INFLUENCE OF IRON AND CYTOKININ ON *CYNODON* SPP. CULTURED AT CHILLING TEMPERATURES

by

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(ABSTRACT)

Bermudagrass (Cynodon spp.), when cultured at the northern limit of adaptation for semitropical grasses, is exposed seasonally to temperatures low enough to limit growth and turf quality. Research was conducted to investigate the influence of foliar iron and cytokinin applications on bermudagrass growth during fall and spring. The relationship of photosynthesis, respiration, and nonstructural carbohydrate composition to chilling temperatures was also studied.

Foliar applications of Fe in late-summer and fall extended bermudagrass performance during low temperature periods of fall. Frequent Fe applications aided the retention of green bermudagrass turf during prolonged exposure to chilling temperatures. Iron applied the previous season stimulated post-dormancy recovery. Benzyladenine (BA) applied alone was not as effective as Fe for promoting green bermudagrass color retention during fall and BA had few ef-

fects on spring growth when applied the previous season. Applications of BA in conjunction with Fe were beneficial for retention of green bermudagrass color during late fall when clear plastic covers were used to prevent frost injury. A 6- to 8-week longer bermudagrass growing season occurred when clear plastic covers were used to prevent frost injury. Iron and BA did not significantly affect the total nonstructural carbohydrate (TNC) levels in Midiron bermudagrass rhizomes and stolons at the onset of dormancy in field studies.

Midiron bermudagrass had higher photosynthetic and respiration rates than Tifgreen bermudagrass after 4 days exposure to chilling (10/7°C day/night) temperatures in controlled environment studies. Midiron recovered higher photosynthetic rates than Tifgreen when returned to a warm (30°C) environment after exposure to chilling temperatures. The TNC in leaves of Midiron and Tifgreen increased 88 and 160%, respectively, during 5 days at chilling temperatures. The inability to transport carbohydrate from and the subsequent accumulation of high photoassimilate levels in leaves was associated with the inability of bermudagrass to fully recover high photosynthetic rates following chilling. Reduced respiratory activity was apparently responsible for the accumulation of high TNC levels in leaves.

In contrast to photosynthesis, respiration was reversibly inhibited by short term exposure of bermudagrass to chilling temperatures. Rapid recovery of high respiratory activity may be important for maintenance of aesthetically pleasing bermudagrass turf following chilling.

Foliar applied Fe or BA generally caused darker green Midiron and Tifgreen turf after exposure to chilling temperatures in a controlled environment, although the enhancement of physiological activity differed with chemical applied and cultivar. Iron stimulated recovery of photosynthetic and respiratory activity in both cultivars after exposure to chilling temperatures. However, during chilling Midiron CO_2 exchange was more responsive to Fe applications.

Benzyladenine increased photosynthesis in Tifgreen but not in Midiron and did not significantly affect respiration in either cultivar. Neither Fe nor BA had a consistent effect on TNC levels in bermudagrass leaves, rhizomes, or stolons.

These investigations indicate that cultivar selection may play a major role in determining turf quality at chilling temperatures. Iron may modify bermudagrass physiology and enhance performance of bermudagrass exposed to chilling temperatures.

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INTRODUCTION

The region of the United States located between the northern humid and the southern humid regions is classified as a climatic transition zone. Neither cool- nor warm-season turfgrasses are well adapted to this area. Many cultivars of both cool- and warm-season turf species do not survive the extremes of the environment that occur within the transitional zone. The increased public interest in outdoor active and spectator sports, on both the amateur and professional levels, demands that athletic field turf be resistant to wear injury, have high recuperative potential, be dense and resilient enough to provide participant safety, and have high aesthetic appeal.

Bermudagrass [Cynodon dactylon (L.) Pers.] a warm-season perennial, is often grown on athletic fields in the southeastern United States, because it forms a dense resiliant sod that is resistant to wear injury and has high recuperative potential. However, in transition zone states, such as Virginia, seasons when athletic fields experience the most use may not coincide with periods of maximum bermudagrass growth. Growth of warm-season perennial grasses is greatly reduced when exposed to cool (<15°C) but non-freezing temperatures typical of the fall and spring seasons.

Prolonged exposure to such chilling temperatures, results in an alteration of plant physiological functions, a loss of pigmentation, and the plant eventually enters a state of dormancy.

Advances have been made in the characterization of the physiological response of plants to chilling temperatures and in classifying plants according to low temperature sensitivity. Fewer advances have been made in the development of agricultural strategies that can improve the performance of warm-season turfgrass species in areas close to the northern limits of their adaption. The objectives of this research were to determine the influence of foliar Fe and cytokinin application on: (1) bermudagrass performance during fall and on post-dormancy recovery in spring, (2) photosynthetic and respiratory CO_2 exchange of bermudagrass during exposure to chilling temperatures, and (3) nonstructural carbohydrate composition of bermudagrass in relation to chilling temperatures.

REVIEW OF LITERATURE

Temperature is one of the major environmental constraints governing the distribution of wild and cultivated plants (Berry and Bjorkman, 1980; Graham and Patterson, 1982). Growth of warm-season perennial grasses is greatly reduced by exposure to temperatures of 10 to 15°C (West, 1969; West, 1970; Lyons and Breidenback, 1979). Many temperate zone species, however, may grow well at these same temperatures. The reduced growth rate of tropical and subtropical grasses that occurs when they are exposed to chilling temperatures is the result of physiological disruptions (Karnok and Beard, 1983).

Temperatures of 10 to 15°C down to 0°C are often referred to as a chilling stress for grasses of tropical and subtropical origin. According to Levitt (1980), the term stress in biology almost always has the connotation of possible injury. Although the loss of green pigmentation from warm-season turfgrass species during exposure to chilling temperatures is considered an injury by turf managers trying to maintain an aesthetically pleasing turf, almost all of the effects of chilling on bermudagrass growth can be explained on the basis of the temperature coefficient (Q_{10}) of various enzyme catalyzed reactions. Direct injury of bermu-

dagrass from exposure to chilling temperatures has not been demonstrated. Therefore, temperatures of 10 to 15°C down to 0°C will hereafter be referred to as chilling temperatures rather than to chilling stress.

Effects of Chilling Temperatures on Photosynthesis

Photosynthesis is one of the first physiological functions affected by chilling temperatures (Taylor and Rowley, 1971; Berry and Bjorkman, 1980). The optimum temperature for photosynthesis in warm-season perennial grasses is between 32 and 38°C. Photosynthetic rates decline dramatically as temperatures are lowered below this optimum. Miller (1960) measured photosynthetic rates of common bermudagrass [Cynodon dactylon (L.) Pers.] and Seaside creeping bentgrass (Agrostis palustris Huds.) over a temperature range of 15 to 40°C. Bermudagrass photosynthetic rates decreased 45% when temperatures were lowered from 35 to 15°C. Bentgrass photosynthetic rates decreased only 25% when the temperature was lowered from the level of maximum photosynthesis (25°C) to 15°C. Photosynthetic capacity of pangolagrass (Digitaria decumbens Stent.) was reduced to approximately two-thirds of the initial capacity at 30°C after 12 hours at a 10°C night temperature (West, 1970).

Species as well as cultivars within species vary in respect to physiological sensitivity to chilling temperatures.

Taylor and Rowley (1971) investigated the effects of chilling on photosynthetic behavior of dallisgrass (Paspalum notatum Pers.) and sorghum (Sorghum vulgare Pers.). Photosynthesis declined immediately in both dallisgrass and sorghum when temperatures were first lowered from 25 to 10°C. Three-days exposure to 10°C reduced photosynthetic activity of dallisgrass 30 to 40%, while sorghum was essentially inactive.

Rogers et al. (1977) determined that zoysiagrass (Zoysia spp.) maintained higher photosynthetic rates during autumn at chilling temperatures than bermudagrass. Karnok and Beard (1983) reported that 18 hours at 7°C reduced photosynthesis of 'Pee Dee' and 'Ormond' bermudagrass 54 and 68%, respectively, and 'Texas Common' and 'Floratam' St. Augustinegrass (Stenotaphrum secundatum (Walt.) Kuntze] by 80 and 84%, respectively. After 2 weeks at a 7/5°C (day/night) temperatures, photosynthetic rates at 7°C were approximately 11, 2, 2, and 0% of pre-chill rates for Ormond, Pee Dee, Texas Common, and Floratam, respectively.

Enzymes which catalyze biological processes such as photosynthesis, may be reversibly denatured by low temperatures due to weakening of hydrophobic bonds and unfolding of the protein molecule, or to dissociation of large enzymes into subunits (Levitt, 1980). Phosphoenolpyruvate carboxy-

lase (PEPCase), the initial CO_2 acceptor in C_4 plants, is cold sensitive (Uedan and Sugiyama, 1976). Thus, reductions in photosynthetic CO_2 assimilation may initially be caused by reduced PEPCase activity when bermudagrass is exposed to chilling temperatures.

Chilling causes starch accumulation in chloroplasts of some plant species (West, 1969; West, 1970; Karbassi et al., 1970; Hilliard and West, 1970; Taylor and Graig, 1971; Forde et al., 1975; Rogers et al., 1977). Starch accumulation in chloroplasts presumably inhibits the photosynthetic process because of feedback inhibition of photosynthetic enzymes (Neales and Incoll, 1968; West, 1970) and eventual damage to the chloroplast ultrastructure (Taylor and Graig, 1971). Chilling temperatures may repress the mobilization of starch from chloroplasts by reducing the activity of amylolytic enzymes (West, 1970; Karbassi et al., 1970).

Photosynthate accumulation in chloroplasts may not only be disruptive to the photosynthetic process but may also limit metabolites needed for growth (Hilliard and West, 1970). Dunn and Nelson (1974) reported that total nonstructural carbohydrates (TNC) accumulated in bermudagrass stolons but remained relatively unchanged in rhizomes during fall as air temperatures declined. The authors suggested that reduced growth rates at chilling temperatures favor ac-

cumulation of carbohydrate in bermudagrass stolons. Because rhizome TNC remained relatively unchanged but declined in stolons during late winter, the authors concluded that stolons are the most important carbohydrate storage organ for bermudagrass.

Although photosynthesis is markedly reduced, bermudagrass may accumulate TNC in storage tissue when exposed to chilling temperatures because growth is slow. Therefore, maintenance of photosynthetic activity during chilling may stimulate accumulation of carbohydrate and thus increase growth potential following dormancy periods. Rogers et al. (1977) reported that zoysiagrass (Zoysia spp.) accumulated less starch in leaves, maintained higher photosynthetic rates, and subsequently accumulated more TNC in storage tissues than bermudagrass during fall. The authors suggested that high TNC may account for the superior ability of zoysiagrass to survive winter dormancy periods.

High light levels and low temperatures interact to cause a decline in bermudagrass leaf chlorophyll content (Youngner, 1959). Photooxidation of chlorophyll exceeds chlorophyll synthesis at low temperatures (Shirley, 1938) resulting in a net loss of chlorophyll. However, alterations in chlorophyll content during short term chilling are not apparently responsible for decreased photosynthetic ac-

tivity in chill-sensitive plants. Taylor and Rowley (1971) reported that chlorophyll a and b levels in sorghum, dallisgrass, and corn (Zea maize L.) were essentially unaltered by 2.5 days at 10°C yet photosynthetic rates dropped immediately when temperatures were lowered from 25 to 10°C. Changes in chlorophyll a and b were not detected until permanent photosynthetic damage occurred. Similarily, Bagnall (1979) reported that the photosynthetic rate of sorghum dropped rapidly with temperature down to 3°C, although chlorophyll content declined only after two days of constant 7°C.

Effects of Chilling Temperatures on Respiration

Respiratory behavior at chilling temperatures has received less attention than photsynthesis. Respiration of cotton (Gossypium hirsutum L.) leaves increased during 12 hours at 2.8°C, but extended chilling reduced respiration (Amin, 1969). Dark respiration of cucumber (Cucumis sativus L.) leaves at 20°C was unaltered by up to 6 hours pre-exposure to 1°C (Van Hasselt and Van Berlo, 1980).

Karnok and Beard (1983) reported that respiration rates of chill-sensitive and chill-resistant bermudagrass and St. Augustinegrass cultivars declined after 12 hours at 5°C but remained within 60% of pre-chilling rates. After 2 weeks of chilling, respiration rates of Ormond and Pee Dee bermudagrass, and Texas Common St. Augustinegrass were similar to

rates measured following the initial 12 hour chilling night. Floratam St. Augustinegrass exhibited no respiratory activity after 2 weeks of chilling.

Previous reports indicate that respiration may be much less affected by chilling temperatures than is photosynthesis. Photosynthate accumulation in leaves of chill-sensitive plants exposed to low temperatures would appear to be due only to an inability to hydrolyze starch. Therefore, growth of bermudagrass at chilling temperatures would be controlled by a lack of assimilate transport from leaves to growing points. However, insufficient information is available to support this suggestion.

Iron Nutrition

Gris (1843) demonstrated that plants deprived of an adequate supply of iron (Fe) failed to develop chlorophyll and became chlorotic. However, it was not until 1860 that Sachs established the essentiality of Fe for normal growth of higher plants (Brown, 1961a). Although Fe nutrition of plants has been studied for more than a century, most research has focused on the correction of Fe deficiency in calcareous soils (Berger, 1962). Iron is seldom deficient in soils as it comprises 4.2% of the earth's crust (Bould, 1963) and ranks fourth in abundance among the chemical elements after oxygen, silicon, and aluminum (Sauchelli, 1969).

Agronomic crops require less than 0.5 ppm Fe from the soil plow layer. Therefore, Fe deficiency is not due to absence of Fe in soils but rather to Fe availability (Mengel and Kirkby, 1979), and the ability of plants to absorb and translocate sufficient Fe (Snyder, 1975).

The soluble inorganic forms of Fe are Fe^{3+}, $Fe(OH)^{2+}$, $FeOH_4^{+}$, and Fe^{2+}. All are extremely low in comparison with total soil Fe content (Mengel and Kirkby, 1979). Total Fe in solution is controlled by the solubility of hydrous Fe^{3+} oxides that give rise to Fe^{3+} and its hydrolysis species (Lindsay, 1972) and is highly pH dependent (Troug, 1948). The Fe^{3+} activity in solution decreases 1000-fold for each unit increase in pH above 4.0 (Lindsay, 1972).

Although pH is a primary factor governing the presence of soluble Fe in soil solution, other factors that interfere with the absorption or utilization of Fe may cause the plant to become deficient and chlorosis to develop (Brown, 1961). Wallace and Lunt (1960) stress the following causative factors for Fe deficiency and subsequent chlorosis in plants: (a) low iron supply, (b) calcium carbonate in soil, (c) bicarbonate in soil or irrigation water, (d) excessive soil moisture, (e) high soil phosphorus levels, (f) high levels of heavy metals, (g) high nitrate nitrogen levels, (h) unbalanced cation ratios, (i) poor soil aeration, (j) certain

organic matter additions to soils, (k) viruses, (l) root damage by nematodes or other organisms, and (m) low or high temperatures. These factors were also considered in reviews by Brown (1956 and 1961) and more recently by Snyder (1975). Past reviews focused attention on soils and essential nutrient interaction effects on Fe supply to plants. The effects of temperature on the utilization of Fe by plants has received minor attention in past reviews.

Plant Absorption and Translocation of Iron

The availability of Fe to plants from the soil or growing medium is influenced by a number of factors previously disclosed. Although soils may contain sufficient amounts of Fe, absorption and utilization of Fe depend on specific mechanisms within plant roots and processes controlling transport to other plant tissues (Kannan and Pandey, 1982). Absorption and translocation of Fe is strongly temperature-dependent because of the requirement of active metabolism for these processes in plants. The use of metabolic inhibitors and temperature gradients provides evidence for the role of metabolism in the absorption of Fe (Moore, 1972).

Nutrient solutions containing 2,4-dinitrophenol (DNP), an uncoupler of phosphorylation, inhibited Fe uptake by pea (Pisum sativum L.) at both high and low transpiration rates (Branton and Jacobson, 1962). Low transpiration rates also reduced Fe levels in leaves. Kanan and Wittwer (1967) reported that absorption of ^{59}Fe by enzymically isolated cells from fully expanded tobacco (Nicotiana tabacum L.) leaves was 2.5 to 3.0 times higher at 25 than at 5°C. A 51% inhibition of Fe uptake was caused by DNP. Succinate in the growth medium stimulated Fe adsorption, as did light. These findings indicate that active Fe absorption is an energy dependent process.

Teng and Prichett (1970) reported that total Fe uptake by whole centipedegrass [Eremochloa ophiuroides (Munro.) Hack.] rapidly decreased when night temperatures fell to 7 and -1°C from 18 to 25°C. The concentration of Fe in individual plant parts was higher in plants grown at low than at warm temperatures. Iron may have actually accumulated in plants prior to low temperature exposure and subsequently became concentrated by lack of normal growth and development at low night temperatures. The authors disclosed that leaves of centipedegrass grown at low temperatures were only 30 to 50% as large as those grown at warm temperatures.

Khadr and Wallace (1964) reported that ^{59}Fe uptake was lower at 15 than at 25 or 35°C in rough lemon (Citrus jambhiri L.) but not in trifoliate orange (Poncirus trifoliata L.). Decreasing the temperature from 35 to 15°C significantly decreased the leaf content of Fe in both species.

Iron complexed with citrate is transported from roots to leaves (Tiffin, 1966a, 1966b, 1967, 1970), which is certainly of metabolic origin (Moore, 1972). Therefore, transport of Fe to leaves would require citrate synthesis in roots.

In well aerated soils, Fe^{2+} contributes little to the total soluble inorganic Fe content (Lindsay, 1972; Mengel and Kirkby, 1979). Absorption of Fe by plants depends on the

reduction of Fe^{3+} to Fe^{2+} in close proximity to the root because Fe^{2+} is the ionic species absorbed (Brown et al., 1961; Ambler et al., 1970; Chaney et al., 1972; Christ, 1974).

Plants possess an Fe-stress response mechanism which functions during periods of low Fe availability. However, plant species and cultivars within species vary in degree of response; monocotyledonous plants are less responsive to Fe stress than dicotyledons (Brown, 1953; Chaney et al., 1972; Christ, 1974; Foy et al., 1981; Williams et al., 1982). According to Brown (1978), Olsen et al. (1981, and 1982), the Fe-stress response mechanism occurs in phases as follows: (1) a release of hydrogen ions by roots into soil solution results in lower pH and increased Fe solubility (Marschner et al., 1974; Olsen and Brown, 1980a, 1980b), (2) a reductant, such as caffeic acid, or riboflavin (Wallace, 1982; Olsen et al., 1982) is released from roots, (3) Fe^{3+} is reduced to Fe^{2+} at the root surface, and (4) Fe^{2+} is absorbed. The response mechanism is under some degree of metabolic control, since pH of nutrient solutions may rise after Fe-stress is alleviated, and plants may again respond if Fe supply is diminished (Olsen and Brown, 1980a, 1980b).

The root region with greatest reductive capacity and of greatest Fe absorption is between the area of elongation and

maturation (Ambler et al., 1970) which coincides with the region of high metabolic activity. Brown et al. (1961) reported that reducing capacity of soybean (Glycine max L.) roots was enhanced by illumination and concluded that photosynthate supply may be important to the reductive capacity of the root.

The above facts indicate that active Fe absorption is an energy dependent process. Therefore, environmental factors that influence metabolism and subsquently the plant energy balance may affect the Fe nutritional status of plants.

Biochemical Functions of Iron in Plants

Iron is the micronutrient most commonly deficient in turf (Beard, 1973). Iron deficiency symptoms appear first as an intervenal chlorosis in young actively growing leaves. Green plants require a continuous supply of Fe because Fe does not readily move from older to newer leaf tissue (Brown, 1961b; Brown, 1978). Therefore, Fe previously obtained by plants during favorable conditions for uptake will not support the needs for plant growth and development during periods of reduced Fe availability.

Iron content of plants varies from 10 to 30 ppm for "deficient" plants, 60 to 300 ppm in a "normal range", and "excess" iron levels may reach 400 to 1000 ppm. Iron levels as high as 2000 ppm in bermudagrass were noted by Madison

(1971). Complex interrelationships between Fe and other nutrients makes plant analysis data difficult to interpret (Snyder, 1975). Attempts to quantitate the relationship between degree of chlorosis and leaf tissue Fe concentration have met with varying success. O'Toole (1966) reported that chlorotic leaves of grasses had higher Fe content than healthy leaves. Similarily, chlorotic pear (Prunus spp.) leaves were reported to have higher Fe content than healthy tissue by Oberkowsky (1933). He supported the concept that only a small amount of Fe in leaves is metabolically "active". DeKock et al. (1960a, 1960b) maintained that factors such as the ratio of total P to total Fe, free amino acid content, organic acid content, N nutrition, and plant growth hormones may affect the metabolic activity of Fe and the expression of deficiency symptoms.

The capacity of Fe to form stable chelate complexes with molecules containing O, S, or N and ability to exist in more than one oxidation state are the two important characteristics which underlie the numerous physiological effects of Fe (Rains, 1976). Iron functions in enzyme systems in which heme functions as a prosthetic group (Mengel and Kirkby, 1979). Some Fe containing heme compounds in plants include: cytochromes b, b_6, c, c_2, and f, cytochrome oxidase, catalase, and peroxidase; and non-heme compounds ferredoxin

and ferrichrome (Price, 1970). Activities of catalase, ferredoxin, and cytochrome c were strongly depressed, whereas activity of cytochromes a and a_3, and ferrichrome were slightly decreased by Fe deficiency (Price, 1968).

