HIGH TEMPERATURE LIMITATION FOR ENZYMATIC SUGAR CONVERSION IN WHEAT KERNELS

By

NIELS OLSEN MANESS

Bachelor of Science
Texas A&M University
College Station, Texas
1980

Master of Science
Texas A&M University
College Station, Texas
1983

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 1987

HIGH TEMPERATURE LIMITATION FOR

ENZYMATIC SUGAR CONVERSION

IN WHEAT KERNELS

Thesis Approved:

Richard C. Johnson
Thesis Adviser

Eugene A. Krenger, Jr.

Carl D. Michel

Eddie Basler

Norman N. Durham
Dean of the Graduate College

PREFACE

The high temperature restriction for starch biosynthesis from sucrose in wheat kernels identified in the following chapters is probably regulated within the kernel itself. The exact nature of the synthetic restriction for kernel growth imposed by high temperature was not identified. Further work is needed to identify the rate limiting enzyme or group of enzymes in the starch biosynthetic pathway. The mechanism regulating a synthetic restriction could then be identified and possibly utilized for screening the kernel starch synthetic efficiency of cultivars. Some degree of high temperature tolerance is known to exist in selected wheat cultivars. Selection for starch synthetic efficiency in kernels could provide a novel strategy for wheat yield improvement or yield stability in environments where wheat yield is limited by high temperature.

Sincere appreciation is extended to members of my graduate advisory committee for their guidance and assistance during the course of this work. Dr. Richard Johnson, as thesis adviser, provided patient understanding and guidance over the course of the studies. His confidence in myself to direct the research reported in this thesis was invaluable. Dr. Eugene Krenzer

provided the CR7 data logger used in the field study and served as soils representative of the committee. Dr. Eddie Basler served as major adviser for a time and offered many helpful suggestions during the studies. Dr. Earl Mitchell advised me on biochemical aspects of the study and served as a departmental representative outside the Agronomy Department.

The wit and technical assistance of Ms. Linda Brown, Mr. Dave Ferris, Ms. Hirut Kebede, Dr. Jim Heitholt and Mr. Glen (Lefty) Hartman is deeply appreciated. Appreciation is also extended to Dr. Ron McNew for statistical guidance.

I would especially like to express my appreciation to my wife, Nancy, for assisting during her offhours with lab work, for typing and correcting this thesis and for taking time between these chores to have our first child, George. Her understanding and encouragement is forever appreciated. Also, the timeless and undying devotion of Brandy and Kitty has been inspiring.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

HIGH TEMPERATURE LIMITATION FOR ENZYMATIC SUGAR CONVERSION IN WHEAT KERNELS

Running Head: Heat Limitation for Sugar Metabolism in Wheat Kernels.

N.O. Maness
Department of Biochemistry
Oklahoma State University
Stillwater, Oklahoma 74078
Telephone: 405-624-6197

High Temperature Limitation for Enzymatic Sugar Conversion in Wheat Kernels[1].

Manuscript Received: _____

Revised Manuscript Received: _____

N.O. Maness[2], R.C. Johnson and L.I. Croy

Department of Agronomy, Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma 74078

FOOTNOTES:

[1] Research was supported by the Oklahoma Agricultural Experiment Station, Oklahoma State University, Journal paper no. of the Oklahoma Agricultural Experiment Station.

[2] Present address: Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma 74078.

[3] Abbreviations: ADPG, adenosine diphosphoglucose; UDPG, uridine diphosphoglucose; PPFD, photosynthetic photon flux density; DTT, dithiothreitol; PMSF, phenylmethyl-sulfonyl flouride; fw, fresh weight; ODW, oven dry weight.

ABSTRACT

The high temperature restriction for enzymatic conversion of sucrose to starch was characterized in wheat (Triticum aestivum L. cv TAM W-101) kernels. Temperature treatments of 23 C and 35 C were imposed on isolated intact wheat spikes from anthesis to maturity. The activity of four enzymes required for conversion of sucrose to starch was monitored at five day intervals from 10 d to 25 d after anthesis. Sucrose, glucose, fructose and starch contents of kernels were also determined at these growth stages and at maturity. Kernel fresh and dry weight was significantly increased by high temperatures 10 d after anthesis. At this growth stage, endosperm synthetic capacity, as determined by measuring the activity of UDP sucrose synthase, UDP and ADP glucose pyrophosphorylase, was not inhibited in heated kernels. Later in kernel development, however, the rate of kernel filling and of starch accumulation was significantly depressed in kernels growing at high temperatures. The activity of UDP sucrose synthase and both pyrophosphorylase enzymes declined linearly from 15 d to 25 d after anthesis. Their rate of decline at 35 C was significantly greater than at 23 C. Our data indicates

that the ability to provide metabolites required for starch synthesis in wheat endosperm is restricted by high temperature, limiting the ability of endosperm cells to provide metabolites required for starch synthesis and leading to a lower mature kernel dry weight. Since temperature treatments were isolated on spikes only, the decreased synthetic for starch accumulation due to high temperature at 15 to 25 d after anthesis appeared to be regulated within the kernel or spike itself.

INTRODUCTION

Starch accumulation in wheat endosperm is limited by temperatures exceeding 25 C to 30 C (19). Studies utilizing solution cultured spikes of wheat (2) and *in vitro* investigations of kernel development in maize (12) provide evidence that synthetic processes of the caryopsis are restricted more than sucrose supply during high temperature exposure. It is therefore becoming increasingly evident that sucrose utilization rather than sucrose supply plays an important role, perhaps even a dominant role, in regulating starch accumulation when kernels are exposed to temperatures which restrict their growth.

Sucrose enters wheat endosperm cells from the endosperm apoplast by the process of facilitated diffusion involving saturable carriers (9). A decreasing sucrose gradient from the endosperm apoplast and across the plasmalemma of endosperm cells is maintained by sucrose cleavage in the cytoplasm of the cell (7). Two enzymes are responsible for sucrose cleavage during kernel development in wheat. UDP sucrose synthase is apparently responsible for sucrose cleavage inside endosperm cells, while invertase activity is largely restricted to the

outer pericarp of the kernel (5). Invertase appears to be responsible for sucrose cleavage in pericarp tissues at all stages of development and may mediate starch synthesis in these tissues prior to cellularization of the endosperm (5,14).

The cellular compartment for starch biosynthesis in reserve tissues appears to be the amyloplast. MacDonald and Rees (15) compiled the location of enzymes necessary for the conversion of sucrose to starch in soybean suspension cultures. They provided evidence that the conversion of sucrose to starch in reserve cells involves cleavage of sucrose and conversion to triose phosphates in the cytoplasm, transfer of triose phosphates into the amyloplast and synthesis of starch from triose phosphates inside of the amyloplast. Starch synthase and ADPG pyrophosphorylase were confined solely to the amyloplast while invertase, UDP sucrose synthase and UDPG pyrophosphorylase were absent from amyloplasts and probably confined to the cytoplasm. There is evidence that the carbon form entering wheat endosperm amyloplasts is also triose phosphate (13).

The high temperature limitation for kernel growth in wheat following anthesis resides in part, within the kernel itself (20). Production of the enzymes necessary for conversion of sucrose to starch is regulated inside the endosperm tissue (16). The enzymatic conversion of

sucrose to starch in kernels, therefore, must be controlled within the kernel itself, while the quantity of sucrose translocated to the grain is presumably a function of photosynthetic and translocatory tissues. Interactions between the processes of photosynthesis, translocation and assimilate utilization for starch synthesis make it difficult to identify and characterize environmental limitations for starch synthesis resident solely within the kernel. Spike isolation by thermally isolating spikes from other plant parts (8) and by solution culturing spikes removed from the plant (18) have provided important tools to minimize these interactions.

Little is known about which synthetic process limits starch biosynthesis in wheat kernels during high temperature exposure. Heat inactivation of starch synthase has been recently demonstrated at temperatures exceeding 30 C (17). Activity of the soluble form of starch synthase was found to be more heat labile than the starch granule bound form. Heat inactivation of the soluble form was suspected to be caused by a direct effect on the enzyme protein while inactivation of the bound form of starch synthase was thought to be caused by metabolic limitations. The objective of this study was to determine if a potential synthetic restriction, localized within the kernel, for conversion of sucrose to starch could be shown during kernel exposure to high temperature. Results indicate that the high temperature limitation for

starch synthesis is caused by a reduction in capacity of the endosperm to supply metabolic components for starch synthesis.

MATERIALS AND METHODS

Plant Material. Vernalized plants of wheat (Triticum aestivum L. cv TAM W-101) were grown in a controlled environment chamber at the Controlled Environment Research Laboratory, Oklahoma State University. Plants were grown in a 8:2:2:1 mixture of Redi-earth, sand, sandy loam soil and perlite in 15 cm pots. Pots were watered regularly and fertilized weekly with a modified Hoagland solution. Flag leaf temperatures were 18 C during 16 h light periods and 10 C during 8 h dark periods. Light intensity was 650 µmol m^{-2} s^{-1} PPFD at flag leaf level and RH was 70%.

Spikes containing approximately equal numbers of spikelets were tagged at completion of anthesis. Harvest stages were determined as days after tagging; the date of tagging was referred to as day zero. Kernel maturity was defined by complete loss of green pigment from flag leaf, peduncle and spikelets.

Spike Temperature Treatment. Six transparent cylindrical spike treatment tubes (40 liter air volume) were constructed to each accommodate five spikes at each of five locations along the tube (25 spikes/tube). Three tubes

were regulated at 35 C (high spike temperature treatment) while the other three tubes were regulated at 23 C (ambient spike temperature treatment). Each tube constituted a replication, providing three replications for each spike temperature treatment during each growth chamber experiment. The experiment was repeated three times.

Heat for the high spike temperature treatment was supplied by 300 W incandescent light bulbs thermostatically controlled and located within an insulated box. Warmed air was conveyed to the high spike temperature treatment tubes during the 16 h light period only. Ambient air was conveyed to the ambient spike temperature treatment tubes continuously and to the high spike temperature treatment tubes during the 8 h dark period. Air velocity within the tubes was approximately 4 ms^{-1}. Temperature inside the tubes was monitored using thermocouples placed at regular intervals inside each tube and at flag leaf level. Only a 2 C to 3 C temperature gradient existed in the tubes.

Groups of five spikes each were selected at completion of anthesis for placement into each of the five locations of a temperature treatment tube. Spikes were placed into tubes during the dark period to allow spikes to acclimate in an equal thermal environment prior to temperature treatment. A clear plastic flap was fitted securely around the peduncle so that spikes were thermally independent of other plant parts.

Sampling Procedure. Wheat spikes were harvested at 1100 h at five day intervals from 10 to 25 days after anthesis. A final harvest was conducted at grain maturity. Five spikes (one from each location along a tube) were severed from the plant and immediately placed on ice. The three apical and distal fertile spikelets were discarded since florets from these spikelets consistently produce smaller grains due to delayed anthesis and reduced assimilate intake in these kernels (4). The remaining kernels were then pooled, and aliquots utilized for enzyme, carbohydrate and kernel fill determinations.

