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ABSTRACT

CULTURAL AND ENVIRONMENTAL FACTORS INFLUENCING THE STOMATAL DENSITY AND WATER USE RATE OF PENNCROSS CREEPING BENTGRASS

By

Robert C. Shearman

The effects of various cultural and environmental factors on the stomatal density and water use rate of Penncross creeping bentgrass were investigated. The factors included (a) light intensity, (b) temperature, (c) cutting height, (d) soil moisture, (e) irrigation frequency, and (f) nitrogen nutritional level. The relative importance of these cultural and environmental factors in determining the water use rate was determined.

Stomatal density counts were made from clear-nitrocellulose replications of the leaf blade surface using a light microscope at 430 magnifications. A special wind tunnel apparatus was used to determine the water use rate of the turves. The atmospheric drought-stress conditions maintained in the chamber were 33 C, 40% relative humidity, 4300 lux, and a constant wind velocity of 4 miles per hour. The turves were exposed to these conditions for

a 12 hour period. The water use rate was determined from the percent moisture lost during the exposure period.

Higher light intensities resulted in an increase in stomatal density and water use rate. The stomatal density was reduced by 48% when the light intensity was reduced from 25,800 to 3762 lux, while the water use rate declined by 50% for the same treatments. Suboptimal growth temperatures resulted in a reduction in the stomatal density and water use rate. A 30% reduction in stomatal density occurred between 20 and 10 C temperature while the water use rate declined by 20%. No differences were found in stomatal density and water use rate at the 20 and 33 C growing temperature.

The water use rate increased with increased heights of cut. Increasing the cutting height from 0.7 to 2.5 cm resulted in a 53% greater water use rate. Turves cut at 12.5 cm had a water use rate which was double that at 0.7 cm. Increased nitrogen nutritional levels of 0.23, 0.91, and 1.83 kg per 92.9 m^2 resulted in reduced stomatal density and water use rate. The decline in water use rate at the higher nitrogen nutrition levels was for a short-term drought-stress period and not based on an entire growing season.

The stomatal densities were inversely related to the amount of water applied. The water use rates decreased when the water application rate was increased from 1.3 to

10.0 cm per week. Infrequent irrigation resulted in a reduction of the water use rate, while the stomatal density was increased. The water use rate was positively correlated to the percent vegetative cover.

Light intensity, cutting height, and frequency of irrigation had the greatest effect on the water use rate. Nitrogen nutritional level was intermediate in its effect, while temperature and water application rate had the least effect on the water use rate.

The stomatal densities from the various studies ranged from 72 to 125 per square millimeter. A 31% increase was noted between the second and sixth leaf blade on the culm. A three fold increase in stomatal density was found when the upper surface was compared to the lower surface of the leaf blade.

CULTURAL AND ENVIRONMENTAL FACTORS INFLUENCING THE STOMATAL DENSITY AND WATER USE RATE OF PENNCROSS CREEPING BENTGRASS

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INTRODUCTION

Penncross creeping bentgrass (*Agrostis palustris* Huds.) is one of the most widely used seeded grasses on greens in the United States. It has a vigorous growth habit and requires a high intensity culture that results in a thatching tendency if not properly maintained. Cultural practices for maintenance of a quality Penncross turf generally include (a) high nitrogen fertilization, (b) close, frequent mowing, (c) soil cultivation and modification, (d) preventative fungicide applications, and (e) irrigation.

The creeping bentgrasses, such as Penncross, are quite susceptible to drouth stress. The growth rate, intensity of culture, and shallow root system under close mowing necessitates frequent irrigation during moisture stress periods. The amount and frequency of irrigation required are determined by various cultural and environmental factors. Factors affecting the rate of turfgrass growth are probably the most influential in effecting the water use rate. Included are light, temperature, soil moisture, frequency of irrigation, cutting height and frequency, and level of nitrogen nutrition. The relative importance of many of these cultural and environmental factors on the water use rate of turfgrasses has not been determined.

The objective of this investigation was to determine the relative importance of several cultural and environmental factors on the water use rate of Penncross creeping bentgrass. The factors studied were (a) light intensity, (b) temperature, (c) soil moisture content, (d) cutting height, (e) frequency of irrigation, and (f) level of nitrogen nutrition. The influence of these factors on stomatal density was also observed. Correlations between stomatal density and water use rate were determined in relation to the cultural or environmental factors studied.

LITERATURE REVIEW

The Water Use Rate of Turfgrasses

The consumptive use or water use rate is defined as the total amount of water required for turfgrass growth plus the quantity lost by transpiration and evaporation from soil and plant surfaces (3). It has been demonstrated that the evapotranspiration rate of a turf is greater than the evaporation rate from a bare soil (34, 35). The increase in amount of water lost from a turf is due to transpirational losses from the extensive leaf surface area exposed to the atmosphere plus the turfgrass root system which increases the amount of water removed from the soil profile. The water use rate for actively growing turfgrasses can vary from 1.75 to 5.25 cm per week depending on the environmental and cultural conditions and may exceed 7.5 cm per week under high atmospheric moisture stress (3).

A number of factors influence the water use rate of turves including (a) length of the growing season, (b) evapotranspiration rate, (c) rate of growth, (d) the turfgrass species or cultivar, (e) intensity of culture, (f) amount of traffic, (g) soil texture, (h) precipitation, and (i) available soil moisture (3). The total amount of water used per year is partially determined by the length of the growing season. The amount of water used may also vary due to factors influencing the evapotranspiration rate and growth rate throughout the growing season. Both are influenced by the turfgrass species or cultivar, intensity of culture, soil texture, amount of soil moisture, precipitation, and intensity of traffic.

The factors noted for influencing the water use rate such as the species or cultivar, intensity of culture, precipitation, amount of soil moisture, and light intensity have been noted by various workers to have marked influence on stomatal density (9, 13, 14, 21, 22, 23, 24, 25, 26).

Stomatal Relationships to Water Use Rate

Approximately 90 percent of the water lost from a leaf occurs by diffusion through the stomata. Factors influencing the stomatal number and fluctuation in stomatal aperature provide the indirect mechanism for regulation of the transpiration rate (18). The response of stomata to light, carbon dioxide concentration, and internal water stress are predictable (18, 31). Light and water stress are the major factors determining stomatal aperature. The light effect has been explained quantitatively by a photosynthetic reduction of the CO_2 concentration in the intercellular spaces and guard cells (18).

Stomata are essentially closed in the dark. A minimal light intensity is necessary for stomatal opening which varies with the species. Tomatoes (*Lycopersicum esculentum* Mill) have been reported to require at least 2956 lux, while 968 lux causes opening in *Pelargonium sp*. (18). The degree of stomatal opening increases with increased light intensity up to a maximum intensity for the particular species where a maximum stomatal aperature occurs. Very high light intensities may cause a decrease in stomatal opening (18, 30). This could be an indirect effect attributed to high leaf temperatures or a high rate of water loss which causes a tissue water stress.

