PHYSIOLOGICAL AND ANATOMICAL MANIFESTATIONS OF DROUGHT IN THE VEGETATIVE SHOOT OF DROUGHT-SUSCEPTIBLE AND DROUGHT-RESISTANT WHEAT VARIETIES

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

CHAPTER I

GENERAL INTRODUCTION

A broad view of the problem of water stress in plants reveals certain generally accepted basic concepts. Significantly, a limited water supply causes a decrease in synthesis and an increase in hydrolysis which results in decreased growth. The various organs of the plant body are affected differently; the age of the plant at the time the stress is applied influences the response. Plants may be hardened by exposure to intermittent periods of stress, thus enabling them to better withstand the drying.

Much of our present knowledge concerning these conclusions is based upon interdisciplinary treatment of certain areas of investigation. Observations of morphological and anatomical differences in the plant body as well as physiological data have contributed a large part of the experimental evidence. Differences may exist between mesic or xeric species or between intra-specific groups when they are grown in areas of greater or lesser amounts of available water. Certain plants therefore, have been classified as drought resistant varieties and are capable of surviving various periods, and varying degrees of drought. The survival ability depends upon the ability to absorb water against a high soil moisture tension, to reduce water loss, and to endure dehydration (Kozlowski, 1964). In some plants evidence of this ability is expressed by visible morphological adaptations.

Evidence of the manifestation of a change in morphological or anatomical characteristics in the same organ of the same kind of plant when grown in environments of either high or low moisture supply indicates that an alteration of internal water balance has occurred. Water imbalance results in changes in basic physiological processes which are expressed through the various patterns of morphogenesis.

The ability to endure dehydration is considered a property of the protoplasm, and in some cases drought resistant plants may exhibit the characteristic of endurance without manifesting gross morphological or anatomical adaptations. Contrastingly, plants which are susceptible to drought injury may survive certain periods of drought by developing some adaptive structures, but in spite of such development, further desiccation could not be tolerated. In these plants dehydrated protoplasm is unable to withstand the extreme changes in the physiological processes which occur on continued drying.

In many instances the effects of environmental water on certain anatomical relationships have been shown to have a direct relationship to a particular physiological process such as photosynthesis or transpiration (Kozlowski, 1964). The impact of interpretations of this nature on an increased understanding of the mechanisms involved in drought resistance has undoubtedly contributed much to the sustained interest in the problem.

Previous investigations of internal water stress in the wheat plant reveal few reports of the effects on the anatomy of the various plant parts (Maximov, 1929; Penfound, 1931). These workers observed a direct effect of internal water balance on the percentage area of xylem tissue and the total volume of other mechanical elements.

Physiological experimentation on drought-subjected wheat plants have revealed certain functional responses of subcellular structures and molecular systems within certain organs (Todd and Yoo, 1964; Todd and Basler, 1965; Todd and Webster, 1965).

It is conceivable that a study of the differentiation patterns in the primary developing tissues, and a comparison of the anatomy and morphology of the seedling before and after water stress might reveal certain ontogenetic adaptations as visible expressions of particular physiological responses to drought. The established relationship between moisture level and amount of tissues which characteristically have thickened lignified cell walls seemed to indicate an important role of the peroxidase catalyzed reactions which lead to lignin formation.

The present report deals with observations on the drought response of several "hardy" and injury-susceptible varieties of wheat. The intentions of this investigation have been (1) to identify and compare anatomical variations in the leaf of representative varieties of the two behavioural forms of wheat, (2) to compare survival abilities of the plants, and to measure the leaf growth and peroxidase activity before and after subjection to drought stress, and (3) to descriptively analyze the anatomical patterns of the vegetative shoot tip with special reference to procambial and xylem differentiation in a known drought hardy and a drought susceptible variety of wheat before and after subjection to drought.

CHAPTER II

REVIEW OF LITERATURE

The concept of the role of drought resistance in plant growth and survival has been summarily treated in such reviews as those by Levitt (1956), Parker (1956), and Oppenheimer (1960). The UNESCO volume (1961), on arid zone research emphasized the dominant role of water in plant growth. The importance of the relationship of water deficits and physiological processes was established by Hagan (1955), Hagan et al., (1959), and Vaadia et al., (1961).

Controversial treatment of the terminology and mechanisms involved in drought resistance has led to much confusion in the literature. Early considerations pointed to structural adaptations which influenced the rate of water loss (Kramer, 1959b), as being the determinant factor in influencing the ability of the plant to withstand drought. As massive amounts of experimental evidence have accumulated, emphasis has shifted to considerations of the properties of protoplasm to endure dehydration as a basic factor in drought resistance (Kramer, 1959b; Kozlowski, 1964). Iljin's (1931, 1935), concerted efforts on this particular aspect of the problem resulted in his mechanical injury theory as the explanation of the mechanism by which the protoplasm is injured. The visible tearing of the protoplasts he attributed to the associated effects of drying and remoistening rather than to water loss itself. His observations of cellular contraction during desiccation and pulling

in of the wall with the collapsing protoplast were considered as supportive evidence. The importance of a large ratio of cell surface to volume in increasing resistance to dehydration was also emphasized. Although attempts to explain dehydration resistance on the basis of differences in bound water content have appeared in the literature (Todd and Levitt, 1951; Migahid, 1961), Kramer (1959b), claimed that bound water cannot protect protoplasm against dehydration. He pointed out that differences in bound water content are caused by changes in total water content and chemical composition and are not a cause of drought resistance. The relationship of increased protoplasmic viscosity, decreased pore permeability, and increased drought resistance reported by Stocker (1948), has not to this writers knowledge been refuted. His claim of changes in the calcium potassium ratio as the causative agent in protoplasmic viscosity changes however, was not supported by the data of Simonis (1952) and Werk (1954).

Several workers have established that protoplasma from different sources will show different endurance abilities (Milthorpe, 1950; Pisek and Larcher, 1954; Levitt, 1956). Changes in dehydration endurance with season, age, and temperature have been reported by Pisek and Larcher (1954), and Abel (1956), thus pointing significantly to the importance of biochemical interrelationships.

Consensus at the present time admits the inadvisability of sole dependence on either structural adaptations or protoplasmic properties alone as sufficiently explanatory in drought resistance mechanisms, but rather, views the problem from the standpoint of the involvement of many factors. On this basis various types and degrees of drought resistance have been differentiated (Kramer, 1959b), and crop plants

identified as drought resistant are so classified because they are capable of postponing the development of critical internal water deficits. This characteristic is attributed to the possession of a limited or moderate ability to endure dehydration, combined with structural characteristics which decrease the rate of water loss.

In the present paper the term drought-resistance embraces Kramer's twofold consideration, and structural characteristics as outlined by Oppenheimer (1960), are deemed adaptive responses to drought if observed to develop after such stress has been applied. This writer is of course cognizant of the difficulty in separating the normally occurring structural effects of aging from those of stress.

Different varieties of wheat respond differently to internal tissue water deficits (Helmerick and Pfeifer, 1954; Todd and Webster, 1965), and results indicate that there may possibly be stages of growth at which the plants are more susceptible to injury. According to Levitt (1956), older tissues are less resistant to drought than are younger ones. Milthorpe (1950), demonstrated that in young wheat seedlings total moisture could be reduced 98% before emergence of the first leaf without permanent injury. Susceptibility to drought increased with age and was attributed to the increasing proportion of vacuolated to meristematic tissue. Kramer and Kozlowski (1960), however, pointed out the difficulty in ascertaining a true desiccation resistance differential in young and old tissue since water is often translocated from older tissues to younger ones during internal water stress of the whole plant.

Effects of leaf water deficits on some of the more important physiological processes have recently been treated by Kozlowski (1964).

Increased hydrolysis has been reported to occur in water stressed leaves. Significant among the chemical changes indicative of such increase is a reduction in total carbohydrate and a conversion of starch to sugar (Woodhams and Kozlowski, 1954). Vassiliev and Vassiliev (1936), found that wheat leaves exposed to drought differed from thoroughly hydrated plants by being higher in hemicellulose and sugars. Thickened cell walls develop with the occurrence of accelerated conversion of polysaccharides into wall materials (MacDougal and Spoehr, in Shields, 1950). Limited stretching growth results from internal water stress and has been attributed to increased wall thickness (Shields, 1950). Cells with thickened walls are better able to withstand tensions and compression than thin walled cells. The ability to withstand compression has been attributed to lignin deposition between the cellulose microfibrils (Davies, Giovanelli, and A. P. Rees, 1964).

Numerous investigators have pursued an understanding of the biosynthetic pathways involved in the process of lignification in a variety of plants. In a recent comprehensive review (Brown, 1966), it was pointed out that peroxidase has been shown to catalyze the polymerization of many phenols in the presence of hydrogen peroxide to lignin or lignin-like substances. Prevailing evidence supports the view that in higher plants peroxidase is the enzyme responsible for the formation of certain required oligomers, and polymerization of such products in the synthesis of lignin. Todd and Yoo (1964), demonstrated a difference in peroxidase activity of detached wheat leaves when desiccated and when stored over water.

The overall growth pattern of the shoot is a result of sustained activity of the apical meristem at the tip of each shoot. In

meristematic regions subjected to water deficits, growth is materially reduced (Loomis, 1934; Thut and Loomis, 1944; Wilson, 1948). In the normally developing shoot tip distinct physiological and morphological activities make possible the recognition of a series of definable regions (Wardlaw, 1957). These are (1) the distal region "the center or focal point of the meristem on which the integrity and sustained development of the primary axis depend", (2) the subdistal region, in which the inception of growth centers takes place, (3) the organogenic region, subjacent to the subdistal region, in which the outgrowth of leaf primordia takes place, (4) the subapical region, subjacent to the organogenic region and usually characterized by a considerable widening and elongation of the shoot, accompanied by a conspicuous enlargement of the primordia (tissue differentiation which begins at various levels in different apices is well advanced in this region), and (5) the region of maturation. Wide histological diversity is displayed by the shoot apices of various plants. The duplex type as defined by Newman (1961), is descriptive of that observed in angiosperms and is described in terms of Schmidt's (1924), tunica corpus concept. A third aspect of apical meristem organization is the zonation pattern revealing a central, peripheral, and a pith rib meristem zone as described by Foster (1938). Reports indicate that zonation patterns differ from time to time in the individual apex, the zones therefore, are not discrete entities, but evidence differential activity in the shoot apex (Allsopp, 1964).

In a recent review Allsopp (1964), presented a very thorough coverage of experimental work on various aspects of shoot morphogenesis with special reference to apical growth. Some controversy exists in the

literature concerning the degree of autonomy of the shoot apex. It has been well established that the apex can undergo changes in response to influences from distant parts of the shoot, such as the response to the flowering impulse. Other investigations however, both cultural and surgical, emphasize the autonomy of the normal vegetative apex and these workers describe it as a self determining region, apart from the supply of nutrients (Wardlaw, 1947, 1950, 1952; Ball, 1948, 1952; Allsopp, 1965). The surgical experiments of Ball (1960), however, indicated that the shoot apex of higher plants had undergone biochemical differentiation in the direction of loss of synthetic ability. The latter author therefore, supported the view that the pattern of activities of the apical meristem is determined by the subjacent tissues.

All apices show early differentiation of the embryonic cells into epidermis, cortex, vascular tissue, and usually pith (Allsopp, 1964). The differentiation of vascular tissue reveals the fairly distinct processes of procambial development and the differentiation of the vascular tissue from the procambium (Clowes, 1961; Esau, 1965b). In spite of controversial reports concerning the determinate site of vascular differentiation, direct observation has led to general acceptance that the development of procambium is acropetal, in that it progressively extends distally from the procambium already present. This is of course a consequence of apical growth, but is interpreted to support the view that the pattern of differentiation is determined by the mature tissue below (Esau, 1965b). Experimental investigations on the other hand promote the conclusion that the inception of vascular tissue is a basipetal process (Wardlaw, 1965).

Many investigators have reported on the anatomical structure of

shoot apices of Gramineae but in most cases the interest was in the distinguishing features of younger and older apices or in the differences of vegetative and reproductive apices (Rosler, 1928; Kleim, 1937; Thielke, 1962). Sharman (1945), presented some considerations on the biology of the tip of Agropyron repens, and Buvat (1953), and Poux (1959), pointed to the importance of ontogenetic considerations.

According to Opatrna et al., (1963), the wheat apex was studied in detail by Kuperman (1961), who established a scale of 12 differentiating levels based upon morphological changes from germination to the time of grain production. A description of the anatomy as observed in the first four Kuperman Stages has been given by Opatrna, Seidlova, and Benes (1963). This work emphasizes the transition from the vegetative to the reproductive apex.

The present report is concerned with the effects of internal water stress on the anatomy of the vegetative wheat apex with special reference to the procambium and xylem differentiation.

CHAPTER III

ANATOMICAL VARIATIONS IN THE LEAF

Introduction

Plants subjected to periodic dryness respond in various ways. During such exposures, some plants will "resist" the stress while others manifest a degree of "injury." Numerous investigators have reported on the effects of soil moisture and atmospheric humidity on certain anatomical relationships in a variety of plants (Eberhardt, 1903; Yapp, 1912; Lebedincev, 1927; Scheibe, 1927; Maximov, 1929; Penfound, 1931; Cain and Potzger, 1940; Parker, 1952; Simonis, 1952). A comparative analysis of the anatomical features of the leaves of a drought-hardy strain of wheat closely related to a drought-susceptible strain might contribute to an understanding of the differential response between these 2 plant groups. The appearance of anatomical changes after internal water stress could conceivably be correlated with the apparent ability or lack of ability of the plant to withstand the environmental change.

In the present investigation a study was made of the leaf anatomy of a known drought-hardy strain, Triticum aestivum L., cv. Cheyenne (C. I. 8885), and a known non-resistant strain, T. aestivum L. cv. Ponca (C. I. 12128) (Todd et al., 1962). Changes in the anatomy of the second foliar leaves were compared after exposure to various degrees of water stress.

Materials and Methods

Seeds of each variety of wheat were sown in separate 6-inch pots in well moistened sandy loam. After the initial germination period the seedlings were thinned to 25 per pot and maintained in the greenhouse under conditions conducive to good growth for a period of 3 weeks.

Experimental materials consisted of 5 potted samples of each variety, the number 1 pot served as the control and was watered at regular intervals throughout the duration of the experiment. After the preliminary 3-week growth period, applications of water were discontinued to the fifth pot. At 2 day intervals thereafter pot number 4, 3, and 2 respectively, received no more water. The experiment was discontinued when samples number 2-5 had received 2, 4, 6, and 8 days of stress respectively. Four replicate experiments were run. A fifth experiment was designed so that all experimental plants were subjected to a 4-day stress period after which time they were maintained in a well moistened condition for 4-days, and then harvested.

After the indicated treatment three samples of the second leaf from each pot were collected, and the percent relative turgidity was determined. Weatherley's method as modified by Todd et al., (1962), was used. Portions of other samples of second leaves were cut into 1.5-cm lengths. The first 1.5-cm from the leaf apex was discarded, and the other samples were placed in fixative solution, Craf III, as modified by Sass (1951). The tissue was dehydrated by the TBA series as described by Jensen (1962). Transverse and longitudinal serial sections 10-12 μ thick were stained in iron-alum hematoxylin, safranin, orange G, crystal violet, fast green, or Bismarck Brown Y.

Tissue sections were examined with regard to overall histology,

cell patterns, and cellular inclusions. Different kinds of cells, cell sizes, cell wall relationships, and cell position in the system relative to internal and external environment were considered.

Observations

The basic anatomical features seen in the controls of each variety were essentially the same as those described by Percival (1921), for the foliage leaf of wheat. Ponca had more xylem area in relation to leaf thickness, as well as more prominent nuclei, and larger and more abundant chloroplasts than did Cheyenne. Ponca also showed relatively larger substomatal cavities and intercellular spaces. Sclerenchyma tissue was relatively more abundant in Cheyenne.

Visual observation of the whole plants showed regions of chlorotic tissue and severe wilting after more than 4 days of drought. Data in Figure 1 show that between the fourth and sixth day of stress, leaf samples of both varieties exhibited a sharp drop in percent relative turgidity.

Sections from drought-stressed leaves of both varieties showed a loss of tissue organization, and plasmolysis of nuclei and chloroplasts. As internal stress increased, cell wall thickness of all tissues increased progressively in both varieties. Epidermal wall thickenings showed a progressive increase in deposition of wall materials at the outer surface of both lower and upper epidermis. The epidermal walls associated with the mesophyll tissue, however, showed some regions which seemed to lack an affinity for wall-specific strain. Xylem elements and sclerenchyma tissue in both varieties showed angular and lamellar thickenings, but the angular thickenings appeared to be more

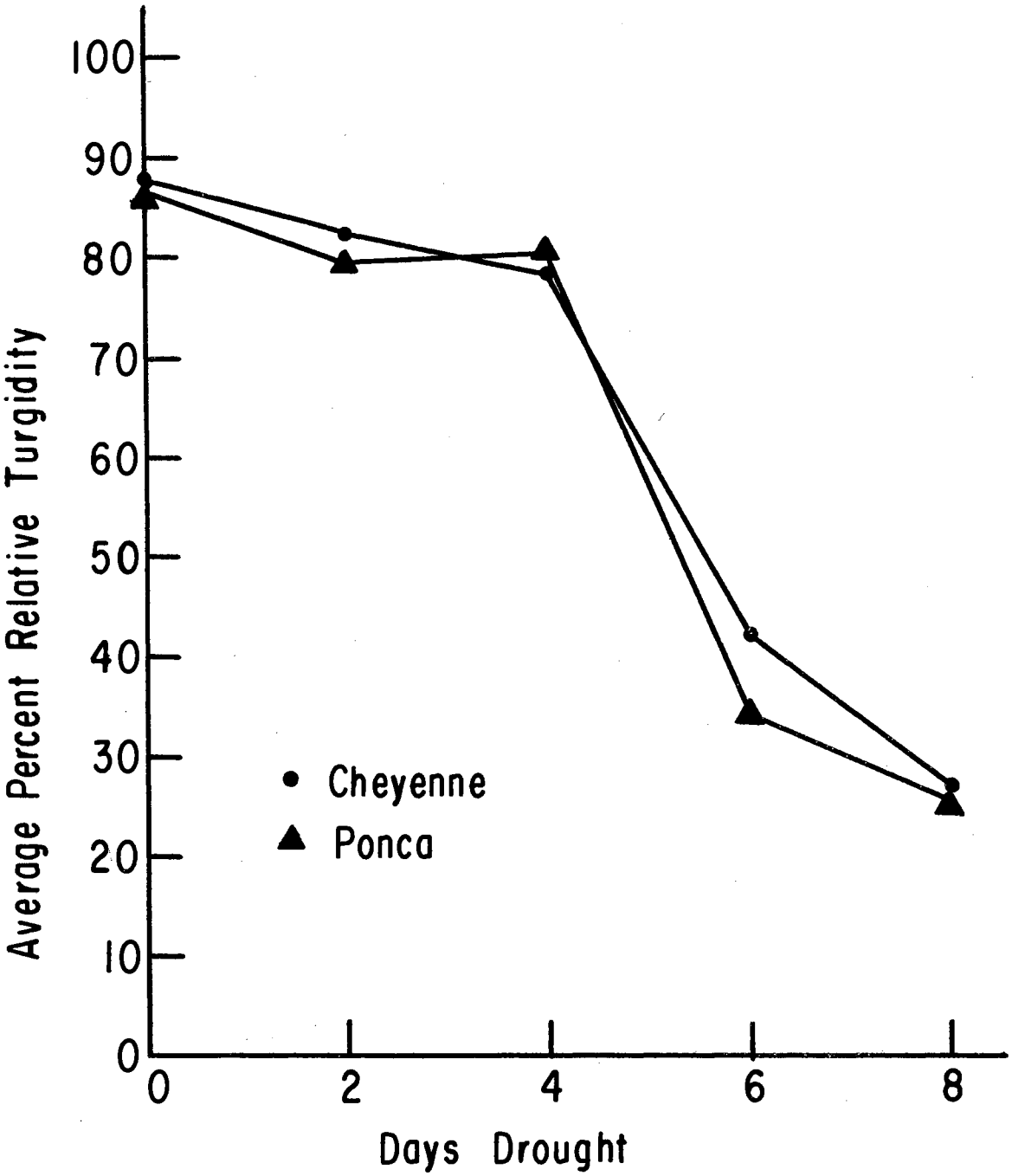


Figure 1. Average percent relative turgidity in leaves of control and stressed plants.

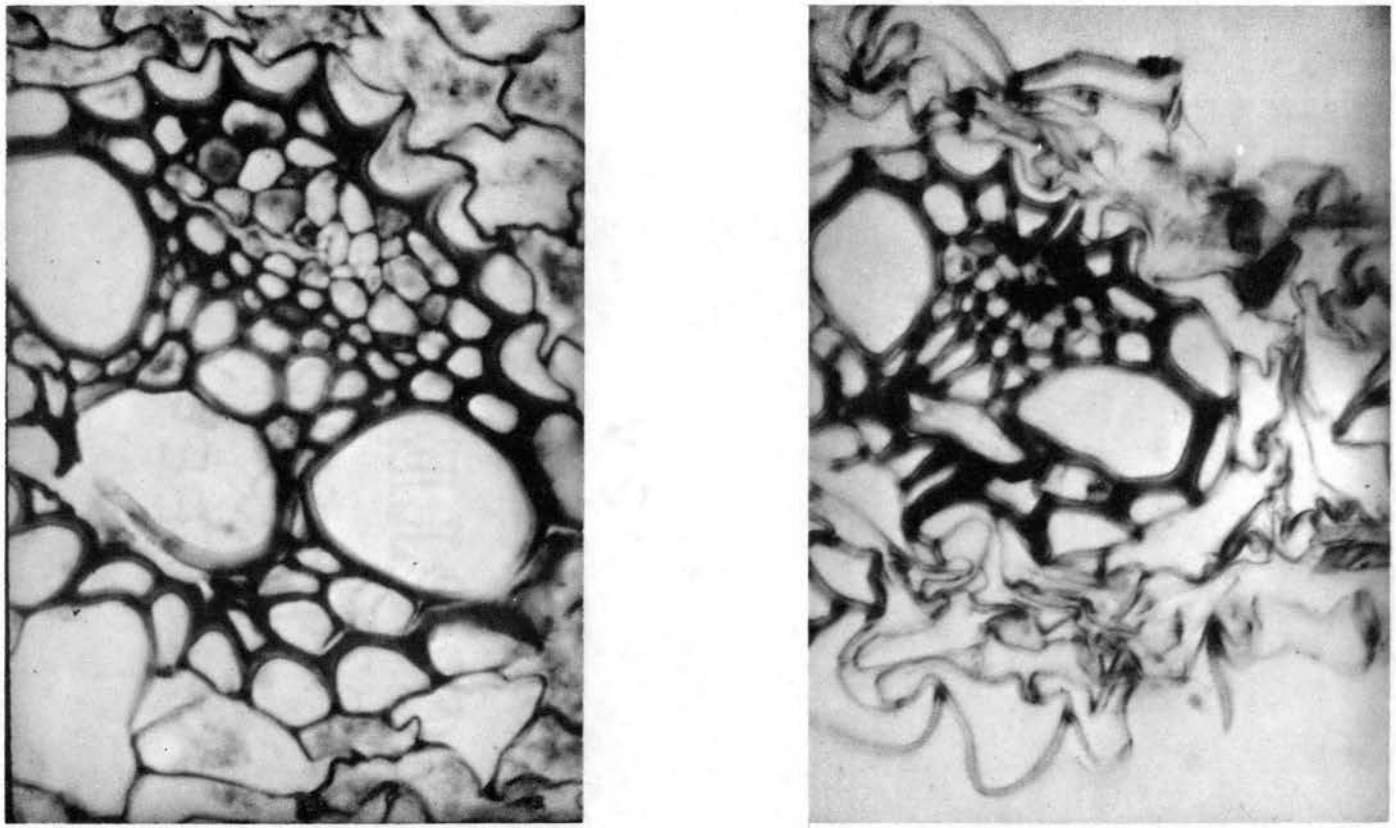
pronounced in Ponca. Xylem vessel thickness of stressed specimens increased markedly, and the retention of stain specific for lignin was negatively correlated with the percent relative turgidity. The difference in degree of response between 8-day stressed plants of the 2 varieties is shown in a section through the midrib (Fig. 2A, B.).

