AN INVESTIGATION OF KERNEL SHRIVELLING IN TRITICALE

A Thesis

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by

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ABSTRACT

Triticale lines selected for variation in degree of kernel shrivelling were studied to test the hypothesis that shrivelled kernels were the result of the rapid breakdown of endosperm starch to sugars following initiation of premature germination processes.

Grain density measurements were highly correlated with bushel weight and also agreed well with the visual rating of shrivelling and, thus, were used as the index of degree of kernel shrivelling. Alpha-amylase activity of mature Triticale grain varied greatly between lines and was inversely correlated with grain density. That is, lines with low density had high amylase content. To study the development of the amylase activity a line with low activity (6A320) and a line with high activity (6A190) were compared at different stages of grain maturity. In both lines amylase activity was high at 10 days followed by a rapid decrease to 38 days at which time activity in 6A190 increased very rapidly as compared to only a slight increase in 6A320.

As expected, reducing sugar content of mature grain reflected the alpha-amylase activities. In addition, developmental patterns of reducing sugars coincided closely with the alpha-amylase patterns for the lines 6A190 and 6A320. Reducing sugar levels in Manitou wheat decreased steadily during early stages and reached a constant low level by maturity.

Starch content at maturity was not correlated with either alpha-amylase or reducing sugars but a correlation of 0.75* was obtained for starch content and grain density. Starch accumulation profiles during grain development were similar for 6531, 6A320 and 6A190 at early stages, however, at approximately 55 percent moisture starch deposition in 6A190

ceased while 6531 and 6A320 continued to increase. Consequently, at maturity 6A320 and 6531 had about 6 and 10 mg. more starch per kernel respectively than 6A190. In Manitou deposition continued until approximately 40 percent moisture. These patterns were very similar to the patterns of dry matter accumulation.

Mean endosperm starch granule size of Triticale lines ranged from 17.81 to 25.61 μ as compared to 18.70 for Manitou and 28.24 for Prolific rye. There was no consistent relationship between starch granule size and degree of kernel shrivelling. The three Triticale lines with the lowest mean starch granule size all had Triticum persicum included in their parentage.

Grains of 16-day old excised heads of 6531 incorporated approximately 10 percent more sucrose-^{14}C into endosperm than 6A190 indicating that the rate of synthesis in endosperm tissue of 6A190 is probably lower than 6531.

No improvement in grain development was observed following the injection of 1 ml. of 10^{-1}M CCC into the culms of 6A190 and 6531 either at flowering or 31 days later. Alpha-amylase activity in CCC treated grains of 6A190, however, was reduced to 78 percent of the untreated controls suggesting that kernel shrivelling cannot be directly attributed to high levels of alpha-amylase.

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1. INTRODUCTION

Artificially synthesized interspecific and intergeneric plant hybrids have been extensively employed to study genetic relationships among species and for this purpose they have proved to be extremely useful. More recently they have also been used as tools in the transfer of desirable genes into cultivated crop species. Triticale, which is a combination of Triticum (wheat) and Secale (rye) genomes, has for some time now received close attention as a potentially useful commercial crop. A comprehensive discussion of the breeding program and basic research at the University of Manitoba directed toward the development of Triticale as a commercial crop from 1954 to the present may be found in a paper by Larter (1968).

In order to develop wheat-rye hybrids to a point where they possess desirable levels of agronomic and quality characteristics a number of problems must be overcome. While a great deal of progress toward improvement has been made several areas need further attention.

One of the persisting problems in the development of Triticale is its poor kernel characteristics. The kernels, rather than being well filled and plump like wheat, are severely shrivelled at maturity, thus resulting in a low weight per bushel. This shrivelling is independent of environment and occurs even under favourable growing conditions (personal observation).

It had been frequently observed that Triticale was more susceptible to germination in the ear prior to or at maturity than locally adapted cereal varieties. In addition, reports from a number of sources indicated that mature Triticale grain was higher in alpha-amylase activity than sound wheat (see for example, Müntzing, 1963).

In view of these findings and observations it was postulated that shrinkage and partial collapse of the endosperm of Triticale might result from the rapid conversion of starch to sugar associated with the onset of precocious germination.

The objectives of this study were to test this hypothesis by determining the nature of the relationships among kernel shrivelling, alpha-amylase activity, and carbohydrate content of Triticale lines.

2. LITERATURE REVIEW

2.1 Breeding and development of Triticale

Triticale is an allopolyploid produced by doubling the chromosome number of the sterile hybrid that results from a cross between wheat, Triticum aestivum L. em Thell (group aestivum) or T. turgidum L. (group durum) and rye, Secale cereale L.

Literature relating to the breeding and development of wheat-rye hybrids from the earliest reports in the later 1800's to the present has recently been reviewed by Briggle (1969).

The first serious breeding work with Triticale was begun in Sweden in 1934 by Müntzing (1939). Working with octaploid (56 chromosome) Triticales he found that all lines were partially sterile and pollen fertility was lower than in standard wheat varieties. Spanish workers headed by Sanchez-Monge (1955) also investigated the usefulness of 56-chromosome Triticales but found that shrivelled grain and sterility were problems. This led him to speculate that 42 chromosomes would be nearer the optimum number for Triticale than 56 chromosomes. However, he found that the grain of hexaploid Triticales resembled that of the octaploid Triticales in that it was shrivelled and of low quality resulting in low flour yields, (Sanchez-Monge, 1958). Using gamma irradiation of the female parent immediately after emasculation followed by pollination with pollen of sister plants Sanchez-Monge (1968) was able to achieve some improvement in endosperm quality. From 77 progenies it was possible to select 5 with smoother seeds. Reciprocal crosses between these 5 lines and their original lines seemed to indicate a plasmagenic influence on endosperm quality.

Canadian Triticale research initiated in 1954 has also been concentrated on the improvement of 42 chromosome types. Considerable progress has been made in the improvement of agronomic characteristics such as plant height and maturity (Larter, et al., 1968). Partial sterility and shrivelling of the grain are limiting factors at present. Cytological instability which exists within Triticale lines is believed to be the cause of the partial sterility. Aneuploids were observed with a frequency of 10-15 percent in a routine somatic chromosome count of bulk seed samples from 30 strains. The majority of the aneuploid types were hypoploid. They observed that any one of the species Triticum timopheevi, T. persicum or Secale montanum, when included in the parentage of a Triticale hybrid, contributed genes for desirable kernel characteristics to the progeny.

2.2 Alpha-amylase development

Schwimmer (1947) found that the amount of alpha-amylase per wheat kernel remained relatively constant throughout the entire course of development and early stages of ripening. However, on a per gram of dry matter basis immature wheat had about 68 times the activity of mature grain. In contrast, the amount of beta-amylase per gram dry weight increased with the development of the grain, being attended at the same time by a decreased solubility in water. Similar results were reported by Olered (1964), namely that alpha-amylase activity per unit of dry matter in wheat was very high during the first stages of kernel development and that there was then a very rapid reduction such that when the grain had reached the "milky stage" the residual alpha-amylase activity was already very small.

Initiation of germination processes in cereal grains are characterized by large increases in alpha-amylase activity. In rye with as little as 0.5 percent visibly germinated seed the amylase value was nearly always high (Tedin and Persson, 1963). Even among the plants with no visibly germinated seeds there were many with very high amylase activity. Likewise, in winter wheat Bingham and Whitmore (1966) observed that there were considerable varietal differences in susceptibility to germination in the ear and such germination was always associated with an increase in alpha-amylase activity. However, even in the absence of germination some varieties exhibited high levels of alpha-amylase and the authors concluded that alpha-amylase activity appeared to be under two types of genetic control. From the point of view of plant breeding it is thus not sufficient only to select against visible sprouting but it is necessary to carry out chemical assays for amylase activity as well. Selection against alpha-amylase activity in rye was shown to be effective.

Müntzing (1963) noted that rye had a stronger tendency to pregermination than wheat and that in octaploid wheat-rye hybrids this tendency seemed to be just as marked as in rye. Variation in alpha-amylase activity was found among different lines of Triticale and the line with the lowest amylase activity was also the one with the best kernel type as determined by visual judgement.

In germinating rice seeds rapid breakdown of endosperm starch began after about four days of germination (Murata, et al., 1968). Alpha-amylase activities were found to parallel the pattern of starch breakdown. Juliano and Varner (1969) studying germinating peas found that starch was degraded slowly in the first 6 days, a period during which alpha-amylase activity was very low. Beta-amylase was present at a

constant level while phosphorylase gradually increased and reached a peak on the fifth day. Beginning on the sixth day there was a more rapid degradation of starch which coincided with alpha-amylase production. The authors consequently concluded that alpha-amylase was the major enzyme involved in the initial degradation of starch into more soluble forms while phosphorylase and beta-amylase assisted in the further conversion to free sugars.

2.3 Gibberellins and amylase activity

The stimulation by gibberellic acid (GA) of alpha-amylase activity and consequently of sugar release from starchy endosperm is well documented and is being utilized on a commercial basis in the malting industry.

Paleg (1960) was one of the first to demonstrate that added GA stimulated the production of amylase enzyme and release of reducing sugars from barley endosperm. Yomo (1958) found that separated barley embryos and endosperms produced less alpha-amylase when cultured separately than when cultured together on a gel of agar. He subsequently showed that the embryo produced a gibberellin-like hormone which diffused from the scutellum and activated the endosperm to produce hydrolytic enzymes, which then in turn degraded the endosperm. When treated with GA, cell walls of barley endosperm first break down at the periphery adjacent to the aleurone layer indicating that the aleurone layer must be the site of GA action (Briggs, 1963). The amino acid analogues DL-ethionine and DL-p-fluorophenylalanine were potent inhibitors of sugar release and also depressed the incorporation of radioactive amino acids into soluble protein. Since this phenomenon is a well known aspect of the inhibition of de novo protein synthesis, Briggs concluded that GA acts by stimulating

de novo enzyme synthesis rather than by activating a preformed enzyme. These observations and conclusions were supported by the work of Varner (1964). Visual inspection of GA-treated barley half-seeds revealed that that part of the starchy endosperm in contact with the aleurone layers was first dissolved indicating that the alpha-amylase was probably coming from the aleurone layer. This was confirmed quantitatively. Following incubation with phenylalanine-^{14}C alpha-amylase was found to be labelled and to constitute a major fraction of the label incorporated into protein. The author questioned whether the entire amylase molecule was produced by de novo synthesis or whether the labelling resulted from addition to or modification of a precursor as is the case in B. subtilis. Using density labelling of barley alpha-amylase with H_2O^{18} Filner and Varner (1967) showed that all of the enzyme was synthesized de novo in response to gibberellic acid.

Radley (1967) showed the scutellum to be the site of production of gibberellins in the early stages of the germination of barley seed. Activity of the scutellum ceased on the third day at which time the axis probably commenced to produce gibberellins. CCC (2-chloroethyl trimethylammonium chloride) was an effective inhibitor of gibberellin production.

All concentrations of GA_3 (0.07-0.09 µmoles/plant) sprayed onto intact leaves of tobacco plants caused a significant reduction of starch levels in the leaves (Lee and Rosa, 1969). Reducing sugar levels increased but were not great enough to account entirely for the loss of starch in GA_3 treated leaves. GA_3 treatment also resulted in a significant increase in amylase activity and the authors attributed the decrease in starch content to this enzyme stimulation.

2.4 Gibberellins and seed dormancy

Black and Naylor (1959) by feeding GA at 100 and 1000 ppm to Avena fatua heads excised at the "milky stage" were able to produce non-dormant seeds. Seeds produced on heads fed only H_2O were completely dormant. Simpson (1965) demonstrated that non-dormant embryos of Avena fatua produced endogenously a gibberellin-like factor. This factor was absent in freshly matured dormant embryos but increased in amount with length of the afterripening period.

2.5 Inhibitors

Abscisic acid at 5×10^{-5}M almost completely inhibited growth response and alpha-amylase synthesis in barley seed (Khan and Downing, 1968). The inhibition was reversed to a large extent by kinetin while GA was far less effective. However, GA_3 in combination with kinetin almost completely reversed the inhibition of alpha-amylase synthesis. Paleg, et al. (1965) observed CCC to be ineffective in restricting release of reducing sugars from excised barley endosperm tissue. Similarly, El-Fouly and Jung (1966) by spraying CCC on wheat plants were unable to alter the levels of amylase activity in the ears. However, amylase activity in leaves and stems was increased by CCC treatment. Larter (1967) found CCC to be effective in reducing the height of barley plants. This is a typical CCC response and indicates an inhibition of GA synthesis or action since gibberellins are known to promote internode elongation of dwarf plants of various species. CCC treatment (10^{-1} of 10^{-3}M) had no significant effect on protein percentage, kernel weight or total beta-amylase activity of grain from the treated barley plants.

2.6 Grain development and chemical composition

Numerous investigations of starch development in cereal grains and corn have been reported in the literature. Frequently these have also included studies of changes in the physico-chemical characteristics of the starches during development.

Jennings and Morton (1963) reported that in the developing wheat grain starch content per endosperm increased rapidly and almost linearly from about day 12 to day 35. The percentage of endosperm dry weight constituted by starch also reached a maximum value about this time. While the rate of starch synthesis in normal barley (Glacier) was higher than that in the high amylose cultivar Glacier (Pentlandfield), in both cases most of the synthetic activity in the developing grain occurred between the third and sixth weeks after anthesis (Merritt and Walker, 1969).