The water balance within the plant is influenced by the interrelated processes of absorption, translocation and transpiration. Changes in water content or hydrature of the leaf are generally expressed as changes in water deficit. The water deficit is considered a very powerful stomatal regulator. When above a critical level, the water deficit will override all other stomatal opening stimuli (3, 18).

In general, studies concerning the temperature influence on stomata have been difficult to evaluate. It is hard to separate the effects of temperature on the (a) diffusion coefficient, (b) water deficit of the leaf tissue, and (c) atmospheric relative humidity. Within the normal-range of temperatures, 10-25 C for cool-season turfgrasses, temperature has little effect on the steady-state stomatal aperature. Temperatures higher than 30-35 C have a closing effect which may contribute to the cause of midday closure of stomata (18). Temperature does not influence differentiation of stomata, but can produce morphological abnormalities in the stomata (1). Carbon dioxide levels increase in the intercellular spaces with increased temperature (18). This response is thought to be due to increased respiration.

Stomata play a vital role in gas exchange between the leaf and the adjacent atmosphere. They function as the primary pathway for water vapor, carbon dioxide, and oxygen exchange (31). Stomata may occur on both surfaces of a leaf (an amphistomatic leaf) or on one surface only, either the upper (an epistomatic leaf) or the lower (a hypostomatic leaf) (16). Although stomata may occur on both leaf surfaces, they are more commonly found on the lower surface of most plants (16, 27, 31). However, this does not seem to be the case for grasses. Several researchers have reported a higher stomatal density on the upper as opposed to the lower surface of such species as blue panicgrass (Panicum antidolate Retz), corn (Zea Mays L.), barley (Hordeum vulgare L.), creeping bentgrass (Agrostis palustris Huds.), and annual bluegrass (Poa annua L.) (13, 27, 23, 24). Other workers have made similar observations for such species as alfalfa (Medicago sativa L.), beans (Phaseolus vulgaris L.), pumpkin (Cucurbita peps L.), and tomatoes (Lycopersicum esculentum Mill.) (10, 16, 27).

Generally speaking, the frequency of stomata may range from 50 to 500 per square millimeter (31). The stomata may be separated by no more than 1 or 2 epidermal cells at the higher frequency. Creeping bentgrass and annual bluegrass were reported to have a frequency of 1 stomata to 2 epidermal cells. The same species grown under infrequent watering had a 1 to 4 ratio, indicating that variability within a species could be caused by exposure to various environmental and cultural factors (23). The stomata are arranged in parallel rows in species of monocotyledons which have veined leaves. The stomata tend to be scattered in species with netted-veination.

Stomatal density can vary with the leaf position and plant maturity. Blue panicgrass (Panicum antidolate Retz.) was shown to have fewer stomata per unit area in the younger leaves at the top of the culm than leaves collected at either the middle or base of the culm (13). Preliminary studies with the same plant showed 30 percent fewer stomata at the seedling stage of growth than at the time of pollination. Work with several cultivars of alfalfa indicated an inverse relationship between stomatal density and leaf position (10). Leaves sampled from the apex of the plant had significantly more stomata per mm^2

than those from the base. Samples from all cultivars had significantly greater stomatal densities on the adaxial leaf surface compared to the abaxial surface. Stomatal frequencies were studied on the lower leaf surface of 649 cultivars from the World Collection of Barley (24). Frequencies decreased progressively from the flag to the lower leaves, with the flag-leaf having approximately twice the stomatal density of the basal leaves. Stomatal frequencies ranged from 36 to 98 per mm^2 with a mean of 64 on the flag leaf of the cultivars observed. A negative correlation was obtained between the stomatal frequency and size. The researchers suggested that the negative correlation between frequency and size of stomata might be the result of a compensatory relationship, such that the total pore area of different cultivars is approximately equal.

Although stomatal density varies significantly for most species with position and surface of the leaf, work with blue panicgrass indicated that the numbers do not vary significantly as to position on the same leaf. Impressions at the base, middle, and apex of the leaf averaged 116, 119, and 119 per mm^2, respectively (13).

Stomatal density varies significantly among cultivars within a species, as well as between species. Among cultivars of alfalfa studied, Ladak maintained the greatest number of stomata regardless of the surface and Sonora the lowest (10). Stomatal densities were significantly different among clones of blue panicgrass (14). Clones selected for high forage production under irrigated conditions had a significantly higher stomatal density than clones which were selected for seedling drought tolerance. A significant negative correlation between drought tolerance and mean stomatal density was found, indicating that clones which had the highest number of stomata per unit area were the least drought tolerant seedlings.

Effect of Light and Temperature on the Water Use Rate

The role of light in influencing the water use rate of turfgrass can be closely associated with its various effects on the stomata, leaf and shoot density, root growth, cuticle development, and carbohydrate reserve.

The water content of plant tissues varies diurnally. The water content is greater during the night, with a maximum in the early morning hours and decreases during the day with a minimum at midday. The osmotic pressure within the tissue varies inversely with the degree of hydration. Increased osmotic pressure during the daylight hours is caused by (a) the buildup of soluble carbohydrates and other organic compounds resulting from photosynthesis, and (b) a decrease in the degree of hydration due to water loss through transpiration exceeding water absorption by the root system (3). The response of stomatal aperature to light and temperature have already been discussed in the previous section, as well as, the importance of the stomata in water loss by transpiration.

Increased light intensity and duration of illumination play roles in increasing the number of stomatal initials and the stomatal frequency (28, 30). An increase in epidermal cell size and a decrease in size of stomata were also noted. Alfalfa (Medicago sativa L.) and birdsfoot trefoil (Lotus corniculatus L.) were reported to have a significantly higher number of stomata when grown in full-sunlight of approximately 96750 lux compared to plants grown under similar conditions but shaded by 92% Saran Shade Cloth (11). Leaves of silverrod (Solidago virgaurea R.) were also found to have greater numbers of stomata per unit area when grown in the sun as opposed to leaves grown in the shade (4). Miskin reported a similar response in barley with increased light intensity, while temperature was reported to have very little effect on stomatal frequency (24). In addition to the effect of increased light intensity on stomatal frequency and size of epidermal cells, cuticle thickness and consistency is also influenced due to the effect of light on the oxidation and condensation of fatty acids involved in the process of cuticle formation (20). Low light intensities tend to result in a thin cuticle, reduced root system, and low carbohydrate reserves. Investigation with Coastal bermudagrass (Cynodon *dactylon* L.) showed that reduced light intensity resulted in a decrease in percentage of plant weight associated with the roots and rhizomes (7). Carbohydrate reserves were also reduced.