Inspection of rewatered plants showed Cheyenne, rather than Ponca, to resemble more closely the control plants. In rewatered Ponca, the leaves did not appear fully turgid and the areas of chlorotic tissue were relatively larger. Sections through the midrib of these leaves are shown in Figure 3A, B. The percent relative turgidity was 96 for Cheyenne and 97 for rewatered Ponca.

Discussion

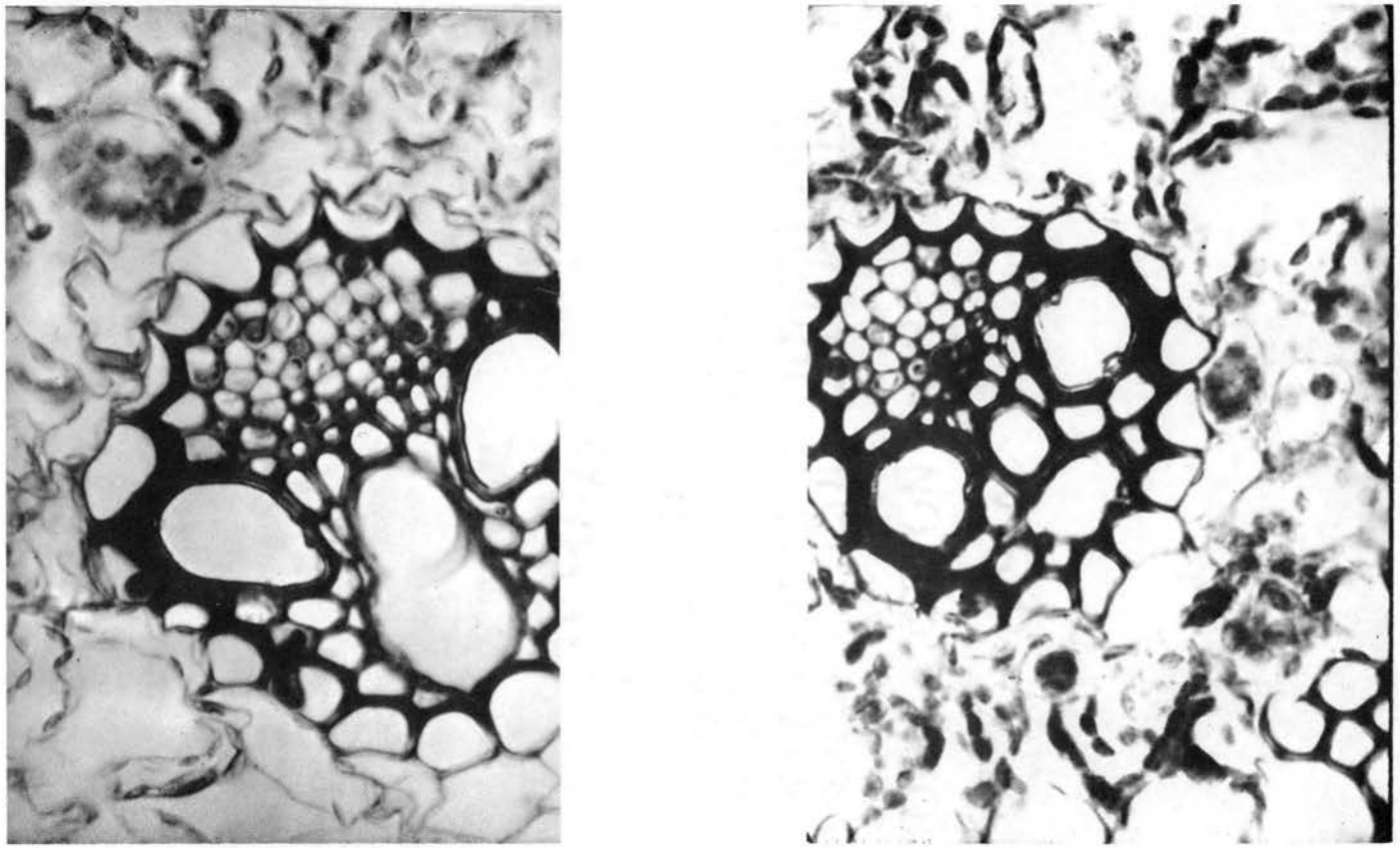
The second leaves of the plants investigated show a difference in response to internal water stress, although the 2 varieties of wheat are closely allied. Anatomical contrasts that may suggest different functional specialization are proportionately greater amounts of xylem elements per leaf volume in Ponca and a greater amount of mechanical tissue in Cheyenne.

Xylem tissue development has been shown to increase with increased water absorption and increased transpiration rates (Penfound, 1931). The importance of reduced transpiration in increasing drought resistance and survival was emphasized by Kramer (1959b). In a recent report Salim and Todd (1965), suggest that the low transpiration rates observed in barley may account in part for the greater amount of drought hardiness in barley when compared to wheat and oats. The difference in relative amounts of xylem to mesophyll tissue between the 2



A B

Figure 2. Transections through midrib of specimens after 8 days of stress. A, Cheyenne; B, Ponca. Note comparative degree of plasmolysis and internal disorganization, relative xylem vessel thickness, and degree of lignin-specific dye (shown as amount of black in xylem elements, and sheath cells) in the two varieties. (X970).



A B

Figure 3. Transections through midrib of specimens rewatered after 4 days of stress. A, Cheyenne; B, Ponca. Note comparative degree of plasmolysis and internal disorganization, relative xylem vessel thickness, and degree of lignin-specific dye (shown as amount of black in xylem elements, and sheath cells) in the two varieties. (X970).

varieties of wheat studied here suggests a difference in economy of water consumption and distribution within the plant body of each. Since transpiration rates were not measured, it was not possible to correlate rate of water loss with xylem volume, however, the data do indicate a direct relationship between drought response and the ratio of xylem to mesophyll tissue volume. Ponca had a high ratio of xylem to mesophyll tissue but lacked the ability to withstand the stress as successfully as did Cheyenne which had a lower ratio of xylem to mesophyll tissue. It is postulated that some inherent factor in the regulation of xylem differentiation may contribute to varying degrees of ability to withstand drought stress.

Cell shrinkage, plasmolysis, and increase in cell-wall thickness in stressed plants confirm earlier findings for some other plant species (Parker, 1952; Simons, 1956). The comparative degree of these phenomena between the varieties of wheat studied indicate a difference in the rate of internal disorganization. The underlying physiology of each plant group may account for variable amounts of cell-wall plasticity and elasticity which cause a differential response of wall resistance to pressure. It has been reported that in soybeans, drought decreases the elastic extensibility of cell-walls (Clark and Levitt, 1956). These authors attributed the survival ability to drought avoidance which was brought about by increased deposition of leaf surface lipids.

Results of this investigation showed that plants with second leaves having a proportionately larger amount of sclerenchyma tissue to mesophyll tissue also resisted the stress treatments more successfully. Maximov (1929), found that an abundance of available water in the soil

favored the development of sclerenchyma tissue in the leaves of sunflower, wheat and waxbean. When these plants were grown in dry soil, the sclerenchyma cells were fewer and thinner walled. In the present study the leaves of both control and stressed plants of Cheyenne developed a greater proportion of sclerenchyma tissue than did those of Ponca. Since this difference was observed as a varietal distinction in the unstressed plants, one might conclude that the differentiating mechanisms operating in Cheyenne provide the leaf with an anatomical pattern which is capable of withstanding certain drying effects.

An increased deposition of lignin seems to be indicated as a drought response. In control samples of both Cheyenne and Ponca, sections in closer proximity to the leaf base showed less retention of lignin-specific dye in xylem elements than did the sections closer to the apex. Since in the grass leaf the more mature tissues are located nearer the leaf apex, one would expect a greater amount of secondary wall materials in this region. A negative correlation between tissue water content and retention of lignin-specific stain in the xylem of stressed leaves was indicated.

The discontinuity of apparent thickening of epidermal cell-walls in the leaves of stressed plants suggests that there is no actual increase in wall material. Instead, a swelling of the wall may result from the binding of more water by previously synthesized wall material. A study of the chemical and physical nature of the cell-wall materials of drought-hardy and drought-susceptible plants seems worthy of pursuit.

Conclusions

Triticum aestivum, L., wheat varieties Cheyenne and Ponca showed changes in tissue organization in the second leaf after subjection to internal water stress. Variations indicative of anatomical disorganization were directly related to the percent relative turgidity of the leaf tissue. When days drought were increased from 2 to 8 the drought effects were more pronounced. Wheat variety Ponca showed more extreme changes and was less able to resume functioning upon rewatering than was variety Cheyenne.

CHAPTER IV

PLANT SURVIVAL LEAF GROWTH AND PEROXIDASE ACTIVITY

Introduction

Plants capable of resuming their functions upon rewatering after subjection to internal water stress must be capable of retaining important metabolic systems during the stress period. The more favorable balance between synthesis and breakdown maintained by drought resistant wheats during water stress suggests that there is either less disburbance in equilibrium between synthetic and hydrolytic enzymes or perhaps a compensatory mechanism may affect the action of particular enzymes required in certain basic systems.

The so called "hardening" of seeds and vegetative plant parts indicates that certain plants are able to increase their ability to withstand a particular stress after application of repeated short exposures to the stress. A comparison of a particular response of the plant after it has been hardened, and the same response in the young developing seedling might contribute to an understanding of certain causal relationships involved.

This investigation is concerned with the relative survival abilities and the relative peroxidase activity in the second leaves of several closely related varieties of wheat. Peroxidase was selected as the enzyme of study for two reasons. First because of its involvement in tissue differentiation (Wardlaw, 1965), and secondly because of its

role in the biosynthesis of lignin which is abundant in xylem, the water conducting tissue of the plant.

In the present study peroxidase activity was assayed in the second leaves of ten varieties of wheat. Tests were performed on the leaves of hardened plants, and on young developing seedlings exposed to drought at 3 different levels of development. The enzyme determinations were concurrent with studies on relative survival abilities of the wheat varieties after 8 weekly cycles of drought and rewatering. In the young seedlings enzyme assays were made after the plants were subjected to one week of drought. Particular attention was devoted to the measurement of relative tissue water content of the second leaves and to the relative amount of growth attained by the leaf during the stress treatment.

Materials and Methods

Two different experiments and a replicate of each were run. In the first, seedlings of ten varieties of wheat were established in a mixture of soil, peat moss, and sand (2:2:1) in twelve 15x23 inch wooden trays approximately 3 inches deep. Triticum aestivum, L., varieties cv. Cheyenne (C.I. 8885), cv. Kanking (C.I. 12719), c. Ponca (C.I. 12128), and cv. Red Chief (C.I. 12109), hard red winter wheats; and cv. Dual (C.I. 13093), cv. Leapland (C.I. 11762), cv. Pennol (12755), cv. Red Coat (C.I. 13170), cv. Seneca (C.I. 12529), and cv. Thorne (C.I. 11856), soft red winter wheats were used. The seeds of the hard red varieties were supplied by the Agronomy Department, Oklahoma State University and those of the soft red varieties were obtained from the University of Maryland Experimental Station.

Each tray contained one row of each variety of wheat (sequence of rows was randomly arranged) and the plants were maintained in a controlled growth chamber with a temperature of $21^{\circ} \pm 1^{\circ}$ C and light supplied daily for 10 hours by 40 watt cool white flourescent lights which gave a light intensity at plant level of 21,500 lux. The plants were watered regularly for a period of three weeks at the end of which time all the plants appeared vigorous and healthy. At that time seedlings were thinned to 20 per row in each flat. Six flats were labeled controls and the plants contained therein were maintained in a well moistened condition throughout the duration of the experiment. The remaining six flats were designated experimentals and these plants received 8 weekly cycles of 6 days drought with rewatering on the seventh day. At the time of rewatering the surviving plants in each flat were counted. When the eighth drought period was completed the final count of surviving plants was made and they were harvested.

Second leaves of control and experimental plants were severed just above the sheath and the following determinations were made.

1. Length measurements were made on ten leaves of control plants and ten leaves of experimental plants of each variety.

2. Percent relative turgidity was determined for the control and experimental groups of each variety from randomly chosen samples. Weatherley's method as modified by Todd *et al.*, (1962), was used.

3. Fresh weight and dry weight values were determined on other samples and used to calculate the percent water content of the fresh leaf tissue.

4. Four leaves of the control and experimental groups of each variety were weighed and fresh weight values were obtained. These four leaves were homogenized in a borosilicate glass tissue grinder in 0.05M phosphate buffer pH 4.5 at a temperature of 4° C. The homogenate was squeezed through 2 layers of cheese cloth and the final volume made up to 2.00 ml. All apparatus and materials used in the preparation were prechilled. The homogenate was centrifuged at 27,000 x g for

40 min at 2 to 3° C. The supernatants were used for peroxidase assays. Peroxidase activity was determined by the method of Seigel (1953). The change in optical density at 420 mμ per 0.25 ml supernatant in 3 min was used as the measure of enzyme activity. Specific activity is given as the change in optical density/mg protein/3 min. One ml of the supernatant was used for protein determination by the Folin-phenol method.

Survival data and data from 1, 2, 3, and 4 above were subjected to a statistical analysis.

In the second experiment seeds of the same ten varieties of wheat used in experiment number one were placed on well moistened germination paper, covered and placed in the growth chamber. At three arbitrarily chosen levels of development namely (1) the first foliage leaf just visible through the coleoptile aperture, (2) the first foliage leaf 1.5-cm beyond the coleoptile aperture, and (3) the first foliage leaf 3.5-cm beyond the coleoptile aperture, the seedlings were removed from the germination paper and placed in a 2:2:1 mixture of soil, peat moss and sand in 1½ x 1½ x 2½ vita-band containers. In this report the above described levels of development are referred to as stage 1, stage 2, and stage 3 respectively. Experimental plants of each variety of wheat at each stage were potted in soil with a total moisture stress at which wheat plants permanently wilt. The controls were potted in well moistened soil. The seedlings were maintained for one week in the controlled growth chamber described previously. The soil of the control plants was kept moist but the experimental plants were not watered. At the end of the week the plants were harvested. Determinations of percent relative turgidity, length measurements, fresh weight and dry weight values, peroxidase activity and protein content were made on second leaf samples as described for experiment number one. The data

were subjected to a statistical analysis.

Results

In the survival studies all of the control plants lived throughout the experiment. The experimental plants showed a continuing decrease in number of live plants at the end of each drought cycle (fig. 4). Analysis of variance for this data is shown in table XI of the appendix. Duncan's Multiple Range test was calculated from the data of drought cycles V and VIII. The results are given in table I. Each test was significant at the 5% level of confidence by the analysis of variance.

The multiple range test shows that the hard red and soft red wheats cannot be separated into two groups on the basis of survival ability. In cycle V field hardy varieties Red Chief, Cheyenne, and Kanking are grouped near the top of the list, and injury susceptible variety Ponca at the bottom of the list. These results substantiate the findings of Todd and Webster (1965), for four and six weeks of drought. Variety Thorne, a soft red wheat is shown by this test to be not significantly different from Red Chief, and Seneca and Dual also soft red varieties are not significantly different from Cheyenne and Kanking. Drought cycle VIII showed a considerable displacement of variety positions and relationships in the range test. Ponca retained its position at the very bottom of the list. Variety Thorne replaced variety Red Chief at the top of the range while Dual and Seneca did not appear significantly different from Red Chief.

Table II shows the mean values for measurements of percent relative turgidity, protein content and peroxidase activity for control

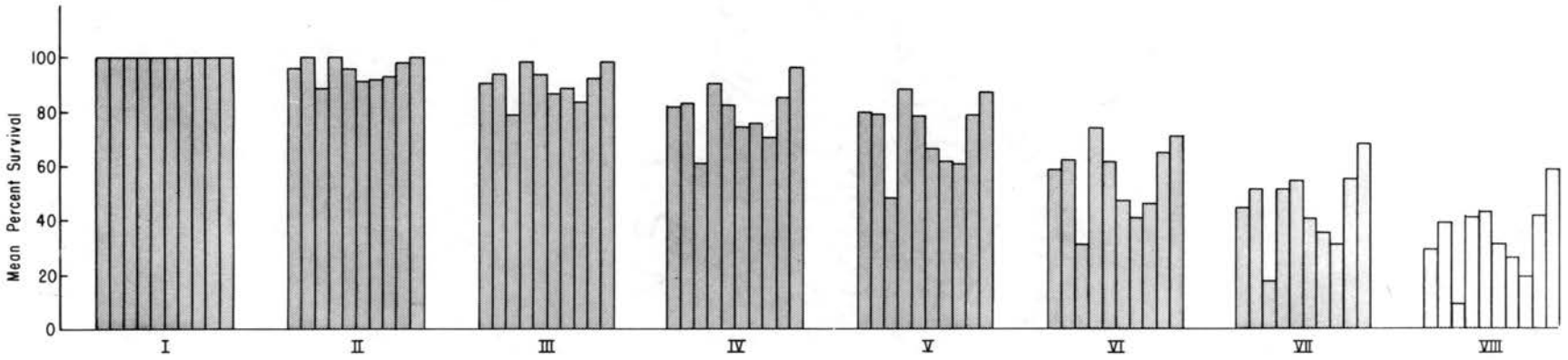


Figure 4. Survival of the original population of 10 wheat varieties after subjection to 8 successive weekly drought periods (I through VIII). Values are averages of 20 plants of each variety replicated 6 times. In each drought period the varieties are (from left to right) Cheyenne, Kanking, Ponca, Red Chief, Dual, Leapland, Pennol, Red Coat, Seneca and Thorne.

TABLE I

COMPARISON BETWEEN SURVIVAL ABILITIES IN DROUGHT CYCLE V AND VIII FOR TEN VARIETIES OF WHEAT

Mean Percent Survival			
Cycle V		Cycle VIII	
Red Chief	87.50	Thorne	59.16
Thorne	86.25	Dual	43.33
Cheyenne	78.73	Seneca	42.50
Kanking	77.91	Red Chief	41.66
Seneca	77.91	Kanking	39.68
Dual	77.08	Leapland	31.25
Leapland	65.00	Cheyenne	29.16
Pennol	60.83	Pennol	26.66
Red Coat	60.00	Red Coat	19.58
Ponca	47.08	Ponca	9.16

(Data shown in Figure 4) Varieties connected by vertical lines were not significantly different at the 5% confidence level from one another in Duncan's Multiple Range Tests.

TABLE II

TABULATED DATA FOR MEAN VALUES OF PERCENT RELATIVE TURGIDITY SOLUBLE PROTEIN CONTENT AND PEROXIDASE ACTIVITY OF THE SUPERNATANT FRACTION OF WHEAT LEAVES AFTER EIGHT WEEKLY CYCLES OF DROUGHT

	% Rel. Turg.		mg Prot./gm dw		*Perox. Act./mg dw		**Perox. Act./mg Prot.	
Variety	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Cheyenne	97.38	95.35	52.47	29.19	0.340	0.343	6.50	11.70
Kanking	91.63	96.00	42.44	29.34	0.274	0.306	6.45	10.45
Ponca	89.63	84.00	54.96	19.43	0.323	0.274	5.95	12.60
Red Chief	89.38	76.74	49.00	39.31	0.251	0.320	5.12	8.12
Dual	94.85	94.66	39.47	36.82	0.179	0.328	4.54	8.89
Leapland	94.91	95.01	39.62	25.95	0.198	0.267	4.99	10.28
Pennol	81.91	82.58	58.06	43.91	0.321	0.340	5.52	10.73
Red Coat	90.43	95.57	49.57	25.63	0.238	0.281	4.80	11.05
Seneca	91.12	85.74	51.44	36.62	0.225	0.296	4.37	8.11
Thorne	91.71	89.35	29.57	35.20	0.221	0.350	7.47	10.01

* Units of peroxidase activity expressed as optical absorbance at 420 mμ at 3 min/mg dry weight
** Units of peroxidase activity expressed as optical absorbance at 420 mμ at 3 min/mg protein

and experimental plants at the end of drought cycle 8. Analysis of variance for these data is given in table XII of the appendix.

Percent relative turgidity values showed differences of small magnitude between the two groups. Variance tests on these data indicated a significance at the 1% level of confidence for treatment differences and at the 5% level of confidence for varietal differences. Comparison of the percent relative turgidity and the percent survival at the end of drought cycle VIII is shown in figure 5. Duncan's Multiple Range Tests on these data are compared in table III. Red Chief is different from all the other varieties in the relative turgidity test and is below Ponca on the list although it is considerably above Ponca in the survival test. Though more extreme here, the data of Todd and Webster (1965), indicated similarly a fairly poor correlation between relative turgidity and survival ability. Ponca and Red Coat had poor survival ability but their relative turgidity values were not significantly different from Thorne and Dual respectively, which headed the list for the survival test.

Peroxidase activity measurements after the eighth weekly drought cycle are shown in figure 6. In all wheat varieties tested there was a marked increase in peroxidase activity per mg protein after the stress treatment (fig. 6, A). The data show smaller amounts of increase in peroxidase activity when measured on a per mg dry weight basis, and for variety Ponca there was a decrease rather than an increase (fig. 6, B). Figure 7 illustrates a comparison between the percent of control peroxidase activity per mg protein and the percent of control peroxidase activity per mg dry weight. The magnitude of the difference between the two measurements varies considerably among

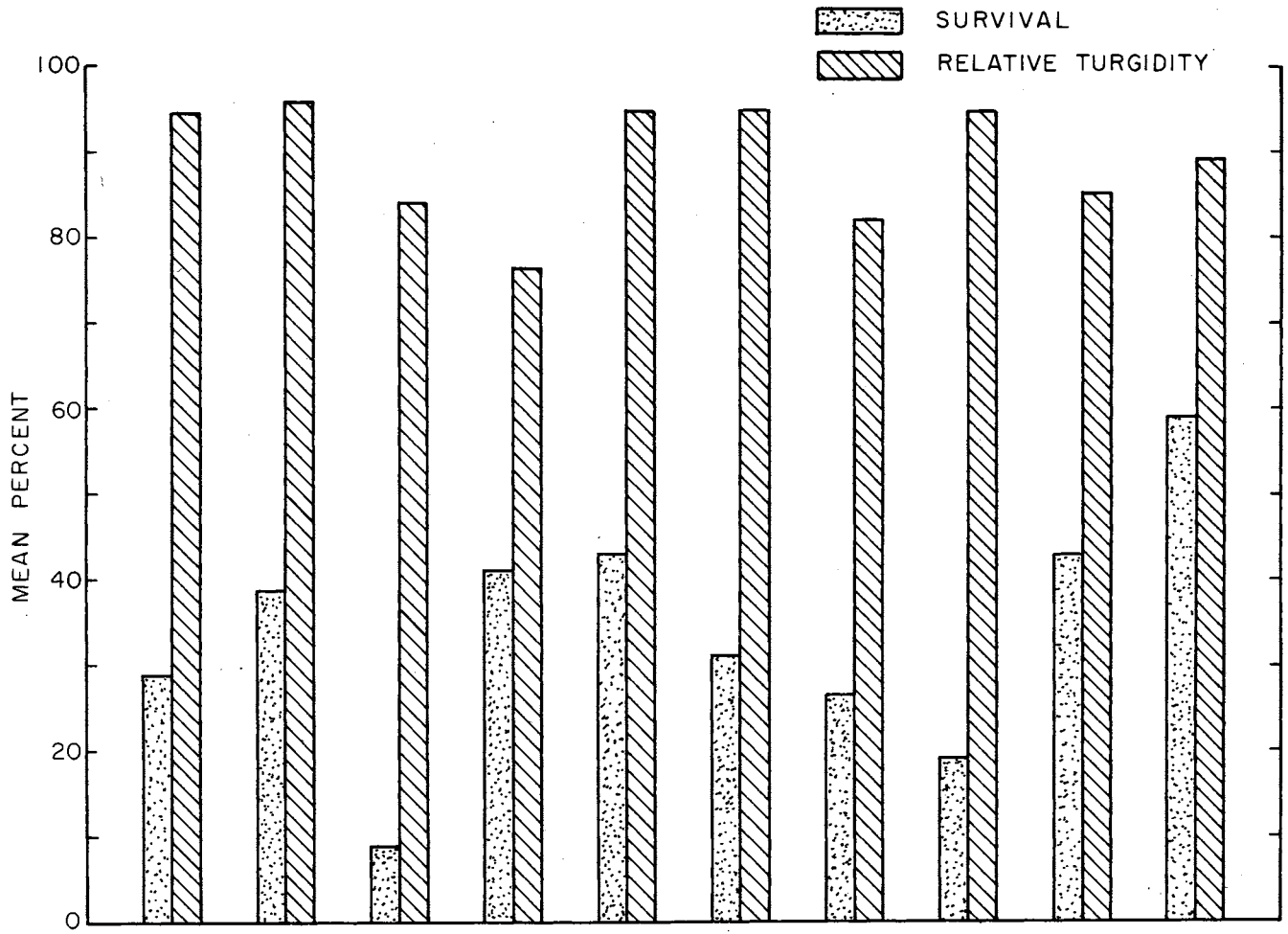


Figure 5. Comparison between mean percent survival and mean percent relative turgidity in 10 varieties of wheat at the end of the eighth weekly cycle of drought. Values are averages of 20 plants of each variety replicated 6 times. Varieties are (from left to right) Cheyenne, Kanking, Ponca, Red Chief, Dual, Leapland, Pennol, Red Coat, Seneca, and Thorne.

