10.5 | 11.9 | 11.2B |
| None | 19.3 | 14.3 | 16.8B | 23.5 | 24.0 | 23.8C | 10.1 | 8.9 | 8.5B | 11.8 | 11.6 | 11.7B |
| avg. | 28.5a§ | 25.3a | -- | 31.8a | 31.1a | -- | 10.1a | 10.0a | -- | 12.6a | 13.2a | -- |
| Fe x material | | NS† | | | NS | | | NS | | | NS | |
| LSD¶ | | 9.6 | | | 7.2 | | | 1.7 | | | 2.5 | |

†NS = not significant.

‡Upper case letters represent statistical differences among materials, averaged over Fe treatments, within the same observation date. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Lower case letters represent statistical difference between Fe treatments across all material treatments within the same observation date. Means not followed by the same letter are significantly different at the 0.05 probability level.

¶Least significant difference at the 0.05 probability level among materials, averaged over Fe treatments, within the same observation date.

tion with and without BA, the triazoles, or MZ63 did not enhance post-transplant rooting of a sod considered to be suitable for harvest.

The sod strength measurements of 4 August indicated only the BA treatment caused a significant increase in sod development. All sod strength values measured were lower than anticipated. The lower numbers were a result of harvesting the sod at a cutting depth of 1.3 cm instead of 2.2 cm.

An overall increase in sod strength was observed with treatments of propiconazole and triadimefon for the measurements made approximately 8 weeks following chemical application in July. The increase in sod strength could have been caused by an increase in tiller and rhizome prodution triggered by the chemical applications. The data indicate a difference in sod strength of Kentucky bluegrass probably could not be observed until somewhere in the period of 4 to 8 weeks after treatment.

Overall analyses of the data from experiment 1 indicated propiconazole or triadimefon applied alone provided the most consistent increases in post-transplant rooting and sod strength of the materials with PGR-like activity (Table 6). MZ63 treatment significantly increased rooting 4 weeks after transplanting, but no sustainable rooting difference compared to the control was obtained at 8 weeks. MZ63 did not enhance sod strength 4 or 8 weeks after treatment. Benzyladenine treatments did not promote root growth according to vertical root lifts on either 11 August or 16 September. A small increase in sod strength was observed on 4 August, but the response was not sustained through the 31 August harvest. Iron treatment did not enhance sod strength or post-transplant rooting over the control regardless of whether applied alone or in combination with BA, MZ63, propiconazole, or triadimefon.

## August applications of Fe, BA, triazoles, or MZ63 (Experiment 2)

***Kentucky bluegrass responses from treatments of Fe, BA, triazoles, and MZ63 seaweed extract on 24 August 1987. Root lift on 29 Sept. and sod strength measured 21 Sept. and 19 Oct. 1987.***

The vertical root lift of 29 September again showed propiconazole or triadimefon increased root growth of Kentucky bluegrass approximately 5 weeks after treatment (Table 7). Benzyladenine resulted in root lift values that were not significantly different from sod receiving no chemical treatment. Iron chelate treatment actually decreased post-transplant root growth of treated Kentucky bluegrass sod whether applied alone or in combination with BA, the triazoles or MZ63 seaweed extract. The decrease in sod rooting was observed for the addition of Fe to any other material as well as for the overall Fe response.

Soil moisture conditions for the 21 September and 19 October sod strength measurements were sufficient to allow cutting of sod at the 2.2 cm depth. The deeper sod cut resulted in greater sod strength values than those reported for the July application. Propiconazole was the only material that significantly increased sod strength (Table 7). However, there was no apparent increase in overall sod strength values from 21 September to 19 October. This indicated the growth promoting response of propiconazole was maintained, but no further enhancement in sod strength occurred between 4 and 8 weeks. Neither BA or triadimefon increased sod strength at the 4 or 8 week harvests when compared to the untreated sod. Once again, Fe plus BA, triazoles, or MZ63 treatments and the Fe treatments alone did not enhance sod strength (Table 7).

Table 7. Post-transplant rooting and sod strength following foliar applications of Fe, BA, and triazoles on 24 August 1987 in experiment 2.

| Material applied | Date of observation: 29 Sept. Fe (mg m$^{-2}$) 0 | 112 | avg. | 21 Sept. Fe (mg m$^{-2}$) 0 | 112 | avg. | 19 Oct. Fe (mg m$^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|
| | kg 900 $^{-1}$ cm$^{-2}$ Root lift | | | kg 30 cm$^{-1}$ Sod strength | | | | | |
| BA | 45.3 | 33.5 | 39.4B‡ | 19.0 | 14.7 | 16.8B | 18.3 | 17.0 | 17.7B |
| Propiconazole | 60.2 | 49.0 | 54.6A | 23.2 | 25.5 | 24.3A | 23.3 | 24.0 | 23.7A |
| Triadimefon | 54.3 | 52.3 | 53.3A | 14.8 | 19.5 | 17.2B | 16.7 | 17.0 | 16.8B |
| None | 40.2 | 32.0 | 36.1B | 17.8 | 19.5 | 18.6B | 18.0 | 20.0 | 19.0B |
| avg. | 50.0a§ | 41.7b | -- | 18.7a | 19.8a | -- | 19.1a | 19.5a | -- |
| Fe x material | | NS† | | | NS | | | NS | |
| LSD¶ | | 9.8 | | | 3.5 | | | 4.6 | |

†NS = not significant.

‡Upper case letters represent statistical differences among materials, averaged over Fe treatments, within the same observation date. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Lower case letters represent statistical difference between Fe treatments across all material treatments within the same observation date. Means not followed by the same letter are significantly different at the 0.05 probability level.

¶Least significant difference at the 0.05 probability level among materials, averaged over Fe treatments, within the same observation date.

## August applications of Fe and triazoles (Experiment 3)

*Kentucky bluegrass growth responses from treatments of Fe and triazoles on 24 August 1987. Root lift on 29 September and sod strength measured 24 September and 19 October 1987.*

This study included the triazole fungicides, triadimefon and propiconazole, their combination with Fe, and the treatment of Fe alone. Vertical root lift data measured on 29 September, approximately 5 weeks after treatment, indicated triadimefon and propiconazole stimulated post-transplant root growth of sod transplanted 1 week after treatment (Table 8). Triadimefon treatments tended to cause the largest increase in rooting when applied alone or with the addition of Fe. Root growth of Kentucky bluegrass sod with the combination of Fe plus propiconazole was not significantly different from application of propiconazole alone, and actually appeared to be reduced. Comparison of the overall root lift means of Fe-treated sod indicated no significant response from Fe treatment.

The data indicated propiconazole was the most active material applied for improving sod strength (Table 8). A significant increase in sod strength was observed with propiconazole in the 24 September harvest. Triadimefon did not increase sod strength over the chemically untreated Kentucky bluegrass. Again, Fe application with and without the triazoles did not increase sod strength.

The 19 October measurements of sod strength also showed propiconazole to be the only treatment that increased sod strength of Kentucky bluegrass (Table 8). The sod strength values for this harvest were once again lower due to dry soil conditions and the

Table 8. Post-transplant rooting and sod strength following foliar applications of Fe and triazoles on 24 August 1987 in experiment 3.

| Material applied | Date of observation: 29 Sept. Fe ($mg\ m^{-2}$) 0 | 112 | avg. | 24 Sept. Fe ($mg\ m^{-2}$) 0 | 112 | avg. | 19 Oct. Fe ($mg\ m^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|
| | $kg\ 900^{-1}\ cm^{-2}$ Root lift | | | $kg\ 30\ cm^{-1}$ Sod strength | | | | | |
| Propiconazole | 54.5 | 43.0 | 48.7A‡ | 21.9 | 23.1 | 22.5A | 19.0 | 16.5 | 17.8A |
| Triadimefon | 50.6 | 54.9 | 52.8A | 19.3 | 21.7 | 20.5B | 16.1 | 15.5 | 15.8B |
| None | 33.5 | 33.6 | 33.6B | 20.7 | 19.0 | 19.8B | 14.9 | 14.4 | 14.6B |
| avg. | 46.2a§ | 43.8a | -- | 20.6a | 21.3a | -- | 16.7a | 15.5a | -- |
| Fe x material | | NS† | | | NS | | | NS | |
| LSD¶ | | 8.6 | | | 2.0 | | | 2.6 | |

†NS = not significant.

‡Upper case letters represent statistical differences among materials, averaged over Fe treatments, within the same observation date. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Lower case letters represent statistical difference between Fe treatments across all material treatments within the same observation date. Means not followed by the same letter are significantly different at the 0.05 probability level.

¶Least significant difference at the 0.05 probability level among materials, averaged over Fe treatments, within the same observation date.

requirement for harvesting at a depth of 1.3 cm. Iron application did not increase sod strength values when applied either alone or with triadimefon or propiconazole.

## *Discussion*

Kentucky bluegrass treated with propiconazole at 42 mg $m^{-2}$ consistently had increased rooting and sod strength values as compared to untreated sod. Increases in rooting and sod strength following propiconazole treatment were observed at sod harvests approximately 4 and 8 weeks after treatment. Triadimefon applied at 150 mg $m^{-2}$ primarily enhanced post-transplant root growth of Kentucky bluegrass, but an enhancement of sod strength was also indicated in experiments 1 and 3 (Tables 6 and 8). The simultaneous promotion of Kentucky bluegrass rooting and sod strength from applications of the triazole fungicides, triadimefon and propiconazole, and the cold water seaweed extract, MZ63, suggested the growth-regulatory effects of these materials probably was not from direct cytokinin-like (CK-like) activity. Increases in sod strength from chemical treatments probably were caused by enhanced lateral shoot (rhizome and tiller) development. In this respect, the growth regulator activity of the fungicides was CK-like, since it has been documented that exogenous cytokinin applications promote lateral bud initiation (Heide, 1965; Ali and Fletcher, 1971; Lee et al., 1974; Goodwin and Morris, 1979). Many scientists also have reported exogenous cytokinin applications less than $10^{-6}$ M stimulated lateral root initiation, but applications greater than $10^{-6}$ M clearly inhibited root initiation and production (Heide, 1965; Goodwin and Morris, 1979; Hinchee and Rost, 1986). Exogenous applications of triazoles at the levels listed prob-

ably would be expected to inhibit rooting if their growth-regulator like activity was CK-like.

These data supported the research of Fletcher and Arnold (1986), who proposed the CK-like activity of triadimefon appeared to be a result of enhanced root growth (no basis for the enhancement presented) and the subsequent production of greater levels of endogenous cytokinins within the root. Fletcher and Arnold (1986) proposed the cytokinins could be transported throughout the plant and possibly stimulate foliar growth through promotion of lateral bud initiation. No work has been done to characterize the possible CK-like activity of propiconazole. However, the similarity of the chemicals suggests propiconazole's growth regulating activity could be the same as that proposed for triadimefon.

MZ63 seaweed extract treatment in experiment 1 increased post-transplant rooting of Kentucky bluegrass sod, but did not enhance sod strength 4 or 8 weeks after treatment. As was reported in Chapter 3, Kentucky bluegrass showed rooting and sod strength stimulation from MZ63 treatment, but the growth responses were variable. The promotion of both sod strength and rooting indicated the growth-regulatory activity of the MZ63 seaweed extract could be the same as that previously proposed for propiconazole and triadimefon. However, MZ63 contains natural auxins and gibberellins as well as natural cytokinins, and specific characterization of its response could be very difficult to determine.

Benzyladenine treatment did not significantly increase rooting of transplanted bluegrass sod in either experiment 1 or 2 (Tables 6 and 7). The lack of Kentucky bluegrass rooting response is consistent with previous reports that showed exogenous BA applications inhibited lateral root formation (Heide, 1965; Goodwin and Morris, 1979; Hinchee and Rost, 1986). Data did not indicate an inhibition of rooting from BA

application, nor did it indicate rooting stimulation. A significant increase in sod strength was reported on 4 August in experiment 2, but the stimulatory effect was not maintained through the 31 August measurement (Table 7). Exogenous BA applications stimulate lateral bud initiation (Heide, 1965; Ali and Fletcher, 1971; Lee et al., 1974; Goodwin and Morris, 1979). The overall lack of growth response of sod treated with BA corresponded with reports previously published by Tao et al. (1983) and Varga and Bruinsma (1973), in which BA applications were determined to act more effectively as anti-senescence materials than as growth substances. Other data obtained (see Chapters 5 and 6) examining the responses of Kentucky bluegrass to Fe, BA, and other materials support these reports.

Neither Fe applications alone or Fe applied in combination with BA, the triazoles, or MZ63 seaweed extract enhanced Kentucky bluegrass growth significantly over the control. Although not significant, Fe application generally decreased root and shoot growth in each experiment. The Fe source used in these studies was a chelated Fe phosphate citrate. Variable growth responses of cool-season turfgrasses to chelated Fe have been previously reported. Yust et al. (1984) reported no differences in clipping yields of Kentucky bluegrass treated with $FeSO_4$ or FeDTPA (diethylene-triaminepentaacetic acid), and no difference in clipping yields of Fe treated or untreated plots as well. Root growth of creeping bentgrass turf was increased with applications of FeDTPA when it was applied in association with high N fertilization in the late spring and summer months (Snyder and Schmidt, 1973; Schmidt and Snyder, 1984). However, the increased root yield was accompanied by a decrease in top growth. They further reported bentgrass receiving foliar applications of FeDTPA in the spring had diminished root growth as compared to foliar applications of $FeSO_4$.

Snyder (1975) proposed foliar applications of the chelate FeDTPA caused the accumulation of chelated iron in the roots of bentgrass and this accumulation limited the activity and translocation of Fe and possibly other elements in the roots. Other researchers have also reported decreased root and shoot growth of plants when chelated Fe compounds were supplied in nutrient solutions and as foliar applications (Wallace et al., 1957; Delap, 1970). The overall lack of growth response and even the decline in rooting and sod strength of Kentucky bluegrass from Fe treatment in our studies indicated summer applications of chelated Fe citrate were not beneficial when applied alone or with BA and the PGR-like materials. The same sequestering effect of the chelate suggested by Snyder (1975) could have caused the decline in Kentucky bluegrass growth following Fe treatment.

The activity and subsequent fate of the various chelating compounds used in Fe fertilizers is still subject to debate. Brown et al. (1961) and Tiffin (1970) reported the chelate clearly remained outside the roots when chelated Fe was supplied as the Fe source in nutrient solutions. Other researchers have reported the chelate could also move into the plant as well as the Fe (Wallace and Hale, 1962; Wallace et al., 1966). The chelated Fe phosphate citrate used in our studies is a similar compound to that reported by Tiffin (1970) to be the primary translocated form of Fe within plants. Tiffin supplied FeEDDHA (ethylenediaminedi-Q-hydroxyphenylacetic acid) in nutrient solution to soybeans and observed the EDDHA remained in the solution following Fe uptake. The Fe collected in xylem exudates was an Fe citrate chelate. This complex would seem to be quite similar to the chelated Fe phosphate citrate used in our studies. However, the similarities in composition between the Fe chelates would not guarantee rapid entry of the Fe phosphate citrate into the plant. As previously indicated, Tiffin (1970) reported the chelating agent EDDHA was not taken up by the roots of soybean,

and Brown (1961) reported chelating agents remained in solution instead of being absorbed. Still, Wallace et al. (1966) demonstrated the chelating agent could be absorbed. No information was available concerning the rate of foliar and root uptake of chelated Fe phosphate citrate. However, the generally negative rooting and sod strength growth responses observed with summer applications of Fe in our studies suggested some level of the chelate complex could have been absorbed by the plant and caused the sequestering phenomenon previously suggested by Snyder (1975). One troubling aspect with the hypothesis of reduced growth due to sequestering of Fe and other elements in our study is the similarity between the chelated Fe that was applied in our studies and that which is reported to be the primary translocated form in plants. Tiffin (1970) did not report any growth reduction from the Fe citrate complex collected from the xylem exudate of soybeans. More research is required to determine if the chelated Fe phosphate citrate compound functions similarly in the plant as the Fe citrate compound identified by Tiffin (1970). A determination of the degree of absorption of the chelated Fe phosphate citrate used in our study would be particularly useful.

Another possible explanation for the decrease in rooting and sod strength of Kentucky bluegrass from the combination treatments of Fe plus BA, triazoles, or MZ63 is the possibility of an adverse cytokinin and auxin interaction. Chelates have demonstrated auxin-like properties when applied in low concentrations (Heath and Clark, 1956a, 1956b; Thimann and Takahashi, 1958; Wallace et al., 1962). While no information was available on the auxin-like activities of chelated Fe phosphate citrate used in our studies, the possibility of an interaction with BA or the other materials with PGR-like activity can not be dismissed.

