AN EVALUATION OF HIGH TEMPERATURE EFFECTS ON ANNUAL BLUEGRASS (POA ANNUA L.)

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ABSTRACT

AN EVAULATION OF HIGH TEMPERATURE
EFFECTS ON ANNUAL BLUEGRASS
(*Poa annua* L.)

by James Alan Fischer

The effects of high temperature on *Poa annua* were studied in a specially designed wind tunnel chamber. Wind speed in the chamber was 11.4 miles per hour, the relative humidity was approximately 100 percent and temperature treatments ranged from 37.2° C. to 44.9° C. Exposure times varied from 10 minutes to 12 hours. Injury was evaluated by gross observations and a histological technique.

High temperature resulted in a systematic injury to plant tissue. Injury occurred first at the junction of the leaf sheath and leaf blade of all affected leaves.

With a longer exposure time or higher temperatures, a longer period of time was required for leaf growth to recommence. The rate of elongation and total elongation were decreased with increasing severity of treatment. With increasing severity of treatment, fewer tillers and roots were produced from the lower portion of the crown. These roots were shorter, more spindly and more highly branched than roots of normal plants.

The order of cellular changes observed was protoplasmic granulation, protoplasmic coagulation, cell wall breakdown and total cell collapse.

Kill was a function of exposure time. A much shorter period of time was required to obtain 50 percent kill at higher temperatures than at lower temperatures.

AN EVALUATION OF HIGH TEMPERATURE EFFECTS ON ANNUAL BLUEGRASS (*POA ANNUA* L.)

By

James Alan Fischer

A THESIS

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DEDICATION

This thesis is dedicated to Janet, whose "I think I can" leads inevitably to "I will."

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. J. B. Beard, Dr. C. R. Olien, and Dr. C. M. Harrison for their assistance, guidance and criticism during the course of this study and the preparation of the manuscript.

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INTRODUCTION

The common grasses may be classified as cool-or warm-season, depending on their optimum temperature for growth. Most of the grasses adapted to the North Central and Northeastern states are cool-season grasses. Heat and moisture stress frequently occur in this area causing growth reduction and, occasionally, death of the cool-season grasses. Poa annua L. is particularly subject to mid-summer kill.

Poa annua is widely distributed on high-maintenance, high-stress, close-cut, irrigated turfgrass areas such as golf course fairways, greens and tees. It is better adapted to close mowing than the bentgrasses or Kentucky bluegrasses on heavy soils which are compacted and water saturated. Annual bluegrass, a prolific seed producer, initiates seeds throughout the spring, summer and fall, even under the 6.3 mm. mowing height common on the putting green. It is estimated that Poa annua comprises approximately 75 percent of the total irrigated golf course turf in Michigan. Approximately one million dollars is spent each year on maintenance of this Poa annua turf.

A serious problem with annual bluegrass is that it often turns brown and dies in mid-summer, sometimes even with adequate irrigation. The cause of death has been attributed to such factors as disease, drouth, wilt or direct high temperature injury. Since Poa annua is a turfgrass of significant

importance in Michigan, a more complete understanding is needed regarding the effects of high temperature.

This study was initiated to establish some biological criteria, through a histological approach, that would aid in qualitatively distinguishing the nature of high temperature stress.

Once these criteria are established for evaluating high temperature stress, stress due to desiccation may be similarly investigated.

REVIEW OF LITERATURE

The temperature giving the maximum growth rate independent of the time factor has been defined as the optimum temperature for growth (17). The optimum temperature is actually a range rather than a single temperature since temperature effects are conditioned by other environmental factors. World plant distribution is largely governed by the prevailing temperature and moisture regimes.

Based on the daily increase in dry weight, Mitchell (20) reported an optimum temperature of 20° C. for perennial ryegrass (Lolium perenne L.), orchardgrass (Dactylis glomerata L.), colonial bentgrass (Agrostis tenuis Sibth.) and velvetgrass (Holcus lanatus L.). The optimum for Dallisgrass (Paspalum dilitatum L.), a warm-season grass, was near 30° C. A rapid decline in growth occurred above the optimum. Growth of the cool-season grasses ceased above 35°C. He also reported a decrease in new tillers above 28° C.

Brown (3) and Harrison (8) reported an optimum near 20° C. for top growth of Kentucky bluegrass (Poa pratensis L.) and 16° C. for root and rhizome growth. Sullivan and Sprague (24) also reported an optimum near 20° C. for perennial ryegrass. Brown reported soil temperature to be more important than air temperature in relation to grass growth.

Darrow (6) grew Kentucky bluegrass at soil temperatures of 15, 23, and 35° C. while exposing the shoots to normal greenhouse temperatures. Growth was best at 15 and 23° C. with a large reduction in growth occurring at 35° C.

This reduction in growth should be distinguished from direct high temperature injury, which is sometimes characterized by a break in the time vs. temperature curve. Levitt (19) termed the reduction in growth as indirect injury and divided it into metabolic and transpirational high temperature injury. The direct non-metabolic effect of high temperature is shown by the inability of the CO_2 or O_2 supply to increase or decrease the heat injury. Such evidence is usually unnecessary since the killing time is usually short and death occurs before the plant has had time to acclimatize.

Direct heat injury may occur under natural conditions. Huber (14) reported that, in many cases, plants reach and even exceed the 45-55° C. range which is usually accepted as the normal temperature limit for most plants. Hopp (13) reported temperatures as high as 37.7 to 41.1° C. inside ripe tomatoes at air temperatures of 26.7 to 28.3° C. The highest leaf temperature recorded by Harder (7) was 44.25° C., 7.75° C. above that of the air. Perhaps the greatest danger of heat injury occurs when the soil is exposed to insolation, and temperature as high as 55 to 75° C. occur.

On over-grazed range areas in the semi-arid West, Julander (15) reported soil temperatures at the 0, 127 and 254 mm. depths reached a maximum of 51.5, 50.0 and 48.5° C.,

respectively, with an air temperature of 37° C. He assumed that the high soil temperature reached during drought on over-grazed range can be a direct cause of death of range grasses.

The phenomena known as scald occurs on waterlogged turfs under conditions of high temperature. Some observers have attributed scald injury to direct high temperature effects. According to Montieth and Dahl (21), it is improbable that the temperature of water in the soil ever reaches a point which would cause injury to plants. They further stated that true scalding of plants--that is, injury from actual exposure to water of high temperature--has not been sufficiently studied to determine whether such injury actually occurs on turf.

Laude (16) reported an increase in heat resistance of plants when exposed to light. The loss of heat resistance in darkness was slower than the gain in resistance in light.

Sachs (23) found that the blades of young, fully grown grass leaves were the first killed after exposure to high temperature. Younger leaves which were not fully grown and bud parts were more hardy. The old, healthy leaves were the most heat resistant.

Several attempts have been made to evaluate the gross effects of high temperature on plants. A percent survival rating at a certain period of time after treatment is commonly used, with the plants grown in an optimum environment.

Heyne and Laude (11) subjected corn seedlings to 54.55° C. for 5 hours at a relative humidity of 25-30% and called their results heat resistance. Soil pots were saturated throughout the experiment. Their test gave good correlation with field drought studies.

Carroll (4) studied the effects of high soil temperature accompanied by high soil moisture, under high and low nitrogen. Plugs of sod of various grasses were heated in a bath. The temperatures used were 50° C. and 60° C., with plants removed as these temperatures were reached in the center of the plug. In a third treatment, the plugs were held at 50° C. for 4 hours. The time required for the center of the sod plugs to reach the treatment temperature was approximately 2 hours. The air above the sod plugs did not reach the temperature of the water surrounding the soil. Poa annua grown under a low nitrogen level showed survival of 100, 20 and 60%, respectively, at 50° C., 60° C., and 50° C. for four hours, and 70, 0 and 55%, respectively, under high nitrogen. Poa pratensis grown under a low nitrogen level showed survival of 100, 2 and 25%, respectively, and 100, 2 and 40% under high nitrogen. Lethal temperature varied with time of exposure. Soil temperature frequently influenced plant survival more than air temperature. The observed injury was due to thermal effects upon the protoplasm, though Carroll made no microscopic examinations.