The function of cytochromes in electron transport and the role of cytochrome oxidase in the terminal step in the respiratory chain is well known. Cytochromes function in oxidation-reduction processes in both respiratory and photosynthetic systems (Rains, 1976). These oxidation-reduction processes are mediated by the interconversion of Fe^{3+} and Fe^{2+} states (Chance et al., 1968).

Evidence indicates that adequate Fe is necessary not only for production of cytochromes and ferredoxin, but also for normal activity of some photosynthetic enzymes, normal development of chloroplast ultrastructure, and chlorophyll synthesis. Chloroplasts contain as much as 80% of the total Fe in plants (Neish, 1939; Spiller and Terry, 1980). Terry and Low (1982) reported that the proportion of Fe in sugarbeet (Beta vulgaris L.) seedling leaves external to the chloroplasts was about 20 to 25%. The chloroplast lamellae contained approximately 58 and 75% of chloroplast Fe in Fe-sufficient and deficient plants, respectively. Chloroplast stroma contained approximately 20% of leaf Fe in Fe-sufficient plants whereas stromal Fe in deficient plants ap-

proached 0%. Iron deficiency causes a reduction in granal lamellae in tomato (Lycopersicon esculentum Mill.), spinach (Spinacea oleracea L.), and corn chloroplasts (Vesk et al., 1966). Stocking (1975) reported that moderate Fe deficiency causes a failure of corn to develop normal grana in mesophyll chloroplasts, but bundle sheath cell chloroplasts were affected less. Under severe deficiency, a drastic reduction of the lamellar systems in both mesophyll and bundle sheath chloroplasts occurred. Stocking (1975) showed that Fe deficiency reduced activity of ribulose bisphosphate carboxylase (RUBPCase) and malic enzyme activity in corn, but PEPCase activity remained high. Terry (1980) reported a decline in RUBPCase activity in Fe-deficient sugarbeet leaves.

There is considerable controversy as to whether Fe plays a direct role in chlorophyll biosynthesis (Price et al., 1972). Although some researchers (O'Toole, 1966; Oberkowsky, 1933; O'Toole, 1966) failed to show a direct relationship between Fe supply and degree of chlorosis, Jacobson and Oertli (1956) and Terry and Low (1982) demonstrated a good correlation between the level of Fe supply and chlorophyll content in leaves. As outlined by Mengel and Kirkby (1979), the steps in chlorophyll synthesis where Fe may function are: (1) the condensation of glycine and succinyl Co-A to form delta-amino-levulinate (Marsh et al., 1963;

Miller et al., 1982), and (2) in the oxidation step from coproporphyrinogen to protoporphyrinogen (Machold and Stephan, 1969).

Because Fe plays a central role in the development of the chloroplast enzymes, electron carriers, and ultrastructure, a decreased photosynthetic activity in deficient plants may occur. Iron fertilization of Fe-deficient poplar (Populus spp.) increased photosynthesis; the response was associated with an increase in chlorophyll following Fe application (Keller and Koch, 1964). Oquist (1973) reported that Fe deficiency depressed photosynthetic activity of blue-green alga (Anacystis nidulans L.) on a per unit chlorophyll basis. Terry (1980) demonstrated that Fe-deficient sugarbeet leaves had less chlorophyll and fewer photosynthetic units per unit leaf area than leaves supplied with sufficient Fe. He suggested, that when leaves were deficient in Fe, low photosynthetic rates occurred because of a decrease in photochemical capacity rather than reduced chlorophyll content per se.

Involvement of cytochromes and cytochrome oxidase in the reduction of oxygen to water in the respiratory electron transport chain is commonly recognized (Rains, 1976). Thus, respiration may be affected by Fe supply. However, evidence to support a relationship of Fe nutrition to respiratory activity is lacking.

Iron and Plant Growth

The essentiality of Fe for plant growth and development is unquestioned. The response of plants to Fe are typically based on interrelationships with other essential nutrients (Snyder, 1975). Because soils are seldom lacking in Fe, and Fe supply to plant roots is affected by a multitude of factors, soil applied Fe studies are virtually meaningless for determining plant Fe needs. Likewise, soil tests may not be a good indicator of Fe fertilization requirements. Considerable information exists on the correction of Fe deficiency on a wide range of agronomic crops. Most deal with the correction of Fe deficiency symptoms with foliar sprays or soil amendments to correct deficiencies on problem soils.

Deal and Engel (1965) reported increased turf density and improved color characteristics of Kentucky bluegrass (Poa pratensis L.) grown at low N-P-K fertility following application of 1.1 or 11.2 kg Fe ha^{-1}. When Fe was supplied to apple rootstocks previously grown in nutrient solution containing low (0.02 ppm) Fe, increases in dry weight of leaves, new shoots, and roots occurred (Delap, 1970). Keller and Koch (1964) reported that severe Fe-deficiency caused decreased leaf size in poplar, thus, photosynthetic assimilatory area was reduced.

Increasing Fe concentration in nutrient solution from 0.1 to 2.5 ppm increased dry matter yield of Barley (Hordeum vulgare L.) at anthesis and straw and kernal yields at maturity (Beauchamp and Rossi, 1972). Snyder and Schmidt (1973) reported enhanced shoot growth of creeping bentgrass (Agrostis palustris Huds.) during early spring following late-fall, early-winter applications of Fe in combination with N fertilizer. When applied in conjunction with high N in late spring, however, Fe significantly reduced clipping yields.

Cytokinins

The discovery of cytokinins occurred in 1955 when Miller, Skoog, von Saltza, and Strong isolated a substance called kinetin (6-furfurylaminopurine) from autoclaved herring sperm DNA (Moore, 1979). Kinetin promoted mitosis and cell division in tobacco callus tissue in vitro (Miller et al., 1955). Letham (1963) reported the first isolation of a naturally occurring cytokinin (zeatin) from immature corn kernels. Since the first isolation and characterization of a naturally occurring cytokinin in plant tissues, cytokinins have been isolated from more than 40 plant species (Weaver, 1972), and it is now confidently assumed that cytokinins are ubiquitous among seed plants (Miller, 1979).

The most biologically active synthetic cytokinins are N^6-substituted adenine derivatives (Skoog et al., 1967). Ring substituents in the N^6-position can confer high cytokinin activity on adenine (Varner and Ho, 1976). The benzyl ring substituent in benzyladenine is the most active biologically (Skoog et al., 1967). Benzyladenine was one of the first commercially synthesized cytokinins (Weaver, 1972). Although cytokinins primarily promote cell division and differentiation, they exhibit a wide range of other physiological effects in plants.

Cytokinin Delay of Senescence

Exogenous cytokinins delay chlorophyll and protein disappearance which usually occurs when leaves are detached from plants. Richmond and Lang (1957) were able to maintain detached cocklebur (Xanthium pennsylvanicum L.) leaves in a fully green condition for up to 20 days by cytokinin treatment. Subsequently, the delay of senescence in detached leaves of other species has been reported (Person et al., 1957; Osborne, 1962; Sugiura et al., 1962). Fletcher (1969) demonstrated the potential for manipulating plant development when he discovered that exogenous cytokinin delayed senescence in intact bean (Phaseolus vulgaris L.) leaves (Towne and Owensby, 1983).

Systemic fungicides commonly used on turf, such as benomyl and triadimefon may exhibit cytokinin-like activity in some plants. The cytokinin activity of benomyl was demonstrated in soybean [Glycine max (L.) Merr.] callus and radish (Raphanus sativus L.) cotyledon assays (Skene, 1972) and by Amaranthus betacyanin assay (Thomas, 1974). Benomyl was similar to kinetin in delaying senescence of detached wheat (Triticum aestivum L.) leaves (Person et al., 1957). Triadimefon delayed senescence in detached barley (Hordeum vulgare L.) leaves (Buchenauer and Grossman, 1977). Kane and Smiley (1983) reported that 'Merion' but not 'Fylking' Kentucky bluegrass leaves previously treated with triadimefon retained more chlorophyll than untreated leaves when floated on distilled H_2O for 6 to 8 days at 25°C.

Although plant senescence is a programmed series of complex biochemical events, the ability of cytokinins to prevent chlorophyll loss and inhibit protein degradation appear responsible for the antisenescent effect (Thimann, 1980). Cytokinin may prevent triggering of the senescence program by maintaining an active metabolic state of mature leaves so that photosynthate export does not decline (Thomas and Stoddart, 1980).

Growth and development processes are sensitive to environmental changes, and plants subjected to stress may show

symptoms of premature senescence (Hall, 1973). Low temperatures may interact with light intensity (Youngner, 1959; Taylor and Rowley, 1971) to elicit senescence-like responses (Thomas and Stoddart, 1980). Lahiri and Singh (1969) reported that short term exposure of millet (Pennisetum typhoides L.) to supraoptimal temperatures accelerated yellowing and protein degradation in leaves.

The acceleration of senescence by environmental stress may be associated with a decline in cytokinin production. Cytokinin biosynthesis was strongly retarded by exposure of corn roots to chilling temperature (Atkin et al., 1971). Itai and Vaadia (1971) reported that water stress caused a reduction in cytokinin content of leaf tissue and xylem exudate of tobacco (Nicotiana rustica L.). Similar reductions in cytokinin content in sunflower (Helianthus annuus L.) root exudates occur (Itai and Vaadia, 1965).

Cytokinins and Photosynthesis

Photosynthetic activity declines during progressive senescence. However, photosynthetic inactivity may preceed chlorophyll decline. The loss of photosynthetic activity is related more to rapid break down of RUBPCase than to chlorophyll loss (Thimann, 1980). Cytokinin can delay the loss of RUBPCase activity by preventing proteolysis of the enzyme (Beevers, 1976; Thimann, 1980).

Apart from senescence inhibition, cytokinin may influence chloroplast differentiation and chlorophyll and RUBPCase biosynthesis. Fletcher and McCullagh (1971) reported that etiolated cucumber cotyledons pretreated with cytokinin had up to 450% more chlorophyll than controls after exposure to 3 hours of light. Cytokinins may restore chloroplast lamellae and reinitiate chlorophyll synthesis in detached yellow leaves of certain plants if genetic codes remain intact (Dyer and Osborne, 1971). Naito et al. (1978) reported that benzyladenine (BA) increased leaf chlorophyll content in intact bean plants when applied at early or middle phenological stages. Parthier et al. (1981) reported that BA increased RUBPCase activity in pumpkin (Cucurbita spp.) cotyledons by stimulating synthesis of the enzyme. Light and BA were required for maximum RUBPCase synthesis. Earlier, Feieraband and de Boer (1978) reported a similar response in rye (Secale cerale L). to BA treatment.

Cytokinin Directed Transport

The concept of plant hormone metiated metabolite transport was first introduced by Went (1939) in support of his "nutrient diversion theory" to explain apical dominance (Patrick, 1976). Shindy and Weaver (1967) reported that BA stimulated ^{14}C-photosynthate transport to shoot tips. Similarly, Gersani and Kende (1982) reported that ^{14}C-leucine and

^{14}C-sucrose moved to localized sites in bean leaves where BA was applied. Approximately 10 to 16 times more radioactivity accumulated in the BA treated area of the leaf.

Cytokinins may affect transport of nutrient elements as well as photosynthetic metabolites. Kinetin at 1.5×10^{-4} M stimulated mass-flow type transport of ^{32}P in phloem of corn leaves to the area of kinetin application (Muller and Leopold, 1966). Hatch and Powell (1970) reported that ^{32}P was mobilized to the point of BA application to apple (Malus sylvestris L.) seedlings. Transport of ^{59}Fe to trifoliate leaves from a primary leaf of bean seedlings was increased by treatment of the trifoliate leaf with kinetin (Kanan and Matthew, 1970). Transport of Fe to stem and root tissue increased but total absorption was not affected.

Cytokinin and Plant Growth

Cytokinin may increase growth and yield in some agronomic crops. Benzyladenine applied at any developmental stage to rice (Oryza sativa L.) increased dry matter accumulation (Biswas and Choudhuri, 1977). Crosby et al. (1981) reported that 2 mM BA applied to the terminal inflorescence at the R_3 developmental stage of field grown soybean increased fruit set and seed yield. Benzyladenine increased phylloclade (vegetative shoot) number of Thanksgiving cactus (Schlumbergera truncata L.) grown under long day conditions (Heins et al., 1981).

Kuraishi et al. (1966) reported that 2 x 10^{-5} M BA did not significantly affect fresh weight of pea (Pisum sativum L.) plants grown under greenhouse conditions. However, plants treated with BA grew well following a 3-hour exposure to -2°C, whereas untreated plants yellowed, decreased in fresh weight, and died 1 week after cold treatment. Benzyladenine applications in mid- or late-July to native tallgrass prairie, dominated by big bluestem (Andropogon gerardi Vitman) and indiangrass [Sorghastrum nutans (L.) Nash], increased total herbage yields the following season (T ne and Owensby, 1983).

Experiment 1. Fall Performance and Spring Growth of Midiron Bermudagrass as Influenced by Iron and Compounds Exhibiting Cytokinin-like Activity.

Materials and Methods

The study was initiated in June 1982 and continued until June 1984 on a 5-year-old Midiron bermudagrass [Cynodon dactylon (L.) Pers.] turf growing on a Groseclose silt loam (a clayey, kaolinitic, mesic Typic Hapludult) soil with a pH of 6.0 at the Turfgrass Research Center at Blacksburg, VA. Nitrogen at 48.8 kg ha^{-1} $month^{-1}$ as NH_4NO_3 was applied from June through September. Phosphorus and potassium as concentrated superphosphate and KCl, respectively, were applied each June at 96.7 kg ha^{-1}. The turf was mowed twice a week to a height of 2.5 cm, and irrigated following fertilizer applications and during low rainfall periods to avoid wilt.

Treatments were applied to 1.8 m by 1.8 m plots and arranged in randomized complete blocks replicated three times. Treatments were Fe as sodium iron diethylenetriamine pentaacetate, triadimefon [1-4-chlorophenoxy)-3, 3-dimethyl-1-(1H-1, 2, 4-triazol-1-yl)-2-butanone], benomyl [methyl-1-(butylcarbamoyl)-2- benzimida-zolecarbamate], and benzyladenine (6-benzylaminopurine) applied at rates shown in Table 1.

Table 1. Rate and number of applications of iron, and compounds with cytokinin-like activity applied to Midiron bermudagrass.

Treatment	Rate	Number of Applications[†]
	mg a.i. m^{-2}	
Fe	61	3
Fe + Benzyladenine	61 + 3	3
Fe + Benzyladenine	61 + 6	3
Be + Benzyladenine	61 + 9	3
Triadimefon	75	3
Triadimefon	150	3
Triadimefon	300	3
Benomyl	230	3
Benomyl	460	3
Benomyl	920	3
Benzyladenine	3	3
Benzyladenine	6	3
Benzyladenine	9	3
Fe + Benomyl	61 + 460	3
Fe + Triadimefon	61 + 150	3
Triadimefon	610	1
Benomyl	1830	1
Control	-	

[†]Applications were made the first week in August, September, and October, or a single application was made the first week in October.

Commercially available or experimental formulations of compounds were used. Chemicals were applied the first week in August, September, and October or a single application was made the first week in October. A compressed-air boom sprayer which delivered 561 L ha^{-1} of chemical solution or suspension was used for applications.

During the third week in October of each year, one-half of each plot was covered with a clear plastic film to allow evaluation of treatment effects during low night temperatures of late fall. Plastic covers were removed in mid-December after all treatments had completely lost green pigmentation. Plastic covers were returned to the same half of plots during the first week in April to hasten post-dormancy growth and allow evaluation of treatment effects at low night air temperatures. Plastic covers were not considered a source of variation in the experimental design but were used to allow the evaluation of chemical treatments on bermudagrass growth at chilling temperatures in the abscence of frost.

Turf quality ratings were taken at approximately 3- to 4-week intervals during the fall. Quality ratings were visual estimations of the combined components of turf density, uniformity, and color and represent a relative comparison of treatment effects. A one to nine scale was used with nine

being the most dense, most uniform, and darkest green. A quality rating of five was considered aesthetically acceptable turf. Visual estimations of percent green bermudagrass ground cover (PGBGC) were taken every 7 to 14 days during October and November to determine the influence of treatments on fall bermudagrass performance. Visual estimations of PGBGC and clipping yields were taken to determine the influence of treatments on growth in spring.

Greenhouse Evaluations of Post-dormancy Growth

Sod-plugs 10-cm in diameter by 3.8-cm deep were taken from each field plot on 21 February 1983 and 28 February 1984 prior to dormancy break and transplanted to 15-cm diameter by 20-cm deep plastic containers filled with a Grosecclose silt loam soil. Sod-plugs were allowed to break dormancy under greenhouse conditions in naturally available light. Irrigation by an automatic mist system twice-a-day provided 1.25-cm water day^{-1}. Counts of new shoots produced and clipping yields were used to determine the influence of treatments on post-dormancy growth. Following 7-weeks of growth in 1983 and 5-weeks of growth in 1984, roots were washed free of soil, cut below the sod-plug, dried at 65° C for 24 hours, and weighed.

On 21 March 1983 and 23 March 1984, prior to dormancy break, sod-plugs 10-cm in diameter by 3.8-cm deep were taken

from field plots and transplanted to 15-cm diameter by 10-cm deep plastic containers filled with a Groseclose silt loam soil. Sod-plugs were allowed to break dormancy under greenhouse conditions but were maintained in darkness by covering with black plastic film. The black plastic film prevented light from reaching the plugs so that any growth would be derived from nonstructural carbohydrates in rhizomes and stolons. Clipping yields were used as an estimation of carbohydrates in rhizomes and stolons.

All data were subjected to an analysis of variance. When a significant F ratio occurred, a least significant difference was calculated.

Results and Discussion

Turf Quality

Visual evaluation of treatment effects on turf quality began immediately following the first chemical application in August. However, treatment effects on turf quality were not significant until October when cooler temperatures caused loss of green color differentially among treatments (Table 2). Turf quality differences were detected in late October 1982 and in the second week of October 1983.

Plots treated with Fe had higher turf quality than the control on 26 October 1982. Benzyladenine (BA) at all rates in conjunction with Fe caused higher turf quality scores than the control on 26 October 1982. However, BA alone had no significant effect on turf quality at any rate applied. Although BA applications in conjunction with Fe improved turf quality scores over Fe application alone, this effect was not significant.

On 10 October 1983, Fe and Fe plus 3.0 mg BA m^{-2} significantly improved turf quality. Higher turf quality than the control was observed on 28 October 1983 in plots treated with Fe or Fe plus BA at all BA rates. The 6 mg BA m^{-2} treatment applied in addition to Fe maintained higher bermudagrass turf quality than treatment with Fe alone.

Table 2. Midiron bermudagrass turf quality during late summer and early fall 1982 and 1983 in response to iron fertilization and compounds with cytokinin-like activity.

Treatment	Rate	Turf Quality Rating†					
		1982			1983		
		1 Sept.	7 Oct.	26 Oct.	7 Sept.	10 Oct.	28 Oct.
	mg a.i. m^{-2}						
Fe	61	6.5	5.7	3.7	6.3	6.0	4.0
Fe + Benzyladenine	61 + 3	7.0	7.0	4.3	6.7	6.7	5.0
Fe + Benzyladenine	61 + 6	6.7	6.7	4.3	6.5	5.7	5.3
Fe + Benzyladenine	61 + 9	6.7	6.7	4.7	6.3	5.7	4.7
Triadimefon	75	6.5	6.0	3.3	6.3	4.3	1.7
Triadimefon	150	6.7	6.0	2.7	6.7	3.0	1.7
Triadimefon	300	6.0	5.0	1.3	6.5	3.3	1.0
Benomyl	230	6.3	5.3	1.0	6.0	4.3	1.0
Benomyl	460	6.5	5.7	2.0	6.0	4.3	1.0
Benomyl	920	6.5	6.7	1.7	6.5	4.0	1.3
Benzyladenine	3	6.7	6.3	2.3	6.3	5.3	1.0
Benzyladenine	6	6.0	5.3	2.7	6.0	5.0	1.0
Benzyladenine	9	6.0	5.3	1.3	6.3	5.0	2.3
Fe + Benomyl	61 + 460	6.3	5.7	3.0	6.3	5.3	1.3
Fe + Triadimefon	61 + 150	6.7	6.3	5.3	6.5	5.3	3.3
Triadimefon	610	6.5	4.3	1.0	6.7	2.7	1.3
Benomyl	1830	6.3	5.0	2.3	6.5	4.3	1.7
Control	-	6.5	5.3	2.0	6.5	4.7	2.0
FLSD 0.05‡		NS	NS	1.4	NS	1.2	1.1

† Quality rated 1 to 9; 9 = darkest green, most dense, and most uniform.

‡ FLSD; F least significant difference; NS, not significant.

Better turf quality than the control was observed in late October of 1982 and 1983 in plots treated with Fe plus 150 mg triadimefon m^{-2}. On 10 October 1983, plots treated with 75 and 150 mg m^{-2} alone had lower turf quality than the control. Turf quality was not significantly affected during late summer or fall by any of the other compounds tested.

Retention of Green Color

Turf quality ratings were a measure of turf density, uniformity, and to a lesser degree color. Percent green bermudagrass ground cover (PGBGC) ratings taken during the fall may be a better assessment of treatment effects on bermudagrass performance, since density of turf was primarily determined by summer management. Also, a primary objective of this study was to determine treatments that would effectively delay the loss of green color and subsequently slow the onset of dormancy.

Significant differences in PGBGC during fall 1982 did not occur until the second week in October (Table 3). Higher PGBGC was maintained from 12 to 26 October 1982 in plots treated with Fe than where no Fe was applied. Higher PGBGC occurred in plots that received Fe plus any rate of BA than in control plots during 12 through 26 October 1982. On 19 October, 6 and 9 mg BA m^{-2} applied in addition to Fe significantly improved PGBGC compared to Fe alone.

