HYDROSTATIC PRESSURE EFFECTS IN STEEPED BARLEY RELATING TO WATER-SENSITIVITY.

by

Gerard Damien Thraves

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science
in
Plant Science
The University of Manitoba

Winnipeg, Manitoba, 1984

HYDROSTATIC PRESSURE EFFECTS IN STEEPED BARLEY RELATING TO WATER-SENSITIVITY

BY

GERARD DAMIEN THRAVES

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

DEDICATION

This work is dedicated to the memory of David Copper B.S.A. (1981) University of Manitoba.

ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who gave of their time and expertise to help me with this thesis project. Special thanks is extended to Dr. William Woodbury whose inspiration proved invaluable and to Mike Sumner for his constant encouragement and assistance. I would also like to express my appreciation to Dr. A.W. MacGregor and Dr. B.R. Irvine for reading the manuscript; to Bert Luit for his microscopy and photographic skills; and to Dr. Meran Owen and Wayne Johnson for their technical assistance.

ABSTRACT

Thraves, Gerard Damien. M.Sc., The University of Manitoba, 1984, Hydrostatic Pressure Effects in Steeped Barley Relating to Water-Sensitivity.

Major Professor: Dr. William Woodbury, Department of Plant Science.

Following hydrostatic pressure treatment the properties of barley grains were observed to be similar to those of water-sensitive grains. In particular, the post-treatment germination environment markedly influenced the perpetuation of germination inhibition. Although hydrostatic pressure appeared to force water into the embryos of steeped grain no discernable disruption was observed. The manner by which hydrostatic pressure imposed germination inhibition was not established. However, it was possible that anoxia and narcosis were the major contributors to a collective inhibition which may have additionally involved other mechanisms normally associated with water-sensitivity.

TABLE OF CONTENTS

Page

Page

Page

LIST OF TABLES

Table | Page

LIST OF FIGURES

LIST OF PLATES

Plate | Page

CHAPTER I

INTRODUCTION

Grain selection criteria allow the maltster to increase the uniformity of germination and reduce malting loss. Interrupted, aerated and acidulated steeping of abraded barley coupled with the use of exogenous gibberellic acid have accelerated germination and reduced respiratory losses. Hydrostatic pressure during steeping or at casting has been reported to disrupt the uniformity of germination and reduce malt extract.

Eyben and Droogenbroeck (1969), Davidson and Jangaard (1978) and Yoshida et al. (1979) observed that hydrostatic pressure during steeping or at steep-out restricted rootlet growth and respiratory activity. Eyben and Droogenbroeck (1969) reported that hydrostatic pressure did not push water into the grain or disrupt the embryonic axis and that the restriction of respiratory activity was not the consequence of asphyxiation. They proposed that pressure interfered with the ability of the aleurone to form amylase and the embryo to synthesize gibberellic acid. In contrast Yoshida et al. (1979) observed that hydrostatic pressure forced water and possibly micro-organisms as far as the nodal region of the embryo. They suggested that the restriction

of respiratory activity and the inhibition of gibberellic acid synthesis was due to the disruption of embryonic organs.

The objective of this study was to further elucidate the cause of hydrostatic pressure inhibition in steeped barley. This was attempted by: 1) observing the ability of water-sensitivity breaking treatments to alleviate hydrostatic pressure inhibition, and 2) establishing by use of electron and light microscopy whether hydrostatic pressure disrupted embryo integrity.

CHAPTER II

LITERATURE REVIEW

This chapter contains five sections each of which is devoted to a distinct subject area. The first section draws attention to the general properties of barley grain and the significance of these properties in the selection of barley for malting. The second reviews some aspects of grain physiology during the steeping process. The third section deals exclusively with the implications of hydrostatic pressure during steeping on grain metabolism, growth, appearance, and malt yield. The fourth section considers the broader consequences of external pressure on seed germination. Finally, the fifth section examines the effects of external pressures on root growth and membrane properties.

2.1 MALTING BARLEY

2.1.1 The Barley Grain

The eventual fusion of the pericarp and the testa which is derived from the inner integument defines the barley

fruit as a caryopsis (Macleod, 1979). The pericarp of hulled barley secretes a sticky polysaccharide which results in the glumes adhering to the pericarp when the grain dries. The mature embryo, which occupies a dorso ventral location in the proximal portion of the grain, is comprised of a scutellum, a coleoptile enclosed plumule, and a radical plus five to eight secondary root initials within a coleorhiza (Hector, 1936). When compared to wheat the embryos of barley are generally larger, have scutella which project further beyond the tip of the coleoptile and have root initials that are more equal in size (Russell, 1960).

The bulk of the mature grain is dead endosperm which consists of hemicellulose walls surrounding starch granules embedded in a protein matrix. Except proximally, where the columnar epithelial layer of the scutellum disc abuts the entire depletion layer, the endosperm is entirely surrounded by two to three living layers of cubical thick walled aluerone cells. The aleurone cells are additionally distinguished from the endosperm by their lack of starch granules and their high oil and protein content (Hough et al. 1971).

2.1.2 Malting Quality

The malting quality of a recommended variety varies with batch and is evaluated on the basis of appearance, moisture content, grain size, germination capacity, germination energy, pre-germination and protein content (Brookes, 1979). Appearance indicates the proportion of half grains, seed contamination, bacterial and fungal activity, and the degree of severe pre-germination (Hector, 1936; Flannigan and Healy, 1983). For prolonged storage barley grains are required to have a moisture content of between 12 and 14%. Higher moisture levels promote deterioration of the grain due to microbial activity (Brookes et al. 1976).

Whilst large grains initially absorb water more rapidily than small grains, the latter usually reach their optimum moisture content for germination sooner (Brookes et al. 1976). Although grain size is highly dependent on variety and harvest conditions, malting barley should have a high degree of size uniformity. Approximately 95% of the grains in a sample should have diameters >2.2 mm and samples with a high proportion of grains with diameters <2.2 mm are usually rejected (Brookes, 1979).

2.1.3 Germination Capacity and Energy

The Tetrazolium and Thunaeus test for viability are used to establish the germination capacity of freshly harvested grain. This capacity must be above 95% for barley of

malting quality (Brookes, 1979; Reeves, 1981). Germination energy measures the percentage of grains that would germinate if malted at the time of the test and is determined by placing 100 grains in a 9 cm diameter petri dish containing 2 Whatman No. 1 filter papers and 4 ml of water. The percentage germination is the number of grains that germinate after 3 days in the dark at 18°C (Brookes, 1979). Freshly harvested barley usually has a germination energy <50%. Profound dormancy is considered to be the difference between germination capacity and energy (Brookes, 1979; Reeves, 1981).

2.1.4 Dormancy

Barley exhibits two types of dormancy. The first, profound dormancy, is the failure of grains to germinate irrespective of water availability. The second, water-sensitivity, exhibits itself under conditions of excess water (Pollock *et al*. 1955ab). The extent of profound dormancy and water-sensitivity is highly dependent on variety and climate, with barley harvested under dry conditions generally exhibiting lower degrees of both dormancy types (Pollock and Pool, 1962; Gordon, 1970a).

Continuous year round malting requires that grain from the previous year's crop be available for malting whilst freshly harvested grain is dried and stored till dormancy (where present) is broken (Brookes, 1979). Essery and

Pollock (1956) reported that the rate of improvement in germination energy (loss of profound dormancy) is higher at 18°C than 0°C for grains of the same moisture content. The rate at which water- sensitivity declines during storage is inversely related to grain moisture content subsequent to drying. Pollock et al. (1955b) observed that both profound dormancy and water- sensitivity can be overcome by mechanical damage of the grain coat or by removal of the husk and pericarp by acid scarification. Pollock et al. (1955b) also observed the following, namely: that piercing the embryo will alleviate profound dormancy but not water-sensitivity; that a higher concentration of hydrogen peroxide is required to overcome profound dormancy than water-sensitivity; and, that a O_2 atmosphere will alleviate water-sensitivity but not profound dormancy. Pollock et al. (1955a) reported on the effect on dormancy by the removal of the pericarp and/or the husk with 50% v.v. sulphuric acid. Profound dormancy and water-sensitivity both appeared to be determined by pericarp integrity. The removal of the husk only mildly alleviated profound dormancy and did not influence water-sensivity. The acid scarification treatment failed to disrupt the testa and testa integrity did not appear to be involved in determining profound dormancy or water-sensitivity. In contrast Briggs and MacDonald (1983) reported that acid decortication increases the permeability of the testa. Pollock and Kirsop (1956) observed that profound dormancy in barley can be

overcome by treating the grain with hydrogen sulphide or thiourea. They concluded that these compounds deactivate pericarp polyphenol oxidase which would otherwise absorb O_2 in the profoundly dormant grain thereby preventing embryo growth.

Dunwell (1981) reported that freshly harvested barley grains have an optimum germination temperature of 10°C and that both the optimum and range of temperature for germination increases as profound dormancy declines. It was also noted, that at all temperatures the husk reduced the level of germination by limiting gas diffusion. This suggested that there is less evidence for an embryo dormancy mechanism in barley than there is for a secondary mechanism involving aleurone, endosperm or testa. MacLeod (1979) stated that the barley embryo lacks none of the biochemical mechanisms required for germination, dormancy being imposed by non-embryonic structures or by the grain environment.

Dormant varieties of barley have been found to contain no detectable level of abscisic acid (ABA), even though ABA is an antagonist to the stimulating influence of gibberellic acid (GA) on dormant grain germination (Dunwell, 1981). Moreover, Dunwell was able to break the dormancy of barley grains by exogenous GA application and concluded that a lack of GA rather than the presence of any inhibitory substance is responsible for post-harvest dormancy. Exogenous gibberellic acid (GA) possibly relieves dormancy in some seeds by the mobilization of food reserves (Palmer,

1974). Palmer noted, however, that barley germination takes place prior to the mobilization of endosperm reserves and, although exogenous GA increases the rate of germination of non-dormant barley, it is unable to stimulate germination of water-sensitive grains. The ability of exogenous GA to break post-harvest dormancy (profound dormancy) in barley appears to act by a mechanism physiologically distinct from the stimulation of aleurone activity (MacLeod, 1979). Exogenous GA induces the embryo of profoundly dormant grains to release the sulphydryl compounds glutathione and cysteine from embryo proteins. Increased levels of these compounds have been associated with the germination of previously dormant barley grain (Belderok, 1968). MacLeod (1979) noted, that gibberellin synthesis requires O_2 which may be limiting in the profoundly dormant grain.

Unlike water-sensitivity, the nature of profound dormancy is more clearly understood and generally this form of dormancy does not pose any problem to the maltster since it is often absent or of short duration. Indeed, profound dormancy of optimum duration is a selection criterion for avoiding pre-harvest sprouting (MacLeod, 1979). Crabb and Kirsop (1969) examined the uptake of O_2 and the release of CO_2 from normal and water-sensitive barley grains under water-sensitive conditions. On the basis of their tests they determined that there is no significant difference between the rate of O_2 consumption by water-sensitive and normal

barley grains and that the resistance to O_2 diffusion through the pericarp, husk and surface film of water is the same for normal and water-sensitive grain. Moreover, the level of fermentation in both grain types was approximately the same and the removal of the pericarp eliminated anaerobic respiration in both normal and water-sensitive grains. The ability of normal embryos to germinate at lower (2%) O_2 tension than water-sensitive embryos indicates that the embryos of water-sensitive grains have a higher O_2 requirement for germination which cannot be sustained under water-sensitive conditions. Crabb and Kirsop (1970) used an embryo extract to induce a dormancy condition in water-insensitive grain under the conditions of the water-sensitivity test. However the embryo extract did not impose dormancy in the germination energy test. The dormancy induced by the embryo extract derived from the embryos of either normal or water- sensitive grains could be alleviated by treatments which break water-sensitivity. Under reduced O_2 tension the embryo extract inhibited the germination of decorticated water-sensitive grains more than decorticated normal grains. Crabb and Kirsop were unable to identify all the ethanol embryo extract components. They did, however determine that the embryo sugars sucrose and raffinose were resposible for at least 30% of the inhibiting nature of the extract and they concluded that these sugars and other unidentified extract components could be inhibiting to the

germination of water-sensitive embryos under conditions of limited O_2 availability.

In the embryo of barley grains germinated under aerobic conditions both sucrose and raffinose are metabolized. However, under anaerobic conditions and during aerated steeping, raffinose metabolism is inhibited. Raffinose metabolism requires high energy phosphate bonds and only takes place in the presence of another respiratory substrate. In barley this starts approximately 14 hours after imbibition, raffinose being completely utilized 24 hours after the start of imbibition. This period of raffinose metabolism is concurrent with the synthesis of complex carbohydrates in the embryo (Macleod, 1957).

Jansson (1962) proposed that water-sensitivity was the result of accumulated indoleacetic acid (IAA) uncoupling oxidative phosphorylation in water-sensitive grains. Gaber and Roberts (1969a) investigated the influence of respiratory uncouplers under conditions of excess and optimum water availability. They observed that the germination inhibition of low water-sensitive is considerably less than high water-sensitive grain under conditions of excess water and in the presence of respiratory uncouplers. In addition, they noted that IAA stimulated germination of water-sensitive grains in excess water and that IAA antagonists failed to alleviate water-sensitivity.

Blum and Gilbert (1957) reported that water-sensitivity was reduced by surface sterilization of the grains with 1% sodium hypochlorite or 0.1% mercuric chloride prior to conducting the water-sensitivity test. They concluded that the sterilization treatment reduced microbial toxin production and competition for O_2. Jansson et al. (1959) suggested that Blum and Gilbert (1957) had erroneously interpreted their surface sterilization results and that their surface sterilized grain performed no better than water-sensitive grain which had not been steeped in water prior to being placed in petri-dishes with excess water. They asserted that control water-sensitive grain pre-steeped exhibits an additional germination inhibition phenomenon distinct from water-sensitivity. Jansson et al. (1959) observed that steeping concentrations of 1 to 0.125% mercuric chloride and 0.1% hypochlorite considerably reduced subsequent petri- dish microbial population counts under water-sensitive conditions, but failed to stimulate the germination of water- sensitive grains. Even in the presence of a high microbial count, 0.9% ferrous sulphate or pericarp removal alleviated water- sensitivity. Furthermore, water-sensitive grain failed to germinate in a continuously aerated renewed water steep where the microbial count was low.

Jansson (1959) soaked normal and water-sensitive grains of English barleys in 0.1% mercuric chloride and water for 1 hour prior to conducting germination energy and water-

sensitivity tests. Following this procedure he observed that water soaking depressed the germination of both normal and water-sensitive grains in the water-sensitivity test and that mercuric chloride soaking reduced germination of water-sensitive grain considerably in both the water-sensitive and germination energy tests. In addition, the inhibition of water-sensitive grain in the germination energy test was overcome by the presence of sulphydryl bearing cysteine. However, Jansson (1960) was able to alleviate water-sensitivity in grains of Swedish barleys by 1 hour soaking treatments in 0.5% mercuric chloride or 0.5% hypochlorite prior to conducting water-sensitivity tests. Blaim (1959) reported on the ability of coumarin to inhibit germination of wheat grains by preventing water uptake by the embryo. Blaim suggested that coumarin acted by reducing the permeability of the protoplast. Jansson (1959) was able to prevent coumarin inhibition of germination by pre-soaking the grains in mercuric chloride for a short duration of time. Jansson (1960) proposed that mercuric chloride binds coumarin, and alleviates water-sensitivity by binding an inhibitory substance which is responsible for water-sensitivity. The ability of mercuric ion to bind sulphydryl groups led Jansson to test sulphydryl inactivating compounds. Potassium ferricyanide was found to alleviate water-sensitivity whilst mercaptoacetic acid reversed the stimulating effect. Jansson suggested that potassium ferricyanide oxidized suylphydryl groups

to disulphide groups (breaking water-sensitivity) whilst mercaptoacetic acid re-established water-sensitivity by splitting disulphide groups to sulphydryl groups. Jansson further proposed that the oxidizing properties of sodium hypochlorite were responsible for its ability to break water-sensitivity. Employing the standard water-sensitivity test Gaber and Roberts (1969b) investigated the effect of various antibiotic combinations on the germination of water-sensitive grains under conditions of excess water. They observed that single bactericide or fungicide treatments were less successful in stimulating germination than a combination of gram positive and gram negative bactericides plus a fungicide. The combination of streptomycin, penicillin and nystatin almost completely eliminated water-sensitivity. This antibiotic combination significantly reduced the microbial population count, O_2 uptake and CO_2 output of the grain environment. Gaber and Roberts concluded that water-sensitive grains contain a larger and more mixed population of micro-organisms below the husk than water-insensitive grains and that under conditions of excess water the micro bial population rapidly expands. Such expansion limits germination by competing with the embryo for O_2 a condition which may eventually cause embryo death.

2.1.5 Pre-germination

Visual assessment of pre-germination does not accurately indicate the total degree of pre-germination as incipiently pre-germinated grains are not detected. Tests which measure grain α-amylase prove most satisfactory in estimating pre-germination (Brookes, 1979). Following heat destruction of β-amylase activity, the α-amylase activity may be detected by placing barley half grains on agar medium containing soluble starch. After 30 min. incubation at 37°C the grains are removed and the dish flooded with iodine solution. The solution is then drained to reveal colourless spots in those areas previously occupied by pre-germinated grains (Lloyd and Martin, 1982).

Heavy rainfall during harvest increases the degree of dormancy and the extent of pre-germination (Gordon, 1970 a,b). Pre-germination results from a pericarp rupture at high (approximately 45%) grain moisture. This allows atmospheric contact with the embryo and may initiate growth provided that grain moisture is above 30% (Gordon, 1970a). Gordon also observed that ruptured grains 28 days after anthesis were able to germinate under both germination energy and water-sensitivity test conditions whilst non-ruptured grains failed to do so; that the number of ruptured grains increases with days following anthesis; and, that drying of pre-germinated grains with significant rootlet growth on the head does not enable the embryo to reinitiate growth

through previously inactive root initials when adequate moisture becomes available. Gordon (1970b) reporting on the requirement of pericarp-testa rupture for pre-germination in barley, wheat, rye and triticale stated that the potential of rupturing is dependent on variety and that within a variety the character is heterogeneric.

2.1.6. Protein Content

Whilst the results of barley nitrogen (N) estimated by the Kjeldahl technique are expressed as total N in the United Kingdom, in North America and Continental Europe total N is multiplied by a factor of 6.25 to give a value for protein content (Moll, 1979). In the United Kingdom two-row barleys considered suitable for ale malts have total N values ranging from 1.3 to 1.6% N_2. In North America, however, higher N (2.0% to 2.2%) six-row barleys are more commonly used in malting (Hough et al, 1971). Traditional pale ale in the United Kingdom was made from low N (1.3% to 1.4%) two-row barley. Nevertheless, in recent years the trend has been towards the use of higher N grain and the production of fewer malt types. Consequently today's pale ale malts are made from barleys with up to 1.65% total N. The high N barleys malted in North America produce malts which are highly enzymic in character and are often used with a high proportion of adjunct (Briggs et al, 1981).

Generally as protein content increases from 9 to 13% expected malt extract can decline from 79.0 to 70.0% (Moll 1979). In addition to low total N the composition of barley storage protein (hordein) is crucial to the malting quality of a variety (Baxter, 1981). Unlike total N, hordein composition is a strong varietal characteristic relatively independent of environment. Increased N content of the best malting varieties is less detrimental to malting quality than when occurring in poorer malting varieties. Barley hordeins of poor malting quality tend to form disulphide bridges during mashing and undergo excessive degradation during boiling. These conditions respectively result in poor mash separation and over dark beer due to amino acid sugar condensation (Baxter, 1981).

2.2 STEEPING

Steeping allows the rapid attainment, with minimum respiratory loss, of the grain moisture content (43 to 46%) required for even modification on the germination floor (Brookes et al, 1976). Steep aeration, air rests and warmer steeping temperatures reduce the time to and may improve the uniformity of chitting which marks the end of steeping. Total steeping time is generally 2 to 3 days at temperatures of 10 to 15°C. Steeping of distally abraded grains and the addition of 0.1 to 0.2 ppm of gibberellic acid to final steeps accelerates the malting process and often reduces

malting time and losses to less than 5 days and 5% respectively (Hudson, 1983). Malting time may further be reduced by the introduction of acidulated steeping (Palmer, 1972, 1974).

2.2.1 Water Uptake During Steeping

Water uptake during steeping, the velocity of which is an exponential function of temperature, is considered to be triphasic (Brookes et al, 1976). The first phase, imbibition, involves a rapid (6 to 10 hours) physical absorption of water by grain colloids. The pericarp cuticle, which is absent in the region of the embryo, is the principle barrier to water entry during this phase which ends with a proximal preferential distribution of water (Brookes et al. 1976). Imbibition is independent of metabolic activity since even though metabolism commences as soon as a viable grain starts to hydrate imbibition also takes place in non-viable grains (Bewley and Black, 1978). Initially on wetting gas collects and escapes from grain surfaces, but, since the phenomenon is shown by non-viable grains, the gas is thought to be released from trapped pockets and colloid surfaces rather than to be respiratory in nature (Haber and Brassington, 1959). The second phase (10 to 12 hours) is characterized by a very slow or complete absence of water uptake, some hydrolysis of starch and the embryo becoming metabolically active (Brookes et al. 1976). This level of metabolic activity is present in both dormant and non-dormant grains (Bewley and Black, 1978).

The third phase involves a linear pattern of water uptake which is directly correlated to the grain's metabolism, the vacuolization of meristematic tissue and elongation culminating in visible germination (Brookes et al. 1976). The three phases of water uptake do not take place in unison throughout the grain and therefore there is a differential level of hydration between tissues (Bewley and Black, 1978).

2.2.2. Respiration During Steeping

Using a Warburg respirometer to monitor steeping barley respiration Davidson and Jangaard (1978) determined that the rate of respiration increase is essentially linear. In contrast Eyben and Droogenbroeck (1969) and Yoshida et al. (1979) used an O_2 electrode to measure the respiration of steeping barley and determined that it is nonlinear and follows a pattern of non-consecutive exponential increments. In explanation, respiratory activity is observed to increase immediately after initial imbibition and reach a plateau after 4 hours. Respiratory activity then remains constant on this first plateau for approximately 10 hours and then accelerates for approximately 15 hours to a second plateau. The second plateau is maintained for approximately 10 hours following which respiratory activity increases exponentially. This final exponential phase is associatd with visible germination.

Brookes et al. (1976) noted the following concerning grain respiration during and after steeping: that the respiratory quotient of grain rises rapidly to 1.5 during the first phase of water uptake; that as water uptake continues the respiratiory quotient declines to approximately 0.98 by the end of the third phase; and, that the intensity of respiration reaches a maximum when the plumule is 3/8 to 5/8 the length of the grain. Moreover, Hough et al. (1971) reported that 50 to 70% of the total respiration during steeping is due to the activity of the embryo and as the moisture content increases so does the respiratory demand for O_2. Irrespective of steeping technique, the presence of a water film on the grain surface limits O_2 consumption but not CO_2 evolution. This anoxia results in the accumulation of ethanol and lactic acid. The presence of these substances may delay chitting in the absence of air rests but would not be expected to reduce viability since barley grain will chit in unaerated steeps (Brookes et al, 1976).

2.2.3. Carbohydrate Metabolism During Steeping

Brookes et al. (1976) reviewing carbohydrate metabolism during steeping reported that the levels of glucose, fructose and sucrose decline during the first 24 hours and then rise. During steeping raffinose is not metabolized. Between 12 and 24 hours after the start of imbibition starch begins to accumulate in the scutellum, this starch being derived from fat droplets present in the scutellum (Bewley and

Black, 1978). As grain moisture content increases α and β - amylase activity intensifies and the amylopectin fraction of the endosperm is first solubilized (Brookes et al. 1976).

2.3 THE EFFECT OF HYDROSTATIC PRESSURE DURING STEEPING ON BARLEY GERMINATION AND MALT QUALITY

Steeping barley exposed to the hydrostatic pressures of deep steep tanks and/or pumping operations exhibits reduced respiratory activity, delayed growth and poor modification (Eyben and Droogenbroeck, 1969; Yoshida et al. 1979). Eyben and Droogenbroeck (1969) mimicked steeping depths of 5 and 10 m by applying continuous hydrostatic pressures[1] of 49 and 98 KPa to aerated steeping barley and observed that with increased steeping depth respiratory activity and germination rate were depressed. They also observed: that subsequent to steeping root growth was less vigorous; that all indices of modification were reduced except soluble nitrogen; and, that final malt yield was less.

---

[1]Pressures from all referenced sources have been converted to the nearest whole number KPa or MPa equivalent where appropriate.

2.3.1 Restriction of Respiratory Activity

Eyben and Droogenbroeck (1969) using hydrostatic pressure treatments of 196 KPa for 30 seconds determined with a Warburg apparatus that the restriction of respiratory activity following treatment initially increases with steeping time. Maximum restriction of respiratory activity occurs at the beginning of the ultimate exponential phase of respiratory activity associated with embryo development. Restriction of respiratory activity following hydrostatic pressure treatment then declines with increasing germination time. Using an oxygen electrode, Yoshida et al. (1979) measured (during steeping) the reduction in magnitude of sequential respiratory activity increments of two 2-row barley varieties. The varieties at moisture contents of 20, 35 and 45% were subjected to single or cumulative hydrostatic pressure treatments of 147 KPa for 10 seconds. They observed that there is a cumulative restriction of respiratory increments with successive treatments during steeping and that the degree of restriction is dependent on variety. Davidson and Jangaard (1978) observed that compared to dry steep-outs, wet steep-outs[2] reduced the respiratory activity of grains by

---

[2]Wet steep-outs unlike dry steep-out involve pumping operations which expose the steeped grain to hydrostatic pressure.

approximately 26%. Following steep-out into a damp chamber, wet steep-out grains failed to regain presteep-out respiratory activity for up to 28 hours.

Eyben and Droogenbroeck (1969) examined the effect of a constant hydrostatic pressure of 196 KPa for 1, 15 and 30 second durations on steeped barley having maximum pressure sensitivity that is when entering the final exponential phase of respiratory activity. They determined that the depression of respiration is the same at constant pressure irrespective of duration and that the rate of respiratory recovery over a period of 4.3 days declines with increased duration. Moreover, the maximum activity following recovery from all durations never equalled the respiratory activity maximum of grains which received no hydrostatic pressure treatment. In a further experiment Eyben and Droogenbroeck used maximum sensitivity grains to determine the effect of higher hydrostatic pressure treatments of constant duration (30 seconds) on subsequent respiratory activity. They observed that the degree of activity restriction is proportional to the magnitude of the applied hydrostatic pressure. Whilst a hydrostatic pressure of 98 KPa delayed recovery of respiratory activities to control levels for 2 days, treatments of 196, 294 and 490 KPa permanently inhibited recovery of respiratory activity to control levels. Eyben and Droogenbroeck concluded that the extent to which respiratory activity of steeped grain is restricted increases with both greater

duration and magnitude of hydrostatic pressure, and that the restriction caused by these two parameters is cumulative.

2.3.2 Proposed Mechanisms by which Respiratory Activity is Restricted

Yoshida et al. (1979) measured increases in the moisture content of embryo and endosperm resulting from a single 10 second, 147 KPa hydrostatic pressure treatment applied to barley grains at either 15, 35 or 60 steeping hours. Embryos showed a higher increase (0.8 to 1.5%) in moisture content than endosperm (0.2 to 0.4%) as a result of pressure treatment. In addition embryos exhibited a greater increase in moisture content during treatment with increased steeping hours. Examination of dissected steeped grains subjected to hydrostatic pressure treatment under black ink revealed that the treatment forces ink into the coleorhiza and nodal region of the embryo. Grains soaked in ink for the same duration without hydrostatic pressure showed no ink penetration (Yoshida et al. 1979). Yoshida et al. proposed that hydrostatic pressure forced water into the embryo and possibly inhibited respiratory activity by destroying embryonic organs. In contrast Eyben and Drogenbroeck (1969) reported that as treated grain shows no change in moisture level, and no cellular disruption, the inhibitory effect of hydrostatic pressure is not due to asphixiation, excess water being

forced into the grains, or damage to the embryonic organs. Davidson and Jangaard (1978) observed that prior to wet steep-out barley rootlets were turgid, whilst following wet steep-out the rootlets were flaccid. Grains with or without rootlets both showed restriction of respiratory activity following hydrostatic pressure treatment. This indicated that root damage could not account for the drop in respiratory activity associated with wet steep-outs.

2.3.3. Growth and Appearance Following Hydrostatic Pressure Treatment

Barley grains appear duller following hydrostatic pressure treatment. In such grains the bleached coloration of the husk is lost and the ivory colour of the coleorhiza is replaced by a greyish tone (Eyben and Droogenbroeck, 1969, Yoshida *et al*. 1979). Grains exposed to hydrostatic pressure show poorer uniformity of growth when cast onto the germination floor. Rootlet growth is generally less vigorous and acrospire growth is often totally inhibited. A high proportion of grains show little or no growth associated with evident signs of putrefaction. The scutella of putrescent grains often have a pink discolouration in the region of the columnar epithelial layer and a fluid mush in place of the endosperm. Yoshida *et al*. suggested that the destruction of embryonic organs may have induced abnormal metabolism and/or

microflora may have been carried into the embryo region by the inrush of water during hydrostatic pressure treatment.

2.3.4 Inhibition of Modification

Eyben and Droogenbroeck (1969) reported that, in addition to restricting respiratory activity, hydrostatic pressure treatment has a deleterious effect on indices of modification. The restriction of respiratory activity delays amylase synthesis and reduces the final yield and diastatic power of the malt. Yoshida et al. (1979) determined differences between fine and coarse extracts[3] for the barley varieties Betzes and Fuji Nijo which had been subjected to a total of 1, 2 or 3 hydrostatic pressure treatments of 98, 147 or 196 KPa at 24 hour steeping intervals. Their results indicate that with a single treatment the difference between extracts generally increases with greater magnitude of hydrostatic pressure. At constant hydrostatic pressure the extract difference increases with the frequency of treatment. Betzes subjected during steeping to a single treatment of 196 KPa or triple treatment of 98 or 147 KPa had extract differences of 4.6, 3.9 and 19.0 respectively, whilst control malt had an extract difference of 1.3. Fuji Nijo, the res-

---

[3]The difference between fine and coarse grind extracts indicates the degree of malt modification. On a dry weight basis these differences are 1.3 for very high modified malt, 2.0 to 2.6 for average malts and greater than 3.3 for poorly modified malts (Moll, 1979).

piratory activity of which is less sensitive to hydrostatic pressure during steeping, showed a decrease in extract difference from 1.1 to 0.1 with increased frequency of the 98 KPa treatment. With the 147 KPa treatment Fuji Nijo extract difference increased with frequency from 0.2 to 3.3.

2.3.5 Proteolytic Activity

Hydrostatic pressure of 294 KPa which had a negative influence on carbohydrate modification failed to influence the formation of proteolytic enzymes, the level of soluble nitrogen, or the Hartong number[4]. These protein modification indices were similar for treatment and control grains (Eyben and Droogenbroeck, 1969). Yoshida et al. (1979) using a single hydrostatic pressure treatment of either 98 or 147 KPa at 24 hours steeping, observed that the levels of formol nitrogen, wort nitrogen, and the Kolbach

---

[4]The Hartong number (index) of a malt gives an indication of the degree of proteolytic and cytolytic modification. The value of the index reflects extract yields from finely ground malt at 20, 45, 65 and 80°C. The extract yield for each of these temperatures is then calculated as a percentage of standard E.B.C. extract yields. The constant 58.1 is then subtracted from the mean of the extract percentages to give the Hartong number. Poorly modified malts generally have index values less than 5 whilst well modified and highly enzymic malts have values greater than 5. (Moll, 1979).

index[5] for worts of Betzes and Fuji Nijo were greater for treated than control samples with the exception of the 98 Kpa treatment sample of Fuji Nijo. Steeping grains, again with the exception of the 98 KPa treatments of Fuji Nijo, which were subjected to additional hydrostatic pressure treatments at 48 and/or 72 steeping hours had wort nitrogen indices below control levels. Wort nitrogen levels progressively decreased with increasing number of treatments. Wort derived from Fuji Nijo which received a 98 KPa hydrostatic pressure treatment at 24 and 48 steeping hours had higher wort nitrogen indices than control wort. Conversely, Fuji Nijo subjected to the same pressure at 24, 48 and 72 hours had lower wort nitrogen indices than controls. With single hydrostatic pressure treatments of 98, 147, or 196 KPa at 24 steeping hours Betzes wort exhibited a progressive decrease in nitrogen indices with greater magnitudes of hydrostatic pressure. Increased frequency and magnitude of hydrostatic pressure during steeping generally decreases wort nitrogen indices, the decrease in these indices being greater for the more pressure sensitive barley Betzes than Fuji Nijo (Yoshida et al. 1979).

---

[5]The Kolbach index is the ratio of soluble protein to total protein. Highly modified malts have greater index values. However, the index is considered an unreliable measure of modification since soluble nitrogen levels are strongly influenced by mashing technique and barley variety (Moll, 1979).

2.3.6 Inhibition of Gibberellic Acid (GA) Synthesis

Eyben and Droogenbroeck (1969) suggested that the mechanical compression of the embryonic axis caused by hydrostatic pressure could possibly inhibit GA formation. Yoshida et al. (1979) subjected both Fuji Nijo and Betzes to hydrostatic pressure treatment of 147 KPa 24 hours before the end of steeping and at steep-out. In the germination box control samples of both varieties had endogenous GA levels which increased to a maximum by the second day and then declined over the following four days. The levels of endogenous GA in treated Betzes remained extremely low until the fourth germination day following which GA levels increased to a maximum on the sixth germination day. This maximum was less than half the second germination day control maximum. Endogenous GA levels in treated Fuji Nijo were significantly depressed but exhibited the same general pattern as control grain, endogenous GA levels reaching a maximum on the second germination day. This maximum approximated a third of the control maximum.

2.3.7 Attenuation of Worts

Attenuation limits of Betzes and Fuji Nijo worts declined with increased magnitude and frequency of hydrostatic pressure treatments during steeping (Yoshida et al. 1979).

Fermentation proceeded normally following a single hydrostatic pressure treatment of either 98 or 147 KPa for 10 seconds during steeping. Treatments of 196 KPa for 10 seconds caused premature flocculation. Premature flocculation was also induced by double and triple 98 and 147 KPa 10 second treatments during steeping. Yeast growth was actually inhibited in Betzes wort by triple 147 KPa 10 second hydrostatic pressure treatment during steeping. Generally hydrostatic pressure during steeping caused premature flocculation to a lesser degree in Fuji Nijo wort and yeast growth was never inhibited (Yoshida et al. 1979). The flocculant character of brewers yeast is determined by genetic interactions with the wort environment such as changes in pH, temperature, O_2 levels, as well as sugar starvation and/or the presence of ethanol and bivalent ions (Hough et al. 1971).

Eyben and Droogenbroeck (1969) reported that proteolytic activity was only restricted by extreme hydrostatic pressure. Yoshida et al. (1979) noted that the low endogenous levels of GA in malts which received hydrostatic pressure treatments during steeping probably reduced protease and glucanase activity in addition to diastatic power. Stewart and Russell (1981) stated that the ability of wort polypeptides to induce flocculation when added to yeast strains growing in protein lacking mediums can be prevented by prior protease hydrolysis of wort polypeptides. The inability of

daughter cells to separate following budding has been alleviated by trypsin and glucanase. These enzymes are thought to aid the mechanical shearing of cell walls by breaking peptide and glucan linkages existing between daughter cells (Stewart and Russell, 1981).

2.3.8 The Effect of Gas Pressure During Steeping on Respiratory Activity and Modification Indices

Eyben and Droogenbroeck (1969) were unable to detect any deleterious effects on germination or malt quality with air pressures less than 294 KPa. Higher air pressures did, however, slightly depress respiratory activity. Gomez (1971) reported that steeped barley subjected to air or N_2 pressures ranging from 98 to 382 KPa for 15 minutes following steeping exhibits restricted respiratory activity, the degree of which increases with the magnitude of pressure treatment. Respiratory restriction is greater for O_2 than N_2 pressurized grain.

Using air, N_2 and CO_2 Gomez (1971) applied gas pressures ranging from 98 to 588 KPa for 15 minutes following steeping. He observed that increasing gas pressure caused an increase in all indices which reflect proteolytic activity but that amylolytic indices differed little from controls. In addition, the degree of increase in proteolytic activity with greater pressure is dependent on the gas used and that

the gas pressure treatments enhanced GA synthesis and reduced modification time.

2.3.9 Reducing Respiratory Losses

Eyben and Droogenbroeck (1969) concluded that hydrostatic pressure during steeping inhibited germination and reduced malt yield by restricting GA synthesis. They also noted that exogenous GA does not stimulate respiration in grains subjected to hydrostatic pressure. With these observations in mind, Eyben and Droogenbroeck were able to reduce malting losses to between 2 and 6% by subjecting steeping barley at maximum pressure sensitivity to a hydrostatic pressure treatment of 490 KPa for 5 minutes. Following treatment 20 to 100 mg of GA per tonne was added at the beginning of germination.

2.4 THE INFLUENCE OF EXTERNAL PRESSURE ON SEED GERMINATION

2.4.1 Hydrostatic Pressure

Enhancement of seed germination by exposing seed to high hydrostatic pressures has proven beneficial with some species (Vidavar and Lue-Kim, 1967). Davies (1928) subjected both *Medicago sativa* and *Melilotus alba* to hydrostatic pressures of 50 MPa and 202 MPa and observed that shorter exposures (1 to 5 minutes) at 202 MPa gave higher total germinations following treatment than longer exposures (2 to

8 hours) at 50 MPa. Following treatment, air dried seed gave higher total germinations than control seed for up to 10 months. Exposures of 30 minutes to 202 MPa were injurious and all hydrostatic pressure treatments increased the permeability of the seed coat.

Using several species, Rivera et al. (1937) subjected seeds with hard impermeable coats to high hydrostatic pressures from 2 to 405 MPa at temperatures of 0, 25 and 50°C for durations of 1 to 20 minutes They observed that hydrostatic pressure treatments failed to significantly improve the germination performance of Medicago sativa and Melilotus alba samples which had a lower precentage of hard seeds. However, hard coated Cladrastis lutea seed subjected at 25°C to a hydrostatic pressure of 68 MPa for 1 to 20 minutes eventually produced 100% germination 24 days after treatment. Control Cladrastis lutea seed showed a complete absence of germination and other hydrostatic pressures were less beneficial or injurious. Microscopic examination of Cladrastis lutea seed following hydrostatic pressure treatment revealed the presence of a coat fracture which was most frequently observed in the region of the hilum.

Subjection of Medicago sativa and Melilotus alba to hydrostatic pressure treatments at 0°C improved germination performance less than treatments at 18°C (Davies, 1927). Final germination percentages of Ditremexa occidentalis which has a hard impermeable seed coat following hydrostatic

pressure treatments of 7, 17 and 69 MPa at 0°C were no different from controls. However, at 25 and 50°C the final germination percentages of Ditremexa occidentalis increased with greater hydrostatic pressure (Rivera et al. 1937).

Taylorson and Hendricks (1979) used dilute solutions of the anesthetics ethanol, methanol, chloroform, ethyl ether and acetone to overcome dormancy in Panicum capillare, Echinochloa crus-galli and Seraria faberi. They concluded that the anesthetics were not osmotically active nor involved metabolically, rather that their ability to stimulate germination involved their respective lipid solubilities. Ethanol has been reported to increase the hydraulic permeability of erythrocyte cell membranes (Seeman et al. 1970). Ethanol and other liquid anesthetics increase membrane permeability by expanding and fluidizing the membrane in addition to increasing the mobility of membrane water by melting membrane bound water of hydrophobic hydration (Kuiper, 1972). Conversely, ethanol has been reported to decrease the hydraulic permeability of Nitella membranes (Kiyasawa, 1975). Hendricks and Taylorson (1980) applied hydrostatic pressures of 0.1 to 4.0 MPa to imbibed Panicum capillare seeds immersed in solutions of either ethanol or ethyl ether for 5 hours to 3 days. They observed that the ability of these anesthetics to promote germination was linearly inhibited with increased pressure to a critical

pressure (approximately 800 KPa) beyond which further increases in pressure failed to induce greater inhibition. Thus they proposed that hydrostatic pressure treatment can either prevent or reverse membrane expansion caused by anesthetics. They also asserted that the increase in membrane volume is the result of a change in protein configuration at the anesthetic site of action and that in the absence of hydrostatic pressure the change of configuration may either activate a membrane bound protein into enzymic action or facilitate ion transport by opening a protein tunnel.

Johnson and Flagler (1950) reported that the narcotic effect of ethanol on tadpoles could be abolished by hydrostatic pressures between 13 and 34 MPa and that in the absence of narcotics the same increase in hydrostatic pressure itself induced narcosis. The increased solubility of N_2 in the plasma membrane of a diver at a depth of 91 m (approximately 1 MPa) is enough to induce severe nitrogen narcosis (Hoar, 1975). Hendericks and Taylorson (1980) used air to transmit hydrostatic pressures of 0.1 to 4.0 MPa to Panicum capillare seeds immersed in anesthetics and observed less than a 50% inhibition of germination before reaching the critical pressure (800 KPa). They concluded that increased O_2 solubility partially offset the pressure inhibition of anesthetic stimulation.

Vidaver and Lue-Kim (1967) measured the influence of hydrostatic pressure and O_2 concentration on Lactuca sativa seed germination both during and subsequent to treatment. They reported that with increased hydrostatic pressure the rate of germination accelerates more rapidly with higher O_2 concentrations up to a limiting concentration beyond which rate is independent of pressure provided hydrostatic pressure remains constant for the duration of germination. Further Vidaver and Lue-Kim observed that hydrostatic pressure reduced germination rate when O_2 concentration was limiting and that greater concentrations of O_2 increased the germination rate in both the presence and absence of hydrostatic pressure. With increasing O_2 concentration, germination took place up to a hydrostatic pressure of 48 MPa. They also noted that air saturated water (O_2 concentration $2x10^{-4}$ M) was able to support germination up to a hydrostatic pressure of 37 MPa.

Monitoring the influence of short hydrostatic pressure treatments (12 to 24 hours) on subsequent germination Vidaver and Lue-Kim reported that aerobically pressurized seed took longer to germinate than anaerobically pressurized seed following hydrostatic pressure treatment. In addition, the reduction in germination was greater with either increased duration and/or magnitude of hydrostatic pressure treatment.

2.4.2 Isotropic Stress

Seed germination can be inhibited by soil isotropic compressive stress (Collis-George and Hector, 1966; Collis-George and Williams, 1968; Shaykewich, 1973). The initial imbibition swelling pressure of seeds is in the order of 40 MPa and declines with water uptake to approximately 1 MPa at germination (Shaykewich, 1973). Collis-George and Williams (1968) employed the equivalency of matric potential and isotropic stress in saturated systems to identify the cause of increased germination inhibition with initial declines in matric potential. They determined that the increasing inhibition which occurred during an initial decline in matric potential from 0 to -32.9 KPa was due to increasing isotropic stress and not to a lowering of water free energy. Williams and Shaykewich (1970) used an unconfined compressive test with declining matric potential from -10 to -1000 KPa in evaluating the change in isotropic stress of four unsaturated soils of known cohesion and shearing angle. They determined that soils with a high degree of moisture retention display the greatest increases in isotropic stress and that matric potential contributes more to isotropic stress in soils of high moisture retention. Further, the proportion of matric potential that contributes to isotropic stress declines with falling potential.

Since seeds at germination exert a swelling pressure of approximately 1000 KPa, Shaykewich (1973) proposed that isotropic stresses in the order of 120 KPa inhibit

seed germination by preventing radicle penetration through the seed coat. Collis-George and Williams (1968) applied an external pressure to a saturated sand system and determined an effective isotropic stress of 32.9 KPa. Compressive pressures of this magnitude have been reported to severely inhibit root elongation in glass bead media (Goss, 1977). Axial growth pressures of 600 to 2500 KPa (Pfeffer, 1893, cited in Gill and Bolt, 1955) and 900 to 1300 KPa (Boone and Veen, 1982) are developed by elongating root tips. External pressures applied at some distance from the seed compresses zones of elastic and plastic compression around the seed. Consequently, the magnitude of the effective isotropic stress at the seed surface is greater than the applied isotropic stress exerted some distance from the seed (Collis-George and Williams, 1968).

2.5 THE EFFECT OF EXTERNAL PRESSURE ON ROOT GROWTH HABIT

2.5.1 Growth Pressure and Impedance

Confined root tips exert axial growth pressures of 600 to 2500 KPa (Pfeffer, 1893, as cited in Gill and Bolt, 1955; Taylor and Ratliff, 1969). Growth, that is, the irreversible plastic extention of cell walls, occurs when a cell's hydrostatic pressure exceeds the confining constraints of the cell wall, the surrounding tissue and the medium impedance (Taylor and Ratliff, 1969; Veen, 1982). Young growing

roots do not form local constrictions. Thus penetration is totally inhibited when the fixed pore diameter is less than the root diameter (Wiersum, 1957). The ability of young growing roots to overcome impedance depends on the availability of O_2 (Gill and Miller, 1956; Tackett and Pearson, 1964; Hopkins and Patrick, 1969) the magnitude of impedance, nutrient availability and the light intensity (Boone and Veen, 1982).

Tackett and Pearson (1964) reported on depth of root penetration in soils of differing bulk density. In low bulk density soil (1.3 and 1.5 g cm^{-3}) depth of root penetration was significantly inhibited at O_2 concentrations below 10% and in intermediate bulk density soils (1.6 to 1.7 g cm^{-3}) penetration depth was increased with greater O_2 concentration. However, in high bulk density soil (1.9 g cm^{-3}) root penetration was minimal and independent of increased O_2 concentration up to 20%. Similarly, Hopkins and Patrick (1969) observed little root penetration in three soils at either high levels of compaction or at low O_2 concentration. Using a glass bead medium Gill and Miller (1956) determined that the ability of corn roots to overcome a range of impedances from approximately 0 to 500 KPa was impaired by reductions in the medium's O_2 concentration from 20 to 1%. Goss (1977) applying moderate external pressures of 20 to 50 KPa to barley roots growing in

a glass bead medium with aerated nutrient solution was able to reduce root elongation by 50 and 80%. Using the same technique Veen (1982) impeded corn crown root elongation by 75% with an external pressure of 40 KPa.