Preparation of Enzyme Extract. All enzyme extractions were conducted at 4 C. Approximately 1 g of fresh immature kernels was weighed, counted and placed into grinding medium consisting of 0.1 M Tris-maleate buffer (pH 7.0), 1 mM EDTA, 1 mM DTT, 1 mM MgCl and 1 µm PMSF in a 1:5 weight to volume ratio. Whole kernels were ground for 1 min. with a polytron tissue homogenizer. A minimal amount of grinding buffer was used to rinse residual extract from the homogenizer. The suspension was dispersed by sonication (300 W, 60 cycles/ min.) for 1 min. to disrupt cellular organelles and centrifuged for 20 min. at 39,000g. Supernatent was saved, the pellet resuspended in 5 ml grinding medium and centrifuged again. The combined supernatents were brought to a 25 ml volume and utilized as crude enzyme preparation for subsequent

enzyme analyses.

Enzyme Assays. UDP sucrose synthase activity was assayed in the direction of sucrose cleavage as UDP-dependent formation of hexose sugars from sucrose. The reaction mixture contained 40 mM Mes (pH 6.5), 0.25 mM UDP, 40 mM sucrose, and 0.5 mM EDTA. The same reaction mixture minus UDP was run simultaneously to correct for invertase activity. Reactions were initiated by addition of 0.1 ml of an appropriate dilution of the crude enzyme preparation. Final reaction volume was 0.5 ml. After incubation at 35 C for 30 min., reactions were terminated by addition of the copper reagent of Nelson's arsenomolybdate procedure (10) and free fructose from the sucrose substrate was measured.

Invertase activity was measured as formation of hexose sugars from sucrose substrate. The reaction mixture contained 0.1 M acetate buffer (pH 4.8), 25 mM sucrose, and 0.5 mM EDTA. Reactions were initiated by addition of 0.1 ml of an appropriate dilution of the crude enzyme preparation. Final reaction volume was 0.4 ml. Reactions were incubated and terminated as described for UDP sucrose synthase.

UDPG pyrophosphorylase and ADPG pyrophosphorylase activities were assayed using a coupled enzyme system measuring glucose-1-P release. The reaction mixtures contained 65 mM Tris-HCl (pH 7.9), 0.5 mM ADPG (UDPG),

0.5 mM NADP, 7.5 mM MgCl, 0.1 unit glucose-6-P dehydrogenase, 0.3 unit phosphoglucomutase and 0.1 ml crude enzyme preparation. Reactions were initiated by addition of 2.5 mM sodium pyrophosphate. After incubation at 30 C for 30 min., NADPH production was measured spectrophotometrically at 340 nm. Final reaction volume was 1.72 ml.

All enzyme reactions were linear with respect to time and enzyme concentration.

Extraction and Analysis of Carbohydrates. Kernels not used for enzyme extraction and mature kernels were accurately weighed and counted to obtain fresh weight measurements and then frozen at -20 C. They were later freeze-dried for 48 to 72 h, oven dried at 70 C for 24 h to denature enzymes, and reweighed to obtain dry weight and water content measurements. Dried kernels were ground to pass a 1 mm screen using a Udy mill and stored in brown bottles at -20 C. Aliquots of dried kernels were extracted with 5 ml 95% (v/v) ethanol at 80 C for 20 min. and centrifuged at 10,000g. The supernatent from four successive ethanol extractions was evaporated in vacuo and resuspended in water for sucrose, glucose and fructose determination using the enzymatic method of Boehringer Mannheim (Boehringer Mannheim Biochemicals, P.O. Box 50816, Indianapolis, Indiana, 46250). Preliminary analyses indicated that greater than 98% of ethanol soluble sugars were recovered using this process.

The residue remaining after ethanol extraction was resuspended in 10 ml water and heated at 90 C for 1 h to gelatinize amylopectin. Samples were cooled and 15 ml 0.2 M acetate buffer (pH 4.5) containing 0.5% (w/v) porcine pancreatic alpha amylase and 2% (w/v) Rhizopus mold amyloglucosidase was added. Samples were incubated at 25 C for 1 h and at 55 C for 24 h, then centrifuged at 39,000g for 15 min. The residue was then extracted in 5 ml water at 60 C for 10 min. The combined supernatents from three water extractions and incubation were utilized for glucose determinations as for ethanol extracts and multiplied by a factor of 0.9 (to account for water gained during starch hydrolysis to glucose) to obtain starch determinations.

Statistical Method. Growth chamber experiments were conducted three times and combined since growth chamber run effects were not significant ($P = 0.05$). The experimental design was a split-split plot, with growth chamber runs as the main plot, spike temperature treatments as sub-plots, and maturity stages as sub-sub plots. Significant temperature x maturity interactions necessitated statistical analyses over maturity stages. Determination of differences in kernel weight, enzyme activity and carbohydrate content between temperature treatments were tested by analysis of variance. The rate of fresh weight, dry weight and starch accumulation, and the rate

of decline of UDP sucrose synthase activity, was determined by the difference between slopes of regressions for harvests between 15 and 25 d after completion of anthesis.

RESULTS

Kernel Fill. Spike temperature treatment had little effect on kernel weight during early kernel development. Kernel weights from spikes treated at 23 C and 35 C were similar from the initial harvest at 10 d to 15 d after anthesis on a fresh weight basis and from 10 d to 20 d after anthesis on a dry weight basis (Fig. 1). Kernel weight was significantly depressed by spike exposure to 35 C at maturity based on both fresh and dry weights ($P=0.01$). Kernel filling duration (d from completion of anthesis to maturity) was six days longer for spikes grown at 23 C (39 d) compared to spikes grown at 35 C (33 d).

Kernel growth rate at 23 C was significantly higher than at 35 C in terms of fresh weight, dry weight and starch accumulation (Table 1). The actual dry weight of kernels grown at 35 C was slightly greater than kernels grown at 23 C at 15 d and 20 d after anthesis (Fig. 1b). However, the rate of dry weight accumulation for kernels grown at 35 C was significantly lower during this time interval (Table 1).

Kernel water content was depressed by spike exposure to 35 C. The percent of water in kernels from both temp-

erature treatments steadily declined as kernel development progressed (Fig. 2a). Kernel percent water was significantly depressed at all stages of development by spike growth at 35 C as compared to 23 C. Kernels from both temperature treatments contained the same weight of water at 10 days after anthesis (Fig. 2b). However, after the 10 d sampling kernels growing at 23 C contained significantly higher quantities of water (P=0.05) than those growing at 35 C.

Changes in Enzyme Activity During Kernel Development. At 10 d after anthesis UDP sucrose synthase activity (μmoles sucrose cleaved gfw^{-1} min^{-1}) was significantly higher (P=0.03) in kernels developing at 35 C than those at 23 C (Fig. 3a). From 15 d to 25 d, no differences in activity between the temperature treatments were detected. The rate of decline in activity of UDP sucrose synthase during this time interval, however, was greater (P=0.10) in kernels growing at 35 C (-7.8 μmoles sucrose cleaved gfw^{-1} day^{-1}) than in kernels growing at 23 C (-5.8 μmoles sucrose cleaved gfw^{-1} day^{-1}). Invertase activity (μmoles sucrose cleaved gfw^{-1} min^{-1}) was consistently and significantly depressed (P=0.05) by kernel exposure to high temperature (Fig. 3b). The sharpest decline in activity of this enzyme occurred between 10 d and 15 d after anthesis. After day 15 the decline in invertase activity was more gradual.

The combined activities of UDP sucrose synthase and invertase represent the total enzymatic sucrose cleavage capacity in kernels. Invertase was less active in sucrose cleavage than UDP sucrose synthase, accounting for less than half of the sucrose cleavage capacity in kernels at 10 d after anthesis and less than one third of this capacity at subsequent developmental stages. From 10 d to 25 d after anthesis, total kernel enzymatic sucrose cleavage capacity declined from 8.1 to 4.2 μmoles sucrose cleaved gfw^{-1} min^{-1} at 23 C and from 6.9 to 3.2 μmoles sucrose cleaved gfw^{-1} min^{-1} at 35 C.

Kernels growing at both 23 C and 35 C contained similar activities for both pyrophosphorylase enzymes [μmoles UDPG (ADPG) cleaved gfw^{-1} min^{-1}] at 10 d after anthesis, but there was a striking difference between the temperature treatments after the 10 d sampling (Fig. 4). In the 23 C treatment, pyrophosphorylase activities increased at 15 d compared to 10 d after anthesis and then decreased. Pyrophosphorylase activities in kernels growing at 35 C declined steadily from 10 to 20 d after anthesis and then stabilized at 25 d after anthesis. Although there were clear temperature effects, the response of both pyrophosphorylase enzymes to temperature treatments was virtually identical (Fig. 4).

Carbohydrate Content During Kernel Development. The

major soluble sugar of kernels at all stages of development was sucrose (Fig. 5a, b and c). Kernels growing at 23 C contained sucrose levels (mg sucrose g ODW^{-1}) significantly greater (P=0.05) at 10 d and 15 d after anthesis than kernels growing at 35 C (Fig. 5a). No difference in sucrose between the temperature treatments was detected at 20 d and 25 d after completion of anthesis. But at the final sampling, mature kernels grown at 35 C contained significantly more sucrose (P=0.05) than those grown at 23 C.

Contents of both glucose and fructose (mg $g\ ODW^{-1}$) were significantly lower (P=0.03) in kernels growing at 35 C than in kernels growing at 23 C. The difference between temperature treatments was greatest during early kernel development (Fig. 5b and c). Fructose was the major sucrosyl hexose sugar; kernel contents of fructose were about three times higher than glucose at most sampling periods (Fig. 5b and c).

Starch content (mg starch $g\ ODW^{-1}$) was significantly greater (P=0.05) in kernels growing at 35 C than in kernels growing at 23 C at 10 d and 15 d after anthesis (Fig. 5d). Kernel starch content from both temperature treatments was equivalent by 20 d after anthesis. In subsequent sampling periods at 25 d after anthesis and at maturity, kernels growing at 23 C contained significantly more starch (P=0.05 and 0.01, respectively) than those growing at 35 C. Kernels grown at 23 C exhibited a

significantly higher rate for starch accumulation ($P=0.04$) between 15 d and 25 d after anthesis than kernels grown at 35 C (Table 1).

DISCUSSION

Kernel ODW was increased by elevating spike temperature from 23 C to 35 C during early maturity stages only. Studies utilizing whole plant (3), spike isolation (8) and solution culture of spikes (2) for temperature treatment have produced similar results. During early kernel development endosperm cell formation and differentiation is apparently accelerated with increasing temperature, initializing starch biosynthetic processes faster in heated kernels. Acceleration of plant organ development by increasing temperature is firmly established (11). In this study kernel capacity to utilize sucrose via UDP sucrose synthase was greater in heated kernels at 10 days after completion of anthesis (Fig. 3a). Pyrophosphorylase enzyme activities did not differ at 10 d so metabolic conversion of sugars for starch synthesis was apparently not restricted by the pyrophosphorylase enzymes either in heated kernels at this growth stage (Fig. 4). Significantly higher accumulation of starch in heated kernels at 10 d after completion of anthesis (Fig. 5d) appeared to be related to temporary enhancement of kernel metabolic capacity as one consequence of advanced kernel development.

Our data indicates that reduced kernel growth rate at 35 C from 15 to 25 d after anthesis can be attributed to deficiencies in kernel metabolic capacity brought about by prolonged spike exposure to high temperature. Although the activity of UDP sucrose synthase was not different between temperature treatments following the 10 day harvest (Fig. 3a), the rate of decline in activity was higher for kernels grown at 35 C than kernels grown at 23 C (P=0.10). The activity of both pyrophosphorylase enzymes was significantly depressed by high temperature after 10 d, diminishing kernel endosperm capacity to metabolize the monosaccharides released by the UDP sucrose synthase reaction for starch synthesis.

