



Effect of high temperature stress on orchardgrass seedlings (*Dactylis glomerata* L.)
by Paul William MacDonald

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
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Abstract:

Orchardgrass (*Dactylis glomerata* L.) seedlings were used to study some effects of high temperature stress. Plants were heat stressed in ovens or in water baths at several temperatures ranging from 45 to 50 C for various lengths of time. In the water bath treatments the plants were inverted and the tops immersed in the water for the desired time duration.

Three phases of investigation were undertaken in this study. In the first phase the effect of heat stressing, clipping back plant tissue artificially, or both, upon the growth and development of orchardgrass seedlings was studied. In the second phase the heat tolerance of orchardgrass in relation to seedling age was studied. In the third investigation the effect of heat stress on photosynthetic rate of 21-day-old orchardgrass seedlings was studied for a one-week period following stress.

In the first phase of study results varied with the amount of tissue killed by stressing. Where more tissue was removed by stressing than by clipping there were significant reductions in plant height, leaf number and tiller number resulting from temperature stress. Clipping resulted in, a reduction in tiller number, and there was a significant interaction between clipping and stressing on tiller number. Where less tissue was killed by stressing than removed by clipping, significant reductions in leaf and tiller number resulted, and cannot be explained. Where only tip burning of the leaves occurred there was a highly significant interaction between clipping and stressing on plant height.

Heat tolerance of orchardgrass seedlings changed with plant age. Over an age range of 13-43 days heat tolerance decreased from 13-31 days and then gradually increased through 43 days. The change in heat tolerance could not be explained.

Highly significant reductions in photosynthetic rate occurred through one day following heat stress, and there was a significant reduction after three days. No reduction was observed one week after heat stress.

It was postulated that the major effect of heat stress on the growth and development of seedlings is the loss of photosynthetic tissue, and that the rate of photosynthesis in heat stressed, but not killed, tissue is reduced for a period of several days but full recovery is noted one week following stress.

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ABSTRACT

Orchardgrass (Dactylis glomerata L.) seedlings were used to study some effects of high temperature stress. Plants were heat stressed in ovens or in water baths at several temperatures ranging from 45 to 50 C for various lengths of time. In the water bath treatments the plants were inverted and the tops immersed in the water for the desired time duration.

Three phases of investigation were undertaken in this study. In the first phase the effect of heat stressing, clipping back plant tissue artificially, or both, upon the growth and development of orchardgrass seedlings was studied. In the second phase the heat tolerance of orchardgrass in relation to seedling age was studied. In the third investigation the effect of heat stress on photosynthetic rate of 21-day-old orchardgrass seedlings was studied for a one-week period following stress.

In the first phase of study results varied with the amount of tissue killed by stressing. Where more tissue was removed by stressing than by clipping there were significant reductions in plant height, leaf number and tiller number resulting from temperature stress. Clipping resulted in a reduction in tiller number, and there was a significant interaction between clipping and stressing on tiller number. Where less tissue was killed by stressing than removed by clipping, significant reductions in leaf and tiller number resulted, and cannot be explained. Where only tip burning of the leaves occurred there was a highly significant interaction between clipping and stressing on plant height.

Heat tolerance of orchardgrass seedlings changed with plant age. Over an age range of 13-43 days heat tolerance decreased from 13-31 days and then gradually increased through 43 days. The change in heat tolerance could not be explained.

Highly significant reductions in photosynthetic rate occurred through one day following heat stress, and there was a significant reduction after three days. No reduction was observed one week after heat stress.

It was postulated that the major effect of heat stress on the growth and development of seedlings is the loss of photosynthetic tissue, and that the rate of photosynthesis in heat stressed, but not killed, tissue is reduced for a period of several days but full recovery is noted one week following stress.

INTRODUCTION

There is a range of temperatures considered near optimal for plant growth and development. Above this range, temperatures are encountered which alter the plant's growth, though permitting survival. With further increases in temperature, a lethal point is reached. The temperatures to be considered here are the supraoptimal but below the lethal.

The effects of high temperatures on plant growth are difficult to study because of the multitude of factors which may affect the response and mask it from the true effect of temperature.

The objectives of this study were to:

1. Investigate the growth and development of heat stressed seedlings and if reduction in growth occurs, determine if it is due to factors other than a loss of photosynthetic tissue.
2. Determine heat tolerance of seedlings in relation to seedling age.
3. Determine the effect of heat stress upon photosynthetic rate.

Orchardgrass (Dactylis glomerata L.) was chosen for study because of its widespread distribution in the Western United States and its importance as a forage crop.

REVIEW OF LITERATURE

The Upper Temperature Limit for Growth

The upper temperature limit for growth is usually accepted as 80-85 C for thermophilic blue-green algae and 58 C for higher plants (22). Resting tissue such as dry seed is known to be able to survive temperatures of 120 C (22). According to Lepeschkin (21), as cited in Levitt's book (22), the time duration a plant is subjected to high temperatures is of fundamental importance, for the heat killing temperature varies inversely with exposure time, and the relation of killing to time is exponential.

Occurrence of Heat Injury Under Natural Conditions

Dutrochet (6), as cited by Levitt (22), reported that a plant's temperature may rise above that of the environment, but never more than 1/3 C except in fleshy organs where the heat from metabolic activity is not dissipated rapidly enough to the environment. Other workers have observed plant temperatures well above that of the surrounding air (7, 34). Hopp (7), for example, observed temperatures as high as 100-106 F inside tomatoes at air temperatures of 80-83 F. Tanner (34) has raised a question as to the value of air temperature as an indicator of plant temperature. Temperatures in the alfalfa plant may range 5-10 C below air temperature at night and 5-10 C above air temperature during parts of the day.

High temperatures which exceed the normal temperature limit of 45-55 C for most plants may occur under natural conditions, and the possibility of heat injury exists under certain conditions (22). Perhaps the

greatest danger of heat injury occurs when the soil is exposed to insolation and reaches temperatures as high as 55-75 C (Lundegårdh (23), as cited by Levitt (22)). Baker (1) stated that surface soil temperatures of 130-160 F have been detected in temperate regions. Laude, et al. (17) obtained similar temperatures in seedbeds at Davis, California.

Method of Measuring Heat Hardiness

Two methods of measuring heat hardiness, which were originally used by Sachs (29) in 1864, as cited by Levitt (22), are still standard for measuring heat hardiness. One is the use of a temperature-controlled heat chamber, in which plants are placed for a standard length of time following which the degree of injury is observed. This method has the advantage of using the whole plant. In the second method, potted plants are inverted and the tops immersed in a constant temperature water bath for a standard length of time, and then observed for growth or microscopically examined for injury. Small pieces or sections of plants may also be used.

According to Sachs (29), in order to prevent drought injury during tests by the first method and to obtain plant temperatures identical with measured air temperatures, maintaining 100% relative humidity is necessary. This precaution will produce injury which is attributed to heat only.

Factors Affecting Response to High Temperature

Numerous factors affect the degree of plant response to high temperature and must be evaluated to properly interpret plant behavior. Some of these factors are previous conditioning of the plant, stage of growth, genotype, and the test conditions. Important test conditions are

temperature of the plant, air and soil, relative humidity, duration of exposure and the soil moisture regime.

Previous conditioning of the plant. The degree of plant injury is affected by the time of day the plant is subjected to heat stress. Laude (16) reported that the degree of injury followed a diurnal cycle, with maximum heat resistance being attained at mid-day and continuing throughout the afternoon. Minimum heat resistance was obtained in the early morning. Loss of resistance was found to be slower than gain of resistance. A measurable amount of heat resistance was acquired with one hour of light following normal darkness in corn, wheat, barley, sorghum and alfalfa. For example, wheat plants kept in total darkness all night and forenoon were injured 95% on the basis of percent tissue kill while plants receiving one hour of daylight prior to stress were injured 15%. He suggested that the increased resistance was due to production of photosynthate but that the amount manufactured in one hour was not sufficient to account for such a marked increase in resistance and that perhaps a photochemical reaction to light causes heat resistance.

Laude and Chaugule (19) demonstrated that heat hardiness may be acquired by exposing plants to temperatures milder than the stress temperatures prior to the heat stress test. Prairie brome grass (Bromus cartharticus Vahl.) was hardened by exposure for 1 hr at 110 F, 2 hr at 120 F and 3 hr at 130 F, respectively, for six, four, and two days prior to heat stress. Hardened and un-hardened plants were then exposed to heated air at 130 F for 4 3/4 hr. Unhardened plants suffered 75% tissue kill and

the hardened plants, 10% tissue kill.

Yarwood (36) found that plants acquired heat tolerance. Leaves were hardened by immersion in a hot water bath for 15-30 sec at 50 C. When hardened and unhardened leaves were stressed at 55 C 12 to 48 hr later, hardened plants were able to withstand the stress temperature three times longer than unhardened plants. This acquired heat tolerance was found to change with time. Acquired heat tolerance was barely apparent 10 min after the 50 C hardening treatment, was greatest about 24 hr later, and lasted at least 72 hr.

Stage of development or age of plant. Laude (20) reported that relatively small differences in stage of development may markedly alter the response to heat. Prairie bromegrass (Bromus cartharticus L.) exhibited a striking change in heat tolerance when subjected to 130 F and 30-35% relative humidity for 4 3/4 hr at intervals from time of emergence to eight weeks old. Shortly after emergence there was a rapid loss of heat tolerance which reached a maximum at 18 days and persisted for about two weeks after which tolerance increased with age. Injury was computed by expressing the length of dead tissue as a percent of the total length. Laude suggested that the response obtained was probably associated with the exhaustion of food reserves of the endosperm at this critical stage of growth. The importance of plant age was pointed out as being very important in studying plant response to high temperature.

Variety or ecotype. Laude (20) found that it is difficult to conduct critical physiological studies within species such as in forage crops because of the lack of genetic uniformity.

Coffman (5), as will be shown later, found that winter oat varieties differed significantly in heat hardiness at different light intensities. He also found that winter-hardy varieties of wheat and barley have more heat resistance than do spring or semi-winter varieties.

Test Conditions

Soil temperature. Soil temperature may affect a plant's response to air temperature. Ketellapper (9) found that soil temperature is largely independent of air temperature and may exert a pronounced effect on top growth, as expressed by dry matter accumulation or tiller number. Soil temperatures may mask or modify the effect of air temperature. Phalaris tuberosa L. was grown under several air temperature regimes and different levels of constant soil temperatures. There was no difference in the dry weights of the tops in different air temperatures at the same root temperatures; however, there was a highly significant difference in the dry weights of the tops subjected to different temperatures at the same air temperature.