# Chapter 5

# Seedling Kentucky Bluegrass Response to Applications of Iron, Benzyladenine, Triazoles, and MZ63 Seaweed Extract

## *Introduction*

Studies were performed to examine the growth responses of seedling Kentucky bluegrass plants treated with iron (Fe), benzyladenine (BA) a synthetic cytokinin, two triazole fungicides with non-target plant growth regulator-like (PGR-like) activity, MZ63 seaweed extract, and various combinations. The objectives of the study were to determine:

i) if applications of BA or the other PGR-like materials are beneficial to Kentucky bluegrass seedling development as determined by various growth parameters.

ii) if the application of Fe alone is beneficial or if the combination of Fe plus BA or other materials might further promote seedling growth.

iii) the approximate length of time required for Kentucky bluegrass seedlings to show a measurable growth response to chemical application.

iv) if either photosynthetic (Ps) rates or cell division and elongation might respond to treatment with Fe, BA, and the other materials.

## *Materials and Methods*

Boxes 75 cm x 45 cm x 27 cm were constructed from plywood. The insides of the boxes were lined in heavy plastic, and two small holes were drilled through the plastic and the sides of the boxes 2.5 cm from the bottom. Water was added to fill the plastic-lined boxes to the 2.5 cm level. The box top had fifty 3.0-cm diameter holes cut in five rows of ten holes each. Fifty 30.5-cm pieces of 2.5-cm diameter flexible plastic tubing were sealed at one end with a square of cheesecloth attached with duct tape. As the tubes were filled with 200 g of a Groseclose silt loam soil (pH 6.4), the soil was firmed by vibrating the tubes with a modified electric jigsaw with a rubber stopper attached on its shaft. Soil bulk densities of the tubes were approximately 1.3 g $cm^{-3}$. Fifty soil-filled

tubes were placed in the holes of each box. The tubes were irrigated from overhead to insure thorough wetting throughout the length of the tube in addition to subirrigation through the base of the tube.

Kentucky bluegrass ('Georgetown') seed were sown at 16 kg $ha^{-1}$ in 10-cm diameter plastic cups filled with a Groseclose silt loam soil (pH 6.4). Seeded cups were irrigated as needed to prevent drying. Individual cups of seedling Kentucky bluegrass at the two to three leaf stage were sprayed with one of the treatments listed in Table 9. Treatments were applied with a compressed-air boom sprayer as previously described (Chapters 3 and 4).

Individual seedlings of Kentucky bluegrass were transplanted into the tubes one day following treatment. The seedlings received no fertilizer applications for the duration of the study and were watered by subirrigation through the bottom of the tube, except during the first week of the study when overhead irrigation was necessary to prevent seedlings from wilting. The experimental design was completely randomized with ten replications for single-measurement experiments 1 and 2, and measurements were made 4 weeks after transplanting. An ANOVA was performed and treatment means were compared by a calculated least significant difference (LSD) value. The experimental designs for single-measurement experiments 3 and 4, and sequential-measurement experiments 5 and 6, were completely randomized two-factor factorials. Data collection for the Kentucky bluegrass plants in experiments 3 and 4 were made 4 and 6 weeks, respectively, after transplanting. Sequential measurements in experiments 5 and 6 were made on three replications at 2 and 4 weeks after transplanting, while four replications were harvested after 6 weeks in the sequential-measurement experiments.

Experiments 1, 4, and 5 were conducted outside the Turfgrass Research Center at Blacksburg, VA during spring and summer months. Supplemental irrigation was applied

Table 9. Various combinations of Fe, BA, triazoles and MZ63 seaweed extract applied to Kentucky bluegrass seedlings at the two-leaf growth stage to determine morphological and physiological responses.

| Material | Level | Iron level |
|---|---|---|
| | mg a.i. $m^{-2}$ | |
| BA | 6 | 0 |
| | | 112 |
| Propiconazole | 42 | 0 |
| | | 112 |
| Triadimefon | 150 | 0 |
| | | 112 |
| MZ63 seaweed extract† | 0.32 ml | 0 |
| | | 112 |
| None | 0 | 0 |
| | | 112 |

†The level of MZ63 cold water seaweed extract (Agrimar Corp., Atlanta GA) is expressed in ml product $m^{-2}$.

as needed. Experiments 2, 3, and 6, were performed in the greenhouse during winter and early. spring. Average greenhouse temperatures were 23°C in the day and 20°C at night. No supplemental lighting was used in any experiment. Overhead irrigation by a misting system was applied as needed to prevent wilting. All seedlings were monitored throughout the experiments for signs of pathogen-incited disease. No observable signs of disease were observed in any experiment.

The treatments in experiment 1 were chelated Fe phosphate citrate applied alone at 112 mg $m^{-2}$, MZ63 cold water seaweed extract at 0.32 ml $m^{-2}$, and triadimefon at 150 mg $m^{-2}$ applied alone and plus Fe at 112 mg $m^{-2}$. The treatments and levels applied to Kentucky bluegrass in experiment 2 were Fe applied alone at 112 mg $m^{-2}$, propiconazole at 42 mg $m^{-2}$ with and without Fe, and triadimefon alone at 150 mg $m^{-2}$. The materials and levels of application applied to seedlings in experiments 3, 4, 5, and 6 were as listed in Table 9.

Comparisons of the treatment means were made by relating only the simple effects of one factor across all levels of the other factor when the ANOVA indicated a significant iron x material interaction, and significant F values for treatment responses. Means of the main effects of a factor averaged across all levels of the other factor were separated by a calculated LSD value for significant treatment responses when there was no significant Fe x material interaction. When Fe x material interaction was significant, the differences in Kentucky bluegrass responses to BA and PGR-like materials with and without Fe were reported for each material applied. Differences in means of BA and the other materials with PGR-like activity with and without Fe were compared with a calculated LSD value when Fe x material interaction was significant.

Entire plants were harvested by removing the tube from the seedling box, detaching the cheese cloth from the base of the tube, and inserting the end of a garden hose nozzle

into the tube base. The intact soil column was forced out of the tube by water pressure, placed on a wire screen, and the soil was gently washed away. Measurements made were the total number of leaves initiated from the original transplant and tillers, the number of lateral buds (both rhizomes and tillers), and root length. Roots and shoots were separated, dried for 12 hours in an oven at 35°C, and dry weights were recorded.

Carbon dioxide exchange rates (CER) of Kentucky bluegrass seedlings were determined in the 6-week sequential-measurement study (Experiment 6). The entire shoot of plants harvested at 2- and 4-week intervals was immediately placed in the Ps measurement chamber and CER determined. However, the large plant size at the 6-week measurement did not permit the entire plant to be placed in the chamber. Therefore, subsamples of the youngest fully expanded leaves of the large plants were harvested and CER measurements were made on these tissues. Subsamples of Kentucky bluegrass leaf tissue were weighed separately from the rest of the harvested material. Photosynthetic rates were adjusted to reflect the entire mass of shoot tissue harvested from the tube after dry weights of the Ps subsample and remaining shoot tissue were determined.

Leaf tissue CER was monitored by measuring the changes in $CO_2$ concentration in an open system. Leaf tisue was placed in a Decagon LD-2 (Decagon Inc., Pullman, WA) leaf disc electrode unit with an effective chamber volume of 5 $cm^3$. This chamber was designed for direct measurement of Ps $O_2$ evolution, but numerous trials indicated its use for carbon dioxide exchange instead of $O_2$ evolution provided Ps data with much less variability. Air flow into the sample chamber and reference line was 0.05 L $min^{-1}$. Differential $CO_2$ concentrations were monitored by passing the air lines through an infrared gas analyzer (model AR-600, Anarad Inc., Santa Barbara, CA). The light source for CER measurements was a 400 W bulb, focused through a 150 mm lens, delivering 750 $\mu$mol $m^{-2}$ $s^{-1}$. The light source was mounted 30.5 cm above the top of the cham-

ber. Carbon dioxide levels were recorded after steady states of carbon dioxide exchange in the light and dark were recorded. Carbon dioxide exchange rates were calculated and expressed in units of $\mu$moles $CO_2$ fixed $m^{-2}$ land area of the tube $s^{-1}$ and $\mu$moles $CO_2$ fixed $g^{-1}$ leaf dry weight $s^{-1}$. Temperature of the LD-2 chamber and of the leaf tissue was maintained at 20°C by pumping cooled water through the water-jacketed chamber.

# *Results*

## Four-week seedling growth following applications of various combinations of Fe, triadimefon and MZ63 (Experiment 1)

***Seedlings treated 28 July 1986 and measured 25 August 1986.***

Iron at 112 mg $m^{-2}$ or MZ63 at 0.32 ml $m^{-2}$ caused significant increases in Kentucky bluegrass leaf number, bud initiation, root length, and root dry weights as compared to the untreated control (Table 10). Triadimefon applied alone resulted in a significantly larger number of leaves for transplants over the untreated controls, but no significant increases for the other parameters measured were observed. The combination of triadimefon plus Fe resulted in significant increases in leaf number and shoot dry weights of Kentucky bluegrass plants over the control plants. All chemical and Fe treatments tended to increase seedling development, although all differences were not statistically different (Table 10).

Table 10. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe, triadimefon, and MZ63 on 28 July 1986 in experiment 1. Growth measurements made 25 August 1986.

| Material applied | Level | Iron level | Leaves | Lateral buds | Shoot dry weight | Root dry weight | Root length |
|---|---|---|---|---|---|---|---|
| | mg —a.i. $m^{-2}$— | | no. — $plant^{-1}$ — | | mg — $plant^{-1}$ — | | cm $plant^{-1}$ |
| MZ63† | 0.32 ml | 0 | 4.5ab* | 0.8ab | 13.5ab | 20.6ab | 20.1a |
| Triadimefon | 150 | 0 | 4.2b | 0.4bc | 13.1ab | 13.4bc | 18.1abc |
| | | 112 | 4.5ab | 0.4bc | 17.7a | 19.3abc | 17.0bc |
| None | 0 | 0 | 3.3c | 0.0c | 9.5b | 12.4c | 15.9c |
| | | 112 | 5.3a | 1.1a | 13.3ab | 22.6a | 18.8ab |
| LSD (0.05) | | | 0.9 | 0.6 | 2.8 | 7.3 | 5.4 |

*Means within the same column not followed by the same letter are significantly different at the 0.05 probability level.

†The level of MZ63 cold water seaweed extract (Agrimar Corp., Atlanta, GA) is expressed in ml product $m^{-2}$.

## Four-week seedling growth responses following applications of various combinations of Fe, propiconazole, and triadimefon (Experiment 2)

*Seedlings treated 17 January 1987 and measured 21 February 1987.*

The combination of propiconazole plus Fe resulted in the most consistent stimulation of Kentucky bluegrass seedling root and shoot development (Table 11). Iron in combination with propiconazole enhanced seedling development for all growth parameters measured as compared to propiconazole applied alone. Triadimefon applied alone caused a significant increase in leaf number and root dry weight of Kentucky bluegrass when compared to the control. Iron applied alone tended to increase Kentucky bluegrass root and shoot development, but the stimulation was not statistically significant. Propiconazole applied alone or with Fe, and triadimefon applied alone increased root length (Table 11).

Few lateral buds were initiated in this experiment regardless of material application. The rate of tillering in cool-season grasses is usually highest in the spring and fall, and rhizome development is at its highest level in the spring, late summer, and fall (Beard, 1973). The combination of short day length in the winter and warm greenhouse temperatures possibly was not conducive to lateral bud initiation.

Table 11. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe and triazoles on 17 January 1986 in experiment 2. Growth measurements made 21 February 1986.

| Material applied | Level | Iron level | Leaves | Lateral buds | Shoot dry weight | Root dry weight | Root length |
|---|---|---|---|---|---|---|---|
| | mg —a.i. $m^{-2}$— | | no. —plant$^{-1}$— | | mg —plant$^{-1}$— | | cm plant$^{-1}$ |
| Propiconazole | 42 | 0 | 5.4ab* | 0.0b | 2.4b | 3.8b | 12.8a |
| | | 112 | 6.0a | 0.4a | 5.6a | 5.8a | 14.5a |
| Triadimefon | 150 | 0 | 5.8a | 0.1ab | 3.5b | 5.8a | 12.9a |
| None | 0 | 0 | 4.6b | 0.0b | 2.1b | 13.4a | 3.3b |
| | | 112 | 5.1ab | 0.0b | 3.5b | 13.6a | 4.1b |
| LSD (0.05) | | | 0.9 | 0.3 | 2.8 | 1.4 | 1.8 |

*Means within the same column not followed by the same letter are significantly different at the 0.05 probability level.

## Four-week seedling growth responses following applications of various combinations of Fe, BA, triazoles, and MZ63 (Experiment 3)

*Seedlings treated 1 April 1987 and measured 29 April 1987.*

MZ63 seaweed extract caused the greatest increase in seedling development of any PGR-like material applied (Table 12). Addition of Fe with MZ63 further enhanced seedling growth of all variables except root dry weight. Propiconazole also caused an increase in Kentucky bluegrass seedling development as compared to the control seedlings. Addition of Fe to propiconazole increased the Kentucky bluegrass seedling responses for every growth parameter measured. The enhancement of seedlings from treatments with BA (with and without Fe) and triadimefon (with and without Fe) were variable. The addition of Fe to BA or triadimefon did not enhance seedling development and even tended to decrease development in some instances (Table 12). Triadimefon tended to increase shoot dry weight, although the differences were not significant. Very little Kentucky bluegrass growth stimulation was observed following BA treatment. Lack of growth response to BA application was consistent with work previously reported by Varga and Bruinsma (1973) and Tao et al. (1983), in which BA was proposed to be more effective as an anti-senescence agent than as a growth promoting material (see Chapter 6).

The addition of Fe, while not always providing a significant growth response, generally promoted Kentucky bluegrass seedling development. Seedlings treated with Fe alone at 112 mg $m^{-2}$ had larger treatment means for leaf number, and tended to have increased root length, and root and shoot dry weights than the plants receiving no material or Fe application (Table 12). The addition of Fe to BA and the other PGR-like

Table 12. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe, BA, triazoles, and MZ63 on 1 April 1987 in experiment 3. Growth measurements made 29 April 1987.

| Material applied | Fe ($mg\ m^{-2}$) 0 | 112 | diff.† | Fe ($mg\ m^{-2}$) 0 | 112 | diff. | Fe ($mg\ m^{-2}$) 0 | 112 | avg. | Fe ($mg\ m^{-2}$) 0 | 112 | avg. | Fe ($mg\ m^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves — no. $plant^{-1}$ — | | | Lateral buds — no. $plant^{-1}$ — | | | Shoot dry wt. — mg $plant^{-1}$ — | | | Root dry wt. — mg $plant^{-1}$ — | | | Root length — cm $plant^{-1}$ — | | |
| BA | 4.8B‡ | 4.2C | -0.6 NS | 0.2B | 0.0C | -0.2 NS | 8.6 | 10.2 | 9.4B | 3.6 | 5.4 | 4.5C | 8.4 | 7.6 | 8.0C |
| Propiconazole | 4.4B | 7.2B | +2.8 * | 0.0B | 1.2B | +1.2 * | 7.8 | 13.8 | 10.8B | 6.6 | 9.0 | 7.8B | 14.2 | 15.8 | 15.0B |
| Triadimefon | 4.8B | 4.2C | -0.6 NS | 0.2B | 0.0C | -0.2 NS | 14.0 | 12.0 | 13.0B | 6.8 | 4.6 | 5.7BC | 9.0 | 9.0 | 9.0C |
| MZ63 | 6.8A | 9.8A | +3.0 * | 1.2A | 2.6A | +1.4 * | 18.2 | 22.6 | 20.4A | 11.8 | 11.4 | 11.6A | 18.2 | 23.6 | 20.9A |
| None | 4.4B | 4.6C | +0.2 NS | 0.2B | 0.2C | 0.0 NS | 8.0 | 10.6 | 9.3B | 2.4 | 7.4 | 4.9BC | 6.8 | 12.0 | 9.4C |
| avg. | 5.0 | 6.0 | -- | 0.4 | 0.8 | -- | 11.3a‡ | 13.8b | -- | 6.2a | 7.6a | -- | 11.3a | 13.6a | -- |
| Fe x material | | * | | | ** | | | NS | | | NS | | | NS | |
| Material | | ** | | | ** | | | ** | | | ** | | | ** | |
| Fe | | * | | | * | | | NS | | | NS | | | NS | |
| LSD¶ | | 2.0 | | | 0.7 | | | 3.9 | | | 3.0 | | | 4.9 | |

*,** Significant at the 0.05 and 0.01 probability levels, respectively. NS = not significant.