Carroll observed the survival of various turfgrasses at air temperatures of 40, 50 and 60° C. with treatment times

of 4 and 6 hours at each temperature. The moisture content of the soil in the pots remained relatively high during the treatment, but the relative humidity of the air passing over the grass leaves was approximately 15% at 50° C. Exposure of all grasses to 40° C. for 4 or 6 hours resulted in only slight injury to the leaf tips at the high and low nitrogen levels. The test at 50° C. for 4 hours showed very little injury to any of the grasses grown under the low nitrogen level, but extensive injury under high nitrogen. The greatest difference among the grasses was obtained at 50° C. for 6 hours. Poa annua showed 60 and 40% survival, respectively, under low and high nitrogen, while Poa pratensis showed 80 and 40% survival.

Mueller and Weaver (22) subjected prairie grass seedlings to temperatures of 57.22 to 62.78° C., a wind speed of 2-6 mph., a relative humidity of 10-29% and exposures of 8-9 hours. Plants were growing in soil in flats when treated. Leaves of the short-grass seedlings were slightly injured by 62.78° C. while the taller-growing species were killed. Factors such as the difference of a few weeks in age and previous exposure to drought had no significant effect on survival.

Julander (15) cut 3.8 cm. pieces from stolons of range grasses, transferred them in lots of 8 into stoppered glass tubes, and immersed them in a constant temperature bath at 48 $\pm$ 0.1° C. for periods of 0, 1/2, 1, 2, 4, 8 and 16 hours. The stolon pieces were then planted and recovery

was estimated after 4 weeks. He found buffalograss and Bermudagrass to be the most heat tolerant, bluestem intermediate, and slender wheatgrass, smooth bromegrass and Kentucky bluegrass low in tolerance.

Several early workers have observed direct high temperature injury to plant tissue under the microscope. Sachs (23) described a heat solidification of protoplasm that may be reversible on cooling. Lepeschkin (18) described four stages of heat coagulation in Spirogyra cells: (1) An imperceptible change in dispersion is detected by an increased permeability to water. The starch grains show just detectible swelling. (2) Starch swells significantly due to a greater increase in permeability and coagulation of the protoplasm surface begins. There is often a movement of the chloroplast ribbon toward the middle of the cell. (3) Complete heat swelling of the starch follows complete coagulation of chloroplasts. (4) The proteins coagulate completely. He observed the formation of fine granules in the superficial protoplasmic layers and noted that the nucleus was the first to coagulate at 43° C. He stated that cell recovery is no longer possible after chloroplasts have begun to coagulate. On the other hand, coagulation of the superficial protoplasmic layer may be reversed.

The following authors were cited by Levitt (19). Doring was able to detect heat swelling of chloroplast starch in living cells and suggested that this may actually injure the protoplasm. Scheibmair observed the first signs of heat

injury in the chloroplasts of mosses. They enlarged and became pale and irregular in contour. Porodko stated that S-shaped curves relating heating time to injury may be obtained. According to Belehradek (1), these are probability curves and simply mean that the organisms of a single kind possess unlike heat killing temperatures in accord with the laws of variability.

The following authors were cited by Belehradek (1). Nagele made the first observation of heat-induced protoplasmic contraction while working with Nitella. According to Policard and Mangenot, mitochondria suddenly disappear in plant cells at 48-50° C. Magestris and Schafer claimed that the cellulose wall of plant cells easily loses its phosphatids and sterols when the temperature rises slightly above 30° C. Kemp and Juul reported that a dividing nucleus is more sensitive to heat injury than when in the resting stage. When heat exposure is too long, assymetric divisions of chromosomes may result, giving rise to permanent modifications and true mutations in higher plants. Milovidov found that chromosomes swell up and finally dissolve after exposure to high temperature. Belehradek and Melichar showed that, in multicellular organisms, the single cellular elements do not die at the same time when subjected to a certain degree of heat. Using leaves of Helodea canadensis, they described a wave of injury proceeding from the base of the leaf towards its apex. Cells near the axis and at the borders were more rapidly destroyed than the rest. However, they stated that the

gradient may actually be due to the injurious effect of cutting, which may be propagated from the base to the tip of the leaf.

Belehradek (1) listed several theories of injury by heat, along with a critical review. These theories were: (1) Heat coagulation of protoplasmic proteins. (2) Heat destruction of enzymes. (3) The asphyxiation theory. (4) The intoxication theory. (5) The theory of lipoid liberation. He concluded that the effects of high temperature on living systems are so complex that no single theory could reduce them all to a uniform basis. He assumed that any of the above theories could become, under certain conditions, the primary factor in the determination of death by heat.

Belehradek and Melichar (2) point out that the temperature coefficient of the length of survival in various living systems is low for moderately high temperatures and higher for very high temperatures. Also, there is often a break in the temperature-time graph in the neighborhood of 35-45° C. They concluded that there are at least two separate processes which can cause injury by heat at various temperature intervals.

Levitt (19) stated that if protein denaturation is the cause of heat injury, there are two possible mechanisms of hardiness: (1) Increased stability of protoplasmic proteins. (2) Increased speed of resynthesis of the proteins. He also listed three similarities among types of hardiness: (1) The environmental conditions that induce dehydration hardiness

(cold, drought, low nitrogen and sometimes other nutrients) also increase heat hardiness. (2) Factors correlated with dehydration hardiness (e.g., low moisture and high sugar content) have also shown a good correlation with heat hardiness, though in both cases exceptions occur. (3) The order of hardiness of different cells to dehydration parallels the order of heat hardiness. On the basis of these similarities Levitt concluded that the same factors prevent the denaturation of the protoplasmic proteins, whether they are exposed to dehydration or heat injury. According to the mechanical theory, heat injury is due to the mechanical weakness of protoplasm which causes it to succumb to thermally induced oscillations.

MATERIALS AND METHODS

Plant Conditioning. All experimental material was obtained from one stoloniferous clone of Poa annua in order to insure genetic uniformity. Several dozen plants, vegetatively propagated from the parental clone, served as the source of plant material. These plants were grown in the greenhouse under continuous light to promote vegetative growth and shoot production. They were watered every second day with a 1/10 concentration of Hoagland's #2 complete nutrient solution.

Uniform shoots at the 3 to 4-leaf stage were selected from the mother plants and planted in sand in 10 cm. diameter pots. Nutrients were supplied every second day with a 1/10 concentration of Hoagland's #2 complete nutrient solution. The plants were grown for 19 days in a growth chamber at a 10-hour photoperiod, a 17.2° C. day-and 15.6° C. night-temperature, a relative humidity of 74± 3% and a light intensity of 900 foot-candles. At 19 days, the plants were at the 7 to 9-leaf stage with 6 to 12 roots.

Treatment Procedures. After 19 days growth, the plants were removed from the growth chamber and taken to the laboratory where they were allowed to reach equilibrium with room temperature (21.1° C.). The plants were removed from the pots and washed in water to remove all sand. The roots were clipped to within 130 mm. of the base of the stem and the

leaves to within 130 mm. of the apical meristem before placement in the treatment chamber. During clipping the plants were kept moist under a spray apparatus. Twelve plants were tested per treatment with two replications.

The plants were treated in a specially designed wind tunnel chamber. The vapor pressure and temperature of the air were produced by an Aminco-Aire unit (Figure 1). This apparatus had heating and cooling elements immersed in a water bath, a low pressure spray apparatus and dry heating coils that permitted control of temperature to within $\pm$ 0.4° C. and relative humidity to within $\pm$ 1.2%. Wind speed in the tunnel was 11.4 mph. In all tests the plants were treated at approximately 100% relative humidity in order to permit evaluation of the effects of heat alone with little or no interaction from moisture stress. The relative humidity recorder, using a lithium chloride sensing element, registered 99.7% relative humidity so there is the possibility that moisture stress might have affected the results.