TABLE III

COMPARISON BETWEEN PERCENT SURVIVAL AND PERCENT RELATIVE TURGIDITY AT THE END OF DROUGHT CYCLE VIII FOR TEN VARIETIES OF WHEAT

Mean % Survival		Mean % Relative Turgidity	
Thorne	59.16	Kanking	92.50
Dual	43.33	Cheyenne	91.15
Seneca	42.50	Leapland	90.62
Red Chief	41.66	Red Coat	89.60
Kanking	39.68	Dual	89.32
Leapland	31.25	Thorne	86.46
Cheyenne	29.16	Seneca	86.30
Pennol	26.66	Pennol	84.04
Red Coat	19.58	Ponca	83.90
Ponca	9.16	Red Chief	70.99

(Data shown in Figure 5) Varieties connected by vertical lines were not significantly different at the 5% confidence level from one another in Duncan's Multiple Range Tests.

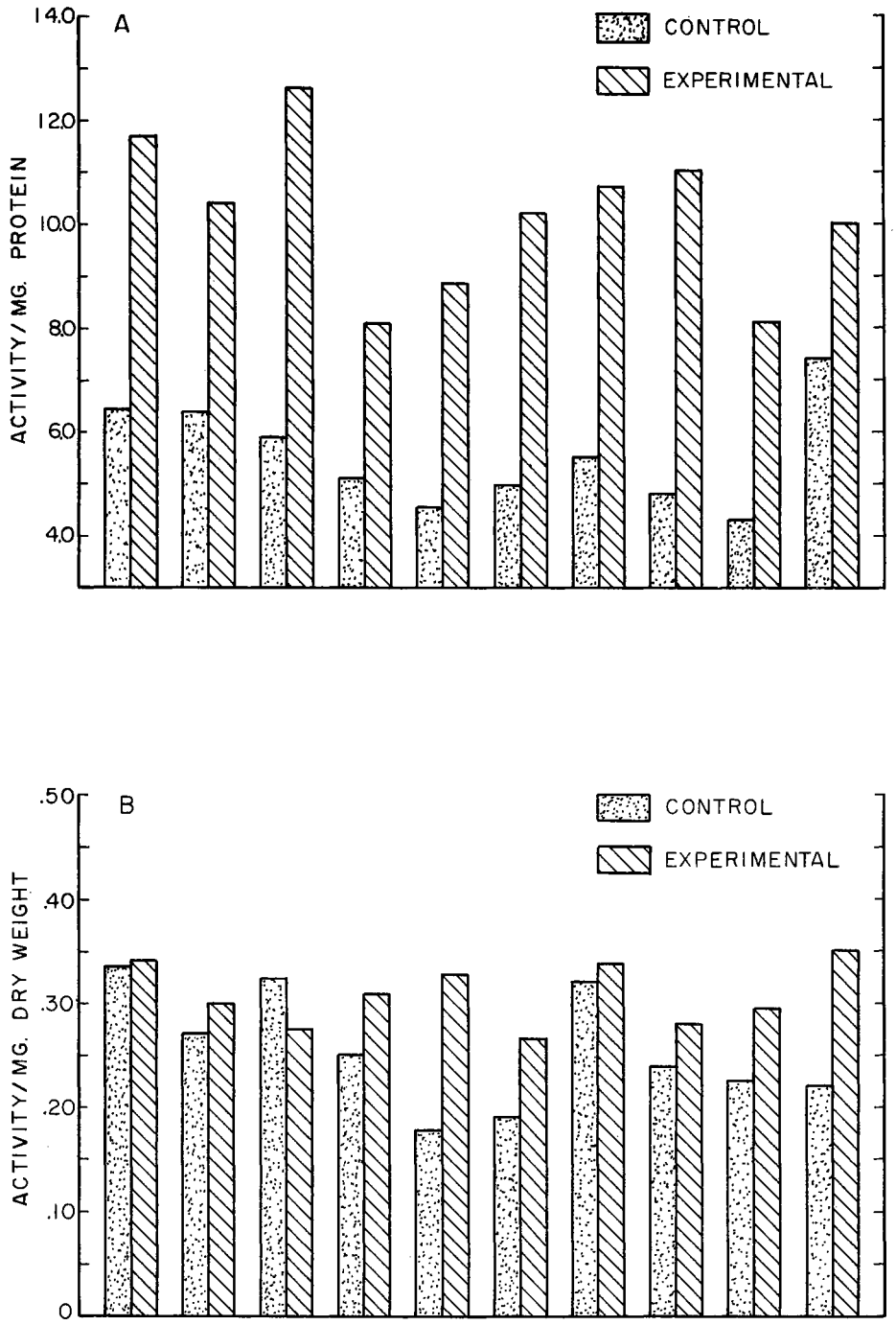


Figure 6. Comparison between peroxidase activity in the second leaves of control and experimental wheat plants after 8 weekly cycles of drought. Wheat varieties are (from left to right) Cheyenne, Kanking, Ponca, Red Chief, Dual, Leapland, Pennol, Red Coat, Seneca and Thorne. Treatment differences for activity/mg dry weight significant at the 5% level of confidence. Treatment and variety differences for activity/mg protein significant at the 5% level of confidence.

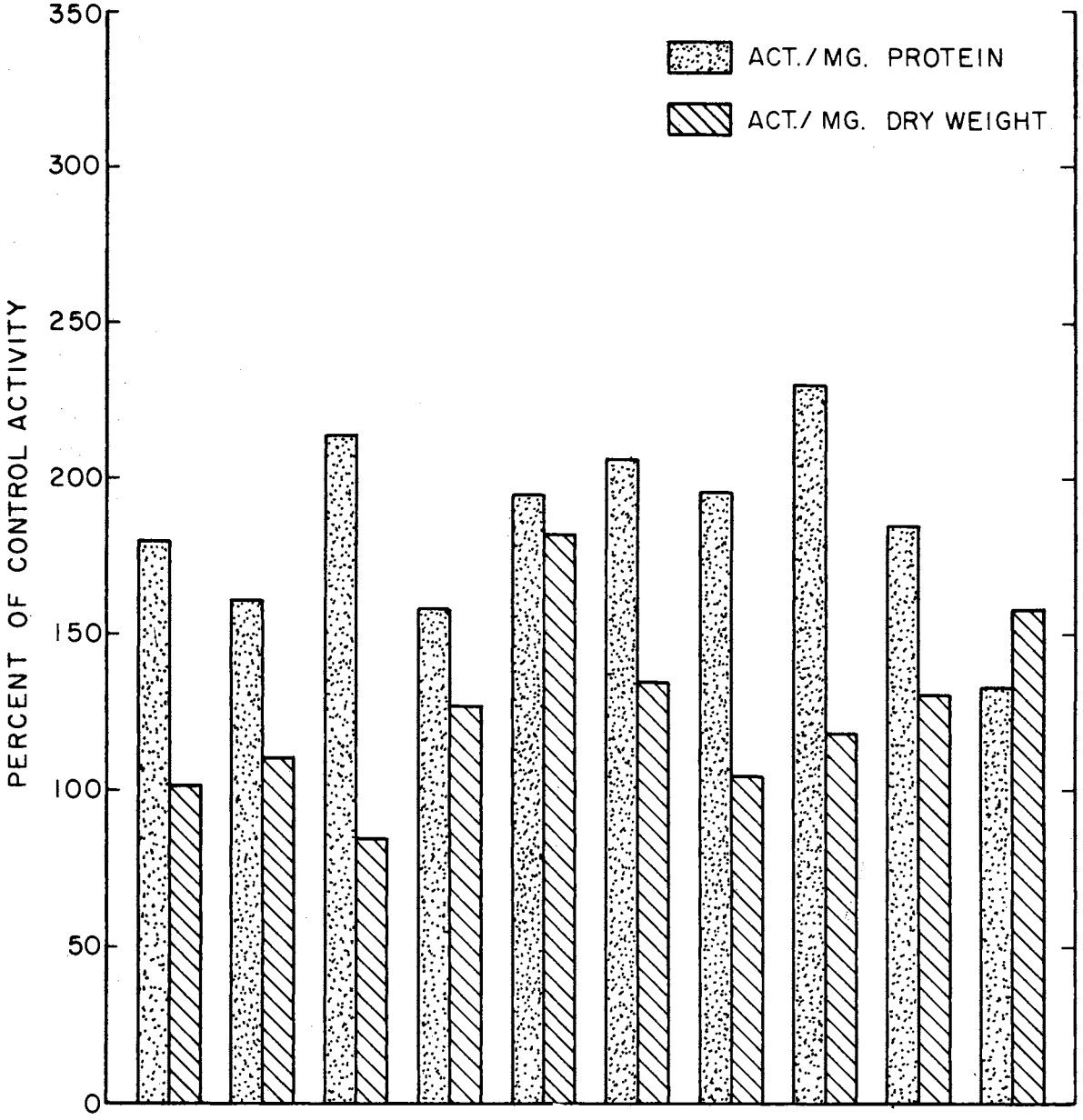


Figure 7. Comparison between percent of control peroxidase activity/ mg protein and percent of control peroxidase activity/mg dry weight in second leaves of 10 varieties of wheat after 8 weekly cycles of drought. Varieties are (from left to right) Cheyenne, Kanking, Ponca, Red Chief, Dual, Leapland, Pennol, Red Coat, Seneca and Thorne.

the varieties of wheat. These differences are plotted in figure 8. The wheat varieties are arranged in order of decrease in magnitude of the difference. It is of particular interest that the ranked positions of the different varieties of wheat as shown in the survival tests in table I are approximately opposite to the respective positions of the varieties as shown in figure 8. This observation suggests that an inverse relationship exists between the relative survival ability and the magnitude of difference between the percent change in peroxidase activity per mg protein and the percent change in peroxidase activity per mg dry weight.

Data for the mean length of second leaves of control and expermental plants after 8 weekly cycles of drought are presented in figure 9. Differences though not extreme, when subjected to the F test showed treatment significance at the 1% level of confidence, and varietal significance at the 5% level of confidence. Analysis of variance of these data is presented in table XIII of the appendix.

In experiment number 2 there was a marked difference between mean percent relative turgidity values for control and experimental plants subjected to 1 week of drought at 3 different levels of development. These data are shown in figure 10. Analysis of variance of these data along with those for measurements of protein and peroxidase activity are shown in table IV of the text for simplification of presentation. The mean values for the variables of table IV are found in tables V, VI, VII and VIII.

Inspection of table IV shows that the effect of stage of development is significant for each variable considered. The effect of treatment is significant for variables percent relative turgidity, and mg

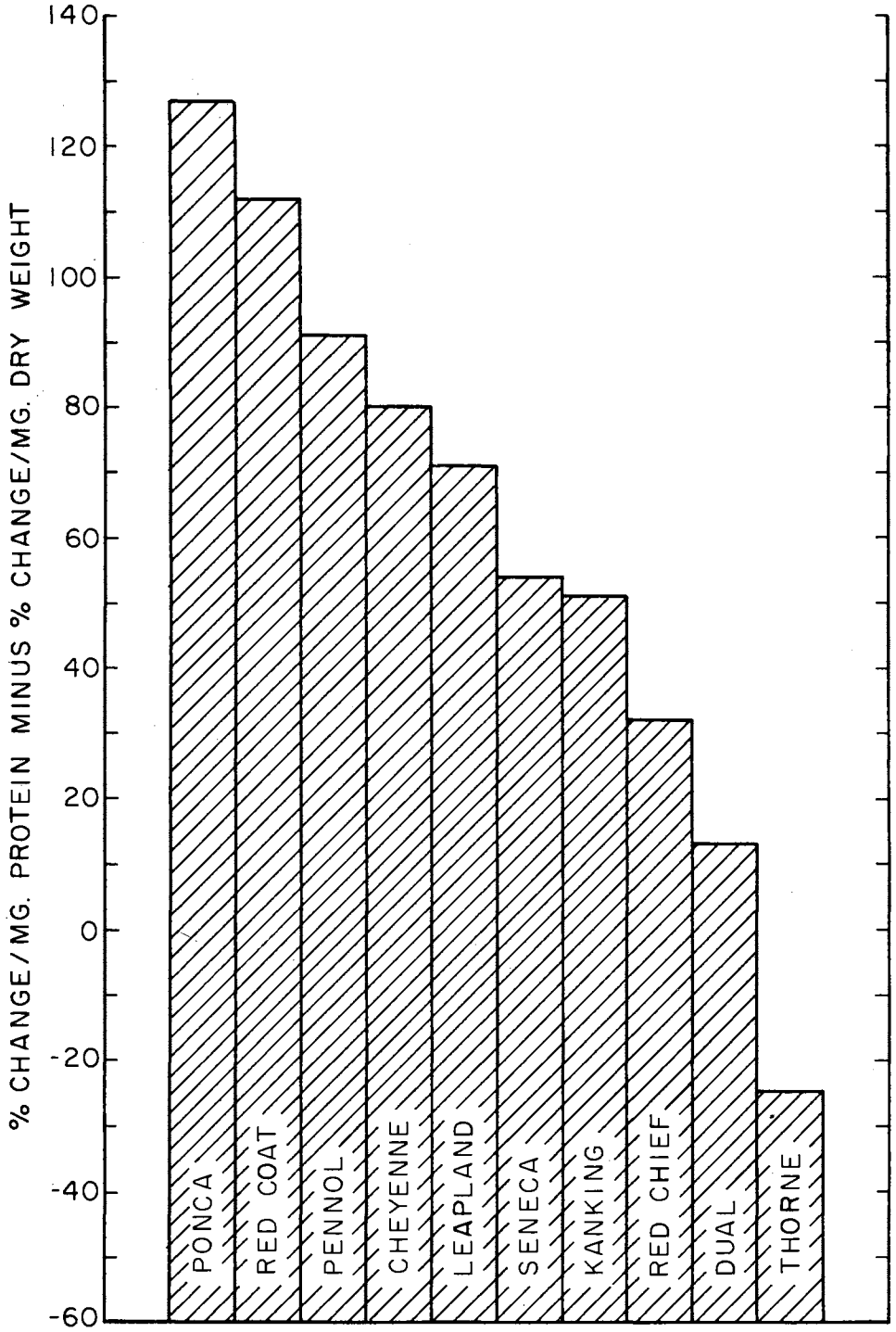


Figure 8. The difference between percent change in peroxidase activity/mg protein and the percent change in peroxidase activity/mg dry weight in the second leaves of 10 varieties of wheat after 8 weekly cycles of drought. Wheat varieties are ranked in order of decreasing magnitude of difference.

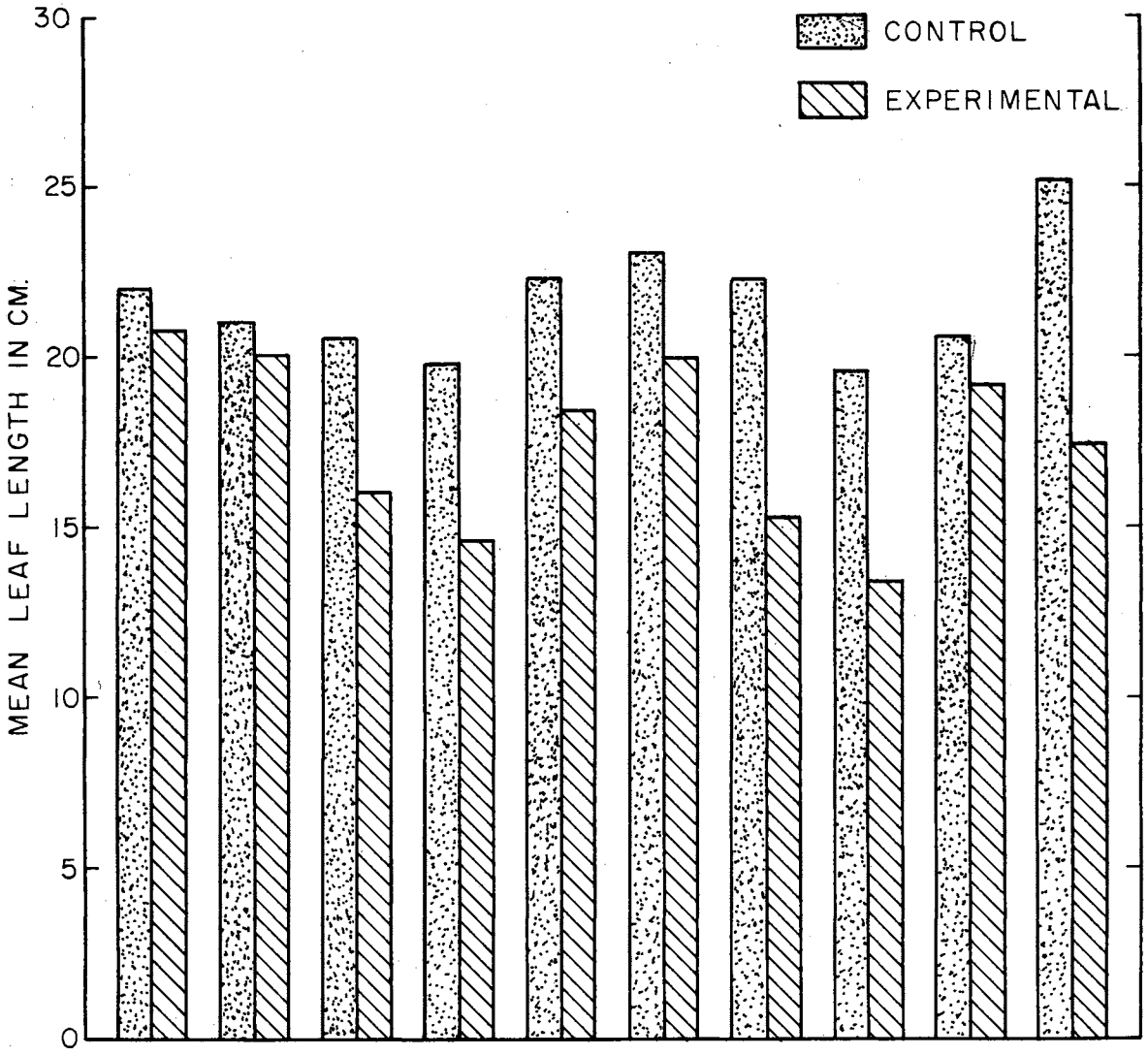


Figure 9. Mean leaf length of second leaves of wheat plants after 8 weekly drought cycles. Values are based on averages for 10 leaves of each variety. Varieties are (from left to right) Cheyenne, Kanking, Ponca, Red Chief, Dual, Leapland, Pennol, Red Coat, Seneca and Thorne.

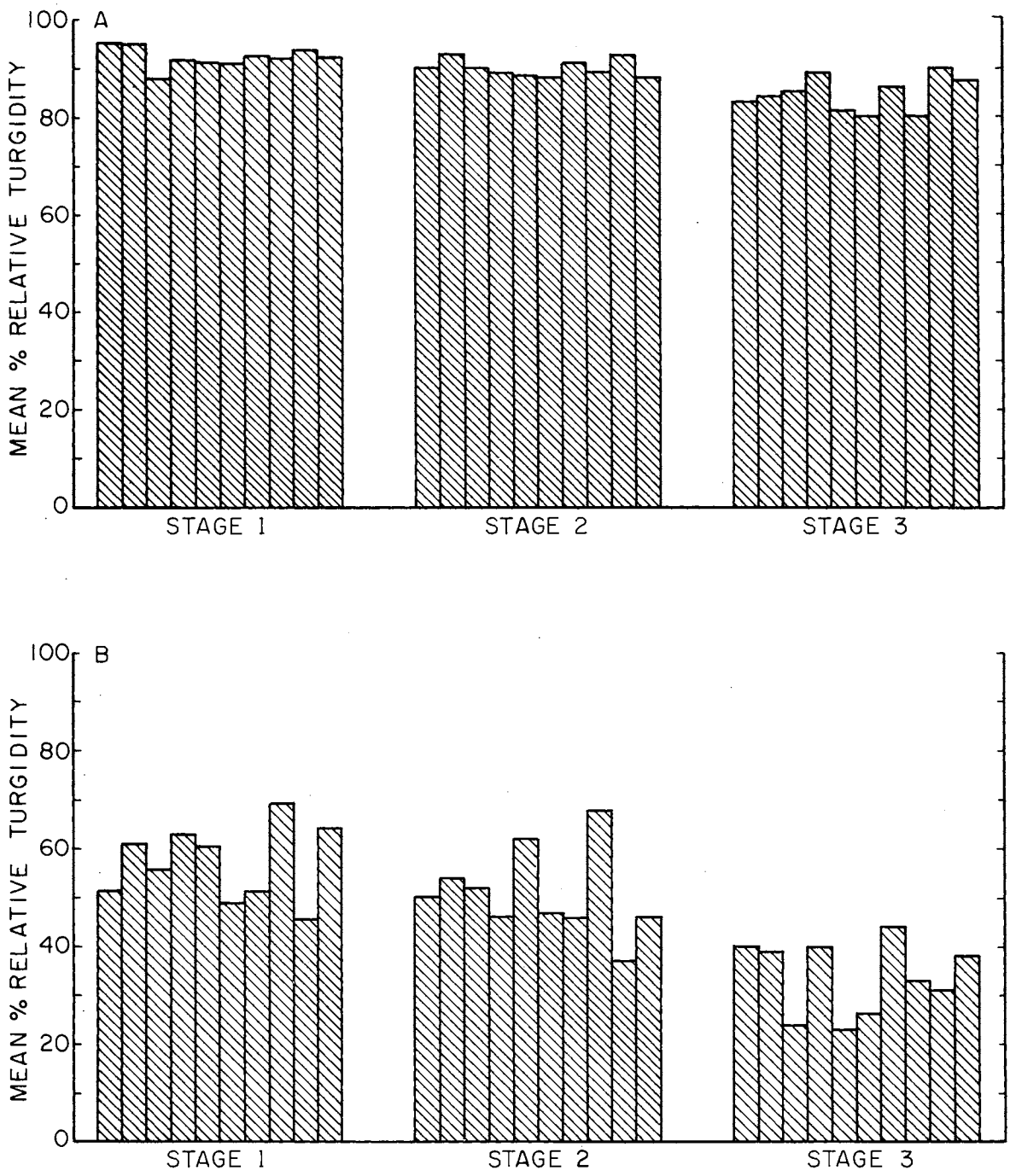


Figure 10. Percent relative turgidity in the second leaves of 10 varieties of wheat after subjection to one week of drought stress when plants were at 3 different levels of development. (A-control, B-experimental). In each stage the varieties are (from left to right) Cheyenne, Kanking, Ponca, Red Chief, Dual, Leapland, Pennol, Red Coat, Seneca and Thorne. (Stages defined in procedures)

TABLE IV

ANALYSIS OF VARIANCE FOR "DATA" OF TABLES V, VI, VII, AND VIII

Source	df	Mean Square			
		% Relative Turgidity	mg Protein per gm dw	Peroxidase Act/mg dw	Peroxidase Act/mg Protein
Replicate	1	60.222	127.650	0.107	141.090
Stage	2	2583.387*	5628.324**	0.291**	1243.561*
Treatment	1	51903.055**	4008.463*	0.275	2059.164
Variety	9	86.298	1055.867*	1.723*	399.000*
Trial x stage	2	40.068	71.421	0.026	51.139
Trial x treatment	1	345.686	4.004	0.101	69.482
Trial x variety	9	44.134	60.646	0.007	15.307
Stage x treatment	2	621.762*	855.443*	0.518*	74.292
Stage x variety	18	68.867*	415.168*	0.323*	111.870*
Treatment x variety	9	109.432*	869.699*	0.382*	226.044*
Replicate x stage x treatment	2	9.159	34.901	0.027	39.210
Replicate x stage x variety	18	11.717	38.618	0.022	8.368
Replicate x treatment x variety	9	19.414	61.418	0.032	14.220
Stage x treatment x variety	18	46.561*	537.167*	0.305*	105.667*
Replicate x stage x treatment x variety	18	13.346	37.112	0.009	9.439
Total	119				

