# A STUDY OF ARTIFICIAL METHODS FOR RAPID DETERMINATION OF THE RELATIVE COLD TOLERANCE OF SIX TURFTYPE BERMUDAGRASSES

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# INTRODUCTION

The demand for improved turfgrasses has greatly increased in recent years. This increased need is the result, at least in part, of the modern age with more time for leisure, outdoor sports and various other activities requiring a good sod for safety and comfort. Serviceability and beauty for highways, airfields, flight strips and stabilization of waterways add to the increased need for better turfgrasses.

Many turfgrasses are now on the market whose area of adaptation is uncertain. Many years are required by present field methods to determine whether a plant is capable of surviving in a given area due to environmental fluctuation from day to day and year to year. Numerous artificial environmental studies have been conducted in an attempt to shorten the time required for determination of the winter hardiness of plants. Much work concerning winter hardiness has been conducted with horticultural and field crops but very little has been done with turfgrasses.

In an attempt to find what factor or group of factors contribute to winter hardiness, this experiment was conducted comparing six varieties of bermudagrass. The plant material was subjected to an artificial cold hardiness test with chemical analyses being made before and after the cold hardening period. Stoma size were measured for possible correlation with cold tolerance. The freezing point and viscosity of extracted cell sap from each variety were also compared.

## LITERATURE REVIEW

Factors which contribute to and methods for determination of cold resistance or winter hardiness in plants have been reported on horticultural and field crops but very little information on this subject is available on turfgrasses.

In an attempt to develop a procedure for rapid determination of the relative cold tolerance of six varieties of turfgrass, Northcutt (15) /1 reported an apparent success in hardening these plants by lowering the temperatures approximately five (5°) degrees Fahrenheit every 48 hours from a maximum of 45° to a low of 25° F. for a period of six hours. No definite trend could be detected in the rate of recovery or percent survival of the plants after being subjected to the various temperatures. He pointed out an interesting but unexplainable result which occurred in that all varieties showed a greater percentage of recovery when subjected to a low temperature of -5° F. than did the samples subjected to a low temperature of 0° or 5° Fahrenheit.

Carroll (3) in 1943, working with turfgrass, found the lethal soil temperature for the majority of the species appeared to be between -10° and -15° C. The cause of the injury appeared to be a direct thermal effect upon the protoplasm.

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/1 Figures in parenthesis refer to Literature Cited.

Harvey (7) in 1918 stated the principal effect of the hardening process for cabbages is a change in the constituents of the protoplasm which prevent their precipitation as a result of the physical changes incident upon freezing. The proteins are changed to forms which are less easily precipitated. This is indicated by an increase in the amino acid content of the cabbage plants on hardening.

Steinmetz (20) in 1926 found the quantity of press juice obtained from 100 grams of material at the respective pressures bears no apparent relation to the hardiness in alfalfa. The total solids in the sap are correlated with total dry matter in the root tissue, but apparently are not correlated with hardiness.

In 1956 Ruelke and Smith (18) stated the principal parts for the storage of available carbohydrates were the primary root in red clover and the stolons and crown in ladino and white clover. Loss of carbohydrates through the winter was greater in the roots than in the above ground parts.

Arakeri and Schmid (1) conducted a study of cold resistance of various legumes and grasses in early stages of growth in 1949. They found all the grasses except brome showed a noticeable reduction in survival from freezing at -10° C. for 8 hours when the seeds had germinated but not emerged from the soil. Conversely, the legumes showed relatively better survival than the grasses at this early stage.

When the grasses had been grown in the greenhouse for one week at which time they were in the one leaf stage, preceeding hardening and freezing, the survival was relatively good. Then again after three weeks of greenhouse growth, the survival of all grasses declined.

Reporting on the changes in carbohydrate content of wheat plants during the process of hardening for drought resistance, Vassiliev and Vassiliev (22) found on the day following the beginning of wilting, the carbohydrate content of the plants of most varieties studied increased in concentration in both forms of sugars (monosaccharides and sucrose) and a somewhat decreased concentration of hemicelluloses with further loss of water.