2.5.2 Growth Habit and Impedance

Wiersum (1957) noticed that with increasing impedance lateral initiation took place closer to the root tip and that the root tip itself had a wrinkled surface. Gill and Miller (1956) concluded that the flattened and gnarled appearance of corn roots grown at approximately 140 KPa was due to the non-symmetrical pressure constraint of the glass bead medium used. In an air gap behind root tips confined in plaster of paris Taylor and Ratliff (1969) noticed slow root bulging and a higher frequency of lateral formation. Using glass beads to establish 70 and 160 μm pore systems, Goss, (1977) observed that the distance at which laterals (150 μm diameter) were erupting through the epidermis was dependent on axial (430 μm diameter) growth resistance and not pore diameter. In both the 70 and 160 μm pore systems, the distance behind the root tip at which laterals were initiated was 30 mm in the absence of external pressure, and 4 mm when the systems were subjected to an external pressure of 40 KPa. With an external pressure lateral development was only inhibited in the 70 μm pore system. In the 160 μm system pressure inhibition of axial growth resulted in laterals being

twice the length of laterals in the same system when not subjected to pressures. Shoot and root dry weight were unaffected by axial impedance in the glass bead system (Goss, 1977). Boone and Veen (1982) reported that in soil abnormally high root densities in zones of low impedance resulted in reduced shoot fresh weight due to exhaustion of potassium. Potassium was most limiting on shoot growth when phosphate was optimal.

2.5.3 The Influence of Impedance on Root Anatomy

Cellulose microfibril orientation at the root tip is random. The establishment of sub-apical elongation region is dependent on the microfibrils developing an orientation perpendicular to the root axis (Green, 1974). Veen (1982) observed that the predominant microfibril orientation of mechanically impeded corn roots is parallel to the root axis and in consequence is inhibiting to axial growth. Wilson et al. (1977) reported that the increased radial diameter of barley roots under an impedance of 20 KPa occurred because of an increase in the number of periclinal divisions of the epidermis, cortex and stele. This increase in radial diameter was also associated with enlarged epidermal and peripheral cortical cell volumes. A 15% increase in epidermal cell volumes beyond 2.5 mm from impeded root tips resulted from greater tangential expansion and reduced radial and longitudinal cell axes. The greater volumes of peripheral

cortical cells was due to radial expansion, the longitudinal axes being reduced. In addition, the endodermal and adjacent cortical cells had greater tangential axes and reduced longitudinal and radial axes. Generally the stele diameter of impeded barley roots was greater than that found in unimpeded roots even though the number of stele cells between 0.2 mm and 0.4 mm from root tips was less in impeded roots.

Impeded barley root axes in a glass bead system developed a waved growth habit of concave and convex surfaces. Goss and Russell (1980) observed that, whilst lateral initiation is more frequent on convex surfaces, root hair development is generally limited to concave root surfaces. They suggested that the convex surfaces were under tension whilst concave surfaces were under compression. The normal pattern of lateral root initiation is acropetal. Root bending is thought to cause wounding of cortical cells on the convex side of the bend. Ethylene produced in the wounded region could be responsible for the initiation and clumping of laterals on the convex side of the root bend (McCully, 1975).

2.5.4 Growth Pressure Verses Impedance, Magnitudes and Mechanisms

The extent of morphological modification imposed on growing roots by external pressures of less than 100 KPa appears to be out of proportion with axial root growth pressures of 900 to 1300 KPa (Goss, 1977; Veen, 1982). Modified

root growth and morphology by moderate external pressures cannot be explained by assuming these external pressures simply oppose the enlargement of vacuoles in elongating cells (Goss and Russell, 1980). Even though radial growth pressures may be in the order of 600 KPa, moderate external pressures of less than 100 KPa induce an increase in radial diameter. This observation demonstrates that external pressure is not influencing root growth habit by simply acting against cell growth pressure (Wilson et al. 1977; Veen, 1982).

Goss and Russell (1980) observed that in the absence of a rootcap, corn axes failed to change their rate of elongation on encountering trivial impedance. Intact axes showed a three to fourfold reduction in elongation for 10 minutes on encountering the same resistance. In addition, following a period of growth under impedance, barley roots took several days to establish the same growth rate of roots never grown under impedance. The post-constraint temporary reduction in cell elongation suggests that the growth of cells formed under impedance is permanently modified. Clowes (1975) noted the proposition that the quiescent centre persists due to pressure exerted by the growth of surrounding cells. In corn the restraint exerted on the quiescent centre by the rootcap is not balanced by the restraint exerted on the centre by the cortex and stele. This unbalanced constraint results in the quiescent centre being hemispherical in shape with the root cap abuting the plane surface whilst

the cortex and stele abut the spherical surface. In normal growth the quiescent centre contributes to the cortex and stele but not to the rootcap.

Lateral initiation closer to the root tip accompanied by increased radial diameter can be induced by ethylene and IAA treatments in the absence of impedance (Goss and Russell, 1980). The tendency for laterals to be closely associated is possibly caused by ethylene production from wounded tissue. This wounding may result from primordium disruption of the pericycle and cortex (McCully, 1975). Integrity of cell membranes is essential, however, if plant tissue is to synthesize ethylene (Satoh and Esashi, 1980). Whereas, under increasing mechanical resistance the number of laterals per main root axis declines in soils, in artificial media the number increases provided lateral extension is not inhibited by pore size (Boone and Veen, 1982). McCully (1975) noted that the apical meristem of main roots inhibits lateral initiation for a distance that varies with apical growth rate and that an inhibitor released by the root apex either inhibits tissue maturation or primordium development. Further, the distance at which laterals develop behind the tip is noticeably shorter in aquatic angiosperms and pteridiophytes and local applications of nitrate stimulate lateral development in that region only. Finally, where laterals are clumped their lengths tend to be increased.

Satoh and Esashi (1980) were able to stimulate ethylene production from cotyledons by exogenous application of D-amino acids. L-amino acids failed to stimulate ethylene synthesis. The most probable precursor of ethylene is L-methionine (Abeles, 1973) yet endogenous L-methionine failed to stimulate ethylene synthesis in cotyledons. It appears, therefore, that exogenous D-methionine which stimulates ethylene synthesis is not converted to L-methionine by cotylendon tissue (Satoh and Esashi, 1980). Satoh and Esashi proposed that a stereospecific receptor site in the cell membrane changes conformation on binding D-amino acid and that the conformational change stimulates ethylene synthesis. Increased impedance could bring about a conformation change at a receptor site. Membrane protein conformations are dependent on hydrophobic interactions which are particularly sensitive to pressure (Hochachka and Somero, 1973).

The evolution of ethylene from mechanically impeded pea epicotyls was found to be proportional to the magnitude of constraint. The ethylene induced a reduction of internodal elongation and an increase in radial diameter (Goesch et al. 1966). Evolution of ethylene from bean roots increased sixfold one hour after encountering impedance and declined twenty to thirty hours after the initiation of impedance (Kays et al. 1974).

Veen (1982) observed a 75% reduction in the length of corn roots grown in a glass bead medium with an externally

applied impedance of 40 KPa. Soil with penetrometer resistance between 0.9 and 1.6 MPa reduced corn root elongation by 50% (Boone and Veen, 1982). Goss (1977) stated that the external pressure applied to the flexible sides of a glass bead confinement chamber is the same as that experienced by a root tip growing between the glass beads. Gill and Miller (1956) concluded, that the pressure applied to a flexible diaphragm impinging on glass beads is less than the actual pressure confining root growth because of arching. Arching describes the displacement of glass beads caused by root growth. For one bead to be displaced in the fixed system many other beads in the system have to be displaced. Using a penetrometer in a glass bead system under constant pressure of 490 KPa, Barley (1963) measured a 600 KPa increase in resistance as depth increased from 1 to 7 cm. Soil penetrometer resistance is generally four times as great as soil impedance experienced by growing roots (Boone and Veen, 1982). The penetrometer penetrates the soil at a much faster rate than a root and does not take the path of least resistance. In addition, the penetrometer does not decrease soil tensile strength through radial expansion. Decreased tensile strength reduces the axial pressure required for elongation. However, the magnitude of mechanical impedance required to induce comparable modifications in root growth behaviour is still approximately forty times greater in the soil than in a glass bead system (Boone and Veen, 1982).

2.5.5 Torturosity of Root Gas Diffusion

Even in the absence of ethylene and mechanical constraint, the O_2 limited root growth of oats in waterlogged soil is preferentially radial (Blackwell and Wells, 1983). Boone and Veen (1982) suggested that in a glass bead system under moderate external pressure reduced O_2 availability is responsible for the modification in root morphology and growth habit. The root cortex of pea (Greenwood, 1967, Armstrong, 1983, Armstrong *et al.* 1983), rice and barley (Barber *et al.* 1962) as well as corn (Saglio, 1983) allow non-tortuous diffusion of O_2 in the gas phase. Barber *et al.* (1962) measured the change in $^{15}O_2$ activity of barley and rice roots 15 cm from the grain when grown in aerated water. These measurements were taken during and after initial exposure of the shoots to $^{15}O_2$. Whilst it was possible to measure the rate of appearance and disappearance of activity in rice, it was only possible to measure the disappearance of activity in barley. The rate of change in activity was found to be dependent on the cross-sectional area of root gas spaces. Whereas, gas space in rice varied between 5 and 30%, in barley it was below 1%. Barber *et al.* concluded that the degree of gas space development in rice is an adaptation for waterlogged conditions, and that unlike rice, the rate of O_2 diffusion through the continuous gas spaces of the barley root

cortex would be inadequate to sustain the respiratory requirement of the meristem.

Bryant (1934) observed that when compared to barley roots grown in aerated Hoaglands solution, roots grown in non-aerated solution showed a 15% increase in diameter, aerenchyma formation and lateral initiation closer to the root tip. Ethylene rather than O_2 starvation has been reported to cause lysigenous arenchyma formation in poorly oxygenated corn roots (Campbell and Drew, 1983). Unimpeded corn root cortical gas space is essential in maintaining the cell charge in root tissue close to the grain. Nevertheless, unimpeded corn root gas space inadequately ventilates the root meristem to sustain growth. In wheat Burstrom (1959) observed that the greater cross-sectional area of gas spaces coupled with radial growth rather than axial is related to the flooding of gas filled root intercellularies. Moreover, there is no significant difference between the respiratory activity of the apices of flooded and non-flooded roots and that the flooding of intercellularies does not induce radial growth in preference to axial growth in an O_2 atmosphere. Burstrom also observed that intercellulary flooding can be induced by auxin inhibition of elongation, calcium deficiency and by squeezing gas out of excised root. Woolley (1983) noted that roots cultured in nutrient solutions maintain intercellular air spaces which under anoxic conditions

immediately flood to give the roots a translucent water soaked appearance.

With increasing root length diffusion path resistance for plant aeration does not increase in linear fashion. Respiratory demand, radial leakage and the development of laterals result in an inability to detect O_2 in the apices of anoxically grown pea roots greater than 7.5 cm in length (Armstrong et al. 1983). The root apex has the maximum respiration rate and the highest O_2 permeability of all root tissue. With increasing root length the apex becomes dependent on the growth medium for O_2 to sustain respiration (Luxmore et al. 1970). Burstrom (1959) in observing that potassium hydroxide flooded the root apical intercellularies of culture grown wheat roots concluded that they contain almost pure CO_2. Further, Burstrom proposed that low apical CO_2 permeability in the absence of auxin and calcium deficiency is responsible for an apical intercellularly CO_2 pressure exceeding 101 KPa. The excess pressure facilitates the formation of continuous gaseous intercellularies. Once intercellularies are flooded the condition is permanent even in an O_2 atmosphere. New intercellularies flood on formation and are larger in dimension than gas intercellularies. The flooding of intercellularies is followed by increased radial growth and a decline in axial elongation.

Fedeel (1964) using a pH electrode investigated the CO_2 tension of natural and chlorophyllous roots grown respectively in aerated nutrient solution and culture medium. He determined that natural root CO_2 concentration (grown in aerated nutrient solution) is greater at the end zone of elongation and in the zone of maturation than at the root tip. Further, he reported that the CO_2 concentration of culture-grown roots is twice that of roots grown in aerated solution and that the epidermis offers considerable resistance to CO_2 diffusion. Culture-grown roots were observed to have greater diameters, larger intercellular spaces and greater cortical cell volumes.

2.5.6 Non-wettable Gas Spaces and Pressure

Woolley (1983) proposed that the high solubility of CO_2 rendered unlikely the maintenance of gaseous intercellularies by CO_2 pressure. Sorokin (1966) on examining oat coleoptiles suggested that two distinct types of intercellularies exist. The first type consisting of water lined air passages continuous with the external atmosphere. The second forms a discontinuous system lined with a plastic lipid membrane which allows the diffusion of CO_2 but not O_2 and N_2. Woolley (1983), after observing that water was unable to penetrate into corn root systems in the absence of pressure to a depth greater than one cell, concluded that the intercellularies of living tissues are lined with a hydrophobic layer. It was noted, however, that oils

with lower viscosity than water failed to penetrate such intercellularies. Sorokin (1966) proposed that when the plastic lipid layer is cut during sectioning it immediately seals off the intercellulary and thereby prevents the escape of the gas. Roland (1978) reported that the formation of intercellular gas spaces in immature tissue of stem and root apices is initiated by the medium deposition of a splitting layer in the middle lamella. The splitting layer is resistant to pectinase and cellulase activity but has distinct phosphatase activity. Immediately following splitting layer depostion cell walls move apart by a precise autolytic process involving no swelling of the middle lamella. The intramural cavity formed is lined continuously with a thin (10 to 20 μm) lipophilic film derived from the splitting layer. This film constitutes a barrier to the aqueous phase. The intramural cavities in immature tissue contain mostly CO_2 and function primarily in CO_2 export. These intramural cavities are formed in continuity with, and eventually contribute to, an irregular gaseous system by anastomosis involving cell wall modification, tearing and disintegration. The mechanism by which apical intramural cavities are formed is distinct from that mechanism involving lamella swelling and the subsequent wall fission by which hydrophilic cavities are formed in other tissues.

Increasing the external pressure applied to roots not only compresses the gas phase but also increases the solubility of the gas phase. The increased solubility of the

gas phase does not influence the degree by which root density increases to the same extent as gas compressibility (Carstensen et al. 1981). The high concentration of CO_2 in the apical intercellular spaces cannot be achieved by independent diffusion. The permeability of the cavity membrane must be influenced by some mechanism which prevents the establishment of an equilibrium solution in cell wall free space (Sorokin, 1966). Under increased pressure the viscosity of lipids increases and this may limit reactions which depend on diffusion (Hochachka and Somero, 1973). However Berezin et al. (1974) using a protein-protein interation (enzyme system) supported in a polyacrylamide gel as a membrane model reported that the rate of interaction initially increases with gel concentration up to a critical concentration. Beyond this the rate of interaction falls to zero. Above the critical concentration mechanical compression (101 KPa) of the gel causes an increase in the rate of interaction proportional to the degree of compression. This rate accelerating effect of compresssion is reversible. Berezin et al. suggested that as the gel concentration nears the critical concentration, the size of the gel pores approaches that of the diffusing protein globule and that the rate of reaction depends on the number of pores with dimensions greater than that of the globule. The number of pores above the critical dimension falls to zero at the critical concentration. Upon compression the dimensions of the incompressible/constant volume gel

alter, such that the area of its surface facet increases whilst its cross-sectional area declines. Berezin et al. proposed that the change of dimension increases the number of gel pores above the critical dimension of the diffusion protein globule and thereby allows diffusion to recontinue. It is conceivable that such a system could chemically amplify weak mechanical stimulation (Berezin et al. 1974). Deformation of leaf cells by bending or rubbing and in the absence of wounding has been reported to double the respiration rate of cherry laurel leaves (Andus, 1935).

Woolley (1983) reported that anoxia and cell death cause the lipophilic apical cavities to become flooded with water. This suggests that the living protoplast must exert an influence on the orientation of molecules in the lipophilic layer. However, this would be inconsistent with the theory of cell wall free space. Kuiper (1972) noted that in roots, membrane hydrostatic permeability has been reported to increase with hydrostatic pressure up to several atmospheres. The decrease in membrane hydrophobicity of root cell membranes which accompanies increased hydrostatic pressure may result from an increase in the exposure of hydrophilic groups. This increased exposure is brought about by conformational changes in lipoprotein and the polarization of ice-like membrane water.

Barber and Gunn (1974) observed that as pore size decreases in a non-rigid glass bead system the total exuda-

tion of amino acids and carbohydrates exuded by barley and corn roots increases. Coster et al. (1977), after noting that the plasma membrane often appears to be mechanically coupled to the cell wall, proposed that strains induced in the cell wall are transmitted to the membrane. These strains could result in a reduction in the thickness of the membrane. Long-term strains are probably accommodated by the introduction of new molecules into the membrane which reinstates the membranes original properties and possibly alleviates the strain effect. The addition of new membrane molecules may not occur however during short-term strains and consequently permanent modification of the membrane could occur.

CHAPTER III

GENERAL MATERIALS AND METHODS

3.1 BIOLOGICAL MATERIALS

3.1.1 Grain Varieties

The principal grains used in this study were Hordeum vulgare var. Argyle and Hordeum vulgare var. Bonanza. Argyle and Bonanza are 6 row blue aleurone barleys. Bonanza is a recommended malting variety grown throughout the Canadian Prairies. Argyle is still undergoing plant scale malting and brewing tests.

The bulk samples of Argyle[6] and Bonanza[7] were sieved[8] into grain fractions 5^+, 6^+, 7^+ and 8^+ (Table 1). The predominant grain size (width) fraction in both varieties was the 6^+ ($\geq$ 2.4 <2.8 mm) fraction. This fraction of both barley varieties was used for all experiments.

---

[6]Canada Grade Registered No. 2
Crop Cert. No. 82-6433879-22
Seed Sealing Cert. No./Lot No. 4-51185

[7]Canada Grade Certified No. 1
Crop Cert. No. 80-7504005-47
Seed Sealing Cert. No./Lot No. 80-M114

[8]Sieves manufactured by Cuthbert Co. Ltd., Winnipeg.

TABLE 1. Weight[1] distribution by grain size of 1 kg samples[2].

Variety	Size Fraction	Sieve Slot Width[3]	Weight of Fraction[1]	% Weight[1]	One Thousand Grain Weights		% Moisture[1]	Grain Density[5]
Bonanza	8+	3.2	6.22	0.62				
Bonanza	7+	2.8	190.00	19.01				
Bonanza	6+	2.4	722.50	72.27	37.47[1]	33.66[4]	10.16	1.28
Bonanza	5+	2.0	81.00	8.10				
Argyle	8+	3.2	0.77	0.08				
Argyle	7+	2.8	91.50	9.16				
Argyle	6+	2.4	846.00	84.66	38.14[1]	34.21[4]	10.30	1.30
Argyle	5+	2.0	61.00	6.10				

[1]Fresh weight (grams).
[2]Samples contained <1g of chaff.
[3]Widths are expressed in millimeters.
[4]Dry weight (grams).
[5]gcm^{-3}.

The two other grains used in the study were Hordeum vulgare var. Scout and Triticum aestivum var. Columbus. Scout is a 2-row yellow aleurone hulless feed barley. Columbus is a Red Spring wheat of bread making quality.

3.2 APPARATUS AND PROCEDURES

3.2.1 Steeping

Prior to steeping, grain was stored in opaque plastic bins at 5°C. The simple steeping apparatus consisted of a clear plastic cylinder (8.5 x 37 cm), closed at one end and immersed (to a depth of 30 cm) in a water bath (20 litres) at 15°C. Steeping of 170 g grain samples in 1.25 L of water was uninterrupted. Cooled compressed (approximately 196 KPa) air, during steeping, continuously entered (67 cm^3 sec^{-1}) the steep via an air port in the submerged closed end. With the exception of steeps where grains received a preliminary surface sterilization, steep water was changed every 10 hours. Grain surface sterilization involved an initial 2 hour steep in water followed by 2 hours of steeping in 1% sodium hypochlorite. The grain was then washed in running water (15 to 16°C) for half an hour before being finally returned to a water steep. Steep water was subsequently changed every 10 hours.

3.2.2 Pressure Treatments

A Torbal model AJ-2 vacuum chamber (Torsion Balance CO, Clifton N.J.) was modified to withstand internal pressure by removing the O-ring and placing a sheet of rubber (1.3 mm thick) between the lid and lip of the (3.26 L) chamber. In addition to the standard lid/chamber clamp, four small G-clamps were used to ensure a pressure tight seal.

An inlet valve in the chamber lid was attached to an air line, the internal pressure of which was controlled by a regulator. A separate outlet valve in the chamber lid was used to evacuate the chamber to atmospheric pressure after treatments. Pressure readings during treatments were taken from pressure gauges built into the chamber lid and on the air line between the lid and regulator.

Hydrostatic pressure treatments were achieved by immersing each grain sample in water contained within a 250 ml beaker. The beaker was then placed inside the pressure chamber. Compressed air was used to mimic hydrostatic pressures of 96.5, 144.7 and 193.0 Kpa.

Due to the nature of the apparatus it was not possible to apply or release pressure instantaneously. Compression and decompression times for treatment magnitudes are given in Table 2.

3.2.3 Germination Environments

Following steeping, treated and control grain samples were set to continue growth in petri-dishes or modified

TABLE 2. Compression and decompression times for treatment pressures.

Hydrostatic Pressure Magnitude[1]	Treatment Duration[2]	Approximate Chamber Volume[3]	Compression Time[2]	Decompression Time[2]
193.0	60	2.8	9	10
193.0	30	2.8	9	10
193.0	20	0.3[4]	3	3
193.0	10	0.3	3	3
144.7	60	2.8	8	9
144.7	30	2.8	8	9
144.7	20	0.3	2.5	2.5
144.7	10	0.3	2.5	2.5
96.5	60	2.8	6.6	8
96.5	30	2.8	6.6	8
96.5	20	0.3	$\leq$2.5	$\leq$2.5
96.5	10	0.3	$\leq$2.5	$\leq$2.5

[1]Hydrostatic pressures were measured in PSI and converted to KPa.
[2]Times are expressed in seconds.
[3]From the total chamber volume of 3.26 litres, the estimated water, beaker and where appropriate ballast volume have been subtracted.
[4]Chamber volume was reduced by approximately 90%, using a gravel and water ballast, for treatment durations of 10 and 20 seconds.

germination boxes. Each petri-dish (9 cm Fisher brand) had 2 filter papers (Whatman No. 1, or in the case of Plates 1 to 6 Whatman 29) placed in its base. The water retentive properties of Whatman 1 and 29 paper types has been reported to produce the same germination energy and water-sensitivity test results for grain samples (O'Farrell 1983). Usually 20 grains were placed in each petri-dish, however in one experiment (reported Section 5.6) 50 grains were placed in each dish. Although water-sensitivity has been noted to be influenced by grain orientation (Hough et al. 1971), grain orientation in all experiments reported in this study was random. Distilled water was placed in each dish, the volume in each case being determined by the experimental objective. All petri-dishes were finally sealed with parafilm before being placed in a dark germination cabinet at 15°C.

Germination boxes (11.5 cm square and 3.5 cm deep) were modified in an attempt to more closely reproduce a malting floor germination environment. Five corks were glued upright to each box base in such a fashion that they supported a 11 x 11 cm piece of wire mesh (2 x 1 mm window screen) placed in each box. The mesh was held in place on the corks by thumb tacks. In all experiments where germination boxes were used, 80 ml of distilled water was placed in each box bringing the water level approximately half way up the height of the corks. Grains (100) were placed on top of the wire mesh where they could continue to germinate at 100% RH

without being in contact with a free film of water. On the internal surface of each box lid a moist 11 cm diameter Whatman No. 3 filter paper was placed. This reduced the possibility that water drops caused by small temperature fluctuations, might form on the internal surface of the lid and fall on the grain lying on the mesh below. Finally all boxes were sealed with parafilm and placed in dark germination cabinet at either 15 or 25°C.

3.3 STATISTICAL ANALYSIS

3.3.1 Method

Data was analysed using the Statistical Analysis System (SAS) Package and the University of Manitoba Amdahl computer.

3.3.2 Analysis

Analysis of variance was conducted on experiments (completely randomized designs) testing for significance between treatments (Hydrostatic pressure or no pressure) and for interactions, pressure magnitude, pressure duration, germination water volume, germination temperature steeping time, germination time and O_2 consumption time.

CHAPTER IV

HYDROSTATIC PRESSURE INHIBITION

4.1 Introduction

The results of three experiments are presented in this chapter. The first experiment investigated the suitability of the chosen steeping technique. The second experiment compared the effect of air pressure and hydrostatic pressure on grain chitting and root growth. The third and final experiment involved an attempt to determine the influence of hydrostatic pressure magnitude and duration on chitting and root growth.

4.1.1 Steeping Technique

The objective of this first experiment was to evaluate the performance of the simple steeping apparatus. For satisfactory modification steeping should end with a grain moisture content of 43 to 46% w.wt. Grain size, protein content, initial moisture and steeping technique all influence the rate at which steep-out moisture is attained. Steeping curves for two barley varieties were obtained in order to determine the optimum steep duration and appropriate pressure treatment time.

4.1.2 Materials and Methods

One hundred and seventy gram samples of Argyle and Bonanza were continuously steeped with aeration at 15°C for 50 hours. Steep water was changed every 10 hours. Mean percent moisture on a wet weight basis was determined during steeping from replicate grain samples. Four replicates each containing 50 grains were taken from the steep at each sampling time.

4.1.3 Results and Discussion

Steeping curves for Argyle and Bonanza are shown in Figure 1. The general form of the curves was similar for both varieties. Water uptake in the aerated steep appeared to be comprised of two distinct periods. The first period (approximately 0 to 10 hours) was characterized by rapid water uptake (imbibition) to moisture contents of 33 to 37%. During the second period (approximately 10 steeping hours onward) water uptake continued at a slower linear rate to a moisture content of approximately 45% at 50 steeping hours. Steep-out moistures for Argyle and Bonanza were achieved after 38 and 28 steeping hours respectively.

Traditionally steeped barley is considered to display a tri-phasic pattern of water uptake (Brookes *et al.* 1976). Other seeds e.g., *Allium cepa*, *Daucus carota* germinated in petri-dishes also show the tri-phasic pattern (Bewley and Black, 1978). Brookes *et al.* (1976) noted that

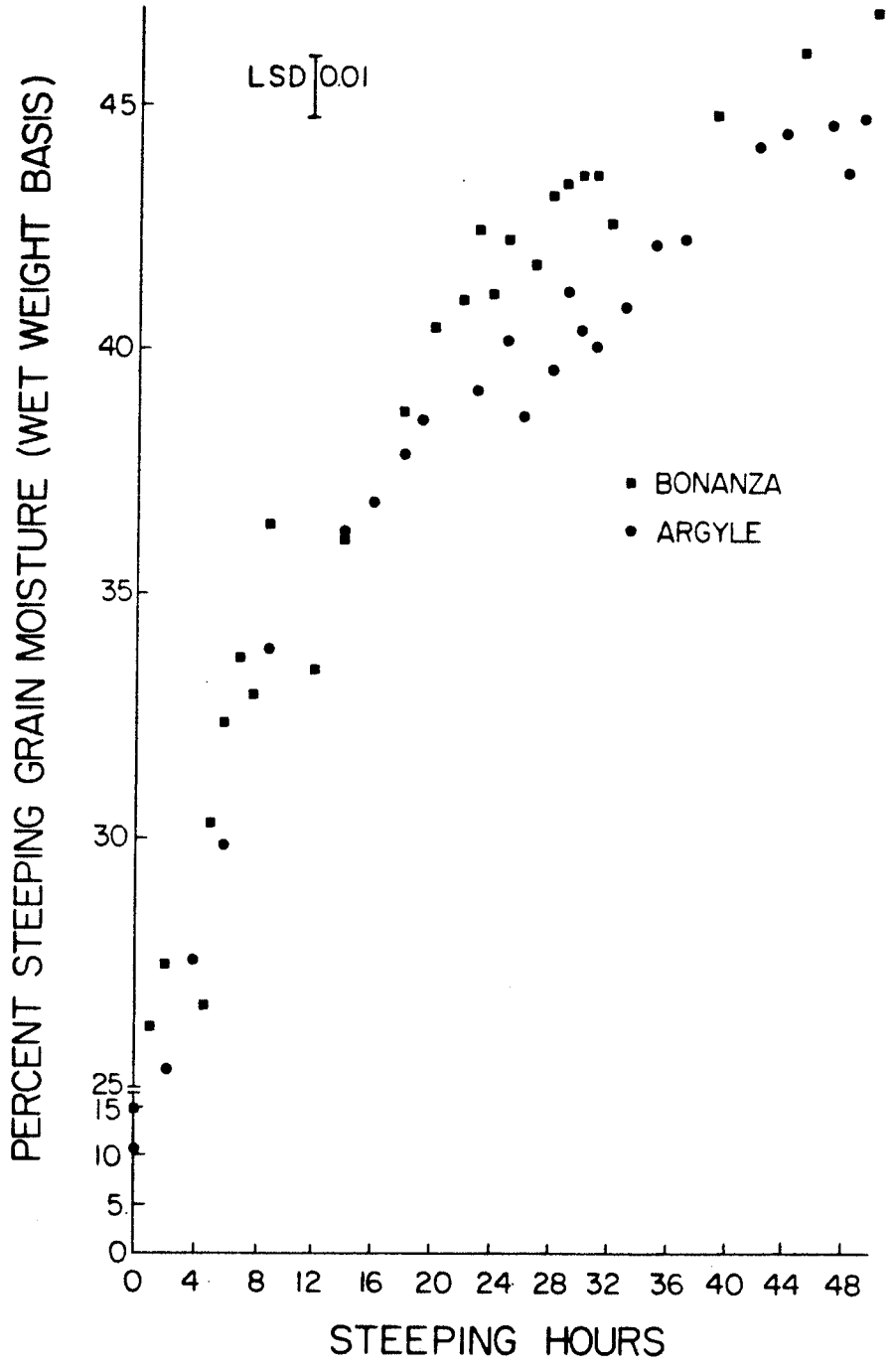


Figure 1. Steeping moisture curves for Argyle and Bonanza. $LSD_{0.01}$ 1.29%

Phase I (imbibition) is a physical process which may last between 6 and 10 hours, during which time grains may attain moisture contents of approximately 32%. Within 6 to 8 hours from the initiation of steeping, both Argyle and Bonanza reached moisture contents that would be associated with the completion of Phase I. In the tri-phasic pattern of water uptake, Phase II (which may last 10 to 12 hours) involves a cessation or slowing down of water uptake. In the simple steeping apparatus the rapid initial period of water uptake which characterized the first 6 to 8 hours was followed by a second period of continuous water uptake at a slower rate. During the period of steeping time where phase II might be expected to have occurred, Argyle and Bonanza reached moisture contents in the range of 37 to 42%. Such moisture contents are usually associated in the tri-phasic pattern with Phase III active water uptake. It appeared that in the aerated steeping apparatus water uptake was continuous and that either Phase II was absent or compressed to a much shorter duration.

The initiation of Phase III has been associated with visible signs of germination (testa/pericarp rupture) (Bewley and Black, 1978). The rupture of the testa coincides with a rise in grain aerobic activity (RQ declines to near 1) following a respiratory lag which is thought to be imposed by the lower O_2 permeability of the intact hydrated testa/pericarp. In the simple steeping apparatus a small fraction

of the barley grains could be seen to have swollen coleorhizas at 22 hours. At 28 hours more than half the grains had swollen coleorhizas and at 44 hours a similar proportion of grains reached the state where their radicles had erupted through the coleorhiza.

During Phase II metabolic events are proposed to occur which are prerequisites for the initiation of Phase III (active water uptake) and visible germination (Bewley and Black, 1978). The steeping curves suggested that Phase II is merely the consequence of anoxia and/or narcosis and that the continuous turbulent aeration of the steep supported active water uptake following imbibition and prior to testa/pericarp rupture. Water associated with the coats of traditionally steeped grain or seeds in petri-dishes is probably rapidly deoxygenated and relatively immobile limiting gas movement to diffusion. Water bound to the testa, pericarp and husk of grain in the aerated steep was likely to be mobile or in contact with mobile water (due to turbulence) allowing gas movement by mass flow. In addition, steep water may have been near saturation for the partial pressure of O_2 in the air stream. Barton and McNab (1956), cited in Hegarty (1978) reported that oxygenation caused excessive water uptake by Phaseolus vulgaris seeds. The possibility that restricted O_2 permeability is responsible for Phase II could perhaps be determined by monitoring the moisture uptake of grains germinating in dilute H_2O_2.

The moisture points for both varieties varied with increased steeping time such that grain moisture appeared to

decline in some instances. Brookes et al. (1976) noted that 3% of a steeping grain's moisture is associated with the husk surface. Before determining wet weights grains were blotted between paper towels to remove excess surface water. Variation in the distribution of grains between towels during blotting as well as varying blotting pressure and duration may have been responsible for some of the extraneous declines in grain moisture with increasing steeping time. However, irrespective of this source of error, Argyle showed a significantly ($p = 0.05$) slower rate of water uptake than Bonanza.

The difference in water uptake between the two varieties could be attributed to their different grain sizes. Although 6^+ (2.4 mm fraction) grain of both varieties was used for steeping, the proportion of small 6^+ grains tended to be greater in Argyle 6^+ fractions than Bonanza 6^+ fractions. This difference between the various proportions of grains of different sizes within a single general size fraction was not reflected in the thousand corn (grain) weight (Table 1) of Argyle (38.14 g) and Bonanza (37.47 g). Grains of Argyle were however, determined to be denser than those of Bonanza (Table 1). Thus the slower water uptake of Argyle was probably the consequence of greater endosperm density and/or tighter less elongated grain husks. Brookes et al. (1976) noted that large grains initially absorb water at a faster rate than small grains and that small grains only have a percent moisture greater than that of large grains after prolonged steeping (88 hours). Grain size therefore appeared to be the determinant factor governing the rate of

water uptake. Ehrich and Kneip (1931) cited in Brookes et al. (1976) reported that barley samples with the lowest nitrogen (N) content absorb water faster and malt sooner than grains with higher contents. The percent protein values for steeped Argyle and Bonanza were 11.8 and 13.1 respectively[9]. The lower nitrogen content of Argyle may have offset some of the difference in water uptake rate between the two varieties determined by grain size alone.

4.2 AIR PRESSURE VERSUS HYDROSTATIC PRESSURE

4.2.1 Introduction

Davidson and Jangaard (1978) reported on the deleterious effects of hydrostatic pressure at steep-out. Because of this it was considered reasonable to attempt first to measure a hydrostatic pressure effect on grain steeped to a moisture content of 43 to 46%. Previous results (Section 4.1.3) indicated that Bonanza steeped for 28 hours would have a moisture content within this range. For comparative purposes an attempt at measuring an air pressure effect was also made. Although Eyben and Droogenbroeck (1969) had been unable to detect any deleterious effects of air pressures at 294 KPa, they had detected a slight depresssion of respiratory activity at higher pressures. Gomez (1971) reported depressions in respiratory activity induced by gas pressures of 98 KPa. In addition, he further noted that these depressions were greater for grains pressurized under O_2 than N_2.

---

[9]Determined by Kjeldahl technique and expressed as a percentage of dry weight.

4.2.2 Materials and Methods

Bonanza grain was steeped, with continuous aeration for 28 hours at 15°C. Samples were then subjected to either an air or hydrostatic pressure treatment of 193 KPa for 60 seconds. From each treatment sample six petri-dishes, each containing 20 grains, 2 Whatman No. 1 filter papers and 4 ml of water were set up. Six control dishes were also set up in a similar manner. All dishes were then placed in a germination cabinet at 15°C to allow growth to continue. Seventy-two hours after treatment (100 total hours) the number of chitted[10] grains and the total root dry weight of each treatment and control replicate was determined.

4.2.3 Result and Discussion

The results of the effect of air pressure and hydrostatic pressure on 100 hour root dry weight and chitted grain number are presented in Plate 1 and Table 3. Plate 1 clearly depicts the inhibitory influence of hydrostatic pressure on grain chitting and root growth. Close observation of control grain (Plate 1) revealed that coleoptile extension under the husk in many instances had reached the full length of the grain. It is an approximate rule of thumb that when the coleoptile has extended the full length of the grain, modification of the endosperm is complete (Hough *et al.* (1971). In contrast, the coleoptiles of grains subjected to hydrostatic pressure were not as extended even in those

---

[10]Chitted is used throughout this thesis to describe grains where the radicle has emerged through the coleorhiza.

NOTICE/AVIS

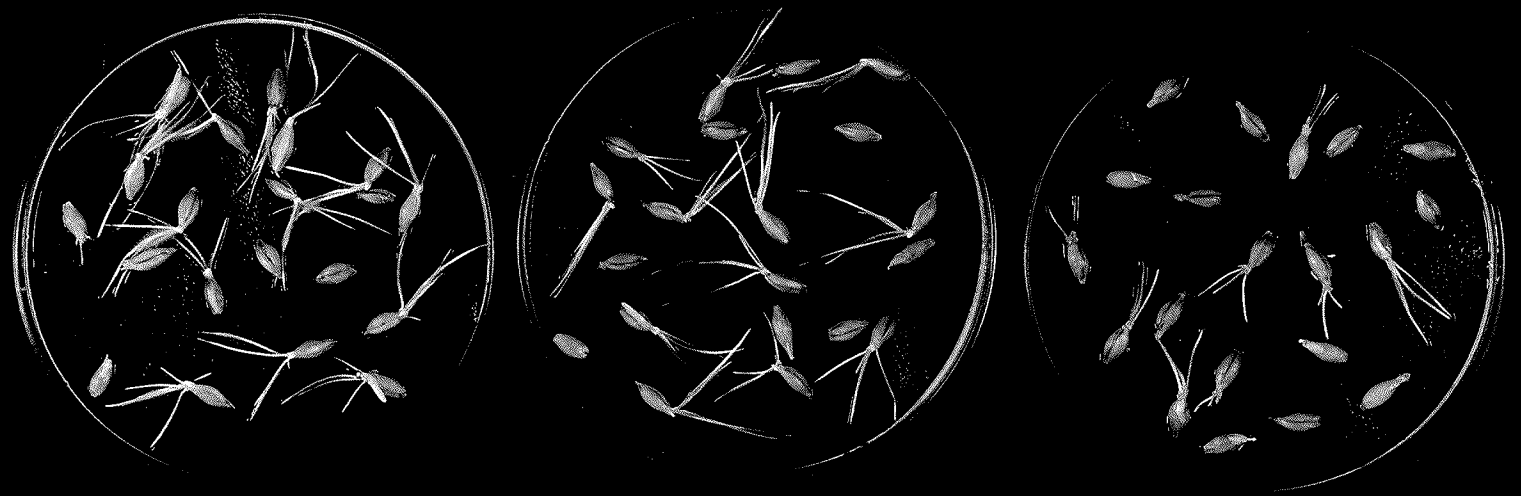
~~PAGE(S)~~ plates 1-34 ~~IS~~/ARE ~~EST~~/SONT black + white photos

PLEASE WRITE TO THE AUTHOR FOR INFORMATION, OR CONSULT THE ARCHIVAL COPY HELD IN THE DEPARTMENT OF ARCHIVES AND SPECIAL COLLECTIONS, ELIZABETH DAFOE LIBRARY, UNIVERSITY OF MANITOBA, WINNIPEG, MANITOBA, CANADA, R3T 2N2.

VEUILLEZ ECRIRE A L'AUTEUR POUR LES RENSEIGNEMENTS OU VEUILLEZ CONSULTER L'EXEMPLAIRE DONT POSSEDE LE DEPARTEMENT DES ARCHIVES ET DES COLLECTIONS SPECIALES, BIBLIOTHEQUE ELIZABETH DAFOE, UNIVERSITE DU MANITOBA, WINNIPEG, MANITOBA, CANADA, R3T 2N2.

Plate 1. The effect of hydrostatic pressure on root growth. Grains were steeped for 28 hours prior to treatment (193 KPa for 60 seconds). The grains were then allowed to continue growth in petri-dishes containing 4 ml of water. Grains are shown 72 hours after treatment (100 total hours).

The Effect Of Hydrostatic Pressure On Root Growth



control

air pressure

hydrostatic pressure

Hordeum vulgare cv. Bonanza

Plate 1

TABLE 3. The effect of hydrostatic pressure and air pressure on the 100 hour root dry weight and chitted grain number of Bonanza steeped for 28 hours at 15°C.

Treatment	Mean Total Root Dry Weight[1]	Mean Root Dry Weight Per Chitted Grain	Mean Number Chitted Grain
(Control)	24.67 a[2]	1.33 a	18.17 a
Air Pressure[3]	16.40 b	1.33 a	12.83 b
Hydrostatic Pressure	3.98 c	0.53 b	7.50 c
CV	12.14	13.44	12.27

[1]Root dry weights are expressed in milligrams and corrected to two decimal places.

[2]Parameters values within a column followed by the same alphabetic subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).

[3]Pressure treatments were conducted at a magnitude of 193 KPa for a duration of 60 seconds.

grains having root development. Plate 1 also depicts the observed inhibitory effect of air pressure on grain chitting. Those grains which chitted following subjection to air pressure did not appear to have retarded seminal root growth or inhibition of coleoptile extention.

Table 3 re-enforces cursory observation of Plate 1. Mean total root dry weights and mean number of chitted grains for each treatment were significantly ($p = 0.01$) different. However, whilst root dry weight per chitted grain following hydrostatic pressure was significantly less than control grains, dry weight per chitted grain following air pressure was not. Apparently air pressure only inhibited grain chitting and not root growth. In comparison, hydrostatic pressure appeared to inhibit both chitting and root growth. The significant reduction in chitted grain weight following hydrostatic pressure may have simply resulted from delayed chitting. However, mean root dry weight per chitted grain following air pressure was not significantly different from that of control grains. Therefore hydrostatic pressure inhibition of root growth, rather than delayed chitting, appeared more likely to be responsible for reduced root growth.

The inhibitory effect on grain chitting of the 193 KPa 60 second air pressure treatment was unexpected since Eyben and Droogenbroeck (1969) were unable to detect any visible effect on germination with a treatment of 294 KPa.

They did not, however, report the duration of their air pressure treatment. Gomez (1971) found that gas pressures of 98 KPa for 15 minutes restricted the respiratory activity of steeped grain, and that the restriction was greater for grains pressure treated under air than N_2. He also observed that the restriction of respiratory activity was temporary, and that it was followed by a rapid acceleration of respiratory activity to levels above that of control grains. This heightened level of respiratory activity was greater for grains treated under air than N_2 and in both cases culminated in treated grains germinating more rapidly than controls.

As air pressure increases the greater partial pressures of O_2 and N_2 results in increased solubility of these gases. At a pressure of 101 KPa in an O_2 atmosphere the level of dissolved O_2 in mammalian tissue becomes toxic in less than half an hour (Hoar, 1975). Increased tissue activity reduces the time for the toxicity to take effect. In plants different divisions and tissue types display varying degrees of tolerance to O_2 toxicity. Angiosperms and tissue with high rates of metabolism are generally the least tolerant.

The mechanism of O_2 toxicity is not clealy understood. However toxic levels of O_2 are thought to block metabolic pathways associated with oxidative phosphorylation (Hoar, 1975).

Nitrogen toxicity is the primary cause of narcosis in mammalian tissue respiring under compressed air. Nitrogen dissolves slowly and requires an hour or more to induce mild narcosis at air pressures of 507 KPa (Hoar, 1975). Hoar noted that N_2 narcosis has been proposed to result from the ability of N_2 to dissolve preferentially in membrane lipid and to act as an anesthetic.

Although the possibility that some degree of O_2 or N_2 toxicity may have been induced during the air pressure treatment of steeped grain the likelihood that this was the primary cause of the inhibition of grain chitting is not great. Unlike mammalian cells, plant cells exert considerable hydrostatic pressures (600 to 2500 KPa, Gill and Bolt, 1955) Nobel (1974). In mammalian tissue the toxic effects of increased gas solubility under compression require 1/2 to 1 hour to take effect even in highly active tissue. Even though root meristems have high respiratory activity, the duration of the air pressure treatment was only 60 seconds. Further, preliminary experiments revealed that barley grain would germinate in H_2O_2 (0.3%) under a constant pressure of 193 KPa.

The duration and rate of release of air pressure may be far more important in inhibiting chitting than the actual gases involved. Under sudden decompression the release of gas bubbles can rupture membranes. The sudden decompression of a diver following 5 hours at 101 KPa will not cause membrane damage. In contrast, sudden decompression following 10 minutes at 404 KPa will disrupt membranes (Hoar, 1975). Greater gas pressure and duration respectively

increase the solubility and quantity of gas dissolved and therefore the potential membrane disruption upon decompression. However, at an air pressure of 101 KPa a duration of 5 hours is insufficient to allow enough air (gases) to dissolve into a divers tissue to cause disruptive bubble formation upon decompression. The steeped barley was subjected to an air pressure of 193 KPa for less than 60 seconds. At this pressure the equilibrium solubility of O_2 and N_2 would be approximately twice that at 101 KPa. In addition, dissolution in H_2O within the grain would be impaired by the testa.

Gas toxicity, sudden decompression and even mechanical compression could each have contributed to the decreased chitted grain number following air pressure treatment. However, none of these potential sources of chitting inhibition explains why only a fraction of the treated grains were inhibited. Perhaps the reduction in chitted grain number was not the consequence of air pressure. Although excess moisture was removed from the surface of the grains before treatment (by blotting between paper towels) free water below the husk may have transmitted a hydrostatic pressure in the region of the embryo, where at the time of treatment the testa/pericarp was ruptured by the swollen coleorhiza. If this was the case however, one would not expect the chitted grain root weight following air treatment to be significantly greater than that of grains subjected to hydrostatic pressure.