Del Rosario, et al. (1968) observed that about 80 percent of the starch in the mature rice kernel had already been synthesized in 14-day old kernels. The aleurone layer was already fully developed in kernels 7 days old. According to Briones, et al. (1968) individual starch granules in rice endosperm increased in size throughout grain development. They noted that the fivefold increase in starch content per grain during development from day 4 to 39 could be due mainly to the increase in size and not in the number of starch granules. As early as 4 days after flowering all starch samples were birefringent. Gelatinization temperature range remained constant at 61° - 65° C during the first two weeks but decreased slightly toward maturity. Amylose content increased from 25.0 to 28.9 percent suggesting that there must be more amylose in the outer starch granule layers than at the center.

Bice, et al. (1945) found wheat endosperm starch to be abundant 8 days after pollination and to increase in amount approximately linearly with time. They also reported an increase in amylose content from 19 percent at 12 days to 25 percent at 24 days. Increase in starch granule size was marked up to 12 or 15 days but there was relatively little subsequent change.

The ratio of amylose to amylopectin in both normal and high amylose barley increased during ripening of the grains (Merritt and Walker, 1969). Average diameter of starch granules in the high amylose barley was smaller than in normal barley at all stages of endosperm development. Similar increases in amylose content during development of barley kernels were reported by Harris and MacWilliam (1958). In a later publication Merritt (1969) showed that during the maturation of normal and high amylose barley the susceptibility of the raw starch to attack by alpha-amylase remained constant until the moisture content of the grain fell below about 60 percent. Thereafter the starch developed a resistance to amylolysis which was directly proportional to the loss of grain moisture. During malting the bulk of the starch solubilized was the amylopectin fraction although amylose was increasingly utilized when germination was prolonged.

Comparing starches from 17 varieties of wheat Medcalf and Gilles (1965) found amylose content to range from 23.4 to 27.6 percent with durum starches tending to be on the high end of the range (26.4 - 27.5 percent). Hard red spring and durum starches were very similar in density. The data suggested that solvents could penetrate durum starches more readily than other wheat starches.

Smooth-seeded peas contained 42 to 43 percent starch composed of 35 percent amylose whereas wrinkled peas had only 31 to 32 percent

starch with an amylose content of 65 percent (Greenwood and Thomson, 1962). In addition, the amylopectin chain length in smooth seeds was 26 to 27 glucose units as compared to 35 to 36 glucose units for the amylopectin from wrinkled seeds.

Reducing sugars in developing wheat grain almost completely disappeared during the maturation phase (Jennings and Morton, 1963). The pattern in durum wheat was similar, however, non-reducing sugars remained at a considerably higher level at maturity (Menger, 1961).

2.7 Starch granule development

Size and shape are starch granule characteristics which vary from species to species and can be used for identification purposes. MacMasters, et al. (1957) described cereal starch granules for this purpose. Wheat, barley and rye starches are similar in that all contain both small, spherical and large, lens-shaped granules. Wheat starch, however, has a larger number of small granules while in rye starch many of the larger granules have a prominent hilum with radiating fissures. Normal starches stain blue or violet with iodine while waxy starches with little or no amylose appear red to red-brown.

Starch granule development in wheat grain was studied photomicrographically by Sandstedt (1946). He found minute starch granules already present in the unfertilized ovary. Pericarp starch developed rapidly with granules reaching maximum size by the fourth or fifth day. However, it practically disappeared due to enzymatic digestion by the time the kernel attained its maximum length. At about the time endosperm cell walls formed (4 or 5 days) minute starch granules were observed in the interior of the endosperm. These developed into the large, lenticular granules

which are characteristic of wheat starch. Small spherical granules began to develop at about the time the kernel attained full length. These completely filled the spaces between the lenticular granules. A more recent report by May and Buttrose (1959) outlines essentially the same general features for starch granule formation in developing barley kernels. Granules initiated up to 15 days from anthesis (Type A) were at first lenticular-shaped, irregular in outline and quite transparent, whereas those initiated between 18 and 30 days (Type B) were spherical, regular in outline and much denser. At all stages type A granules contributed more than 90 percent of the total volume despite the fact that type B granules represented 88 percent of the total number at 36 days. Both starch and endosperm density increased in the later stages of development.

Observations using a scanning electron microscope confirmed the presence of an equatorial groove in large wheat starch granules (Evers, 1969). The absence of this groove in small granules provides evidence in support of different modes of formation of these two types of granules. One shape which was frequently observed was characterized by a deep concavity on the upper major surface of the granules. Presumably the opposite side was similar. The presence of this concavity tends to support the view of May and Buttrose (1959) that the center of the granule is less dense and thus with its weaker crystalline structure would be more susceptible to collapse. In a study of the endosperm of dent corn Wolf, et al. (1952) found that horny endosperm cell walls were about 1.0 μ thick while floury endosperm cell walls were about 1.3 μ thick. Starch granules in floury endosperm cells were large and had relatively smooth surfaces indicating the absence of high pressures. In contrast, granules were smaller and more tightly packed in horny endosperm cells.

2.8 Genetic control of grain carbohydrates

Studies on corn have yielded the greatest amount of information about genes affecting endosperm characteristics, many of which influence amylose to amylopectin ratios. Waxy genotypes which result in zero amylose have been found in a number of species.

Quantitative data for carbohydrate content are given by Creech (1965) for single, double and triple recessive combinations of the genes ae, du, sh_2, su_1, su_2, and wx. The amylose extender gene ae, in addition to changing the amylose content, also caused a substantial increase in sugars and a reduction in starch. Genotypes ae, du, sh_2 and su_1 all had starch granules with smaller mean diameters than normal. This was particularly marked in su_1. An earlier study by Kramer, et al. (1958) in which the same genes were investigated provided information on the amylose content and kernel phenotypes which resulted. Of particular interest to the present study is the gene su_1 which although not changing the amylose content does result in wrinkled kernels, presumably because of the reduction in starch content of the endosperm.

Karper and Quinby (1963) reporting on a sugary endosperm character in sorghum noted that sugary sorghum seeds wrinkled as they matured and that they were about one-quarter smaller than normal seeds. At maturity sugary seeds contained about 4 percent sugars compared to approximately 2 percent in the normal. Genetically, sugary functioned as a simple recessive to normal.

A developmental study of carbohydrates in maize plants carrying su_1 and sh_2 genes singly and in combination was carried out by Jennings and McCombs (1969). After 21 days dry matter content of shrunken lines

began to lag behind that of full lines. Reducing sugars followed a similar pattern in all four lines, namely beginning at about 20 percent of dry matter and decreasing to almost zero at 27 days. Non-reducing sugars decreased in the full lines whereas in shrunken lines they remained at a high level throughout, amounting to about 30 percent of dry matter in the double recessive su_1sh_2. Shrunken genotypes exhibited marked reductions in starch content with large increases in non-reducing sugars accounting for a large portion of the reduction. The sugary-full genotype also had reduced starch content but in this case the difference was made up by about 30 percent water soluble polysaccharides.

3. MATERIALS AND METHODS

3.1 Growth and collection of plant material

Eight Triticale lines were chosen on the basis of a visual assessment of the degree of kernel shrivelling. The lines selected represented a range of kernel types varying from poor to fairly plump. Parentages of these lines are given in Table 3.1. These lines along with the hard red spring wheat variety Manitou, the durum wheat variety Stewart 63 and Prolific spring rye were grown under field conditions at Winnipeg during the summer of 1968. No supplemental fertilizer or irrigation water was applied. Temperatures were below normal and precipitation above normal during the latter half of the 1968 growing season.

Spikes of all lines were tagged at the time of anther extrusion and six spikes of each line were harvested at each of seven different stages of maturity beginning 10 days after initiation of flowering and at weekly intervals thereafter. In addition, a bulk sample of each line was harvested at maturity. Caryopses were removed from the spikes immediately after harvest, weighed to obtain fresh weight and stored at approximately -25°F. At a later date the seed samples were freeze-dried and reweighed.

3.2 Chemical analyses

Grain density was estimated by determining the volume of a seed sample of known weight by displacement of a light paraffin oil. For all chemical analyses grain was ground in a Wiley mill to pass through a number 40 screen.

Protein content (N x 5.7) was estimated by the macro Kjeldahl pro-

Table 3.1. Parentages of Triticale lines studies.

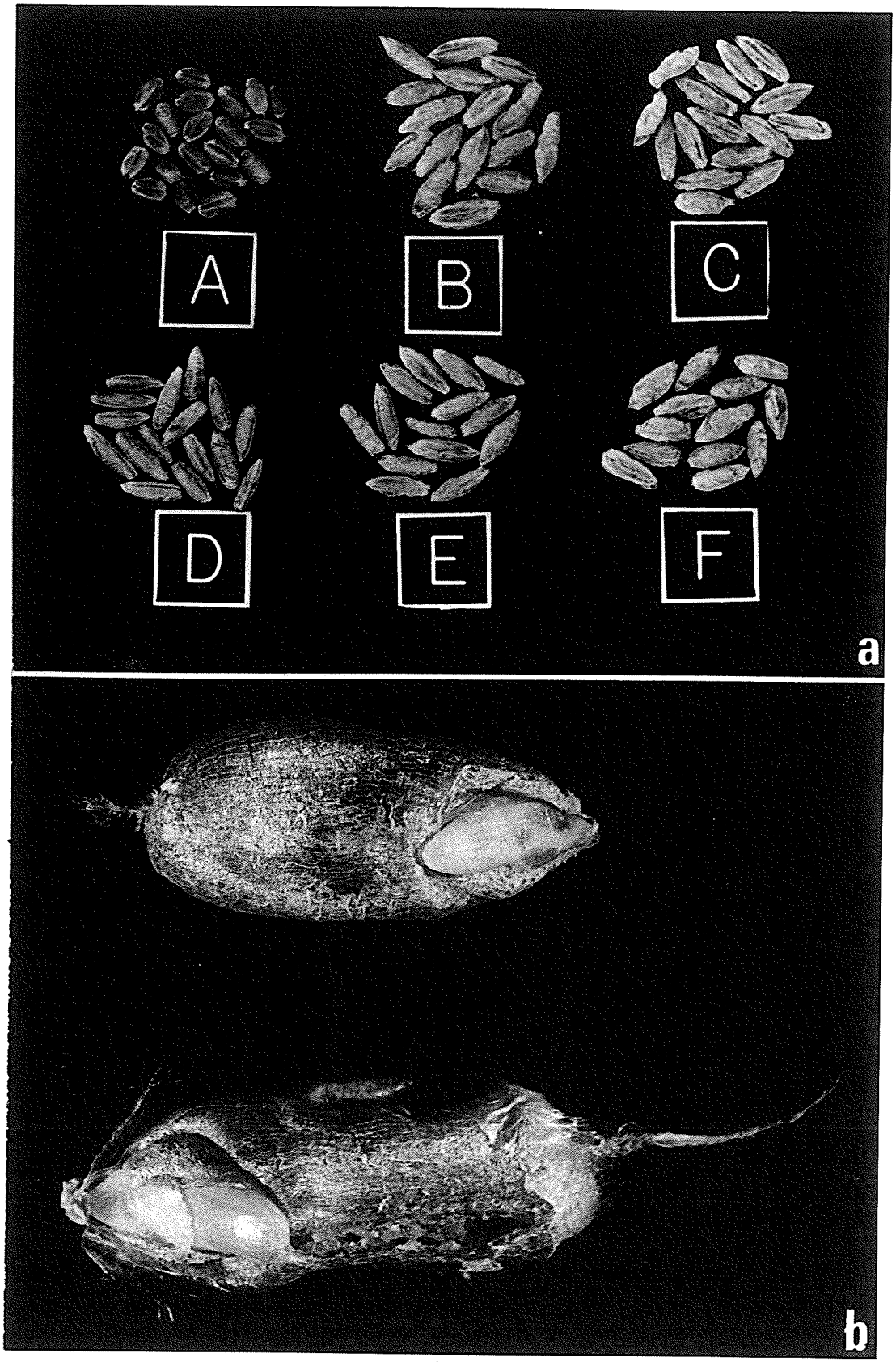
8A92	-	*Triticum aestivum* cv. Kharkov x *Secale montanum*
6531	-	(*T. durum* cv. Stewart x *S. cereale* cv. Prolific) x (*T. dicoccoides* x *S. cereale*)
6A250	-	*T. persicum* x *S. cereale*
6A320	-	AABBRR
6456-3	-	[(*T. durum* cv. Ghiza x rye) x (*T. durum* cv. Carleton x *S. cereale*)] x [(*T. persicum* x rye) x (*Triticum* x rye)]
6517	-	(*T. durum* var. *leucurum* x *S. cereale* cv. Dw. Petkus) x (*T. persicum* x *S. cereale*)
6A190	-	*T. durum* cv. Stewart x *S. cereale* cv. Prolific
6211.2	-	(*T. durum* cv. Ghiza x rye) x (*T. durum* cv. Carleton x *S. cereale*).

Plate 1. (a) Mature grain samples illustrating variations in kernel characteristics.

(A) Manitou (B) 6531 (C) 6A190

(D) Prolific (E) 6A320 (F) 6211.2

(b) Premature sprouting of 45-day old seeds of 6211.2 grown under greenhouse conditions. Pericarp removed to expose embryo.


A
B
C
D
E
F
a
b

cedure. Alpha-amylase activity was determined according to the viscosimetric method of Tipples (1969).

Soluble sugars were extracted from 50 to 100 mg. samples of ground grain with three ml. portions of cold 80 percent ethanol. After evaporation of the ethanol the residue was resuspended in 10 ml. of distilled water. Reducing sugars were measured in the supernatant by the ferricyanide method of Guinn (1967) with glucose as the standard.

