SUCROSE SUPPLY KERNEL DEVELOPMENT AND PREMATURE SPROUTING IN TRITICALE 6A190

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David W. Hatcher

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DAVID W. HATCHER

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ABSTRACT

Hatcher, David W., MSc., The University of Manitoba, October, 1982. Sucrose Supply, Kernel Development, and Premature Sprouting in Triticale 6A190. Major Professor: Dr. R.D. Hill

Developing Triticale 6A190 spikelets were cultured at 8 days post anthesis on 2.5 and 5 percent sucrose solutions and 2.5 percent sucrose with 0.04 percent glutamine to observe the effect on kernel development. A kinetic investigation of sucrose supply to the kernel at three different phases of development was also undertaken. Since Triticale 6A190 is extremely prone to premature sprouting the effect of culturing excised spikelets on 5 percent sucrose was studied to see if any differences in kernel GA_3 sensitivity could be observed.

In all sucrose culture experiments fresh weight increases matched normal kernel growth for 5-6 days while the dry weight paralleled normal seeds for 9-12 days. All cultured kernels displayed an abrupt shift in water content after 5-7 days on solution. Culturing spikelets on 5 percent sucrose did not alter the maximum rate of

respiration achieved by the kernel, 220 nmoles O_2 seed^{-1} hr.$^{-1}$, although it did shorten the period overwhich it was sustained. The sucrose levels declined from initial values in excess of 0.9 mg. sucrose seed^{-1} at days 12-14 post anthesis to 0.42 mg. sucrose seed^{-1} at day 18 before returning to normal levels above 1.0 mg. sucrose seed^{-1} at 20 days post anthesis. This trough could not be avoided by culturing excised spikelets on any of the sucrose solutions. Kernels on either of the 2.5 percent sucrose solutions appeared to reach maturity earlier and did not show a return to normal sucrose levels after the trough. The 5 percent sucrose-cultured kernels displayed continued development after overcoming the sucrose trough. No differences in glucose levels or profiles between normally grown kernels and sucrose-cultured seeds were found. The final starch content of the 5 percent sucrose-cultured kernels, 16.8 mg. starch seed^{-1}, was similiar to that of normal kernels although the deposition profile was altered. The normal seeds achieved their maximum rate of deposition 4 days earlier than the 5 percent sucrose-cultured seeds. The maximum deposition rate in all 4 seed systems was similiar. The 2.5 percent sucrose cultured kernels, with or without glutamine, matched normal seed starch contents until the period of sucrose decline and did not show any increase after this period.

Kinetic investigation of sucrose translocation into the kernel was done by measuring changes in ^{14}C sucrose

specific activities in both seed and stem sections as a function of time. The rate of translocation increased from an initial value of 0.70 ug. $min.^{-1}$ to 1.42 ug. $min.^{-1}$ during the period of sucrose decline. The translocation rate after the sucrose trough returned to 0.75 ug. $min.^{-1}$. A possible explanation for the sucrose trough is presented in relation to the kinetic data.

The studies on the effect of sucrose culturing on alpha amylase release in response to GA_3 indicated that no effect was observed as compared to normally grown kernels of the same, 45 days, age. However, if the factor of desiccation is considered, a possible decrease in alpha amylase can be observed in cultured kernels.

Culturing the spikelets on 5 percent sucrose for up to 45 days did not alleviate the problem of kernel shrivelling.

INTRODUCTION

Triticale cv.6A190 is the result of crossing Prolific rye with Stewart durum. Unfortunately this cultivar displays the undesirable features of severe kernel shrivelling and premature sprouting which have plagued numerous Triticale lines.

Salminen and Hill (1978) have speculated that kernel shrivelling may in part be the result of maximum seed volumes being established relatively early in the seed's development and that some factor at this stage causes a disproportionate volume increase relative to the rate of subsequent seed filling. They indicated that plump lines have a higher rate of dry matter increase which is sustained for a longer period of time than shrivelled lines. Examination by Saari (1977) revealed that 6A190 undergoes a dramatic decrease in sucrose concentration during the early stages of kernel development as well as displaying abnormal sucrose synthase activity. Furthermore, Hill et al (1974) have stated that 6A190 was less efficient than the plump cultivar 6531 in transporting sucrose to the head.

These findings suggest two possible reasons for the observed trough in sucrose concentrations in 6A190. Either some factor is exerting a limiting influence on the

supply of sucrose or some metabolic function within the seed is consuming sucrose at a rate approaching the plant's capabilities of supply.

The recent work of Duffus (1982) , Nicholls (1979), and Cairns and deVilliers (1982) have indicated that sucrose concentration plays an important role in the repression of premature sprouting and alpha amylase release. King et.al. (1979) have indicated that Triticale 6A190 also displays an interesting insensitivity to applied GA_3 in which a simple hormonal explanation of kernel shrivelling and sprouting does not emerge. The unusual pattern of sucrose concentrations within the 6A190 kernel may therefore be linked to it's problem of premature sprouting.

This study was undertaken to investigate the atypical sucrose levels found in 6A190 through a kinetic investigation of sucrose supply and by culturing excised spiklets on varying sucrose solutions to determine the effect on seed development and alpha amylase release.

LITERATURE REVIEW

Chloroplastic Sugar Production and Export

The supply of sugars for the developing kernel is initiated in the chloroplasts through a series of light trapping mechanisms which incorporate carbon dioxide and release a series of products into the cytoplasm. Products such as phosphoglyceraldehyde, (PGA), fructose and glucose six phosphate, (F-6P,G-6-P), fructose diphosphate, (FDP), and uridine diphosphoglucose (UDPG), have been found in the cytoplasm within ten seconds of initiating photosynthesis. (Heber 1974) Sucrose, the major transported sugar however, shows a lag phase of thirty seconds before appearing and always at a concentration which is greater than that found in the chloroplast (Heber 1974).

The fact that all of the previously mentioned sugars except PGA have been shown to be incapable of penetrating the chloroplastic membrane (Heber 1974) has led to the following proposal for the formation of sucrose in the cytoplasm of photosynthetically active mesophyll cells (Heber 1974). Upon exposure to light, dihydroxy acetone phosphate,(DHAP),glyceraldehyde phosphate,(GAP), and FDP levels increase in the chloroplast with an accompanying

light activation of fructose diphosphatase and other enzymes in the carbon reduction mechanism. The increased enzyme activity raises the level of ribulose diphosphate, RuDP, which acts as the carbon dioxide receptor and therefore enhances the incorporation of CO_2 until a steady state is achieved. The DHAP is then exported from the chloroplast via a special transport mechanism located on the inner membrane known as the phosphate translocator. The cytoplasmic DHAP is rapidly equilibriated with GAP through the action of triosephosphate isomerase allowing the two trioses to be coupled through the action of an aldolase to form cytoplasmic FDP. A cytoplasmic fructose diphosphophosphatase, exhibiting a different pH profile from it's chloroplastic counterpart, then converts the diphosphate to the corresponding monophosphate sugar. The cytoplasmic fructose diphosphophosphatase is AMP inhibited allowing it to be active in the light and inactive during periods of darkness when it could seriously interfere with phosphofructokinase of the glycolytic pathway. The FMP is rapidly converted to UDPG through a series of enzyme reactions involving UTP and pyrophosphate liberation. The UDPG then acts as a glucose donor reacting with FMP to form a phosphorylated sucrose molecule through the action of the sucrose phosphate synthetase. The phosphate group is quickly removed via a cytoplasmic phosphatase to yield the free sucrose which is now available for export.

Huber (1981) has shown that 53 % of wheat protoplasts'

photosynthetic capability is directed towards sugar production while only 17 % was diverted to starch synthesis. Differences in percentage allocations were seen within wheat varieties but were generally small when compared to differences within species. High yielding varieties partitioned less carbon to starch and had a higher sugar to starch ratios. Values of 4.5-6.4 to 1 led Huber to suggest that the distribution of end products may be under biochemical control within the mesophyll cell, possibly via the phosphate translocator.

Examination of the translocated sugars from various sources reveals that although sucrose is the prime sugar, and verbascose the largest, all have sucrose as their backbone and differ only in the number of attached galactose residues. Arnold (1968) has postulated that sucrose has evolved as the major mobile energy source due to a series of selective pressures acting over an evolutionary time scale. The nonreducing character and the protection of the glucose residue through derivitization are submitted by Arnold to be the key selective pressures involved.

Sucrose Passage From the Mesophyll to the Phloem

Canny (1973) has suggested that the limitation to movement of photosynthate does not lie in the phloem

itself but rather at either the phloem loading sites or the diffusion of material to these sites. Passage through the extracellular free space (the apoplastic route),as well as transport through the protoplasts and plasmodesmata,(symplastic transport), have been advocated. Current data suggests a combination of both routes with the relative importance of each not yet being fully understood (Geiger et. al. 1974,Geiger 1975, Giaquinta 1976,1977a). Recent work by Lee et al (1980) suggests that an interveinal sucrose gradient does exist in the wheat leaves from the mesophyll cells towards the phloem. Lee and coworkers calculated a flux rate based upon carbon fixation and sucrose accumulation to be 14.16 pmoles per cm^2per sec. This value is very similiar to that determined by Geiger (1974) of 16.12 pmoles per $cm.^2$ per sec. for the translocation of sucrose in sugar beet leaves. Lee et al also calculated a flow rate based upon Fick's equation, which assumes that the total crossectional area of the leaf/ cell is available for sucrose movement, to be 3533 pmoles per $cm.^2$per sec.. Expressing the 14.16 pmoles $cm^{-2}sec.^{-1}$ as a percentage of the value derived from the Ficks equation yielded 0.04 % which has particular significance as it is the same value that Tyree (1970) had calculated to represent the percentage of cell wall area to be involved in symplastic transport. The involvement of the apoplast, initially suggested by Kursanov and Brovchenko (1969) has been confirmed by

Geiger et al (1974) by intercepting translocating materials by flushing the apoplast. They demonstrated that as the rate of photosynthesis increased there was a corresponding rise in the amount of material entering the apoplast.

Phloem Anatomy

The phloem is that tissue in more highly organized plants which is characterized by the posession of certain specialized cells, the sieve elements, and which functions as the major channel of rapid conduction of sugars over long distances (Lamoureux 1975). In addition to sieve elements, phloem tissue contains a variety of specialized parenchyma cells which work in conjunction with the sieve elements to facilitate long distance transport.

The sieve element is an elongated cell with areas on it's wall which contain pores that maintain cytoplasmic connections with adjacent cells. At functional maturity sieve elements have undergone partial autolysis of the protoplast and typically lack intact nuclei,tonoplast, and dictyosomes. However, the plasmalemma remains intact and endoplasmic reticulum, mitochondria, and plastids are still present. P-protein, a characteristic component of dicotyledenous sieve tube members has been reported lacking in the sieve tubes of Zea mays, (Walsh and Evert 1975) Hordeum vulgare, (Evert et al 1971) and Triticum

aestivum, (Kuo et al 1972). Sieve elements typically elongate parallel to the axis of the plant organ and the walls , although similiar to those of young parenchyma initially, noticeably thicken once differentiation begins. The sieve elements often display a pearly lustre on the inside of the cell wall which is referred to as the nacreous wall (Lamoureux 1975). The nacreous wall is however absent in the sieve areas. The sieve element's walls are composed of cellulose, polyuronides, and pectins with the relative proportions of each varying with development, hydration, and species. Lignin is absent from these walls (Lamoureux 1975).

Sieve areas are those parts of the sieve element wall containing the pores which keep the protoplasts of adjacent elements and parenchyma cells in contact. By definition, sieve area refers to the structure of the wall in one cell and the junction of two sieve elements should be considered as being through a sieve pair. Usage of the term sieve area has been by convention allowed to describe either a single wall or pair (Lamoureux 1975). Pores in a sieve area can range from less than 1 micron to 15 microns in diameter with the larger pore thought to represent a higher degree of evolutionary specialization (Lamoureux 1975). Cell pores within a given sieve area are usually the same diameter. Each pore appears to develope at a site where a plasmodesma was present at an earlier stage. The pore

is lined with the B 1-3 glucan, callose. The amount of callose associated with a sieve area varies considerably although these deposits increase as the sieve element reaches the end of it's functional life.

The term sieve tube member refers to one of a series of sieve elements arranged end to end interconnected through specialized sieve areas known as sieve plates. Sieve plates have larger pores than those found in the sieve areas on the lateral cell walls and provide a more complete interconnection between sieve tube members (Esau 1977). The improved connection between sieve tube members allows for enhanced longitudinal conduction. The sieve tube members are thus connected end to end to provide a functional conducting unit known as sieve tubes in the phloem. It's counterpart in the xylem is the vessel.

Companion cells, restricted to the phloem tissue of angiosperms (Benke 1975), are defined as parenchyma cells derived from the same mother cell as the sieve element. The companion cell remains functional for the lifespan of the sieve element. In general, companion cells resemble secretory cells in their ultrastructure and their ability to deliver sugar into a conduit against a gradient suggests a secretory role (Esau 1977). These cells are associated with sieve tube members and the primary pit fields on their walls display characteristic connections with sieve areas on the sieve tube members lateral walls (Lamoureux 1975). Each pore on the sieve

element is connected with several branched plasmodesmata of the companion wall with a median cavity occurring between the walls at their junction (Lamoureux 1975). The connection between companion cell and sieve element is a peculiar structure not seen elsewhere and reflects the difficulties of joining the normal protoplast of the companion cell with the hydrated protoplst of the sieve element (Benke 1975). The numerous plasmatic channels which penetrate the companion cell wall are each 50-60 nm. wide and are lined by plasmalemma (Benke 1975). Endoplasmic reticulum is found close to the entrance of each branch and endoplasmic reticulum-like membranes are found within the median cavities. Endoplasmic reticulum is also seen next to the sieve pore and callose deposits have been observed in both companion cells and sieve elements near their junction (Benke 1975).

The companion cells contain ribosomes, endoplasmic reticulum, and abundant mitochondria. Their abundance supports the concept of considerable metabolic activity occurring in these cells (Benke 1975). The mitochondria are elongated in contrast to the prevalent spherical sieve element mitochondria, and have well developed invaginations of the inner envelope (Benke 1975). In cytochemical studies on the distribution of ATPase activity in the phloem of Cucurbita, Gilder and Cronshaw (1973[a]) observed specific regions of ATPase activity in the wall region between the sieve element and companion

cell of minor veins. Salisbury and Ross (1978) suggest that companion cells, which are considerably larger than sieve elements in minor veins (Esau 1965), may function in phloem loading in the leaves. Most sieve tube members have one or more companion cells associated with them, either singly, or in a longitudinal series connected with each other and other parenchyma cells through the plasmosdesmata (Lamoureux 1975).

Transfer cells are specialized parenchyma cells with internal cell wall protuberances and are thought to be involved in short distance translocation between mesophyll and phloem,and xylem and phloem. Gunning and Pate (1969) classified transfer cells into four different types of which two, type A and B are associated with phloem (Evert 1977). Type A are those associated with sieve elements and have a uniform pattern of cell wall ingrowths and specific connections with the sieve element. As well, they display well organized mitochondria, ribosome rich cytoplasm, dense nucleus, and are believed to be modified companion cells (Gunning et al 1968). Type B however are thought to be modified phloem parenchyma and display wall ingrowths only in the area where it's wall abuts the sieve element. Type C and D are both located in the area of vessels and tracheids.

The wall ingrowths multiply the surface area of the plasmalemma by as much as 20 fold (Gunning et al 1970).

Two main roles have been proposed for the transfer cells of minor veins; to collect and pass on photosynthates, and to retrieve and recycle solutes that enter the leaf apoplast in the transpiration stream (Evert 1977). Pate and Gunning (1972) suggest the wall membrane apparatus of the transfer cell to be an adaptation facilitating apoplast-symplast exhanges of solutes across the plasmalemma. The localization of ATPases next to the wall ingrowths support their involvement in active transmembrane transport of solutes (Maier and Maier 1972). However, minor vein transfer cells are almost entirely restricted to dicotyledons and within this group are confined mainly to herbaceous species (Pate and Gunning 1972).

Kuo et al (1972) studying the transverse veins of wheat leaves revealed that they consist of a single sieve tube and a single vessel each of which is accompanied by one or more parenchyma cells. Blackman (1971) states that these phloem associated parenchyma cells are ontogenetically companion cells. The transverse veins lack both a mestome sheath and a parenchyma sheath except near their junction with longitudinal veins where they become encased in parenchyma cells (Kuo et al 1972). In this area of continuity the mestome sheath of the longitudinal vein is modified, consisting of shorter and wider cells arranged around a "gap" through which the transverse vein penetrates (Kuo et al 1972). Further investigation of the longitudinal veins of wheat by

Kuo et al (1974) revealed that the transverse veins which lack a mestome sheath play only a minor role in sugar uptake, loading no more than approximately 10 % of the leaf photosynthate. They concluded that only the symplastic pathway is the possible route for assimilate movement across the mestome sheath in wheat. Their studies revealed that most plasmodesmata occur in the mestome sheath where it's cells abut the metaphloem and are absent in the region of xylem vessels. Furthermore, 85 % of these connections are to late maturing intermediate bundles which is in agreement with the structural and physiological changes reported by Fellows and Geiger (1974). Fellows and Geiger indicate that phloem loading and sieve tube maturation are preparatory steps to photosynthate export and that these changes occur initially at the leaf tip and spread basipetally.

Phloem Loading

Phloem loading is the process by which the major translocated species are selectively and actively taken up by the sieve tubes, espescially in the minor veins of source regions (Geiger 1975). Each phloem mobile substance does not require a carrier system for entry, as solutes entering via passive diffusion can be translocated in significant quantities if a sufficient concentration gradient towards the phloem exists (Geiger 1975).

Geiger suggests that from energetic considerations, at least one solute must be actively loaded to provide the osmosticum for a mass flow mechanism based upon a water flux down a potential gradient.

Giaquinta (1976) was able to demonstrate through the use of the nonpenetrating sulfhydryl reagent, PCMBS, that a surface carrier protein, located in the plasmalemma, was involved in phloem loading. He suggested that sugars travel in the symplasm through the mesophyll and enter the apoplast at the phloem region. He further submitted that the apoplastic water surrounding the phloem, which is not in direct communication with the transpiration stream,(Gunning et al 1974) would not be an obstacle to solute uptake. Giaquinta's work of 1979 revealed the involvement of a proton cotransport system for sucrose loading powered by a membrane ATPase in sugar beet leaves. This concept has received further support by studies done on broadbeans,(Delrot 1981,Delrot and Bonnemain 1981, Delrot 1980) Ricinus,(Komor et al 1980) maize scutellum,(Humphreys 1978) castor bean cotyledons, (Hutchings 1978) and soybean cotyldeons (Lichtner and Spanswick 1978,1979).

The model proposed by Giaquinta (1980) has sucrose entering the free space near the phloem region and interacting with a specific sucrose carrier protein containing an essential sulfhydryl group. The transport of solute across the sieve element-companion cell complex membrane is

in response to an electrochemical proton gradient established by a vectorial proton translocating ATPase in the phloem membrane. Thus the sucrose transport into the phloem is "secondary" to the proton movement. The high potassium levels in the phloem are attributed to either a passive influx of K^+ ions in response to the negative potential created on the inside membrane by the proton pump or by a proton potassium carrier mediated exchange.

Two models which are variations of proposed neutral solute transport across fungal and bacterial membranes have been suggested by Giaquinta (1980) to describe the manner of interaction between the energy transducing system and the electrochemical gradient. The first model suggests that sucrose bond with an uncharged carrier to form a neutral complex which is then protonated by an apoplastic proton on the outside surface of the membrane. The positively charged complex then crosses to the inner surface where it releases the proton before unleashing the sucrose molecule. The second model is similiar except that the carrier is initially negatively charged and must first be protonated to form the uncharged complex before allowing sucrose to bind. Subsequent movement to the inner surface and sucrose release are required before the proton can be released and the negatively charged carrier regenerated. The work of Amar et al (1978) and Copps et al (1976) in which proton concentrations and electro-

chemical gradients have caused vertical and lateral displacement of proteins, in conjunction with proposed conformational changes related to energy transductions in chloroplasts,(Dilley and Giaquinta 1976) lend support to these models. Giaquinta (1980) suggests that it is conceivable that the proton gradient across phloem membranes causes conformational changes in the membrane related to sucrose loading.

Giaquinta (1980) was able to demonstrate that the phloem loading mechanism could be controlled by internal sieve tube sucrose concentrations. High internal sucrose levels noticeably altered the rate of sucrose entry into the phloem although both non-metabolizable 3-O-methylglucose and the osmoticum mannitol in high internal concentrations did not effect the rate. The effect of high internal substrate concentration is believed to prevent dissociation of the sucrose-carrier complex at the inner surface, thus reducing the amount of "free" carrier at the outer surface.

The question of the sucrose/H^+ co-transport system being energetically feasible is still under review in terms of the electrochemical proton gradient being the sole driving force. Komor (1977) working with castor bean cotyledons showed that a pH change of 1.5 units could drive a 100 fold increase in sucrose. However, Guy et al (1981) working with Pisum sativum have indicated that protonation alone is not sufficient to convert the sugar

transport system to it's fully activated, high affinity form. They suggest that a further light dependant factor, acting synergistically with protonation, may be required.

Tranlocation Studies

The lack of a quantitative description of translocation is at least in part due to the complexity of the process (Minchin and Troughton 1980). Initial studies of translocation rates undertaken by Mason and Maskel (1928) were able to demonstrate that the sucrose concentration decreased exponentially as a function of distance from the source. They also observed that the sucrose flux was linearily related to the sucrose concentration gradient. Utilizing values derived from their experiments they calculated a diffusion coefficient for sucrose which was 40,000 times greater than that of sucrose in water and which must be accounted for in any current theory of phloem translocation (Minchin and Troughton 1980).