The arguments for and against gas toxicity, decompression disruption and mechanical damage apply also, but to a lesser degree, to potential explanations for the inhibitory effect of hydrostatic pressure. The hydrostatic pressure treatments were mimicked by applying compressed air to grain immersed in a beaker (250 ml) of water. The presence of the water would increase further the time to gas solubility equilibrium for a given pressure above that which would be required under direct air pressure. Increasing the pressure (on the water column) would however, increase the solubility of N_2 in lipid more than water (Hoar, 1975). Thus N_2 already dissolved in the water before the application of pressure may have preferentially dissolved into grain lipids on the application of pressure. In mammalian tissue the narcotic effect of N_2 however requires an hour or more to become established at air pressures of 507 KPa (Hoar, 1975). Thus possible contributions of rapid disruption and gas toxicity to chitting inhibition were therefore less for hydrostatic pressure treatments than air pressure treatments. In addition, the inhibition of grain chitting and root growth subsequent to treatment was significantly greater for grains subjected to hydrostatic pressure than for grains subjected to air pressure.

Eyben and Droogenbroeck (1969) were unable to detect any increase in grain moisture or embryonic disruption following hydrostatic pressure treatment. In contrast Yoshida et al. (1979) demonstrated that ink could be forced

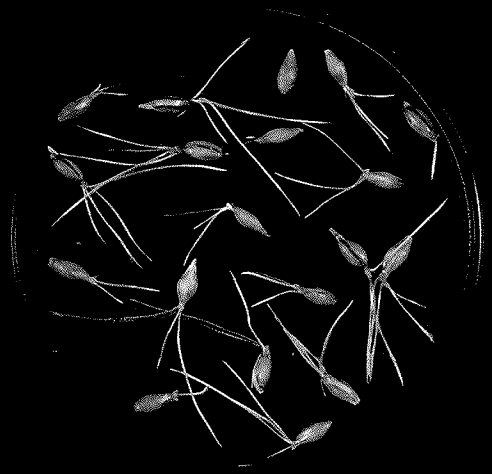
into the nodal region of the embryo by hydrostatic pressure (Chapter 5, Plate 7). They proposed that embryonic disruption was probably responsible for the inhibitory effects of hydrostatic pressure. Embryonic disruption and/or the presence of water in the nodal region may be responsible for the poorer root growth performance of hydrostatically treated grains in comparison to air treated and control grains. However, there is no reason to assume that during decompression that all the water pushed into the nodal region during treatment remains there.

Plate 2 depicts grains that were subjected to hydrostatic pressure (193 KPa for 60 seconds at 44 steeping hours) following radicle eruption through the coleorhiza. It can be seen that both root growth and coleoptile extension were inhibited in treated grains. Yoshida et al. (1976) reported that hydrostatic pressure inhibited coleoptile development. Harper and Lynch (1980) reported that in germinating barley there is a strong positive correlation between the length of the longest root and the length of the coleoptile. This was apparent for treated grains (Plate 2) as only those grains with substantial root development had coleoptiles equal to the full length of the grain. Although root growth generally appeared to be inhibited after treatment, seminals which had not emerged at the time of treatment did develop. Therefore it appears unlikely that the poor root growth performance and inhibition of coleoptile development following hydrostatic pressure was due to mechanical disruption of the embryo.

Plate 2. Hydrostatic pressure inhibition of coleoptile emergence. Grains were steeped for 44 hours. Grains showing radicle eruption (chitted) were selected for treatment (193 KPa for 60 seconds). The grains were then allowed to continue growth in petri-dishes containing 4 ml of water. Root growth and coleoptile development are shown 98 hours after treatment (142 total hours).

Hydrostatic Pressure Inhibition Of Coleoptile Emergence

control



hydrostatic pressure

Hordeum vulgare cv. Bonanza

Plate 2

4.3 THE EFFECT OF HYDROSTATIC PRESSURE MAGNITUDE AND DURATION ON GRAIN CHITTING AND ROOT GROWTH

4.3.1 Introduction

Eyben and Droogenbroeck (1969) reported that the extent by which respiratory activity was restricted by hydrostatic pressure was dependent on the magnitude of the treatment and independent of the duration. They determined that the degree of restriction increased with greater magnitudes of treatment whilst recovery from restriction increased with longer durations. Yoshida et al. (1979) observed that the restriction of respiratory activity induced by successive hydrostatic pressure treatments was cumulative. With the ability of hydrostatic pressure to reduce respiratory activity in mind an experiment was conducted to observe whether pressure magnitude and duration would influence the degree of grain chitting and root growth.

4.3.2 Materials and Methods

Bonanza following continuous aerated steeping for 28 hours at 15°C was subjected to either 96, 144 or 193 KPa hydrostatic pressure treatments for 10, 20, 30 or 60 seconds. Subsequent to treatment three germination boxes (replicates) each containing 100 grains were set up from treated and control samples. The boxes were then placed in a germination cabinet at 15 or 25°C for a further 72 hours. After 100 total hours the root dry weight and chitted grain number for each box was determined.

4.3.3 Results and Discussion

The 100 hour mean total root dry weights for all treatments are shown in Figure 2. Hydrostatic pressures of 144 and 193 KPa significantly depressed root dry weights at both 15 and 25°C. For 95 KPa treatments root dry weights at 15°C were only significantly less for the 10 and 20 second treatment durations. Greater magnitudes of hydrostatic pressure treatment resulted in further significant reductions in final root dry weights. In contrast, increased treatment duration (10 to 60 seconds) failed to significantly influence final root weights.

At 25°C root growth of control grains was significantly greater than that at 15°C. In a similar manner root growth at 25°C following the 144 KPa treatment was greater than that at 15°C. However, there was a significant interaction between the 193 KPa treatment and temperature. That is, following the 193 KPa treatment the final root dry weights (after 72 hours growth) at 25 and 15°C were not significantly different. This would suggest that fewer grains chitted at 25°C than 15°C after the 193 KPa treatment. Generally the variability in root dry weights between replicates at 25°C (CV = 22.77) was greater than that at 15°C (CV = 11.25).

In Figure 3 the number of chitted grains for each treatment are presented. Chitting of control grains was not significantly ($p = 0.01$) different irrespective of

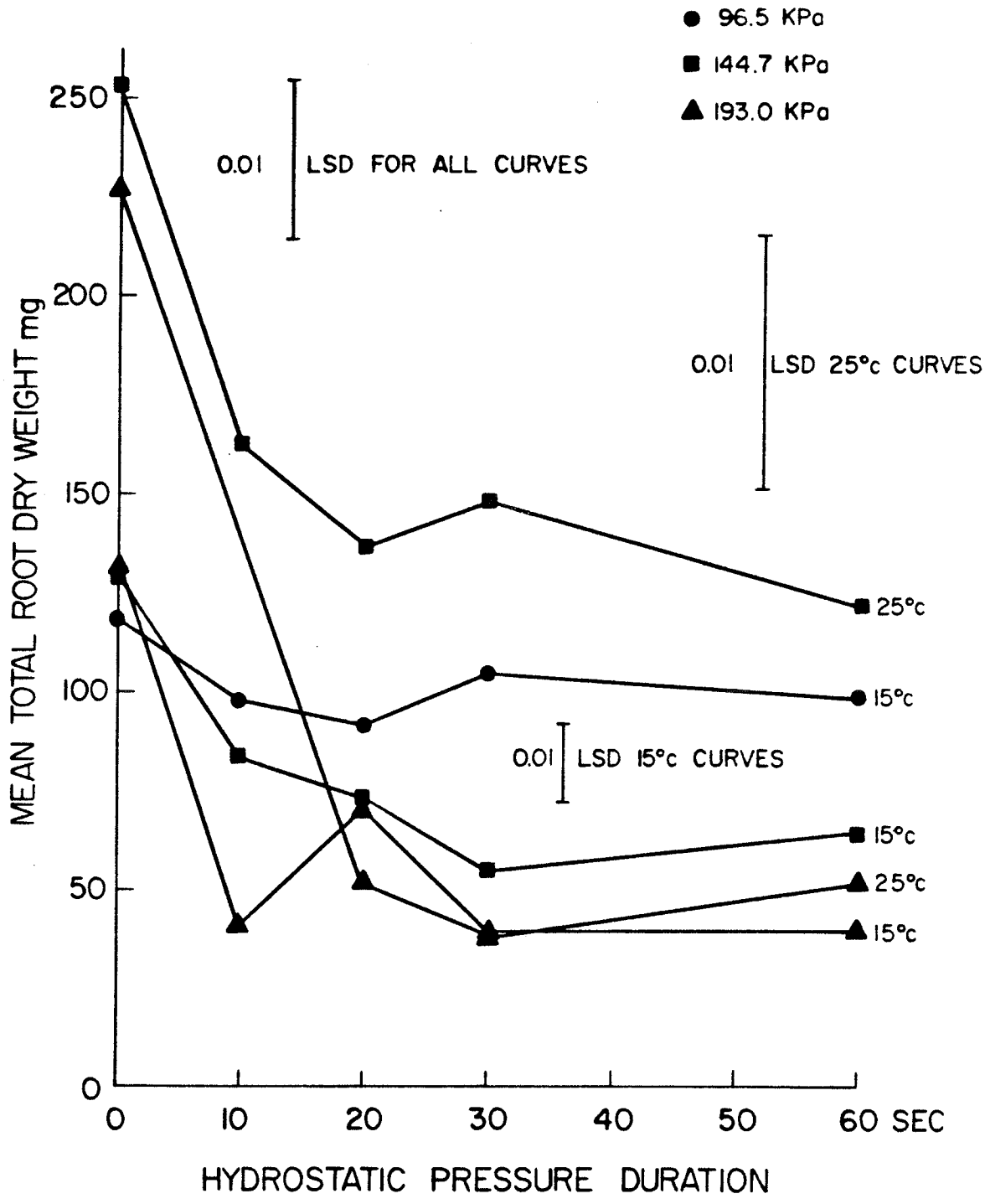


Figure 2. The influence of hydrostatic pressure magnitude and duration on total root growth.
$LSD_{0.01}$ for all curves 40.32 mg
$LSD_{0.01}$ 25°C curves 51.93 mg
$LSD_{0.01}$ 15°C curves 15.16 mg

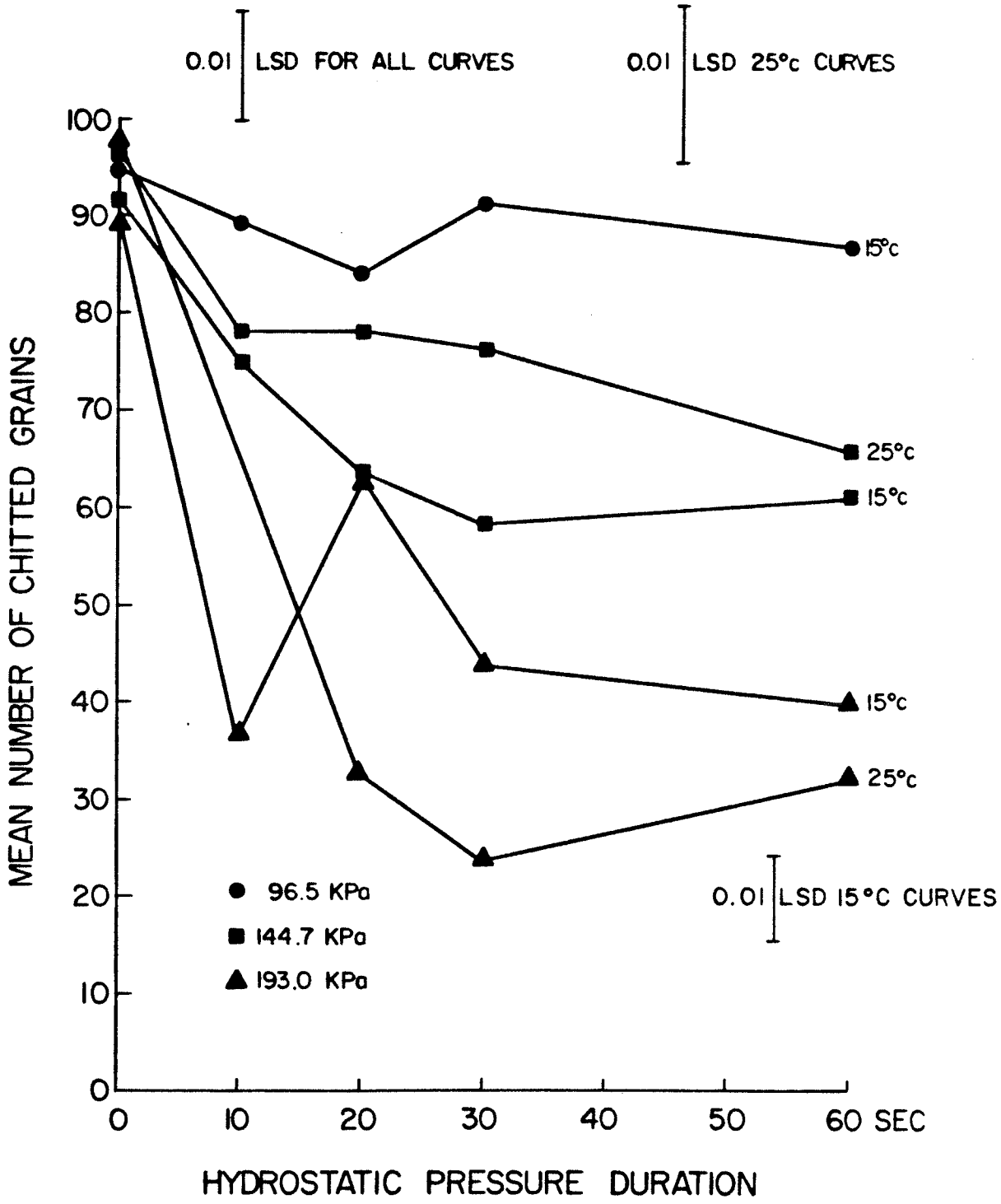


Figure 3. The influence of hydrostatic pressure magnitude on grain chitting.
$LSD_{0.01}$ for all curves 11.43
$LSD_{0.01}$ 25°C curves 16.34
$LSD_{0.01}$ 15°C curves 8.51

temperature. Hydrostatic pressure treatments of 144 and 193 KPa significantly depressed the number of chitted grains at both temperatures. For the 96 KPa treatment, only the 20 second duration produced a significant depression in the final number of chitted grains. With higher treatment pressures greater significant reductions in grain chitting occurred. However as with total root weight there was no significant interaction between magnitude and duration.

Interaction took place between magnitude and temperature. Whilst no significant differences occurred between the chitting of control grains, significantly more treated grains chitted at 25°C than 15°C following the 144 KPa treatment. Whilst following the 193 KPa treatment significantly more grains chitted at 15 than 25°C. In addition the variation in grain chitting at 25°C (CV = 10.79) was greater than that at 15°C (CV = 5.32).

It appeared that differences in total root dry weights for treatments may have simply reflected the number of grains that chitted following each treatment. In order to determine whether hydrostatic pressure actually reduced the rate of root growth and/or delayed chitting, total root dry weights for each treatment were divided by their respective chitted grain numbers. The results of this manipulation are present in Figure 4.

Root growth per chitted grain was significantly ($p = 0.01$) greater at 25°C than at 15°C. Although hydrostatic pressure reduced grain root growth at 25°C this effect was

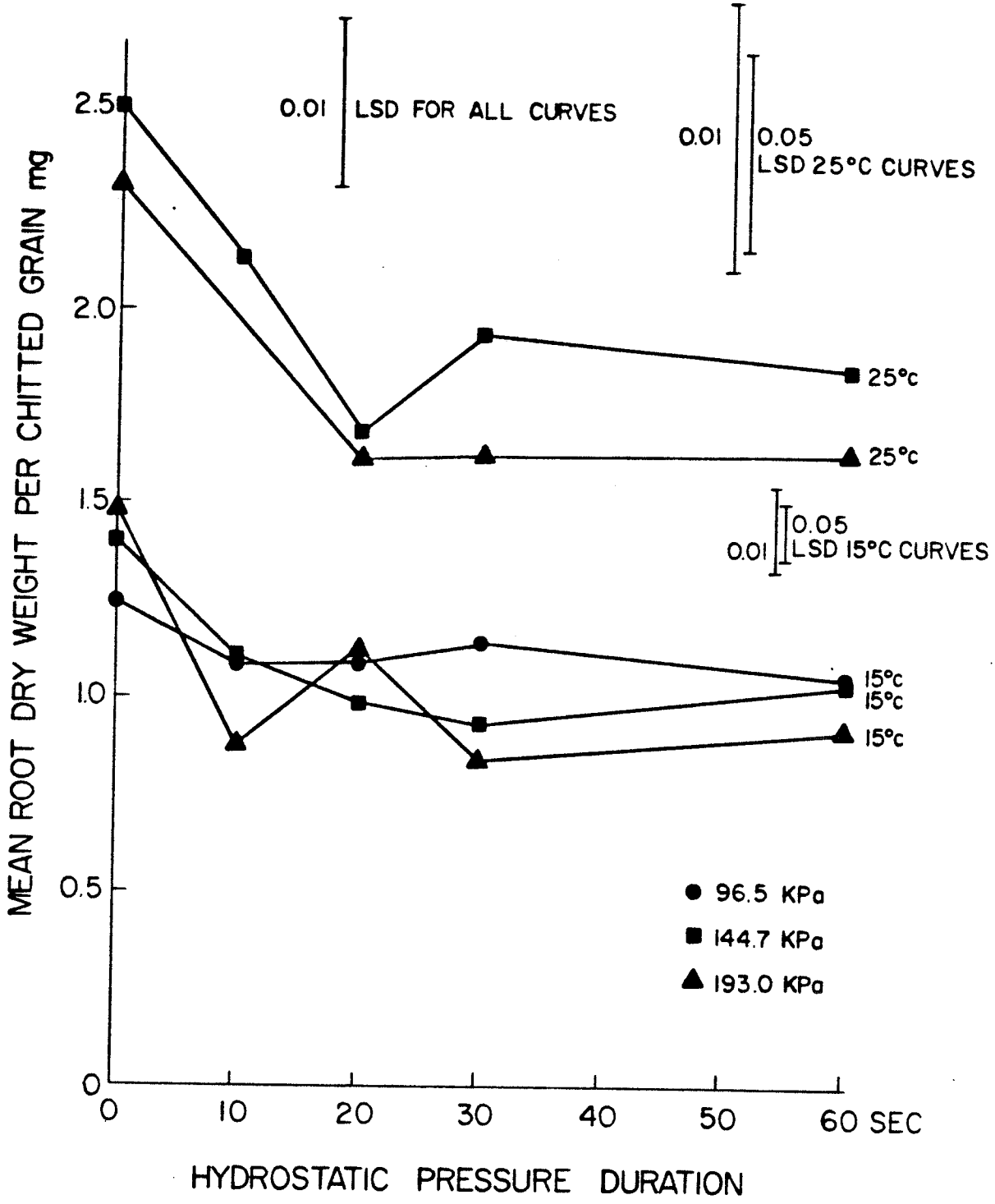


Figure 4. The influence of hydrostatic pressure magnitude and duration on root growth per chitted grain.
$LSD_{0.01}$ for all curves 0.44 mg
$LSD_{0.01}$ 25°C curves 0.71 mg
$LSD_{0.01}$ 15°C curves 0.22 mg

not significant ($p = 0.01$). In contrast, hydrostatic pressures of 144 and 193 Kpa did significantly reduce final root dry weights of grains grown at 15°C. However there was no significant increase in root growth inhibition with greater magnitudes of hydrostatic pressure treatment. These results indicated that at 25 and 15°C higher pressures only increased chitting inhibition and that subsequent root growth was far more dependent on temperature than treatment pressure. At both temperatures the lower root dry weights of treated grains may have resulted from slower growth rate and or delayed chitting.

It is important to note however, that this conclusion is based on a mean root dry weight per chitted grain. It was observed that the root growth of those treated grains that chitted was extremely variable. Often the radicles of treated grains would only emerge 2 to 3 mm beyond the ruptured coleorhiza before ceasing growth. In other treated grains what appeared to be the two seminals adjacent to the radicle erupted first, again these often grew only a few millimetres. The coleorhiza and radicles of such grains often tended to have a brown discolouration. In contrast, some treated grains appeared to grow normally. Of these some had discoloured coleorhiza and radicles whilst others did not. The considerable variation (Plate 1) may have resulted from differing degrees of susceptibility at the time of treatment. Thus the mean root growth per chitted

grain perhaps only represents a mean root growth between inhibited and non or slightly inhibited grains. Certainly in contrast to the conclusion drawn here hydrostatic pressure did affect the ability to sustain root growth subsequent to chitting in some grains. In addition at 25°C root growth in control boxes at 100 hours was very dense. It is possible that root growth of control grains was inhibited by space competition, and that competition was responsible for the apparent non significant reduction of root growth per treated grain at 25°C.

In addition the results presented tend to suggest that the 96 KPa treatment pressure was close to the minimum pressure required to induce a significant inhibition. It appears therefore that an inhibition determining resistance which reflected one or more grain properties had to be overcome. The increased inhibition that occurred as a result of greater treatment magnitudes might be explained by assuming that 1) the minimum pressure required to induce inhibition varied within treated samples and was perhaps determined by subtle differences in the stage of germination found amongst grains, or alternatively 2) that increased magnitude overcame additional resistances which reduced the likelihood of grains recovery (chitting). Eyben and Droogenbroeck (1969) reported that with greater magnitudes of hydrostatic pressure treatments respiratory activity was increasingly reduced. Thus it is perhaps reasonable to assume from their observations and the results presented here, that the degree of respiratory activity determines the chitting potential.

4.4 Summary and Conclusions

Water uptake in the simple continuously aerated steep was much faster than would normally occur in commercial aerated or traditional steeps. Generally, excessive aeration and turbulent action increases respiratory losses and leaching, both of which reduce malt yield. However, using the apparatus it was possible to observe the influence of grain size on moisture uptake. In addition the steep did not appear to abnormally affect post-steeping growth or the susceptibility of grain to hydrostatic pressure treatments. Thus, although the use of interrupted aeration and/or air rests would have more closely mimicked commercial steeps, the benefits gained from such additional experimental complexity would have probably been marginal.

It was observed that in comparison to air pressure, hydrostatic pressure reduced grain root growth in addition to chitting. This observation was confirmed for root growth per grain at 15°C but not at 25°C. Whilst increasing the magnitude of hydrostatic pressure treatment induced greater chitting inhibition root growth per grain was not influenced. In addition chitting and root growth failed to be affected by increased treatment duration between 10 and 60 seconds.

CHAPTER V

AVOIDANCE AND ALLEVIATION OF HYDROSTATIC PRESSURE INHIBITION

5.1 Introduction

During preliminary experiments it was observed that following hydrostatic pressure the proportion of grains that failed to chit was influenced by the grain environment. Grains which failed to chit for up to 100 hours in petri-dishes chitted when allowed to dry. Kirsop and Pollock (1957) reported that water-sensitive grain steeped for 20 hours would germinate if placed in air. In addition they reported that once growth was initiated re-steeping failed to inhibit further development. From these observations Kirsop and Pollock (1957) developed an interrupted steeping technique which enabled them to successfully steep water-sensitive grain. The technique involved an initial 16 hour steep followed by a 24 hour air rest at 100% RH. The grain was finally re-steeped for an additional 24 hours following the air-rest. Hough *et al.* (1971) noted that the removal of surface films stimulates water-sensitive grain to germinate. Brookes *et al.* (1976) drew attention to the embryo's ability during the early stages of germination to absorb water from surface films.

It was also observed during preliminary experiments that following hydrostatic pressure, re-steeping in aerated steeps inhibited chitting whilst re-steeping oxygenated steeps did not. Yoshida et al. (1979) reported that increased frequency of hydrostatic pressure treatment during steeping enlarged the proportion of grains that failed to chit. Hough et al. (1971) noted that water-sensitive grain fails to chit in aerated steeps. Moreover Crabb and Kirsop (1969) were able to stimulate germination of water-sensitive grains in the presence of surface films in an O_2 atmosphere.

The apparent similarity in behaviour of water-sensitive and hydrostatic pressure inhibited grain stimulated the following series of experiments.

5.2 THE COMPARATIVE EFFECTS OF A HYDROGEN PEROXIDE HEAD VERSUS HYDROSTATIC PRESSURE ON ROOT GROWTH AND GRAIN CHITTING

5.2.1 Introduction

Pollock et al. (1955b) reported on the ability of dilute H_2O_2 to stimulate germination of both dormant and non-dormant grain. Further they reported that the concentration required to break water-sensitivity was less than required to break profound dormancy.

5.2.2 Materials and Methods

Bonanza was continuously steeped with aeration at 15°C. Following various steep durations, grain samples were subjected to either hydrostatic pressure or presssure under a head of hydrogen peroxide (0.3%). Three germination boxes (replicates) each containing 100 grains were made up from each treatment sample. With respective control boxes, boxes were set to germinate at either 15 or 25°C. After 100 total hours (steeping plus germination time) the total number of chitted grains and root dry weight for each replicate was determined.

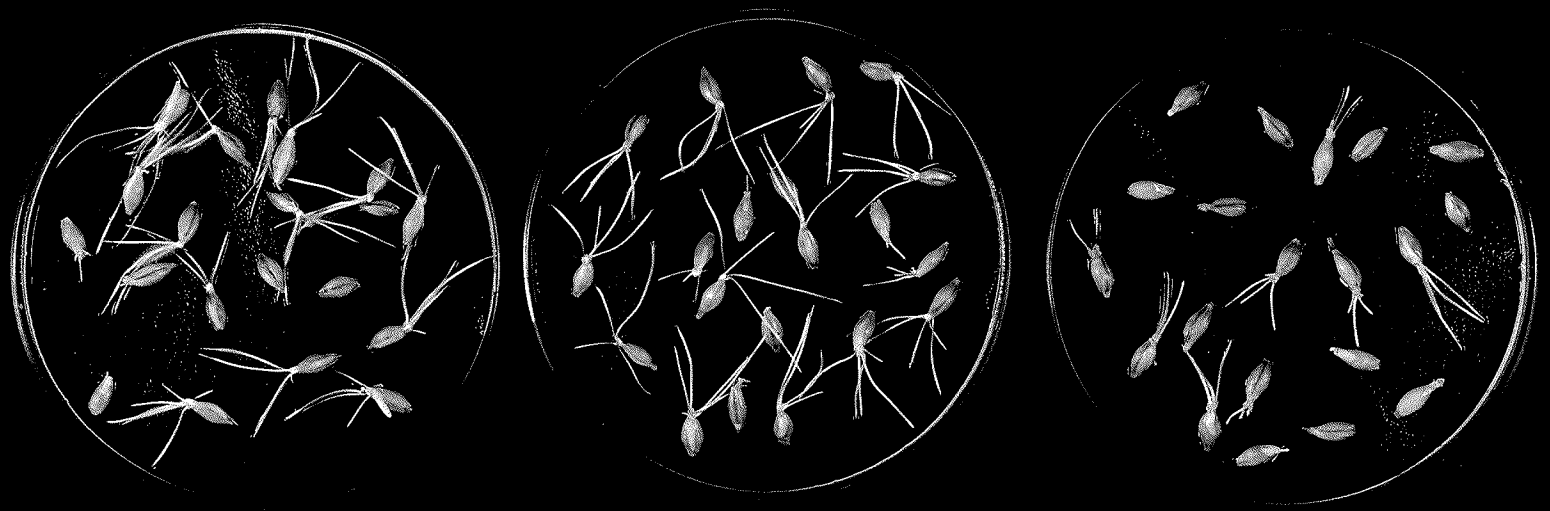
5.2.3 Results and Discussion

The results for the 15 and 25°C germination temperatures are presented in Tables 4 and 5 respectively. Plate 3 depicts the contrasting influence of treatments at 28 steeping hours on the 100 hour root growth and grain chitting.

Increased steep duration significantly ($p = 0.01$) reduced final root dry weight of all treatments at both germination temperatures. Chitting inhibition of control and H_2O_2 treated grain however failed to significantly increase with greater steep durations. In contrast, chitting inhibition of grain subjected to hydrostatic pressure at steep-out significantly increased with steep duration. Thus it appears that for all grain types the steeping environment was more inhibitory to root growth than the germination environments.

Plate 3. The comparative effects of hydrostatic pressure and compression under H_2O_2 (0.3%) on grain chitting and root growth. Grains were steeped for 28 hours before treatment (144 KPa for 30 seconds). The grains were then allowed to continue growth at 15°C in petri-dishes containing 4 ml of water. Root growth and coleoptile development are shown 72 hours after treatment (100 total hours).

Root Growth Following Hydrostatic Pressure Treatment Under A Head Of Hydrogen Peroxide



control

hydrogen peroxide head

hydrostatic pressure

Hordeum vulgare cv. Bonanza

Plate 3

TABLE 4. The comparative effect of a hydrogen peroxide head versus hydrostatic pressure with increasing steeping time on Bonanza 100 hour root dry weight and percent chitted grains when steeped and germinated at 15°c.

Treatment Time[2]	Mean Total Root Dry Weight[1]			Mean Root Dry Weight Per Chitted Grain[1]			Mean Percent Chitted Grains		
	Control	Hydrogen Peroxide Head[3]	Hydrostatic Pressure[4]	Control	Hydrogen Peroxide Head	Hydrostatic Pressure	Control	Hydrogen Peroxide Head	Hydrostatic
18	132.17 ab[5]	107.13 bcd	103.27 cd	1.42 ab	1.15 bcde	1.30 abc	92.67 abc	92.33 abc	79.67 d
22	140.23 a	117.43 abc	83.30 de	1.47 a	1.27 abcd	1.00 def	94.67 a	92.33 abc	82.67 bcd
28	132.57 ab	125.90 ab	71.33 ef	1.45 a	1.41 ab	1.13 cde	91.67 abc	92.00 abc	62.67 e
36	89.87 cde	104.09 bcd	51.33 fg	0.95 efg	1.08 cde	0.79 fg	91.67 abc	97.00 a	65.33 e
48	79.90 def	64.90 ef	26.33 g	0.79 fg	0.68 g	0.32 h	94.33 a	93.67 ab	81.33 cd
72[6]	88.40 cde	65.20 ef	24.50 g	0.92 efg	0.69 g	0.37 h	-	-	-
CV		13.85			11.14			5.15	

[1]Root dry weights are expressed in milligrams and corrected to two decimal places.
[2]Treatment times respresent continuous steeping hours before teatment.
[3]The concentration of hydrogen peroxide used was 0.3%.
[4]Pressure treatments were conducted at a magnitude of 144 KPa for a duration of 30 seconds.
[5]Parameter values for treatments followed by the same alphabetical subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).
[6]Root dry weights for grain treated at 72 hours were not determined until 120 total hours.

Brookes et al. (1976) noted that during steeping the barley grain embryo reaches the optimum moisture content for germination before the endosperm becomes adequately hydrated for complete modification and maximum malt yield. In traditional steeps grain anoxia suppresses embryo growth allowing the optimum grain moisture content (43 to 46%) to be attained with reduced respiratory loss. The results presented in Figure 1 indicated that Bonanza reached the optimum steep-out moisture after approximately 28 steeping hours. Thus grains steeped for less than 28 hours would probably have shown poor modification and higher respiratory losses due to less suppression of embryo growth. It was observed that the aerated steep would support root growth, however the extent of this growth was less than that which would occur in the 15°C germination environment over the same period of time. It seems reasonable to assume that whilst the aerated steep did not provide the same degree of growth suppression that would occur in a traditional steep, respiratory activity was nevertheless inhibited to an extent that tissue RQ's were probably greater than one. The above perhaps explains why control grain steeped for 22 hours (Table 4) had a higher final root dry weight than grain steeped for 28 hours.

The continued significant decline in final root dry weight, irrespective of treatment, was probably contributed to by two other factors in addition to anoxic suppression of

embryo growth. Firstly, with the exception of grains treated at 72 hours, no grains used showed radicle eruption. The decline in final root dry weight was therefore promoted by the selection of slow developing grains. Secondly, Hough et al. (1971) noted that the prolonged steeping and/or excessive water uptake brought about by vigorous aeration may induce a condition similar to water-sensitivity, that can be broken by the removal of surface films of water. Consequently grain vigour in germination environments might have been expected to be reduced by steeping beyond 28 hours.

For all treatment times, with the exception of 18 hours, total root weight of control and H_2O_2 treated grain were significantly ($p = 0.01$) greater than that of grain exposed to hydrostatic pressure. In addition, control weights were never significantly greater than those of H_2O_2 treated grains for respective treatment times with the exception of the 72 hour time (Table 5). In the case of the latter however, the final root weight of H_2O_2 treated grain was still significantly greater than that of grains subjected to hydrostatic pressure.

Root weight per chitted grain (Table 4) was not significantly ($p = 0.01$) different for all treatments at 18 hours. Whilst at 22 hours control weight was significantly greater than that of pressure treated grains, control weight was not significantly different from that of H_2O_2 treated grains. For the 28, 48 and 72 hours treatment times root

weights per control and H_2O_2 treated grains were not significantly different. At the same times, however, the root growth of pressure treated grains was significantly less than controls. In contrast, the final root dry weight for grains subjected to hydrostatic pressure at 36 hours was not significantly different from that of control grains. After treatment at this steeping time, however, the root weight of H_2O_2 treated grains was significantly greater than that of pressure treated grains.

For respective treatment times (with the exception of the 22 hour treatment time Table 4) the mean percentage of chitted grain at 15 and 25°C following hydrostatic pressure treatment was significantly ($p = 0.01$) less than that of control and H_2O_2 treated grains. Percent chitting for control and H_2O_2 treated grain was not significantly different at any treatment time. Whilst following treatment at 22 hours (Table 4), chitting of H_2O_2 treated grain was not significantly different from that of pressure treated grain, control chitting in contrast was significantly greater.

Coleoptile emergence (Table 5) of control and H_2O_2 treated grain was significantly ($p = 0.01$) greater than that of hydrostatic pressure treated grain at all treatment times. With the exception of the 72 hour treatment time coleoptile emergence of control and H_2O_2 treated grains were not significantly different.

TABLE 5. The comparative effect of a hydrogen peroxide head versus hydrostatic pressure with increasing steeping time on Bonanza 100 hour root dry weight, percent chitted grains and coleoptile emergence when steeped at 15°C and germinated at 25°c.

Treatment Time[2]	Mean Total Root Dry Weight[1]			Mean Percent Chitted Grains			Mean Number of Emergent Coleoptiles		
	Control	Hydrogen Peroxide Head[3]	Hydrostatic Pressure[4]	Control	Hydrogen Peroxide Head	Hydrostatic Pressure	Control	Hydrogen Peroxide Head	Hydrostatic
28	223.63 a[5]	212.53 a	121.13 cd	93.33 a	92.00 a	68.67 bc	91.67 a	92.00 a	60.33 bc
36	185.17 ab	154.83 bc	74.07 ef	95.00 a	95.00 a	55.00 dc	93.00 a	85.00 a	47.33 c
48	135.67 cd	101.37 de	38.33 fg	88.00 a	78.67 ab	39.33 de	76.00 ab	62.33 bc	16.00 d
72	155.40 bc	102.67 de	13.17 g	97.00 a	89.00 a	22.33 e	85.33 a	59.00 bc	2.67 d
CV		15.14			10.50			14.50	

[1]Root dry weights are expressed in milligrams and corrected to two decimal places.
[2]Treatment times respresent continuous steeping hours before teatment.
[3]The concentration of hydrogen peroxide used was 0.3%.
[4]Pressure treatments were conducted at a magnitude of 144 KPa for a duration of 30 seconds.
[5]Parameter values for treatments followed by the same alphabetical subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).
[6]Root dry weights for grain treated at 72 hours were not determined until 120 total hours.

Hydrostatic pressure and H_2O_2 treatment at 18 steeping hours (Table 4) depressed final root weight at 15°C to below control levels. Preliminary experiments had indicated that unless the coleorhiza was swollen (testa/ pericarp ruptured) ink could not be pushed into the nodal region by a hydrostatic pressure of 193.0 KPa for 3 minutes. The majority of grain in the aerated steep did not have swollen coleorhizas at 18 steeping hours. Therefore during treatments H_2O_2 or water probably failed to penetrate into the embryonic region. However, water may have been forced under the grain husk thereby slowing down germination by limiting O_2 diffusion to the embryo. If this were the case, however, H_2O_2 below the husk would be expected to stimulate germination. Either one of these liquids occupying pretreatment gas pockets below the husk could have limited the diffusion of CO_2 away from the grain. Before testa/pericarp rupture the steeped barley grain has an RQ>1 and inhibition of CO_2 diffusion would restrict respiration. Hough et al. (1971) notes that high concentrations of CO_2 reduce grain fermentation and that sparging steeps with CO_2 or N_2 slows down growth subsequent to casting. The growth inhibition, however, following treatments at 18 hours may have been independent of the compressing liquid nature, the inhibition being the consequence of a sudden pressure shock transmitted to the embryonic tissue. Reductions in membrane thickness resulting from short term strains have been proposed (Coster et al. 1977).

After 22 steeping hours a greater proportion of grains had swollen coleorhizas. Thus, H_2O_2 or water could under pressure penetrate the embryo region of a large proportion of grains. Possibly only those grains with a ruptured pericarp/testa treated under H_2O_2 were not inhibited. If this were the case it would explain why 22 steeping hour control growth was significantly greater than that of grains subjected to hydrostatic pressure. It might also explain why the root growth of H_2O_2 treated grain was less than that of control grains at this treatment time.

Orphanos and Heydecker (1968) reported that soaking injury in Phaseolus vulgaris was associated with the flooding of a cavity between the cotyledons when the seeds were totally submerged. They also reported that the condition was made worse by aeration. Yoshida et al. (1979) observed that pressure forced ink into the nodal region of the embryo. With this in mind it is interesting to note that the difference in control and H_2O_2 treated grain total root growth increases with steeping time from 48 to 72 hours at both 15 and 25°C. Perhaps there is a defined pathway by which ink and H_2O_2 under pressure reaches the nodal region. Excessive water uptake induced by prolonged aerated steeping may cause such a pathway to become occluded by steep water which limited the penetration of H_2O_2 under pressure. Orphanos and Heydecker (1968) reported that H_2O_2 (1%) completely eliminated soaking injury and reduced the level of free water

in the cavity between the cotyledons of Phaseolus vulgaris.

It appears that where grain coleorhizas were swollen either the physical effects of compression under water do not occur under H_2O_2, or that the oxidizing property of H_2O_2 compensates for such effects during and/or subsequent to treatment. However, although not as deleterious as hydrostatic pressure treatments, H_2O_2 treatments generally reduced total root growth even though chitted grain numbers were not significantly different from controls.

Whilst not discounting any physical effects of compression for which H_2O_2 treatment was probably unable to compensate, the possibility that the H_2O_2 treatment modifies root growth subsequent to treatment must be considered. Pollock et al. (1955b) reported that 1% H_2O_2 (Thunaeus test) considerably retarded root growth to a degree that often made chitting difficult to discern. Collis-George and Williams (1968) noted that H_2O_2 (approximately 0.34%) caused retarded root hair development and degeneration of the radicle in the meristematic region. Plate 3 clearly demonstrates that the (0.3%) H_2O_2 presssure treatments failed to severely retard root growth or cause degeneration of meristems. However, close examination of Plate 3 reveals that the rootlets of H_2O_2 treated grains lack hairs whilst hairs were present on rootlets of both control and hydrostatic pressure treated grains. It appears therefore

that H_2O_2 treatment modified root growth, this might not however, have been entirely responsible for the lower root weights. Possibly the mechanism of H_2O_2 inhibition at the 18 hour treatment time (Table 4) was offsetting the pressure compensatory effects of H_2O_2 observed at other treatment times.

Corresponding root dry weights and number of emergent coleoptiles at 25°C for control and treatment grains were consistent with Harper and Lynch's (1980) positive correlation of root and coleoptile length. This consistency was especially noticeable in the H_2O_2 treatment at 72 hours. Compared to control growth the H_2O_2 treated sample showed a significant depression in total rootlet growth in unison with a significant delay in coleoptile emergence.

5.3 HUSK AND PERICARP INVOLVEMENT IN PERPETUATING HYDROSTATIC PRESSURE INHIBITION

5.3.1 Introduction

Dunwell (1981) reported that the barley husk inhibits germination by limiting gas diffusion. Pollock (1955b) observed that the removal of the husk failed to alleviate water-sensitivity and only mildly relieved profound dormancy. Preliminary experiments indicated that water pushed under the husk during hydrostatic pressure treatment was not the prin-

ciple source of inhibition. Wheat (Plate 4) and naked barley (Plate 5) were susceptible to treatment (193.0 KPa for 60 seconds) at 28 steeping hours. Wheat chitting (72 hours after treatment) appeared to be totally inhibited. Even though root growth was inhibited, chitting of treated naked barley (72 hours after treatment) appeared to be greater than that of hulled grains (Plates 1 and 3). Thus it was apparent that the husk could have been contributing to the persistence of chitting inhibition in treated hulled barley.

Pollock et al. (1955b) reported that mechanical damage to the grain coat or the removal of the pericarp by acid scarification alleviated both profound dormancy and water-sensitivity. They concluded that pericarp integrity was essential to the maintenance of both dormancy types. In the following experiment acid decorticated grain was steeped and subjected to hydrostatic pressure in an attempt to determine the involvement of the pericarp subsequent to treatment.

5.3.2 Materials and Methods

Bonanza samples (170g) were decorticated in 1 litre of cold 50% v.v. sulphuric acid. Initially the barley samples were decorticated for 1 hour following which a fresh acid solution was used for an additional 1/2 hour. During decortication the grains were continually agitated by a magnetic stirrer. Subsequent to decortication the grains were

Plate 4. Hydrostatic pressure inhibition of chitting and root growth in wheat. Grain were steeped for 28 hours before treatment (192 KPa for 60 seconds). The grains were then allowed to continue growth in petri-dishes containing 4 ml of water. Root growth and coleoptile development are shown 72 hours after treatment (100 total hours).

Hydrostatic Pressure Inhibition Of Root Growth In Wheat

control

hydrostatic pressure

Triticum aestivum cv. Columbus

Plate 4

Plate 5. Hydrostatic pressure inhibition of chitting and root growth in naked barley. Grains were steeped for 28 hours before treatment (193 KPa for 60 seconds). The grains were then allowed to continue growth in petri-dishes containing 4 ml of water. Root growth and coleoptile development are shown 72 hours after treatment (100 total hours).

Hydrostatic Pressure Inhibition Of Root Growth In Naked Barley

control

hydrostatic pressure

Hordeum vulgare cv. Scout

Plate 5

washed in copious volumes of water for up to half an hour. Whilst being washed husk and coat remnants were flushed and rubbed from the surface of the grains. After washing grains were immersed in a 2 litre stirred suspension of 2% calcium carbonate for 1/2 hour. Following this the grains were washed for a further 15 minutes in running water. Finally the grains were transferred to a 15°C steep with constant aeration. Initially the steep water was changed after 2 hours with subsequent changes being made every 10 hours.

Bonanza (hulled) was steeped with aeration at 15°C following which steep water was replaced by a 1% sodium hypochlorite solution. Surface sterilization during steeping was allowed to continue for an additional 2 hours following which the grain was washed in running water (approximately 15 to 16°C) for an additional 1/2 hour. The grain was finally returned to a water steep with the water being subsequently renewed every 10 hours.

Both hulled and decorticated Bonanza were subjected to a hydrostatic pressure of 193 KPa for 30 seconds at 28 steeping hours. Treated and control grains were then set to continue growth in either germination boxes or petri-dishes. Six germination boxes (replicates) of each control and treated grain types (hulled or decorticated) were set up. Each germination box contained 100 grains. In a similar manner six petri-dishes for each control and treated grain type were set up. Each petri-dish contained 2 Whatman No.1

filter papers and 4 ml of water, on top of which was placed 20 grains. The germination boxes and petri-dishes were finally placed in a germination cabinet at 15°C for an additional 72 hours. After a total of 100 hours (steeping plus germination time) the number of chitted grains and total root dry weight was determined for each germination box and petri-dish.

5.3.3 Results and Discussion

Total root dry weights and percentage chitted grains for controls and treatments are presented in Table 6. Table 7 includes root dry weights per chitted grain. The Duncan's multiple range test has been used in Table 8 to compare root dry weight per chitted grain and percent chitted grains in the two growth environments. Plate 6 depicts the contrasting chitting and root growth behaviour of control and treated acid decorticated grain.

Total root dry weights and percent chitting (Table 6) of hulled and decorticated control grains were not significantly ($p = 0.01$) different for respective growth environments. Root growth and chitting of treated grains was significantly less than that of control grains in both environments. In petri-dishes root growth and grain chitting of treated hulled grains was significantly less than that of treated decorticated grains. In germination boxes however,

TABLE 6. The effect at 28 steeping hours of hydrostatic pressure on the 100 hour total root dry weight and percent chitting of hulled and acid decorticated Bonanza grown in petri-dishes and germination boxes at 15°c subsequent to treatment.

Grain Type	Treatment	Petri-dish Mean Total Root Dry Weight[1]	Petri-dish Mean Percent Chitted Grains	Germination Box Mean Total Root Dry Weight	Germination Box Mean Percent Chitted Grains
Hulled	(Control)	24.07 a[2]	90.83 ab	130.97 a	91.17 a
Hulled	Hydrostatic Pressure[3]	3.98 c	37.50 c	36.90 b	41.83 c
Decorticated	(Control)	22.70 a	95.83 a	12.32 a	93.17 a
Decorticated	Hydrostatic Pressure	11.22 b	81.67 b	47.63 b	75.00 b
CV		10.48	8.08	11.77	6.82

[1]Root dry weights are expressed in milligrams and corrected to two decimal places.
[2]Parameter values within a column time followed by the same alphabetical subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).
[3]Pressure treatments were conducted at a magnitude of 193 KPA for a duration of 30 seconds.

