THESIS

COLD HARDINESS STUDIES OF BERMUDAGRASS AND *PASPALUM VAGINATUM* SW.

Submitted by

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ABSTRACT OF THESIS

COLD HARDINESS STUDIES OF BERMUDAGRASS
AND PASPALUM VAGINATUM SW.

The relative cold hardiness of 8 bermudagrass Cynodon sp. and 2 Paspalum vaginatum Sw. cultivars was evaluated in growth chamber by dry weight of regrowth obtained from these turfgrasses.

Samples of the turfgrasses which were cold stressed in the field were taken into the greenhouse at regular intervals from fall through winter to spring. The degree of injury of samples subjected to low temperature in the laboratory was estimated via conductivity measurements.

The field study related closely to laboratory analyses. There were significant differences between cultivars with respect to cold hardiness. The bermudagrasses appear to be more cold hardy than P. vaginatum cultivars. Of the turfgrasses studied, Brookings appears to be the most cold hardy followed by NEJC, Tifgreen, Pee Dee 102, Tifdwarf, LaJunta, Santa Ana, Tifway, Futurf and Adalayd in decreasing order of cold hardiness.

All the cultivars tested were chilling sensitive, but Santa Ana and Tifway were less so than the other grasses tested. The results of the field and chilling injury tests suggest that Brookings is

photoperiod sensitive and that it breaks dormancy later than the other cultivars tested.

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INTRODUCTION

Bermudagrass is the most widely used turfgrass in the southern parts of the United States. It is recognized to have excellent wear tolerance and recuperative potential. Use of many cultivars of this turfgrass is limited in the northern extremes of southern U. S. because of high susceptibility to winter injury.

In 1969 Ahring and Irving reported wide varietal differences in the susceptibility of bermudagrasses to cold. In 1978 Portz used a cold chamber technique to screen seventeen cultivars of bermudagrass for cold hardiness.

The large numbers of bermudagrass cultivars and the variability in their cold hardiness makes it imperative that the levels of cold hardiness of existing cultivars be determined. The possibility of improving the winter hardiness of bermudagrass cultivars is promising and this facet of turfgrass science should be further explored.

The purpose of this study is to provide quantitative and qualitative data on the variability and timing of cold hardiness. It was also done to determine the low temperature killing points of cultivars of bermudagrass. The results could serve as important tools in the breeding and selection of winter-hardy types of bermudagrass.

Paspalum vaginatum Sw. is known to perform well in the subtropical areas of Florida and Georgia (Wilson and Latham Jr., 1969).

Work on turf-type cultivars of this grass is scanty. It appears to have a good potential as a turfgrass and its adaptations need to be better understood. Therefore, its potential as a turfgrass for the northern areas of southern United States is of interest and its degree of winter hardiness is of general interest to those in the turfgrass industry.

LITERATURE REVIEW

FREEZING INJURY

If the external temperature is below the freezing point of water, the stress which occurs in plants due to cold is known as freezing stress. Levitt (1972) defined freezing stress as the freezing potential of low temperature stress. Injury from this type of stress may result in extracellular or intracellular freezing.

Extracellular freezing is a process accompanied by redistribution of water. During this process, water is removed from the cells to the extracellular spaces where it freezes. This dehydrates the cells. In addition to this dessication stress, the cells contract and this contraction may cause mechanical damage to the protoplast. As ice crystals are formed, desiccation becomes more pronounced and the protoplasm shrinks; thus tearing and disruption occurs in tender tissues. In more resistant tender tissues, injury is more progressive (Parker, 1963). During equilibrium freezing, i. e. during very slow freezing, the bulk water tends to freeze first and water more closely associated with tissue components freeze last, if at all. In hardy plants, injury does not occur until all the protoplast are severely contracted from frost dehydration and this occurs only at relatively low temperatures (Olien, 1967). Injury from extracellular freezing is not due to desiccation and the accompanying mechanical stress alone. Sukumaran and Weiser (1972)

observed that the extent of injury caused by even very slow freezing on frost-resistant and frost-susceptible leaves of potato was greater than that caused by equivalent desiccation energy. This is particularly true for frost-susceptible leaves. These workers suggested that other factors apart from simple desiccation may be involved in injury due to frost. Also, Olien (1971) working on the leaf tissue of barley observed that maximum injury induced by severe desiccation at sub-freezing temperature was only about two-thirds of that induced by severe freezing tests. He concluded that either the presence of ice in the tissue or the lower temperature of the tissue containing ice contributes to the ease with which the cells are injured and this adds to the desiccation stress. It has been suggested (Heber and Santarius, 1964) that cell death due to freezing is a result of injury to membrane-bound protein and this occurs during the withdrawal of water. The death of the cells may be in the form of loss of protein solubility, protein dissociation into sub-units or altering of protein structure. Lipid phase separation may occur, consequently, lipoprotein membranes are subject to damage at low temperatures.

Intracellular freezing is a non-equilibrium process which involves a sudden drop in the content of the liquid between the protoplasts. The amount of water frozen is not a function of temperature. This process occurs when water accumulates in excess of that closely associated with the cell wall and protoplast. Intracellular freezing is

mainly due to fast temperature change and rapid heat removal. Thousands of tiny ice crystals are formed within the protoplast. A breakdown of cellular compartmentalization results from a mechanical disruption of the protoplasmic structure. Enzymes are thus released into the already injured tissue and this usually causes death of the tissues involved (Olien, 1967). According to Levitt (1972) there are four moments of injury. These are: (1) during freezing, (2) while frozen, (3) during thawing, and (4) after thawing.

FREEZING RESISTANCE

Freezing resistance in plants may be due to freezing avoidance or freezing tolerance.

Freezing avoidance is not a general characteristic of freezing-resistant plants. There are, however, a few exceptions. Turfgrasses under a snow cover are able to avoid the widely fluctuating temperatures which occur above ground. Under a snow cover of 2 inches (5 cm), soil temperature may be as high as 15 ^{o}C above that of air temperature (Carroll, 1943). In this case, the plants themselves do not possess any true survival mechanism with respect to their own environment. Also, as a survival mechanism, some plant tissues "deep supercool" to temperatures as low as -40 ^{o}C in mid-winter. This is not a general phenomenon and it has not been observed in herbaceous species. Even in deciduous forest species, where it occurs it is usually in some but not all of their tissues (Burke et al., 1976).

High cell sap concentration can also prevent ice formation by lowering the temperature at which freezing may occur (Levitt, 1972).

Plants that survive by freezing tolerance are able to withstand extreme dehydration of their cells as the water crystallizes extracellularly. In other words, freezing tolerance is tolerance of extracellular freezing (Levitt, 1972).

COLD HARDENING

Environmental Factors. Certain environmental factors must be present for a plant to reach its genetic potential for cold hardiness.

One such factor is a low average daily air and soil temperature in the range of (34°F) 1.1°C and (40°F) 4.4°C. A noticeable change in response to freezing tests occur after a few days of pre-conditioning. A maximum change may require about 3 weeks (Olien, 1967).

A specific short photoperiod to which a particular variety or cultivar is adapted, is also necessary for reaching maximum cold hardiness. This is evidenced by the work of Hodgoson (1964) on alfalfa. He stated that the response to seasonal change in photoperiod may be the reliable built-in device in plants which foretells them of impending low temperature. The fact that short days induce resistance to low temperature was also mentioned by Ahring and Irving (1969). They stated that the assimilation or accumulation of substances derived from photosynthesis under short days may be a prerequisite for further hardening of bermudagrass under field conditions. Johnston and

Dickens (1974) observed that a combination of low night temperature ($4^{o}C$) and short photoperiod (8 hr light) resulted in maximum hardiness of centipedegrass.

The hardening process involves metabolic activity and the plant must have an energy reserve and access to light. This suggests that light intensity is also a critical factor in the metabolic activities which go on during the development of cold hardiness. Plants grown in carbon dioxide-free air are not capable of achieving normal low temperature hardiness (Beard, 1973).