†Mean differences between Fe treatments within the same material treatment for a measurement were analyzed by t-tests. A * represents statistical difference at the 0.05 probability level and NS = not significant.

‡Upper case letters represent statistical differences among materials, averaged over Fe treatments, within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Lower case letters represent statistical difference between Fe treatments across all material treatments within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

¶Least significant difference at the 0.05 probability level among materials, averaged over Fe treatments, within the same measurement.

materials provided no significant increases in root length and root and shoot dry weights of Kentucky bluegrass compared to BA, the triazoles, or MZ63 applied alone. Still, the treatment means for root length and root and shoot dry weights for the combination treatments of Fe plus the PGR-like materials tended to be higher than the means for the materials applied alone.

A significant Fe x material interaction was observed for leaf number and lateral bud initiation. The interaction was due to the variable responses observed from enhanced seedling development from the addition of Fe to MZ63 and propiconazole, and the lack of response from the addition of Fe to BA and triadimefon.

## Six-week seedling growth responses following applications of various combinations of Fe, BA, triazoles, and MZ63 (Experiment 4)

***Seedlings treated 1 April 1987 and measured 13 May 1987.***

A significant Fe x material interaction was indicated for all growth parameters except shoot dry weight (Table 13). The F value for material response differences was significant for all variables, but the F value for Fe was significant only for root dry weight.

MZ63 seaweed extract and propiconazole tended to cause the largest increases in seedling development (Table 13). As in experiment 3, these chemicals were the most active compounds in stimulating both root and shoot growth. However, the addition of Fe to MZ63 and propiconazole resulted in very different growth responses. The

Table 13. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe, BA, triazoles and MZ63 on 1 April 1987 in experiment 4. Growth measurements made 13 May 1987.

| Material applied | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | diff.† | Fe (mg m$^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves — no. plant$^{-1}$ — | | | Lateral buds — no. plant$^{-1}$ — | | | Shoot dry wt. — mg plant$^{-1}$ — | | | Root dry wt. — mg plant$^{-1}$ — | | | Root length — cm plant$^{-1}$ — | | |
| BA | 8.2B‡ | 6.4B | 7.3 | 1.0B | 1.0B | 1.0 | 30.2B | 28.6BC | 29.4 | 9.0B | 11.6B | + 5.6 NS | 12.0B | 14.2AB | 13.1 |
| Propiconazole | 4.6C | 9.4A | 7.0 | 0.0B | 2.8A | 1.4 | 17.2BC | 46.4AB | 31.8 | 4.6BC | 18.8A | + 14.2 * | 8.4BC | 18.2A | 13.3 |
| Triadimefon | 4.0C | 4.8B | 4.4 | 0.0B | 0.4B | 0.2 | 19.0BC | 23.6C | 21.3 | 4.6BC | 7.4B | + 2.8 NS | 7.0BC | 11.0B | 9.0 |
| MZ63 | 14.4A | 10.8A | 12.6 | 4.4A | 2.8A | 3.6 | 93.8A | 60.0A | 76.9 | 28.8A | 23.4A | -5.4 NS | 25.2A | 18.2A | 21.7 |
| None | 3.6C | 6.8B | 5.2 | 0.0B | 0.8B | 0.4 | 12.6C | 26.6C | 19.6 | 2.4C | 8.2B | + 5.8 NS | 5.0C | 10.6B | 7.8 |
| avg. | 7.0a§ | 7.6a | -- | 1.1a | 1.6a | -- | 34.6a | 37.0a | -- | 9.9 | 13.9 | -- | 11.5a | 14.4a | -- |
| Fe x material | | * | | | ** | | | * | | | * | | | * | |
| Material | | ** | | | ** | | | ** | | | ** | | | ** | |
| Fe | | NS | | | NS | | | NS | | | * | | | NS | |
| LSD¶ | | 3.0 | | | 1.1 | | | 16.9 | | | 5.9 | | | 5.5 | |

*,** Significant at the 0.05 and 0.01 probability levels, respectively. NS = not significant.

†Mean differences between Fe treatments within the same material treatment for a measurement were analyzed by t-tests. A * represents statistical difference at the 0.05 probability level and NS = not significant.

‡Upper case letters represent statistical differences among materials within the same Fe treatment for the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Lower case letters represent statistical difference between Fe treatments across all material treatments within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

¶Least significant difference at the 0.05 probability level among materials, averaged over Fe treatments, within the same measurement.

combination of propiconazole plus Fe appeared to stimulate root and shoot growth as compared to propiconazole applied alone, but the addition of Fe to MZ63 actually tended to decrease root and shoot growth as compared to bluegrass treated with MZ63 alone. This response resulted in the significant Fe x material interaction reported in this study.

Triadimefon and BA tended to promote Kentucky bluegrass root and shoot growth, although treatment means were not always significantly different from the seedlings receiving no BA or PGR-like material treatment (Table 13). The treatment of Fe applied alone stimulated Kentucky bluegrass seedling growth for all shoot and root growth parameters measured when compared to untreated seedlings. Statistical significance of the differences in growth of Fe treated Kentucky bluegrass and the untreated seedlings was probably masked by the highly significant Fe x material interaction observed.

## Six-week sequential-measurements of seedling growth following applications of various combinations of Fe, BA, triazoles, or MZ63 (Experiment 5)

***Seedlings treated 12 August 1987. Measurements made 26 August, 9 September, and 23 September 1987.***

There were no significant F values for Fe x material interaction, BA and PGR-like material response differences, or Fe response differences of Kentucky bluegrass at either the 2-week or 4-week measurements (Tables 14 and 15). The F values for Fe x material interaction at the 6-week harvest were significant for the growth parameters of lateral

bud initiation, root dry weight, and shoot dry weight (Table 16). The F values for BA and the other PGR-like material treatment differences were significant for the number of Kentucky bluegrass leaves and the number of lateral buds initiated.

Chemicals that caused the largest increases in seedling growth at the 6-week measurement were propiconazole and MZ63. In this study, the addition of Fe to propiconazole or MZ63 generally appeared to decrease the growth responses of Kentucky bluegrass for all variables measured 6 weeks after transplanting (Table 16). However, the addition of Fe to triadimefon caused Fe to triadimefon tended to increase growth for all variables measured. Growth parameters of plants treated with BA tended to be larger than untreated seedlings, but were not significantly different. Iron treatment at 112 mg $m^{-2}$ appeared to promote growth for all parameters except lateral bud initiation as compared to untreated Kentucky bluegrass seedlings. Root lengths were not responsive to applications of Fe, BA, triazoles, or MZ63 seaweed extract.

## Morphological and physiological seedling responses following applications of various combinations of Fe, BA, triazoles, and MZ63 (Experiment 6)

***Seedlings treated 7 January 1988. Measurements made on 21 January, 4 February, and 18 February 1988.***

The F values for Fe x material interaction and material response were not significant for any growth parameter of Kentucky bluegrass seedlings measured 2 weeks after transplanting (Tables 17 and 18). The F values for the Fe response were not significant

Table 14. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe, BA, triazoles, and MZ63 on 12 August 1987 in experiment 5. Two week growth measurements made on 26 August 1987.

| Material applied | Fe (mg $m^{-2}$) 0 | 112 | avg. | Fe (mg $m^{-2}$) 0 | 112 | avg. | Fe (mg $m^{-2}$) 0 | 112 | avg. | Fe (mg $m^{-2}$) 0 | 112 | avg. | Fe (mg $m^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves — no. $plant^{-1}$ — | | | Lateral buds — no. $plant^{-1}$ — | | | Shoot dry wt. — mg $plant^{-1}$ — | | | Root dry wt. — mg $plant^{-1}$ — | | | Root length — cm $plant^{-1}$ — | | |
| BA | 3.7 | 2.3 | 3.0 | 0.0 | 0.0 | 0.0 | 6.3 | 5.3 | 5.8 | 3.3 | 4.3 | 3.8 | 7.3 | 6.0 | 6.6 |
| Propiconazole | 4.0 | 3.0 | 3.5 | 0.4 | 0.0 | 0.2 | 6.7 | 4.3 | 5.5 | 4.0 | 4.0 | 4.0 | 10.0 | 7.7 | 8.8 |
| Triadimefon | 3.0 | 3.0 | 3.0 | 0.0 | 0.0 | 0.0 | 4.7 | 4.7 | 4.7 | 3.3 | 3.0 | 3.2 | 9.7 | 9.3 | 9.5 |
| MZ63 | 2.7 | 3.3 | 3.0 | 0.0 | 0.0 | 0.0 | 5.7 | 5.0 | 5.4 | 4.3 | 4.0 | 4.2 | 6.3 | 12.3 | 9.3 |
| None | 2.3 | 3.0 | 2.6 | 0.0 | 0.0 | 0.0 | 3.0 | 3.3 | 3.2 | 2.3 | 4.7 | 3.5 | 8.7 | 7.3 | 8.0 |
| avg. | 3.1 | 2.9 | -- | 0.1 | 0.0 | -- | 5.2 | 4.5 | -- | 3.4 | 4.0 | -- | 8.4 | 8.5 | -- |
| Fe x material | | NS† | | | NS | | | NS | | | NS | | | NS | |
| Material | | NS | | | NS | | | NS | | | NS | | | NS | |
| Fe | | NS | | | NS | | | NS | | | NS | | | NS | |

†NS = not significant.

Table 15. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe, BA, triazoles and MZ63 on 12 August 1987 in experiment 5. Four week growth measurements made on 9 September 1987.

| Material applied | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves — no. plant$^{-1}$ — | | | Lateral buds — no. plant$^{-1}$ — | | | Shoot dry wt. — mg plant$^{-1}$ — | | | Root dry wt. — mg plant$^{-1}$ — | | | Root length — cm plant$^{-1}$ — | | |
| BA | 3.7 | 3.7 | 3.7 | 0.0 | 0.0 | 0.0 | 5.3 | 6.0 | 5.6 | 4.3 | 3.3 | 3.8 | 12.0 | 14.0 | 13.0 |
| Propiconazole | 4.7 | 5.0 | 4.8 | 0.0 | 0.3 | 0.2 | 6.7 | 6.7 | 6.7 | 4.3 | 3.7 | 4.0 | 15.3 | 10.7 | 13.0 |
| Triadimefon | 3.7 | 4.7 | 4.2 | 0.0 | 0.0 | 0.0 | 5.7 | 8.7 | 7.2 | 5.0 | 7.0 | 6.0 | 12.3 | 19.3 | 15.8 |
| MZ63 | 4.3 | 4.0 | 4.2 | 0.3 | 0.0 | 0.2 | 8.7 | 5.7 | 7.2 | 6.3 | 7.3 | 7.0 | 13.3 | 12.0 | 12.6 |
| None | 3.7 | 3.3 | 3.5 | 0.0 | 0.0 | 0.0 | 6.7 | 7.3 | 7.0 | 4.0 | 5.3 | 4.6 | 13.3 | 13.0 | 13.2 |
| avg. | 4.0 | 4.1 | -- | 0.1 | 0.1 | -- | 6.6 | 6.9 | -- | 4.8 | 5.3 | -- | 13.2 | 13.8 | -- |
| Fe x material | | NS† | | | NS | | | NS | | | NS | | | NS | |
| Material | | NS | | | NS | | | NS | | | NS | | | NS | |
| Fe | | NS | | | NS | | | NS | | | NS | | | NS | |

†NS = not significant.

Table 16. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe, BA, triazoles, and MZ63 on 12 August 1987 in experiment 5. Six week growth measurements made on 23 September 1987.

| Material applied | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves — no. plant$^{-1}$ — | | | Lateral buds — no. plant$^{-1}$ — | | | Shoot dry wt. — mg plant$^{-1}$ — | | | Root dry wt. — mg plant$^{-1}$ — | | | Root length — cm plant$^{-1}$ — | | |
| BA | 8.3 | 10.3 | 9.3B† | 1.7B‡ | 2.3BC | 2.0 | 27.3 | 33.7 | 30.5 | 15.0 | 26.0 | 20.5 | 23.3 | 22.7 | 23.0 |
| Propiconazole | 16.0 | 14.0 | 15.0A | 4.7A | 3.7AB | 4.2 | 58.0 | 31.3 | 44.6 | 40.0 | 20.3 | 30.2 | 26.7 | 26.7 | 26.7 |
| Triadimefon | 7.7 | 14.7 | 11.2AB | 2.3B | 4.7A | 3.5 | 14.0 | 47.7 | 30.8 | 16.3 | 48.3 | 32.3 | 22.7 | 24.0 | 23.4 |
| MZ63 | 10.3 | 5.7 | 8.0B | 3.0AB | 1.0C | 2.0 | 40.0 | 21.7 | 30.8 | 29.0 | 20.3 | 24.6 | 27.3 | 20.3 | 23.8 |
| None | 6.0 | 8.8 | 7.4B | 1.7B | 1.3C | 1.5 | 17.0 | 26.7 | 21.8 | 5.3 | 23.3 | 14.3 | 21.7 | 25.0 | 23.4 |
| avg. | 9.7 | 10.7 | -- | 2.7 | 2.6 | -- | 31.3 | 32.2 | -- | 21.1 | 27.6 | -- | 24.3 | 23.7 | -- |
| Fe x material | | NS | | | * | | | NS | | | * | | | * | |
| Material | | * | | | * | | | NS | | | NS | | | NS | |
| Fe | | NS | | | NS | | | NS | | | NS | | | NS | |
| LSD§ | | 4.6 | | | 1.9 | | | -- | | | -- | | | -- | |

* Significant at the 0.05 probability levels. NS = not significant.

†Letters represent statistical differences among materials, averaged over Fe treatments, within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

‡Letters represent statistical differences among material treatments within the same Fe treatment for the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Least significant difference at the 0.05 probability level among materials, averaged over or within Fe treatments, for the same measurement.

except for that of shoot dry weight. The addition of Fe to the BA and PGR-like materials increased seedling shoot dry weight at the 2-week interval. No significant enhancement of Ps either on a gram dry weight basis or land area basis was evident (Table 18).

Significant differences in growth were observed at the 4-week measurement (Tables 19 and 20). The F values indicated significant differences in responses to Fe application for all growth parameters measured except root length and Ps rate on a gram dry weight basis. The addition of Fe to any material except triadimefon tended to stimulate Kentucky bluegrass growth as compared to the materials applied alone. A significant Fe x material interaction was observed at the 4-week harvest for shoot dry weight. This interaction appeared to result from the decrease in shoot dry weight from the Fe plus triadimefon treatment, while the addition of Fe to the other materials increased shoot dry weights. A significant response to material application was observed for the growth parameters of lateral bud initiation and shoot dry weight (Table 19). The largest promotion of Kentucky bluegrass seedling growth was caused by either MZ63 or propiconazole in combination with Fe. The combination of BA plus Fe tended to increase root and shoot growth, but the increases in growth were not significantly different from the seedlings receiving Fe alone. The treatment of Fe applied alone tended to stimulate Kentucky bluegrass growth when compared with the treatment receiving no Fe, BA, triazole or MZ63 applications.

Both Fe and BA or other PGR-like materials stimulated Ps rates that were made on a land area basis (Table 20). The largest increase in Ps rates from applications of materials with PGR-like activity was observed with MZ63 seaweed extract. Propiconazole treatment also tended to increase the Ps response of Kentucky bluegrass, although the difference was not significantly different from untreated seedlings. The

Table 17. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe, BA, triazoles, and MZ63 on 7 January 1988 in experiment 6. Two week growth measurements made on 21 January 1988.

| Material applied | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves — no. plant$^{-1}$ — | | | Lateral buds — no. plant$^{-1}$ — | | | Shoot dry wt. — mg plant$^{-1}$ — | | | Root dry wt. — mg plant$^{-1}$ — | | | Root length — cm plant$^{-1}$ — | | |
| BA | 4.3 | 4.0 | 4.2 | 0.0 | 0.0 | 0.0 | 2.7 | 4.3 | 3.5 | 2.7 | 4.3 | 3.5 | 13.0 | 13.3 | 13.2 |
| Propiconazole | 3.3 | 4.0 | 3.6 | 0.0 | 0.0 | 0.0 | 3.0 | 4.0 | 3.5 | 2.3 | 3.0 | 2.6 | 8.0 | 8.7 | 8.4 |
| Triadimefon | 4.0 | 3.7 | 3.8 | 0.0 | 0.0 | 0.0 | 2.7 | 4.0 | 3.4 | 2.3 | 3.0 | 2.6 | 12.0 | 11.0 | 11.5 |
| MZ63 | 4.3 | 3.7 | 4.0 | 0.0 | 0.0 | 0.0 | 2.7 | 5.0 | 3.8 | 3.0 | 3.3 | 3.2 | 8.0 | 8.0 | 8.0 |
| None | 3.7 | 4.3 | 4.0 | 0.0 | 0.0 | 0.0 | 3.3 | 3.7 | 3.5 | 2.0 | 3.0 | 2.5 | 6.7 | 8.0 | 7.4 |
| avg. | 3.9 | 3.9 | -- | 0.0 | 0.0 | -- | 2.9b† | 4.2a | -- | 2.5 | 3.3 | -- | 9.5 | 9.8 | -- |
| Fe x material | | NS | | | NS | | | NS | | | NS | | | NS | |
| Material | | NS | | | NS | | | NS | | | NS | | | NS | |
| Fe | | NS | | | NS | | | * | | | NS | | | NS | |

*Significant at the 0.05 level of probability. NS = not significant.
†Lower case letters represent statistical difference between Fe treatments across all material treatments within the same measurement.