Kernel water content was depressed by spike warming (Fig. 2). Bhullar and Jenner (2) suggested that endosperm cell size is limited as a consequence of depressed water supply in heated kernels, resulting in reduced volume per kernel to accumulate starch. Reduction in number and size of starch granules appears to be one manifestation of kernel exposure to high temperatures during development (3). The similarity in response of starch accumulation (Fig. 5d) to UDP sucrose synthase activity (Fig. 3a) between temperature treatments provides correlative evidence that UDP sucrose synthase may play a critical role for regulation of sucrose utilization and, indirectly, starch biosynthesis when wheat

kernels are grown at high temperatures. Significantly higher accumulation of sucrose in heated kernels at maturity (Fig. 5a) may be indicative of depressed kernel capacity to utilize sucrose prior to kernel maturity.

Heat inactivation of starch synthase in wheat endosperm slices and in whole kernels has been demonstrated at temperatures above 30 C (17). Activity of the soluble form of starch synthase was found to be more heat labile than the bound form. Reduction in activity of the soluble form was suspected to be caused by a direct effect of heat on the enzyme protein while heat inactivation of the bound form of starch synthase was thought to be caused by metabolic factors. The metabolic capacity of heated kernels in this study was apparently enhanced at 10 d after completion of anthesis by increased UDP sucrose synthase activity (Fig. 3a). Further conversion of metabolites for starch synthesis in kernels from each temperature treatment was not restricted by pyrophosphorylase enzyme activity at this growth stage (Fig. 4). It is possible that bound starch synthase activity was stimulated by this increase in kernel capacity to provide metabolites and thus maintained endosperm starch synthesis in heated kernels at temperatures that were inhibitory to the activity of the soluble form. As kernel growth progressed, declines in the rate of sucrose cleavage via UDP sucrose synthase (Fig. 3a) and in the

activity of both pyrophosphorylase enzymes (Fig. 4) probably diminished the metabolic capacity in endosperm of kernels grown at 35 C compared to kernels grown at 23 C. Invertase activity was significantly depressed by the high temperature treatment at all stages of kernel development diminishing the metabolic capacity of pericarp tissues. This significant loss in metabolic capacity of kernels could have caused the significant depression in rate of starch accumulation observed for heated kernels (Table 1) by imposing a restriction on the amount of metabolites available for starch synthase.

There is evidence that genetic variability exists for the capacity to accumulate starch in wheat cultivars during high temperature exposure (1,3). Our data strongly indicates that the endosperm synthetic capacity kernels of wheat kernels is limited by high temperature, especially during middle to late stages of kernel development. The extent to which each individual starch biosynthetic enzyme exerts a limitation on starch synthesis is unclear. Further work is needed to clarify which enzymatic reaction, or group of reactions, in the conversion of sucrose to starch may be limiting kernel starch synthesis at high temperature. Once the limiting step is identified, successful genetic exploitation for kernel synthetic capacity may improve wheat yield stability through increased kernel synthetic efficiency in

environments where high temperature stress limits kernel development.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Glen Hartman for assistance in construction of spike temperature treatment equipment and for maintenance on the growth chamber and Dr. Jim Heitholt for his assistance in the conduct of experiments and input on the manuscript.

LITERATURE CITED

1. Bagga AK, HM Rawson 1977 Contrasting responses of morphologically similar wheat cultivars to temperatures appropriate to warm temperature climates with hot summers: A study in controlled environment. Aust J Plant Physiol 4:877-887
2. Bhullar SS, CF Jenner 1983 Responses to brief periods of elevated temperature in ears and grains of wheat. Aust J Plant Physiol 10:549-560
3. Bhullar SS, CV Jenner 1985 Differential responses to high temperatures of starch and nitrogen accumulation in the grain of four cultivars of wheat. Aust J Plant Physiol 12:363-375
4. Bremner PM, HM Rawson 1978 The weights of individual grains of the wheat ear in relation to their growth potential, the supply of assimilate and interaction between grains. Aust J Plant Physiol 5:61-72
5. Chevalier P, SE Lingle 1983 Sugar metabolism in developing kernels of wheat and barley. Crop Sci 23:272-277
6. Dale EM, TL Housley 1986 Sucrose synthase in developing wheat endosperms differing in maximum

weight. Plant Physiol 82:7-10

7. Dennis DT, MF Greyson 1987 Fructose 6-phosphate metabolism in plants. Physiol Plant 69:395-404

8. Ford MA, I Pearman, GN Thorne 1976 Effects of variation in ear temperature on growth and yield in spring wheat. Ann Appl Biol 82:317-333

9. Ho LC, RM Gifford 1984 Accumulation and conversion of sugars by developing wheat grains V. The endosperm apoplast and apoplastic transport. J Exp Bot 35:58-73

10. Hodge JE, BT Hofreiter 1962 Determination of reducing sugars and carbohydrates. In RL Whisler, ML Wolfrom, eds, Methods in Carbohydrate Chemistry, Vol 1 Academic Press, New York, pp 380-394

11. Johnson IR, JHM Thornley 1985 Temperature dependence of plant and crop processes. Ann Bot 55:1-24

12. Johnes RJ, S Quattar, RK Crookston 1984 Thermal environment during endosperm cell division and grain filling in maize: Effects on kernel growth and development in vitro. Crop Sci 24:133-137

13. Keeling PL, RH Tyson, IG Bridges 1983 Evidence for the involvement of triose phosphates in the pathway of starch biosynthesis in developing wheat grain. Biochem Soc Trans 11:791-792

14. Kumar R, R Singh 1980 The relationship of starch

metabolism to grain size in wheat. Phytochemistry 19:2299-2303

15. MacDonald FD, T ap Rees 1983 Enzymatic properties of amyloplasts from suspension cultures of soybean. Biochem Biophys 755:81-89

16. Reeves CD, HB Krishnan, TW Okita 1986 Gene expression in developing wheat endosperm. Accumulation of gliadin and ADP glucose pyrophosphorylase messenger RNAs and polypeptides. Plant Physiol 82:34-40

17. Rijven AHGC 1986 Heat inactivation of starch synthase in wheat endosperm tissue. Plant Physiol 81:448-453

18. Singh BK, CF Jenner 1983 Culture of detached ears of wheat in liquid culture: modification and extension of the method. Aust J Plant Physiol 10:227-236

19. Sofield I, LT Evans, MG Cook, IF Wardlaw 1977 Factors influencing the rate and duration of grain filling in wheat. Aust J Plant Physiol 4:785-797

20. Wardlaw IF, I Sofield, PM Cartwright 1980 Factors limiting the rate of dry matter accumulation in the grain of wheat grown at high temperature. Aust J Plant Physiol 7:387-400

Table 1. Comparison of fresh weight, dry weight and starch accumulation rates from 15 to 25 d after anthesis in kernels from spikes exposed to 23 C and 35 C.

Spike temperature	Accumulation Rate		
	Fresh weight	Dry weight	Starch
	mg kernel^{-1} d^{-1}		
23 C	2.02	1.83	1.24
35 C	1.03	1.25	0.76
	Analysis of Variance		
SE	0.07	0.08	0.07
F Ratio	90.47	27.88	23.34
Significance level	0.01	0.03	0.04

FIGURE LEGENDS

Fig. 1. Fresh (a) and dry (b) weights of kernels exposed to 23 C (●) and 35 C (○). Values are the mean of three growth chamber runs +/- SD of three spike temperature treatment replications (n=9). Asterisks indicate differences between temperature treatments according to F test at a 0.05 level of significance.

Fig. 2. Percentage (a) and weight (b) of water in kernels from the 23 C (●) and 35 C (○) spike temperature treatment. Values are the mean of three growth chamber runs +/- SD of three spike temperature treatment replications (n=9). Asterisks indicate differences between temperature treatments according to F test at a 0.05 level of significance.

Fig. 3. UDP sucrose synthase (a) and invertase (b) activities during kernel development at 23 C (●) and 35 C (○). Values are the mean of three growth chamber runs +/- SD of three spike temperature treatment replications (n=9). Asterisks indicate differences between temperature treatments according to F test at a 0.05 level

of significance.

Fig. 4. UDPG (a) and ADPG (b) pyrophosphorylase activities in kernels during development at 23 C (●) and 35 C (○). Values are the mean of three growth chamber runs +/- SD of three spike temperature treatment replications (n=9). Asterisks indicate differences between temperature treatments according to F test at a 0.05 level of significance.

Fig. 5. Sucrose (a), glucose (b), fructose (c) and starch (d) contents of kernels grown at 23 C (●) and 35 C (○). Values are the mean of three growth chamber runs +/- SD of three spike temperature treatment replications (n=9). Asterisks indicate differences between temperature treatments according to F test at a 0.05 level of significance.

