Biochemical And Molecular Analyses Of Cold Acclimation In Bermudagrass [Cynodon Spp.]

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EXECUTIVE SUMMARY

Disruption of membrane integrity, as a result of low temperature-induced water removal, is a primary cause of cold injury to plant cells. The membrane lipid bilayer provides the necessary environment for proper functioning of integral and peripheral membrane proteins. Membrane "fluidity" is thought to be a prerequisite for unimpaired survival at low temperature. Membrane fluidity is affected by the degree of unsaturation (i.e., number of double bonds) in the fatty acid (FA) side-chains of the membrane lipids. These double bonds induce "kinks" in the long hydrocarbon side chains, thus resisting molecular compaction, and maintaining fluidity. As such, increase in (FA) unsaturation reduces the temperature at which membranes undergo damaging, dehydration induced, phase transitions.

'Midiron' and 'U3' bermudagrass were exposed to conditions that induce cold acclimation, and crown and stolon nodes were harvested for total membrane lipid isolation. (FA) analysis of total lipids revealed a significant increase in the important tri-unsaturated species (i.e., linolenic acid; C1 8:3), over shorter and saturated species, and an overall increase in the double-bond index. These changes were more pronounced in 'Midiron' (relatively cold tolerant) than in 'U3' (relatively cold sensitive), during cold acclimation (Figures A and B).

Biochemical analysis of the total membrane lipids identified important glycolipid (GL) and phospholipid (PL) constituents. Three glycolipids species were detected in equimolar amounts in both genotypes (i.e., sterylglucosides, acylated sterylglucosides and cerebrosides). The amounts of these three glycolipids did not vary during cold acclimation. Among the membrane phospholipids we confirmed the presence of four classes: phosphatidy1choline (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylglycerol (PG). PC was in greatest abundance, while the others were found to be in equimolar concentrations (i.e., PC >> PE = PS = PG). Their relative amounts did not change significantly during cold acclimation in either genotype. (FA) changes, within the PL fraction, basically paralleled those found for total polar lipids where there was a decrease in shorter chain and/or saturated FAs, and an increase in the longer chain unsaturated (FA) (i.e., 18:3). This overall change in PL saturation level was quantified by calculating the double bond index (DBI). The DBI for 'Midiron' was significantly greater than that for 'U3' by mid-way through the acclimation period, and continued to diverge significantly through the remainder of the four week experiment.

We extended our (FA) analysis to another turfgrass species (Paspalum vaginatum Swartz.). Data from these experiments basically paralleled those of bermudagrass. For example, the more cold tolerant genotype, P1509018-1, showed a significant increase in C18:3 as compared to the more cold sensitive genotype, P1299042 (Figs. C, D and E). We feel our findings document a general trend in the manner in which warm season turfgrass responds to low temperature, and acclimates to this environmental stress.

The enzymes responsible for increasing the level of unsaturation are the (FA) desaturases (i.e., omega-3 omega-6 and omega-9 We characterized the genes encoding these enzymes by genomic Southern blot hybridization. Results from these analyses indicate that the (o3 and o)9 desaturases are encoded by small gene families (e.g., no more than two members each). In addition, the data revealed diagnostic restriction fragment length polymorphism (RFLPs), not only between the two species, but also between the different cultivars within each species (i.e., six bermudagrasses and three paspalums) -- indicating genetic variability in (FA) biosynthesis.

ANNUAL REPORT (11/1/96 to 10/31/97)

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PROJECT:	Biochemical and Molecular Analyses of Cold Acclimation in Bermudagrass [*Cynodon* spp.]
OBJECTIVES:	(1) Characterize membrane-specific changes in fatty acid and lipid composition during cold-acclimation.
	(2) Characterize the expression of genes involved in fatty acid and lipid biosynthesis during cold acclimation, using heterologous gene probes.
	(3) Clone fatty acid biosynthesis genes (e.g., desaturases) expressed in bermudagrass in response to low temperature.

The primary goal of this project is to gain a more complete understanding of the process of cold acclimation in bermudagrass, by characterizing the changes in membrane composition and gene expression that accompany exposure to low temperature. Previous experience has shown crowns and stolon nodes to be biologically and physiologically appropriate tissue for the proposed studies, confirmed the utility of 'Midiron' and 'U3' germplasm in these analyses, and provided insight as to the important enzymes regulating fatty acid and lipid composition of cellular membranes.