Tysdal (21) in 1933 studying the influence of light, temperature and soil moisture on the hardening process in alfalfa, found the length of day had a very important influence on the hardening process. Alternating temperatures during the hardening process markedly increased cold resistance. Plants subjected to $0^{\circ}$ F. temperatures for 16 hours and then placed in a warm greenhouse ($20^{\circ}$ C.) during the day for 8 hours developed much greater hardiness than those kept continuously at $0^{\circ}$ F. It is suggested that alternating temperatures or decreasing day length, or both might be used under an artificially controlled environment for hardening plants that are to be frozen by artificially produced low temperatures.

In 1916 Bouyoucos and McCool (2) used a method of determining the freezing point of cell sap by crushing the plant tissue and placing it in the freezing tube. They reported the concentration of the cell sap is greater when determined directly in the plant than in the extracted cell sap.

A method of determining hardiness in plants was reported by Dexter (5) in 1930. This experiment indicated that the degree of resistance of plants to injury by cold weather may be measured by the diffusion of

electrolytes and other substances from chilled or frozen tissues into water after such tissues have thawed. The amount of diffusion has been determined with alfalfa roots by conductivity measurements which has been supplemented by colormeteric tests for chlorides. Dexter found that within the limits of this investigation there exist correlations between known hardiness of alfalfa roots and the degree of retention of electrolytes by the tissue after freezing.

Klages (10) found more wheat seedlings survived on the high than on the low moisture soils when exposed to low temperatures.

In 1927, Hill and Salmon (8) found that winter wheat plants growing in a dry soil were injured much more severely by artificially produced low temperatures than similar plants in a wet soil. Plants dried to the point of wilting and watered just before freezing survived as well as those which had been abundantly supplied with water at all times. This is easily explained by the high specific heat of water which in a wet soil prevents a rapid change in temperature, the net result being that those plants in dry soils are exposed to a lower temperature than those in a wet soil.

A method for the determination of comparative hardiness in seedling alfalfa plants by controlled hardening and artificial freezing was reported by Pelter and Tysdal (16) in 1932. Alfalfas were seeded in cypress flats in alternate rows with a control alfalfa of known hardiness, and allowed to grow under optimum conditions in the greenhouse for one month. They were then transferred to the hardening chamber, held at a temperature of $2^{\circ}$ to $4^{\circ}$ C. for two weeks. Before the seedlings were frozen, the soil was brought to a high and uniform moisture content.

The flats with the seedlings were then exposed in the freezer room for a number of hours to a temperature at some point between -10° and -20° C. The length of exposure to low temperatures was so gauged that about 50 percent of the control alfalfa survived. After freezing, the seedlings were removed to the greenhouse and two weeks later survival counts were made. The actual percentages of survival of the alfalfas were calculated in terms of the control alfalfa, and comparisons between alfalfas were made by this standard.

Roots of nonhardened alfalfa plants, with abundant carbohydrate reserves, freeze less easily than roots of plants that have been deprived of light for some time, reported Weiner (23) in 1929.

Rose (17) in Missouri reported that the hardening process in plants is accompanied by a marked increase in water retaining power, and that this water retaining power is due chiefly to the imbibitional forces of the cell.

According to the results obtained by Scarth and Levitt (19) in 1937, the following are features of hardened as compared with unhardened cells, with the plants named. Resistance to injury by deplasmolysis is greater in cabbage. The relative volume of protoplasm and vacuole changes but little, if any at all, in the apple cortex and onion. The expressed juice of hardened cabbage plants shows the precipitation of colloids over a wider range on the pH scale, H-ion concentration slightly lower and the buffering capacity was unchanged. The artificial change in the H-ion concentration of the sap in life does not affect hardiness in cabbage.

Levitt (11) in 1956 found the freezing point of plant tissue is

also variable and must not be confused with the freezing point of the expressed juice, which is constant as long as the concentration is unaltered. The temperature remains constant at the freezing point for some time, provided the rate of cooling is not too rapid.

Celarier and Mehra (4) reported an impression method for the study of the guard cells of the stomata. This method is based on the principles of impression long used in paleontology, and with the use of some of the modern plastics gives very desirable results. Somewhat similar methods have been used by plant pathologists Long and Clements (12) in 1934 and Husain (9) in 1956 to detect the open or closed condition of the stomata.