* F value significant at the 5% level of confidence
** F value significant at the 1% level of confidence

TABLE V

MEAN PERCENT RELATIVE TURGIDITY IN SECOND LEAVES OF CONTROL AND EXPERIMENTAL PLANTS AFTER ONE WEEK OF DROUGHT AT THREE LEVELS OF DEVELOPMENT

Variety	Stage 1		Stage 2		Stage 3	
	Control	Experimental	Control	Experimental	Control	Experimental
Cheyenne	95.83	51.80	90.00	50.00	83.00	40.05
Kanking	95.62	61.00	92.75	54.10	84.00	39.40
Ponca	88.16	55.90	90.02	52.27	85.00	24.62
Red Chief	91.93	63.75	89.80	46.30	89.00	40.19
Dual	91.26	60.66	89.55	62.05	81.00	23.61
Leapland	91.15	49.61	88.00	47.21	80.00	26.18
Pennol	92.67	51.46	91.00	46.18	86.00	44.03
Red Coat	92.30	69.53	89.41	68.41	80.00	33.20
Seneca	93.98	45.56	92.80	37.21	90.00	31.58
Thorne	92.43	64.11	88.00	46.40	87.50	38.50

Stages 1, 2, and 3 defined in materials and methods

TABLE VI

MEAN SOLUBLE PROTEIN CONTENT IN MILLIGRAMS PER GRAM DRY WEIGHT IN SECOND LEAVES OF CONTROL AND EXPERIMENTAL PLANTS AFTER ONE WEEK OF DROUGHT AT THREE LEVELS OF DEVELOPMENT

Variety	Stage 1		Stage 2		Stage 3	
	Control	Treated	Control	Treated	Control	Treated
Cheyenne	39.17	51.80	25.19	40.70	31.58	36.23
Kanking	95.17	53.00	39.69	24.01	31.85	47.48
Ponca	44.72	37.91	18.85	23.35	17.74	15.22
Red Chief	98.39	29.74	23.53	14.27	46.49	35.13
Dual	41.51	22.22	18.62	9.13	24.65	14.03
Leapland	38.65	34.09	26.82	20.91	33.63	51.56
Pennol	38.68	17.43	13.64	14.63	31.35	33.57
Red Coat	28.47	13.82	22.49	5.25	28.81	12.28
Seneca	20.52	43.20	16.00	15.69	62.33	14.32
Thorne	108.43	26.33	30.45	15.91	64.41	15.79

Stages 1, 2 and 3 defined in procedures

TABLE VII

MEAN PEROXIDASE ACTIVITY PER MILLIGRAM DRY WEIGHT IN SECOND LEAVES OF CONTROL AND EXPERIMENTAL PLANTS AFTER ONE WEEK OF DROUGHT AT THREE LEVELS OF DEVELOPMENT

Variety	Stage 1		Stage 2		Stage 3	
	Control	Treated	Control	Treated	Control	Treated
Cheyenne	0.08	0.09	0.14	0.12	0.21	0.10
Kanking	0.07	0.03	0.07	0.12	0.07	0.11
Ponca	0.12	0.19	0.12	0.14	0.11	0.14
Red Chief	0.24	0.14	0.13	0.14	0.14	0.15
Dual	0.09	0.06	0.08	0.06	0.08	0.12
Leapland	0.12	0.16	0.13	0.13	0.11	0.19
Pennol	0.15	0.13	0.13	0.19	0.12	0.13
Red Coat	0.12	0.11	0.16	0.09	0.13	0.11
Seneca	0.14	0.26	0.12	0.16	0.08	0.16
Thorne	0.35	0.19	0.17	0.18	0.13	0.23

Stages 1, 2 and 3 defined in procedures
Peroxidase activity is expressed as optical absorbance at 420 mμ at 3 min per 1 mg dry weight

TABLE VIII

MEAN PEROXIDASE ACTIVITY PER MILLIGRAM SUPERNATANT PROTEIN IN SECOND LEAVES OF CONTROL AND EXPERIMENTAL PLANTS AFTER ONE WEEK OF DROUGHT AT THREE LEVELS OF DEVELOPMENT

	Stage 1		Stage 2		Stage 3	
Variety	Control	Treated	Control	Treated	Control	Treated
Cheyenne	2.04	1.91	5.97	2.96	3.84	3.00
Kanking	1.80	1.72	1.89	4.99	7.46	2.44
Ponca	2.74	5.06	6.51	5.76	6.28	9.60
Red Chief	2.38	4.84	5.49	9.76	2.98	4.20
Dual	2.08	2.52	4.53	7.00	3.34	9.20
Leapland	3.00	4.57	4.96	6.07	3.39	3.67
Pennol	3.78	7.75	9.60	13.12	3.76	3.87
Red Coat	4.28	6.43	7.27	17.44	4.64	8.72
Seneca	6.70	6.17	7.23	10.72	1.70	11.46
Thorne	3.30	7.40	5.56	12.12	2.04	14.80

Stages 1, 2 and 3 defined in procedures
Peroxidase activity is expressed as optical absorbance at 420 mμ at 3 min per 1 mg protein

protein/gm dry weight. Variety effects are significant for variables mg protein/gm dry weight, peroxidase activity/mg dry weight, and peroxidase activity/mg protein. Significant interactions for all variables are: (1) stage x variety, (2) treatment x variety, and (3) stage x treatment x variety. Interaction between stage and treatment is significant for variables, percent relative turgidity, mg protein/gm dry weight, and peroxidase activity/mg dry weight.

The significant stage x variety interaction indicates that the differences between varietal responses vary with stage where responses are measured over all control and experimental plants. Alternately, the differences among responses of each stage of development vary for the ten varieties of wheat tested where responses are measured as totals over all control and experimental plants.

The significant treatment x variety interaction implies that the differences between varietal responses vary with the treatment where responses are measured over all 3 stages of development. Alternately, the differences among responses to treatment vary for the ten varieties of wheat where responses are measured as totals over all three stages. Specifically the differences in values for a particular variable, when averaged over all stages, among the different varieties of wheat are not the same for the two treatments. The stage x treatment x variety interaction implies that the treatment x variety differs with the stage of development.

Since wheat varieties Thorne, Red Chief, Red Coat and Ponca paired at opposite ends of the list for survival test in experiment number 1 these four varieties of wheat were selected to illustrate comparisons which may lead to more meaningful interpretation of the data. Figure

11 shows the interaction effects on percent relative turgidity for the four varieties of wheat. The differences between percent relative turgidity values of control and experimental plants is seen to vary with the level of development of the plants. There is a marked decrease in percent relative turgidity at each stage of development after the stress treatment. This decrease is much greater in stages 2 and 3 than in stage 1 for varieties Thorne and Red Chief while varieties Red Coat and Ponca show the decrease to be much greater in stage 3 than in stages 1 and 2. The magnitude of the differences between treated and non-treated plants at each stage of development is clearly shown in figure 11-B, D, F and H.

Figure 12 shows the interaction between treatment and stage of development on peroxidase activity per mg protein for varieties Thorne, Red Chief, Red Coat and Ponca. In all four varieties the stress treatment resulted in an increase in peroxidase activity at stages 1 and 3. Varieties Thorne, Red Chief and Red Coat also show an increase in activity at stage 2, but variety Ponca shows a decrease in activity at stage 2. The magnitude of the difference in treatment effects at each stage shows stage 2 to be affected most in varieties Thorne and Red Coat, and stage 3 was most affected in variety Thorne. The magnitude of effect is generally greater in Thorne and Red Coat than in the other two varieties.

Figure 13 shows the interaction between treatment and stage of development when peroxidase activity was calculated on a per mg dry weight basis. Variety Thorne showed a marked decrease in activity at stage 1, and a small increase of activity in stages 2 and 3. Red Chief showed a considerable decrease at stage 1 and a slight increase at

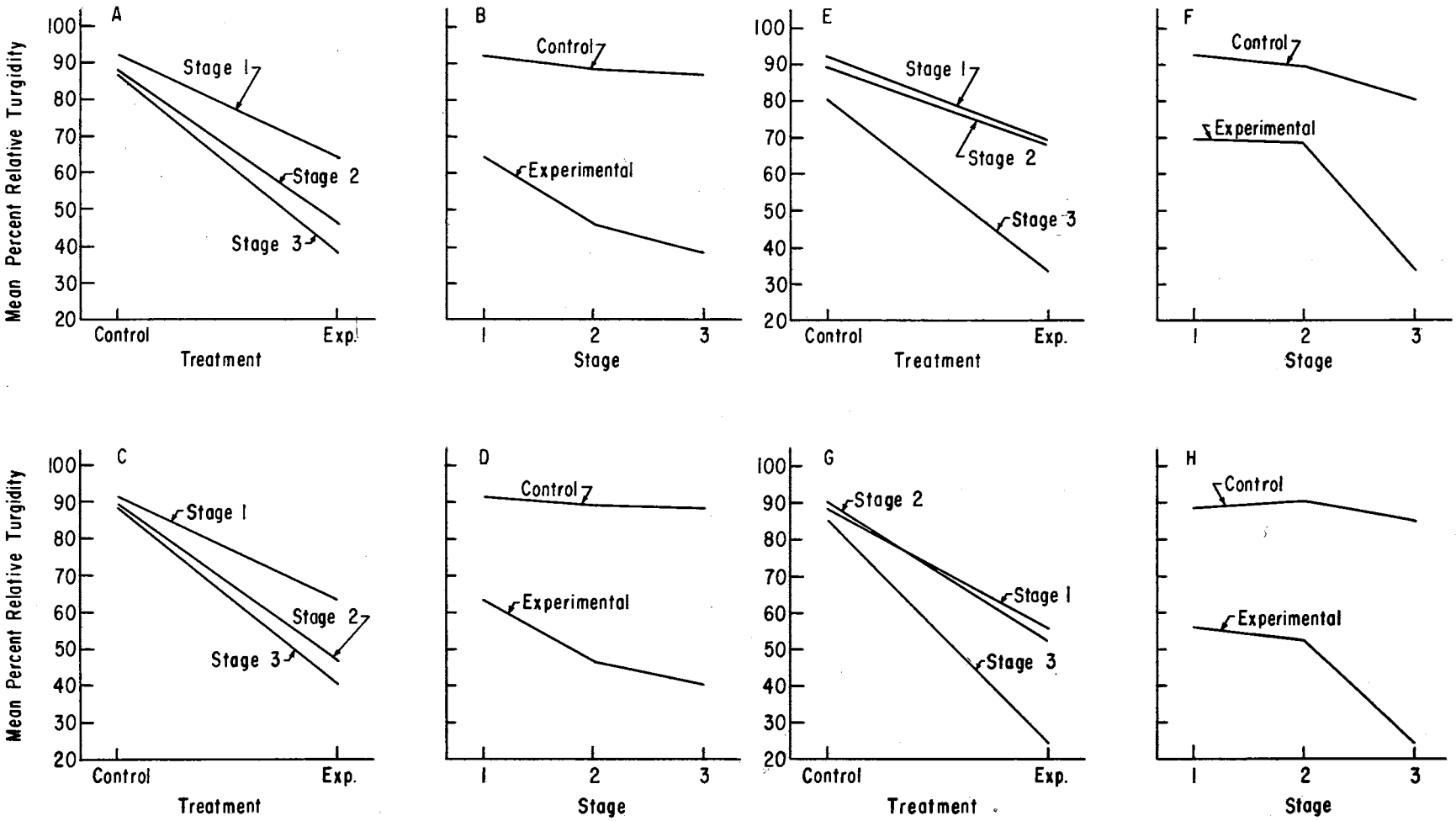


Figure 11. Interaction between treatment and stage of development on the mean percent relative turgidity in second leaves of four varieties of wheat subjected to one week of drought. A-B, Thorne, C-D, Red Chief, E-F, Red Coat and G-H, Ponca.

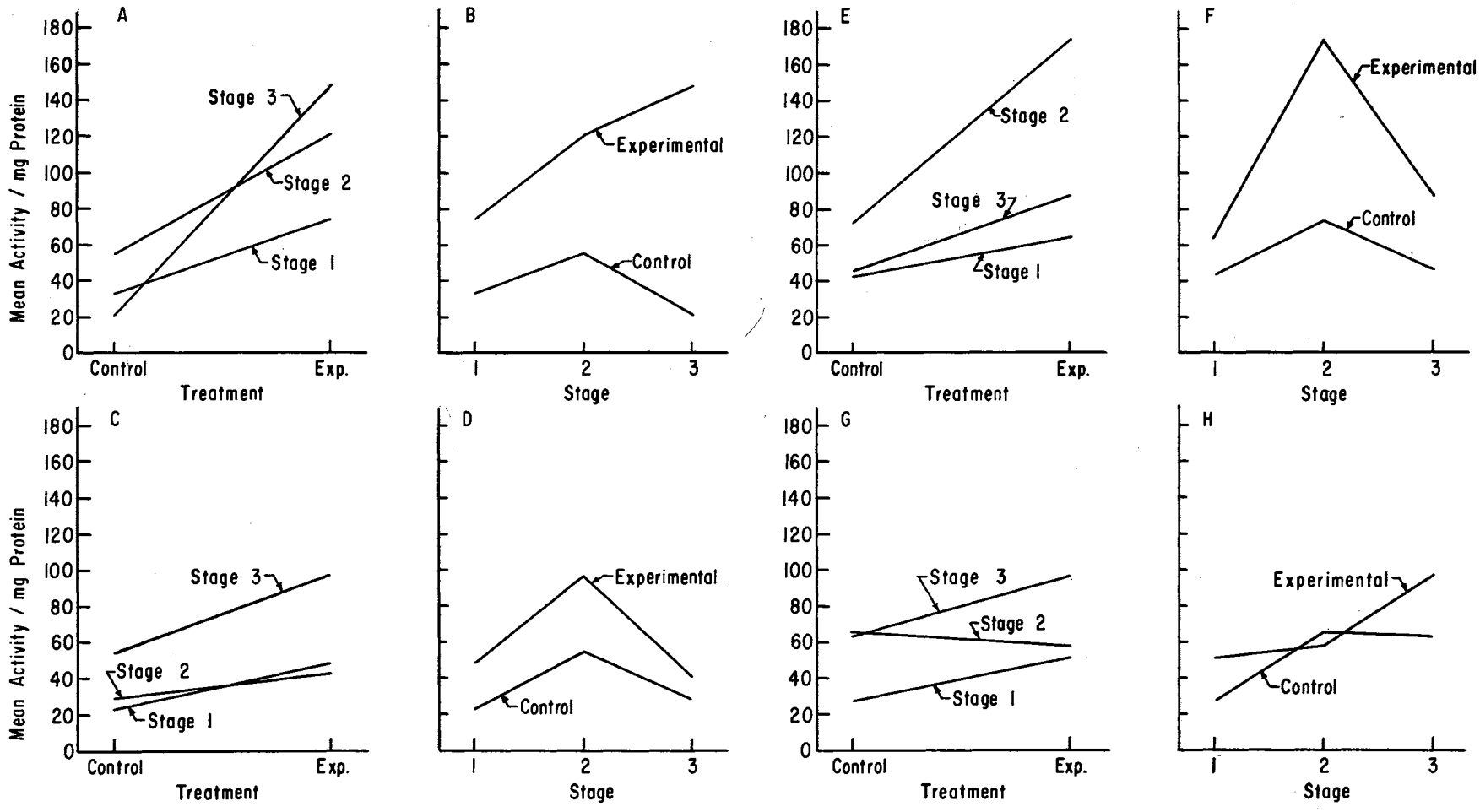


Figure 12. Interaction between treatment and stage of development on the mean peroxidase activity/mg protein in second leaves of four varieties of wheat after subjection to 1 week of drought. A-B, Thorne, C-D, Red Chief, E-F, Red Coat, G-H, Ponca.

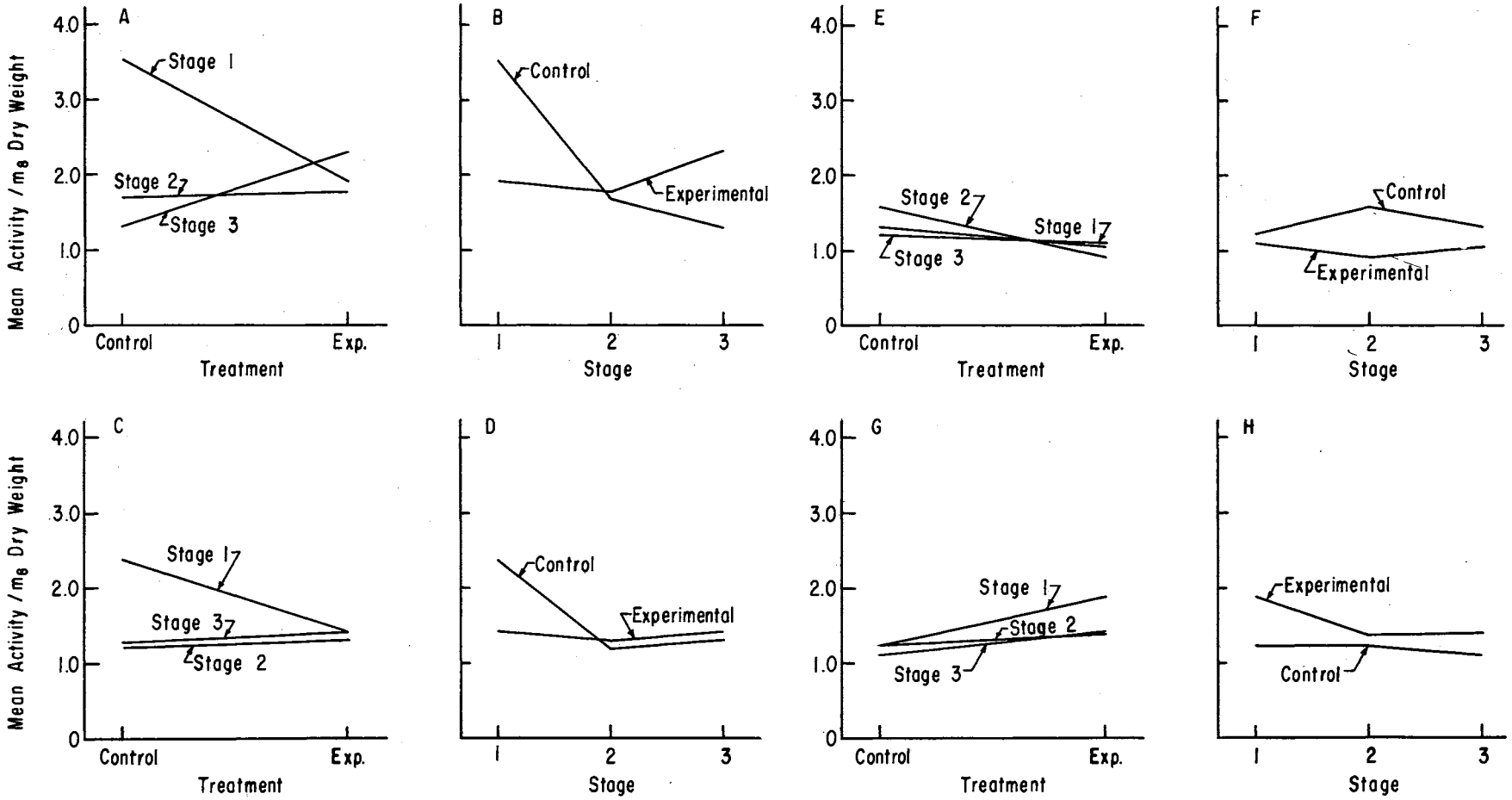


Figure 13. Interaction between treatment and stage of development on the mean peroxidase activity/mg dry weight in second leaves of four varieties of wheat after subjection to 1 week of drought. A-B, Thorne, C-D, Red Chief, E-F, Red Coat, G-H, Ponca.

stages 2 and 3. The difference in effect between stages 2 and 3 was also very slight. Red Coat showed a small decrease in activity in all 3 stages after subjection to drought. Control stage 2 had more activity than either control stage 1 or 3, and experimental stage 2 had less activity than either experimental stage 1 or 3. Ponca was different than the other 3 varieties in that the control for each stage of development had less activity than the experimental for each level of development. The increase in activity after the stress treatment was greater at stage 1 than at stages 2 or 3.

Mean length of the second leaves of plants potted at 3 different stages of development, varied between treatments, among the stages of development, and among varieties tested. The data for the mean length of second leaves of control and experimental plants of each variety are presented in figure 14.

Analysis of variance for the data of figure 14 is given in table XIV of the appendix. Treatment and varietal differences are both significant at the 5% level of confidence. Since leaf length increase is being used as a measure of growth in this study, figure 14 indicates that the amount of leaf growth among the ten varieties of wheat is different. In all cases the effect of water stress on the amount of growth is extreme. Leaf growth is decreased more than 50% in all varieties except Red Coat.

Table IX shows the data of leaf length differences in control and experimental plants at each level of development for the ten varieties of wheat. F tests were significant at the 5% level of confidence for stage, treatment and variety, and for interaction between stage and treatment, stage and variety, and for stage within treatment within

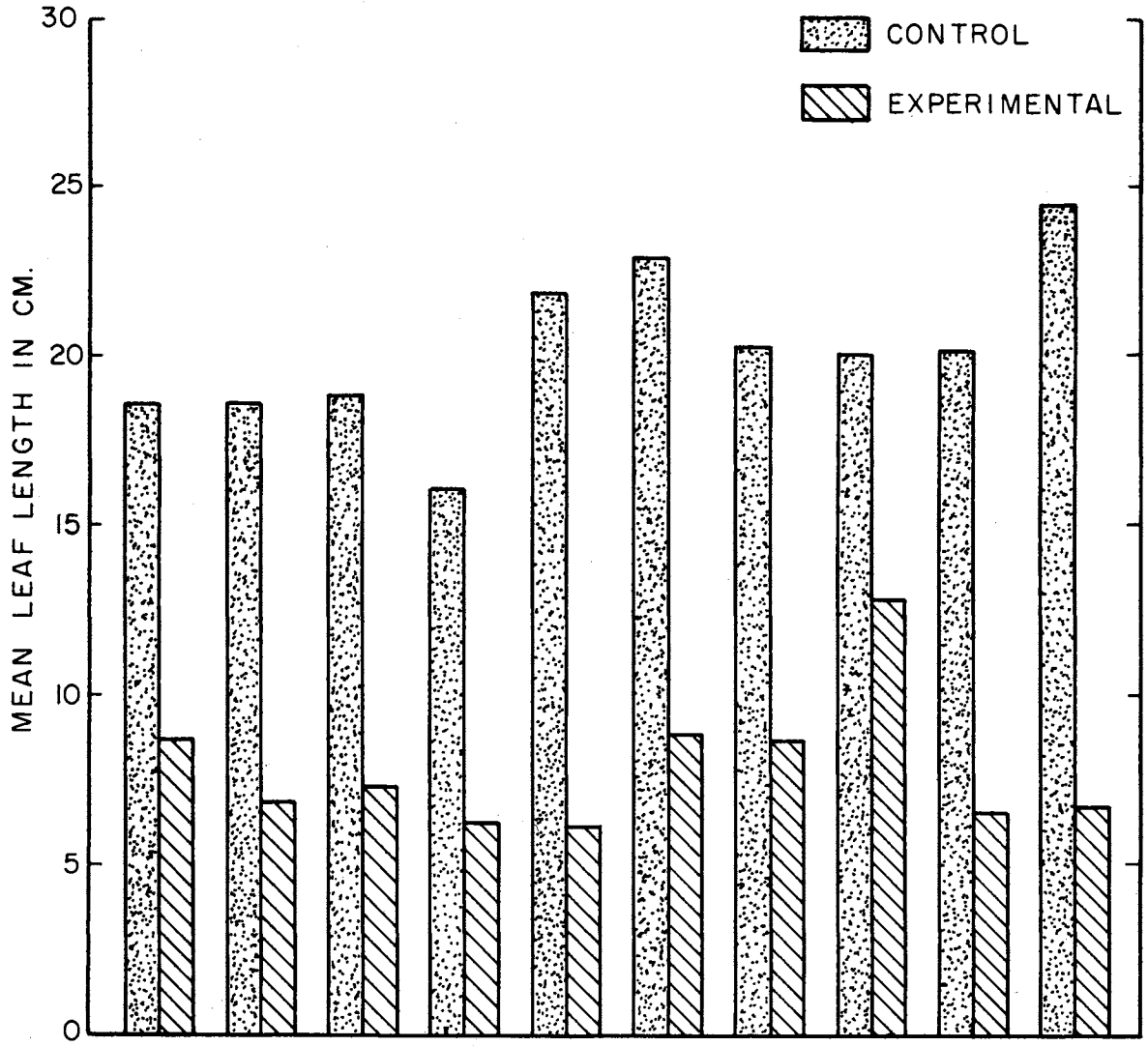


Figure 14. Mean length of second leaves of 10 varieties of wheat after subjection to one week of drought stress when the plants were at different levels of development. Varieties are (from left to right) Cheyenne, Kanking, Ponca, Red Chief, Dual, Leapland, Pennol, Red Coat, Seneca and Thorne. Values based on averages of 10 leaves of each variety. (Stages of development defined in procedures).