Starch was estimated by a modification of the method of Donelson and Yamazaki (1968). Twenty mg. samples of ground grain were suspended in 4 ml. distilled water and the starch gelatinized by placing the test tubes in vigourously boiling water for two minutes. The tubes were then cooled rapidly to 30° C and 5 ml. acetate buffer (pH 4.7) was added and placed in a 30° C water bath. One ml. of alpha-amylase solution (0.020 g Mann alpha-amylase; $>$ 19,900 B.U./gm.) was added and incubation carried out for 1.5 hours at which time enzymatic hydrolysis was stopped by the addition of 1 ml. of 50 percent TCA. After neutralization with NaOH, centrifugation and appropriate dilution, reducing sugars produced by the hydrolysis were measured according to the method of Guinn (1967). Pure Lintner starch (Fisher Scientific Co.) was used as the standard and corrections were made for free reducing sugars present in the samples before hydrolysis.

3.3 Measurement of starch granule size

Fifteen gm. samples of mature grain were steeped for 24 hrs at 4° C in 30 ml. water which was 0.01M with respect to $HgCl_2$ to inhibit amylase activity. After steeping, the grain was thoroughly ground in a Braun mixer and starch washed out through several layers of cheesecloth.

Following centrifugation the top layer of starch was removed and the protein and cellular debris separated by washing on a number 400 Tyler stainless steel sieve (0.037 mm. mesh). The starch washed through the sieve was readded to the sample and following resuspension and centrifugation the remaining top layer of protein contamination was scraped off and discarded. The starch was dried at low temperature in an air oven and stored in the refrigerator.

Granule size measurements were made using a Model B Coulter Counter equipped with a Model M Volume Converter. The solvent employed was 5 percent NH_4CNS in isopropanol (W/W). Because the starch samples failed to disperse completely in the solvent they were first dispersed in approximately 0.5 ml. distilled water and the slurry then added to the solvent (approximately 200 ml.) For each sample, readings were taken at 15 different instrument settings corresponding to particle diameters, ranging from 3.4 to 85.4 μ. (Williams, 1970).

3.4 Respiration determinations

Oxygen uptake was measured using a Warburg Respirometer maintained at 25° C. Whole seeds were placed in 1.5 ml. phosphate buffer (pH 5.7) and 0.5 ml. ethanolamine was placed in the center well of the flask to absorb liberated CO_2. To determine whether photosynthesis was a significant factor with immature green seeds comparisons were made between flasks covered with aluminum foil and flasks left uncovered. Since no differences were observed all further measurements were made without the foil covering on the flasks.

3.5 Sucrose-^{14}C feeding experiment

Triticale and Manitou wheat heads were excised 16 days after flowering and the upper and lower spikelets removed leaving only the central 10 - 12 primary and secondary florets intact. Individual heads were placed in 15 ml. centrifuge tubes containing 5 ml. of a 5 percent aqueous sucrose solution to which sucrose-^{14}C was added to give a final count of 10^6 dpm per ml. The experiment was carried out in triplicate. Sucrose feeding was continued for 24 hours at 80-85° F under continuous fluorescent light. Seeds were then removed from the spikes and weighed to determine fresh weight. Six seeds of each of the Triticale spikes were dissected to separate the seed coat and embryo from the endosperm. The remaining seeds were air-dried for dry weight determination. Because of the very tight adherence of seed coat to endosperm it was not possible to dissect the wheat kernels. Instead they were cut in half along the crease and the starchy endosperm squeezed out in a small volume of distilled water.

After maceration with a blunt glass rod, dissected samples were hydrolysed in 2 ml. of 1.5N H_2SO_4 by placing the stoppered tubes in a boiling water bath for two hours. When cool they were neutralized with NaOH and an aliquot of the supernatant counted in a Nuclear Chicago Model 720 Liquid Scintillation Counter to measure ^{14}C activity.

Total carbohydrate was estimated using the phenol-sulfuric acid procedure.

3.6 CCC treatment

Plants were grown in the greenhouse in six inch clay pots with three

plants per pot. Natural daylight was supplemented with fluorescent tubes to give a daylength of 16 hours. Water and fertilizer were added as required. In the first experiment 1 ml. of 10^{-1}M CCC (2-chloroethyl trimethyl ammonium chloride) was injected with a hypodermic needle into the culm below the spike at the time of flowering. In the second experiment the same amount of CCC was similarly injected 30 days after the initiation of flowering. Controls in both experiments consisted of intact, untreated plants grown under the same conditions. Seeds were harvested at maturity and dry weight per seed determined.

3.7 Decreasing sink size

Plants were grown under the same conditions as for the CCC treatments. At flowering all spikelets from the upper and lower portions of the spike were removed. In addition, the central florets were removed from the remaining spikelets leaving only the primary and secondary florets intact. Seeds were harvested at maturity and dry weight per seed determined. Heads of control plants were left intact but for comparison purposes only weights of lateral seeds from the central portion of the spike were used.

4. RESULTS AND DISCUSSION

4.1 Physical characteristics of experimental lines

Since the material used in the investigation was initially chosen only on the basis of a kernel type rating by visual inspection it was desirable to have some more objective means of classifying the Triticale lines as to their degree of shrivelling. Weight per measured bushel varied from 42.0 to 50.4 pounds (Table 4.1) but there was some question whether this was an adequate measure of kernel shrivelling because of large differences in seed size and it seemed that grain density might be a more accurate parameter. In fact, however, when the Triticale lines were ranked for both characters the relative orders were almost identical and a correlation value of 0.933** for grain density and bushel weight was obtained. This is in agreement with the argument of Hlynka and Bushuk (1959) that kernel size of the grain in itself does not influence the weight per bushel but that density of the grain and shape of the kernel are important factors. Grain density measurements agreed well with the initial visual classification of lines and were used as the criterion of the degree of shrivelling throughout the investigation.

4.2 Pattern of seed development

Changes in dry matter during grain development of three Triticale lines and Manitou wheat are illustrated in Figure 4.1. For the sake of clarity the other five Triticale lines and Stewart 63 and Prolific were not included. Complete data for all eleven experimental lines are given in Table 8.1 of the appendix.

Dry matter per kernel increased rapidly during the early stages of

Table 4.1. Physical grain characteristics of experimental lines at maturity.

Experimental line	Bushel wt. (lbs.)	Seed density (g./cc.)	1000 Kernel wt. (g.)	Seed volume (cc.)
Manitou	60.8	1.3560	30.69	0.0226
Stewart 63	63.0	1.3612	46.37	0.0341
Prolific	51.6	1.2307	29.18	0.0237
8A92	49.9	1.2967	37.11	0.0286
6531	50.4	1.2703	52.52	0.0413
6A250	46.2	1.2608	25.51	0.0202
6A320	46.2	1.2189	38.23	0.0313
6456-3	47.4	1.2125	37.55	0.0310
6517	44.6	1.1604	38.47	0.0331
6A190	42.2	1.1065	43.61	0.0394
6211.2	42.0	1.0810	42.87	0.0397

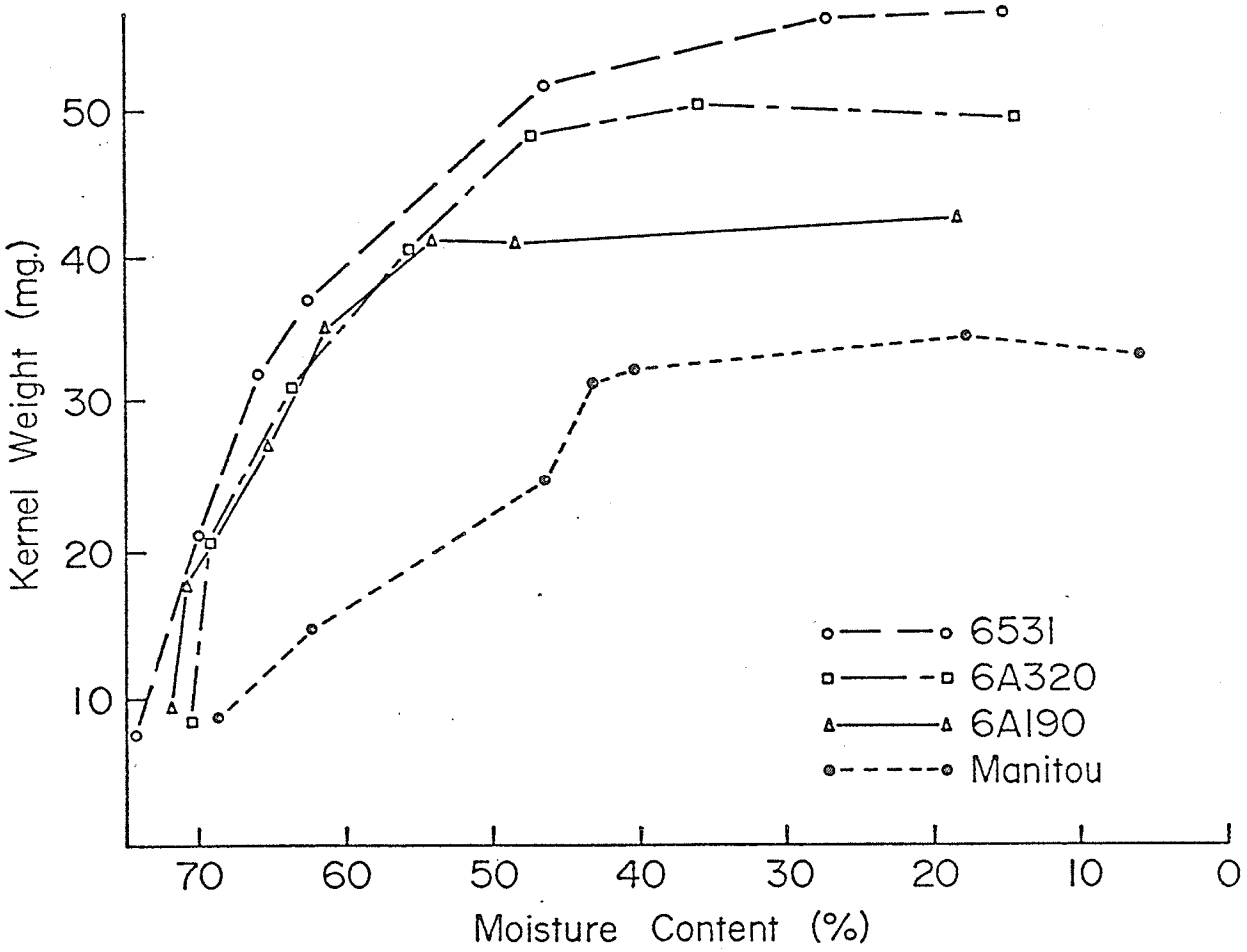


Figure 4.1. Changes in dry matter during grain development of Manitou wheat and three Triticale lines.

development of Triticale grains (first 31 days after flowering). After this time there was a levelling off in the rate of increase and in some of the lines a slight decrease during the final stages of maturation. Variations in seed weight between lines were evident throughout development and became more pronounced at later stages of development. To take into account variations in the rate of maturity of the lines moisture content of the seed rather than days after anthesis was used as the abscissa. Considering the lines 6A190 and 6531 it is evident that at the early stages the rates of increase in dry matter were similar, however, 6A190 ceased to increase at about 55 percent moisture while 6531 continued to increase to about 45 percent moisture. Consequently, there was a difference of about 10 mgs. in the final kernel weight while seed volumes were very similar, being 0.0413 cc. for 6531 and 0.0394 for 6A190. Clearly, these differences then represent the differences in seed density and degree of shrivelling between the lines. It would appear from Figure 4.1 that there is a disturbance in the synthetic process in grains of 6A190 during the latter part of their development.

4.3 Alpha-amylase activity

Alpha-amylase activity was generally high in mature Triticale grain. However, considerable variation existed among the eight lines under investigation (Table 4.2). The variability was non-random in that lines with better kernel type had lower alpha-amylase activity while the lines with poorer kernel characteristics yielded values at the upper end of the range. From the plot (Figure 4.2) it is evident that an inverse relationship existed between alpha-amylase activity and grain density in the lines studied. A highly significant correlation coefficient of -0.909** was obtained.

A comparison of alpha-amylase activities in plump and shrivelled kernels within the Triticale line 6456-3 revealed alpha-amylase levels were approximately five-fold higher in shrivelled seeds than in plump ones. Measured as mg. starch hydrolysed per g. seed, extracts of shrivelled seeds produced an amylase activity value of 470.9 compared to a value of 88.0 for plump seeds. A bulk sample of 6456-3 produced a value of 105.4. Thus, it is clear that the association between amylase activity and kernel shrivelling exists both between Triticale lines and within lines.

Results from investigations carried out by Müntzing (1963) are in agreement with these findings concerning amylase levels. In his material the Triticale line with the lowest alpha-amylase activity was also one of the lines with better kernel type according to a visual assessment.

All Triticale lines had higher amylase activity than Manitou although the lowest (6A250) approached the value for Manitou. It is perhaps significant to note that the amylase activities of Stewart 63 and Prolific were also considerably higher than Manitou. This was especially true of the rye. Triticale appears to be more like its rye parent than its wheat

Table 4.2. Biochemical characteristics of mature grain of experimental lines.[1]

Experimental line	Protein (%)	Alpha-amylase (units/g.)	Reducing sugars (mg./g.)	Starch (%)
Manitou	17.9	0.93	1.232	63.5
Stewart 63	15.6	5.12	1.872	62.7
Prolific	16.0	14.00	2.387	63.6
8A92	20.5	10.96	1.544	60.2
6531	15.0	14.52	2.011	61.5
6A250	14.2	3.31	2.316	63.4
6A320	17.0	7.65	1.889	57.2
6456-3	16.5	18.49	2.393	57.7
6517	17.7	43.45	3.334	60.0
6A190	17.6	45.91	3.590	57.7
6211.2	16.6	43.65	4.206	54.6

[1]All data are expressed on a dry matter basis.