## METHODS AND MATERIALS

Six commercially available turftype bermudagrasses were selected from the turfgrass nursery of the Oklahoma Agricultural Experiment Station located at Stillwater, Oklahoma to be used in this study. The varieties used were:

| Latin name | Common name |
|---|---|
| Cynodon transvaalensis | African bermuda |
| Cynodon dactylon | Common bermuda |
| Cynodon magennisii | Sunturf bermuda |
| Cynodon dactylon X C. transvaalensis | Tiffine bermuda |
| Cynodon dactylon X C. transvaalensis | Tifgreen bermuda |
| Cynodon dactylon | U-3 bermuda |

All varieties were subjected to freezing temperatures following pre-treatments of (1) hardened by shading; (2) hardened by cool temperatures and (3) no hardening of any type.

Thirty-two samples of each of the six varieties were dug with an $1\frac{1}{4}$ inch plug cutter. These samples were then washed free of all soil so that only the bare tissue was used in this study. Sixteen samples of each variety were separated into above and below ground parts, while the other 16 were left intact. All samples were placed in individual $3\frac{1}{4}$" X $3\frac{1}{4}$" X 1 1/8" plastic boxes after washing to facilitate handling.

Two pairs of four inch plugs of each variety were dug in addition. One plug of each pair was separated into above and below ground parts and the other was left intact. All the soil was removed from the samples by washing. The tissue from one pair of the large sized plugs consisting

of (a) above, (b) below and (c) above and below ground parts intact from each variety was placed immediately into separate laminated bags containing dry ice in an attempt to stop all enzymatic action as soon as possible. This plant material was then taken to the Department of Biochemistry for chemical analyses. The tissue from the other pair of plugs after being separated into three components as described above was placed into individual 5" X 7" X 2" plastic boxes and then subjected to a treatment of lowering temperatures from a maximum of 68° F. to a low of 40° F. for specific periods of time ranging from 10 to 14 hours to simulate assumed natural hardening conditions. The temperatures and intervals of time to which the tissue was exposed are shown in Table I. This plant material was removed from the hardening process after eight days and taken directly to the Department of Biochemistry for chemical analyses.

All samples from the large sized (4 inch) plugs were analyzed chemically for percent of soluble solids, reducing sugars, sucrose and total sugars.

In an attempt to determine the effect of short days, as compared with long days, on the plants' ability to withstand cold temperatures, an area two feet X eight feet was marked off on each varietal plot and covered with black plastic each evening at 6:30 P.M. and removed at 8:30 A.M. the next day. This was done so the plant material under the plastic would have only a 10 hour light period. This shading process was conducted for eight days prior to digging two (4 inch) plugs of each variety and separating one plug into above and below ground parts leaving the other intact. These samples were taken to the Department of Biochemistry where chemical analyses were made. Also, four ($1\frac{1}{4}$ inch)

TABLE I

THE LOCATION OF MECHANICAL EQUIPMENT USED, AND PERIOD OF TIME TISSUE OF SIX TURFTYPE BERMUDAGRASS VARIETIES WAS EXPOSED TO LOWERING NOCTIPERIOD TEMPERATURES AND CONSTANT DAYLIGHT TEMPERATURE FROM THE DATE OF DIGGING THROUGH THE TWELFTH DAY IN THE ARTIFICIAL COLD HARDENING PROCESS

| Day | Period | Temperature | Hours | Location* |
|---|---|---|---|---|
| 1 | Dark | 55° F. | 14 | WIF |
| | Light | 68° F. | 10 | SLG |
| 2 | Dark | 55° F. | 14 | WIF |
| | Light | 68° F. | 10 | SLG |
| 3 | Dark | 50° F. | 14 | WIF |
| | Light | 68° F. | 10 | SLG |
| 4 | Dark | 50° F. | 14 | WIF |
| | Light | 68° F. | 10 | SLG |
| 5 | Dark | 45° F. | 14 | WIF |
| | Light | 68° F. | 10 | SLG |
| 6 | Dark | 45° F. | 14 | WIF |
| | Light | 68° F. | 10 | SLG |
| 7 | Dark | 40° F. | 14 | WIF |
| | Light | 68° F. | 10 | SLG |
| 8 | Dark | 40° F. | 14 | WIF |
| | Light | 68° F. | 10 | SLG |
| 9 | Dark - Freezing | 30° F. | 2 | WIF |
| | Dark | 40° F. | 12 | SLF |
| | Light | 68° F. | 10 | SLG |
| 10 | Dark - Freezing | 20° F. | 2 | WIF |
| | Dark | 40° F. | 12 | SLF |
| | Light | 68° F. | 10 | SLG |