Plate 6. Hydrostatic pressure inhibition of chitting and root growth in acid decorticated barley. Grains were steeped for 28 hours before treatment (193 KPa for 30 seconds). The grains were then allowed to continue growth in petri-dishes containing 4 ml of water. Root growth and coleoptile development are shown 72 hours after treatment (100 total hours).

TABLE 7. The effect at 28 steeping hours of hydrostatic pressure on the 100 hour total mean root dry weight per chitted grain and percent chitting of hulled and acid decorticated Bonanza grown in petri-dishes and germination boxes at 15°c subsequent to treatment.

Grain Type	Treatment	Petri-dish Mean Root Dry Weight Per Chitted Grain[1]	Petri-dish Mean Percent Chitted Grains	Germination Box Mean Root Dry Weight Per Chitted Grain	Germination Box Mean Percent Chitted Grains
Hulled	(Control)	1.33 a[2]	90.83 ab	1.44 a	91.17 a
Hulled	Hydrostatic Pressure[3]	0.53 b	37.50 c	0.88 b	41.83 c
Decorticated	(Control)	1.19 a	95.83 a	1.32 a	93.17 a
Decorticated	Hydrostatic Pressure	0.69 b	81.67 b	0.64 c	75.00 b
CV		10.58	8.0	7.84	6.82

[1]Root dry weights are expressed in milligrams and corrected to two decimal places.
[2]Parameter values within a column followed by the same alphabetical subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).
[3]Pressure treatments were conducted at a magnitude of 193 KPA for a duration of 30 seconds.

Acid Decorticated Barley

control

hydrostatic pressure

Hordeum vulgare cv. Bonanza

Plate 6

only chitting of treated hulled grains was significantly less than that of treated decorticated grains.

The variability of behaviour of treatment grains and the resultant lack of certainty in interpreting root dry weight per chitted grain has been noted in Section 4.3.3. Hulled and decorticated control root dry weights per chitted grain (Table 7) were not significantly ($p = 0.01$) different for respective growth environments. Hydrostatic pressure significantly reduced the root growth of hulled and decorticated grains in both environments. Treated hulled and decorticated grains showed no significant difference for root growth in petri-dishes. In germination boxes however, treated decorticated grains had significantly less root growth than treated hulled grain.

Chitting of control grain (Table 8) was not significantly influenced by the two germination environments. Hydrostatic pressure significantly depressed grain chitting in both environments. However, treated decorticated grains - chitted significantly better in both environments than treated hulled grains. Therefore, treated grain chitting appeared to be influenced by the presence or absence of the hull and not the grain environment.

In contrast to the above, root growth per chitted grain (Table 8) for control and treatments <u>was</u> influenced by grain environment. Decorticated control grains showed significantly ($p = 0.01$) less root growth in petri-dishes than

TABLE 8. The effect at 28 steeping hours of hydrostatic pressure on the 100 hour mean root dry weight per chitted grain and percent chitting of hulled and acid decorticated Bonanza grown in petri-dishes and germination boxes at 15°c subsequent to treatment.

Grain Type	Treatment	Post-Steeping Environment	Mean Root Dry Weight[1] Per Chitted Grain	Mean Percent Chitted Grains
Hulled	(Control)	Petri-dish	1.33 ab[2]	90.83 a
Hulled	Hydrostatic Pressure[3]	Petri-dish	0.53 e	37.50 c
Decorticated	(Control)	Petri-dish	1.19 b	95.83 a
Decorticated	Hydrostatic Pressure	Petri-dish	0.69 d	81.67 b
Hulled	(Control)	Germination Box	1.44 a	91.17 a
Hulled	Hydrostatic Pressure	Germination Box	0.88 c	41.83 c
Decorticated	(Control)	Germination Box	1.32 ab	93.17 a
Decorticated	Hydrostatic Pressure	Germination Box	0.64 de	75.00 b
CV			9.14	7.49

[1]Root dry weights are expressed in milligrams and corrected to two decimal places.
[2]Parameter values within a column followed by the same alphabetical subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).
[3]Pressure treatments were conducted at a magnitude of 193 KPA for a duration of 30 seconds.

hulled control grain in germination boxes. However, the growth of decorticated control grains in germination boxes was not significantly different from that of hulled control grains in both germination environments. Treated hulled grains showed significantly greater growth in germination boxes than petri- dishes. In addition treated hulled grain growth in boxes was significantly greater than that of treated decorticated grains in both environments. Whilst no significant difference between treated decorticated grain growth occurred in the two environments decorticated root growth per grain in petri-dishes was significantly greater than that of treated hulled grains in the same environment.

Grains placed in either petri-dishes or germination boxes had surface films of water. However, grains placed in petri-dishes were in contact with free water whilst grains in germination boxes were not. Bookes et al. (1976) noted that the embryo tends to absorb water from the grain coat during the early stages of germination. Thus, initially the husk/pericarp of control grains in germination boxes probably dried somewhat whilst control grain coats in petri-dishes remained unchanged. Despite this no significant chitting differences occurred between hulled grain in either of the two environments. Further, the presence or absence of a husk/pericarp and/or a surface film of water failed to influence the chitting of control grains. In contrast the pre-

sence of a husk on treated grain significantly depressed chitting whilst the presence of a surface film did not.

The husk (Dunwell, 1981) and the pericarp (Pollock and Kirsop, 1956) have been reported to limit the diffusion of O_2. Crabb and Kirsop (1969) observed that the resistance to O_2 diffusion through a surface film of water, the husk, and pericarp was the same for normal and water-sensitive grain. Following pericarp/testa rupture grain respiration has been noted to be predominantly aerobic (Bewley and Black, 1978). Crabb and Kirsop (1969) reported that the removal of the pericarp eliminated anaerobic respiration in both normal and water-sensitive grains. Grain subjected to hydrostatic pressure had swollen coleorhizas, therefore the pericarp/testa would not be expected to be limiting O_2 diffusion. It appeared therefore, that in contrast to control grains, O_2 diffusion resistance of the pericarp significantly contributed to treated grain chitting inhibition.

Plate 6 clearly depicts that total root growth and root growth per chitted grain were significantly inhibited following treatment. Plate 6 also demonstrates that the treatment reduction of total root growth did not simply reflect total chitting inhibition. In contrast, Plates 1 and 3 indicate that for treated hulled grains the reduction of total root growth was contributed to by complete chitting inhibition. However, even with the latter consideration it

is not possible to determine the relative contributions of 1) delayed chitting, and 2) growth inhibition subsequent to chitting to the reduced root growth of treated grains (Tables 7 and 8). It is interesting to note however, that the root growth of decorticated control grains in petri-dishes was significantly less than that of other controls. Perhaps the absence of sub-husk gas pockets contributed to this inhibition? No significant difference in grain root growth occurred between decorticated treatments in either environments. This suggests that no additional inhibition took place when growing rootlets contacted petri-dish free water. Treated hulled grains in germination boxes had the greatest treatment rootlet growth per chitted grain. In contrast, their growth in petri-dishes was poorest. Perhaps in germination boxes, drying of treated grain husks re-established gas pockets and reduced husk thickness resulting in better gas exchange.

5.4 THE INFLUENCE OF FREE WATER ON ROOT GROWTH AND CHITTING OF GRAINS SUBJECTED TO HYDROSTATIC PRESSURE

5.4.1 Introduction

The results in the previous section indicated that the presence of a free water film on or around control and treated grains failed to significantly influence chitting. Preliminary experiments had indicated, however, that if treated grain was allowed to dry, chitting inhibition could

be broken. Water-sensitivity is a dormancy form which is only expressed when the available water exceeds the minimum necessary for germination. In the following experiment the role of free water in hydrostatic pressure inhibition was investigated.

5.4.2 Materials and Methods

Petri-dishes with seven water volumes (0, 1, 2, 3, 4, 5 and 6 ml) were used to germinate control and treated Bonanza which had been steeped at 15°C with aeration for 28 hours. Treated grains were subjected to a 193 KPa hydrostatic pressure treatment for 60 seconds. For each water volume, 6 control and 6 treatment petri-dishes (replicates) each containing 20 grains placed on top of 2 Whatman No. 1 filter papers were set up. The petri-dishes were then placed in a 15°C germination cabinet for an additional 72 hours. This experimental procedure was repeated using a germination temperature of 25°C. After a total of 100 hours the total root dry weight and number of chitted grains was determined for each replicate.

5.4.3. Results and Discussion

The results for steeping and germination at 15°C are presented Table 9. For each water volume, with the exception of 0 ml, control total and chitted grain root growth was significantly ($p = 0.01$) greater than that for respective

TABLE 9. The effect of free water subsequent to hydrostatic pressure treatment at 28 steeping hours on the 100 hour root dry weight and chitted grain number of Bonanza steeped and germinated in petri-dishes at 15°c.

Treatment	Volume of Water	Mean Total Root Dry Weight[1]	Mean Percent Inhibition of Total Root Dry Weight	Mean Root Dry Weight per Chitted Grain	Mean Number of Chitted Grains
(Control)	0 ml	13.83 e[2]	58.21 b	0.70 fg	19.50 a
Hydrostatic Pressure[3]	0	5.80 f		0.36 g	15.67 b
(Control)	1	29.37 b	21.12 d	1.48 c	19.83 a
Hydrostatic Pressure	1	22.72 dc		1.14 cde	19.83 a
(Control)	2	37.90 a	33.84 dc	1.99 a	19.00 a
Hydrostatic Pressure	2	25.08 dc		1.39 cd	19.33 a
(Control)	3	35.82 a	39.90 c	1.96 a	18.17 a
Hydrostatic Pressure	3	21.12 d		1.16 cde	18.00 ab
(Control)	4	37.78 a	80.13 a	1.91 ab	19.83 a
Hydrostatic Pressure	4	8.80 ef		0.93 ef	9.33 c
(Control)	5	29.17 b	82.17 a	1.54 bc	19.17 a
Hydrostatic Pressure	5	5.15 f		0.82 ef	6.50 d
(Control)	6	27.37 bc	76.95 a	1.46 cd	19.00 a
Hydrostatic Pressure	6	6.37 f		1.05 ef	6.17 d
CV		17.01	19.27	19.47	9.34

[1]Root dry weights are expressed in milligrams and corrected to two decimal places.
[2]Parameter values within a column followed by the same alphabetical subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).
[3]Pressure treatments were conducted at a magnitude of 193 KPA for a duration of 60 seconds.

treatments. Control total and chitted grain root growth declined significantly below 2 ml and above 4 ml. In contrast, control chitted grain number did not significantly change with varying water volume. In addition, chitted grain numbers for treatments were only significantly different (less) than controls for water volumes 0, 4, 5 and 6 ml. In 2 and 3 ml petri-dishes no significant differences occurred between control or between treatment parameters. However, with the exception of chitted grain number, treatment root growth parameters at 2 and 3 ml were significantly less than controls. Thus hydrostatic pressure at 2 and 3 ml either inhibited root growth or delayed chitting by making the grain sensitive to excess water. In the 1 ml petri-dishes no significant differences occurred between (with the exception of total root growth) controls and treatments for all parameters, although at this volume and at 0 ml control root growth appears to have been limited by available water.

The results for steeping at 15°C and germination at 25°C are presented in Table 10. For water volumes from 0 to 5 ml control total root growth was significantly ($p = 0.01$) greater than respective treatments. In addition chitted grain root growth for controls between 0 and 4 ml was significantly greater than treatments. The number of chitted control grains did not significantly change with increased water volume. At 1, 2, and 3 ml treated chitted grain numbers were not significantly different from respective

TABLE 10. The effect of free water subsequent to hydrostatic pressure treatment at 28 steeping hours on the 100 hour root dry weight and chitted grain number of Bonanza steeped at 15°c and germinated in petri-dishes at 25°c.

Treatment	Volume of Water	Mean Total Root Dry Weight[1]	Mean Percent Inhibition of Total Root Dry Weight	Mean Root Dry Weight per Chitted Grain	Mean Number of Chitted Grains
(Control)	0 ml	40.57 b[2]	70.90 a	2.08 bc	19.50 ab
Hydrostatic Pressure[3]	0	11.56 cd		0.67 e	17.33 c
(Control)	1	66.37 a	36.08 b	3.34 a	19.83 a
Hydrostatic Pressure	1	41.02 b		2.10 bc	19.50 ab
(Control)	2	65.03 a	24.65 b	3.25 a	20.00 a
Hydrostatic Pressure	2	46.32 b		2.39 b	19.33 abc
(Control)	3	40.68 b	38.88 b	2.32 b	18.83 abc
Hydrostatic Pressure	3	24.58 c		1.32 cde	17.50 bc
(Control)	4	64.87 a	83.11 a	3.27 a	19.83 a
Hydrostatic Pressure	4	10.42 cd		1.14 de	9.17 d
(Control)	5	21.85 c	73.32 a	1.15 de	18.83 abc
Hydrostatic Pressure	5	5.78 d		1.73 bcd	3.67 e
(Control)	6	20.02 cd	68.81 a	1.05 de	19.00 abc
Hydrostatic Pressure	6	6.10 d		1.59 bcd	3.83 e
CV		27.12	23.18	24.93	7.60

[1]Root dry weights are expressed in milligrams and corrected to two decimal places.
[2]Parameter values within a column followed by the same alphabetical subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).
[3]Pressure treatments were conducted at a magnitude of 193 KPA for a duration of 60 seconds.

controls. However, at these volumes total and grain root growth for treatments was significantly less than the controls.

Table 11 presents a comparison of total root growth inhibition and chitted grain number at 15 and 25°C. Inhibition at either temperature was not significantly different for respective water volumes. Chitted grain numbers at 15 and 25°C were only significantly different for treatments at 5 and 6 ml. At these volumes inhibition at 25°C was significantly greater than that at 15°C.

The significant declines at both temperatures in chitted grain number at 4 ml to below control values was followed by a further significant decline at 5 and 6 ml. Controls at these volumes were not significantly different at either temperature. Treatment therefore appears to have made the grain sensitive to excess water. With water volumes of 3 ml and upwards a meniscus bounded each grain. Thus it would seem reasonable to assume that increased treatment chitting inhibition of treated grain may have been associated with the presence of a surface water film. However, at 0 ml chitting of treated grain was significantly less than that of the control grains at both germination temperatures. Grains placed in the 0 ml petri- dishes did not have menisci, as water was drawn from the grains into the filter paper. Further, treated grain chitting was significantly greater at 1 ml than at 0 ml.

TABLE 11. The effect on 100 hour root dry weight inhibition and chitted grain number of hydrostatic pressure on Bonanza steeped at 15°C for 28 hours before treatment and germinated in petri-dishes at 15 and 25°c following treatment.

Treatment	Volume of Water	Mean Percent Inhibition of Total Root Dry Weight		Mean Number of Chitted Grains	
		15°c	25°c	15°c	25°c
(Control)	0 ml	58.21 bc	70.90 ab	19.50 ab[1]	19.50 ab
Hydrostatic Pressure[2]	0			15.67 c	17.33 bc
(Control)	1	21.12 d	36.08 d	19.83 ab	19.83 ab
Hydrostatic Pressure	1			19.83 ab	19.50 ab
(Control)	2	33.84 d	24.65 d	19.00 ab	20.00 a
Hydrostatic Pressure	2			19.33 ab	19.33 ab
(Control)	3	39.90 dc	38.88 dc	18.17 ab	18.83 ab
Hydrostatic Pressure	3			18.00 ab	17.50 abc
(Control)	4	80.13 a	83.11 a	19.83 ab	19.83 ab
Hydrostatic Pressure	4			9.33 d	9.17 d
(Control)	5	82.17 a	73.32 ab	19.17 ab	18.83 ab
Hydrostatic Pressure	5			6.50 e	3.67 f
(Control)	6	76.95 ab	68.81 ab	19.00 ab	19.00 ab
Hydrostatic Pressure	6			6.17 e	3.83 f
CV		21.34		8.52	

[1]Parameter values within a column followed by the same alphabetical subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).
[2]Pressure treatments were conducted at a magnitude of 193 KPA for a duration of 60 seconds.

With increasing water volume above 4 ml treatment total and grain root growth at 15°C, although significantly less than respective controls, did not decrease. However, over the same increase in volume control total and grain root growth significantly decreases whilst chitting remains unchanged. This pattern also occurred for total root growth and grain chitting at 25°C. Therefore it appears possible that the excess water either delayed chitting or inhibited root growth in these controls. Further, it is interesting to note that for the 6 ml volume at 25°C total root and root weights per grain for treated and controls were, in contrast to chitting, not significantly different. For the same volume at 15°C all treatment and control parameters were significantly different. This would tend to indicate that at least at this volume root growth of each treated grain was less inhibited.

5.5 ROOT GROWTH SUBSEQUENT TO HYDROSTATIC PRESSURE

5.5.1 Introduction

Davidson and Jangaard (1978) monitored root growth in a damp chamber at room temperature for 24 hours. They observed that the root growth (chitting) of pressure treated grains was less vigorous than that of controls. Yoshida *et al*. (1979) reported that following hydrostatic pressure root growth was restricted and variable. Specifically, they

determined the root growth (final root dry weight) of isolated embryos after 72 hours. They reported that the root growth of treated embryos was as much as 50% less than that of controls. Eyben and Droogenbroeck (1969) stated that hydrostatic pressure delayed germination and caused irregular radicle growth.

Restricted root growth following treatment implies that hydrostatic pressure reduces the rate of root growth subsequent to chitting. However, reduced final root weights may simply result from delayed chitting. In the previous sections it was not possible to determine whether the reduced grain root growth following pressure treatment was the consequence of reduced root growth and/or delayed chitting.

5.5.2 Materials and Methods

Bonanza and Argyle were steeped continuously with aeration at 15°C. After 28 steeping hours samples of both varieties were subjected to a hydrostatic pressure treatment of 193 KPa for 60 seconds. Control and treated grains were set to germinate at 15°C in petri-dishes. Each petri-dish contained either 4, 2 or 1 ml of water, 2 Whatman filter papers and 20 grains. At each sampling time the total root weight and number of chitted grains of each replicate was determined. Six control and treatment replicates were used at each sampling time.

5.5.3 Results and Discussion

Figure 5 shows the total root weight increases of Bonanza control and treated grains grown with 2 and 4 ml of water. At 55 hours control and treatment weights were not significantly ($P=0.01$) different. However, by 72 hours control weights were significantly greater than treatments irrespective of water discipline. In addition, no significant differences occurred between treatments at 72 hours. From 96 hours onward 2 ml treatment weights were significantly greater than those of 4 ml treatments. Controls at 96 hours were not significantly different. However, from 130 hours onwards 2 ml weights were significantly greater than 4 ml weights. The non-significant decline in total root dry weight for 4 ml controls at 180 hours can only be explained by assuming fewer grains chitted in these replicates.

Control Bonanza root weight per chitted grain (Figure 6) was only significantly ($P=0.01$) different at 180 hours, where 4 ml control weight was significantly less than that of the 2 ml control. At 55 and 72 hours control and treatment weights were not significantly different. With the exception of the 2 ml treatment and 4 ml control weights at 180 hours, control weights were significantly greater than treatments from 96 hours onward. Treatment weights were never significantly different irrespective of water volume.

Figure 7 presents the total root weights for Argyle treatment and controls. At 50 hours weights for control and

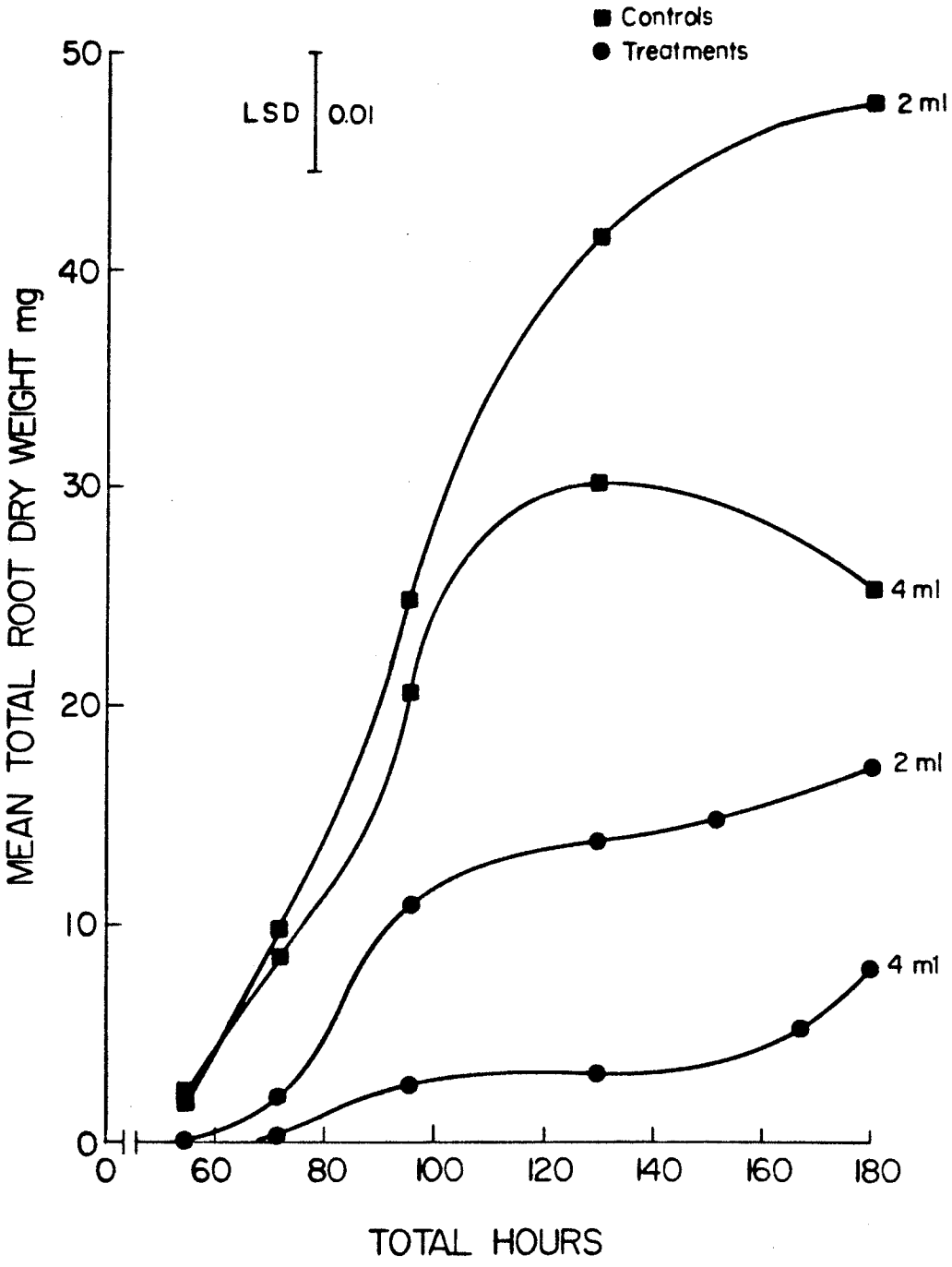


Figure 5. Bonanza total root growth.
$LSD_{0.01}$ 5.56 mg

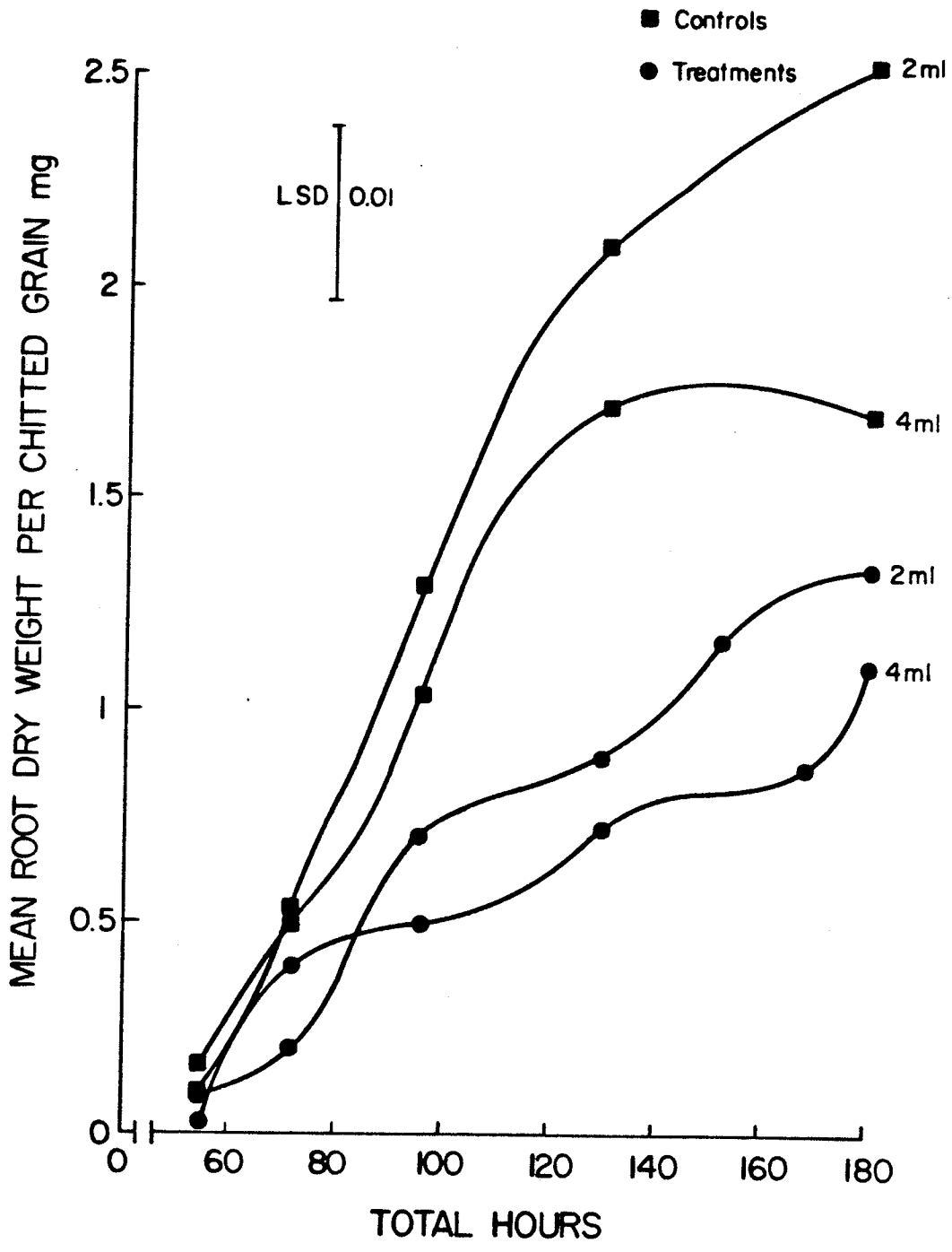


Figure 6. Bonanza root growth per chitted grain. $LSD_{0.01}$ 0.43 mg

treatments were not significantly (P=0.01) different. From 80 hours onwards, with exception of the 1 ml treatment and controls, control root weights were significantly greater than treatments irrespective of water volume. For the 1 ml volume, control weights were only significantly greater than that of treatments following 130 hours. After 130 hours 4 ml control weights were significantly less than the 2 ml control weights. At other sampling times control weights were not significantly different. Treatment weights were not significantly different at 80 hours. Following 80 hours however, 1 and 2 ml treatment weights were significantly greater than 4 ml treatment weights at 100 and 146 hours respectively. In addition, from 108 hours onwards 1 ml treatment weights were significantly greater than those of 2 ml treatments.

Root weights per chitted grain for Argyle are presented in Figure 8. Treatment and control weights were not significantly (P=0.01) different at 50 and 80 hours. From 80 hours onwards however, control weights at 2 and 4 ml were significantly greater than respective treatment weights. Control and treatment weight for the 1 ml discipline were only different at 158 hours. Controls were not significantly different for up to 130 hours. Following this time however, 4 ml control weights were significantly less than those of the 1 and 2 ml controls. Treatment weights at 2 and 4 ml were not significantly different from 50 to 140 hours. After 140 hours 2 ml treatment weights were, however, significantly

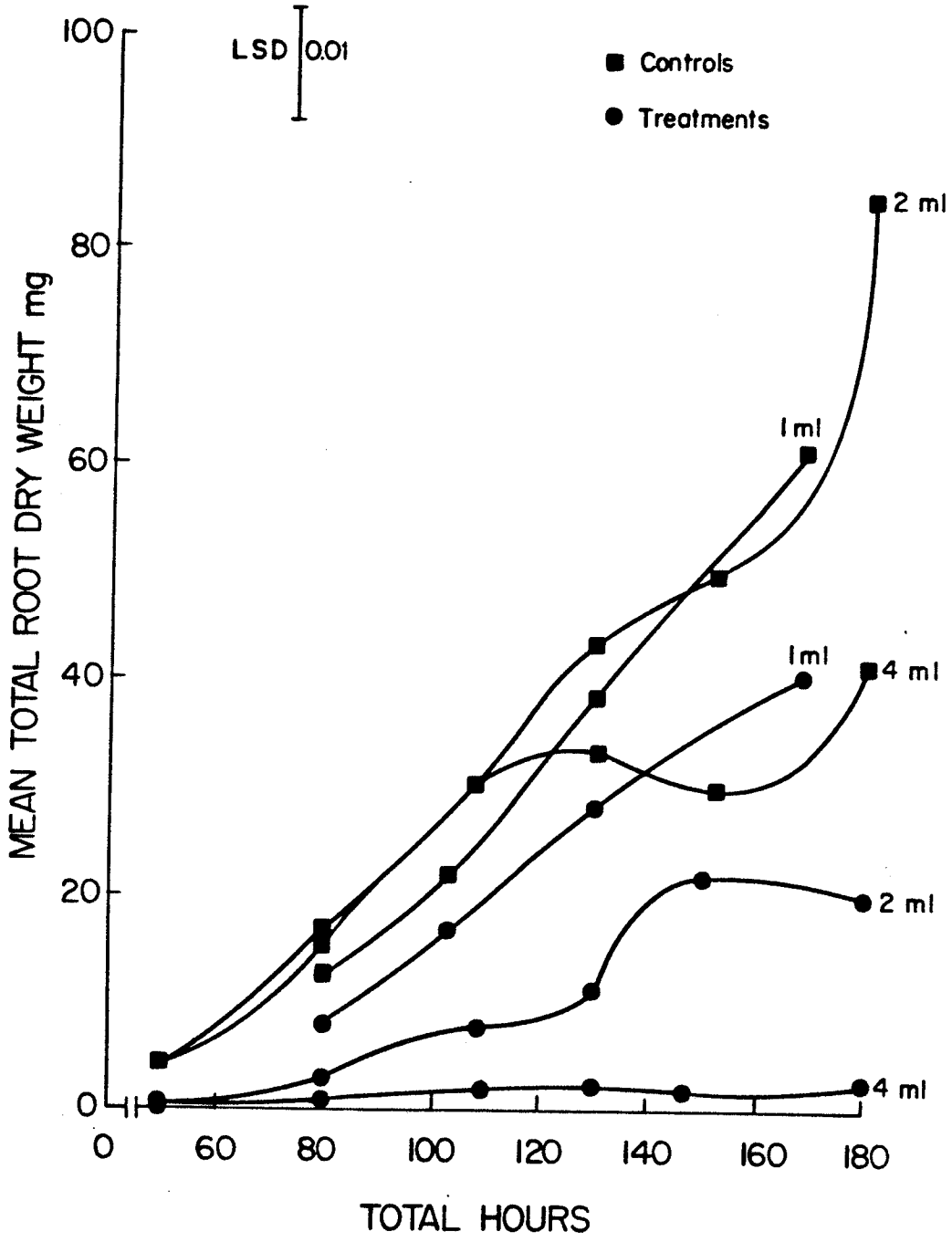


Figure 7. Argyle total root growth.
$LSD_{0.01}$ 10.55 mg

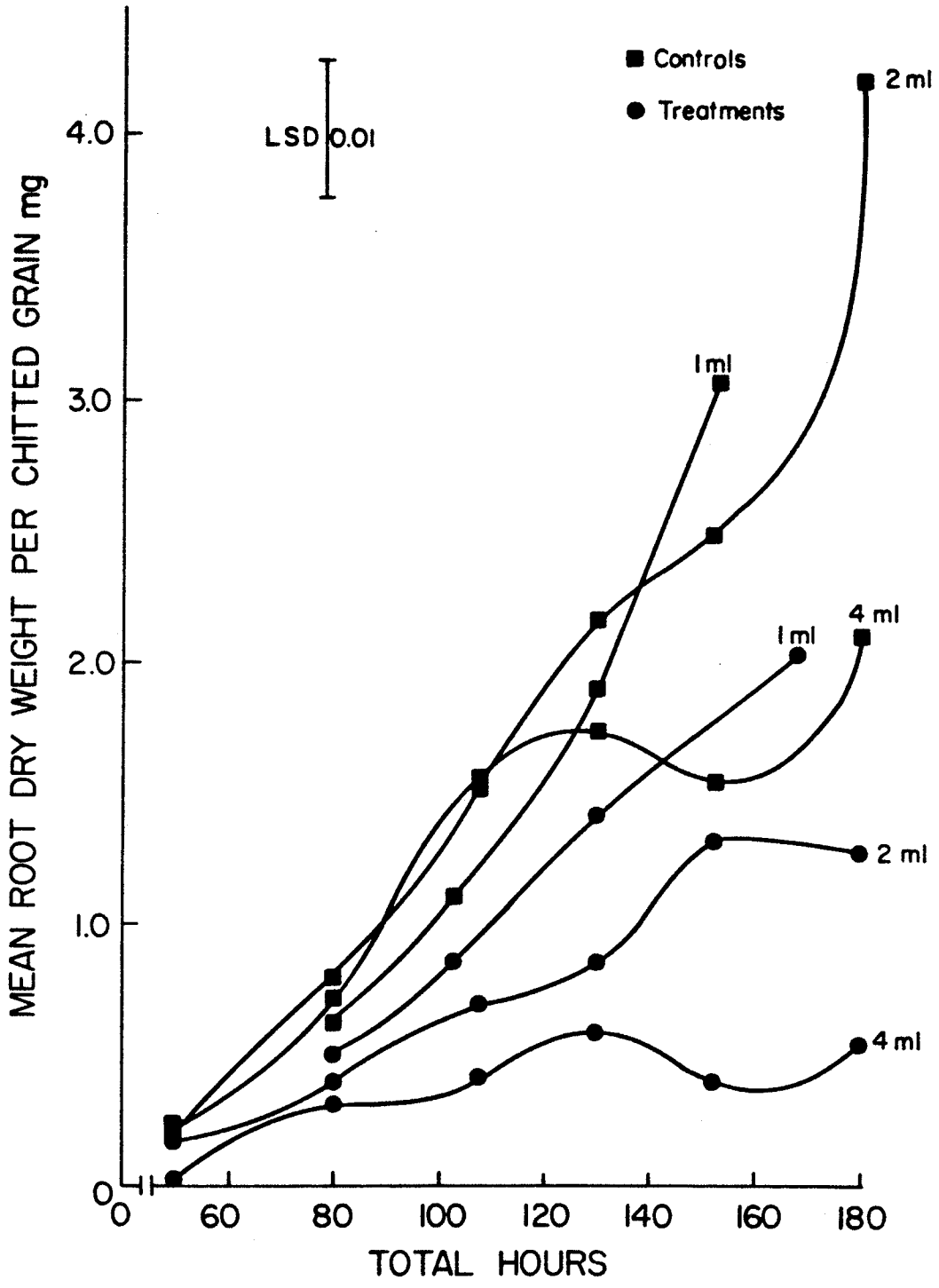


Figure 8. Argyle root growth per chitted grain.
$LSD_{0.01}$ 0.55 mg

greater than those for 4 ml treatments. Root weights for grains chitted at 1 ml were significantly greater than weights for 4 ml grains after 108 hours. In addition 1 ml treatment weights were significantly greater than 2 ml treatment weights at 130 hours.

Figures 6 and 8 clearly demonstrate that with increased germination time rootlet weight of control grain became significantly greater than that of treated grain under conditions of excess water. Control and treated chitted grain growth (root dry weight) for both Bonanza (Figure 6) and Argyle (Figure 8) were not significantly different for up to 72 and 80 hours respectively. However, with increasing germination time control growth became significantly greater than treatment growth in the presence of excess water. This divergence in growth per chitted grain could have only resulted from a slower root growth rate and/or delayed seminal development in treatments. The slower rate and/or the delayed seminal emergence in treatments only appeared however, to persist under conditions of excess water.

5.6 THE ROLE OF MICROORGANISMS IN PERPETUATING CHITTING INHIBITION FOLLOWING HYDROSTATIC PRESSURE TREATMENT

5.6.1 Introduction

Blum and Gilbert (1959) determined that the bacterial count around water-sensitive grains in petri-dishes was 1000 times greater under conditions of excess water.

Further, by prior grain surface sterilization with 1% NaOCl they were able to prevent germination inhibition of water-sensitive grain even in excess water. Jansson et al. (1959) also reported that surface sterilization reduced bacterial counts under the conditions of the water-sensitivity test. However, they were unable to improve the germination performance in excess water of water-sensitive grain irrespective of sterilization. Gaber and Roberts (1969b) successfully germinated water-sensitive grain in an excess solution of antibiotics. They concluded that water-sensitive grains contain (below the husk) a larger and more diverse population of micro-organisms that, under conditions of excess water, inhibit germination.

Yoshida et al. (1979) reported that some grains following hydrostatic pressure treatment develop signs of putrefaction. In addition, they noted that putrid grains develop a pink discolouration in the region of the scutellum and often have their endosperm replaced by a fluid mush. During preliminary experiments it was observed that grains several days after treatment had a putrid odour. In addition treated grains that failed to chit often had what appeared to be an exuded droplet of fluid associated with coleorhiza.

5.6.2 Materials and Methods

Bonanza was steeped with continuous aeration at 15° for 28 hours. Treated grain was subjected to 193 KPa pressure for 60 seconds while immersed in water or in antibiotic solution. The antibiotic solution contained penicillin, streptomycin and nystatin at concentrations of 800, 800 and 400 ppm respectively. This antibiotic combination proved most potent in preventing the expression of water-sensitivity by sensitive grains in excess solution (Gaber and Roberts, 1969b). Treated and control grains were set to continue germination in petri-dishes and germination boxes at 15°C for an additional 92 hours. Petri-dishes contained 2 Whatman No.1 filter papers and either 8 ml of water or antibiotic solution. Fifty grains were placed into each petri-dish; germination boxes contained 100 grains. Three replicates of each treatment and control were used to determine the mean percent chitting at 120 hours.

5.6.3 Results and Discussion

The mean percent chitted grain for each treatment is presented in Table 12. Control chitting was not significantly ($P=0.01$) different irrespective of germination environment. Chitting of grains for all treatments was significantly below control levels. Chitting of grains subjected to hydrostatic pressure prior to being germinated in water was significantly less than that of grains germinated

TABLE 12. The comparative effect of an Antibotic Head versus hydrostatic pressure at 28 steeping hours on 120 total hour mean percent chitted grain number.

Treatment	Post-Treatment Growth Environment	Mean Percent Chitted Grain
(Control)	Petri-dish with water[1]	90.00 a[2]
Hydrostatic Pressure[3]	Petri-dish with water	30.00 e
Antibiotic Head[4]	Petri-dish with water	40.00 cde
Hydrostatic Pressure	Petri-dish with antibiotic Solution[5]	36.67 de
Antibotic Head	Petri-dish with antibiotic solution	42.00 dc
(Control)	Germination box	94.67 a
Hydrostatic Pressure	Germination box	47.33 bc
Antibiotic Head	Germination box	54.67 b
CV		7.61

[1]Petri-dishes each contained 50 grains 2 Whatman No 1 filter papers and 8 ml of water.

[2]Mean percentages with the same alphabetical subscript are not significantly different at alpha 0.01 (Duncan's multiple range test).

[3]Pressure treatments were conducted at a magnitude of 193 KPa for a duration of 60 seconds.

[4]The antibiotic solution contained streptomycin, penicillin and nystatin at concentrations of 800, 800 and 400 ppm respectively.

[5]Each petri-dish contained 8 ml of antibiotic solution in place of water.

in antibiotic solution following exposure to the antibiotic head. Chitting of other treated grains in petri-dishes was not significantly different. In germination boxes grain subjected to the antibiotic did have higher chitting numbers than grain subjected to hydrostatic pressure, however, this difference was not significant.

The results clearly demonstrate that unlike H_2O_2 the antibiotic solution did not prevent or alleviate the chitting inhibition induced by pressure treatment. This observation indicated that the bactericidal properties of H_2O_2 did not determine H_2O_2 ability to alleviate hydrostatic pressure inhibition. However, the antibiotic solution, whether used as a head or as germination solution, did appear to slightly relieve a small fraction of the chitting inhibition which persisted in non-antibiotic treatments. Chitting of treated grain was at its greatest in the germination box environment. Thus, it was apparent that the presence of a film of moisture on the treated grain surface was more inhibitory to chitting than the presence of micro-organisms. In the germination box environment treated grain chitting in the absence of excess water was greater following the antibiotic head than the hydrostatic pressure treatment. Thus possibly micro-organisms in and around the grain were inhibitory to chitting even in the absence of excess water. Blum and Gilbert (1959) and Gaber and Roberts (1969b) proposed that micro-organisms promoted water-sensitivity by producing toxins and competing with the embryo for a limited

O_2 supply. It is clear here that their toxin production and O_2 competition was not the primary cause of chitting inhibition in treated grain.

5.7 SUMMARY AND CONCLUSIONS

The ability of hydrostatic pressure to inhibit grain chitting and subsequent root growth appeared to be significantly enhanced by testa/pericarp rupture. Sensitivity to treatment failed to decline with increased steeping and irrespective of radicle emergence. The significant inhibition of root growth and grain chitting following hydrostatic pressure treatment was not shown by grains subsequent to compression under H_2O_2. Hydrogen peroxide treatments did appear, however, to interfere (absence of root hairs) with normal root development. In addition, the final root weights of grains subjected to H_2O_2 treatments tended to be generally less (not significantly) than that of control grains.

The husk of the barley grain appeared only to play a minor role in the perpetuation of treated grain chitting inhibition. In contrast, the integrity of grain pericarps considerably influenced the degree of chitting inhibition. Acid decortication significantly improved the chitting of treated grains both in the presence (petri-dishes) and absence (germination boxes) of grain menisci.

The availability of free water appeared to contribute (less than pericarp integrity) to the persistence of chitting and root growth inhibition of treated grains.

Excess water (returning grains to a steep) prevented recovery. Intermediate water volumes delayed treated grain chitting and consequently reduced final root weights. The chitting and root growth performance of treated grains exposed to low water volumes was observed not to be significantly different from that of control grains. However, the chitting ability of treated grains placed in a dry environment (dry filter paper) was significantly less than control grains in the same environment and treated grains in low water petridishes.

Antibiotic treatments failed to restore treated grain chitting to control levels. However, the chitting of treated grains germinated in antibiotic solution and/or compressed under antibiotic solution was significantly greater than that of grains subjected to hydrostatic pressure before being germinated in water or antibiotic solution. It appears therefore, that micro-organisms did contribute to chitting inhibition to a limited extent and that this contribution could be alleviated and/or prevented provided antibiotic entered the grain.

Grains subjected to hydrostatic pressure appeared to exhibit many of the properties of water-sensitive grains. However, the treated grains differed from water-sensitive grains in several respects. Firstly, all treated grains had already germinated (had swollen coleorhiza). Secondly, whilst low volumes of free water did completely alleviate chitting inhibition, absence of free water did not. Thirdly,

decortication failed to totally obliterate chitting inhibition. In contrast, water-sensitivity can be broken by the removal of surface water films (interupted steeping), testa/pericarp rupture (swollen coleorhiza) and acid decortication. Therefore it would seem reasonable to conclude that the grains subjected to hydrostatic pressure exhibited a form of water-sensitivity that was severe and that in addition the determining site of imposition was more closely associated with the embryo.

CHAPTER VI

THE MORPHOLOGY AND ANATOMY OF THE COLEORHIZA REGION

6.1 Introduction

Yoshida et al. (1979) proposed that both the destruction of embryonic organs and microflora pushed into the embryonic region could be responsible for the restriction of respiratory activity, abnormal metabolism and putrefaction. Eyben and Droogenbroeck (1969) reported that hydrostatic pressure failed to push water into the embryo and that cellular disruption did not occur. In addition, they rejected the notion that anoxia could be responsible for respiratory restriction. Yoshida et al. (1979 observed, and Plate 7 depicts, that ink can be forced into the embryonic region by hydrostatic pressure. Further, they determined that the moisture content of the endosperm and embryo increased as a consequence of hydrostatic pressure treatment. It is apparent from the results presented in Chapter 5 that anoxia may have played a significant role in hydrostatic pressure inhibition. However, anoxia may have resulted from mechanical damage sustained during compression and decompression. Davidson and Jangaard (1978) observed that prior to wet steep-out roots were turgid, whilst following

Plate 7. Control grains were immersed in ink for 60 seconds. Treated grains were immersed in ink and subjected to a hydrostatic pressure of 192 kPa for 60 seconds. The ink (Osmiroid free flowing black) solution contained 1 part ink to 14 parts water.

Plate 7a. Dorsal view of intact embryos, 1) control, 2) treated.