Turfgrasses have an optimum temperature range for growth and development. For most of the cool-season turfgrass species, the optimum is in the soil temperature range of 15 to 20 C. Plants exposed to temperatures above or below their growth optimum are subject to decreased growth. The greater the deviation from the optimum temperature, the greater is the chance that growth will cease and death occur. Temperatures which encourage more rapid shoot growth rates tend to increase the water use rate. Temperatures within the 10-25 C range have been reported to have little effect on stomatal aperature, while higher temperatures of 30-35 C have a closing effect (18). Studies with sunflower (*Helianthus annus* L.) plants have shown an increase in transpiration with increased soil temperatures (33). The water use increases with temperatures up to a maximum. The decrease in the water use rate at higher temperatures is associated with stomatal closure, and with the fact that root permeability to water is decreased at the higher soil temperatures.

Effect of Clipping Height on the Water Use Rate

The water use rate is thought to increase with the increase in height of the grass plant. This is associated with the increase in total leaf area exposed to desiccating conditions and with the increase in the extent of the root system. There has been very little reported regarding the effect of cutting height on the water use rate of turf-grasses.

The evapotranspiration rates for perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) were greater when the plants were allowed to grow to 25-30 cm, than when they were clipped at 2.5-5.0 cm (25). Workers using Kentucky bluegrass turves clipped at various levels reported that soil water extraction was directly proportional to the height of cut (21). These workers assumed that water extraction from the soil was directly proportional to the number of absorbing roots.

Effect of Irrigation Frequency and Soil Moisture on the Water Use Rate

The turfgrass plant absorbs water from the soil through the root system. Water is absorbed primarily through the root hairs which are located just above the root tips. The root hair zone varies in magnitude with species, environmental conditions existing during devel-opment, and the age of the root (3).

Frequent, light irrigations reduce drought tolerance due to a decrease in the extent of the root system (23, 28). Increased soil moisture has also been reported to decrease the number of root hairs, and the associated absorptive capability of the root system. Creeping bentgrass and annual bluegrass watered 2 and 6 times a week for a period of 5 weeks were more prone to wilting with the more frequent irrigation treatment (23). Roots from plants in the flats watered 6 times per week were somewhat shorter than those less frequently watered. However, as soil moisture stress increases water use efficiency, dry matter production and transpiration of the grass plants decrease. Clones of blue panicgrass were observed to be more efficient in water use when soil moisture levels were maintained near field capacity (14).

Investigations with soybeans (*Glycine max* L.) showed that as soil moisture was reduced below field capacity the evapotranspiration was reduced. Soybeans grown under drought and nondrought soil conditions had different evapotranspiration rates. The ratio of nondroughted to droughted was 0.71 (16).

Soil moisture levels have various indirect effects on the plant, which are associated with the water use rate. Sunflower (*Helianthus annus* L.) and common smartweed (*Polygonum hydropiper* L.) had larger but fewer stomata per unit area when grown under dry soil conditions. Also, the leaves were wider and thicker (28). A reduction in water loss from soybeans grown under droughty conditions was associated with an accumulation of lipids on the leaf surface (9). These workers found that water loss from droughted plants continued to decrease even after the stomata were observed to close. This decrease in water loss was associated with increased cuticle thickness. The cutin layer in annual bluegrass and creeping bentgrass was found to be almost nonexistent on plants maintained under excessive moisture levels (23).

Leaves of frequently watered grasses have more air space between cells of the mesophyll tissue than those of infrequently watered plants (23, 28). The addition of fertilizer was noted to add to this increase in air space between cells of the mesophyll tissue. Grasses grown under semi-arid soil conditions had very little air space between the cells. This variation in air space is important. The increase in air space between cells allows for more surface area from which the water may evaporate and be lost as water vapor through the stomata and cuticle.

Effect of Turfgrass Nutrition on Water Use Rate

Factors which have a direct effect on the shoot growth rate of the turfgrass plant will also have a direct effect on the water use rate. Turfgrass nutrition plays an important role in the amount of moisture extracted from the soil by the plant. Nitrogen fertilization has been reported to increase the water use rate of turfgrasses (3, 5, 8, 12, 19, 22, 32). Early reports from literature reviewed before 1915 indicated that the water requirements of plants was reduced by the use of fertilizers, and that the reduction was greater on poor soils, than on fertile soils (5).

Studies were conducted in Israel concerning the influence of irrigation frequency and nitrogen fertilization on the water use rate of kikiyugrass (Pennisetum clandestinum Hochst.), a turfgrass species. The objective was to find a combination of nitrogen fertilization and irrigation practices which would maintain desirable turfgrass quality, but require a minimal amount of water. The conclusions were that turfgrasses receiving nitrogen applications of 2.1 kg. per 1000 m^2 per month (1.4 pounds of nitrogen per 1000 sq. ft. per month) and irrigated every 25 days did not produce any greater yield than nonfertilized plants irrigated every 7 days (22). The regular use of nitrogen did not produce excessive growth but maintained adequate color and shoot density when water was not applied at too high a frequency. They also concluded that less frequent irrigation only conserved water. Nitrogen fertilization of irrigated grasses increased the evapotranspiration rate (19). Studies with alfalfa, Kentucky bluegrass, and quackgrass (Agropyron repens Beaun) resulted in similar findings (12, 32). Growing conditions that prevent rapid plant growth and promote the accumulation of dry matter would have a greater ability to withstand drouth. Rhizomes of quackgrass from fertilized soil were found to be more severely injured by drought than those from unfertilized soil (12). Studies with various turfgrass species, such as Kentucky bluegrass, Canada bluegrass (Poa compressa L.), rough bluegrass (Poa trivialis L.), annual bluegrass, and colonial bentgrass (Agrostis tenuis L.) indicated that plants grown at high nitrogen levels were less able to withstand soil drought than those grown at low nitrogen levels (8).

The water use efficiency is reported to increase when the level of nitrogen nutrition is increased (19, 32). This is the result of a decrease in the amount of water required to produce a unit of dry matter. Sprague reported that the water requirement per unit of dry matter produced is increased when growth is limited by nitrogen fertilization or a reduction in stored reserves (32). The water use rate is of greater consequence for turfgrasses than the efficiency for dry matter production.

MATERIAL AND METHODS

Source of Penncross Creeping Bentgrass

The investigations were conducted with Penncross creeping bentgrass (*Agrostis palustris* Huds.) using the same certified seed lot (Lot No. HQ-692161) throughout all studies. The seed test of the lot (April, 1969) showed a purity of 98.47 percent and a germination of 94 percent. There were no other crops or weed seeds present.

Establishment Procedures

The turves were established in 0.473 liter (16 oz.) wax cottage-cheese containers. A soil mixture of 80% white, silica sand and 20% sandy loam topsoil was used in all studies except those involving the influence of nutrient levels, where only silica sand was used. The containers were filled with the soil mixture to within 1.25 cm of the rim allowing an 8.75 cm growing medium. The surface area of the containers was 99.4 square centimeters. Drainage was provided by holes punched in the bottom of the wax containers.