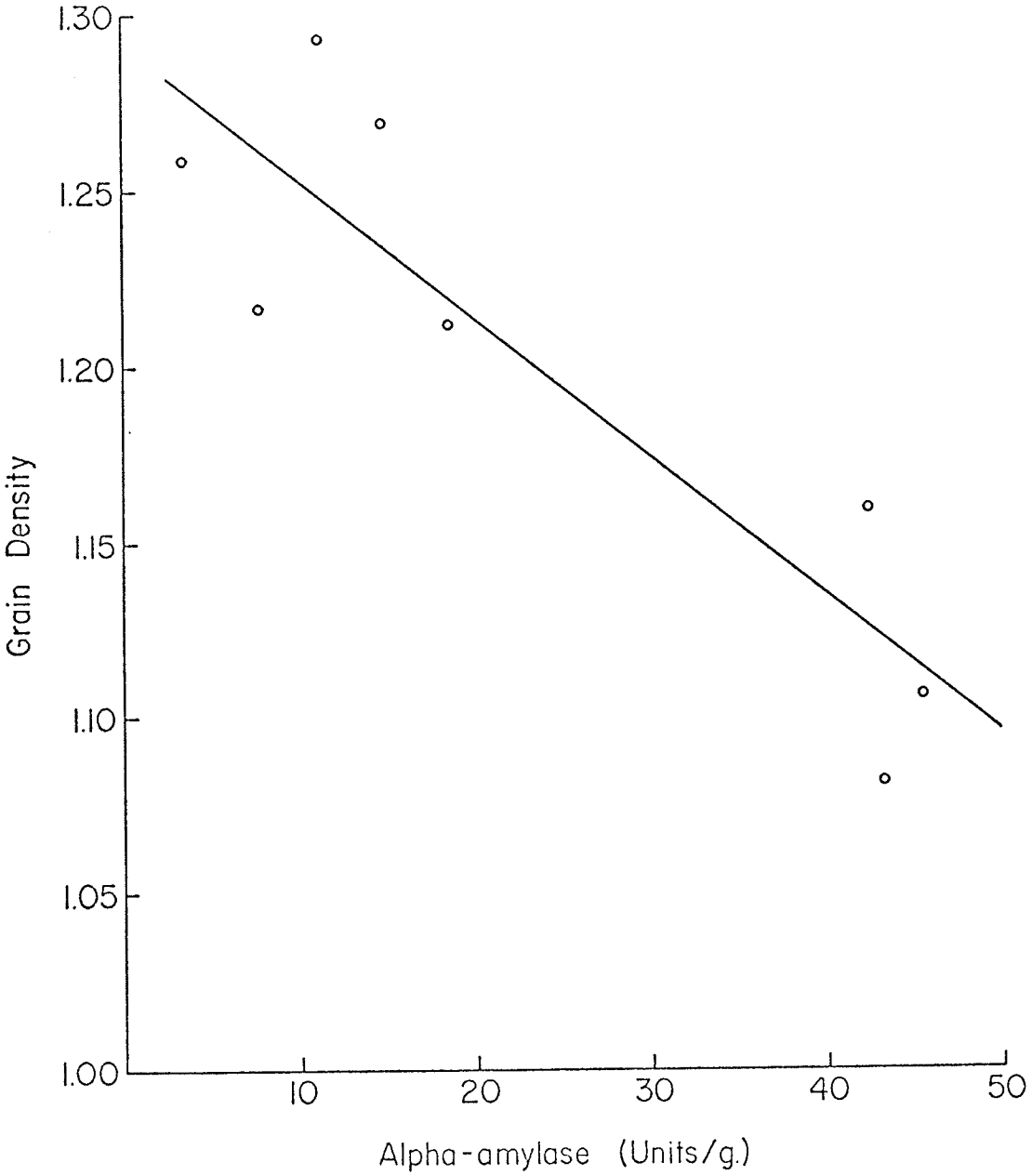


Figure 4.2. Relationship between grain density and alpha-amylase activity in mature grain of eight Triticale lines.

parent with respect to alpha-amylase activity. Similar observations were reported by Müntzing (1963).

Of the eight Triticale lines investigated none yielded intermediate alpha-amylase values although several were intermediate in grain density. The lines 6517, 6A190 and 6211.2 were grouped near the upper end of the range while the other five were distributed toward the lower end. These results would appear to suggest that two levels of alpha-amylase may exist within the Triticale grains. The two wheat varieties and Prolific rye varied in amylase activity but were relatively low in relation to the highest values for Triticale. They cannot properly be included in a discussion of kernel shrivelling because these variations in amylase are not associated with any apparent differences in kernel development and may not be applicable to the situation existing in the Triticale lines. In cereal grains alpha-amylase has been shown to be produced de novo in the aleurone layer surrounding the endosperm in response to GA stimulation (Varner, 1964). The embryo has been found to be the source of this GA production (Radley, 1967). On this basis, a continuous variation in amylase rather than several discrete levels would be expected to occur in the endosperm with the actual amount being dependent on the amount of GA available.

To examine the development of the alpha-amylase activity a line with low activity (6A320) and a line with high activity (6A190) were compared at different stages of maturity. The findings are presented graphically in Figure 4.3. Again percent moisture was used as the index of maturity rather than days after initiation of flowering. Initially alpha-amylase activity was high in both lines followed by a rapid decrease during the early stages of grain development. However, at approximately 50 to 55 percent moisture content activity in 6A190 increased rapidly as compared

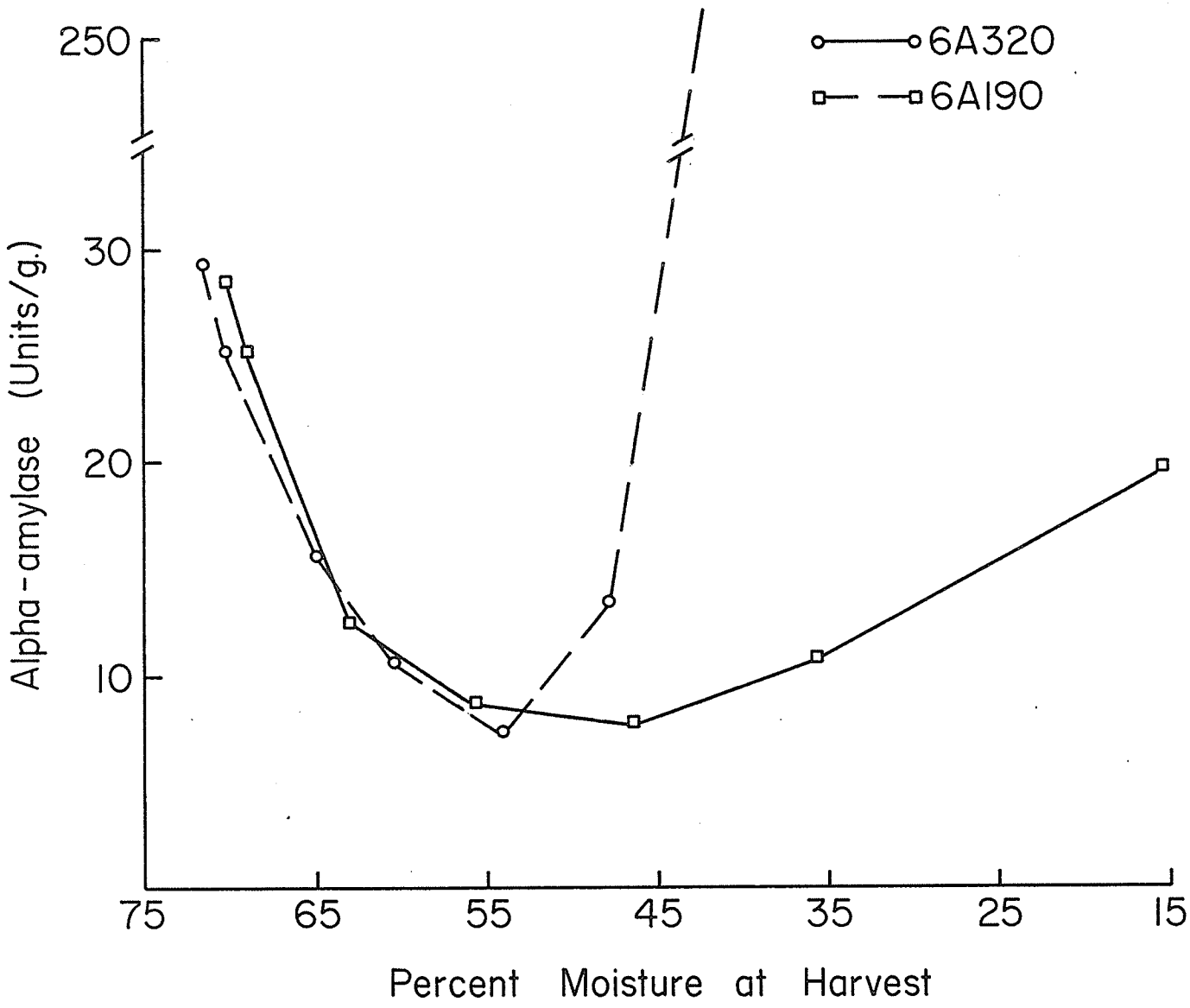


Figure 4.3. Changes in alpha-amylase activity during grain development of Triticale lines 6A320 and 6A190.

to 6A320. Consequently, at maturity 6A190 had about 13 times the activity of 6A320.

The alpha-amylase patterns at early stages of grain development were quite similar to those found for barley. Activity of alpha-amylase in barley grains rose sharply between 7 and 11 days and then fell as rapidly between 11 and 16 days. Thereafter, it fell slowly and the mature barley showed only low alpha-amylase activity (LaBerge and MacGregor, 1969).

These results for Triticale are consistent with the hypothesis that dormancy in grains of 6A190 had been broken and that the events of precocious germination had begun. Alpha-amylase activity is known to be low in sound grain and to increase rapidly with the initiation of germination processes. On this basis it would appear that dormancy in 6A320 had been only partially overcome at this point.

4.4 Reducing sugars

Reducing sugar content in the Triticale lines at maturity ranged from 1.544 to 4.206 mg./g. (Table 4.2). Differences in alpha-amylase activity were, in general, reflected in the reducing sugar values. The correlation coefficient obtained was 0.903**. Similarly, the developmental pattern of reducing sugar content closely approximated the alpha-amylase patterns in the lines 6A320 and 6A190 (Figure 4.4).

The amount of reducing sugars per kernel was fairly constant throughout the development of Manitou wheat kernels. A very gradual decrease was evident with a levelling off during the final stages of maturation. Patterns for the Triticale lines 6531 and 6A320 were similar except that rather than levelling off at later stages there was a slight increase in sugar content. This increase was very marked in 6A190 and coincided with the increase in alpha-amylase activity at this time. Sugar content per kernel was higher at all stages for Triticale than Manitou but this was due, in part at least, to larger seed size in the Triticale lines. The results for Manitou are comparable to those reported for the wheat variety Gabo by Jennings and Morton (1963).

The actual data for the lines illustrated in Figure 4.4 and for two additional lines are given in Table 8.3 of the appendix.

While there were considerable differences in reducing sugar content among the Triticale lines the absolute amounts present were not large even in the highest lines and it is doubtful whether they would be sufficient to account for the hydrolysis of amounts of endosperm starch sufficient to cause the observed kernel shrivelling. It is possible though that sugars were metabolized as they were released and that consequently the amounts accumulated at any given time were never large.