Similar effects of soil temperature were found by Brown (3), as cited by Laude (20), with Poa pratense L., Poa compressa L., Dactylis glomerata L. and Cynodon dactylon L. Pers., and by Benedict (2) with guayule plants. Benedict suggested that high soil temperatures could possibly influence root growth, meristematic growth, and production of substances by the root

which affect the tops.

Laude (20) also pointed out that abnormally high soil temperatures may be reached in the root zone under controlled environmental conditions unlike those normally found in the field.

Relative humidity. Relative humidity requires careful control in high temperature studies. Kinbacher (11) heat stressed three winter oat varieties for 8 hr at 110 F at 50, 75, or 100% relative humidity. One week after stress the amount of dead tissue for each variety increased proportionately with humidity. Also, the same amount of injury could be obtained at different temperatures by adjusting the relative humidity. For example, the same injury resulted when plants were stressed at 110 F and 90% relative humidity as at 120 F and 50% relative humidity. This was believed to be the effect of greater transpirational cooling at the higher temperature resulting in equivalent leaf temperatures at both temperatures. As pointed out by Levitt (22), there is a danger of drought injury rather than heat injury alone when heated air chambers are used, especially at low relative humidities.

Light intensity. Sapper (30), as cited in Levitt (22), found that heat hardiness of plants was not reduced by keeping them in the dark as long as three days. However, these results conflict with those of Laude (20) and Coffman (5). Laude (20), as discussed earlier, showed that even one hour of light prior to heat stress reduced the amount of injury to the plants of several species. Coffman (5) found that heat resistance appeared to be related to light intensity on the day of stress. Oats resisted

heat better after exposure to bright sunlight than when shaded prior to a treatment of $48-51\frac{1}{2}$ C for 45 min to two hr.

Effect of High Temperature on Growth

Emergence. High seedbed temperatures may reduce and/or delay seedling emergence. Laude et al. (17) subjected several species of perennial grasses planted at a depth of one-half inch to six hr of soil temperatures ranging from 108-127 F on different days prior to emergence. It was found that greater reductions in emergence occurred as the time of heat exposure approached the expected time of emergence. Decreasing tolerance to high soil temperatures was suggested as the cause. Reduction in emergence was associated with a delay in time of emergence of seedlings that survived pre-emergence heat exposure and were considerably shorter plants. They state that the delayed emergence and restricted growth of stressed seedlings should place them at a disadvantage with species not so affected.

Post emergence. The grass seedling may be subjected to high soil temperature and high air temperature following emergence. Bucharin (4) found that soil surface temperatures in excess of 54.5 C resulted in severe burning of wheat leaves at the point of soil contact. Laude (20) found similar responses in barley subjected to air temperature of 130 F for 4 $\frac{3}{4}$ hr when the first foliage leaf was emerging through the coleoptile.

Later development. High temperature stress in the seedling stage affects later development of the plant. Laude (20) subjected red bromegrass

(Bromus rubens L.) in the two-leaf stage to 130 F for five hr at 40% relative humidity. Although there was only tip burning of the leaves which disappeared when the plants were placed in a favorable environment, twenty-four days after the stress significant reductions in plant height, number of leaves and number of tillers had occurred. These effects were less pronounced over a longer period of time; however, the heading date was delayed.

Jones (8) found similar effects when he subjected one-quarter inch to one-half inch corn seedlings to 45, 50, and 55 C for one hour and transplanted them to the field. All heat-treated seedlings were shorter in height, less vigorous in growth, and later flowering than the control plants. Healthy-looking tassels were produced, but anthers were small and shrivelled and the pollen was sterile on heat stressed plants.

Mitchell (24) reported that plants grown continuously at temperatures above optimum gave a response similar to that obtained when they were subjected to brief extreme heat exposures. The daily increases in dry weight of top growth were measured for 4 grasses and 3 legumes grown at constant temperatures ranging from 45 F to 95 F at 10 degree intervals. The greatest increase in dry weight was in the 65-75 F range for most of the species and declined at higher temperatures. At 95 F the rate of increase was negligible for some species.

Under certain conditions cessation of growth is possible. Laude (18) found that the condition of summer dormancy may be induced by the combined stimulus of high temperature and long daylength in Poa scabrella (Thurb.) Benth. ex Vasey even when supplied with adequate moisture.

Dormancy could be broken by moderating temperature and supplying moisture.

Effect of High Temperature Injury on Metabolism

Certain metabolic changes associated with high temperature responses have been studied, but many await investigation.

Respiratory measurements were used to demonstrate gradual injury produced at high temperatures by Kuijper (12), as cited by Levitt (22). In Crepis biennis respiration, as measured by CO₂ evolution, decreased with time even at 30 C. There was a marked decrease in reserves of starch and protein as a result of the high respiration rate in Lupinus, Pisum and Triticum. Lundegårdh (23), as cited by Levitt (22), suggested that starvation may occur at high temperatures since the temperature optimum for respiration is higher than for photosynthesis. Assimilation in potato leaves was zero at 45-50 C when measured over short periods of time, or at 37-43 C when measured over long periods of time.

Effect on the cell. Bucharin (4) studied the characteristics of severely burned cells of wheat seedlings. He found increased viscosity of the protoplasm (five to six times greater than the controls). Injured cells would not plasmolyze and their nuclei and protoplasm would stain with neutral red. In controls only the vacuole of uninjured cells would stain. Osmotic activity of slightly injured cells was disrupted. Surface burns were found to occur when the protective covering of the leaf (epidermis cuticle) had not been completely formed.

Effect on seed and seedlings. Siegel (31) postulated that high temperature may stimulate germination by increasing chemical activity, accelerating mobilization of reserves, and enhancing release of energy. Factors affecting germination responses to heat are specific differences in the nature of tissue proteins, insulation of the embryo by maternal tissues, and factors such as age, previous history, water content and others. Contributing factors to stunting of seedlings grown from seed which was subjected to high temperatures were thought to be thermal inactivation of enzymes, denaturation of tissue proteins, or some affect on growth substances.

Kydrev and Kolev (14) subjected dry wheat seed to temperatures ranging from 50-100 C for one hour. Water content declined and germination was impaired only at moisture contents less than 8%. Treated seeds absorbed more water than the controls. Amylase and lipase activity were augmented only in the 60 C treatment. Protease activity increased only with the 50 C treatment, and was inactivated at higher temperatures. Heat resistance was increased in plants from treated seeds. Young plants in the three leaf stage required a progressively longer exposure to dry heat to attain a prescribed degree of wilting than did the controls.

Chemical composition. Sullivan and Sprague (33) determined the chemical composition of perennial ryegrass grown at relatively high temperatures. Clonal material was grown under four regimes of alternating temperatures, the daily maximum being 60, 70, 80, or 90 F for four hours and the daily minimum being 10 F less in each case. With increasing temperatures,

fructosan, a major reserve carbohydrate, was depleted. Other changes noted in the roots and stubble at higher temperatures were: rapid loss in sucrose, increase in the percentage of cellulose, lignin, and pentosan, and higher total nitrogen with a greater proportion being soluble.

The roots were reported to be more seriously injured than the stubble. This was thought to be a result of exhaustion of carbohydrate and an increase in ammonium salts or similar nitrogenous compounds.

Petinov and Razmaev (27, 28) reported the heat-induced changes in the nitrogen and carbohydrate of wheat and corn seedlings in the five-leaf stage. The plants were hardened under gradually increasing daytime temperatures for six days and then exposed to 48 hr of heat and 85-90% relative humidity. Total nitrogen in the leaves declined immediately, but it subsequently increased to exceed the content in the controls. Likewise, total nitrogen in the roots was reported to increase. An increase of simple sugars in both leaves and roots was observed following heat exposure. Respiration rate was observed to drop temporarily during the heating of the leaves and this was attributed to enzyme inactivation.

Enzymes. Enzyme inactivation or disruption has long been considered one aspect of heat injury. Identification and study of particular metabolic pathways which are particularly heat sensitive may be highly rewarding. Molotkovskii (25) studied changes in adenosine triphosphatase activity in pea and squash mitochondria and cytoplasm. A decrease in adenosine triphosphatase activity was observed as temperatures were increased using exposures of one and one-half to three hr between 43 and 50 C, and a sharp

increase in inorganic phosphorus occurred concurrently. It was postulated that there may have been an uncoupling of oxidative phosphorylation by some specific inhibitors. It was also observed that adenosine triphosphatase was able to retain activity in the presence of excess ATP and was depressed in the presence of excess ADP, suggesting that ATP exerted a stabilizing effect on the enzyme. It was also suggested that the decreased adenosine triphosphatase activity may be related to the change in the ATP/ADP ratio rather than a direct effect of temperature.

Metabolites. Another approach to the study of high temperature responses of plants is the concept that metabolite shortages which may have resulted from the effect of non-optimal conditions may be reduced or overcome by supplying essential metabolites from an external source. When such applications to the plant are more beneficial at non-optimal temperatures than at optimal, the applied substance is viewed as being potentially involved in the response and may be studied for the mechanisms involved. Langridge and Griffing (15) applied vitamins, yeast extract, riboside mixtures, and casein hydrolysate to races of Arabidopsis thaliana L. Heynh. Three of five races shown to be damaged by high temperatures gave significantly increased growth at 31.5 C with vitamins, yeast extract, or nucleic acids. Only some of the plants damaged by high temperature responded to these supplements. They reasoned that this may have been due to failure to apply the proper metabolite or to antagonism existing among the components of an applied mixture.

Ketellapper (10) was able to reduce the amount of dry weight loss in

plant tops under unfavorable warm temperatures by spraying the leaves with metabolites. They found that a B-vitamin mixture may completely prevent reduction in growth in lupins.

Kurtz (13) obtained similar results with mutant strains of Neurospora. For example, riboflavin restored growth in a mutant strain of Neurospora crassa which does not grow at temperatures above 28 C, and adenine and pyrimidines were found to cure climatic ills in other temperature-sensitive mutants.

Petinov and Molotkovskii (26) reported that a reduction in heat injury resulted from applications of organic acid solutions sprinkled on the plants prior to heat exposure. Citric and malic acids were among the most effective of those tested. It was suggested that the protective action most probably results from the neutralization of ammonia, the toxic action of which is intensified at high temperatures.

MATERIALS AND METHODS

Seed Source and Preparation

The orchardgrass seed used in these experiments was from clonal material or was of the commercial Potomac orchardgrass variety.