INTRODUCTION

Environmental stress occurs in many forms such as high or low temperature, water excess or deficit, extremes in solar radiation, soil nutrient imbalances, etc. The biochemical and biophysical changes that occur in chilling sensitive plants exposed to reduced temperatures, together with the subsequent expression of low temperature stress symptoms, are collectively referred to as cold injury. The physiological changes include alterations in cytoplasmic streaming, enzyme activity, respiration, photosynthesis as well as effects on membrane permeability, structure and composition.

It is clear that disruption of cellular membrane integrity, as a result of low temperature-induced water removal leading to membrane lipid bilayer phase transitions, is a primary cause of cold injury. The lipid bilayer provides the necessary environment for proper functioning of bound or soluble enzymes associated with a particular membrane system (e.g., plasma membrane, chloroplast thylakoids, etc.). Maintenance of a fluid state for membrane lipids is thought to be one of the prerequisites for

unimpaired survival at low temperature. Physical properties such as flexibility and molecular motions of membrane diacylglycerols (e.g., polar phospholipids), depend, to a large extent, on the degree of unsaturation (i.e., number of double bonds) in their fatty acid (FA) side chains. Double bonds produce bends or kinks, that collectively enable membranes to resist damage at low temperature. Low temperature damage results from the detrimental effects of molecular compaction, partitioning of membrane lipids or proteins (de-mixing), and the formation of flexibility-limiting molecular contacts between adjacent lipid molecules. Thus, changes in FA saturation can affect biophysical properties such as the temperature at which membranes undergo dehydration induced phase transitions -- which may not be reversible, and result in lethal damage.

A better understanding of the nature and basis for tolerance to low temperature and the process of cold acclimation in warm season turfgrass will be very useful for programs focused on germplasm improvement, whether through breeding or biotechnology.

Analysis of Membrane Lipid Composition During Cold Acclimation

[Ms. Jenith Cyril (Doctoral student)]

Changes in bermudagrass lipids and fatty acid content in response to low temperature

Physiological studies have correlated changes in the composition of membrane lipids with survival of exposures to cold and freezing conditions. For example studies using leaves from plants such as *Arabidopsis*, wheat, oats or rye have documented significant increases in phospholipid content during acclimation to low and subsequently lethal temperatures. Therefore, and expanding on our earlier results with bermudagrass, we have investigated the composition of membrane lipids (and their specific fatty acid components) in the crown and stolon node tissue of 'Midiron' and 'U3'.

Samples/tissues were harvested at 0, 7, 14, and 21 days of cold treatment (8/4°C; day/night; 300 umol/m^2/s PPF), as well as "control" (28/20°C) tissues harvested at 0, 7 and 21 days. These tissue samples were processed for the isolation of total lipids, and the purification of total polar lipids, their sub-group component classes and identification of all fatty acids constituents within the lipid groups.

Total lipids are broadly classified into three major groups. These are the phospholipids (including phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol and phosphatidic acid); the glycolipids (including monogalactosyldiglycerides, digalectosyldiglycerides, sterylglucosides, acylated sterylglucoside and cerebrosides); and the neutral lipids (including sterols and triacylglycerols). Total lipids from bermudagrass crowns (and stolon or rhizome nodes) were fractionated into neutral lipids, phospholipids and glycolipids using Sep-Pak cartridges, and then further separated into their constituent classes by thin layer chromatography. Individual lipids within each class can be identified by co-chromatography with commercially available authentic standards, and the use of specific spray detection reagents to reveal their presence and relative abundance on the thin layer chromatography plates.

The neutral lipids represent a relatively minor components of the total membrane lipids isolated from crowns/stolon nodes. The neutral lipids were fractionated, and four species were identifies. However, due to their minor importance, they were not analyzed further in our studies to date.

Among the phospholipid group we confirmed the presence of four lipid classes. These were determined to be: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylglycerol (PG). PC was in greatest abundance, while the others were found to be in equimolar concentrations (i.e., PC>>PE = PS = PG). Neither phosphatidic acid (PA) nor phosphatidylinositol (PI) were detected in any significant amounts. Not surprisingly, our evidence indicates that these phospholipid components are the same for both 'Midiron' and 'U3'. Furthermore, their relative amounts did not change significantly during cold acclimation, nor did detectable levels of PI or PA appear. And this is true for both genotypes. However, the fatty acid content of the phospholipid fraction did change.