Biddulph and Cory (1957) summarized the earlier studies on the velocity of transport in sieve tubes as being of three types. These were: calculations of downward velocity derived from the mass of material transported per unit time through a cross-sectional area (Dixon and Ball 1922); inferred velocities obtained from calculations based upon increases in the concentration of translocates at

specific distances after definite time intervals (Day 1952); and finally, the direct detection of a substance introduced at one point and recovered at another after a set time interval (Vernon and Arnoff 1952). It is interesting to note that the values calculated by Dixon and Ball (1922) of 50 cm./hr. caused a temporary rejection of the phloem as a transport pathway as it was felt that such a high velocity was incompatible with phloem tissue (Biddulph and Cory 1957).

Early investigations of mass transfer rates were calculated through dry weight gains in fruits and tubers. (Minchin and Troughton 1980). This form of study yields only average values calculated over long time periods and does not reflect any short term variations. However, Geiger and Sovonick (1970), utilizing in vivo independant measurements of mass transfer rates and transport speeds via $^{14}C0_2$ pulse label tracers under conditions of petiole cooling, were able to demonstrate that these two parameters were highly correlated.

The use of radioactive tracers in velocity studies was investigated in the 1940's by Biddulph (1941) on the diurnal migration of phosphorous in bean leaves. Since that time there have been numerous studies undertaken on a variety of plants using a wide range of radioactive isotopes. The use of radioisotopes in phloem translocation studies can be grouped into two broad categories (Canny 1975). These are: distance moved by a specified level of tracer activity in

a known time or, the time required for a specified level of tracer to travel a known distance. Fisher (1975) points out that there are three major factors effecting the kinetics of movement of labelled photosynthates. These are: the loss of tracer from the translocation stream, the range of translocating velocities, and finally, the rate at which labelled material enters the translocation pathway.

Biddulph and Cory (1957) were able to undertake a critical comparison of the translocation rate of three tracers simultaneously through the detection of individual isotopes in successive bean stem segments. The velocity of each tracer was calculated from the total distance each had moved from the point of application, the leaf epidermis, in a fifteen minute period. The three isotopes were applied simultaneously by a spray procedure of tritiated water containing ^{32}P phosphate and in a $^{14}CO_2$ environment. An extrapolated distance was not used in the calculation as the activity within the last segment was near the limits of detection. Utilizing this technique they derived velocities of 87 cm./hr. for the tritiated water and ^{32}P while ^{14}C had a velocity of 107 cm./hr. These values were noted to include the time required for movement into the phloem. The differences in the velocities were stated to lend support to the proposal by Vernon and Arnoff (1952) and Swanson and Whitney (1953) of independant velocities for simultaneously moving substances.

Moorby et al (1963) pointed out several advantages for

the use of ^{11}C tracers in plant studies. They indicated that ^{14}C suffers from the disadvantage of being a weak B particle emitter and as such can be assayed only after processing the plant material. As well, the prolonged half-life of ^{14}C makes it impossible for the plant to be used in more than one experiment. They argued that the high emissions of ^{11}C allow it to be counted in vivo and due to it's short half-life,20.4 mins., repeated experiments with the same plant can be undertaken. Utilizing this technique Moorby et al exposed a soybean leaf to $^{11}CO_2$ and counted the radioactivity at four different positions on the stem with external scintillation counters. They then plotted radioactivity versus time for each of the four positions. Extrapolation of each of these lines to zero radioactivity gave the exact time of appearance for a known distance and allowed an average velocity to be calculated.

In 1965 Geiger and Swanson utilized $^{14}CO_2$ as the source of label and in a pulse label system measured the radioactivity appearing over time in a separate immature sugar beet leaf which was acting as a photosynthetic sink. The plot of the levels of radioactivity in the sink with time allowed the rate of entry to be calculated using the first derivative of the radioactive accumulation time profile.

Fisher (1970) fed soybean plants $^{14}CO_2$ and measured the radioactivity as a function of distance down the stem.

He plotted the log of the radioactivity versus distance for the various time periods after exposure to the labelled CO_2 yielding a series of curves that did not significantly differ in appearance. Velocities were calculated by determining the extrapolated distance travelled at log cpm equals zero. This value was then divided by the time at which the plot was determined and yielded an average of 0.84 cm./min.

A variety of other modifications of these experiments have been undertaken to determine translocation rates in other plants. Some novel approachs include Ford and Peel (1967) who utilized aphids placed at various distances down the stem. This allowed them to overcome the processing problem by simply analyzing the honeydew excreted by the aphids. Van Die et al (1973) used the systematic bleeding of the Yucca plant to measure ^{14}C in the exudate with time at a known distance.

The Vascular System of an Ear of Wheat

Sucrose moves to the developing kernel through the vascular system of the ear. There are no apparent differences in terms of vascular arrangement along the rachis. Evans et al (1970) have found that each spikelet added one major vascular bundle to the complement of bundles

within the rachis. However there is no simple relationship between the number of vascular bundles and the number of spikelets (Evans et al 1970). There are, on the other hand,distinct differences in the vascular system supplying individual florets within a spikelet (Hanif and Langer 1972). The segment of the rachilla at florets one and two is much shorter and broader than the other rachillae on the spikelet and their unique structure is thought to put these florets in an advantageous position. Hanif and Langer have shown that the first three florets are supplied by principal vascular bundles while the more distal florets receive material through a subvascular system attached to the bundle supplying the third floret.

Serial and longitudinal sections of the attachment region of the grain to the rachilla reveals that the traecheary elements of the pericarp are not in direct continuity with those of the rachilla (Zee and O'Brien 1970). The traecheary elements of the pericarp bundle and those of the rachilla are separated by an area of thick walled cells which form a central cylinder. The core is surrounded by numerous sieve elements and transfer cells and extends up to 0.5 mm. into the kernel. It meets the vascular bundle of the pericarp, lying in the crease, at the base of the coleorhiza. The core of cells provides a barrier for the movement of solutes from the rachilla to the grain via the free space transport. Zee and O'Brien suggest that these cells are modified tracheary elements whose different-

iation is abnormal and whose net effect is to severely limit the flow of solution to the pericarp bundle. The role of this core area, as it is seen in ryegrass, oats, and bromegrass, may be in aiding solute transfer to the sieve tubes which extend the entire length of this region.

The vascular bundle running the entire length of the crease region is the sole source of nutrients to the kernel (Frazier and Appalanaidu 1965). There are fewer xylem elements than phloem, with the xylem being heavily lignified at the base of the kernel. The number of xylem elements decrease towards the apex of the kernel while the phloem is extensively developed along the entire length (Frazier and Appalanaidu 1965). The route of nutrient movement has been shown to be up through the vascular bundle and then moving across the chalaza and out into the kernel through the transfer cells of the nucellar projection (Frazier and Appalanaidu 1965, Hughes 1976).

Zee and O'Brien (1970) suggest that the control mechanism which regulates the growth of the developing grain is more likely to be associated with the differentiation of the cells of the pigment strand than with changes within the cells of the attachment region. They have shown that the pigment strand undergoes a series of changes throughout the kernel's development.

Initially, up to twelve days after anthesis, these cells do not appear to be different from the adjacent parenchyma cells of the pericarp and numerous plasmodesmata

traverse the cell walls. There is free passage of water and solutes from the vascular bundle to the endosperm via either symplastic or apoplastic routes. Dramatic changes occur during days 12 to 18 as the cell walls thicken and develop pronounced pit fields in which lie plasmodesmata. At this time an adcrusting substance,composed of lipids and polyphenols appears on the wall areas which lie outside the pit fields. The appearance of the adcrusting material sharply reduces apoplastic movement and most solutes pass through the plasmodesmata. The adcrusting substance continues it's spread into the pitfields and slowly limits the symplastic network. There is an accompanying degeneration of the cytoplasm of the cells at this time. Finally the pigment strand cells are crushed,forming a solid core of cells each of which is enclosed by a lignified wall. The passage of water and nutrients to the grain is severely restricted although there is some space between the crushed cells which allows water to enter for germination.

The method for the movement of sugars into the endosperm through the aleurone layer is currently under debate. Bechtel and Pomeranz (1977) and Morrisson et al (1978) report no transfer cells in the aleurone layer of wheat, while Ayre and Angold (1979) have published micrographs of the wheat kernel's crease region aleurone cells showing wall ingrowths characteristic of transfer cells. However, once in the endosperm, the sucrose moves apoplastically

down a concentration gradient and is rapidly taken up by the cells and converted to starch (Jenner 1976).

Sugar Distribution Within the Ear

The carbon in the kernel is largely derived from assimilate accumulated by the ear, flag leaf, and the green stem immediately below the ear (Carr and Wardlaw 1965). A direct relationship exists between the weight of the grain per ear and the photosynthetic area above the flag leaf node (Simpson 1968). Export from the flag leaf is distributed in equal amounts above and below the node of insertion although material moving downward does not pass the second internode (Wardlaw and Porter 1967). Sugar concentrations increase in the stem internodes until a maximum is reached a few days after anthesis (Wardlaw and Porter 1967). A decline from this maximum then occurs until at maturity little or no material remains (Wardlaw and Porter 1967). The concentration of sucrose within the phloem is considered to be approximately 10 % (Evans et al 1970). Studies on the patterns of evolutionary development have shown that ears of wild diploid progenitors of wheat may be solely self supporting but with evolutionary advances there has been a marked increase in the amount of assimilate that is translocated through the stem to

meet the needs of kernel growth (Evans et al 1970). Evans and his coworkers (1970) have shown that modern wheats have larger stems, more vascular bundles, and that the maximum rate of import of assimilate per ear per day is greatest in modern hexaploids. They state that this increase is a direct function of the increased phloem area and have calculated a specific mass transfer rate of 3.3 g. per cm.^{2} of phloem per hour. Although the photosynthetic capacity of the flag leaf slowly declines after anthesis,the capability of the ear to incorporate carbon dioxide increases dramatically over days 4 to 15. In awned varieties the photosynthesis achieved by the ear was nearly twice that of non-awned varieties (Carr and Wardlaw 1965).

The partioning of photosynthate between various organs of a plant has a pronounced effect on it's growth and development. The distribution of assimilate is governed by both the strength of the sink and it's proximity to the source although the size of the sink may exert more than a proportional influence as compared to distance (Cook and Evans 1978). Cook and Evans have shown that a large sink biased the partitioning of assimilate under all conditions. However, the strength of the bias was strongly influenced by the degree of photosynthesis occurring in the ear. The preferential supply of larger sinks over smaller was most visible when distant sources, under photosynthetic stress, were the suppliers (Cook and

Evans 1978). The work of Cook and Evans also indicated there was movement of assimilate between spikelets during stress periods. A common feature of a mature ear of wheat is that the central spikelets are larger than those in the basal region which in turn are larger than those found in the upper spikelets (Brenner and Rawson 1978). Work done by Rawson and Evans (1970) indicates that the central spikelets may have a greater advantage for the import of flag leaf photosynthate and that the supply of material becomes progressively more limiting as it approachs the terminal spikelets. The partial sterilization of the central spikelets, causing increased grain weight in both areas above and below this region, led them to speculate that the early differentiation of the central spikelets over the others allows them to compete more effectively for flag leaf photosynthate. However, Radley and Thorne (1981) have shown that if the kernels are removed from the developing ear the effect on the final grain weight is highly dependent upon variety. Certain varieties will respond with an increased weight while others show a definite decline.

Within a spikelet, grain weight increases from the basal floret to the second floret and then steadily declines towards the apical florets. Studies on the effect of position within a spikelet on assimilate supply revealed that a large sink requirement was needed in order to maximize grain growth on any floret, espescially in the

central region of the ear (Brenner and Rawson 1978). However, when assimilate supply was slightly reduced, all florets displayed a decrease in grain weight although severe reductions seemed to effect kernels in the third or more apical florets to a greater extent. Weight loss was observed in the two basal florets but it was equally divided and displayed no distance effect. The more apical kernels however revealed an increasing weight loss with distance. The key to the regulating mechanism is as yet unknown but the nature of the subvascular bundles supplying florets four and five (Hanif and Langer 1972) are suspected to be involved. Brenner and Rawson (1978) have postulated that the vascular system within a spikelet is physically adequate to support high growth in all grains. They believe the maintenance of a sufficient assimilate gradient to overcome the resistance offerred by the vascular tissue to the more distal florets is a major factor effecting the growth in the intact spikelet.

The maintenance of sugar gradients to the developing kernel has been studied extensively in liquid culture (Jenner and Rathjen 1972,1977,Jenner 1970,1973). Detached ears of wheat grown on a 2-80 mg./ml. solution of sucrose displayed a pattern in which from 2-30 mg./ml. the level of sucrose in the grain increased and remained level till 50mg./ml at which time the level of sucrose diminished. Jenner (1970) was able to demonstrate that at an external concentration of 25 mg.ml. the kernels had an internal

concentration of 16.7 mg./ml. water in the seed, which is very similiar to that found in normal controls. The relationship seen in early developing cultured plants between external sucrose levels and internal concentrations was maintained as the plant matured although by 15 days post anthesis the relationship only extended to those plants on 20 mg./ml. Higher concentrations evoked no increase in the levels within the kernels. However, studies done on the sucrose content of the floral organs and rachis indicated a strong corellation between internal sucrose concentrations and that of the external culture medium over the range of 0-100 mg./ml (Jenner 1970). It has been postulated that the photosynthate entering the ear is transported via the transpiration stream to the floral organs where the water is lost through transpiration (Jenner 1968). The solutes then accumulate forming a large concentration gradient at the base of the kernel. This proposal is supported by the fact that as compared to ear cultures, culturing individual spikelets on sucrose reduces by 50 % the amount of starch synthesized. Removal of the glumes causes an additional 50 % reduction in starch production as compared to spikelet culture or a 75 % reduction as compared to excised ears (Jenner 1968).

Canny (1973) has proposed that metabolic energy is required for the transportation of sugars. This proposal has been substantiated by Jenner (1976) through the use of 0.2 mM iodoacetate which depressed the levels of sucrose

within the kernel of cultured ears without effecting the concentration in the rachis. The use of higher concentrations of iodoacetate, 1.0 mM , significantly depressed the sugar levels in both the rachis and the kernel yet exerted no effect on the levels found in the floral organs. The differing response seen in the kernel and rachis versus the floral organs was also evident when respiratory inhibitors,dinitrophenol or sodium fluoride, were incorporated into the culture medium. Jenner postulates that the sucrose accumulated in the floral organs by the transpiration stream, is loaded into the phloem for transport into the grain by a series of enzymatic reactions, one of which displays saturation kinetics. He also states that the sieve tube loading may be effected by these inhibitors (Jenner 1976).

Factors such as size and state of cultured ear, temperature, relative humidity, and the air velocity over the ear would all be expected to influence the degree of sucrose gradient formed. Jenner further states that the critical section for transporting sucrose into the kernel is common to both cultured and naturally grown ears.

There is a divergence of opinion as to the actual state in which sucrose enters the grain. Sakri and Shannon (1975) propose that sucrose hydrolysis is a prerequisite for the sucrose movement from the phloem into the endosperm. The hydrolysis is thought to be caused by a cell wall invertase and isomerization is avoided as the necessary

enzymes are found only in the cytoplasm. They also imply that the movement of the sugar through the apoplast is in the form of phosphorylated hexoses. Opposition to this proposal is headed by Jenner (1974) who recognizes the existence of the extracellular invertase but argues that this enzyme is not a regulating factor in the entry of sucrose. He however does concur on the possibility of phosphorylated hexose involvement and suggests that these sugars can be interconverted. He has also been able to demonstrate that although neither glucose or fructose inhibit the uptake of sucrose, both are taken up 45 % faster than sucrose (Jenner 1974).

Jenner's (1979) study on the effect of shading for brief periods immediately after anthesis has demonstrated that shading for up to ten days severely effects both the size and weight of the grains although no effect is seen if the shading is stopped after the first five days. Asna (1969) has shown that shading up to fifteen days after anthesis can influence grain development, but after this period there is no appreciable influence. Surprisingly, even though shading resulted in lighter grains, there was no difference in the amount of protein in shaded versus non-shaded plants at either ten days after anthesis or at harvest. The effect of shading on the concentration of sucrose in the rachis was highly dependant upon variety. Jenner (1979) was able to demonstrate that although sucrose levels fell during shading, the response upon illumination varied

from no difference to a continuing increase in concentration while controls of the same variety continued to decline. Jenner (1979) suggests that there is a mechanism influencing the capacity of the grain to accumulate dry matter. He postulates that since protein content is not effected by shading while sucrose content and starch deposition are, that either two separate mechanisms exist for controlling capacity or that accumulation of starch and protein are independently controlled.

Starch Formation and Characteristics

The granules of starch formed in cereals have characteristic shapes and sizes which vary with species (MacMasters et al 1957). Wheat contains starch granules which are small and spherical to those which are large and lens shaped. Although starch granules have been observed in the endosperm as early as 4-5 days after anthesis, granules within the pericarp have already reached their maximum size by this time. However, as the kernel grows, most of the material within the pericarp is hydrolyzed before the grain reaches it's maximum length. The large lenticular shaped granules are the result of granules formed during days 1-15 and are known as type A (Klassen 1970). The smaller, spherical granules, which begin to appear between days 18-30 are known as type B and tend to

fill in the spaces left by the larger granules (Klassen 1970). The type A granules contribute more than 90 % of the total volume yet type B accounts for 88 % of the total number of granules by day 36 (Klassen 1970). Jennings and Morton (1963) reported that the starch content per endosperm increased rapidly and almost linearily over the time period 12 to 35 days post anthesis. The amylose content of wheat starch has been found to range from 23.4 to 27.6 % (Metcalf and Gilles 1963).

The work by DeFekete el al (1963) and Recondo and Leloir (1961) demonstrated that ADP-glucose and UDP-glucose had the ability to donate a glucose moiety to starch although the ADP-glucose transfer occured at a faster rate. These findings led to the acceptance of ADP-glucose -α-1,4,-glucan 4-α-glucosyl transferase (starch synthase) as the enzyme responsible for starch synthesis in plants (Preiss et al 1973, Turner and Turner 1975). Although starch synthase is the major catalyst of alpha 1,4 linkages, the enzyme starch phophorylase has also been shown to be capable of transferring a glucose moiety from glucose-1-phosphate to an existing glucan (Pontis and Salerno 1980).

Turner (1970) has proposed a pathway for the production of starch in wheat which is still generally accepted. The key enzymes in the proposed pathway are uridine diphosphate glucose pyrophosphorylase and particularily adenosine diphosphate glucose pyrophosphorylase. These enzymes both display very large increases in activity during periods of

maximum starch deposition although UDPG pyrophosphorylase activity is always considerably greater than ADPG pyrophosphorylase by a factor of ten. Turner proposes that the key for regulation of starch synthesis is supplied by ADPG pyrophosphorylase. This proposal is strongly supported by the work done by Kumar and Singh (1981). They suggest 3-phosphoglycerate to be the primary product of immature green wheat pericarps which are capable of photosynthesis. While the pericarp is still photosynthetically active, it will supply the endosperm with 3-PGA which has been shown to activate ADPG pyrophosphorylase (Dickinson and Preiss 1969) thus causing an increase in starch deposition. This proposal would explain why starch production declines with ripening even though adequate assimilate supply is available. Further support for this theory is seen in the work of Jenner and Rathjen (1977) in which cultured endosperms (peeled grains) absorb progressively more sucrose as their age increased indicating that there was no reduction in the permeability or absorptive capacity of the endosperm associated with ripening. However, they did find that cultured endosperm stopped producing starch well before normal controls. These trends would support the concept of an alteration in the metabolic activities within the grain with maturity. Jenner and Rathjen (1977) feel that the metabolic activities associated with the accumulation of dry matter could be regulated by ontogenetic factors engendered internally

rather than simply responding to signals from other parts of the plant.

Investigation by Saari (1977) of various Triticale lines and wheats have indicated that the enzyme sucrose synthase, which is required in Turner's proposal for the initial conversion of sucrose to UDP glucose, also undergoes a dramatic increase in activity over the period of considerable starch formation. Hexokinase however, which is responsible for the conversion of fructose to fructose-6-phosphate, shows only a steady increase in activity with maturity and has been proposed to be more involved in the supply of energy through the glycolytic pathway (Saari 1977).

Liquid Culture

Short term experiments of culturing detached ears of wheat in mediums containing amino acids and sucrose were undertaken by Graham and Morton (1963) and Jenner (1970) but longer periods of culture were hampered by the problem of microbiological contamination of the medium. Millerd et al (1975) were able to culture peas for a maximum of five days and all plants displayed continuing growth. Donovan and Lee (1977) developed a method of culturing detached ears of wheat for twelve days with normal kernel development. Their culture media consisted

of $CaCl_2$, $KHPO_4$, $MgSO_4$, thiamin, myoinositol, and an amino acid mixture containing all twenty-one basic acids. The plants were then placed in the medium for twelve days starting at the eigth day after anthesis. Cultured grain development parallelled field grown material in terms of dry weight, nitrogen, starch, and protein. In media deficient in either sucrose and or amino acids the grain's weight development proceeded at a slower rate. Protein synthesis occurred in media with or without sucrose present although starch synthesis was inhibited by the absence of amino acids. Donovan and Lee's work of 1978 demonstrated that normal development could be achieved if the nitrogen source was supplied either as the complete twenty-one amino acids or as glutamine, asparagine, or ammonium nitrate. They also found that although no differences in kernel dry weight or starch accumulation occurred when the nitrogen source was increased relative to the sucrose, the nitrogen level per grain and percent nitrogen did increase.

Lesar and Peterson (1981) were able to successfully culture excised oat panicles in liquid medium for twelve days as well. Their data revealed that the rate of grain filling was near normal when the sucrose supply was maintained at 55.6 mM but at 13.9 mM it was insufficient to sustain the normal growth rate. They stated that an excess of supply does not increase the rate of grain filling above the normal limits although the premature drying of glumes and flag leaf during the latter stages of culture,

days 9-12, could influence the transpiration stream. Lesar and Peterson concluded that liquid culture technique appears to be a viable method to study the effects of supplied substrates on grain composition.