Cultural Factors. The normal hardening process involves metabolic activities, hence a certain minimum level of nitrogen is required. Above this level, increased nitrogenous fertilizer application decreases the frost hardiness of turfgrasses. Nitrogen stimulates growth and in such actively growing plants the total water content of the protoplasm is increased. This results in reduced low temperature hardiness. The carbohydrate content of actively growing plants is also depleted and the possible role of sugars in frost hardiness and that of starch as an energy source is lost. Carroll and Welton (1939) observed that heavy applications or a late application of nitrogenous fertilizer during the hardening period reduces the resistance of Kentucky bluegrass to low temperature. Hendrix and Gilbert (1976) made similar observations on bermudagrass. Cordukes et al. (1966) mistakenly fertilized Kentucky bluegrass, ryegrass and bentgrass just before hardening. When

these turfgrasses were subjected to cold treatment the release of electrolytes via conductivity analyses was very high. This is indicative of high levels of injury. Baker and David (1963) also mentioned that winter kill to grasses is greater on swards that carry a large amount of leaf through the autumn. However, applications of nitrogenous fertilizers made after the grass has hardened can be beneficial. Such grasses overwinter well and resume growth earlier in spring (Smithberg and White, 1975). Also, Dickens et al. (1978) noted that nitrogen fertilizer applications made to centipedegrass enhanced spring recovery in the field.

Gilbert and Davis (1971) contended that a maximum level of hardiness is not developed in bermudagrass under a low nitrogen fertilizer regime alone. A balanced ratio of N-P-K is required. High rates of nitrogen fertilizer reduced winter hardiness of common bermudagrass, but this reduction was less when coupled with high levels of potassium (Hendrix and Gilbert, 1976). The data of work done by Beard and Rieke (1966) on Kentucky bluegrass emphasizes the fact that maximum winter survival depends not just on high K or low N levels, but rather on a balanced nutrient relationship.

The height of cut is an important factor in the winter survival of turfgrasses. This was shown by Beard and Rieke (1966) on Kentucky bluegrass. Kentucky bluegrass was severely injured by low temperature when the height of cut was less than $1\frac{1}{2}$ inches (3.75 cm).

Decreased survival of centipedegrass also occurred when the height of cut was less than 3.7 cm (Dickens and Johnston, 1977). Beard (1973) stated that the reduced leaf area under shorter cutting heights resulted in reduced synthesis and accumulation of carbohydrate. The role of carbohydrate in cold hardiness is therefore lost to turfgrasses under shorter cutting heights. The reduced quantity of plant materials is also less effective in insulating the turfgrass against extremely low temperatures.

While an adequate soil moisture level is needed to reduce injury due to winter soil drought, excessive irrigation rates is detrimental. Growth is stimulated thereby reducing carbohydrate accumulation and also increasing the hydration level of the turfgrass. This increases the susceptibility of the turfgrass to frost kill. Metcalf et al. (1970) observed that a small change in the hydration level of the crown of 3 wheat and 3 barley cultivars resulted in large differences in cold survival. They observed that much damage occurs during the cold weather following a mid-winter thaw when the crown tissues have a high moisture content.

Inadequate drainage facilities also result in accumulation of water in the crown tissue following a mid-winter thaw. This also results in an increased hydration level of the turfgrass and increased susceptibility to winter kill. Surface drainage is especially important in winter as the soil becomes frozen and therefore impervious to water.

The data obtained by Ruelke (1961) showed that growth regulators can affect the frost hardiness of perennial grasses. Low temperature injury to bermudagrass is effectively reduced by low application rates of maleic hydrazide. This is especially true when there are prolonged warm periods followed by repeated frosts.

Dunn and Nelson (1978) observed a delay in the spring regrowth of bermudagrass under a deep thatch. They did not, however, find a significant relationship between thatch depth and winter injury. Beard (1973) contended that accumulation of thatch increases the susceptibility of turfgrass to low temperature kill. Under this condition, the meristematic tissue is above the soil and it becomes exposed to air temperature which fluctuates far more than that of the soil. The grass may also be killed by snow mold in the thatch.

The low temperature survival of young turfgrass seedlings is low until the four-leaf stage is reached. The work done by Hendrix and Gilbert (1976) on bermudagrass indicated that plantings made early in autumn can be as much as 20% more cold tolerant than later plantings. In winter oats, winter hardiness increased with the age of the plant (Pfeifer and Kline, 1960).

HARDENING PERIOD CHANGES

Physiological - There is a profound alteration in plant metabolism during the hardening period; however, there have been conflicting

reports as to whether these changes are correlated with the degree of low temperature hardiness in plants.

Davis and Gilbert (1970) noticed several changes in the soluble protein fraction in the overwintering parts of Tifdwarf and Tifgreen (T-328). The changes included a decrease in the density and a wider separation of two protein bands which were closer together in the unhardened tissue. Four new, compact, well-defined bands were also present. The soluble protein content of the roots of both hardy and non-hardy alfalfa increased during the hardening period (Gerloff et al., 1967). This may not, however, reflect the hardening capacities of the varieties as there were no varietal differences in the soluble protein content. Several other workers (Jung and Smith, 1961; Dunn and Nelson, 1974; and Zech and Pauli, 1960) have observed increases in the soluble nitrogen content of plants during the fall hardening period. There were differences in the degree of correlation between this fraction and the level of cold hardiness obtained in the different plants they worked with. Dunn and Nelson (1974) went further by stating that at maximum level of cold hardiness the least hardy cultivar will have the maximum level of total nitrogen and α-amine nitrogen. It was not declared, however, whether this fraction is directly involved in the hardening process. It is apparent from these results that specific protein changes do occur during the hardening period but no cause and effect relationship was proved.

In general, total carbohydrate content increases during fall, reaches its highest level in late fall and early winter, and decreases in spring. Increases have been observed in the levels of sucrose and reducing sugar during the fall hardening process. This is accompanied by a reduction in starch level. The starch level increases in late winter when cold acclimation occurs (Dunn and Nelson, 1974; Jung and Smith, 1961; Zech and Pauli, 1960; and Carroll and Welton, 1939). These changes in sugar content occur in woody and herbaceous perennials as well as in winter annuals. It led earlier workers to try and induce cold hardiness by feeding plants with sugar. The differences in cold hardiness obtained were almost always small. Efforts were even made to rank plant species or cultivars in relative cold hardiness levels on the basis of relative sugar content. This has been successful in only isolated cases (Levitt, 1972). Roger et al., (1975), however, noticed a relatively low level of sugar accompanied by a high starch content during the overwintering period in Meyer Zoysia. Carroll and Welton (1939) stated that the possible role of increased sugar content in the cold hardiness of plants is that it increases the osmotic pressure of the cell sap. This added pressure, they contended, increases the resistance of the cell to the withdrawal of water during freezing. They also gave as a more probable role, the protective action of sugar against frost precipitation of protein. Levitt (1957) stated that sugar is a secondary factor in frost hardiness. The amount

of dehydration that will injure the plant is determined by a primary hardiness factor; therefore, sugars cannot induce in plants tolerance to freeze induced dehydration. Rather, by accumulating sugars in the vacuole, the sugars decrease the amount of ice formed thereby lowering the temperature at which frost killing dehydration will occur. The extent of the effect of sugars, however, depends on, and is proportional to, the frost dehydration that the plant can withstand without injury. For a tender plant, the sugar concentration has a negligible effect on the frost killing point while for a hardy plant, the frost killing point is appreciably lowered.

It has been shown that cell sap concentration increases as freezing tolerance increases in plants. This has been found during cold hardening, when species or varieties differing in cold hardiness are compared and when solutes are fed artificially to potentially cold hardy plants (Levitt, 1972). Although cell sap concentration may be a factor in frost hardiness, there are other obviously influential factors, since a direct relationship has not been established between cell sap concentration and cold hardiness levels (Dexter, 1935).

It has been observed by several workers (Kuiper, 1970; de la Roche et al., 1972) that the degree of unsaturation of fatty acids increases during cold hardening. Kuiper (1970) observed that phosphatidyl ethanolamine is predominantly esterified with polyunsaturated fatty acid. He also noted that the percentage of this lipid, phosphatidyl

ethanolamine in the leaves of a cold hardy variety of alfalfa increases as the treatment temperature is decreased from 20°C to 15°C. This may, however, indicate a relationship to chilling rather than to freezing tolerance since 15°C is too high a temperature to be used in testing for frost hardiness. Winter wheat seedlings grown at 2°C synthesized larger amounts of phospholipids than those grown at 24°C (de la Roche, et al., 1972). The significance of these observations is that, during cold acclimation, lipids become less saturated and, therefore, the membranes will be more permeable to water. This reduces the likelihood of intracellular freezing. The membranes are also less likely to be irreversibly damaged by freezing temperatures.