Table 3. Fall performance of Midiron bermudagrass during 1982 in response to applications of iron and compounds with cytokinin-like activity.

Treatment	Rate	Percent Green Bermudagrass Ground Cover						
		Not covered				Under plastic covers		
		7 Oct.	12 Oct.	19 Oct.	26 Oct.	1 Nov.	19 Nov.	23 Nov.
	mg a.i. m^{-2}							
Fe	61	76.7	70.0	60.0	46.7	85.0	70.0	61.7
Fe + Benzyladenine	61 + 3	80.0	76.7	65.0	53.3	90.0	73.3	71.7
Fe + Benzyladenine	61 + 6	86.7	80.0	73.3	53.3	91.7	71.7	68.3
Fe + Benzyladenine	61 + 9	86.7	80.0	70.0	46.7	86.7	71.7	55.0
Triadimefon	75	80.0	52.3	46.7	33.3	81.7	68.3	63.3
Triadimefon	150	80.0	48.3	45.0	36.7	71.7	61.7	55.0
Triadimefon	300	70.0	46.7	43.3	33.3	73.3	53.3	53.3
Benomyl	230	73.3	53.3	46.7	35.0	73.3	43.3	21.7
Benomyl	460	76.7	46.7	41.7	25.0	71.7	48.3	31.7
Benomyl	920	76.7	46.7	38.3	21.7	70.0	51.7	23.3
Benzyladenine	3	83.3	60.0	51.7	36.7	71.7	70.0	65.0
Benzyladenine	6	73.3	50.0	43.3	32.3	76.7	65.0	50.0
Benzyladenine	9	73.3	48.3	41.7	23.3	73.3	51.7	31.7
Fe + Benomyl	61 + 460	76.7	70.0	56.7	48.3	76.7	41.7	25.0
Fe + Triadimefon	61 + 150	80.0	70.0	61.7	53.3	73.3	66.3	45.0
Triadimefon	610	70.0	43.3	38.3	23.3	71.7	45.0	35.0
Benomyl	1830	70.0	40.0	33.3	21.7	66.7	41.7	26.7
Control	-	73.3	56.7	50.0	31.7	81.7	63.3	51.7
FLSD 0.05[†]		NS	10.2	9.7	12.3	NS	15.3	16.9

[†]FLSD, F least significant difference; NS, not significant.

Higher PGBGC was observed throughout the observation period when any rate of BA was applied in addition to foliar Fe applications. When applied in addition to Fe, BA at 6 mg m^{-2} generally maintained the highest PGBGC score.

Benomyl at 460 mg m^{-2} applied in conjunction with Fe aided the maintenance of PGBGC during fall 1982. However, the Fe plus 460 mg benomyl m^{-2} treatment had PGBGC similar to plots that received only Fe. When applied alone, benomyl at all rates tended to reduce PGBGC when compared to the control. Benomyl at 920 mg m^{-2} significantly reduced PGBGC on 19 October. PGBGC of plots that received 460 mg benomyl m^{-2} was significantly lower than plots treated with Fe at all dates during October 1982.

The response of Midiron to triadimefon was similar to that of benomyl under natural conditions. Triadimefon at 150 mg m^{-2} applied in conjunction with Fe improved PGBGC during fall compared to untreated turf. On 12 and 19 October 1982, plots treated with 150 mg triadimefon m^{-2} had significantly lower PGBGC than plots treated with Fe. A single application of triadimefon or benomyl at a high rate in early October caused a reduction in PGBGC compared to the control on 12 and 19 October 1982. The adverse effects of these treatments was also evident on 26 October 1982.

Table 4. Fall performance of Midiron bermudagrass during 1983 in response to applications of iron and compounds with cytokinin-like activity.

Treatment	Rate	Percent Green Bermudagrass Cover							
		Not covered					Under plastic covers		
		6 Oct.	13 Oct.	24 Oct.	4 Nov.	14 Nov.	23 Nov.	28 Nov.	10 Dec.
	mg a.i. m^{-2}								
Fe	61	71.7	71.7	60.0	53.3	20.0	83.3	80.0	65.0
Fe + Benzyladenine	61 + 3	76.7	73.3	66.7	58.3	21.7	91.7	83.3	67.3
Fe + Benzyladenine	61 + 6	81.7	73.3	71.7	60.0	20.0	83.3	76.7	63.3
Fe + Benzyladenine	61 + 9	78.3	73.3	68.3	58.3	21.7	91.7	80.0	66.0
Triadimefon	75	50.0	48.3	50.0	23.3	2.3	65.0	55.0	40.3
Triadimefon	150	46.7	46.7	45.0	11.7	1.0	48.3	36.7	21.7
Triadimefon	300	56.7	53.3	48.3	11.7	2.7	53.3	38.3	19.0
Benomyl	230	55.0	51.7	43.3	14.7	1.0	60.0	51.7	38.3
Benomyl	460	55.0	53.3	43.3	15.0	2.3	58.3	56.7	41.7
Benomyl	920	48.3	43.3	40.0	13.3	1.0	53.3	48.3	34.3
Benzyladenine	3	66.7	63.3	53.3	43.3	5.7	83.3	68.3	50.7
Benzyladenine	6	58.3	56.7	51.7	33.3	10.0	86.7	68.3	54.7
Benzyladenine	9	54.7	53.3	51.7	21.7	2.7	76.7	65.0	55.0
Fe + Benomyl	61 + 460	78.3	68.3	66.7	56.7	16.7	80.0	70.0	60.3
Fe + Triadimefon	61 + 150	61.7	61.7	58.3	35.0	5.7	63.3	51.7	32.3
Triadimefon	610	61.7	56.7	53.3	16.7	1.0	38.3	31.7	14.7
Benomyl	1830	63.3	60.0	53.3	21.7	1.3	51.7	55.0	39.0
Control	-	55.0	55.0	51.7	25.0	2.3	65.0	60.0	46.7
FLSD 0.05 †		11.7	8.8	9.4	11.6	4.0	13.7	16.4	15.7

† FLSD, F least significant difference.

Significant differences in PGBGC were detect during the first week in October 1983 (Table 4). Iron applications significantly enhanced PGBGC compared to the control except on 24 October 1983. Application of BA at all rates significantly improved PGBGC when applied in addition to Fe. Higher PGBGC occurred in plots that received 6 mg BA m^{-2} plus Fe compared to Fe treatment alone on 24 October. Application of BA to plots that also received Fe generally improved bermudagrass performance over Fe treatment alone throughout the observation period. Plots treated with 3 mg BA m^{-2} in the absence of Fe generally had higher PGBGC than control plots at all observation dates and was significantly higher on 6 October. The moderate and high rate of BA had no significant effect on PGBGC when applied without Fe treatment.

Compared to the control, Fe plus 460 mg benomyl m^{-2} significantly improved PGBGC at all observation dates. However, application of benomyl in addition to iron produced no better turf than Fe application alone. Benomyl at 230, 460, and 1830 mg m^{-2} had no significant effect on PGBGC compared to untreated turf. Benomyl at 920 mg m^{-2} reduced PGBGC ratings at all dates between 6 October and 14 Nobember 1983.

Triadimefon had little significant influence on turf performance during October 1983. However, triadimefon at 75 and 150 mg m^{-2} reduced PGBGC ratings compared to the control

on 4 November. Triadimefon in addition to Fe did not improve PGBGC ratings during 1983 as was observed in 1982. The triadimefon plus Fe treatment may have not been as effective in 1983 because nighttime air temperatures were generally lower in October 1983 than in October 1982 (Appendices 1 and 2).

Frost Protection with Clear Plastic Covers

All plots approached complete dormancy by the first week in November 1982 and second week in November 1983. However, when protected from direct frost injury by covering with clear plastic, Midiron maintained green pigmentation until the first week in December 1982 (Table 3) and third week of December 1983 (Table 4).

During 1982, only the 3 mg BA m^{-2} plus Fe treatment maintained significantly higher PGBGC than the control when Midiron was grown under clear plastic covers. On 23 November 1982, PGBGC was decreased as the rate of BA increased. Benzyladenine alone at 9 mg m^{-2} reduced PGBGC when compared to untreated turf on 23 November.

In contrast to 1982 data, the improved PGBGC in plots treated with Fe, as well as Fe plus BA continued until 10 December 1983 when turf was protected from direct frost with plastic covers. However, no consistent improvement in PGBGC was observed for any rate of BA applied in addition to Fe

compared to Fe application alone. Compared to the control, all rates of BA plus Fe caused significantly higher PGBGC ratings on 23 November 1983. This response was evident until 10 December 1983 under plastic. Thus, Fe plus BA may not only improve performance of bermudagrass under natural conditions but may also be beneficial when protective covers are subsequently used during late fall to extend bermudagrass growth.

Benomyl at all rates caused significantly lower PGBGC than the control on 24 November 1982. Applying Fe in conjunction with benomyl did not offset the adverse effects of benomyl in 1982 but did in 1983.

Triadimefon applied in multiple applications had no significant influence on PGBGC during 1982 under plastic. A single application of 610 mg triadimefon m^{-2} in October significantly reduced PGBGC below the control on 19 November and adversely affected PGBGC ratings taken on 23 November 1983. Generally, all rates of triadimefon applied caused lower PGBGC compared to the control during 1983. The PGBGC ratings were significantly lower on 10 December where 3 monthly triadimefon applications of 150 and 300 mg m^{-2}, and a single October application of 610 mg m^{-2} was applied. The adverse effect of triadimefon at 610 mg m^{-2} was evident on 23 and 28 November.

During 1982, Fe plus 150 mg triadimefon m^{-2} maintained higher PGBGC than Fe plus 460 mg benomyl m^{-2}. However, during 1983, Fe plus benomyl maintained higher PGBGC than the Fe plus triadimefon treatment. The exact cause of this reversal in response is not known, but may have occurred because of differences in air temperature among the two years. Air temperatures were generally not as cold in October 1982 as in October 1983 and PGBGC among the Fe plus benomyl and Fe plus triadimefon treatments was similar under natural conditions. However, in 1983, when temperatures were low during October, PGBGC was higher in the Fe plus benomyl than in the Fe plus triadimefon treatment during late-October and early November. These data indicate that benomyl may be more compatable than triadimefon for use in combination with Fe when temperatures are low and bermudagrass is not grown under protective covers. These data also indicated that protection during early fall by certain chemical treatments may persist when bermudagrass is protected from frost by covers in late fall.

Post Dormancy Growth

Although Fe and Fe plus BA treatments prolonged bermudagrass green color retention in fall, these treatments had little significant effect on recovery from dormancy in spring 1983 based on PGBGC ratings (Table 5).

Table 5. Post-dormancy growth of Midiron bermudagrass during spring 1983 in response to previous season applications of iron and compounds with cytokinin-like activity.

Treatment	Rate	Percent Green Bermudagrass Ground Cover						Clipping yields
		Not covered			Under plastic cover			
		11 May	18 May	30 May	13 April	27 April	11 May	24 May
	mg a.i. m^{-2}							g $plot^{-1}$
Fe	61	40.0	50.0	71.7	16.7	45.0	58.3	29.3
Fe + Benzyladenine	61 + 3	41.7	53.3	71.7	33.3	55.0	61.7	27.7
Fe + Benzyladenine	61 + 6	50.0	51.7	73.3	28.3	51.7	66.7	31.7
Fe + Benzyladenine	61 + 9	45.0	60.0	71.7	20.0	51.7	66.7	33.3
Triadimefon	75	48.3	66.7	73.3	48.3	56.7	63.3	38.3
Triadimefon	150	53.3	63.3	75.0	50.0	55.0	65.0	27.7
Triadimefon	300	51.7	61.7	76.7	16.7	55.0	63.3	31.7
Benomyl	230	25.0	45.0	70.0	11.7	30.0	43.3	29.0
Benomyl	460	15.0	31.7	68.3	10.0	16.7	28.3	31.7
Benomyl	920	10.0	40.0	63.3	8.3	13.3	26.7	31.7
Benzyladenine	3	36.7	58.3	68.3	33.3	50.0	66.7	38.3
Benzyladenine	6	41.7	45.0	71.7	18.3	53.3	61.7	28.3
Benzyladenine	9	38.3	53.3	75.0	23.3	43.3	61.7	31.0
Fe + Benomyl	61 + 460	13.3	38.3	68.3	11.7	30.0	41.7	26.7
Fe + Triadimefon	61 + 150	45.0	61.7	75.0	36.7	40.0	51.7	34.3
Triadimefon	610	46.7	55.0	65.0	16.7	63.3	70.0	25.0
Benomyl	1830	20.0	45.0	70.0	10.0	23.3	33.3	21.7
Control	-	41.7	48.3	68.3	25.0	40.0	55.0	25.0
FLSD 0.05[†]		11.0	17.6	NS	20.8	13.9	13.2	NS

[†]FLSD, F least significant difference; NS, not significant.

Plots treated with Fe and Fe plus BA generally had higher PGBGC than controls during spring 1984 (Table 6). Significantly higher PGBGC occurred on 6 June 1984 in plots treated with Fe plus BA.

Triadimefon at 75 and 150 mg m^{-2} generally improved post-dormancy recovery in both years. On 18 May 1983 and 6 June 1984 PGBGC was significantly higher than the control when 75 mg triadimefon m^{-2} was applied during the previous growing season. The 150 mg triadimefon m^{-2} treatment significantly increased PGBGC scores on 6 June 1984. Triadimefon at 300 mg m^{-2} generally improved post-dormancy recovery during 1983. However, when 300 mg triadimefon m^{-2} was applied the previous season, the spring 1984 PGBGC was less than the control until 6 June. However, the 610 mg triadimefon m^{-2} treatment stimulated early recovery of Midiron during spring of 1983 and 1984 and significantly greater PGBGC was observed on 6 June 1984.

On 11 May 1983, all benomyl treatments had significantly lower PGBGC than the control. This response was evident on 18 May but not on 30 May 1983. Apparently, Midiron overcame the adverse effects of benomyl and obtained PGBGC similar to the control by late May. In contrast, recovery from winter dormancy in 1984 was generally better where benomyl was applied.

Table 6. Post-dormancy growth of Midiron bermudagrass during spring 1984 in response to previous season applications of iron and compounds with cytokinin-like activity.

Treatment	Rate	Percent Green Bermudagrass Ground Cover						Clipping Yield
		Not covered				Under plastic covers		
		7 May	21 May	28 May	6 June	7 May	21 May	15 May
	mg a.i. m^{-2}							g $plot^{-1}$
Fe	61	28.3	48.3	51.7	71.7	46.7	86.7	33.7
Fe + Benzyladenine	61 + 3	28.3	51.7	61.7	81.7	63.3	90.0	40.3
Fe + Benzyladenine	61 + 6	30.0	50.0	60.0	81.7	56.7	86.7	33.7
Fe + Benzyladenine	61 + 9	35.0	50.0	60.0	80.0	53.3	81.7	25.7
Triadimefon	75	31.7	50.0	60.0	70.3	56.7	88.3	25.0
Triadimefon	150	23.3	50.0	60.0	80.0	46.7	91.7	35.7
Triadimefon	300	26.7	38.3	48.3	70.0	40.0	83.3	27.7
Benomyl	230	25.0	48.3	58.3	75.0	51.7	83.3	34.3
Benomyl	460	26.7	40.0	56.7	65.0	60.0	90.0	33.0
Benomyl	920	35.0	48.3	58.3	71.7	48.3	81.7	36.7
Benzyladenine	3	23.3	41.7	53.3	75.0	50.0	83.3	39.0
Benzyladenine	6	26.7	43.3	53.3	73.3	53.3	86.7	31.3
Benzyladenine	9	26.7	36.7	46.7	66.7	53.3	88.3	28.7
Fe + Benomyl	61 + 460	38.3	48.3	58.3	76.7	68.3	91.7	31.0
Fe + Triadimefon	61 + 150	33.3	53.3	63.3	83.3	63.3	91.7	33.3
Triadimefon	610	33.3	46.7	56.7	78.3	51.7	88.3	26.7
Benomyl	1830	23.3	45.0	55.0	75.0	46.7	80.0	24.7
Control	-	20.0	41.7	50.0	66.7	50.0	83.7	28.7
FLSD 0.05[†]		NS	NS	NS	9.5	NS	NS	10.1

[†]FLSD, F least significant difference; NS, not significant.

There were no significant differences between weight of clippings harvested from plots on 24 May 1983, although clipping yields were 53% higher from turf treated with 3 mg BA m^{-2} than from untreated turf. Plots treated with 3 mg BA m^{-2} applied alone or with Fe had significantly higher clipping yields on 15 May 1984.

Biswas and Choudhuri (1977) reported BA increased growth and dry matter accumulation in rice (Oryza sativa L.). Towne and Owensby (1983) reported that 0.55 mg BA m^{-2} applied in mid- or late-July significantly increased total herbage yields of native prairie grasses the following year. These authors suggested that synthetic cytokinin could be cycled through plant-storage organs during dormancy and reactivated with the resurgence of growth. Midiron clipping weights were increased the following spring by the lowest rate of BA applied the previous season in the present study. That higher rates of BA did not elicit increased clipping yields agrees with data reported by Towne and Owensby (1983).

Midiron broke dormancy 3 to 4 weeks earlier in the spring when covered with clear plastic than when not covered. In 1983, BA applied alone or with Fe generally improved PGBGC following dormancy break under plastic. Application of 3 mg BA m^{-2} in conjunction with Fe produced

significantly higher PGBGC than the control on 27 April 1983. A similar trend was observed in 1984 under plastic covers.

Triadimefon at 75 and 150 mg m^{-2} applied the previous season stimulated earlier growth under plastic in 1983 as evidenced by significantly higher PGBGC ratings on 13 and 27 April 1983. Plots treated with 610 mg triadimefon m^{-2} had higher PGBGC than the control on 27 April and 11 May 1983. However, during 1984 no significant growth stimulation was observed for these treatments.

The stimulation of spring growth by triadimefon observed in 1983 was not observed in 1984 possibly because of much colder temperatures which occurred during the 1983-1984 winter (Appendices 1 and 2) and the possibility that triadimefon may not protect against freezing injury.

Benomyl at all rates tested slowed post-dormancy recovery of Midiron grown under plastic covers in 1983 but not in 1984. Iron applied in addition to benomyl did not alter the adverse effects of benomyl on post-dormancy recovery observed in 1983.

Post Dormancy Growth Under Greenhouse Conditions

Growth of Midiron was good for all treatments when allowed to break dormancy under greenhouse conditions. Recovery from dormancy in a warm greenhouse environment may not

be indicative of growth under natural conditions where bermudagrass would be subjected to intermittent chilling temperatures. However, growth of Midiron under greenhouse conditions in the absence of light following dormancy illustrates the importance of carbohydrate energy reserves on development of bermudagrass canopies in spring. During 1983 and 1984, post-dormancy growth was rapid in all treatments for the initial 2 weeks but thereafter clipping yields declined dramatically (Tables 7 and 8). The substantial reduction in clipping yields probably occurred because carbohydrate energy reserves were rapidly depleted. Darkness prevented photosynthesis and therefore growth occurred at the expense of carbohydrates in rhizome and stolon tissue. Because carbohydrates may be exhausted by post-dormancy recovery when photosynthetic rates are low, severely weakened bermudagrass turf could result from late cool temperature periods or frost.

The Fe and Fe plus BA treatments did not significantly affect post-dormancy foliar growth of Midiron in the dark or light. However, in both 1983 and 1984 shoot counts and foliage yields of Midiron grown with natural lighting were consistently higher in the Fe and the Fe plus BA treatments compared to untreated turf.

Table 7. Post-dormancy growth of Midiron bermudagrass sod-plugs during 1983 following winter dormancy in response to previous season applications of iron and compounds with cytokinin-like activity.

Treatment	Rate	Clipping[1] Yields			Shoot[‡] Counts		Clipping[†] Yields		Root[‡] Yields
		31 Mar.	5 Apr.	11 Apr.	26 Feb.	1 Mar.	17 Mar.	28 Mar.	11 Apr.
	mg a.i. m^{-2}	---------mg pot^{-1}---------			--shoots pot^{-1}--		-----mg pot^{-1}-----		mg pot^{-1}
Fe	61	106.7	126.7	26.3	7.0	25.0	433	467	185
Fe + Benzyladenine	61 + 3	76.7	110.0	27.7	11.3	24.3	517	467	345
Fe + Benzyladenine	61 + 6	90.0	100.0	27.0	8.3	20.0	567	517	213
Fe + Benzyladenine	61 + 9	76.7	103.3	20.3	6.7	25.0	400	467	251
Triadimefon	75	116.7	176.7	36.0	7.0	31.3	533	467	258
Triadimefon	150	63.3	90.0	21.0	9.0	36.7	667	400	273
Triadimefon	300	93.3	76.7	47.0	8.7	20.7	433	500	252
Benomyl	230	73.3	120.0	34.7	2.7	17.7	467	367	274
Benomyl	460	126.7	136.7	33.0	2.0	20.7	433	533	191
Benomyl	920	120.0	160.0	28.3	2.3	25.3	633	367	163
Benzyladenine	3	120.0	123.3	19.7	8.0	39.7	500	500	184
Benzyladenine	6	73.3	123.3	26.7	8.0	34.3	367	367	177
Benzyladenine	9	120.0	96.7	21.7	2.7	26.7	467	300	133
Fe + Benomyl	61 + 460	103.3	143.3	34.0	2.3	20.0	467	333	142
Fe + Triadimefon	61 + 150	73.3	86.7	24.0	1.0	13.7	400	367	201
Triadimefon	610	156.7	133.3	28.7	7.3	46.3	600	667	416
Benomyl	1830	110.0	116.7	29.3	9.0	43.7	500	500	199
Control	-	80.0	116.7	29.3	3.7	20.7	367	433	202
FLSD 0.05[§]		51.3	NS	NS	NS	NS	252	NS	129

[†] Plants maintained in darkness.