Table 18. Photosynthetic responses of Kentucky bluegrass seedlings treated at the two-leaf stage with combinations of Fe, BA, triazoles, and MZ63 on 7 January 1988 in experiment 6. Two week measurements made 21 January 1988.

| Material applied | Fe ($mg\ m^{-2}$) 0 | 112 | avg. | Fe ($mg\ m^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|
| | —$\mu mol\ m^{-2}\ s^{-1}$ ‡— | | | —— $\mu mol\ g^{-1}\ s^{-1}$ —— | | |
| BA | 0.21 | 0.28 | 0.24 | 0.047 | 0.033 | 0.040 |
| Propiconazole | 0.33 | 0.21 | 0.27 | 0.046 | 0.037 | 0.042 |
| Triadimefon | 0.28 | 0.11 | 0.20 | 0.041 | 0.023 | 0.032 |
| MZ63 | 0.17 | 0.30 | 0.24 | 0.033 | 0.031 | 0.032 |
| None | 0.20 | 0.16 | 0.18 | 0.029 | 0.023 | 0.026 |
| avg. | 0.24 | 0.21 | -- | 0.039 | 0.030 | -- |
| Fe x material | | NS† | | | NS | |
| Material | | NS | | | NS | |
| Fe | | NS | | | NS | |

†NS = not significant.

‡Photosynthetic rates are expressed in units of $\mu mol\ CO_2\ m^{-2}$ land area $s^{-1}$ and $\mu mol\ CO_2\ g^{-1}$ shoot dry weight $s^{-1}$, respectively.

Table 19. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe, BA, triazoles, and MZ63 on 7 January 1988 in experiment 6. Four week growth measurements made 4 February 1988.

| Material applied | Fe ($mg\ m^{-2}$) 0 | 112 | avg. | Fe ($mg\ m^{-2}$) 0 | 112 | avg. | Fe ($mg\ m^{-2}$) 0 | 112 | diff.† | Fe ($mg\ m^{-2}$) 0 | 112 | avg. | Fe ($mg\ m^{-2}$) 0 | 112 | avg.· |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves —no. $plant^{-1}$— | | | Lateral buds — no. $plant^{-1}$ — | | | Shoot dry wt. — mg $plant^{-1}$ — | | | Root dry wt. — mg $plant^{-1}$ — | | | Root length — cm $plant^{-1}$ — | | |
| BA | 4.7 | 5.7 | 5.2 | 0.0 | 0.0 | 0.0B‡ | 3.3A§ | 6.0B | +2.7 NS | 3.7 | 6.7 | 5.2 | 17.3 | 17.3 | 17.3 |
| Propiconazole | 5.3 | 9.0 | 7.2 | 0.0 | 2.0 | 1.0A | 5.3A | 12.7A | +7.4 * | 7.6 | 12.3 | 10.0 | 20.7 | 15.3 | 18.0 |
| Triadimefon | 5.3 | 4.6 | 5.0 | 0.0 | 0.0 | 0.0B | 8.3A | 3.0B | -5.3 NS | 7.3 | 3.7 | 5.5 | 16.0 | 11.0 | 13.5 |
| MZ63 | 5.7 | 9.7 | 7.7 | 0.3 | 1.7 | 1.0A | 6.7A | 13.3A | +6.6 * | 5.7 | 15.3 | 10.5 | 17.7 | 17.0 | 17.4 |
| None | 5.0 | 7.0 | 6.0 | 0.0 | 0.3 | 0.2AB | 4.0A | 5.3B | +1.3NS | 5.0 | 5.0 | 5.0 | 16.7 | 20.3 | 18.5 |
| avg. | 5.2b¶ | 7.2a | -- | 0.06b | 0.8a | -- | 5.5 | 8.1 | -- | 5.9b | 8.6a | -- | 17.7 | 16.2 | -- |
| Fe x material | | NS | | | NS | | | * | | | NS | | | NS | |
| Material | | NS | | | * | | | * | | | NS | | | NS | |
| Fe | | * | | | * | | | * | | | * | | | NS | |
| LSD# | | NS | | | 0.9 | | | 3.8 | | | 3.9 | | | NS | |

* Significant at the 0.05 level of probability. NS = not significant.

†Mean differences between Fe treatments within the same material treatment for a measurement were analyzed by t-tests. A * represents statistical difference at the 0.05 probability level and NS = not significant.

‡Letters represent statistical differences among materials, averaged over Fe treatments, within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Letters represent statistical differences among materials, within the same Fe treatment, for the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

¶Lower case letters represent statistical difference between Fe treatments across all material treatments within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

#Least significant difference at the 0.05 probability level among materials, averaged over Fe treatments, within the same measurement.

Table 20. Photosynthetic responses of Kentucky bluegrass seedlings treated at the two-leaf stage with combinations of Fe, BA, triazoles, and MZ63 on 21 January 1988 in experiment 6. Four week measurements made 4 February 1988.

| Material applied | Fe (mg $m^{-2}$) 0 | 112 | avg. | Fe (mg $m^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|
| | —$\mu$mol $m^{-2}$ $s^{-1}$ †— | | | —— $\mu$mol $g^{-1}$ $s^{-1}$ —— | | |
| BA | 0.41 | 0.77 | 0.59B‡ | 0.058 | 0.061 | 0.060 |
| Propiconazole | 0.52 | 1.13 | 0.82AB | 0.050 | 0.046 | 0.048 |
| Triadimefon | 0.42 | 0.40 | 0.41B | 0.027 | 0.043 | 0.035 |
| MZ63 | 0.97 | 2.00 | 1.48A | 0.069 | 0.068 | 0.068 |
| None | 0.41 | 0.63 | 0.52B | 0.048 | 0.056 | 0.052 |
| avg. | 0.51b§ | 0.95a | -- | 0.048 | 0.053 | -- |
| Fe x material | | NS | | | NS | |
| Material | | * | | | NS | |
| Fe | | * | | | NS | |
| LSD¶ | | 0.74 | | | NS | |

* Significant at the 0.05 probability level. NS = not significant.

†Photosynthetic rates are expressed in units of $\mu$mol $CO_2$ $m^{-2}$ land area $s^{-1}$ and $\mu$mol $CO_2$ $g^{-1}$ shoot dry weight $s^{-1}$, respectively.

‡Upper case letters represent statistical differences among materials, averaged over Fe treatments, within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Lower case letters represent statistical difference between Fe treatments across all material treatments within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

¶Least significant difference at the 0.05 probability level among materials, averaged over Fe treatments, within the same measurement.

addition of Fe to the chemicals significantly enhanced Ps rates on a land area basis when the main effects of Fe treatment across BA, triazole, or MZ63 applications were compared (Table 20).

The F values for Fe x material interaction for the data at the 6-week measurement were not significant for any growth parameter measured (Tables 22 and 22). Significant differences in Kentucky bluegrass seedling response were indicated for the BA and PGR-like material applications for leaf number, lateral bud initiation, root and shoot dry weight, and Ps rate on a land area basis.

The seaweed extract, MZ63, significantly increased all Kentucky bluegrass growth parameters except root length and Ps rate on a gram dry weight basis (Tables 21 and 22). Triadimefon, BA, and propiconazole generally enhanced the growth responses of Kentucky bluegrass, but the increases were not significantly different from the seedlings receiving no material application. An increase in Ps rate on a land area basis was observed for all Kentucky bluegrass plants treated with BA, the triazoles or MZ63 seaweed extract (Table 22). No increase in Ps rate on a gram dry weight basis was indicated.

The addition of Fe to BA, triazoles, or MZ63 further increased the growth responses of Kentucky bluegrass at the 6-week measurement in experiment 6 (Tables 21 and 22). Iron application alone at 112 mg $m^{-2}$ caused an increase in root dry weight and Ps rate on a land area basis, but did not significantly increase growth responses for other parameters as compared to the plants receiving no material or Fe application.

Table 21. Seedling growth responses of Kentucky bluegrass treated at the two-leaf stage with combinations of Fe, BA, triazoles, and MZ63 on 7 January 1988 in experiment 6. Six week growth measurements made 18 February 1988.

| Material applied | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. | Fe (mg m$^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves — no. plant$^{-1}$ — | | | Lateral buds — no. plant$^{-1}$ — | | | Shoot dry wt. — mg plant$^{-1}$ — | | | Root dry wt. — mg plant$^{-1}$ — | | | Root length — cm plant$^{-1}$ — | | |
| BA | 8.7 | 14.3 | 11.5B† | 1.7 | 2.3 | 2.0AB | 19.0 | 38.3 | 28.7AB | 11.3 | 20.7 | 16.0B | 20.7 | 28.0 | 24.4 |
| Propiconazole | 7.3 | 10.7 | 9.0B | 0.7 | 1.7 | 1.2B | 22.0 | 30.7 | 26.4AB | 22.0 | 21.0 | 21.5B | 25.3 | 22.0 | 23.7 |
| Triadimefon | 7.0 | 14.0 | 10.5B | 0.7 | 3.3 | 2.0AB | 17.7 | 31.0 | 24.4AB | 16.7 | 27.7 | 22.2B | 19.3 | 27.3 | 23.3 |
| MZ63 | 14.3 | 18.3 | 16.3A | 3.0 | 3.7 | 3.4A | 39.0 | 41.3 | 40.2A | 28.3 | 38.7 | 33.5A | 26.3 | 22.3 | 24.3 |
| None | 8.7 | 8.7 | 8.7B | 1.0 | 1.3 | 1.2B | 12.3 | 15.3 | 13.8B | 12.3 | 18.0 | 15.2B | 24.0 | 25.7 | 24.9 |
| avg. | 9.2b‡ | 13.2a | -- | 1.4b | 2.5a | -- | 22.0b | 31.3a | -- | 18.1b | 25.2a | -- | 23.1 | 25.1 | -- |
| Fe x material | | NS | | | NS | | | NS | | | NS | | | NS | |
| Material | | • | | | • | | | • | | | • | | | NS | |
| Fe | | • | | | • | | | • | | | • | | | NS | |
| LSD§ | | 4.7 | | | 1.4 | | | 16.2 | | | 10.9 | | | NS | |

• Significant at the 0.05 level of probability. NS = not significant.

†Upper case letters represent statistical differences among materials, averaged over Fe treatments, within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

‡Lower case letters represent statistical difference between Fe treatments across all material treatments within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Least significant difference at the 0.05 probability level among materials, averaged over Fe treatments, within the same measurement.

Table 22. Photosynthetic responses of Kentucky bluegrass seedlings treated at the two-leaf stage with combinations of Fe, BA, triazoles, and MZ63 on 21 January 1988 in experiment 6. Six week measurements made 18 February 1988.

| Material applied | Fe ($mg\ m^{-2}$) 0 | 112 | avg. | Fe ($mg\ m^{-2}$) 0 | 112 | avg. |
|---|---|---|---|---|---|---|
| | — $\mu mol\ m^{-2}\ s^{-1}$ † — | | | —— $\mu mol\ g^{-1}\ s^{-1}$ —— | | |
| BA | 1.6 | 5.3 | 3.4AB‡ | 0.045 | 0.067 | 0.056 |
| Propiconazole | 3.2 | 3.8 | 3.5AB | 0.078 | 0.069 | 0.074 |
| Triadimefon | 2.2 | 5.2 | 3.7AB | 0.065 | 0.086 | 0.076 |
| MZ63 | 6.1 | 5.1 | 5.6A | 0.087 | 0.066 | 0.076 |
| None | 1.7 | 2.2 | 2.0B | 0.080 | 0.076 | 0.078 |
| avg. | 3.0b§ | 4.3a | -- | 0.071 | 0.073 | -- |
| Fe x material | | NS | | | NS | |
| Material | | * | | | NS | |
| Fe | | * | | | NS | |
| LSD¶ | | 2.3 | | | NS | |

* Significant at the 0.05 probability level. NS = not significant.

†Photosynthetic rates are expressed in units of $\mu mol\ CO_2\ m^{-2}$ land area $s^{-1}$ and $\mu mol\ CO_2\ g^{-1}$ shoot dry weight $s^{-1}$, respectively.

‡Upper case letters represent statistical differences among materials, averaged over Fe treatments, within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

§Lower case letters represent statistical difference between Fe treatments across all material treatments within the same measurement. Means not followed by the same letter are significantly different at the 0.05 probability level.

¶Least significant difference at the 0.05 probability level among materials, averaged over Fe treatments, within the same measurement.

# *Discussion*

Applications of BA, propiconazole, triadimefon or MZ63 seaweed extract generally promoted Kentucky bluegrass seedling growth as indicated by increased lateral bud initiation, and larger root and shoot dry weights. Root elongation appeared to be less responsive to the treatments, but seedlings apparently developed more extensive, fibrous root systems that resulted in greater root mass. Growth promoting activity of the triazole fungicides, propiconazole and triadimefon, apparently were non-target growth regulator-like responses since there was no evidence of a pathogen-incited disease that could have been controlled by the fungicidal activity of the materials.

Prior research has indicated a stimulation of Ps activity following applications of BA, triadimefon, or propiconazole (Adedipe et al., 1971; Kettlewell et al., 1982; Chernyad'ev et al., 1984 and 1986; Dei, 1983; Davies et al., 1984; Gautam et al., 1984;). The reported stimulation of Ps was caused by enhanced Ps enzyme activity and increased chlorophyll (Chl) concentration. No enhancement of Ps of treated Kentucky bluegrass seedlings on a gram shoot tissue dry weight basis was observed in our study. However, the Ps rate on a land area basis was increased following applications of various combinations of Fe, BA, triazoles, and MZ63. The increased Ps rate on a land area basis appeared to be the result of increased leaf and lateral bud initiation and development. This stimulation could have been caused by CK-like activity of the materials that stimulated cell division and differentiation (Richmond and Lang, 1957; Letham, 1963; Skoog et al., 1967; Weaver, 1972; Jones, 1973; Wareing and Philips, 1981). As previously discussed in Chapter 4, the enhancement of lateral bud initiation and enhanced shoot growth is consistent with reported CK-like growth responses. However, the stimulation of seedling Kentucky bluegrass root initiation and development observed in our studies did

not suggest direct CK-like activity of the growth regulating chemicals. It is possible the enhanced growth of seedling Kentucky bluegrass from applications of triadimefon, propiconazole, and MZ63 seaweed extract were caused by increased root initiation and development and a subsequent increase in cytokinin synthesis and transport within the plant as proposed by Fletcher and Arnold (1986). Enhanced root and shoot growth of seedling grasses would cause the seeded area to mature as a sod more quickly. Stimulation of leaf and lateral bud development would increase leaf area available for Ps and further promote plant growth and development as the sod matures.

MZ63 seaweed extract and the systemic triazole fungicide, propiconazole, were the most active growth regulator-like materials. In particular, MZ63 was very effective in increasing root and shoot growth and development. Applications of triadimefon and BA stimulated growth, but the differences between treated and untreated plants were not always significant. The lack of root and shoot growth promoting activity of BA had been reported earlier by Varga and Bruinsma (1973) and Tao et al. (1983). Research indicated BA was more effective as an anti-senescence compound than as a plant growth promoter. The data gathered here support this hypothesis (also see Chapter 6).