The fatty acid (FA) constituents of the phospholipids (PL) were analyzed using standard procedures as described in previous investigations. Again, there were four major components present in the PL fraction from both genotypes: palmitic acid (16:0), stearic acid (18:0), linoleic acid (18:2) and linolenic acid (18:3). The changes within the PL fraction basically paralleled those found for total polar lipids, where there was a decrease in shorter chain and/or saturated FAs and a concomitant increase in the longer chain, most unsaturated FA (i.e., 18:3). This overall change in PL-FA saturation level can be quantified by calculating the double bond index (DBI) for each genotype at each tissue-harvest date. The DBI for 'Midiron' was significantly greater than that for U3 by mid-way through the acclimation period, and continued to diverge significantly through the remainder of the three week experiment. Overall, this is attributed to the more rapid and greater increase in the synthesis/amount of 18:3, as compared to the other FAs, in 'Midiron' versus U3.

Among the glycolipids isolated from the total polar lipid fraction, extracted from the two bermudagrass cultivars, we detected the presence of only three lipid classes. Our analysis identified the presence of sterylglucosides, acylated sterylglucosides and cerebrosides. These three glycolipids were detected in equimolar amounts in crown/stolon node tissues of both 'Midiron' and U3. As with the phospholipid analysis, the amounts of these three glycolipids did not vary during cold acclimation, nor were new species identified. Again, as with the PLs, the constituent FAs of the glycolipids (GL) did change during cold acclimation. To a first approximation they too paralleled those changes found when analyzing the FAs in total polar lipids. Interestingly, although the DBI of the FAs from the GL increased during cold acclimation for both 'Midiron' and U3, the DBIs were not significantly different between these two genotypes. This appears to be the result of the combination of a significant increase in 18:3 for 'Midiron' over U3, countered by an increase in 18:2 for U3 accompanying a slight decrease in 18:2 of 'Midiron'.

Overall, it is clear that changes in membrane FA composition, which are known to mitigate many of the deleterious effects of low temperature (e.g., structural lipid packing, reduced membrane fluidity, bilayer phase transition, partitioning, etc.), are detected in two major lipid fractions; the PLs and GLs. However, whether one fraction plays a more influential role is unclear at this time. For example, the primary effect may be the result

of activity in the PL fraction, due to its significant increase in 18:3 as well as in the DBI (over time and between genotypes). However, the GL fraction contains a larger proportion of 18:3 FA than does the PL [e.g., in 'Midiron', 43% (GL) versus 25% (PL) after 21 days of cold acclimation].

Alterations in fatty acid composition of Paspalums in response to low temperature

Seashore Paspalum (*Paspalum vaginatum* Swartz.) has traditionally been used along roadways and sidewalks. Most recently it is being used on golf course fairways, tees and greens. Different accessions and ecotypes of this warm season turfgrass vary in the degree of tolerance to cold temperatures. We studied the changes in membrane fatty acids that occur in three cultivars during acclimation. Not only did we want a better understanding of biochemical responses in the paspalum cultivars, we were very interested to learn how this turfgrass species responded to low temperature with respect to what we have documented for Bermudagrass. Three genotype ('Adalayd', PI299042 and PI509018-1) were kindly provided by Dr. R. R. Duncan, University of Georgia, Griffin, GA. These were grown and treated identically as the bermudagrass cultivars.

Fatty acid changes were analyzed in the acclimating stolon node and crown tissues. Palmitic acid (C16:0) comprised a little over 25% of the total membrane polar fatty acids in these tissues. Under control conditions, no significant changes in the fatty acid composition was observed. During low temperature exposure, in the cold sensitive genotype, PI299042, the levels of C16:0 declined insignificantly throughout the treatment period (see summary Figure C). Similarly, for 'Adalayd" and PI509018-1 (the cold tolerant genotype), overall, there was no significant difference in C16:0 content from the beginning to the end of the three week low temperature treatment (see summary Figs. D and E).

Stearic acid (C18:0) is also found in stolon nodes and crowns of Paspalum, but as a relatively small percentage of the total membrane polar lipid fraction (e.g., less than or equal to 5%) in the three Paspalum genotypes studied. Under control conditions, the level of C18:0 showed a decline after the second week. This change however was not statistically significant. There was no major change in the C18:0 fatty acid composition in the cold sensitive genotype, PI 299042, during low temperature treatment (Fig. C). The intermediate and tolerant genotypes, 'Adalayd' and PI509018-1 (respectively), showed slight increases in the amount of C18:0 during cold treatment (Figs. D and E), with 'Adalayd' having a statistically significant change in the first week. However, because stearic acid represents such a small proportion of the total membrane FA content, an absolute change from ~4% to 5% is not believed to be biologically meaningful.