With notable exceptions, the total water content of plants which acclimate decreases as cold hardiness is developed. The exceptions are succulents whose degree of hydration may be as high as 95% and they still develop a high degree of cold hardiness (Levitt, 1972). The concept of bound water - the water fraction presumed to be held so tightly by the plant that it does not freeze, and its possible relationship with frost hardiness has generated much controversy. Even the fraction of total water content that can be described as bound is subject to controversy as it depends on the methods used in removing water from the plant. Different forces will be capable of removing different fractions of total water content (Levitt, 1959). In his investigation, Levitt (1959) described as bound water, that fraction of water which is not removed

when in equilibrium with $Mg(ClO_2)_2$ at 0° to $5^{\circ}C$ but is removed at $105^{\circ}C$. He concluded that at least some protoplasmic components undergo an increase in hydration capacity during the hardening period. Several workers have determined that there is no obvious relationship between bound water and frost hardiness. Carroll (1943) worked on a number of turfgrasses which differ widely in cold hardiness. He observed that there were negligible differences among them with respect to bound water content during the hardening period. This led him to conclude that bound water cannot be used as a reliable index for measuring the cold hardiness of turfgrasses. Also, Gusta et al. (1975) working on non-acclimated winter cereal, acclimated hardy cereal and acclimated spring wheat, did not observe any relationship between unfreezable (bound) water per unit dry matter and cold hardiness. White et al. (1975) obtained similar freezing curves (ΔTm, K) for two perennial ryegrass varieties which differ in cold tolerance. (ΔTm is the melting point depression and K is the liquid water which does not freeze.)

During the cold hardening period, turfgrasses become darker green, reduced in size, reduced in leaf area, less succulent and more prostrate in growth habit (Beard, 1973).

FACTORS AFFECTING LOW TEMPERATURE KILLING POINT

The low temperature killing point varies widely in turfgrass species as evidenced by variation in their frost hardiness levels

(Arakeri and Schmid, 1949; Baker and David, 1963; Carroll, 1943; and Miller, 1966). On a scale of excellent through good, medium, poor to very poor, Beard (1973) ranked bentgrass as excellent and bermudagrass as poor.

Differences also occur among turfgrass varieties and cultivars in the low temperature killing points. The differences are sometimes marginal but still indicate a difference in their survival rates. This marginal difference may be critical during less severe winters (Davis and Gilbert, 1970; Dunn and Nelson, 1974; Ahring and Irving, 1969).

A rapid freezing or thawing causes plants to be killed at higher temperatures as does repeated freezing and thawing. A rapid freezing rate results in intracellular ice formation which is lethal to plants; whereas, a slow cooling rate allows ice to be formed extracellularly where it can be accommodated (Burke et al., 1976).

A partially injured tissue may fail to recover if it is immediately exposed to high temperature or excessive early spring fertilization which stimulates growth (Beard, 1973).

DETERMINING COLD HARDINESS

Field Trials - The earliest method for determining cold hardiness in turfgrasses was to leave the plants on the field through winter. Those that survived were termed hardy while those that did not were termed tender. Some fall between these two extremes and were rated

according to the percentage field survival. This method often requires long periods of time and the weather conditions in any one season may be too mild in testing the hardiness of the plant. In other seasons, the weather conditions may be too severe and both hardy and tender plants may be killed. The particular temperature at which the plant is killed cannot also be ascertained if the above method is used (Dexter et al., 1930).

Artificial Freezing

This is a much quicker method sometimes used in measuring, quantitatively, the cold hardiness levels in turfgrasses and other plant species and cultivars. Materials used consist of the meristematic regions of the crown. The leaf is less critical in the winter survival of turfgrasses and as such is less valuable in freezing tests. Ahring and Irving (1969) used rhizomes which were washed and sectioned into lengths containing 5 nodes. Davis and Gilbert (1970) used round plugs 5 cm diameter and 10 cm deep while Dunn and Nelson (1974) used both stolons and rhizomes. Sprigs washed from the upper portions of the soil (0-1.25 cm deep) were used as stolons while those washed from the lower depths (1.25-12.5 cm) were used as rhizomes. Plant sections may be allowed to cold acclimate in the field or subjected to a temperature between -1.1 and -4.4^{o}C for a period of 21 to 28 days to induce hardening. Davis and Gilbert (1970) took samples from the field at thirty-day intervals from September 1965 to May 1966.

Samples taken in September 1965 were non-hardened while those taken in May 1966 had already deacclimated. Ahring and Irving (1969) used a single naturally cold acclimated sample. Levitt (1972) stated that samples should be taken and subjected to the low temperature treatment from fall through winter to spring. Such sampling will reflect the plant's readiness to harden in the fall, the level of hardiness during periods of widely fluctuating temperatures and the tolerance of the plant to late frost. In all cases with very slight modifications, samples are subjected to a particular low temperature in a freeze chamber. It is allowed to equilibrate at that temperature for about one hour and then the temperature is set for the next lower treatment. The rate of temperature drop is usually about $2^{o}C$/hr. The control samples are held in a refrigerator set at a constant temperature of $+4.4^{o}C$. As soon as each sample is removed from the freezer it is placed in the control refrigerator and allowed to thaw slowly for about 48 hours (Ahring and Irving, 1969). As a precaution against appreciable supercooling, the material should be sprinkled with snow when a temperature of about -1^{o} or $-2^{o}C$ is attained during the freezing process.

Estimation of Injury

After low temperature treatment, sprigs are placed in moist vermicullite and allowed to regrow in the greenhouse, the day temperature in the greenhouse is usually between 27^{o} and $32^{o}C$ while the

night temperature is between 16° and 24°C. After a period of 3-4 weeks, visual determinations are made of the sprigs which have produced regrowth and these are rated accordingly (Dunn and Nelson, 1974).

When plant tissues are injured or killed by cold or by any other means, the cell becomes disorganized. It loses its capacity to regulate the diffusion of its contents and this leads to the exosmosis of electrolytes. The quantity of electrolytes that leak out of the cell correlate well with the degree of injury and can be estimated by conductivity measurements (Dexter et al., 1930).

The electrical resistance of plant tissues has been noted to decrease with increase in the level of injury (Wilner et al., 1960). After a low temperature treatment points of electrode are clamped into the plant tissue and the electrical resistance is measured using a Wheatstone Bridge.

Another method of estimating injury is based on the fact that injured or dead cells reduce the colorless triphenyl tetrazolium chloride (TTC) to red. Varying shades of the red color are obtained depending on the level of injury attained by test material. The results of these tests were earlier being interpreted visually. Such a visual rating is not quantitative and is subject to bias. Recently, Ahring and Irving (1969) used a refined TTC method and found that a 25% decrease in TTC absorbance after the freeze treatment correlated with that of

the survival check in bermudagrass. This they proposed serves as a good index of viability.

COLD HARDINESS OF BERMUDAGRASS

Davis and Gilbert (1970) observed that bermudagrass hardens over an extended period of time and that protein changes occur during this period. They also observed that Tifgreen attained a higher level of cold hardiness than Tifdwarf. Using the TTC test, Ahring and Irving (1969) found that coastal bermudagrass will not harden much below freezing under the climatic conditions of Oklahoma. Other cultivars used in the study include Common, Midland, Greenfield, Afghanistan 8153 and Yugoslavia 9959. They observed that cold hardiness increased in that order. Dunn and Nelson (1974) also observed that bermudagrass cultivars vary in winter injury response and that chemical changes occur during the hardening period. The effect of nitrogenous and potash fertilizer application on the winter survival of bermudagrass has also been studied (Adams and Marvin, 1960). Portz (1978) used cold chamber technique to screen seventeen cultivars of bermudagrass on the basis of cold tolerance. Dunn and Nelson (1978) worked on the relationship between thatch, rhizomes and carbohydrate and winter injury in Reno and Midway bermudagrasses.