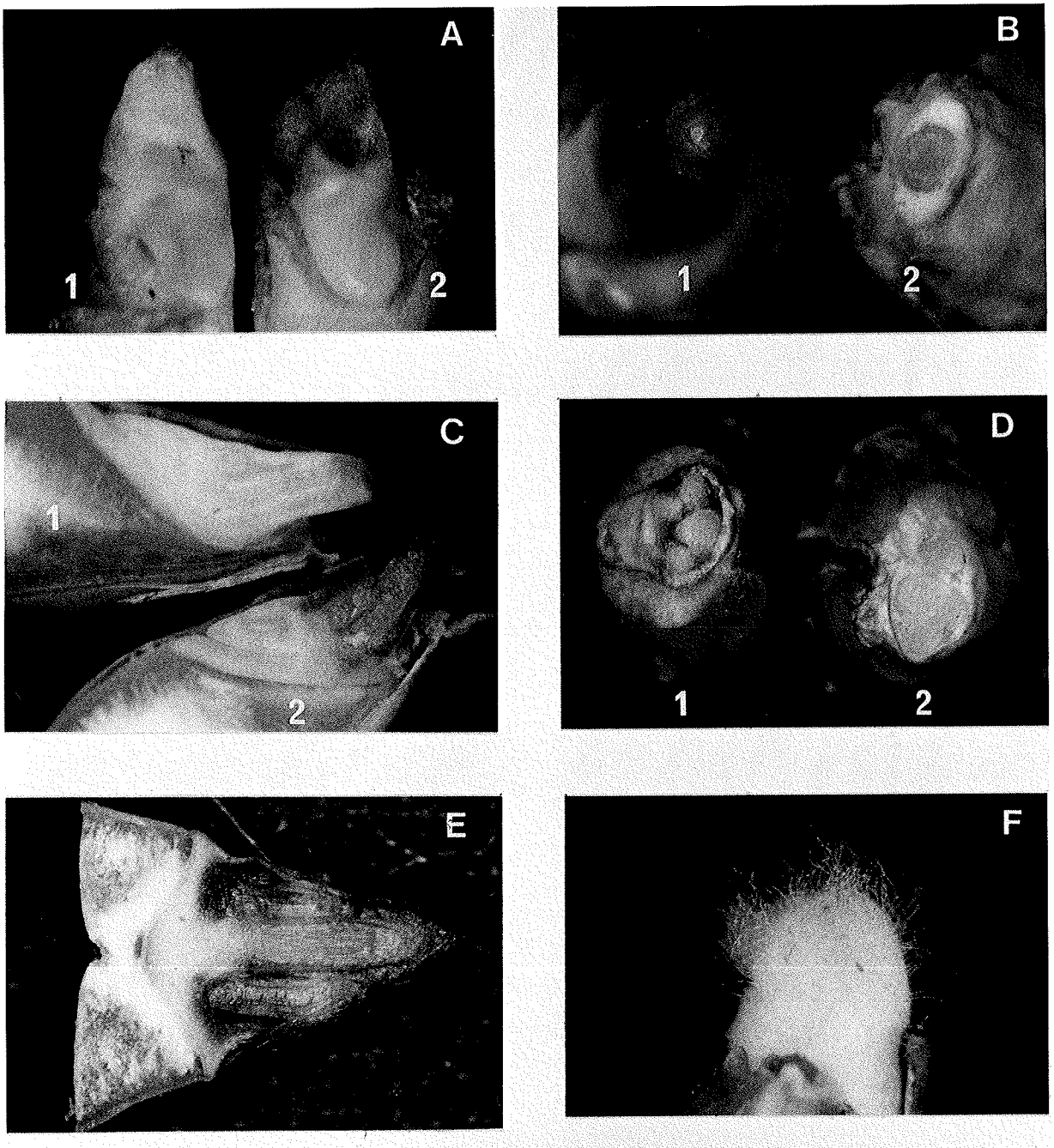
Plate 7b. Cross-section through distal coleorhiza region 1) treated, 2) control.

Plate 7c. Longitudinal-section (parallel to ventral furrow) through embryos, 1) control, 2) treated.

Plate 7d. Cross-section through proximal coleorhiza region, 1) treated, 2) control.

Plate 7e. Longitudinal-section (perpendicular to ventral furrow through treated embryo.

Plate 7f. Root hairs on dorsal coleorhiza surface following eruption of seminals. The grain was germinated on wet paper at room temperature.



Hordeum vulgare cv. Argyle

Plate 7

steep-out roots were flaccid. Kuiper (1972) noted that root membrane hydrophobicity declines with increased hydrostatic pressure up to several atmospheres. Using a Cambridge Stereoscan MKIIA scanning electron microscope, the coleorhiza morphology of Bonanza and Arygle was observed to determine the influence of treatment on structural integrity.

6.1.1 Materials and Methods

Bonanza was steeped with aeration at 15°C for 30 hours. Treated grains were subjected to a hydrostatic pressure of 145 KPa for 30 seconds. Control and treated grains were then fixed by a procedure outlined in O'Brien and McCully (1981). Initially grains were washed in distilled water. Primary fixation was carried out in a solution of 3% gluteraldehyde in 0.05 M dibasic sodium phosphate buffer (pH 6.8) at room temperature for 24 hours. Grains were then washed in four changes of 1:1 distilled water/buffer solution over 1 hour. Secondary fixation was then carried out in a buffered 1% solution of osmium tetroxide for 2 hours on ice. Following fixation in OsO_4 the grains were again washed in a 1:1 solution of distilled water/buffer before being finally rinsed in distilled water.

Grains were then dehydrated in acetone as recommended (Sorvall handbook). This involved dehydration up through an acetone series (25, 50, 70, 95 and 100%) with 1 hour and four acetone/water solution changes at each step in

the series. When dehydration could not be carried out continuously grains were left overnight in 70% acetone. Following dehydration grain husks were removed before each grain was sectioned perpendicularly to the furrow. The embryo ends of the grains were then critical point dried (CPD) Anderson (1951) in a Sorvall critical point drier. The CPD grains were then mounted on studs and coated with gold (approximately 100Å) using a Balzer sputter coater. SEM was then used to examine 3 treated and 3 control grains.

6.1.2 Results and Discussion

The form of control coleorhizas was as depicted in Plate 8. The distal region of all coleorhizas examined showed some degree of surface disruption. Proximally however, coleorhizas appeared to have a relatively uniform surface with only the occasional disruption site. Closer examination (Plate 9a) of the surface of coleorhizas revealed that a ruffled surface film was present through which the underlying parenchyma cells could be discerned. Proximally discrete apertures were observed to occur in the film above parenchyma lacunae. Intercellular spaces, (lacunae), determined by the polyhedral geometry of parenchyma cells, have been observed in germination seeds (Gershoy *et al*. 1976). The apertures in the proximal coleorhiza region were most frequently discrete (Plate 9b), however, occasionally they were fused by what appeared to be film tearing (Plate 9a).

Plate 8. Control coleorhiza critical point dried after 30 steeping hours. (var. Bonanza)

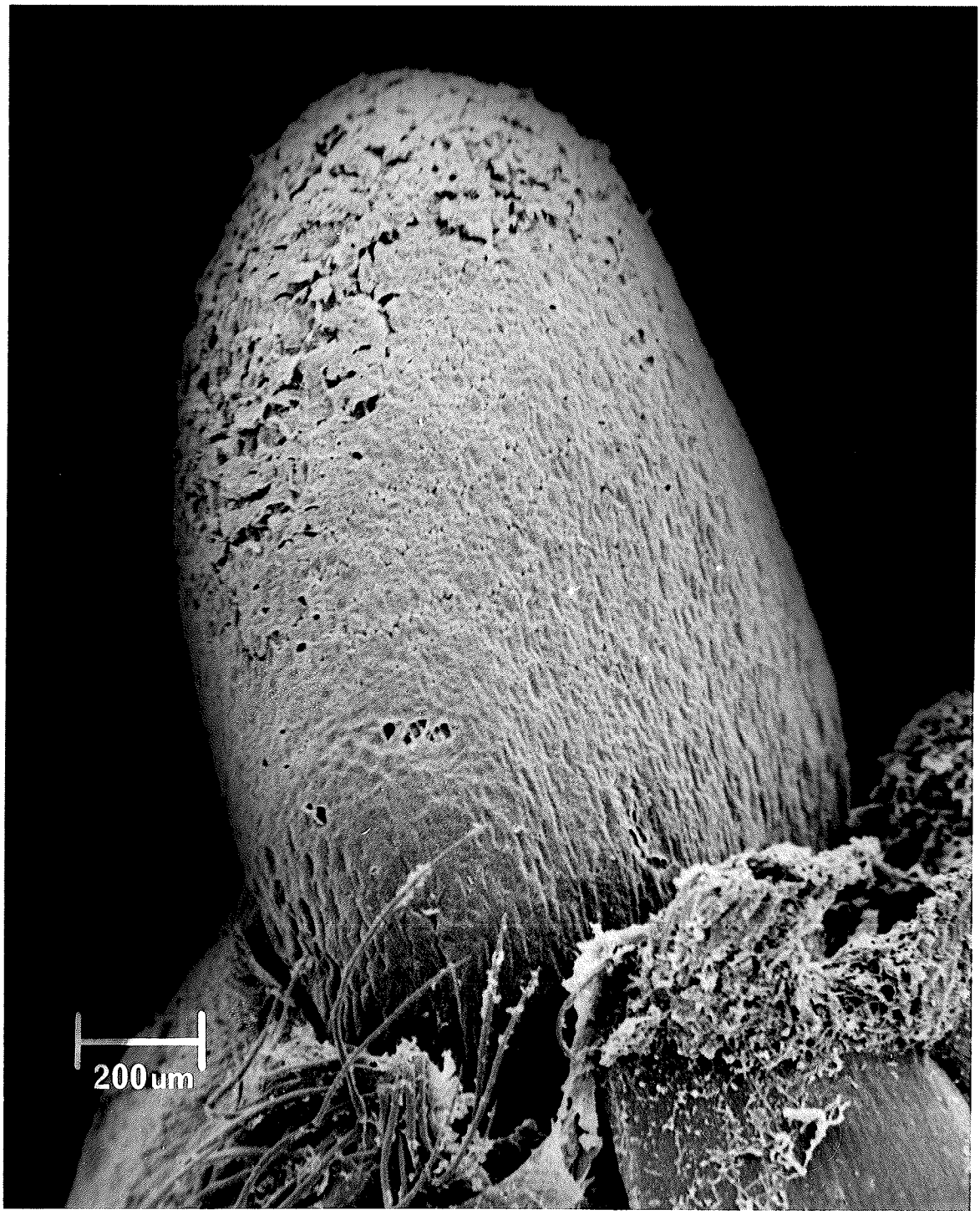
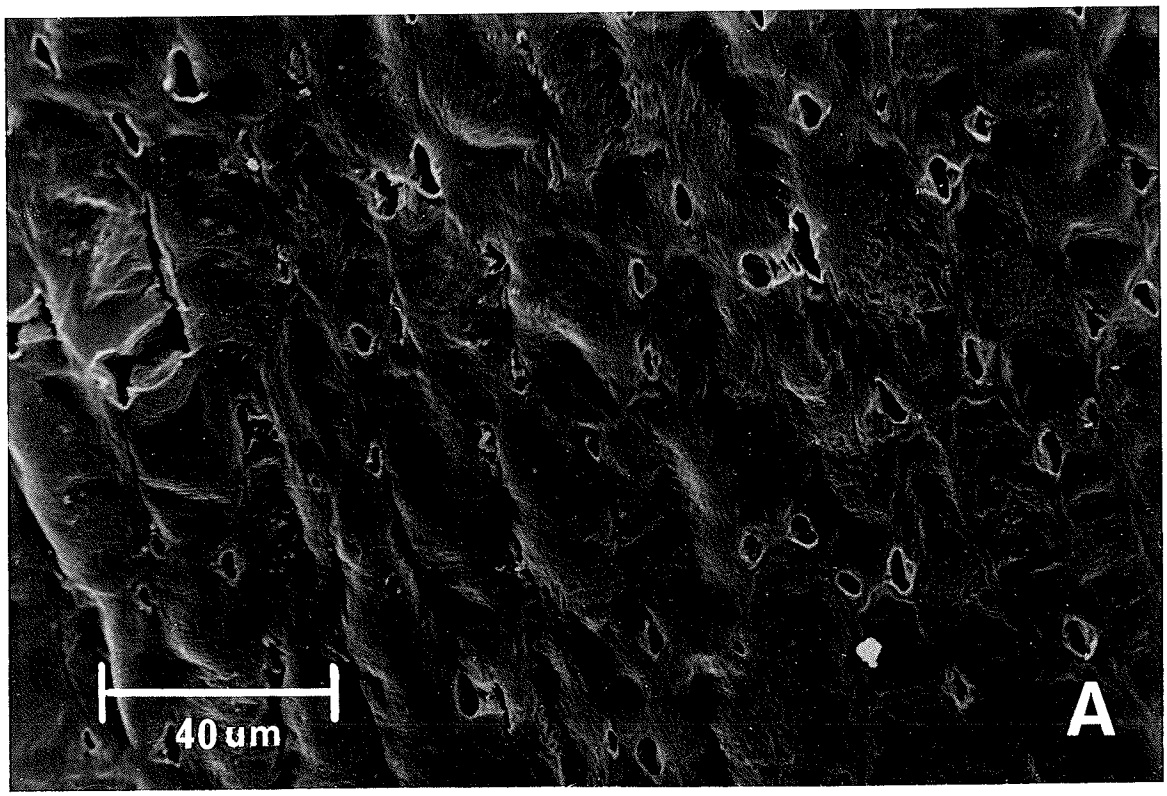
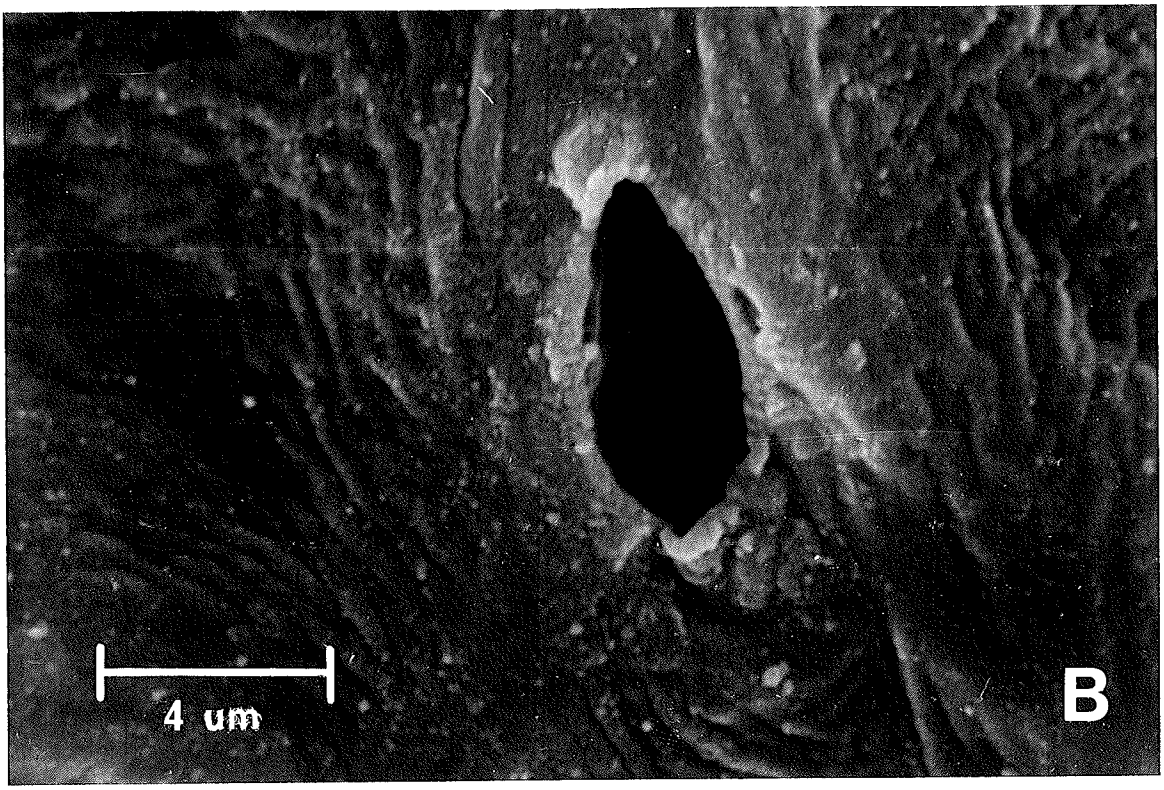

200um

Plate 9a. Control grain, critical point dried coleorhiza proximal surface detail after 30 steeping hours (var. Bonanza).

Plate 9b. Control grain, discrete aperture and surface film of the critical point dried coleorhiza proximal surface after 30 steeping hours (var. Bonanza).


40 um
A


4 um
B

The general form and surface features of treated coleorhizas did not appear to be different from controls. Treated coleorhizas were also disrupted distally (Plate 10a). In addition the surface film with discrete apertures (Plate 10b and 11a) was also present on treated coleorhizas. Both treated and control coleorhizas had bacteria adhering to their surfaces (Plates 11a and 11b). Bacteria were also present within lacunae (Plate 10b).

The distal surface disruption and proximal film apertures may have been artifacts of the preparation technique. Critical point drying would have been the least likely source of distortion since the liquid/gas interface does not exist at the critical point. Fixation and dehydration would have been far more likely sources of disruption.

6.2 CPD VERSUS AIR DRYING, FREEZE DRYING AND WATER STABILIZATION

6.2.1 Introduction

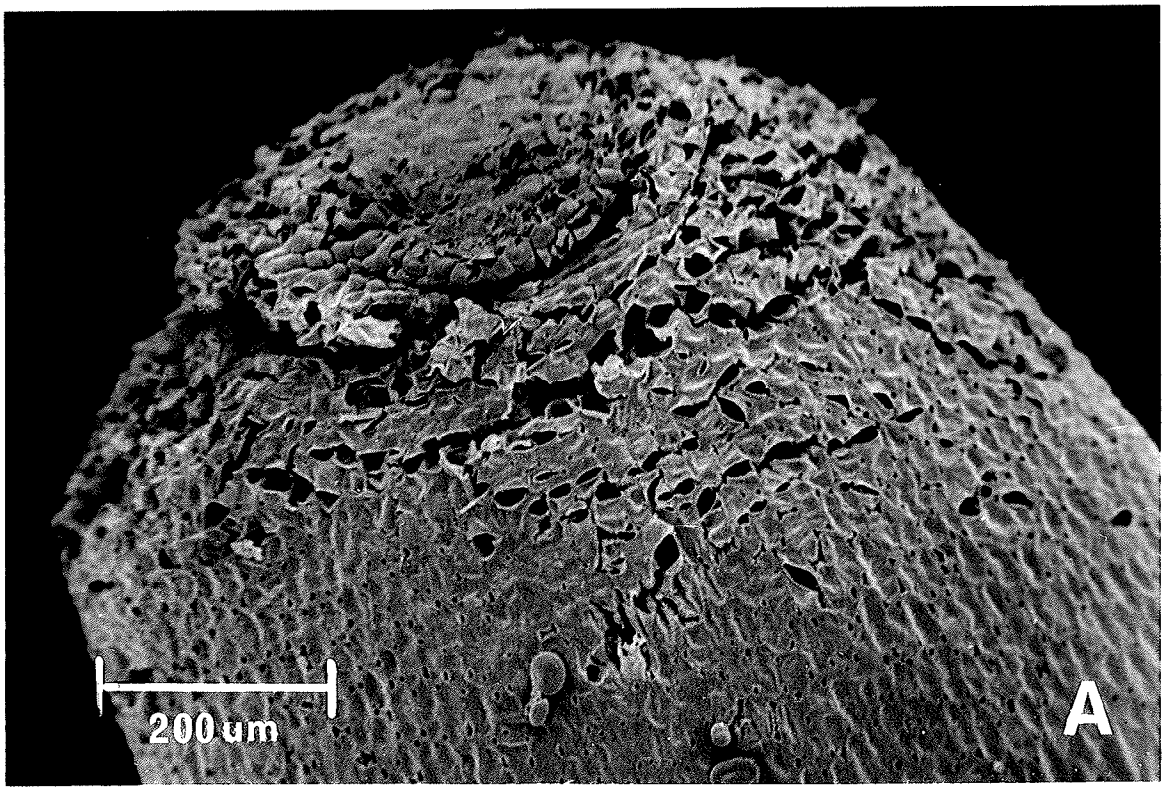
In order to obtain a measure of the relative contributions of fixation, dehydration, and critical point drying to coleorhizas distortion, several SEM preparation techniques were used.

6.2.2 Materials and Methods

Bonanza was steeped with aeration at 15°C. Grains were removed from the steep at 30, 35, 44 and 46 steeping hours. Thirty hour grains were prepared for SEM by the tech-

Plate 10a. Treated grain, distal surface detail of critical point dried coleorhiza after 30 steeping hours. (var. Bonanza).

Plate 10b. Treated grain, proximal surface detail of critical point dried coleorhiza after 30 steeping hours (var. Bonanza).


200 um
A

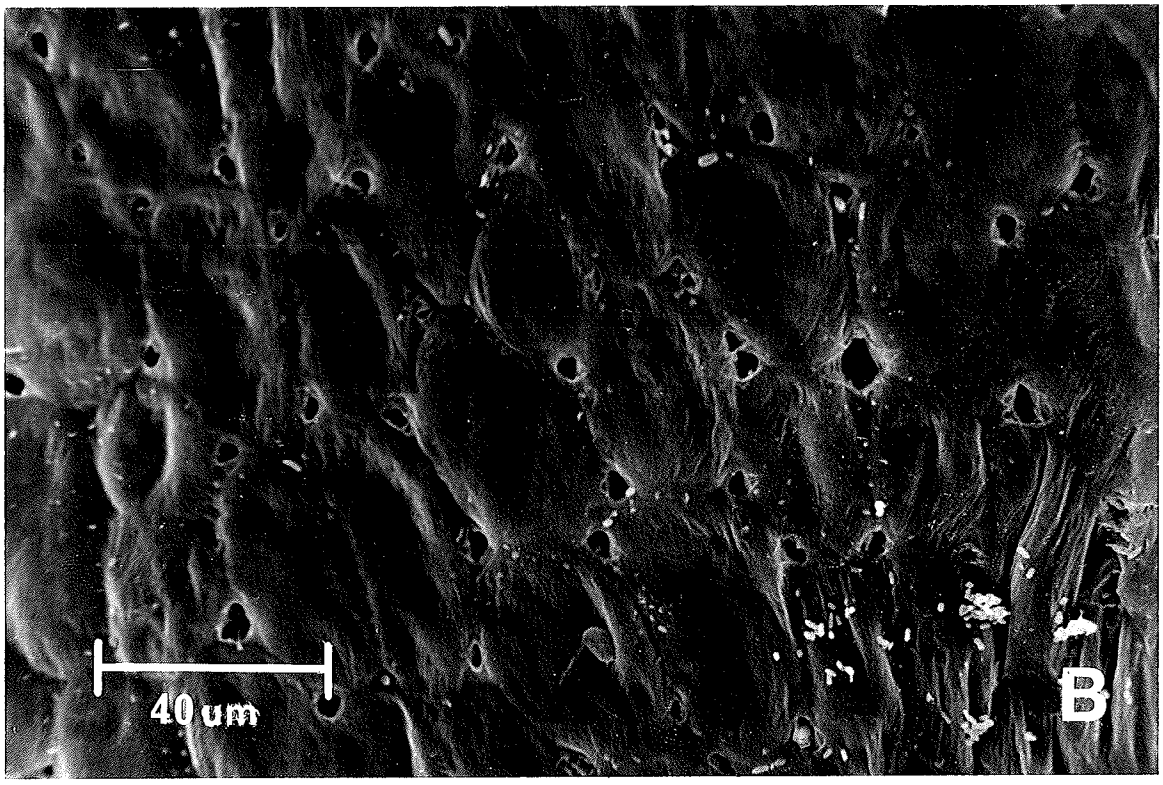
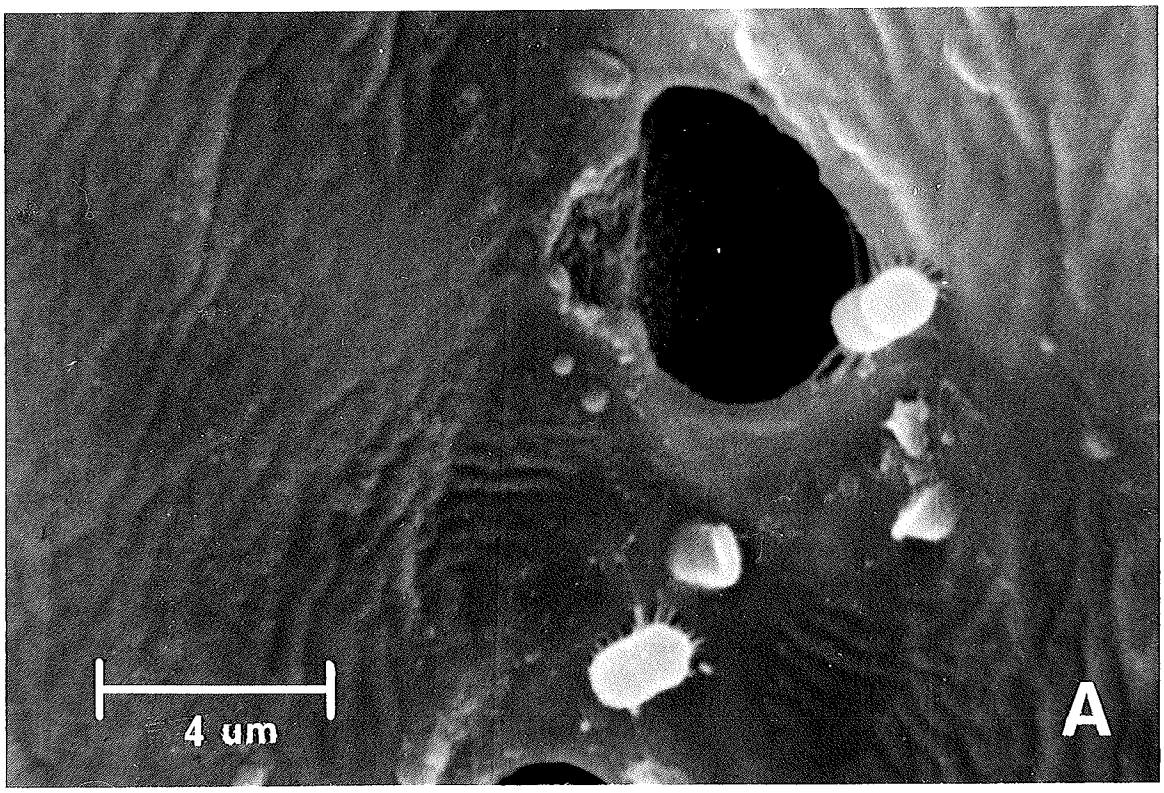
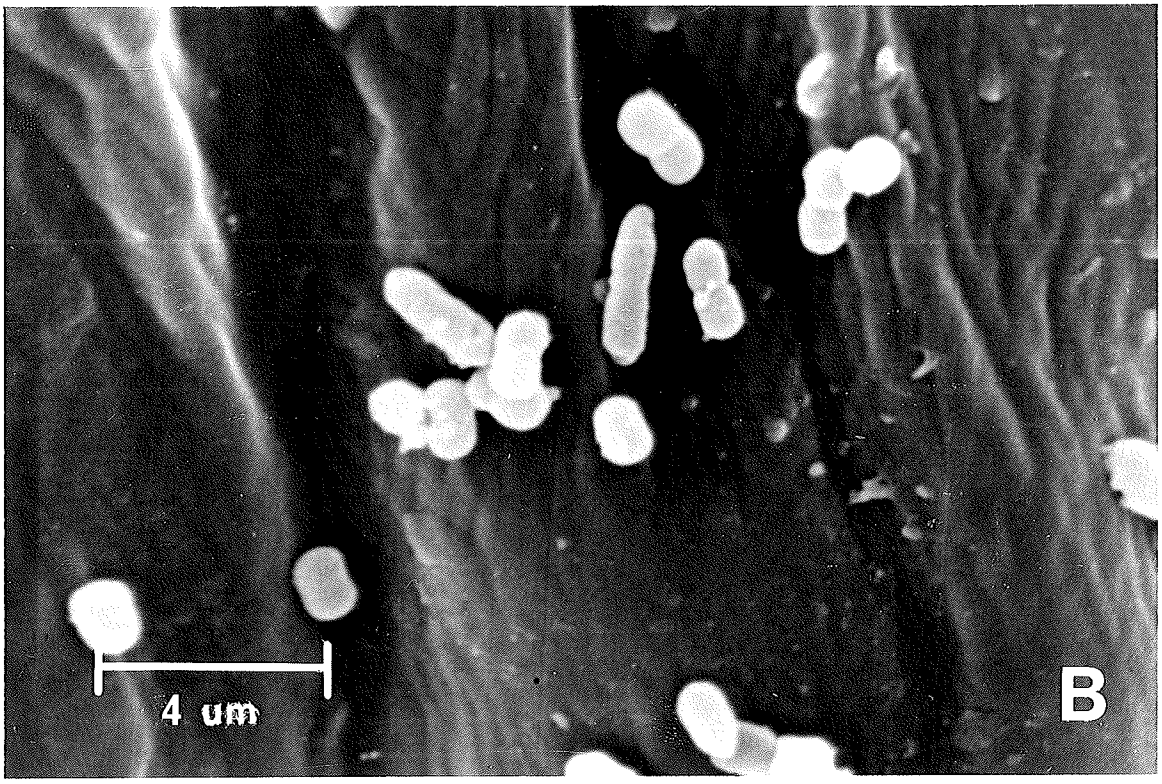

40 um
B

Plate 11a. Treated grain, discrete coleorhiza surface aperture and associated bacteria.

Plate 11b. Treated grain, coleorhiza ruffled surface film and bacteria.


4 um
A


4 um
B

nique outlined in section 6.1.1. Grains sampled at 35 hours were frozen for 10 hours before being freeze dried (FD) for 24 hours. One 44 hour grain sample was dehusked and allowed to air dry (AD) for 24 hours before being vacuum dried (VD) for an additional 24 hours. Another 44 hour sample was frozen for 24 hours before being VD for 24 hours. Grain steeped for 46 hours was VD for 24 hours.

Bonanza grains (50) were set to germinate at 25°C in a petri-dish. After 20 hours grains with swollen coleorhizas were frozen in liquid nitrogen (water stabilization). For each preparation technique the coleorhiza surface of 2 grains was examined.

6.2.3 Results and Discussion

The coleorhizas of CPD grain (Plate 12a) and those of grain frozen in liquid nitrogen (Plate 14d) showed the least distortion. Of the other techniques AD (Plate 12c and VD (Plate 13a) appeared to produce less distortion of the total stucture than techniques which involved freezing (Plates 13c and 14a).

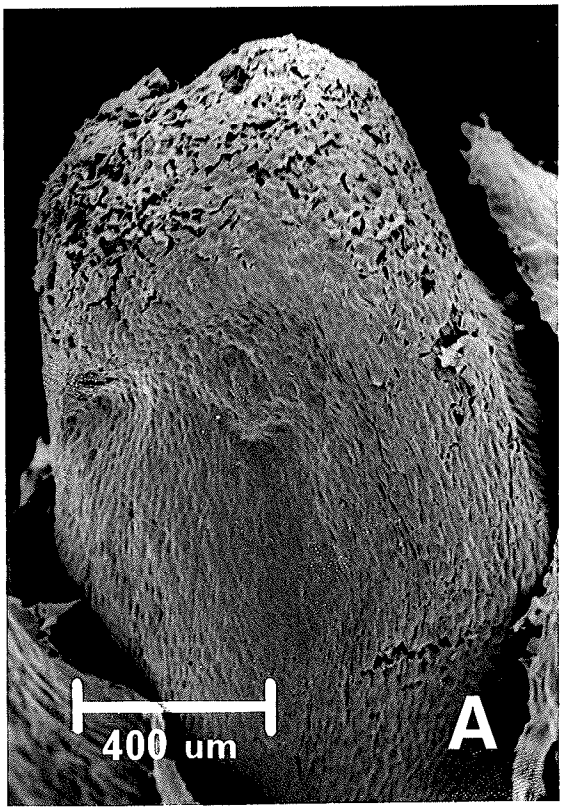
The distortion of surface detail was the least for CPD grain (12b). Film apertures of AD (Plate 12d) and FD (Plate 14b) grains were mostly discrete in proximal regions. The coleorhiza surface film of grain frozen in liquid N_2 appeared to have shrunk as a result of preparation. The extent of surface lacunae is far more visible in these grains (Plate 14d). Simple VD of grains caused adjacent apertures to become both distorted and fused (Plate 13b). Vacuum drying of frozen grain caused severe disruption of the coleorhiza surface (Plate 13d).

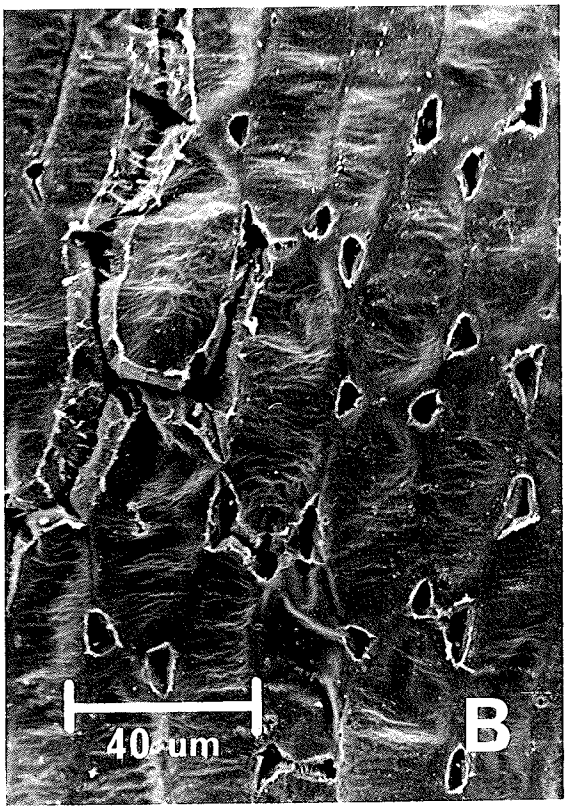
Plate 12a. Coleorhiza critical point dried after 30 steeping hours.

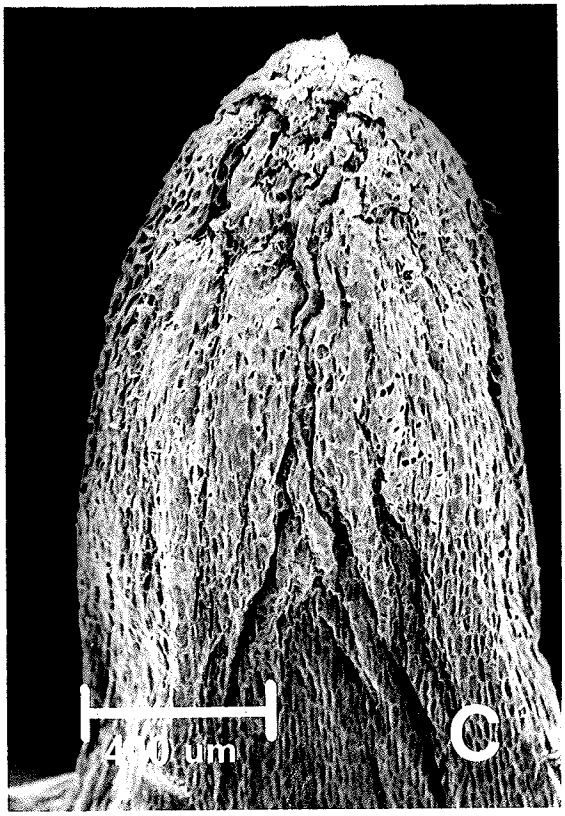
Plate 12b. Distal surface detail of critical point dried coleorhiza shown in plate 12a.

Plate 12c. Coleorhiza air and vacuum dried after 44 steeping hours.

Plate 12d. Surface detail of air and vacuum dried coleorhiza shown in plate 12c.


400 um
A


40 um
B


C

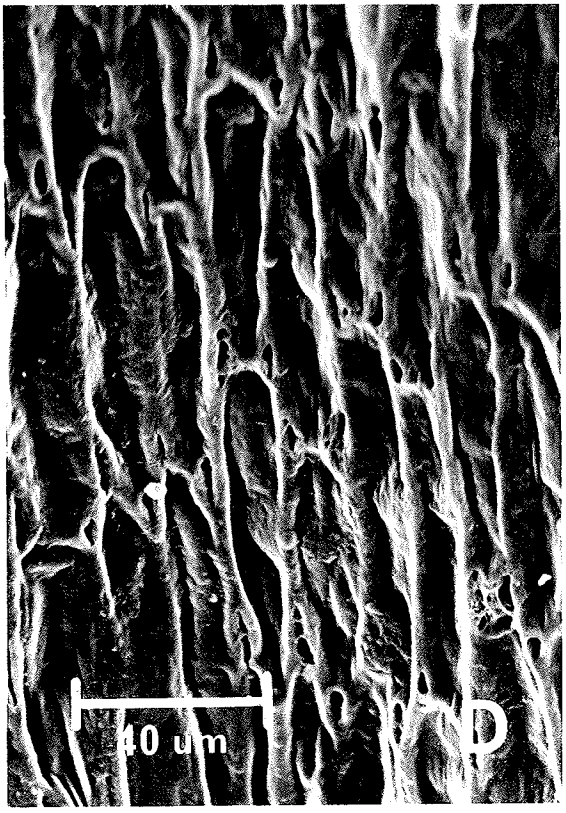
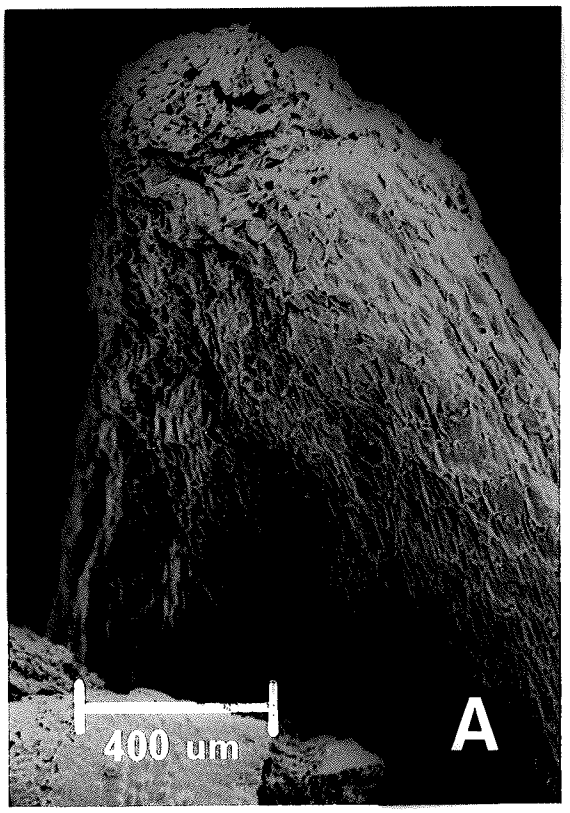

40 um
D

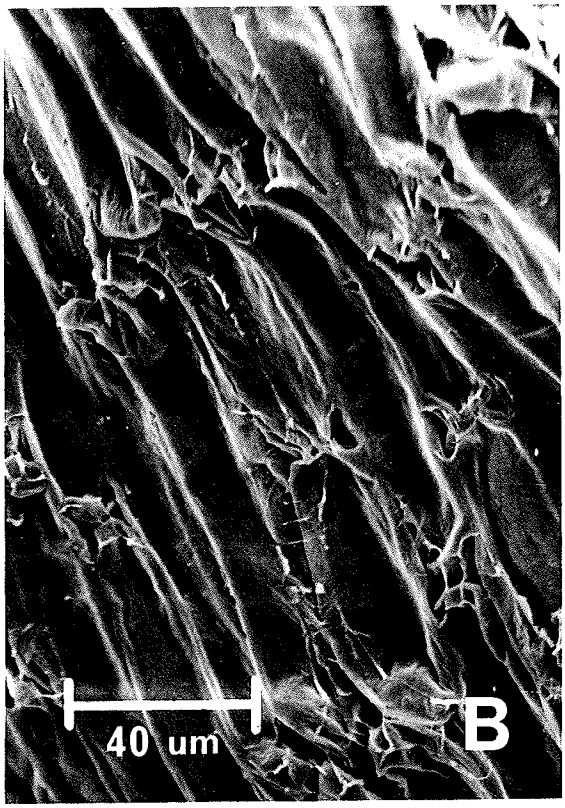
Plate 13a. Coleorhiza vacuun dried after 46 steeping hours.

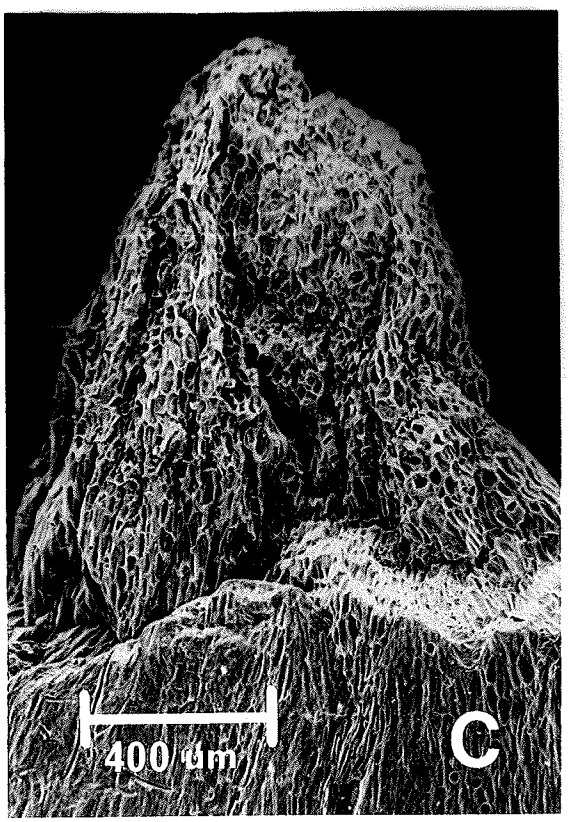
Plate 13b. Surface detail of vacuum dried coleorhiza shown in in plate 13a.

Plate 13c. Coleorhiza frozen and vacuum dried after 44 steeping hours.

Plate 13d. Surface detail of frozen and vacuum dried coleorhiza shown in plate 13c.


400 um
A


40 um
B


400 um
C


40 um
D

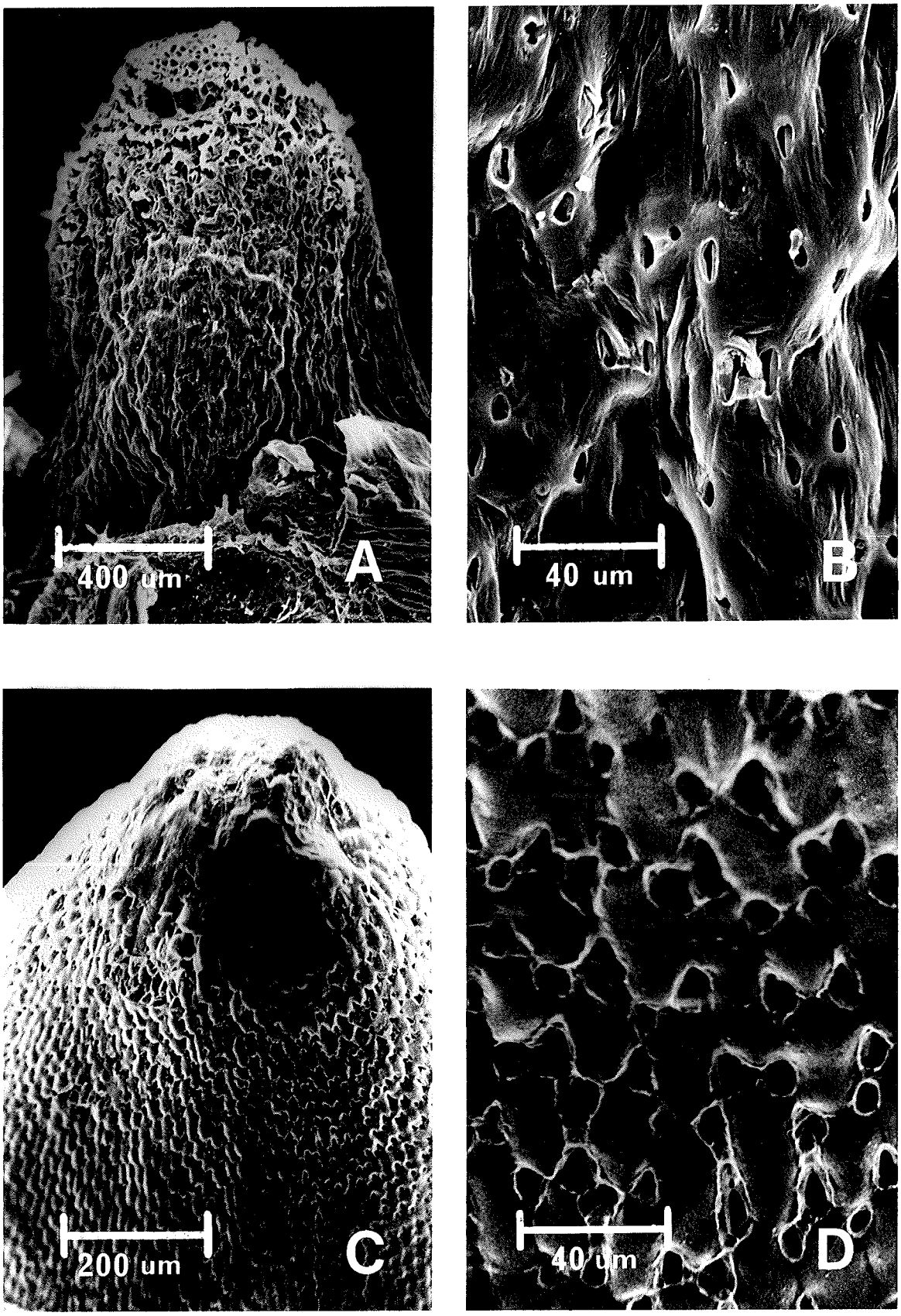
Unlike the distal ends of CPD coleorhizas (Plates 8, 10a and 12a) the distal coleorhiza portion of petri-dish germinated grains frozen in liquid N_2 (Plate 14c) were not disrupted. This suggested that the disruption of the distal ends of CPD coleorhizas did not occur during testa/pericarp rupture. The occurrence of discrete film apertures in CPD, FD, AD and liquid N_2 frozen coleorhizas suggested that if these were the consequence of preparation technique, distortions during fixation and dehydration were not necessary to induce their formation. In addition, the presence of apertures in the surface film of CPD and FD coleorhizas indicated that surface tension forces were not responsible for their formation. Where surface tension forces did act (Plates 12d and 13b) surface distortion was much greater. However, the coleorhizas surface of FD grains (Plate 14b) was undulating whilst the surfaces of CPD and liquid N_2 frozen coleorhizas (Plates 12b and 14d) were not. In contrast to slow freezing (Plate 14b) rapid freezing in liquid nitrogen (Plate 14d) avoids the distortion which may result as a consequence of large ice crystal formation. The rate of thermal energy loss during rapid freezing prevents the formation of large distorting crystals. Depending on the rate of rapid cooling and the moisture content of the tissue, water may assume either the vitreous state were there is inadequate time for molecules to assume ice-like configurations (nucleation points) or form innocuous ice crystals due to the limited movement of

Plate 14a. Coleorhiza freeze dried after 35 steeping hours.

