HEAT SHOCK PROTEIN (HSP) FORMATION IN ANNUAL BLUEGRASS
Preliminary Report for the USGA Research Committee

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Introduction

Attached is a brief report describing our initial heat screen for annual bluegrass (Poa annua L.). We accomplished our initial goal of obtaining a relative heat tolerant and heat sensitive biotype of annual bluegrass. A 12 C separation between the most heat tolerant (Avon Oaks Country Club, Avon, Ohio) and the most sensitive (Chemlawn R&D, Douglasville, Georgia) biotype was observed. The screen was repeated during two consecutive years. However, annual bluegrass heat tolerance seemed to depend on the time of the year the screen was done. Early summer screens resulted in good separation of the biotypes. This separation disappeared during fall screens. It appeared as Fall approached the heat tolerance of the biotypes decreased and in some instances the heat sensitive biotypes were more heat tolerant than the heat tolerant biotypes initially screened. Why this occurred is open to speculation beyond the scope of this study.

An interesting observation was made that may explain why annual bluegrass is sensitive to drought and pest stresses. When we subjected biotypes to heat treatments, followed by a two week recovery period, the heat treated biotypes that survived appeared visually the same as the non-treated biotypes (controls). However, if these biotypes were subjected to a minor moisture stress (a level at which no visual affect was observed on the controls), the heat treated biotypes died. From this observation it appears that heat predisposes annual bluegrass to moisture stress. Further, work is needed to quantify this observation. Although it may help explain the sensitivity of annual bluegrass to summer diseases such as anthracnose that are caused by relatively weak pathogens. Again this is conjecture on my part but it does bring up the possibility of future research projects.

The two biotypes form Avon Oaks C.C., Ohio and Douglasville, Georgia along with "Victa" Kentucky bluegrass and a tall fescue cultivar have been established in suspension culture to provide a sterile system to evaluate heat shock protein (HSP) formation in these grasses. We are currently in the process of determining if these proteins do form and in what quantity.

I feel this work will be successful and completed by the summer of 1987. At this time, I think the use of HSP for determining heat tolerance in breeding programs is still feasible. Preliminary work at Cornell University has shown differences among corn hybrids with regard to HSP formation. It may turn out to be a method for "finger printing" turfgrass cultivars. The project is progressing well and will hopefully yield information important to the turfgrass community.

EFFECT OF HIGH TEMPERATURE STRESS ON THE SURVIVAL RATE OF ANNUAL BLUEGRASS BIOTYPES

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Annual bluegrass (Poa annua L.) has been classified as a weed in most agronomic situations. However, its ability to tolerate mowing heights as low as 3 mm makes it a predominant grass species of golf courses in the northern United States and Canada. It is an important golf course turf since it is one of the few turfgrasses able to withstand the low mowing height necessary for greens maintenance.

Poa annua distribution is worldwide: Europe, North Africa, North Asia, Australia, South and North America, and Antartica (7). It is thought to be a hybrid between Poa supina, a creeping perennial found in the mountainous regions of Central and Northern Europe, and Poa infirma, an upright growing annual from the lowlands of the Mediterranean region (11). Its growth pattern as a winter annual was recongnized by Carl Linnaeus, who named it Poa annua in 1743. A wide range of supspecies exist that differ in their appearance, growth habits, apomictic tendiencies and response to environmental stresses. Forty-eight subspecies have been described and most certainly there are many more (16).

Two commonly occurring subspecies of annual bluegrass are Poa annua spp annua and Poa annua spp reptans. The subspecies annua has a tufted, bunch type growth habit; has few adventitious roots; is largely outcrossing; and prone to a large amount of seed production. It is found largely in non-irrigated conditions and dies after seed production (3).

Poa annua spp reptans represents the other extreme. It is largely apomictic, has sparse seed production, is found in irrigated conditions, and is perennial in nature. Its growth habit is creeping, therefore it has more leaf and node numbers and secondary tillers than the annua subspecies (3).

Although Poa annua enjoys a worldwide distribution, it is poorly adapted to high and low temperatures (2). High air temperature stress produces two types of symptoms. Leaf tip die back occurs during long term or chronic exposure to temperatures of 35 C day and 23.9 C night. Short term or acute exposure to higher temperatures results in leaves changing from dark green to purple and ultimately collapse of the plant (16). A two hour exposure to 42 C has been shown to be lethal to Poa annua (18). Heat stress damage has been described as a function of time per thermal unit or $T = 1 - b \log z$, when T = killing temperature, z = killing time, and a and b are constants for intercept and slope respectively (9). Some reviews of plant temperature stress have implied that long term elevated growth temperatures are comparable to high temperature shock (9,10). There is much controversy regarding this subject in the literature citations. The conclusion is dependent upon which physiological index is used to evaluate plant response.