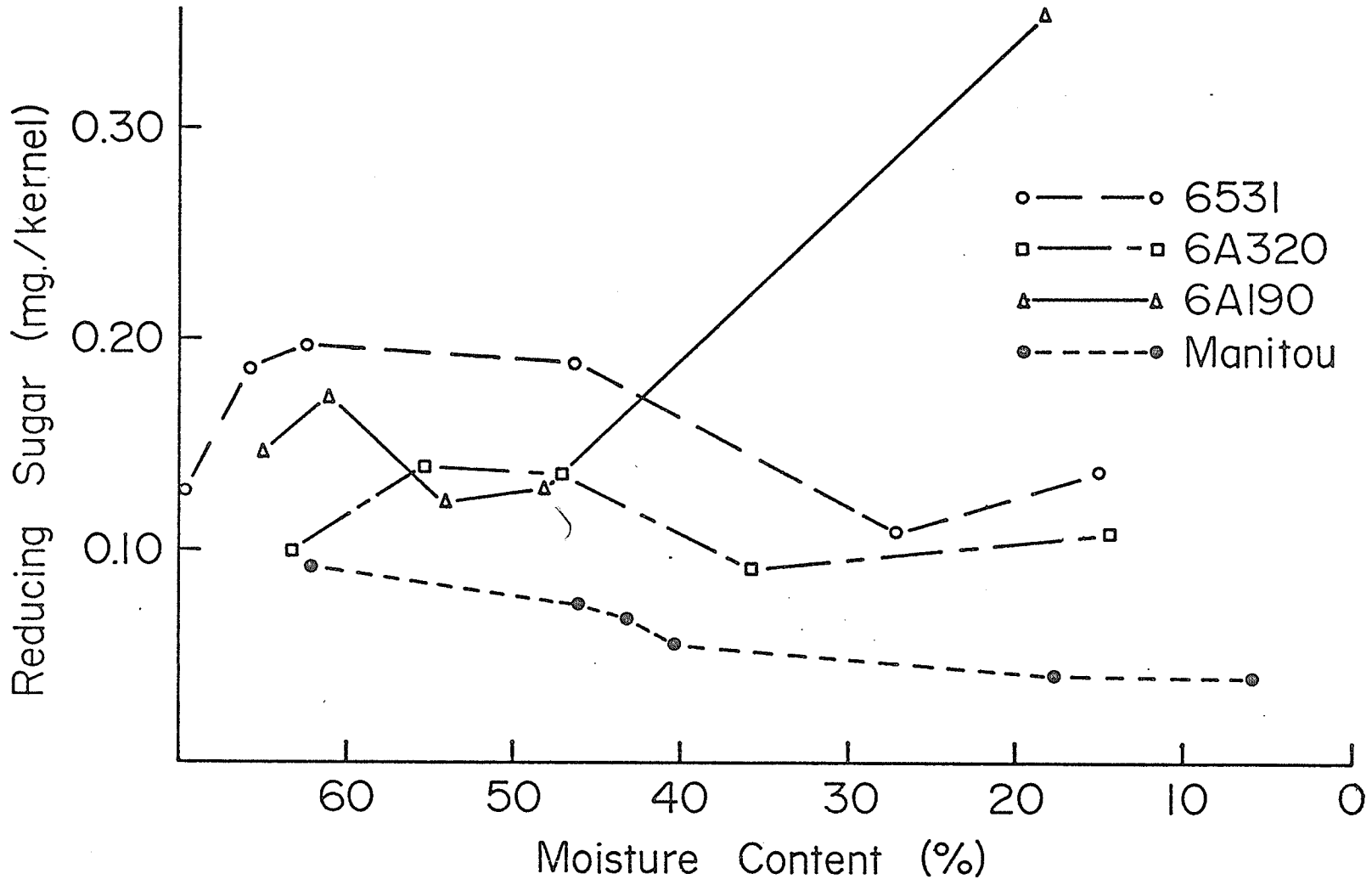


Figure 4.4. Changes in reducing sugar content during grain development of Manitou wheat and three Triticale lines.

Experimental evidence (Table 4.3) indicated that respiration rates of the Triticale lines at the first three sampling dates were not greatly different from those of Manitou wheat. In each case respiration rate declined as seeds became more mature. At the last sampling date the Triticale lines, with the possible exception of 6456-3, were noticeably higher than Manitou. In 6A190 a slight increase was evident between the third and fourth dates.

Expressed on a per seed basis (Table 4.4) the Triticale lines, because of their larger seed size, had higher respiration values than Manitou. 6456-3 was most similar in pattern to Manitou while the other three Triticale lines did not show the continuous decline during development. The catabolism of significant amounts of endosperm starch should be associated with considerable increases in the rate of respiration of the tissue. It is significant to note that respiration values for 6A190 and 6531, lines which vary widely in kernel type as well as alpha-amylase activity, were quite similar. This suggests that catabolic degradation of starch may not wholly account for kernel shrivelling in Triticale.

While duplicate samples run on the same day usually agreed quite closely, there was considerable variation between heads analysed on different days. Several factors could contribute to this variability. Changes in greenhouse temperature and light intensity as affected by the amount of cloud cover as well as time of watering could influence the respiration rate. Since watering of pots was carried out every second day the time period between watering and collection of head samples could vary by as much as 24 hours. It is not known what differences would exist in tillers of the same plant which are ontogenetically diverse but this could possibly also contribute to the observed variations.

Table 4.3. Changes in respiration rate of isolated grains of Manitou wheat and Triticale (μl O_2/g. dry matter/hour).

	Days after flowering			
	31	38	45	52
Manitou	188.0 (124.4-377.4)*	128.4 (93.7-148.9)	99.1 (83.3-109.8)	47.8 (46.3-49.2)
6A190	169.1 (100.3-247.6)	-	85.0 (63.2-120.4)	116.8 (104.0-140.6)
6531	151.0 (137.1-158.0)	119.2 (111.0-130.1)	109.9 (83.9-141.3)	95.0 (87.0-102.9)
6456-3	229.9 (168.2-289.9)	174.9 (120.8-239.5)	158.2 (134.7-199.6)	75.0 (73.5-76.5)
6211.2	172.5 (159.5-185.4)	136.1 (109.9-181.2)	-	101.2 (85.7-116.7)

*Range of values

Table 4.4. Changes in respiration rate of isolated grains of Manitou wheat and Triticale (μl O_2/seed/hour).

	Days after flowering			
	31	38	45	52
Manitou	4.63 (3.50-6.70)*	3.60 (2.50-4.20)	2.90 (2.55-3.10)	1.28 (1.20-1.35
6A190	6.76 (4.20-9.47)	-	4.17 (3.40-4.93)	6.54 (6.05-7.43)
6531	6.73 (6.35-7.03)	6.00 (5.22-6.78)	6.69 (5.40-8.03)	6.14 (5.58-6.70)
6456-3	6.47 (3.66-11.20)	4.96 (4.45-6.90)	5.26 (3.85-6.43)	2.94 (2.93-2.95)
6211.2	6.16 (5.88-6.44)	6.80 (5.45-9.03)	-	6.68 (5.45-7.90)

*Range of values.

4.5 Starch content

Total starch content of Triticale lines at maturity ranged from 54.6 to 63.4 percent (Table 4.2). There was no significant agreement between either alpha-amylase activity and starch content or reducing sugars and starch content. However, a significant correlation of 0.746* was obtained for grain density and percent starch (Figure 4.5).

Patterns of starch deposition during seed development as illustrated in Figure 4.6 indicated that up to about 55 percent moisture the Triticale lines 6A320, 6531, and 6A190 were quite similar in the amounts of starch produced per kernel. However, after this point there was essentially no further synthesis in 6A190 while starch levels in 6A320 and 6531 continued to increase with the result that at maturity they had approximately 6 and 10 mgs. more starch per kernel, respectively, than 6A190. These differences in starch accumulation were consistent with the differences existing in grain density. In addition, the results coincided very closely with the patterns of dry matter accumulation for these lines. Thus, the slow rate of starch deposition in 6A190 relative to 6531 and 6A320 appears to be the contributing factor resulting in low dry matter content in this line.

In Manitou, starch deposition continued until approximately 40 percent moisture suggesting that in the Triticale lines with poor kernel characteristics a premature termination of starch synthesis occurs. In the better Triticale lines this was not as evident but even in these starch accumulation ceased at an earlier stage than in Manitou.

The actual data on starch accumulation for the lines illustrated in Figure 4.6 and two other lines are given in Table 8.4 of the appendix.

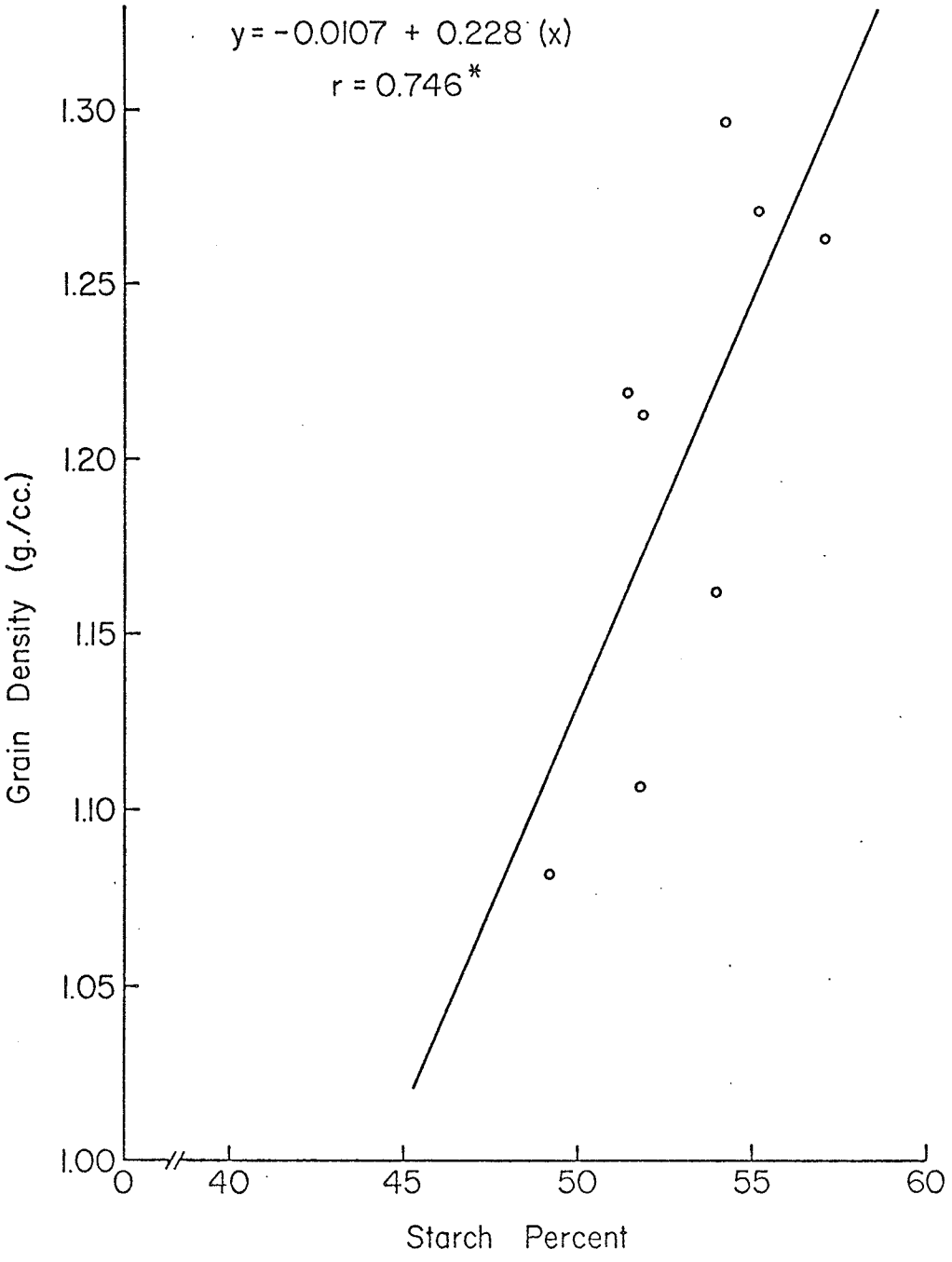


Figure 4.5. Relationship between grain density and starch content of mature grain of eight Triticale lines.

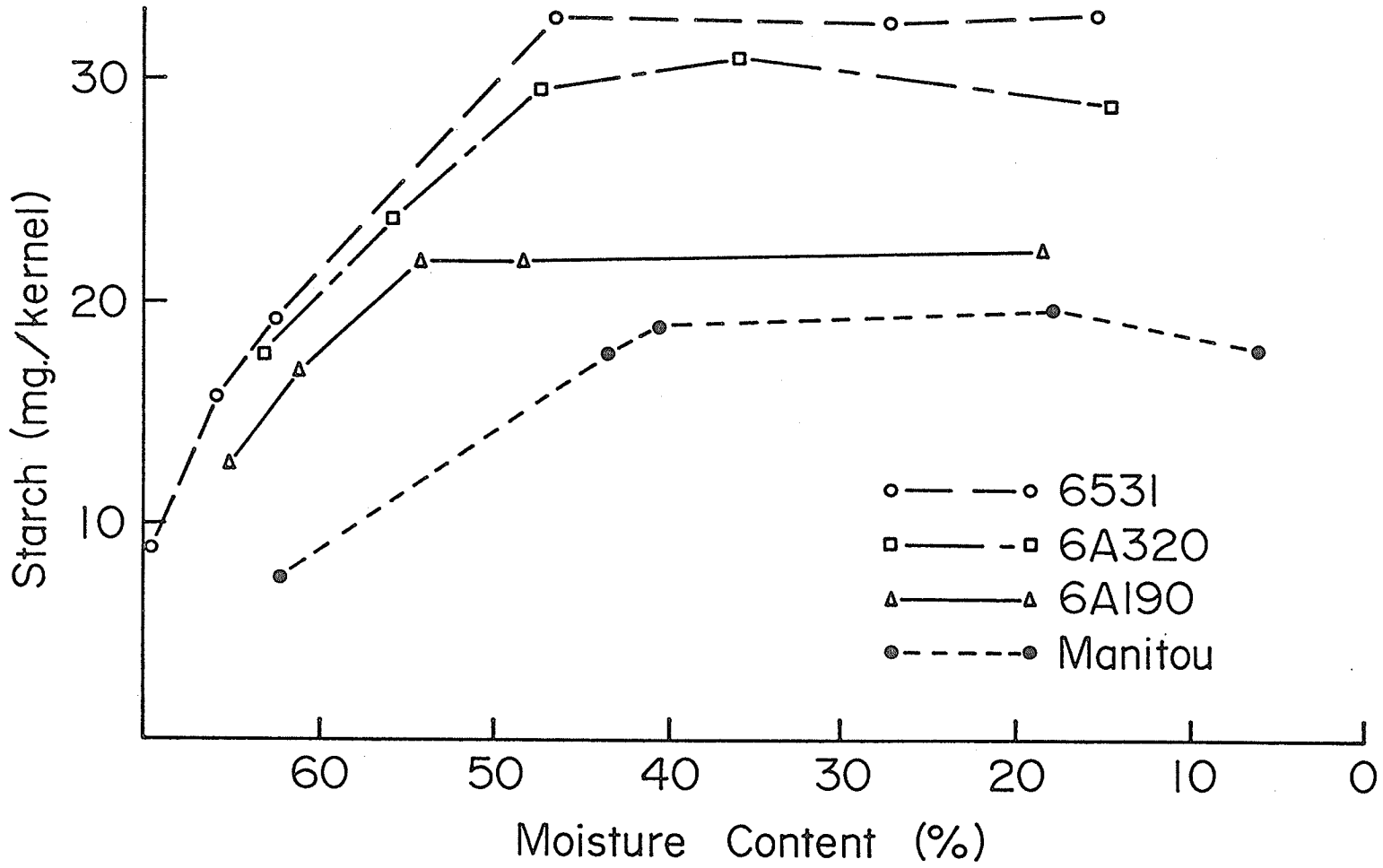


Figure 4.6. Changes in starch content during grain development of Manitou wheat and three Triticale lines.