Whole plants were used in order to evaluate the response of all plant parts exposed to the same treatment conditions. Twelve plants could be treated in the wind tunnel chamber at one time. They were suspended on 4 clips beneath a small revolving platform which insured uniform exposure (Figure 2). Exposure times were measured with a stop watch.

Treatment temperatures used were 37.2, 37.6, 37.8, 39.5, 40.2, 41.2, 42.0, 42.3, 43.4 and 44.9° C. Exposure times

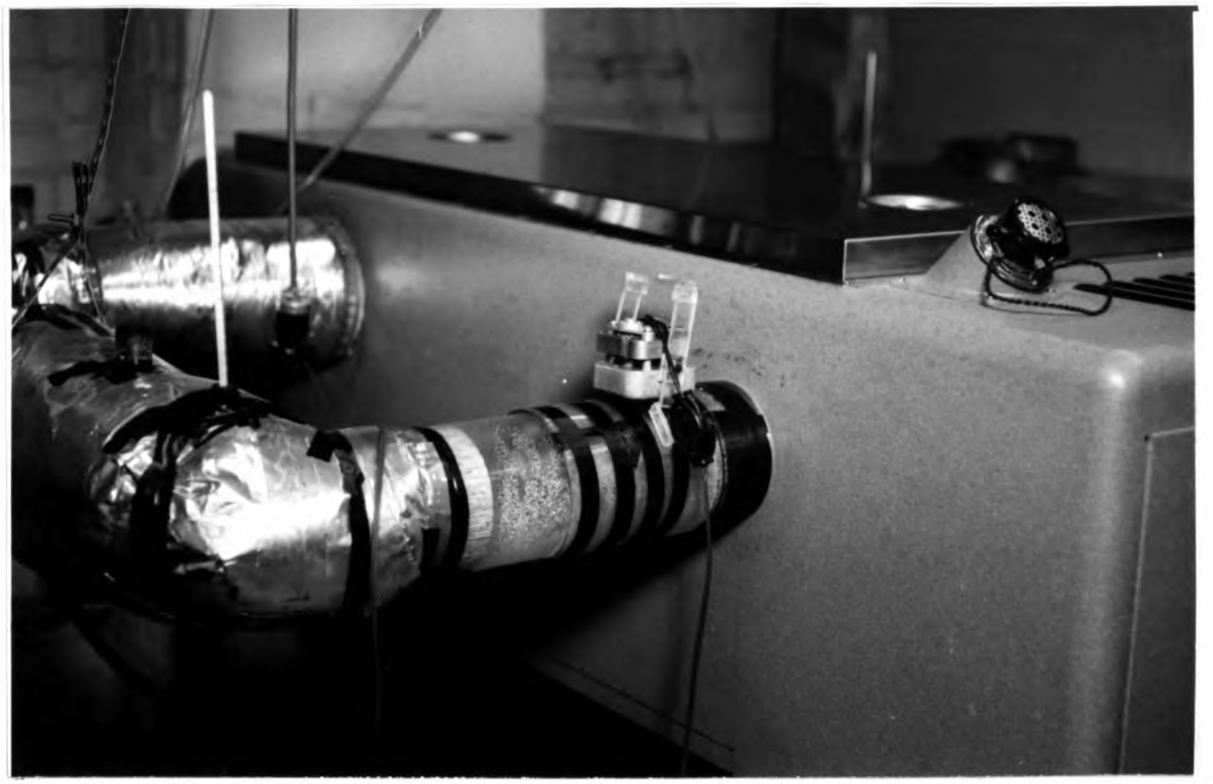


Figure 1.--Aminco-Aire unit with attached wind tunnel. The motorized revolving platform is in position in the treatment chamber in the center of the picture. The fan (not shown) is located in the insulated wind tunnel on the left side of the picture. The air is conditioned in the Aminco-Aire unit before passing into the treatment chamber.

Figure 2.--Revolving platform with clips for holding plant material. View is just above the treatment chamber.

varied from 12 hours at the lowest treatment temperature to 10 minutes at the highest treatment temperature.

Immediately after treatment, the plants were placed in sand in 19 x 28 cm. trays and watered with nutrient solution. Stakes were placed behind each plant to measure the date and rate of elongation. The flats were then placed in plastic freezer bags with the end left open to insure a more favorable moisture regime for regrowth of the plants. The trays were placed in a growth chamber with a 20-hour photoperiod, a 18.3° C. day- and 12.8° C. night-temperature, a relative humidity of 20% and a light intensity of 2000 foot-candles. The sand temperature and air temperature under the plastic bags was 17.2° C. which is close to the optimum for root and top growth of Poa annua (9).

Evaluation of Heat Injury. The grass plants were observed at intervals after treatment to determine the nature and location of injury due to heating. The number of new roots and date of emergence were noted. Leaf elongation data was taken every 24 hours and an attempt was made to correlate this data with observed injury.

A hand-sectioning technique was used to obtain leaf cross-sections. First the oldest 4 or 5 leaves were stripped from plant and gross injury, if any, was observed. Then the remaining leaves were cross-sectioned, working from the tip of the apical meristem upward. This was accomplished by using a small wooden block and double-edged Adams razor blades

which had previously been cleaned with xylene. Care was taken to keep the tissue wet at all times.

The sections were transferred from the razor blade to a drop of water on a glass slide with a small camel's-hair brush. A cover slip was placed over the sections and they were observed under the microscope.

Kohler illumination was used and photomicrographs were taken to keep visual records of the injury. Photomicrographs were taken with a 35 mm. Kodak camera using professional, fine-grained, Panatomic-X black-and-white film.

RESULTS

Gross Observations. The plants had a total of 8-9 leaves at the time of treatment. Eight days after clipping, the check plants produced 1 to 2 new young leaves. In future discussions, these leaves will be numbered from the youngest to the oldest leaf.

A trend in the order of susceptibility of the leaves to heat injury was observed throughout the temperature range studied. Leaf browning and leaf decay were used as two of the criteria of gross injury. These were based on microscopic observations, where the brown and decayed areas always corresponded with tissues showing damage and collapse.

The oldest leaves (numbers 7, 8 and 9) turned yellow and then brown from 3 to 5 days after the plants were subjected to any temperature treatment. Similar leaves from check plants normally turned brown in 5 to 9 days. Aging is considerably hastened by any exposure to high temperature. Since these leaves were old, their capacity for photosynthesis was reduced and their contribution to the survival of a heat injured plant was probably slight or negative.

The first signs of injury to the younger leaves occurred at the junction of the leaf sheath and leaf blade of leaves 2 and 3. This area did not become visibly differentiated until the sheath and blade had elongated sufficiently for the

tip to be just visible between the next 2 older leaves. When leaf 2 was collapsed at the junction of the leaf sheath and leaf blade, the sheath usually elongated to its normal length. The blade did not elongate but withered and turned brown from its base to its tip. At the same level of treatment, leaf 3 was also collapsed at the junction of the leaf blade and leaf sheath. The leaf sheath elongated to its normal length, but the leaf blade did not elongate as much as leaf 3 of a check plant.

With a more severe heat treatment, leaf 4 was damaged at the junction of the leaf sheath and leaf blade. This leaf was almost mature at the time of treatment and was in an active stage of growth. The elongation of leaf 4 was initially measured on the stakes. With check plants, leaf 3 usually surpassed the length of leaf 4 at 5 to 7 days after clipping (Figure 3). When the heat treatment was severe enough to damage leaf 4 at the junction of the leaf sheath and leaf blade, leaves 2 and 3 were usually damaged from the apical meristem to a few mm. above the junction of the leaf sheath and leaf blade.

Leaves 5 and 6 were fully matured at the time of clipping and rarely elongated after clipping. These leaves were the last to be injured by high temperatures.