The seed was spread uniformly across the soil surface at a rate of approximately 0.228 kg. per 92.9 m^2, and was covered with a thin, 0.6 cm layer of a sand and soil mixture. Several layers of cheese cloth were placed on the soil surface to maintain favorable moisture conditions during establishment. Each study was seeded 10 days prior to initiation of the various treatments. The establishment period under an automatic mist-irrigation system in the greenhouse was similar for all the plants. Temperature and light conditions were not monitored during establishment. The plants were 1.88 cm in height when the experimental treatments were initiated.

Cultural Practices

Similar cultural practices such as cutting height, fertilization, and irrigation were utilized for each study. Variations in cultural practices will be discussed with each study where appropriate. The cutting height was maintained at 5.0 cm with the turves being clipped at weekly intervals. The cutting height study was the only exception to this procedure.

The nutrient solution used was a modified Hoagland's solution (17), having an N-P-K ratio of 4:1:2. It was adjusted to a pH of 7.0 by the addition of sodium hydroxide. The nutrient solution application rate approximated 0.912 kg. of nitrogen per 92.9 m^2 per month. Nutrient solution applications were made twice weekly. All the plants were watered daily with the exception of the watering frequency study. Those studies involving the influence of fertility, irrigation frequency, and amount of water applied were the only exceptions.

The two basic types of data collected in all studies were stomatal density and water use rate. The procedures for preparation of stomatal mounts and determination of water use rate were standard throughout all studies.

Procedure for Stomatal Mount Preparation

Leaves for stomatal impressions were randomly chosen from the same position on the culm of the plants in each treatment container. They were selected at a similar stage of development, the sixth leaf. The method for making stomatal impressions was similar to that reported by Sampson (29) with the following modifications. The silicone rubber monomer procedure was eliminated. Impressions were made directly from the surface of each leaf using a nitrocellulose preparation (clear fingernail polish). This gave a satisfactory negative print of the turfgrass leaf surface.

Each leaf blade was firmly attached to a sheet of Parafilm with a clear cellulose adhesive taped to the leaf base and tip. The nitrocellulose preparation was spread evenly across the leaf surface and allowed to dry for 6 hours. After drying, the impressions were ready for peeling. The tapes applied to the base and tip of each leaf acted as tabs which aided in peeling the transparent replica from the leaf blade.

The transparent replica was then transferred to a polished microscope slide and placed under a slip cover. The edges were firmly bound with clear cellulose adhesive tape. Transfers were made swiftly to prevent the specimen's replica from curling. Curled, clouded, or otherwise undesirable replications were discarded.

Stomatal density counts were made at 430 magnifications. The actual area observed was 0.159 square millimeters, but counts were converted to numbers per mm^2. Counts were based on the following considerations: (a) all counts were made from the margin of the leaf toward the mid-vein; (b) they were taken from the middle of the leaf; (c) density counts were made on the abaxial and adaxial surfaces; and (d) any stomata within or touching the field were included in the density count. The number of replications for the stomatal density counts varied with the individual study, but were within the range of 4 to 10.

Percent Moisture Loss Determination

Wet weight determinations were made 12 hours after the containers were watered to saturation. The 12 hour drainage period was adequate to approach field capacity, for the sandy soil mixtures used in the investigations.

Field capacity is achieved when there is no longer any rapid downward movement of water through the soil (6). Moisture movement continues to take place but at a much slower rate. The movement is due primarily to capillary forces effective in the micropores. The macropores would greatly exceed the micropores in the sandy soil mixture used, thereby decreasing the amount of time necessary to obtain a moisture level simulating field capacity.

The total moisture at the beginning of each study ranged from 20 to 33%. The percent moisture was constant within each study. Once the steady state of moisture percentage was approached, the containers were readied for exposure to the desiccating conditions of the wind tunnel. Preparation for the wind tunnel involved covering the bottoms of the containers with polyethylene to prevent water losses through the drainage holes, and recording wet weights prior to the exposure period.

The containers were then placed in a special wind tunnel apparatus with a controlled environment of (a) 33 C, (b) 40% relative humidity, (c) 4300 lux light intensity and (d) a constant air-flow of 4 miles per hour. These conditions were chosen after some preliminary experiments in order to maximize the water use rate and thus minimize the duration of the exposure period. The latter was important in experiments involving large numbers of replications. The exposure period selected was 12 hours. This was ample time for marked differences in the water use rates to occur between treatments.

The containers were placed in the wind tunnel apparatus on a rotating platform that allowed all containers to be uniformly exposed to the environmental conditions within the chamber. The relative humidity and temperature within the chamber were monitored with a Hygrodynamics hygrometer (Model no. 15-3001) that was accurate to ±1.5%. A light source was supplied from overhead fluorescent lighting and an additional incandescent light source placed immediately above the chamber.

The containers were again weighed at the termination of the 12 hour exposure period, and the weights recorded (termination weight). The oven dry weights were then determined by placing the turves in a 105 C forced-air drying oven for 24 hours. Initial studies showed that containers exposed to the same conditions for 36 and 48 hours had the same oven dry weight as those exposed for 24 hours. The percent moisture lost was calculated as follows:

$$\frac{\text{Wet Weight-Termination Weight}}{\text{Wet Weight-Oven Dry Weight}} \times 100 = \text{Percent Moisture Lost}$$

Wet weight minus the termination weight was used to delineate the amount of moisture lost during exposure to conditions of the wind tunnel apparatus. The total moisture content was the difference between the wet weight and the oven dry weight. The percent moisture lost was determined by dividing the total moisture content into the amount of moisture lost during the exposure period and multiplying by 100. These calculations were made on each observation within the individual studies.

Description of Studies Conducted

Six individual studies were conducted during the course of this investigation. The environmental and cultural factors investigated in terms of their influence on stomatal density and water use rate were: (a) light intensity, (b) temperature, (c) amount of water applied, (d) frequency of watering, (e) cutting height and (f) nitrogen fertilization.

Environmental Studies

Study I.--The effect of light intensity on stomatal density and water use rate.

Three light regimes were used in this study to determine the effect of light intensity on the stomatal density and water use rate of Penncross creeping bentgrass. The light intensity treatments were (a) 3762 lux, (b) 25,800 lux, and (c) sunlight. Each treatment had 10 replications totaling 30 observations for the study. The experimental design was completely randomized.

Those turves under light intensities of 3762 and 25,800 lux were placed in separate, environmentally controlled growth chambers set at a constant day-night temperature of 20 C and a 16 hour photoperiod. Those exposed to full sunlight were placed in an open area near the greenhouse where the light intensities ranged from 29,025 to 96,750 lux. They were exposed to varying day-night temperatures with an average daily temperature range of 15-20 C and a photoperiod of approximately 16 hours.

The preconditioning period was terminated after 3 months. Leaf samples for stomatal measurements were taken at this time. The water use rate was determined by the use of the wind tunnel apparatus and determining the percent moisture lost by the method previously described. The data was appropriately analyzed, and the correlation between stomatal density and water use rate determined.

Study II.--Determining the effect of temperature on stomatal density and water use rate.