The major fatty acid found in Paspalum was C18:2 (linoleic acid). It accounted for ~50 % of the total membrane lipid fraction. Overall in the control plants, no significant changes were observed in the C18:2 levels during the three week experiment. During cold treatment however each genotype showed a distinct response. In PI299042 there was a slight and gradual increase in the relative amount of C18:2 that was initiated in the first week (Fig. C). In contrast, 'Adalayd' and PI509018-1 both showed a decrease. C18:2 in 'Adalayd' decreased rapidly during the first two weeks of cold treatment, then leveled off (Fig. D). The decrease for PI509018-1 was more gradual in the first week,

but the level of C18:2 decreased more rapidly and dropped to a lower value in this genotype by the second week, and it too leveled off by the third week (Fig. E).

Linolenic acid (C18:3) is another major fatty acid found in the total membrane polar lipids of the Paspalum genotypes examined. It comprised almost 25% of this fraction. There were no significant changes in the amount of this fatty acid in the control plants. Similarly, there was very little change in the amount of C18:3 in either PI299042 or 'Adalayd' during the three week low temperature exposure. 'Adalayd' did, however, show a trend toward a small increase as exposure to low temperature continued (Figs. C and D). In contrast, there was a significant increase in the percent of C!8:3 present in PI509018-1, by the second week of the experiments (Fig. E). Interestingly, the timing of this increase (day 14) lagged slightly behind (day 7) the decrease observed for C18:2. As such, the lowest amount reached for C18:2 coincided with the highest amount attained for C18:3 (day 14 for both).

The basic hypothesis predicts that the level of cold tolerance will be, at least in part, reflected in the amount and degree of FA unsaturation. As such, plants that are capable of withstanding lower temperatures have been found to contain higher amounts of unsaturated FAs in membrane lipids. The more the increase in unsaturated fatty acid in the membranes, the more fluid the membrane, thus preventing damage as result of cold temperatures. We have observed that this is basically true for the cultivars of Seashore Paspalum studied here. The amount of linolenic acid (C18:3) has almost a seven fold increase in the cold exposed tissues of PI509018-1, the more cold tolerant genotype. This increase appears to have occurred at the expense of linoleic acid (C18:2), which was found to decrease dramatically during the first and second weeks of cold treatment. In the more cold sensitive cultivar, PI299042, such an increase in the amount of linolenic acid was not observed, which could be one of the reasons that this genotype does not withstand exposure to low temperatures as well. 'Adalayd' appears to be somewhat intermediate between PI509018-1 and PI299042 in its ability to alter membrane lipid FA composition. As cold acclimation/cold hardiness is a complex phenotype, the tolerance of 'Adalayd' may reflect other genotypic capabilities not yet investigated (e.g., increased production of cryoprotective solutes).

The double bond index (DBI) provides a mathematically derived comparative measure of the degree of unsaturation at any particular time point for which the relative amounts of each FA species is known. By the end of the experiment there was no significant difference in DBI between 'Adalayd' and 'PI299042' (1.63 and 1.62, respectively). However, the maximum DBI attained by 'PI509018-1' was 0.1 units greater (i.e., 1.73) over this same time.

As outlined above, we have observed very similar alterations in membrane polar lipid FA composition in the paspalum (Figs. C, D and E) and bermudagrass (Figs. A and B) genotypes evaluated. Therefore, and although we intend to characterize the FAs from a few other bermudagrass cultivars (e.g., Arizona common, Midway, Quickstand and/or Vamont), we believe that our findings represent a general trend in the way warm season turfgrasses respond to low temperature, and acclimate to this environmental stress. Despite the similarities in the observed changes in FA composition, the actual value differences between bermudagrass and paspalum may be instructive as well. For example, the change in C18:3 in 'Midiron' is more rapid and its level increases to a greater extent than is observed in PI509018-1, 'Adalayd' or PI299042, and U3.

Based on these results, we continue to be convinced of the important role played by the fatty acid desaturases, enzymes involved in adding double bonds to the FA chains of membrane lipids, in low temperature acclimation. We have evaluated the presence of the genes, encoding these desaturases, at a molecular level (see the following section). At present, we are directing our efforts toward cloning the gene for the ω3 desaturase(s) [which codes for a soluble enzyme that introduces the third double bond --- to convert linoleic acid (C18:2) to linolenic acid (C18:3)], by PCR based cloning methods.