Alpha Amylase Characteristics

Alpha amylases (∝1-4 glucan-4-glucanohydrolases, E.C. 3.2.1.1) are endoenzymes which hydrolyze the internal glycosidic linkages of starch to produce smaller glucose oligomers which assume the alpha configuration at the anomeric carbon of glucose (Banks and Greenwood 1975). The role of alpha amylase in grains is to provide the germinating embryo with an energy source through the mobilization and solubilization of the starch reserves found primarily in the endosperm. There are two major groups of alpha amylase isozymes present in wheat which are separable by electrophoretic techniques. The alpha I group , pI 4.6 - 5.0, is acidic and is present throughout kernel development while alpha II, pI 6.2, is neutral and found only after the onset of germination (Silvanovich and Hill 1977). In Triticale cv 6A190 however the alpha II band is present throughout the growth cycle yet investigation of it's parental cultivars revealed that it is absent in Prolific rye and only faintly seen in Stewart durum (Silvanovich and Hill 1977). The alpha II group although

appearing only at germination in most cases, contributes the larger portion of enzymatic activity in the germinating seed. Isoelectric focussing of each group revealed that each could be further resolved into a number of electrophoretically distinct species (Nishikawa and Nobuhara 1971, MacGregor 1976).

Numerous investigations of cereal alpha amylases have shown that they are monomeric in nature having a molecular weight ranging over 40-58,000 (Greenwood and Milne 1968, MacGregor 1978, Tkachuk and Kruger 1974, Silvanovich and Hill 1977). The pH activity profiles indicate an optimum at pH 5.5 although the alpha I isozyme tends to be more acid stable than alpha II (MacGregor 1978). The alpha I is however less heat stable than alpha II (Silvanovich and Hill 1977) and both require calcium to maintain their tertiary structure and maximum enzyme activity.

The action of alpha amylase can be studied *in vitro* in both suspension and solution (M^{C}Laren and Packer 1970) yet the rates of hydrolysis during these experiments were much slower than those reported *in vivo* (Walker and Hope 1963). The reason for this discrepancy has not yet been explained. Starchs from different sources also display differing degrees of susceptability to enzymatic attack and it is thought that these observations could be related to their varying amylopectin contents (Ball and Schwimmer 1974, Leach and Schoch 1961, Banks and Greenwood 1975).

Alpha Amylase in Triticale cv 6A190

During the ten to twenty day period following anthesis in Triticale 6A190 the amylase activity is primarily confined to the pericarp. The localization of the enzyme explains why the large number of spherical granules of starch located in the pericarp initially, are completely dissolved by the tenth day. The amylase activity declines in Triticale 6A190 after day twenty in the pericarp but displays a dramatic increase in the aleurone and endosperm by twenty-eight days after anthesis (Deido et al 1975). Wheat and rye generally have lower enzyme activities in the pericarp than Triticale with their duration being shorter as well. Triticale 6A190 has been shown to have extremely high levels as compared to other cultivars. A study of 15 Triticale lines showed that 6A190 had the highest activity (Salminen and Hill 1978). An inherent characteristic of 6A190 is the considerable variability in enzymatic activity displayed between individual seeds. Material at day seventeen had a coefficient of variation of 0.32 % yet by day thirty-eight the variation had increased to 113 % (Deido et al 1975). Klassen (1970) also noted the high degree of variability and attributed it to the problem of seed aneuploidy.

Microscopic examination of maturing 6A190 seeds indicated that the enzymes' activity could be correlated with specific lesions in the endosperm and aleurone cells. Two major lesions seen were: outer layers of the kernel often contained necrotic tissue lacking a defined structure with the adjacent endosperm cells containing partially degraded starch granules or, degeneration of the aleurone cells with adjacent subaleurone cells being free of starch (Deido et al 1975). Further examination revealled that the amylase released was not exclusively associated with the aleurone layer as the endosperm adjacent to the aleurone showed no starch damage while those near the necrotic tissue did.

Work by King et al (1979) has shown that there is an increase in endogenous alpha amylase as the kernel loses water with an accompanying degradation of starch in the endosperm. King and his coworkers were also able to demonstrate that the high levels of giberrellic acid early in the kernel's development did not appear to control the later production of alpha amylase. They suggested that a simple hormonal action between giberrellic acid and abscisic acid does not occur as the loss of control over dormancy and high alpha amylase activity is not reflected by abnormal levels of abscisic acid.

Premature Sprouting

Initial studies on the problem of premature sprouting were concerned with the production of alpha amylase in the aleurone layer of the grain in response to secreted gibberellic acid (Briggs 1978). The typical understanding of the situation was that under condition of sufficient moisture the embryo would secrete the necessary GA (Jones 1973). However, Gibbons (1979,1980) has demonstrated that the role of the aleurone is secondary to that of the embryo and scutellum in the initial production of alpha amylase in germinating barley. He was able to demonstrate through the use of a fluorscein isothiocyanate conjugated antibody system,FITC, that within thirty hours of initiating germination alpha II amylase can be seen in the endosperm tissue immediately adjacent to the scutellum (Gibbons 1979). He stated that the alpha II amylase appears to be synthesized at a site near or within the scutellar parenchyma and is rapidly transported to the tips of the scutellar epithelial before being passed into the layer of crushed cells at the scutellum-endosperm junction. His work of 1980 indicates that there is a pattern of cell wall breakdown similiar to that of alpha amylase distribution with some indications that the wall breakdown preceded the arrival of the amylase. Gibbons's results (1980) reveal that the transport of both

the wall hydrolases and the amylase is facilitated by the cells surrounding the nucellar sheath which are themselves resistant to breakdown (Briggs 1972). Utilizing his FITC system Gibbons was able to demonstrate the expansion of a broad front of amylase spreading out from the scutellum, which by 76 hours had filled one third of the endosperm. It is only at the 76 hour mark that slight alpha amylase production can be detected in the aleurone.

Duffus (1982) suggests that the emphasis on alpha amylase release is somewhat late in the germination cycle and it is likely that the onset of premature sprouting is associated with events in the embryo itself. Through experimental results (Duffus 1980) it has been shown that it is possible to suppress precocious germination in isolated barley embryos if the medium contained 0.35 M sucrose. Duffus (1982) proposed that the information required to suppress the germination is derived from the environment rather than the embryo. In the initial stages of endosperm development sucrose and ABA levels are elevated but decline with maturity. Duffus (1982) speculates that there may be some tandem effect involving sucrose levels and ABA sensitivity. The earlier work of Cameron-Mills and Duffus (1979) on embryo sucrose uptake indicates that at 50 mM active transport predominates while at higher concentrations, above those found in the endosperm at this stage, passive diffusion plays an increasing role. Duffus (1982) proposes that the principal role of sucrose entering the

embryo is to provide a high osmotic pressure. Any rate increase with respect to utilization or decrease in uptake rate might induce germination. The involvement of sucrose levels and precocious germination have also been indicated in the work of Crouch and Sussex (1981) on rapeseed. They suggested that ABA and sucrose prevent germination by allowing embrogeny to continue.

The role of sucrose in premature germination was initially indirectly indicated by Radley (1967) who found that incubating isolated barley scutellums is 2 % sucrose solution severely reduced the amount of GA_3 secreted while intact embryos exhibitted no effect. Jones and Armstrong (1971) found that both glucose and maltose , hydrolysis products of germinating barley seeds, accumulate in the subaleurone layer and achieve concentrations of 570 mM. They were able to demonstrate that both maltose and glucose in the 0.2-0.4 M range effectively inhibited alpha amylase production via osmotic regulation in isolated barley embryos. They stated that osmotic regulation provided a more sensitive means of control than a hormonal system although the manner of it's action was unresolved.

Experiments by Nicholls (1979) in which wheat panicles were cultured on 0.4 and 4.0 % sucrose solutions for ten days revealed that the caryopsis from these ears failed to produce alpha amylase when incubated with GA_3. He proposed that with respect to GA_3 induced alpha amylase production in the aleurone, the wheat caryopsis remains insensitive

until some time dependent critical stage of differentiation is passed. Nicholls suggests that some facet of metabolism is responsible for transforming the aleurone to it's responsive state.

A similiar result was observed in Avena fatua caryopsis cultured on sucrose solutions (Cairns 1982[a]). Cairns was able to demonstrate that seeds cultured on sucrose displayed progressively greater dormancy and increased GA_3 insensitivity with increasing concentrations of sucrose in the culture media. Surprisingly, deembryonated seeds incubated in 300 mM sucrose or maltose failed to respond to applied GA_3 while glucose and fructose cultured seeds did. Further investigation of this phenomena (Cairns and deVilliers 1982[b]) revealed that glucose and fructose in the range 0-300 mM actually stimulated alpha amylase synthesis in de-embryonated kernels. Sucrose demonstrated only a slight stimulation at 100 mM and was inhibitory at higher concentrations. Raffinose was found to inhibit amylase production at all concentrations. The stimulating action of all saccharides at low concentrations, except raffinose,on the sensitivity of the aleurone to GA_3 has led Cairns and deVilliers to speculate that the sugars are acting as physiologically active substances rather than as an osmotica as suggested by Jones and Armstrong (1971). They state it is amylase synthesis,and not the activity, which is effected by the sugars through some form of negative feedback with the mechanism still unresolved.

Like Duffus (1982), Cairns and deVilliers state that the variation in saccharide concentration available to the embryo and surrounding tissue could form a link in a system which controls both alpha amylase production and dormancy, either directly or indirectly. As well, they draw a similiar to Nicholls (1979) in which they state that it appears that the stage of after ripening will effect the response of the embryo to sucrose.

Ortega-Delgado and Vega-Vazquez (1982) have also observed a relationship between sucrose levels and sprouting problems in corn. They have indicated that in varieties of corn studied, those which were prone to premature sprouting displayed a continued decline in sucrose levels with maturation. Non-sprouting varieties however,did not reveal this decline.

MATERIALS AND METHODS

Growth Conditions

Triticale cv. 6A190 (X Triticosecale Wittmack) seeds were germinated on filter paper prior to planting in a soil mixture consisting of equal portions of earth, sand, and peatmoss. A small amount of fertilizer was added to the mixture prior to planting and at six weeks into growth. Two seeds were placed in each 6 inch pot and the plants were allowed to grow in the greenhouse until approximately one month prior to anthesis. The plants were then transferred to a growth cabinet where the temperature was maintained at 22°C / 18°C with a 16 hour day and 8 hour night respectively. The plants were monitored daily and were tagged upon emergence of their anthers. At 8 days post anthesis designated plants with long stems were cut and placed in a water solution. The stems were recut under water and transferred to individual test tubes containing 5 mls. of sucrose solution. Aluminum foil covered the test tubes and the cut stems were forced through, thus forming a tight cover/seal about the stem and minimizing airborne contamination. The stems were recut under water every second day, with

approximately 0.5 cm. being removed, and placed in a fresh sucrose solution. Liquid uptake was calculated from the change in sucrose solution levels,correcting for evaporation, and recorded.

Respiration Rate Determinations

Beginning at 8 days post anthesis 25 kernels from both normally grown plants and 5 % sucrose cultured plants were monitored for oxygen uptake with a Clarke-type oxygen electrode (Rank Brothers, Cambridge, England). Plants were grouped in a series of 5 with 1 kernel from each plant being pooled to provide 5 samples of 5 seeds each for both systems. Seeds for each sample were picked just prior to analysis, quickly weighted, and immediately transferred to an oxygen electrode well containing 2 mls. of a K_2PO_4 - NaOH buffer at pH 7.2. The buffer was held at 25°C by a circulating water bath. A Yellow Springs Instruments standard sensitivity membrane was used with the polarizing voltage adjusted to 0.7 volts. A small spinning magnetic bar in conjunction with rapid manipulation of liquid levels within the well removed any bubbles attached to the seeds. Respiration rates were monitored over a 10 minute period and the seeds were then quickly frozen, lyophilized, and stored in a freezer.

Sugar Extraction

Groups of 4-5 lyophilized seeds were immersed in 10 mls. of boiling 75 %v/v ethanol and homogenized for 30 seconds with a polytron (PT 20 head). The material was then centrifuged at 20,000 g for 5 mins. in a Sorvall RC2-B centrifuge and the supernatant collected. The resultant pellet was then resuspended in a second 10 mls. of boiling 75 %v/v ethanol and the procedure repeated. The two supernatants were combined and evaporated to dryness in a hot water bath under a stream of air. The residue was resuspended in 1 ml. of hot water and passed through a 1 X 5 cm. column containing 1 gm. of hydrated Biosil A 200. The column was washed with water and the first 4 mls. of eluate were collected, frozen, and lyophilized. Analysis of 1-5 mg./ml. sugar standards showed that this column yielded a 95-100 % recovery of all sugars. Examination of radioactive samples indicated a recovery of 93 % from the ethanol extraction to collection of eluted material. All samples were stored in a freezer until analyzed.

Starch Analysis

The pellet resulting from centrifugation during the

sugar extraction was removed from the centrifuge tube by washing with 2 mls. of 0.2 M KOH. An additional 2 mls. of KOH was used to rinse the centrifuge tube and was combined with the initial material. The samples were then placed in a boiling water bath for 20 mins. to solubilize the starch. Cooled starch solutions were adjusted to pH 5.5 by the addition of concentrated acetic acid. Sodium acetate-acetic acid buffer (0.5 M, pH 5.5) was added to bring the final volume to 5.0 mls.. A 50 ul. aliquot of sodium acetate-acetic acid buffer containing 0.4 mg. of Rhizopus amyloglucosidase (Sigma) was added to each sample. Each test tube was sealed with parafilm and transferred to a 37°C water bath for 72 hours. Duplicate samples of each starch preparation, as well as standards, were assayed for glucose using the Worthington Statzyme Reagent Set. The only modification to the listed procedure was the utilization of 1.5 mls. of reagent to 10 ul. of sample as compared to a recommended 3.0 mls. of reagent to 20 ul. sample. All glucose values for starch content were adjusted for glycosidic cleavage by multiplying assayed values by 0.92 .

Sugar Analysis

Sugar analysis was accomplished with a Beckman 100 A HPLC pump system attached to a Whatman 4.1 X 250 mm. PAC-10 column. The solvent employed was a 87.5:12.5 v/v acetonitrile - water mixture dispensed at a flow rate of 2.0 mls./

$min.^{-1}$. A 4.1 X 40 mm. guard column containing 1.0 gms. of Whatman CO:PELL ODS was located directly in front of the analytical column. A Pharmacia refractive index monitor with a 8 ul. flow through cell was employed as a detector and kept at room temperature by a circulating water bath. The detector was linked to a Spectro-Physics 4100 programable integrator which supplied both peak heights and peak areas for calibration. The sample size of 20 ul. was governed by a 20 ul. injection loop attached to an Altex injection valve. Dilution of samples was varied in order to remain within calibrated values.

^{14}C Sucrose Incubation and Analysis

Saari's (1977) examination of Triticale 6A190 indicated that the seed displayed a period during it's development in which sucrose levels fell considerably before returning to near normal levels. In order to investigate kinetically any differences in the supply pattern, the 6A190's kernel development has been divided into three phases. Phase I refers to the period prior to the decline in sucrose, phase II, to the period of the trough, and phase III, to the period after the trough in which sucrose levels have recovered. The various periods of development were monitored by picking random kernels from grouped plants and determining their carbohydrate content. Phase II was

Figure 1. The separation of sugars utilizing the HPLC technique.

Legend: I = sugar standards

II = plant extracts

A = solvent front

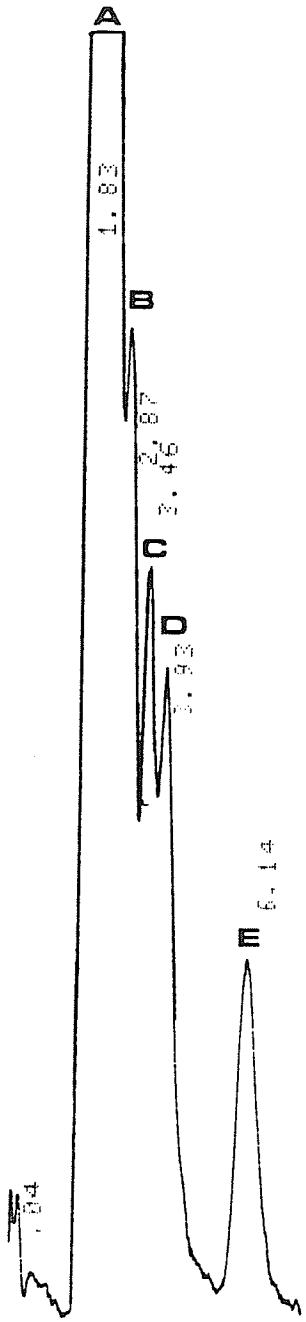
B = ribose

C = fructose

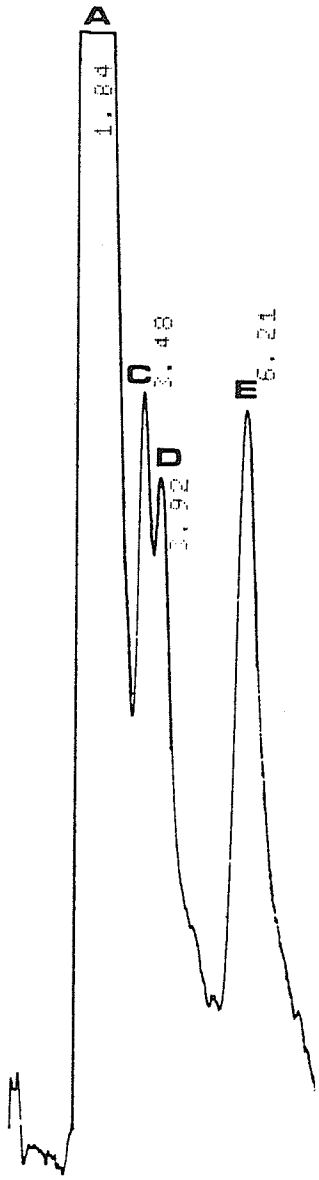
D = glucose

E = sucrose

I



II



selected when a large drop in carbohydrate content was observed.

Plants for the three stages of kernel development were excised approximately 20 cm. from the base of the head, placed in a small flask of water, and quickly transferred to the laboratory. The stems were then recut under water, at a 45 ° angle, 7.5 cm. below the base of the ear, and immediately placed in a small vial of radioactive 1 % sucrose. The 1 % ^{14}C sucrose solution was prepared from a 673 mCi/mmol. stock solution of uniformly labelled sucrose supplied by New England Nuclear. (NEC-100X) The specific activity of the 1 % sucrose was approximately 1700 dpm/ug.

Five plants were deposited in each vial and then placed in a dark chamber,held at room temperature, for varying periods of incubation. Incubation periods for Phase I material were 40,70,100,and 130 minutes while Phase II employed 25,50,75,and 100 minute intervals. Phase III excised stems were incubated for 40,80,120,and 160 minutes. Once the allotted incubation period was completed the excised stems were quickly removed from the sucrose solution and the first 6 cms. below the base of the ear were removed. These stem sections were individually tagged, weighted, frozen and lyophilized. Four seeds were collected from each ear from the 4th basal floret, if necessary from the 5th, and immediately subjected to the previously described sugar extraction. An additional kernel from each head was

collected and pooled for each phase. These pooled seeds were weighed ,frozen,lyophilized, and reweighted. Individual stem sections which had been lyophilized were cut into small pieces after weighing and subjected to the same method of sugar extraction outlined earlier. HPLC analysis of both stem and seed material allowed for the separate collection of hexose peaks and sucrose material directly into scintillation vials. Ten mls. of NEN Aquasure were added to each vial which was placed into a Searle Mark III Liquid Scintillation System. Sugar and background samples were counted for 10 mins.

Radioactive starch samples were handled in the same manner as previously described. One ml. aliquots of cooled, pH adjusted hydrolysate were mixed with 3 mls. of Aquasure for counting.

Alpha Amylase Production in Response to Applied GA_3

Two seeds from each plant cultured on 5 % sucrose or grown normally were collected at 30, 35, 40 and 45 days after anthesis. Each seed was individually weighed before being deembryonated. One seed from each pair was placed in 0.5 mls. of 10^{-2} M $CaCl_2$ bathing solution while the other was placed in a similiar solution which also contained 10^{-5} M GA_3. All seeds were allowed to sit in the bathing solution for 24 hours at room temperature. Each seed and

and it's bathing solution were then transferred to a centrifuge tube containing 4.5 mls. of 10^{-3} M $CaCl_2$ and 0.2 M NaCl and homogenized for 30 seconds with a polytron PT 20 head. The mixture was then spun at 20,000 g for 5 minutes and the supernatant collected.

Analysis of amylase activity employed a modified Briggs assay(Weselake 1981) in which B-amylase was not incorporated into the assay medium. The B-limit dextrin from waxy maize starch at 0.075 % concentration was employed as the substrate. Activity is reported in IDC units which by definition is the amount of enzyme required to change the absorbance of an iodine complexed B-limit dextrin solution from 0.6 to0.4 in 100 minutes. (Briggs 1961) The assay was conducted at 35 ° C in 0.05 M sodium acetate buffer (pH 5.5 , 0.001 M $CaCl_2$) with the incubation period being varied to keep the normalized optical density values between 0.6 and 0.4 . A Zeiss PMQ II spectrophotometer was used for all absorbance readings.

Total Carbohydrate

Aliquots of ethanol extracts were made up to 2.0 mls. with distilled water. A 50 ul. aliquot of 2 N phenol was added to each test tube prior to the rapid introduction of 5.0 mls. of concentrated sulfuric acid. Samples were allowed to sit 30 minutes and the absorbance was read at

485 nm. . Ethanol had no effect on the absorbance readings and concentrations were taken directly from standard curves prepared using sucrose as the carbohydrate source.