MATERIALS AND METHODS

The grasses used in this study were increased in the greenhouse during the fall of 1977 and spring of 1978. Pee Dee 102, Santa Ana, Tifgreen, Tifway, Tifdwarf, LaJunta (from LaJunta, Colorado), NEJC (from North East Junior College Campus, Sterling, Colorado), Brookings (from Brookings, South Dakota) bermudagrass, and Futurf and Adalayd Paspalum vaginatum Sw. were used in the tests. Land preparation for field planting included roto-tilling, harrowing, leveling and a pre-planting application of 2 lb N/1000 sq. ft. (910 gm/.009ha). The grasses were planted in the field on May 31, 1978 in a randomized complete block design with three replications. Each plot measures .9m x 3.6m with a spacing of .15m between the plots. After establishment 1/2 lb (227.6 gm) of 2, 4-D plus 1/2 lb (227.6 gm) of dicamba per acre (.405ha)was applied to the plots in order to control broadleaf weeds. At this dosage, some damage was noticed on Pee Dee 102 and Tifway. A 2% solution of glyphosate was applied as often as necessary to keep the cultivars from growing into one another. On July 10, 1978 the plots were again fertilized using 1 lb N/1000 sq. ft. (455 gm/.009ha). After establishment, the entire area was top-dressed once. Daily maximum and minimum air temperatures were recorded. Soil temperature was also recorded three times a day.

FIELD STUDY

The objective of this study was to determine the regrowth of turfgrasses which were cold stressed in the field. The time during the winter when any or all of the turfgrasses were injured beyond recovery would also be ascertained. The literature asserts that field study of cold hardiness in turfgrasses is unsuitable (Dexter et al., 1930). The method they described, however, is such that the turfgrasses were left on the field throughout the winter and evaluations were made only at the end of that period. This was modified by taking samples out of the field at intervals and evaluating their regrowth in the greenhouse. Starting from December 1, 1978 and every two weeks thereafter, a core measuring approximately 10 cm diameter and 5 cm deep was taken out of each plot. They were placed in flats in a sand medium and the flats were arranged in a randomized complete block design. They were placed in the greenhouse under a day temperature of 28^{o}C and a night temperature of 20^{o}C. They were watered every day. The cultivars were rated visually on the basis of the regrowth obtained from the samples after 6 weeks. A scale of 0 to 10 was used where 0 = no regrowth and 10 = complete regrowth.

Results and Discussion. Means of the ratings of survival of the cultivars from samples taken on December 1 are represented in an LSD graph (Fig. 1). When the bars of the LSD graph overlap, no significant difference occurs. At this time, except for Brookings all the cultivars recovered very well and there was no significant difference (5%) in their visual regrowth ratings. Fig. 1 revealed a significant difference between the visual regrowth ratings for Brookings and for the other cultivars. Fig. 2 shows the LSD graph of the visual regrowth ratings for the samples taken on December 18, 1978. Pee Dee 102, Tifgreen, Tifdwarf, NEJC and to a lesser extent LaJunta recovered very well. The recovery of Santa Ana, Tifway and Brookings, according to the visual regrowth ratings were low while Futurf and Adalayd were practically dead. Fig. 2 revealed a significant difference (5%) between the cultivars. The LSD graph of the visual regrowth ratings for the samples taken on December 30 is shown as Fig. 3 . Pee Dee 102, Tifgreen, and Tifdwarf ranked very high in the ratings while LaJunta and NEJC ranked intermediate, and Brookings, Tifway and Santa Ana ranked very low. No samples of Futurf and Adalayd were taken on this date, and on subsequent dates because they were already dead. The LSD graph for the samples taken on January 15 (Fig. 4) revealed a significant difference between the cultivars in terms of

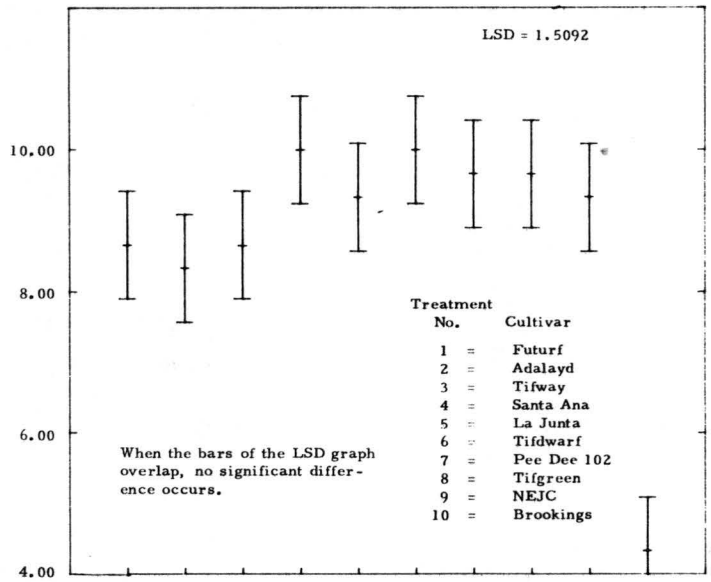
visual regrowth ratings. LaJunta, Tifway and Santa Ana had very little or no regrowth. Pee Dee 102, Tifgreen and Tifdwarf received low visual regrowth ratings. In contrast to the poor regrowth exhibited by Brookings in the previous samples, it showed an improvement in its regrowth capability and ranked highest in the ratings. As shown in Fig. 5 , there is a significant difference in the visual regrowth ratings of the cultivars under test for the samples taken on January 30. Brookings ranked highest, NEJC and Tifgreen were intermediate while Pee Dee 102, and Tifdwarf ranked lowest on the visual regrowth ratings scale. No samples of Tifway, LaJunta and Santa Ana were taken on this and subsequent dates because they were already dead in the field. This was shown by their regrowth rating in the January 15 samples. Samples of Tifgreen, NEJC and Brookings were taken on February 14. There was no regrowth observed for the Tifgreen, while that of NEJC was very poor (mean visual regrowth ratings = 1.33). Brookings had almost complete regrowth. On subsequent dates, no further samples of any of the cultivars except Brookings was taken. The means of the visual regrowth ratings for all the samples of Brookings is shown on Fig. 6 .

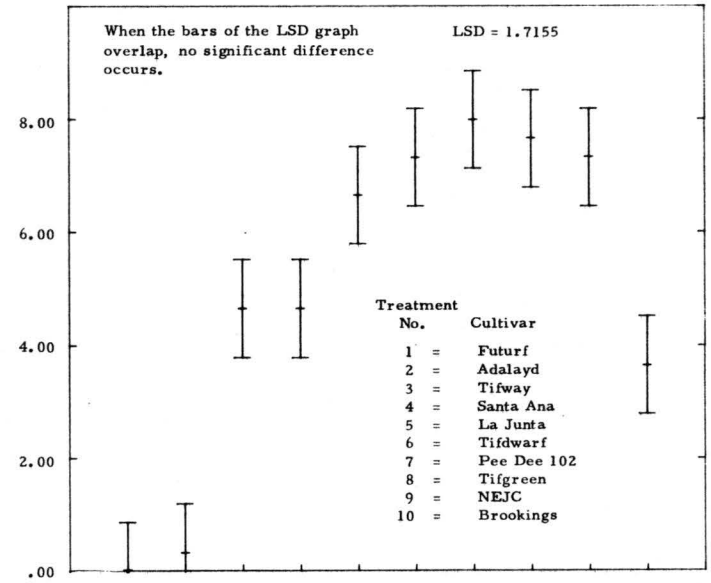
The visual regrowth ratings for all the cultivars and for all the sampling dates revealed the different times during the winter when a cultivar was felt to be dead. Futurf and Adalayd did not live through December. Tifway, Santa Ana, and LaJunta did not live through January. The last sampling data for Pee Dee 102, and Tifdwarf was

Figure 1. Means of visual regrowth ratings for samples taken December 1, 1978 (LSD = 1.5092).

Figure 2. Means of visual regrowth ratings for samples taken December 18, 1978 (LSD = 1.7155).

Figure 3. Means of visual regrowth ratings for samples taken December 30, 1978 (LSD = 1.7514).