Fatty Acid Desaturase Genes in Bermudagrass and Seashore Paspalums

[Ms. Jenith Cyril and Mr. John Wells (Research Technician)]

Southern blot hybridization analysis allows for the identification of whole genes or gene fragments, within genomic DNA, having similar sequence to that of the gene probe(s) used in the hybridization. In our work, hybridization was performed using as probes three heterologous desaturase genes, cloned from four species. Our purposes is to analyze the presence or absence of these gene-related sequences in the nuclear genome of turfgrass species and cultivars, estimate the number and size of the related gene sequences, identify polymorphic differences (which may be species or cultivar specific), and screen for the suitability of these sequences in RNA (gene expression) analyses that are to follow.

Southern hybridization analysis was performed on six, greenhouse grown, bermudagrass cultivars (i.e., U-3, Midiron, Arizona common, Midway, Quickstand and Vamont), and three paspalum cultivars (i.e., Adalayd, PI299042 and PI509018-1). DNA was extracted from 2 grams of leaf tissue using a solution of hexadecyltrimethylammonium bromide, followed by phenol extraction to remove protein contaminant, and ethanol precipitated to concentrate and recover the purified DNA. Following digestion with restriction enzymes (*Hind*III, *Pst*I and *Eco*RI), the resulting DNA fragments were size fractionated by electrophoresis through a 1.0% agarose gel, and then attached to nylon membranes by vacuum transfer and UV cross linking. The DNA bound to the membranes was hybridized to radiolabeled heterologous gene probes of ω3 desaturase from *Brassica napus* and *Triticum aestivum,* ω6 desaturase from *Arachis hypogea* and ω9 desaturase from *Oryza sativa.* to identify complementary "turfgrass" sequences. Positive results then allow for an estimation of the number of related genes present in the genome. Comparisons of each hybridization pattern (number of hybridizing DNA fragments and their molecular sizes) from one enzyme digestion to another allows for the estimation of gene number (i.e., the number of genes with related or nearly identical protein coding sequence). Some genes are found as single copies, but most are present in gene families, some of which can be very large (and therefore difficult to characterize and work with).

With the exception of ω6 desaturase from peanut, all of the tested gene probes hybridized to distinct DNA fragments in the turfgrass genomes. This is not unexpected because analysis of nucleotide and protein databases indicated low sequence homology between ω6 desaturase genes across even relatively narrow taxonomic/phylogenetic boundaries (much less between monocots and dicots). This

likely explains the lack of discrete hybridization signal when the peanut w6 desaturase gene clone was used to probe the bermudagrass and paspalum nuclear DNAs.

Overall, hybridizations using the ω3 desaturase gene clones from *B. napus* and wheat produced DNA banding patterns that were very similar in size distribution and number. In bermudagrass, fragments (four to nine depending upon cultivar) ranged in size from 4.5 kb to 0.9 kb with both HindIII and PstI, while probing EcoRI digests revealed single fragments of 4.0 kb. This would indicate the possibility of no more than two members in the ω3 desaturase gene family in bermudagrass. Probing Seashore Paspalums with the same ω3 gene clones identified three to five fragments between 6.0 kb and 0.8 kb, and also indicates a gene family of two members.

Analysis of the bermudagrass genome probings using the ω9 desaturase gene clones, resulted in the identification of only two *Hind*III digestion fragments (1.0 kb and 0.5kb), four to five PstI digestion fragments ranging from 3.4 kb down to 0.8 kb, and two EcoRI digestion products of 4.0 kb and 2.3 kb. Principally, because of the short length of the *Hind*III fragments, it is likely that only a single ω9 desaturase gene is present in bermudagrass. Omega 9 desaturase probing of the three paspalums showed multiple bands of less than 2.4kb with both HindIII and EcoRI, and single fragments of 2.5kb with PstI. Therefore, as the case with bermudagrass, it is likely that there is only a single ω9 desaturase gene in the seashore paspalums.

The small gene families indicated for the ω3 and the ω9 desaturases, identified for both bermudagrass and seashore paspalum, are convenient for both genetic and molecular manipulations. Cloning these paired or unique genes will be relatively straight forward. Analysis of their specific expression patterns (e.g., normal and low temperature induced) will not likely be confounded by the presence of numerous transcripts from many other related genes.