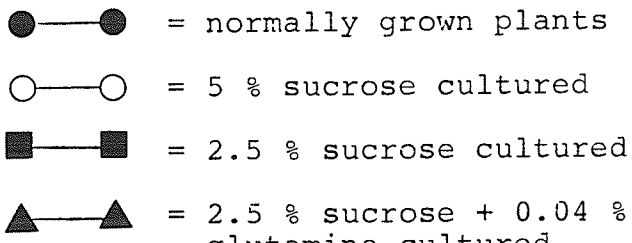
RESULTS

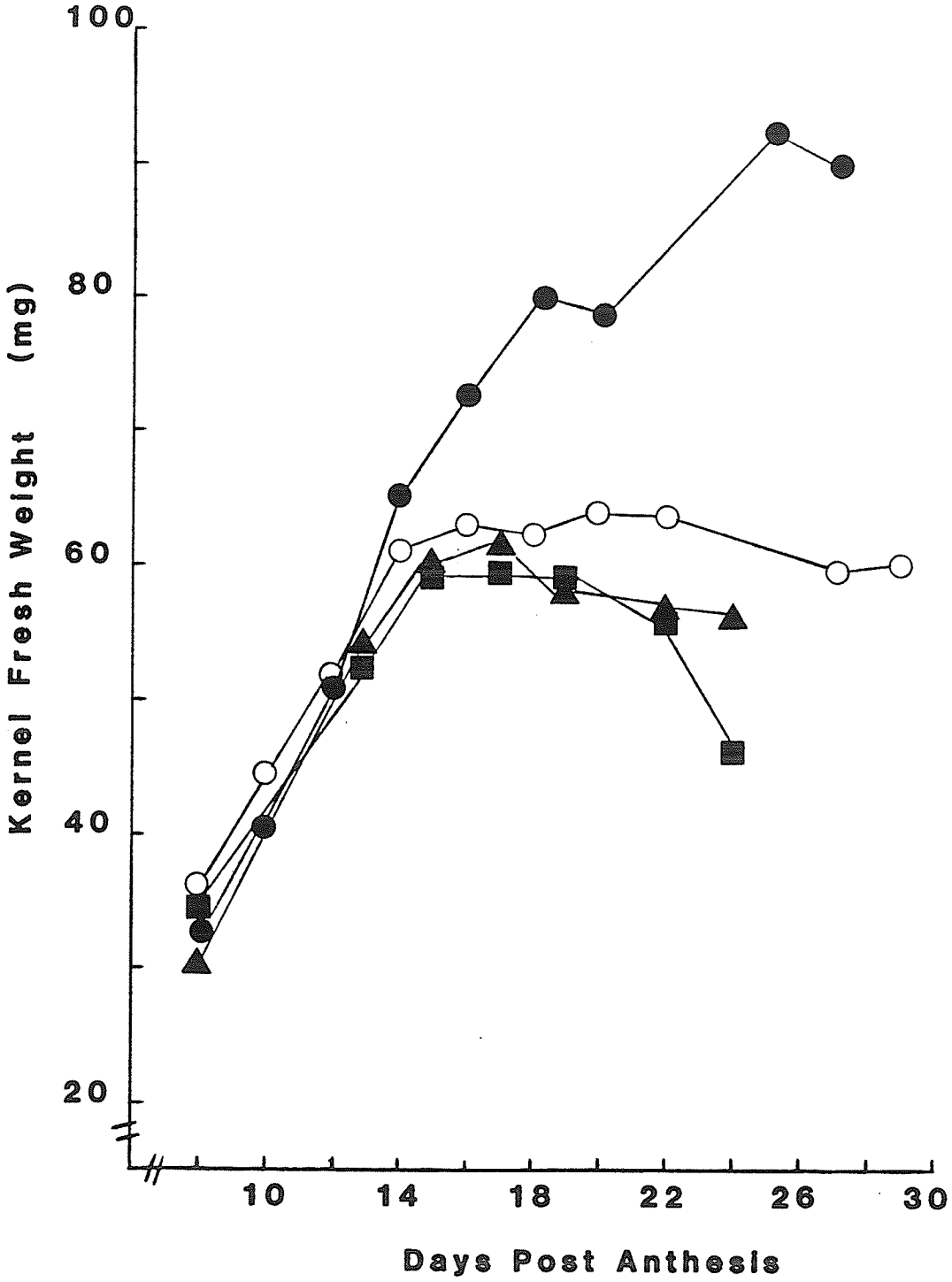
Kernel Weight and Water Content

Examination of all plants grown on sucrose culture and those grown normally indicates a linear increase in fresh weight in all cases over the time period extending 8 to 15 days post anthesis. The rate of increase at this stage is approximately 4.4 mg. kernel^{-1}day^{-1}. (Figure 2) However, after this date, there is considerable divergence in fresh weight profiles as the normally grown plants continue their increase while the sucrose cultured kernels exhibit either a maintenance of a constant weight, as is the case for 5 percent sucrose-cultured seeds, or display a steady decline, as seen in both 2.5 percent culture systems. The normally grown plants display a difference in fresh weight in excess of 33 percent by day 27 after anthesis.

A large portion of the differences in fresh weights between normally grown kernels and those cultured in sucrose is reflected in the water content of the seeds. The water content of sucrose cultured seeds display a similar pattern with those of normally grown plants, revealing a progressive increase in water content over the time period

Figure 2. Fresh weight change with development



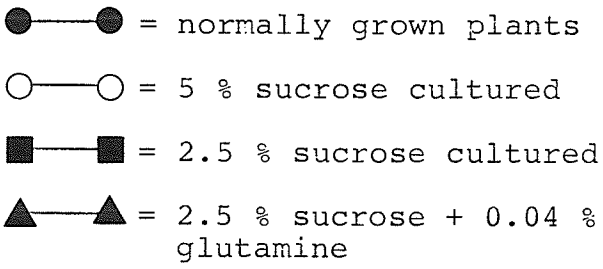

100
80
60
40
20
Kernel Fresh Weight (mg)
10
14
18
22
26
30
Days Post Anthesis

8-15 days post anthesis. A maximum of 40-43 mg./kernel is achieved by sucrose-cultured kernels between days 14-16 after anthesis. (Figure 3) The sucrose cultured kernels then undergo an abrupt decrease in water content which is not reflected in any portion of the normally grown seed's profile. Furthermore, the rate of water loss is similar in all three systems extending from their maximum over the next 7 days at a rate of approximately 12 mg./day. The maximum water content of the cultured seeds is only 75 percent of that displayed by the normally grown kernels. At 24 days after anthesis both the 5 percent sucrose and 2.5 percent sucrose solution with 0.04 percent glutamine exhibit only 60 percent of a normal seeds'moisture content at this date while the 2.5 percent sucrose without glutamine displays only 42 percent.

The change in the percentage of water in a seed exhibits a linear decrease with maturity in both the normal and sucrose-cultured kernels although the slopes are somewhat different. (Figure 4)

All kernels display a similar linear increase in dry weight over the 8-17 day period although the 2.5 percent cultured systems tended to be somewhat lower than the others. (Figure 5) At 17 days post anthesis the 2.5 percent sucrose with 0.04 percent glutamine cultured kernels show an abrupt decrease in dry weight before undergoing a slow recovery which does not return to the original weight until day 22. The 2.5 percent sucrose system exhibits a similar trend

Figure 3. The change in water content within the kernels with development.



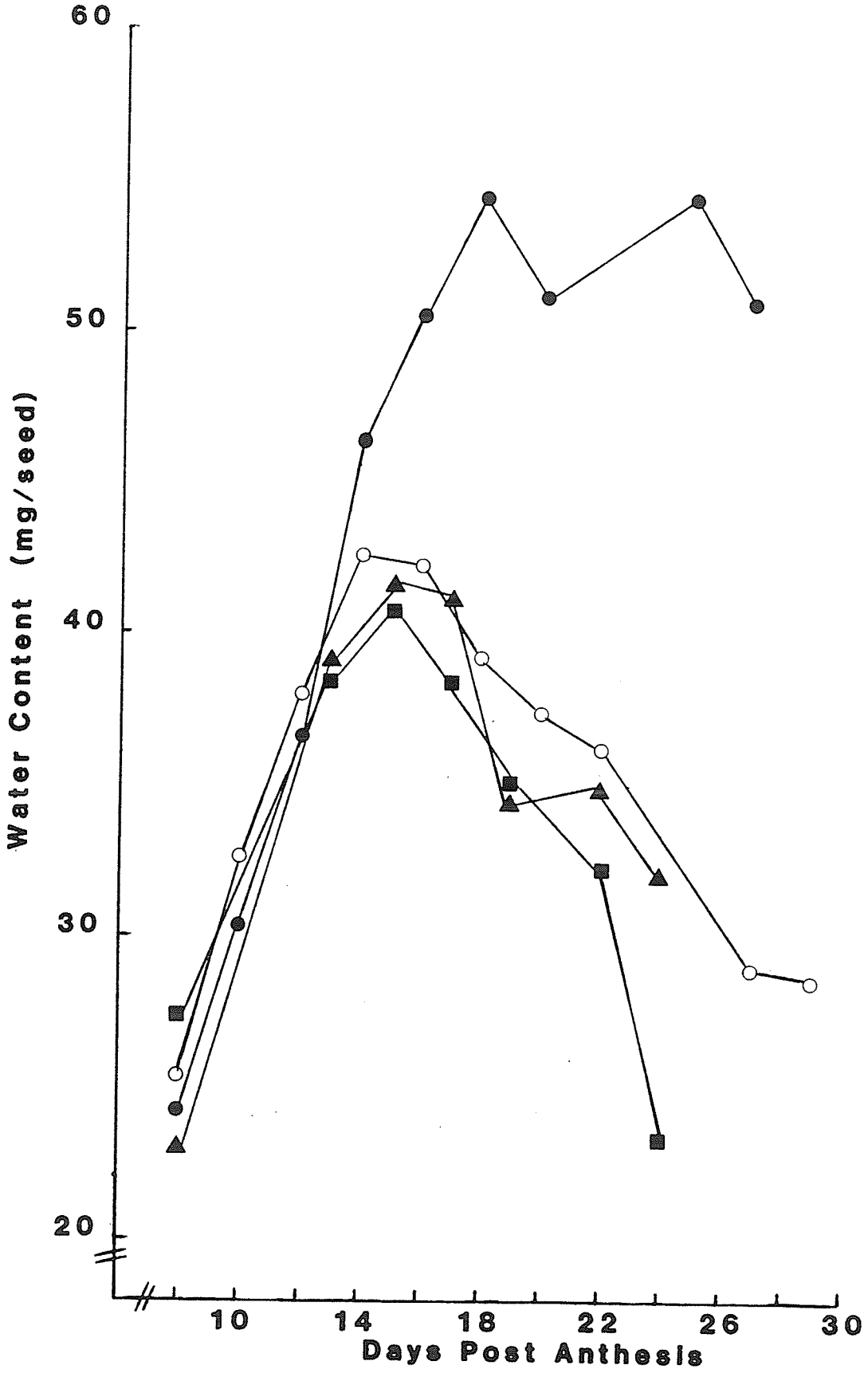
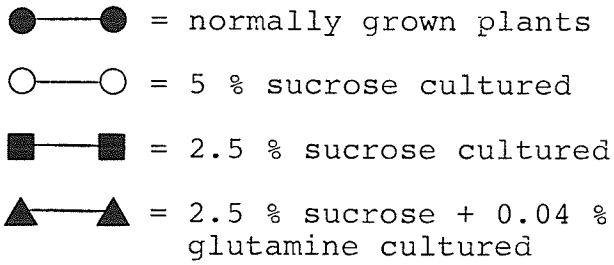
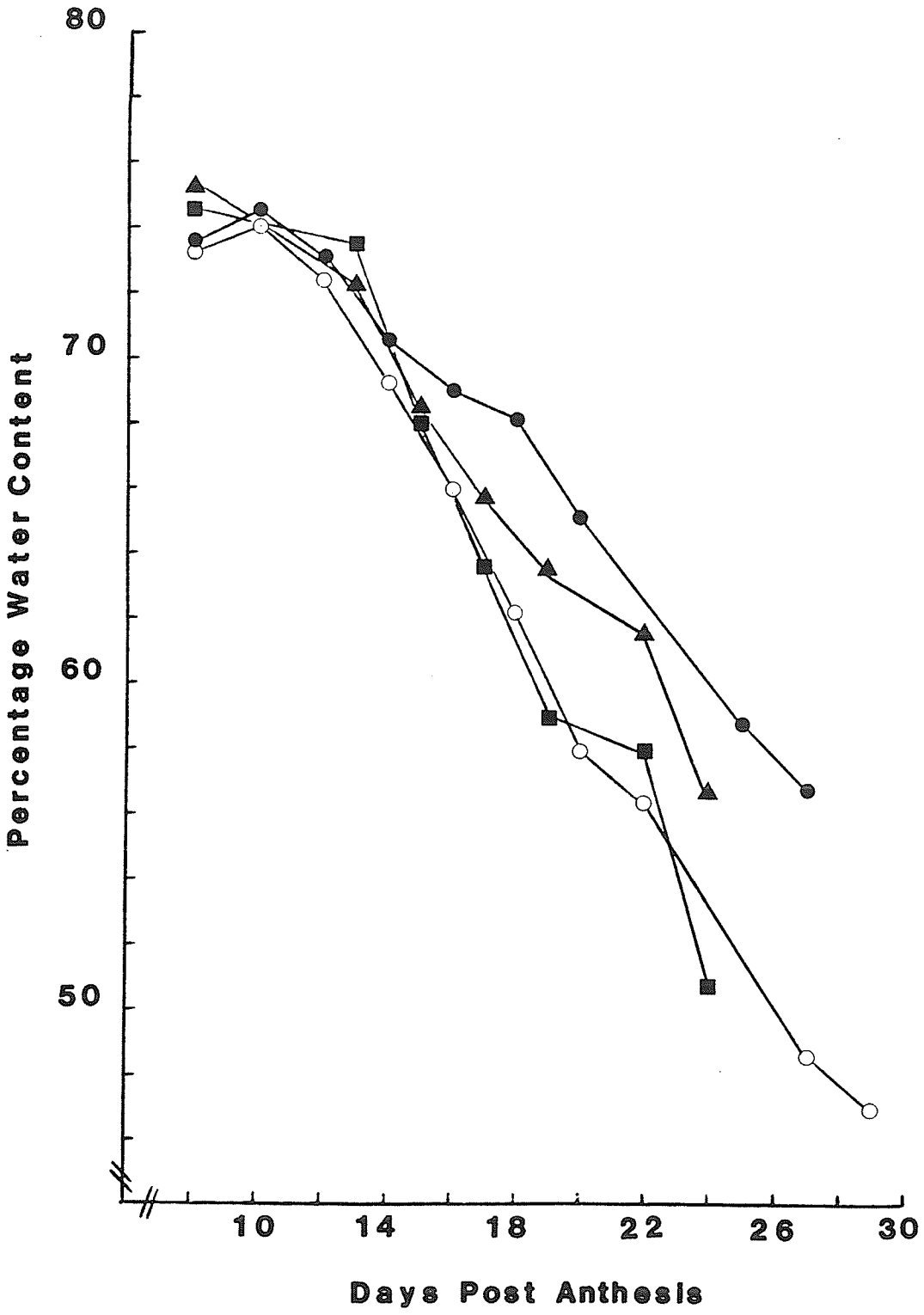

60
50
40
30
20
Water Content (mg/seed)
10
14
18
22
26
30
Days Post Anthesis

Figure 4. The percentage of water within a kernel with development.



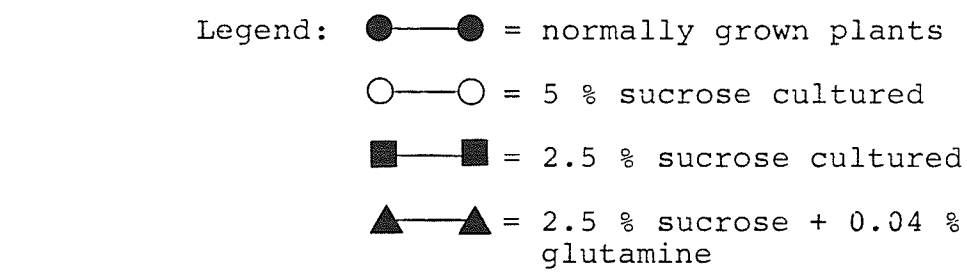

80
70
60
50
Percentage Water Content
10
14
18
22
26
30
Days Post Anthesis

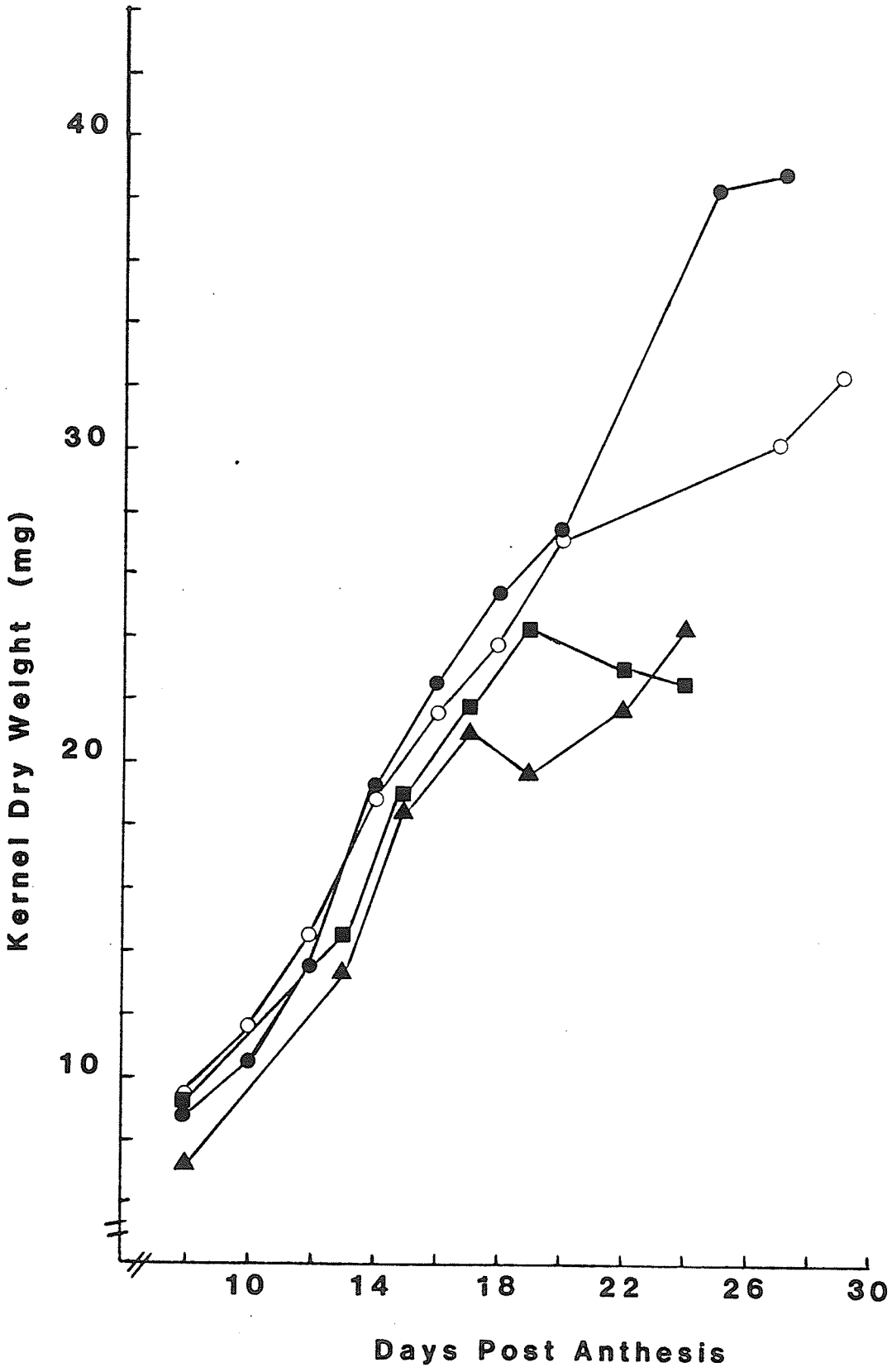
although it maintains a linear increase in dry weight until 19 days post anthesis before undergoing a slow decline in weight. The 5 percent sucrose-cultured kernels match the pattern shown by normally grown seeds until 20 days post anthesis, at which time, although continuing to increase in dry matter, they do so at a rate considerably slower than that seen in normal plants. The 5 percent sucrose-cultured kernels, at this stage, increase in dry weight at a rate of approximately 0.6 mg. day^{-1} as compared to 1.7 mg. day^{-1} for normal seeds. It is interesting to note that in both cases the dry matter increase is approximately twice that displayed by the starch deposition in the respective kernels. The maximum dry weight achieved by either of the 2.5 percent sucrose-cultured systems was found to be 66 percent of the normal kernel at day 24 while the 5 percent cultured kernel achieved approximately 83.5 percent of the normal dry matter at 29 days after anthesis.

Respiration Rate

Respiration is the process whereby organic compounds undergo controlled oxidation to release energy for the maintenance and development of the plant. (Bidwell 1979) The developing kernel actively respires as large amounts of energy are required for the numerous anabolic pathways essential for the embryo to remain viable during the period

Figure 5. Kernel dry weight with maturity




40
30
20
10
Kernel Dry Weight (mg)
10
14
18
22
26
30
Days Post Anthesis

of dormancy and subsequent germination. Examination of this key process was undertaken to study both the effects of sucrose culturing as well as to observe if any significant deviation occurred relevant to the unusual sucrose metabolism of 6A190.

Analysis of respiration on a per seed basis indicates that both 5 percent sucrose-cultured and normally grown seeds achieved a similar maximum of 220 versus 212 nmoles oxygen $seed^{-1}hour^{-1}$ respectively athough the time at which this was achieved differs. (Figure 6) The 5 percent sucrose-cultured kernels reached their maximum by day 14 while normally grown seeds attained their maximum at 20 days after anthesis. Examination of the respiration profiles reveals that both systems display a steady increase to the respective maximums with no significant differences illustrated between the two curves except at the period of maximum respiration and in the case of the normal seeds, slightly after the maximum. Further review of these profiles with respect to the standard error for each point indicates that the normally grown seeds have a broader, more prolonged period of higher respiration than the 5 percent cultured seeds. A very noticeable sharp decrease in respiration rates can be seen in both cases beginning at day 25 for normal seeds and slightly later at day 27 for sucrose-cultured kernels. It should be noted that the maximum respiration rates achieved on a per seed basis correspond to periods of lowest starch deposition in

both experiments.