LSD = 1.5092
10.00
8.00
6.00
4.00
When the bars of the LSD graph overlap, no significant difference occurs.
Treatment No. Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings


When the bars of the LSD graph overlap, no significant difference occurs.
LSD = 1.7155
8.00
6.00
4.00
2.00
.00
Treatment No. Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings

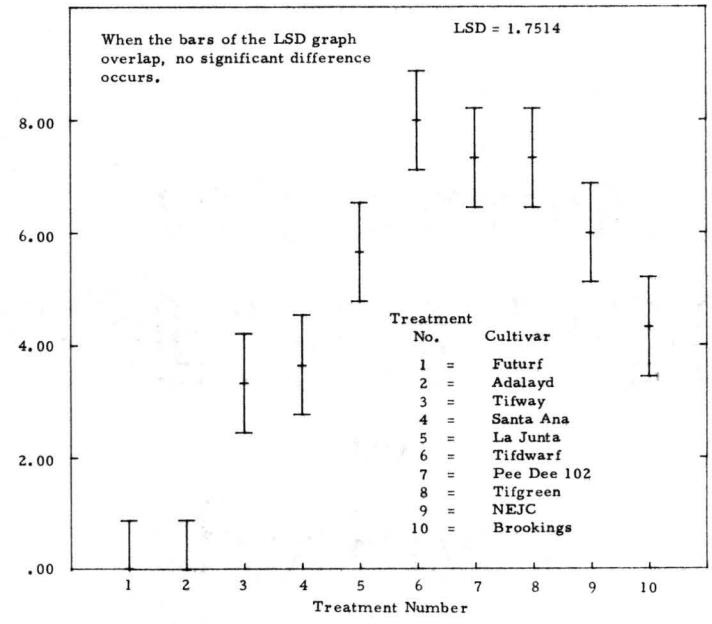
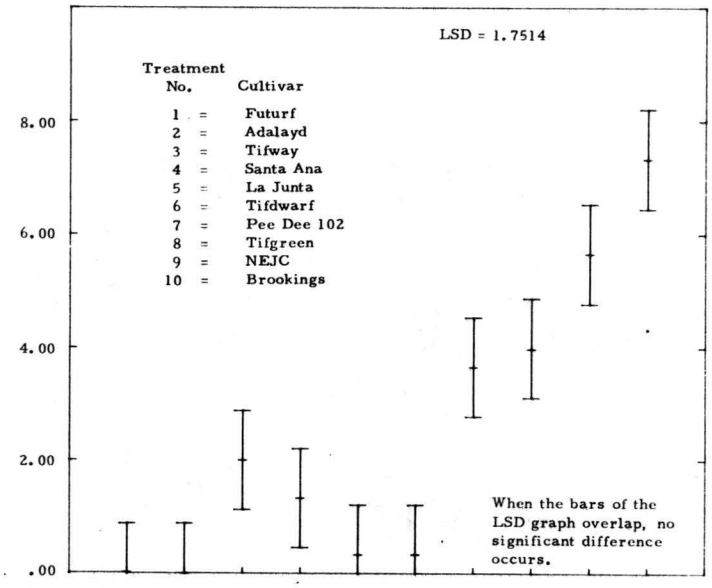
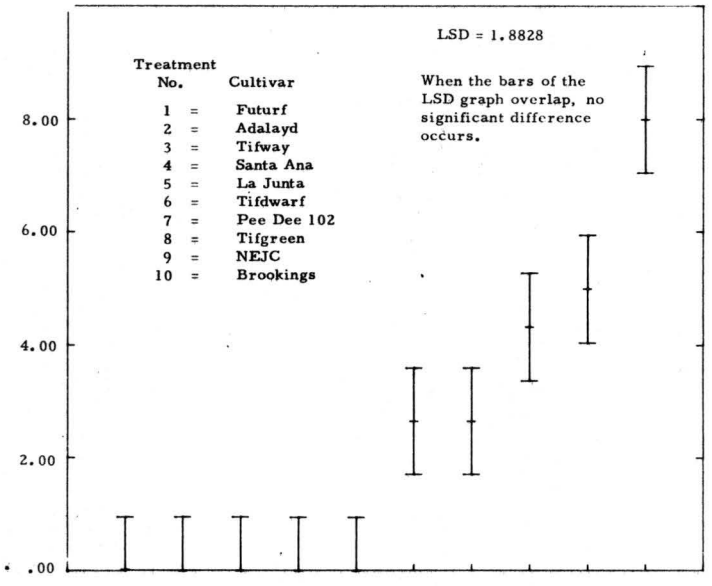

When the bars of the LSD graph overlap, no significant difference occurs.
LSD = 1.7514
8.00
6.00
4.00
2.00
.00
Treatment No. Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings
1 2 3 4 5 6 7 8 9 10
Treatment Number

Figure 4. Means of visual regrowth ratings for samples taken January 15, 1979 (LSD = 1.7514).

Figure 5. Means of visual regrowth ratings for samples taken January 30, 1979 (LSD = 1.8828).

Figure 6. Means of visual regrowth ratings for samples taken February 14, 1979 (LSD = 1.7155).


LSD = 1.7514
Treatment No. Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings
When the bars of the LSD graph overlap, no significant difference occurs.
8.00
6.00
4.00
2.00
.00


LSD = 1.8828
Treatment No. Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings
When the bars of the LSD graph overlap, no significant difference occurs.
8.00
6.00
4.00
2.00
.00

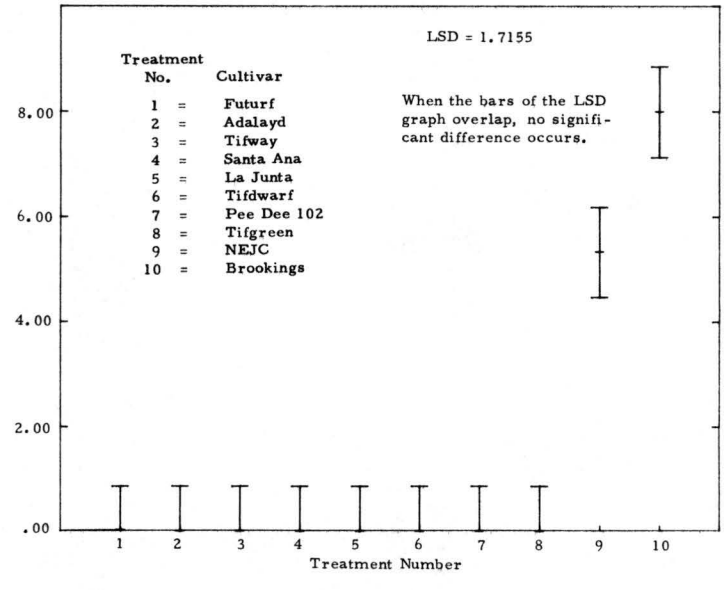
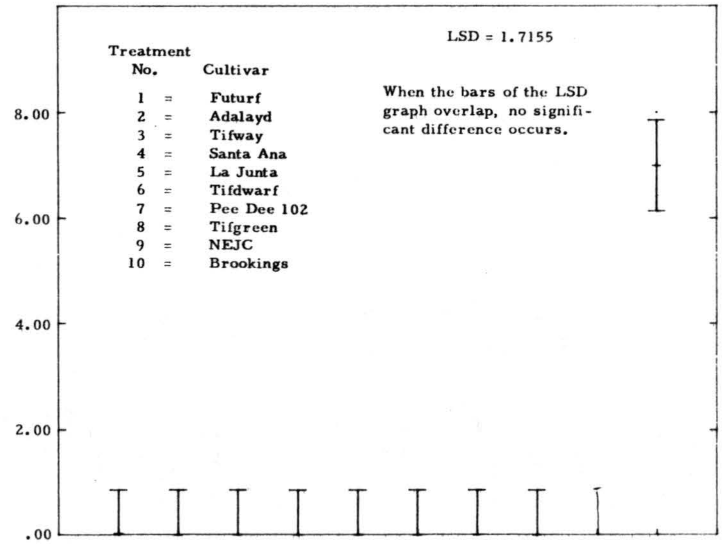
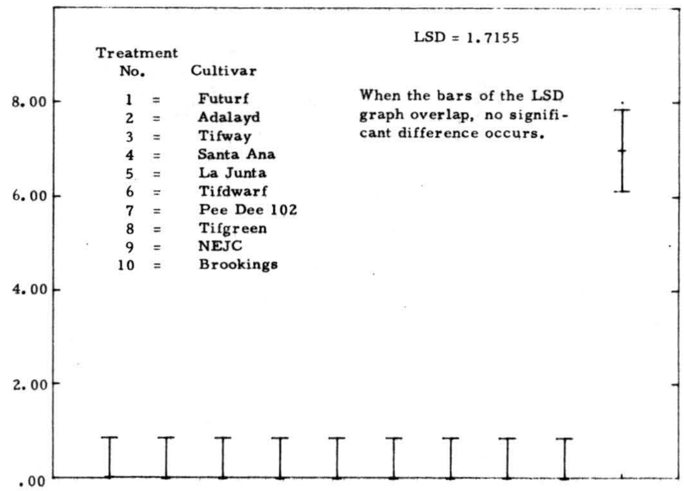