TABLE IX

MEAN LEAF LENGTH IN CENTIMETERS OF SECOND LEAVES OF CONTROL AND EXPERIMENTAL PLANTS AFTER ONE WEEK OF DROUGHT AT THREE LEVELS OF DEVELOPMENT

Variety	Stage 1		Stage 2		Stage 3	
	Control	Experimental	Control	Experimental	Control	Experimental
Cheyenne	18.75	10.35	19.15	9.50	17.90	6.10
Kanking	21.00	8.80	18.50	8.55	16.50	3.15
Ponca	20.25	7.15	18.10	8.00	18.40	6.75
Red Chief	18.90	8.00	17.00	5.65	12.60	5.00
Dual	23.00	7.10	19.65	7.65	23.00	3.60
Leapland	24.00	11.65	27.00	10.80	17.95	4.00
Pennol	22.10	11.50	21.50	7.20	17.40	7.15
Red Coat	16.90	12.50	20.00	14.60	23.40	9.15
Seneca	21.00	6.40	20.25	6.50	19.25	6.25
Thorne	24.40	7.40	25.40	6.90	23.50	6.00

Stages 1, 2, and 3 defined in procedures

variety. Interaction between treatment and stage of development for varieties Thorne, Red Chief, Red Coat and Ponca is shown in figure 15. These data illustrate that in all four of the varieties the direction of response is the same for each stage of development, but the magnitude of the response varies for the different levels of development. In varieties Thorne and Red Chief stage 2 was affected to a greater extent than were stages 1 and 3. Variety Red Coat showed the greatest effect at stage 3, and variety Ponca at stage 1. It is interesting that the control plants of the different stages had significant differences in amount of growth. The magnitude of difference of course was much smaller than that between control and experimental plants. The differences between control stages however, was comparable to the differences between experimental stages for a particular variety. This observation seems to suggest that a response to the handling of the plants is being expressed. In varieties Thorne, Red Coat and Ponca the stage of development at which the response to stress was most drastic was also the level of development at which the control plants showed the most growth. If the stage showing the most growth within the given period of time is considered as the most active level of development of the 3 stages being tested, then it seems indicated that the most active stage is most susceptible to stress. The response of variety Red Chief indicated that stage 3 was less affected by the stress. Interpretation is difficult here however, due to the obvious influence of transplating on the growth of the seedlings.

Discussion

The varieties of wheat studied in this investigation demonstrated

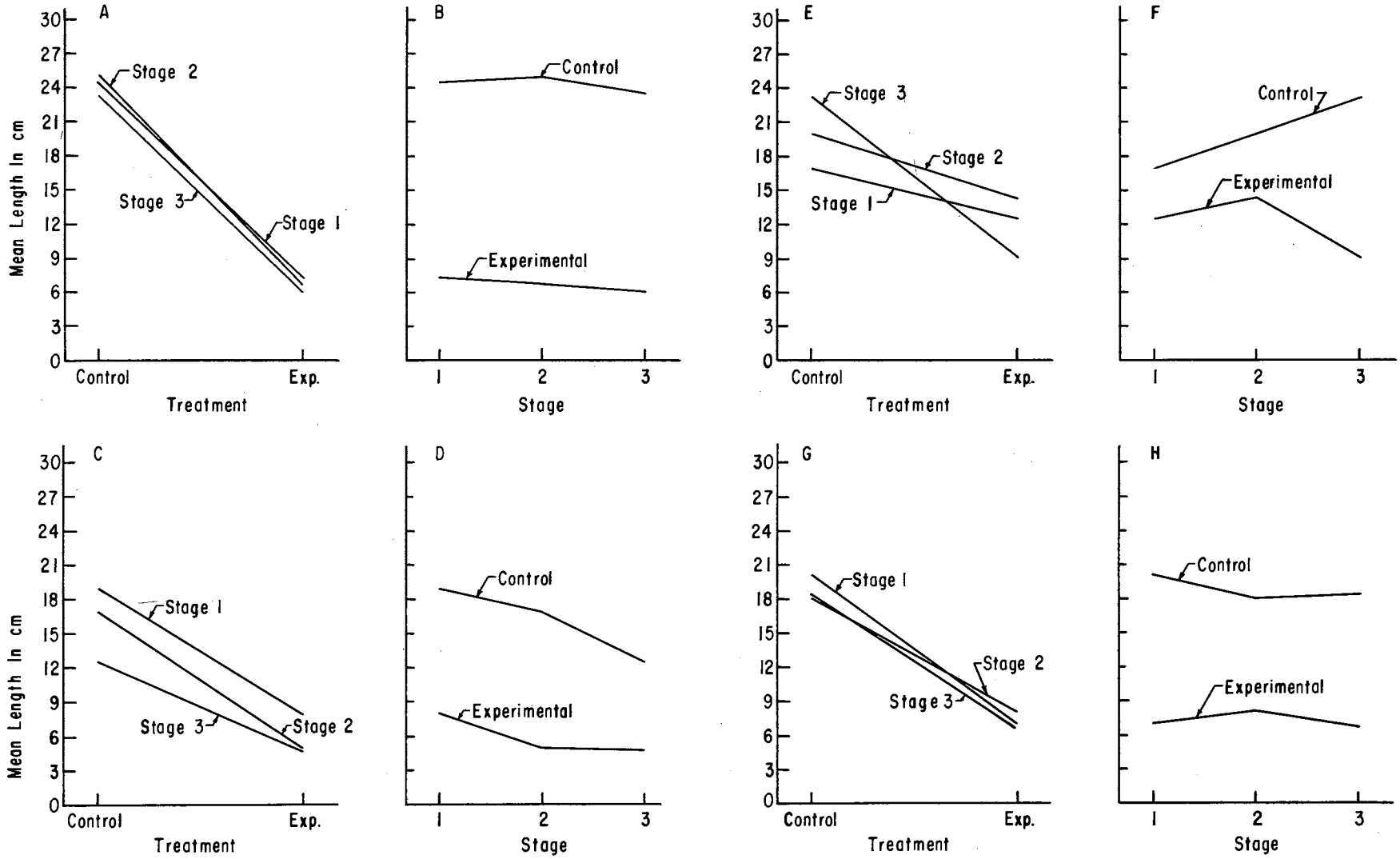


Figure 15. Interaction between treatment and stage of development on the mean leaf length for four varieties of wheat subjected to one week of drought. A-B, Thorne, C-D, Red Chief, E-F, Red Coat, G-H, Ponca.

varying abilities to withstand comparable periods of drought stress. Several varieties of soft red and hard red winter wheat of the same species, Triticum aestivum, L., were chosen for study because the two groups are characteristically field grown under two distinct environmental conditions. In the United States hard red winter wheat is produced in the great plains areas under climatic conditions of moderate to severe winters, relatively hot dry summers, and an annual rainfall of 30 inches or less. The grain of this type of wheat is described as having a strong gluten. Soft red winter wheat is grown in the northeastern wheat producing states where there is a higher humidity and the annual rainfall is 30 inches or more. This type of wheat has been described as being capable of withstanding greater amounts of moisture and having less resistance to drought (Swanson, 1938). The gluten of soft red winter wheat is characterized as being more pliable than that of hard red winter wheat.

The relative survival abilities exhibited by the ten varieties of wheat being studied here as determined by a screening test suggested by Todd and Webster (1965), have enabled this investigator to classify the different varieties of hard and soft red winter wheat as drought hardy or drought susceptible when grown under controlled environmental conditions. Statistically significant data have shown the relative survival abilities of varieties Red Chief, Kanking, Cheyenne and Ponca to agree essentially with the data of Todd and Webster (1965). Because the survival tests showed that the soft red and hard red varieties of winter wheat tested here cannot be separated into the two classifications of hard and soft on the basis of ability to survive internal water stress, interpretations of the experimental results have been

made from the point of view of drought hardy varieties as opposed to drought susceptible varieties. No attempt has been made to make comparisons between the group of soft red wheats and that of hard red wheats.

Interpretation of comparisons between the results of tests on plants of the age used in the survival studies and the results of tests on the young seedlings of experiment number 2 in this study must be made with reservations because of the interaction of aging effects. A statistical comparison between the data of the two experiments was not attempted since the conditions and design of each were so different. Nevertheless, with the above indicated reservations in mind certain salient features of the two experiments pertaining to the variables considered are treated together in the discussion. It is believed that such an approach is most feasible to a better understanding of the mechanisms of response in drought hardy and drought injury-susceptible varieties of wheat.

In spite of the inherent differences between soft red and hard red winter wheats the observed responses of the several varieties of wheat used here indicate that under controlled environmental conditions some varieties of soft red winter wheat will endure the effects of drought as well or better than certain hard red varieties of wheat. This observation is well illustrated by the data of figure 4. The survival tests showed that the known field hardy hard red variety Cheyenne did not respond as well as soft red varieties Dual, Seneca, and Thorne to the early applications of stress. After the second week of drought Red Chief alone, of the field hardy hard red varieties showed survival ability comparable to that of soft red variety Thorne

through drought cycle number 6. After the sixth week of stress, the performances of the soft red varieties Dual, Seneca, and Thorne were superior to all the other varieties tested. There was a continuing loss of plants with each successive drought cycle. In the case of those plants which were able to resume functioning upon rewatering after each drought cycle the assumption is that the protoplasm of these plants was better able to withstand the desiccation than was that of the plants that were not able to resume functioning. It is apparent from the data in figure 4 that the rates of decrease in number of live plants at each drought cycle varied for any specific variety. Since the plants were randomly arranged in the plots, and it is presumed that the roots of each had equal opportunity to compete for the available water, the latter observation suggests that the percent of the remaining plants at the end of each cycle that retained desiccation resistant protoplasm fluctuated from one week to the next. It seems indicated that an adaptive mechanism operates in response to water deficit and it is functional at different levels in different plants of the same variety as well as functioning in different numbers of plants of different varieties.

Percent relative turgidity measurements at the end of drought cycle 8 showed no apparent correlation between the level of tissue water content of the remaining plants and the relative percent survival of the original population of plants of the several varieties of wheat. It is worthy of note that when varieties Cheyenne and Ponca were compared for changes in leaf anatomy in chapter III of this study it was found that upon rewatering the extent of disorganization was greatest in variety Ponca, but the percent relative turgidity was 97 while in

variety Cheyenne it was 96. Todd and Webster (1965), also observed that certain field hardy varieties of wheat did not maintain the same relative positions in Duncan's Multiple Range lists for tests on percent survival and percent relative turgidity.

It seems indicated that certain plants may be capable of competing for available water and may even retain it in the tissues but the ability to use the water to the best advantage is not present. Since tissue water content is being measured here the results may be more meaningful if considered in the light of Stocker's (1960), observation that it is not the amount of water, but rather the dynamic state of water contained in the plant that is important to the best functioning of the vital processes.

The small amount of difference between the percent relative turgidity in control and experimental plants of the hardened group presents a striking contrast to the differences observed between control and experimental plants which were subjected to one week of drought stress at different levels of development (table V, and fig. 10). When the drought effects were considered at each stage of development the data showed that at stage one the percent relative turgidity dropped as low as 46 in variety Seneca. The lowest at stage 2 was 37%, again exhibited by variety Seneca and at stage 3 variety Dual was the lowest with 23% relative turgidity. There was considerable variation among the levels of tissue water content for the various varieties at each stage of development. At no stage were the relative varietal levels comparable to those observed in the hardened plants.

Evidence for statistically significant interaction between the stage of development and the effects of drought on the percent

relative turgidity were presented in the section on results. The detailed account of this interaction in varieties Thorne, Red Chief, Red Coat, and Ponca, (fig. 11), showed a similar pattern of interaction for varieties Thorne and Red Chief, and a similar pattern for varieties Red Coat and Ponca. From this observation it seems logical to assume that each of the two varieties exhibiting the similar patterns of interaction between stage of development and response to drought has a similar mechanism of handling the available water at the specific stage in question. One might expect just such a distinction between the 2 pairs of varieties, since it has already been shown that each pair possesses some distinctive property which causes a strikingly different response to drought. This explanation of course does not, and is not intended to account for any association between the relative levels of water in the respective wheat varieties after the hardening process.

Peroxidase activity in the second leaves of plants subjected to 8 weekly cycles of drought was higher than the activity of the leaves of control plants. When the activity was measured per mg protein content, the increase was greater than 50% in all varieties except variety Thorne. Increases in peroxidase activity per mg dry weight were not as great. There was little or no change evidenced for varieties Cheyenne, Kanking and Pennol, and in variety Ponca there was a decrease. The demonstration that the difference between the percent change of peroxidase activity per mg protein and the percent change in peroxidase activity per mg dry weight resulted in a ranking of the wheat varieties in an approximate inverse order to that of the survival ranks. This suggests an interaction between protein and dry weight

levels with peroxidase activity at varying degrees of drought resistance.

From the results described it is possible to consider the roles of protein level, dry weight level, and peroxidase activity in drought resistance. First it is important to emphasize that increased development of mechanical tissues appears to be an adaptation that enables the plant to increase its particular level of drought resistance. It would be expected that increased lignin biosynthesis would accompany the occurrence of increased amounts of mechanical tissue. Experimental evidence has been produced to support the essential role of peroxidase in the formation of lignin from shikimic acid which is formed from glucose, and 3 and 4 carbon sugars (Brown and Neish, 1955). Though shikimic acid is the common precursor for lignin and some amino acids, particularly the aromatic forms, it has been indicated that adequate supplies of phosphorus to supply energy favors the pathway of formation and polymerization of amino acids to form protein rather than the pathway to lignin biosynthesis (Miller and Anderson, 1965). These authors further suggested that conditions favoring the pathway to protein synthesis also favored the activity of auxins. Increased auxin activity has been shown to be followed by a decreased peroxidase activity (Siegel et al., 1960).

The evident interplay described above suggests the hypothesis that in drought subjected plants peroxidase activity increases because the increase in available sugars (Vassiliev and Vassiliev, 1936), leads to increased levels of shikimic acid which is directed to lignin formation. The assumption of the pathway to lignin formation rather than to amino acid and protein synthesis seems justified by the

established findings of decreased protein synthesis in the leaves of droughted plants. Further it has been shown that RNA level after an initial increase, was followed by a pronounced decrease (about 0.2% of the dry matter in 20 days) in tomato plants subjected to soil drought (Kessler, 1961). In drought subjected wheat leaves the nucleic acid content in the supernatant fraction decreased about one-half with a 50% loss of water (Todd and Basler, 1965). The marked increase in peroxidase activity per mg protein reported here for the leaves of hardened wheat plants indicates that after treatment a considerable portion of the pH 4.5 soluble protein is peroxidase.

Dry weight increase would be expected to occur concurrently with increased lignin deposition. Thus the peroxidase activity relative to dry weight would show a lesser amount of increase than when measured relative to protein. In the event that the increased lignin deposition was great enough to cause the stressed plants to have a higher percentage of dry weight, then the peroxidase activity per mg dry weight in the control plants would be higher than that in the experimentals. Interestingly enough, variety Ponca, the most drought sensitive of all the wheats tested here exhibited a higher peroxidase activity per mg dry weight in the leaves of control plants than in the leaves of droughted plants. A related point of interest is that variety Ponca and other drought susceptible plants showed a greater tendency for continued shoot growth after the application of moisture stress which caused the cessation of root growth than did drought hardy varieties (Salim, Todd and Schlehuber, 1965).

When data were considered on 4 leaf basis (Table XVII), the hard red varieties showed a decrease, or no change at all in peroxidase

activity. One would expect that in drought hardy varieties the increase in peroxidase activity per mg dry weight after stress would be higher than the increase in drought susceptible plants. Observations on varieties Dual, Ponca, Seneca, and Thorne clearly support this prediction. Results of varieties Pennol, Red Chief, and Red Coat are rather difficult to interpret, and the results of varieties Cheyenne, and Kanking show a deviation from the expected pattern. At the present time no suitable explanation can be given for this.

A particular point of interest is the apparent relationship between relative survival ability and the difference in percent increase of peroxidase activity per mg protein and the percent increase in peroxidase activity per mg dry weight. It is postulated that if the rate of lignin synthesis exceeds the rate of protein hydrolysis the plants have high survival ability. As the rate of protein hydrolysis approaches and exceeds the rate of lignin synthesis, the relative survival ability declines. It is conceivable that in the drought hardy plant a built in mechanism controls the relative rates. In injury susceptible plants there would exist no such mechanism to maintain a favorable balance and the plants though increasing the rate of lignin synthesis in response to drought, would have protein hydrolysis occurring at a greater rate than the lignin synthesis.

When measurements on the plants studied here were subjected to this test of relationship the wheat varieties were placed in a approximate inverse order to their placement in the test for survival ability. The slight deviation from placement of exact inverse order could be accounted for by lack of statistically significant differences between the varieties involved (table III).

Peroxidase activity measurements in young seedlings yielded data extremely difficult to interpret. For the most part there appeared to be no consistent pattern in either control or experimental plants. It is impossible to make comparisons between measurements in these plants, and measurements in the plants used in the survival studies because of the interaction between the stage of development and treatment effects in the seedling plants. Moreover, the extreme differences in levels of maturity of the tissues between the two groups make attempts at comparisons of biochemical complexes impossible.

Significantly the data accumulated from measurements on the seedling plants indicated that the varieties of wheat tested here had different amounts of peroxidase activity in the leaves of the control plants at each stage of development. The differences were more extreme when calculated on a per mg protein basis than when calculated on a per mg dry weight basis (tables VII and VIII). The data when considered on a per 4 leaf basis (tables XVIII, XIX and XX in appendix), showed that peroxidase activity decreased after stress at each of the three stages of development. This observation supports the findings of Todd and Yoo (1964), for detached wheat leaves held over $CaCl_2$.

The ages of the plants at the end of the experiment were approximately 9, 10, and 12 days respectively for developmental stages 1, 2, and 3. In most cases only leaves number 1 and 2 were visible, but the evidence of leaf number 3 on some plants indicated that the rates of growth varied. Leaf length measurements (fig. 14), show the difference in growth rates between varieties, and the data in table IX attests to the differential growth rates among the stages of development.

Interactions between synthetic and respiratory functions of such

actively growing leaves would have to be reckoned with in consideration of the causal effects resulting in the extreme variations in protein, dry weight, and peroxidase activity apparent in both control and experimental plants. The significant interaction between treatment and stage of development (table IV), suggests the reflection of adaptive mechanisms at the various stages of development. The presence or absence of sufficient water may affect the level of action, or even the ability of such mechanisms to maintain an active role in the growth of the plant.

Conclusions

Of ten varieties of winter wheat subjected to repeated exposures to drought under controlled environmental conditions soft red and hard red varieties could not be separated into two groups on the basis of survival ability. Correlation between relative survival ability of the original population and the percent relative turgidity of the second leaves of surviving plants was fairly poor. Peroxidase activity in the second leaves of control plants was significantly lower than that of plants which had been exposed to 8 weekly cycles of drought. On the basis of the marked increase in peroxidase activity per mg protein it was concluded that after hardening treatment a considerable portion of the pH 4.5 soluble protein was peroxidase. Changes in peroxidase activity when considered as a percent of control activity relative to protein and dry weight content indicated a relationship between these measurements and the relative survival ability of the wheat varieties.

Seedling plants subjected to 1 week of drought when at different

levels of development showed significant interaction between the stage of growth and treatment on levels of peroxidase activity, leaf growth and percent relative turgidity. Variations in the magnitude and the direction of change for these variables indicate the operation of adaptive mechanisms at different levels of development.

CHAPTER V

PROCAMBIAL AND XYLEM DIFFERENTIATION IN THE SHOOT TIP AFTER SUBJECTION TO DROUGHT STRESS

Introduction

Indications of differential xylem and mechanical tissue development, and apparent related differential levels of peroxidase activity in drought "hardy" and drought "injury-susceptible" varieties of wheat suggests that xylem initiation in the two groups of plants warrants an investigation. The stem apex gives rise to the primordia of the differentiating tissues of the shoot. A study of the anatomy of the vegetative shoot tip of wheat plants after subjection to drought stress may possibly reveal certain stress induced anomalies or alterations in xylem inception patterns.

The pattern of early differentiating xylem elements in the vascular plate separating the primary root and stem in normal wheat seedling morphology and development has been demonstrated (Avery, 1930; Boyd, and Avery 1931; McCall, 1934). It was shown that as internodal elongation occurred, vascular elements showed discontinuation between portions arising from the plate and the newer bundles being derived from the upper portions of the stem (McCall, 1934).

The amount of differentiating xylem tissue has been shown to be directly related to the amount of sugar diffusing through the tissue (Wetmore and Rier, 1965). The work of the latter authors though

pertaining to callous tissue of Syringa vulgaris, is in agreement with the postulates of earlier investigations (Jacobs and Morrow, 1957, 1958; and Torrey, 1955, 1958, 1963), and may apply to some other plants. Previous citation of the work of Vassiliev and Vassiliev (1936), has called attention to the finding that significant alterations occur in carbohydrate metabolism of drought subjected wheat plants.

An account of tracheary element development in the area of first differentiation in the stem of the wheat plant after subjection to water stress seems justified. In the plant subjected to drought an increase in the amount of available sugar to the shoot tip may have some significant effect on the control of xylem differentiation. Moreover, the differential effects of drought stress on a "hardy" and an "injury-susceptible" variety of wheat may be demonstrated by a differential response of xylem differentiation.

Materials and Methods

Triticum aestivum, L., resistant variety c.v. Cheyenne (C.I. 8885) and non-resistant variety c.v. Ponca (C. I. 12128)(Todd et al., 1962), were used for this study. Seeds of each variety of wheat were sown in separate 6-inch pots in well moistened sandy loam. After the initial germination period the seedlings were thinned to 25 per pot and maintained in a controlled growth chamber for a period of 3 weeks (chamber described in materials and methods of chapter IV).

Experimental material consisted of 5 potted samples of each variety, the number 1 pot served as the control and was watered at regular intervals throughout the duration of the experiment. After the

preliminary 3-week growth period, applications of water were discontinued to the fifth pot. At 2 day intervals thereafter pot number 4, 3, and 2 respectively, received no more water. The experiment was discontinued when samples number 2-5 had received 2, 4, 6, and 8 days of stress respectively. The plants were harvested and the crown area and shoot apex were removed according to the dissection technique of Sharman (1945). Some of the dissected tips were fixed in FAA, dehydrated through TBA alochol series and mounted in paraffin blocks. Serial sections 8-10 μ thick were stained by Foster's (1934) tannic acid-ferric chloride method. Microscopic studies were made on the overall anatomical organization with special reference to the procambium and tracheary elements. Photomicrographs were made to illustrate points of interest.