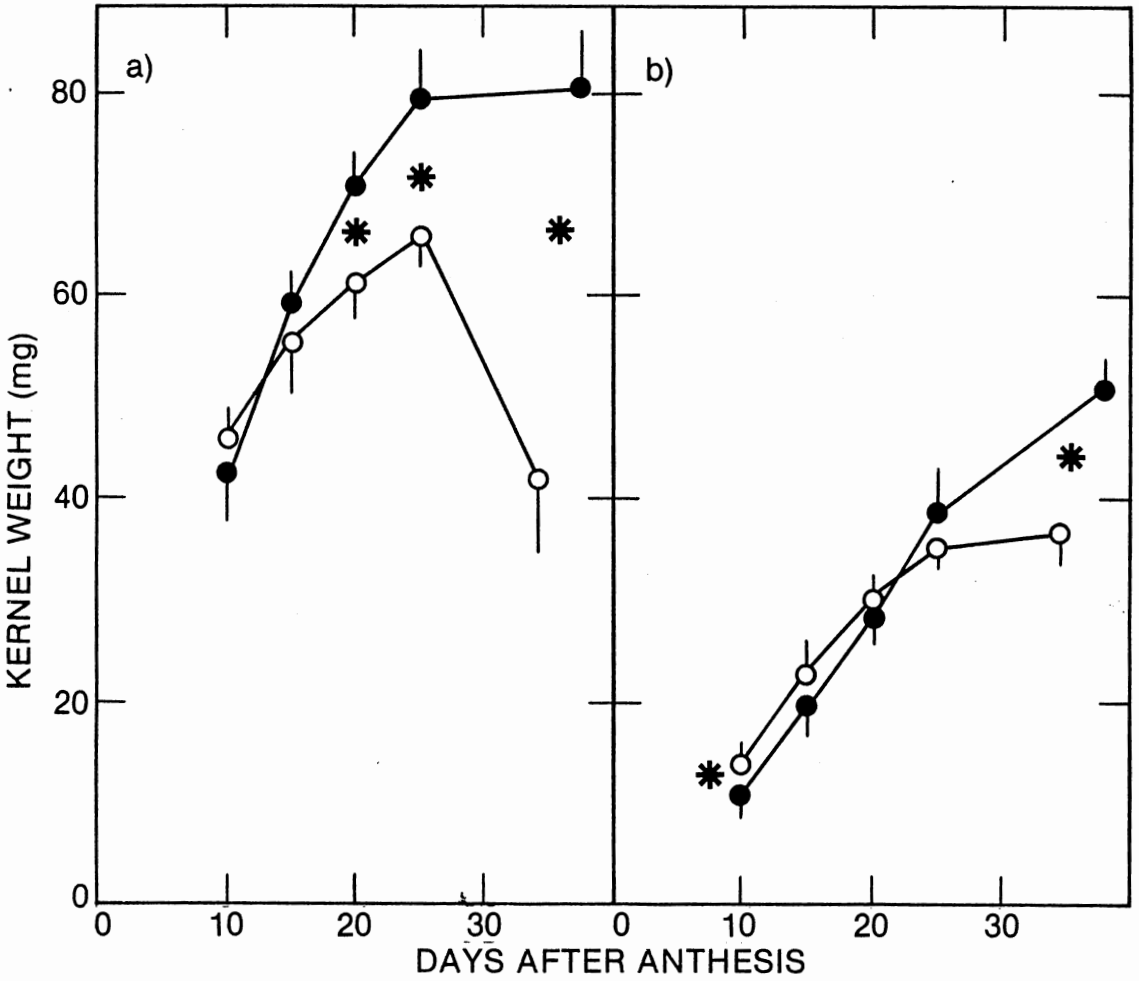

Figure 1. Kernel weight

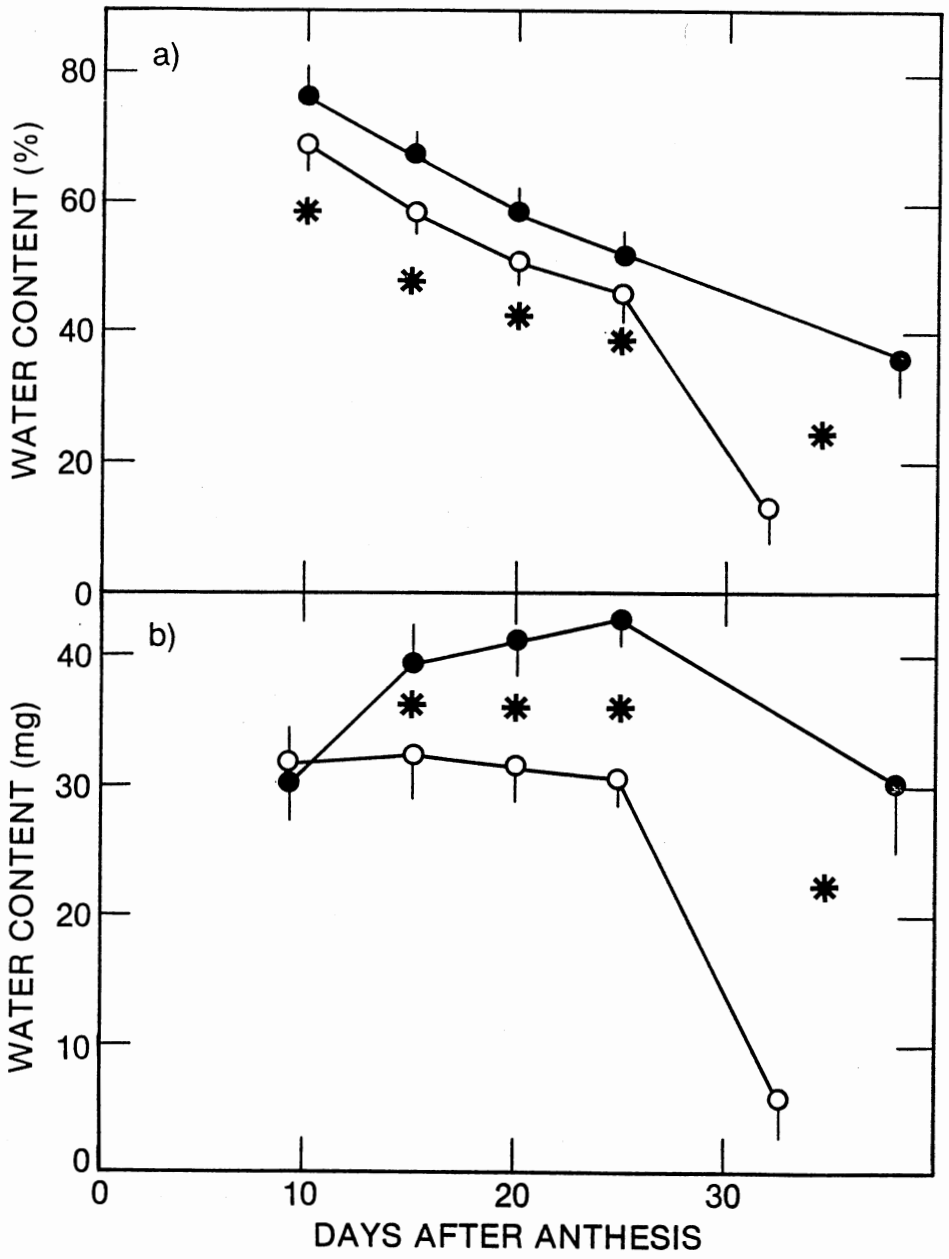

Figure 2. Kernel water content

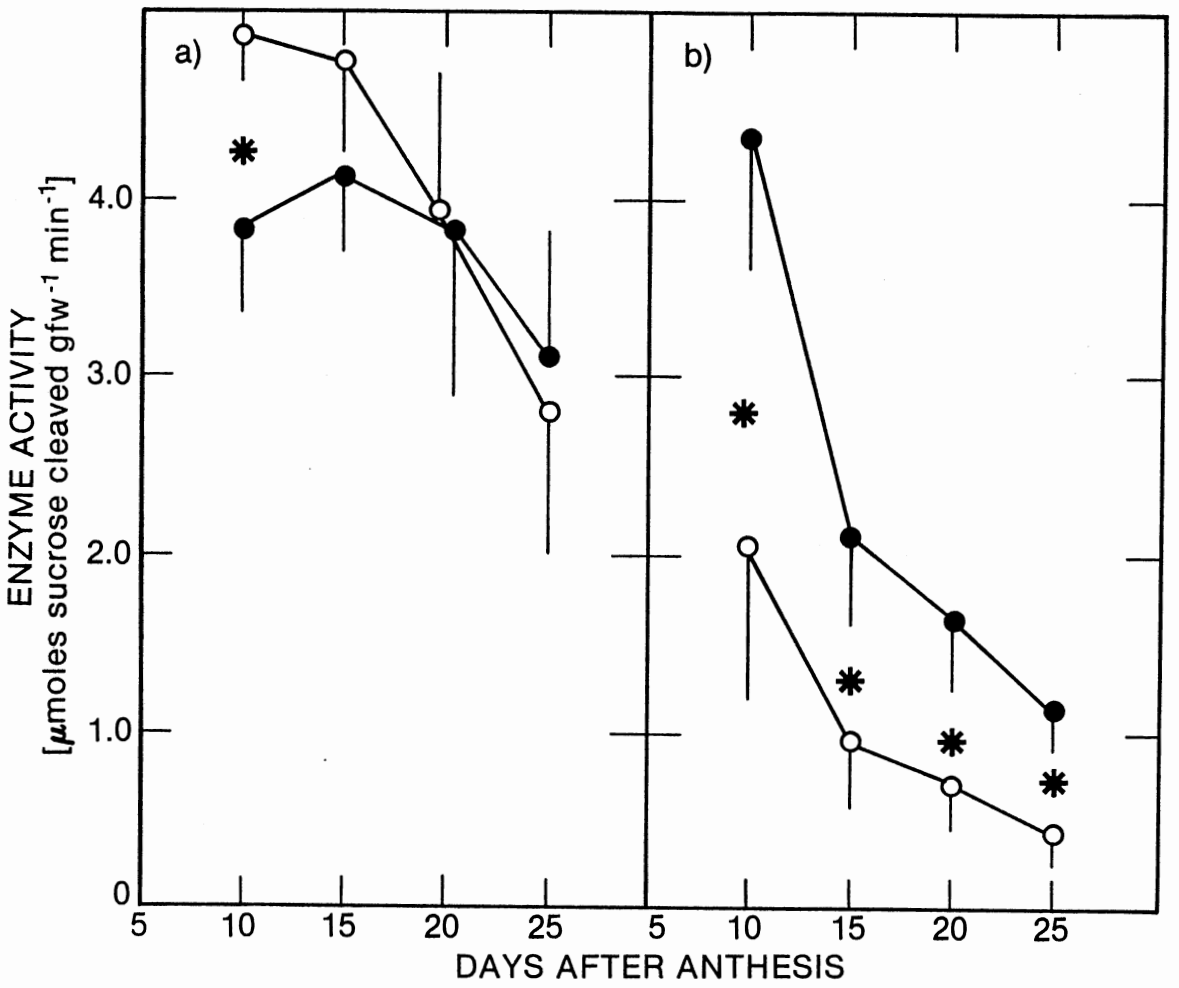

Figure 3. Enzyme activity of UDP sucrose synthase and invertase

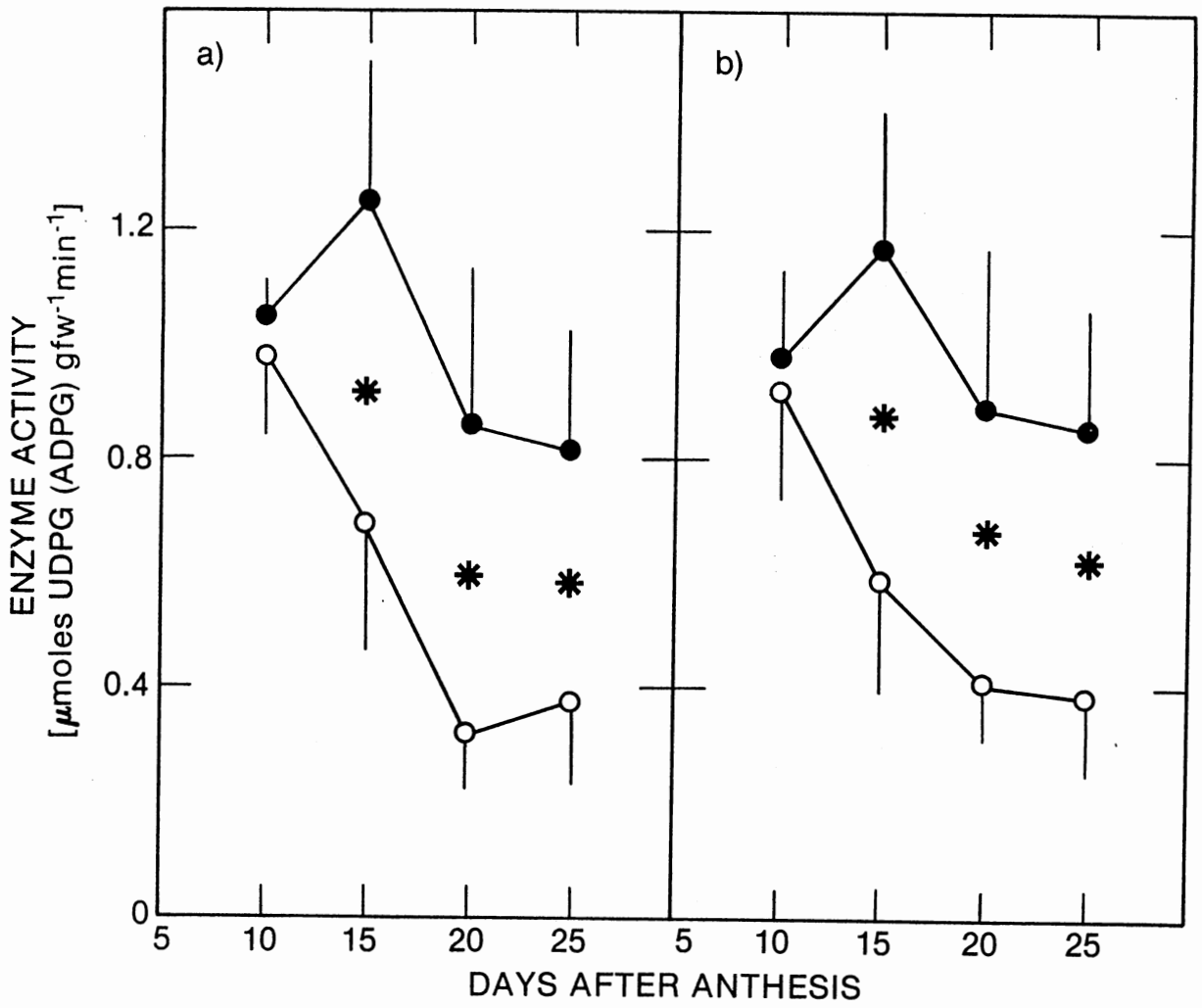

Figure 4. Enzyme activity of UDPG (ADPG) pyrophosphorylase

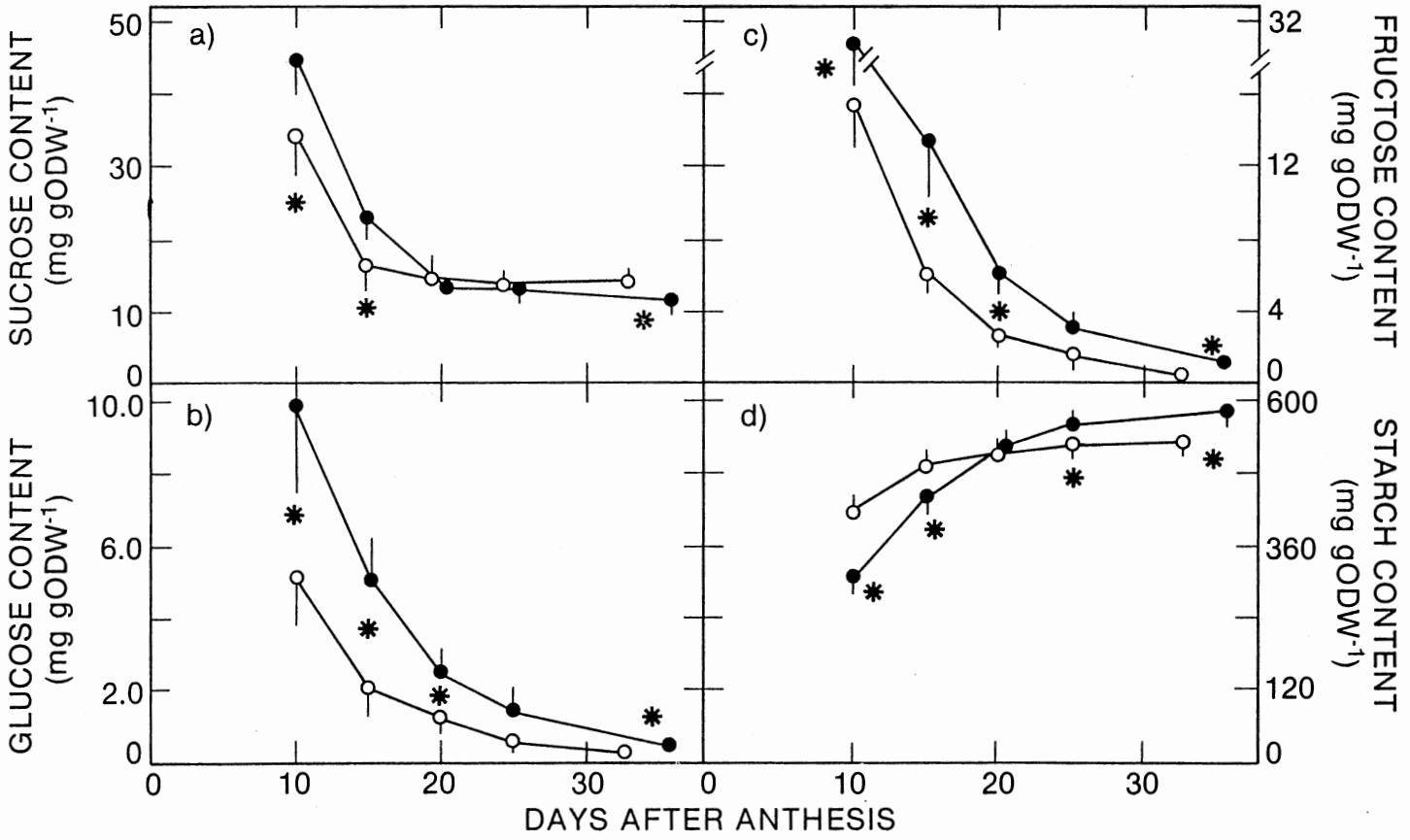

Figure 5. Sucrose, glucose, fructose and starch contents

CHAPTER II

SEASONAL AND DAILY VARIATION FOR ENZYMATIC SUGAR CONVERSION IN WHEAT KERNELS

Running Head: Variation for Enzymatic Sugar Conversion in Wheat Kernels.

N.O. Maness

Department of Biochemistry

Oklahoma State University

Stillwater, Oklahoma 74078

Telephone: 405-624-6197

Seasonal and Daily Variation for Enzymatic Sugar Conversion in Field Grown Wheat Kernels.[1]

Manuscript Received: _____

Revised Manuscript Received: _____

N.O. Maness[2], R.C. Johnson and J.J. Heitholt

Department of Agronomy, Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma 74078

FOOTNOTES:

[1] Research was supported by the Oklahoma Agricultural Experiment Station, Oklahoma State University, Journal paper no. of the Oklahoma Agricultural Experiment Station.

[2] Present address: Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma 74078.

[3] Abbreviations: ADPG, adenosine diphosphoglucose; UDPG, uridine diphosphoglucose; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl flouride; fw, fresh weight; ODW, oven dry weight.

ABSTRACT

Starch biosynthesis in wheat (Triticum aestivum) kernels is apparently controlled largely within the kernel itself. Wheat plants (cv TAM W-101) were grown in the field during two seasons and temperatures were monitored at spike level from anthesis until maturity. The activity of four enzymes required for the conversion of sucrose to starch was monitored on daily cycles of 0700 h, 1100 h, 1500 h and 1900 h at five day intervals from 10 d to 30 d after anthesis. Sucrose, glucose, fructose and starch contents of kernels were also determined at these stages and at 2400 h and at kernel maturity. In either season endosperm synthetic capacity, determined by the activities of UDP sucrose synthase and UDP (ADP) glucose pyrophosphorylase, was less in kernels which accumulated the greater amount of thermal units. Mature kernel fresh weight, dry weight and starch content was least for kernels exposed to higher temperature during early growth. While results do not implicate a single enzyme as "rate limiting" for starch synthesis in kernels grown at high temperature, they do suggest that a synthetic restriction similar to those existing in controlled environment and in vitro limits kernel growth in

a field environment as well.

INTRODUCTION

Dry weight accumulation in wheat kernels is a function of both the duration and the rate for accumulation of storage reserves during kernel development. The major storage reserve in wheat kernels is starch, comprising about 70% of the mature kernel weight (12). Starch is synthesized in the endosperm of the kernel through a series of biochemical reactions starting from sucrose, which is produced in photosynthetic tissues and translocated to the kernel.

Temperature plays a dominant role in regulating the duration and rate of kernel starch accumulation in wheat. The duration of kernel filling is consistently decreased with increase of temperature above 20 C (19). However, rate of kernel filling increases with temperature to 25 C but then declines at higher temperatures (6). Because the rate of kernel filling no longer increases to compensate for shortened duration at temperatures approaching 25 C, kernel weight at maturity is reduced.

It has become evident that sucrose utilization in wheat kernels, rather than sucrose supply from photosynthetic tissues, plays an important and perhaps dominant role in regulating starch accumulation when kernels are