Treatment with Fe alone consistently stimulated root and shoot growth as compared to the untreated plants. It is possible the enhanced growth responses observed were a result of the inability of the untreated seedlings to take up enough Fe from the soil. While Fe levels in the soil are relatively high, the availability of the Fe can be quite low (Marschner, 1979). Generally, Fe must be reduced from the ferric ($Fe^{3+}$) state to the ferrous ($Fe^{2+}$) state prior to absorption by the plant (Ambler et al., 1970, Chaney et al., 1972). Plants differ in their ability to facilitate this reduction process. Brown (1961) reported the primary mechanisms plants utilize for the reduction process are the release of $H^+$ or other reducing compounds from roots, direct reduction of $Fe^{3+}$ to $Fe^{2+}$

on the roots, and increasing organic acid contents in their roots. Romheld and Marschner (1986) reported differing mechanisms of Fe uptake by graminaceous and non-graminaceous plants under Fe deficiency stress. Non-graminaceous species were characterized by having an inducible plasma membrane-bound reductase that promoted release of $H^+$ similar to the mechanism proposed by Brown (1961). Romheld and Marschner (1986) further indicated graminaceous species had enhanced release of phytosiderophores (chelating agents that are proposed to be non-proteinogenic amino acids) from the roots and a highly specific uptake system for the phytosiderophore. Kentucky bluegrass seedlings might initially lack the capability to absorb sufficient Fe because of inadequate root development and the inability to reduce $Fe^{3+}$ to $Fe^{2+}$. Foliar application of the chelated Fe could have facilitated Fe uptake by the treated plants and resulted in the observed growth responses.

The growth responses of Kentucky bluegrass to the combination treatments of Fe, BA, triazoles, or MZ63 seaweed extract were quite varied. For example, the addition of Fe to propiconazole enhanced root and shoot growth as compared to propiconazole applied alone in experiments 2, 3, and 4, but the combination had no additional effect or even decreased Kentucky bluegrass growth across the 2-, 4-, and 6-week measurements in experiment 5. These data indicate the response variability is not due to the timing of the application, because the variable Kentucky bluegrass response to treatments occurred in all studies regardless of the time of application. Previous field studies at the Turfgrass Research Center at Virginia Tech had indicated the possibility of reduced turf growth following the application of a chelated Fe application (Snyder and Schmidt, 1973; Schmidt and Snyder, 1984). The chelated Fe could have retarded plant growth and development. The highly variable responses from the combinations of Fe and the other materials and the positive growth response of the plants receiving Fe alone

suggested the possibility of a more complex interaction between the chelated Fe and the chemical itself. Chelates have been reported to have auxin-like activity (Heath and Clark, 1956a, 1956b; Thimann and Takahashi, 1958; Wallace et al., 1962;) An adverse interaction between the auxin-like chelate and the growth promoting activities of BA, triazoles, or MZ63 seaweed extract could have occurred. The probability of such an interaction would be dependent on the degree of uptake of the chelate itself and the specific auxin-like activity of the chelate. No information was available on the level of uptake or auxin-like activity of chelated Fe phosphate citrate. Research is needed to examine these properties of the chelated Fe and determine if the highly variable growth responses of treated Kentucky bluegrass were due to an interaction with BA or the other PGR-like materials. Prior work has indicated endogenous concentrations of cytokinins relative to those of auxins and other plant growth substances at a particular time were important in initiating a plant growth response (Hinchee and Rost, 1987). Exogenous levels of cytokinins greater than $10^{-6}$ M have been reported to inhibit root initiation (Goodwin and Morris, 1979), but cytokinin concentrations less than $10^{-6}$ have been reported to interact with endogenous auxins and actually stimulate lateral root initiation (Wightman et al., 1980).

Results indicated the minimal time required for an observable Kentucky bluegrass seedling response to the Fe and chemical treatments was approximately 4 weeks. No significant differences in plant growth were evident at the 2-week measurement date in the sequential harvest studies. Small, but non-significant differences in the growth of treated Kentucky bluegrass growth responses were observed 4 weeks after transplanting. Large differences between treated and untreated plants were observed at week 6 of the sequential measurement studies. These data corresponded to the elapsed time needed for the BA, triazoles, or MZ63 materials to trigger a measurable Kentucky bluegrass

growth response in the field (see Chapters 3 and 4). Field data on an established Kentucky bluegrass sod indicated a significant rooting response generally could be observed after 4 weeks, while an increase in Kentucky bluegrass sod strength following treatment required 6 to 8 weeks.

# Chapter 6

# Excision-induced Senescence of Kentucky Bluegrass Leaves as Monitored by Chlorophyll Fluorescence, Carbon Dioxide Exchange, and Leaf Color

## *Introduction*

Research was conducted to examine the anti-senescence properties of foliar applications of Fe, benzyladenine (BA), two triazole fungicides, propiconazole and triadimefon, and various combinations of these materials on excised Kentucky bluegrass leaves. The objectives of these experiments were to determine if:

i) foliar applications of Fe, BA, or the triazole fungicides with non-target plant growth regulator-like (PGR-like) activity delayed excision-induced leaf senescence as

monitored by carbon dioxide exchange, chlorophyll (Chl) fluorescence, and visual leaf color ratings.

ii) the combinations of Fe plus BA or the triazoles enhance the delay in Kentucky bluegrass leaf senescence as compared to applications of the materials alone.

iii) Chl fluorescence is a viable technique to monitor leaf senescence.

## *Materials and Methods*

Experiments were performed in October and repeated in December 1987. Kentucky bluegrass ('Georgetown') plugs were taken from the field with a 5-cm diameter plugger 4 weeks prior to the initiation of the first (October) study. The plugs were planted in 7.6-cm diameter styrofoam cups filled with a Groseclose silt loam soil (a clayey, kaolinitic mesic Typic Hapludult) with a pH of 6.2. Holes were punched in the bottom of each cup and the plugs were subirrigated by placing the cups on a plastic covered greenhouse bench containing 2.5 cm of water.

Treatments applied to Kentucky bluegrass were Fe as the chelated Fe phosphate citrate, triadimefon and propiconazole (systemic triazole fungicides), BA (a synthetic cytokinin), and the combination of the three organic materials with Fe as shown in Table 23. Commercially available or experimental formulations of compounds were used. Each treatment was applied to five plugs resulting in five replications. Treatments were

applied with a compressed-air boom sprayer that delivered 123 L $ha^{-1}$ at a pressure of 276 kPa. Plugs were sprayed twice to insure sufficient leaf coverage.

The youngest, fully expanded leaves of Kentucky bluegrass were excised from individual plants in the plugs 1 day after spraying. Leaf tips were removed, and 3 $cm^2$ of leaf tissue were harvested from each sample of the youngest fully expanded leaves. Excised leaf tissue was floated on distilled water in uncovered Petri dishes. The Petri dishes were kept in the dark in a growth chamber maintained at 22°C. The experiment was designed as a completely randomized 2 x 4 factorial observing the effects of BA or the triazoles applied with and without Fe at 112 mg $m^{-2}$ across 4 measurement dates.

Measurements of carbon dioxide exchange rates (CER) and Chl fluorescence of detached leaves were initiated 1 day after excision (DAE). Measurements on the same leaf tissue were repeated 4, 7, and 10 DAE. The CER of leaf tissue was monitored by measuring the change in $CO_2$ concentrations in an open system as previously described in Chapter 5.

Chlorophyll (Chl) fluorescence transients were measured immediately before determination of CER. A baseline was determined prior to placing tissue samples in the chamber by recording the fluorescence signal from the empty chamber. Baselines for succeeding measurements were checked each day fluorescence values were measured to monitor stability of the equipment and signal. Leaf tissue samples were removed from the darkened growth chamber just prior to measurement, blotted dry on tissue paper, and placed in the LD-2. Dark respiration CER was allowed to reach its steady-state level and then fluorescence measurements were made. The probe assembly and photodiode of the Decagon fluorescence detector (FD-1) were inserted into a windowed side port of the LD-2 chamber. The assembly was fitted with a 740 nm optical filter placed between the sample chamber and photodiode. This filter permitted passage of

Table 23. Various combinations of Fe, BA, and triazoles applied as anti-senescence treatments to Kentucky bluegrass.

| Material | Level | Iron |
|---|---|---|
| | mg a.i. $m^{-2}$ | |
| BA | 6 | 0 |
| | | 112 |
| Triadimefon | 150 | 0 |
| | | 112 |
| Propiconazole | 42 | 0 |
| | | 112 |
| None | 0 | 0 |
| | | 112 |

long wavelength red light (the fluorescence region) while removing undesired actinic light. Fluorescence signals were passed to a control box with a digital display by an output amplifier and were recorded on a strip chart recorder. The Decagon LS-1 low heat LED light source was used for fluorescence measurements. The light was fitted directly on top of the perspex window of the LD-2. This light provided illumination at 660 nm. After a steady-state fluorescence signal was obtained, the LED was removed, and CER measurements were initiated by turning on the 400 W light source.

Peak (P) and terminal (T) fluorescence values (in relative units) were determined from the curves recorded on the strip chart. Percent fluorescence decay (PFD) was calculated by the formula (100 - (P - T)/P) to provide a measure of Chl activity.

Visual leaf color ratings were made at the time fluorescence and CER data were collected. Ratings of color were on a 1 to 9 scale with 9 being green and 1 being brown. These ratings were made to provide an indication of the degree of Chl degradation in the leaves.

Repeated time analyses using Wilke's criterion for the hypotheses of time effects and time x treatment interactions were performed, since repeated measurements were made on the same leaf material (Morrison, 1976). Data for CER and fluorescence measurements were analyzed by contrasts and Dunnett's procedure for each measurement date when time effects were significant.

# *Results*

The ANOVA of data on CER, Chl fluorescence, and leaf color from multivariate analyses using Wilke's criterion showed time to be highly significant and the time x treatment interaction to be non-significant. Therefore, contrasts and Dunnett's procedure were used as statistical analyses for each date of measurement.

## Leaf Senescence as Monitored by Chlorophyll Fluorescence

A typical fluorescence transient curve following a dark to light transition is illustrated in Figure 2. The fluorescence signals can be attributed primarily to Chl *a* fluorescence of photosystem 2 (PS II) and the attached light harvesting complex (Papageorgiou, 1975; Krause and Weis, 1984). Peak (P) and terminal (T) values of fluorescence output were recorded in our studies. The rise in fluorescence to point P has been attributed to a transient impedance in photosystem one (PS I) (Krause and Weis, 1984). The block is thought to be due to inefficient electron acceptors in PS I and an initial shortage of oxidized NADP. The decline in fluorescence value to point T has been attributed to "fluorescence quenching" (Krause and Weis, 1984). Duysens and Sweers (1963) have proposed that electron acceptor Q in the path between PS II and PS I quenches Chl fluorescence in its oxidized state and this contributes to the decline in fluorescence output from P to T. Krause (1973) also reported the establishment of a proton gradient across the thylakoid membranes provides a potent fluorescence quenching mechanism when the transthylakoid membrane gradient was high. In other

words, the initially higher level of fluorescence results from a temporary impedance to flow of electrons. When electrons begin to flow more readily, the energy of excited PS II pigments can be passed to PS I and not be re-emitted as fluorescence. High levels of fluorescence decay indicate the establishment of a steady flow of electrons and therefore, good photophosphorylation and NADP reduction.

The trends for increase or decrease of P and T with time in the experiments were inconsistent. Mean P values pooled across all treatments for the October study showed a general decrease across the time period, but the values declined slightly and then increased again in the December study (Tables 24 and 25). No previous reports on the behavior of P fluorescence values during excised-induced leaf senescence were available. Shaw et al. (1986) reported applications of Ps inhibiting herbicides to excised leaves resulted in higher P values as herbicide concentrations were increased. Schreiber et al. (1978) reported a rise in P values of ozone-treated bean leaves was suppressed as exposure times were increased. The methods of inducing senescence by herbicide treatment probably do not relate well to the experimental method of using leaf excision to promote senescence.

Terminal values pooled across all treatments for the October study generally decreased across time, but steadily increased over the 10-day measurement period in the December study (Tables 24 and 25). Shaw et al. (1986) reported increased T values following herbicide applications. Heat and chilled-stressed leaves were also shown to have increased T levels of Chl fluorescence (Schreiber and Berry, 1977; Melcarek and Brown, 1977; Smillie, 1979). Larger T values apparently indicate an impedance in electron flow between PS II and NADP reduction.

The lack of consistency in the P and T fluorescence values made it very difficult to interpret data based on P and T values alone. A reduction in the level that P values

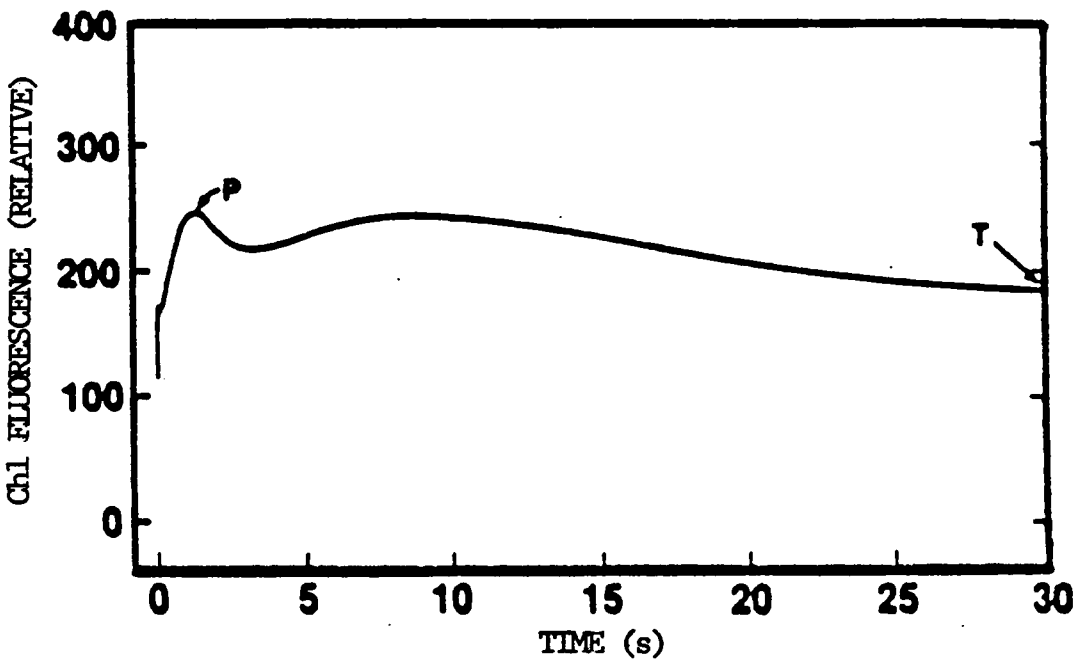


Figure 2. A chlorophyll fluorescence induction curve following a dark-light transition (after Shaw et al., 1986).

Table 24. Mean peak (P), terminal (T), percent fluorescence decay (PFD) values and carbon dioxide exchange rates (CER) of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in October 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Variable | 1 | 4 | 7 | 10 |
| Peak (P) | 21.6 | 21.8 | 20.2 | 17.1 |
| CV,%† | 14.9 | 13.9 | 16.3 | 15.5 |
| Terminal (T) | 8.5 | 7.3 | 7.5 | 7.4 |
| CV,% | 14.9 | 10.7 | 16.2 | 20.5 |
| PFD (100 - (P - T)/P) | 59.5 | 65.8 | 62.4 | 56.0 |
| CV,% | 17.5 | 8.6 | 5.3 | 12.6 |
| CER‡ | 3.8 | 3.5 | 1.9 | 0.76 |
| CV,% | 39.5 | 26.1 | 34.8 | 49.6 |

†Coefficient of variation.
‡Carbon exchange rates are expressed in $\mu$mol $m^{-2}$ $s^{-1}$.

Table 25. Mean peak (P), terminal (T), percent fluorescence decay (PFD) values and carbon dioxide exchange rates (CER) of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in December 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Variable | 1 | 4 | 7 | 10 |
| Peak (P) | 18.1 | 16.4 | 16.7 | 17.0 |
| CV,%† | 15.7 | 14.8 | 12.6 | 16.7 |
| Terminal (T) | 5.6 | 6.0 | 6.8 | 7.1 |
| CV,% | 13.6 | 13.9 | 17.3 | 19.5 |
| PFD (100 - (P - T)/P) | 68.5 | 63.0 | 58.5 | 58.6 |
| CV,% | 5.4 | 5.4 | 15.7 | 14.5 |
| CER‡ | 2.2 | 1.6 | 0.92 | 1.73 |
| CV,% | 37.6 | 47.1 | 41.6 | 57.6 |

†Coefficient of variation.
‡Carbon exchange rates are expressed in $\mu$mol $m^{-2}$ $sec^{-1}$.

reach has been interpreted to reflect a decline in the water-splitting activity and subsequent electron transport of PS II (Schreiber et al., 1978). However, the variable trends for P values across measurement dates do not permit such an interpretation (Schreiber, 1983). Data for P and T parameters for both studies are presented, but the importance and interpretation of significant differences in peak and terminal fluorescence values observed across the measurement dates are suspect (Tables 26 through 33).