[‡] Plants maintained with light/dark periods.

[§] FLSD, F least significant difference; NS, not significant.

Table 8. Post-dormancy growth of Midiron bermudagrass sod-plugs during 1984 following winter dormancy in response to applications of iron and compounds with cytokinin-like activity.

Treatment	Rate	Clipping Yields†			Shoot Counts‡		Clipping Yields‡		Root Yields‡
		2 Apr.	9 Apr.	13 Apr.	5 Mar.	9 Mar.	23 Mar.	30 Mar.	6 Apr.
	mg a.i. m^{-2}	mg pot^{-1}			shoots pot^{-1}		mg pot^{-1}		mg pot^{-1}
Fe	61	112.0	103.3	28.3	9.0	26.4	150	463	217
Fe + Benzyladenine	61 + 3	80.0	101.7	31.7	10.0	33.3	137	487	363
Fe + Benzyladenine	61 + 6	93.3	96.7	31.7	8.7	25.0	177	413	293
Fe + Benzyladenine	61 + 9	83.3	110.0	26.7	7.0	28.3	160	540	227
Triadimefon	75	101.7	156.7	41.7	6.7	30.0	160	470	245
Triadimefon	150	73.3	83.3	25.0	8.7	41.7	180	567	237
Triadimefon	300	80.0	76.7	53.3	9.0	26.7	140	593	273
Benomyl	230	86.7	121.7	46.7	2.0	18.0	67	513	287
Benomyl	460	110.0	133.3	33.3	2.7	21.7	183	567	183
Benomyl	920	121.7	156.7	21.7	2.7	23.5	187	437	143
Benzyladenine	3	126.7	133.3	21.7	9.0	42.0	183	513	197
Benzyladenine	6	93.3	121.7	25.0	8.7	33.3	133	437	193
Benzyladenine	9	96.7	83.3	26.7	3.3	25.0	117	360	157
Fe + Benomyl	61 + 460	110.0	151.7	38.3	3.3	22.4	167	507	227
Fe + Triadimefon	61 + 150	86.7	93.3	29.3	2.3	17.4	187	507	233
Triadimefon	610	145.0	131.7	29.3	8.7	33.3	147	493	233
Benomyl	1830	116.7	121.7	28.7	10.0	31.0	180	443	230
Control	-	76.7	120.0	26.7	3.3	22.6	130	407	240
FLSD 0.05§		46.7	NS	NS	NS	14.3	NS	NS	121

† Plants maintained in darkness.

‡ Plants maintained with light/dark periods.

§ FLSD, F least significant difference; NS, not significant.

This response appears unrelated to carbohydrate levels since clippings produced in continuous darkness were similar for the Fe, Fe plus BA, and control treatments. Chlorotic leaves were observed during spring in field grown Midiron bermudagrass that received no Fe applications the previous season. The general enhancement of growth measured in greenhouse and observed in field studies for the Fe and Fe plus BA treatments may have occurred because of improved photosynthetic capacity at an early stage following dormancy break. Terry (1980) reported that chlorotic sugar beet (Beta vulgaris L.) leaves had lower photosynthetic rates than leaves with high chlorophyll content. Enhanced photosynthetic capacity just after dormancy break could provide assimilate needed for rapid establishment of bermudagrass turf canopies.

Application of 3 mg BA m^{-2} in conjunction with Fe significantly increased root weights over those of untreated turf. Root weights generally decreased as the rate of BA increased.

Midiron treated with 3 mg BA m^{-2} produced 50 and 65% more growth in the dark following dormancy than untreated Midiron on 31 March 1983 and 2 April 1984, respectively. The response to BA was significant on 2 April 1984. Higher rates were not as effective. These data suggest that low rates of BA applied during the fall may enhance carbohydrate storage

or slow the rate of carbohydrate utilization during the dormancy period. Thus, high carbohydrate energy reserves would be available for rapid spring growth following dormancy.

Shoot counts, and clipping yields of Midiron grown under greenhouse conditions with natural light periods following dormancy were generally higher for the 3 mg BA m^{-2} treatment. Significantly higher numbers of new shoots were counted on 9 March 1984 when Midiron received 3 mg BA m^{-2} the previous season.

Significantly greater clippings on 17 March 1983 and shoot counts on 9 March 1984 from the light treatment were measured for turf treated with 150 mg triadimefon m^{-2}. A single October application of 610 mg triadimefon m^{-2} caused higher initial post-dormancy growth in darkness. Yields were significantly higher on 31 March 1983 and 2 April 1984. Shoot counts and clipping yields from the light treatment were also enhanced in 1983 and 1984 with this single October application of triadimefon. Triadimefon at 610 mg m^{-2} significantly increased rooting in 1983 but not in 1984. This same rate of triadimefon significantly hastened the loss of PGBGC in the field during fall 1983 and to a lesser extent during late fall 1984. Triadimefon at 610 mg m^{-2} may have prevented growth of Midiron during late fall. Thus, carbohydrates may have been conserved and used for more rapid

post-dormancy growth. The other triadimefon treatments had no consistent effect on Midiron growth under greenhouse conditions following dormancy.

Growth of Midiron under greenhouse conditions following dormancy was not significantly affected by benomyl treatments with the exception that 920 mg benomyl m^{-2} had higher clipping yields on 17 March 1983 from the light treatment.

Conclusion

The results of this study indicate that foliar Fe fertilization during late summer and fall can extend bermudagrass performance during fall without adverse effects on growth the following spring. Benzyladenine applications may not enhance bermudagrass turf quality or performance when Fe is not also applied to turf. Triadimefon may potentially be useful for stimulating post-dormancy growth of bermudagrass turf and deserves further evaluation. However, triadimefon may have limited benefit on fall bermudagrass performance. Midiron bermudagrass treated with benomyl generally did not perform well in fall or spring. However, the negative effects of benomyl were alleviated by Fe.

The use of clear plastic covers offers a means for preventing frost injury to bermudagrass turf. A 6- to 8-week longer growing season could be realized on bermudagrass turf if protective covers were utilized. Protective covers could provide year-long green bermudagrass turf in some climatic regions if included as part of management of athletic fields used primarily for professional sports. However, because of time and labor constraints the use of such covers may not be of practical value for routine turf use. Fe fertilization alone and in conjunction with synthetic cytokinin applications during late summer and fall may provide a practical

means for prolonging color retention and thus extending fall bermudagrass performance. The use of Fe and BA could also enhance turf color if protective covers are included as a part of fall and spring bermudagrass management. Post-dormancy growth of bermudagrass may be enhanced by Fe and cytokinin applications.

Experiment 2. Fall Performance and Post-Dormancy Growth of Midiron Bermudagrass in Response to Nitrogen, Iron, and Benzyladenine.

Materials and Methods

The 2-year study was initiated in June 1983 on a 6-year-old Midiron bermudagrass [Cynodon dactylon (L.) Pers.] turf growing in a Groseclose silt loam (a clayey, kaolinitic, mesic Typic Hapludult) soil with a pH of 5.7 at the Turfgrass Research Center at Blacksburg, VA. A multiple split-plot experimental design with four replications was used. Whole plots were 4.5 by 3 m and consisted of monthly nitrogen fertilization treatments during June through September of 2.4 and 4.8 g m^{-2} from NH_4NO_3.

Sub-plots were Fe treatments of none, single, and split applications of 0, and 120 mg m^{-2} $month^{-1}$ from sodium ferric diethylenetriamine pentaacetate during 15 July through 15 October. Sub-sub plots were 1.5 by 1.5 m and consisted of benzyladenine (6-benzylaminopurine) treatments of 0 and 6.2 mg m^{-2} applied every 2 weeks (12.4 mg m^{-2} $month^{-1}$) during 15 August through 15 October. Fe and benzyladenine (BA) were applied using a compressed air boom sprayer which delivered 561 L aqueous solution ha^{-1}.

Nitrogen and Fe treatments were randomly arranged in rows and BA treatments were randomly arranged in column strips across N and Fe treatments within replications producing complete factorialization of treatments.

The plots were maintained at a 2.5-cm height by mowing one or two times per week. Irrigation was applied as needed to maintain favorable growing conditions. Phosphorus and K as concentrated superphosphate, and KCl, respectively, were applied in June 1983 and 1984 at 9.7 g m^{-2}.

Visual ratings of percent green bermudagrass ground cover (PGBGC) taken at approximately weekly intervals were used as an indication of treatment effects on bermudagrass performance during the fall. Visual ratings of PGBGC taken at approximately weekly intervals following dormancy break in spring and clipping yields taken in May 1984 and 1985 were used to assess treatment effects on post-dormancy recovery.

Two soil cores 10 cm in diameter by 10 cm deep were taken at random from each plot on 13 September, 16 October, and 14 November 1984. After washing soil from each core, samples were stored at -8° C until rhizome and stolon tissue could be collected by hand separation. Rhizome and stolon tissue was dried for one hour at 100° C, then for 24 hours at 65° C in a forced-air oven. Tissue was then ground to

pass a 40-mesh screen in a Cyclone Sample Mill (UD Corporation, Bolder, Colorado).

Rhizome and stolon tissue were subsequently analyzed for nonreducing and reducing sugars and starch by the methods of Smith (1968) with modifications as outlined below. Water soluble sugars (WSS) were extracted by addition of 20 ml distilled H_2O to 100-ml test tubes containing 100-mg tissue samples and heating at 100° C for 1 hour in a water bath. Following this initial extraction, a 4-ml aliquot of extract was removed and replaced by 4 ml of 0.8 N H_2SO_4. Samples were returned to a 100° C water bath. After 1 hour, a 4-ml aliquot was again removed, and 8 ml of enzyme-buffer solution was added. The enzyme-buffer solution contained 4 ml of 15% takadiastase (Charase 40000, Miles Laboratories) and 4 ml buffer (2:3, v:v, 0.2 N acetic acid:0.2 N sodium acetate, pH 4.9). Samples were then incubated for 24 hours at 37° C after which a 4-ml aliquot was removed.

Reducing power of each 4-ml aliquot was immediately determined by automated colorimetric analysis following reaction with p-amino hydroxybenzoic acid hydrazide. Nonreducing sugar content of tissue was taken as the difference in reducing power following incubation with dilute H_2SO_4 minus reducing power of the hot water extract. Starch content was taken as the difference in reducing power following en-

zyme digestion minus reducing power of the hot water extract. Carbohydrate content was expressed as glucose equivalents per kg dry weight of tissue.

All data were subjected to an analysis of variance. When a significant F ratio occurred, a least significant difference (LSD) was calculated.

Results and Discussion

Fall Performance

The experimental area was observed throughout the spring, summer and fall periods for visual differences which may have occurred in response to treatments. No obvious visual differences in turf response to application levels of Fe or BA occurred until September or October when ambient air temperatures reached chilling and/or frost conditions (Appendices 2 and 3). Plots that received high (4.8 g N m^{-2}) N had darker green more dense turf during June through early-September than did plots fertilized with low (2.4 g N m^{-2}) N.

Fall bermudagrass color retention was prolonged by high summer N in 1983 and 1984 (Table 9 and Table 11). Plots receiving high summer N had significantly higher PGBGC on 30 September and 24 October 1983 and from 6 October through 7 November 1984. Reeves et al. (1970) reported that Tifgreen bermudagrass fertilized with N during the fall retained a darker green color longer but was more susceptible to frost injury than turf that received only summer N. Data of the present study indicate that N applied during late summer can influence bermudagrass performance during the fall. Low temperatures cause reduced bermudagrass growth.

Table 9. Midiron bermudagrass green ground cover during fall 1983 as influenced by nitrogen, iron, and benzyladenine.

Iron	Benzyladenine	Percent Green Ground Cover				
		Date of Visual Rating				
		30 Sept.	6 Oct.	13 Oct.	24 Oct.	4 Nov.
——mg m^{-2} $month^{-1}$——		——————2.4 g N m^{-1} $month^{-1}$——————				
0	0	55.0	55.0	55.0	53.8	18.8
	12.4	60.0	61.3	58.8	57.5	31.3
Mean		57.5	58.1	56.9	55.6	25.1
120	0	60.0	60.0	61.3	58.8	41.3
	12.4	61.3	63.8	63.8	60.0	43.8
Mean		60.6	61.9	62.5	59.4	42.5
120 split	0	60.0	68.8	71.3	66.3	43.8
	12.4	65.0	71.3	73.8	70.0	48.8
Mean		62.5	70.0	72.5	68.1	46.3
N Mean		60.2	63.3	64.0	61.0	37.9
		——————4.8 g N m^{-2} $month^{-1}$——————				
0	0	62.5	63.8	62.5	62.5	31.3
	12.4	70.0	66.3	66.3	67.5	41.3
Mean		66.3	65.0	64.4	65.0	46.3
120	0	66.3	67.5	67.5	65.0	46.3
	12.4	76.0	70.0	68.6	67.5	53.8
Mean		68.1	68.8	68.1	66.3	50.0
120 split	0	70.0	75.0	74.1	74.5	51.2
	12.4	75.0	78.8	75.9	75.3	52.6
Mean		72.5	76.9	75.0	74.4	51.9
N Mean		69.0	70.2	69.2	68.5	46.0
FLSD 0.05 (Fe)*		3.9	5.0	3.7	3.4	4.6
FLSD 0.05 (BA)‡		4.3	3.3	NS	NS	5.2

* FLSD, F least significant difference for comparison of Fe means.

‡ FLSD, F least significant difference for comparison of BA means; NS, not significant.

Table 10. F-test for Midiron bermudagrass green ground cover during fall 1983 as influenced by nitrogen, iron, and benzyladenine.

Parameter	Date of Visual Rating				
	30 Sept.	6 Oct.	13 Oct.	24 Oct.	4 Nov.
Nitrogen (N)	**	NS	NS	*	NS
Iron (Fe)	*	***	***	***	***
Benzyladenine (BA)	*	*	NS	NS	*
N x Fe	NS	NS	NS	NS	NS
N x BA	NS	NS	NS	NS	NS
Fe x BA	NS	NS	NS	NS	NS
N x Fe x BA	NS	NS	NS	NS	NS

*, **, *** Significant at the 5, 1 and 0.1% level of probability, respectively. NS, not significant.

Table 11. Midiron bermudagrass green ground cover during fall 1984 as influenced by nitrogen, iron, and benzyladenine.

Iron	Benzyladenine	Percent Green Ground Cover, Date of Visual Rating: 20 Sept.	4 Oct.	10 Oct.	15 Oct.	30 Oct.	7 Nov.
mg m^{-2} $month^{-1}$		2.4 g N m^{-1} $month^{-1}$					
0	0	93.8	81.3	53.8	45.0	45.0	18.8
	12.4	91.3	83.8	58.8	58.8	51.3	17.5
Mean		92.5	82.5	56.3	48.1	48.1	18.1
120	0	96.0	91.2	81.3	70.0	66.3	33.8
	12.4	95.0	90.0	85.0	72.5	70.0	36.3
Mean		95.5	89.4	83.1	71.3	68.1	35.0
120 split	0	95.0	90.0	73.8	62.5	56.3	35.0
	12.4	95.0	90.0	81.3	66.3	61.3	32.5
Mean		95.0	90.0	77.5	64.4	58.8	33.8
N Mean		94.3	87.3	72.3	61.3	58.3	29.0
		4.8 g N m^{-1} $month^{-1}$					
0	0	93.8	90.0	71.3	51.3	62.5	27.5
	12.4	92.5	91.3	77.5	57.5	63.8	25.0
Mean		93.1	90.6	74.4	54.4	63.1	26.3
120	0	95.0	92.5	83.8	78.8	77.5	47.5
	12.4	95.0	95.0	91.3	87.5	82.5	45.0
Mean		95.0	93.8	87.5	83.1	80.0	46.3
120 split	0	95.0	93.8	85.0	71.3	75.0	50.0
	12.4	95.0	95.0	90.0	81.3	80.0	48.8
Mean		95.0	94.4	87.5	76.3	75.5	49.4
N Mean		94.4	92.9	83.1	71.3	73.5	40.6
FLSD 0.05 (Fe) †		1.5	2.7	5.6	7.4	5.1	4.2
FLSD 0.05 (BA) ‡		NS	NS	5.3	NS	NS	NS

† FLSD, F least significant difference for comparison of Fe means.

‡ FLSD, F least significant difference for comparison of BA means; NS, not significant.

Table 12. F-test for Midiron bermudagrass green ground cover during fall 1984 as influenced by nitrogen, iron, and benzyladenine.

Parameter	Date of Visual Rating					
	20 Sept.	4 Oct.	10 Oct.	15 Oct.	30 Oct.	7 Nov.
Nitrogen (N)	NS	**	**	*	*	**
Iron (Fe)	**	***	***	***	***	***
Benzyladenine (BA)	NS	NS	*	NS	NS	NS
N x Fe	NS	NS	*	NS	NS	NS
N x BA	NS	NS	NS	NS	NS	NS
Fe x BA	NS	NS	NS	NS	NS	NS
N x Fe x BA	NS	NS	NS	NS	NS	NS

*, **, ***Significant at the 5, 1, and 0.1% level of probability, respectively. NS, not significant.

Therefore, N taken up by roots may be utilized less rapidly for production of new tissue. Although water soluble N was applied no later than the second week in September in both years of this study, differences in PGBGC among N application levels were evident until the first week in November in 1983 and 1984. Nitrogen applied to bermudagrass during fall when temperatures are low may not be utilized efficiently and may contribute to high tissue nitrogen levels. Since high tissue NO_3-N levels may decrease low temperature tolerance (Dunn and Nelson, 1974) N fertilization of bermudagrass during fall should be avoided.

Significantly higher PGBGC during 30 September through 4 November 1983 was observed in plots treated with a split application of 120 mg Fe m^{-2} $month^{-1}$ than in plots receiving no Fe (Table 9). A single monthly application of 120 mg Fe m^{-2} significantly improved PGBGC ratings on 13 October and 4 November compared to the 0 mg Fe m^{-2} treatment.

Higher PGBGC was maintained during 20 September through 7 November 1984 by either single or split monthly applications of Fe compared to turf that received no Fe (Table 11). In contrast to 1983, a single monthly Fe application generally provided better maintenance of green color during fall 1984 than the split Fe application treatment. This response was more pronounced at low than at high summer N. From 6

through 24 October 1983, PGBGC in plots receiving split application of Fe was significantly higher than where single monthly Fe application was used. The different response to timing of Fe application probably occurred because of seasonal differences in fall temperatures in 1983 compared to 1984 (Appendix 2 and 3). Air temperatures were much cooler and low temperatures were persistent longer in fall 1983 than in 1984. These data indicate that frequent applications of Fe may more effectively extend the maintenance of green bermudagrass turf during fall in years when chilling temperatures are more severe and prolonged.

Benzyladenine (BA) improved green color retention during fall in 1983 and 1984. Significantly higher PGBGC ratings on 30 September, 6 October, and 4 November 1983, and on 10 October 1984 were observed in plots treated with BA. Although BA applications may improve green color retention of bermudagrass exposed to chilling temperatures, application of Fe appears to be required in conjunction with BA to maintain acceptable levels of green bermudagrass ground cover longer into the fall.

Post-dormancy Growth

Summer N application level did not significantly affect post-dormancy recovery of Midiron bermudagrass in 1984 (Table 13) or 1985 (Table 15). Adams and Twersky (1960) reported that increasing summer N fertilization rates decreased winter survival of coastal bermudagrass. Increasing K application rates with increasing N offset the adverse effects of high N on winter survival. Midiron has a good to excellent winter survival history. Winter survival and post-dormancy recovery of Midiron were not affected by N application level probably because the range of N rates used was narrow and within moderate rates for fertilization of bermudagrass turf.

A single application of 120 mg Fe m^{-2} $month^{-1}$ during the previous season improved post-dormancy recovery of Midiron in 1984 and 1985 as evidenced by significantly higher PGBGC ratings for this treatment. (Table 13 and Table 15). The single monthly Fe application stimulated more rapid development of green bermudagrass ground cover in spring 1984 than the split Fe application treatment. However, no significant difference in PGBGC ratings in spring 1985 occurred among the single and split Fe application levels.

Table 13. Midiron bermudagrass green ground- cover and clipping yields during spring 1984 as influenced by nitrogen, iron, and benzyladenine applied during the previous growing season.

Iron	Benzyladenine	Percent Green Ground Cover, Date of Visual Rating: 7 May	14 May	21 May	10 June	25 June	Clipping Yield: 25 May
mg m^{-2} $month^{-1}$		2.4 g N m^{-2} $month^{-1}$					g $plot^{-1}$
0	0	27.5	37.5	45.0	60.0	75.0	28.8
	12.4	25.0	36.3	48.8	63.8	77.5	27.3
Mean		26.3	36.9	46.8	61.9	76.3	28.0
120	0	28.8	47.5	51.3	66.3	81.3	29.5
	12.4	27.5	42.5	52.5	67.5	81.3	27.3
Mean		28.1	45.0	51.9	66.9	81.3	28.4
120 split	0	22.5	35.0	43.8	58.8	75.0	24.8
	12.4	28.8	40.0	48.8	63.8	78.8	29.8
Mean		25.6	37.5	46.3	61.3	76.9	27.3
N Mean		26.6	39.8	48.3	63.3	78.1	27.9
		4.8 g N m^{-2} $month^{-1}$					
0	0	17.5	35.0	41.3	56.3	71.3	34.8
	12.4	23.8	38.8	46.3	61.3	76.3	32.0
Mean		20.6	35.0	43.8	58.8	73.8	33.4
120	0	30.0	47.5	60.0	72.5	86.3	37.0
	12.4	31.3	48.8	61.3	73.8	87.5	32.0
Mean		30.6	48.1	60.6	73.1	86.9	34.5
120 split	0	22.5	38.8	47.5	62.5	76.3	32.8
	12.4	26.3	41.3	52.5	67.5	81.3	25.5
Mean		24.4	40.0	50.0	65.0	78.8	29.1
N Mean		25.2	41.0	51.5	65.6	79.8	32.3
FLSD 0.05 (Fe)†		4.7	5.5	6.2	4.4	3.3	NS
FLSD 0.05 (BA)‡		NS	NS	6.1	4.8	4.1	NS

† FLSD, F least significant difference for comparison of Fe means.