exposed to high temperatures. Studies utilizing solution cultured spikes of wheat (3) and in vitro investigations of kernel development in wheat (2) and maize (14) provide evidence that synthetic processes of the endosperm in kernels are restricted more than sucrose supply when kernels are exposed to high temperature. Recent evidence for maize kernel sugar transport suggest that sugar uptake by the endosperm may be primarily regulated by the endosperm capacity for starch synthesis as well (8). Since production of enzymes necessary for starch biosynthesis is regulated inside the endosperm tissue (17), the metabolic conversion of sucrose to starch must be regulated within the kernel itself; the quantity of sucrose translocated to the grain, however, is a function of photosynthetic and translocatory tissues.

The biosynthesis of starch from sucrose appears to occur in two separate cellular compartments inside plant reserve tissues (7). MacDonald and Rees (16) compiled the cellular location of enzymes necessary for starch biosynthesis from sucrose in soybean suspension cultures. They found that sucrose was converted to triose phosphates by glycolysis in the cytoplasm, triose phosphates were transported into the amyloplast via the phosphate translocator, and then triose phosphates were converted into starch inside the amyloplast. Among the enzymes potentially involved in starch synthesis, ADPG pyrophos-

phorylase, starch synthase and alkaline pyrophosphatase were found only in the amyloplast (9, 16) while UDP sucrose synthase, invertase and UDPG pyrophosphorylase were absent from amyloplasts and probably confined to the cytoplasm (16). The carbon form entering wheat endosperm amyloplasts also appears to be triose phosphates (15). Sucrose cleavage is mediated by UDP sucrose synthase in the endosperm and by invertase in the pericarp of wheat kernels (5).

In the following study, wheat kernels were sampled over a daily cycle from field grown plants in two different seasons to determine whether synthetic restrictions for kernel growth, known to occur in controlled environment and in vitro, are present under field conditions. Results indicate that endosperm synthetic capacity is decreased in kernels from spikes accumulating the greater number of thermal units. The apparent synthetic restriction for kernel growth caused by high temperature was mainly expressed over the course of days rather than hours, suggesting that metabolic limitations for starch synthesis are produced gradually in the kernel. Rapid alterations of resident enzymes may not cause the major restriction for kernel growth when kernels are exposed to high temperature.

MATERIALS AND METHODS

Plant Material. Wheat (*Triticum aestivum* cv TAM W-101) plants were grown at the Agronomy Research Farm, Oklahoma State University, Stillwater, Oklahoma during the 1985 and 1986 growing seasons. Each of four replications consisted of two plots which contained five 3 m rows spaced 23 cm apart. Seeding rate was 1 seed per 2 cm. Plots were fertilized according to soil analysis recommendations; 130 kg ha^{-1} phosphorus was applied preplant and 120 kg ha^{-1} nitrogen was applied in split applications (preplant and early jointing). Irrigation was applied with sprinklers as needed to avoid moisture stress.

Spikes containing approximately equal numbers of spikelets were tagged at completion of anthesis. Harvest stages were determined as days after tagging, the date of tagging was referred to as day zero. Only one spike was sampled from each plant and only plants from the middle three rows of each plot were selected for sampling. Kernel maturity was defined by complete loss of green pigment from the flag leaf, peduncle and spike.

Temperature Measurement. Air temperature was monitored

hourly in each of the four replications using a CR-7 Measurement and Control System (Campbell Scientific, Logan, Utah) and thermocouples shielded from direct sunlight were suspended at spike level. Measurements were initiated at anthesis and continued until maturity. Heat accumulation at each sampling date was converted to thermal units (TU) (13) using the following equation:

$$TU = \sum \frac{\text{max C} - \text{min C}}{2} ;$$

where max C and min C are maximum and minimum daily temperatures. A base temperature for growth of 0 C was assumed.