The influence of temperature on stomatal density and water use rate of Penncross creeping bentgrass was evaluated in this study. The three temperatures used were 10, 20, and 33 C with eight replications per treatment totaling 24 observations. The treatment temperatures were based on soil temperatures which were monitored by soil probe thermometers. The three controlled environment growth chambers used had 12 hour photoperiods with a

21,500 lux light intensity. Each temperature treatment was held constant throughout the preconditioning period.

Upon termination of the 2.5 month treatment period, the stomatal density and water use rate were determined for each growing temperature treatment using the procedures previously outlined. The correlation coefficient was calculated for stomatal density and water use rate.

Cultural Studies

Study III.--The effect of cutting height on the water use rate of grasses.

The effect of three cutting heights on the water use rate of Penncross creeping bentgrass was evaluated in this study. The cutting heights were 0.7, 2.5, and 12.5 centimeters. They were replicated 4 times giving a total of 12 observations for the study.

The establishment procedure was the same as that described in the general discussion. After establishment the plants were maintained in a controlled environment growth chamber, having a 12 hour photoperiod of 21,500 lux and a constant day-night temperature of 20 C. The cultural practices were the same as those previously described, with the exception of the cutting height treatments. Clipping treatments were begun when the plants reached 3.75 centimeters and were continued throughout the study. Plants were clipped weekly, except those at the 12.5

centimeter cutting height which were not clipped during the preconditioning period.

The study was terminated after 3 months and the plants exposed to the standard desiccating conditions of the wind tunnel apparatus. The percent moisture loss as influenced by the treatments was determined according to the procedure previously described.

Study IV.--Determination of the effect of water application rate on stomatal density and water use rates.

The influence of water application rate on stomatal density and water use rate was evaluated in this study. The study was established by the methods previously described. The turves were placed in a controlled environment growth chamber with a constant day-night temperature of 20 C and a 12 hour photoperiod of 21,500 lux. The three treatments were 1.3, 2.5 and 10.0 centimeters of water applied per week. It was determined that 210 milliliters applied weekly were equivalent to 2.5 centimeters of water applied per 99.4 cm^2 per week. Therefore, 15, 30, and 120 milliliters per day were applied for each of the three treatments. Preliminary tests demonstrated that an application of 120 milliliters of water was necessary to obtain drainage from the container. The treatments were replicated six times giving a total of 18 observations for the study.

Cultural practices were similar to those mentioned in the general discussion, with modifications in the application of the nutrient solution. The nutrient solution was applied as growth and color dictated. It was applied to all the containers at the 15 milliliter application rate. Additional water was used to adjust the differences in application rates for the 30 and 120 milliliter treatments.

The turves were sampled for stomatal density counts after 3 months of treatment. They were then placed in the special wind tunnel apparatus and the water use rate was determined.

Study V.--The effect of irrigation frequency on stomatal density and water use rate.

The irrigation frequency study was established and maintained under similar conditions to those for the water application rate study. The treatments consisted of (a) watering only when visual wilt occurred, (b) watering 3 times per week to saturation, and (c) watering daily to saturation. There were 9 replications of each treatment for a total of 27 observations. Samples were taken for stomatal density data at the end of the 3 month treatment period. The plants were then exposed to the standard wind tunnel conditions for the purpose of determining water use rates.

Study VI.--Determination of the effect of nitrogen on stomatal density and water use rate.

The stomatal density and water use rate were determined for plants of Penncross creeping bentgrass which had been subjected to three levels of nitrogen nutrition (Table 1). All the treatments received the same micronutrient levels which were applied in concentrations similar to those used in a complete Hoagland's nutrient solution (17).

The nutrient solution was applied daily throughout the 3 month treatment period. The growing conditions, cutting height and frequency were the same as those previously described in the cultural practice section. Stomatal density counts were taken when the treatment period was terminated. The turves were exposed to the standard conditions of the wind tunnel after the stomatal density samples were taken. The correlation coefficient was determined for the relationship between the stomatal density and the water use rate.

An auxiliary study was conducted, under the same establishment procedures, to determine the clipping weight production, root organic matter production, and shoot density for the same nitrogen nutritional levels. Clipping production was determined on a fresh weight basis. The root organic matter production was determined by ashing the roots in a muffle furnace at 700 C for 4 hours. Shoot

Table 1. N-P-K nutrient levels for nutritional experiment expressed as kilograms per 92.9 square meters.

	Nutrient Level					
	N		P		K	
Treatment Numbers	(kg/92.9 m^2)	(lbs./1000 ft^2)	(kg/92.9 m^2)	(lbs./1000 ft^2)	(kg/92.9 m^2)	(lbs./1000 ft^2)
1	0.23	0.5	0.23	0.5	0.46	1.0
2	0.91	2.0	0.23	0.5	0.46	1.0
3	1.83	4.0	0.23	0.5	0.46	1.0

densities were determined from a grid system with 2.5 cm^2 units. The densities were determined from an average of three counts per pot.

Initial soil moisture content was determined for each study. The soil moisture content varied between 18-33% for the various studies. No significant variation in initial soil moisture content was noted between treatments of the same study.

RESULTS AND DISCUSSION

Study 1. The Effects of Light Intensity on Stomatal Density and Water Use Rate

The effect of light intensity on the water use rate of Penncross creeping bentgrass is shown in Table 2. The three treatments were 3762 lux, 25,800 lux and full sunlight. Water use rates expressed as percent moisture loss were 26.3, 39.1, and 46.1, respectively for the three treatments. Table 3 shows the results for stomatal densities obtained from the same study. The stomatal densities were 78.6, 113.2, and 125.8 stomata per mm^2 for the three treatments. The water use rate was positively correlated to the stomatal density (0.88).

Table 2. The effect of three light intensities on the water use rate of Penncross creeping bentgrass expressed as percent moisture lost during a 12 hour exposure period at 33 C, 40% relative humidity, and 4300 lux.

Light Intensity Treatments (lux)	Percent Moisture Lost (average for 10 replications)	Multiple Comparison Test
3,762	26.3	a
25,800	39.1	b
sunlight	46.1	c
	$s_{\bar{x}} = 1.22$	

Values with the same letter are not significantly different at the 1% level (Duncan's Multiple Range Test).

Table 3. The influence of three light intensities on the average stomatal density for upper and lower leaf surfaces of Penncross creeping bentgrass expressed as number per mm^2.

Light Intensity Treatments (lux)	Stomatal Number per mm^2 (average for 10 replications)	Multiple Comparison Test
3,762	78.6	a
25,800	113.2	b
sunlight	125.8	c
	$s_{\bar{x}} = 0.31$	

Values with the same letter are not significantly different at the 1% level (Duncan's Multiple Range Test).

Previous research with dicotyledons showed that increased light intensity and duration of illumination play a role in increasing the number of stomatal initials therefore, increasing the stomatal density (28, 30). The results of this experiment showed a similar response. The increased stomatal density resulting from higher light intensities was associated with an increase in the water use rate.