TABLE I (continued)

| Day | Period | Temperature | Hours | Location* |
|---|---|---|---|---|
| 11 | Dark - Freezing | 10° F. | 2 | WIF |
| | Dark | 40° F. | 12 | SLF |
| | Light | 68° F. | 10 | SIG |
| 12 | Dark - Freezing | 0° F. | 2 | WIF |
| | Dark | 40° F. | 12 | SLF |
| | Light | 68° F. | 10 | SIG |

*Location: WIF = Walk in Freezer
SIG = Seed Lab Germinator
SLF = Seed Lab Freezer

samples of shaded material of each variety were dug, along with four samples of unshaded material. These samples were left intact after washing, put in plastic boxes and placed immediately into the freezing chamber and maintained in darkness at 30° F. for two hours, 40° F. for 12 hours, then placed in the seed lab germinator for 10 hours of light at 68° F. as shown by the ninth day procedure of Table I. The same procedure was followed for the next three consecutive days, the only difference being the freezing temperatures were lowered as shown in Table I for the 10th, 11th and 12th day treatment.

At the end of each of the 24 hour periods following the dark freezing temperatures as shown in Table I, four samples of each of the following; above ground, below ground, all intact, shaded and nonshaded, were taken out of the seed lab germinator. These boxes were placed in the seed lab greenhouse where two tablespoonsful of moistened vermiculite were added to each sample. All samples were watered as needed. Every seven days for the next three weeks there were survival counts made on each of the samples based on new vegetative growth being evident.

A comparison of guard cell size of each of six varieties of bermudagrasses was made, assuming there would be a relative relationship to stomata size. The procedure as described by Celarier and Mehra (4) was used, in which fresh blade samples of each variety were pressed lightly so the leaves would remain flat in order to make stoma and guard cell comparisons. The surface of the blade was then painted with a cellulose nitrate in acetone solution, allowed to dry, the plastic impression was then peeled off and placed on a slide for detailed study.

Of each variety, ten guard cells were measured on both the upper

and lower surfaces of the blades. By the use of an oil emersion lens, 10 X 97 power, five counts were made of the number of guard cells which were in the oil emersion field of view.

To determine the freezing point of the cell sap from each of the six varieties studied, one square foot of sod of each was dug and the sap extracted by the use of a hydraulic Carver press employing pressure up to 16,000 pounds per square inch. The sap was filtered to remove all vegetative debris.

A Beckmann thermometer was used in the determination of the freezing point of the cell sap as described by Loomis and Shull (13).

An Ostwald viscosimeter was employed to determine the viscosity of the cell sap of each variety as directed in the procedure described by Morrow (14).

## RESULTS AND DISCUSSION

It would appear from the results obtained in this study that shortening the day length for eight consecutive days was of little or no benefit as a hardening process. By comparing these shaded samples with other samples of the same varieties which had no previous hardening and were exposed to the same low temperatures as shown in Table I, there was very little difference in the amount of survival.

The failure to obtain 100 percent survival from those plants which were shaded and then subjected to a temperature of 30° F. for two hours is believed to be the result of extreme heat and scalding of the tissue when placed in the greenhouse to break dormancy and initiate new growth.

The abrupt drop in percent survival from 20° F. to 10° F. and the 100 percent increase in survival of most varieties following exposure to 0° F. temperature as shown in Tables II and III is of interest but unexplainable. Perhaps there is a conversion or blocking of the carbohydrate fraction plus other unknown physiochemical variations which are more injurious to the plant at this point, when it attempts to make new growth, than when the plant tissue is subjected to lower temperatures but with apparently adequate, available carbohydrates for this purpose. Maybe a chemical reaction is progressing at a slow rate from around 20° F. to 10° F. and only partially complete whereas with a small extension of time, even with a further drop in temperature to 0° F., the reaction is completed permitting production of new growth in an orderly manner.