Not surprisingly, clear polymorphic differences were observed between the two turfgrass species: bermudagrasses and seashore paspalum. This was true for all enzyme digestions and all desaturase gene probes, where usually one to three polymorphic fragments existed for each digest. Furthermore, there were no common fragments for a given enzyme/gene probe combination shared between bermudagrass and seashore paspalum.

Of real interest were the "within species" comparisons, where several DNA polymorphisms were detected. Such polymorphisms are indicators of genetic variability between the cultivars. With the *Hind*III digest probed with wheat ω3, each bermudagrass could be distinguished. U-3 and Arizona common both had a 4.5kb polymorphic fragment that was polymorphic when compared to the other bermudagrass cultivars. On the other hand, Arizona common was missing a 3.4kb fragment when compared to U-3. 'Midiron' had unique 3.0kb and 2.9kb fragments, but shared a 4.3kb fragment with 'Quickstand'. 'Midway' lacked a 2.4kb fragment present in Arizona common, 'Quickstand' and 'Vamont'.

Similarly, in the *Pst*I digest of bermudagrass DNAs probed with wheat ω3, all six cultivars could be distinguished. U-3 had a unique 3.0kb fragment. 'Midiron' and 'Midway' shared a 1.6kb fragment, but 'Midway' also showed a unique 1.0kb fragment. Arizona common, 'Vamont' and 'Quickstand' showed somewhat similar fragment

patterns. However, 'Quickstand' had a unique 1.9kb fragment, and Arizona common had a 0.8kb fragment not found in 'Midway' or 'Vamont'. In the *EcoR*I digest probed with wheat ω3, Midiron had a unique 4.3kb fragment. As expected, identical results were observed using the ω3 desaturase gene probe from *B. napus* on bermudagrass DNA digests.

Omega 9 desaturase, from rice, presented a similar picture but, bermudagrass cultivars could not be distinguished on the *Hind*III digest membrane. Arizona common and 'Vamont' were polymorphic by the absence of a 3.0kb fragment, but were not distinct from each other when comparing *Pst*I digested DNA. Using *EcoR*I, 'Midiron', 'Midway' and 'Quickstand' shared a 4.3 polymorphic fragment compared to other bermudagrass cultivars. 'Vamont' had a unique 1.3kb fragment.

Analysis of the three Seashore Paspalums was also made. Probing with the ω3 desaturase gene clone(s) detected no polymorphism with *Hind*III digested DNA. In probing *Pst*1 digested DNA, PI509018-1 showed unique 2.4 and 2.0 fragments that could be used to distinguish it from 'Adalayd' and PI299042 --- both of which shared a common 2.1kb fragment. 'Adalayd' was distinct from PI509018-1 on the *EcoR*I membrane, which revealed polymorphic fragments of 2.9kb and 1.3kb. The rice ω9 probing showed a polymorphism only in the *Hind*III digest, where PI509018-1 had a unique 2.3kb fragment.

Gene copy reconstructions of one and ten copy equivalents were used on all membranes, and they served as positive controls. In all cases, fragments in the digested turfgrass genomic DNA showed a similar hybridization intensity to the single copy loading equivalent of the "heterologous" gene probe, indicating the absence of multiple, tandemly repeated copies of these gene sequences in the bermudagrass or seashore paspalum genomes. The mere presence of these sequences does not imply gene activity. Confirmation by RNA gel blot analysis or ribonuclease protection assays will be required and is ongoing. The presence of specific desaturase genes, as indicated by Southern blot analysis, is a preliminary step in their isolation and characterization of their function relative to cold hardiness in turfgrass species.

FATTY ACID CHANGES IN 'MIDIRON' (COLD TOLERANT)