In all experiments orchardgrass seed was planted in two x two inch jiffy pots at a depth of one-half inch in greenhouse soil, and watered regularly with standard nutrient solution.

Growth and Development of Heat Stressed Seedlings

Three studies were conducted to measure the effects of heat stress on orchardgrass seedling development.

In the first study, orchardgrass seedlings were grown at 21.1 C at a light intensity of 1600 ft-c and a 15 hr photoperiod. Thirteen days after planting the seedlings were thinned to two per pot. Half of the plants were stressed in a Cenco oven at 54.5 C for 35 min. Following stress half of the stressed plants and half of the check plants were clipped back to one-half inch above the soil surface with 10 min following treatment. The purpose of the clipping treatment was to remove dead tissue from stressed plants and thus determine if the effect of heat stress was due to factors other than loss of photosynthetic tissue. Each treatment was replicated five times.

The plants were replaced in the growth chamber following treatments. One week later they were transplanted into four x four inch plastic pots. Measurements on plant height, leaf number and tiller number were taken periodically for 63 days following stress.

The second experiment was conducted as the first except that the stress conditions were modified. The seedlings were stressed at 51.7 C for 40 min. The plants to be clipped were clipped three-quarter inch above the soil surface. Clipping removed all killed tissues on the stressed plants. Measurements were taken as before for 60 days following stress.

In the third experiment the pre-stress conditions were altered. It appeared that if the seeds were germinated in light the first node was too high causing the seedling to have a weak base. This problem was eliminated by starting growth in complete darkness for the first three days at 18.9 C, and then changing to low light intensity of 500 ft-c for three days. The plants were then moved into the greenhouse and 12 days after planting subjected to the following treatments. Half of the plants were heat-stressed in the oven at 55 C for 20 min. The controls were placed in darkness during the stress period so as not to give these any photosynthetic advantage over the stressed plants. Following the stress treatment half of the controls and half of the stressed plants were cut back to three-quarter inch above the soil surface. This experiment was replicated five times. Measurements were taken as before for 56 days following stress.

Heat Tolerance of Seedlings

The purpose of the second phase of investigation was to determine the heat tolerance of seedlings in relation to seedling age.

A preliminary study was made to determine the amount of time

required to kill 50% of the tissue of 12-day-old seedlings stressed at temperatures of 45, 50 and 55 C.

Seedlings were grown six days in the constant temperature room at 18.9 C, transferred to the greenhouse, and later thinned to two plants per pot.

A constant temperature water bath was used to stress the plants. The jiffy pots were inverted and placed on wide mesh screens so that the tops of the plants were immersed in the water to within one-half inch of the soil surface. The time durations used for each temperature were as follows:

<u>Temperature C</u>	<u>Time (min)</u>		
45	1	2	5
50	1	2	3
55	$\frac{1}{2}$	1	2

Twenty-four hours following stress the amounts of injury to the plants were recorded.

A second preliminary experiment was carried out using the same temperature treatments, a greater range of treatment times, and seedlings of five ages. The purpose of this experiment was to obtain data on the relation of heat tolerance to seedling age and to obtain a more satisfactory range of heat injury.

Potomac orchardgrass was grown for six days at 18.9 C in total darkness and then transferred to a growth chamber and grown under a constant temperature of 21.1 C, a 15 hr photoperiod and 1500 ft-c light intensity until the day of stress. Planting dates were staggered in order to have plants of five different ages on the day of stress.

Twelve-, fifteen-, eighteen-, twenty-one-, and twenty-four-day-old seedlings were stressed in the water bath at temperatures of 45, 50 and 55 C for four different periods of time. Each treatment was replicated five times. The four time periods used at each temperatures were as follows:

<u>Temperature</u>	<u>Time (min)</u>			
45	1	3	5	10
50	$\frac{1}{2}$	1	2	3
55	$\frac{1}{4}$	$\frac{1}{2}$	1	3

Following the treatments the plants were placed back in the growth chamber. On the fifth day following stress, the plants were rated for injury (the estimated amount of tissue killed as a percent of the total length of the leaf). A nine point scale was used:

<u>Reading</u>	<u>Percentage of Tissue Killed</u>
1	no kill
2	slight kill (<10%)
3	25%
3.5	37.5%
4	50%
4.5	62.5%
5	75%
5.5	87.5%
6	100% (complete kill)

Four experiments were conducted to determine heat tolerance of orchardgrass with respect to seedling age. In all experiments plants ranged from 13 to 43 days of age at three-day intervals on the day of stress.

Seedlings were stressed for three min at 50 C in the water bath by the same procedure as above. Following stress the plants were placed back in the growth chamber and evaluated for injury five days later using the above scale. These experiments were replicated four to six times.

In the first experiment the seedlings were grown at 21.1 C, 1500 ft-c light intensity and 15 hr photoperiod. In the second experiment growth chamber difficulty forced a change in the pre-stress conditions. Seedlings were grown in darkness for six days and then in light at 250 ft-c and 15 hr photoperiod for nine days and then transferred to a growth chamber and grown at 22.8 C, 1200 ft-c light intensity and 16 hr photoperiod. In the third experiment the seedlings were grown at 21.1 C, 3500 ft-c light intensity and a 15 hr photoperiod. And in the fourth experiment seedlings were grown under the same conditions as in the first experiment except that the plants were grown in four x four inch plastic pots rather than jiffy pots.

Photosynthetic Rate of Orchardgrass Seedlings Following Heat Stress

Five experiments were conducted to determine the effect of heat stress on the photosynthetic rate of orchardgrass seedlings.

The seedlings were grown in the constant temperature room at 18.9 C as before and transferred to a growth chamber and grown at 21.1 C, 1500 ft-c, 3500 ft-c light intensity and 15 hr photoperiod in the last four experiments.

Twenty-one-day-old seedlings were stressed in a water bath for 60 sec at 50 C by the same procedure as in phase II.

In the first two experiments a Warburg apparatus, equipped with a light bar, was used to measure photosynthetic rate at 30 C and 2000 ft-c; and in the last three experiments a Gilson Differential Respirometer was used to measure photosynthesis at 30 C and 1400 ft-c light intensity.

A light-dark-light-dark sequence of 15 min each was used with readings of O_2 exchange taken every five min. A $CO_3^{--}-HCO_3^-$ buffer of pH 8.65 was used to keep the CO_2 concentration constant at 0.03% (35). Total apparent photosynthesis was determined by summing the volumes of O_2 evolved or utilized during the last 10 min of each light period and dark period, respectively. The oven dry weights of the tissue sample were used to calculate the volume of oxygen produced per milligram of tissue per hour. Photosynthesis was measured one to five hr, 24 hr, one week, and three weeks following stress in the first experiment, and one to five hr, 24 hr, three days and one week following stress in the last four experiments.

One-inch sections of plant material which were stressed but not burned and corresponding sections of check plants were used to measure photosynthetic rate. In the last four experiments plant leaves were marked with India ink at the lowest point of heat stress on treated plants and corresponding points on check plants. The purpose of marking the leaves was to distinguish the stressed portions of leaves from the new growth following treatment. At each sampling date only marked leaves of stressed and check plants were used to measure photosynthetic rate. Each experiment was replicated three or four times.

For testing hypothesis of statistical results, accepted methods described by Snedecor (32) were used.

RESULTS AND DISCUSSION

Growth and Development of Heat Stressed Seedlings

In the three experiments where the effects of removing tissue by stressing, clipping, or both, upon growth were studied, results varied with the amount of tissue killed by stressing. In study one, more tissue was removed by stressing than by clipping. In study two less tissue was removed by stressing than by clipping, and in study three there was only tip burn. (See Figure 1) The detailed results of these treatments upon growth and development of orchardgrass seedlings appear in Appendix Figures 1 - 9.

Plant height, leaf number and tiller number of these three studies at 45, 43, and 41 days of age, respectively, appear in Table IA and the mean square values for treatments and interactions appear in Table IB. In study one these measurements were reduced more by heat stress than by clipping. There was a significant reduction in plant height (5% level) and highly significant reductions in leaf and tiller number (1% level) due to heat stress. There was also a significant reduction in tiller number due to clipping and a significant interaction between clipping and stressing on tiller number. In this first study a greater loss in photosynthetic tissue occurred from heat stress than from clipping.

In the second experiment, where tissue was not burned back to the point of clipping by temperature stress, significant reductions in leaf and tiller number occurred (Table IB). Surprisingly, clipping had no effect upon these variables either for stressed or non-stressed plants. Reasons for this lack of clipping effect are obscure.

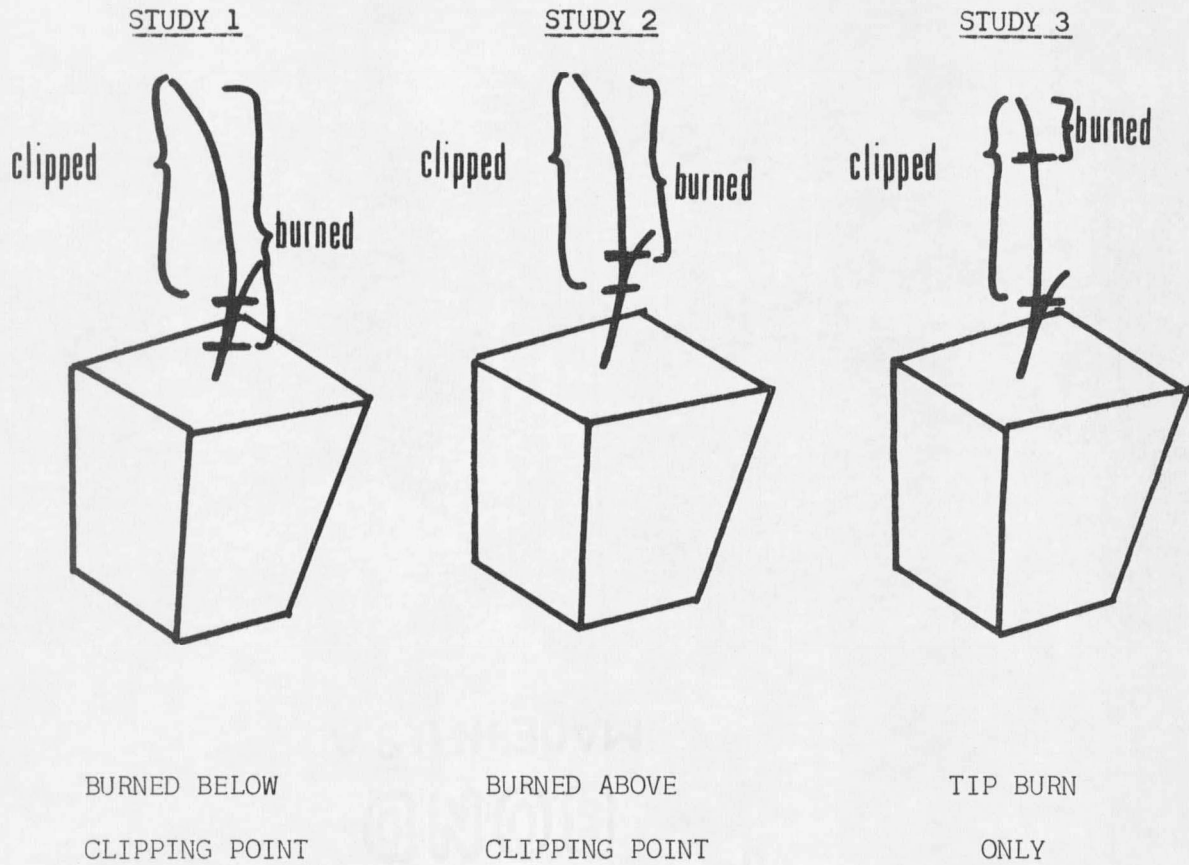


Figure 1. Illustration of amount of tissue damaged in relation to clipping point in three heat stress studies on thirteen-day-old orchardgrass seedlings.