‡ FLSD, F least significant difference for comparison of BA means; NS, not significant.

Table 14. F-test for Midiron bermudagrass green ground cover and clipping yields during spring 1984 as influenced by nitrogen, iron, and benzyladenine applied during the previous growing season.

	Date of Visual Rating					Clipping Yield
Parameter	7 May	14 May	21 May	10 June	25 June	25 May
Nitrogen (N)	NS	NS	NS	NS	NS	NS
Iron Fe)	*	***	**	***	***	NS
Benzyladenine (BA)	NS	NS	*	*	*	NS
N x Fe	NS	NS	NS	NS	*	NS
N x BA	NS	NS	NS	NS	NS	NS
Fe x BA	NS	NS	NS	NS	NS	NS
N x Fe x BA	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 5, 1, and 0.1% levels of probability, respectively.

Table 15. Midiron bermudagrass green ground cover and clipping yield during spring 1985 as influenced by nitrogen, iron, and benzyladenine applied during the previous growing season.

Iron	Benzyladenine	Percent Green Ground Cover, Date of Visual Rating: 25 Apr.	30 Apr.	6 June	Clipping Yield: 7 May
mg m^{-2} $month^{-1}$		2.4 g N m^{-2} $month^{-1}$			g $plot^{-1}$
0	0	27.5	52.5	61.3	28.2
	12.4	30.0	52.5	61.3	26.4
Mean		28.8	52.5	61.3	27.3
120	0	32.5	58.8	65.0	28.8
	12.4	32.5	56.3	66.3	26.5
Mean		32.5	57.5	65.6	27.6
120 split	0	30.0	51.2	63.4	29.0
	12.4	33.8	56.3	68.3	28.4
Mean		31.9	53.8	66.3	28.7
N Mean		31.0	54.6	64.4	27.9
		4.8 g N m^{-2} $month^{-1}$			
0	0	22.5	40.0	55.0	25.4
	12.4	23.3	41.3	55.0	27.9
Mean		23.1	40.6	55.0	26.6
120	0	32.5	53.8	66.3	25.9
	12.4	33.8	52.5	67.5	27.6
Mean		33.1	53.1	66.9	26.8
120 split	0	30.0	55.0	66.3	27.3
	12.4	36.3	62.5	70.0	27.8
Mean		33.1	58.8	68.1	27.6
N Mean		29.8	50.8	63.3	27.0
FLSD 0.05 (Fe) †		5.3	8.7	7.2	NS
FLSD 0.05 (BA) ‡		NS	NS	NS	NS

† FLSD F least significant difference for comparison of Fe means.

‡ FLSD, F least significant difference for comparison of BA means; NS, not significant.

Table 16. F-test for Midiron bermudagrass green ground cover and clipping yield during spring 1985 as influenced by nitrogen, iron, and benzyladenine applied during the previous growing season.

Parameter	Date of Visual Rating			Clipping Yield
	25 April	30 April	6 May	7 May
Nitrogen (N)	NS	NS	NS	NS
Iron (Fe)	*	*	**	NS
Benzyladenine (BA)	NS	NS	NS	NS
N x Fe	NS	NS	NS	NS
N x BA	NS	NS	NS	NS
Fe x BA	NS	*	NS	NS
N x Fe x BA	NS	NS	NS	NS

*, ** Significant at the 5 and 1% levels of probability, respectively.
NS, not significant.

Post-dormancy recovery of Midiron was improved in spring 1984 in plots treated with 12.4 mg BA m^{-2} $month^{-1}$ compared to plots that received no BA. The response to BA was significant on 21 May and 10 and 25 June 1984. However, in 1985 no significant differences among BA application levels occurred at any observation date.

Clipping yields were not significantly affected in May 1984 or 1985 by any level of N, Fe, or BA. The significantly higher PGBGC ratings for the single monthly Fe treatment compared to no Fe without a corresponding increase in clipping yields appears contradictory. McCaslin and Watson (1977) reported that Fe applied in late September to common bermudagrass approaching the onset of dormancy had no effect on color ratings taken the following spring. Bermudagrass turf treated with Fe the previous season was as chlorotic as the turf that received no Fe applications. McCaslin and Watson (1977) suggested that no "carry-over effects" of Fe occurred. Midiron treated with Fe during summer and fall appeared to have darker green leaves in spring 1984 and 1985 than Midiron that received no Fe applications. This observation indicates that Fe applied the previous season may reduce chlorosis exhibited in bermudagrass turf in early spring under some environmental conditions. McCaslin and Watson (1977) applied Fe to bermudagrass approaching dorman-

cy when foliar absorption may have been poor and complex reactions in the calcareous soil may have inhibited root Fe uptake. Because clipping yields did not differ significantly among Fe application levels, the differences in Midiron PGBGC ratings during spring may have occurred primarily due to chlorosis in plots that received no Fe applications the previous season.

Nonstructural Carbohydrates

When data were combined over all N, Fe, and BA application levels, total nonstructural carbohydrate (TNC) levels in rhizomes changed relatively little between 13 September and 14 November 1984 (Table 17). However, stolon TNC levels increased by approximately 27% between 13 September and 16 October. Although TNC levels in stolons declined between 16 October and 14 November, TNC levels were 15% higher on 14 November than on 13 September.

Starch levels in stolons accounted for the most dramatic increase of the nonstructural carbohydrate fractions during 13 September to 16 October. However, during the 16 October to 14 November period, stolon starch levels decreased by 37%, and non-reducing and reducing sugars increased by 187, and 105%, respectively. Rhizome starch levels decreased by 22%, and nonreducing and reducing sugars increased by 100, and 124%, respectively, during 16 October through 14 November.

Table 17. Non-structural carbohydrate fractions in Midiron bermudagrass stolons and rhizomes during fall 1984.

Date of Sampling	Non-structural Carbohydrate Fractions			
	Water Soluble Sugars			
	Non-reducing	Reducing	Starch	Total
	g kg^{-1}			
	Stolons			
13 September	7.6	44.6	230.7	282.9
16 October	10.3	53.7	294.7	358.7
14 November	29.6	110.0	186.7	326.3
FLSD 0.05	4.0	7.3	27.1	26.3
	Rhizomes			
13 September	7.4	47.1	311.0	365.5
16 October	11.1	51.3	298.7	361.1
14 November	22.2	114.7	233.7	370.6
FLSD 0.05 †	3.8	13.6	22.7	NS

† FLSD 0.05, F least significant difference for comparison of date of sampling means within a non-structural carbohydrate fraction.

Dunn and Nelson (1974) reported similar increases in TNC in three other bermudagrass cultivars. In U-3, Midway, and Westwood bermudagrass, starch levels gradually decreased, and reducing and nonreducing sugars increased slightly during late-September to early-December (Dunn and Nelson, 1974). Rogers, et al. (1975) reported that Meyer zoysiagrass (Zoysia japonica Steud.) rhizomes and stolons accumulated TNC during September and remained near 50% of dry weight until December. Reducing sugars remained low and unchanged, while total sugars gradually increased during fall.

Conversion of starch to sugar and accumulation of maximum sugar levels in perennials acclimated to cold temperatures has been suggested to favor low temperature survival by plants (Guinn, 1971; Aldon and Herman, 1971; Parker, 1962; Sauai and Yashan, 1968). Accumulation of sugars in rhizome and stolon tissue of Midiron bermudagrass may explain the relatively good winter survival exhibited by this cultivar.

Stolon TNC levels were generally lower at each sampling date in plots receiving high N relative to low N applications (Table 18). On 16 October, plots treated with high N had significantly lower stolon TNC than those treated with low summer N.

Table 18. Total non-structural carbohydrates in Midiron bermudagrass stolons during fall 1984 as influenced by nitrogen, iron, and benzyladenine.

Iron	Benzyladenine	Total Non-structural Carbohydrates								
		13 Sept.			16 Oct.			14 Nov.		
		g N m^{-2} $month^{-1}$			g N m^{-2} $month^{-1}$			g N m^{-2} $month^{-1}$		
mg m^{-2} $month^{-1}$		2.4	4.8	Mean	2.4	4.8	Mean	2.4	4.8	Mean
		g kg^{-1}								
0	0	285.0	281.4	283.2	366.9	376.8	371.8	315.3	335.8	325.5
	12.4	289.3	258.2	273.8	372.3	361.2	366.8	348.9	318.7	333.8
Mean		287.2	269.8	278.5	369.6	369.0	369.3	332.1	327.2	329.7
120	0	273.8	280.1	276.9	361.2	344.8	353.0	343.2	313.6	328.4
	12.4	291.3	283.0	287.2	366.2	347.1	357.0	345.3	311.4	323.4
Mean		282.5	281.5	282.0	363.7	346.2	355.0	344.3	312.5	328.4
120 split	0	302.3	291.4	296.8	346.6	349.0	347.8	317.4	326.6	322.0
	12.4	288.3	271.2	279.8	353.6	358.0	355.8	321.0	318.3	319.7
Mean		295.3	281.3	288.3	350.1	353.5	351.8	319.2	322.4	320.8
N Mean		288.3	277.5		361.6	356.2		331.8	320.7	
FLSD 0.05 (Fe)†		NS	NS	NS	NS	NS	2.9	NS	NS	NS
FLSD 0.05 (BA)‡		NS	NS	NS	NS	NS	NS	NS	NS	NS

† FLSD, F least significant difference for comparison of Fe means.

‡ FLSD, F least significant difference for comparison of BA means; NS, not significant.

Table 19. Total non-structural carbohydrates in Midiron bermudagrass rhizomes during fall 1984 as influenced by nitrogen, iron, and benzyladenine.

Iron	Benzyladenine	Total Non-structural Carbohydrates								
		13 Sept.			16 Oct.			14 Nov.		
		g N m^{-2} $month^{-1}$			g N m^{-2} $month^{-1}$			g N m^{-2} $month^{-1}$		
		2.4	4.8	Mean	2.4	4.8	Mean	2.4	4.8	Mean
mg m^{-2} $month^{-1}$		g kg^{-1}								
0	0	376.4	372.9	374.7	359.7	368.8	364.3	367.8	371.0	369.4
	12.4	359.1	367.6	363.4	367.6	380.9	374.3	378.6	369.3	374.0
Mean		367.8	370.3	369.0	363.7	374.9	369.3	373.2	370.2	371.7
120	0	370.1	382.5	376.3	348.8	383.0	365.9	364.0	381.0	372.5
	12.4	381.2	352.9	367.0	347.2	367.6	357.4	376.1	372.5	374.3
Mean		375.7	367.7	371.7	348.0	375.3	361.6	370.1	370.2	373.4
120 split	0	380.2	354.3	367.6	359.5	341.0	350.2	366.9	351.8	359.3
	12.4	363.7	325.0	344.3	351.2	357.4	354.3	377.9	370.8	374.4
Mean		372.0	339.7	355.9	355.4	349.2	352.3	372.4	361.3	366.8
N Mean		371.9	359.2		355.7	366.4		371.9	369.4	
FLSD 0.05 (Fe) †		NS	NS	NS	9.3	19.2	7.1	NS	NS	NS
FLSD 0.05 (BA) ‡		NS	NS	NS	NS	NS	NS	NS	NS	NS

† FLSD, F least significant difference for comparison of Fe means.

‡ FLSD, F least significant difference for comparison of BA means;
NS, not significant.

Table 20. F-test for total non-structural carbohydrates in Midiron bermudagrass stolons and rhizomes during 1984 as influenced by nitrogen, iron, and benzyladenine.

	Stolons			Rhizomes		
	Sampling date			Sampling date		
Parameter	13 Sept.	16 Oct.	14 Nov.	13 Sept.	16 Oct.	14 Nov.
Nitrogen (N)	NS	*	NS	NS	**	NS
Iron (Fe)	NS	*	NS	NS	*	NS
Benzyladenine (BA)	NS	NS	NS	NS	NS	NS
N x Fe	NS	NS	NS	NS	*	NS
N x BA	NS	NS	NS	NS	NS	NS
Fe x BA	NS	NS	NS	NS	NS	NS
N x Fe x BA	NS	NS	NS	NS	NS	NS

*, ** Significant at the 5 and 1% level of probability, respectively; NS not significant.

However, on 16 October rhizomes from plots receiving high N had significantly higher TNC than plots receiving low summer N (Table 19). No significant difference in stolon or rhizome TNC occurred on 14 November among N application levels.

Iron application level had no significant effect on stolon TNC on 13 September. On 16 October, stolon TNC levels were significantly lower when Fe was applied as single or split monthly applications than when no Fe was applied. Split application of Fe caused a greater inhibition of storage carbohydrate accumulation in stolons than a single monthly application of Fe when data were combined over all N and BA application levels. Although single or split monthly Fe applications inhibited TNC accumulation in stolons during 13 September to 16 October there were no significant differences in stolon TNC levels on 14 November.

Stolon TNC levels decreased in all treatments during 16 October through 14 November. This may have occurred because of unseasonably warm temperatures (Appendix 3) during late October that may have promoted growth at the expense of TNC in stolons. Stolon TNC levels decreased slightly more between 16 October and 14 November when no Fe was applied than when Fe was applied in either single or split applications. Plots that received Fe had greater PGBGC during late October and many have maintained a photosynthetic advantage over the

more chlorotic 0 mg Fe m^{-2} $month^{-1}$ application level. Thus, recently assimilated CO_2 would have been more available for growth in the Fe treatments and less mobilized from stolons.

There were no significant differences in rhizome TNC among Fe application levels on 13 September (Table 19). A significant N by Fe interaction occurred on 16 October. Rhizome TNC levels decreased during 13 September to 16 October in plots receiving single or split monthly Fe applications in conjunction with low summer N. Rhizome TNC increased slightly or remained relatively unchanged in plots receiving no Fe and low summer N or high summer N and any Fe application level. Combined over all N and BA application levels, rhizome TNC on 16 October were significantly lower where Fe was applied in split monthly applications than when no or a single monthly Fe application was used. Rhizome TNC levels increased from 16 October to 14 November in plots receiving low N regardless of Fe application level, and at high N when the split Fe application was used. Rhizome TNC levels remained relatively unchanged during 16 October to 14 November where no or single monthly Fe applications were made in conjunction with high summer N. Therefore, no significant difference in rhizome TNC occurred among Fe application levels on 14 November.

There appeared to be no relationship between TNC levels in Midiron storage tissues and post-dormancy growth enhancement by Fe treatment. Enhanced post-dormancy recovery may have occurred because the onset of dormancy in fall was delayed. Chalmers and Schmidt (1979) reported that prolonged dormancy periods reduced the viability of Tifgreen bermudagrass stolon and rhizome nodal buds.

On 14 November when Midiron had just entered a state of dormancy, stolon TNC levels were generally lower where BA was applied in conjunction with Fe when data were combined over all N levels. Rhizome TNC levels on 14 November, however, were generally higher in plots treated with BA regardless of Fe application level.

Conclusion

The results of this study indicate that when used in conjunction with moderate summer N fertilization, foliar applied Fe can improve the performance of bermudagrass during fall and hasten post-dormancy recovery in spring. The extension of bermudagrass performance during fall by Fe application does not adversely affect carbohydrate storage levels in bermudagrass rhizomes and stolons present at the onset of dormancy. Benzyladenine was less effective than Fe for extending bermudagrass color retention during fall. Applications of Fe alone in conjunction with moderate summer N fertilization may be more cost effective for turf managers desiring to extend bermudagrass green color retention during fall.

Experiment 3. CO_2 Exchange and Leaf Chemical Composition of Tifgreen Bermudagrass Exposed to Chilling Temperatures as Influenced by Iron and Benzyladenine.

Materials and Methods

Mature sods of Tifgreen bermudagrass [Cynodon dactylon (L.) Pers. x C. transvaalensis Burtt-Davy] 10-cm in diameter by 3.8-cm thick were obtained during March and April from the Turfgrass Research Center at Blacksburg, VA. Each sod was transplanted to 10-cm diamter by 15-cm deep plastic pots containing a Groseclose silt loam (a clayey, kaolinitic mesic Typic Hapludult) soil and with a pH of 5.7 and established under greenhouse conditions for approximately 4 weeks. Greenhouse temperatures during the establishment period ranged from 26 to 35°C. New sod was potted on 2-week intervals to insure similar ages for testing in each replication. The turfs were irrigated with 200 ml tap water twice-a-week and received 4.8 g N m^{-2} from a 20-20-20 (N-P-K) every 4 weeks throughout the study. The grass canopies were maintained at a 3.0 cm height by clipping twice weekly, and clippings were removed.

Following the establishment period, 21 pots of sod were transferred to a controlled environment chamber programmed for a 12-hour photoperiod and 30/28°C (day/night) temperature. A photosynthetic photon flux density (PPFD) of 450 μmol m^{-2} sec^{-1} at the turf surface was provided by fluorescent and incandescent lamps. Relative humidity was maintained between 70 and 80%. After a 2-week acclimation period to growth chamber conditions, the turfed pots were randomly assigned to one of seven groups containing three pots each.

Treatments of 120 mg Fe m^{-2} from sodium iron diethylenetriamine pentaacetate and benzyladenine (6-benzylaminopurine) at 12.4 mg m^{-2}, and a control were applied to turf within each group. Rates are of total Fe and benzyladenine (BA) applied in two applications at 2-week intervals. Fe and BA were applied to foliage in 1.2 L distilled water m^{-2} using an atomizer. The control was sprayed with distilled water only. A separate group was randomly selected for each CO_2 exchange rate (CER) determination before (30/28°C day/night) chilling, after 3 days exposure to chilling (10/7°C day/night) temperatures, and following a brief recovery (2 hours at 30°C) after 3 days of chilling. A separate group was also randomly selected from which leaf tissue was collected for chlorophyll and protein determina-

tions, and for amylolytic enzyme activity assay both before and after 3 days exposure to chilling temperatures.

The pots of turf to receive chilling treatment were clipped to a 3.0 cm height and transferred during the light period to an identical controlled environment chamber 1 week after the second chemical treatment application was made. Photoperiod (12 hours light), PPFD, relative humidity, and day temperature were initially the same as those maintained during the acclimation period. Chilling was imposed at the beginning of the dark period, by lowering temperatures approximately 5°C per hour until a 7°C ambient air temperature was obtained. A day/night air temperature of 10/7°C and a 12 hour photoperiod at 450 μmol m^{-2} sec^{-1} (PPFD) was maintained for the entire chilling period.

CO_2 Exchange Rate Determinations

CO_2 exchange rate of the whole turf was monitored by measuring the change in CO_2 concentration in an open differential system (Jarvis, 1971). A 10-cm diameter by 10-cm tall clear plastic assimilation chamber that fit snuggly into the plastic pot containing the grass was placed over the turf canopy. Air flow rates ranging from 3.0 to 6.0 liters min^{-1} passed through the assimilation chamber with 0.5 l min^{-1} of reference and sample air passing through an Anarad (Model AR-600, Anarad, Inc., Santa Barbara, CA) infrared

gas analyzer. Analyzer output was recorded by a Leeds and Northrup Azar recording potentiometer. Temperature inside the assimilation chamber was monitored by a copper-constantine thermocouple placed 0.5 cm above the turf surface and was controlled by the growth chamber thermostat to within 2°C. A 1000-W metal halide lamp was used to provide 850 µmol m^{-2} sec^{-1} (PPFD) at the top of the turf canopy during daytime CER determinations.

One week after the second chemical treatment, CER was determined immediately before chilling at 30°C in the light (daytime) and 28°C in the dark (nighttime) after the turf was clipped to a 3.0 cm height. After 3 days at chilling temperatures, daytime CER was determined at 10°C and nighttime CER at 7°C. One group exposed to 3 days of chilling stress was returned to the acclimation chamber and allowed to recover for 2 hours in light at 450 umol m^{-2} sec^{-1} (PPFD) and 30°C. CER was again measured at 30°C in light and 28°C in darkness. All CER determinations were made within 4 hours of the commencement of the light period. On the day after CER determinations were made, the above ground herbage of the turfs was harvested for each group within 4 hours of the commencement of the light period.

Tissue separations were made to determine percent leaves in each turf canopy. Leaf area was measured using an

electronic leaf area meter (Model AAM Automatic Area Meter, Far East Mercantile Corp., Distributor New York, NY) and leaf dry weight to leaf area ratios (specific leaf weight, SLW) determined. Total herbage yield was measured and leaf area index (LAI) of each turf canopy was calculated from yield, SLW, and percent leaf tissue values so that CER could be expressed on a leaf surface area basis.

Leaf Carbohydrate Analysis

Leaf tissue samples collected following CER determinations were dried for 24 hours at 65°C, ground to pass a 40-mesh screen, and stored at -8°C until analyzed for non-structural carbohydrates. Carbohydrate analysis was similar to the method described by Smith (1968) as modified by Wolf and Ellmore (1973) for automated analysis. Sugars were removed with hot (100°C) distilled water and analyzed directly. Starch was extracted from leaf tissue residues by hydrolysis with takadiastase (Charase 40000, Miles Laboratories). Reducing equivalence of all extracts was measured using glucose as the standard.