LSD = 1.7155
Treatment No. Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings
When the bars of the LSD graph overlap, no significant difference occurs.
8.00
6.00
4.00
2.00
.00
1 2 3 4 5 6 7 8 9 10
Treatment Number

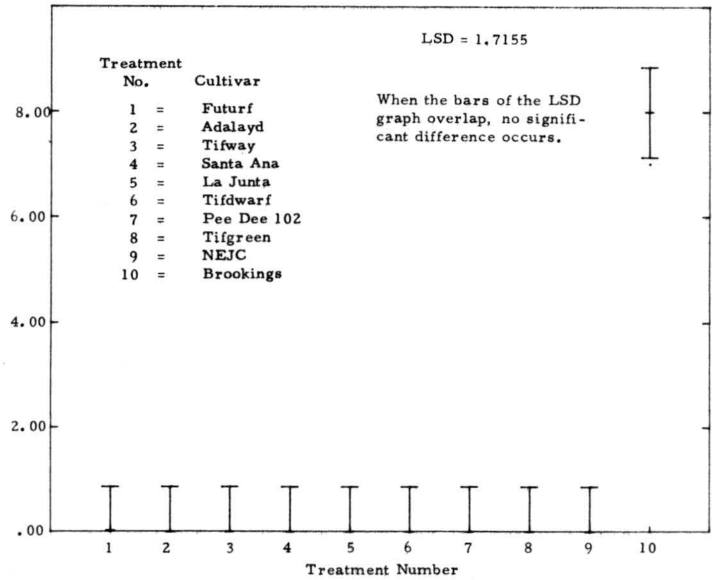
Figure 7. Means of visual regrowth ratings for samples taken February 26, 1979 (LSD = 1.7155).

Figure 8. Means of visual regrowth ratings for samples taken March 15, 1979 (LSD = 1.7155).

Figure 9. Means of visual regrowth ratings for samples taken March 30, 1979 (LSD = 1.7155).


LSD = 1.7155
Treatment No.
Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings
When the bars of the LSD graph overlap, no significant difference occurs.
8.00
6.00
4.00
2.00
.00


LSD = 1.7155
Treatment No.
Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings
When the bars of the LSD graph overlap, no significant difference occurs.
8.00
6.00
4.00
2.00
.00


LSD = 1.7155
Treatment No.
Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings
When the bars of the LSD graph overlap, no significant difference occurs.
8.00
6.00
4.00
2.00
.00
1
2
3
4
5
6
7
8
9
10
Treatment Number

January 30. Samples of Tifgreen taken on February 14 showed no regrowth at all while the last sampling date for NEJC was February 26. Sampling was discontinued in each case as the particular cultivar had very little or no regrowth from samples taken on the preceding sampling date. Brookings is the only variety which lived through the winter. These observations correlate with the artificial freezing test carried out as part of this study. Brookings showed poor regrowth from the samples taken on the earlier sampling dates. Artificial freezing test carried out at the same time showed that Brookings was capable of surviving the temperature prevailing in the field. At later sampling dates, the visual regrowth ratings for Brookings went up considerably. It is suggested that the poor regrowth obtained from samples taken in December could be due to shorter daylengths prevalent at that time of the year. The regrowth of samples of Brookings taken on later dates improved and this coincided with longer days as there was no supplemental light in the greenhouse during the period of the experiment. Also, Brookings probably goes into dormancy in response to low temperature and it breaks dormancy later than for any of the other cultivars being studied. This suggests that it has longer chilling unit requirement.

With the development of spring it was noticed that Pee Dee 102, Tifgreen and NEJC came up in small patches in the field. NEJC, however, recovered on a much wider scale than either Tifgreen or Pee Dee 102. The patches of live NEJC, Tifgreen and Pee Dee 102 appear to

have been under a thicker snow cover than found on other areas of the experimental plot; therefore, they were more protected by the snow against low temperature. Snow has an extremely low thermal conductivity and it protects turfs not only against low temperature but also serves in reducing the frequency of freezing and thawing. The idea that the live patches were probably under a thicker snow cover is brought out by the fact that the live patches were observed only at a particular portion of the experimental plot.

Conclusion. Bermudagrass generally appears to be more capable of surviving the winter than the cultivars of P. vaginatum. Of the bermudagrass cultivars, Brookings appears to be the most capable of surviving severe winters while Tifway appears to be the least capable. NEJC, Tifgreen and Pee Dee 102 may also be able to survive less severe winters in Fort Collins, Colorado (Table I).

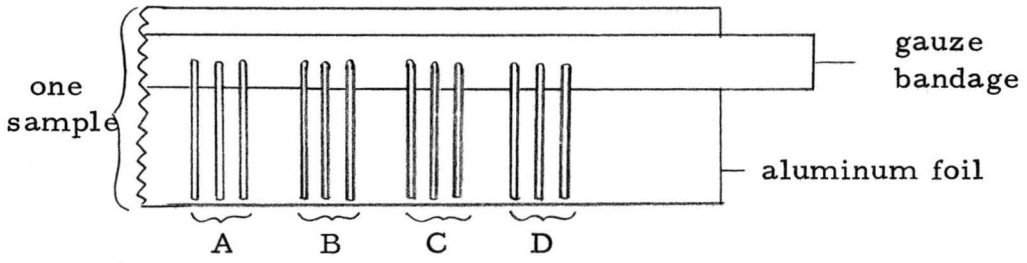
ARTIFICIAL FREEZING

This quick and quantitative method was used to determine the degree of cold hardiness attained during the winter. Sampling was done from fall through winter into spring in order to ascertain (1) period of acclimation, (2) period of deacclimation, (3) maximum level of cold hardiness obtainable and the stability of this level of cold hardiness, and (4) tolerance of plants to late frost. Starting from mid-October and every month thereafter, stolons were taken from the field from each cultivar. The stolons were washed and sectioned into pieces

Table I. Climatological data for Fort Collins, Colorado 1973-1977 derived from the National Oceanic and Atmospheric Administration, The Environmental Data Service, Ashville, North Carolina.

		Temp Average Min. °C			Temp Average Min. °C
Nov	1973	-3.9	Feb	1973	-7.5
	1974	-3.8		1974	-5.9
	1975	-5.6		1975	-8.9
	1976	-5.4		1976	-4.9
	1977	-4.1		1977	-5.7
	1978	-4.7		1978	-7.4
Dec	1973	-6.7	Mar	1973	-2.8
	1974	-8.7		1974	-2.7
	1975	-6.9		1975	-4.3
	1976	-8.1		1976	-5.5
	1977	-6.7		1977	-4.3
	1978	-12.5		1978	-2.1
				1979	-2.1
Jan	1973	-9.6	Apr	1973	-1.3
	1974	-10.9		1974	1.2
	1975	-8.6		1975	-1.1
	1976	-9.9		1976	1.8
	1977	-12.2		1977	2.7
	1978	-10.9		1978	2.4
	1979	-15.0		1979	1.6

containing four to six nodes. Four pieces of each cultivar were laid out on a thin aluminum foil with the end of the pieces making contact with a strip of gauze bandage laid out at one end of the aluminum foil.



A, B, C, D, represent different cultivars

The aluminum foil is then wrapped around the cultivars to make a sample. Twelve such samples were prepared. Each sample was moistened with distilled water, kept in a weighted tube and corked. They were kept in thermos flasks which contained methanol. Three tubes were kept in each flask. The control samples were kept in a separate flask and held between 4.4°C and 6.1°C in the control chamber. The other samples were subjected to standard freezing tests using a methanol bath. The temperature of the bath was brought to 0°C which was the highest temperature treatment. After thirty minutes, the 0°C treatment sample was removed. Subsequently, samples were removed at 2°C intervals over the range 0 to -20°C. The temperature of the bath was allowed to drop 1°C at a time and held at that temperature for thirty minutes, thus the rate of drop is less than 2°C per hour. At -2°C all the samples in the bath were

sprinkled with snow to prevent appreciable supercooling. Upon removal from the bath every sample was kept in the control chamber. After the last sample was removed from the bath, all the samples were left in the control chamber for 24 hours.