TABLE II

PERCENT SURVIVAL AFTER EXPOSURE TO FREEZING TEMPERATURES OF SIX TURFTYPE BERMUDAGRASS VARIETIES WHICH WERE HARDENED BY SHADING

| Varieties | % Survival | Varieties | % Survival |
|---|---|---|---|
| 30° Exposure | | 20° Exposure | |
| 1. U-3 | 100 | 1. U-3 | 100 |
| 2. Tifgreen | 100 | 2. Tifgreen | 100 |
| 3. Common | 100 | 3. Common | 100 |
| 4. Tiffine | 0 | 4. Tiffine | 100 |
| 5. Sunturf | 0 | 5. Sunturf | 100 |
| 6. African | 0 | 6. African | 100 |
| 10° Exposure | | 0° Exposure | |
| 1. Common | 25 | 1. U-3 | 100 |
| 2. U-3 | 0 | 2. Tifgreen | 100 |
| 3. Tifgreen | 0 | 3. Tiffine | 100 |
| 4. Tiffine | 0 | 4. Common | 100 |
| 5. Sunturf | 0 | 5. African | 50 |
| 6. African | 0 | 6. Sunturf | 50 |

TABLE III

PERCENT SURVIVAL AFTER EXPOSURE TO FREEZING TEMPERATURES OF SIX TURFTYPE BERMUDAGRASS VARIETIES WHICH HAD NO PREVIOUS HARDENING

| Varieties | % Survival | Varieties | % Survival |
|---|---|---|---|
| 30° Exposure | | 20° Exposure | |
| 1. U-3 | 100 | 1. U-3 | 100 |
| 2. Tifgreen | 100 | 2. Tifgreen | 100 |
| 3. Tiffine | 100 | 3. Tiffine | 100 |
| 4. Common | 100 | 4. Common | 100 |
| 5. Sunturf | 100 | 5. Sunturf | 100 |
| 6. African | 100 | 6. African | 100 |
| 10° Exposure | | 0° Exposure | |
| 1. U-3 | 50 | 1. Tifgreen | 100 |
| 2. Tiffine | 50 | 2. Tiffine | 100 |
| 3. Tifgreen | 25 | 3. Common | 50 |
| 4. Common | 25 | 4. Sunturf | 50 |
| 5. African | 25 | 5. U-3 | 25 |
| 6. Sunturf | 0 | 6. African | 25 |

The percent survival of all varieties which were hardened by shading and those which had no previous hardening is shown in Tables II and III respectively.

The results obtained from the survival of samples which were cold hardened prior to freezing showed a 100 percent recovery of all varieties at 30° F. with a gradual decline in survival with each increment of lower temperatures.

This would indicate that U-3, Tifgreen and Tiffine become more winter hardy by an artificial cold hardening process than Common, African and Sunturf. Why Sunturf was killed at 20° F. and had 75 per cent recovery at the 10° F. exposure as shown in Table IV is not known.

By comparing the results obtained from the survival of the above and below ground parts, which were separated and cold hardened prior to freezing, we find the above ground parts of all varieties survived to a greater extent than did the respective below ground parts.

This indicates perhaps that there is more reserve plant food in the above ground parts which permits new growth to occur than is contained in those parts below ground.

The above ground parts of U-3 bermuda when compared with the other varieties showed the highest percent survival. In addition, the below ground parts of U-3 also recovered from three of the four freezing temperatures as shown in Table V. None of the other varieties were as consistent in this respect.

When comparing all of the samples used in the artificial cold hardening process, there seems to be a rather definite trend as to which grass is the most winter hardy. The results indicate that U-3

TABLE IV

PERCENT SURVIVAL AFTER EXPOSURE TO FREEZING TEMPERATURES OF SIX TURFTYPE BERMUDAGRASS VARIETIES WHICH WERE HARDENED BY AN ARTIFICIAL COLD PROCESS

| Varieties | % Survival | Varieties | % Survival |
|---|---|---|---|
| 30° Exposure | | 20° Exposure | |
| 1. U-3 | 100 | 1. U-3 | 100 |
| 2. Tifgreen | 100 | 2. Tifgreen | 100 |
| 3. Tiffine | 100 | 3. Tiffine | 100 |
| 4. Common | 100 | 4. Common | 25 |
| 5. African | 100 | 5. African | 25 |
| 6. Sunturf | 100 | 6. Sunturf | 0 |
| 10° Exposure | | 0° Exposure | |
| 1. U-3 | 100 | 1. Common | 25 |
| 2. Tifgreen | 100 | 2. U-3 | 0 |
| 3. Tiffine | 100 | 3. Tifgreen | 0 |
| 4. Sunturf | 75 | 4. Tiffine | 0 |
| 5. Common | 75 | 5. Sunturf | 0 |
| 6. African | 50 | 6. African | 0 |