Amylolytic Enzyme Activity (AEA) Assay One g samples of leaf tissue were removed from each pot within 4 hours of the commencement of the light period both before and following 3 days at chilling temperatures and was stored at -8°C for not

more than 7 days before AEA was assayed. A separate sample of leaf tissue was collected from each treatment, weighed fresh, dried for 24 hours at 65°C and reweighed to determine percent dry weight of tissue. Percent dry weight and SLW previously determined for each treatment were used to express AEA on a leaf surface area basis. Assay procedures were previously described by Karbassi et al.(1970) and are outlined below.

Leaf tissue samples were homogenized in an Omni-mixer with 30 ml ice-cold $CaCl_2$ (0.0001 M). The reaction mixture containing 4 ml of succinate buffer (0.05 M, pH 5.2) and 2 ml of starch solution was the same as previously described by Karbassi, et al. (1970). Reaction at 25°C was initiated by addition of 4 ml of leaf homogenate (enzyme source) to the reaction mixture. At predetermined times (Rogers et al., 1977), 1.5 ml aliquots were removed from reaction vessels and mixed with 5 ml of acidic I_2-KI solution. Absorbance of the starch-iodine complex at 625 nm was determined spectrophotometrically. Amylolytic enzyme activity was expressed as the change in optical density m^{-2} leaf surface hr^{-1}.

Chlorophyll Determination and Turf Color

Fully expanded leaves (200 mg) were collected from the canopy surface of each treatment both before and after 3 days of chilling, and stored at -8°C. Chlorophyll was extracted by grinding leaves in a mortar and pestle with 3 ml 80% acetone (v:v, acetone:distilled H_2O). Samples were decanted to a 50-ml volumetric flask and taken to volume with 80% acetone and stored in a dark cabinet at 25°C for 24 hours. Absorbance of the extracts was measured spectrophotometrically at 645 and 663 nm. Chlorophyll concentration was calculated according to the methods of Arnon (1949) and expressed as mg chlorophyll cm^{-2} leaf surface from a knowledge of leaf fresh weight, percent dry weight, and SLW. Visual ratings were made every 7 days after chemical treatments were imposed and following chilling to determine effects of treatments on turf color.

Statistical Analysis

The experiment was replicated 4 times. All data were subjected to an analysis of variance appropriate for a completely random experimental design (Steele and Torrie, 1980). When a significant F ratio occurred for a treatment effect, a least significant difference (LSD) was calculated.

Results and Discussion

Daytime CER per unit leaf surface averaged over all treatments was reduced approximately 73% by 3 days exposure of Tifgreen bermudagrass to chilling temperatures (Table 21). After chilling, daytime CER returned to within only 50% of pre-chill CER rates following 2 hours at 30°C. The low temperature (10°C) at which daytime CER were measured after exposure of Tifgreen to chilling would have a direct inhibitory effect on photosynthetic enzyme activity (Taylor et al., 1974). However, Tifgreen failed to recover high daytime CER following exposure to chilling temperatures when returned to 30°C for two hours.

Photosynthate accumulation in chloroplasts has been suggested as an explanation for reduced photosynthetic rates of chill-sensitive plant species exposed to chilling temperatures (Neales and Incole, 1968). Failure of starch to be hydrolyzed and translocated out of chloroplasts presumably causes product inhibition of the photosynthetic mechanism and eventual damage to the chloroplast ultrastructure (West, 1970; Karbassi et al., 1970; Taylor and Graig, 1971; Forde et al., 1975; Rogers et al., 1977). Low temperatures may repress the mobilization of starch from chloroplasts by reducing the activity of amylolytic enzymes (Karbassi et al., 1970).

Table 21. Daytime CO_2 exchange rate (CER) of Tifgreen bermudagrass before (30°C), after (10°C), and following recovery (2 h 30°C) from 3 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

Treatment	Rate	CER †		
		Temperature°C		
		30	10	2 h 30
	$mg\ m^{-2}$	——— $mg\ CO_2\ dm^{-2}\ hr^{-1}$ ———		
Control	-	6.76	1.69	3.30
Iron	120.0	7.93	2.41	4.15
Benzyladenine	12.4	8.82	2.17	4.37

†CER per unit leaf surface area.

*, **Significant at the 5 and 1% levels of probability respectively; NS, not significant; temperature, **; treatment, *; temperature x treatment, NS; FLSD 0.05 for comparison of temperature or treatment means = 0.95.

Rogers et al. (1977) reported a decline in amylolytic enzyme activity (AEA) during fall in field grown bermudagrass. In the present study, AEA was significantly reduced in Tifgreen leaves by exposure to chilling temperatures for 3 days (Table 22).

Although the decline in Tifgreen AEA during exposure to chilling was associated with an 86% increase in leaf starch levels when averaged over all treatments, water soluble sugars (WSS) increased 307% during the chilling period (Table 22). These data indicate that Tifgreen leaves could hydrolyze starch but were not readily able to translocate carbohydrates out of assimilating tissues. The high levels of TNC in leaf tissue appear responsible for the inability of Tifgreen to rapidly recover high photosynthetic rates when returned to a warm environment after exposure to chilling temperatures.

Averaged over all treatments, nighttime CER was 66% lower after 3 days at chilling temperatures than before chilling (Table 23). These data indicate that exposure of bermudagrass to chilling temperatures not only disrupts the assimilation of carbon but also interferes with utilization of assimilates due to reduced respiratory activity. The high level of WSS in Tifgreen leaves after the chilling period supports this suggestion.

Table 22. Leaf nonstructural carbohydrate composition and amylolytic enzyme activity in Tifgreen bermudagrass before (30/28°C) and after 4 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

		Nonstructural Carbohydrates						Amylolytic Enzyme activity	
		Simple Sugars		Starch		Total			
		Day/Night Temperature °C							
Treatment	Rate	30/28	10/7	30/28	10/7	30/28	10/7	30/28	10/7
	mg m^{-2}	g kg^{-1}						Δ OD m^{-2} leaf hr^{-1}	
Control	-	30.1	134.3	41.0	73.3	71.2	207.6	3.16	1.33
Iron	120.0	30.6	125.7	39.3	61.5	69.9	187.2	2.52	1.08
Benzyladenine	12.4	32.6	119.7	36.5	82.7	66.6	202.3	4.86	2.02
Mean		31.1	126.6	38.9	72.5	69.2	199.0	3.51	1.48
FLSD 0.05		11.7		14.4		19.7		1.50	
Temperature (Temp)		***		***		***		**	
Treatment (Treat)		NS		NS		NS		*	
Temp. x Treat.		NS		*		NS		NS	

†FLSD, F least significant difference for comparison of temperature or treatment means.

*, **, ***Significant at the 5, 1, and 0.1% levels of probability, respectively; NS, not significant.

Table 23. Nighttime CO_2 exchange rate (CER) of Tigreen bermudagrass before (28°C), after (7°C), and following recovery (2 h 30°C) from 3 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

		CER†		
		Temperature °C		
Treatment	Rate	28	7	2 h 30
	$mg\ m^{-2}$	——— $mg\ CO_2\ dm^{-2}\ hr^{-1}$ ———		
Control	-	0.87	0.22	0.85
Iron	120.0	0.95	0.34	0.97
Benzyladenine	12.4	0.86	0.32	0.85

†CER per unit leaf surface area.

***Significant at the 0.1% level of probability; NS, not significant; temperature, ***; treatment, NS; temperature x treatment, NS; FLSD 0.05 for comparison of temperature means = 0.40.

Although daytime CER was low, significant accumulation of starch and WSS occurred probably because low nighttime CER prevented utilization of carbohydrates.

Nighttime CER for all treatments measured following the recovery period were similar to rates measured before chilling. The ability of bermudagrass to regain high respiration rates following exposure to chilling temperatures may be beneficial for replacement of leaves damaged at chilling temperatures.

Daytime CER at all temperatures was generally higher in Tifgreen treated with Fe than in the control prior to chilling (Table 21). A significantly higher daytime CER was measured for the Fe treatment before chilling temperatures were imposed. Benzyladenine (BA) treated turf had higher daytime CER than the control when data were combined over all temperature levels. Daytime CER was similar for the Fe and BA treatments.

Leaves of Fe treated turf generally accumulated less TNC at chilling temperatures and starch levels were significantly lower (Table 22). Since plants treated with Fe had somewhat lower AEA than the control, enhancement of starch hydrolysis can not account for the lower TNC that occurred. Nighttime CER was generally higher in plants treated with Fe (Table 23) and thus enhanced respiratory activity may account for the lower leaf TNC levels after chilling.

Leaf AEA was significantly higher in Tifgreen treated with BA than in the control before and after the chilling period (Table 22). However, leaf starch content was generally higher and sugars were lower following chilling in plants treated with BA. Benzyladenine had no significant effect on nighttime CER (Table 23). Maintenance of high AEA at chilling temperatures may be of little importance to mobilization of assimilates from leaves unless growth respiration creates a demand for photoassimilate.

Chlorophyll content declined during the chilling period in all treatments (Table 24). Tifgreen treated with BA had a significantly higher chlorophyll content before exposure to chilling temperatures than the Fe or control treatments. However, BA did not delay chlorophyll loss during 3 days of chilling. Similarily, Towne and Owensby (1985) reported that BA applied to big bluestem (Andropogon gerardi Vitman) during mid-June to late-July caused no delay in the progressive decline in leaf chlorophyll content during late-summer and fall.

After 3 days at chilling temperatures, plants treated with Fe had significantly lower chlorophyll content than those treated with BA.

Table 24. Chlorophyll and crude protein content of Tifgreen bermudagrass leaves before (30/28°C) and after 3 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

Treatment	Rate	Chlorophyll Temperature °C 30/28	Chlorophyll Temperature °C 10/7	Crude Protein Temperature °C 30/28	Crude Protein Temperature °C 10/7
	mg m^{-2}	— μg cm^{-2} —		— g kg^{-1} —	
Control	-	32.4	26.8	168.1	131.3
Iron	120.0	29.6	24.3	193.8	134.3
Benzyladenine	12.4	41.7	32.3	186.3	123.8
Mean		34.6	27.8	182.7	129.8
FLSD 0.05 †		6.5		25.8	
Temperature (Temp)		*		***	
Treatment (Treat)		*		NS	
Temp * Treat		NS		*	

†FLSD, F-least significant difference for comparison of temperature or treatment means.

*,*** Significant at the 5 and 0.1% levels of probability, respectively; NS, not significant.

However, the chlorophyll content of Tifgreen treated with Fe did not differ significantly from the control. Turf color ratings were not significant different among treatments when Tifgreen was grown at 30/28°C (day/night) temperatures until 1 week (day 21) after the second chemical treatment application (Table 25). Iron and BA treatments produced darker green color than controls on day 21 and on day 25 after exposure to chilling temperatures. Green pigmentation loss from mature Tifgreen leaves appeared more rapid in controls than in Fe or BA treatments and may account for the lighter green color observed in the controls. When a chlorophyll index (Madison, 1963) was calculated for each sample and daytime CER was expressed on a per unit chlorophyll basis (Table 26), plants treated with Fe had significantly higher CER at all temperatures than the BA or control treatments. Oquist (1973) reported that insufficient Fe in the culture medium depressed photosynthetic activity of the blue-green alga Anacystis nidulans on a per unit chlorophyll basis. Terry (1980) demonstrated that Fe-deficient low chlorophyll containing sugarbeet (Beta vulgaris L.) leaves had fewer photosynthetic units per unit leaf area than leaves supplied with sufficient Fe.

Table 25. Color of Tifgreen bermudagrass before and after 3 days exposure to chilling temperatures as influenced by iron and benzyladenine.

Treatment	Rate	Turf Color† Day of Visual Rating				
		1	7	14	21	25
	mg m^{-2}					
Control	-	7.5	7.4	7.6	7.3	6.2
Iron	120.0	7.4	7.9	8.0	8.2	8.0
Benzyladenine	12.4	7.6	8.0	8.1	8.2	8.1
FLSD 0.05‡		NS	NS	NS	0.7	1.2

†Visual rating of turf color on a scale of 1 to 10 the darkest green; day 1 through day 21 at 30/28°C and day 25 after 3 days at 10/7°C (day/night) temperatures.

‡FLSD, F least significant difference for comparison of treatment means, NS, not significant.

Table 26. Daytime CO_2 exchange rate (CER) per unit chlorophyll of Tifgreen bermudagrass before (30°C), after (10°C), and following recovery from 3 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

		CER		
		Temperature °C		
Treatment	Rate	30	10	2 h 30
	mg m^{-2}	mg CO_2 mg chlorophyll^{-1} hr^{-1}		
Control	-	2.07	0.61	1.31
Iron	120.0	2.66	0.98	1.69
Benzyladenine	12.4	2.13	0.61	1.34
Mean		2.29	0.73	1.45

**Significant at the 1.0% level of probability; NS, not significant; temperature, **; treatment, **; temperature x treatment, NS; FLSD 0.05 for comparison of temperature or treatment means = 0.20.

He suggested that when leaves were deficient in Fe, low photosynthetic rates occurred because of a decrease in photochemical capacity, rather than to reduced chlorophyll content per se.

Crude protein content in all treatments declined significantly during 3 days at chilling temperatures (Table 24). The Fe treatment had significantly higher protein content before chilling but was not different from the control after exposure to chilling temperatures. Although BA increased crude protein levels in Tifgreen leaves before chilling, BA did not prevent translocation of N from leaves during the chilling period.

Total above ground herbage yields after chilling were not significantly different from herbage yields before exposure to chilling temperatures (Table 27). These data illustrate the inhibitory effects of chilling temperatures on Tifgreen bermudagrass growth since and yields did not increase during the chilling period. Significantly higher yields occurred in the BA, than in the Fe or control treatments before and after exposure to chilling temperatures.

Specific leaf weight (SLW) generally increased in all treatments during exposure of Tifgreen to chilling temperatures and may reflect the increase in leaf TNC that occurred.

Table 27. Yield and yield components of Tifgreen bermudagrass before (30/28°C) and after 4 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

Treatment	Rate	Yield		Percent Leaves		Specific Leaf Weight		Leaf Area Index	
		Day/Night Temperature°C							
		30/28	10/7	30/28	10/7	30/28	10/7	30/28	10/7
		---g m^{-2} ------				----- g m^{-2} ----			
Control	-	232.2	223.0	0.54	0.54	25.38	30.29	4.94	3.98
Iron	120.0	222.8	220.5	0.55	0.55	24.41	27.66	5.02	4.38
Benzyladenine	12.4	273.8	271.7	0.56	0.55	29.40	33.73	5.21	4.43
Mean		242.9	238.4	0.55	0.55	26.4	30.56	5.06	4.26
FLSD 0.05 †		26.5		NS		NS		NS	
Temperature (Temp)		NS		NS		NS		NS	
Treatment (Treat)		***		NS		NS		NS	
Temp. x Treat.		NS		NS		NS		NS	

† FLSD, F-least significant difference for comparison of temperature or treatment means.

*** Significant at the 0.1% level of probability; NS, not significant.

The lower mean SLW after chilling for the Fe treatment compared to the control may be associated with the fact that less TNC accumulated in leaves of Tifgreen treated with Fe.

After chilling, the Fe and BA treatments generally had a higher leaf area index (LAI) than the control. The lower LAI in all treatments after exposure to chilling temperatures may be related more to increases in SLW than to senescence of leaf tissue. Leaf area index decreased approximately 19, 15, and 15% for the control, BA, and Fe treatments, respectively, whereas SLW increased approximately 19, 15, and 13%, respectively.

Conclusions

The results of this study support the hypothesis that photoassimilate accumulation in chloroplasts may inhibit the recovery of high photosynthetic activity in bermudagrass following exposure to chilling temperatures. The accumulation of high levels of WSS in leaves suggests that metabolism of carbohydrates other than starch is also disrupted by exposure of bermudagrass to chilling temperatures. Reduced respiratory activity appeared responsible for the dramatic accumulation of nonstructural carbohydrates in leaves during the chilling period.

In contrast to photosynthesis, respiration is reversibly inhibited by short term exposure of bermudagrass to chilling temperatures. Recovery of high respiratory activity following exposure to chilling temperatures may be important for growth of bermudagrass leaves and maintenance of aesthetically pleasing turf.

Iron applications may not only provide more aesthetically pleasing Tifgreen bermudagrass turf during exposure to chilling temperatures, but may also enhance physiological activity as well. Foliar applications of Fe to bermudagrass may not cause increased leaf chlorophyll content but may provide a more efficient photosynthetic mechanism, since photosynthetic rates on a per unit chlorophyll basis were

increased by Fe. Therefore, Fe applications may prolong CO_2 assimilation as high light levels and chilling temperatures cause a net loss of chlorophyll.

Benzyladenine may enhance turf color and photosynthetic activity of bermudagrass exposed to chilling temperatures. However, the reason for higher photosynthetic rates of Tifgreen following BA treatment is unclear. Photosynthesis was higher for the BA treatment at warm temperatures possibly because chlorophyll content was increased by BA. However, BA treated turf maintained a photosynthetic advantage after chilling, although chlorophyll content was similar for the control and BA treatments. The ability of BA treated turf to recover higher photosynthetic rates following exposure to chilling temperatures suggests that photosynthetic enzyme activity may have been preserved by BA. Further research is needed to clarify the effects of BA on CO_2 assimilation in bermudagrasses exposed to chilling temperatures.

Experiment 4. CO_2 Exchange, Chemical Composition, and Morphology of Tifgreen and Midiron Bermudagrass Exposed to Chilling Temperatures as Influenced by Iron and Benzyladenine.

Materials and Methods

Mature sods of Tifgreen bermudagrass [Cynodon dactylon (L.) Pers. x C. transvaalensis Burtt-Davy], and Midiron bermudagrass [C. dactylon (L.) Pers.] 10 cm in diameter by 4 cm thick were obtained from the Turfgrass Research Center at Blacksburg, VA during September and October. Each sod was transplanted to a 10 cm diameter by 15 cm deep plastic pot containing a Groseclose Silt Loam (a clayey, kaolinitic, mesic Typic Hapludult) soil with a pH of 5.7. New sod was potted on 2-week intervals to insure similar ages for testing in each replication. Sods were established under greenhouse conditions for approximately 4 weeks. Greenhouse temperatures and irrigation were as described in Experiment 3. The turfs were fertilized with 2.4 g N m^{-2} from a 20-20-20 (N-P-K) soluble fertilizer every 2 weeks and maintained at a 3 cm height by clipping two to three times a week throughout the study.

Following the establishment period, 20 pots of each cultivar (40 pots total) were transferred to a controlled environment chamber for a 2-week acclimation period. Growth chamber conditions during the acclimation period were the same as those maintained in Experiment 3.

After acclimation to growth chamber conditions the pots were randomly assigned to one of five groups containing four pots of each cultivar. Chemical treatments were 0, and 120 mg Fe m^{-2} from sodium iron diethylenetriamine pentaacetate, and benzyladenine (6-benzylaminopurine) at 0, and 12.4 mg m^{-2} applied in two equal applications at 2-week intervals. Fe and BA were applied in 1.2 L distilled H_2O m^{-2} using an atomizer. The controls were sprayed with distilled water.

A separate group was randomly selected for each CO_2 exchange rate (CER) determination before (30/28°C day/night) chilling, after 4 days exposure to chilling (10/7°C day/night) temperatures, and following recovery (2 hours at 30°C) after 4 days of chilling. One group was randomly selected from which leaf tissue was collected before chilling, and another group selected to provide leaf tissue exposed to 4 days at chilling temperatures for chlorophyll determinations and amylolytic enzyme activity assay. A complete factorial arrangement of treatment variables included temperature, cultivar, Fe, and BA as factors.

Chilling treatment was imposed and CER determined by methods previously described (Experiment 3). On the day after CER determinations were made, the above ground herbage of the turfs was harvested within 4 hours of the commencement of the light period. Total dry herbage yield, percent leaves, and specific leaf weight were determined so that a leaf area index for each turf could be calculated and CER expressed on a leaf surface area basis (Experiment 3).

Rhizomes and stolons in each pot were washed free of soil and frozen at -8°C until rhizome and stolon tissue could be separated by hand. Rhizome, stolon, and stem tissue were dried for 1 hour at 100°C and then for 24 hours at 65°C. Leaf tissue was dried for 24 hours at 65°C. All tissue samples were ground to pass a 40-mesh screen and stored at -8°C until analyzed for TNC by methods outlined in Experiment 2.

Amylolytic enzyme activity assays and chlorophyll determinations were made using previously described methods (Experiment 3). Visual ratings were made every 7 days after the acclimation period and following chilling to determine the effects of treatments on turf color.

The experiment was replicated 4 times. All data were subjected to an analysis of variance appropriate for a completely random experimental design (Steele and Torrie,

1980). When a significant F ratio occurred for a treatment effect, a least significant difference (LSD) was calculated.

Results and Discussion

CO_2 Exchange

Daytime CO_2 exchange rate (CER) for all treatments was significantly reduced by 4 days exposure to 10/7°C (day/night) temperatures (Table 28). Midiron had a higher daytime CER than Tifgreen at each temperature. Following short-term recovery from chilling, CER of Midiron returned to within 70%, whereas CER of Tifgreen returned to within only 27% of the daytime CER measured before exposure to chilling temperatures.