Estimation of Injury. Literature (Dexter et al., 1930) suggested that the degree of injury from cold temperature in turfgrasses is well correlated with the exosmosis of electrolytes following exposure to cold. Such outward diffusion of electrolytes can be estimated by conductivity measurements.

Two pieces of each cultivar which had been previously frozen, were put in a test tube and $2\frac{1}{2}$ ml of distilled water was added. The test tubes were placed in a refrigerator set at 25°C and kept there for 24 hours. After this period, each test tube was mechanically shaken to enable the contents to mix thoroughly. The exosmosis of electrolytes was then measured using a conductivity meter. The stolons were then killed by putting the test tubes in a steam bath set at 80°C and left there for 30 minutes. The exosmosis of electrolytes from the killed tissues were then determined using the conductivity meter. The percentage injury at each temperature treatment was determined as

$$\frac{\text{exosmosis of electrolytes at given temperature}}{\text{exosmosis of electrolytes from killed tissue}} \times 100$$

A plot of the percentage injury against temperature was made and the killing point is determined as the mid-point in the inflection of the curve.

Results and Discussion. The low temperature killing points for the cultivars of bermudagrass and Paspalum vaginatum Sw. included in this study dropped (i.e. the plants become hardier) from fall to winter. For Brookings which is the only cultivar that actually survived the winter, cold hardiness reached a maximum in mid-winter and decreased in spring. The general trend (Table II)is that cold hardiness increased gradually between October and November, 1978 and then increased rather sharply between November and December, 1978. The recorded maximum levels of cold hardiness for Pee Dee 102, Santa Ana, Tifdwarf, LaJunta, Tifway, Futurf and Adalayd were attained in December. For Tifgreen and Brookings the recorded maximum levels of cold hardiness were attained in January. For NEJC the maximum cold hardiness level was attained in February. The last sampling data for the two P. vaginatum cultivars Futurf and Adalayd and for Tifway bermudagrass was December. For Pee Dee 102, Santa Ana, Tifgreen, Tifdwarf and LaJunta the last sampling data was January. The last sampling date for NEJC was February while sampling for Brookings continued throughout the duration of the experiment. No data was recorded for the various cultivars beyond the stated dates because the cultivars were already dead in the field.

Daily soil temperatures taken on the experimental plot at a depth of 5 cm ranged between +2°C and -8.9°C for December, 1978. A temperature range between -7.8°C and -8.9°C occurred on as many

Table II. Low temperature killing point for 8 bermudagrass and 2 Paspalum vaginatum cultivars.

	Oct 18 1978	Nov 15 1978	Dec 14 1978	Jan 13 1979	Feb 14 1979	Mar 16 1979	Apr 15 1979	May 14 1979
Futurf	-2	-3	-7					
Adalayd	-3	-3	-7					
Tifway	-3	-4	-7					
LaJunta	-2	-4	-9	-9				
Santa Ana	-4	-5	-9	-9				
Pee Dee 102	-2	-5	-11	-11				
Tifdwarf	-3	-5	-11	-11				
Tifgreen	-2	-6	-9	-11				
NEJC	-2	-3	-11	-11	-13			
Brookings	-2	-6	-13	-17	-17	-11	-6	-6

as 14 days for December, 1978. These low temperatures must have accounted for the death of Futurf and Adalayd P. vaginatum and Tifway bermudagrass. The recorded maximum cold hardiness level -7°C attained by these three cultivars was obtained from the samples taken from the field on December 14, 1978. Up to that date, the soil temperature never went below -5.5°C. Soil temperature remained persistently low through January with the lowest temperature being -11.1°C. A soil temperature range between -7.8°C and -10°C were recorded either in the morning or in the evening of 28 days in January. As for Futurf, Adalayd and Tifway for December, Pee Dee 102, Santa Ana, Tifgreen, Tifdwarf and LaJunta did not live through January. The recorded maximum cold hardiness levels for Pee Dee 102, Tifdwarf and Tifgreen being -11°C and for Santa Ana, and LaJunta -9°C. These cold hardiness levels were obtained from the samples taken on January 13, 1979 and the minimum soil temperature on the experimental plot before January 13, was -10°C. The soil temperature did not fall below -11.1°C throughout January but it stayed very close to it. The death of Pee Dee 102, Tifgreen and Tifdwarf in the field must have resulted from a long exposure to a temperature very close to their low temperature killing points (-11°C). Santa Ana and LaJunta with a low temperature killing point of -9°C were killed in the field in January. The lowest soil temperature for February was -11.1°C. This was recorded on February 1, 1979. The soil temperature ranged between -4.4°C and -11.1°C until February 10.

After that the soil temperature went up to between -1.1°C and -2.2°C for the rest of February except on February 23 when a soil temperature of -6.7°C was recorded. The last sampling date for NEJC was February 14, and the maximum cold hardiness level, -13°C, was recorded for that sample. The death of NEJC in the field may have resulted from the repeated freezing and thawing which occurred three times during February. Brookings, however, lived through the winter and attained its maximum level of cold hardiness, -17°C, in January 1979. This level was maintained through February but it dropped sharply with the coming of spring.

From Table I (the climatological data from Fort Collins, Colorado from 1973-1979) it can be observed that the average minimum temperature for December, 1978 (-13.6°C) was much lower than for the same period for the year 1973 to 1977. This observation also holds true for January 1979 (-15°C) with respect to average minimum temperature for the month of January. The winter of 1978 to 1979 was more severe than for the previous five years. It appears that some bermudagrass cultivars might live through several milder winters but be killed by an unusually cold one.

Conclusion. Bermudagrass cold acclimates over a long period of time and it probably does not reach its maximum cold hardiness level until mid-winter. This renders it susceptible to cold temperatures which may occur before the maximum mid-winter cold hardening.

A surviving plant has to adapt rather fast so that it can withstand cold and alternating temperatures. This is especially true for areas where the temperature change is rapid (Gerloff et al., 1967). In many instances it takes only about 3 weeks of a temperature near +1.6°C for cold hardy plants to reach their maximum level of cold hardiness (Olien, 1967). Unfortunately, this is not the case with bermudagrass nor with P. vaginatum cultivars. Cultivar differences, however, do occur in the response of bermudagrass to cold. Of the bermudagrasses studied, Brookings appears to be the most cold hardy followed by NEJC while Tifway appears to be the least cold hardy. Tifgreen, Tifdwarf, Pee Dee 102, Santa Ana, and LaJunta are intermediate and of these the last two appear to be less cold hardy than the first three. Bermudagrasses appear to be superior to the turf-type cultivars of P. vaginatum in their response to cold. The two cultivars of P. vaginatum - Futurf and Adalayd studied were found to be as poor as Tifway bermudagrass with respect to cold hardiness. Brookings which appears to be the most cold hardy of all the cultivars studied, is very coarse and has fewer nodes; and therefore it cannot be used for high quality turf. Many homeowners would even object to its use on home lawns. NEJC is less coarse and while it may not be used on high quality turf, it may be acceptable to homeowners. Tifgreen, Tifdwarf and Pee Dee 102 are high quality turfgrasses and may live through milder winter conditions than existed during this study.