Two approaches to screening for heat tolerance at the whole plant level have been taken. Plant samples may be submitted to moderately high temperatures for an extended period of time (weeks) or to high temperatures for a short length of time (minutes). Prolonged exposure to moderate temperatures allows selection for efficient CO_2 fixation, superior carbohydrate partitioning, low CO_2 compensation points and decreased light and dark respiration (11,4). High temperature is thought to evaluate membrane stability, protein synthesis and protein stability under stress (19).

Wallner et al. (15) have presented evidence that electrolyte leakage from heat stressed leaf pieces can predict the drought resistance of several turfgrass species. In contrast, Wehner and Watchke (18) found no significant differences in afflux of cell solutes among heat tolerant and susceptible turf species within a range of 43 C to 49 C. Neither cell leakage nor protein synthesis inhibition correlated with the rate of recovery determined by shoot growth. A 50% loss of all solutes was not realized until leaf pieces had been heated at 50 C for 150 minutes or 55 C for 20 minutes (15). Protein synthesis of Poa annua was reduced to 25% and 9% by 30 minute exposure to 43 C and 45 C respectively and was not significantly different from heat tolerant turf species (19). Wehner argues that his screen does not examine direct injury as previously thought (19). Although his laboratory studies correlated well with the heat tolerance evidenced in field studies (20).

North American Poa annua populations have been evaluated for heat tolerance by Cordukes (6) and Wehner (20). Both researchers evaluated Poa annua for recovery from brief high temperature (47 C) exposure. Five percent of the Canadian populations were assigned a good recovery rating. The Illinois collection displayed a 60% reduction in dry weight when compared with the control. There was a biotype which demonstrated great sensitivity to high temperature. A 4 C difference in absolute temperature tolerance was found between the most and least temperature tolerant biotypes.

The purpose of this study was to screen various biotypes from around the country to find two biotypes that vary the most in heat tolerance. These biotypes would then be used in studies to investigate possible mechanisms of heat stress.

Methods and Materials

Plugs 8.8 cm in diameter were cut on the edge of golf greens from courses located in the United States (Figure 1). One hundred individual plants were isolated from each plug by hand, planted into 5.7 cm square pots containing soiless planting mix (Reddiearth) and grown in the greenhouse at 21 $\pm$ 10 C. Plants were screened for high temperature tolerance using the method described by Wehner (18). Plants were pre-acclimated for two days in a growth chamber held at 26.7 C for 16 hours light and 21.1 C for 8 hours dark prior to the temperature screen. A tiller from each plant was included in each temperature treatment. There were five plants from each biotype included in the screen and the screen was repeated three times during the summer. Treatments consisted of 30 minute exposures to hot water baths maintained at 26, 38, 40, 42, 44 and 46 C. The plant material was cooled and transplanted in 98 cell trays filled with Reddiearth. The trays were arranged randomly in the growth chamber. The growth chamber was maintained as previously described. Plants were watered daily. Fresh weights were taken of new growth after a two week interval.

Fresh weight of treated tillers was divided by the fresh weight of the control for each plant. The adjusted fresh weight means were regressed over temperature treatment and the area under the response curve was found for each biotype in each replicate. LT 50's, the lethal temperature where 50% of the population dies, were calculated for each biotype. The data were analyzed by analysis of variance and biotype means were separated by LSD.

figure 1. List of Poa annua specimens and locations.

Code	Location
10	Harts G.C., Merion, Indiana
20	Anderson G.C., Anderson, Indiana
24	Findley C.C., Finley, Ohio
28	Michigan State University Turf Program, Lansing, MI
31	Chemlawn R & D, Douglasville, Georgia
37	Shawnee Hills G.C., Portsmouth, Ohio
60	Foster Park G.C., Fort Wayne, Indiana
71	Pebble Beach G.C., Carmel, Califorinia
74	Erie C.C., Cincinnati, Ohio
75	Little Scioto G.C., Portsmouth, Ohio
76	Cincinnati C.C., Cincinnati, Ohio
77	Shawnee Hills G.C., Portsmouth, Ohio
78	Ironton C.C., Ironton, Ohio
79	Elk G.C., Portsmouth, Ohio
80	Running Fox G.C., Cincinnati, Ohio
81	Jaycees G.C., Chillicothe, Ohio
83	Kenwood C.C., Cincinnati, Ohio
84	Unknown
89	Halcount G.C., Winston-Salem, North Carolina
92	Avon Oaks C.C., Avon, Ohio
93	Wooster C.C., Wooster, Ohio
94	Unknown from Findley area
95	Unknown
97	Wyethville C.C., Wyethville, Virginia
102	Shaker Heights C.C., Cleveland, Ohio

Results

Unlike Poa pratensis L. (17) there appears to be no correlation between Poa annua biotypes selected from warm regions and heat tolerance (Figure 2). Wehner's study of heat tolerance of Poa annua collected in Illinois found no correlation between location and heat tolerance (personal communication). Regions with high temperature and no irrigation generally promote the annual type. Heat avoidance is the primary mechanism of survival at high temperatures. Several southern grasses capable of withstanding high temperature enter a quiescent period until temperatures more favorable for growth occurred (3).