Expression of the respiration rate on a weight basis, either fresh weight (Figure 7) or dry weight (Figure 8) indicates the same general decline in respiration rates with maturity although the differences in the two systems are more pronounced when expressed on a fresh weight basis. Scrutiny of the respiration rates on a fresh weight basis indicates that over the period of day 12 to 14 the normal seeds undergo a dramatic decrease fro the 5 percent sucrose kernels and maintain a significant difference until 19 days after anthesis. This period of divergence corresponds in the normal seeds with the period of maximum starch deposition. Analysis of this period indicates that when standard error bars are introduced, the respiration rate is almost constant. Although the 5 percent sucrose-cultured kernels do not display the marked divergence from linearity seen in normally grown seeds, they do exhibit two periods of almost no change in respiration rate. These are days 16-18, and 20-22. Days 16-18 are part of the period of increasing starch deposition while days 20-22 correspond to the period of maximum deposition.

Review of respiration rate profiles expressed in the three different manners draws attention to the rate observed at day 20. It is the date of maximum respiration on a per seed basis, and in both manners of weight expression, the point of divergence from pre-existing patterns.

Figure 6. Respiration rates with maturity

Legend ●——● = normally grown plants

○——○ = 5 % sucrose cultured

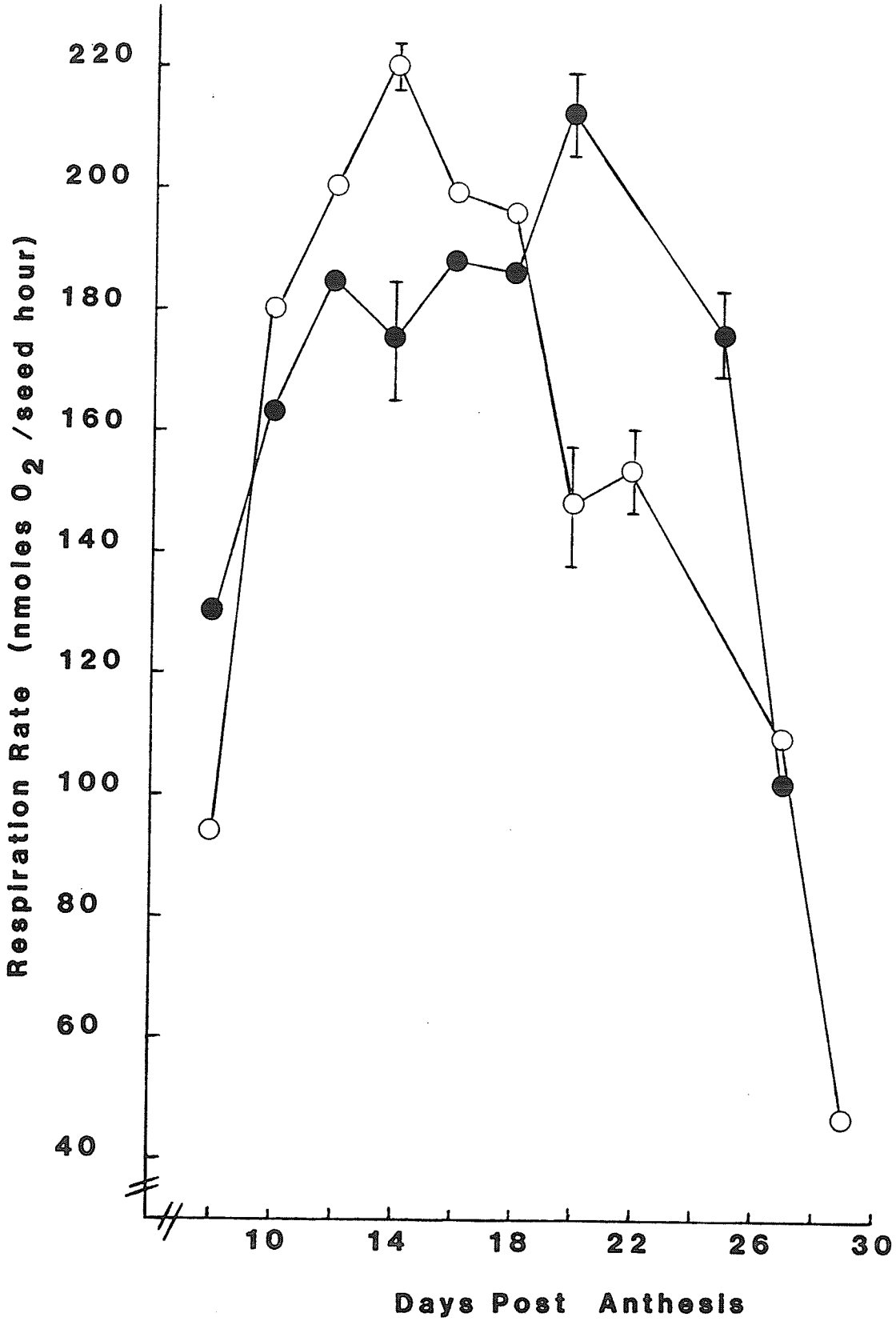
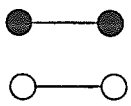

220
200
180
160
140
120
100
80
60
40
Respiration Rate (nmoles O_2 /seed hour)
10
14
18
22
26
30
Days Post Anthesis

Figure 7. Respiration rates of Triticale 6A190 kernels expressed on a fresh weight basis as a function maturity.



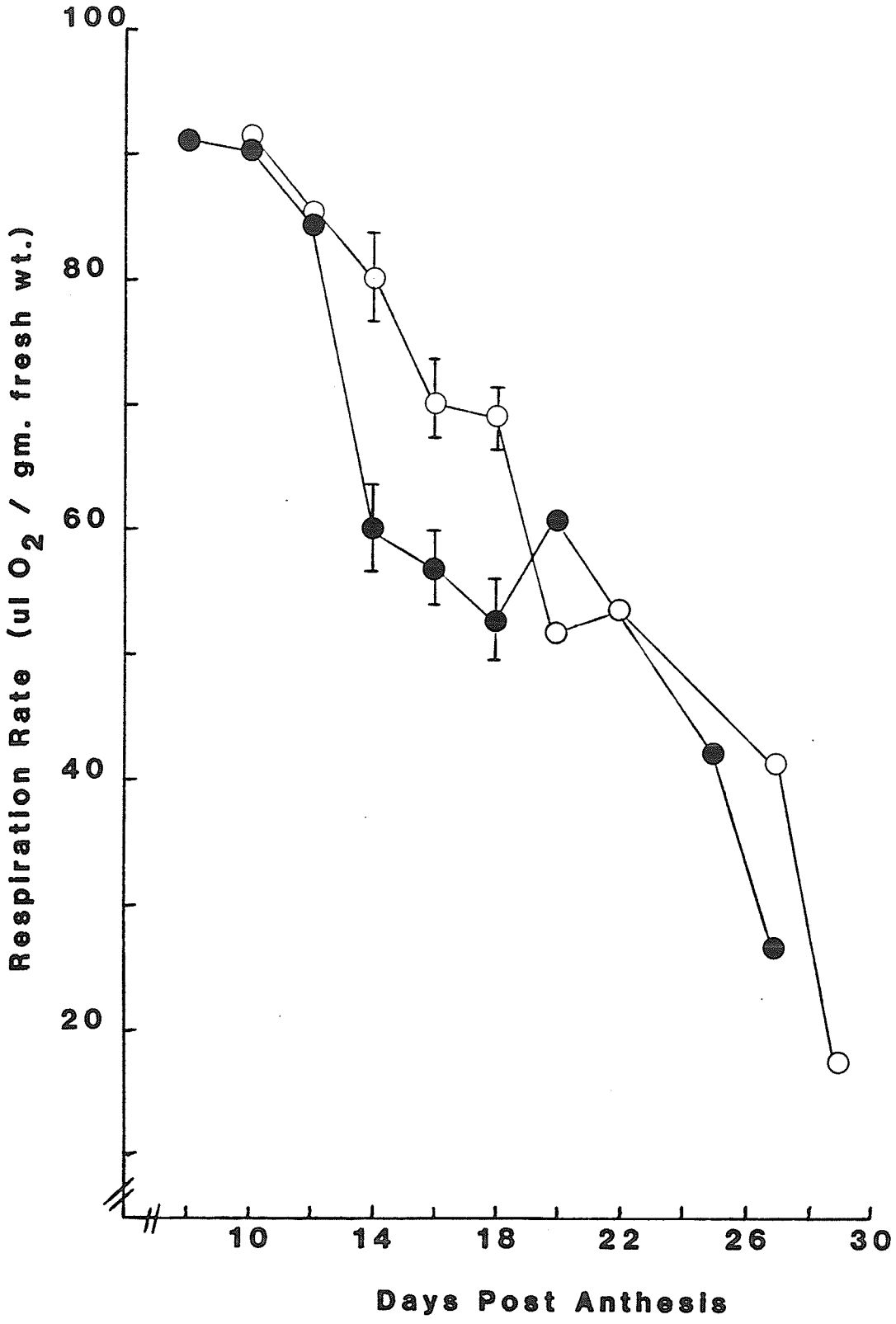
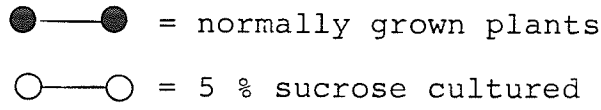
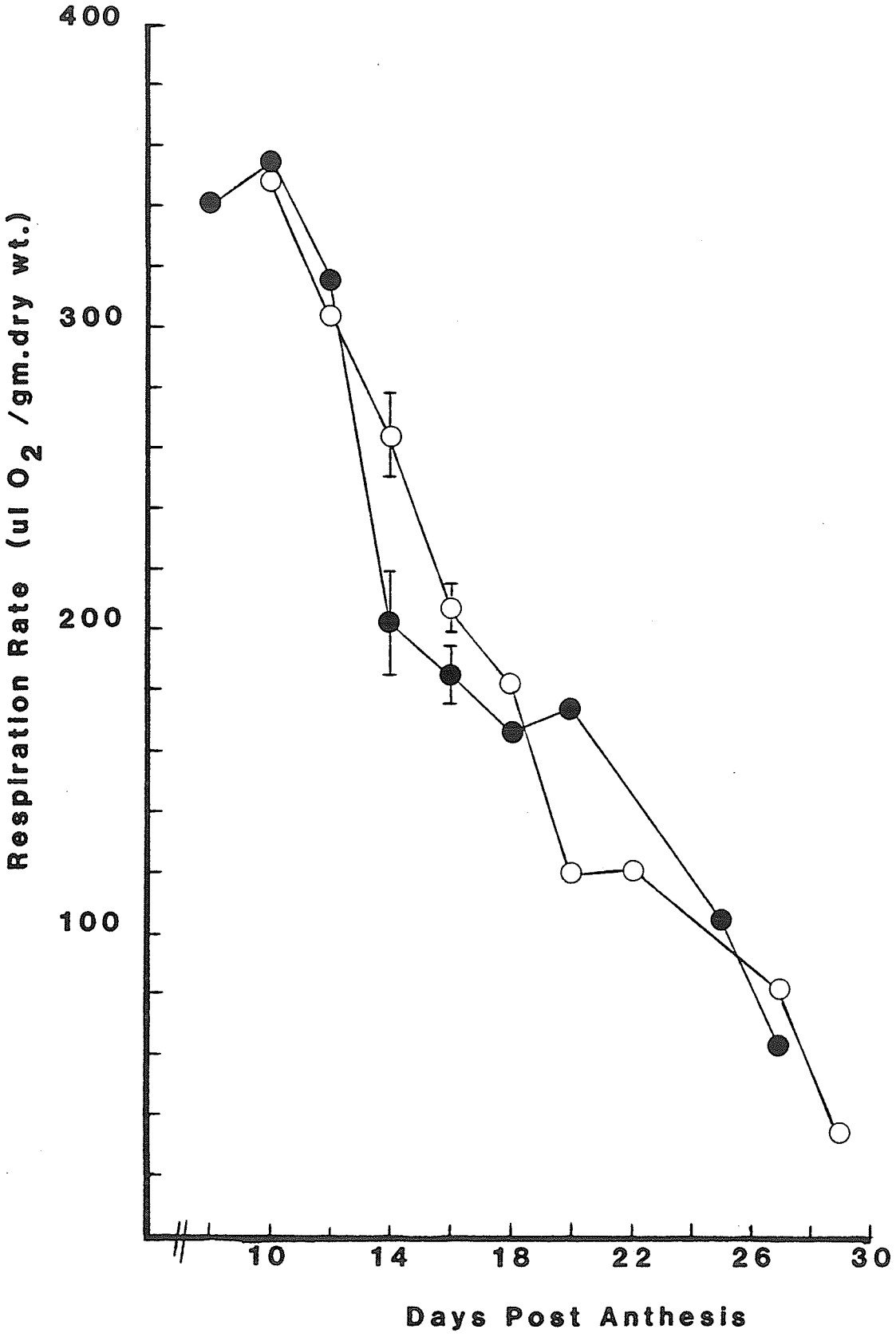

100
80
60
40
20
Respiration Rate (ul O_2 / gm. fresh wt.)
10
14
18
22
26
30
Days Post Anthesis

Figure 8. Respiration rates of Triticale 6A190 kernels expressed on a dry weight basis as a function of time.




400
300
200
100
Respiration Rate (ul O_2 /gm.dry wt.)
10
14
18
22
26
30
Days Post Anthesis

Starch Content

The culturing of ears on 5 percent sucrose solution yielded no differences in the final starch content per kernel as compared to normally grown seeds. The normal seeds had maximum starch values of 16.2 mg.per seed while 5 percent sucrose-cultured kernels displayed 16.8 mg. per seed. The seeds from culture on either of the 2.5 percent sucrose solutions, with or without glutamine, did, however show a divergence from the regular pattern reaching maximums of 11.0 and 11.7 mg.per seed respectively at 24 days post anthesis. These values are 74 and 78 percent respectively of the normal seeds' starch content at the same time period.

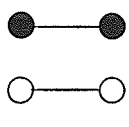
Although the normally grown kernels and 5 percent sucrose-cultured seeds exhibited no differences in their final starch contents, the manner in which they were achieved did vary. Both systems underwent a similar linear growth rate of approximately 0.7 mg. per day over days 8-14. However, from days 14-21, the normal seeds demonstrated a convex nonlinear increase while the 5 percent sucrose kernels exhibited a concave nonlinear rise. Although the shape of the profiles differed, the maximum rate of starch deposition, occurring on different days, was similar. The normally grown seeds revealed a maximum rate of 2.4 mg.per day on days 14-16 while the 5 percent sucrose

counterparts displayed a 2.6 mg. per day deposition rate on days 20-22 after anthesis. (Figures 9 &10)

The initial period of starch deposition for both 2.5 percent sucrose seeds, with or without glutamine, was similar to that seen in both normal and 5 percent sucrose-culture systems. At 13 days post anthesis both 2.5 percent sucrose systems displayed a dramatic increase in starch deposition. The 2.5 percent sucrose without glutamine followed the pattern displayed by normal seeds almost exactly, even to the point of showing a decline in starch content at a corresponding time period. However, unlike the normal seed, the 2.5 percent sucrose kernels were unable to resume their sharp increase in starch deposition and showed only a slow increase in levels over the next 5 days. The 2.5 percent sucrose with glutamine(0.04%) achieved the same starch content as it's counterpart but demonstrated a "rest" period during rapid starch deposition. Maximum rates of starch deposition were similar to those seen in normal and 5 percent sucrose kernels. All four cases did reveal a "rest" period in which starch levels either decreased or remained level after a period of rapid laydown.

Expressing starch formation as a function of moisture content indicated that there were considerable differences in conditions under which maximum deposition occurred. (Figure 11) Maximum starch formation was initiated at approximately 70.5 percent moisture content in normal seeds yet a corresponding rate in 5 percent sucrose kernels is not

Figure 9. Starch content of Triticale 6A190 with maturity.



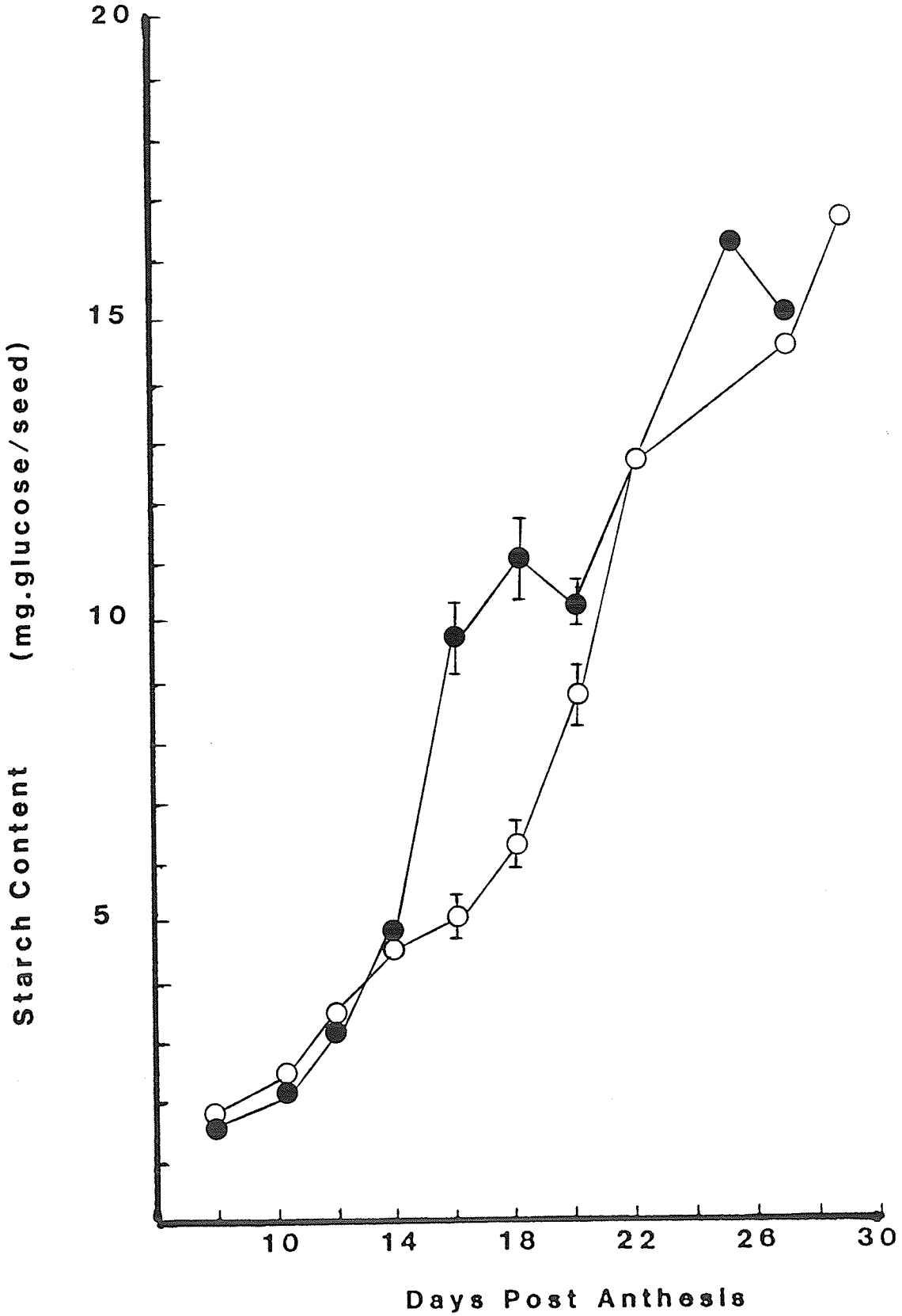
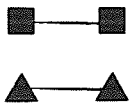

20
15
10
5
Starch Content (mg.glucose/seed)
10
14
18
22
26
30
Days Post Anthesis

Figure 10. Starch content of Triticale 6A190 with maturity.