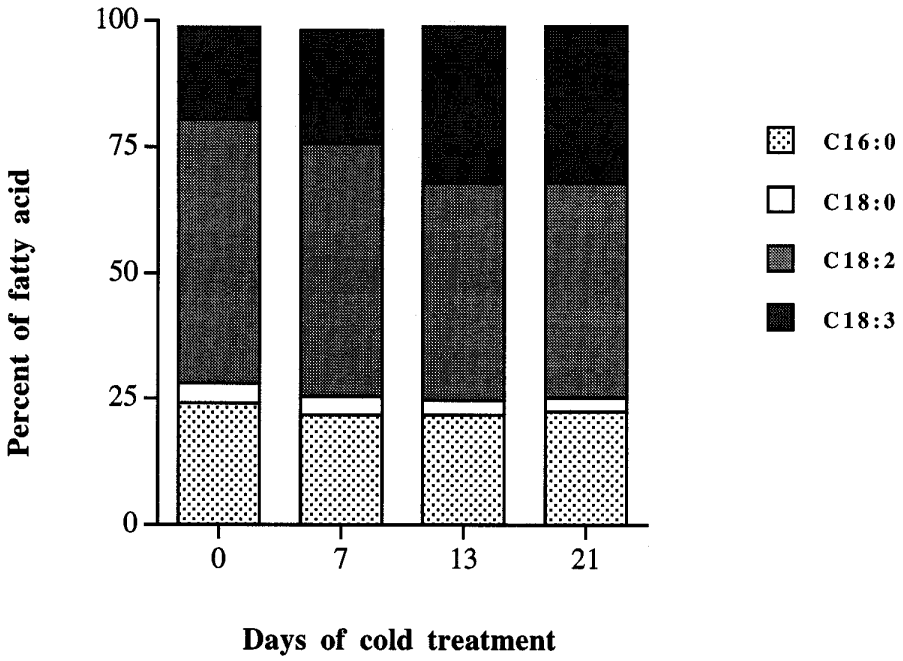

Figure A

FATTY ACID CHANGES IN 'U3' (COLD SENSITIVE)

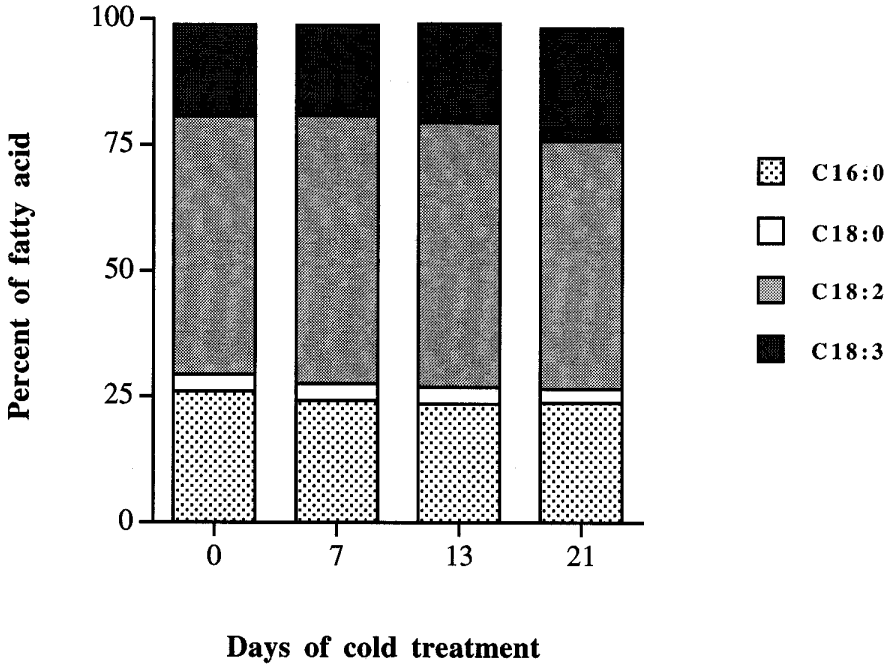

Figure B

FATTY ACID CHANGES IN PI299042 (COLD SENSITIVE)

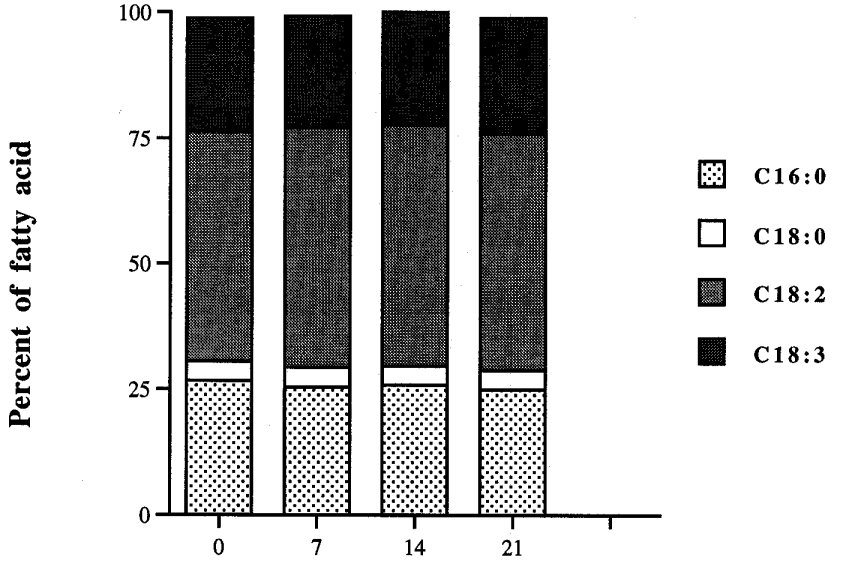

Figure C

FATTY ACID CHANGS IN 'ADALAYD' (INTERMEDIATE)