Sampling Procedure. Wheat spikes were sampled on five day intervals at 0700 h, 1100 h, 1500 h and 1900 h from 10 to 30 d after anthesis for kernel fill, enzyme activity and carbohydrate determinations. An additional sampling at 2400 h was conducted for kernel fill and carbohydrate determinations only. Mature spikes were sampled at 1100 h. Groups of five spikes within each replication were severed from the plant at time of harvest and immediately placed on ice. Three apical and distal fertile spikelets were discarded since the florets of these spikelets consistently produce smaller kernels due to differences in date of flowering and assimilate availability (4). Kernels from the remaining spikelets in each replication were then pooled and aliquots uti-

lized for kernel fill, enzyme activity and carbohydrate determinations.

Preparation of Enzyme Extract. All enzyme extractions were conducted at 4 C. Approximately 1 g of fresh immature kernels was weighed, counted and placed into grinding medium consisting of 0.1 M Tris-maleate buffer (pH 7.0), 1 mM EDTA, 1 mM DTT, 1 mM MgCl and 1 µm PMSF in a 1:5 weight to volume ratio. Whole kernels were ground for 1 min. with a polytron tissue homogenizer. A minimal amount of grinding buffer was used to rinse residual extract from the homogenizer. The suspension was dispersed by sonication (300 W, 60 cycles/min.) for 1 min. to disrupt cellular organelles and centrifuged for 20 min. at 39,000g. Supernatent was saved, the pellet resuspended in 5 ml grinding medium and centrifuged again. The combined supernatents were brought to a 25 ml volume and utilized as crude enzyme preparation for subsequent enzyme analyses.

Enzyme Assays. UDP sucrose synthase activity was assayed in the direction of sucrose cleavage as UDP-dependent formation of hexose sugars from sucrose. The reaction mixture contained 40 mM Mes (pH 6.5), 0.25 mM UDP, 40 mM sucrose, and 0.5 mM EDTA. The same reaction mixture minus UDP was run simultaneously to correct for invertase activity. Reactions were initiated by addition of 0.1 ml of an appropriate dilution of the crude enzyme prepara-

tion. Final reaction volume was 0.5 ml. After incubation at 35 C for 30 min., reactions were terminated by addition of the copper reagent of Nelson's arsenomolybdate procedure (10) and free fructose from the sucrose substrate was measured.

Invertase activity was measured as formation of hexose sugars from sucrose substrate. The reaction mixture contained 0.1 M acetate buffer (pH 4.8), 25 mM sucrose, and 0.5 mM EDTA. Reactions were initiated by addition of 0.1 ml of an appropriate dilution of the crude enzyme preparation. Final reaction volume was 0.4 ml. Reactions were incubated and terminated as described for UDP sucrose synthase.

UDPG pyrophosphorylase and ADPG pyrophosphorylase activities were assayed using a coupled enzyme system measuring glucose-1-P release. The reaction mixtures contained 65 mM Tris-HCl (pH 7.9), 0.5 mM ADPG (UDPG), 0.5 mM NADP, 7.5 mM MgCl, 0.1 unit glucose-6-P dehydrogenase, 0.3 unit phosphoglucomutase and 0.1 ml crude enzyme preparation. Reactions were initiated by addition of 2.5 mM sodium pyrophosphate. After incubation at 30 C for 30 min., NADPH production was measured spectrophotometrically at 340 nm. Final reaction volume was 1.72 ml.

All enzyme reactions were linear with respect to time and enzyme concentration.

Extraction and Analysis of Carbohydrates. Kernels re-

maining after enzyme extraction and mature kernels were accurately weighed and counted to obtain fresh weight measurements and then frozen at -20 C. They were later freeze-dried for 48 to 72 h, oven dried at 70 C for 24 h to denature enzymes, and reweighed to obtain dry weight and water content measurements. Dried kernels were ground to pass a 1 mm screen using a Udy mill and stored in brown bottles at -20 C. Oven dried aliquots were extracted with 5 ml 95% (v/v) ethanol at 80 C for 20 min. and centrifuged at 10,000g. The supernatent from four ethanol extractions was evaporated in vacuo and resuspended in water for sucrose, glucose and fructose determination using the enzymatic method of Boehringer Mannheim (Boehringer Mannheim Biochemicals, P.O. Box 50816, Indianapolis, Indiana, 46250). Preliminary analyses indicated that greater than 98% of ethanol soluble sugars were recovered using this process.

The residue remaining after ethanol extraction was resuspended in 10 ml water and heated at 90 C for 1 h to gelatinize amylopectin. Samples were cooled and 15 ml 0.2 M acetate buffer (pH 4.5) containing 0.5% (w/v) porcine pancreatic alpha amylase and 2% (w/v) Rhizopus mold amyloglucosidase was added. Samples were incubated at 25 C for 1 h and at 55 C for 24 h, then centrifuged at 39,000g for 15 min. The residue was then extracted in 5 ml water at 60 C for 10 min. The combined supernatents

from three water extractions and incubation were utilized for glucose determinations as for ethanol extracts and multiplied by a factor of 0.9 (to account for water gained during starch hydrolysis to glucose) to obtain starch determinations.

Statistical Method. Differences in daily time of sampling were determined using least significant difference tests at the 0.05 probability level. Determination of differences between seasons and kernel maturity stages were tested by analysis of variance. The experimental design within each season was a randomized complete block in a split plot arrangement of treatments with kernel maturity stage as the main plot and daily time of sampling as the subplot. For comparisons between seasons, the experimental design consisted of a randomized complete block in a split plot in time arrangement of treatments with season as the main plot, kernel maturity stage as the subplot and daily time of sampling as the sub-subplot.

RESULTS

Temperature Measurement. Thermal unit accumulation and average daily temperature during kernel filling was distinctly different between each season (Table 1). Daily average temperature in 1985 was lower than 1986 during the first 15 d after anthesis; daily temperatures averaged 18 C to 22 C and thermal unit accumulation was about 36 units lower than 1986. After 15 d in 1985 temperatures rose sharply and remained high to maturity; daily average temperatures ranged from 25 C at 20 d to 31 C at 30 d. In contrast, average daily temperature during 1986 was 25 C for the first 15 d of kernel filling and ranged from 25 C to 29 C at later stages of kernel filling. Thermal unit accumulation was higher in 1986 than 1985 at 10 d, 15 d and 20 d after anthesis but lower than 1985 at subsequent sampling periods (Table 1). Kernel filling duration (d from anthesis to maturity) was 34 d in 1985 and 32 d in 1986.

Kernel Fill. Seasonal differences existed for fresh and dry weight accumlation in kernels (Table 2). Based on fresh weight, kernels were significantly heavier ($P = 0.01$) throughout 1985 as compared to 1986. On a dry

weight basis, however, kernels from 1986 were heavier (P = 0.03) at 10 d after anthesis than kernels from 1985. No differences in dry weight between the seasons were detected at 15 d and 20 d after anthesis. From 25 d after anthesis to maturity, kernel dry weights were significantly greatest (P = 0.01) in 1985 than 1986. Mature kernel fresh weights were 67.0 mg kernel^{-1} in 1985 compared to 57.2 mg kernel^{-1} in 1986. Mature kernel dry weights were 41.0 mg kernel^{-1} in 1985 compared to 35.4 mg kernel^{-1} in 1986.

Variation in percent of water and weight of water in kernels was generally not significant during the day in either season (Table 3). During both seasons the percent of water in kernels declined steadily as stage of development progressed. The percent of water in kernels was significantly lower (P = 0.01) during 1986 as compared to 1985 prior to 25 d. The weight of water in kernels initially increased to 15 d after anthesis followed by a plateau and then a steady decline by 25 d after anthesis to maturity (Table 3). The percent of water and weight of water in kernels at maturity was 38.8 percent and 26.0 mg kernel^{-1} during 1985 and 21.7 percent and 9.8 mg kernel^{-1} during 1986.

Changes in Enzyme Activity During Kernel Development. UDP sucrose synthase activity (μmoles sucrose cleaved gfw^{-1} min^{-1}) was slightly higher (P = 0.07) in 1986 at 10

d after anthesis than in 1985 (Table 4). Activity of this enzyme at 15 d and 20 d after anthesis was significantly greater ($P = 0.001$) in 1985 than in 1986. By 25 d activities between the seasons were equivalent and by 30 d kernels from 1986 exhibited significantly higher ($P = 0.002$) UDP sucrose synthase activity compared to kernels from 1985. During the first 20 d of kernel filling in both seasons kernels exhibited lowest UDP sucrose synthase activity at the 1500 daily sampling period (Table 4). At subsequent sampling periods in 1985 and at 30 d in 1986, UDP sucrose synthase activity declined over the course of the day.

Invertase activity (μmoles sucrose cleaved gfw^{-1} min^{-1}) was significantly higher ($P = 0.003$) in kernels from 1985 than kernels from 1986 at all stages of kernel development (Table 4). Although daily variation in activity of this enzyme existed during various kernel developmental stages, no consistent pattern over seasons was detected. In both seasons invertase activity declined at each successive sampling date.

UDPG and ADPG pyrophosphorylase activities [μmoles UDPG (ADPG) gfw^{-1} min^{-1}] were significantly higher ($P = 0.002$) during 1985 in kernels during the first 20 d of kernel filling as compared to kernels from 1986 (Table 5). However, activities at subsequent kernel filling stages of 25 d and 30 d after anthesis were significantly

higher (P = 0.04) in 1986 rather than 1985. Pyrophosphorylase enzyme activities generally declined with advancing stage of kernel development with the exception of 1986 at 15 d after anthesis. During most stages of kernel development pyrophosphorylase activities declined steadily over the course of the day (Table 5).

Carbohydrate Content During Kernel Development. Sucrose was the major soluble sugar in kernels during most stages of kernel development (Table 6). Kernel sucrose contents ($mg\ g\ ODW^{-1}$) were significantly greater (P = 0.001) at 10 d and 15 d after anthesis in 1985 than the same kernel filling stages in 1986. Kernel sucrose contents were equivalent between seasons at 20 d after anthesis. By the 25 d harvest and at harvest stages thereafter sucrose contents were again significantly greater (P = 0.001) in kernels from 1985. Sucrose contents of kernels from 1986 were relatively stable decreasing only slightly throughout kernel development (Table 6). Significant variation for time of daily sampling existed in each season for sucrose content in kernels. No consistent pattern for this variation was observed.

Kernel contents of both glucose and fructose ($mg\ g\ ODW^{-1}$) were significantly greater (P = 0.001) prior to 25 d after anthesis in 1985 than in 1986 (Table 6). At 25 d and 30 d after anthesis, kernel contents of both sugars were equivalent between seasons. At all kernel maturity

stages, kernel glucose and fructose contents were higher during daylight hours than at the 2400 h daily sampling period (Table 6). Fructose was the major sucrosyl hexose sugar; kernel fructose contents were about three times higher than glucose at 10 d, 15 d and 20 d after anthesis.

Kernel starch accumulation (mg g ODW^{-1}) was significantly greater ($P = 0.05$) at 10 d after anthesis in 1986 than in 1985 (Table 7). Starch contents of kernels at the 15 d harvest were equivalent between seasons. By 20 d after anthesis and sampling periods thereafter starch contents were significantly greater ($P = 0.01$) in kernels from 1985 as compared to 1986. Significant accumulation of starch in kernels at successive daily sampling periods occurred between 10 d and 15 d after anthesis (Table 7). Kernel starch accumulation appeared to follow no consistent daily pattern after these growth stages. Mature kernel starch contents were significantly greater ($P = 0.05$) in 1985 (598.0 mg g ODW^{-1}) than in 1986 (566.4 mg g ODW^{-1}).