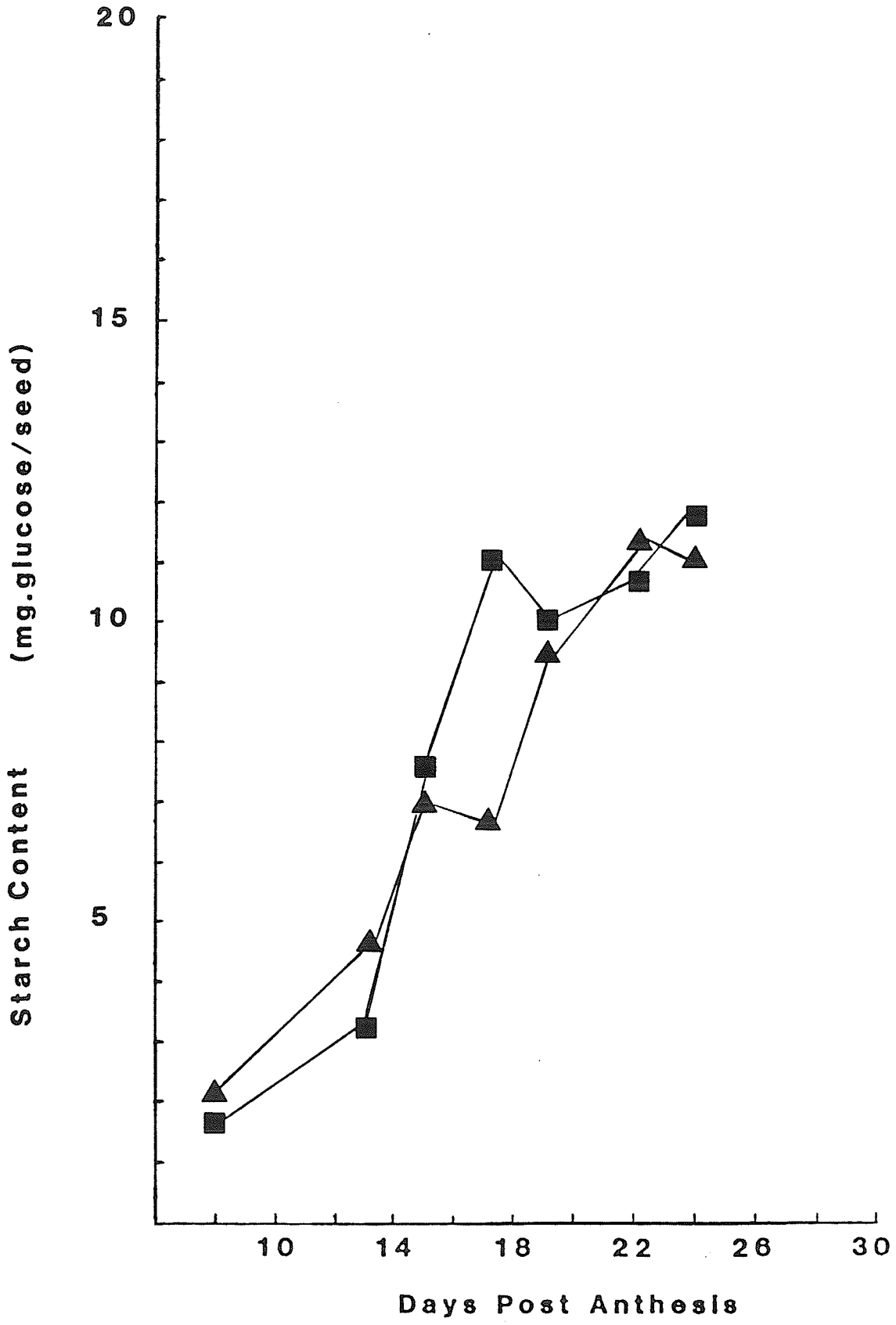
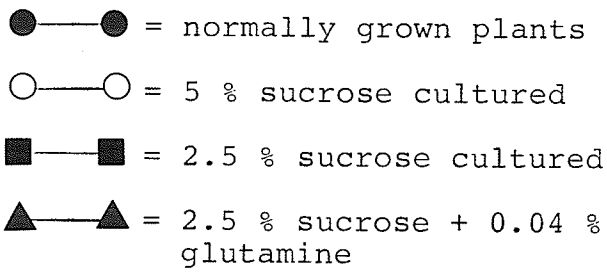
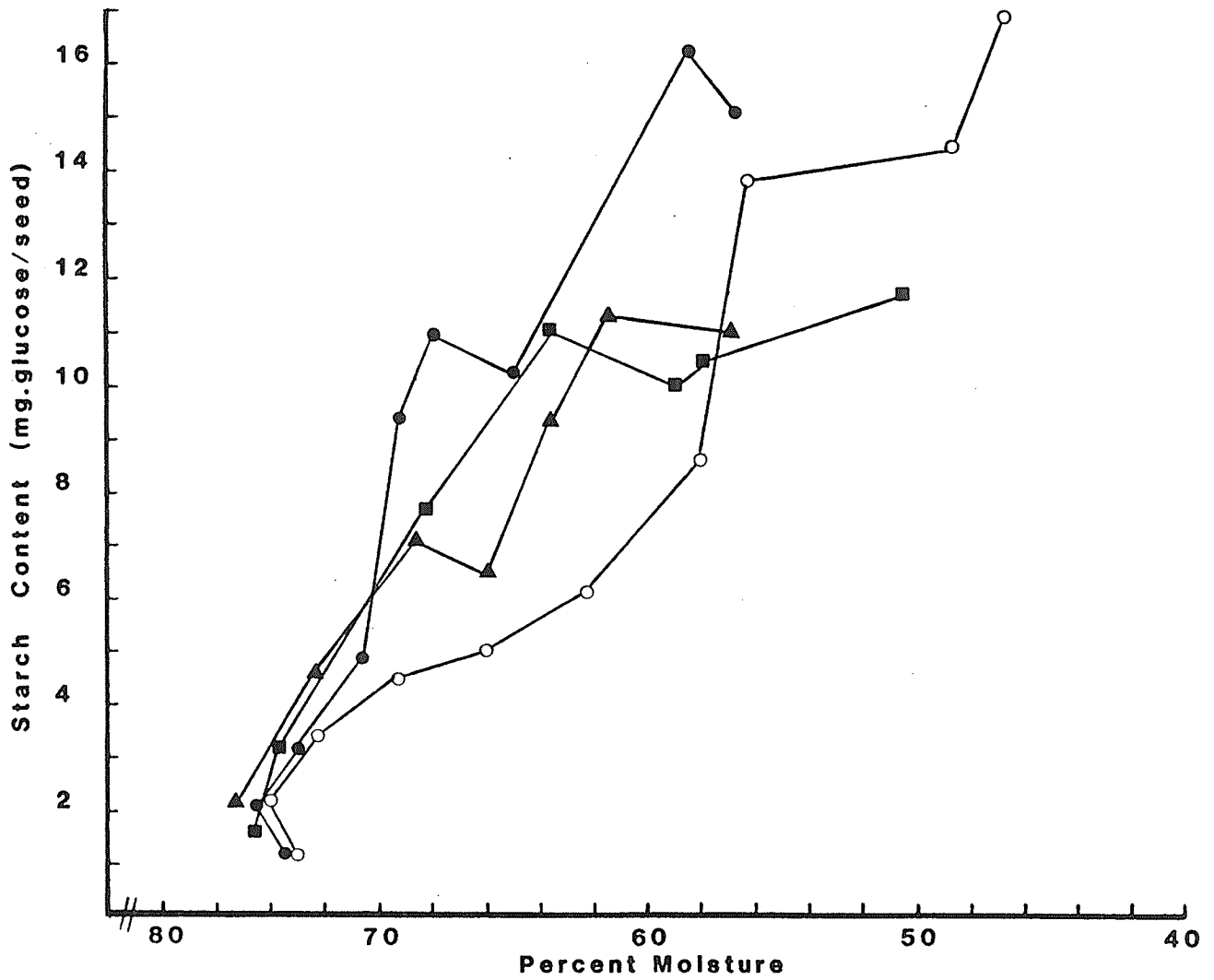

20
15
10
5
Starch Content (mg.glucose/seed)
10
14
18
22
26
30
Days Post Anthesis

Figure 11. Starch content as a function of moisture content in Triticale 6A190 kernels grown under different conditions.



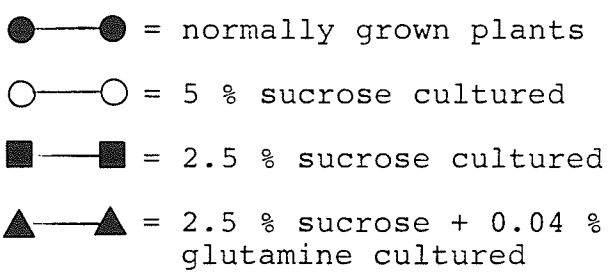

Starch Content (mg.glucose/seed)
16
14
12
10
8
6
4
2
80
70
60
50
40
Percent Moisture

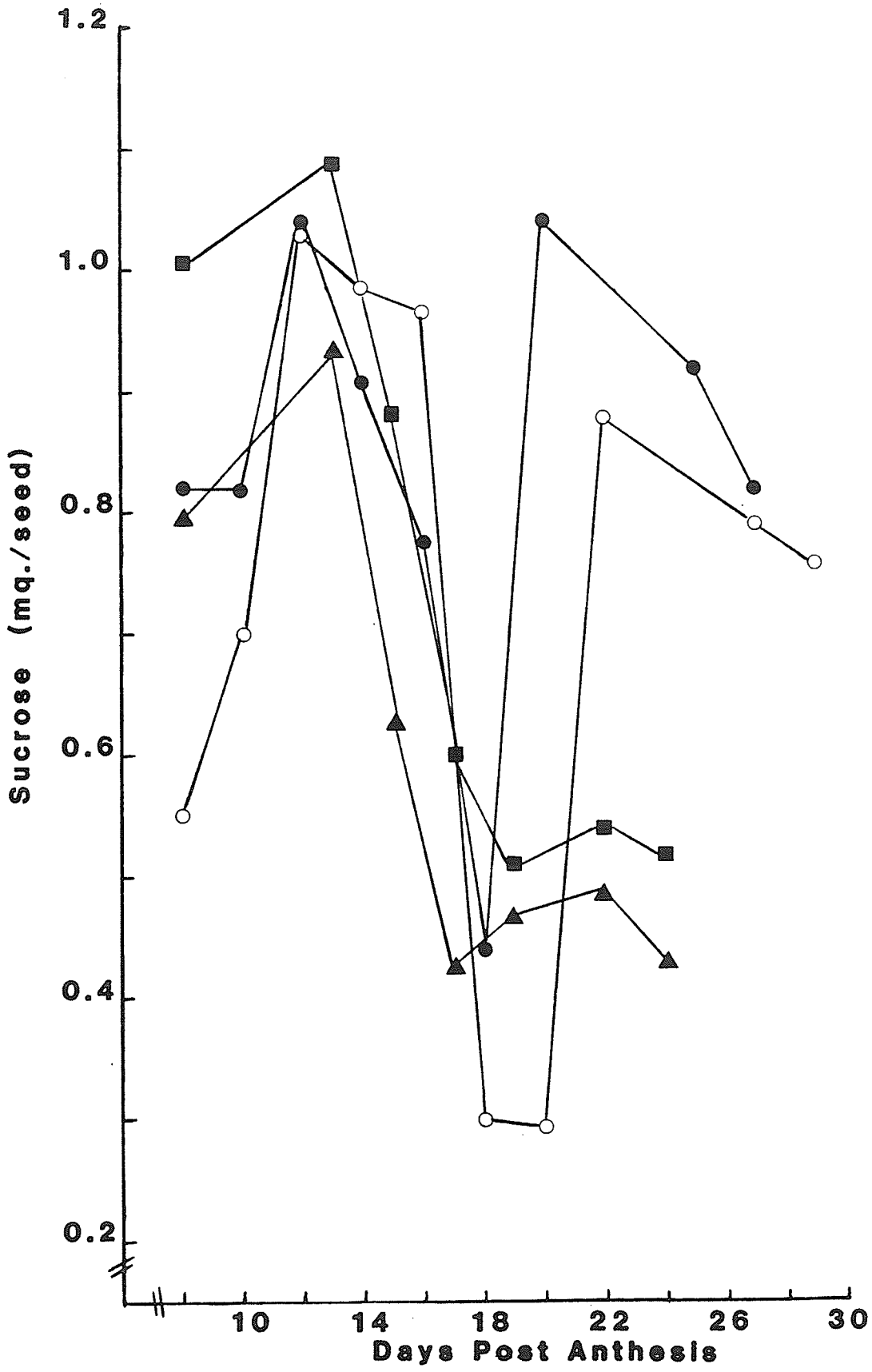
observed until 58 percent. The 2.5 percent sucrose alone shows a steady linear increase in starch content until circa 64 percent at which time the content in the seed remains relatively constant as the moisture declines. A similiar pattern is reflected in the 2.5 percent sucrose with glutamine although the "rest" period mentioned earlier is evident at 69-66 percent.

Sucrose and Glucose Levels

In all of the growing conditions analyzed the sucrose levels rose during the initial period following anthesis to values in excess of 0.9 mg. per seed. (Figure 12) These levels were maintained for varying periods, 2-4 days, before displaying a characteristic dramatic decrease in concentration. Normally grown kernels demonstrated a 57 percent loss in sucrose over a 6 day period before rising to original levels in excess of 1.0mg.per seed. The culturing of 6A190 plants on any of the sucrose solutions was unable to avoid this loss of sucrose. The 5 percent sucrose system showed a 70 percent decrease over a 2 day period, remaining at minimum levels of 0.35 mg.per seed for an additional 2 days before returning to a concentration slightly less than before the trough. Examination of the trends in sucrose levels, particularily in the 5 percent system, indicated that the trough in sucrose concentration interrupts a

Figure 12. Sucrose content with maturity of Triticale 6A190 kernels grown under varying conditions.




1.2
1.0
0.8
0.6
0.4
0.2
Sucrose (mg./seed)
10
14
18
22
26
30
Days Post Anthesis

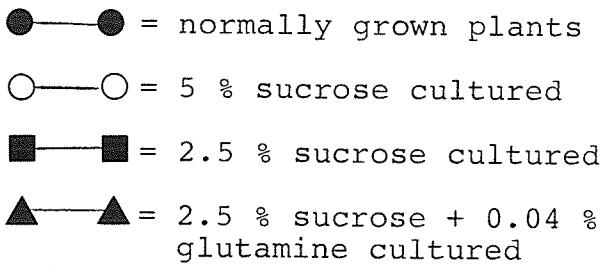
general trend of decline in content within the kernel with maturity.

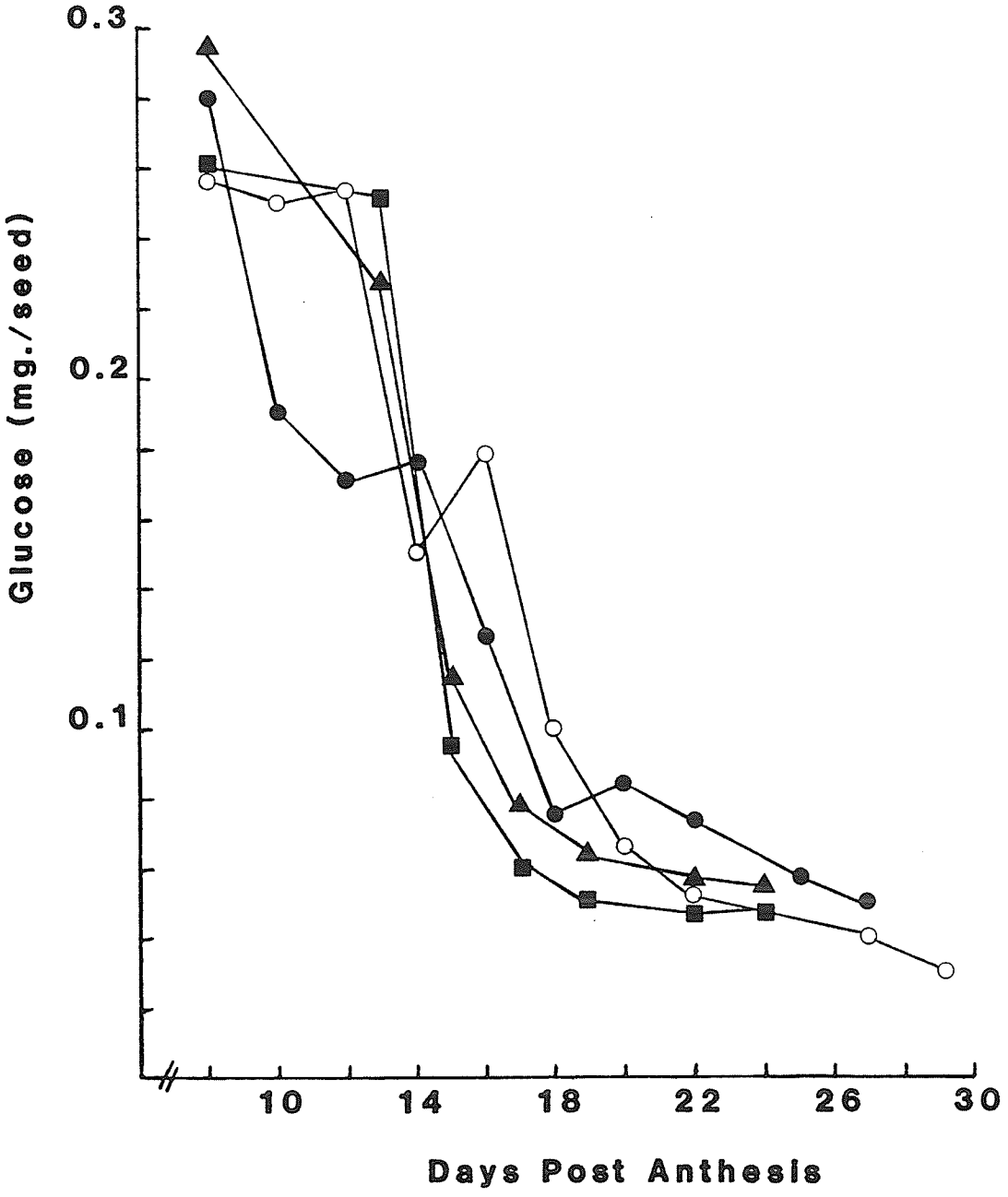
Both 2.5 percent sucrose systems, with or without glutamine displayed the same characteristic drop in sucrose content. The culture system with glutamine exhibited a 54.5 percent loss, while without it, a 53 percent loss was seen. In each case the decline was initiated at 13 days post anthesis reaching minimum contents within 4-6 days. However, unlike normal and 5 percent sucrose-cultured kernels, these seeds demonstrated no return to previous high levels.

In all four systems the period of sucrose loss corresponded significantly with the period of increased starch deposition. The inability of both 2.5 percent sucrose-cultured seeds to return to normal sucrose levels may account for the apparent levelling in starch contents seen at this time.

All four growing conditions reflected similar patterns in glucose content per kernel. Each displayed a rapid nonlinear decrease from initial values of 0.26-0.30 mg.per seed. (Figure 13) Loss of glucose levelled off at values extending over 0.04-0.06 mg. per kernel. The loss of glucose viewed in every system parallels the same general pattern seen for sucrose loss. The glucose content approaches it's minimum plateau as the sucrose content reaches it's minimum concentration. The patterns of glucose decrease correspond well with periods of maximum starch deposition and dry matter increase.

Figure 13. Glucose content of Triticale 6A190 kernels grown under varying conditions.




0.3
0.2
0.1
Glucose (mg./seed)
10
14
18
22
26
30
Days Post Anthesis

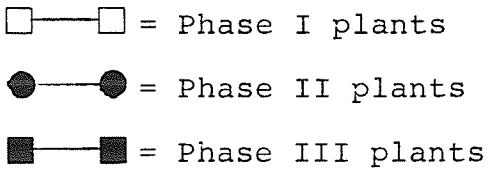
^{14}C Sucrose Uptake

Examination of the specific activity patterns as a function of time indicated that the concentration of radioactivity in the stem section displayed a definite nonlinear increase in Phase III although Phase I appeared linear. Phase II data was insufficient to make a definite comment for although giving the impression of being slightly curved, a linear relationship can not be rejected. (Figure 14) The patterns displayed in the seeds however show that in all three phases a linear relationship between specific activity and time was evident. (Figure 15) Analysis of Figure 16 indicates that the appearance of radioactivity in the starch as a function of time is also nonlinear.

Closer examination of the specific activity in the seeds reveals that the values of Phase I and III are very similiar in magnitude for the corresponding time periods. Those of Phase II are considerably larger. Furthermore, the slope of the lines for Phase I and III, 0.286 and 0.203 dpm $ug.^{-1}min.^{-1}$ respectively, are also quite similiar. The slope of the line for Phase II is almost three times greater at 0.651 dpm. $ug.^{-1}min.^{-1}$.

Calculations of the sucrose content in the samples indicated that in Phase I and III sucrose concentrations were at expected values. The early selection of the Phase I material at day 8 to ensure avoiding the sucrose trough

Figure 14. Specific activity of sucrose within the stem as a function of the period of incubation.



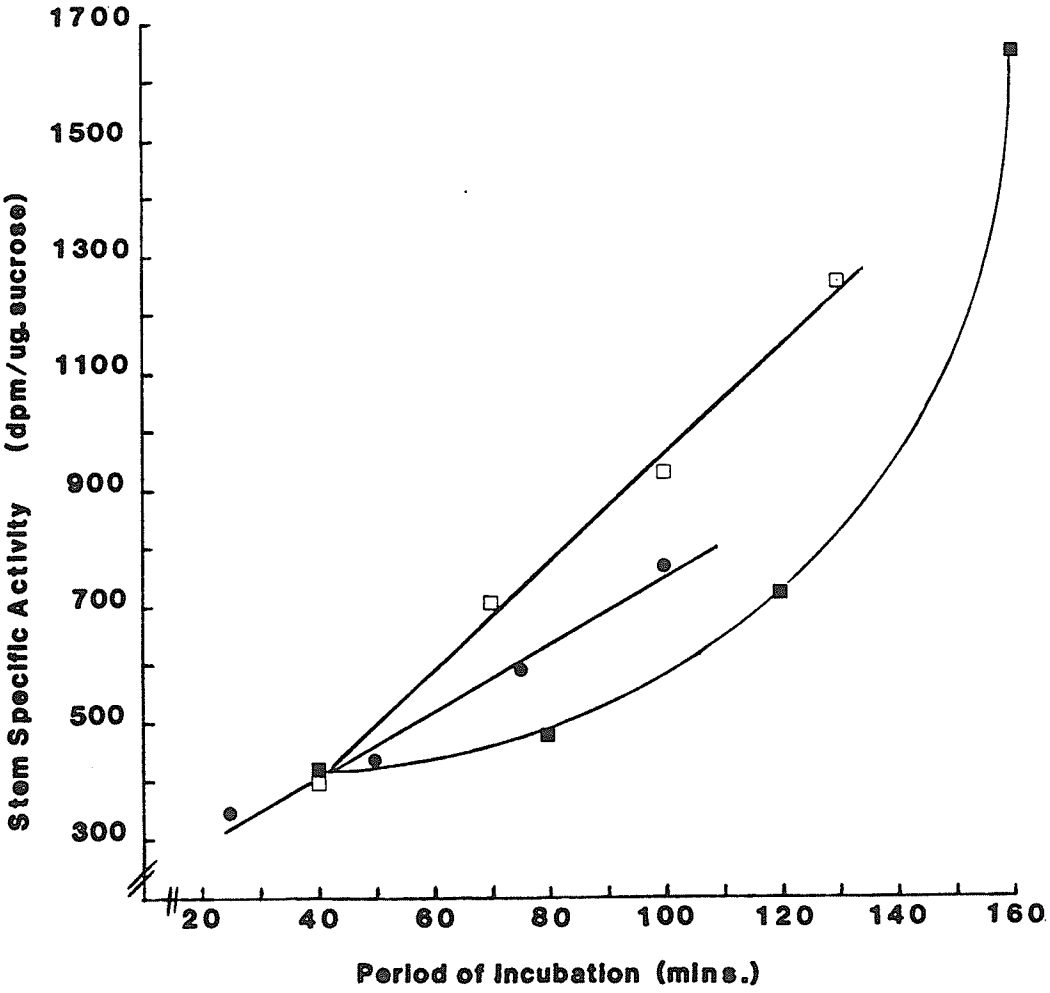
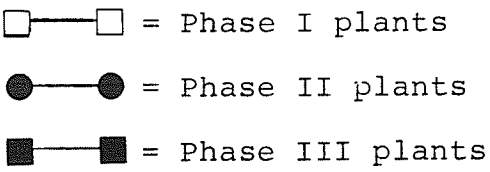

1700
1500
1300
1100
900
700
500
300
Stem Specific Activity (dpm/ug.sucrose)
20
40
60
80
100
120
140
160
Period of Incubation (mins.)