Table IA. The effect of heat stress and clipping treatments on orchard-grass seedlings as measured by plant height, leaf number and tiller number. Readings taken 41-45 days following treatment.

Treatment	Degree of stress		
	Burned below clipping point	Burned above clipping point	Tip burn only
<hr/>			
	<u>Height (cm)</u>		
check	33.9	33.6	21.8
check-clipped	29.6	27.8	29.0
stressed	23.0	23.3	25.8
stressed-clipped	26.6	28.0	20.2
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	<u>Number of Leaves</u>		
check	55.6	32.3	31.2
check-clipped	41.3	30.0	21.8
stressed	19.6	19.3	30.2
stressed-clipped	25.6	18.0	26.2
<hr/>			
	<u>Number of Tillers</u>		
check	11.7	6.3	7.6
check-clipped	7.3	5.7	6.0
stressed	4.7	4.0	8.0
stressed-clipped	4.7	4.3	7.4
<hr/>			

Table IB. Mean square values of the clipping, stressing and clipping x stressing interaction sources of variation for plant height, leaf number and tiller number. Readings taken 41-45 days following treatment.

Source of variation	Degree of stress		
	Burned below clipping point	Burned above clipping point	Tip burn only
<u>Height (cm)</u>			
Clipping	0.67	1.00	3.00
Stressing	147.33*	79.83	29.00
Clipping x Stressing	47.66	78.50	205.00**
Error	11.45	29.61	17.83
<u>Number of Leaves</u>			
Clipping	52.16	10.17	225.00*
Stressing	2002.62**	469.83*	15.00
Clipping x Stressing	309.55	0.66	36.00
Error	51.63	66.06	31.75
<u>Number of Tillers</u>			
Clipping	14.17*	0.16	6.00
Stressing	70.17**	10.17	4.00
Clipping x Stressing	12.66*	1.00	2.00
Error	1.50	0.97	2.58

* and ** indicate significance at the .05 and .01 levels, respectively.

In the third experiment where only tip burning of the leaves occurred, there was a significant reduction in leaf number due to clipping. Neither clipping nor stressing resulted in significant reductions in height or tiller number; however, there was a highly significant interaction between clipping and stressing on plant height.

From these three studies it appears that the major effect of heat stress is the loss of initial photosynthetic tissue. There is little evidence that the effects of heat injury are translocated to other parts of the plant.

The decreases in leaf and tiller number from heat stress in orchardgrass in this study were similar to those observed by Laude (20) for red brome. With red brome, however, the effects gradually disappeared with time. This was not the case with orchardgrass. In Laude's studies photosynthetic tissue loss was not measured, but he attributed his results directly to gross heat stress effects. The results from the experiments here indicate that the major effect of heat stressing is the loss of photosynthetic tissue. Only the data for leaf number of the clipped but not stressed plants in study two do not agree with this hypothesis.

In this first phase of investigation there were many problems with technique. Inadequate control of air temperature, soil temperature, and soil moisture made it impossible to isolate the heat factor from the others present.

The oven air temperature was difficult to control when the oven door was opened. The soil temperature was not measured, but it is doubtful that it ever reached that of the air temperature. Soil moisture was at

field capacity prior to heat stress; however, it was obvious that considerable drying had taken place during the stress period. The relative humidity was probably lower than saturation since there was no control over it. This may have influenced the response obtained from the heat stress. According to Kinbacher's work (11), less heat injury should have resulted to the orchardgrass seedlings under the stress conditions used in this investigation than if the relative humidity had been 100%. However, Laude (20) stressed bromegrass in the two leaf-stage for five hr at 130 F and 40% relative humidity and obtained less injury than obtained in these studies at 20 min. Under his conditions the orchardgrass seedlings would have probably been completely killed unless the relative humidity here was higher than 40% resulting in more injury than at a lower relative humidity.

Heat Tolerance of Orchardgrass Seedlings

In the second phase of study 12-day-old orchardgrass seedlings were heat stressed at 45, 50 or 55 C for various lengths of time. It was difficult to obtain differential kill at all temperatures. The results in Table II show that 45 C did not produce any visible injury 24 hr following stress even after five min of stress. At 50 C a wide range of tissue kill was obtained over a short period of time. Percentage of tissue killed increased from 10 to 100% from one-three min of stress. All plants were killed at 55 C at all time durations used.

These data were used to design an experiment to determine the heat tolerance of orchardgrass seedlings in relation to seedling age. The results from two of the time durations used are reported in Table III.

Table II. Percentage of tissue killed on 12-day-old orchardgrass seedlings 24 hr following stress.

Temperature C	Time stressed (min)				
	$\frac{1}{2}$	1	2	3	5
	% Injury				
45	--*	0	0	--	0
50	--	10	75	100	--
55	100	100	100	--	--

* Blanks indicate time durations not used.

Table III. Percentage of tissue killed on orchardgrass seedlings in relation to seedling age. (measured five days following stress)

Seedling age (days)	Temperature C					
	45		50		55	
	Duration of stress (min)		Duration of stress (min)		Duration of stress (min)	
	1	3	1	3	1	3
12	0	0	10	10	13	25
15	0	0	2	10	30	22
18	0	4	13	10	25	40
21	4	8	19	50	55	70
24	4	10	4	85	75	100

It appears that 45 C was within the tolerable range for short time duration since it produced no visible injury five days after stress. Injuries produced at 50 and 55 C, were less than in the previous experiment.

Heat tolerance in orchardgrass changed considerably over a short period of time. For example, at 50 C and three minutes stress, a 12- or 15-day-old seedling had 10% tissue kill whereas a 24-day-old seedling had 85% tissue kill (Table 3). Thus, in a nine day period the heat tolerance decreased 75%.

In most cases heat injury was found to increase with plant age and with duration of stress in plants ranging in age from 12 to 24 days. No explanation can be given for the low percent tissue kill in 24-day-old seedlings stressed at 50 C for one min.

In four subsequent experiments plants in a wider age range were used. Only one temperature and time duration were used (50C and three min). The means from all four experiments averaged together are presented in Figure 2.

The younger seedlings again appear to be more heat tolerant than those slightly older. Heat tolerance decreased from 13 through 31 days, and then gradually increased through 43 days of age.

These results are similar to those obtained by Laude (20) on prairie bromegrass. However, he observed a peak of low heat tolerance lasting approximately two weeks from 14 days to 28 days after which tolerance increased with age through 56 days. Laude associated these responses with endosperm exhaustion and the transition toward independence from the seed and before the young plant is completely established.

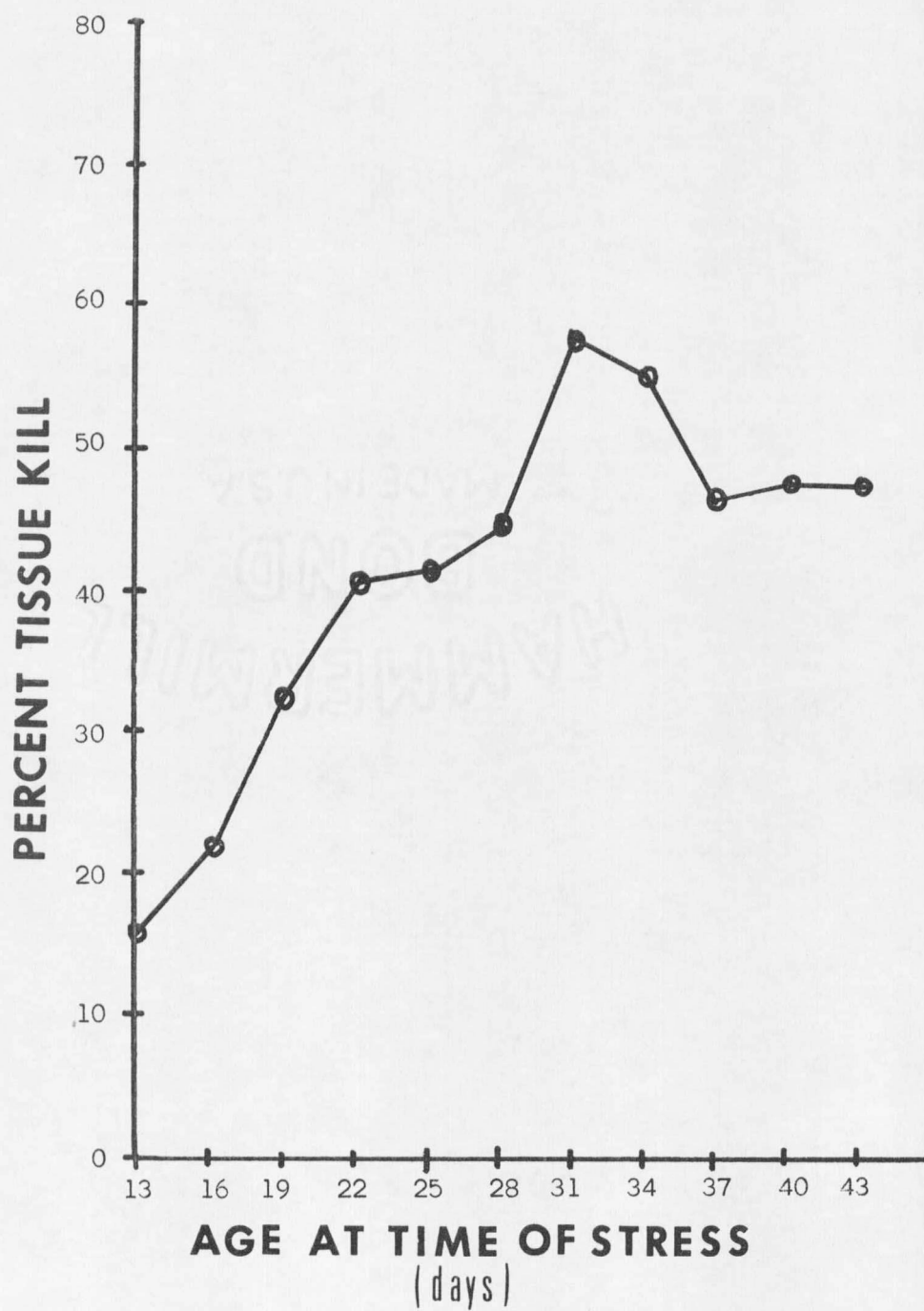


Figure 2. Heat tolerance of orchardgrass seedlings in relation to seedling age. (means of 4 experiments)