Light intensity also affects other plant responses which could have influenced the water use rates observed in this study. The total leaf area and shoot growth were reduced at the lowest light intensity compared to plants grown at the two higher intensities. Bermudagrass leaves, shoots, and root numbers were reduced at lower light intensities in a previous study (7). A reduction in leaf area exposed to desiccating conditions is usually associated with a decrease in transpiration. The reduced root production observed at low light intensities could also limit the water absorbing capabilities of the grass plant.

Study 2. The Effects of Temperature on the Stomatal Density and Water Use Rate

Light intensity and water deficit are major factors determining stomatal aperature. Since the greatest portion of water lost by the plant is through the stomata these are very important factors influencing the water use rate of a plant. The temperature influence on the water use rate of plants is not as clearly defined. Little effect on stomatal aperature has been reported within the normal temperature range for cool season turfgrasses (18). Temperatures higher than 30-35 C tend to have a closing effect on the stomata (18, 33). There has been considerable difficulty in attempting to evaluate the influence of temperature because of the confounding influence on the diffusion coefficient, the water deficit of leaves, and the relative humidity. These problems were minimized by the controlled wind tunnel technique used in these investigations.

Three temperature treatments, 10, 20, and 33 C, were included in this experiment. The water use rates were 51.9, 61.9, and 61.3 percent for the respective treatments (Table 4). Plants grown at 10 C had a substantially reduced water use rate compared to the 20 and 33 C treatments. There was no difference in the water use rates between plants grown at 20 and 33 C. The effect of these same three temperatures on stomatal density are shown in Table 5. The 10 C treatment had a stomatal density of 72.3 per mm^2. The stomatal density of the 10 C treatment was significantly different from the 20 and 33 C temperature treatments. There was no difference in stomatal density at the 20 and 33 C temperatures.

Table 4. The effect of three temperature treatments on the water use rate of Penncross creeping bentgrass expressed as percent moisture lost during a 12 hour exposure period at 33 C, 40% relative humidity and 4300 lux.

Temperature Treatments (centigrade)	Percent Moisture Lost (average for 8 replications)	Multiple Comparison Test
10	51.9	a
20	61.9	b
33	61.3	b
	$s_{\bar{x}} = 2.79$	

Values with the same letter are not significantly different at the 1% level (Duncan's Multiple Range Test).

Table 5. The effect of three temperature treatments on the average stomatal density for upper and lower surfaces of Penncross creeping bentgrass leaf blades expressed as number of stomata per mm^2.

Temperature Treatment (centigrade)	Stomatal Number per mm^2 (average for 8 replications)	Multiple Comparison Test
10	72.3	a
20	99.4	b
33	103.1	b
	$s_{\bar{x}} = 3.57$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

There was no significant correlation between stomatal density and water use rate. The correlation coefficient was 0.46. However, water use rate and stomatal density both increased between 10 and 20 C.

Study 3. The Effect of Cutting Height on Water Use Rate

It is generally thought that higher cutting heights result in an increased water use rate of turfgrasses, but little is known regarding the magnitude of this relationship. The effects of 0.7, 2.5, and 12.5 cm cutting heights on the water use rate of Penncross creeping bentgrass were investigated in this study. The results of this study are shown in Table 6. The water use rate expressed as percent moisture lost, increased as the cutting height increased (Table 6).

Table 6. The effect of three heights of cut on the water use rate of Penncross creeping bentgrass expressed as percent moisture lost during a 12 hour exposure period at 33 C, 40% relative humidity and 4300 lux.

Height of Cut Treatment (cm)	Percent Moisture Lost (average for 4 replications)	Multiple Comparison Test
0.7	21.4	a
2.5	32.8	b
12.5	45.0	c
	$s_{\bar{x}} = 1.48$	

Values with the same letter are not significantly different at the 1% level (Duncan's Multiple Range Test).

Raising the cutting height from 0.7 to 2.5 cm resulted in approximately a 50% increase in the water use rate. Higher heights of cut encourage more extensive and deeper rooting (3). The leaf area exposed to desiccating conditions is greater at the higher cutting heights. This would enhance the extent of water loss by transpiration. Also, the increase in extent of the root system would allow the plant to draw moisture from a greater portion of the soil profile.

Study 4. The Effect of Three Water Application Rates on the Stomatal Density and Water Use Rate

Three application rates of water, 1.3, 2.5 and 10.0 centimeters, were applied weekly to Penncross creeping bentgrass turves. Significant differences in water use rates were recorded between those plants receiving 1.3 cm and 10.0 cm of water per week (Table 7). Plants receiving 2.5 cm had a water use rate which was not significantly different from the plants receiving either the lowest or the highest water application treatments. There was a trend toward decreasing water use rate between the plants receiving 1.3 and 10.0 cm water application rates per week.

Table 7. The effect of three water application rates on the water use rate, expressed as percent moisture lost, of Penncross creeping bentgrass during a 12 hour exposure period at 33 C, 40% relative humidity and 4300 lux.

Water Application Rate (cm/week)	Percent Moisture Lost (average for 6 replications)	Multiple Comparison Test
10.0	47.2	a
2.5	50.6	ab
1.3	55.0	b
	$s_{\bar{x}} = 1.68$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

The stomatal density was inversely related to (a) the amount of water applied weekly and (b) the water use rates (Table 8). No significant correlation (0.55) was found between the stomatal density and the water use rate. Plants receiving 1.3 cm of water per week had significantly fewer stomata per unit area than those receiving 2.5 and 10.0 cm. There was no difference between the latter two treatments. A trend of decreasing stomatal density with decreasing soil moisture was observed. Visual estimates of vegetative cover were made for each treatment and replication in this study. The vegetative cover was significantly decreased at the lowest moisture treatment (Table 9). Visual observations of depth and extent of rooting indicated an increase at the lowest water application rate.

Table 8. The effect of three water application rates on the average stomatal density for upper and lower surfaces of Penncross creeping bentgrass leaf blades expressed as number of stomata per mm^2.

Water Application Rates (cm/week)	Number of Stomata per mm^2 (average for 6 replications)	Multiple Comparison Test
10.0	120.1	a
2.5	117.6	a
1.3	102.5	b
	$s_{\bar{x}} = 4.40$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

Table 9. Visual estimates of the percent vegetative cover for Penncross creeping bentgrass turfs receiving three rates of water application.

Water Application Rates (cm/week)	Percent Vegetative Cover (average for 6 replications)	Multiple Comparison Test
10.0	95.8	a
2.5	88.3	a
1.3	51.7	b
	$s_{\bar{x}} = 3.14$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

Study 5. The Effects of Three Irrigation Frequencies on the Stomatal Density and Water Use Rate

The effects of three irrigation frequencies, (a) watered only when wilt appeared, (b) watered 3 times per week, and (c) watered 7 times per week, on the stomatal density and water use rate of Penncross creeping bentgrass were investigated. The pots were watered until the soil was saturated at each watering. Plants watered only when wilt appeared had a reduced water use rate compared to those irrigated 3 and 7 times per week (Table 10). The latter two treatments were not significantly different.