Iron significantly increased daytime CER when data were combined for all temperature, cultivar and benzyladenine (BA) levels (Table 28). The daytime CER was higher before chilling in Tifgreen turf treated with Fe. Iron, generally, did not cause higher daytime CER in Midiron before chilling temperatures were imposed. However, the Fe treatment caused higher Midiron daytime CER after the chilling period. Treatment with Fe allowed attainment of higher daytime CER in both cultivars following recovery from chilling.

Nighttime CER was lower for all treatments following 4 days at chilling temperatures (Table 29). In contrast to daytime CER, nighttime CER following a short term recovery period, was similar to rates measured before chilling temperatures were imposed. Midiron had higher nighttime CER

Table 28. Daytime CO_2 exchange rate (CER) of two bermudagrass cultivars before (30°C), after (10°C), and following recovery (2 h 30°C) from 4 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

		CER		
		Temperature °C		
Iron	Benzyladenine	32	10	2 h 30
mg m^{-2}		mg CO_2 dm^{-2} leaf hr^{-1}		
		Midiron		
0	0	6.89	1.76	4.36
	12.4	6.78	1.64	4.72
Mean		6.84	1.70	4.54
120	0	7.41	2.20	5.38
	12.4	6.37	2.35	5.15
Mean		6.89	2.28	5.27
Cultivar Mean		6.87	1.99	4.91
		Tifgreen		
0	0	5.24	0.97	1.10
	12.4	6.44	1.14	1.66
Mean		5.84	1.06	1.38
120	0	6.64	1.25	1.81
	12.4	6.90	0.97	2.17
Mean		6.77	1.11	1.99
Cultivar Mean		6.31	1.09	1.69

FLSD 0.05 for comparison of temperature, cultivar, iron, or benzyladenine means = 0.43.

Table 29. Nighttime CO_2 exchange rate (CER) of two bermudagrass cultivars before (28°C), after (7°C) and following recovery (2 h 30°C) from 4 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

		CER		
		Temperature °C		
Iron	Benzyladenine	28	7	2 h 30
mg m^{-2}		mg CO_2 dm^{-2} leaf hr^{-1}		
		Midiron		
0	0	0.82	0.26	0.74
	12.4	0.63	0.28	0.61
Mean		0.73	0.27	0.68
120	0	0.71	0.37	0.91
	12.4	0.64	0.36	0.83
Mean		0.68	0.37	0.87
Cultivar Mean		0.71	0.32	0.78
		Tifgreen		
0	0	0.44	0.21	0.31
	12.4	0.44	0.24	0.48
Mean		0.44	0.23	0.40
120	0	0.62	0.24	0.44
	12.4	0.49	0.26	0.58
Mean		0.56	0.25	0.51
Cultivar Mean		0.50	0.24	0.46

FLSD 0.05 for comparison of temperature, cultivar, iron, or benzyladenine means = 0.14.

Table 30. F-test for daytime and nighttime CO_2 exchange rates (CER) of two bermudagrass cultivars as influenced by temperature, iron, and benzyladenine.

Parameter	CER	
	Daytime	Nighttime
Temperature (T)	***	***
Cultivar (C)	***	***
Iron (Fe)	**	*
Benzyladenine (BA)	NS	NS
T x C	***	NS
T x Fe	NS	NS
T x BA	NS	NS
C x Fe	NS	NS
C x BA	NS	NS
Fe x BA	NS	NS
T x C x Fe	NS	NS
T x C x BA	NS	NS
T x Fe x BA	NS	NS
C x Fe x BA	NS	NS
T x C x Fe x BA	NS	NS

*, **, *** significant at the 10, 1, and 0.1% level of probability, respectively. NS, not significant.

than Tifgreen at all temperatures. Although higher nighttime CER occurred in turf treated with Fe, when data were combined over all other factor levels, the primary effect of Fe was the enhancement of nighttime CER recovery after chilling.

Benzyladenine (BA) generally increased daytime and nighttime CER of Tifgreen (Tables 28 and 29). However, nighttime CER of Midiron was generally lower for the BA treatment. Benzyladenine had no consistent effect on daytime CER of Midiron.

Nonstructural Carbohydrates and Amylolytic Enzyme Activity

Total nonstructural carbohydrates (TNC) in leaves of Midiron and Tifgreen increased 88, and 160%, respectively, during 5 days at chilling temperatures (Table 31). Leaf TNC levels were similar among cultivars before chilling was imposed. After 5 days of chilling, leaf TNC were higher in Tifgreen than in Midiron.

Reducing and nonreducing sugars accounted for the greatest increase of the TNC components (Table 33). Chilling temperatures initially cause accumulation of starch in leaves of other grasses possessing the C_4 pathway of photosynthesis (Taylor and Rowley, 1971). Tifgreen accumulated high leaf starch levels during 4 days at chilling temperatures in an earlier study of the present research (Table

Table 31. Total nonstructural carbohydrates in leaves, stems, stolons, and rhizomes of two bermudagrass cultivars before (30/28°C) and after 5 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

		Leaves		Stems		Stolons		Rhizomes	
		Temperature °C †							
Iron	Benzyladenine	30/28	10/7	30/28	10/7	30/28	10/7	30/28	10/7
mg m^{-2}		g kg^{-1}							
		Midiron							
0	0	79.2	162.9	136.8	213.4	175.9	229.3	202.7	215.9
	12.4	85.1	162.2	154.6	198.2	178.5	209.7	242.3	193.6
Mean		82.1	162.6	145.7	205.8	177.2	219.5	222.5	204.8
120	0	85.4	154.1	143.3	214.6	223.9	230.6	234.8	229.8
	12.4	92.0	165.0	154.4	192.9	203.2	223.3	233.0	252.4
Mean		88.7	159.6	148.9	203.7	213.6	226.9	233.9	241.1
Cultivar Mean		85.4	161.1	147.3	204.8	195.4	223.2	228.2	222.9
		Tifgreen							
0	0	97.0	254.9	200.5	216.3	389.9	404.4	434.6	406.4
	12.4	102.3	269.8	216.3	208.2	370.8	384.1	398.6	403.2
Mean		99.7	264.6	208.4	212.3	380.4	394.3	416.6	404.8
120	0	97.0	272.6	190.0	237.1	350.5	421.8	387.5	387.6
	12.4	107.9	251.1	211.1	251.9	378.5	389.6	402.8	401.3
Mean		102.5	261.8	200.5	244.5	364.5	405.7	395.1	394.5
Cultivar Mean		101.1	263.2	204.5	228.4	382.2	390.3	405.9	399.6
FLSD 0.05 ‡		36.6		38.6		93.5		109.5	

† Day/night temperature.

‡ FLSD, F - least significant difference for comparison of temperature, cultivar, iron, or benzyladenine means; NS, not significant.

Table 32. F tests for nonstructural carbohydrates in two bermudagrass cultivar leaves, stems, stolons, and rhizomes as influenced by temperature, iron, and benzyladenine.

Parameter	Leaf	Stem	Stolon	Rhizome
Temperature (T)	**	**	NS	NS
Cultivar (C)	**	**	**	**
Iron (Fe)	NS	NS	NS	NS
Benzyladenine (BA)	NS	NS	NS	NS
T x C	***	***	NS	NS
T x Fe	NS	NS	NS	NS
T x BA	NS	NS	NS	NS
C x Fe	NS	NS	NS	NS
C x BA	NS	NS	NS	NS
Fe x BA	NS	NS	NS	NS
T x C x Fe	NS	*	NS	NS
T x C x BA	NS	NS	NS	NS
C x Fe x BA	NS	NS	NS	NS
T x C x Fe x BA	NS	NS	NS	NS

*, **. Significant at the 5, and 1% levels of probability, respectively; NS, not significant.

Table 33. Nonstructural carbohydrates in leaf, stem, stolon, and rhizome tissue of two bermudagrass cultivars before (30/28°C) and after 5 days exposure to 10/7°C (day/night) temperatures.

		Non-structural Carbohydrates					
		Water Soluble Sugars					
		Reducing		Non-reducing		Starch	
		Day/night Temperature °C					
Tissue	Cultivar	30/28	10/7	30/28	10/7	30/28	10/7
		g kg^{-1}					
Leaf	Midiron	8.1	20.3	27.9	96.3	49.4	44.5
	Tifgreen	7.2	34.1	29.1	168.6	64.9	60.5
Mean		7.7	27.2	28.5	132.5	57.2	52.5
FLSD 0.05+		5.9		27.4		13.2	
Stem	Midiron	11.2	31.7	38.5	115.2	97.5	57.9
	Tifgreen	8.4	20.4	35.8	86.0	160.3	121.9
Mean		9.8	26.9	37.2	100.6	128.9	89.9
FLSD 0.05		8.8		26.7		53.5	
Stolon	Midiron	9.0	9.5	69.4	87.9	117.0	125.8
	Tifgreen	5.4	7.4	54.3	71.1	322.5	318.8
Mean		7.2	8.5	61.9	79.5	219.8	218.8
FLSD 0.05		2.6		15.7		87.2	
Rhizome	Midiron	6.7	6.7	60.6	82.1	160.9	134.2
	Tifgreen	4.9	5.3	44.9	58.6	356.0	335.8
Mean		5.8	6.0	52.8	70.4	258.5	235.0
FLSD 0.05+		1.4		16.7		95.0	

+FLSD, F-least significant difference for comparison of temperature or cultivar means; NS, not significant.

Table 34. F tests for nonstructural carbohydrates in leaf, stem, stolon, and rhizome tissue of two bermudagrass cultivars as influenced by temperature.

		Nonstructural Carbohydrates		
		Water Soluble Sugars		
Tissue	Parameter	Reducing	Non-reducing	Starch
Leaf	Temperature (T)	***	***	NS
	Cultivar (C)	***	***	***
	T x C			
Stem	Temperature (T)	***	***	***
	Cultivar (C)	***	**	***
	T x C	*	*	NS
Stolon	Temperature (T)	NS	***	NS
	Cultivar (C)	***	**	***
	T x C	NS	NS	NS
Rhizome	Temperature (T)	NS	***	NS
	Cultivar (C)	***	***	***
	T x C	NS	NS	NS

*, **, *** Significant at the 5, 1, and 0.1% levels of probability, respectively. NS, not significant.

22). Although amylolytic enzyme activity (AEA) was significantly reduced by chilling (Table 35), Midiron and Tifgreen were able to convert starch to translocatable carbohydrate forms during the chilling period. Leaf starch levels before and after chilling were similar.

Chilling temperatures inhibited carbohydrate transport from leaves of both cultivars as evidenced by the high leaf nonreducing sugar content after the chilling period. Whether the inability to translocate photosynthate from assimilating tissues at chilling temperatures is responsible for reduced bermudagrass growth or assimilate accumulation is caused by decreased sink demand is unclear. Because respiration was significantly reduced by chilling, assimilate accumulation in leaves was probably caused by decreased sink demand. The reduced ability of both cultivars to transport carbohydrate from and the subsequent accumulation of high assimilate levels in leaves was apparently responsible for the inability of both cultivars to fully recover high photosynthetic rates following exposure to chilling temperatures.

Midiron stem tissue had lower TNC levels than Tifgreen before chilling was imposed (Table 31). However, no significant differences in stem TNC occurred among cultivars after the chilling period. Stem TNC increased by approximately 39 and 12% in Midiron and Tifgreen, respectively,

Table 35. Amylolytic enzyme activity (AEA) and chlorophyll content of two bermudagrass cultivars before (30/28°C) and after 4 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

Iron	Benzyladenine	AEA		Chlorophyll (a+b)	
		Day/Night Temperature °C			
		30/28	10/7	30/28	10/7
mg m^{-2}		ΔOD m^{-2} leaf hr^{-1}		μg cm^{-2}	
		Midiron			
0	0	2.78	1.97	37.3	43.2
	12.4	2.84	2.12	42.1	44.5
Mean		2.81	2.05	39.7	43.9
120	0	2.56	1.84	40.5	44.1
	12.4	2.79	2.01	37.8	38.6
Mean		2.68	1.93	39.1	41.3
Cultivar Mean		2.75	1.99	39.4	42.6
		Tifgreen			
0	0	3.31	1.29	63.4	45.1
	12.4	4.19	1.73	56.1	46.3
Mean		3.75	1.51	59.8	45.7
120	0	3.05	1.18	61.2	44.5
	12.4	3.98	1.68	61.9	48.6
Mean		3.52	1.43	61.6	46.5
Cultivar Mean		3.64	1.47	60.7	46.1
FLSD 0.05+					

+FLSD, F least significant difference for comparison of temperature, cultivar, iron, or benzyladenine means.

Table 36. F test for amylolytic enzyme activity (AEA) and chlorophyll content of two bermudagrass cultivars as influenced by temperature, iron, and benzyladenine.

Parameter	AEA	Chlorophyll
Temperature (T)	***	NS
Cultivar (C)	NS	**
Iron (Fe)	NS	NS
Benzyladenine (BA)	NS	NS
T x C	*	**
T x Fe	NS	NS
T x BA	*	NS
C x Fe	NS	NS
C x BA	NS	NS
Fe x BA	NS	NS
T x C x FE	NS	NS
T x C x BA	NS	NS
C x Fe x BA	NS	NS
T x C x Fe x BA	NS	NS

*, **, *** Significant at the 5, 1, and 0.1% level of probability, respectively. NS, not significant.

during 5 days at chilling temperatures. These data suggest that lower leaf TNC in Midiron occurred because photoassimilate was more readily translocated from leaves to stems in Midiron than in Tifgreen.

Amylolytic enzyme activities of Midiron and Tifgreen after chilling were 72, and 40%, respectively, of activities measured before chilling (Table 35). Generally, higher AEA was measured in Midiron than in Tifgreen after the chilling period, although leaf starch levels were similar among cultivars. Amylolytic enzyme activity alone cannot account for lower TNC content in Midiron leaves. Higher nighttime CER coupled with a more active starch hydrolysis mechanism apparently enhanced translocation and utilization of photoassimilates in Midiron.

Stolon and rhizome TNC were higher in Tifgreen than in Midiron before and after exposure to chilling temperatures (Table 31). Because Midiron is less tolerant of frequent mowing than Tifgreen, frequent removal of Midiron leaf tissue before chilling was imposed probably caused low Midiron stolon and rhizome TNC levels.

Stolon TNC levels were generally higher and rhizome TNC lower after chilling stress compared to TNC levels before chilling. Stolon and rhizome nonreducing sugars increased, whereas starch and reducing sugars were not significantly changed by exposure to chilling temperatures (Table 33).

Acclimation to low temperatures (hardening) in perennial plants is associated with accumulation of maximum sugar content (Parker, 1972; Sakai and Yoshida, 1968; Guinn, 1971; Alden and Herman, 1971). If sugar accumulation in storage tissues is an indication of cold hardiness in bermudagrass, then Midiron appears to harden more rapidly than Tifgreen since nonreducing sugar content of stolons and rhizomes was significantly higher in Midiron than in Tifgreen after 5 days at chilling temperatures.

There was a significant temperature by cultivar by Fe interaction effect on stem TNC (Table 31). Tifgreen treated with Fe accumulated more TNC in stems than untreated turf whereas TNC accumulation in Midiron stems was similar among Fe levels during the chilling period. Iron had no significant effect on TNC accumulation in Tifgreen stolons and inhibited TNC accumulation in Midiron stolons during exposure to chilling temperatures (Table 31). Iron had no significant effect on rhizome TNC levels (Table 31).

Amylolytic enzyme activity was generally lower for the Fe treatment (Table 35). However, no significant differences in leaf starch or TNC levels occurred after the chilling period in response to Fe. Iron had no consistent effect on TNC fractions in any tissue sampled.

Although higher AEA occurred in turf treated with BA (Table 35), leaf TNC and TNC fractions were not significantly different from untreated turf (Tables 31 and 33). Stem TNC were generally lower in turf treated with BA. Benzyladenine had no significant effect on stolon and rhizome TNC levels or TNC fractions of any tissue sampled.

Chlorophyll Content and Turf Color Ratings

Tifgreen had significantly higher chlorophyll content than Midiron before chilling (Table 35). However, chlorophyll content of the two cultivars was similar after chilling temperatures were imposed. Chlorophyll content of Tifgreen was significantly reduced by 4 days of chilling, whereas Midiron chlorophyll content was relatively unchanged.

Dipaola et al. (1980) evaluated the effects of chilling temperatures on chill-sensitive Pee Dee, and chill-resistant Ormond Bermudagrass. Leaf chlorophyll content slightly decreased in Pee Dee and increased slightly in Ormond during 4 days exposure to chilling temperatures. Although no significant difference in chlorophyll content was detected among cultivars, Ormond had a significantly darker green color than Pee Dee after the 4-day chilling treatment.

Table 37. Color ratings of two bermudagrass cultivars before and after 4 days exposure to chilling temperatures as influenced by iron and benzyladenine.

Iron	Benzyladenine	Turfgrass Color† Day of Visual Rating 1	7	14	21	26
mg m^{-2}						
		Midiron				
0	0	8.3	8.0	8.0	7.9	7.0
	12.4	8.4	8.2	8.3	8.2	7.0
Mean		8.4	8.1	8.2	8.1	7.5
120	0	8.2	8.3	8.1	8.3	7.9
	12.4	8.4	8.3	8.2	8.1	8.2
Mean		8.3	8.3	8.2	8.2	8.1
Cultivar Mean		8.4	8.2	8.2	8.2	7.8
		Tifgreen				
0	0	7.9	7.8	7.8	7.5	6.2
	12.4	8.0	7.9	7.7	7.7	6.8
Mean		8.0	7.9	7.8	7.6	6.6
120	0	8.0	8.0	7.8	7.9	7.0
	12.4	7.9	8.0	7.9	8.0	7.3
Mean		8.0	8.0	7.9	8.0	7.2
Cultivar Mean		8.0	8.0	7.9	7.8	6.9
FLSD 0.05‡		NS	NS	NS	NS	0.5

†Visual rating of turf color on a scale of 1 to 10 with 10 the darkest green; day 1 to 21, 30/28°C, and day 26, 10/7°C day/night temperatures.

‡FLSD, F least significant difference for comparison of temperature or treatment means; NS, not significant.

Table 38. F-tests for color ratings of two bermudagrass cultivars.

Parameter	Day of Visual Rating				
	1	7	14	21	26
Cultivar (C)	NS	NS	NS	NS	**
Iron (Fe)	NS	NS	NS	NS	*
Benzyladenine (BA)	NS	NS	NS	NS	*
C x Fe	NS	NS	NS	NS	NS
C x BA	NS	NS	NS	NS	NS
Fe x BA	NS	NS	NS	NS	NS
C x Fe x BA	NS	NS	NS	NS	NS

*, ** Significant at the 5 and 1% levels of probability, respectively; NS, not significant.

Similarly, Midiron had darker green color than Tifgreen after 4 days at chilling temperatures (Table 37), although no difference in chlorophyll content among cultivars was detected in leaves from the upper canopy surface.

Neither Fe nor BA, had a significant effect on chlorophyll content (Table 35). However, Fe treated turf had a darker green color after the chilling period than untreated turf (Table 37). In general, BA caused darker green turf after exposure to chilling temperatures (Table 37).

Although Fe had no significant effect on chlorophyll content, photosynthetic activity on a per unit chlorophyll basis was generally higher for the Fe treatment (Table 39). These data support results of previous work with Tifgreen bermudagrass (Table 26).

Yield and Yield Components

There were no significant differences in above ground herbage yield among temperature, cultivar, or BA treatment levels (Table 41). The lack of significance in yield among temperature levels, illustrates the negative effect of chilling temperatures on bermudagrass growth. Iron reduced above ground herbage yields of both cultivars. However, the effects of iron on growth occurred before chilling temperatures were imposed.

Table 39. Daytime CO_2 exchange rate (CER) per unit chlorophyll of two bermudagrass cultivars before (30°C), after (10°C), and following recovery (2 h 30°C) from 4 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

		CER		
		Temperature °C		
Iron	Benzyladenine	30	10	2 h 30
mg m^{-2}		mg CO_2 mg chlorophyll^{-1} hr^{-1}		
		Midiron		
0	0	2.15	0.80	1.35
	12.4	1.81	0.73	1.10
Mean		1.98	0.76	1.23
120	0	2.01	0.98	1.87
	12.4	1.89	1.04	1.45
Mean		1.95	1.01	1.66
Cultivar Mean		1.97	0.89	1.44
		Tifgreen		
0	0	1.11	0.24	0.34
	12.4	1.47	0.25	0.51
Mean		1.29	0.25	0.43
120	0	1.42	0.26	0.75
	12.4	1.32	0.24	0.62
Mean		1.37	0.25	0.69
Cultivar Mean		1.33	0.25	0.56

FLSD 0.05 for comparison of temperature or treatment means = 0.37.

Table 40. F-tests for daytime carbon dioxide exchange rates (CER) per unit chlorophyll of two bermudagrass cultivars.

Parameter	CER
Temperature (T)	***
Cultivar (C)	***
Iron (Fe)	NS
Benzyladenine (BA)	NS
T x C	*
T x Fe	NS
T x BA	NS
C x Fe	NS
C x BA	NS
Fe x BA	NS
T x C x Fe	NS
T x C x BA	NS
T x Fe x BA	NS
C x Fe x BA	NS
T x C x Fe x BA	NS

*, ***. Significant at the 5 and 0.1% levels of probability, respectively; NS, not significant.

Table 41. Yield and yield components of two bermudagrass cultivars before (30/28°C) and after 5 days exposure to 10/7°C (day/night) temperatures as influenced by iron and benzyladenine.