The growth response is characterized as the ratio of fresh weight of the temperature treatments to the control. The areas under the fresh weight response curves were not significantly (P=0.05) different for biotypes. This finding conflicts with Wehner who used plant growth as an index of temperature tolerance (18). There was a considerable range of areas among biotypes; unfortunately there was also considerable variability in the data. Comparison of fresh weights among controls revealed significant differences among biotypes.

It appeared that for several biotypes 38 to 42 C were the optimal temperatures for growth. The significant block effect reflected the decrease in temperature tolerance experienced by all biotypes as the summer progressed. This trend was documented by Wehner (20). No difference in heat tolerance was discernable among biotypes in October.

Mean survival was regressed over treatment and LT 50's were calculated. Significant differences were found among biotypes and blocked. Separation of means produced two distinct groups of high and low temperature tolerance (Table 1). Three response patterns were found which are best described as linear, quadratic and stepped. The stepped consists of comparable survival rates until a critical temperature is exceeded then there is no survival. Since there was only a two degree difference between treatments this indicates a critical maximum temperature.

A low overall survival rate in certain genotypes biased estimations of LT 50's. Estimates of LT 50's were generally higher when treatment response was corrected for control. The ranking of biotypes with high and low LT 50's was the some whether response was correlated by control or not. Estimates of LT 50's which are not corrected by controls will by used in this report.

The most heat tolerant biotype in this study was sampled from Avon Oaks C.C., Cleveland, Ohio. LT 50 was greater than 46 C. The lowest tolerant biotype was sampled from Douglasville, Georgia.

Conclusions

It was surprising that heat tolerance did not correlate with the location the plants were sampled. Examination of growth habit might be helpful in determining the mechanism of heat tolerance/heat avoidance among southern specimens of _Poa annua_. It is understandable that cooler climes may have genes for heat tolerance which have not had the opportunity for expression and selection. It is curious that plants sampled from the South and transition zone made such a poor showing.

Growth response proved to be a poor index for selection of heat tolerant material. Nelson's research on tall fescue sheds some light on why this is true. He found that tillering and leaf expansion rate are negatively correlated (14). Sampling of biotypes which display strong tillering may not compare favorably with those which channel their growth in leaf expansion. Measurements of individual plant growth rather than changes of grass contributed significantly to the experimental error. In addition, growth, particularly measured as fresh weight, is profoundly affected by available moisture. This is a probable source of error since use of the small chambers made it difficult to maintain optimum moisture levels. Sampling of new growth after temperature treatments often resulted in tiller death.

Survival, expressed as LT 50 appears to be a much more reliable measure of heat tolerance. Less labor is involved and less plant material is lost. Future screens should include a 48 C temperature treatment, since a few biotypes LT 50's exceeded the 46 C maximum in the experiment.

It appears that a temperature optima ranging from 38 to 42 C may exist for several Poa biotypes. Survival and growth were enhanced in this temperature range. It is logical that when temperature is not growth limiting; growth, a function of chemical reactions, would be promoted with increasing temperature. Certain biotypes displayed a critical maximum temperature at which survival was not possible. Perhaps a critical enzyme is not functional or water transport is impeded at higher temperatures.

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Figure 2. Mean tolerance of 25 annual bluegrass biotypes to high temperature stress.

Biotype number	Mean survival*
92	47.000
76	46.100
97	45.750
24	45.133
94	44.450
75	44.200
60	43.900
28	43.750
37	43.150
80	42.675
71	42.500
93	42.417
83	41.850
10	41.833
77	41.500
95	41.083
78	41.033
81	40.500
102	40.400
74	39.950
79	39.633
84	39.467
89	39.267
20	36.900
31	35.167
LSD (0.05)	5.151

* Temperature at which 50% of the population survived (LT50).

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USGA EXECUTIVE SUMMARY

THE OHIO STATE UNIVERSITY - Dr. Karl Danneberger, Principal Investigator

Mechanisms for heat tolerance in annual bluegrass

Heat screen for annual bluegrass biotypes

Twenty-five poa annua biotypes collected from the continental United States were screened for high temperature tolerance. A 12°C difference was detected between the least and most tolerant biotypes. The most heat tolerant biotype in this study was sampled from Avon C.C., Cleveland,Ohio and the least from Douglasville, Georgia. No correlation between location and tolerance was found. High temperature treated biotypes were more prone to drought stress than non-treated. It was interesting to note that the experiment was repeatable during the summer months but no difference in heat tolerance was detected among biotypes when screened during the Fall (October).

Future objectives

The two biotypes from Avon, Cleveland and Douglasville, Georgia along with "Victa" Kentucky bluegrass and a tall fescue cultivar are being propagated in suspension culture. This plant material will be used to determine if and in what quantity heat shock proteins (HSP) are formed. These proteins will be evaluated for feasibility and practicality as a rapid screening method of determining high temperature tolerance in turfgrass genetic material.