Plate 14b. Surface detail of freeze dried coleorhiza shown in in plate 14a.

Plate 14c. Coleorhiza frozen in liquid N_2.

Plate 14d. Surface detail of coleorhiza frozen in liquid N_2.


400 um
A
40 um
B
200 um
C
40 um
D

water molecules to nucleation points (O'Brien and McCully, 1981). Therefore it would appear that the surface distortion (Plate 14b) caused by large ice crystal formation was not responsible for the discrete apertures. Indeed, where surface distortion did not occur following rapid cooling (Plate 14d) film apertures were not discrete. The surface film was however, influenced by differing preparation techniques. The AD and VD films (Plates 12d and 13b) appeared to be buckled upwards over lamellae. However, AD films had discrete apertures despite the action of surface tension forces. The coleorhiza surface film may be the most sensitive coleorhiza feature, however, it is reasonable to assume it is more elastic than a cell wall since few tears occurred in its surface even when grains were AD and FD. In contrast, the combined distortion of large ice crystals and surface tension forces resulted in large holes in coleorhiza (Plates 13c and 13d).

6.3 EMBRYO DORSAL MORPHOLOGY

6.3.1 Introduction

The coleorhiza surface film was present on steeped and petri-dish germinated grains. Esau (1977) noted that the coleorhiza has been defined as degenerated primary root. Dayan *et al*. (1977) reported on the nature of the mucilagenous layer (mucigel) associated with the surface of barley

roots. They observed that the mucigel had bounding membranes that enclosed a heterogenous matrix of granular regions and fibrillar layers. The thickness of the coleorhiza surface film can be perceived in Plate 14b as a result of the surface distortion. Bacteria have been observed to produce mucilage on areas of wheat roots free of plant root mucigel (Rovira and Campbell, 1974). In addition Rovira and Campbell reported that bacteria adhere to the sheet mucigel of plant origin by fibrous mucilage of bacterial orgin (See Plate 11a).

Dayan et al. (1977) drew attention to the difficulty of extracting solely mucigel components from barley roots sterilized with antibiotic. Their warm water extract (roots shaken in water at 35°C for 3 hours) caused the least disruption of root tissue. Only a fraction of the mucigel was soluble in the warm water extract. The soluble fraction was determined to be comprized of low molecular weight polysaccharides.

Thus it is possible that the coleorhiza and/or associated bacteria could secrete the surface film. In the following experiment the coleorhiza surface was examined prior to imbibition.

6.3.2 Materials and Methods

Grains of Bonanza and Argyle were decorticated in sulphuric acid as described in section 5.3.2. The grains

were then fixed, dehydrated and CPD in a similar manner to that outlined in section 6.1.1. The testa immediately above the embryo of each grain was removed with a fine pair of tweezers, SEM was then used to examine the exposed dorsal surface detail.

6.3.3 Results and Discussion

The dorsal surfaces of Bonanza and Argyle embryos were similar in appearance (Plate 15). The coleorhiza tips (Plate 15 area A and Plate 16A) appeared to have a crown of clearly discernable rectangular surface cells. In contrast, more proximal coleorhiza regions (Plate 15 area B, Plate 16B and Plate 17) were covered with a highly convoluted surface film through which the underlying cell form could not be discerned. In the mid region of the dorsal surface of (Plate 15 area C) the embryos had a distinct surface depression. The polyhedral form of the surface cells lining the depression (Plate 16C) were not obscured by the surface film which was less prominent in this region. In contrast to the coleorhiza surface, the coleoptile surface (Plate 15 area D) did not posses the convoluted surface film. In addition, the cells in this region appeared to be elongate rectangular and have thick walls (Plate 16D).

The crown of rectangular cells circumscribing the coleorhiza tip (Plate 16A) could be interpreted as a root cap vestige of the degenerate primary root. The amorphous

Plate 15. Dorsal surface of critical point dried embryo, (var. Argyle). See plate 16 for surface detail of areas a, b, c and d. For structure e see plate 18.

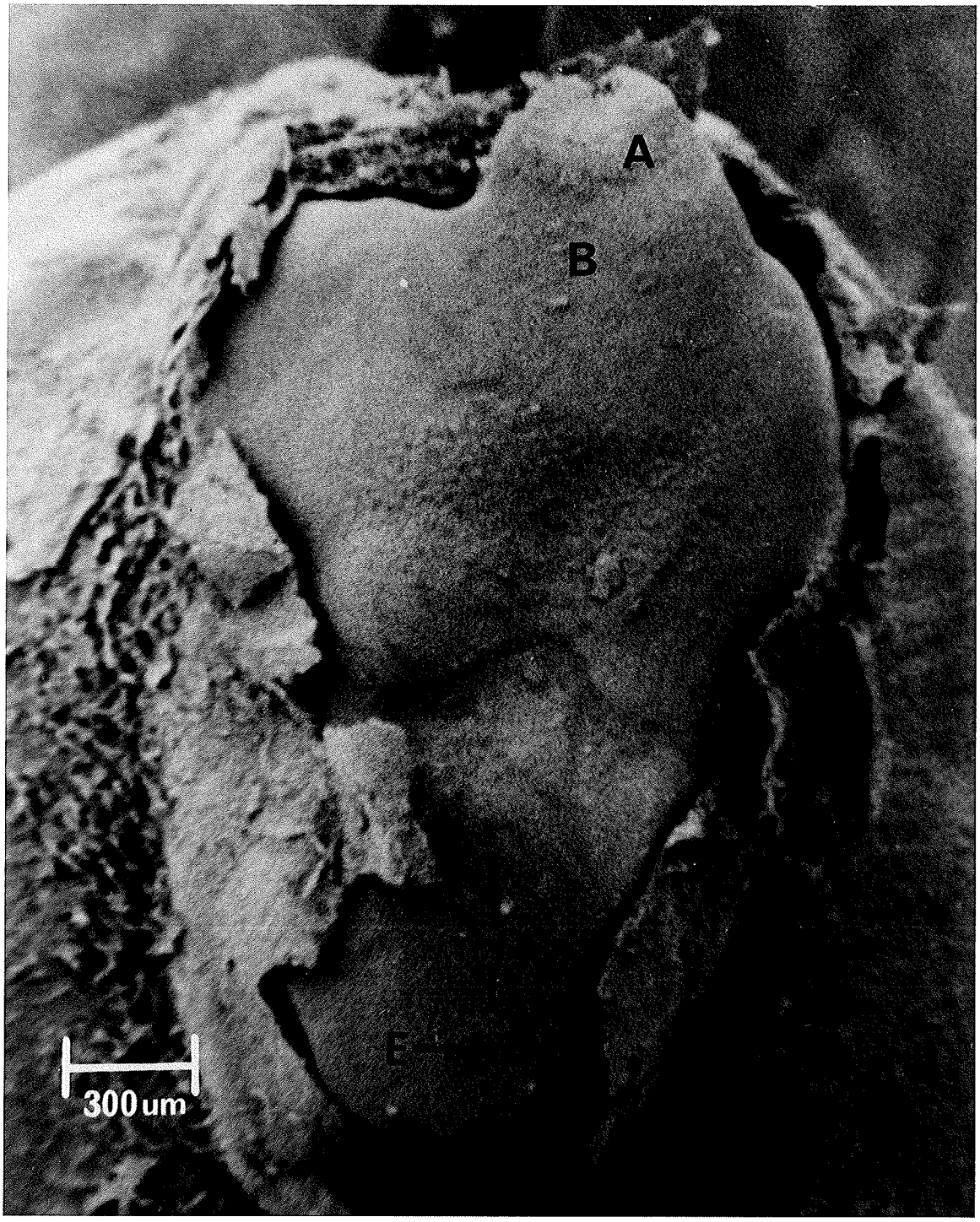
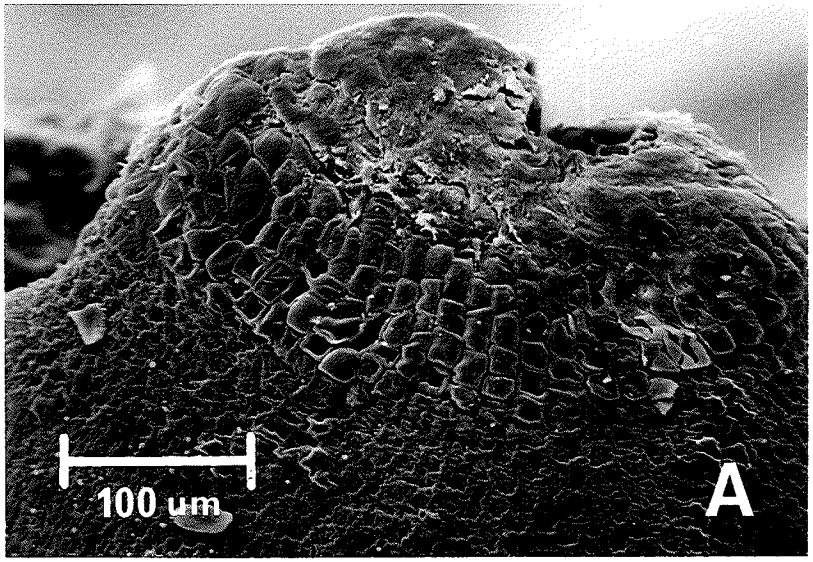

A
B
E
300 um

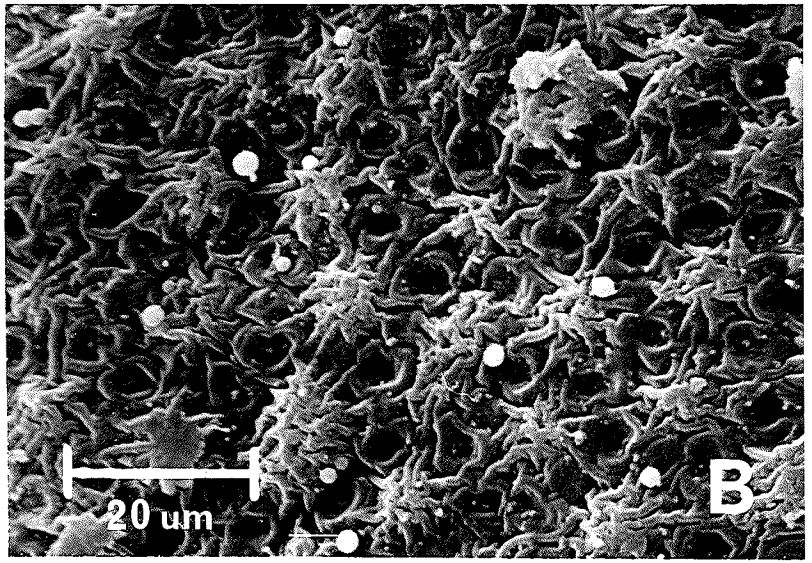
Plate 16a. Distal portion (coleorhiza) of critical point dried embryo (var. Argyle).

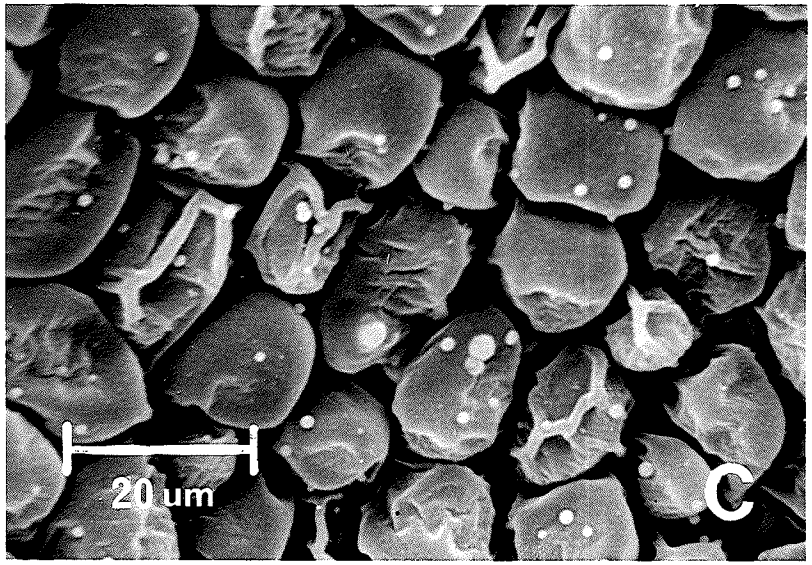
Plate 16b. Highly convoluted coleorhiza surface film of critical point dried embryo (var. Argyle).

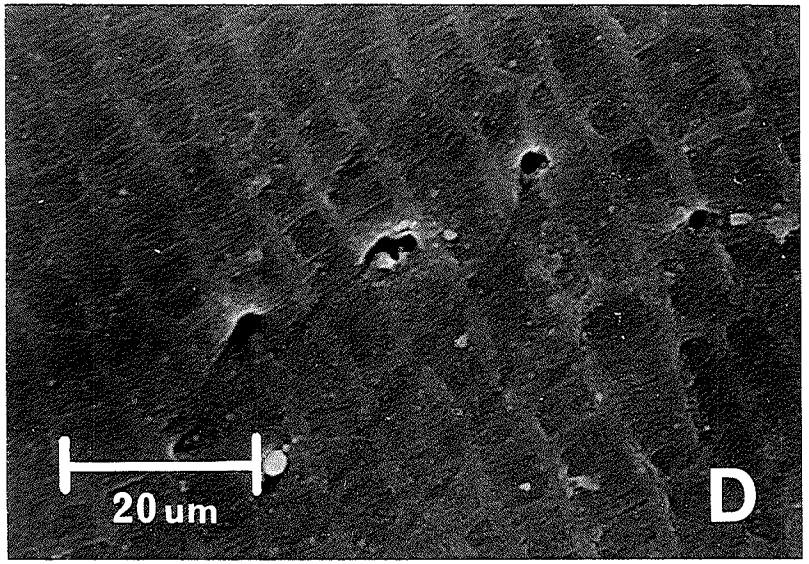
Plate 16c. Cell detail of distinct dorsal surface depression in mid region of critical point dried embryo (var. Argyle).

Plate 16d. Coleoptile surface detail of critical point dried embryo (var. Argyle).


100 um
A


20 um
B


20 um
C


20 um
D

deposit distal to the crown of cells might therefore be root-cap exudate. Collins (1918) referred to the distinct crown of cells on the barley coleorhiza apex as the embryonic appendage. It was noted that the appendage lies immediately beneath the micropyle and that its cells swell rapidly on imbibition. Symons (1982) suggested that the papilla (embryonic appendage) possibly has an influence on the germinability of wheat. His observations of coleorhiza/papilla development and the pattern of grain coat splitting led him to propose that the papilla amplifies (by 15 fold) and focuses the coleorhiza swelling pressure responsible for initial coat splitting. Further, he noted that the cells of the papilla do not vacuolate till grain drying and proposed that this slow pace of development could have a limiting influence on germination ability.

The presence of the convoluted surface film prior to imbibition (Plates 16b and 17) indicated that the film probably unfolds to accommodate coleorhiza swelling. The occurrence of proximal film apertures and distal film tearing just prior to chitting (Plate 12b) indicated that new film material probably was not deposited subsequent to imbibition.

Esau (1977) noted that the coleoptile possesses an opening near its apex and that stomatal and hydathodal openings occur on the inner and outer epidermal surfaces. The coleoptile opening (Plate 15 area e and Plate 18) was observed on the dorsal surface of all embryos examined. Controll-

Plate 17. Highly convoluted coleorhiza surface film of critical point dried embryo (var. Bonanza).

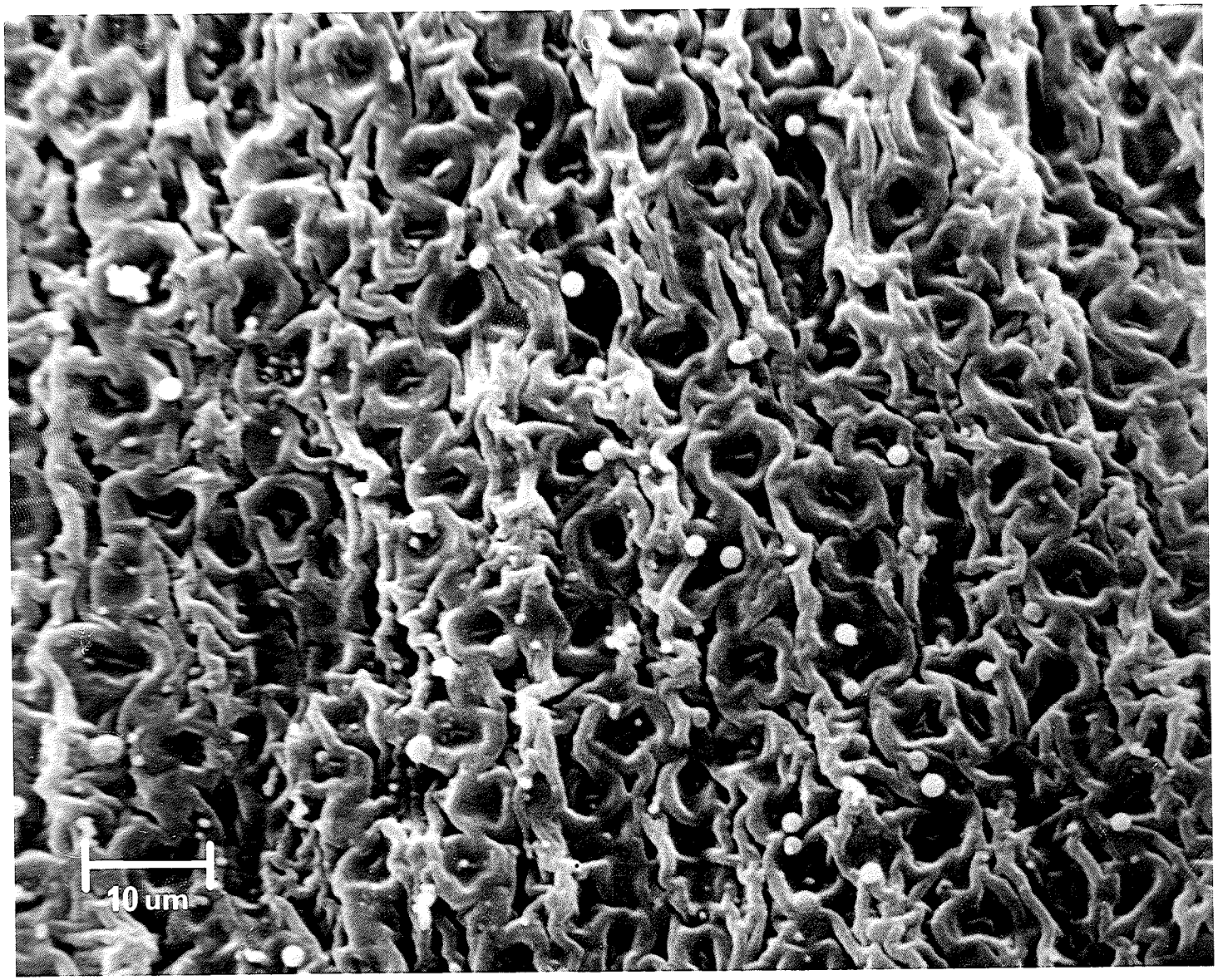
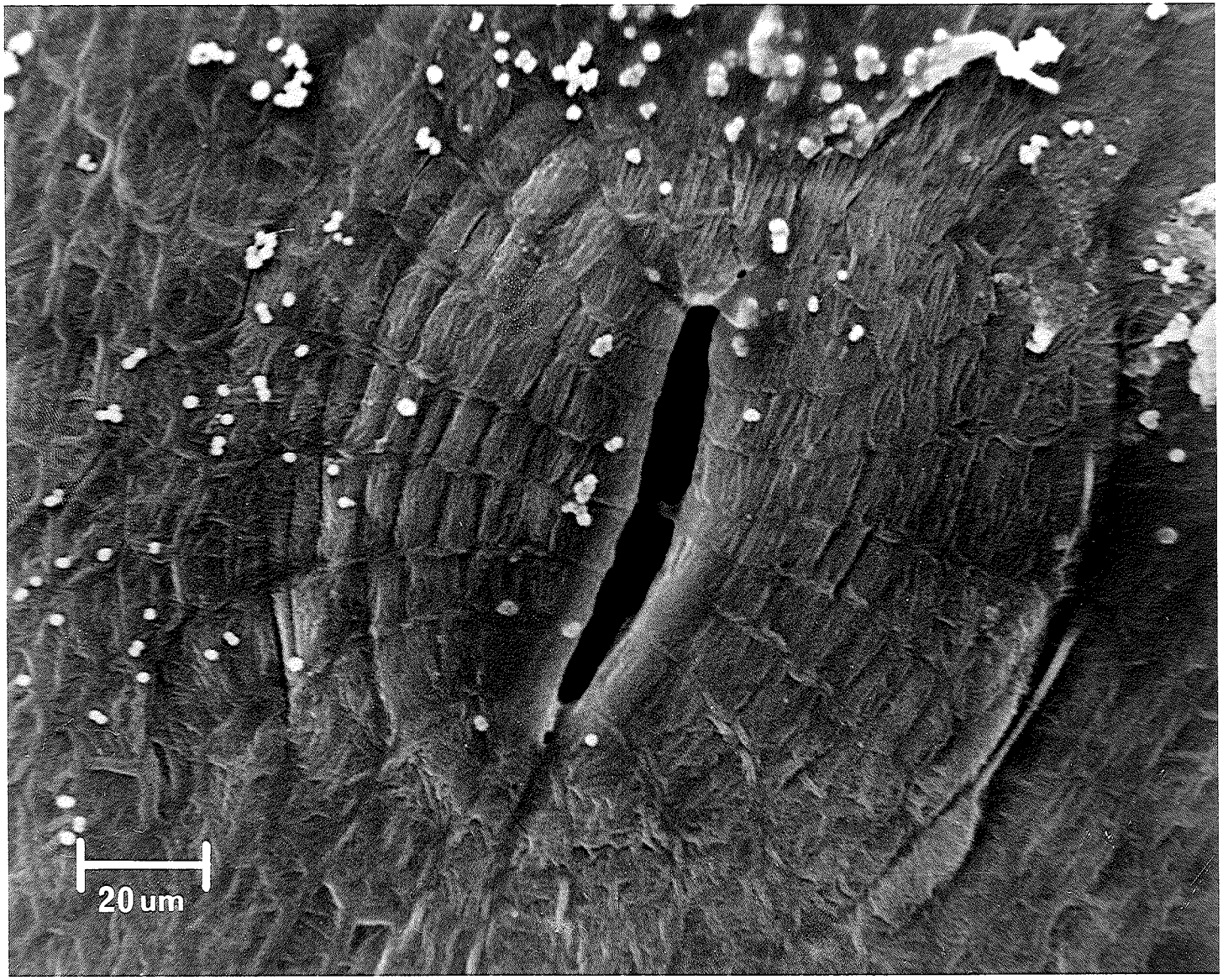

10 um

Plate 18. Opening near coleoptile apex of critical point dried embryo (var. Argyle).


20 um

ed apertures (stomates) were not observed on the coleoptile dorsal surfaces. Those apertures that did occur (Plate 16D) were uncontrolled and intercellular. Sorokin (1966) reported that in oat coleoptiles such apertures connected the external atmosphere with an intercellular network of water lined air passages.

6.4 COLEORHIZA AND RADICLE MORPHOLOGY OF CHITTED GRAINS

6.4.1 Introduction

The results presented in Section 4.3 indicated that prior to chitting (radicle eruption) a hydrostatic pressure of 96 KPa failed to significantly inhibit subsequent root growth. During preliminary experiments however, it was observed that a treatment of 96 KPa would inhibit root growth if applied just following radicle eruption. In contrast, a treatment of 48 KPa subsequent to chitting did not inhibit root growth. These preliminary experiments indicated that coleorhiza integrity increased the magnitude of the pressure treatment required to induce inhibition of root growth.

It was also observed that the coleorhizas of grains set to germinate in petri-dishes could develop root hairs subsequent to chitting (Plate 7). In the following experiment the coleorhizas of steeped chitted grains were examined.

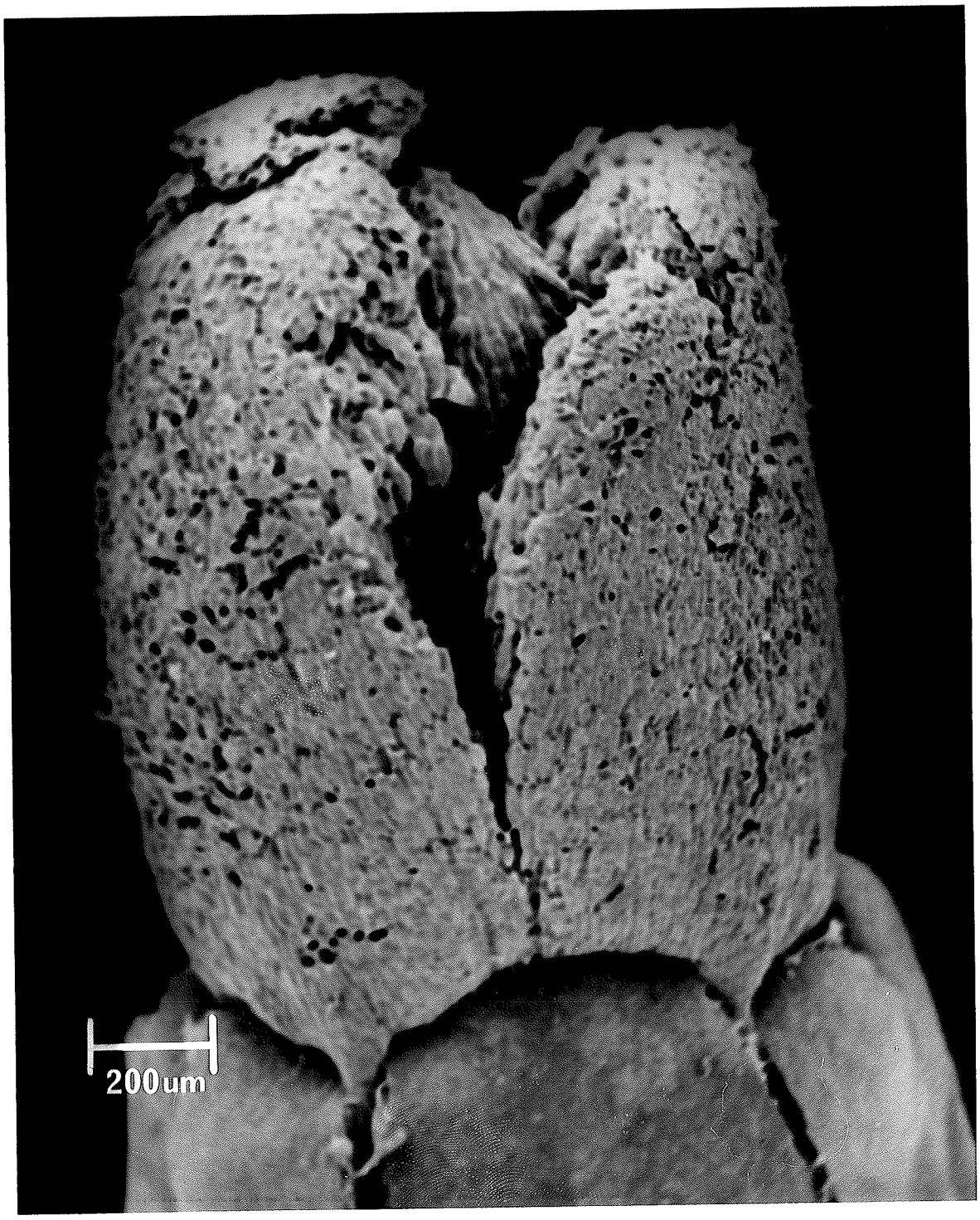
6.4.2 Materials and Methods

Bonanza and Argyle were steeped with aeration at 15°C for 46 hours. A sample of chitted grains was prepared for SEM as outlined in Section 6.1.1. In addition several grain coleorhizas were bisected along a tangential plane above the longitudinal axis. The upper portions of the coleorhizas were then removed along with the radicle and seminals embedded in the lower portions. The lower portions of the coleorhizas were then prepared for SEM as outlined in Section 6.1.1.

6.4.3 Results and Discussion

Plate 19 shows the split that develops in the coleorhiza due to radicle growth (eruption). Generally by this stage of development distal coleorhiza surface cell files were quite disrupted. More proximally however, colerohiza integrity was not visibly influenced by the eruption of the radicle. The coleorhiza surface cells appeared to have lost the polyhedral form displayed at 28 steeping hours and generally were far more elongated. In addition, longitudinal intercellular spaces were more frequent. The coleorhiza surface film was often absent above cell junctions and fewer discrete apertures were observed. The persistence of the film even after 46 hours steeping suggested that a considerable fraction of its components was not water soluble. In addition the absence of film above the more numerous intercellular spaces indicated that either new film was not being

Plate 19. Radicle erupting (chitting) through coleorhiza of critical point dried grain, (var. Bonanza).


200um

deposited or that the rate of deposition was inadequate to compensate for the increase in coleorhiza volume.

The tips of coleorhiza occasionally bore a portion of tissue that appeared to be structurally detached from the rest of the tissue at the surface (Plates 10a, 19, 20). The detachment of tissue generally took place along a circumscribing distal furrow. This distal furrow or fold was observed occasionally (Plate 20) on coleorhizas at an earlier stage of development. It is conceivable that this furrow delineates the embryonic appendage. Symons et al. (1984) note however that the coleorhizal papilla (embryonic appendage) is easily detached. It was also observed that often a portion of coleorhiza tissue was carried forth by the emerging radicle.

Plate 21 depicts the erupting radicle and its associated root cap. Root cap cells immediately behind the radicle tips often appeared to be crushed and ruptured. Although amorphous debris was present on radicle tips no mucilage or bacteria were observed. Distally, root cap cells were intact and when attached to the surface appeared to be closely packed to adjacent cells. Each root cap cell was generally cylindrical and greatly elongated. The elongation of cells increasing (50 to 300 μm) with greater displacement from radicle tips. In contrast the protoderm cells beyond the root cap appeared rectangular and elongate (50 to 100 μm). The protoderm was devoid of intercellular spaces but did appear to bear a film.

Plate 20. Distal furrow or fold of coleorhiza of critical point dried grain steeped for 30 hours, (var. Bonanza).

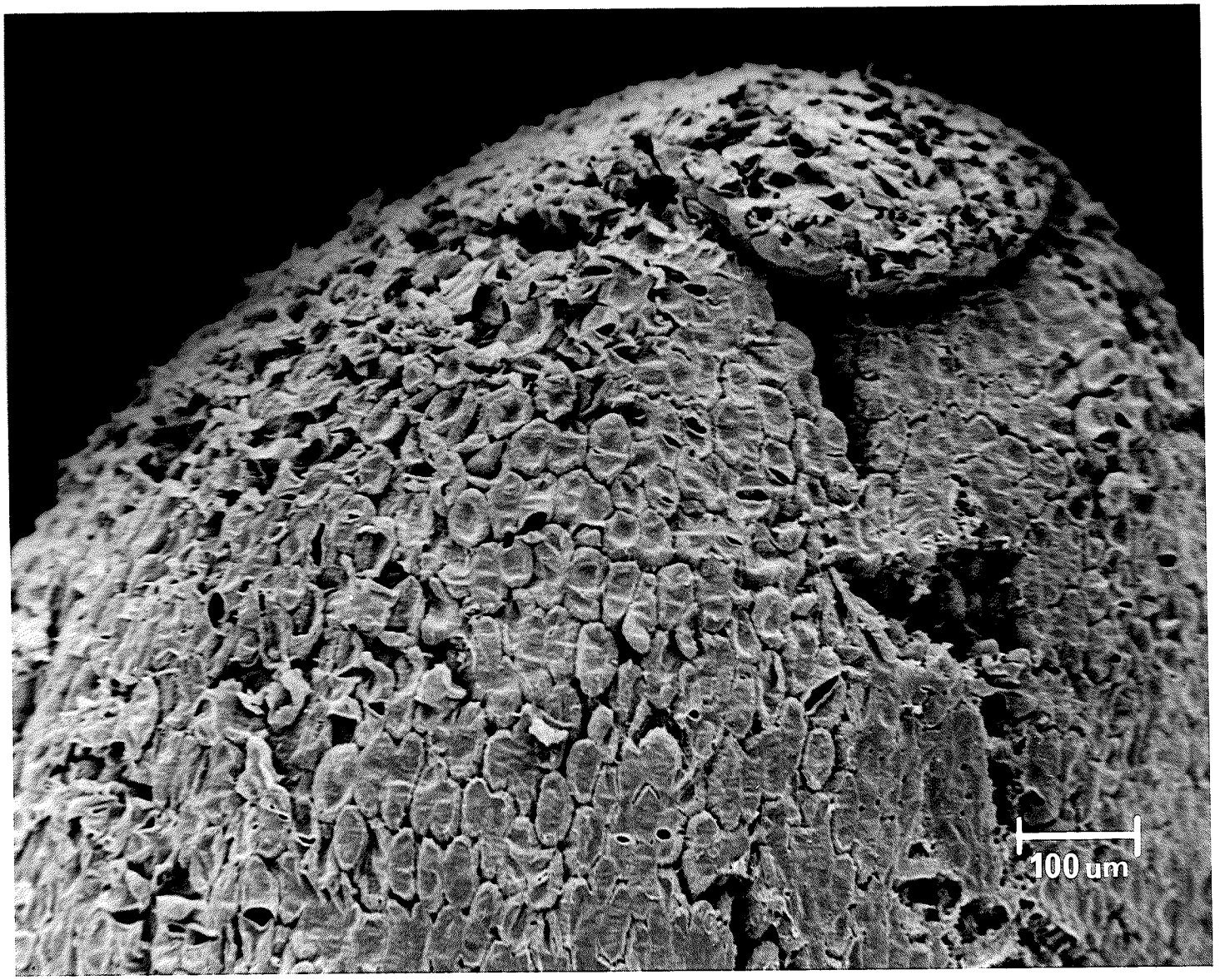
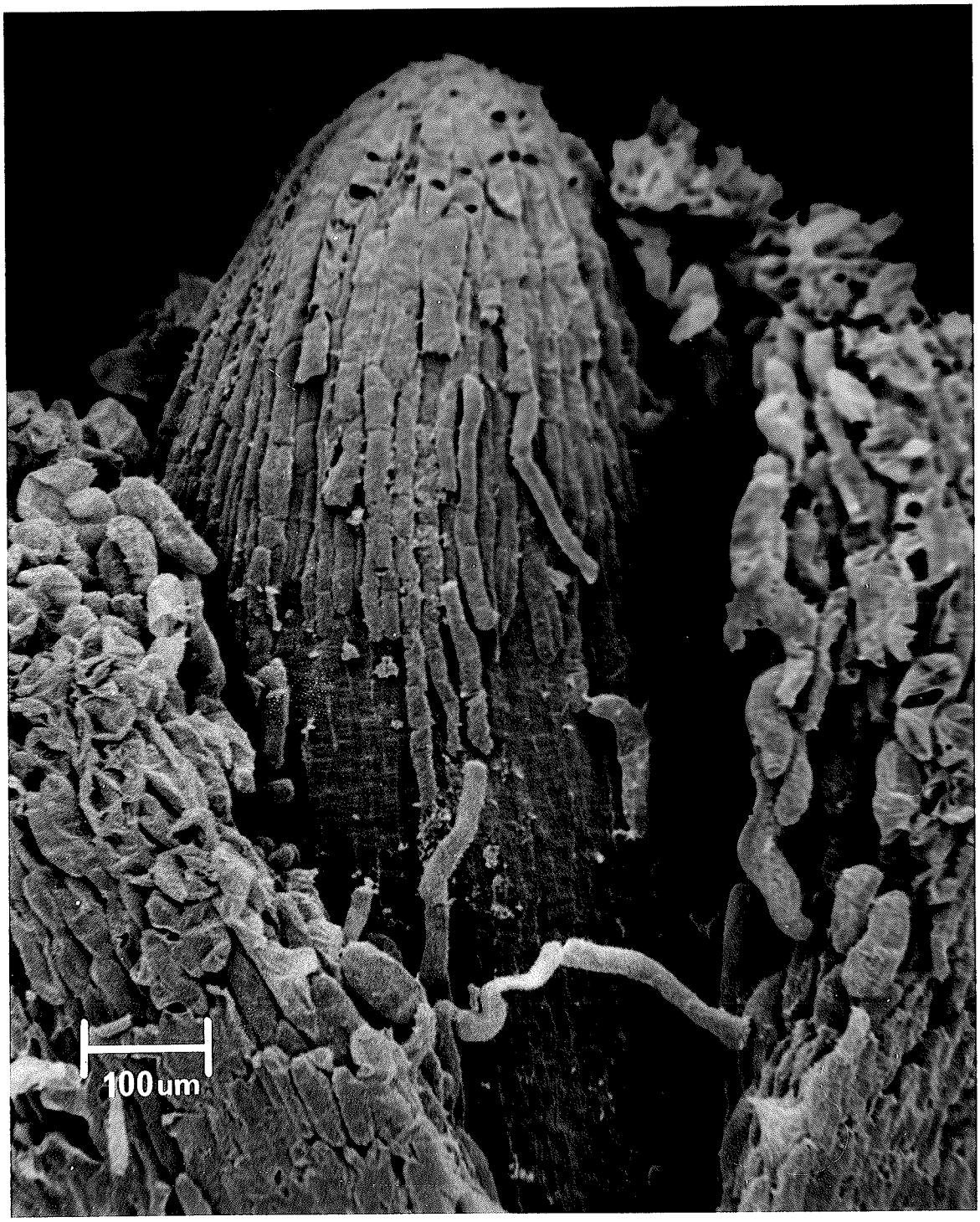

100 um

Plate 21. Erupting radicle (chitting) and associated root cap of critical point dried grain, (var. Bonanza).


100 um

The internal surface of coleorhiza chambers which previously enclosed root initials were lined with cells (Plate 22) which appeared to be identical to those of the root cap. Large intercellular spaces were present that could be seen to extend for several cell layers. Besides the frequent pieces of debris of which many appeared to be cell fragments, bacteria were noticeably absent on the internal surfaces of coleorhizas. It is possible that the cells on the internal surfaces observed were merely left behind by the root cap. However, the entire length of the radicle chamber was lined with the elongated cylindrical cells.

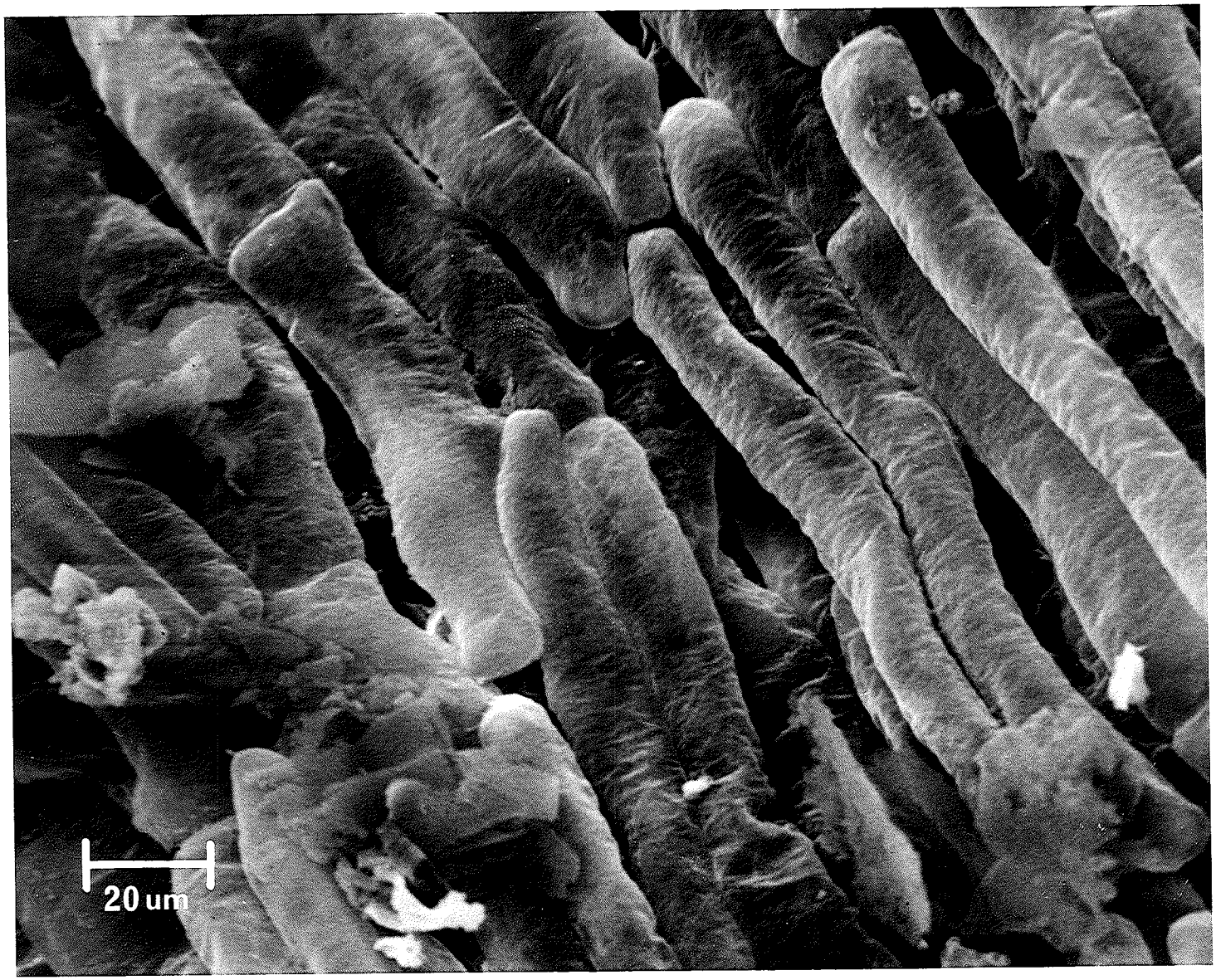
6.5 COLEORHIZA ANATOMY

6.5.1 Introduction

The coleorhiza is a non-vascular (Hector, 1936) parenchymatous (Percival, 1921) structure. Gershoy et al. (1976) reported that in germinating embryos ramifying gas channels are defined by the contact geometry at the junctions of three or more enlarged rounded polyhedral parenchyma cells. The presence of ramifying channels in continuum with the surface lacunae observed (Section 6.1.2) may provide the major pathway by which ink under pressure reaches the nodal region of the embryo (Plate 7).

Hydrostatic pressure treatment (Section 6.1) did not appear to modify coleorhiza surface morphology.

Plate 22. Cell detail of internal surface of colerohiza chamber of critical point dried grain, (var. Argyle).


20 um

Modifications could, however, have been obscured by SEM preparation techniques. Yoshida et al. (1979) proposed that the inhibition of respiration and growth following treatment may be due to embryonic disruption. Such disruption might not be discernible from surface examination. In an attempt to determine whether cellular disruption occurs during treatment barley embryos were sectioned.

6.5.2 Materials and Methods

Argyle was surface sterilized as in Section 5.3.2. Following sterilization the grain was steeped with aeration at 15°C for 26 hours. Treated grain was subjected to a hydrostatic pressure of 193 KPa for 30 seconds. Embryos were removed by cutting through the grains with a razor below the scutellum disc. The embryos were then immediately fixed following the same procedure as that outlined in Section 6.1.1.

Following fixation the embryos were dehydrated up through a (25, 50, 75, 95 and 100%) ethanol series. Each dehydration step lasted 1 hour during which time the dehydrant was renewed every 15 minutes. Finally the embryos were stored overnight (room temperature) in 100% ethanol. Following dehydration ethanol was displaced through a propylene oxide/ethanol series (25, 50, 75 and 100%). The first three steps in the series were carried out over a period of 30 minutes. The final displacement step involved 3 changes of 100% propylene oxide over a period of 1 1/2 hours.

Finally the embryos were embedded in Spurr's standard resin (Spurr, 1969). The first three steps in the embedding procedure involved immersion of the embryos for 1 hour in resin/ propylene oxide solutions (25, 50 and 75% resin). The embryos were then left overnight in 100% Spurr's resin. Infiltration with fresh resin everyday was continued for a further 4 days. Eventually the embryos were placed in foil dishes and covered with fresh resin. The dishes were then transferred to a vacuum oven at 70°C for 24 hours during which time the resin polymerized.