Percent fluorescence decay (PFD) values determined from the decline in fluorescence output from P to T provided the lowest coefficients of variation for the measurements made in this study (Tables 24 and 25). Shaw et al. (1986) reported other fluorescence parameters had lower coefficients of variation in a herbicide bioassay, but the parameters were calculated using the value for the inflection point (point I) on the fluorescence transient curve (Figure 2). Point I was not observed on the fluorescence transients recorded in the experiments performed here. Shaw et al. (1986) indicated a digital converter was sometimes required to detect point I of the fluorescence curves. No converter was available for our studies. Shaw et al. (1986) reported a PFD measurement provided meaningful data for herbicide bioassays, but its variability was higher than some other measurable fluorescence parameters. Our studies appeared to indicate PFD measurements provide a measure of the efficiency of Ps light utilization and electron transfer between photosystems II and I. A large PFD value should reflect the ability of the Ps apparatus of the leaf tissue to overcome the transient impedance of electron flow and utilize light energy for carbon assimilation instead of re-emitting the light as fluorescence.

No significant differences in PFD of Kentucky bluegrass leaves between any treatments were found for days 1 and 4 in the October study (Table 34). However, BA alone, BA plus Fe, and Fe applied alone each provided significantly larger PFD's than the

Table 26. Comparisons of mean peak (P) fluorescence values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles, and untreated leaves in October 1987.

| Treatment | Days after leaf excision | | | |
|---|---|---|---|---|
| | 1 | 4 | 7 | 10 |
| | P | | | |
| BA | 25.6 | 26.2 | 22.6 | 18.8 |
| BA + Fe | 20.0 | 22.6 | 22.0 | 19.0 |
| Propiconazole | 24.6 | 21.8 | 18.2 | 18.6 |
| Propiconazole + Fe | 24.2 | 20.4 | 17.2 | 17.2 |
| Triadimefon | 15.8 | 23.6 | 20.2 | 19.2 |
| Triadimefon + Fe | 20.0 | 17.0 * | 20.4 | 11.6 * |
| Fe | 23.0 | 21.0 | 21.0 | 15.2 |
| Control | 20.0 | 21.8 | 20.2 | 17.4 |
| LSD (0.05) | 4.9 | 4.6 | 4.9 | 4.0 |

*Means within the same column are significantly different from the control at the 0.05 level according to Dunnett's test.

Table 27. Contrasts of mean peak (P) fluorescence values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in October 1987.

| Contrast | Days after leaf excision | | | |
|---|---|---|---|---|
| | 1 | 4 | 7 | 10 |
| | P | | | |
| Propiconazole | 24.6 | 21.8 * | 18.2 | 18.6 |
| vs. BA | 25.0 | 26.2 | 22.6 | 18.8 |
| Triadimefon | 15.8 ** | 23.6 | 20.2 | 19.2 |
| vs. BA | 25.0 | 26.2 | 22.6 | 18.8 |
| Triadimefon | 15.8 ** | 23.6 | 20.2 | 19.2 |
| vs. Propiconazole | 24.6 | 21.8 | 18.2 | 18.6 |
| Materials + Fe | 21.4 | 20.0 | 19.9 | 15.9 |
| vs. Fe | 23.0 | 21.0 | 21.0 | 15.2 |
| Triadimefon + Fe | 20.0 | 17.0 * | 20.4 | 11.6 * |
| vs. Fe | 23.0 | 21.0 | 21.0 | 15.2 |
| BA + Fe | 20.0 | 22.6 | 22.0 | 19.0 |
| vs. Fe | 23.0 | 21.0 | 21.0 | 15.2 |
| Propiconazole + Fe | 24.2 | 20.4 | 17.2 | 17.2 |
| vs. Fe | 23.0 | 21.0 | 21.0 | 15.2 |

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

Table 28. Comparisons of mean peak (P) fluorescence values of excised Kentucky bluegrass leaves treated with various combinations of Fe, BA, and triazoles, and untreated leaves in December 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Treatment | 1 | 4 | 7 | 10 |
| | P | | | |
| BA | 17.6 | 17.0 | 15.8 | 17.8 |
| BA + Fe | 19.2 | 19.2 | 17.8 | 17.8 |
| Propiconazole | 18.2 | 17.8 | 18.0 | 17.2 |
| Propiconazole + Fe | 16.8 | 16.8 | 15.6 | 16.0 |
| Triadimefon | 19.0 | 13.6 * | 14.8 | 17.6 |
| Triadimefon + Fe | 18.6 | 17.0 | 18.0 | 17.4 |
| Fe | 19.8 | 17.0 | 16.4 | 17.4 |
| Control | 15.6 | 17.4 | 17.0 | 15.0 |
| LSD (0.05) | 4.3 | 3.7 | 3.2 | 4.3 |

*Means within the same column are significantly different from the control at the 0.05 level according to Dunnett's test.

Table 29. Contrasts of mean peak (P) fluorescence values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in December 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Contrast | 1 | 4 | 7 | 10 |
| | P | | | |
| Propiconazole | 18.2 | 17.8 | 18.0 | 17.2 |
| vs. BA | 17.6 | 17.0 | 15.8 | 17.8 |
| Triadimefon | 19.0 | 13.6 * | 14.8 | 17.6 |
| vs. BA | 17.6 | 17.0 | 15.8 | 17.8 |
| Triadimefon | 19.0 | 13.6 ** | 14.8 * | 17.6 |
| vs. Propiconazole | 18.2 | 17.8 | 18.0 | 17.2 |
| Materials + Fe | 18.2 | 16.2 | 17.1 | 17.1 |
| vs. Fe | 19.8 | 17.0 | 16.4 | 17.4 |
| Triadimefon + Fe | 18.6 | 17.0 | 18.0 | 17.4 |
| vs. Fe | 19.8 | 17.0 | 16.4 | 17.4 |
| BA + Fe | 19.2 | 17.6 | 17.8 | 17.8 |
| vs. Fe | 19.8 | 17.0 | 16.4 | 17.4 |
| Propiconazole + Fe | 16.8 | 14.0 | 15.6 | 16.0 |
| vs. Fe | 19.8 | 17.0 | 16.4 | 17.4 |

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

Table 30. Comparisons of mean terminal (T) fluorescence values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles, and untreated leaves in October 1987.

| Treatment | Days after leaf excision | | | |
|---|---|---|---|---|
| | 1 | 4 | 7 | 10 |
| | T | | | |
| BA | 9.0 | 7.8 | 8.2 | 7.0 |
| BA + Fe | 8.6 | 7.4 | 7.4 | 7.6 |
| Propiconazole | 9.2 | 7.2 | 7.2 | 7.4 |
| Propiconazole + Fe | 8.8 | 6.8 * | 6.8 | 7.4 |
| Triadimefon | 7.2 | 7.8 | 7.8 | 6.2 * |
| Triadimefon + Fe | 8.0 | 7.0 | 7.8 | 6.2 * |
| Fe | 8.8 | 6.6 * | 6.8 | 6.6 |
| Control | 8.2 | 8.0 | 8.4 | 8.6 |
| LSD (0.05) | 1.9 | 1.8 | 1.8 | 2.3 |

*Means within the same column are significantly different from the control at the 0.05 level according to Dunnett's test.

Table 31. Contrasts of mean terminal (T) fluorescence values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in Octobver 1987.

| Contrast | Days after leaf excision | | | |
|---|---|---|---|---|
| | 1 | 4 | 7 | 10 |
| | T | | | |
| Propiconazole | 9.2 | 7.2 | 7.2 | 7.4 |
| vs. BA | 9.0 | 7.8 | 8.2 | 7.0 |
| Triadimefon | 7.2 * | 7.8 | 7.4 | 8.4 |
| vs. BA | 9.0 | 7.8 | 8.2 | 7.0 |
| Triadimefon | 7.2 * | 7.8 | 7.4 | 8.4 |
| vs. Propiconazole | 9.2 | 7.2 | 7.2 | 7.4 |
| Materials + Fe | 8.5 | 7.1 | 7.3 | 7.1 |
| vs. Fe | 8.8 | 6.6 | 6.8 | 6.6 |
| Triadimefon + Fe | 8.0 | 7.0 | 7.8 | 6.2 |
| vs. Fe | 8.8 | 6.6 | 6.8 | 6.6 |
| BA + Fe | 8.6 | 7.4 | 7.4 | 7.6 |
| vs. Fe | 8.8 | 6.6 | 6.8 | 6.6 |
| Propiconazole + Fe | 8.8 | 6.8 | 6.8 | 7.4 |
| vs. Fe | 8.8 | 6.6 | 6.8 | 6.6 |

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

Table 32. Comparisons of mean terminal (T) fluorescence values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles, and untreated leaves in December 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Treatment | 1 | 4 | 7 | 10 |
| | T | | | |
| BA | 5.8 | 6.0 | 6.2 | 6.8 |
| BA + Fe | 5.6 | 6.2 | 6.4 | 6.2 |
| Propiconazole | 5.0 | 6.2 | 7.0 | 6.8 |
| Propiconazole + Fe | 6.0 | 5.6 | 6.4 | 7.8 |
| Triadimefon | 6.0 | 5.2 * | 6.2 * | 7.2 |
| Triadimefon + Fe | 5.8 | 6.4 | 7.8 | 7.2 |
| Fe | 5.6 | 5.8 | 6.2 * | 7.4 |
| Control | 5.2 | 6.8 | 8.4 | 7.8 |
| LSD (0.05)% | 1.2 | 1.3 | 2.2 | 2.1 |

*Means within the same column are significantly different from the control at the 0.05 level according to Dunnett's test.

Table 33. Contrasts of mean terminal (T) fluorescence values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in December 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Contrast | 1 | 4 | 7 | 10 |
| | T | | | |
| Propiconazole | 5.0 | 6.0 | 7.0 | 6.8 |
| vs. BA | 5.8 | 6.0 | 6.2 | 6.8 |
| Triadimefon | 6.0 | 5.2 | 6.2 | 7.2 |
| vs. BA | 5.8 | 6.0 | 6.2 | 6.8 |
| Triadimefon | 6.0 * | 5.2 | 6.2 | 7.2 |
| vs. Propiconazole | 5.0 | 6.0 | 7.0 | 6.8 |
| Materials + Fe | 5.8 | 6.1 | 6.9 | 7.1 |
| vs. Fe | 5.6 | 5.8 | 6.2 | 7.4 |
| Triadimefon + Fe | 5.8 | 6.4 | 7.8 * | 7.2 |
| vs. Fe | 5.6 | 5.8 | 6.2 | 7.4 |
| BA + Fe | 5.6 | 6.2 | 6.4 | 6.2 |
| vs. Fe | 5.6 | 5.8 | 6.2 | 7.4 |
| Propiconazole + Fe | 6.0 | 5.6 | 6.4 | 7.8 |
| vs. Fe | 5.6 | 5.8 | 6.2 | 7.4 |

* Significant at the 0.05 probability levels.

control at 7 DAE. Only the BA treatment caused the PFD to be significantly higher than the control 10 DAE. All PFD values for leaves treated with a PGR-like material plus Fe were larger than the control except for triadimefon plus Fe at day 10 (Table 34).

Comparisons among treatments in the October study indicated significant differences in PFD 4, 7, and 10 DAE (Table 35). The combination of triadimefon and Fe resulted in significantly lower PFD values for excised leaves when compared to Fe applied alone at 4, 7, and 10 DAE. No explanation is apparent for the low PFD values of the triadimefon plus Fe treated leaves in the October study. Single treatments with Fe resulted in significantly larger PFD values at each measurement date and were significantly greater than the group of treatments consisting of BA or triazoles plus Fe. This indicated no benefit was gained by the combination of BA or triazoles plus Fe. Leaves treated with BA consistently had the highest PFD values for any of the chemicals applied across the 10-day measurement period (Table 35). Previous studies have indicated BA is markedly more effective in delaying leaf senescence rather than promoting plant growth when compared to other cytokinins or materials with CK-like activity (Letham, 1967; Varga and Bruinsma, 1973; Tao et al., 1983). Our results supported these reports.

Mean PFD values of excised Kentucky bluegrass leaves for every treatment but triadimefon plus Fe increased at day 4 before declining steadily 7 and 10 DAE in the October study (Table 35). Excision-induced leaf senescence as determined by a decline in PFD progressed rapidly after day 4. Benzyladenine alone consistently provided the largest PFD values across measurement dates. The fungicide treatments of propiconazole alone and triadimefon alone also tended to have larger PFD values than the control leaves 4, 7, and 10 DAE, although no significant differences were

Table 34. Comparisons of mean percent fluorescence decay (PFD) values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles, and untreated leaves in October 1987.

| Treatment | Days after leaf excision | | | |
|---|---|---|---|---|
| | 1 | 4 | 7 | 10 |
| | PFD | | | |
| BA | 63.7 | 69.9 | 63.7 * | 62.4 * |
| BA + Fe | 52.8 | 67.3 | 66.3 * | 59.6 |
| Propiconazole | 62.3 | 67.0 | 59.2 | 60.1 |
| Propiconazole + Fe | 63.5 | 66.5 | 60.4 | 52.0 |
| Triadimefon | 54.0 | 66.9 | 62.9 | 56.4 |
| Triadimefon + Fe | 59.1 | 55.5 | 61.7 | 45.9 |
| Fe | 61.6 | 68.6 | 67.4 * | 56.5 |
| Control | 58.9 | 63.2 | 58.3 | 50.6 |
| LSD (0.05) | 15.7 | 8.5 | 5.0 | 10.6 |

*Means within the same column are significantly different from the control at the 0.05 level according to Dunnett's test.

Table 35. Contrasts of mean percent fluorescence decay (PFD) values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in October 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Contrast | 1 | 4 | 7 | 10 |
| | PFD | | | |
| Propiconazole | 62.3 | 67.0 | 59.2 * | 60.1 |
| vs. BA | 63.7 | 69.9 | 63.7 | 62.4 |
| Triadimefon | 54.0 | 66.9 | 62.9 | 56.4 |
| vs. BA | 63.7 | 69.9 | 63.7 | 62.4 |
| Triadimefon | 54.0 | 66.9 | 62.9 | 56.4 |
| vs. Propiconazole | 62.3 | 67.0 | 59.2 | 60.1 |
| Materials + Fe | 58.5 | 63.2 | 62.8 | 54.2 |
| vs. Fe | 61.6 | 68.6 | 67.4 | 56.5 |
| Triadimefon + Fe | 59.1 | 55.5 ** | 61.7 ** | 45.9 * |
| vs. Fe | 61.6 | 68.6 | 67.4 | 56.5 |
| BA + Fe | 52.8 | 67.3 | 66.3 | 59.6 |
| vs. Fe | 61.6 | 68.6 | 67.4 | 56.5 |
| Propiconazole + Fe | 63.5 | 66.5 | 60.4 ** | 52.0 |
| vs. Fe | 61.6 | 68.6 | 67.4 | 56.5 |

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

indicated(Tables 34 and 35). No benefit in senescence delay was evident with the combination of propiconazole or triadimefon with Fe in the October study.

Comparisons with the control treatment indicated significant differences in PFD 1, 7, and 10 days after leaf excision in the December study (Table 36). Leaves treated with Fe alone and propiconazole alone had larger PFD values as compared to the control 1 DAE. No significant differences were indicated 4 DAE, although the PFD values for the treatments of Fe applied alone and propiconazole alone were larger than PFD values for the control leaves. The BA plus Fe combination was the only treatment with significantly larger PFD values as compared to the control 7 DAE. Still, all leaves treated with the combinatins of triazoles and Fe tended to have larger PFD values. All treated Kentucky bluegrass leaves had larger PFD values than the control leaves 10 DAE.

Mean PFD values of excised leaves showed a consistent decline across measurement dates in the December study (Table 36). This indicated the plant was losing the ability to utilize light energy as efficiently as it previously had. Again, bluegrass treated with BA alone and BA plus Fe consistently had the largest PFD values across dates. Leaves treated with Fe alone and propiconazole alone provided the greatest PFD 1 and 4 DAE, but the high PFD values were not maintained 7 and 10 DAE. Triadimefon applied alone and the combination of triadimefon plus Fe caused leaves to have significantly larger PFD values as compared to the control 10 DAE in the December study (Table 36).