The youngest leaf and the apical meristem required a more severe heat treatment to cause leaf collapse and inhibition of leaf production from the apical meristem than did leaves 2 through 6. The point at which 50 percent of the plants treated were unable to produce new leaves from the

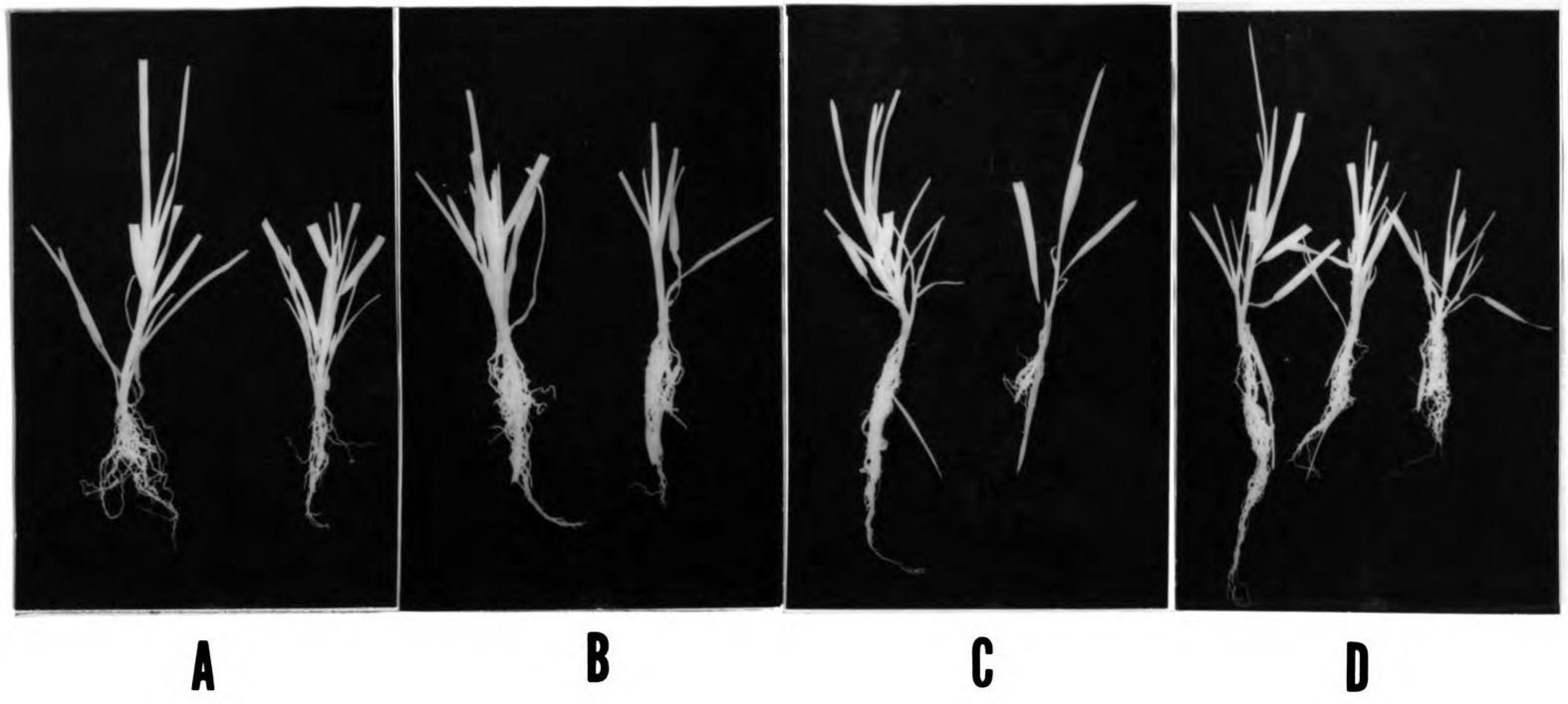


Figure 3.--Comparisons of root, tiller and leaf growth of heat-treated and check plants of Poa annua.

A. Check plant on left. Plant on right treated at 38.4° C. for 5 hours. Both at 11 days after treatment. No new leaf production from apical meristem of heat-treated plant, but new roots and tillers produced from lower portion of crown.
B. Plant on left treated at 37.8° C. for 8 hours and on right at 37.8° C. for 10 1/4 hours. Both at 12 days after treatment. Plant on left producing new roots, tillers and leaves. Plant on right producing new roots but no new leaves or tillers.
C. Check plant on left. Plant on right treated at 42.0° C. for 50 minutes. Both at 17 days after treatment. The heat-treated plant produced a few new roots, but no new leaves or tillers.
D. Check plant on left. Plant in middle treated at 38.4° C. for 3 1/2 hours and the plant on right treated at 38.4° C. for 4 hours. All at 15 days after treatment. Note reduced root, leaf and tiller growth with increased exposure time. New roots, tillers and leaves produced by all plants.

apical meristem after 8 days was chosen as the 50 percent kill point in this study.

The lower portion of the crown of Poa annua was the last area to be killed by exposure to high temperature. The heat treatment might be severe enough to kill all the existing leaves and prevent new leaf production, but new roots might still be produced from the lower portion of the crown. These roots were fewer in number, later in emerging, more spindly and more highly branched than roots of check plants. Often, new tillers would arise from the lower portion of the crown after 6 to 9 days even though no new leaves were produced from the apical meristem (Figure 3).

With a longer exposure time at a certain temperature, or a higher temperature at the same exposure time, a longer period of time was required for leaves 3 and 4 to begin elongation (Figure 4). Also, the total amount of elongation was less as was the rate of elongation. An exception to the last response occurred when leaf 4 was not too severely injured by the treatment. In this case, elongation was delayed for 1 to 3 days, but was very rapid for the next 24 to 48 hours (Figure 4). The treatment X time interaction was highly significant (Appendix Table 1).

Derivation of 50 Percent Kill Curve. The number of plants killed was noted for each temperature with varying exposure times. The results were then presented on a graph with the percent kill plotted against exposure time for each of the eleven temperatures used. Figure 5 shows the response of plants of Poa annua to treatment temperatures of 41.2 and