Endosperm exhaustion of orchardgrass occurs between 14-16 days at 20 C. ^{1/} The young seedling is therefore independent of the seed at 14-16 days of age. At this age the seedlings had the highest heat tolerance measured. It appears that the seedling enters a period of decreasing heat tolerance lasting for approximately 15 days during the initial stages of independence after which heat tolerance begins to increase.

It could be pointed out that there was much variability in the results obtained from these four experiments and that the graph in Figure 2 represents an average of all the data obtained.

Photosynthetic Rate of Orchardgrass Seedlings Following Heat Stress

The data from all five experiments on the study of the effect of heat stress on the photosynthetic rate of orchardgrass seedlings were averaged and are presented in Table IV. Each value represents the mean of all replications for each treatment at any given time of sampling. The data from the first experiment for photosynthesis at three weeks following heat stress are not included in this table since there were no significant differences after one week or at three weeks.

The data presented in Table IV show that there was a highly significant reduction (at the .005 level) in photosynthetic rate for at least 24 hr following heat stress, and a significant reduction in photosynthetic

^{1/} Murray, Glen A. 1963. Relationship of endosperm exhaustion to growth and survival of orchardgrass seedlings (*Dactylis glomerata* L.) subjected to cold stress. MS Thesis. Montana State College. 45p.

Table IV. Photosynthetic rate of orchardgrass leaf sections at different time intervals following heat stress.

Time of sampling (measured from time of applying heat stress)	Photosynthetic rate $\mu\text{lO}_2/\text{mg/hr}$		% Reduction
	Non-heat-stress leaf sections	Heat-stressed leaf sections	
1-5 hr	21.7	6.6	69.6***
24 hr	19.6	11.3	42.3***
3 days	22.6	15.7	30.5*
1 week	17.3	16.9	2.3

*** and * indicate significance at the .005 and .05 levels, respectively.

rate (at the 5% level) three days after stress. No significant reduction in photosynthetic rate occurred one week following stress.

It appears that heat stressing of orchardgrass seedlings caused a temporary reduction in the rate of photosynthesis which lasted over a period of time. A recovery of 27.3% of its photosynthetic capacity occurred the first day and an additional 11.8% over the second and third days following stress. There was no significant reduction in photosynthetic rate one week after heat stress, and it appeared that the heat-stressed tissue had fully regained its photosynthetic capacity.

In the first phase of investigation it was postulated that the major effect of heat stress on the growth and development of orchardgrass seedlings was the loss of initial photosynthetic tissue. From the additional information gained in the last phase of investigation it may now be postulated that the major effect of heat stress on the growth and development of orchardgrass seedling is the loss of initial photosynthetic tissue and the reduction in the rate of photosynthesis of heat stressed, but not killed, tissue which lasts over a period of several days.

SUMMARY

Orchardgrass seedlings from clonal material or from the commercial Potomac variety were used to study some of the effects of high temperature stress. Seedlings were grown under controlled environmental conditions in the greenhouse or in growth chambers. Plants were stressed in ovens or in water baths.

The effect of loss of leaf tissue by heat stressing in an oven, clipping, or both, upon the growth and development of orchardgrass seedlings was studied in three experiments. Results varied with the amount of tissue killed by stressing. Significant reductions in plant height, leaf number and tiller number occurred as the result of temperature stress but not by clipping where more tissue was removed by stressing than by clipping.

Where less tissue was removed by stressing than by clipping there were significant reductions in leaf and tiller number resulting from temperature stress, but not from clipping. This was not expected and the reasons are obscure.

Significant reductions in leaf number due to clipping occurred where there was only tip burning of the leaves. No reductions in height or tiller number resulted from either clipping or temperature stress. There was a highly significant interaction between clipping and stressing on plant height.

It was hypothesized that the major effect of heat stress on the growth and development of orchardgrass seedlings was the loss of initial photosynthetic tissue.

Heat tolerance of orchardgrass seedlings was studied using various temperatures and durations of stress on plants of different age. Twelve-day-old seedlings were not visibly injured by 45 C even after five min stress in a water bath, a wide range of injury was obtained at 50 C at one to three min stress, and all plants were killed at 55 C at all time durations used.

Heat tolerance changed rapidly over a short period of time. Heat tolerance decreased 75% in seedlings ranging in age from 12-15 days to 24 days when stressed three min at 50 C.

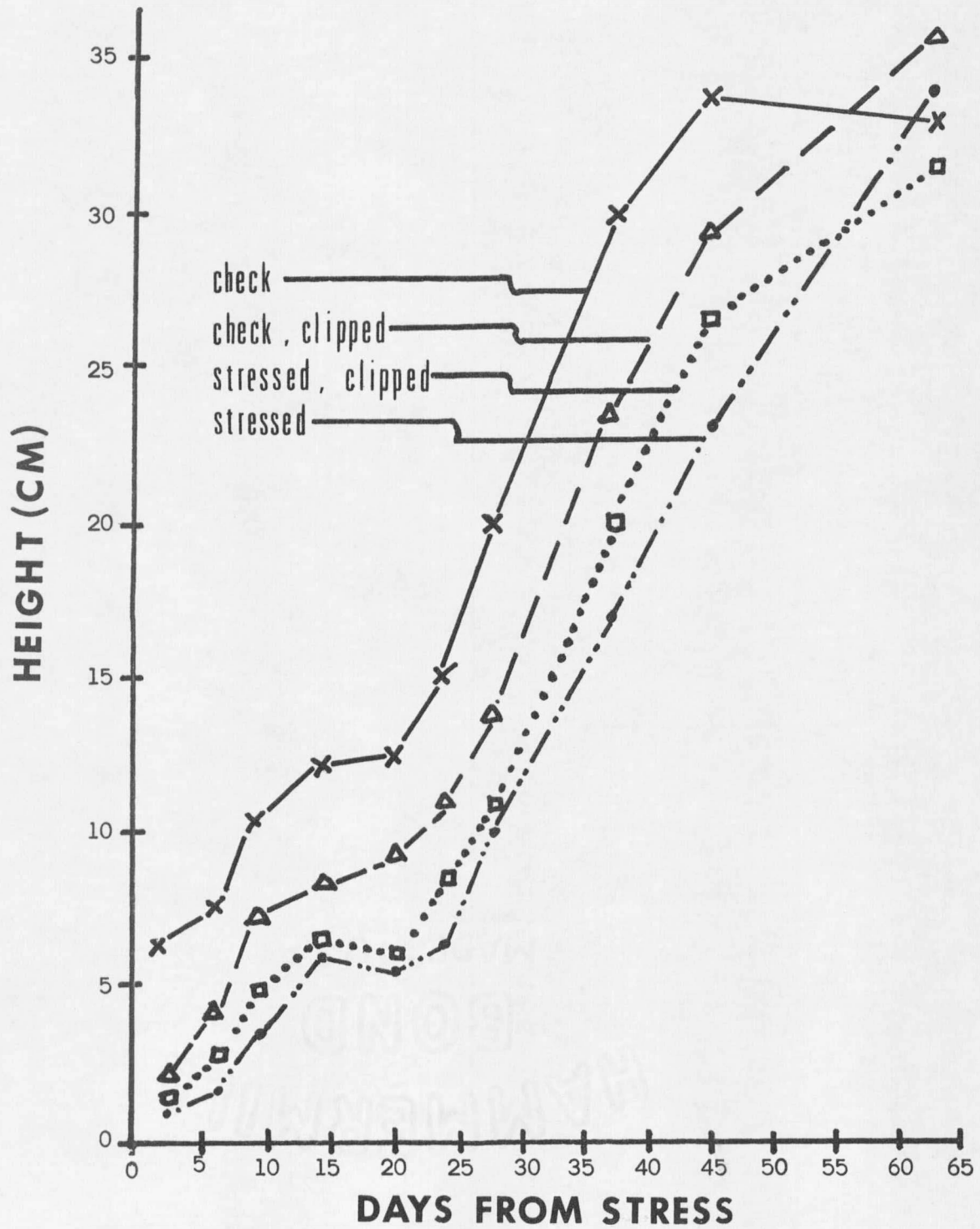
In plants of a wider age range (13-43 days of age) the younger seedlings appeared to be the most heat tolerant. Heat tolerance decreased from 13 to 31 days of age and then gradually increased through 43 days of age. The loss of heat tolerance by young seedlings could not be explained.

The effect of heat stress on the photosynthetic rate of orchardgrass seedlings was studied on 21-day-old seedlings stressed for one minute at 50 C in a water bath. Highly significant reductions in photosynthetic rate occurred through 24 hours following heat stress and was significant after three days. Heat stressed seedlings gradually regained their photosynthetic capacity after heat stress and full recovery was observed one week after stress.