Significantly larger PFD values of leaves treated with the combinations of BA or triazoles plus Fe were observed 1 DAE in the December study (Table 37). Percent fluorescence decay values for leaves receiving Fe applications alone were larger than the material plus Fe grouping at day 1. This difference apparently could be attributed to the highly significant difference between treatments of Fe applied alone and propiconazole plus Fe. Kentucky bluegrass leaves treated with propiconazole applied alone had sig-

Table 36. Comparisons of mean percent fluorescence decay (PFD) values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles, and untreated leaves in December 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Treatment | 1 | 4 | 7 | 10 |
| | PFD | | | |
| BA | 66.6 | 64.3 | 60.9 | 61.3 * |
| BA + Fe | 70.5 | 64.5 | 64.2 * | 65.3 * |
| Propiconazole | 72.4 * | 65.1 | 61.0 | 59.5 * |
| Propiconazole + Fe | 64.1 | 60.0 | 59.1 | 58.6 * |
| Triadimefon | 68.2 | 61.0 | 57.0 | 59.3 * |
| Triadimefon + Fe | 68.7 | 62.4 | 56.2 | 62.2 * |
| Fe | 71.7 * | 65.8 | 61.1 | 58.3 * |
| Control | 65.9 | 60.9 | 48.6 | 45.6 |
| LSD (0.05) | 5.7 | 5.2 | 13.9 | 12.7 |

*Means within the same column are significantly different from the control at the 0.05 level according to Dunnett's test.

nificantly larger PFD than the synthetic cytokinin, BA, but PFD values were not different from PFD values obtained from triadimefon treatment (Table 37). Again, the combination of Fe plus BA or the triazoles did not delay leaf senescence any more than single treatments of Fe or the triazoles applied alone as monitored by fluorescence parameters. Four DAE, the only treatments significantly different in PFD values were indicated by leaves treated with Fe alone and propiconazole plus Fe. No differences for any contrast were obtained for measurements 7 and 10 DAE.

Treated Kentucky bluegrass leaves had similar responses in each study. Applications including BA were the most effective senescence retarding treatments in both experiments. Propiconazole-treated Kentucky bluegrass leaves generally had larger PFD values than triadimefon-treated leaves across all measurement dates, suggesting propiconazole was the more effective triazole material for delaying excision-induced leaf senescence. Iron applied alone caused a marked decrease in the rate of excision-induced senescence and maintained significantly greater PFD across measurement dates in both studies. Although values were not always significantly different, all treated leaves generally showed a decline in senescence rates (as indicated by higher PFD values) as compared to the untreated control 7 and 10 DAE. The only exception was observed 10 DAE when leaves treated with triadimefon plus Fe tended to have lower PFD values than the untreated leaves (Table 34).

## Leaf Senescence as Monitored by Carbon Dioxide Exchange

Treatments with propiconazole and propiconazole plus Fe caused significantly higher leaf CER than the control leaves 1 and 4 DAE (Table 38). No treatments caused

Table 37. Contrasts of mean percent fluorescence decay (PFD) values of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in December 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Contrast | 1 | 4 | 7 | 10 |
| | PFD | | | |
| Propiconazole | 72.4 * | 65.1 | 61.0 | 59.5 |
| vs. BA | 66.6 | 64.3 | 60.9 | 61.3 |
| Triadimefon | 68.2 | 61.0 | 57.0 | 59.3 |
| vs. BA | 66.6 | 64.3 | 60.9 | 61.3 |
| Triadimefon | 68.2 | 61.0 | 57.0 | 59.3 |
| vs. Propiconazole | 72.4 | 65.1 | 61.0 | 59.5 |
| Materials + Fe | 67.8 * | 62.3 | 59.8 | 59.4 |
| vs. Fe | 71.7 | 65.8 | 61.1 | 57.3 |
| Triadimefon + Fe | 68.7 | 62.4 | 56.2 | 62.2 |
| vs. Fe | 71.7 | 65.8 | 61.1 | 57.3 |
| BA + Fe | 70.5 | 64.5 | 64.2 | 65.3 |
| vs. Fe | 71.7 | 65.8 | 61.1 | 57.3 |
| Propiconazole + Fe | 64.1 ** | 60.0 ** | 59.1 | 50.6 |
| vs. Fe | 71.7 | 65.8 | 61.1 | 57.3 |

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

significantly larger CER than the control at day 7, although all treatments except triadimefon plus Fe tended to have larger CER. Leaves treated with BA plus Fe, BA alone, propiconazole alone, and Fe alone had significantly larger CER than the control leaves 10 DAE. The same general trends for treatment responses were observed for PFD values in the October study, although leaves treated with BA alone produced the only significant PFD values as compared to the control (Table 34).

Contrasts of CER as influenced by treatments in the October study showed few significant differences (Table 39). The combination of propiconazole plus Fe caused significantly higher CER than Fe applied alone 1 DAE. Leaves treated with Fe alone had significantly greater CER than the triadimefon plus Fe combination. This paralleled the observed differences in PFD between these treatments (Tables 34 and 35). No significant differences between BA or the triazoles and the combinations with Fe were observed in the October study.

Carbon dioxide exchange rates of leaves treated with the various combinations of BA, triazoles, and Fe were not significantly different from control leaves 1 DAE, but significance was indicated 4, 7, and 10 DAE (Table 40). The CER of propiconazole-treated leaves was significantly larger than the control 4 DAE, while treatments of propiconazole plus Fe, and BA plus Fe caused significantly greater leaf CER than the control 7 and 10 DAE. Mean CER values in the December study seemed to indicate the addition of Fe to BA or the triazoles resulted in the leaves maintaining somewhat higher Ps rates than the leaves treated with BA or the triazoles. This was not evident in experiment 1. Still, all treatments of BA or triazoles, either with or without Fe, tended to have larger CER than the controls.

Contrasts among treatment combinations for the December study indicated the only significant difference in leaf CER occurred 7 DAE when the BA plus Fe treatment had

Table 38. Comparisons of mean carbon dioxide exchange rates (CER) of excised Kentucky bluegrass leaves treated with various combinations of Fe, BA, and triazoles, and untreated leaves in October 1987.

| Treatment | Days after leaf excision | | | |
|---|---|---|---|---|
| | 1 | 4 | 7 | 10 |
| | $\mu$mol $m^{-2}$ $s^{-1}$ | | | |
| BA | 4.31 | 3.99 | 2.30 | 1.10 * |
| BA + Fe | 3.90 | 3.01 | 2.50 | 1.15 * |
| Propiconazole | 4.29 | 4.29 * | 2.16 | 0.90 * |
| Propiconazole + Fe | 5.83 * | 3.81 | 2.27 | 0.71 |
| Triadimefon | 3.30 | 3.79 | 1.97 | 0.71 |
| Triadimefon + Fe | 2.89 | 2.71 | 0.57 | 0.16 |
| Fe | 3.26 | 3.88 | 2.34 | 1.01 * |
| Control | 2.34 | 2.75 | 1.51 | 0.30 |
| LSD (0.05) | 2.25 | 1.39 | 1.03 | 0.57 |

* Means within the same column are significantly different from the control at the 0.05 level according to Dunnett's test.

Table 39. Contrasts of mean carbon dioxide exchange rates (CER) of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in October 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Contrast | 1 | 4 | 7 | 10 |
| | $\mu$mol $m^{-2}$ $s^{-1}$ | | | |
| Propiconazole | 4.29 | 4.29 | 2.16 | 0.90 |
| vs. BA | 4.31 | 3.99 | 2.30 | 1.10 |
| Triadimefon | 3.33 | 3.79 | 1.97 | 0.71 |
| vs. BA | 4.31 | 3.99 | 2.30 | 1.10 |
| Triadimefon | 3.33 | 3.79 | 1.97 | 0.71 |
| vs. Propiconazole | 4.29 | 4.29 | 2.16 | 0.90 |
| Materials + Fe | 4.21 | 3.18 | 1.78 | 0.67 |
| vs. Fe | 3.26 | 3.88 | 2.34 | 1.01 |
| Triadimefon + Fe | 2.89 | 2.71 | 0.57 ** | 0.16 ** |
| vs. Fe | 3.26 | 3.88 | 2.34 | 1.01 |
| BA + Fe | 3.90 | 3.01 | 2.50 | 1.15 |
| vs. Fe | 3.26 | 3.88 | 2.34 | 1.01 |
| Propiconazole + Fe | 5.83 ** | 3.81 | 2.27 | 0.71 |
| vs. Fe | 3.26 | 3.88 | 2.34 | 1.01 |

** Significant at the and 0.01 probability levels.

Table 40. Comparisons of mean carbon dioxide exchange rates (CER) of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles, and untreated leaves in December 1987.

| Treatment | Days after leaf excision | | | |
|---|---|---|---|---|
| | 1 | 4 | 7 | 10 |
| | $\mu$mol $m^{-2}$ $s^{-1}$ | | | |
| BA | 3.46 | 2.80 | 1.33 | 1.77 |
| BA + Fe | 4.40 | 2.13 | 2.59 * | 1.97 * |
| Propiconazole | 3.63 | 2.96 * | 1.54 | 1.38 |
| Propiconazole + Fe | 3.97 | 2.18 | 1.75 | 2.23 * |
| Triadimefon | 2.98 | 1.72 | 0.96 | 0.99 |
| Triadimefon + Fe | 4.27 | 2.39 | 1.68 | 1.49 |
| Fe | 4.27 | 2.11 | 1.70 | 1.33 |
| Control | 3.60 | 1.33 | 0.83 | 0.64 |
| LSD (0.05) | 2.17 | 1.58 | 0.92 | 1.29 |

*Means within the same column are significantly different from the control at the 0.05 level according to Dunnett's test.

significantly greater CER than bluegrass leaves treated with chelated Fe applied alone (Table 41). Although no significant differences were indicated, treatments including propiconazole or BA provided the highest CER. Triadimefon treated leaves generally had lower CER than BA or propiconazole treatments. Combinations of Fe plus BA or the triazoles did not cause significant differences in CER as compared to Fe treatments alone. However, the addition of Fe to the materials tended to maintain slightly higher leaf CER than the leaves treated with BA or the triazoles alone 7 and 10 DAE (Table 41).

## Leaf Senescence as Monitored by Leaf Color Ratings

The color of the detached Kentucky bluegrass leaves was monitored across measurement dates as senescence progressed. These ratings provided an indication of the degree of Chl degradation of the leaves. Color comparisons of leaves treated with various combinations of Fe, BA, and triazoles with the untreated leaves indicated no significant differences in leaf color until 7 and 10 DAE (Table 42). Leaves treated with BA (with or without Fe), and Fe applied alone retained more of their green color than the control leaves 7 and 10 DAE in the October study (Table 42). These results were similar to those reported for the PFD and CER data (Tables 34 and 38). Every treatment except for triadimefon plus Fe had significantly greener leaf color ratings at day 10 as compared to the control.

Color ratings for triadimefon plus Fe treated leaves in the October study were significantly lower than ratings of leaves treated with Fe alone for measurements made 4, 7, and 10 DAE (Table 43). This corresponded to the low CER values and smaller PFD

Table 41. Contrasts of mean carbon dioxide exchange rates (CER) of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in December 1987.

| Contrast | Days after leaf excision | | | |
|---|---|---|---|---|
| | 1 | 4 | 7 | 10 |
| | $\mu$mol $m^{-2}$ $s^{-1}$ | | | |
| Propiconazole | 3.62 | 2.96 | 1.54 | 1.37 |
| vs. BA | 3.46 | 2.80 | 1.33 | 1.77 |
| Triadimefon | 2.98 | 1.72 | 0.96 | 0.99 |
| vs. BA | 3.46 | 2.80 | 1.33 | 1.77 |
| Triadimefon | 2.98 | 1.72 | 0.96 | 0.99 |
| vs. Propiconazole | 3.62 | 2.96 | 1.54 | 1.37 |
| Materials + Fe | 4.21 | 2.23 | 2.00 | 1.89 |
| vs. Fe | 4.27 | 2.11 | 1.69 | 1.33 |
| Triadimefon + Fe | 4.27 | 2.39 | 1.68 | 1.49 |
| vs. Fe | 4.27 | 2.11 | 1.69 | 1.33 |
| BA + Fe | 4.40 | 2.13 | 2.59 * | 1.97 |
| vs. Fe | 4.27 | 2.11 | 1.69 | 1.33 |
| Propiconazole + Fe | 3.97 | 2.18 | 1.75 | 2.23 |
| vs. Fe | 4.27 | 2.11 | 1.69 | 1.33 |

*Significant at the 0.05 level of probability.

Table 42. Comparisons of mean color ratings of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles, and untreated leaves in October 1987. October, 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Treatment | 1 | 4 | 7 | 10 |
| | Rating† | | | |
| BA | 8.2 | 7.8 | 7.8 * | 6.8 * |
| BA + Fe | 8.0 | 8.2 | 7.6 * | 6.6 * |
| Propiconazole | 8.2 | 8.0 | 7.2 | 6.2 * |
| Propiconazole + Fe | 8.6 | 8.0 | 7.0 | 5.6 * |
| Triadimefon | 8.0 | 8.2 | 7.0 | 6.0 * |
| Triadimefon + Fe | 8.4 | 7.2 | 6.6 | 3.8 |
| Fe | 8.4 | 8.2 | 7.8 * | 6.4 * |
| Control | 8.4 | 7.8 | 6.6 | 4.0 |
| LSD (0.05) | 0.7 | 0.7 | 0.8 | 0.7 |

* Means within the same column are significantly different from the control at the 0.05 probability level according to Dunnett's test.
†Color ratings are based on a scale of 9 = green, 1 = brown.

values obtained in these experiments (Tables 36 and 40). Benzyladenine treated leaves had significantly higher color ratings than leaves treated with triadimefon alone 7 and 10 DAE, and BA treated leaf color was significantly greater than propiconazole alone treated leaves 10 DAE (Table 43). Color ratings for leaves treated with Fe alone were significantly greater when contrasted with the grouping of all chemical plus Fe treated leaves 7 and 10 DAE. This indicated no apparent benefit in the combination of BA or triazoles plus Fe as compared to Fe alone. Fe applied alone also significantly enhanced leaf color as compared to the leaves treated with propiconazole plus Fe at day 10. The general trend for the October study appeared to indicate no benefit in sustaining green leaf color was obtained from the addition of Fe to BA or the triazoles.

No significant differences in the color ratings between the treated and control leaves were observed 1 DAE in the December study (Table 44). Four DAE, leaves treated with BA alone and BA plus Fe had higher color ratings than the control leaves. Leaves treated with BA plus Fe, propiconazole, propiconazole plus Fe, triadimefon plus Fe, and Fe alone maintained significantly greener color than the control leaves 7 DAE. Triadimefon alone treated leaves rated the same color as the control leaves at day 7. All treatments except triadimefon applied alone had higher color ratings 10 DAE in the December study. This corresponded well to the data reported for the PFD values for the December study (Table 36). The trends for color ratings followed the same trends for CER values, though the significant differences among treatments were not the same (Table 40).

Few significant differences in color ratings of leaves treated with any of the combinations of BA, triazoles, and Fe were indicated in the December study (Table 45). Leaves treated with propiconazole had significantly higher color ratings than triadimefon-treated leaves 7 DAE, and leaves treated with BA applied alone had greater

Table 43. Contrasts of mean color ratings of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in October 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Contrast | 1 | 4 | 7 | 10 |
| | Rating† | | | |
| Propiconazole | 8.2 | 8.0 | 7.2 | 6.2 * |
| vs. BA | 8.2 | 7.8 | 7.8 | 6.8 |
| Triadimefon | 8.0 | 8.2 | 7.0 ** | 6.0 ** |
| vs. BA | 8.2 | 7.8 | 7.8 | 6.8 |
| Triadimefon | 8.0 | 8.2 | 7.0 | 6.0 |
| vs. Propiconazole | 8.2 | 8.0 | 7.2 | 6.2 |
| Materials + Fe | 8.3 | 7.8 | 7.1 ** | 5.3 ** |
| vs. Fe | 8.4 | 8.2 | 7.8 | 6.4 |
| Triadimefon + Fe | 8.4 | 7.2 ** | 6.6 ** | 3.8 ** |
| vs. Fe | 8.4 | 8.2 | 7.8 | 6.4 |
| BA + Fe | 8.0 | 8.2 | 7.6 | 6.6 |
| vs. Fe | 8.4 | 8.2 | 7.8 | 6.4 |
| Propiconazole + Fe | 8.6 | 8.0 | 7.0 * | 5.6 ** |
| vs. Fe | 8.4 | 8.2 | 7.8 | 6.4 |

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
†Color ratings based on a scale of 9 = green, 1 = brown.