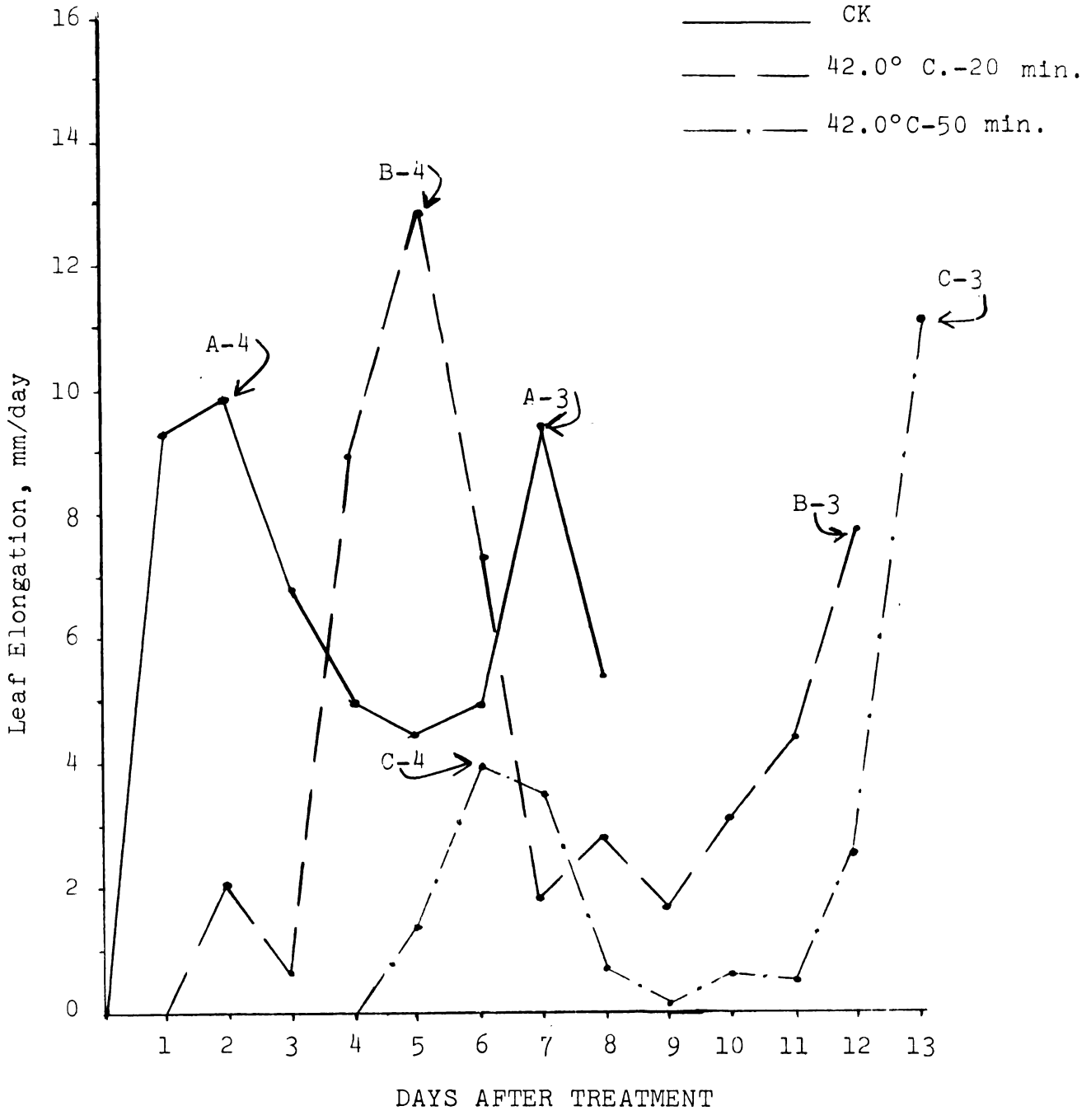


Figure 4.--Effect of increasing exposure time to high temperature on the date and rate of elongation of leaves 3 and 4.

A. Check plants; B. plants treated at 42.0° C. for 20 minutes; C. plants treated at 42.0° C. for 50 minutes; 3 and 4 refer to the maximum rate of elongation of leaves 3 and 4. Elongation of leaf 4 of B and C was delayed by 1 and 4 days, respectively. Note the stimulative effect of heat on elongation of B-4 at 5 days. An average of ten plants were observed.

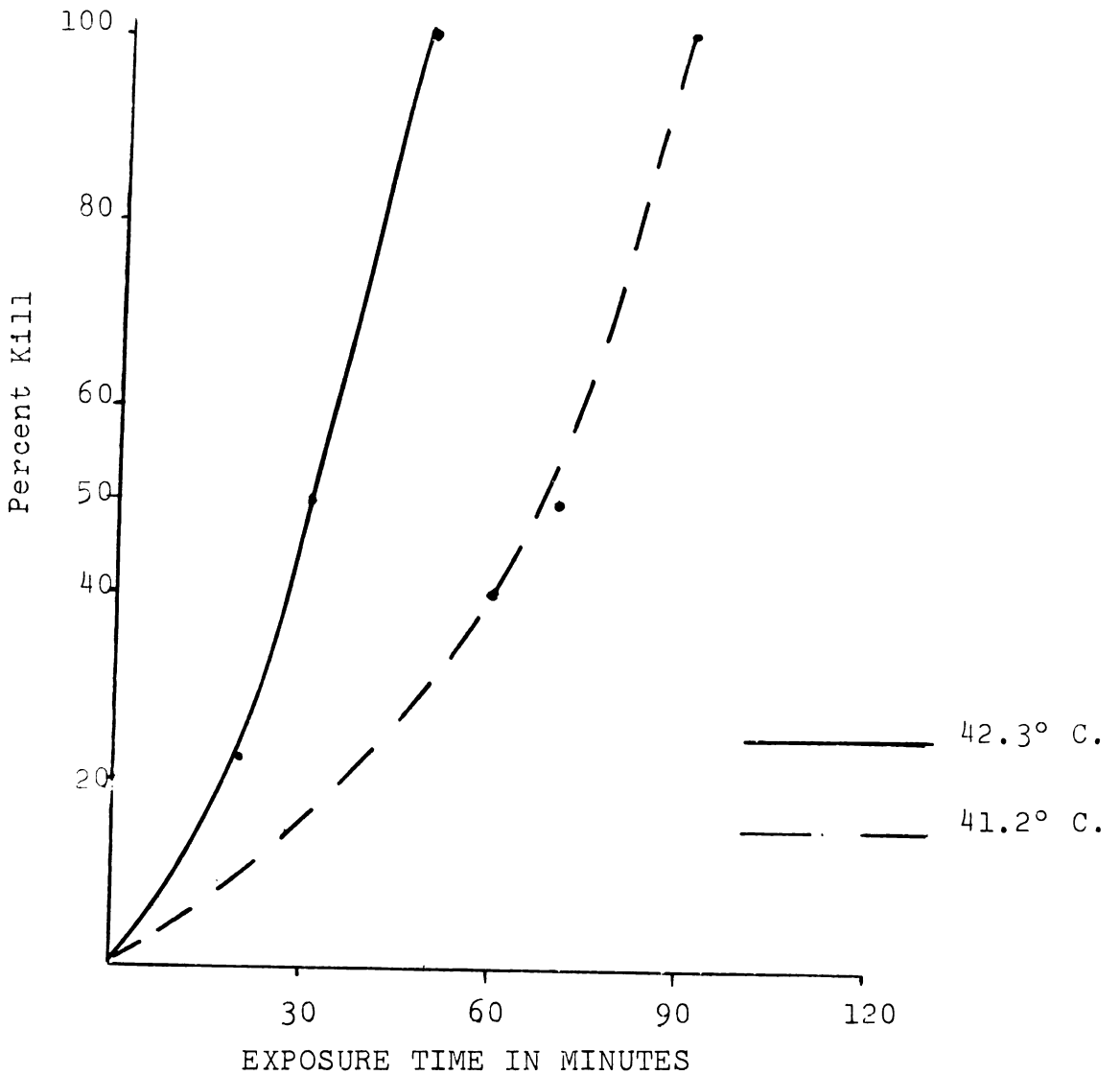


Figure 5.--Response of Poa annua to treatments of 41.2° C. and 42.3° C. at approximately 100 percent relative humidity. The criterion of kill was no new leaf production from the apical meristem at 8 days after treatment. A shorter exposure time was required for kill at the higher temperature as shown by the steeper slope of the curve. An average of 10 plants were observed.

42.3° C. at approximately 100 percent relative humidity. A shorter exposure time was required for kill at the higher temperature as shown by the steeper slope of the curve.

The 50 percent kill point for each of the eleven temperatures was plotted on a graph of time versus temperature to obtain the overall 50 percent kill curve (Figure 6). Kill was shown to be a function of exposure time. Longer exposure times were required to obtain 50 percent kill at lower treatment temperatures.

Microscopic Observations. Figure 7 shows the collapse of leaves 1 through 4 at the junction of the leaf sheath and leaf blade at 6 1/2 days after treatment for 50 minutes at 42.0° C.

Injury was observed to occur randomly in spots in the mesophyll at the junction of the leaf sheath and leaf blade of affected leaves. The first sign of injury was a granular appearance of the protoplasm (Figure 8). Later, the granular, particulate appearance of the protoplasm appeared more pronounced and strands were visible across the protoplasm (Figure 9). Then the protoplasm coagulated against the cell wall which may have begun to break down and become wavy at this point (Figure 9).

The final stage of collapse appeared when the epidermal cells from opposite sides of the leaf almost touched as in Figure 10. The epidermal cells themselves usually did not collapse but they appeared devoid of cellular contents at this final collapse stage.