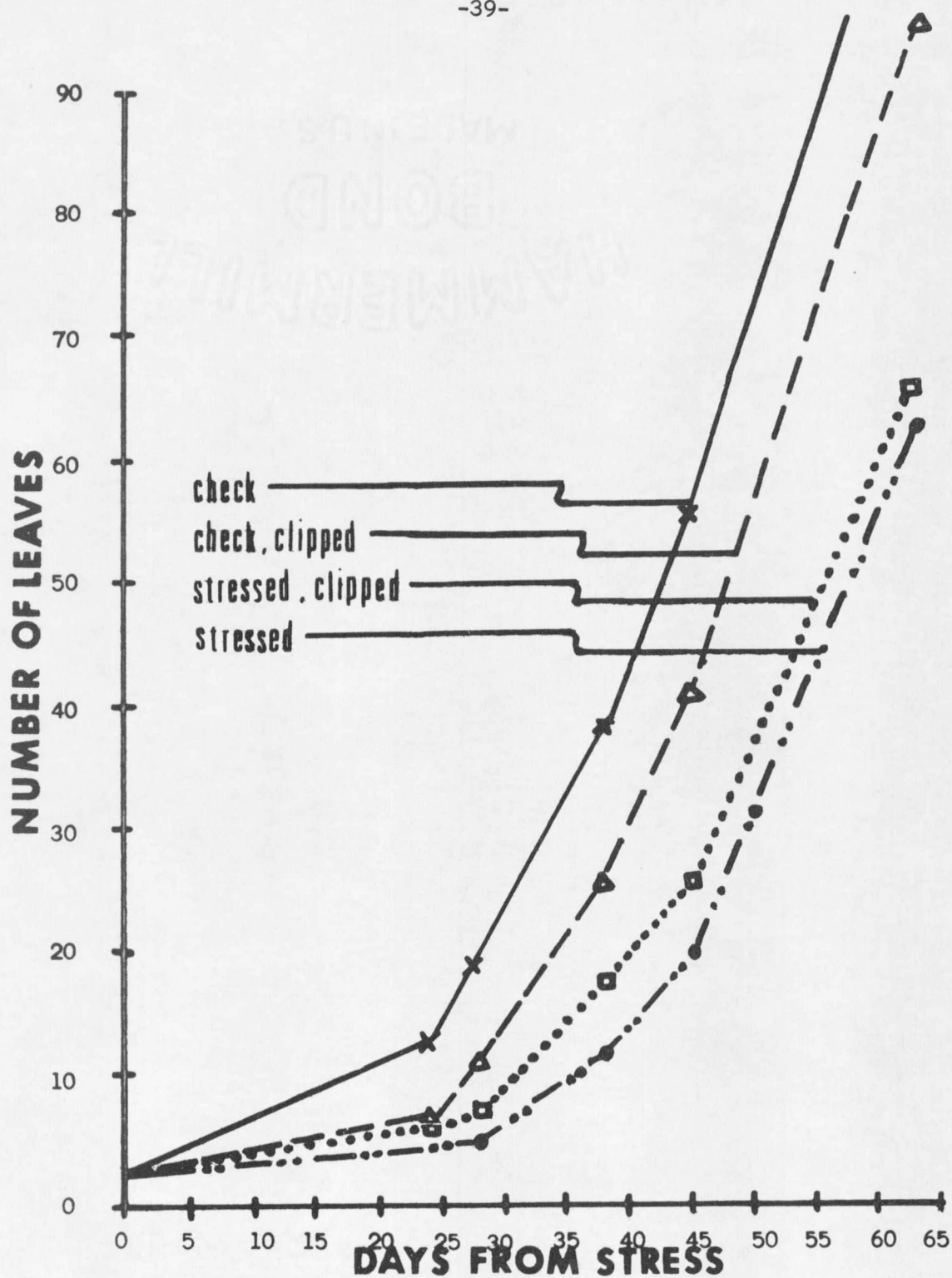
It was therefore hypothesized that the major effect of heat stress on the growth and development of heat-stressed orchardgrass seedlings as evidenced by reductions in growth of seedlings is the loss of initial photosynthetic tissue and, in addition, the reduction in the rate of

photosynthesis of heat-stressed, but not killed, tissue which lasts over a period of several days.

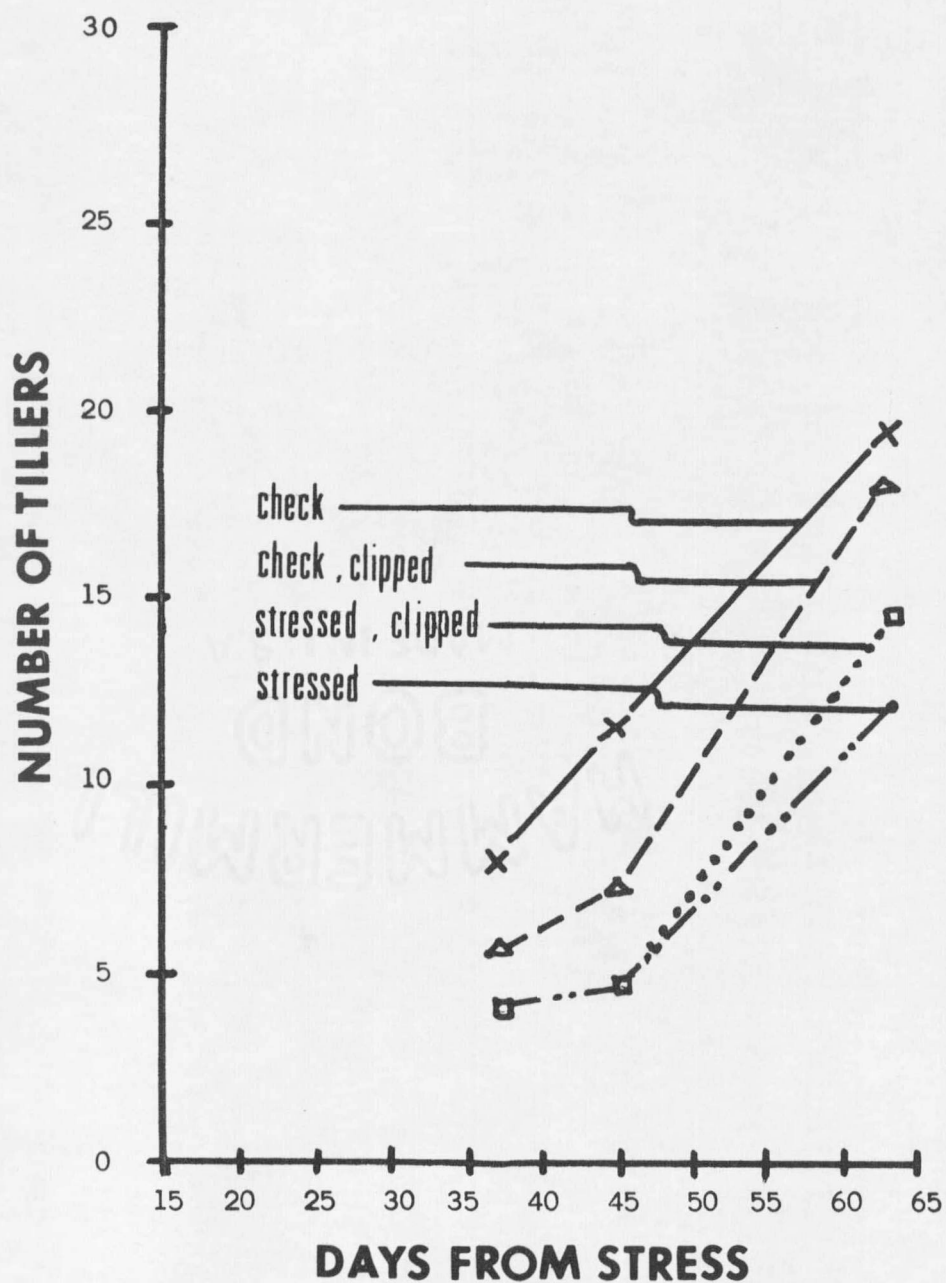
APPENDIX



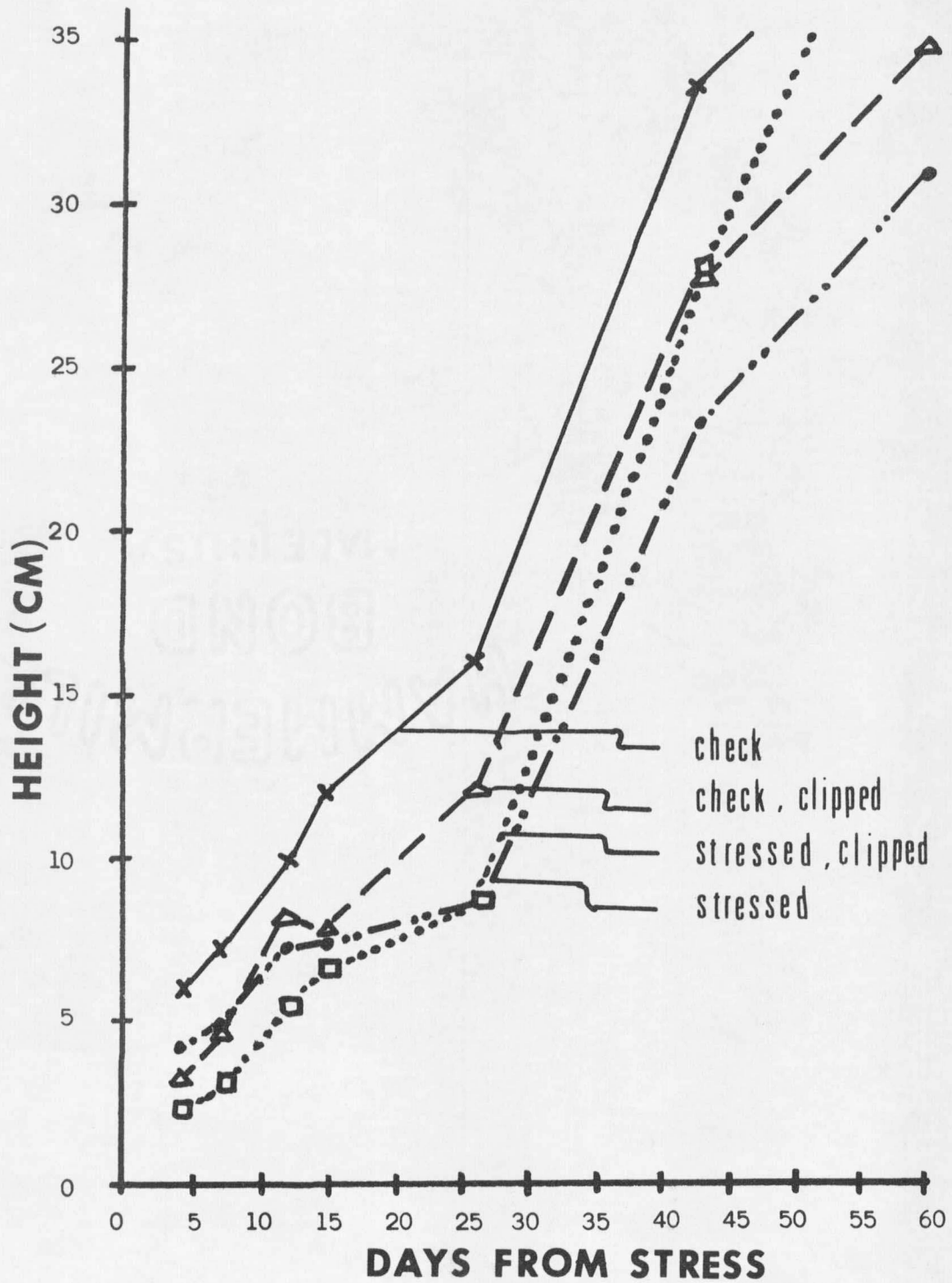
Appendix Figure 1. Height of orchardgrass seedlings following heat stress of 54.5 C for 35 minutes.