Embedded embryos were sectioned using glass blades and a Reichert Om U2 microtome. The sections (1 μm thick) were floated into a water filled boat immediately behind the cutting edge. From the boat, sections were transferred to drops of 10% acetone on gelatin coated (O'Brien and McCully, 1981) glass slides. The sections were flattened by passing the slides above an alcohol flame.

The sections were stained for 15 minutes with 1% toluidine blue in 1% aqueous borax. Following staining the sections were washed for 10 to 15 minutes in running distilled water. Sections were mounted in distilled water before being viewed with a Nikon microscope (optiphot model XF-EF). Photographs were taken using 35 mm Kodak Panatomic-X film (ASA 32).

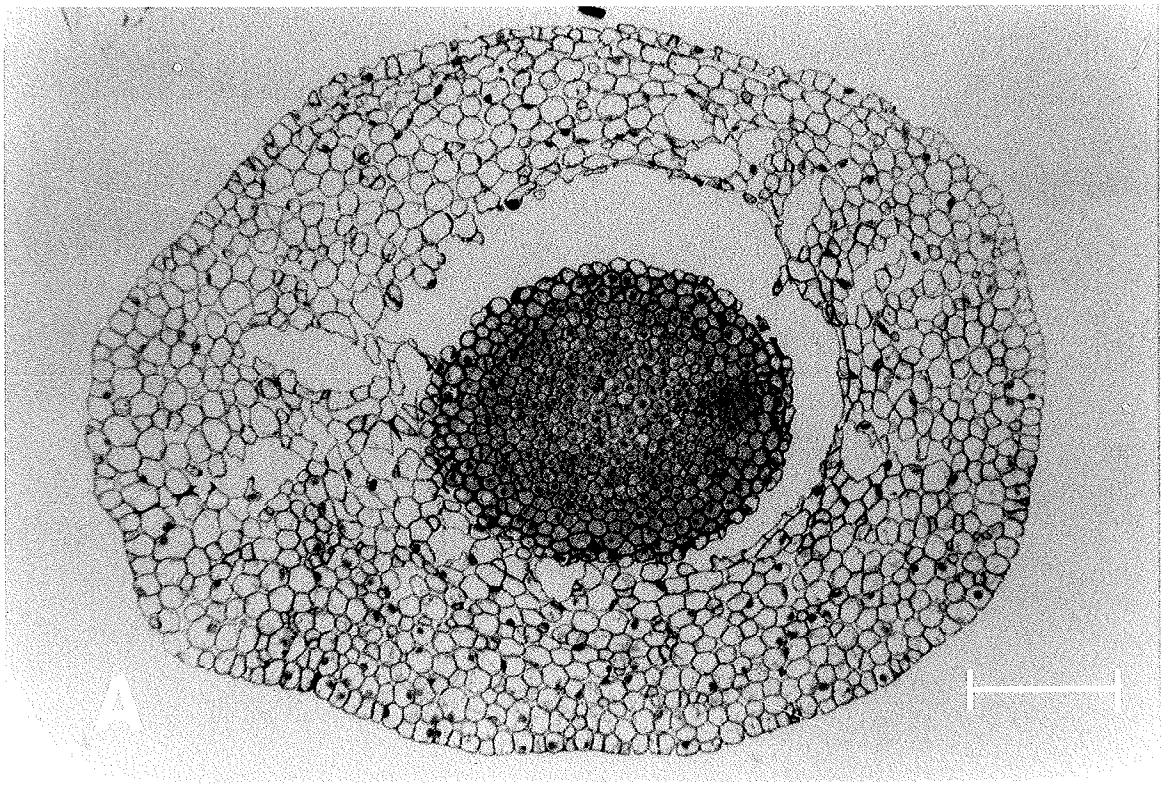
6.5.3 Results and Discussion

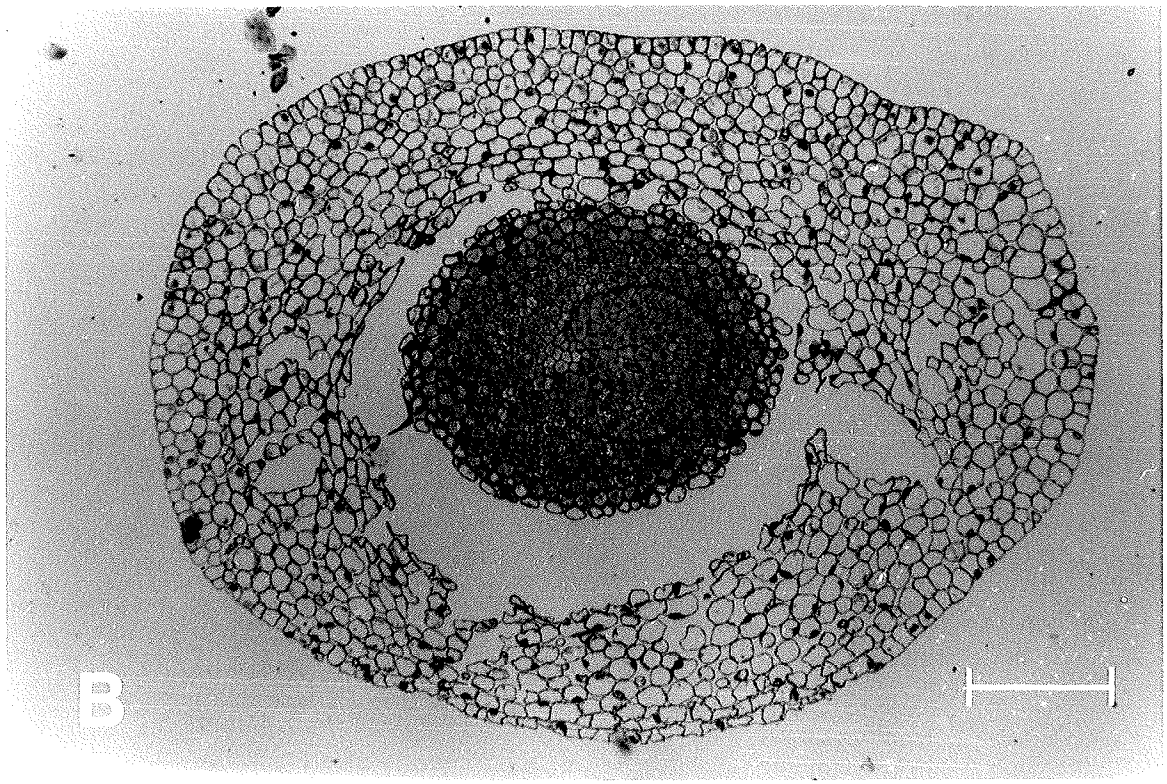
During examination of numerous sections no apparent differences were observed between the tissue of control and treated grains. With the exception of the outer bounding layer (coleorhiza surface cells) of parenchyma, the internal cell layers were loosely packed. Although most intercellular spaces observed were the consequence of cell geometry, numerous other large spaces were not. In cross section the surface bounding cells were frequently cubical and smaller than cells below the surface. In contrast, the underlying cells appeared to be rounded and enlarged with numerous facets. Longitudinal sections confirmed that the cells which comprised the distal portion of the coleorhiza were polyhedral in form. Coleorhiza cells in areas adjacent to the radicle were revealed in longitudinal section to be elongated. In addition, the degree of elongation tended to increase with depth, such that cells closest to the radicle were often as elongated as radicle-cap cells.

Cross sections (Plate 23a and b) of the distal end of the coleorhiza exposed a large vestibule between the radicle-cap and the discontinuous internal surface of the coleorhiza. The dimensions of the vestibule were inclined to be greatest below the dorsal surface of the coleorhiza. Ventrally radicle-cap cells made intermittent contact with adjacent coleorhiza cells. The vestibule often appeared to be continuous with other coleorhiza lumens, some of which

Plate 23a. C.S. showing loose packing of coleorhiza cells, vestibule and radicle cap. (var. Argyle). Bar = 150 μm.

Plate 23b. C.S. showing radicle meristem and cap. (var. Argyle). Bar = 166 μm.


A


B

extended to within a few cell layers of the surface. In contrast to the coleorhiza cells the cells of the radicle-cap had dense cytoplasms and were tightly packed in such a manner that the cap appeared to lack intercellular spaces. Despite this tight packing, the outer cell layer of the radicle-cap cells appeared to be discontinuous.

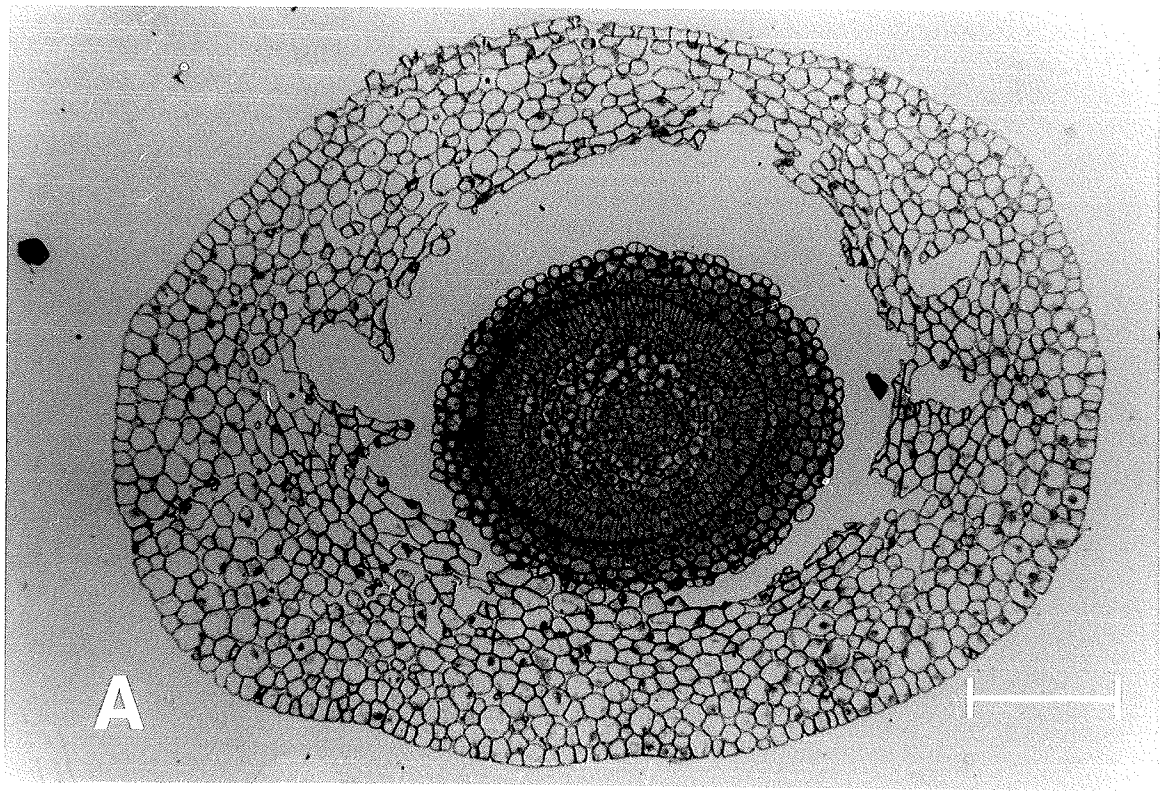
Further basipetal cross sections (Plate 24a and b) revealed that the vestibule persisted below the level of the radicle-cap. Here however, the internal cell layer of the coleorhiza was less disrupted and fewer lumen extended off the main vestibule. In addition, the packing of coleorhiza cells was observed to be tighter in this region. This was especially noticeable in the ventral region of the coleorhiza where the radicle impinged upon the internal surface.

Proximally the rounded outline of the distal region gave way to an oval form (Plate 25a and b). In this region two discrete lumens (Plate 25a) were observed to flank the radicle vestibule which was becoming increasingly reduced. Basipetally the flanking lumens were partially occupied by the caps of other initials. In this region the coleorhiza tissue ventral to the radicle was compressed (Plate 26a and b). The cells in this region had a squamous outline and appeared to interlock like pieces of a jigsaw puzzle such that few intercellular spaces were present.

The final cross-sections (Plate 27) revealed that the vestibule around the radicle became increasingly reduced

Plate 24a. C.S. coleorhiza, radicle cap, protoderm, cortical ground meristem and procambium. (var. Argyle). Bar = 154 μm.

Plate 24b. C.S. coleorhiza and radicle. Note late metaxylem. (var. Argyle). Bar = 153 μm.


A

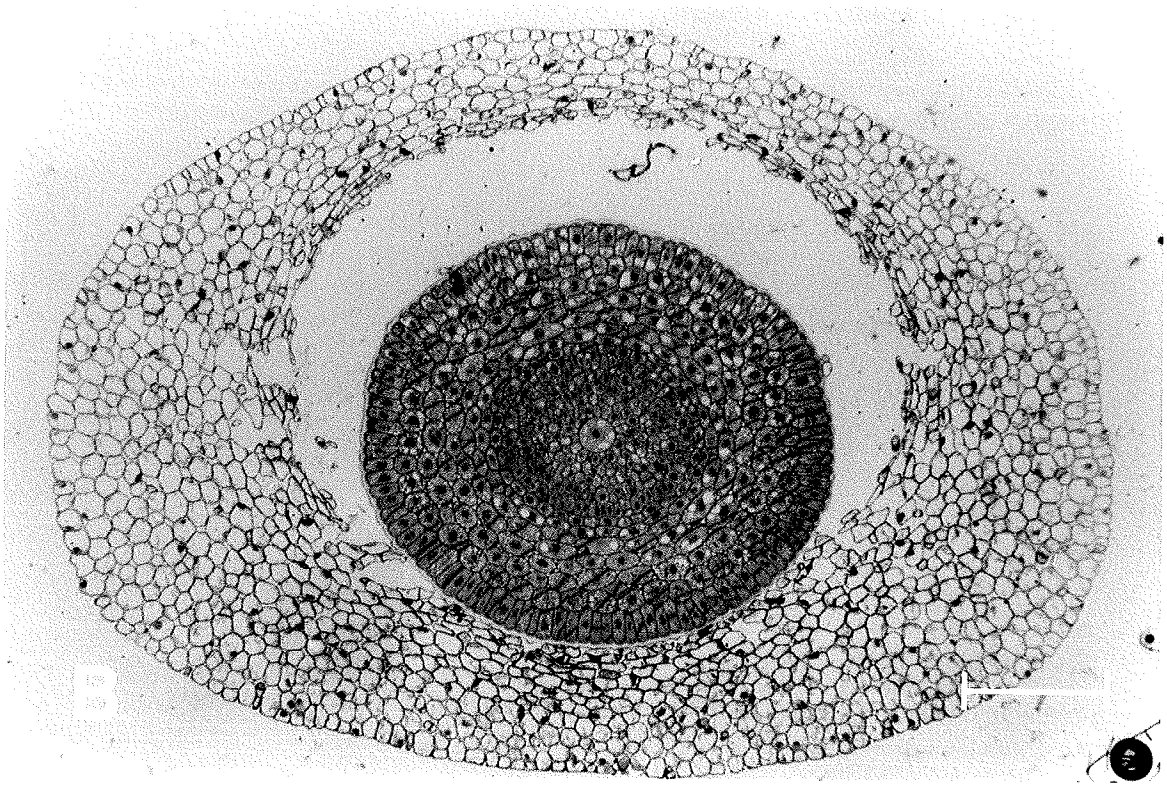
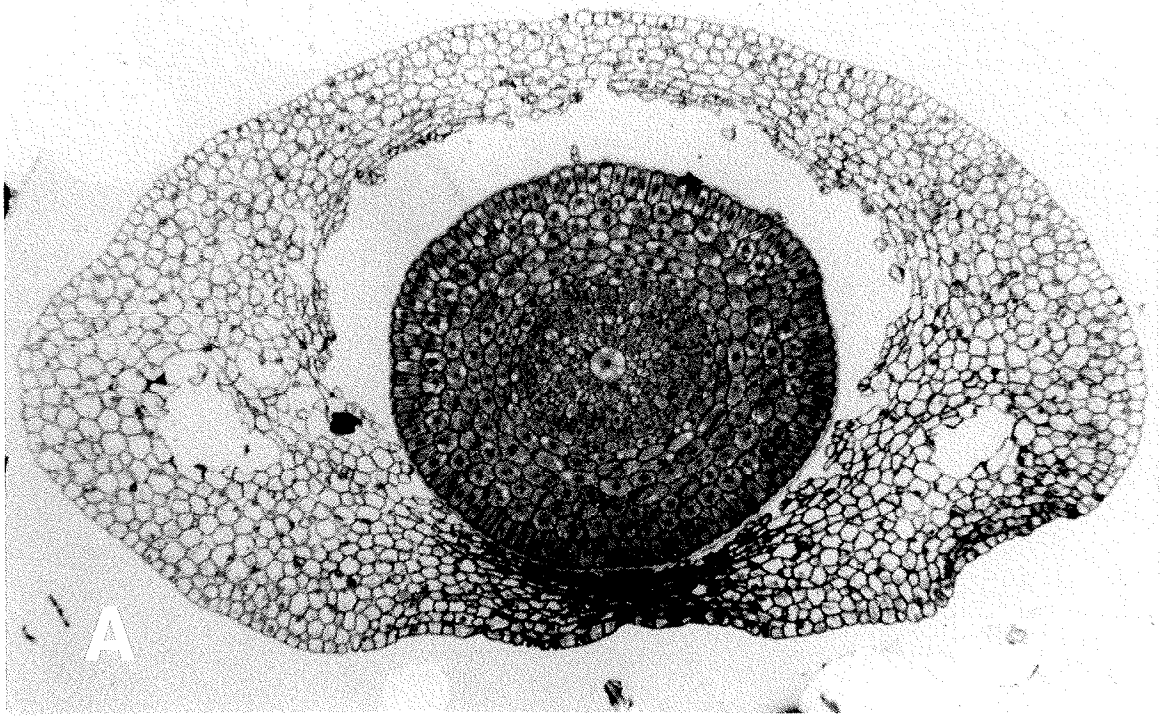

B

Plate 25a. C.S. showing discrete seminal lumen and diminished radicle vestibule. (var. Argyle). Bar = 153 μm.

Plate 25b. C.S. showing root caps of seminals occupying enlarged lumen adjacent to radicle vestibule. (var. Argyle). Bar = 171 μm.


A

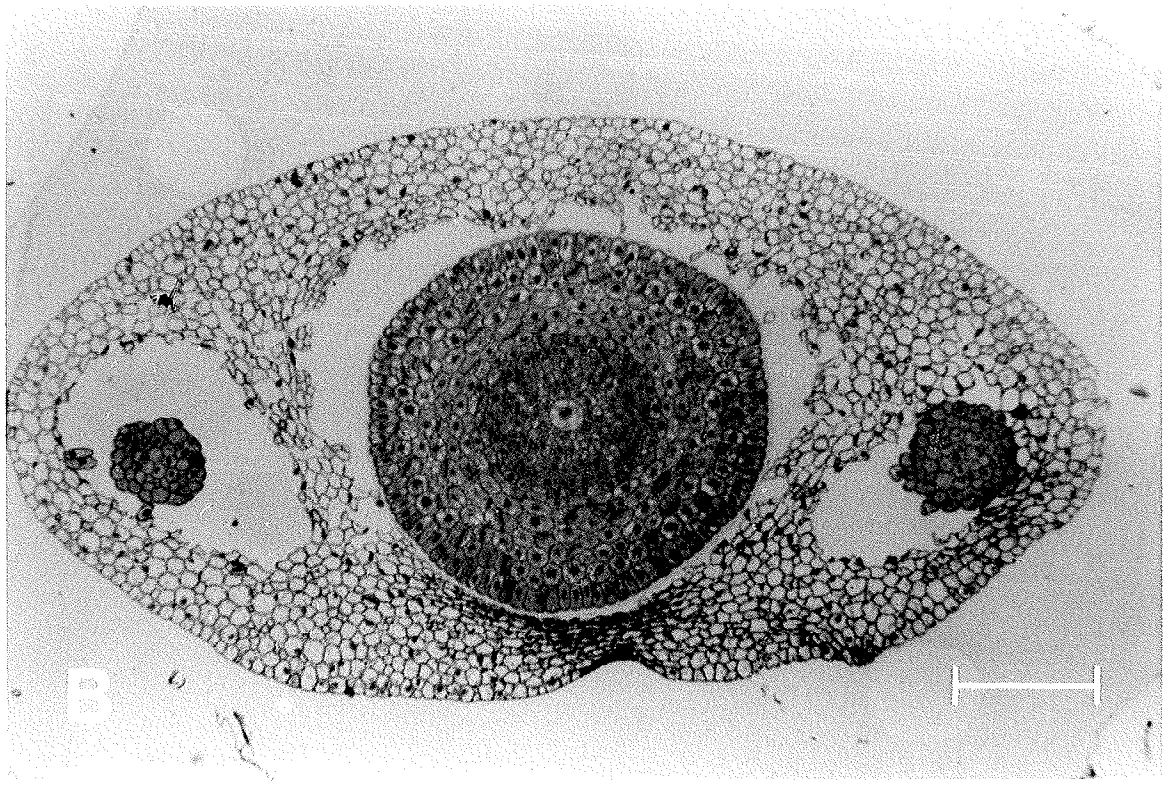
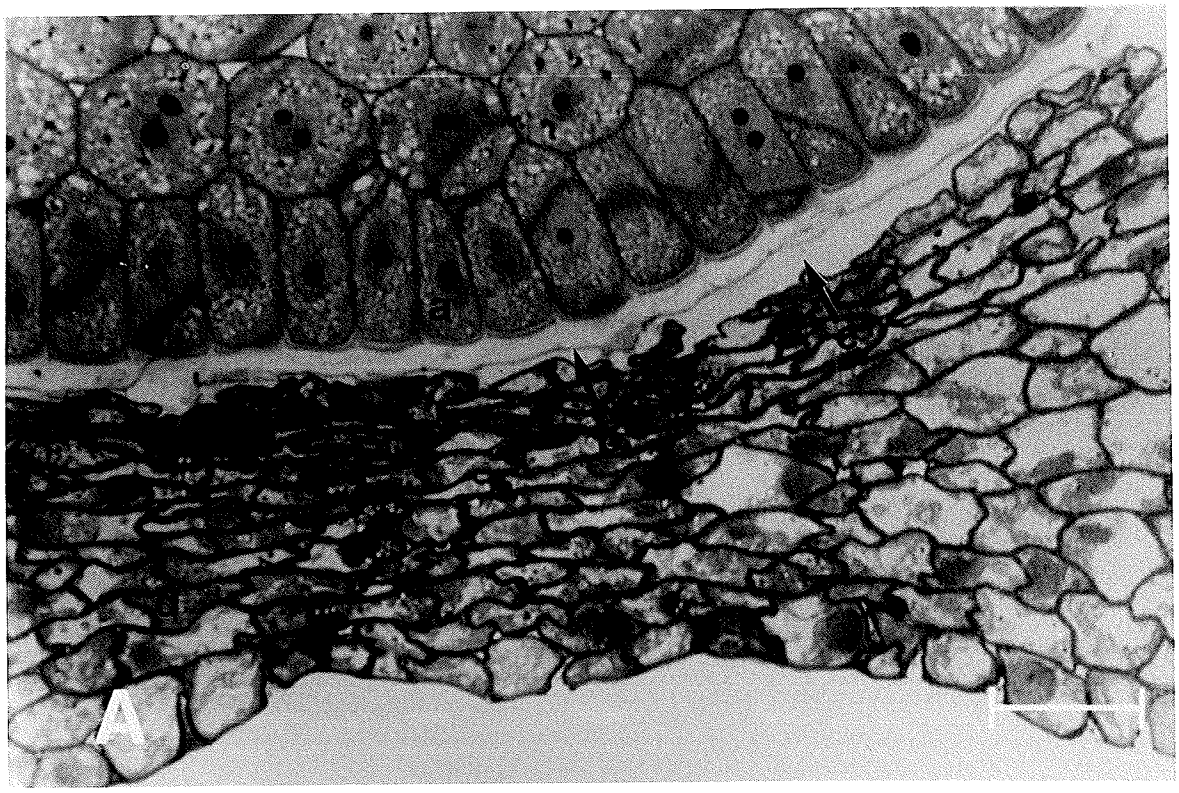

B

Plate 26a. C.S. ventral coleorhiza region.
a) protoderm
b) mucigel
c) interface
d) cells with squamous appearance.
(var. Argyle).
Bar = 36 μm.

Plate 26b. Phase contrast of plate 26a.
Bar = 36 μm.


A

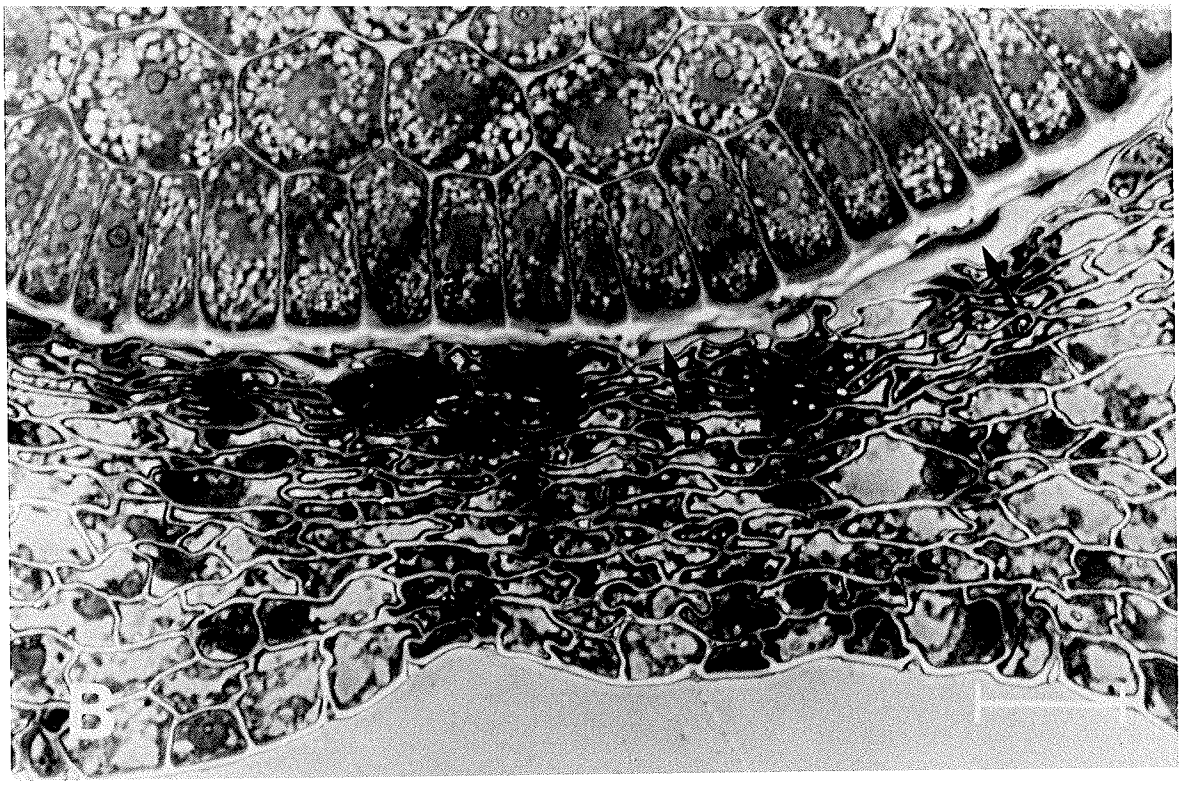
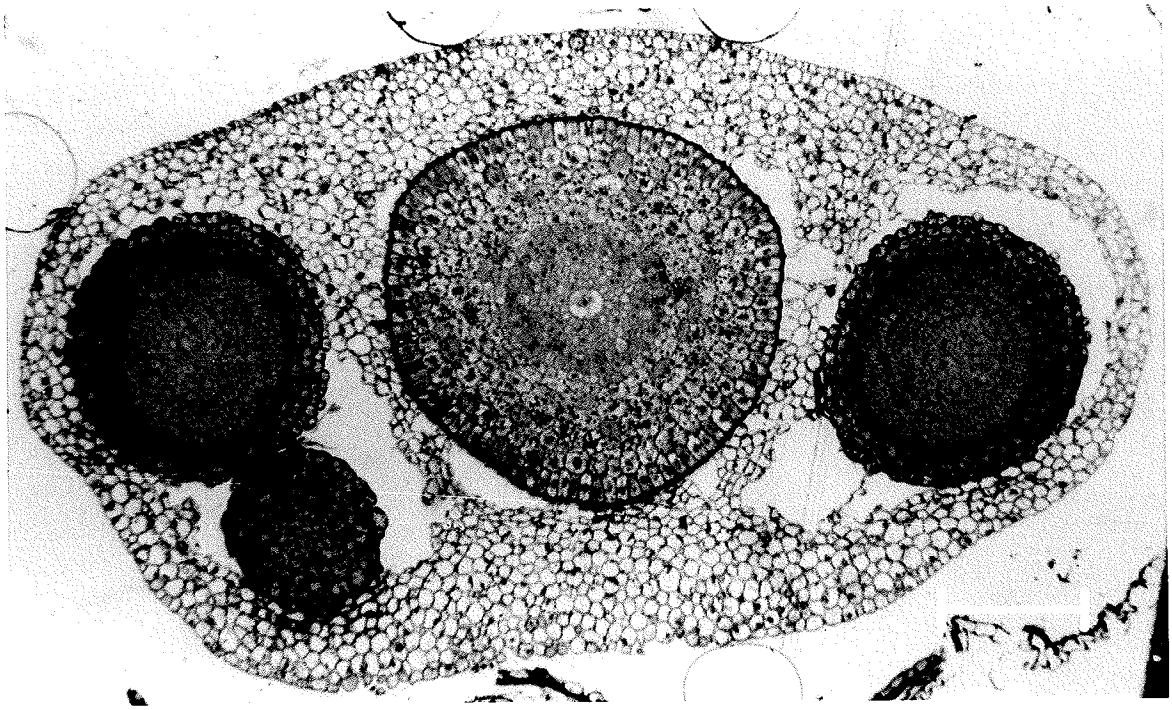

B

Plate 27. C.S. coleorhiza showing coleorhiza tissue in contact with most of the radicle surface. (var. Argyle). Bar = 204 μm.



basipetally. Similarly the flanking lumens became increasingly occupied by the adjacent seminals. It appears (Plate 27) that the adjacent seminals could have been disrupting the coleorhiza.

In longitudinal section (Plate 28) the relative densities of coleorhiza and radicle tissue were more apparent. The distal end of the coleorhiza was comprised of thick walled cells which appeared to bear the remnants of compressed cells (Plate 29a). This compressed layer may have been the remains of the pressure papilla which Symons (1982) noted to be very sensitive to preparation technique.

The cells of the coleorhiza tissue appeared generally to have a large single vacuole which occupied most of the cell volume. In contrast, the cells of the radicle-cap (Plate 29b) had numerous smaller vacuoles that presumably would have eventually coalesced. In addition, intercellular spaces were not observed in the radicle-cap tissue. The quiescent centre (Clowes, 1984) and the distal portion of the procambial cylinder (Plate 30a) also lacked intercellular spaces. Apical intercellular spaces did occur however, between adjacent files of cortical initials. Roland (1978) described the formation of such cavities in root apices. He reported that the process involved a precise autolytic parting of a splitting layer that is deposited between adjacent cell walls.

The cells at the radicle base (Plate 30b) were arranged in discrete stacks. Each stack appeared to have arisen from basipetal and acropetal anticlinal divisions of a

Plate 28. L.S. distal portion of coleorhiza. (var. Argyle). Bar = 150 μm.

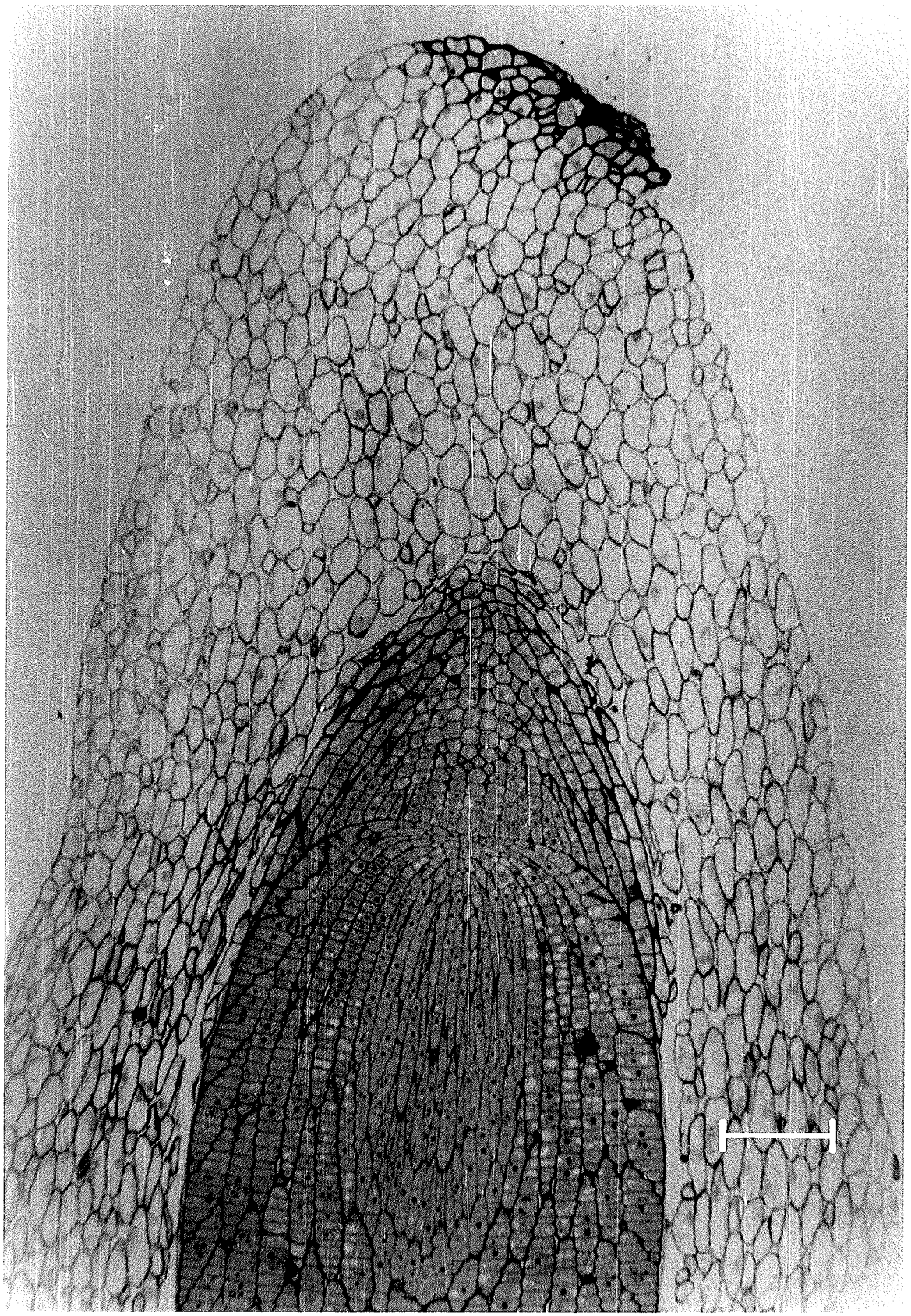
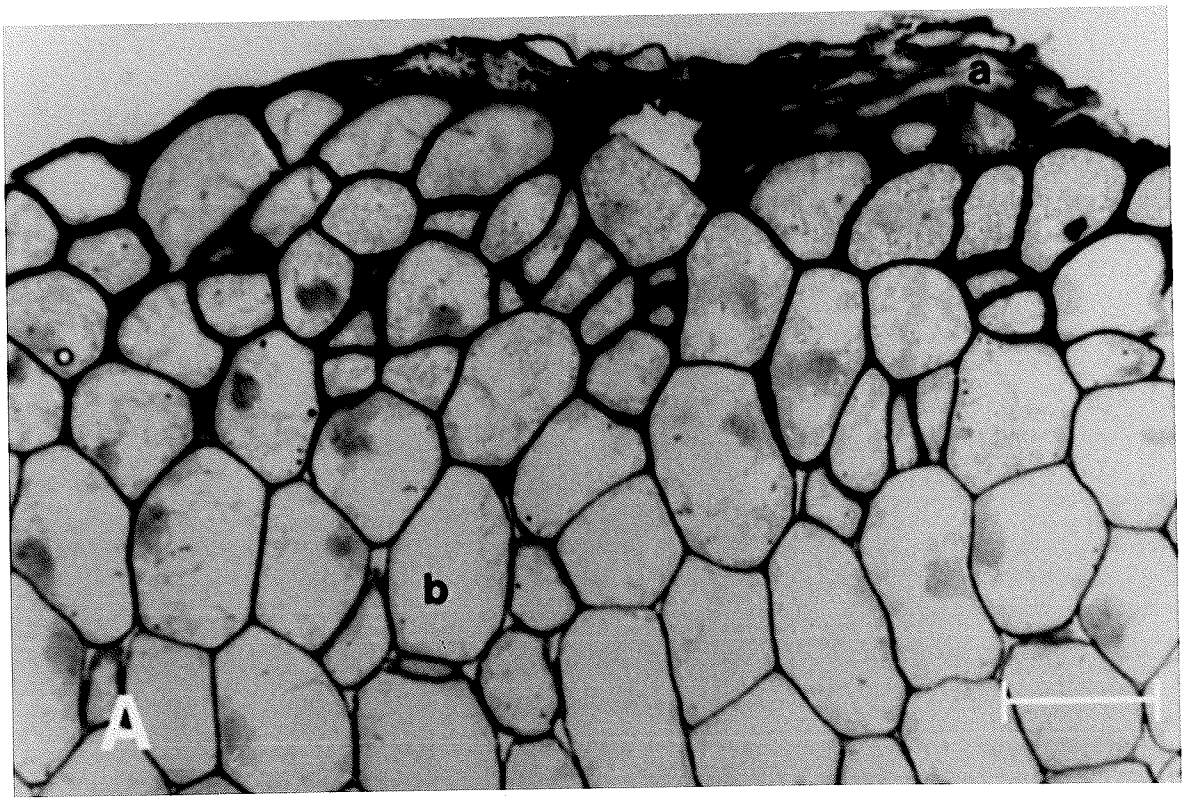


Plate 29a. L.S. tip of coleorhiza.
a) possible remnants of papilla
b) Large polyhedral cell of tip.
(var. Argyle). Bar = 35 μm.

Plate 29b. L.S. radicle cap in close proximity to coleorhiza tissue.
a) coleorhiza tissue with large cell vacuoles and intercellular spaces
b) radicle cap cells with dense cytoplasms numerous small vacuoles.
(var. Argyle). Bar = 29 μm.


a
b
A

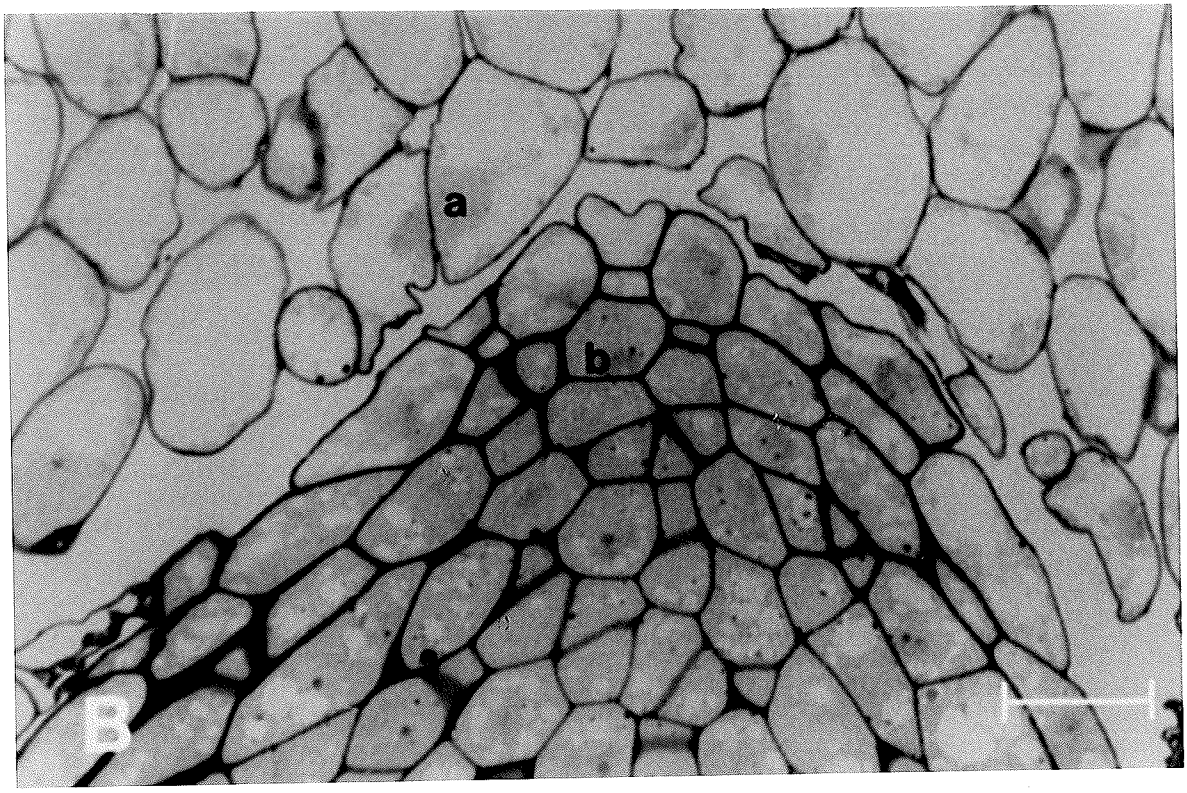
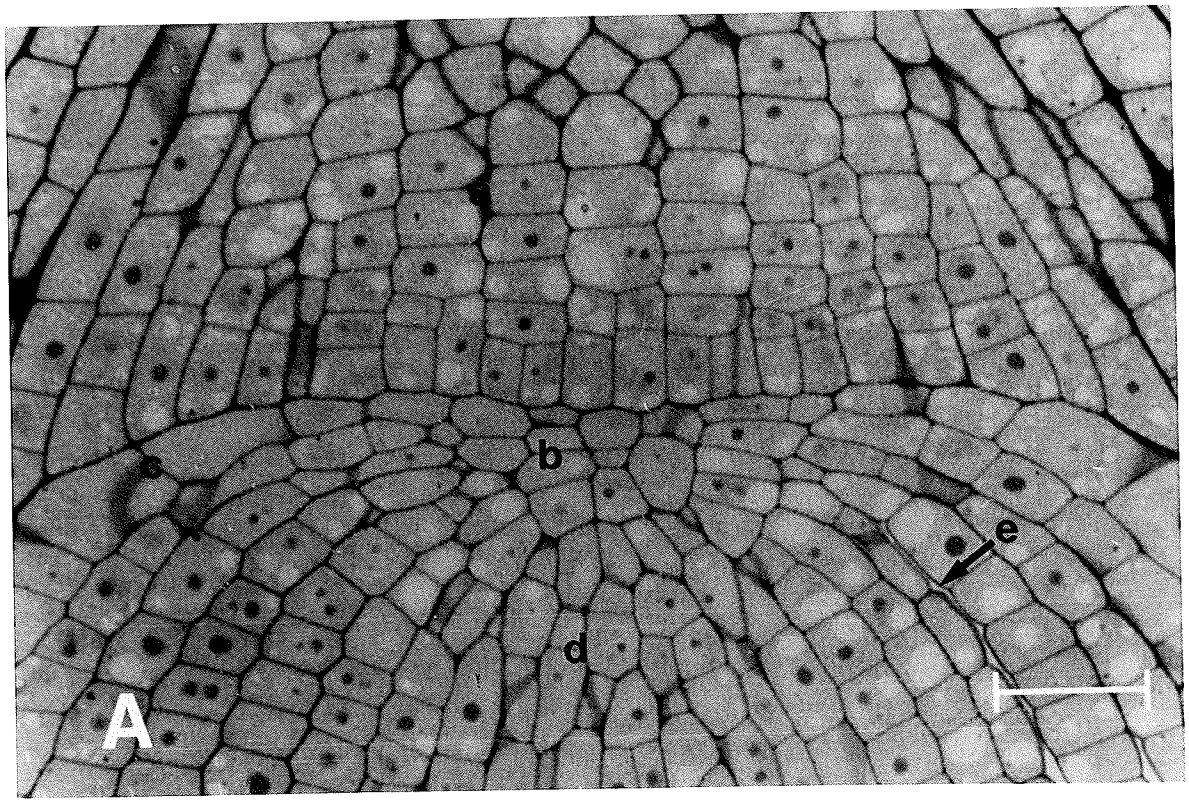
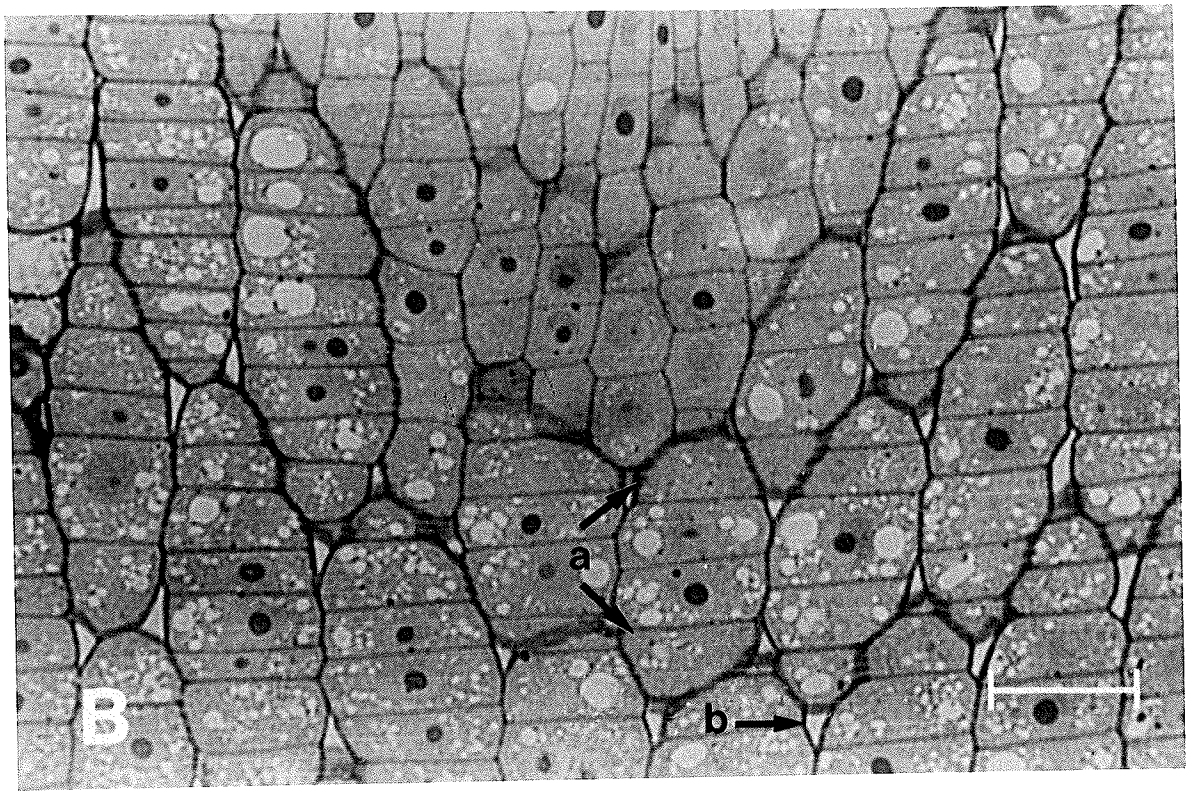

a
b
B

Plate 30a. L.S. radicle apical meristem.
a) radicle cap
b) quiescent centre
c) protoderm initials
d) provascular cambium
e) apical intercellulary
(var. Argyle). Bar = 36 μm.