If starch percentage is reduced in Triticale grains other components must occupy a larger fraction of the total to make up for the decrease. Protein content varied from a high of 20.5 percent for 8A92 to a low of 14.2 percent for 6A250 compared to 17.9 percent for Manitou (Table 4.2). From a summation of starch and protein percentages it is clear that decreases in starch in Triticale lines are not compensated for by increases in protein, the result being that the summed values are lower for Triticale than for wheat and rye. Contributing to these differences is the fact that shrivelled Triticale grains have a higher proportion of pericarp-testa. Consequently, both fibre and ash percentages would be higher. In addition, slightly greater amounts of hexose sugars also account for some of the difference.

4.6 Premature sprouting of Triticale grains

A random sample of ten mature heads of each Triticale line was collected in the field and the percent sprouted grains assessed visually. In seven of the eight lines there was evidence of premature sprouting ranging from 0.5 to 6.4 percent (Table 4.3). 6531 was the only line without any sprouted grains. Alpha-amylase determinations and degree of premature sprouting were in agreement in five of the Triticale lines. That is, low alpha-amylase activity was associated with a low percentage of sprouted grains and high alpha-amylase was associated with a higher percent of sprouted grain. However, 8A92 and 6A320 had a higher percentage and 6A190 a lower percentage of sprouted grains than would have been predicted on the basis of alpha-amylase activity.

Bingham and Whitmore (1966) found that in soft wheat varieties sprouting was always associated with high amylase values but that even in some varieties in which there was no visible evidence of sprouting the amylase values were often quite high.

Similarly, in rye Tedin and Persson (1963) observed high amylase activity in plants which did not exhibit any germinated seeds. Thus, a direct relationship between alpha-amylase activity and premature sprouting need not necessarily be expected to exist in all Triticale lines.

Table 4.5. Variation in premature sprouting of grain of Triticale lines grown under field conditions.

Experimental line	Total No. of grains	No. of sprouted grains	Percent sprouted grains
8A92	315	20	6.4
6531	548	0	0.0
6A250	645	3	0.5
6A320	502	23	4.6
6456-3	567	7	1.2
6517	565	34	6.0
6A190	438	6	1.4
6211.2	450	16	3.6

4.7 Starch granule size distribution

Common wheat starch consists of two types of starch granules (May and Buttrose, 1959). Large (Type A) granules comprise the major portion of starch volume. In addition, there are smaller (Type B) granules which, although being much more numerous, comprise only a small fraction of the total volume. Rye starch has fewer of these small granules (MacMasters, et al., 1957). The small granules, although only contributing a small portion of the total volume, do serve to pack all available space and may contribute significantly to endosperm density. An indication of this is the fact that Manitou wheat had a seed density of 1.3560 while the density of Prolific rye was only 1.2307. Mean starch granule diameters for the Triticale lines and wheat and rye are given in Table 4.6.

The mean value for Prolific rye was 28.24 μ as compared to only 18.70 μ for Manitou wheat. In the Triticale lines mean starch granule diameter ranged from 17.81 to 25.61 μ. Five of the lines were near the average for wheat and rye while the other three were very similar to wheat. There was no consistent agreement between starch granule size and seed density in the Triticale lines. For example, 6A190 and 6531 which had seed densities of 1.1065 and 1.2703, respectively, were almost identical with respect to mean starch granule size.

The frequency distributions for Manitou, Stewart 63, Prolific and 6A190 are shown for illustration purposes in Figure 4.7. The particle size distribution in 6A190 was peculiar in that it exhibited a major peak at a diameter of approximately 18 μ corresponding to Stewart 63 and Manitou and with a definite shoulder apparent in the profile at a larger particle diameter which best coincides with the peak of Prolific starch. This

Table 4.6. Starch granule size distribution patterns for wheat, rye and Triticale.

Mean granule diameter (μ)	Starch granule frequency (%)					
	Manitou	Prolific	Stewart 63	8A92	6531	6A250
3.4	0.8	0.8	0.3	0.3	0.6	0.1
4.2	1.5	0.5	0.9	0.5	0.6	0.5
5.4	2.7	0.8	2.6	0.8	1.0	1.3
6.8	4.4	1.2	3.4	1.3	2.0	2.8
8.5	4.6	1.7	4.5	2.2	3.4	4.9
10.7	6.9	2.9	6.5	4.4	5.8	10.0
13.5	18.1	6.6	14.0	10.4	12.0	21.5
16.9	27.1	12.4	22.1	18.5	18.3	26.7
21.4	17.2	18.8	19.3	19.9	18.8	17.3
27.0	7.4	19.9	10.7	16.6	14.0	6.2
34.0	3.8	15.9	5.0	10.1	8.4	2.6
42.8	2.7	8.7	4.3	7.5	5.4	2.0
54.0	0.8	5.3	3.2	4.0	3.9	1.4
67.9	1.0	2.9	1.9	2.1	3.6	1.4
85.4	0.8	1.4	1.2	1.6	2.1	1.2
Average	18.70	28.24	21.78	25.61	25.20	19.07

Table 4.6. Continued

Mean granule diameter (μ)	Starch granule frequency (%)				
	6A320	6456-3	6517	6A190	6211.2
3.4	0.5	0.1	0.2	0.3	0.2
4.2	0.4	0.4	0.5	0.1	0.4
5.4	0.9	1.0	1.3	0.2	1.0
6.8	1.4	2.5	3.5	1.4	2.1
8.5	2.7	4.8	6.8	2.6	3.6
10.7	5.9	9.2	11.5	6.4	6.7
13.5	13.1	19.8	22.6	13.8	13.9
16.9	21.3	26.6	26.1	20.0	21.9
21.4	20.1	20.4	15.4	18.8	19.5
27.0	12.8	7.6	5.2	10.9	12.5
34.0	7.0	2.5	2.1	8.9	5.9
42.8	5.2	2.0	1.7	8.4	4.5
54.0	4.1	1.2	1.4	4.6	3.2
67.9	2.9	1.0	1.0	2.0	2.6
85.4	1.5	0.8	0.8	1.6	2.0
Average	24.26	18.99	17.81	25.11	23.47

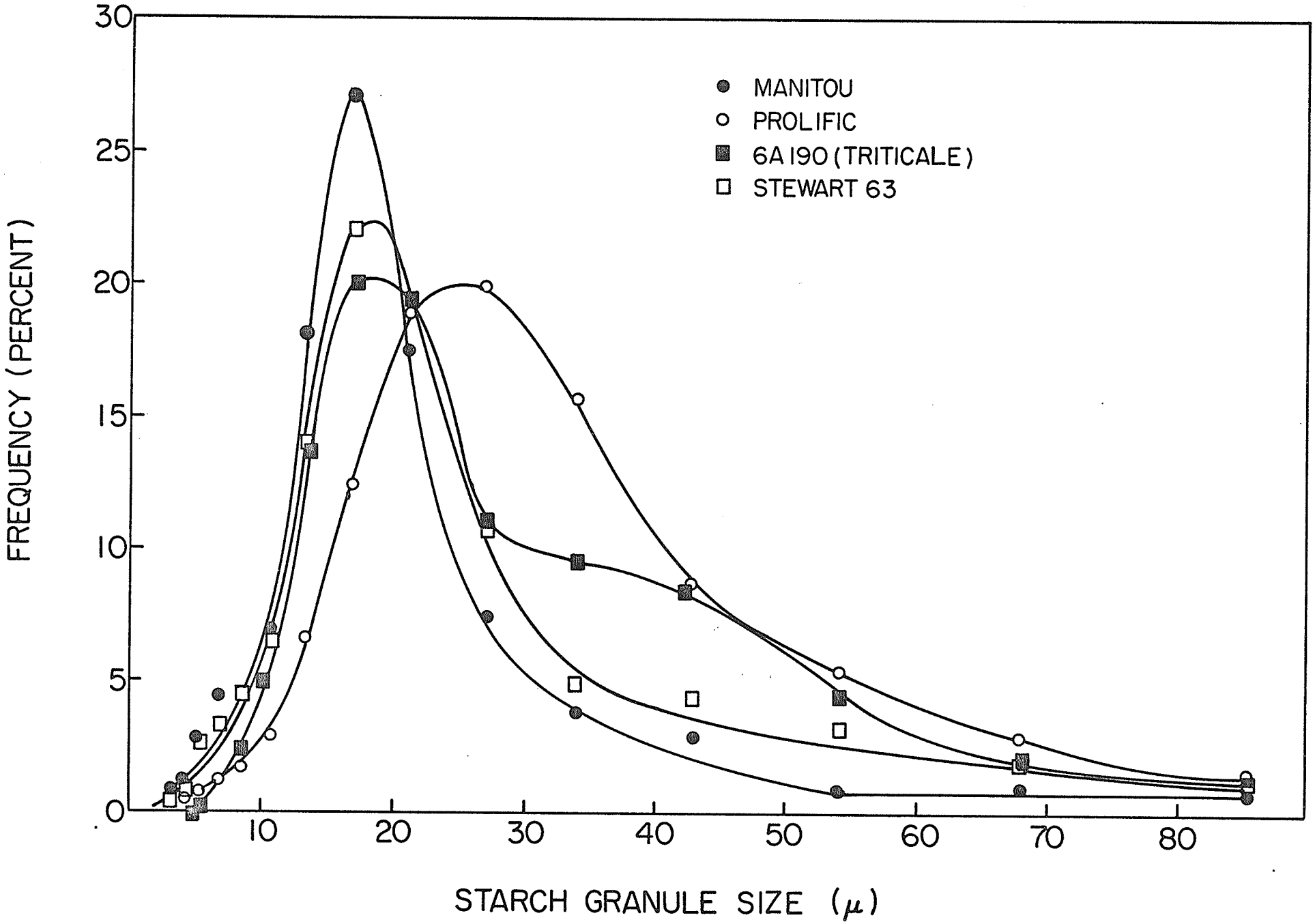


Figure 4.7. Frequency size distributions of endosperm starch granules of wheat, rye and Triticale.

unusual size distribution was not a general characteristic of Triticale. In fact, of the eight lines tested, only 8A92 gave a similar profile.

Russian workers studying shrivelled seeds of 42 chromosome wheat-rye amphiploids stated that unlike normal caryopses the shrivelled ones lacked fine-grained starch in their endosperm cells (Zhyla and Shulyndin, 1969). They attributed this to a disturbance of synthetic processes during the stage when small starch granules are initiated. They further concluded that this disturbance arose as a result of incompatibility between the wheat and rye genomes in the amphiploids.

On the basis of the results obtained in this study there was no apparent correlation between starch granule size and degree of kernel shrivelling in the eight Triticale lines examined.

It is interesting to note that of the eight Triticale lines used in the study the three whose mean starch granule diameters were similar to wheat all had Triticum persicum included in their parentage. In none of the other five lines was this species present as one of the parents. Larter, et al. (1968) have made the observation that Triticum timopheevi, Triticum persicum or Secale montanum when included in the parentage of a Triticale hybrid transmitted genes for desirable kernel characteristics to the progeny. It appears that at least part of this desirable effect of T. persicum may arise as a result of its influence on the starch granule size distribution patterns.

Since most of the Triticale lines are of complex parentage interactions and modifications among genetic material also become more complex. The three lines 6A250, 6456-3, and 6517 in which Triticum persicum is present vary in the complexity of their parentage (see Table 3.1). 6A250 which has the highest density arose from a single cross involving only

T. persicum and Secale cereale while 6517 and 6456-3 have four and eight parents, respectively. The incorporation of this additional genetic material could have introduced deleterious factors which resulted in poor seed characteristics in spite of the beneficial effect of T. persicum on starch granule size.

4.8 Incorporation of sucrose-^{14}C

Transverse cross-sections of immature grains revealed that in Triticale the pericarp-testa was folded and separated from the endosperm whereas in Manitou wheat it adhered quite tightly to the endosperm (see Plate 2). In addition, the central cavity at the crease was much larger in Triticale than in wheat. Both of these faults were more pronounced in 6A190 than in 6531.

Thus, it appeared that one of several situations existed in the Triticale grains. Either endosperm development was retarded while growth of the pericarp-testa was normal or endosperm development was normal while growth of the pericarp-testa was abnormally stimulated. A combination of reduced endosperm growth and stimulated pericarp-testa development could also result in the observed effect. To investigate rates of growth of endosperm relative to the pericarp-testa a feeding experiment with sucrose-^{14}C was conducted using spikes excised 16 days after anthesis. The results of this investigation are summarized in Table 4.7.

The line with better kernel characteristics (6531) incorporated approximately 10 percent more ^{14}C into endosperm tissue than 6A190, a line with more severely shrivelled kernels. Incorporation into the pericarp-testa was about 4 percent greater in 6A190 than in 6531. The endosperm to pericarp-testa ratio of ^{14}C was 2.66 for 6531 while for 6A190 it was only 2.32. This difference was just below significance at the 5 percent level.