Table 44. Comparisons of mean leaf color ratings of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles, and untreated leaves in December 1987.

| | Days after leaf excision | | | |
|---|---|---|---|---|
| Treatment | 1 | 4 | 7 | 10 |
| | Rating† | | | |
| BA | 8.6 | 7.8 * | 6.6 | 6.4 * |
| BA + Fe | 8.4 | 8.0 * | 7.0 * | 6.6 * |
| Propiconazole | 8.8 | 7.6 | 6.8 * | 6.0 * |
| Propiconazole + Fe | 8.6 | 7.2 | 7.0 * | 6.6 * |
| Triadimefon | 8.4 | 7.6 | 6.2 | 5.4 |
| Triadimefon + Fe | 8.2 | 7.6 | 7.0 * | 6.2 |
| Fe | 8.8 | 7.6 | 7.2 * | 6.8 * |
| Control | 8.6 | 7.0 | 6.2 | 4.6 |
| LSD (0.05) | 0.8 | 0.8 | 0.6 | 1.0 |

* Means within the same column are significantly different from the control at the 0.05 probability level according to Dunnett's test.
†Color ratings based on a scale of 9 = green, 1 = brown.

color ratings 10 DAE than leaves treated with propiconazole or triadimefon. Throughout the December study, the addition of Fe to BA or the triazoles tended to cause leaves to maintain greener leaf color 7 and 10 DAE. This response was not evident in the October study. Leaves treated with BA (with and without Fe) or Fe applied alone generally maintained the highest color ratings as leaf senescence progressed (Table 45).

## *Discussion*

The technique of excising leaves to "induce senescence" is a procedure that continues to be debated by researchers. The metabolic activities of leaves resulting from a wounding response following leaf excision are probably very different from the metabolic activities of attached leaves. Therefore, the wounding response is a negative aspect to the technique of inducing leaf senescence by leaf excision. However, Thimann (1980) points out research with excised leaves prevents complications that could arise from metabolite transfer in and out of the leaf from other tissues and plant organs, and interference from other endogenous plant growth substances such as cytokinins and auxins.

The rate of senescence of excised leaves has been reported to increase when the leaves are placed in the dark (Thimann and Satler, 1979b; Thimann, 1980). Senescence of excised leaves placed in the dark was delayed by treatments with kinetin and BA (Thimann and Satler, 1979b). For this reason, we chose to place the excised Kentucky bluegrass leaves in the dark to accelerate senescence. However, the youngest fully expanded leaves used in our studies had been treated with foliar applications of the chem-

Table 45. Contrasts of mean color ratings of excised Kentucky bluegrass leaves treated with combinations of Fe, BA, and triazoles in December 1987.

| Contrast | Days after leaf excision | | | |
|---|---|---|---|---|
| | 1 | 4 | 7 | 10 |
| | Rating† | | | |
| Propiconazole | 8.8 | 7.6 | 6.8 | 6.0 * |
| vs. BA | 8.6 | 7.8 | 6.6 | 6.4 |
| Triadimefon | 8.4 | 7.6 | 6.2 | 6.0 * |
| vs. BA | 8.6 | 7.8 | 6.6 | 6.4 |
| Triadimefon | 8.4 | 7.6 | 6.2 * | 6.0 |
| vs. Propiconazole | 8.8 | 7.6 | 6.8 | 6.0 |
| Materials + Fe | 8.4 | 7.6 | 7.0 | 6.5 |
| vs. Fe | 8.8 | 7.6 | 7.2 | 6.8 |
| Triadimefon + Fe | 8.2 | 7.6 | 7.0 | 6.2 |
| vs. Fe | 8.8 | 7.6 | 7.2 | 6.8 |
| BA + Fe | 8.4 | 8.0 | 7.0 | 6.6 |
| vs. Fe | 8.8 | 7.6 | 7.2 | 6.8 |
| Propiconazole + Fe | 8.6 | 7.2 | 7.0 | 6.6 |
| vs. Fe | 8.8 | 7.6 | 7.2 | 6.8 |

* Significant at the 0.05 level of probability.
†Color ratings based on a scale of 9 = green, 1 = brown.

icals while still attached to the plant, 1 day before leaf excision, instead of being excised and floated on a solution of the chemicals as described by Thimann and Satler (1979b). Therefore, the anti-senescence responses of the leaves to chemical and Fe treatments appeared to be in response to the amount of chemical or Fe absorbed through the foliage or possibly remaining on the exterior of the leaf at excision.

The possibility existed that the acceleration of senescence observed in our studies, as determined by declining PFD, decreasing CER, and leaf yellowing, was not actually due to the senescence process. The observed changes in leaf appearance, Ps levels, and Chl activity could also have been caused by a wounding response of the leaves following excision, or possibly an etiolation effect on the leaves following placement in the dark. Further research is needed to determine the degree to which leaf wounding and subsequent placement of the leaves in constant darkness affects leaf metabolic activity, and to determine if the decline in PFD, CER, and green leaf color are actually senescence phenomena. For the purposes of our research, we felt it was appropriate to describe the observed changes in PFD, CER, and leaf color of excised Kentucky bluegrass leaves as excision-induced senescence phenomena (as described by Thimann, 1980), with the understanding that the responses could be due in part to wounding or etiolation effects.

Treatments with the synthetic cytokinin, BA, or propiconazole were effective to some degree in delaying sensecence of excised Kentucky bluegrass leaves as determined by PFD, CER, and leaf color measurements. Foliar applications of Fe applied alone also exhibited anti-senescence properties as monitored by Chl fluorescence, carbon dioxide exchange, and leaf color of excised leaves. Results appeared to indicate Fe, BA, or triazole applications maintained Ps activity by possibly delaying Chl degradation (as observed from the higher green leaf color ratings) or by maintaining Chl activity and efficiency of the light harvesting complex (as indicated by larger PFD and CER values).

The addition of Fe to BA or the triazoles generally did not significantly enhance the delay of excision-induced leaf senescence over applications of Fe, BA, or triazoles applied alone. Anti-senescence activity of the Fe plus BA or triazole treatments varied between the October and December studies. The addition of Fe appeared to slightly enhance the anti-senescence activity of BA and the triazoles in the December study, but the addition of Fe apparently did not further delay excision-induced senescence in the October study.

Benzyladenine, the synthetic cytokinin, was the most active anti-senescence treatment applied to Kentucky bluegrass as determined by measuring PFD, CER, and leaf color of excised leaves. The results concurred with data previously reported indicating BA was more effective as an anti-senescence material than as an agent for promoting plant growth (also see Chapters 3, 4, and 5) (Letham, 1967; Varga and Bruinsma, 1973; Tao et al., 1983). Adedipe et al. (1971) reported BA-treated beans had higher Chl and carotenoid levels and suggested the retardation of leaf senescence was due to a maintenance of Ps activity. Benzyladenine-treated Kentucky bluegrass leaves were determined to retain significantly larger amounts of Chl than untreated leaves (Kane and Smiley, 1983). Based on visual color ratings, BA-treated leaves maintained greener color and possibly had less Chl degradation than untreated leaves in the experiments reported here. Benzyladenine treatments also tended to maintain higher CER and PFD 7 and 10 DAE.

Foliar applications of propiconazole and, to some extent, triadimefon, delayed leaf senescence of excised Kentucky bluegrass leaves. These chemicals have been reported to delay leaf senescence, as indicated by enhanced green leaf color and increased Ps rates in wheat and soybeans (Buchenauer et al., 1981; Kettlewell and Davies, 1982; Ballard et al., 1984; Gautam et al., 1984). Kane and Smiley (1983) reported triadimefon and etaconazole (a triazole very similar in structure and activity to propiconazole) treated

Kentucky bluegrass leaves had larger total non-structural carbohydrate levels and greater Chl retention than untreated leaves. The anti-senescence response exhibited by these materials could be due to CK-like activity. However, the proposed explanations of growth regulator activity of triadimefon and propiconazole treatments presented in previous chapters would not provide a satisfactory explanation for the anti-senescence activity of the chemicals. Fletcher and Arnold (1986) have suggested the growth promoting activity of triadimefon is a result of stimulated root growth and increased endogenous cytokinin synthesis and translocation. Leaves used in the studies reported here were excised 1 day after foliar application. Little or no effect from a stimulation in root growth and enhanced cytokinin synthesis was likely to have been realized. Anti-senescence activity of propiconazole and triadimefon, be it CK-like or not, must have come primarily from foliar absorption of the chemicals. The anti-senescence response appeared to result from the treated leaves' capacity to maintain the activity of the Ps apparatus as indicated from increased PFD, CER, and green leaf color ratings of treated leaves as compared to the controls.

Propiconazole tended to be a more effective anti-senescence chemical than triadimefon. Propiconazole treated leaves (with and without Fe) generally had larger PFD values, higher CER, and better visual color ratings than leaves treated with triadimefon (with and without Fe). Carbon exchange rates of propiconazole treated leaves were comparable and in some cases even favorable to those reported for BA across all measurement dates. However, BA treated leaves generally maintained larger PFD values and higher leaf color ratings 7 and 10 DAE.

Fe applied alone was very active as an anti-senescence treatment. Iron treatments resulted in larger PFD, greater CER, and higher color ratings than the untreated control. Garg and Hemantaranjan (1987) reported chelated Fe sources retarded leaf senescence

of french bean. Garg and Hemantaranjan (1987) further indicated the chelated Fe compounds stimulated Chl *a* and *b* production, enhanced Ps rates, and increased auxin (indole-3-acetic acid) concentrations. Previous reports indicated chelates applied at low concentrations demonstrated auxin-like properties (Heath and Clark, 1956a, 1956b; Thimann and Takahashi, 1958; Wallace et al., 1962). Auxins and auxin-like compounds have been shown to retard leaf senescence to a degree (Sacher, 1957 and 1959; Osborne and Hallaway, 1960 and 1964). However, other researchers indicated auxins generally were not potent retarding leaf senescence (Shibaoka and Thimann, 1970; Wittenbach, 1977). Further research is required to determine if the chelated Fe phosphate citrate compound used in our studies had any significant auxin-like anti-senescence activity.

The promotion of green leaf color following Fe application in our studies was most evident. Iron treatments provided some of the highest color ratings for leaf tissue at each measurement date. The greening effect of Fe on turfgrasses has been well documented (Deal and Engel, 1965; Schmidt and Snyder, 1984; White, 1985; Yust et al., 1985). The promotion of green color has been attributed to the requirement of Fe as a precursor in the biosynthesis of Chl (Chabarak and Mantell, 1979; Miller et al., 1982).

Percent fluorescence decay measurements of excised Kentucky bluegrass leaves following a dark induction period provided a rapid, reliable technique to monitor leaf senescence. The PFD values generally declined as excision-induced leaf senescence progressed, apparently indicating the ability of the leaf tissue to trap light energy and utilize it for photochemistry was being reduced. Trends for the decline in PFD and the reduction in CER and leaf color ratings were similar. This further substantiated the validity of using Chl fluorescence to monitor senescence of excised leaves. The fluorescence technique could be less time consuming and labor intensive than assays involving extractions and determinations of levels of Chl, nucleic acids, and proteins.

Data reported for fluorescence signals of detached leaves treated with Ps-inhibiting herbicides indicated values for peak and terminal fluorescence increased as herbicide concentrations were increased (Shaw et al., 1986). The higher peak and terminal fluorescence values following herbicide treatment appeared to be due to an impedance in electron transport between the photosystems. Data from the senescence experiments reported here indicated peak and terminal values changed very slowly across time instead of rapidly rising as did the values for excised leaves treated with Ps-inhibiting herbicides (Shaw et al., 1986). The gradual changes in fluorescence values and the resulting steady decline in PFD reflected an overall reduction in light harvesting capacity of the pigments and a subsequent reduction in utilization of the energy for photochemistry. No blockage of either PS I or II was indicated by the fluorescence plots throughout the senescence experiments reported here. The leaves appeared to lose the ability to efficiently utilize light for Ps as excision-induced senescence progressed.

# Chapter 7

# Summary

Field, greenhouse, and laboratory studies were conducted to examine the morphological and physiological responses of Kentucky bluegrass (*Poa pratensis L.*) to foliar applications of iron (Fe), benzyladenine (BA), triazoles and MZ63 seaweed extract. Foliar applications of BA at 6 mg $m^{-2}$, MZ63 at 0.32 ml $m^{-2}$, propiconazole at 42 mg $m^{-2}$ and triadimefon at 150 mg $m^{-2}$ enhanced post-transplant rooting and sod strength of established Kentucky bluegrass in the field. Foliar applications of these chemicals also tended to increase root and shoot dry weights, total number of leaves per plant, total number of buds initiated, and photosynthetic (Ps) rate on a land area basis for seedling Kentucky bluegrass. The increased Ps rate appeared to be in response to a stimulation of leaf and lateral bud initiation and development following treatment. Materials that most consistently enhanced growth and development of Kentucky bluegrass were MZ63 cold water seaweed extract and propiconazole. Triadimefon generally promoted growth and development of Kentucky bluegrass, but the growth re-

sponses tended to be variable. The synthetic cytokinin, BA, also stimulated bluegrass growth and development, but foliar applications of BA generally provided the least growth response of any material applied.

Foliar applied Fe phosphate citrate at 112 mg $m^{-2}$ in repeated applications in fall and spring or early spring enhanced post-transplant rooting and sod strength of established Kentucky bluegrass sod as compared to single applications of Fe at 112 mg $m^{-2}$ However, applications of Fe alone or in combination with the cytokinin or growth regulator-like chemicals in the summer generally decreased rooting and sod strength of established Kentucky bluegrass sod. Growth and development of seedling bluegrass treated with Fe at 112 mg $m^{-2}$ was promoted. Significant growth responses following foliar Fe application suggested seedling plants were unable to absorb sufficient amounts of Fe from the soil. The inability of seedling plants to absorb the necessary Fe could have been due to the lack of an extensive root system permitting sufficient Fe uptake to satisfy the plants requirements. Foliar applications of the chelated Fe appeared to correct this problem and stimulate Kentucky bluegrass growth as compared to the untreated control plants. Research is needed to determine the level of chelated Fe phosphate citrate that is absorbed by the roots or directly through the leaves and the factors that influence the absorption following foliar application. Such research could offer explanations for the variable growth responses observed in the field.

Morphological and physiological growth responses of Kentucky bluegrass following treatment with the various combinations of Fe, BA, triazoles, or MZ63 seaweed extract were highly variable. In some studies, the addition of Fe with the other materials apparently stimulated Kentucky bluegrass growth, but in other studies it seemed to decrease growth. An adverse interaction between the growth promoting activities of Fe and the other materials could have occurred.

Benzyladenine was the most effective anti-senescence treatment applied to Kentucky bluegrass leaves as monitored by carbon dioxide exchange (CER), percent fluorescence decay (PFD), and leaf color ratings. The anti-senescence activity of BA was much more pronounced than its growth promoting activity, as had previously been reported (Varga and Bruinsma, 1973; Tao et al., 1983). Foliar applications of Fe or propiconazole also delayed leaf senescence of excised Kentucky bluegrass leaves. The anti-senescence activity of triadimefon was variable. Additional research is required to examine the anti-senescence activity of these materials on intact Kentucky bluegrass leaves. If senescence of intact leaves could be delayed, treated plants could maintain a larger leaf area to absorb and utilize light for Ps.

The growth promoting activities of MZ63, propiconazole, and triadimefon, and the anti-senescence properties of propiconazole and triadimefon were, in many respects, cytokinin-like (CK-like). However, the stimulation of Kentucky bluegrass rooting following treatment with these materials suggested the growth regulator activity was more than strictly CK-like. Exogenous cytokinin treatments have been shown to inhibit root initiation and development (Heide, 1965; Goodwin and Morris, 1979; Hinchee and Rost, 1986). MZ63 seaweed extract contains natural cytokinins, auxins, and gibberellic acids. The variable growth promoting response of this material could be due to the combined growth promoting effects of these three plant growth substances. Growth regulator-like activities of triadimefon and propiconazole have been proposed to be CK-like (Buchenauer et al., 1981; Kettlewell et al., 1982; Ballard et al., 1984; Gautam et al., 1984). Root and shoot growth promoting activity of triadimefon and propiconazole observed in our studies supported the hypothesis presented by Fletcher and Arnold (1986), which stated the CK-like activity of triadimefon was a result of stimulated root growth leading to increased biosynthesis of endogenous cytokinins that could be transferred within the plant. However, this hypothesis would not explain the CK-like anti-

senescence activity of propiconazole and triadimefon observed with excised Kentucky bluegrass leaves The triazoles possibly reduced the rate of chlorophyll degradation (and ultimately leaf senescence) and maintained higher CER, higher PFD, and more green leaf color.

Recording PFD from peak fluorescence values to terminal fluorescence values appeared to be a rapid and reliable technique to monitor excision-induced leaf senescence of Kentucky bluegrass leaves. Percent fluorescence decay data appeared to indicate the efficiency of the photosystems and light harvesting complex in absorbing and utilizing light energy for Ps. These studies showed larger PFD values apparently indicated a greater capacity for excised Kentucky bluegrass leaves to capture and utilize light energy for Ps.

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