Iron	Benzyladenine	Total Yield		Percent Leaves		Specific Leaf Weight		Leaf Area Index	
		Day/Night Temperature °C							
		30/28	10/7	30/28	10/7	30/28	10/7	30/28	10/7
mg m^{-2}		g m^{-2}				g m^{-2}			
		Midiron							
0	0	289.1	302.9	36.5	37.7	23.60	29.71	4.67	3.89
	12.4	381.3	320.3	37.3	40.4	24.24	28.77	5.58	4.49
Mean		335.2	311.6	36.9	39.1	23.92	29.24	5.13	4.19
120	0	317.2	301.3	37.0	36.5	24.18	30.14	4.96	3.65
	12.4	309.8	314.1	38.0	38.2	22.46	30.25	5.30	4.10
Mean		313.5	307.7	37.4	35.6	23.32	30.20	5.13	3.88
Cultivar Mean		324.4	309.7	37.2	37.3	23.62	29.7	5.13	4.04
		Tifgreen							
0	0	319.4	321.2	49.2	50.3	33.27	30.32	4.72	5.33
	12.4	366.7	329.6	48.6	50.3	31.91	30.28	5.25	5.36
Mean		343.1	325.4	48.9	50.3	31.91	30.28	5.25	5.36
120	0	298.1	285.1	47.3	49.8	31.33	29.14	4.50	4.87
	12.4	301.2	275.0	47.5	48.7	29.66	30.79	4.82	4.35
Mean		299.7	280.1	47.4	49.3	30.50	29.97	4.66	4.61
Cultivar Mean		321.4	302.8	48.2	49.8	31.21	30.13	4.96	4.99
FLSD 0.05[+]		21.3		3.43		2.34		0.62	

[+]FLSD, F least significant difference for comparison of temperature, cultivar, iron or benzyladenine means.

Table 42. F-tests for yield and yield components of two bermudagrass cultivars.

Parameter	Total Yield	Percent Leaves	Specific Leaf Weight	Leaf Area Index
Temperature (T)	NS	NS	*	NS
Cultivar (C)	NS	***	*	NS
Iron (Fe)	*	NS	NS	NS
Benzyladenine (BA)	NS	NS	NS	NS
T x C	NS	NS	*	**
T x Fe	NS	NS	NS	NS
T x BA	NS	NS	NS	NS
C x Fe	NS	NS	NS	NS
C x BA	NS	NS	NS	NS
Fe x BA	NS	NS	NS	NS
T x C x Fe	NS	NS	NS	NS
T x C x BA	NS	NS	NS	NS
C x Fe x BA	NS	NS	NS	NS
T x C x Fe x BA	NS	NS	NS	NS

*, **, *** Significant at the 5, 1, and 0.1% levels of probability, respectively. NS, not significant.

Percent leaf tissue was not affected by temperature, Fe, or BA treatments (Table 41). However, Tifgreen had a significantly higher percentage of leaf tissue than Midiron both before and after the chilling period.

Specific leaf weight (SLW) increased when Midiron was exposed to chilling temperatures (Table 41). However, SLW was relatively unchanged by exposure of Tifgreen to chilling. Specific leaf weight was significantly higher for Tifgreen than for Midiron before chilling but was similar among cultivars after 4 days at chilling temperatures. Iron and BA had no significant effect on SLW.

Midiron had a leaf area index (LAI) similar to Tifgreen before exposure to chilling temperatures (Table 41). However, Midiron had a significantly lower LAI than Tifgreen after chilling. The lower LAI of Midiron was probably unrelated to senescence of leaves at low temperatures but rather to changes in SLW used to calculate LAI. Although TNC content in Tifgreen leaves increased dramatically during the chilling period, SLW did not increase as might be expected. Reductions in cholorophyll content at chilling temperatures probably prevented changes in SLW. Crude protein loss was proportional to chlorophyll reduction in Tifgreen leaves during a 3 day chilling period in an earlier study (Experiment 3). Therefore, reduction of crude protein content dur-

ing 4 days of chilling may also have offset the effects of increased leaf TNC on SLW. Midiron SLW increased probably because TNC accumulated in leaves without subsequent loss of chlorophyll during exposure to chilling temperatures.

Conclusions

Midiron and Tifgreen should be classified as chill-tolerant and chill-sensitive, respectively, based on physiological responses to chilling temperatures. The greater ability to assimilate and transport carbon during and following recovery from exposure to chilling temperatures may allow Midiron to grow more rapidly than Tifgreen. Thus, Midiron may provide turf superior to Tifgreen during exposure to chilling temperatures.

The inability to transport carbohydrate from and the subsequent accumulation of high photoassimilate levels in leaves was apparently responsible for the inability of bermudagrass to fully recover high photosynthetic rates following exposure to chilling temperatures. Photosynthesis may be permanently impaired, but respiration appeared to be reversibly inhibited by short term exposure of bermudagrass to chilling temperatures.

Although Fe maintained the aesthetic qualities of both chill-sensitive and chill-tolerant cultivars during the chilling period, the enhancement of physiological activity differed with cultivar. However, foliar applied Fe stimulated recovery of physiological activity in both cultivars tested after exposure to chilling temperatures. Iron enhanced the aesthetic qualities of bermudagrass without limiting TNC in rhizomes and stolons.

Benzyladenine enhanced the aesthetic qualities of Midiron and Tifgreen exposed to chilling temperatures. However, BA may be of greater importance to enhancement of physiological activity in chill-sensitive than in chill-tolerant bermudagrass cultivars.

SUMMARY

Field and greenhouse studies were conducted to determine the effects of foliar applications of Fe and cytokinin on bermudgrass (Cynodon spp.) performance during fall and on growth in spring. The influence of foliar applied Fe and cytokinin on photosynthetic and respiratory CO_2 exchange of bermudagrass during exposure to chilling temperatures was determined in controlled environment chamber studies. Non-structural carbohydrate composition of bermudagrass tissues in relation to chilling temperatures as influenced by Fe and cytokinin was also investigated.

Foliar applications of Fe in late-summer and fall extended bermudagrass performance during low temperature periods of fall without adverse effects on post-dormancy growth. The synthetic cytokinin benzyladenine (BA) applied alone was not as effective as when applied with foliar Fe.

The systemic fungicides triadimefon and benomyl were evaluated as possible cytokinin sources because these chemicals are commercially available and can be readily obtained by turf managers. Although triadimefon stimulated post-dormancy growth, no improvement in green bermudagrass color retention was observed during fall. Further research to determine if triadimefon applied after dormancy could stimulate

earlier post-dormancy growth appears warranted. Midiron bermudagrass treated with benomyl did not perform well in fall or spring. Therefore, applications of benomyl to bermudagrass should be avoided at times when chilling temperatures may occur.

Clear plastic covers prevented frost injury and resulted in a 6- to 8-week longer bermudagrass growing season. Although time and labor constraints may limit the use of protective coverings to specialty turf areas, year-long green bermudagrass turf may be realized in some climatic regions if protective covers were included as part of management. Iron and BA applications may further enhance turf color when used in conjunction with protective covers.

Foliar applied Fe improved bermudagrass fall performance and post-dormancy recovery regardless of summer nitrogen fertilization rate. Frequent Fe applications may be required to extend the maintenance of bermudagrass color when chilling temperatures are severe and prolonged. Applications of BA in conjunction with foliar Fe fertilization may aid retention of green bermudagrass turf during prolonged exposure to chilling temperatures.

Improved bermudagrass performance during fall caused by Fe does not adversely affect TNC levels in stolons and rhizomes at the onset of dormancy. The enhancement of post-dor-

mancy recovery by Fe was not related to TNC in rhizomes and stolons but possibly to prevention of chlorosis usually caused by an inability of bermudagrass to take up sufficient Fe from cool moist soils or because Fe delayed the onset of dormancy and subsequently shortened the dormancy period.

Based on physiological responses to chilling temperatures Midiron should be classified as more chill-tolerant than Tifgreen. The greater ability to assimilate and transport carbon at chilling temperatures may allow Midiron to maintain turf superior to Tifgreen during exposure to chilling temperatures.

The accumulation of high photoassimilate levels in leaves is apparently responsible for the inability of bermudagrass to fully recover high photosynthetic rates following exposure to chilling temperatures. The accumulation of high levels of water soluble sugars in leaves suggests that metabolism of photoassimilates other than starch is also affected by chilling temperatures. Reduced respiratory activity appeared responsible for the accumulation of nonstructural carbohydrates in leaves when bermudagrass was exposed to chilling temperatures.

In contrast to photosynthesis, respiration was reversibly inhibited by short term exposure of bermudagrass to chilling temperatures. Rapid recovery of high respiratory

activity may be important for maintenance of aesthetically pleasing bermudagrass turf following exposure to chilling temperatures.

Although foliar Fe applications improved the aesthetic qualities of Tifgreen and Midiron during chilling, the enhancement of physiological activity differed with cultivar. However, foliar applied Fe stimulated recovery of photosynthetic and respiratory activity in both cultivars tested after exposure to chilling temperatures. The improved fall Midiron bermudagrass performance observed in field studies caused by Fe may have occurred because Fe enhanced assimilation and utilization of CO_2 for growth.

Foliar applications of Fe did not cause increased chlorophyll content. However, photosynthetic rates on a per unit chlorophyll basis were enhanced by Fe. Iron applications may prolong CO_2 assimilation as high light levels and chilling temperatures cause a net loss of chlorophyll. Thus, the aesthetic qualities of bermudagrass may be improved by Fe applications without limiting TNC in stolons and rhizomes.

Benzyladenine generally improved the aesthetic quality of Midiron and Tifgreen exposed to chilling temperatures. However, BA influenced photosynthetic rates of Tifgreen more than Midiron. Benzyladenine applied in addition to Fe may be of greater importance to enhancement of photosynthetic ac-

tivity in chill-sensitive than in chill-tolerant bermudagrass cultivars. However, BA may have little significant effect on TNC levels in rhizomes and stolons. The ability of BA treated Tifgreen to recover higher photosynthetic rates following exposure to chilling temperatures without preventing chlorophyll loss suggests that photosynthetic enzyme activity may have been preserved by BA. However, further research is needed to clarify the effects of BA on CO_2 assimilation in bermudagrass exposed to chilling temperatures.

These investigations indicate that foliar applications of Fe may be used to modify bermudagrass physiology and enhance performance of bermudagrass exposed to chilling temperatures. Cultivar selection may also play a major role in determining turf quality at chilling temperatures.

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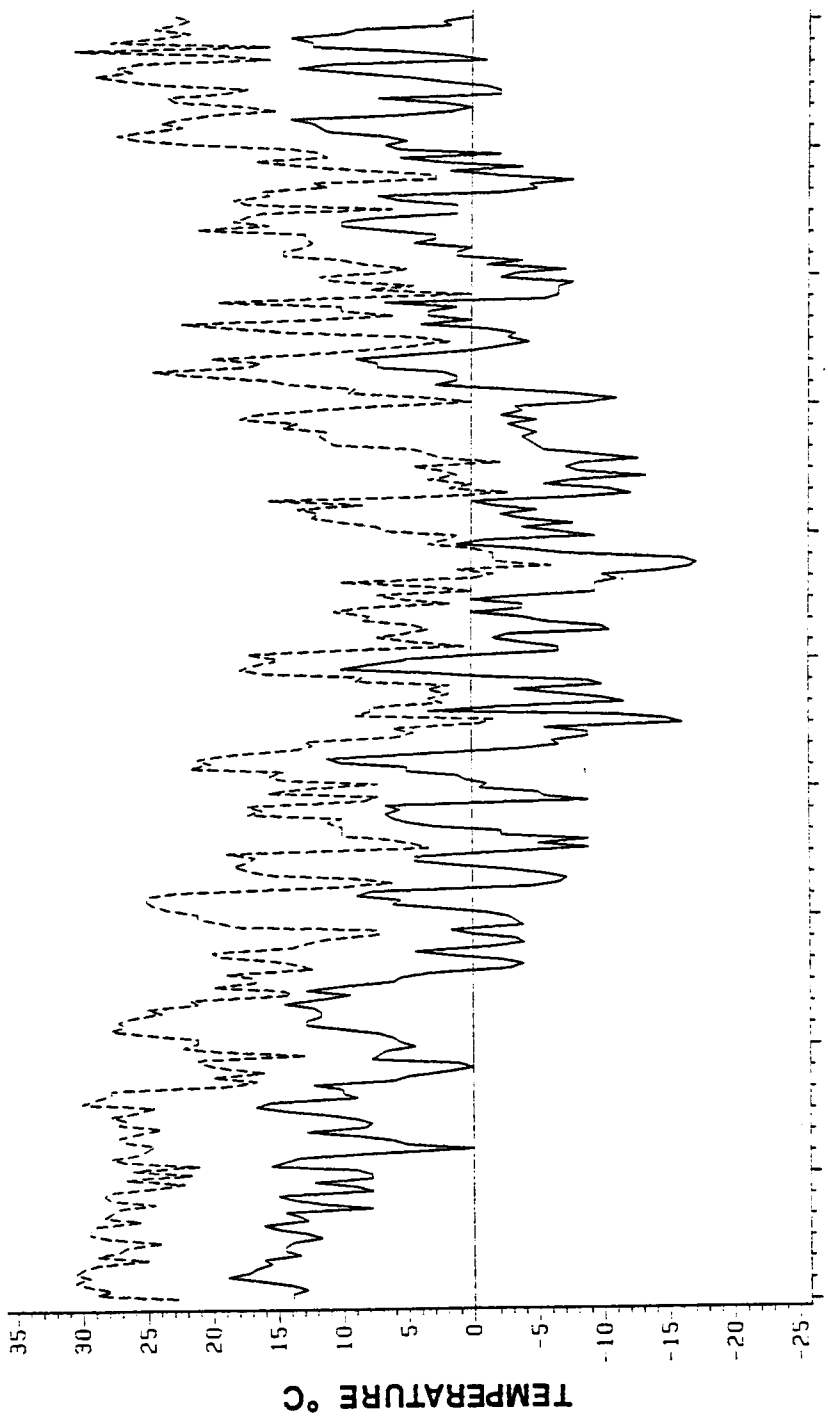
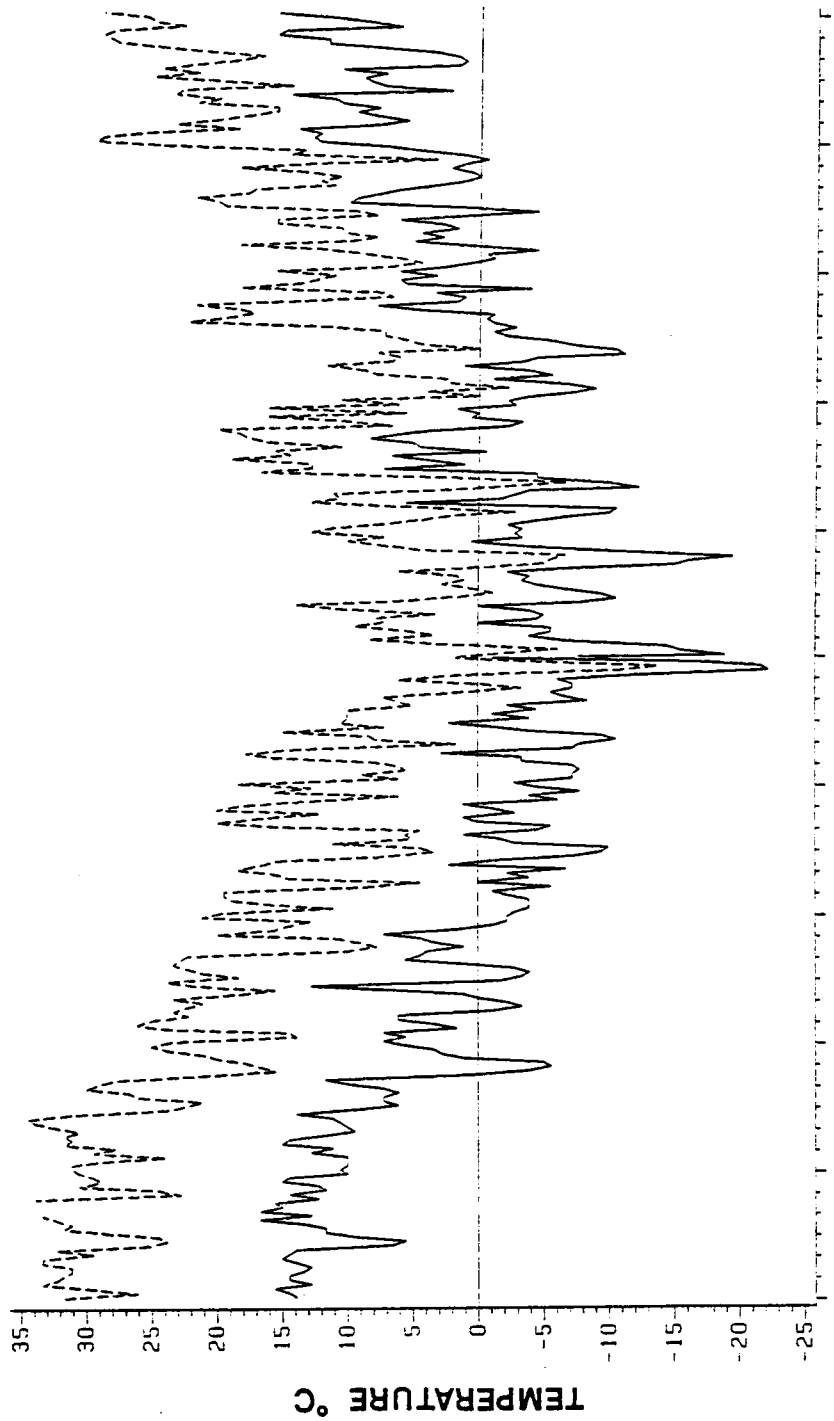
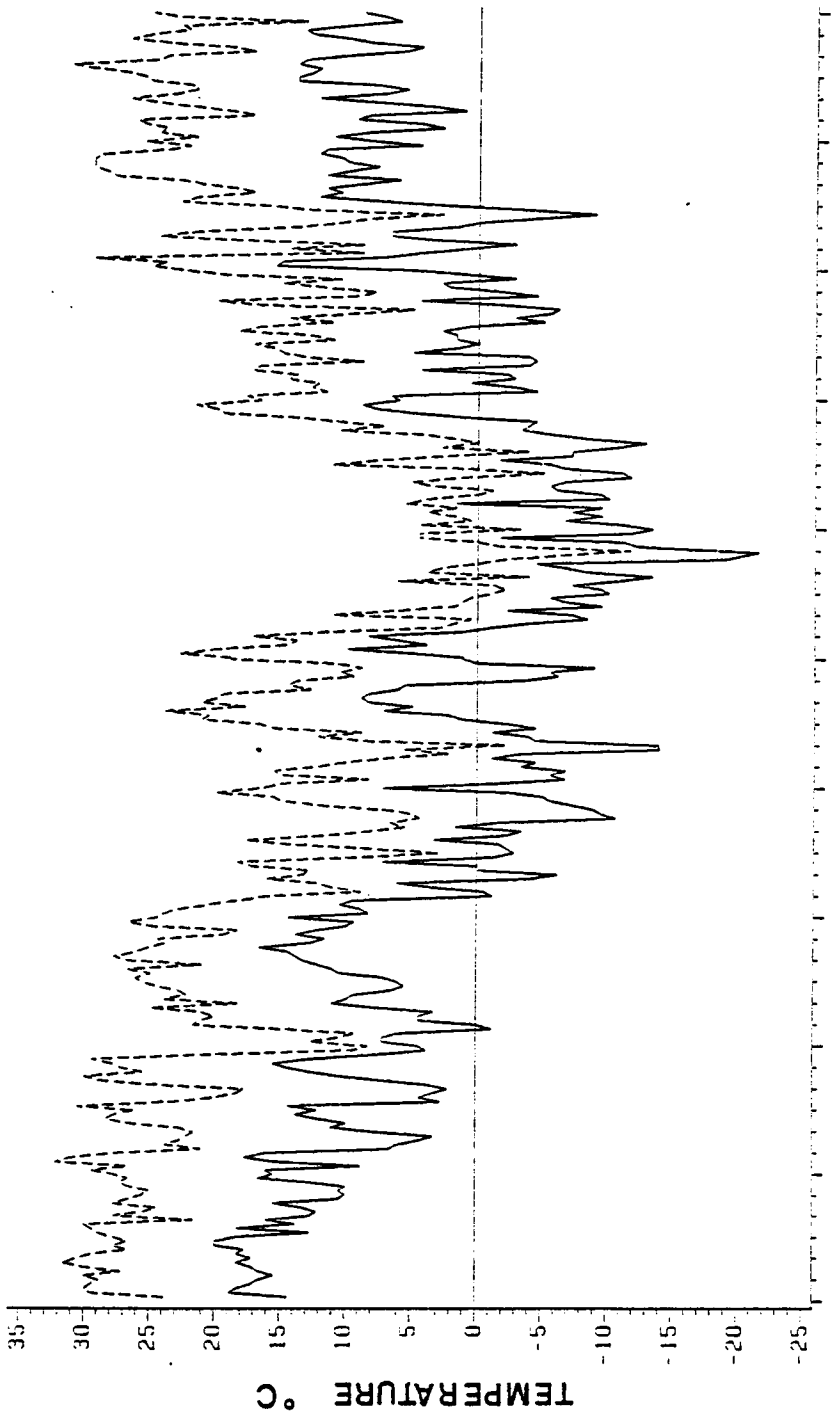
APPENDIX



Appendix 1. Maximum (---) and minimum (—) daily temperatures during August 1982 through May 1983.



Appendix 2. Maximum (---) and minimum (—) daily temperatures during August 1983 through May 1984.



Appendix 3. Maximum (---) and minimum (—) daily temperatures during August 1984 through May 1985.

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