DISCUSSION

Accumulation of ODW in wheat kernels can be divided into a lag phase lasting from anthesis to the beginning of linear ODW accumulation (about 10 d after anthesis) followed by a linear phase (10 d to 25 d after completion of anthesis) and then a maturation phase (11). The duration of any of the three phases is regulated by temperature; as temperature rises above that level which is optimal for kernel growth the duration of any phase is reduced (20). Thermal unit accumulation (Table 1) was higher during the first 20 d of kernel filling in 1986 compared to 1985. By 25 d after anthesis to maturity accumulated thermal units were greater in 1985 than in 1986. The lag phase of kernel growth apparently occurred prior to 10 d after anthesis in each season. Linear phase of kernel growth, defined by linear accumulation of ODW between kernel growth stages, was longer in 1985 than 1986, occurring from about 10 d to 25 d after anthesis in 1985 compared to 10 d to 20 d after anthesis in 1986 (Table 2). The maturation phase was shorter in 1985 than 1986, occurring from about 25 d to 34 d after anthesis (9 d) in 1985 and from about 20 d to 32 d after anthesis in 1986 (12 d). Higher temperatures accompanied by higher

thermal unit accumulation occurred during the lag phase of kernel growth in 1986 as compared to 1985 (Table 1). High temperatures during the lag phase are known to cause accelerated endosperm cell formation and differentiation which initiates endosperm starch biosynthesis earlier. Kernel ODW (Table 2) and starch content (Table 7) were greater only at 10 d after anthesis in 1986 as compared to 1985. Endosperm capacity to utilize sucrose via UDP sucrose synthase was slightly favored in 1986 over 1985 at 10 d after anthesis (Table 4). Initiation of starch biosynthetic processes earlier in kernels grown in 1986 was probably caused by advanced development as a consequence of kernel exposure to higher temperatures during the lag phase of kernel growth in 1986 than in 1985.

By 20 d after anthesis and sampling stages thereafter kernels exposed to the high lag phase temperature of 1986 were lighter (Table 2) and contained less starch (Table 7) than kernels exposed to more favorable lag phase temperatures in 1985. The percent of water and weight of water in kernels were also substantially lower in 1986 than in 1985 (Table 3). Endosperm cell size and perhaps cell number may be limited because of this reduced water supply caused by high temperature occurring during the lag phase, limiting the potential of endosperm for starch accumulation of later phases of kernel growth (1, 14). The lower lag phase temperatures of 1985 may

have favored increased endosperm cell volume for starch deposition at later kernel growth stages due to increased kernel water content, providing increased sites for starch synthesis and accumulation by maturity in these kernels over kernels grown in 1986.

Starch synthetic capacity in the endosperm of kernels defined by the activities of UDP sucrose synthase (Table 4) and of UDPG and ADPG pyrophosphorylase (Table 5), responded negatively to higher seasonal thermal unit accumulation during the linear and maturation phases of kernel development. Thermal unit accumulation in 1985 was less than 1986 until 25 d after anthesis (Table 1). The activities of UDP sucrose synthase (Table 4) and both pyrophosphorylase enzymes were higher in 1985 than 1986 at 15 d and 20 d after anthesis. By 25 d and 30 d after anthesis thermal unit accumulation in 1985 had increased over 1986, and activities of these enzymes had declined in 1985 to levels lower than those observed in 1986. Significantly higher accumulation of sucrose in kernels grown in 1985 at 25 d and 30 d after anthesis (Table 6) may be indicative of reduced endosperm capacity to utilize sucrose for starch synthesis during the later phases of 1985 as compared to 1986. The increase in synthetic capacity of kernels in the later phases of the 1986 season apparently occurred too late to significantly influence kernel ODW or starch content at maturity.

Bhullar and Jenner (2) recently suggested that two separate yet related kernel processes are apparently depressed when wheat kernels are subjected to high temperature: a comparatively low temperature optimum for starch synthesis and an irreversible reduction in endosperm capacity to accumulate starch. In this study endosperm synthetic capacity was apparently favored in kernels growing at the lower temperature in each season. Kernel capacity to accumulate starch, determined by the volume of endosperm available for starch synthesis (3), was probably favored in kernels grown in 1985 due to increased kernel water content (Table 3). Kernels grown in 1985 probably achieved the higher mature starch content due to of increased sites for starch biosynthesis, even though starch synthetic capacity of kernels was favored in 1986 at 25 d and 30 d after anthesis.

The inhibitory effects of high temperature on endosperm synthetic capacity (Table 4 and 5) and starch accumulation (Table 7) were most pronounced over the course of days than hours. This delayed metabolic expression of high temperature suggests that reduction in activity of enzymes was probably not caused by rapid alteration of the properties of resident enzymes. It is possible that the loss of endosperm synthetic capacity accompanying high temperature was due to reduced enzyme synthesis and/or increased enzyme degradation, or due to

inactivation of one or more enzymes required for starch synthesis from sucrose. Further work is needed to determine the exact mechanism whereby endosperm synthetic capacity is restricted by high temperature. Identification of this mechanism would enhance successful genetic incorporation of favorable mechanisms for yield stability through increased kernel synthetic efficiency in environments where high temperature stress limits kernel development.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Eugene Krenzer for loan of the CR7 measurement and control system used in the experiments. Gratitude is also extended to Ms. Linda Brown and Mr. Dave Ferris for technical assistance with the experiments.

LITERATURE CITED

1. Bhullar SS, CF Jenner 1983 Responses to brief periods of elevated temperature in ears and grains of wheat. Aust J Plant Physiol 10:549-560
2. Bhullar SS, CF Jenner 1986 Effects of temperature on the conversion of sucrose to starch in the developing wheat endosperm. Aust J Plant Physiol 13:605-615
3. Bhullar SS, CF Jenner 1986 Effects of a brief episode of elevated temperature on grain filling in wheat ears cultured on solutions of sucrose. Aust J Plant Physiol 13:617-626
4. Bremner PM, HM Rawson 1978 The weights of individual grains of the wheat ear in relation to their growth potential, the supply of assimilate and interaction between grains. Aust J Plant Physiol 5:61-72
5. Chevalier P, SE Lingle 1983 Sugar metabolism in developing kernels of wheat and barley. Crop Sci 23:272-277
6. Chowdhury SI, IF Wardlaw 1978 The effect of temperature on kernel development in cereals. Aust J Agric Res 29:205-223
7. Dennis DT, MF Greyson 1987 Fructose 6-phosphate

metabolism in plants. Physiol Plant 69:395-404

8. Griffith SM, RJ Jones, ML Brenner 1987 In vitro sugar transport in Zea mays L. Kernels I. Characteristics of sugar absorption and metabolism by developing maize endosperm. Plant Physiol 84:467-471

9. Gross P, T ap Rees 1986 Alkaline inorganic pyrophosphatase and starch synthesis in amyloplasts. Planta 167:140-145

10. Hodge JE, BT Hofreiter 1962 Determination of reducing sugars and carbohydrates. In RL Whisler, ML Wolfrom, eds, Methods in Carbohydrate Chemistry, Vol 1 Academic Press, New York, pp 380-394

11. Housley TL, CST Daughtry 1987 Fructan content and fructosyltransferase activity during wheat seed growth. Plant Physiol 83:4-7

12. Jenner CF, AJ Rathjen 1985 Factors regulating the accumulation of starch in ripening wheat grain. Aust J Plant Physiol 2:311-322

13. Johnson RC, ET Kanemasu 1983 Yield and development of winter wheat at elevated temperatures. Agron J 75:561-565

14. Jones RJ, S Quattar, RK Crookston 1984 Thermal environment during endosperm cell division and grain filling in maize: Effects on kernel growth and development in vitro. Crop Sci 24:133 137

15. Keeling PL, RH Tyson, IG Bridges 1983 Evidence for the involvement of triose phosphates in the pathway of

starch biosynthesis in developing wheat grain. Biochem Soc Trans 11:791-792

16. MacDonald FD, T ap Rees 1983 Enzymatic properties of amyloplasts from suspension cultures of soybean. Biochem Biophys Acta 755:81-89

17. Reeves CD, HB Krishnan, TW Okita 1986 Gene expression in developing wheat endosperm. Accumulation of gliadin and ADP glucose pyrophosphorylase messenger RNAs and polypeptides. Plant Physiol 82:34-40

18. Rijven AHGC 1986 Heat inactivation of starch synthase in wheat endosperm tissue. Plant Physiol 81:448-453

19. Sofield I, LT Evans, MG Cook, IF Wardlaw 1977 Factors influencing the rate and duration of grain filling in wheat. Aust J Plant Physiol 4:785-797

20. Wiegand CL, JA Cuellar 1981 Duration of grain filling and kernel weight of wheat as affected by temperature. Crop Sci 21:95-101

Table 1. Thermal unit (TU) accumulation and average temperature following anthesis for kernels from 1985 and 1986.

Days after anthesis	Season			
	1985		1986	
	TU	Temperature[a]	TU	Temperature[a]
10	165	22.2	201	23.4
15	266	23.1	303	25.3
20	392	20.8	416	24.2
25	529	25.2	519	23.2
30	669	31.4	619	22.1
Maturity	751	30.6	660	26.0

[a]Temperature is the average daily temperature for each respective sampling period.

Table 2. Daily patterns in weight accumulation of wheat kernels at various growth stages during 1985 and 1986.

Daily cycle	Days after anthesis									
	1985					1986				
	10	15	20	25	30	10	15	20	25	30
	mg kernel fw^{-1}									
0700	40.6	52.4	63.9	66.8	71.8	39.3	47.6	52.7	55.8	60.6
1100	42.9	51.5	59.5	63.2	69.5	37.1	43.5	55.3	58.2	61.6
1500	40.7	52.3	62.6	64.7	67.6	39.7	50.0	56.8	57.0	59.4
1900	42.3	53.5	62.1	64.9	64.7	38.1	43.7	56.9	54.2	58.5
2400	--	55.0	63.9	68.1	66.9	38.9	50.1	55.2	58.4	61.7
MEAN[a]	41.6	52.9	62.4	65.5	68.1	38.6	47.0	55.4	56.7	60.4
$LSD_{0.05}$[b]	NS	1.7	2.4	4.1	NS	NS	3.1	3.5	3.6	NS
	mg kernel ODW^{-1}									
0700	11.7	19.2	29.4	36.9	41.8	13.7	20.2	26.2	30.2	34.8
1100	12.1	19.4	28.0	34.5	40.7	13.1	18.4	27.7	31.4	35.9
1500	12.2	19.5	29.6	35.6	40.9	14.2	19.8	28.2	31.7	34.5
1900	12.8	20.7	29.9	36.2	41.5	14.5	19.5	28.4	29.4	34.8
2400	--	21.2	30.8	37.9	41.0	14.6	21.8	27.8	31.4	36.3
MEAN[a]	12.2	20.0	29.5	36.2	41.2	14.0	19.9	27.7	30.8	35.3
$LSD_{0.05}$[b]	0.7	0.8	1.1	1.9	NS	NS	1.8	NS	NS	NS

[a]Means are averages taken over daily cycles.

[b]$LSD_{0.05}$ represents the least significant difference at the 5% level of probability between daily times of sampling. NS, nonsignificant difference at the 5% level of probability.

Table 3. Daily patterns in water content of wheat kernels during various growth stages in 1985 and 1986.