Figure 15. Specific activity of sucrose within the kernels of 6A190 as a function of the period of incubation.



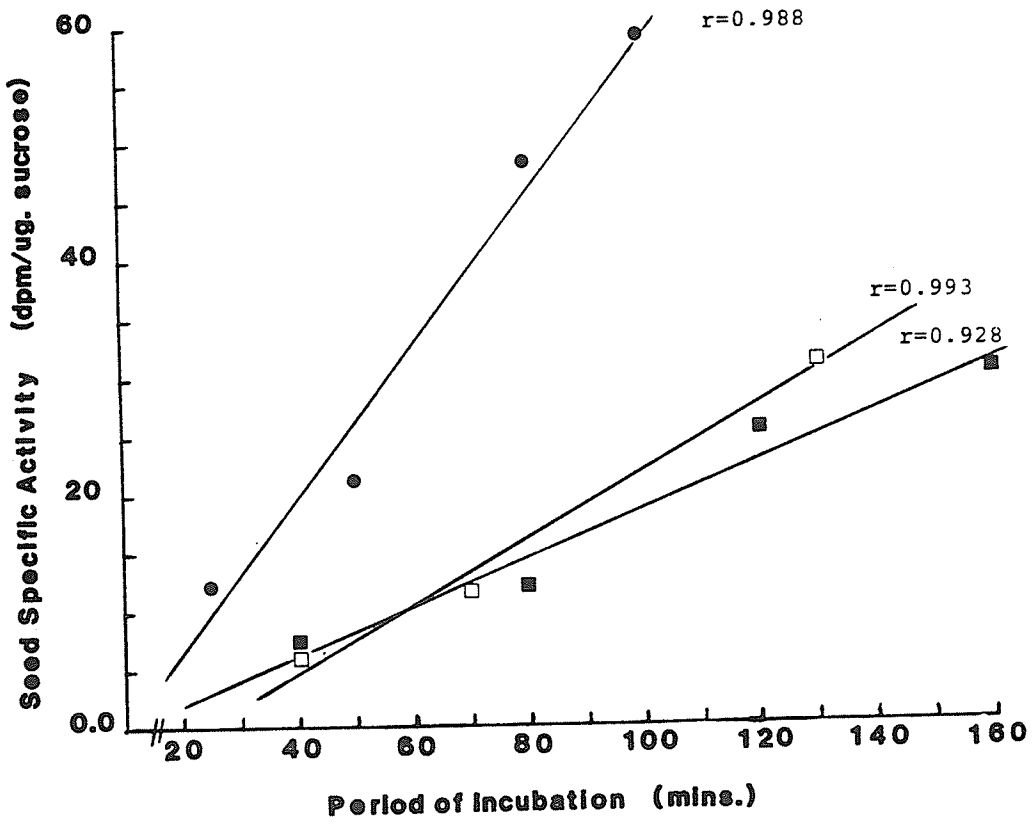
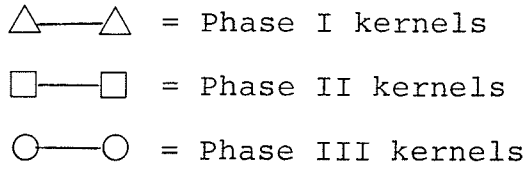
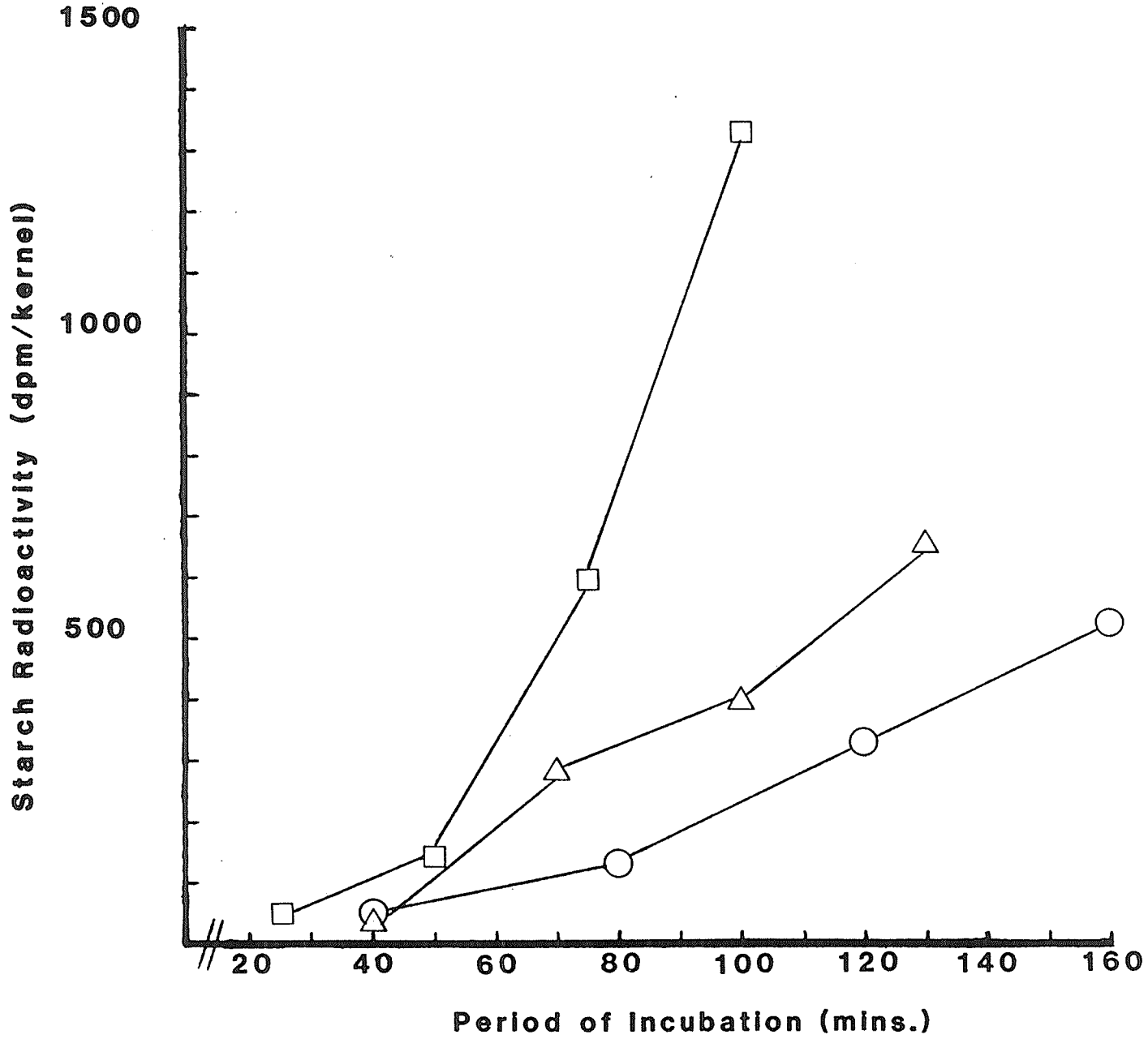

60
40
20
0.0
Seed Specific Activity (dpm/ug. sucrose)
r=0.988
r=0.993
r=0.928
20
40
60
80
100
120
140
160
Period of Incubation (mins.)

Figure 16 The appearance and accumulation of radioactivity in the starch component of Triticale 6A190 kernels as a function of time of incubation.




1500
1000
500
Starch Radioactivity (dpm/kernel)
20
40
60
80
100
120
140
160
Period of Incubation (mins.)

may account for the slightly lower values than expressed in previous analysis. (Table 1) The 0.809 mg. sucrose per seed in Phase III clearly shows that the kernels have recovered from Phase II. It is unfortunate that the Phase II material was not exactly at the minimum sucrose concentrations. This is not surprising however as it is impossible to get 20 plants and their kernels at the same physiological state simultaneously.

Calculation of the velocities listed in Table 1 were done through a modification of the equation put forth by Fisher (1975)

$$\frac{dR_1}{dT} = (k_{12}\frac{R_2}{S_2} + \ldots k_{1n}\frac{R_n}{S_n}) - (k_{21} + \ldots k_{n1})\frac{R_1}{S_1} \qquad (1)$$

where:

R_n = cpm in the n^{th} compartment
S_n = amount of substance in the n^{th} compartment (gms.)
k_{1n} = transport from n^{th} compartment into 1^{st} compartment (gms./ min.)
k_{n1} = transport from 1^{st} compartment into n^{th} compartment (gms./ min.)

Derivation of the Fisher formula relative to the cultured spikelet system can be accomplished in the following manner. The change in radioactivity within the seed can be expressed as:

$$\frac{dB}{dT}^* = k_1A^* - k_2B^* \qquad (2)$$

TABLE 1. Physiological and radioactivity status of the plant material used in the kinetic investigation.

PHASE	TIME (MINS)	AVERAGE SUCROSE IN SEED (MG.)	AVERAGE SPECIFIC ACTIVITY PER SEED (DPM/UG.)	AVERAGE SPECIFIC ACTIVITY IN STEM (DPM/UG.)	VELOCITY (UG./MIN.)
I	40	0.709	5.86	399	0.515
I	70	0.809	11.4	705	0.367
I	100	0.717	----	925	-----
I	130	0.612	31.0	1256	0.143
II	25	0.579	12.1	347	1.13
II	50	0.635	21.0	435	1.00
II	75	0.644	43.9	588	0.77
II	100	0.454	58.7	770	0.42
III	40	0.846	7.54	418*	0.418
III	80	1.00	11.6	473	0.439
III	120	0.728	25.2	720	0.213
III	160	0.663	30.1	1653	0.083

* suspect value as only two samples gave accurate sugar levels in the stems

where:

B^* = radioactive sucrose in the seed (dpm)
A^* = radioactive sucrose in the stem (dpm)
k_1 = translocation constant into the seed sucrose pool ($min.^{-1}$)
k_2 = translocation constant out of the seed sucrose pool ($min.^{-1}$)

Steady state kinetics state that:

$$k_1 A = K = k_2 B \tag{3}$$

where:

A = mass of sucrose in the stem (ug.)
B = mass of sucrose in the seed (ug.)
K = translocation rate constant (ug./min.)

Mathematical manipulation, by multiplying and dividing by A and B of their respective segments of equation(2) yields:

$$\frac{dB^*}{dT} = k_1 A \frac{A^*}{A} - k_2 B \frac{B^*}{B} \tag{4}$$

Since equation (3) indicates $k_1 A = K = k_2 B$, K can be substituted in equation (4)

$$\frac{dB^*}{dT} = K \frac{A^*}{A} - K \frac{B^*}{B} \tag{5}$$

Reduced to:

$$\frac{dB^*}{dT} = K \left(\frac{A^*}{A} - \frac{B^*}{B} \right) \tag{6}$$

Dividing both sides of the expression by B yields:

$$\frac{dB^*/B}{dT} = \frac{K}{B} \left(\frac{A^*}{A} - \frac{B^*}{B} \right) \tag{7}$$

In this form the more accurate specific activities can be

utilized to determine the translocation rates. The velocities indicated in Table 1 show that the maximum velocity was attained in Phase II at 1.13 ug./ min. as compared to 0.515 ug./min. for Phase I and 0.439 ug./min. for Phase III. The velocities listed in Table 1 also reveal that there is a general decline in velocities observed with increasing periods of incubation. Utilizing these maximum velocities a maximum delivery rate of sucrose to the kernel can be calculated on a daily rate. These are: for Phase I 0.74 mg.sucrose/ day and similiarily 0.63 mg. sucrose/ day for Phase III. Phase II showed a full 2 fold increase in delivery over the other phases at 1.63 mg. sucrose / day. A review of these values and their implication is found in the following discussion section.

Alpha Amylase Release in Response to Sucrose Culture

King et al (1979) concluded that further study with Triticale 6A190 was required in the area of GA control over both shrivelling and sprouting as the kernel dries out. They suggested that the GA regulating these processes could be produced in the embryo. Nicholls (1979) speculated that in wheat caryopsis changes in metabolism emanating from the interruption to the supply of sucrose to the caryopsis may be involved in the development of the ability of the

aleurone to respond to GA_3. As well, Gibbons (1982) and Duffus (1982) have suggested the involvement of the embryo and it's sucrose control in precocious germination. These studies,combined with the work of Saari (1977) who observed a severe disruption in sucrose levels in 6A190 during development, made it desirable to observe the effect of culturing 6A190 on 5 percent sucrose and the alpha amylase release in response to applied GA_3.

No differences were seen in alpha amylase levels at 30 days post anthesis between normally grown seeds and those grown in liquid culture. Application of GA_3 to the bathing solutions of either system did not invoke a significant response.

At 35 days after flowering however, marked differences were observed between sucrose cultured seeds and normal kernels in both regular seed activity and that induced by GA_3. Sucrose cultured seeds with no GA_3 in the bathing solution displayed the lowest activity of the four experiments while those with GA_3 revealed the greatest amylase activity. Surprisingly, the normally grown kernels did not exhibit these differences as amylase values in both cases were very similar. Although not displaying differences amongst themselves, the normal kernel's activity was located between the two extremes displayed by the sucrose-cultured seeds and were significantly different from either.

Differences observed at 35 days after anthesis were not apparent a 40 days. No differences between any of the

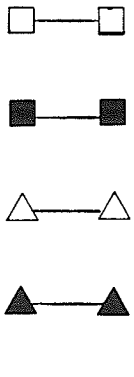
four experiments were detected. However, at day 45, a noticeable increase in the activity of normal seeds was seen as compared to sucrose cultured kernels. Furthermore, a very significant difference between normal seeds without GA_3 and those with GA_3 could be detected although the reason for the discrepancy is unknown. Contrary to the divergence seen in the normal seeds, the sucrose cultured systems both displayed lower activities of the same magnitude.

Examination of the general trends indicated a gentle increase in amylase activity in normally grown kernels with no GA_3 induced differences until after day 40. At this time there is a signigicant surge in activity in both systems, espescially the in the system without GA_3. This is the first time a significant difference between the two normally grown kernels is observed.

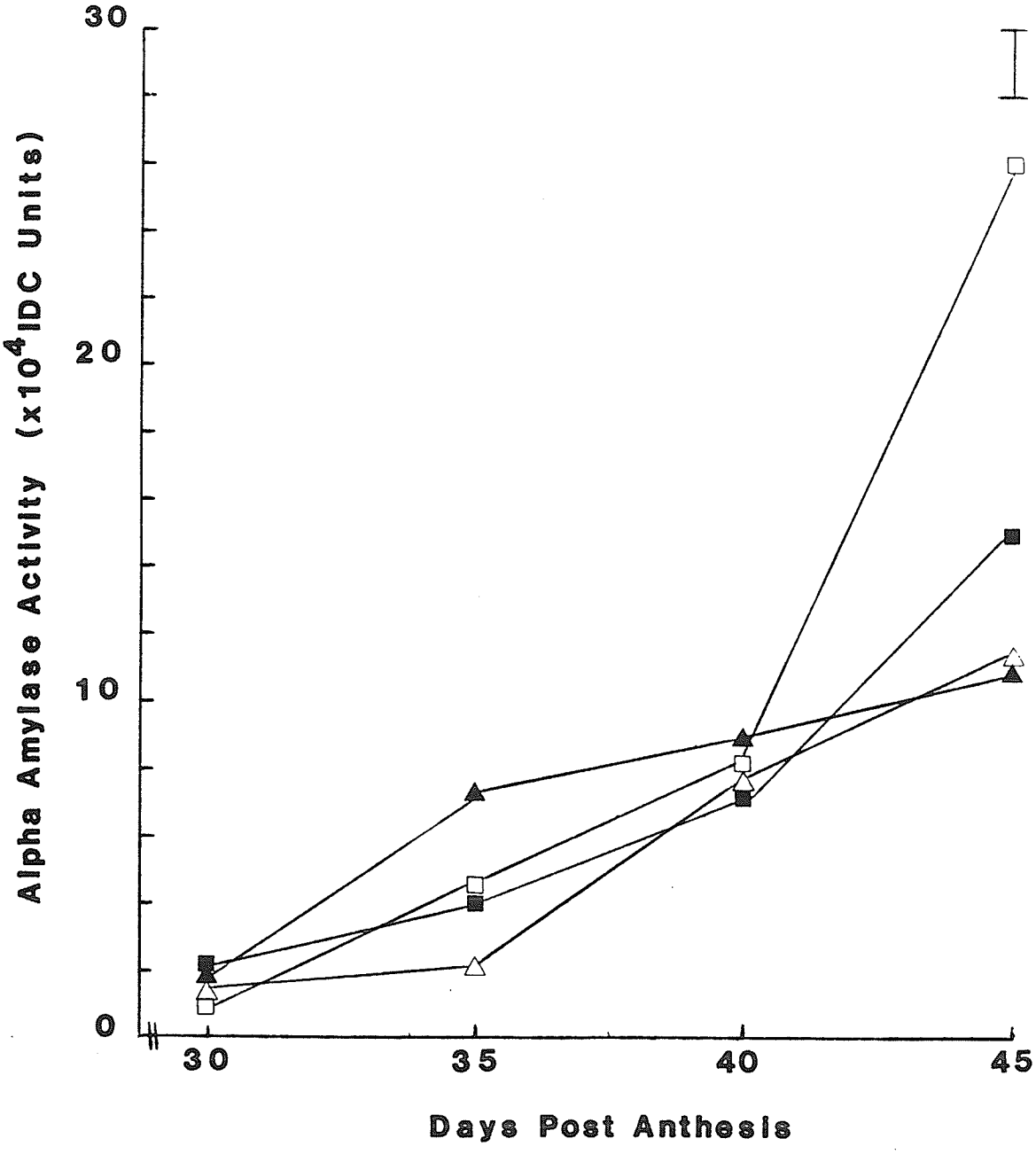
Analysis of the sucrose cultured systems reveals a slightly different trend. Both sucrose cultured systems show similiar amylase activities at day 30 yet the GA_3 induced system reveals a dramatic surge at day 35 after which it rises only slowly. The sucrose cultured kernels without GA_3 appear to be slower in invoking an amylase release not doing so till day 40. An increase at day 45 is also observed. (Figure 17)

These trends would appear to indicate that the sucrose cultured seeds tend to have lower amylase levels at maturity with no significant response to GA_3 being seen after day 35.

Figure 17 Alpha amylase release from Triticale 6A190 kernels in response to incubating in a bathing solution , with or without GA_3 present, for 24 hours.