The results suggest that the line 6A190 is characterized by both reduced endosperm growth and stimulated pericarp-testa development as compared to 6531. Carbohydrate per endosperm in 6A190 was 14.15 mg. whereas

Plate 2. Transverse cross-sections of immature grains.

(a) Manitou (b) 6A190 (c) 6531

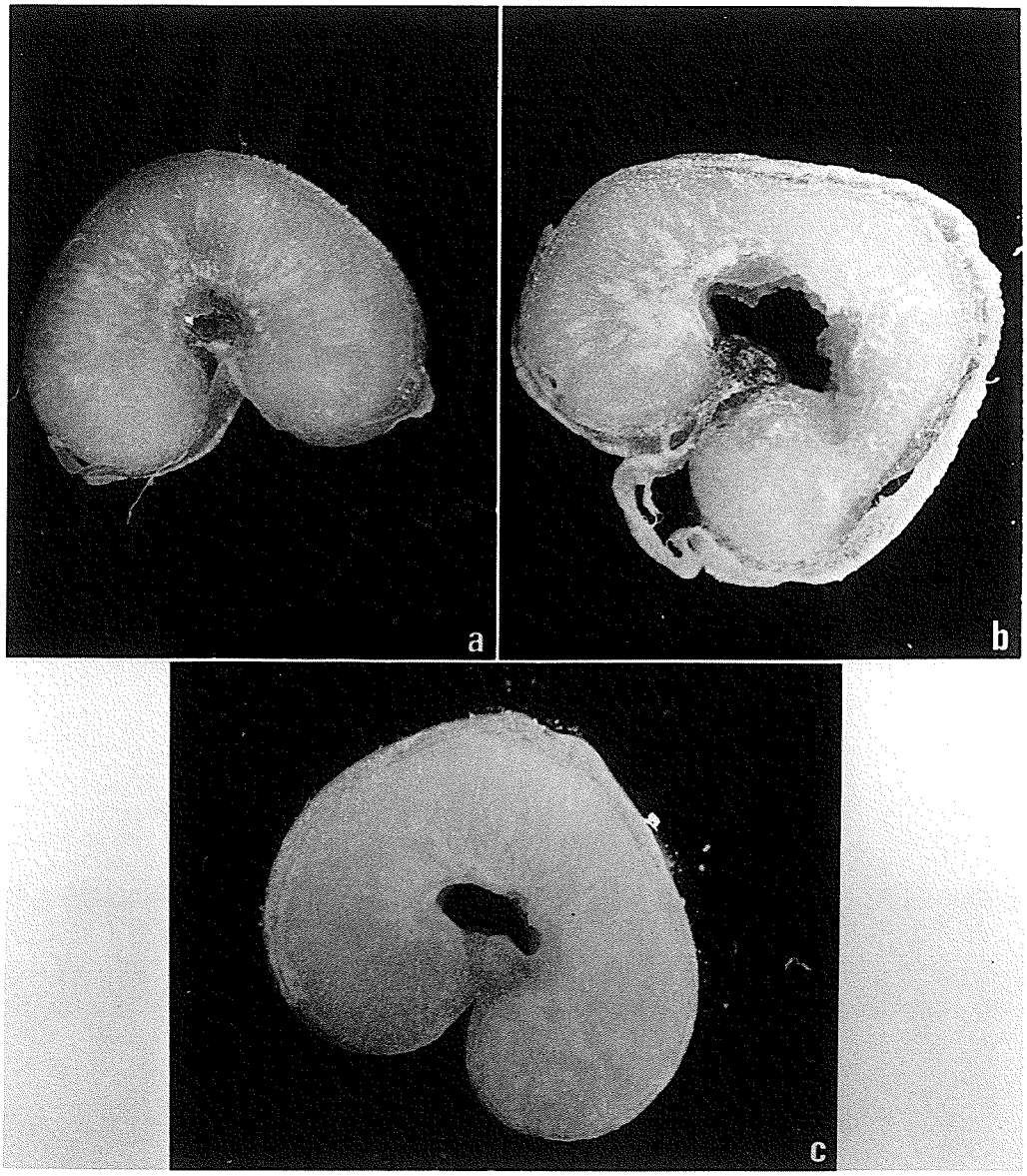

a
b
c

Table 4.7. Sucrose-^{14}C incorporation into grain of excised 16-day old heads of two Triticale lines.

	6531	6A190	6A190 as % of 6531
^{14}C incorporation (dpm)			
Endosperm	26,989	24,481	90.7
Pericarp-testa	10,169	10,558	104.1
Total	37,158	35,039	94.3
Endosperm/Pericarp-testa	2.66	2.32	
Total Dry Matter (mg./seed)			
Endosperm	20.77	18.67	89.9
Pericarp-testa	6.42	6.07	94.6
Total	27.19	24.74	91.0
Total carbohydrate (mg./seed)			
Endosperm	16.96	14.15	83.4
Pericarp-testa	2.30	2.20	95.7
Total	19.26	16.35	84.9

it was 16.96 for 6531 pointing out the fact that endosperm development in 6A190 prior to the time of excision of the heads was also reduced which is in agreement with the starch accumulation profiles (Figure 4.6). Amounts of carbohydrate per pericarp-testa were similar for 6531 and 6A190 (2.30 and 2.20 mg., respectively) but in 6A190 the pericarp-testa constituted a greater percentage of the total carbohydrate than was the case in 6531. Apparently there is a change in the distribution of carbohydrate measured as ^{14}C from sucrose in 6A190 with a larger proportion going to the pericarp-testa. However, this increase is not sufficient to compensate for the reduced amount proceeding to the endosperm so that the net result is that total incorporation into kernels of 6A190 is reduced relative to 6531.

From consideration of the results it appears that there probably was reduced carbon source availability in the endosperm of 6A190 as compared to 6531 and that this may also be coupled with enhanced growth of the pericarp-testa in kernels of 6A190.

This conclusion is in agreement with general observations on the development of F_1 seeds of interspecific and intergeneric hybrids. In many instances crosses of this nature are difficult to make because seeds fail to develop fully *in vivo*. Even though fertilization is readily achieved there is little or no endosperm development and embryos degenerate after about a week to 10 days. The problem is circumvented by excising the immature embryos and starting the plants on an artificial nutrient medium.

Sisodia and McGinnis (1970) suggested that problems associated with wheat-rye hybrids could arise from nuclear-cytoplasmic interactions and imbalances. They further pointed out that a harmonious relationship be-

tween cytoplasm and nucleus needs to be both quantitative and qualitative. In hexaploid Triticale the cytoplasmic to nuclear ploidy ratio is 4 to 6 whereas in octaploid Triticale the C:N ratio is 6:8. Established species would be expected to have a 1:1 C:N ploidy ratio following many generations of evolution and adjustment. This may help to explain why Pissarev (1963) when crossing octaploid Triticale with hexaploid Triticale using the octaploid as female obtained 52 percent seed set with well-shaped F_1 seeds. In the reciprocal cross seed set was only 14.9 percent and seeds were weak and shrivelled. In the first instance the C:N ploidy ratio would be 6:7 whereas in the reciprocal cross the ratio would be 4:7 which is further removed from the theoretically optimum 1:1 relationship.

Larter (personal communication) has observed that in Triticale lines there is an improvement in meiotic stability and consequently in fertility with increased numbers of generations of selfing. It can be postulated that this is a direct result of a continuing process of adjustment between the nucleus and cytoplasm.

4.9 Effect of CCC treatment

If poor kernel type in Triticale was the result of high levels of alpha-amylase an improvement in kernel characteristics would be expected if the amounts of alpha-amylase present in the seed could be reduced. Development of alpha-amylase activity is known to be a gibberellin-induced reaction and associated with the initiation of germination processes. Gibberellin levels, in turn, have been shown to be reduced by application of CCC (Michniewicz, 1965; Simpson, 1966). It was, therefore, desirable to investigate what effect CCC treatment would have on the development of Triticale kernels.

Injection of CCC at the time of flowering resulted in a slight decrease in seed weight of Manitou and 6A190 while in 6531 seed weight was reduced to 87.1 percent of the untreated control (Table 4.8).

A second experiment in which the CCC was applied 31 days after flowering produced almost identical results (Table 4.9). Thus, in no case was there any improvement in seed weight as a result of the CCC treatments. Alpha-amylase activity in grain from spikes of 6A190 treated with CCC 31 days after flowering was reduced to 78 percent of that in grain from untreated controls. The fact that this reduction was not associated with a beneficial effect on seed development suggests that kernel shrivelling cannot be directly attributed to high levels of alpha-amylase. It should be noted however, that even the reduced levels of alpha-amylase in 6A190 were still considerably higher than in Triticale lines with better kernel characteristics.

It can be argued that, in addition to reducing alpha-amylase activity, the inhibition of gibberellin biosynthesis by CCC also interferes with other growth promoting effects of GA. In this way, the expected improve-

Table 4.8. Effect of CCC application at time of flowering on seed development of wheat and Triticale.

Line	Treatment	No. of Seeds	Mean Wt. per Seed (mg.)	Percent of Control
Manitou	Control	954	23.00	
	CCC	940	22.58	98.2
6531	Control	778	36.46	
	CCC	862	31.76	87.1
6A190	Control	256	43.57	
	CCC	524	42.90	98.5

Table 4.9. Effect of CCC application 31 days after flowering on seed development of wheat and Triticale.

Line	Treatment	No. of Seeds	Mean Wt. per Seed (mg.)	Percent of Control
Manitou	Control	894	32.88	
	CCC	952	32.12	97.7
6531	Control	688	67.78	
	CCC	757	63.65	93.9
6A190	Control	526	54.24	
	CCC	549	53.78	99.2

ment in seed growth as a result of decreased alpha-amylase might be effectively counteracted. However, this possibility is largely ruled out by the fact that CCC treatment had no effect on seed weight in Manitou. Mature grain of Manitou is normally very low in alpha-amylase (see Table 4.2) so that if CCC had a detrimental effect on seed development it should be fully expressed.

4.10 Effect of sink size on kernel development

To determine whether seed development was being restricted by the amount of photosynthate available the number of florets per spike was reduced in order to decrease competition among seeds. In this way if there was a limited amount of photosynthate a reduction in the size of the sink might be expected to result in an improvement in seed development.

Results summarized in Table 4.10 revealed that in Manitou and 6531 there were very slight increases in seed weight compared to their controls. However, in 6A190 the mean weight of seeds from emasculated heads was only 88.4 percent of the control. Under greenhouse conditions 6A190 exhibits a fairly high degree of sterility which is illustrated by the fact that it produced an average of 20.2 seeds per head as compared with 25.5 for 6531 and 29.8 for Manitou. This low fertility is a general characteristic of many Triticale lines and is especially true of 6A190. In effect then, because of their reduced seed set the 6A190 controls were already developing under conditions of reduced competition and thus were not adequate as controls. Flag leaf area and head area have been shown to be important contributors to grain yield of wheat varieties (Kriedemann, 1966; Simpson, 1968). Removal of some of the florets causes a decrease in the photosynthetic area of the head and is most likely a contributing factor to the reduction in seed weight in 6A190.

Table 4.10. Effect of a reduction in the number of florets per spike on seed development of wheat and Triticale.

Line	Treatment	No. of Seeds	Mean Wt. per Seed (mg.)	Percent of Control
Manitou	Control	336	36.34	
	Florets removed	621	36.74	101.1
6531	Control	276	71.93	
	Florets removed	317	72.70	101.1
6A190	Control	198	60.00	
	Florets removed	135	53.06	88.4

Since no improvement in seed weight was achieved as a result of reducing the sink size there is no evidence available to support the view that limiting photosynthate is responsible for poor seed development in Triticale.

4.11 Aneuploidy in relation to kernel characteristics

It is interesting to speculate whether poor seed development and low fertility as exist in 6A190 can be related back to a common cause. Sterility in Triticale has been attributed to meiotic instability as reflected in a frequency of 10 to 15 percent aneuploids observed in bulk seed samples from thirty strains (Larter, et al., 1968). Also, an improvement in fertility has been noted with increase in the number of generations which a Triticale line is removed from its original synthesis (Larter, personal communication).

While a limited amount of information is available on the effect of aneuploidy on chemical composition and seed development in cereals, it appears that, in general, the consequences are of a negative nature. Barley trisomic plants, for example, are generally weaker and produce smaller seeds than normal disomic plants (Tsuchiya, 1967). With the exception of one line all durum wheat monosomics obtained by Mochizuki (1968) were characterized by poor endosperm formation. In fact, he found that the frequency of monosomics could be greatly increased by screening plants derived from shrivelled seeds. From these results he reasoned that deficiency of a single chromosome in tetraploid wheat results in poor endosperm development.

The majority of the Triticale aneuploids observed (Larter, et al., 1968) were hypoploid with a lower frequency of hyperploids. With the loss of one or more chromosomes a portion of the genetic material is missing and if this includes genes required for endosperm development an impairment of endosperm development would result. The findings of Mochizuki (1968) would tend to indicate that almost every chromosome in tetraploid wheat carries at least one of these genes.

Using whole chromosome substitution lines Kuspira and Unrau (1957) found that at least seven chromosomes of common wheat carry genes affecting kernel weight. Of these, chromosome 1B had the greatest effect.