Daily cycle	Days after anthesis									
	1985					1986				
	10	15	20	25	30	10	15	20	25	30
	percent water									
0700	71.1	63.4	54.0	44.8	41.7	65.2	57.5	50.3	45.8	42.5
1100	71.7	62.4	52.9	45.4	41.4	64.6	57.8	50.0	46.1	41.7
1500	70.1	62.6	52.8	45.0	39.5	64.3	59.5	50.4	44.4	41.9
1900	69.9	61.2	51.9	44.1	35.3	61.7	55.4	50.2	45.7	40.6
2400	--	61.4	51.8	44.4	38.8	62.5	56.6	49.7	46.2	41.2
MEAN[a]	70.7	62.2	52.7	44.7	39.3	63.7	57.4	50.1	45.6	41.6
$LSD_{0.05}$[b]	NS	0.8	1.3	NS	NS	NS	NS	NS	NS	NS
	mg water $kernel^{-1}$									
0700	28.9	33.2	34.5	30.0	29.9	25.6	27.4	26.5	25.6	25.8
1100	30.8	32.2	31.5	28.7	28.8	24.0	25.1	27.6	26.9	25.7
1500	28.6	32.7	33.0	29.1	26.7	25.5	30.2	28.6	25.3	24.9
1900	29.6	32.7	32.2	28.7	23.2	23.5	24.3	28.6	24.8	23.8
2400	--	33.8	33.1	30.2	26.0	24.3	28.4	27.4	27.0	25.4
MEAN[a]	29.5	32.9	32.9	29.3	26.9	24.6	27.1	27.7	25.9	25.1
$LSD_{0.05}$[b]	NS	NS	1.7	NS	NS	NS	NS	1.4	NS	NS

[a]Means are averages taken over daily cycles.

[b]$LSD_{0.05}$ represents the least significant difference at the 5% level of probability between daily times of sampling. NS, nonsignificant difference at the 5% level of probability.

Table 4. Daily patterns in UDP sucrose synthase and invertase activity of wheat kernels at various growth stages during 1985 and 1986.

Daily cycle	Days after anthesis									
	1985					1986				
	10	15	20	25	30	10	15	20	25	30
	umoles sucrose cleaved gfw^{-1} min^{-1}									
UDP SUCROSE SYNTHASE										
0700	4.51	5.41	4.31	3.42	2.24	5.78	4.36	3.79	3.04	2.24
1100	5.49	5.62	4.72	3.08	1.93	5.49	4.40	3.00	4.15	2.29
1500	5.03	4.78	4.10	3.28	1.65	5.18	3.34	2.80	3.29	2.35
1900	5.48	5.27	4.91	2.72	1.45	5.96	4.22	3.99	3.05	2.07
MEAN[a]	5.13	5.27	4.51	3.13	1.82	5.60	4.08	3.40	3.38	2.24
$LSD_{0.05}$[b]	0.52	0.32	0.53	0.57	0.34	NS	0.53	0.44	0.29	0.26
INVERTASE										
0700	4.54	3.68	1.92	0.85	0.54	4.48	1.32	1.71	0.53	0.27
1100	5.24	3.86	2.10	0.92	0.49	4.05	1.73	1.58	0.66	0.34
1500	5.84	3.82	2.28	1.47	0.40	4.73	1.18	1.52	0.55	0.48
1900	6.21	4.21	2.65	0.93	0.33	4.33	1.22	0.89	0.70	0.24
MEAN[a]	5.46	3.89	2.24	1.04	0.44	4.40	1.36	1.43	0.61	0.33
$LSD_{0.05}$[b]	0.53	NS	0.44	0.37	0.18	0.30	0.43	0.31	NS	0.08

[a]Means are averages taken over daily cycles.

[b]$LSD_{0.05}$ represents the least significant difference at the 5%level of probability between daily times of sampling. NS, nonsignificant difference at the 5% level of probability.

Table 5. Daily patterns in UDPG pyrophosphorylase and ADPG pyrophosphorylase activity of wheat kernels at various growth stages during 1985 and 1986.

Daily cycle	Days after anthesis									
	1985					1986				
	10	15	20	25	30	10	15	20	25	30
	umoles UDPG (ADPG) gfw^{-1} min^{-1}									
UDPG PYROPHOSPHORYLASE										
0700	1.72	1.53	1.16	0.51	0.54	0.93	0.59	0.87	0.62	0.71
1100	1.65	1.59	0.98	0.50	0.46	0.84	0.46	0.70	0.66	0.59
1500	1.40	1.48	0.81	0.52	0.40	0.72	0.31	0.79	0.63	0.43
1900	1.51	1.27	0.82	0.46	0.34	0.54	0.66	0.74	0.67	0.33
MEAN[a]	1.57	1.47	0.94	0.50	0.44	0.76	0.51	0.78	0.65	0.52
$LSD_{0.05}$[b]	0.14	NS	0.08	NS	0.03	0.18	0.08	NS	NS	0.11
ADPG PYROPHOSPHORYLASE										
0700	1.40	1.71	1.20	0.62	0.56	0.94	0.69	0.98	0.74	0.72
1100	1.30	1.59	1.03	0.66	0.51	0.90	0.51	0.82	0.79	0.56
1500	1.33	1.50	0.87	0.56	0.41	0.94	0.54	0.69	0.55	0.58
1900	1.38	1.41	0.90	0.55	0.30	0.75	0.67	0.70	0.80	0.40
MEAN[a]	1.35	1.55	1.00	0.60	0.45	0.88	0.60	0.80	0.72	0.57
$LSD_{0.05}$[b]	NS	0.14	0.09	NS	0.03	0.13	0.04	0.21	0.14	0.09

[a]Means are averages taken over daily cycles.

[b]$LSD_{0.05}$ represents the least significant difference at the 5% level of probability between daily times of sampling. NS, nonsignificant difference at the 5% level of probability.

Table 6. Daily patterns in sugar content of wheat kernels at various growth stages during 1985 and 1986.

Daily cycle	Days after anthesis									
	1985					1986				
	10	15	20	25	30	10	15	20	25	30
	mg sucrose g ODW^{-1}									
0700	30.1	19.3	9.2	12.0	11.4	11.3	10.2	10.2	10.2	9.1
1100	23.1	17.9	8.6	10.4	10.9	12.0	8.8	10.4	8.7	8.5
1500	25.9	16.6	9.3	11.0	12.5	12.4	10.2	9.5	9.5	8.5
1900	25.1	15.7	11.8	11.8	13.5	11.5	11.1	9.7	9.2	8.7
2400	--	16.1	12.4	11.1	12.0	10.4	8.6	8.8	9.5	9.2
MEAN[a]	26.1	17.1	10.3	11.3	12.1	11.5	9.8	9.7	9.4	8.8
$LSD_{0.05}$[b]	NS	1.6	1.2	0.8	1.3	NS	1.3	0.6	0.8	NS
	mg glucose g ODW^{-1}									
0700	8.9	2.4	0.9	1.0	0.8	2.7	1.3	0.6	0.6	0.4
1100	7.6	3.6	1.6	0.8	0.5	2.6	1.1	0.8	0.8	0.6
1500	6.6	2.5	1.7	1.1	0.8	2.8	1.0	1.2	0.7	0.4
1900	6.0	3.7	1.9	0.7	0.9	2.2	1.7	1.0	0.9	0.4
2400	--	3.6	1.4	0.8	0.6	1.3	0.7	0.7	0.6	0.5
MEAN[a]	7.3	3.2	1.5	0.9	0.7	2.3	1.2	0.9	0.7	0.5
$LSD_{0.05}$[b]	NS	0.2	0.3	0.1	0.2	0.6	0.3	0.1	0.1	NS
	mg fructose g ODW^{-1}									
0700	26.4	9.3	3.3	2.0	1.5	12.9	4.6	2.5	1.6	1.1
1100	25.5	11.7	4.9	1.7	0.8	14.0	5.0	2.9	2.0	1.0
1500	20.6	9.2	4.6	2.1	1.3	12.1	4.8	2.7	1.8	0.9
1900	19.9	10.2	4.5	1.8	1.1	10.5	5.3	2.3	1.8	0.9
2400	--	10.8	4.1	1.7	0.9	8.5	3.4	1.9	1.6	0.9
MEAN[a]	23.1	10.2	4.3	1.9	1.1	11.6	4.6	2.5	1.8	1.0
$LSD_{0.05}$[b]	5.3	0.9	0.5	0.2	0.3	2.6	1.1	0.4	0.3	NS

[a]Means are averages taken over daily cycles.

[b]$LSD_{0.05}$ represents the least significant difference at the 5% level of probability between daily times of sampling. NS, nonsignificant difference at the 5% level of probability.

Table 7. Daily patterns in starch content of wheat kernels at various growth stages during 1985 and 1986.

Daily cycle	Days after anthesis									
	1985					1986				
	10	15	20	25	30	10	15	20	25	30
	mg g ODW^{-1}									
0700	437.9	489.6	588.6	611.5	606.3	463.2	516.2	538.3	552.3	578.5
1100	442.2	507.3	587.6	596.1	580.5	498.6	516.2	553.2	577.9	565.9
1500	446.5	511.8	577.6	601.2	587.9	473.5	508.4	542.1	556.3	559.1
1900	450.9	531.0	580.1	615.3	575.4	466.1	529.6	546.8	545.0	556.1
2400	--	518.1	593.2	595.4	588.3	509.9	508.5	546.5	566.3	559.8
MEAN[a]	444.4	511.5	585.4	603.9	587.1	482.3	515.8	545.4	559.6	563.9
$LSD_{0.05}$[b]	NS	12.1	NS	9.7	NS	18.2	NS	NS	18.8	16.0

[a]Means are averages taken over daily cycles.

[b]$LSD_{0.05}$ represents the least significant difference at the 5% level of probability between daily times of sampling. NS, nonsignificant difference at the 5% level of probability.

VITA

Niels Olsen Maness

Candidate for the Degree of

Doctor of Philosophy

Thesis: HIGH TEMPERATURE LIMITATION FOR ENZYMATIC SUGAR CONVERSION IN WHEAT KERNELS

Major Field: Crop Science

Biographical:

Personal Data: Born in Nacogdoches, Texas, July 3, 1958, the son of E.G. and Kathryn Maness. Married August 22, 1981 to Nancy Eugena Cavallin.

Education: Graduated from Nacogdoches High School, Nacogdoches, Texas in June, 1976; received Bachelor of Science Degree from Texas A&M University in Agronomy in May, 1980; received Master of Science Degree from Texas A&M University in Agronomy in December, 1983; completed requirements for the Doctor of Philosophy Degree at Oklahoma State University in December, 1987.

Professional Experience: Teaching Assistant, Department of Agronomy, Texas A&M University, January, 1981 to May, 1981 and January, 1982 to May, 1982. Plant Biochemistry Laboratory Technician, Texas A&M University, June, 1982 to July, 1983. Teaching Assistant, Oklahoma State University, April, 1986. Research Associate, Department of Biochemistry, Oklahoma State University, March, 1986 to present.

Professional Organizations: Sigma Xi, American Society of Plant Physiologists, American Society of Agronomy, Crop Science Society of America.

Honor Societies: Phi Kappa Phi, Gamma Sigma Delta, Golden Key National Honor Society

Awards: Newly Elected Associate Member Award, Sigma Xi, Texas A&M University Chapter, 1985. Out-

standing Ph.D. Candidate in Agronomy Department, Oklahoma State University, 1986.