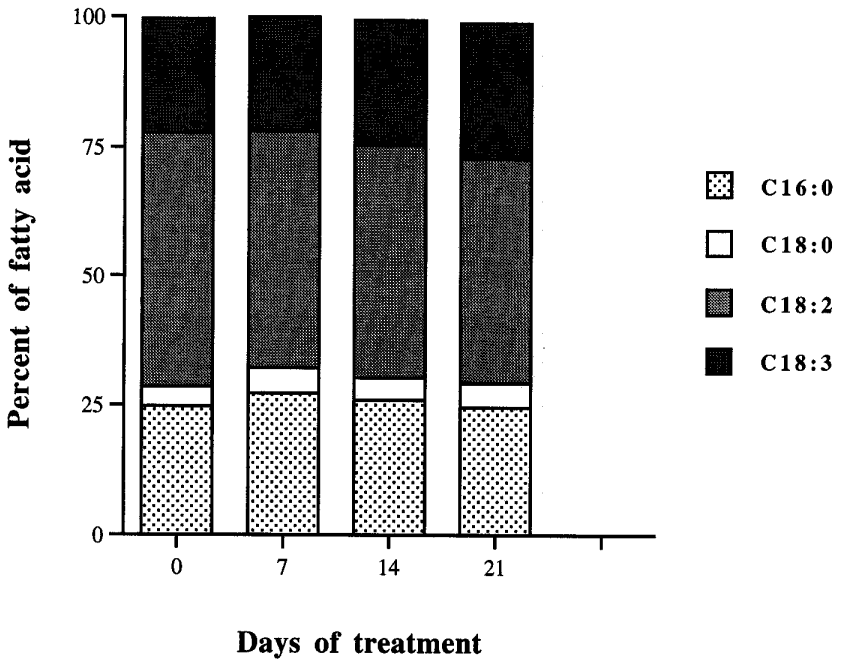

Figure D

FATTY ACID CHANGES IN PI509018-1 (COLD TOLERANT)

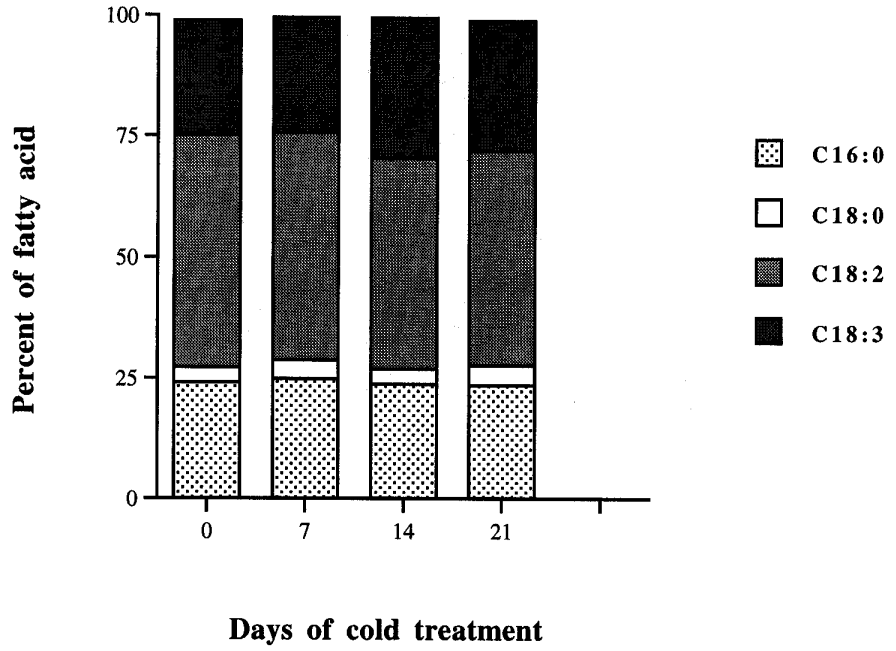

Figure E