Legend
= normally grown seeds without GA_3
= normally grown seeds with GA_3
= 5 % sucrose-cultured seeds without GA_3
= 5 % sucrose-cultured seeds with GA_3

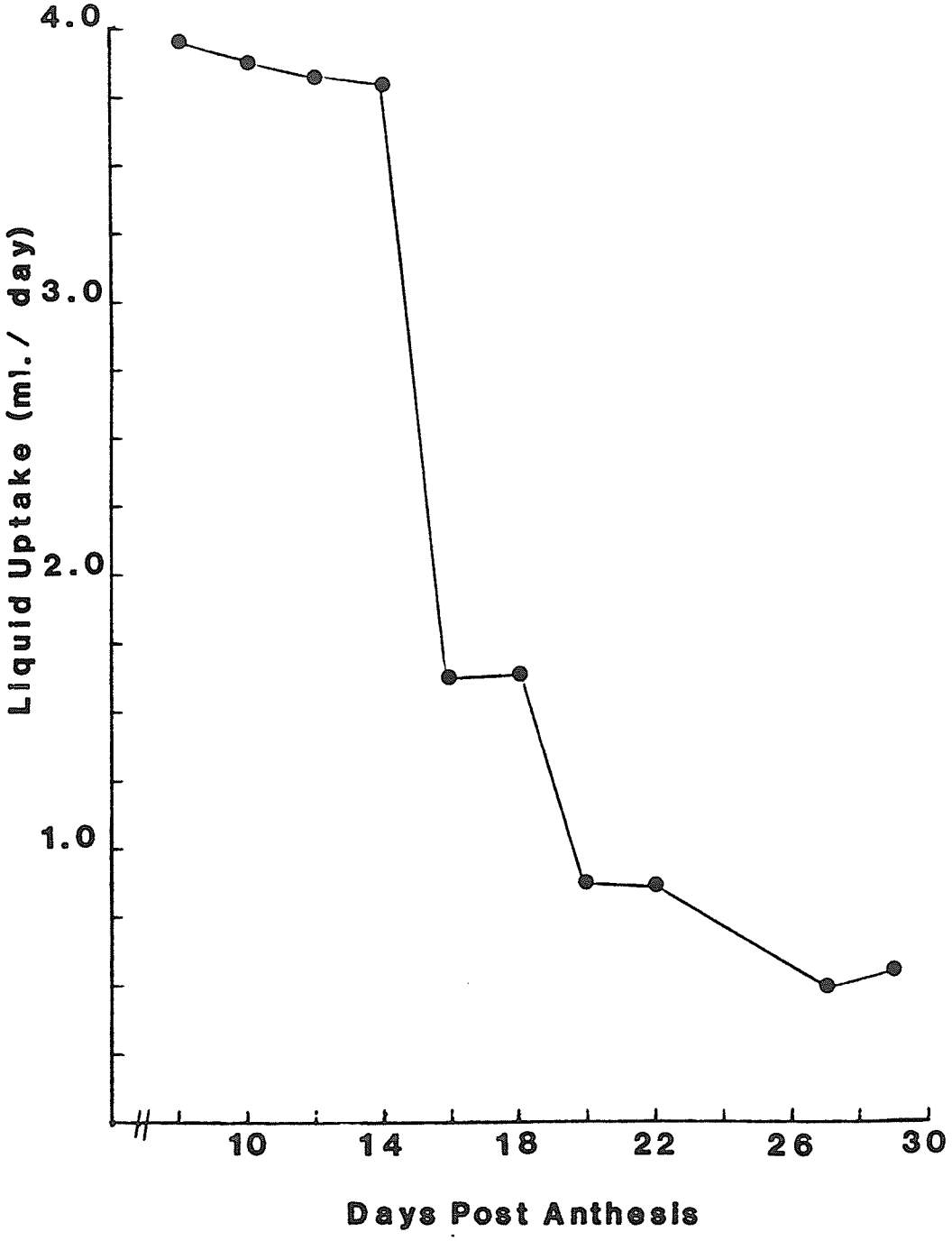

30
20
10
0
Alpha Amylase Activity (x10^{4}IDC Units)
30
35
40
45
Days Post Anthesis

DISCUSSION

Analysis of the combined results indicate that it is possible to maintain Triticale 6A190 excised spikelets on a liquid culture of 5 percent sucrose for twenty-one days although the pattern of growth diverges considerably with that seen in normal kernels with increasing periods of culture. Review of the current literature indicates investigation of liquid culture on plant growth has not been extended past 12 days (Lesar and Peterson 1981, Donovan and Lee 1977,1978).

The most readily observable feature of the liquid culture experiment was that the awn, glumes, and lemmas of the excised spikelets tended to yellow and dry out prior to normal heads. Yellowing was initiated at the tips of the awns 6-8 days after culturing began and proceeded slowly downward through the remaining floral portions. A similiar observation was voiced by Lesar and Peterson (1981) who also described a significant decrease in media uptake in the final 3 days of culture in their experiment. The decrease in media uptake is clearly visible in these experiments as seen in Figure 18. The yellowing of the floral organs would suggest that the cultured plants were under water stress.

Figure 18. Average daily liquid uptake for excised Triticale 6A190 spikelets on a 5 percent sucrose solution.


4.0
3.0
2.0
1.0
Liquid Uptake (ml./ day)
10
14
18
22
26
30
Days Post Anthesis

The kernels of all three sucrose cultured spikelets displayed a pattern of growth similiar to normally grown plants for the initial 5-6 day period. However, although no differences were seen in dry weights, the fresh weights then began to differ considerably from normal kernels. The change in fresh weight was the result of a substantial shift in water content. It is unfortunate that neither Lesar and Peterson (1981) nor Donovan and Lee (1977,1978) supply fresh weight data for comparison. In all sucrose-cultured systems the water content reached a similiar maximum at almost identical time periods although both 2.5 percent sucrose-cultured spikelets came from a different crop than either the 5 percent sucrose-cultured spikelets or the normally grown plants. The similiar patterns would imply that a critical component for continued water conduction was missing from the culture medium. This proposal is supported by the abrupt decrease in solution uptake seen in Figure 18. Lesar and Peterson (1981) and Donovan and Lee (1977,1978) both incorporated a series of vitamins and essential elements into their media which were not present in the Triticale culture solutions. It may be possible that the cultured spikelets were utilizing components already in the stem and not present in the medium for the initial 5-6 day period before exhausting this supply and initiating the sharp decline in liquid uptake. It is also possible that the decline may be due to the artificial nature of the system or by wound tissue

restricting the flow. The involvement of wound tissue was kept to a minimum by constant recutting of the stem to remove any possible restrictions.

Examination of the respiration rates of the cultured seeds with those normally grown supports the possible importance of the water content within the seeds. Although there are no apparent differences in respiration rates, the shortened period of maximum respiration of the 5 percent sucrose kernels corresponds with the water content within the seed.

The data indicates that neither of the 2.5 percent sucrose-culture solutions were capable of maintaining a substantial increase in dry weight after 9-11 days on culture. The 5 percent sucrose-cultured kernels were capable of sustaining an increase although the rate was reduced to 30 % of normal seeds by 12 days on solution. The reason for this inability of the 2.5 percent sucrose-culture solutions to maintain growth conflicts with what is reported by Lesar and Peterson (1981) who showed similiar patterns between normal and 55.6 mM sucrose cultured kernels. A possible explanation for this feature may lie in the strange metabolic pattern of Triticale 6A190. Saari (1977) reported a sudden decrease in sucrose concentration in this cultivar during a period of rapid dry matter accumulation. One of the objectives of this experiment was to observe whether or not sucrose culture could overcome the trough in sucrose concentration within the kernel. The data shown in Figure 12 clearly indicate

that none of the culture solutions could avoid this decline. It is important to realize that although the 5 percent solution did not avoid the trough , this concentration did return sucrose concentrations to normal values which was not the case for either of the two 2.5 percent sucrose solutions. It may be possible that at 2.5 percent the sucrose concentration is insufficient to overcome the metabolic demands placed on it. This proposal is supported by the observation that in both 2.5 percent sucrose-culture systems the dry matter increase is similiar in rate and amount to normal seeds until the sucrose concentration declines to minimum values at day 17 for the solution with and day 19 for the solution without glutamine. From this period onward the sucrose concentration within the seed does not return to normal and dry matter increases minimally. It would not be unwarranted to suggest that with the cessation of starch, and dry matter accumulation, and with sucrose concentrations remaining low, these seeds may have reached physiological maturity.

The capability of maintaining a dry weight increase is severely retarded if amino acids are not present in the medium (Donovan and Lee 1977). These authors suggest that amino acid omission causes a slowing in starch synthesis due to inadequate enzyme formation. The results from 6A190 on 5 percent sucrose do indicate a slower production of starch although the overall accumulated content or the rate of maximum deposition show no differences with normal kernels.

It is interesting to review the discrepancy seen in

Figure 5. between the dry weights of kernels cultured on 5 percent sucrose and those normally grown. At 29 days after anthesis the 5 percent sucrose-cultured seeds' dry weight average 32.4 mg. while normally grown seeds have achieved a weight of 38.8 mg.. Surprisingly, this large difference is not reflected in the starch contents of the two different seed systems as both are very similar. Comparison of these starch values with those found in Klassen et al. (1971) indicate that at this stage both sets of kernels have achieved 73.2-76 percent of their total starch content and therefore does not indicate any abnormal starch levels. The difference between the two systems may be reflected in their protein content. Klassen and coworkers (1971) list 6A190 as having 17.6 % protein content. It is worth noting that the dry weight of the 5 percent sucrose-cultured seeds is 83.5 percent of that of the normally grown kernels. Although these figures can account for the differences, it is misleading for the entire difference to be attributed to protein,as to do so would imply that there is no protein whatsoever in the cultured kernels.

Wardlaw (1971) stated that grain growth in water stressed plants ceased prematurely and resulted in a reduction in grain yield. However, the differences in growth did not become evident until 24 days post anthesis. It is possible that a similiar phenomena is existing in these experiments. Wardlaw (1971) further suggests that the stress accelerates senescence. It would appear that in the

2.5 percent cultured kernels reached premature maturity and ceased further growth. The 5 percent seeds are displaying decreased growth as they are approaching senescence. It is possible that since the starch content is similiar between normal and 5 percent sucrose-cultured kernels the decline in growth is affecting the formation of cellular material,such as cell walls, rather than starch production.

The period of rapid starch accumulation corresponds well with the period of dry matter increase and sucrose decline. The decline in sucrose is therefore not surprising if it is assumed that the rate of sucrose supply becomes insufficient to support the increased starch synthesis. Support for such a proposal can be seen in the work of Saari (1977) in which sucrose synthase activity in 6A190 undergoes a sharp rise during a corresponding period of rapid dry matter accumulation. There is a discrepancy in this arguement in that the 5 percent sucrose-cultured kernels display a sucrose concentration minimum over days 18-20 while showing a starch deposition rate only approaching maximum values. The maximum deposition rate is not achieved until days 20-22 after anthesis when sucrose levels are returning to normal. This discrepancy can possibly be explained by the problem of getting all kernels of a plant at the same stage of development. It is quite possible that the seeds analyzed had already passed through the sucrose minimum at an earlier date. Such a suggestion is not without support as

it can be seen that the sucrose decline in normal kernels can be a single point.

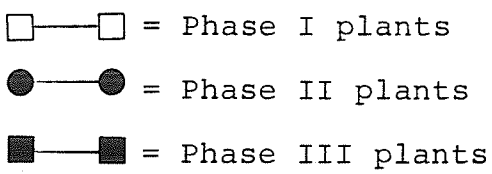
Examination of the ^{14}C sucrose uptake data indicates a pattern of decreasing velocity with increasing time of incubation. This decrease may be accounted for by the altered conditions under which the experiment was done. It must be emphasized that normal sucrose transport to the kernel is via the phloem (Frazier and Appalanaidu 1965) which according to Evans (1970) has a sucrose concentration of 10 percent. However, in the excised spikelet culture technique the radioactive sucrose is mainly transported to the kernel in the xylem. Jenner (1979) reported that under these conditions it is only at the base of the grain, where the xylem discontinuity is found,(Zee and O'Brien 1970) does transfer back to the phloem occur and normal movement into the kernel is reestablished. Jenner (1979) believes that culturing allows the final entry of sucrose to be the same as normal but no comment is put forth on the possible effects on the rate of assimilate movement.

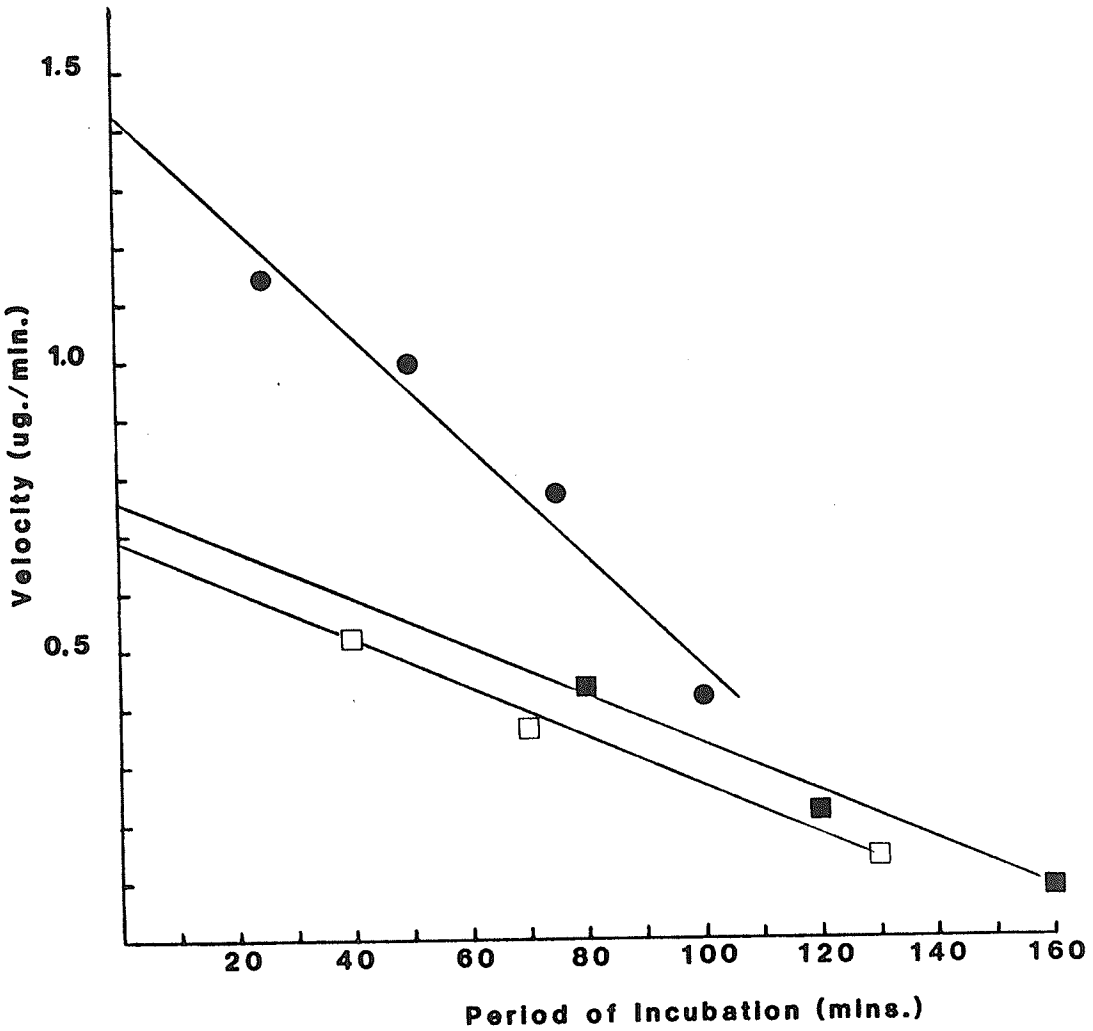
Strong emphasis must also be given to the radioactive sucrose being incorporated in a 1 percent sucrose solution and not a normal 10 percent solution. The reason a 1 percent solution was utilized was strictly economic as to incorporate sufficient radioactivity into a 10 percent solution would be economically prohibitive. It may therefore be possible that as time increased the concentration gradient at the base of the kernel was decreased by the incorporation of

a 1 percent sucrose solution from the xylem. Analysis of the data therefore suggests that if the velocity in all three phases is decreasing with time a realistic initial velocity may be obtained by extrapolating to time zero. Values derived from such an extrapolation are for Phase I, 0.7, for Phase II, 1.42, and for Phase III 0.75 ug. sucrose per minute. It is quite evident that during Phase II there is a 2 fold increase in the relative supply of assimilate in response to increased demand. (Figure 19)

Examination of dry matter increases for plants in Phase I indicate that the kernels on average underwent a 1.1 mg.per day increase. Transformation of Phase I translocation rates indicate a sucrose supply in the order of 1.01 mg.per day. During Phase II, the period of maximum dry weight increase, a 2.8 mg. per kernel per day gain was observed. The corresponding translocation rate of 2.04 mg. sucrose per day indicates a significant deficiency in sucrose movement. Consideration must be given to the fact that the earliest velocities were determined at 25 mins. after immersion into solution. The events which occur during the initial period of "xylem loading" and the corresponding movement in this artificial system would effect the determination of velocities. The unaccountable factors influencing the calculated velocities would have a more pronounced effect on the Phase II study as it was done over a much shorter time frame which was closer to the initial immersion. Examination of Phase II

Figure 19. Sucrose translocation rates as a function of time for Triticale 6A190 kernels in three different periods of growth.




1.5
1.0
0.5
Velocity (ug./min.)
20
40
60
80
100
120
140
160
Period of Incubation (mins.)

velocity patterns reveals a somewhat curved nature and if the extrapolated velocity was determined from a line tangent to this curve a translocation rate of 2.38 mg. sucrose per day into the kernel is obtained. The phase III data does indicate that the translocation rate has decreased to a value similar to that in Phase I and is insufficient to account for the 1.6 mg. per day increase in kernel dry weight observed.

It is important to keep in perspective when comparing dry weight increases and sucrose delivery that the two sets of data were derived from two different crops. Although growing conditions in the greenhouse and growth room were kept as close as possible, physiological responses may be slightly different. The plants used for ^{14}C uptake were monitored by following the total carbohydrate per kernel in random samples. The Phase I material was utilized at 8 days post anthesis to avoid the sucrose trough, while Phase II spikelets were placed on radioactive sucrose when they displayed a large drop in sugar content. Phase III spikelets were placed on sucrose well past the trough. Since the plants in Phase II had such a short period of sucrose decline it is not unreasonable to suggest that out of 20 plants utilized not all kernels would be at the same stage. It is probable that the period of maximum demand was bracketed and included in the samples. Examination of Table 1 indicates that the seeds in question did not achieve minimum sucrose levels seen in the normal kernels followed

in the previous experiments. As such, since the velocities are averages of 5 plants for each time period the translocation rate determined must be considered a composite value.

Although sucrose is the major translocated substance it is by no means the only component of the phloem. Protein content must be considered in the overall dry weight increases displayed by the kernels. In Phase I the daily supply of sucrose calculated from the maximum extrapolated velocities accounts for 91.8 percent of the dry matter increase. In Phase II sucrose supply accounts for 85 percent while in Phase III, 67.5 percent. The decreasing capability of the sucrose supply to account for the dry matter increases is consistent with the involvement of protein. It is only at 5-6 days post anthesis that the earliest traces of protein deposits are found in wheat and deposition begins in earnest only at approximately 15-18 days after flowering (Jennings and Morton 1963). Examination of the rate of starch deposition in Phase I averages 0.6 mg. per day, well below the 1.01 mg. sucrose per day delivered. However, during Phase II, starch accounts for only 85 percent of the dry matter increase during a period of maximum depostion. The deposition rate at this time is 2.4 mg. per day. This value is similar to the tangent to the curve extrapolated value of 2.38 but does show a deficiency on the linearily extrapolated value of 2.04 mg. sucrose per day.

Inspection of the respiration rates of the seeds shown in Figure 6 reveals a broad peak with an average value of 200 nmoles O_2 $hr.^{-1}seed^{-1}$. Calculation of a corresponding sucrose utilization on a per day basis indicates a consumption of 0.16 mg.sucrose. The abnormal trough observed in sucrose levels may therefore be due to the inability of the delivery system to supply sufficient sucrose during a period of high demand. It would appear that even the 2 fold increase in delivery is mainly consumed in starch synthesis forcing the kernel to draw upon it's equilibriated internal sucrose to supplement it's respiration and other anabolic requirements.

The increased delivery rate displayed in these experiments must be considered to be for an artificial system yet they imply the cause of the sucrose decline is not directly due to sugar supply but another facet of the kernel's growth. Saari (1977) has indicated that during the period of rapid dry matter increase there is a large increase in sucrose synthase activity. Since 6A190 displays a period of abnormally large synthase activity confined to a short period (Saari 1977) it would seem to imply that the starch synthesis system exceeds the capability of the plant to supply nutrients, forcing the decline in sucrose. Some form of feedback inhibition must then come into effect on the synthase as it's activity rapidly declines. This allows the sucrose translocation rate,which is working at maximum capability,to supply

sufficient sugar to meet all anabolic and respiratory requirements and allow internal sucrose concentrations to return to normal.

Comparison of the responses seen in normally grown kernels with those cultured on 5 percent sucrose does suggest that sucrose may have some effect on alpha amylase levels although the manner of action is still unknown. Examination of activity levels at 45 days after anthesis indicates that there is no significant differences(t test) between normal and sucrose-cultured seeds. However, this statement is correct only if certain qualifications are utilized. The culturing of spikelets on sucrose results in a severe reduction of water content as shown earlier. The results stated by Wardlaw (1971) suggest that water stress can cause premature senescence. King et al. (1979) have indicated that desiccation is a prerequisite for invoking a response to applied GA_3 in 6Al90. It is therefore not unwarranted to suggest that at all days examined the sucrose cultured seeds are in a greater state of desiccation than their counterparts. The data shown in Figure 17 indicates that although in a state which should be more responsive, the sucrose cultured seeds, both those with and without GA_3 , are not showing a greater response. Furthermore, at day 40 all four seed systems are at the same amylase level yet both the normally grown seed systems show a significant increase in amylase at day 45 which is not reflected in either of the sucrose-cultured

seed systems. It would appear that although numerically the sucrose is not invoking an insensitive response, when the increased desiccation is considered, an effect is definitely implied. Such a statement would initially appear contradictory since desiccation is a prerequisite for GA responsiveness. However, King and coworkers (1979) do suggest that desiccation may not be the sole trigger for amylase production and release in the maturing kernel. Support for this suggestion is voiced by Nicholls (1979) who concluded from his studies of the sensitivity of wheat caryopsis to GA_3 that desiccation was not necessary for the development of the ability of the aleurone to respond to GA_3. King and coworkers (1979) had observed that kernel desiccation did not always predate the appearance of alpha amylase. Nicholls (1979) also concluded that transformation of the aleurone to the responsive state was time dependant and required some facet of metabolism. He further speculated that rapid dehydration of the kernels reduces the development of the responsive state. The rapid loss of water within the kernels on 5 percent sucrose would be in agreement with this statement. Nicholls as well suggested that from his finding the maintenance of sucrose supply to the cultured ear of some genotypes inhibited the formation of a responsive state and that the embryo was not necessarily involved in the responsive state formation, directly or indirectly. The results of the experiment presented in this paper, when considered with Nicholls' findings, do indeed

imply an effect by sucrose in alpha amylase production in maturing Triticale 6A190 kernels. However, although the arguments presented appear to provide a satisfactory explanation , another dilema arises. How does the 5 percent sucrose-culture exert its' effect when the sucrose in the phloem at 10 percent (Evans 1970) does not?

In conclusion, this study, in conjunction with the results of King et al (1979) and Nicholls (1979), highlights the need for further research on the patterns of metabolic components within Triticale 6A190 as it matures. The comment by King and coworkers (1979) that a simple hormonal explanation is not sufficient appears to be very accurate.

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