The decline in water use rate was positively correlated (0.99) with the decrease in the vegetative cover (Table 11). A 47.8% reduction in vegetative cover was noted for those turves which were watered only when wilt was apparent. This was significantly different from the 16.1 and 7.2 percent reduction recorded for the irrigation frequencies 3 and 7 times per week. There was no significant reduction in vegetative cover between the 3 and 7 times per week irrigation frequency. A reduction in the extent of rooting was also noted in the turves receiving the least frequent irrigation treatments. The more frequently irrigated plants appeared to have developed the most extensive root system. The plants receiving 3 and 7 irrigations per week had the fewest stomata (Table 12). Plants watered only when wilt appeared had a significantly greater number of stomata than the more frequently irrigated treatments. Leaves of those plants watered only at wilt were much narrower and reduced in area.

Table 10. The effect of three irrigation frequencies on the water use rate expressed as percent moisture lost during a 12 hour exposure period at 33 C, 40% relative humidity, and 4300 lux.

Irrigation Frequency Treatments (times/week)	Percent Moisture Lost (average for 9 replications)	Multiple Comparison Test
At wilt	35.1	a
3	49.3	b
7	49.7	b
	$s_{\bar{x}} = 1.14$	

Values with the same letter are not significantly different at the 1% level (Duncan's Multiple Range Test).

Table 11. Visual estimates of percent vegetative cover for Penncross creeping bentgrass turfs receiving three frequencies of irrigation.

Irrigation Frequency Treatments (times/week)	Percent Vegetative Cover (average for 9 replications)	Multiple Comparison Test
At wilt	52.2	a
3	83.9	b
7	92.8	b
	$s_{\bar{x}} = 3.48$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

Table 12. The effect of three irrigation frequencies on the average stomatal density for upper and lower surfaces of Penncross creeping bentgrass leaves expressed as number of stomata per mm^2.

Irrigation Frequency Treatments (times/week)	Stomatal Number per mm^2 (average for 9 replications)	Multiple Comparison Test
At wilt	117.6	a
3	90.6	b
7	93.7	b
	$s_{\bar{x}} = 2.68$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

There was no significant correlation between the water use rate and the stomatal density. However, the correlation between percent vegetative cover and water use rate was significant (0.98). Turves receiving irrigation only at wilt had a significant reduction in percent vegetative cover compared to those receiving 3 and 7 irrigations per week. The decline in water use rate was associated with the low shoot density of the turf maintained at this irrigation frequency. Although the water use rate was reduced, the loss of turfgrass quality at this irrigation frequency was undesirable.

Study 6. The Effect of Nitrogen Nutrition on Stomatal Density and Water Use Rate

The nitrogen treatments utilized in this experiment were (a) 0.23, (b) 0.91, and (c) 1.83 kg. per 92.9 m^2 (0.5, 2.0, and 4.0 pounds of actual nitrogen per 1000 sq. ft.) per growing month. The corresponding nitrogen-phosphorus-potassium ratios were as follows: (a) 1:1:2, (b) 4:1:2, and (c) 8:1:2. Turves grown under these nitrogen levels were exposed to a stress period of 12 hours in which the conditions were 33 C, 40% relative humidity, and 4300 lux. The percent moisture losses were 46.2, 40.2, and 34.0 for the respective treatments, indicating a reduction in water use rate with increased nitrogen levels (Table 13).

Table 13. The effect of three nitrogen nutritional levels on the water use rate expressed as percent moisture lost during a 12 hour exposure period at 33 C, 40% relative humidity and 4300 lux.

Level of Nitrogen Nutrition (klg./92.9 m^2)	Percent Moisture Lost (average for 6 replications)	Multiple Comparison Test
.228 (0.5#/1000 sq. ft.)	46.2	a
.912 (2.0#/1000 sq. ft.)	40.2	b
1.825 (4.0#/1000 sq. ft.)	34.0	c
	$s_{\bar{x}}$ = 1.18	

Values with the same letter are not significantly different at the 1% level (Duncan's Multiple Range Test).

The stomatal density decreased with increased fertilization (Table 14). Plants receiving the 1.83 treatment had a significantly lower stomatal number per mm^2 than those receiving 0.23 kg. The average width of the leaf at the midportion increased at the higher-N-treatments. The leaf widths recorded were (a) 1.58, (b) 2.96, and (c) 3.03 mm for the three nitrogen nutritional levels. The stomatal density increased as the leaf width decreased. There was a positive correlation (0.60) between the stomatal density and water use rates of the turves studied.

Water use rate has been reported to increase with an increase in the rate of nitrogen fertilization (3, 5, 8, 12, 19, 22, 32). The increase in water use rate, in such cases, has been associated with an increased growth rate and dry matter production. However, the water use becomes more efficient on the basis of the amount of water necessary to produce a pound of dry matter, when nitrogen fertilization is increased.

Table 14. The effect of three nitrogen nutritional levels on the average stomatal density for upper and lower surfaces of Penncross creeping bentgrass leaves expressed as number of stomata per mm^2.

Levels of Nitrogen Nutrition ($kg/92.9\ m^2$)	Stomatal Number per mm^2 (average for 6 replications)	Multiple Comparison Test
.228 (0.5#/1000 sq. ft.)	88.1	a
.912 (2.0#/1000 sq. ft.)	83.6	ab
1.825 (4.0#/1000 sq. ft.)	78.6	b
	$s_{\bar{x}} = 2.48$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

The clipping weight production, shoot density, and root organic matter production were observed for the three nitrogen levels in an auxiliary study. The average fresh weight of clippings did increase with increased nitrogen. The fresh weights were 1.34, 2.84, and 2.48 gm for the respective treatments (Table 15). A reduction in fresh weight of clippings was noted for the 0.23 kg treatment.

However, no difference was noted between the 0.91 and 1.83 kg nitrogen levels. Shoot densities increased with increased nitrogen nutrition (Table 16).

Table 15. Total of clipping weights recorded as grams yielded above the 5 cm cutting height for the dates of harvest of November 22, December 5, and December 19, 1970.

Treatment Numbers	Level of Nitrogen Nutrition ($kg/92.9\ m^2$)	Clipping Weights (average for 3 replications)	Multiple Comparison Test
1	0.23	1.34	a
2	0.91	2.84	b
3	1.83	2.48	b
		$s_{\bar{x}} = 0.48$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

Table 16. Shoot density counts recorded as shoots per 2.5 cm^2 for Penncross creeping bentgrass receiving three levels of nitrogen nutrition.

Treatment Numbers	Level of Nitrogen Nutrition ($kg/92.9\ m^2$)	Shoot Density (average for 4 replications)	Multiple Comparison Test
1	0.23	32.5	a
2	0.91	51.0	b
3	1.83	50.6	b
		$s_{\bar{x}} = 0.41$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

The lower nitrogen rate promoted the development of an extensive root system (Table 17), while it discouraged dry matter production and shoot density. The root organic matter production at the 0.23 kg nitrogen level was nearly 2 times greater than that at the two higher nitrogen levels. The increase in root organic matter production and stomatal density associated with this nutritional level would tend to lend support to these findings.