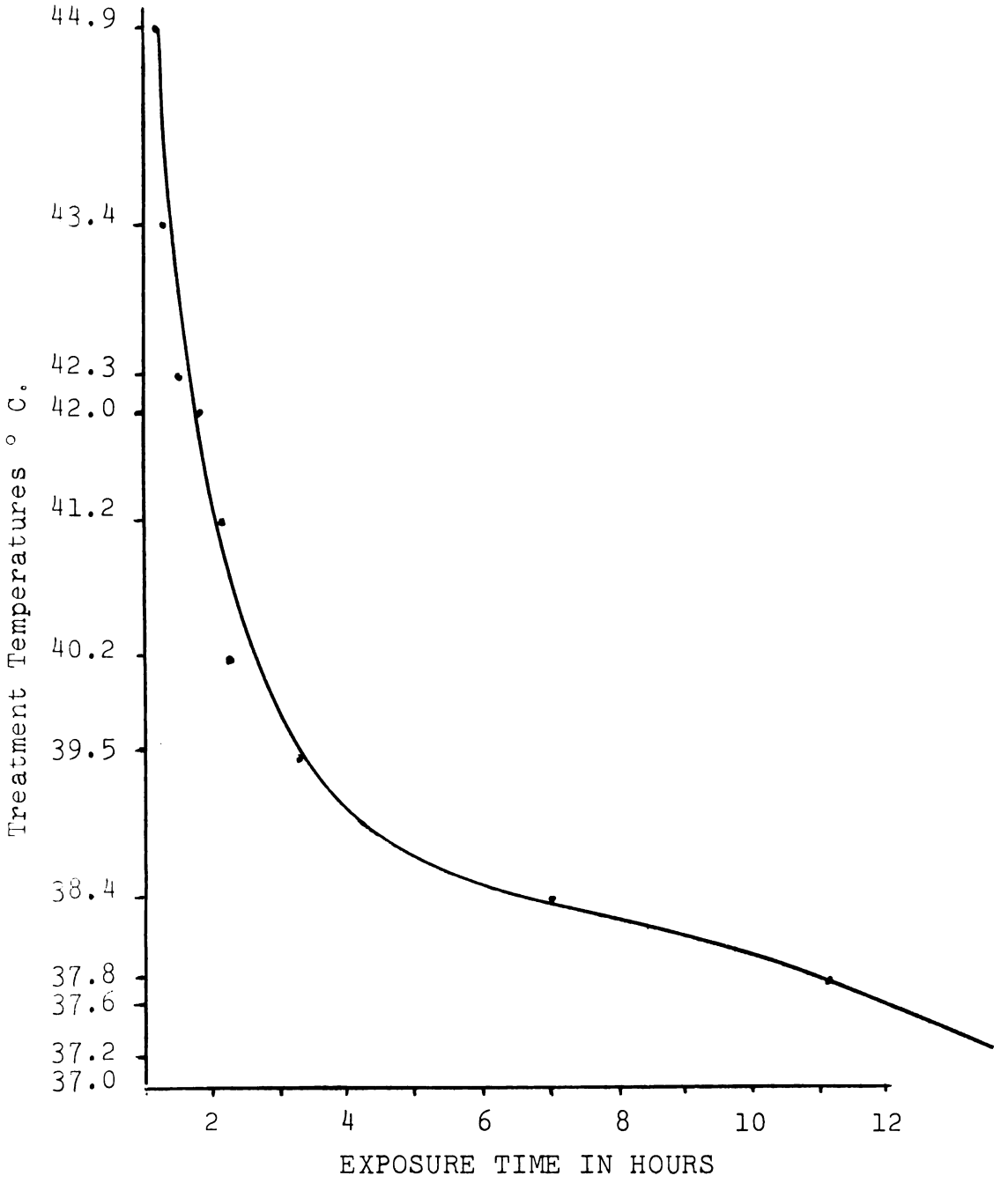


Figure 6.--Fifty percent kill of Poa annua subjected to 11 treatment temperatures at approximately 100 percent relative humidity. Kill is a function of exposure time at each treatment temperature. Longer exposure times were required to obtain 50 percent kill at lower treatment temperatures.

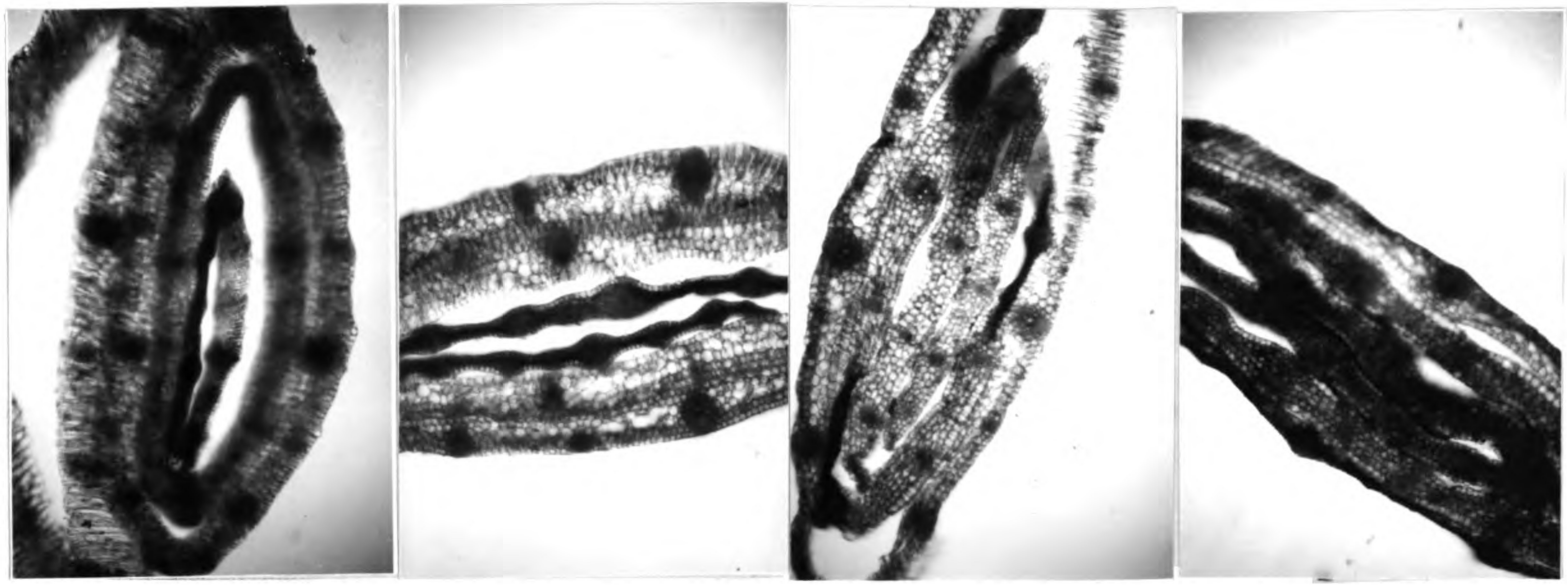


Figure 7.--Cross sections of Poa annua treated for 50 minutes at 42.0° C. Plants observed at 6 1/2 days after treatment. 100X magnification.

A. Leaves 1 through 4 just above the apical meristem. Leaf 1 is collapsed.
B. Leaves 2 through 4 with leaf 2 collapsed at the junction of the leaf sheath and leaf blade.
C. Leaves 2 through 4 with leaf 3 collapsed in spots at the junction of the leaf sheath and leaf blade.
D. Leaves 2 through 4 with one-half of leaf 4 collapsed at this point just below the junction of the leaf sheath and leaf blade.