Results

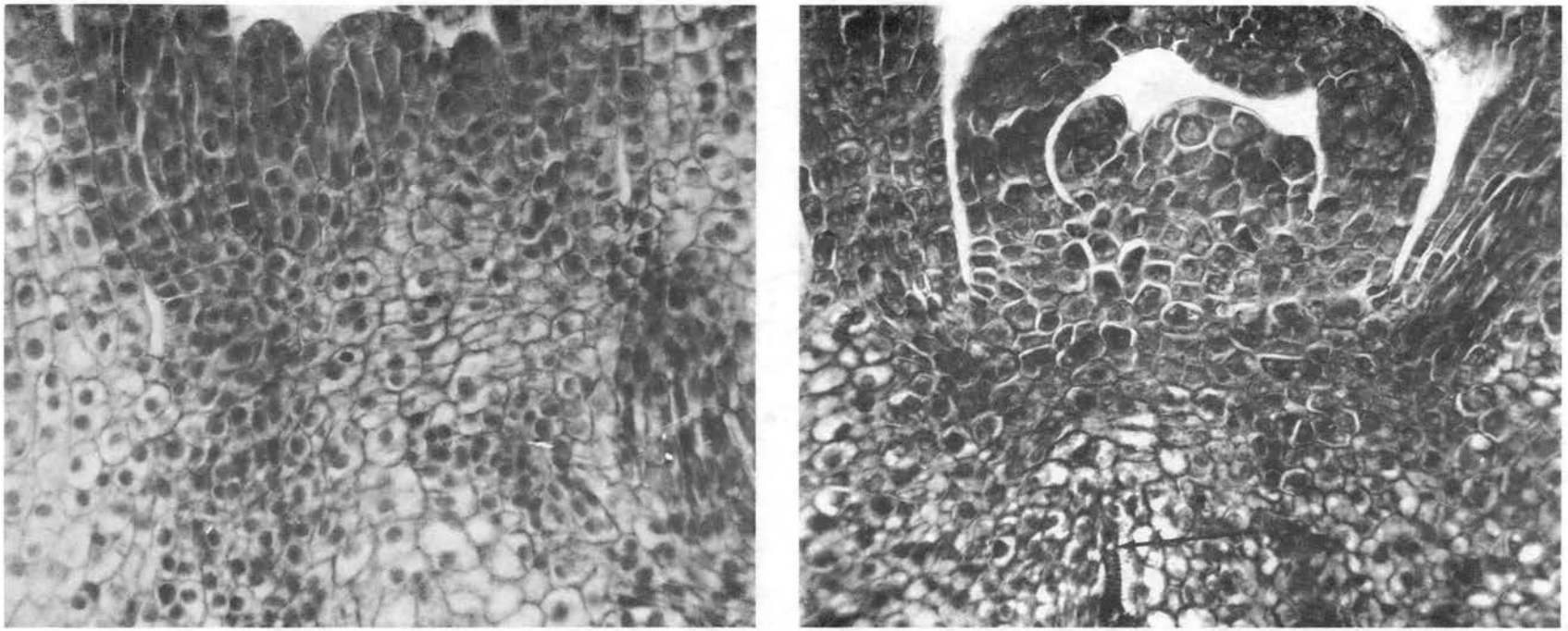
The shoot tip as defined in this report included the stem tissues distal to the last differentiated node, and the apex with its leaf primordia. Densely cytoplasmic elongated cells which were traceable to the apex, leaf primodia, or leaf traces were identified as procambium. Xylem elements were classified as annular, sclariform, reticulate, or pitted, according to the descriptions of Esau, (1953), and where possible, were traced to the respective leaf in order to aid in determination of the point of inception and the direction of development.

Descriptions of the apex anatomy were recorded from median longitudinal sections. Measurements of apex height and diameter were taken above the youngest leaf primordia. Analysis of procambium

differentiation and xylem inception were based on observations of sequential sections and reconstruction of overall pattern. The terminology used is that of Sharman (1945), with the exception of cited descriptions, at which place the original terminology of the author cited is used.

Preliminary studies were made on the apices of 72 hour seedlings to determine the basic anatomical pattern of the two varieties of wheat being studied. Five apices of each of the two varieties of wheat were examined. There was considerable variation in the size and zonation patterns of the apices of each variety. Results showed that the average width of Cheyenne apices was 85.0 μ and the average length was 27.2 μ. The average width of Ponca apices was 81.6 μ and the average length was 13.6 μ. Elongation of the crown internode was greater in variety Cheyenne (187 μ as opposed to 136 μ in Ponca), and it was apparent that the rate of growth was greater in the overall tip than was the growth rate in Ponca. Figure 16 shows apices from 72 hour seedlings of both varieties. The first 3 assimilating leaves are visible in each. A uniseriate tunica is apparent, and elongate densely protoplasmic corpus cells are clearly shown in variety Cheyenne. Apex zonation is less distinct in variety Ponca. An acropetally developing tracheary element is seen at the arrow in the subapical region in variety Ponca. The lack of visible xylem in this region of the Cheyenne tip is probably due to the relatively greater amount of elongation. Elongating densely protoplasmic procambial cells are visible at the base of developing leaves.

Procambial differentiation in the apex was difficult to discern in longitudinal section. In transverse sections the first visible



A B

Figure 16. Apices from 72 hour seedlings showing median l. s. cut parallel with the plane of the leaf blades. A, Cheyenne; B, Ponca. Note lack of distinct zonation in variety Ponca and acropetally developing tracheary element. Variety Cheyenne shows a uniseriate tunica and elongate, densely protoplasmic corpus cells. (X356).

procambium in the apex proper was approximately 0.8 μ below the origin of the youngest leaf primordium. Attempts to trace the procambium into the primordium were unsuccessful, and it was concluded that the inception of the procambium was at the base of the developing leaf primordium (fig. 17).

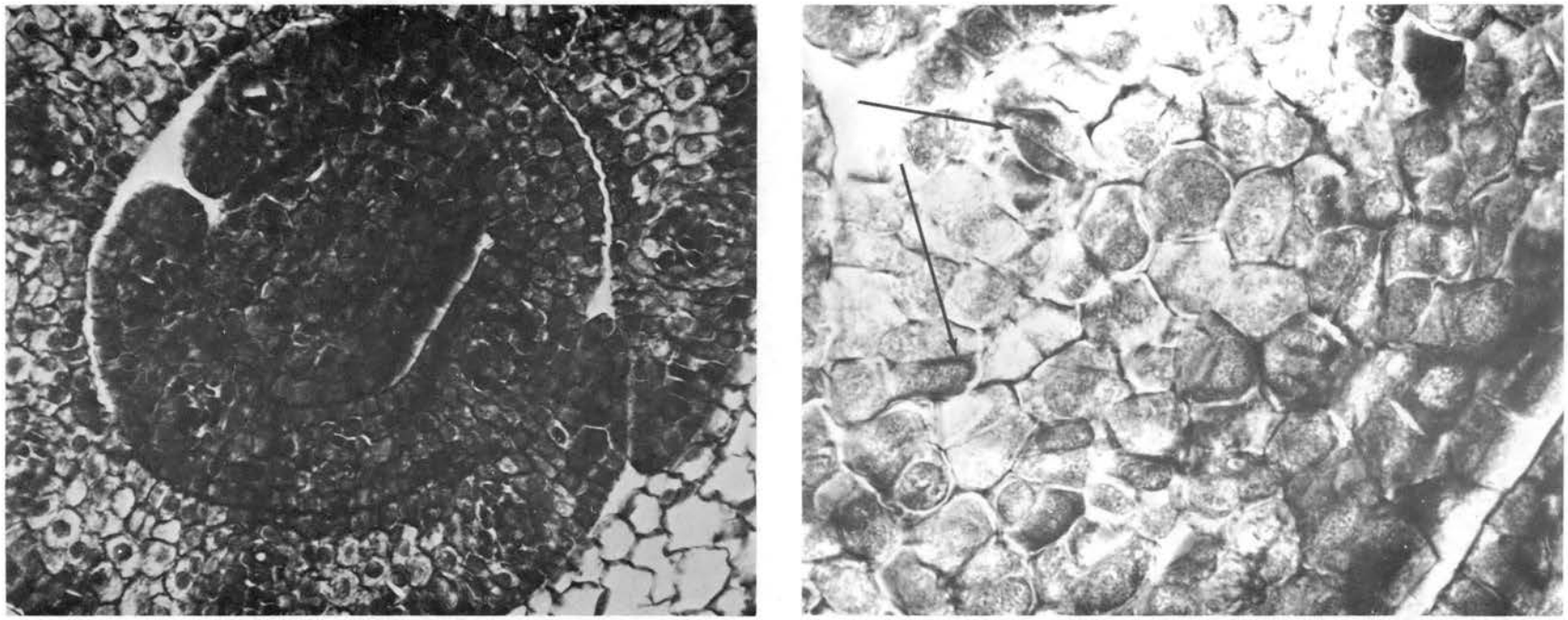
In general the anatomical patterns of the 72 hour seedling apex of wheat varieties Cheyenne and Ponca were comparable to that described as the first stage of organogenesis of the vegetative apex of the wheat plant, (Opatrna, et al., 1963).

When the experimental procedures in this investigation were completed the plants were 28 days old. The seeds had not been vernalized, and a 10 hour photoperiod was employed throughout the growing period. Apices from control plants showed an anatomical structure comparable to that of the first, or in some cases, the second stage of organogenesis as described by Opatrna et al., (1963). According to these authors:

> The second stage of organogenesis is characterized by the initiation of assimilating leaves by the shoot apex. At the beginning of this stage, i.e. during the initiation of the primodia of the lower leaves, the outer cells of the central core form a second superficial layer, the hypodermis. During the initiation of the primordia of the middle leaves, several larger cells are found at the apical part of the hypodermis. Under these is a group of conspicious small cells with relatively large nuclei. At the end of this stage, during the initiation of the primordia of the last leaves, the differences in the size of the cells at the tip of the apex are less obvious. At about the level of the youngest leaf primordium, the cells of the central core begin to become vacuolized and very elongated.

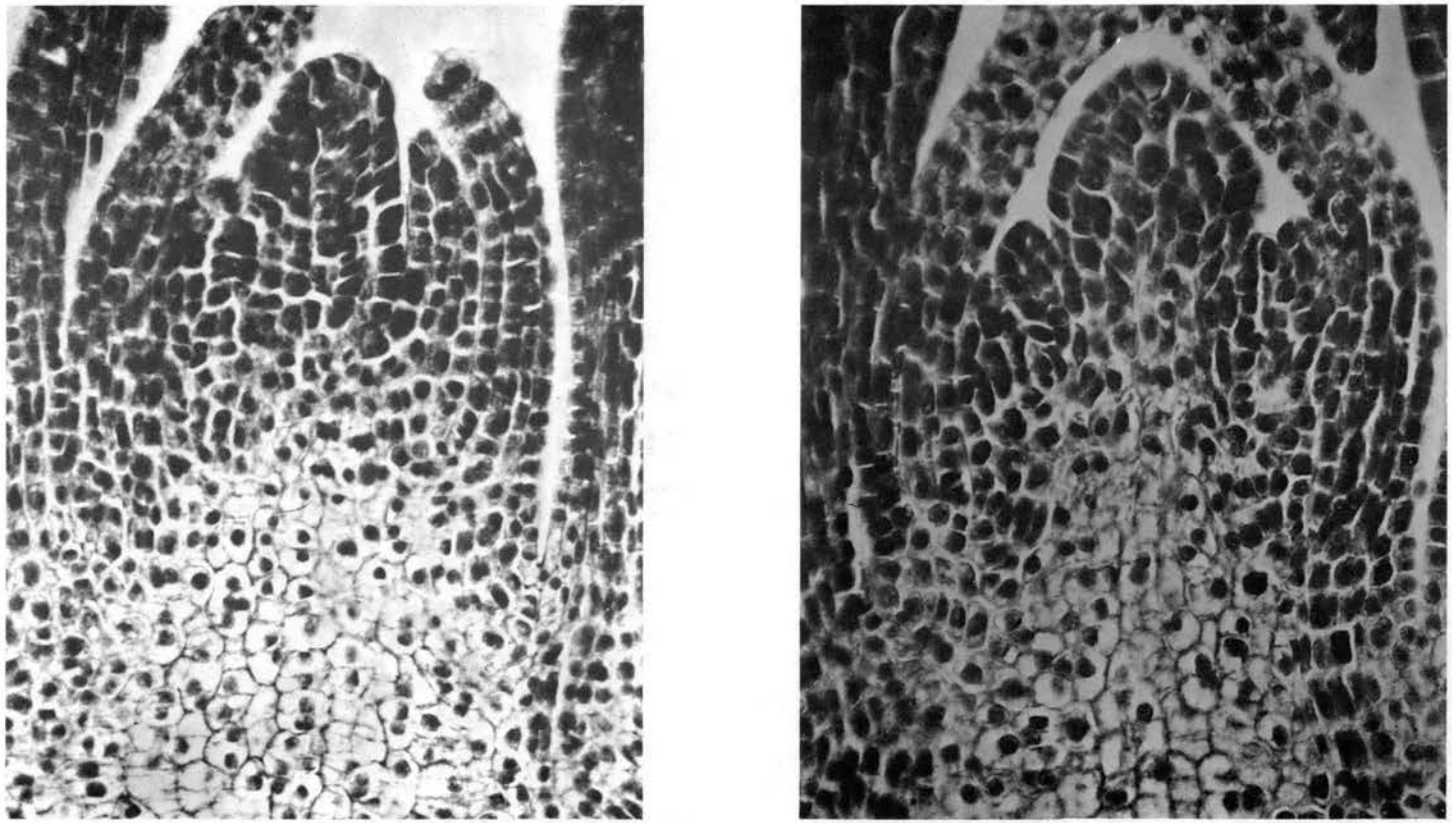
Figure 18 shows a representative apex from the control plants of varieties Cheyenne and Ponca.

Though there was variation among the control apices, common



A B

Figure 17. A, transection 18 μ below the origin of the youngest leaf primordium from an apex of a 72 hour seedling of variety Cheyenne. (X356). B, enlargement showing procambial divisions at arrows. (X594).



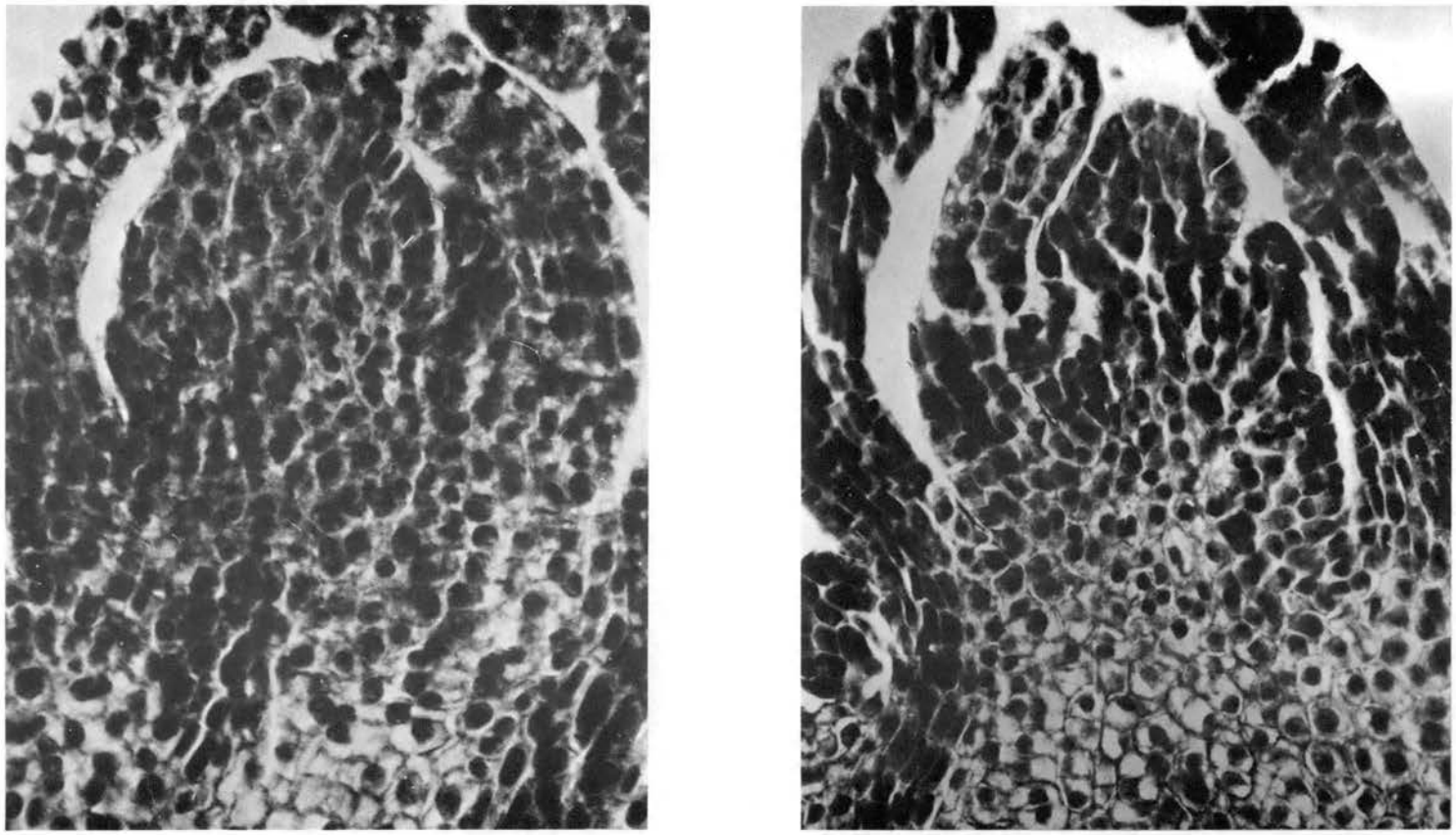
A B

Figure 18. Apices of untreated 4 week old wheat plants showing median l. s. cut parallel to plane of leaf blade. A, Cheyenne; B, Ponca. (X125).

features were (1) an anticlinally dividing uniseriate tunica composed of flattened cells with small nuclei, (2) a corpus composed of elongate cells showing both anticlinal and periclinal divisions, (3) a large vacuolate area subjacent to the corpus, composed of mainly isodiametric cells which showed evidence of mitotic division, and (4) a sub-apical region showing laterally and longitudinally oriented rows of procambium, and cross sectional views of leaf traces.

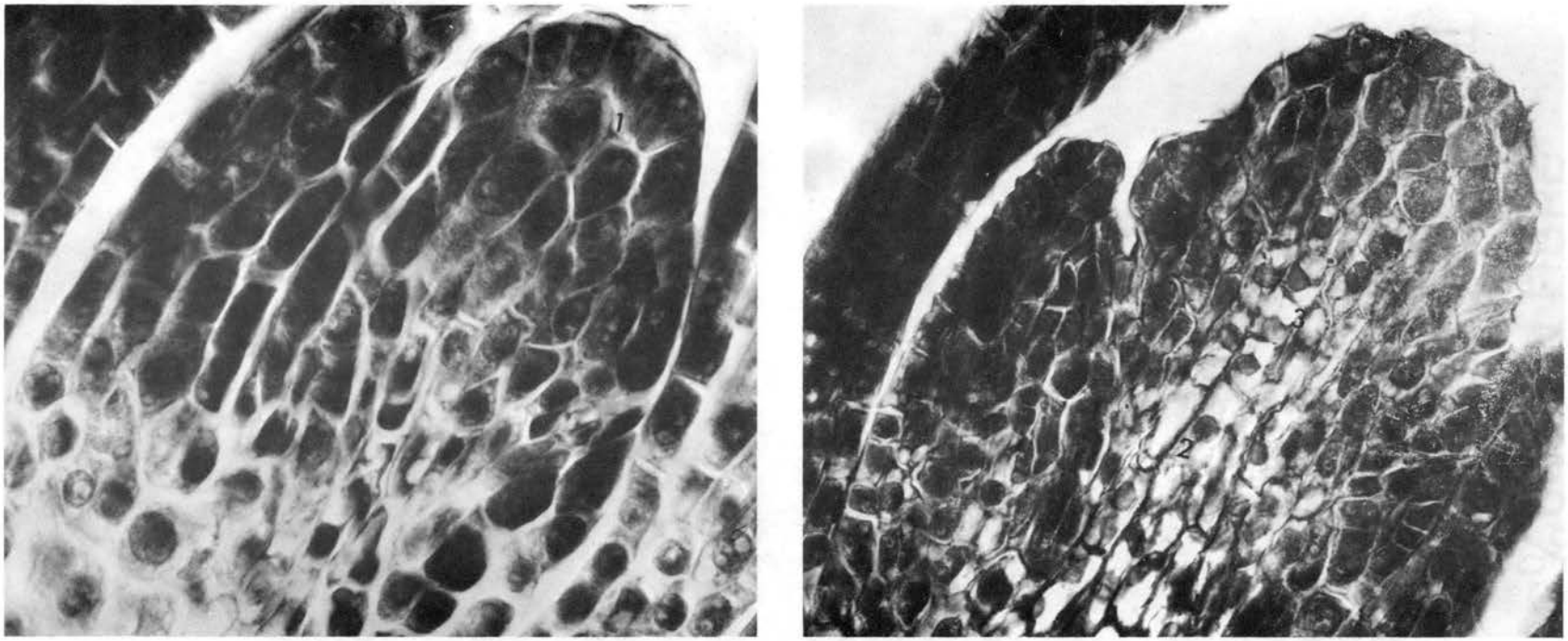
Apices from experimental plants also exhibited anatomical variation. Some of these apices fitted the description of the previously described second stage of organogenesis. Others showed considerable elongation and other characteristics of the third stage of organogenesis as described by Opatrna et al., (1963). These workers describe the third stage as being covered by the dermatogen and hypodermis and in some instances a sub-hypodermis is present. The cells in the hypodermis are smaller than they were in the second stage, and the cells lying under these apical cells show increase in size and evidence of mitosis. Figures 19 and 20 show apices from experimental plants of varieties Cheyenne and Ponca observed in this study.

Outstanding characteristics of the shoot tip from treated plants were (1) thickened cell walls in zones of the apex and in the sub-apical region, (2) apparent absence of protoplasts in some cells of the sub-apical region, (3) in some cases the sub-apical region was heavily granulated to a depth of 136 μ, and showed abundant periclinal and anticlinal divisions, while in other apices vacuolation was seen just sub-distal to the corpus cells, (4) plasmolysis of some cells, (5) lack of distinct zonation in some apices, and (6) increased lateral bud formation. All of the above listed characteristics were not usually



A B

Figure 19. Apices from 4 week old wheat plants after subjection to 4 days drought showing median l. s. cut parallel to plane of leaf blade. A, Cheyenne; B, Ponca. (X125).



A B

Figure 20. Apices from 4 week old wheat plants after subjection to 6 days drought showing median l. s. cut parallel to plane of leaf blade. A, Cheyenne; B, Ponca. Note (1) plasmolysis, (2) thickened cell walls and (3) vacuolation sub-distal to corpus cells. (X356).

apparent in any one shoot tip, nor could any one characteristic be identified with a specific period of drought, or a specific variety of wheat. Nevertheless, manifestations of anatomical changes after drought stress indicated that the response of the individual plants showed variable degrees of tissue maturation in the apical and subapical regions of the shoot tip.

Measurements of apex length and width from control and experimental apices are compiled in table X. Inspection of table X reveals several points. First, the average sizes of the apices from experimental plants of wheat variety Cheyenne are larger than those of variety Ponca, second, the average length and width of the control apices of both varieties of wheat are the same, third, after 2 days drought the apices of both varieties of wheat show an increase in both length and diameter, and in variety Cheyenne the increase in length is considerable, fourth, after 4 days of drought the apices of variety Cheyenne are shorter than the 2 day drought specimens and after 6 and 8 days drought respectively, the apices show continued decrease in length; although the width dimensions also change during this period the apices are elongated, and fifth, after 4 and 6 days drought the apices of variety Ponca though somewhat longer than the control apices, show continued increase in width. After 8 days drought the average length is the same as that of controls, but the width shows considerable decrease.

Procambium was recognizable as longitudinal or laterally oriented columns of cells in longitudinal view in the region below the base of the tip, and in and at the base of the leaf primordia. In some tips the procambial cells surrounded the vacuolated region, while in others

TABLE X

LENGTHS AND WIDTHS OF VEGETATIVE SHOOT APICES OF 28 DAY OLD CONTROL AND EXPERIMENTAL PLANTS

Days Drought	Number of Apices Measured	Avg. Length in microns	Avg. Width in microns
	Variety Cheyenne		
0	5	68.00	85.00
2	3	166.00	102.00
4	5	153.00	170.00
6	4	143.00	98.00
8	3	136.20	119.00
	Variety Ponca		
0	5	68.00	85.00
2	4	74.80	102.00
4	4	85.00	112.20
6	3	85.00	136.00
8	4	68.00	40.80

(Magnification x 430)

leaf gaps intervened and there was no visible connection between the longitudinal procambial columns. When the distances of first visible procambium (that not continuous with a visible leaf primordium) in mid-longitudinal section from the tip of the apex were measured and plotted against days drought the curves shown in figure 21 were derived for the two varieties of wheat. The latter figure shows that in control apices the distance between the tip of the apex and the first visible procambial cells was greater in variety Cheyenne than in variety Ponca. After the plants were subjected to drought there was a decrease in this distance in the shoot tips of both varieties. As the period of drought exposure was increased there was a concomitant decrease in the distance in variety Cheyenne. Wheat variety Ponca showed a decrease in the distance through the fourth day of drought, but after the sixth and eighth days of drought there was a marked increase in the distance. Measurements on apices after the eighth day of drought showed that the distance between the tip of the apex and the procambium was greater than the comparable measurement in the shoot tips of control plants.