TABLE V

PERCENT SURVIVAL AFTER EXPOSURE TO FREEZING TEMPERATURES OF SIX TURFTYPE BERMUDAGRASS VARIETIES WHICH WERE SEPARATED INTO ABOVE AND BELOW GROUND PARTS AND HARDENED BY ARTIFICIAL COLD TEMPERATURES

| Below Ground Parts | | | Above Ground Parts | |
|---|---|---|---|---|
| | Varieties | % Survival | Varieties | % Survival |
| | 30° Exposure | | 30° Exposure | |
| 1. | U-3 | 25 | U-3 | 100 |
| 2. | Sunturf | 25 | Tifgreen | 75 |
| 3. | African | 25 | Common | 25 |
| 4. | Tifgreen | 0 | African | 25 |
| 5. | Tiffine | 0 | Sunturf | 0 |
| 6. | Common | 0 | Tiffine | 0 |
| | 20° Exposure | | 20° Exposure | |
| 1. | U-3 | 25 | U-3 | 100 |
| 2. | Tifgreen | 0 | Tifgreen | 100 |
| 3. | Tiffine | 0 | Tiffine | 50 |
| 4. | Sunturf | 0 | Common | 50 |
| 5. | African | 0 | Sunturf | 0 |
| 6. | Common | 0 | African | 0 |
| | 10° Exposure | | 10° Exposure | |
| 1. | Sunturf | 75 | U-3 | 100 |
| 2. | U-3 | 25 | Tifgreen | 100 |
| 3. | Tiffine | 25 | Tiffine | 100 |
| 4. | African | 25 | Sunturf | 100 |
| 5. | Tifgreen | 0 | Common | 50 |
| 6. | Common | 0 | African | 25 |
| | 0° Exposure | | 0° Exposure | |
| 1. | Tifgreen | 0 | Tifgreen | 25 |
| 2. | U-3 | 0 | U-3 | 0 |
| 3. | Tiffine | 0 | Tiffine | 0 |
| 4. | Sunturf | 0 | Sunturf | 0 |
| 5. | Common | 0 | Common | 0 |
| 6. | African | 0 | African | 0 |

is the most winter hardy followed by Tifgreen, Tiffine, Common, Sunturf and African respectively.

The percent survival of all samples of the six turftype bermudagrass varieties after exposure to all freezing temperatures are shown in Table VI.

The chemical analyses which consist of the percent soluble solids, reducing sugars, sucrose and total sugars, were made on the following samples: above and below ground parts, all (not separated), the non-hardened and shaded material.

It was found from the chemical analyses that the nonhardened material usually had the highest percent of soluble solids followed by the hardened, then shaded material as shown in Table VII.

When comparing the guard cells of each variety, they were found to be smaller on the upper surface of the leaf than on the lower surface, from two to five units. The number of stoma observed in an oil emersion field of view was fewer on the upper than the lower surface of the leaf.

Sunturf had the smallest guard cells followed by Tifgreen, U-3, Tiffine, Common and African, as shown in Table VIII.

When comparing the viscosity and freezing point of extracted cell sap, the most viscous cell sap was the hardest to freeze with the exception of that from Common and U-3 as shown in Table IX.