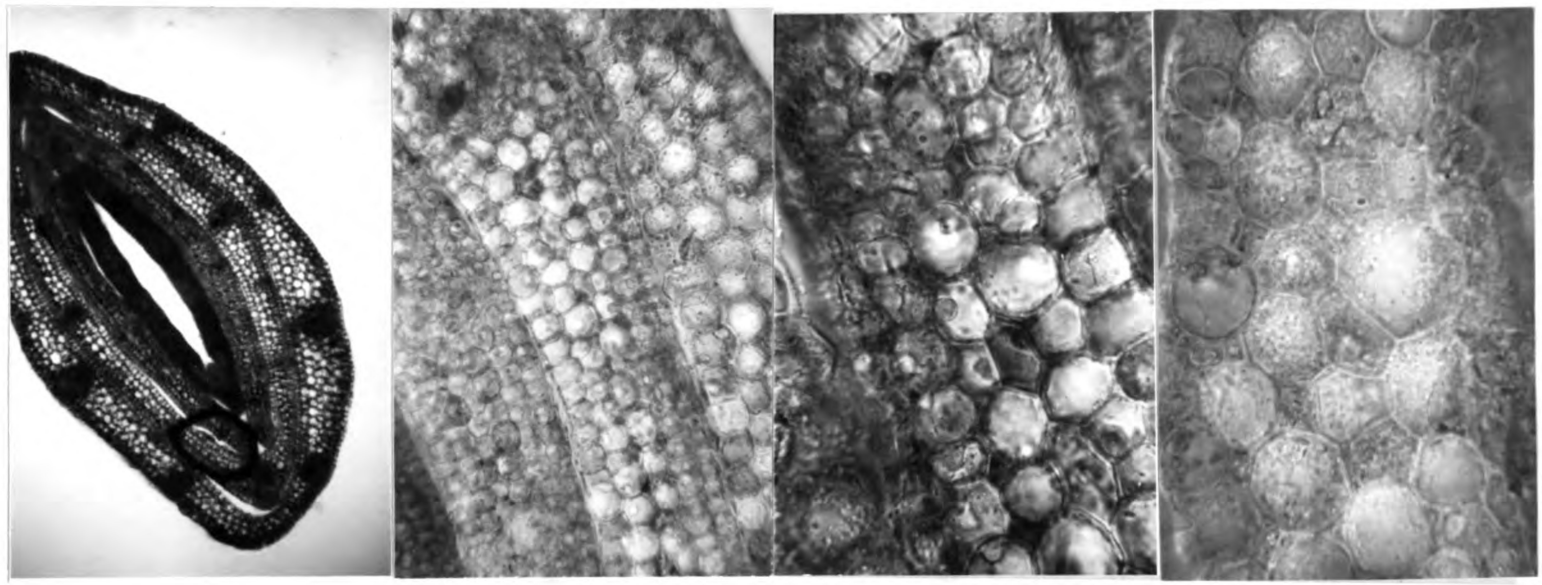


Figure 8.--Cross sections of Poa annua comparing a check plant with a plant treated for 10 1/2 hours at 37.6° C. at approximately 100 percent relative humidity. Both at 4 days after treatment.

A. 100X of leaves 1 through 4 of check plant.
B. 430X of leaves 2 through 4 of check plant.
C. 970X of leaf 3 of check plant. Protoplasm appears clear with relatively few granules. Position of cross section is just below the junction of leaf sheath and leaf blade.
D. 970X of leaf 3 of plant treated for 10 1/2 hours at 37.6° C. Noted the slightly granular appearance of the protoplasm of the cells in the mesophyll. Position of cross section is just below the junction of the leaf sheath and leaf blade.

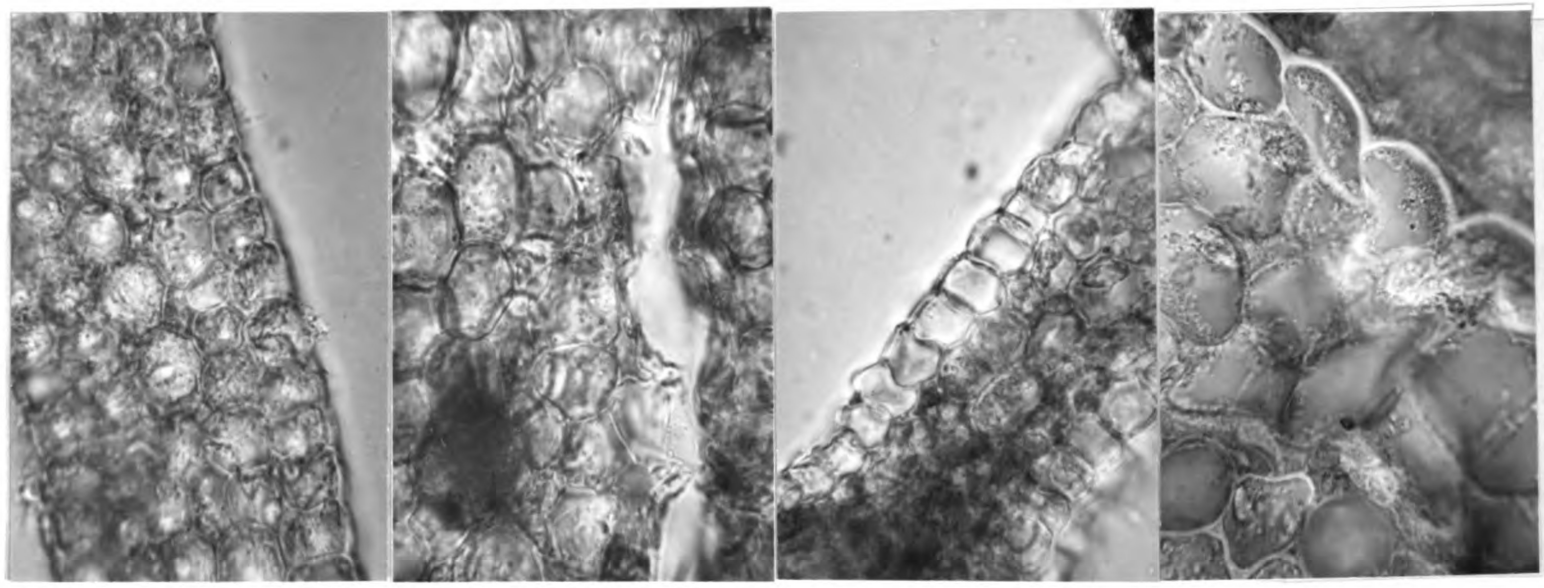
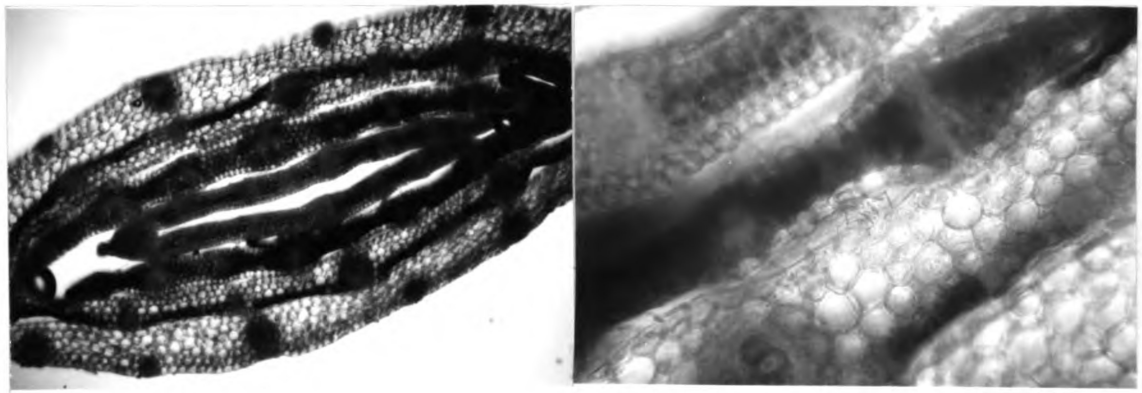


Figure 9.--Cross sections of Poa annua treated at 41.2° C., 40.2° C., and 38.4° C. at various exposure times. Relative humidity was approximately 100 percent. Cross sections show granular protoplasm and cell wall breakdown of leaf 3 at the junction of the leaf sheath and leaf blade. Magnification is 970X.

A. Three days after treatment for 50 minutes at 41.2° C. Note the granular protoplasm of cells in the mesophyll.
B. Six days after treatment for 1 1/4 hours at 41.2° C. Note the granular protoplasm and protoplasmic coagulation of cells in the mesophyll.
C. Six days after treatment for 1 1/2 hours at 40.2° C. Note the granular protoplasm, wavy cell walls and partial collapse of cells in the center of the mesophyll.
D. Three and one-half days after treatment for 11 hours at 38.4° C. Note the protoplasm coagulated against the wavy cell walls in the cells of the mesophyll.