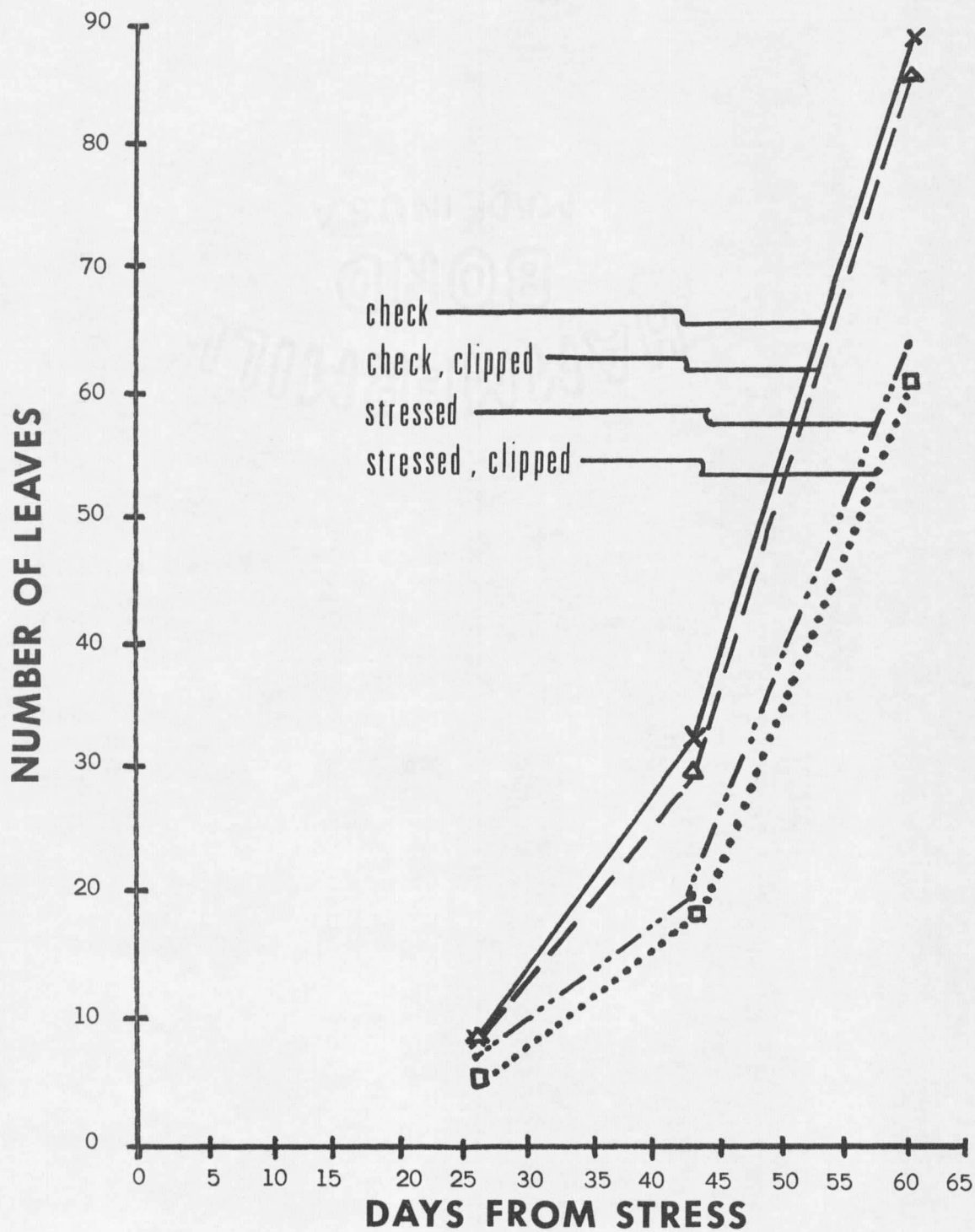
Appendix Figure 2. Number of leaves of orchardgrass seedlings following heat stress of 54.5 C for 35 minutes.



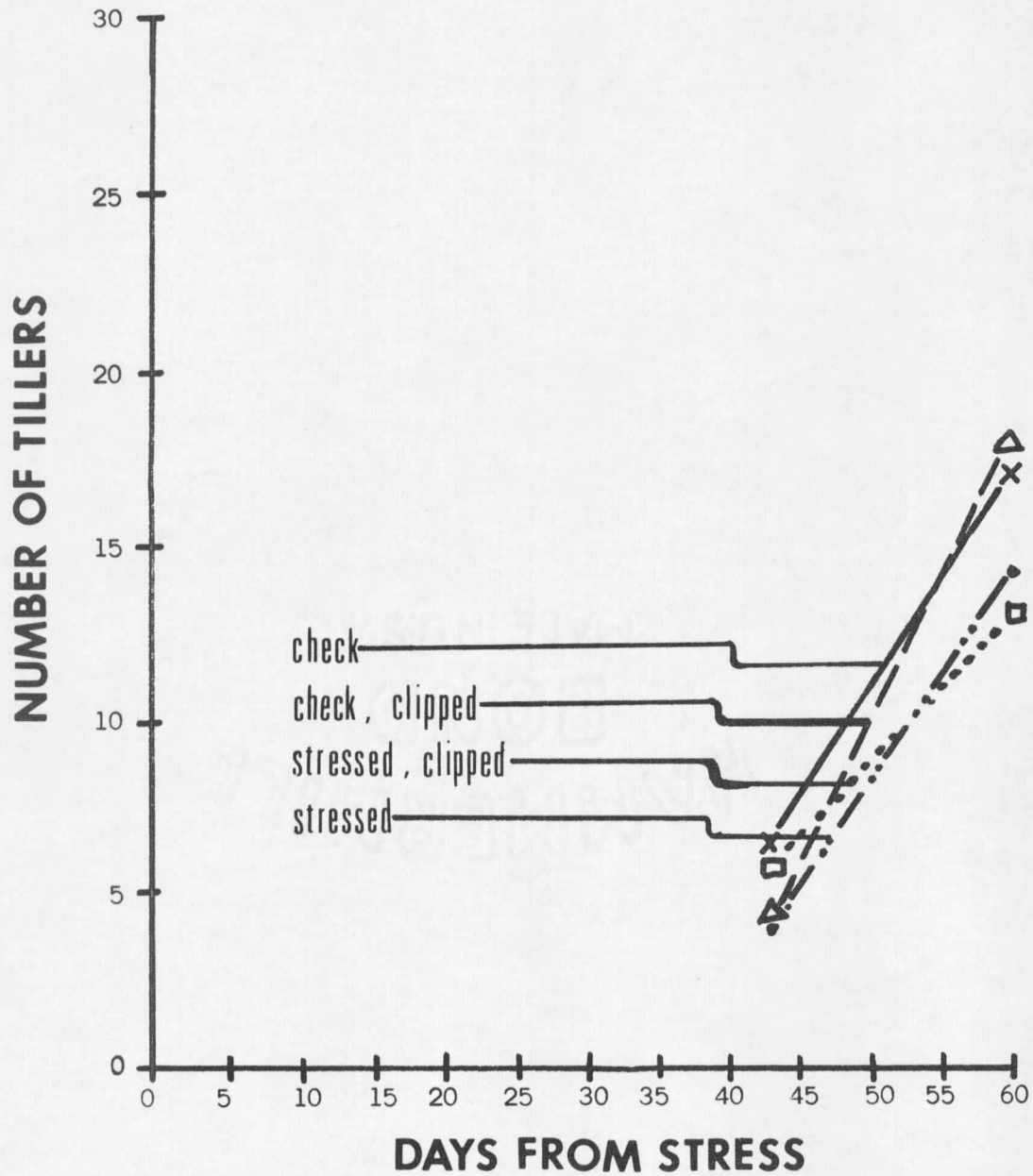
Appendix Figure 3. Number of tillers of orchardgrass seedlings following heat stress of 54.5 C for 35 minutes.