In order to check whether there was any relationship between kernel shrivelling and aneuploidy in Triticale samples of plump and shrivelled seeds were selected from within the line 6456-3. Seeds were germinated and mitotic chromosome counts obtained from root tip squashes. The results (Table 4.11) were rather striking. Of the plump kernels only one out of a total of 50 or 2.0 percent did not have the normal 42 chromosome complement. It had a somatic chromosome number of 41. Within the shrivelled group of kernels the frequency of aneuploids was much higher constituting 32.0 percent of the total. Of these 75 percent were hypoploids with 41 being the predominant chromosome number in this group. It thus, appears that at least a portion of the kernel shrivelling is associated with abnormalities within the chromosome complement. However, since a fair proportion of the shrivelled seeds had a normal chromosome complement, additional factors causing shrivelling must also be operative. Nuclear-cytoplasmic interactions discussed earlier no doubt are included with these elements.

The selection of plump seeds proved to be an extremely efficient means of screening for euploids in this line and if the same effect occurs in other lines it should be a very useful aid in efforts to improve cytological stability of Triticale.

Table 4.11. Frequency of aneuploids in plump and shrivelled seeds selected from a bulk sample of 6456-3 Triticale.

Chromosome constitution	6456-3 Plump	6456-3 Shrivelled
42	49	34
41	1	9
40 + telo	0	1
40	0	1
39	0	1
43	0	3
42 + telo	0	1
No. of aneuploids	1	16
Percent aneuploids	2.0	32.0

5. GENERAL DISCUSSION

During the process of improving Triticale for use as a crop of commerce, a number of limiting factors have been encountered. Two of the detrimental characteristics not yet fully corrected are reduced fertility and poor seed development. While the problem of reduced fertility has been the subject of a considerable amount of cytological and genetic investigation, practically no work had previously been carried out on the nature and causes of kernel shrivelling as it occurs in Triticale grains. The only information available was that Triticale grains were prone to premature germination and that alpha-amylase activity in mature grain tended to be higher than in sound wheat. Consequently, the approach taken to the problem was to select a series of Triticale lines exhibiting varying degrees of kernel shrivelling and to measure in them various chemical parameters with particular emphasis on alpha-amylase levels and carbohydrates. This information would then provide the necessary basis from which additional investigations could be undertaken.

Information accumulated during this study indicates that catabolic processes such as the alpha-amylase mediated breakdown of endosperm starch are not the sole causes of kernel shrivelling in Triticale lines. A definite association between alpha-amylase activity and degree of shrivelling both between lines and within lines was clearly demonstrated. The question is whether or not the levels of amylase activity observed could give rise to the amount of kernel shrivelling which is characteristic of Triticale. Evidence to support the view that shrivelling cannot be wholly attributed to the degradative action of amylase came from several areas of investigation.

The first of these is the fact that alpha-amylase activity did not begin to increase rapidly until 38 days after pollination while in some seeds the first visual indications of shrivelling were already evident at 16 days. Upon removal of the pericarp-testa from these seeds, cavities or faults devoid of tissue were visible in the endosperms. These must be the result of factors other than the action of amylase. This, plus the observation of the large central cavity at the crease (see Plate 2) would suggest that the endosperm tissue was never developed rather than starch having been synthesized and then subsequently degraded by enzymes.

Reducing sugar levels observed were never very high which is contrary to what would be expected if starch breakdown were taking place. Supporting this were the respiration determinations. These were not greatly different from Manitou except at the last sampling date. In addition, it is significant that the Triticale lines 6531 and 6A190, which vary considerably in kernel type and alpha-amylase levels had very similar respiration rates.

Furthermore, no improvement in seed development was obtained upon treatment with CCC in spite of the fact that alpha-amylase was reduced in grain from treated spikes. The concept that the reduction in alpha-amylase was offset by an inhibition of other GA-dependent growth processes is largely invalidated by the fact that there was no reduction in seed weight of Manitou wheat as a result of CCC treatment. Here alpha-amylase was already very low so that if there was any negative effect on growth from CCC it should have been expressed fully.

Approximately 10 to 15 percent of barley endosperm starch is degraded during a germination period of 5 to 7 days in the malting process.

During this time the acrospire develops to nearly the full length of the kernel and alpha-amylase activity increases to 2000 to 4000 times that in mature ungerminated grain. Respiration rate in barley has been shown to increase very rapidly during the early stages of germination (Abdul-Baki, 1969) so that after 11 hours of imbibition it is already at a level of about 20 µl 0_2/seed/hour. These embryonic and enzymatic changes are much more extensive than those which are evident in Triticale grains. It is thus, difficult to envisage starch degradation of a similar order occurring in Triticale endosperms. The greatest amount of visible germination observed under field conditions was only approximately six percent (Table 4.5) and of these many were in the early stages exhibiting mainly enlarged embryos with acrospire growth just beginning. No doubt there would be some starch breakdown associated with these modifications but additional factors would be necessary to account for the reduced amounts of endosperm starch in Triticale.

From an overall consideration of the results of the various aspects of the investigation it is concluded that poor kernel development in Triticale is the result of abnormalities in starch synthesis together with some starch breakdown at the latter stages of growth due to increased levels of alpha-amylase.

These effects, in turn, can probably be attributed in part to a high incidence of aneuploidy and abnormal nuclear-cytoplasmic interactions.

The manner of expression or mode of action of these factors at the biosynthetic or metabolic level could possibly occur in one of several ways. An immediate hypothesis would be that specific enzymes necessary for synthesis are either not produced in sufficient quantities or else are not fully functional. This is the type of result which could be

readily associated with a chromosome deficiency and the absence of genes controlling the production of these enzymes.

Effects of a more general nature such as could be ascribed to incompatibility between wheat and rye genomes or to nuclear-cytoplasmic relationships could result in imbalances in total cellular function causing abnormal cell divisions to occur or complete failure of division. The cavities observed at early stages of development in endosperm tissue might arise in this manner. Conceivably, the remainder of the endosperm could then be reasonably normal and the causes of shrivelling more anatomical in nature than biochemical. Cavities in particular areas of the endosperm would be more readily explained on this basis than on the basis of localized enzyme deficiencies or poor transport of precursors to these areas.

The high frequency of aneuploidy or abnormalities in the chromosome complement found in shrivelled grains of Triticale supports the observation that Triticale lines which have a high degree of sterility tend to produce seed of poor quality. It would suggest that both of these characteristics, i.e. sterility and shrivelling, arise from a common cause. In contrast to the high frequency of aneuploids in shrivelled seeds was the fact that simply by selecting plump seeds there was almost absolute selection pressure against aneuploids. This in itself should prove useful as an aid in improving the efficiency of screening for genetic stability in Triticale. Also, it points out the fact that improvement in kernel type will probably be achieved in conjunction with an improvement in fertility.

Efforts to improve Triticale grain quality in the breeding program have in the past largely been based on the visual selection of lines on the basis of seed type. Evidence from the Canadian as well as the Mexican breeding programs indicates that a considerable measure of improvement is possible by this means. Continued selection pressure in favor of plump

seeds and high fertility together with cytological screening for stable 42 chromosome plants should result in further improvement in the future.

The concept of nuclear-cytoplasmic relationships is probably also of key importance and is worthy of further consideration. The stabilization of reproductive processes and improvement in general adaptability will, no doubt, be accompanied by an enhancement of kernel development. This is a process of an evolutionary nature and as such occurs slowly but there are ways in which it might be accelerated. One is by crossing octaploid and hexaploid Triticale lines using the octaploid as the female and selecting hexaploid progenies. In this way the cytoplasm should be essentially that of hexaploid wheat and consequently a 1:1 cytoplasmic to nuclear ploidy ratio would be expected. Pentaploid hybrids from reciprocal crosses between hexaploid and tetraploid wheats are also being employed in order to investigate the effects of 6x and 4x cytoplasm on the performance of hexaploid Triticale (Kyio, personal communication).

No genetic information was obtained during the study which would give direction in the choice of parents for the production of new wheat-rye amphiploids. Lines of less complex parentage would have been useful in establishing genetic control of kernel characteristics.

One area of investigation which might be pursued in the future is a more detailed cytological and histological study of endosperm development, paying particular attention to starch deposition and starch granule growth. This has not received much attention but is probably necessary as a supplement to the biochemical data obtained during grain development in order to more fully interpret the results. Cytological and histological examinations of kernel sections should yield information regarding the structural nature of endosperm tissue and hopefully show

whether low grain density arises as a result of other factors in addition to the abnormalities in gross structure of the kernel as illustrated in Plate 2.

Further biochemical investigations would emphasize enzymes involved in starch biosynthesis. Estimation of quantitative changes in starch synthesizing enzymes such as ADPG - and UDPG - pyrophosphorylase during grain development should help to elucidate the efficiency of the functioning of the biosynthetic apparatus.

The study of the effects of growth hormones and inhibitors with the objective of achieving preferential stimulation or inhibition of enzymes and determining the resultant effect on grain development could be expanded to a separate investigation of the various levels of the growth regulators. Information was obtained using CCC to inhibit alpha-amylase production but no doubt more could be learned by pursuing this area further.

6. CONTRIBUTIONS TO KNOWLEDGE

Eight Triticale lines of diverse parentage and varying in degree of kernel shrivelling were selected for an investigation of the nature and causes of poor kernel development.

Alpha-amylase activities were determined for mature grain of the lines and comparisons made with the values for the hard red spring wheat variety Manitou, the durum wheat variety Stewart 63 and the spring rye variety Prolific. For the Triticale lines alpha-amylase activity and kernel shrivelling as measured by grain density were shown to be correlated both between lines and within lines. Changes in alpha-amylase activity during grain development were studied in two lines of Triticale which exhibited large differences in amylase levels at maturity. The lines were shown to be similar at early stages of development with the differences beginning to become evident at about 38 days after anthesis.

Information was obtained on changes in reducing sugars and starch content during development and maturation of grains of a number of Triticale lines. No studies of this nature had previously been reported for Triticale.

Endosperm starch granule size distribution patterns were determined for the eight Triticale lines and compared to those for Manitou, Stewart 63 and Prolific. Five of the Triticale lines had mean granule sizes intermediate between wheat and rye while the other three were similar to wheat. Mean starch granule size was found to have no apparent relationship to kernel shrivelling in the Triticale lines.

Treatment of developing spikes of Triticale with CCC to inhibit alpha-amylase production was found to have no positive effect on kernel

development. Similarly, a reduction in the number of florets per spike did not result in the remaining kernels on the spike undergoing enhanced growth.

Plump and shrivelled seeds were selected from within the Triticale line 6456-3 and chromosome numbers determined. It was found that the frequency of aneuploidy in shrivelled seeds was 32 percent compared to only 2 percent in the plump seeds.

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8. APPENDIX

Table 8.1. Changes in dry matter (mg./kernel) during development of grains of wheat, rye and Triticale grown under field conditions.

	Days after anthesis						
	10	17	24	31	38	45	52
Manitou	8.99	14.96	24.82	31.64	32.48	34.89	33.30
Stewart 63	10.66	17.43	33.67	43.52	47.43	50.88	45.69
Prolific	6.58	12.10	19.75	27.25	35.11	35.35	35.37
8A92	5.12	17.05	21.22	29.01	36.65	38.89	37.92
6531	7.87	21.22	32.17	37.30	51.87	56.46	56.74
6A250	5.83	17.21	23.65	28.32	29.75	28.14	27.44
6A320	8.58	20.95	31.51	41.73	48.57	51.58	49.64
6456-3	7.02	16.91	27.81	33.47	37.86	38.47	33.97
6517	10.79	21.72	27.09	36.36	40.71	40.63	42.94
6A190	9.46	17.73	27.68	35.46	41.31	41.09	42.76
6211.2	11.28	22.02	31.77	38.82	50.04	44.63	53.56

Table 8.2. Changes in moisture content (percent) during development of grains of wheat, rye and Triticale grown under field conditions.

	Days after anthesis						
	10	17	24	31	38	45	52
Manitou	68.7	62.2	46.3	43.2	40.4	17.8	5.8
Stewart 63	69.1	61.4	52.0	45.7	39.9	29.4	5.0
Prolific	71.3	68.9	64.3	53.4	49.9	36.6	30.0
8A92	71.4	66.0	63.7	51.6	46.2	43.3	5.1
6531	74.1	69.7	65.9	62.5	46.4	27.1	15.0
6A250	72.4	64.9	60.9	54.9	45.7	25.1	2.4
6A320	70.6	69.0	63.5	55.8	47.1	35.9	14.5
6456-3	73.8	71.0	63.7	55.7	50.3	39.2	9.4
6517	72.0	69.9	61.8	54.7	50.6	43.6	31.1
6A190	71.9	70.9	65.0	61.0	54.0	48.2	18.1
6211.2	71.6	69.5	62.2	55.9	47.8	28.8	19.3

Table 8.3. Changes in reducing sugars (mg./kernel) during development of grains of wheat and Triticale grown under field conditions.

	Days after anthesis					
	17	24	31	38	45	52
Manitou	6.319	3.073	2.303	1.819	1.279	1.262
6531	6.248	5.866	5.434	3.659	2.177	2.463
6A320	-	3.479	3.364	2.836	1.796	2.214
6456-3	8.777	5.025	4.160	3.977	2.497	3.568
6A190	-	3.479	3.364	2.836	1.796	2.214
6211.2	8.568	4.901	2.848	3.112	2.291	3.044

Table 8.4. Changes in starch content (mg./kernel) during development of grains of wheat and Triticale grown under field conditions.

	Days after anthesis					
	17	24	31	38	45	52
Manitou	7.43	13.33	17.29	18.65	19.40	17.34
6531	8.74	15.43	18.80	32.41	32.15	32.79
6A320	-	17.39	23.33	29.45	30.43	28.20
6456-3	7.82	15.09	20.01	22.33	24.82	19.09
6A190	-	12.50	16.60	21.78	21.45	22.04
6211.2	9.22	17.03	20.99	29.05	24.84	29.70