TABLE VI

PERCENT SURVIVAL OF ALL SAMPLES OF SIX TURFTYPE BERMUDAGRASS VARIETIES AFTER EXPOSURE TO ALL FREEZING TEMPERATURES

| Varieties | % of Total Samples Which Survived At All Temperatures |
|---|---|
| 1. U-3 | 62.50 |
| 2. Tifgreen | 61.25 |
| 3. Tiffine | 51.25 |
| 4. Common | 47.50 |
| 5. Sunturf | 40.00 |
| 6. African | 33.75 |

TABLE VII

CHEMICAL ANALYSIS OF SIX TURFTYPE BERMUDAGRASS VARIETIES SHOWING PERCENT OF SOLUBLE SOLIDS, REDUCING SUGARS, SUCROSE AND TOTAL SUGARS FROM ALL TREATMENTS

| Variety | | | Percent | | | |
|---|---|---|---|---|---|---|
| | | | Sol. Solids | Red. Sugars | Sucrose | Total Sugars |
| U-3 | NH [1] | AB | 3.68 | .15 | 1.20 | 1.35 |
| | SH | AB | 3.08 | .11 | 1.00 | 1.11 |
| | CH | AB | 3.76 | .21 | .78 | .99 |
| U-3 | NH | BG | 4.54 | .20 | 1.95 | 2.15 |
| | SH | BG | 3.02 | .15 | 1.05 | 1.20 |
| | CH | BG | 4.21 | .13 | 3.95* | 4.08* |
| U-3 | NH | All | 3.49 | .18 | 1.25 | 1.43 |
| | SH | All | 3.31 | .13 | 1.10 | 1.23 |
| | CH | All | 3.60 | .14 | .81 | .95 |
| Sunturf | NH | AB | 3.39 | .17 | .83 | 1.00 |
| | SH | AB | 3.30 | .12 | .86 | .98 |
| | CH | AB | 2.68 | .15 | .43 | .58 |
| Sunturf | NH | BG | 4.43 | .12 | .65 | .77 |
| | SH | BG | 2.71 | .10 | .81 | .91 |
| | CH | BG | 3.08 | .15 | .69 | .84 |
| Sunturf | NH | All | ---- | --- | --- | --- |
| | SH | All | 3.24 | .13 | 1.02 | 1.15 |
| | CH | All | 1.72 | .12 | .30 | .42 |
| Tiffine | NH | AB | 3.26 | .14 | .74 | .88 |
| | SH | AB | 2.72 | .17 | .57 | .74 |
| | CH | AB | 2.45 | .09 | .24 | .33 |
| Tiffine | NH | BG | 3.47 | .18 | 1.00 | 1.18 |
| | SH | BG | 3.09 | .18 | 1.17 | 1.35 |
| | CH | BG | 3.45 | .12 | .70 | .82 |
| Tiffine | NH | All | 3.07 | .17 | .46 | .63 |
| | SH | All | 2.28 | .15 | .93 | 1.08 |
| | CH | All | 2.95 | .11 | .50 | .61 |

TABLE VII (continued)

| Variety | | | Percent | | | |
|---|---|---|---|---|---|---|
| | | | Sol. Solids | Red. Sugars | Sucrose | Total Sugars |
| Tifgreen | NH | AB | 3.24 | .15 | .78 | .93 |
| | SH | AB | 2.92 | .12 | .51 | .63 |
| | CH | AB | 2.96 | .15 | .72 | .87 |
| Tifgreen | NH | BG | 3.56 | .12 | 1.26 | 1.38 |
| | SH | BG | 3.20 | .16 | 1.40 | 1.56 |
| | CH | BG | 2.72 | .14 | 1.05 | 1.19 |
| Tifgreen | NH | All | 3.59 | .14 | 1.01 | 1.15 |
| | SH | All | 3.00 | .11 | .85 | .96 |
| | CH | All | 2.63 | .09 | .71 | .80 |
| African | NH | AB | 2.87 | .19 | .49 | .68 |
| | SH | AB | 2.12 | .09 | .47 | .56 |
| | CH | AB | 2.35 | .09 | .55 | .64 |
| African | NH | BG | 3.55 | .24 | .98 | 1.22 |
| | SH | BG | 3.79 | .14 | 1.57 | 1.71 |
| | CH | BG | 3.07 | .13 | .93 | 1.06 |
| African | NH | All | 3.27 | .21 | .54 | .75 |
| | SH | All | 2.50 | .09 | .75 | .84 |
| | CH | All | 2.51 | .12 | .72 | .84 |
| Common | NH | AB | 3.15 | .15 | .71 | .86 |
| | SH | AB | 3.35 | .11 | .66 | .77 |
| | CH | AB | 3.38 | .18 | 1.18 | 1.36 |
| Common | NH | BG | 3.66 | .14 | .76 | .90 |
| | SH | BG | 2.46 | .13 | 1.02 | 1.15 |
| | CH | BG | 3.18 | .10 | 1.06 | 1.16 |
| Common | NH | All | 3.72 | .15 | 1.02 | 1.17 |
| | SH | All | 3.17 | .13 | .66 | .79 |
| | CH | All | 2.95 | .14 | .94 | 1.08 |