A B

Figure 10.--Cross sections of Poa annua 4 days after treatment at 42.0° C. for 50 minutes. Relative humidity was approximately 100 percent. Cross sections show final stage of collapse of leaf 2 at the junction of the leaf sheath and leaf blade.

A. 100X of leaves 1 through 4. The position of the cross section was at the junction of the leaf sheath and leaf blade of leaf 2. Note the total collapse of the mesophyll of leaf 2 and the partial collapse of the mesophyll of the leaf sheath of leaf 3.

B. 430X of leaves 2 and 3 of the section shown in A. Note the total collapse of the mesophyll of leaf 2 and the partial collapse of the mesophyll of the leaf sheath of leaf 3. The epidermal cells appear devoid of cell contents.

DISCUSSION OF RESULTS

The responses of Poa annua were probably due to direct high temperature stress, although there was the possibility of an interaction with desiccation stress since the tests were run at slightly less than 100 percent relative humidity. In the past, little or no effort has been made to distinguish between the direct effects of high temperature and those of desiccation.

Heyne and Laude (11), Carroll (4) and Mueller and Weaver (22) subjected the leaves of grasses growing in saturated soil to high temperatures with an atmosphere of 10 to 30 percent relative humidity. Although they called their results heat resistance, there probably was an interaction between high temperature and desiccation stress at these low humidities. During the time required to reach temperature equilibrium, the plant tissues were subject to potential desiccation injury at these low humidities. The evaporative cooling of leaves at these low humidities could prolong the time required for temperature equilibrium to be reached. Their tests gave good correlation with field drought studies. Heat and water stress are rarely distinguished in field drought investigations.

The plant part most sensitive to heat injury was the junction of the leaf sheath and leaf blade. This is an area of active cell division and/or elongation, depending

upon leaf age. It follows that cells which were actively dividing or elongating were most susceptible to high temperature injury. The fact that leaf 1 and the apical meristem were among the last tissues to be injured might be due, in part, to the insulating effect of the outer leaves. The finding in this study agree with those of Sachs (23), who stated that young leaves were more sensitive to heat injury than very young leaves, and that buds were more sensitive than older plant parts.

Post-treatment leaf elongation was slower and later in starting with longer exposure time or a higher temperature. More widespread cell damage at the junction of the leaf sheath and leaf blade resulted in fewer cells remaining which were capable of cell division or elongation. Following a period of initial shock, the very rapid elongation of leaf 4 after mild heat treatment was probable due to a stimulative effect on cell division or cell elongation. Dangeard (5) found that heat-induced cessation of cell elongation may be reversed after several days at room temperature. Hilbrig (12) stated that the longer plants are exposed to high temperatures, the longer it takes them to recommence growth.

Kill was a function of exposure time at each temperature studied. Longer exposure times were required for kill as the temperature was lowered.

The effects of heat on the cells of the mesophyll were observed as protoplasmic granulation, followed by protoplasmic coagulation, cell wall breakdown and total cell

collapse as the treatment became more severe. These changes may have resulted from a direct physical effect of high temperature on the protoplasmic constituents. Cellular responses to high temperature generally agreed with those described in the literature (1, 19). However, the sectioning technique employed in this study did not permit as detailed an investigation into heat-induced protoplasmic changes as was desired. A more refined sectioning technique, possibly combined with a vital staining technique, might be useful in further studies.

It should be emphasized that this investigation is just the first step in a study of the effects of high temperature on living tissue.

CONCLUSIONS

Exposure of whole plants of Poa annua to an atmosphere of approximately 100 percent relative humidity and temperatures above 37.2° C. resulted in injury and death of plant tissues. These effects probably were the result of direct high temperature injury, although there was the possibility of some interaction with moisture stress.

The order of susceptibility to high temperature was as follows: leaves 2 and 3 most sensitive; leaf 4; leaves 5 and 6; leaf 1 and the apical meristem; and the lower portion of the crown. This last area included all meristematic areas from just below the apical meristem to the base of the stem. Leaves 7, 8 and 9 were injured by all temperature treatments. Injury occurred first at the junction of the leaf sheath and leaf blade of all affected leaves.

With a longer exposure time or higher temperatures, a longer period of time was required for leaf growth to recommence. The rate of elongation and total elongation were decreased with increasing severity of treatment because fewer cells remained which were capable of cell division or elongation. With increasing severity of treatment, fewer tillers and roots were produced from the lower portion of the crown. These roots were shorter, more spindly and more highly branched than roots of normal plants.

The order of cellular changes observed was protoplasmic granulation, protoplasmic coagulation, cell wall breakdown and total cell collapse. These probably were caused by direct high temperature effects on protoplasmic constituents.

Kill was a function of exposure time at each temperature studied. A much shorter period of time was required to obtain 50 percent kill at higher temperatures than at lower temperatures.

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APPENDIX

Table 1.--Analysis of variance of leaf elongation of check and heat-treated plants of Poa annua for 8 days after treatment.

Source of Variation	DF	SS	MS	Observed F
Total	239	4528		
Treatment	2	1321	660.5	122.3**
Time	7	375	53.6	9.9**
Plants (within treatment)	27	155	5.7	1.1
Treatment X Time	14	1648	117.7	21.8**
Error	189	1029	5.4	

**Significant at the 1% level.

The above is an analysis of the leaf elongation of check plants and plants treated for 20 minutes at 42.0° C. and 50 minutes at 42.0° C. Relative humidity was approximately 100 percent. There were 10 plants per treatment. The treatment X time interaction was significant at the 1% level, as illustrated graphically by the peaks in Figure 4.

would be evaluated by following the content of liquid water in the space between protoplasts, plant weight, and by differential thermocouples. The stresses would be evaluated during the experiment by the electrophoretic technique used in winter hardiness studies. Injury would be evaluated by histological techniques. The 50% kill points would be determined by survival in a controlled environment chamber. Dr. J. B. Beard, Crop Physiologist Project Leader, Dr. C. R. Olien, Plant Physiologist and Dr. A. E. Erickson, Soil Physicist, will cooperate on the project in a joint effort.

Following the development of the method described above, studies will be initiated to establish quantitative relationships between internal vapor pressure, tissue water content, extracellular water content and killing temperatures of several varieties of cereals and grasses.

Several genetically pure varieties of winter cereals and grasses would be grown in a controlled environment chamber, hardened and adjusted to 65% moisture. The vapor pressure which kills 50% of several varieties of winter cereals through the entire range of temperature which the plants can survive will be determined. The line representing 50% kill in a vapor pressure-temperature graph should change radically or be discontinuous where the type of killing stress changes. The free energy values obtained from an intensity plot of this type might be helpful in identifying the components of the protoplasts affected by each type of stress (drought, heat and crystallization).

The funds for the equipment used in this study were provided by the North Central Regional Committee (NC - 71). The basic experimental plan was formulated by Dr. J. B. Beard, Dr. A. E. Erickson and Dr. C. R. Olien. This Master's thesis is an independent contribution to the overall objectives outlined in the NC - 71 research proposal below.

The final draft of the research proposal was submitted by the above three researchers in May, 1963. A revised final draft of the research proposal was submitted in December, 1964 (see below).

Drought Injury and Resistance in Plants

A new technique will be developed and evaluated whereby the physical stresses which destroy critical plant tissue by desiccation can be distinguished from stresses involving crystallization or heat effects. The development of a technique for separation of these three stress effects is essential in understanding the drought problem.

The stresses concerned with are biophysical in nature and injury occurs in a short time once the stress is sufficiently severe. The test chamber would involve a high velocity air stream with precise control of temperature and humidity. At each temperature plants would be exposed in the air stream while the vapor pressure was lowered from saturation in small intervals. After the plant came to equilibrium at each interval, a few would be transplanted back to an environment suitable for growth. Attainment of equilibrium

After the physical stresses and injuries have been well quantified, investigations on the metabolic effects occurring prior to kill will be pursued.