Table 17. Root organic matter production recorded as grams of organic matter produced for Penncross creeping bentgrass receiving three levels of nitrogen nutrition.

Treatment Numbers	Level of Nitrogen Nutrition ($kg/92.9\ m^2$)	Root Organic Matter Production in Grams (average for 4 replications)	Multiple Comparison Test
1	0.23	1.59	a
2	0.91	0.69	b
3	1.83	0.90	b
		$s_{\bar{x}} = 0.25$	

Values with the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

The Effect of Leaf Blade Surface and Position on the Stomatal Density

The stomatal density of the upper and lower leaf surfaces of Penncross creeping bentgrass were compared at each of two leaf positions (Table 18). The leaf positions compared were the second and sixth leaf from the base of the culm. Stomatal densities differed significantly between surfaces at both leaf positions. The upper surface had a greater number of stomata per mm^2 in each case. The sixth leaf had the greatest number of stomata per unit area for both surfaces when compared to the second leaf position.

Table 18. The effect of leaf blade position and surface on the stomatal density expressed as number of stomata per mm^2 Penncross creeping bentgrass.

Leaf Blade Position and Surface	Average for 18 Replications	Multiple Comparison Test *	Multiple Comparison Test †
sixth upper	123.9	a	a
sixth lower	44.7	b	
second upper	90.6	a	b
second lower	37.7	b	

*surface $s_{\bar{x}} = 1.99$
†position $s_{\bar{x}} = 2.22$

Values with the same letter are not significantly different at the 1% level (Duncan's Multiple Range Test).

The average stomatal density for the lower compared to the upper leaf position was significantly reduced, indicating a greater number of stomata on the younger leaves.

Similar studies with blue panicgrass indicated that younger leaves at the top of the culm had fewer stomata per unit area than leaves collect at the middle or base of the culm (13). It was also shown that the total number of stomata were reduced at the time of pollination as compared to the seedling stage. Studies with various cultivars of barley showed that the stomatal density decreased progressively from the flag leaf to the lower leaves (24). It would appear that the relationship between stomatal density and leaf blade position are not consistent among the grasses and may vary between species.

The stomatal density for blue panicgrass did not vary significantly in reference to position on the same leaf blade (13). Counts were determined to be similar whether taken at the base, middle, or tip of the leaf. Similar results were obtained in this study. However, all counts were taken from the middle of the leaf blade. The stomatal density may vary from 50 to 500 per mm^2 for plants in general (31). The stomatal densities from the various studies within this investigation ranged from 72 to 125 per square millimeter.

The Relative Importance of Cultural and Environmental Factors Influencing Stomatal Density and Water Use Rate

Reductions in light intensity, cutting height, and frequency of irrigation resulted in the most significant influence on the water use rate. Turves growing at 3762 lux had a 50% reduction in water use rate compared to turves grown at 25,800 lux. The light intensity of 3762 lux is near the compensation point reported for many turfgrasses (3). Turves clipped at 2.5 cm had a 53% greater water use rate than those cut at 0.7 cm. The water use rate doubled between turves cut at 0.7 cm and 12.5 cm. Turves receiving irrigation only when wilt occurred had a water use rate that was reduced 43% compared to those receiving irrigations 3 and 7 times per week.

The most significant factor influencing the stomatal density in this investigation was light intensity. Literature previously cited indicated that light intensity and duration of illumination play a role in increasing the stomatal density (28, 30). Soil moisture content, irrigation frequency, and temperature were intermediate in their influence on the stomatal densities. The nitrogen nutritional level had the least significant effect on stomatal density of the factors studied.

The relationships between stomatal density and water use rate were investigated. High light intensities increased the stomatal density which was positively correlated ($r = 0.88$) with an increase in water use rate. Infrequent irrigations resulted in an increased stomatal density. However, the water use rate was inversely related to the stomatal density and was significantly lower at the least frequent irrigation treatment. The decline in water use rate was positively correlated (r = 0.98) to a reduction in percent vegetative cover. This increase in stomatal density at the least frequent irrigation treatment probably resulted in an increased water use rate on a per plant basis; however, this increase was overshadowed by the reduction in percent vegetative cover.

The nitrogen nutritional level was intermediate in its effect on the water use rate, but had the least significant effect on the stomatal density of all the factors investigated. Nitrogen influenced other factors within the plant. The degree of hydration is increased with increased levels of nitrogen fertilization (19, 32). The increased tissue hydration results in a decreased water deficit in relation to the microenvironment surrounding the leaf blade. The changes in water content or hydrature are expressed as changes in water deficit. The water deficit is considered a very powerful stomatal regulator which may override all other stomatal opening stimuli (3, 18). In addition to increasing the degree of tissue hydration, the nitrogen nutritional level increases air space between the cells of the mesophyll. The increased air space between cells allows for more surface area from which water may evaporate and be lost as water vapor the stomata and cuticle.

The temperature and water application rate had the least effect on the water use rate. A reduction in water use rate of 20% occurred with decreased temperature. An 18% reduction occurred with increased rates of water application. The stomatal density was reduced by 30% for the temperature treatments and 20% with the water application rates.

These studies indicate that cultural factors such as cutting height, irrigation frequency, and nitrogen fertilization have the greatest influence on reducing the water use rate of turfgrasses. Manipulation of one factor within a maintenance program may not be significant. However, a combination of cultural factors could significantly reduce the water demanded by a turf. Manipulation of these factors where irrigation applications are limited in respect to water source, capacity and facilities could be very important for maintenance of desired turfgrass quality. Light intensity was the environmental factor that had the greatest influence on the water use rate. Adjustment of irrigation practices to the existing light intensity conditions, such as shade versus open sunlight and cloudy versus sunny days is important in maintaining quality turf.

CONCLUSIONS

The following conclusions can be made regarding the influence of various cultural and environmental factors on the stomatal density and water use rate of Penncross creeping bentgrass:

1. Stomatal density and water use rate increase with increasing light intensity.
2. Growing temperatures below the optimum for shoot growth cause a reduction of stomatal number and water use rate.
3. The water use rate increases as the cutting height is increased.
4. The water use rate is reduced with infrequent irrigation. However, a critical moisture balance is necessary to maintain desirable turfgrass quality.
5. Stomatal density and root organic matter production are reduced at higher nitrogen nutritional levels. Clipping yield and shoot density are increased as the level of nitrogen nutrition increases.
6. Water use rate declines with increased levels of nitrogen nutrition for a short term atmospheric, drought-stress period of 12 hours.

7. Stomatal density is influenced by leaf blade surface and position. The stomatal numbers are greater on the upper than on the lower surface of the leaf blade. Stomatal density is reduced on mature leaves when compared to younger leaves located higher on the culm.

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