/1 NH = Not Hardened
SH = Shaded Treatment
CH = Cold Hardened
AB = Above Ground Parts
BG = Below Ground Parts
All = Sample Not Separated

*Obvious error

TABLE VIII

AVERAGE GUARD CELL SIZE OF SIX TURFTYPE BERMUDAGRASS VARIETIES AND THE NUMBER OF STOMA OBSERVED IN AN OIL EMERSION FIELD

| Variety | Average Length of Guard Cells Upper Surface of Leaf | Average Length of Guard Cells Lower Surface of Leaf | No. of Stomata Observed per 10 X 97 Oil Emersion Field | |
|---|---|---|---|---|
| | | | Upper | Lower |
| 1. Sunturf | 20 units | 23 units | 14 | 16 |
| 2. Tifgreen | 22 units | 25 units | 5 | 7 |
| 3. U-3 | 22 units | 25 units | 9 | 11 |
| 4. Tiffine | 35 units | 37 units | 8 | 9 |
| 5. Common | 35 units | 40 units | 5 | 7 |
| 6. African | 37 units | 40 units | 5 | 6 |

TABLE IX

THE FREEZING POINT AND COMPARATIVE VISCOSITY OF EXTRACTED CELL SAP OF SIX TURFTYPE BERMUDAGRASS VARIETIES

| Variety | Freezing Point | Viscosity |
|---|---|---|
| 1. Common | - .072° C. | 1.0547 |
| 2. Tiffine | - .0145° C. | 1.0709 |
| 3. Tifgreen | - .012° C. | 1.0668 |
| 4. U-3 | - .0095° C. | 1.0162 |
| 5. Sunturf | - .008° C. | 1.0466 |
| 6. African | - .005° C. | 1.0445 |
| 7. Distilled water | - .0° C. | 1.0000 |

## SUMMARY AND CONCLUSIONS

An experiment was conducted at the Oklahoma Agricultural Experiment Station in 1957 and 1958 with six different varieties of turftype bermudagrass in an attempt to develop a procedure for rapid determination of the relative cold tolerance of these turfgrasses.

The bermudagrass varieties used in this study were: African, Common, Tiffine, Tifgreen, Sunturf and U-3. Tissue of each was subjected to a form of hardening by artificial cold temperatures and shortened day length by shading. These samples were then compared on the basis of survival following exposure to cold temperatures with tissue which had not been hardened. To further examine the species and determine what tissue imported a greater relative cold tolerance to the variety, samples of each were separated into above and below ground parts and subjected to the same treatments as cited above. Chemical determinations were made on the percent of soluble solids, reducing sugars, sucrose and total sugars in each of the varieties prior to and following prefreezing treatments.

As a possible rapid means of determining relative cold tolerance between varieties, the comparative viscosity and relative freezing point of extracted cell sap was determined for each. In addition, the size of the guard cells was measured and the number of stoma counted on the upper and lower surfaces of the blade.

The results of this study indicate that more plant survival was

obtained from the nonhardened material than was obtained by shading or artificially cold hardened. However, within the six varieties tested, based on the survival from all samples exposed to all of the temperatures, the most winter hardy varieties appear to be U-3 followed by Tifgreen, Tiffine, Common, Sunturf and African respectively.

There was no apparent relationship between the constituents of the plant tissue and the cold tolerance of these turfgrasses. It was noted, however, that the nonhardened material usually had the highest percentage of soluble solids followed by the hardened then shaded material, respectively.

When the size of the guard cells was compared to the apparent cold tolerance of the six turfgrass varieties, there appeared to be a rather good relationship between small sized guard cells and plant survival after exposure to freezing temperatures with the exception of Sunturf.

When the comparative viscosity and the freezing point of extracted cell sap were compared, the anticipated results were obtained in that the more viscous cell sap was the hardest to freeze with the exception of that from Common and U-3 bermuda.

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