It is significant to note that in general in the larger apices the distance between the tip of the apex and the procambium was less than that in the smaller apices. This observation may suggest that the distances of the procambium are relative to the sizes of the apices. This explanation however, does not account for the marked difference between Cheyenne and Ponca controls, nor does it account for the decrease in distance with the decrease in size of 8 day droughted apices of variety Cheyenne; and the increase in distance of 6 and 8 day droughted apices of variety Ponca.

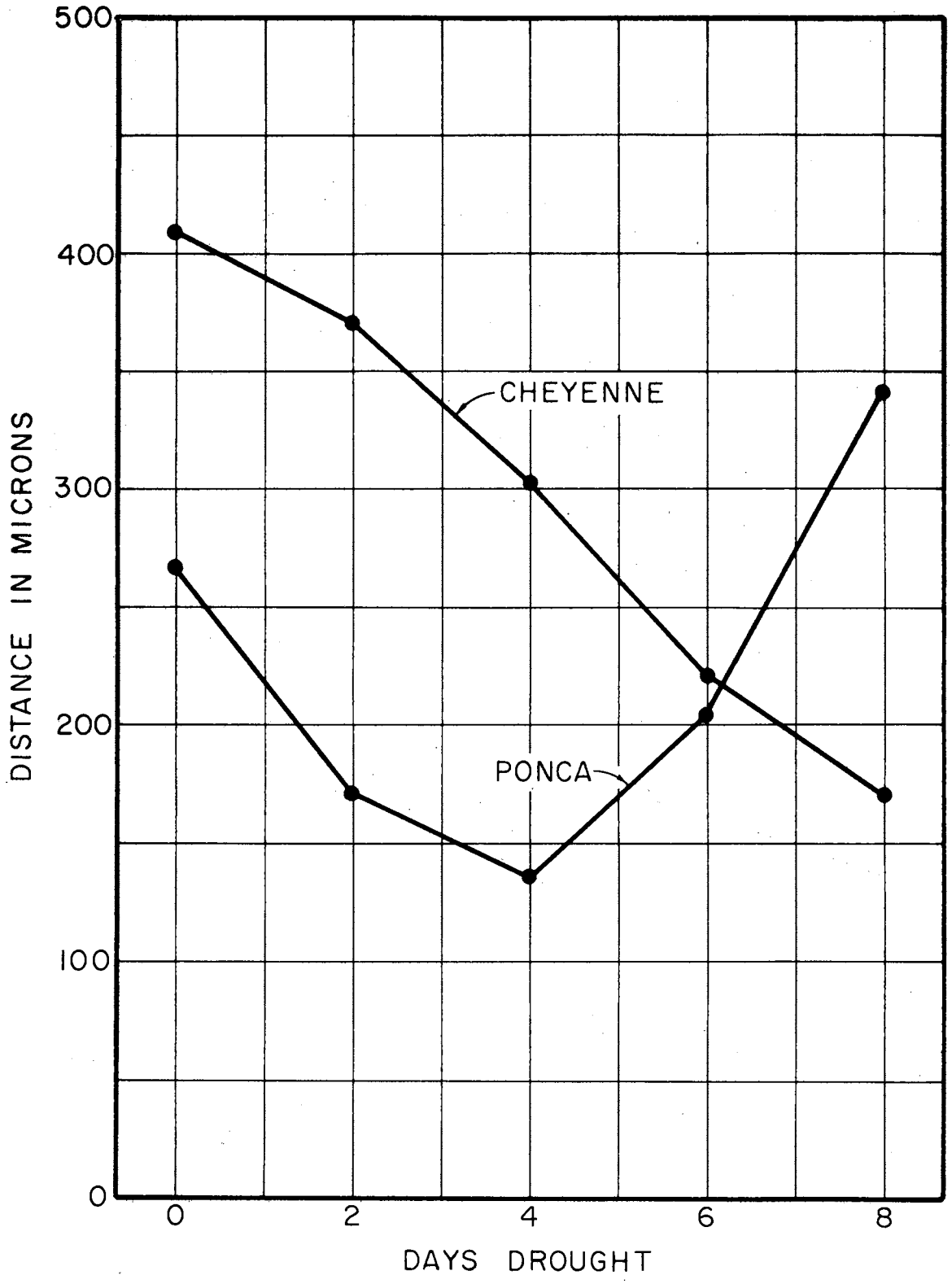
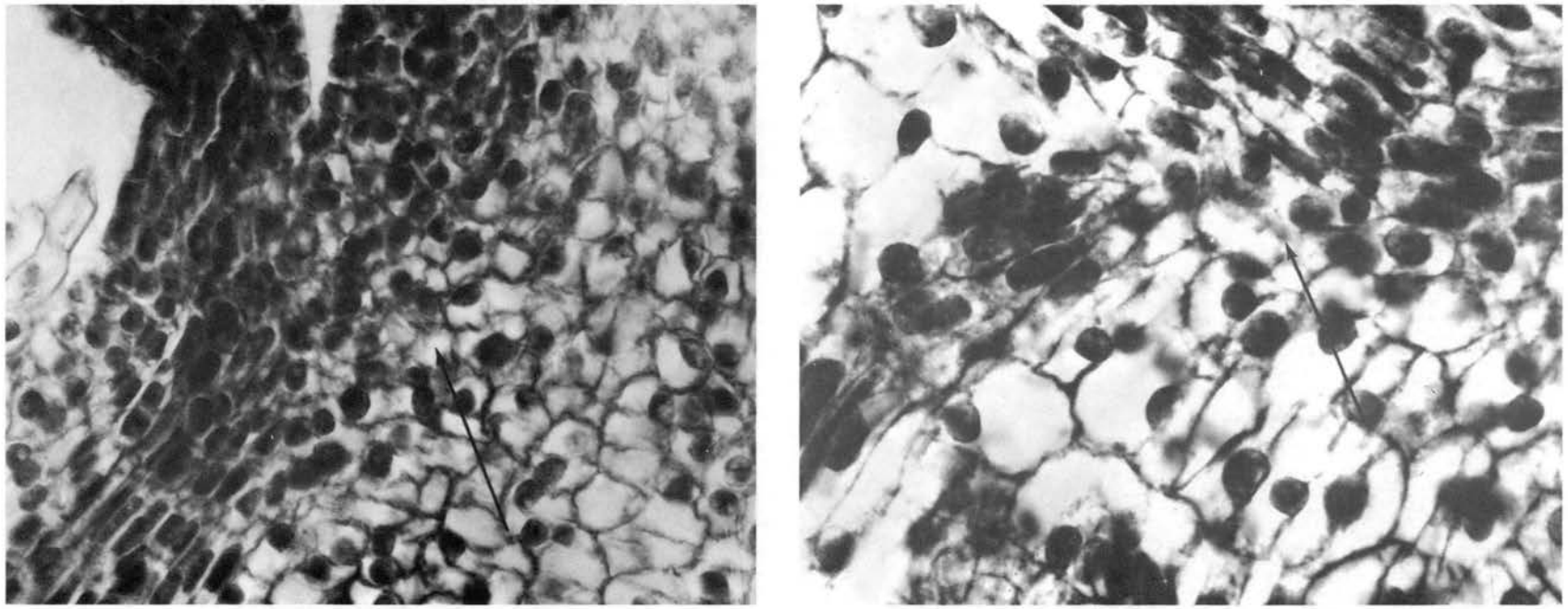


Figure 21. Mean distance between the tip of the apex and differentiated procambium in median longitudinal section in vegetative shoot tips of 28 day old wheat plants. (X 430).

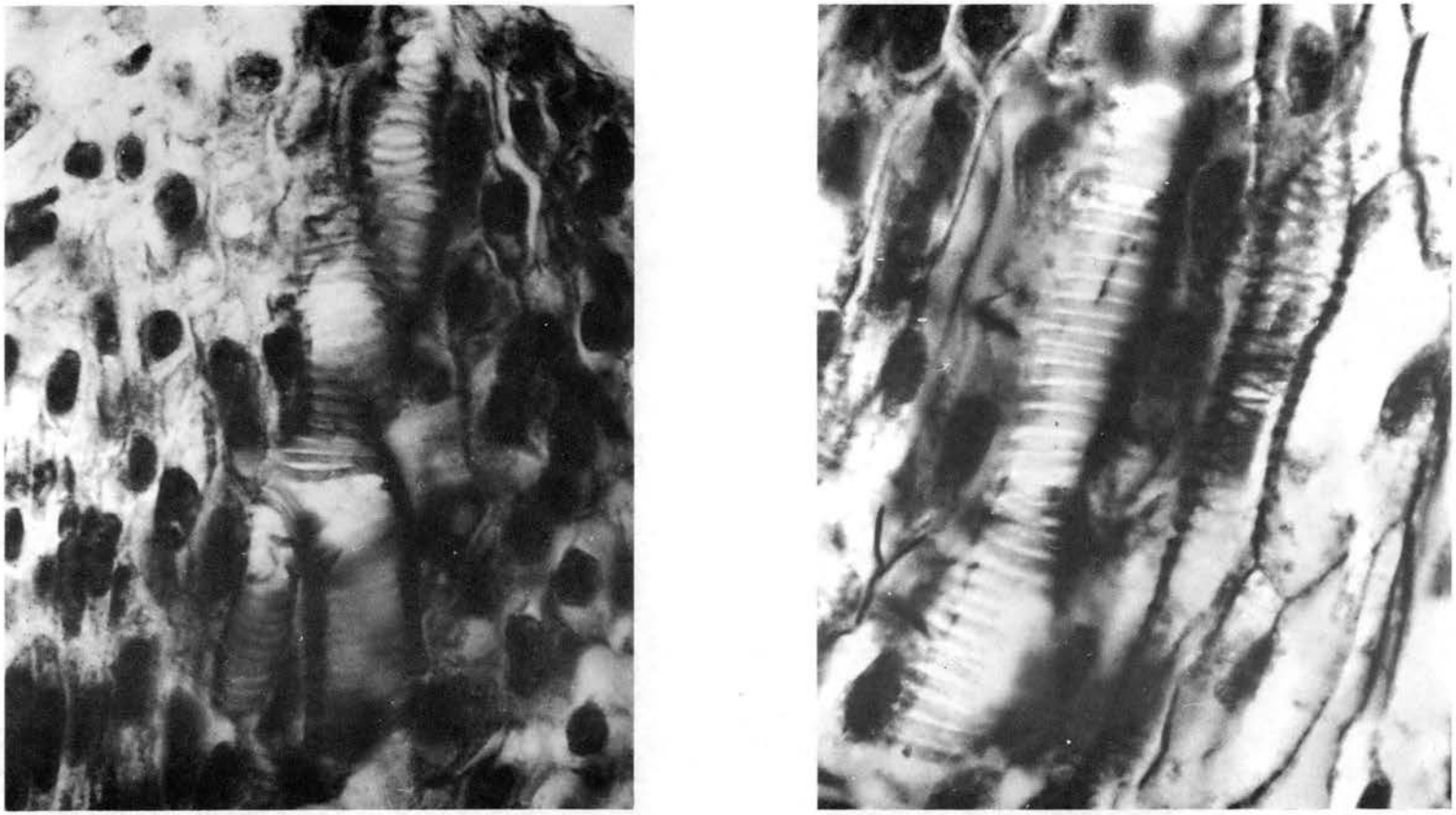
Differentiated xylem could be detected at varying levels from the tip of the apex in the different shoot tips that were examined. In some, the xylem was continuous with that of the lower node and measurements or descriptions were not recorded since the interest here is in the inception of xylem above the last differentiated node. In cases where there was an obvious discontinuity between the lower and upper xylem tissue an attempt was made to ascertain the point of inception of the xylem by defining the most acropetally differentiated element, or by tracing the differentiated elements to a particular leaf trace or leaf primordium.

With few exceptions in all of the apices examined the visible tracheary elements were traceable to the lower node or to a particular leaf. The summary of one such tracing is shown in figures 22 and 23. The leaf gap is depicted at the arrow 14 microns from the mid-longitudinal section (fig. 22, A). Twenty one microns lateral to the mid-line (fig. 22, B), the arrow indicates the trace continuous into the leaf. Two rows of annular protoxylem can be traced to the leaf insertion, 119 microns from the mid-longitudinal plane (fig. 23A), and in figure 23, B, 1 row of annular, and 2 rows of helical xylem can be seen 493 microns down from the insertion of the leaf. Xylem differentiation is apparently occurring in basipetal direction from the base of the developing leaf, and in acropetal direction from lower portions of the stem. The more mature xylem shown in figure 23-B was located 238 microns down from the insertion of the older leaf below, and is thus described as being acropetally differentiated from the lower node. The discontinuation which was apparent at 20 μ lateral to the mid-longitudinal section then, was due to the intervening leaf



A B

Figure 22. Longisections cut parallel to plane of leaf blade through 4 week old Cheyenne apex after subjection to 4 days drought. A, leaf gap shown at the arrow 14 μ from mid-longitudinal section. B, the arrow indicates the trace continuous into the leaf at 21 μ from the mid-longitudinal plane. (X125).



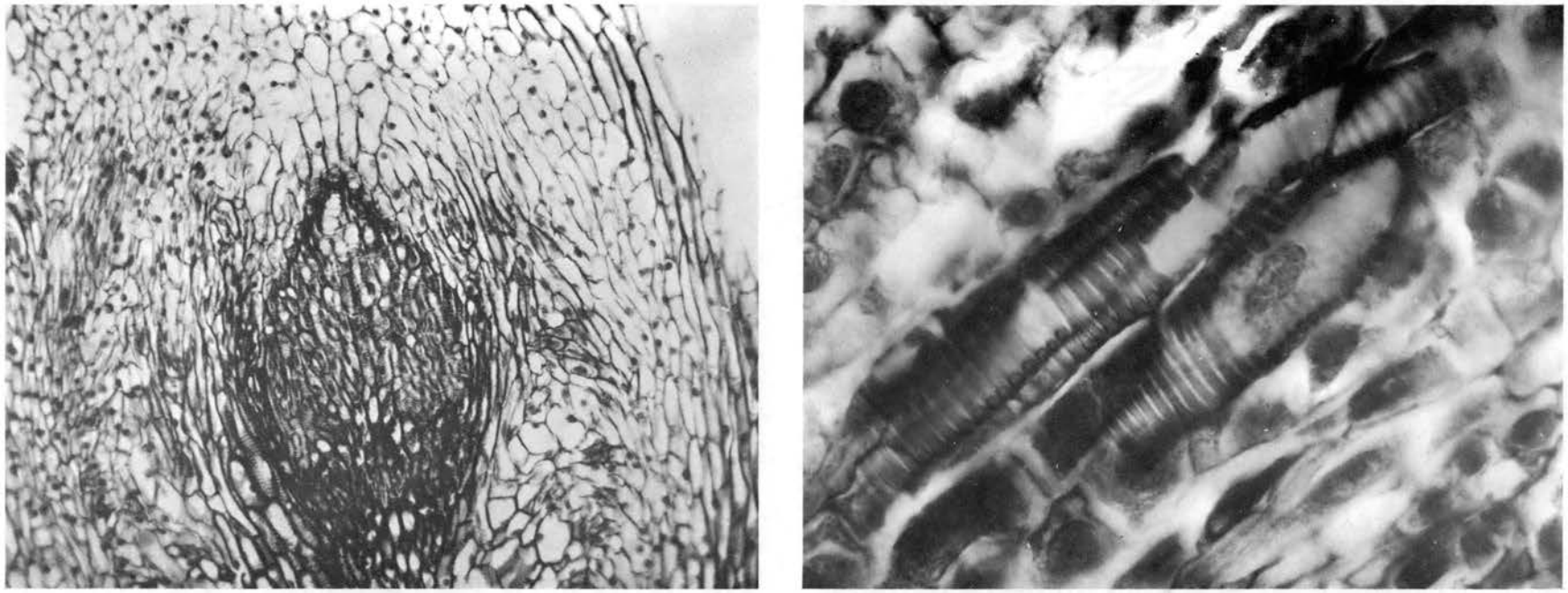
A B

Figure 23. Longisections 119 u from A in figure 22. A, two rows of annular protoxylem traced to leaf insertion. B, 1 row of annular and 2 rows of helical xylem can be seen 493 u down from leaf insertion (differentiating acropetally). (X356).

gap rather than to a lack of differentiated tissue at that level in the stem. A section from this same shoot tip (fig. 24, A), shows a longitudinal section through the most apically differentiated node 420 μ lateral to midline. Many of the numerous xylem elements seen in this view were directly traceable to younger leaves in more median planes. This pattern of extensive xylem differentiation in the most apically differentiated nodal area was common to all the shoot tips examined. Annular tracheary elements which appear to be differentiating without basipetal or acropetal connection are shown in figure 24, B. Adjacent sections revealed no connecting elements that were traceable to particular leaves. The apex from which figure 24-B was made was a 2 day droughted plant of variety Ponca. This illustration of apparent isolated differentiation of xylem is again illustrated in figure 25-A, which shows an annular element at the adaxial base of a leaf primordium in an apex of variety Cheyenne after subjection to 4 days drought. Such instances of isolated xylem were rarely encountered and did not appear to be correlated with the treatment to which the plants were subjected. Figure 25-B shows annular xylem differentiating acropetally through the sub-apical region from the node below in a 4 day droughted specimen of variety Cheyenne. This apparent wave of xylem differentiation from the lower node was common to all of the apices studied.

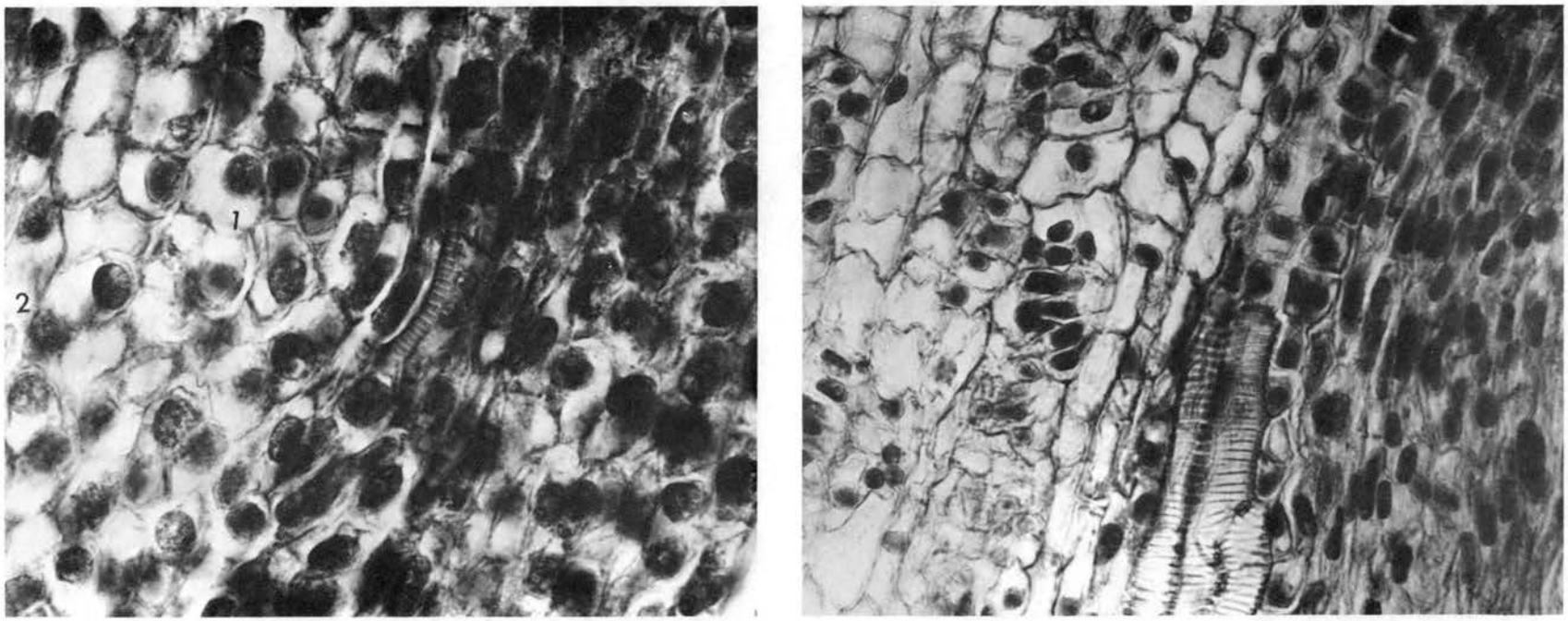
In apices from drought subjected plants some cell walls were thickened, (fig. 26, A-B). In the sub-apical region wall thickness between 3 adjacent cells measured as much as 5.8 μ (X450), and lignin specific stain was shown to be retained in isolated areas, (fig. 27, A-B).

There were no distinctive patterns of anatomical variation that



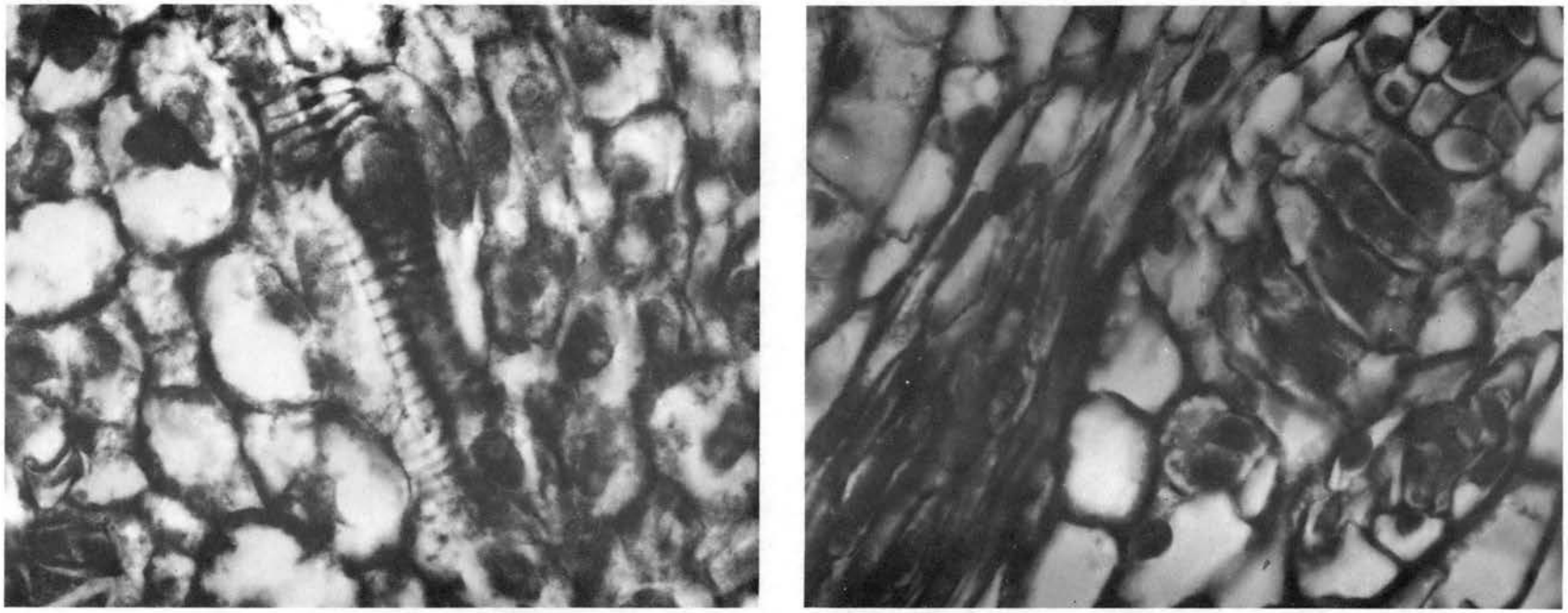
A B

Figure 24. A, Longisection through the most apically differentiated node 420 μ lateral to mid-line from a 4 week old Cheyenne wheat plant after subjection to 4 days drought. Some of the xylem elements were traceable to younger leaves. (X125). B, Longisection from an apex of a 4 week old Ponca wheat plant after subjection to 2 days drought. Xylem elements in sub-apical region showing no basipetal or acropetal connection. (X594).