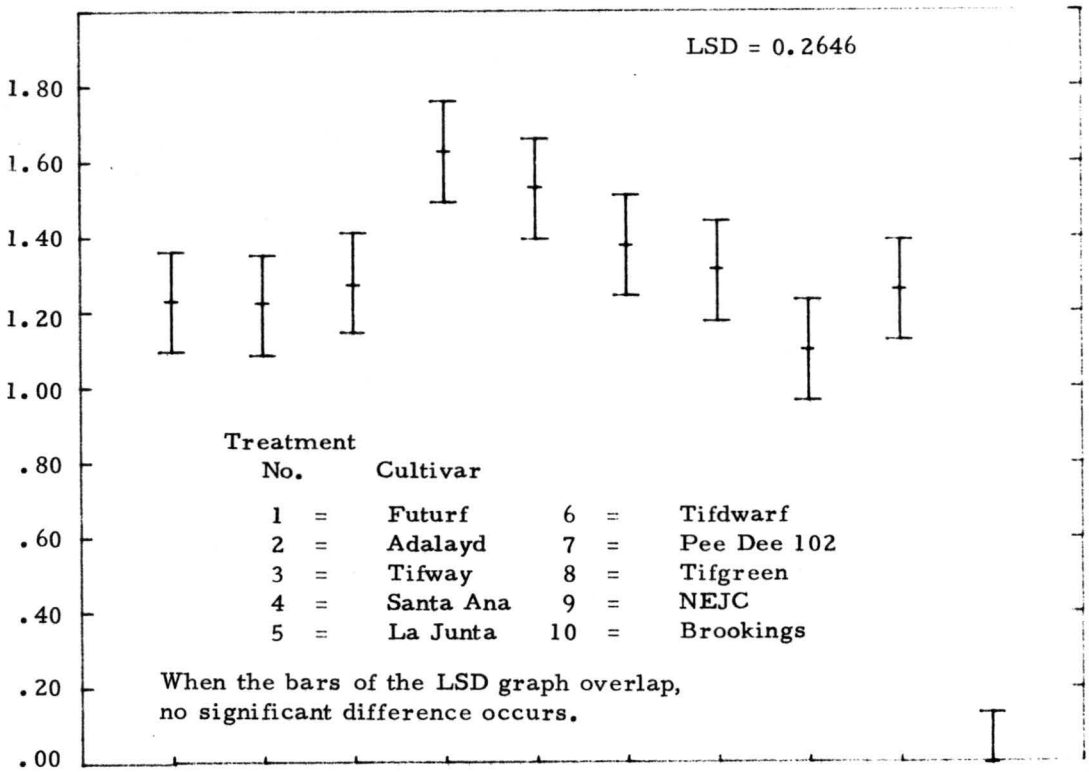
CHILLING STRESS STUDY

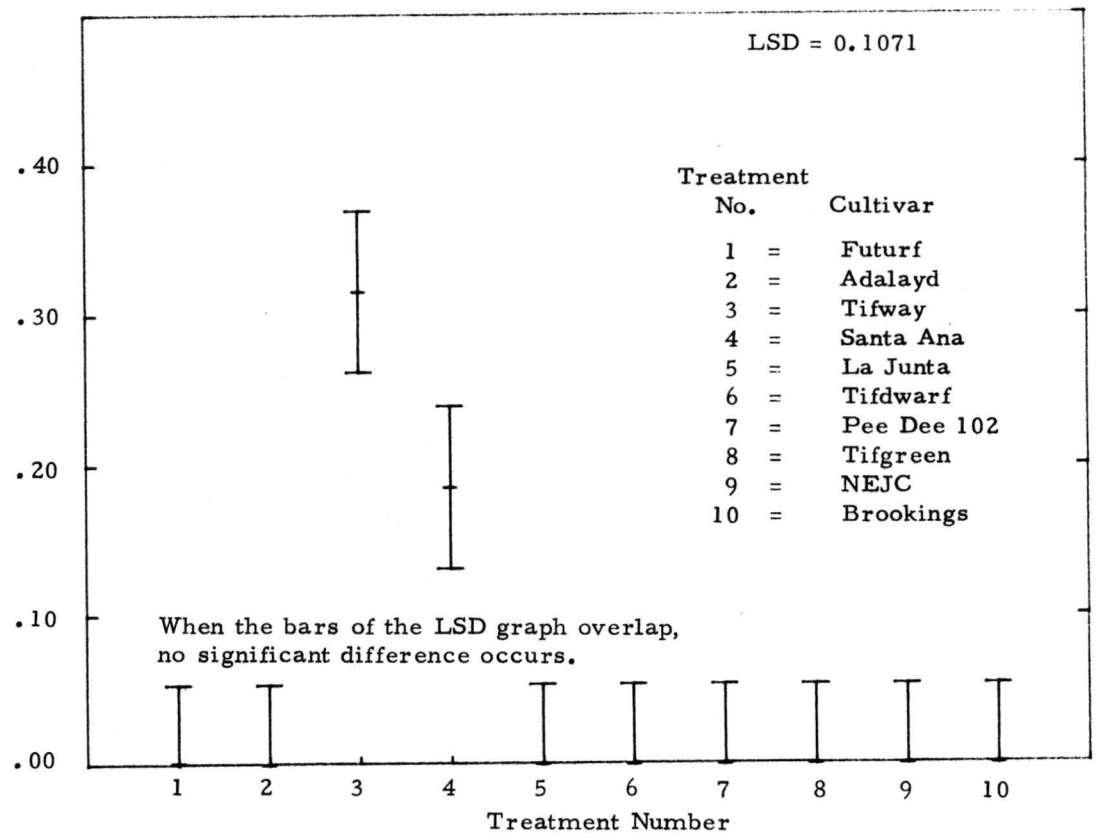
The purpose of this study was to determine the susceptibility of the turfgrasses to chilling. The greenhouse grown grasses were initially planted in pots (16cm x 10cm x 6cm) filled with sand. After establishment the grasses were cut back to level with the top of the pot. The pots were then transferred to the growth chamber and the temperature treatments were 15°, 10° and 5°C both day and night and 12 hours of light was provided. The 5°C treatment was later discontinued as it was putting too much stress on the growth chamber and causing light to go off and on inconsistently. After 6 weeks, samples from the 15° and 10°C treatments were harvested, oven-dried at 105°C for 48 hours and weighed. The experiment was repeated for Brookings using 15° and 10°C both day and night and 15 hours of light.

Results and Discussion. Figure 10 shows for the 10°C treatment, the means of the dry weight of the regrowth of the cultivars after 6 weeks presented in an LSD graph. Figure 11 is for the 15°C treatment. When the bar of the LSD graph overlap, no significant difference occurred. None of the cultivars used in the 10°C treatment except Santa Ana and Tifway produced any regrowth. Figure 10 showed that there were significant differences (5%) in the mean of the dry weight of the regrowth of the cultivars at the 10°C treatment. There were also significant differences (5%) in the means of the dry

Figure 10. Means of the dry weight of the regrowth under 15°C.

Figure 11. Means of the dry weight of the regrowth under 10°C.


LSD = 0.2646
1.80
1.60
1.40
1.20
1.00
.80
.60
.40
.20
.00
Treatment No. Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings
When the bars of the LSD graph overlap, no significant difference occurs.


LSD = 0.1071
.40
.30
.20
.10
.00
Treatment No. Cultivar
1 = Futurf
2 = Adalayd
3 = Tifway
4 = Santa Ana
5 = La Junta
6 = Tifdwarf
7 = Pee Dee 102
8 = Tifgreen
9 = NEJC
10 = Brookings
When the bars of the LSD graph overlap, no significant difference occurs.
1 2 3 4 5 6 7 8 9 10
Treatment Number

weight of the regrowth of the cultivars at the 15°C treatment (Fig. 11). There was no significant difference, however, between the regrowth of either Futurf or Adalayd and those of Pee Dee 102, Tifgreen, Tifdwarf, Tifway and NEJC. All the cultivars used in this test had some regrowth except Brookings. When the hours of light were increased from 12 to 15 with the temperature of the growth chamber still held at 15°C there was considerable regrowth of Brookings. The mean of the dry weight of the regrowth was 2.59 gm.

Conclusion. From the results of the 10°C treatment, it appears that all the cultivars used in the test are susceptible to chilling stress. Santa Ana and Tifway which are the only cultivars that produced any regrowth at this temperature have been observed from a previous experiment in this study not to be particularly cold hardy. Tifway was observed to be the least cold hardy of the bermudagrass cultivars used. It therefore appears that while Tifway is the most susceptible to freezing stress, it is the least susceptible to chilling stress of all the bermudagrass cultivars used in this study. However, the P. vaginatum cultivars - Futurf and Adalayd appear to be very susceptible to both chilling and freezing stress. The observation that Brookings produced no regrowth at either the 10°C or 15°C treatment but did produce regrowth when 15 hours of light was combined with 15°C suggests a photoperiodic response. It may also be a dormancy factor in which

case Brookings goes into dormancy in response to the low temperature and requires long chilling units in order to break dormancy. Similar observations were made in the field study when minimal regrowth was obtained from samples of Brookings put in the greenhouse in December.

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