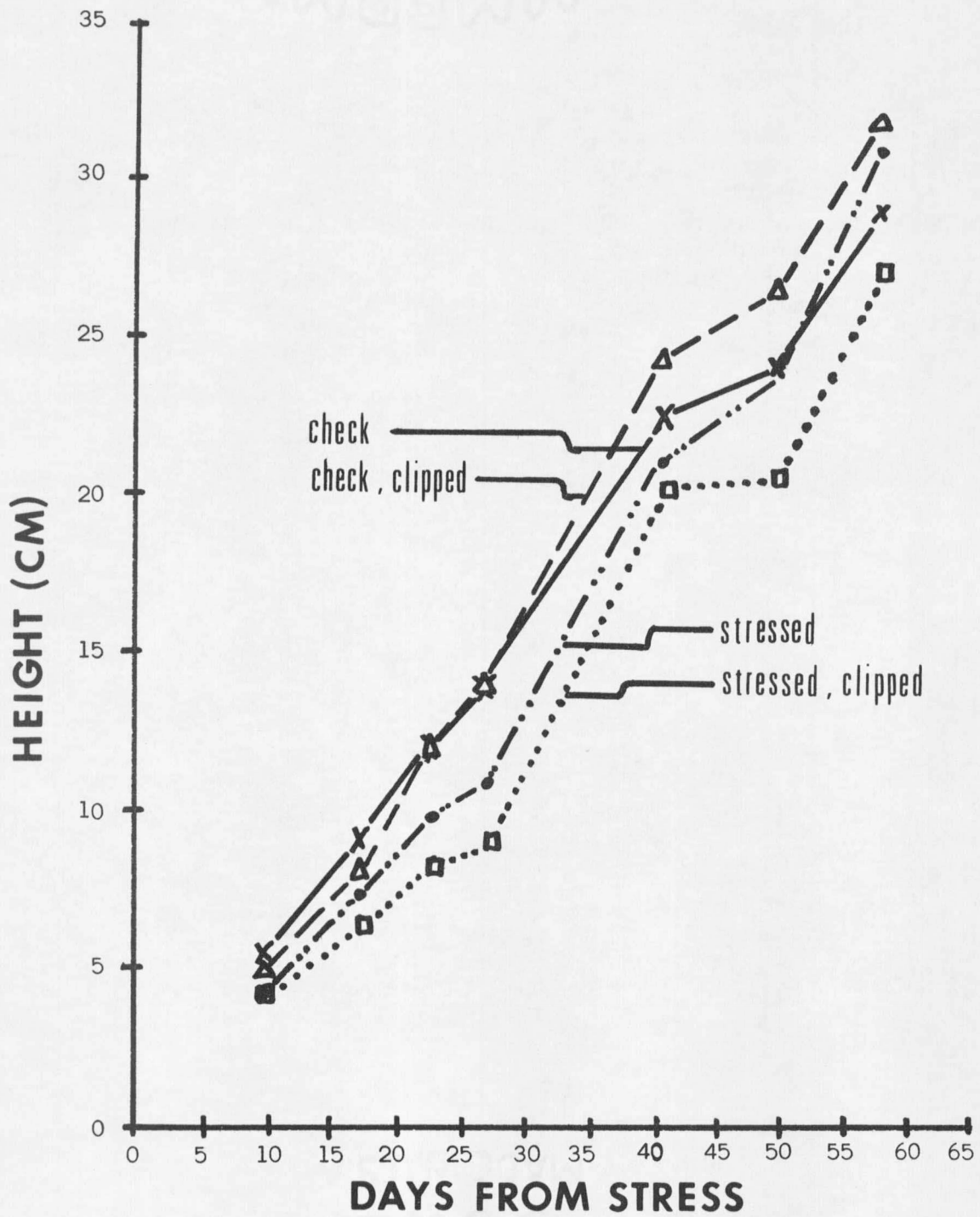
Appendix Figure 4. Height of orchardgrass seedlings following heat stress of 51.7 C for 40 minutes.



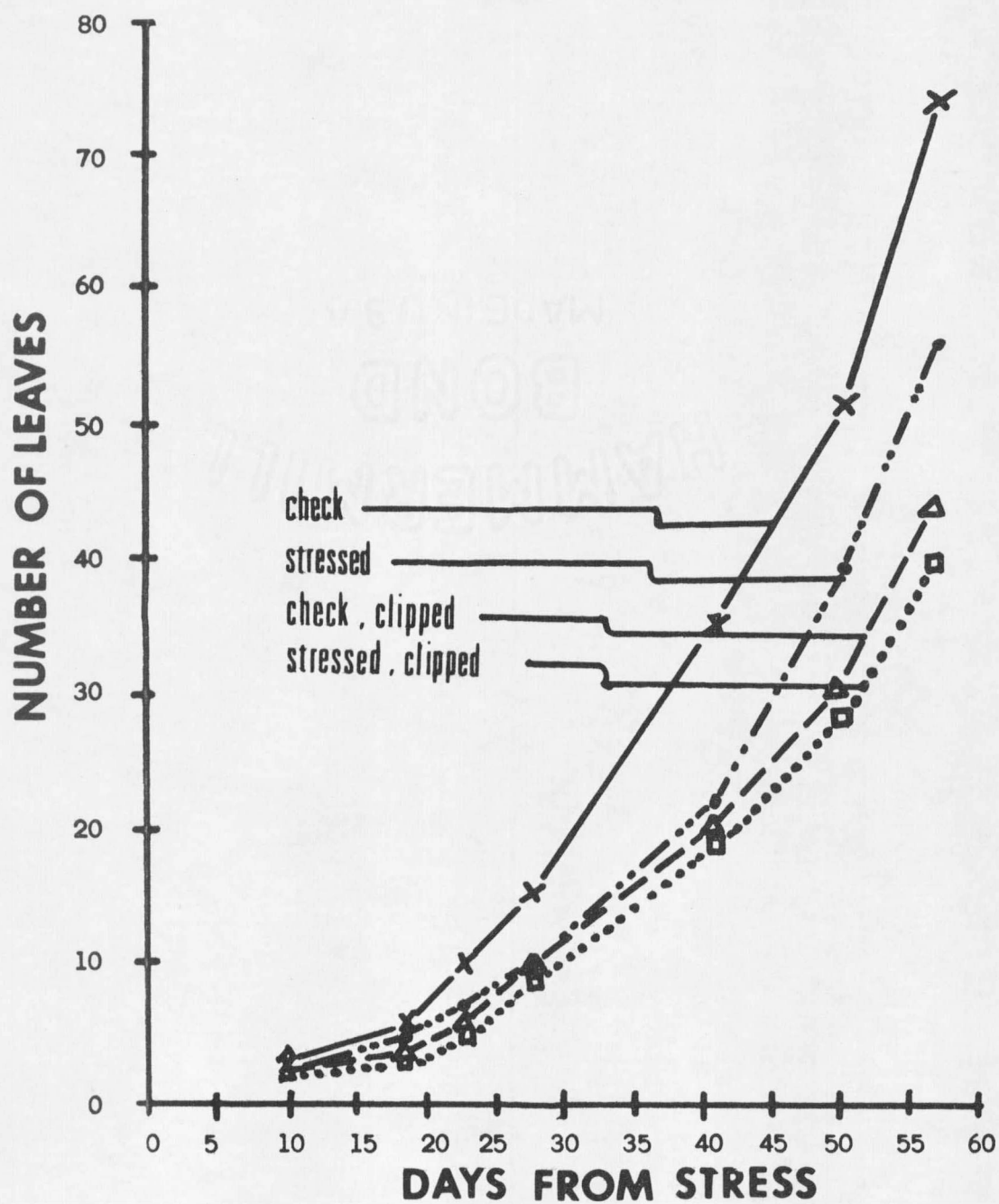
Appendix Figure 5. Number of leaves of orchardgrass seedlings following heat stress of 51.7 C for 40 minutes.



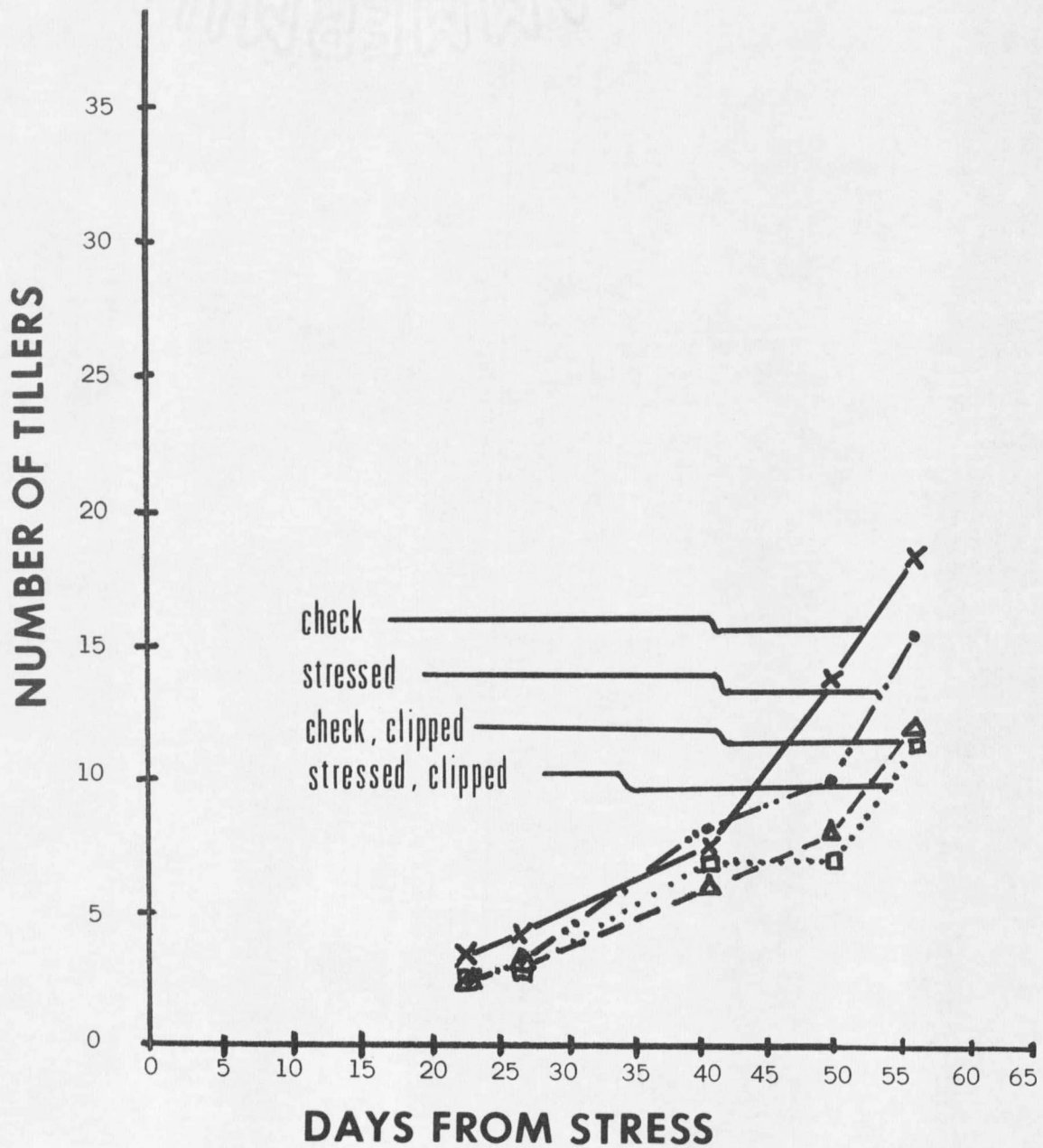
Appendix Figure 6. Number of tillers of orchardgrass seedlings following heat stress of 51.7 C for 40 minutes.



Appendix Figure 7. Height of orchardgrass seedlings following heat stress of 55 C for 20 minutes.



Appendix Figure 8. Number of leaves of orchardgrass seedlings following heat stress of 55 C for 20 minutes.



Appendix Figure 9. Number of tillers of orchardgrass seedlings following heat stress of 55 C for 20 minutes.

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