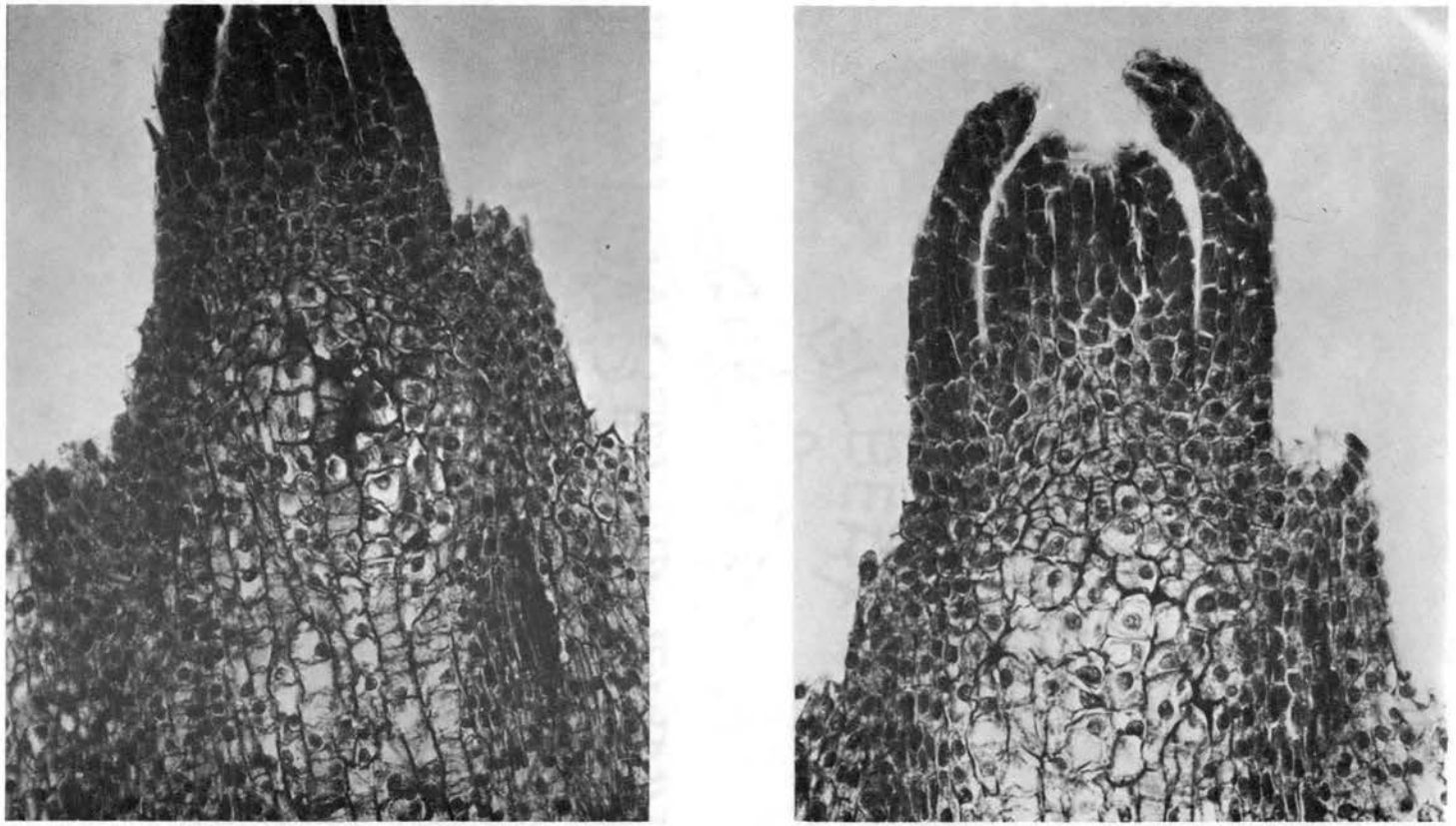
A B

Figure 25. Longisection cut parallel to the plane of the leaf blade from an apex of wheat variety Cheyenne after subjection to 4 days drought. A, arrow shows annular xylem element at adaxial base of leaf, (1) origin of youngest leaf primordium, (2) sub-apical vacuolated area. B, acropetally differentiating annular xylem 253 μ down from the most distal portion of vacuolated area. (X356).



A B

Figure 26. Longisections through apices of 8 day droughted wheat plants. A, Cheyenne; B, Ponca. View of area 310 μ down from apex tip showing thickened cell walls, pitted wall development in provascular region, and xs of leaf trace. (X594).



A B

Figure 27. Mid-longisections cut parallel to the plane of the leaf blade from apices of 4 week old wheat plants after subjection to 8 days drought. A, Cheyenne; B, Ponca. Note thickened cell walls and retention of lignin specific stain shown as black areas between cells. (X356).

showed differentiation of the stress effects between the two varieties of wheat studied.

At the site of axillary bud differentiation it was difficult to discern the direction of procambial differentiation when the traces appeared continuous with main axis traces. When there was a gap between main axis traces and the axillary bud, the tissue beneath the bud appeared vacuolated and parenchymatic (fig. 28).

Discussion

The anatomical descriptions of shoot tip organization in wheat varieties Cheyenne and Ponca reveal no outstanding distinction in pattern between the growing points of the two plant forms. Although examination of the shoot tip of young germinating seedlings showed that variety Cheyenne grew at a more rapid rate than did variety Ponca, by the time the seedlings were established in 4 weeks of vegetative growth the average size of the stem apices of both varieties was the same. At this age the shoot apices were characteristic of the first or second stages of organogenesis of the wheat apex as described for the vernalized wheat seedling (Opatrna, et al., 1963). The latter authors indicated that the chronological age of the plants they characterized as being in stage one was less than 5 days after sowing. The second stage of organogenesis extended from 5 days through 15 days after sowing and the third stage was depicted at 20 to 25 days after sowing.

It is significant to note that in this study the apices of control plants were comparable to those of plants of much younger chronological age in the work of the former investigators. The apices from droughted plants on the other hand, though of the same 28 day chronological age

Figure 28. Axillary bud shown in mid-longisection from the apex of a 4 week old wheat plant of variety Cheyenne after subjection to 2 days drought. (X356).

as the controls showed organization comparable to that of apices from plants 9, 15, and 20 days older than the vernalized seedlings that exhibited characteristics of the first stage of organogenesis.

The latter observation seems to suggest that within the 8 day period of drought exposure the apices of treated plants "aged" to varying degrees. It has been suggested that in shoot growth studies the best measurement of physiological age could be found in the constant succession of leaves and internodes (Dormer, 1965). The term "plastochron" has long been used as the scale interval for the development of successive leaves. The former author however, discredits what he calls the "chronological plastochron" as a true measure of physiological age, and suggests the use of the "correlative plastochron", which represents the amount by which one consequence lags behind the other.

When changes in the leaf were expressed in regard to plastochrons, it was shown that values such as dry weight, oxygen uptake, and chlorophyll synthesis of individual leaves were consistent (Michelini, 1958). The changes in form of the apex during a plastochron has been shown to be related to the development of the respective leaves.

Further inspection of table X reveals an interesting point. Since the control apices are in minimal area phase, it can be assumed that the initiation of the last leaf has just been completed, (Kaufman, 1959). The apices of the experimental plants then have apparently been stimulated to a spurt of growth. In variety Cheyenne, after 2 and 4 days of drought treatment the apices are evidently still in the first stages of leaf initiation. After the sixth day of drought, the apices appear to have the minimal area form, but they are still larger than

the controls. Stimulated growth activity, triggered by decreased water supply in some parts of the plant body would cause an increase in numbers of cells in the apex (this was evidenced in anatomical examination, particularly in the region of the corpus), and an increase in size of some already exisiting cells (also evidenced in large vacuolated cells of the ground meristem).

In the event that an equal growth in all areas of the tunica prevented the manifestation of the leaf primordia, there would then occur a lag in the plastochronic development. Such a lag period would obviously result in the "waiting" cells taking on characteristics of more mature cells, and generally becoming more differentiated. Apices from experimental plants did show this trait of greater cellular maturity and differentiation than did those of control plants. A point of particular interest is the relationship of the drought period and the location of procambium. When we consider Wardlaw's (1965) description of the basipetal differentiation of prevascular tissue it becomes apparent that the cells of the ground meristem region located subjacent to the corpus when stimulated to premature differentiation become parenchymatous. Parenchymatization then being followed by a blocking out of the prevascular procambium. One of the results of this premature differentiation of ground meristem then would be a decrease in distance from the tip of the apex to incipient vascular tissue. Further consideration of this point seems to suggest that if the stimulus for prevascular inception is received in a polar direction from the apex or leaf primordia, arrested development or lag in the plastochron may permit the differentiation of the ground meristem to proceed to a point beyond which the region was capable of forming

procambial tissue. Such an extreme effect would result in an increase in distance between the tip and the procambial cells. This explanation may account for the curve for variety Ponca (fig. 21).

No evidence has been presented here for a stimulation for either an increase or decrease in fully differentiated xylem tissue in the apex or sub-apical region of the vegetative shoot tip of wheat varieties Cheyenne or Ponca. The reported effect on procambial inception and development however, suggests that an examination of cellular organization at the electron microscope level would enable one to observe some of the earliest manifestations of drought effects on xylem differentiation.

Conclusions

Wheat plants of Triticum aesitivum, L., varieties Cheyenne and Ponca showed accelerated tissue differentiation in the shoot tips after subjection to drought stress. There was no apparent difference in response between the two varieties. At the light microscope level the amount and pattern of xylem differentiation was not altered.

CHAPTER VI

SUMMARY

The anatomical patterns of second leaves of 4-week old wheat seedlings, Triticum aestivum, L., varieties Cheyenne and Ponca were changed after the plants were subjected to 2, 4, 6, and 8 days of internal water stress. Anatomical variations were (1) thickened cell walls in all tissues, (2) plasmolysis of nuclei and plastids, (3) increased amounts of sclerenchyma and xylem tissue, and (4) apparent increased deposition of lignin. The observed changes were progressive with increased drought exposure and the severity of response was greatest in variety Ponca.

Of ten closely related varieties of winter wheat, T. aestivum, L., subjected to a screening test suggested by Todd and Webster (1965), hard red types and soft red types could not be separated into two respective groups on the basis of drought survival ability when grown under controlled environmental conditions. Statistical analysis of the survival data permitted the ranking of the ten varieties of wheat according to relative survival ability. When the second leaves of the plants were measured for length, percent relative turgidity, and peroxidase activity, statistical analysis of the data showed significant differences among the varieties of wheat. There was no evidence for a correlation between relative survival ability of the original plant population and the percent relative turgidity of surviving plants for

the ten varieties of wheat at the end of 8 weekly cycles of drought. Peroxidase activity showed a marked increase after stress when calculated on a per mg. protein basis. Plants subjected to one week of drought when at 3 different levels of development showed marked differences in second leaf percent relative turgidity, growth, and peroxidase activity. Statistical analysis of the data indicated significant interaction between treatment and stage of development for the various varieties. It was postulated that differential adaptive mechanisms among the three stages of growth accounted in part for the different responses.

The shoot tips of drought hardy Cheyenne and drought susceptible Ponca showed anatomical changes after 3-week old seedlings were subjected to 2, 4, 6, and 8 days drought. Anatomical changes included (1) thickened cell walls, (2) loss of protoplasts in cells of the sub-apical region, (3) plasmolysis, (4) heavy lignin deposits in isolated regions of the sub-apical region, and (5) increased lateral bud formation. There was an initial increase in overall size of the apices, but as the drought period was extended there was a decrease in overall apex size. There was no apparent difference in the effect between the two varieties of wheat.

Procambial differentiation was closer to the tip of the apex in droughted plants than in controls of variety Cheyenne. In variety Ponca after 2 and 4 days drought the procambium was closer to the tip of the apex than in controls, but after 6 days of drought the distance had increased. After 8 days drought the distance increased beyond that observed in the controls. There was no evidence at the light microscope level that xylem differentiation in the sub-apical region of the shoot

tip either decreased or increased in either of the varieties of wheat tested.

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APPENDIX

TABLE XI

ANALYSIS OF VARIANCE FOR "DATA" OF TABLE I AND FIGURE 4

Source	df	MS
Trials	1	18.212
Replicates	5	2.365
Treatment	1	15864.165*
Variety	9	168.380*
Week	7	1406.231*
Trial x replicate	5	2.330
Trial x treatment	1	18.256
Trial x variety	9	5.368
Trial x week	7	10.538
Replicate x treatment	5	2.380
Replicate x variety	45	0.928
Replicate x week	35	0.772
Treatment x variety	9	168.389*
Treatment x week	7	1406.244
Variety x week	63	8.574*
Trial x replicate x treatment	5	2.323
Trial x replicate x variety	45	1.041
Trial x replicate x week	35	0.796
Trial x treatment x variety	9	5.363
Trial x treatment x week	7	10.531
Trial x variety x week	63	1.330
Replicate x treatment x variety	45	0.926
Replicate x treatment x week	35	0.769
Replicate x variety x week	315	0.378
Treatment x variety x week	63	8.572*
Trial x replicate x treatment x variety	45	1.041
Trial x replicate x treatment x week	35	0.797
Trial x replicate x variety x week	315	0.338
Trial x treatment x variety x week	63	1.331
Replicate x treatment x variety x week	315	0.378
Residual	315	0.338
Total	1919	

* F value significant at the 5% level of confidence

TABLE XII

ANALYSIS OF VARIANCE FOR "DATA" OF TABLE II

Source	df	Mean Squares			
		% Relative Turgidity	mg Protein per gm dw	Peroxidase Act./mg dw	Peroxidase Act./mg Protein
Replicate	1	6.724	0.130	0.001	0.876
Treatment	1	32.256**	2557.904*	2.602*	1881.186*
Variety	9	102.621*	284.163*	1.757	51.066*
Trial x treatment	1	0.338	1.865	0.001	1.963
Replicate x variety	9	8.717	5.072	0.000	5.168
Treatment x variety	9	27.081*	221.543*	0.394*	23.345*
Trial x variety x treatment	9	2.870	4.070	0.001	4.836
Total	39				

* F value significant at the 5% level of confidence
** F value significant at the 1% level of confidence

TABLE XIII

ANALYSIS OF VARIANCE FOR "DATA OF TABLE XV AND FIGURE 9

Source	df	MS
Replicate	1	160.402
Treatment	1	1402.126**
Variety	9	84.362*
Leaf	9	4.306
Replicate x treatment	1	15.643
Replicate x variety	9	7.328
Replicate x leaf	9	5.289
Treatment x variety	9	12.932
Treatment x leaf	9	4.247
Variety x leaf	81	3.457
Replicate x treatment x variety	9	15.237
Replicate x treatment x leaf	9	3.537
Replicate x variety x leaf	81	5.364
Treatment x variety x leaf	81	4.046
Replicate x treatment x variety x leaf	81	3.677
TOTAL	399	

* F value significant at the 5% level of confidence
** F value significant at the 1% level of confidence

TABLE XIV

ANALYSIS OF VARIANCE FOR "DATA" OF TABLE IX AND FIGURE 14

Source	df	MS
Replicate	1	103.194
Stage	2	863.253*
Treatment	1	43460.660*
Variety	9	185.964*
Leaf	9	3.592
Trial x stage	2	10.746
Trial x treatment	1	7.666
Trial x variety	9	2.991
Trial x leaf	9	10.964
Stage x treatment	2	86.358*
Stage x variety	18	35.219*
Stage x leaf	18	4.141
Treatment x variety	9	158.577
Treatment x leaf	9	3.777
Variety x leaf	81	3.109
Replicate x stage x treatment	2	1.296
Replicate x stage x variety	18	1.745
Replicate x stage x leaf	18	4.423
Replicate x treatment x variety	9	0.405
Replicate x treatment x leaf	9	3.431
Replicate x variety x leaf	81	3.204
Stage x treatment x variety	18	11.665*
Stage x treatment x leaf	18	1.377
Stage x variety x leaf	162	2.910
Treatment x variety x leaf	81	3.306
Replicate x stage x treatment x variety	18	2.608
Replicate x stage x treatment x leaf	18	2.151
Replicate x stage x variety x leaf	162	3.471
Replicate x treatment x variety x leaf	81	3.771
Stage x treatment x variety x leaf	162	3.633
Residual	162	2.872
Total	1199	

* F value significant at the 5% level of confidence

TABLE XV

MEAN LEAF LENGTH IN CENTIMETERS OF SECOND LEAVES OF CONTROL AND EXPERIMENTAL PLANTS AFTER EIGHT WEEKLY DROUGHT CYCLES

Variety	Control	Treated
Cheyenne	22.00	19.70
Kanking	21.00	20.00
Ponca	20.50	16.00
Red Chief	19.85	14.75
Dual	22.35	18.40
Leapland	23.00	19.90
Pennol	22.25	15.25
Red Coat	19.60	13.35
Seneca	20.65	19.15
Thorne	25.20	17.45

TABLE XVI

AVERAGE NUMBER OF PLANTS OF TWENTY FOR SIX FLATS WHICH SURVIVED WEEKLY DROUGHT CYCLES

Variety	Drought Cycle							
	1	2	3	4	5	6	7	8
Cheyenne	20.00	19.17	17.92	16.17	15.75	11.58	8.92	5.83
Kanking	20.00	20.00	18.58	16.33	15.58	12.25	10.25	7.92
Ponca	20.00	17.75	15.58	11.92	9.42	6.08	3.42	1.83
Red Chief	20.00	20.00	19.50	17.92	17.50	14.75	10.25	8.33
Dual	20.00	19.08	18.42	16.25	15.42	12.08	10.83	8.67
Leapland	20.00	18.17	17.08	14.67	13.00	9.25	8.00	6.25
Pennol	20.00	18.25	17.42	14.92	12.17	8.08	7.08	5.33
Red Coat	20.00	18.42	16.58	13.83	12.00	9.08	6.33	3.92
Seneca	20.00	19.50	18.33	16.83	15.58	12.92	11.00	8.50
Thorne	20.00	20.00	19.50	19.00	17.25	15.00	13.75	11.83

Twenty of twenty plants which served as control survived throught the duration of the experiment

TABLE XVII

AVERAGE VALUES FOR MEASUREMENTS OF SOLUBLE PROTEIN AND SUPERNATANT PEROXIDASE ACTIVITY PER FOUR LEAVES FROM WHEAT PLANTS AFTER EIGHT WEEKLY CYCLES OF DROUGHT

	mg Fresh Weight		mg Dry Weight		mg Protein		*Peroxidase Activity	
Variety	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Cheyenne	242.5	193.5	30.50	27.41	1.60	0.80	1.30	1.18
Kanking	291.0	236.0	43.12	32.38	1.83	0.95	1.49	1.24
Ponca	286.5	201.0	36.39	32.96	2.00	0.73	1.47	1.13
Red Chief	248.5	224.5	40.82	31.80	2.00	1.25	1.28	1.27
Dual	374.0	223.0	55.09	33.95	2.18	1.25	1.24	1.39
Leapland	337.5	282.0	51.74	40.46	2.05	1.05	1.28	1.35
Pennol	250.5	186.0	34.44	24.50	2.00	1.08	1.38	1.44
Red Coat	224.5	203.0	42.86	34.13	2.13	0.88	1.28	1.20
Seneca	283.5	237.5	39.85	32.74	2.05	1.20	1.12	1.21
Thorne	334.0	263.5	43.96	32.65	1.30	1.15	1.22	1.43

* Peroxidase activity as optical absorbance at 420 mμ per 0.25 ml of original 2.00 ml of supernatant

TABLE XVIII

AVERAGE VALUES FOR MEASUREMENTS OF SOLUBLE PROTEIN AND SUPERNATANT PEROXIDASE ACTIVITY PER FOUR LEAVES FROM *STAGE ONE WHEAT PLANTS AFTER ONE WEEK OF DROUGHT

	mg Fresh Weight		mg Dry Weight		mg Protein		**Peroxidase Activity	
Variety	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Cheyenne	197.5	46.50	56.16	17.85	2.20	0.92	0.56	0.21
Kanking	208.5	48.00	44.13	19.81	4.20	1.05	0.42	0.09
Ponca	197.5	52.00	52.77	17.67	2.36	0.67	0.81	0.41
Red Chief	245.0	63.50	25.41	21.52	2.50	0.64	0.76	0.38
Dual	307.0	42.50	77.82	29.30	3.23	0.65	0.65	0.21
Leapland	312.0	90.00	62.88	30.80	2.43	1.05	0.91	0.61
Pennol	280.0	57.00	53.78	24.67	2.08	0.43	0.98	0.41
Red Coat	231.0	93.00	46.71	41.96	1.33	0.80	0.71	0.58
Seneca	240.5	39.50	59.95	13.89	1.23	0.60	1.02	0.46
Thorne	306.0	74.00	22.41	30.38	2.43	0.80	1.00	0.74

* Stage one defined in procedures

** Peroxidase activity as optical absorbance at 420 mμ per 0.25 ml of original 2.00 ml of supernatant

TABLE XIX

AVERAGE VALUES FOR MEASUREMENTS OF SOLUBLE PROTEIN AND SUPERNATANT PEROXIDASE ACTIVITY PER FOUR LEAVES FROM *STAGE TWO WHEAT PLANTS AFTER ONE WEEK OF DROUGHT

	mg Fresh Weight		mg Dry Weight		mg Protein		**Peroxidase Activity	
Variety	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Cheyenne	183.5	69.50	37.71	28.28	0.95	1.15	0.69	0.42
Kanking	191.0	61.00	47.12	26.65	1.87	0.64	0.44	0.39
Ponca	265.0	42.50	68.97	17.99	1.50	0.42	1.06	0.30
Red Chief	180.0	28.00	31.88	17.52	0.75	0.25	0.51	0.30
Dual	276.0	36.00	76.78	27.37	1.43	0.25	0.81	0.21
Leapland	327.5	68.00	70.11	25.83	1.88	0.54	1.16	0.41
Pennol	252.0	41.00	58.67	17.09	0.80	0.25	0.96	0.41
Red Coat	219.5	102.50	45.80	47.63	1.03	0.25	0.90	0.55
Seneca	306.0	57.50	74.99	21.03	1.20	0.33	1.08	0.41
Thorne	355.5	48.50	56.82	22.00	1.73	0.35	1.20	0.49

* Stage two defined in procedures

** Peroxidase activity as optical absorbance at 420 mμ per 0.25 ml of original 2.00 ml of supernatant

TABLE XX

AVERAGE VALUES FOR MEASUREMENTS OF SOLUBLE PROTEIN AND SUPERNATANT PEROXIDASE ACTIVITY PER FOUR LEAVES FROM *STAGE THREE WHEAT PLANTS AFTER ONE WEEK OF DROUGHT

	mg Fresh Weight		mg Dry Weight		mg Protein		**Peroxidase Activity	
Variety	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Cheyenne	182.5	38.00	43.38	15.18	1.37	0.55	0.65	0.20
Kanking	180.5	26.00	44.57	13.69	1.42	0.65	0.39	0.19
Ponca	208.5	32.00	56.37	16.43	1.00	0.25	0.78	0.29
Red Chief	179.5	26.00	43.02	9.11	2.00	0.32	0.75	0.16
Dual	313.5	24.00	83.15	17.82	2.05	0.25	0.86	0.26
Leapland	222.0	185.00	52.92	8.34	1.78	0.43	0.76	0.20
Pennol	321.0	25.50	87.07	14.00	2.73	0.47	1.28	0.22
Red Coat	258.0	70.00	58.32	36.65	1.68	0.45	0.97	0.49
Seneca	215.5	43.00	58.55	17.46	3.65	0.25	0.59	0.35
Thorne	332.0	32.00	67.54	14.57	4.35	0.23	1.11	0.41

* Stage three defined in procedures

** Peroxidase activity as optical absorbance at 420 mμ per 0.25 ml of original 2.00 ml supernatant

VITA

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Doctor of Philosophy

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