Plate 30b. L.S. radicle base. The (column) base of the provascular tissue can be seen top centre of plate.
a) cell stack which appears to have arisen from the division of a single cell.
b) triangular intercellular space.
(var. Argyle). Bar = 31 μm.


b
e
d
A


a
b
B

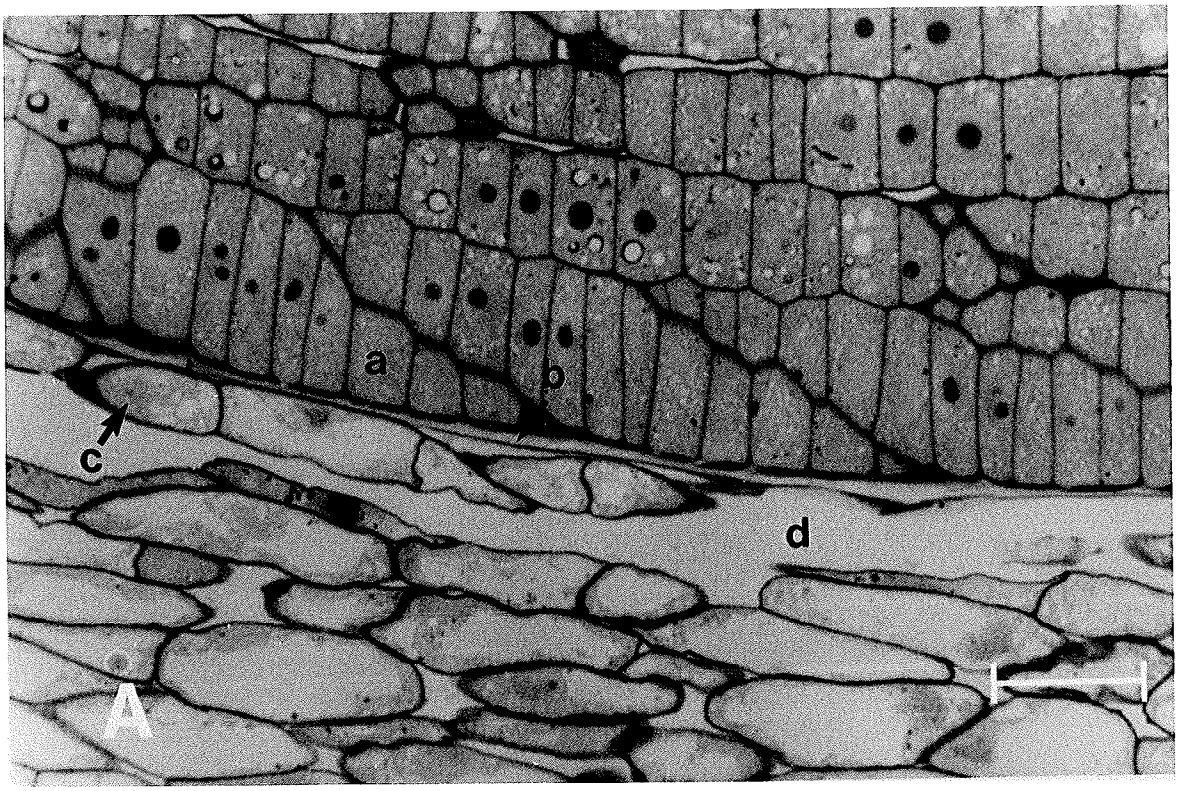
single cell. Where three of these stacks impinged an approximately triangular intercellular space occurred.

In the longitudinal sections examined the large vestibule surrounding the radicle was not seen. However, a continuous intertissue space at the radicle/coleorhiza interface was observed (Plate 31a and b). It is possible that the longitudinal sections were made through the region where the radicle impinges on the ventral internal surface of the coleorhiza, that is, where the radicle vestibule dimension was at a minimum. Alternatively, in the two grains from which longitudinal sections were made, the radicle may have already swollen to occupy most of the vestibule. Irrespective of this however, the interface gap between the radicle and coleorhiza tissue continued to the base of the radicle where it was continuous with the intercellular spaces of the scutellum (Plate 32).

During examination of cross sections the radicle was observed to be completely surrounded by a mucilagenous layer (Plate 24b, 26a and b) whereas the radicle-cap was not (Plate 25a). Occasionally the mucilagenous layer appeared to traverse the vestibule (Plate 33) and make contact with adjacent coleorhiza cells. The root mucigel as distinct from root-cap mucilage has been reported (Esau, 1965, Dayan et al. 1977) to result from the gelatinization of cell wall material between the rootcap and protoderm. The absence of the mucilagenous layer on the radicle-cap in contrast to its

Plate 31a. L.S. radicle coleorhiza interface.
a) protoderm
b) mucigel
c) radicle cap cell
d) intertissue space
(var. Argyle). Bar = 35 μm.

Plate 31b. Phase contrast of Plate 31a.
Bar = 35 μm.


a
b
c
d
A

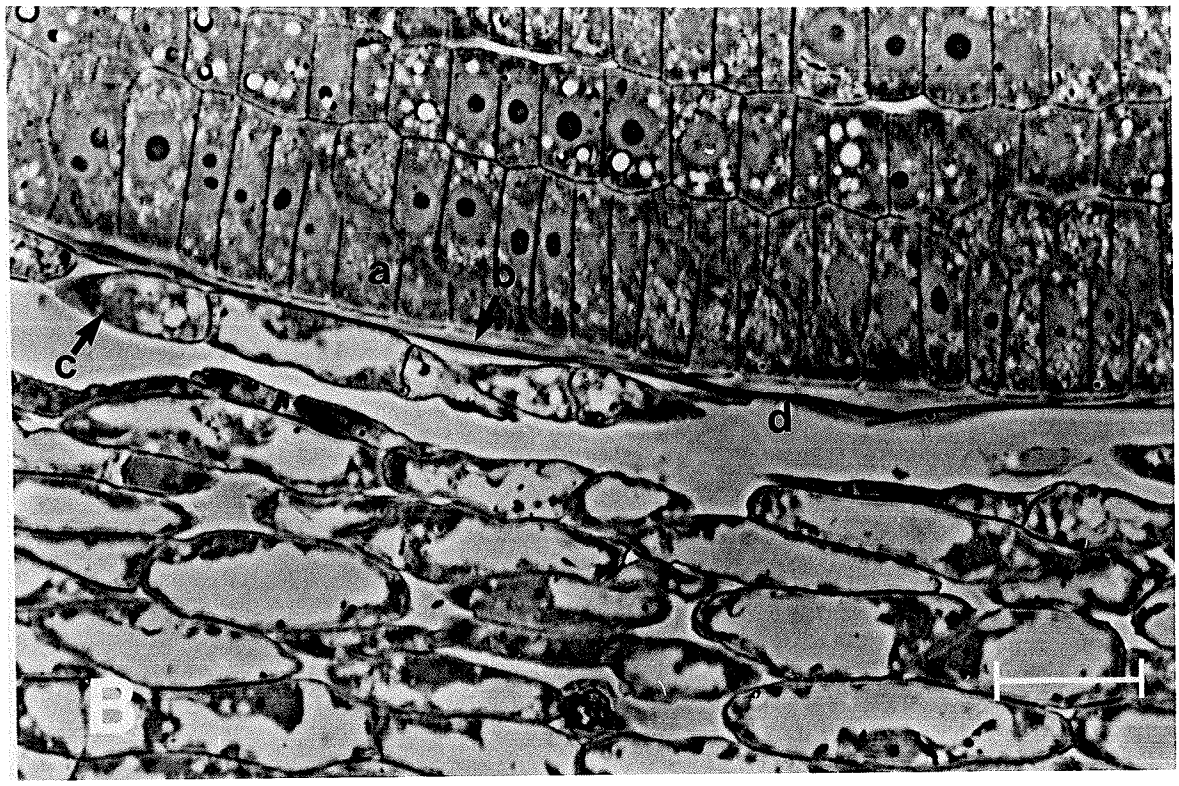

a
b
c
d
B

Plate 32. L.S. scutellum and endosperm. Showing scutellum intercellularies, columnar epithelial layer, depletion layer and endosperm matrix. (var. Argyle). Bar = 200 μm.

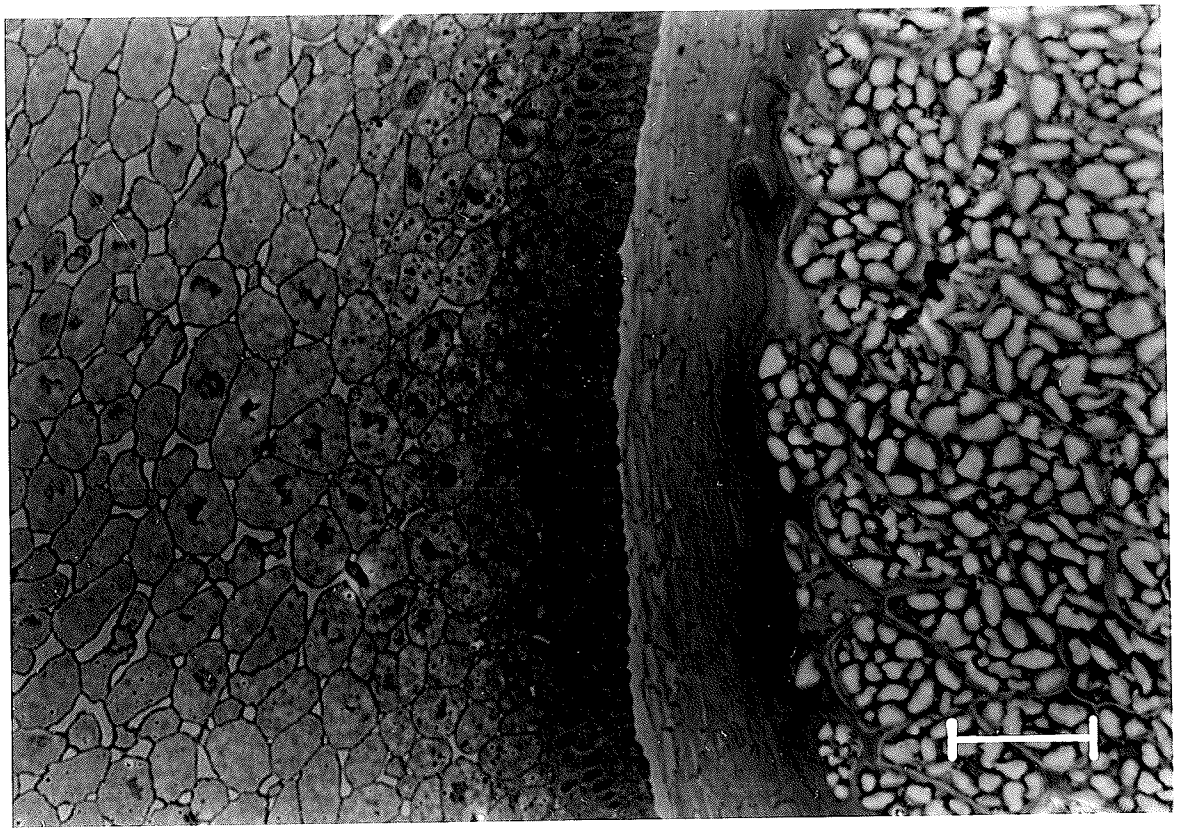
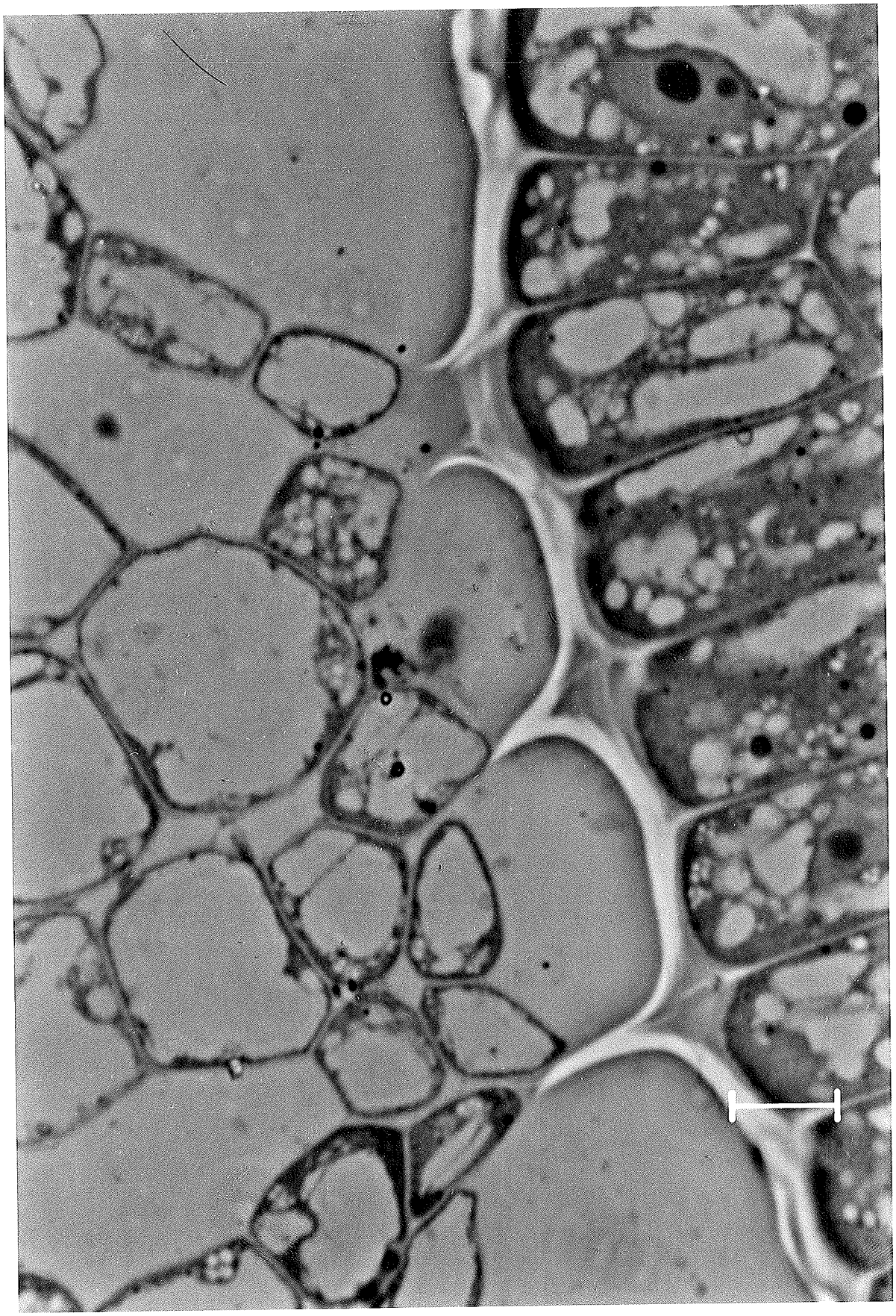


Plate 33. C.S. radicle and coleorhiza tissue. Showing radicle mucigel.
(var. Argyle). Bar = 12 μm.



presence between radicle-cap cells and the protoderm (Plates 31a and b) has been reported on root primordiums (Esau, 1977). Where the mucigel traverses the vestibule, the movement of apoplastic water from the coleorhiza to the radicle may be facilitated.

6.6 SUMMARY AND CONCLUSIONS

The scanning electron micrographs of CPD grains indicated that hydrostatic pressure failed to discernibly influence coleorhiza integrity. The distal surfaces of both treated and control coleorhizas were quite disrupted and bore ruptured cells. The origin of this disruption was uncertain, however, several possible sources were noted. These were 1) testa/pericarp rupture, 2) abnormal distribution of swelling strains due to excessive water uptake during aerated steeping, 3) sudden shrinking strains during the first dehydration step, and 4) grain impact caused by the turbulent action of steep aeration. Of these sources the last two appeared to be the most likely.

Proximally the surface of coleorhizas bore discrete apertures determined by cell geometry. The average radius of these apertures was approximately 40 μm. From the equation[11] an excess pressure of approximately 37 KPa would

---

[11] excess pressure, $p_1 = \frac{2\gamma}{r}$

where r = aperture radius (approximately 40×10^{-6}m)
and γ = water surface tension (7.4×10^{-2}Nm^{-2})

be required for water to gain entry. Aperture hydrophobicity would however, increase the minimum excess pressure required for water to gain entry.

In cross section the coleorhiza tissue appeared to contain numerous intercellular spaces. In addition a vestibule was observed between coleorhizal internal surfaces and seminal roots. Unlike the outer bounding cell layer of the cell layer of the coleorhiza, the internal cell layer was discontinuous. The discontinuity and large vestibule may have simply been the consequence of coleorhiza swelling. Alternatively however, ethylene induced lysigenous aerenchyma formation during steeping might have been responsible for the vestibule and coleorhizal discontinuity.

The results presented do not establish whether a gas or water phase occupies the coleorhiza intercellularies and vestibule. Woolley (1983) drew attention to the inexplicable presence in plants of gas phase intercellularies in equilibrium with water. In addition, he noted that anoxia could cause such intercellularies to flood. Hough et al. (1971) stated than even in aerated steeps the level of dissolved O_2 inadequately supports complete aerobism. Hydrophobic monolayers have been proposed (Sorokin, 1966; Roland, 1978; Woolley, 1983) to line gas filled intercellularies, it would seem likely that the internal surface of the coleorhiza bore such a layer and that this mechanism would prevent vestibule flooding during swelling. The presence of a gas phase

has been reported in barley embryos following imbibition (Allerup, 1969) as well as in barley coleoptile and corn scutellum tissue slices (Ohmura and Howell, 1960).

Hydrostatic pressure treatments (Chapters 4 and 5) probably forced water (ink, Yoshida, et al. 1979) into a previously gas filled embryonic vestibule. The excess pressure required appears, based on aperture radii and the minimum chitting inhibition pressure (96 KPa Section 4.3), to have been determined by aperture hydrophobicity and tissue hydraulic conductivity (tortuosity), a property of root tissue which has been reported (Fiscus, 1977) to vary with tissue density and rise to a constant level with increased hydrostatic pressure.

CHAPTER VII

RESPIRATORY ACTIVITY

7.1 Introduction

Eyben and Droogenbroeck (1969), Davidson and Jangaard (1978) and Yoshida et al. (1979) all reported that hydrostatic pressure restricted the respiratory activity of steeped grain. Eyben and Droogenbroeck (1969) observed that the degree of restriction increased with greater magnitudes of treatment and that the recovery period was prolonged by greater treatment duration. Yoshida et al. (1979) determined that the respiratory restrictions associated with independent treatments were cumulative and that treated grain may never re-establish pre-treatment respiratory levels. The results presented in Chapter VI indicated that hydrostatic pressure treatments (up to 193 KPa for 60 seconds) failed to disrupt the integrity of the coleorhiza and radicle meristem. Although tonoplasts appeared to be intact, temporary (Kuiper, 1972) or permanent (Coster et al. 1977) modification of membrane properties may have taken place during treatment. Such modifications may have affected the ability of cells to function normally. In the following experiments the viability and O_2 consumption of treated grains were investigated.

7.1.1 The Tetrazolium Viability Test

The tetrazolium test is frequently used to determine the viability of barley grains (Brookes, 1979) as well as other plant tissues (Delouche *et al*. 1962). The colourless solution of the tetrazolium salt (2,3,5-triphenyl tetrazolium chloride) is reduced to an insoluble red formazan by the activity of dehydrogenase enzymes (Bewley and Black, 1982). The loss or absence of dehydrogenase activity is often associated with a concurrent loss of respiratory activity and seed viability (Delouche *et al*. 1962). Bewley and Black (1982) note however, that dehydrogenase activity does not ensure viability.

7.1.2 Materials and Methods

Argyle was surface sterilized for 2 hours (as outlined in Section 5.3.2) before being steeped with aeration at 15°C for a further 28 hours. Treated grains were subjected to a hydrostatic pressure of 193 KPa for 30 seconds. Thirty hour samples of treated and control grains were then subjected to the tetrazolium test. Additional grain samples were set to continue germination at 15°C for a further 30, 90, 120 or 180 hours in petri-dishes before being subjected to the test. Each petri-dish (replicate) contained 20 grains, 2 Whatman No. 1 filter papers and 4 ml of water. For each germination time, 6 replicates of each grain type were set up.

At testing time the grains of each replicate were sectioned longitudinally. This section was made slightly to the side of the ventral furrow to ensure the radicle was left intact in one grain half. Replicate grain halves (with intact radicles) were then placed in individual glass vials containing (0.5%) 2,3,5-triphenyl tetrazolium chloride. The vials were then placed in an oven at 40°C for 1 hour. Following removal from the oven the embryo regions were examined for stain development. A mean stain development number for each embryo region was then determined from the 6 (20 grains per replicate) replicates of each grain type.

7.1.3 Results and Discussion

The results for embryo region stain development are presented in Table 13. Four millilitres of water was used in each petri-dish, as this volume was shown to be inhibitory to the continued germination of treated grains (Section 5.4). Generally the intensity of stain development was less for treated grains at all germination times. However, with the exception of the radicles of a few treated grains all other embryo regions did stain. The absence of staining in treated grains was only significant ($p = 0.05$) from 120 hours onward. The absence of radicle staining after 120 hours did, however, significantly increase with germination time such that the greatest number of non-stained radicles occurred in the final 180 hour treatment samples.

TABLE 13. Tetrazolium[1] staining of Argyle embryos subjected to hydrostatic pressure[2] at 28 steeping hours prior to being germinated in petri-dishes[3].

Treatment	Mean Number of Stained Embryo Regions					Number of Chitted Grains
	Coleorhiza	Radicle	Node	Scutellum	Plumule	
30 Hour (Control)	19.7[4]	19.7 a[5]	20.0	20.0	20.0	0.0
Treatment	19.3	19.5 a	20.0	20.0	19.6	0.0
60 Hour (Control)	20.0	20.0 a	20.0	20.0	20.0	-
Treatment	20.0	19.5 a	20.0	20.0	20.0	-
120 Hour (Control)	20.0	20.0 a	20.0	20.0	20.0	19.8
Treatment	20.0	17.3 b	20.0	20.0	20.0	7.3
150 Hour (Control)	20.0	19.7 a	20.0	19.8	20.0	19.3
Treatment	19.5	16.3 c	19.5	19.5	20.0	11.0
210 Hour (Control)	19.8	19.8 a	19.8	19.8	19.8	19.3
Treatment	19.8	13.5 d	19.8	19.8	19.8	9.2
CV	2.5	4.4	1.6	1.7	1.8	10.4

[1]0.5% 2,3,5-triphenyl tetrazolium chloride.
[2]193 KPa for 30 seconds at 28 steeping hours.
[3]Each petri-dish (replicate) contained 20 Argyle grains, 2 Whatman No.1 filter papers and 4 ml of water.
[4]Each mean was determined from six replicates of 20 grains.
[5]Mean numbers followed by the same alphabetic subscript are not significantly different at alpha 0.05 (Duncan's multiple range test).

Plate 34 portrays five inhibited treated grains following 210 hours germination time. Four of the treated grains shown in the photograph have radicles which failed to stain. Delouche et al. 1962 notes that grains where more than 3/4 of the radicle fails to stain are non-germinable (non-viable). These results indicated that one possible outcome of hydrostatic pressure treatment was the loss of radicle dehydrogenase activity. However, it is important to note that in all significant treatment cases the number of non-stained radicles never exceeded 33%.

The significant increase in non-stained treated radicles with germination time indicated that where radicle tissue lost its viability, it was not the immediate consequence of treatment. The treatment may have reinstated pre testa/pericarp rupture anoxia (respiratory lag, Phase II, Bewley and Black, 1978) or perpetuated steep-imposed anoxia that exists following coat rupture even in aerated steeps (Hough et al. 1971). Thus treated grains in the germination environment may have remained in Phase II whilst control grains progressed into respiration Phases III and IV. The treatment imposed anoxia (Phase II) would promote the accumulation of ethanol and lactic acid in the steeped barley. The ability of the grain to tolerate the extended anoxia (avoid alcohol narcosis) would depend on the grain's glycolytic rate, lactic acid O_2 debt carrying capability (Crawford, 1977) and the potential for alcohol oxidation (Cossins and Turner, 1962). The accumulation of ethanol within steeped

Plate 34. Five inhibited treated grains after a total of 210 hours germination time.
(var. Argyle).

barley grain has been reported to be a potential source of germination inhibition (Brookes et al, 1976). In contrast to rice, barley grains have been determined to be incapable of converting ethanol to ethanal even under aerobic conditions (Cossins and Turner, 1962). Crawford (1977) proposed that the anoxia tolerance of rice grain is derived from its low glycolytic rate and its ability to accumulate a lactic acid O_2 debt. In intolerant grains anoxia stimulates an increased glycolytic rate which results in greater lactic acid and ethanol accumulation. Further, the accumulation of lactic acid reduces the pH and favours increased decarboxylase activity. The accumulated ethanol eventually fluidizes cell membranes further reducing the cell charge and permanently inactivating mitochondria. The mitochondrial inactivation results in the inability of grain to establish Phase III respiration when returned to aerobic conditions.

Thus reduced membrane hydrophobicity induced by ethanol accumulation might have been responsible for the loss of radicle viability (Plate 34). However, even if ethanol dehydrogenase was not active the activity of other dehydrogenases could have reasonably been expected to have been detected. Perhaps the narcotic effects of ethanol accumulation had progressed to such a degree that protein degeneration and cessation of cell metabolism occurred before the tetrazolium test was carried out.

The majority of inhibited treated grain radicles did stain (centre grain Plate 34). However, irrespective of this dehydrogenase activity the radicles of these inhibited treated grains often failed to grow beyond eruption through the coleorhiza (chitting). Bewley and Black (1982) noted that the presence of such activity does not ensure viability. In Section 5.5 it was shown that treatment resulted in a slower root growth rate and/or delayed seminal root development. Ethanol narcosis (mitochondrial damage) could have been responsible for the growth inhibition of chitted treated grains having dehydrogenase activity. It has been reported that ethanol can inhibit radicle and coleoptile growth in germinating corn (Nashed and Girton, 1958). However, Brookes et al. (1976) drew attention to the fact the grain ethanol accumulation fails to inhibit the germination of traditionally steeped barley. Further, the simple aerated steeping apparatus used did support root growth, although to a lesser extent than germination environments, even though aerated commercial steeps fail to prevent grain anoxia (Hough et al. 1971).

Jackson et al. (1982) criticized Crawford's (1977) contention that flooding-intolerant roots under anoxic conditions suffer from ethanol narcosis. The accumulation of ethanol resulting from stimulated glycolysis and alcohol dehydrogenase activity. From their experiments on Pisum sativum. L Jackson et al. (1982) reported that

exogenous ethanol applications up to 100 X's the concentration of endogenous (Xylem sap 2.1 mol m^{-3}) or flooded soil (3.9 mol m^{-3}) concentrations failed to stimulate flooding injury in roots, isolated leaf protoplasts, or detached leaves under aerobic or anaerobic conditions. Cannel and Jackson (1981) however, proposed that ethanol (3.8 mM) induces leakage of amino acids and sugars. Jackson et al. (1982) proposed that flooding tolerance may reflect the ability of plant tissue to adapt morphologically. In this regard they suggested that the ability to form aerenchyma (gas filled channels) may determine the ability to overcome anoxia. Barber et al. (1962) examined the cross-sectional area of gas spaces in the roots of barley and rice plants grown in aerated water. They determined that in rice the space area varied between 5 and 30% whilst in barley it was less than 1%. From this they concluded that the degree of gas space development in rice roots is an adaptation for waterlogged conditions.

Evidence for the existence of intercellular and intertissue spaces has been presented in Section 6.5. If these spaces are gas filled they might become occluded with water during hydrostatic pressure treatment. The occlusion of the radicle vestibule and coleorhiza intercellular spaces may encourage anoxic conditions around the radicle prior to its eruption through the coleorhiza. The potential would then exist for the radicle intercellularies to become flooded

(Woolley, 1983). Roland (1978) proposed that these apical intercellularies function primarily in CO_2 export. Increased inhibition of CO_2 movement away from the grain could contribute to germination inhibition (Kidd, 1913ab, Radley, 1976). Burstrom (1959) noted that once apical intercellularies flood the condition is permanent even following a return to aerobic conditions. Further, Burstrom observed that the flooding results in a decline in axial root growth.

7.2 OXYGEN CONSUMPTION OF GRAINS SUBJECTED TO HYDROSTATIC PRESSURE

7.2.1 Introduction

The tetrazolium viability test indicated that under conditions of excess water the majority of inhibited treatment embryos retained dehydrogenase activity. Preliminary observations (Section 5.4) revealed that treated grain would re-initiate growth if allowed to dry slightly. Although the tetrazolium test provides evidence for the presence of respiratory activity it does not distinguish between respiration types. The findings of Eyben and Droogenbroeck (1969), Davidson and Jangaard (1978) and Yoshida et al. (1979) demonstrated that hydrostatic pressure treatments can restrict aerobic respiratory activity both during and subsequent to steeping. In the following experiment the O_2 consumption of pressure treated grains germinated under conditions of excess water was compared with that of control grains.

7.2.2 Materials and Methods

Argyle was steeped as outlined in section 7.1.2. Treated grains were subjected to a hydrostatic pressure treatment of 193 KPa for 30 seconds. The O_2 consumption of 3 treatment and control grain replicates was measured at 30 steeping hours. Each replicate consisted of 200 grains. Additional replicates were set to continue germination at 15°C for a further 20, 50, 70, 95 or 125 hours in petri-dishes. Each petri-dish contained 20 grains, 2 Whatman No.1 filter papers and 4 ml of water. The 200 grains for each O_2 consumption replicate were obtained from 10 treatment or control petri-dishes.

Preliminary experiments had indicated that most of the O_2 demand of germinating barley was associated with rootlets. In order to obtain a representative measure of grain O_2 demand, grain roots and exposed plumules were removed.

The O_2 consumption of each grain replicate (200 grains) was measured in air saturated distilled water at 15°C using 300 ml BOD bottles and an Orion O_2 electrode, model 97-08. During measurements the BOD bottle was immersed in a water bath maintained at 15°C. In addition, grains were continually stirred by a magnetic bar stirrer.

7.2.3 Results and Discussion

The O_2 consumption pattern at 3 minute intervals of treated and control grains at 30 steeping hours is

presented in Figure 9. Hydrostatic pressure significantly (P=0.01) depressed consumption over the 90 minute measurement period. Although treated and control grain O_2 consumption significantly declined with time no significant interaction between treatments and time took place. The pattern of declining comsumption with time observed at 30 steeping hours was also displayed by both grain types at other germination times. In all cases there was a rapid reduction in dissolved O_2 accompanied by a declining consumption rate over the first 20 to 30 minutes. This period was followed by a more gradual decline in dissolved O_2 which reflected the still declining but erratic consumption rate over the last 60 minutes.

Because two distinct periods appeared to occur during the measurement periods, O_2 consumption rates for the total 90, first 30 and last 60 minutes were plotted for all germination times (Figures 10, 11 and 12). Hydrostatic pressure at 30 steeping hours significantly (P = 0.01) depressed O_2 consumption rate over the whole germination period. In addition the rates of treated and control grains significantly increased with time. However, at any single germination time the depression in consumption rate of treated grains was with the exception of a few points (50, 75, 125 hours figure 10 and 125 hours figure 11) not significant.

Observations of O_2 consumption indicated that treatment had not totally inhibited aerobic respiration. Yoshida et al. 1979 proposed that the restriction of

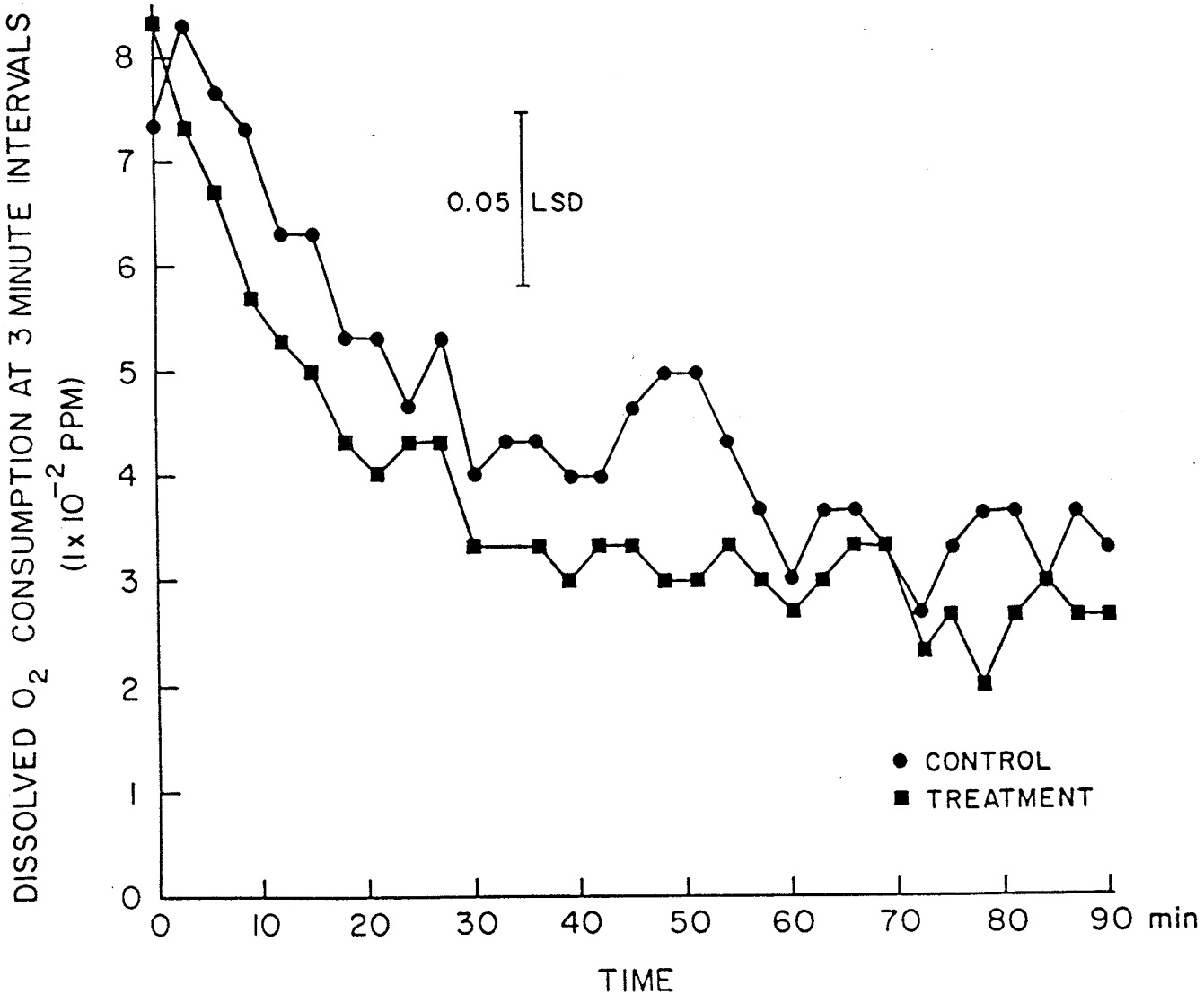


Figure 9. Oxygen consumption at 30 steeping hours. $LSD_{0.05} = 1.66 \times 10^{-2}$ PPM MIN^{-1}.

respiratory activity following treatment may have been due to embryonic damage. However, the ability of H_2O_2 and restricted moisture in the germination environment to prevent and/or alleviate treatment effects (Chapter 5) coupled with the absence of discernible embryonic disruption (Chapter 6) indicated that the restriction was imposed by some other mechanism.

Observations (Chapter 6) indicated that a vestibule exists between the internal surface of the coleorhiza and the embryonic roots. In addition numerous intercellular spaces determined by the polyhedral geometry of parenchyma cells were present within coleorhizas. Gershoy *et al.* (1976) reported that embryos contain numerous gas-filled intercellularies which aid the diffusion of respiratory gases. The hydrostatic pressure treatment could have partially or totally occluded gas diffusion pathways with water. The diffusion coefficients at 25 C for O_2 and CO_2 in air (0.226 and 0.181 $cm^2\ sec^{-1}$) are approximately 1 x 10^4 times greater than their respective values in water (2.60 x 10^{-5} and 2.04 x 10^{-5} $cm^2\ sec^{-1}$) at the same temperature. From these coefficent values and Fick's equation[12] it can be appreciated that the displacement of a grain gas phase with a water phase might considerably inhibit aerobic activity.

The nature of the apparatus used to measure O_2 consumption avoided the establishment of gas diffusion

---

[12] $dn = -DA \frac{dc}{dx} dt$

were dn is the amount of gas that diffuses in time dt across an area A where the concentration gradient is dc/dx and D is the diffusion coefficient.

gradients around the grains. Air saturated water at 15°C and approximately 1 atm was used for all recordings. The dissolved O_2 volume was therefore approximately 33% (10 ppm) of the total dissolved gas volume. In air saturated water at 25°C the volume of dissolved O_2 would be approximately 21% (8 ppm). Therefore at least initially the available O_2 in the bulk water around the grains would not have been inhibiting to phase III (RQ=1) respiratory activity within both grain types. The reduced consumption of treated grains was therefore assumed to reflect a greater resistance to gas diffusion within the respiring tissue of the grain.

During the 90 minute period over which O_2 consumption was monitored every 3 minutes, consumption significantly declined for both grain types (Figure 9). In order to provide a possible explanation for this observation several simplifying assumptions will be used. Firstly, since initially the grains were placed in air saturated water the ppO_2 outside the grains was greater than that in the gas phase surrounding respiring embryonic tissue. Secondly, that the partial pressures of gases within the gas phase of treated and control grains was initially the same and declined in such a manner (during measurement) that the differences between partial pressures remained constant whilst aerobic activity was not inhibited by low ppO_2 in the bulk water surrounding the grains. Thirdly, that the considerably greater values of gas diffusion constants for the gas phase

relative to the water phase resulted in diffusion resistance through the gas phase of treated and control grains being negligible. Finally, that the partition gradient for gases ($^{dc}/dx$) across the water/gas interface was constant for both grain types whilst aerobic activity was not inhibited.

Although constant stirring was used to prevent the establishment of diffusion gradients between the electrode and the grains, both grain types would nevertheless have had a bound water layer associated with their surfaces. Thus in both grain types O_2 diffusion to the embryo gas phase was probably limited by the low O_2 diffusivity in water. In the case of control grains a bound water/coleorhizal intercellulary gas interface (phase partition) probably existed at or just below the surface of the coleorhiza. Yoshida et al. (1979) reported and Plate 7 depicts that ink under pressure can be forced into the nodal region of the embryo. This observation indicates that during hydrostatic pressure treatment the phase partition was pushed below the coleorhizal surface into the embryonic vestibule, effectively increasing the depth of the bound water. Previously it was assumed the $^{dc}/dx$ for the partition of treated and control grains was constant. From Fick's equation it can be seen that the rate of O_2 diffusion through the grain water phase would not change whilst $^{dc}/dx$ remained constant. However,

adopting the assumption that in the gas phase of both grain types the partial pressure of gases was the same, then water phase gradients of control grains would have been greater than that of treated grains. The greater water phase gradient of control grains would account for the more rapid consumption of O_2 surrounding these grains (Figure 9). Generally it appears possible that treatment increased the depth (dx) of the water phase decreasing the gradient and therefore the rate of O_2 diffusion. With this in mind it is interesting to note that Eyben and Droogenbroeck (1969) reported that with greater magnitudes of hydrostatic pressure respiratory activity was increasingly restricted. Possibly greater pressure moves the phase partition deeper into the embryonic tissue (increasing dx water phase) resulting in a decreased concentration gradient across the water phase.

With time (Figure 9) respiration reduced the level of dissolved O_2 in the bulk water. This would have resulted in a reduction in the water phase gradient. For the same decline in dissolved O_2, the water phase gradients of treated and control grains would have declined equally. However, because of the shorter water phase resistance in control grains, their gradients and O_2 consumption will have remained constantly greater than that of treated grains providing dissolved O_2 was not limiting respiration. Thus the reduction of O_2 consumption observed (Figure 9) was

probably the results of the declining water phase gradients. Eventually the declining gradients could have limited the rate of O_2 diffusion to such a degree that aerobic activity was inhibited.

Previously it was assumed that partition gradients would have remained constant provided conditions remained aerobic. However considering the following (Amoore, 1961), partition gradients probably declined before the level of bulk O_2 imposed anoxia. Amoore (1961) observed the hyperbolic relationship between the QO_2 of excised (Pisum sativum var. Meteor) root tips at 25°C and O_2 tension. The maximum rate of O_2 consumption (QO_2 -9.4 L O_2 mg^{-1} dry wt h^{-1}) was obtained with tensions of 40 to 50%. In air the QO_2 increased to -7.8. Hoar (1975) described the critical O_2 pressure above which the rate of O_2 uptake (respiratory activity) is independent of ppO_2 as the incipient limiting tension. Further, he described the minimum ppO_2 required to maintain basal metabolism as the incipient lethal tension. Between these tensions the rate of O_2 uptake is dependent on the ppO_2 (respiratory dependence). In air saturated water at 1 atm and 15°C O_2 comprises approximately 33% (10 ppm) of the dissolved gas volume. Thus irrespective of grain type aerobic respiration from the beginning of measurement was probably limited by the O_2 tension. During respiratory dependence respiration rate would have continued to decline along with falling O_2 tension (dissolved O_2).

Partial pressures of O_2 below the incipient lethal tension induce a condition of anoxia (RQ>1) within the

tissue which if persistent could result in narcosis and cell death. At 1 atm air saturated water at 25°C has an O_2 concentration of 8 ppm. Thus while the O_2 concentration remained above 8 ppm the rate of O_2 diffusion into control grain would not have been expected to be less than that at the incipient lethal level. However, in treated grains the greater O_2 diffusion resistance imposed by the flooded intercellularies and vestibule would have effectively raised the ppO_2 at which the lethal tension occurred. Thus in addition to having a higher rate of aerobic activity than treated grains at the same level of dissolved O_2, control grain basal metabolism could have been sustained at a lower level of dissolved O_2. In this regard it is interesting to note that Crabb and Kirsop (1969) were able to germinate non-dormant barley embryos at lower O_2 tensions than water-sensitive embryos. In addition, Vidaver and Lue-Kim (1967) observed that continuous hydrostatic pressure only reduced the germination rate of Lactuca sativa when the dissolved O_2 concentration was limiting. They were able to increase the rate of germination with greater O_2 concentrations, up to a limiting concentration, irrespective of continuous constant hydrostatic pressures up to 48 MPa.

At the beginning of measurements (Figure 9) the level of dissolved O_2 would have been nearer to or possibly below the incipient lethal tension of treated grains. High RQ values and alcohol narcosis might reasonably be expected to have been experienced by these grains first. Any difference in RQ values between control and treated grains would

invalidate the previous assumption that the partial pressures of gases within grain gas phases were the same. Provided total pressure remained constant, grains with higher RQ's would have a lower gas phase ppO_2. For the same increase in RQ the change (steeper) in partition gradients would have been greater in control grains. However, since treated embryos probably entered anoxia first, O_2 consumption by these grains would decline less rapidly than control grains not experiencing anoxia (Figure 9). Control grain would be expected to show this slower decline in O_2 consumption later than treated. Once the dissolved O_2 in the bulk water fell below the respective lethal tensions increasing RQ values, declining pH and alcohol narcosis would influence O_2 diffusion in addition to ppO_2 in the bulk water. Oxygen consumption by controls would be expected to remain greater than that of treated grains for the same level of dissolved O_2 whilst ppO_2 remained the single most important factor influencing the O_2 diffusion rate.

Figure 11 presents the O_2 consumption rates for the first 30 minutes of measurement time for each germination period. During this time control grains would have been least likely to be experiencing anoxia as the concentration of dissolved O_2 generally did not fall below 8 ppm until the last 60 minutes of measurement (Figure 12). However, irrespective of the relative degree of anoxia within both grains, treatment significantly ($p = 0.01$) reduced the rate

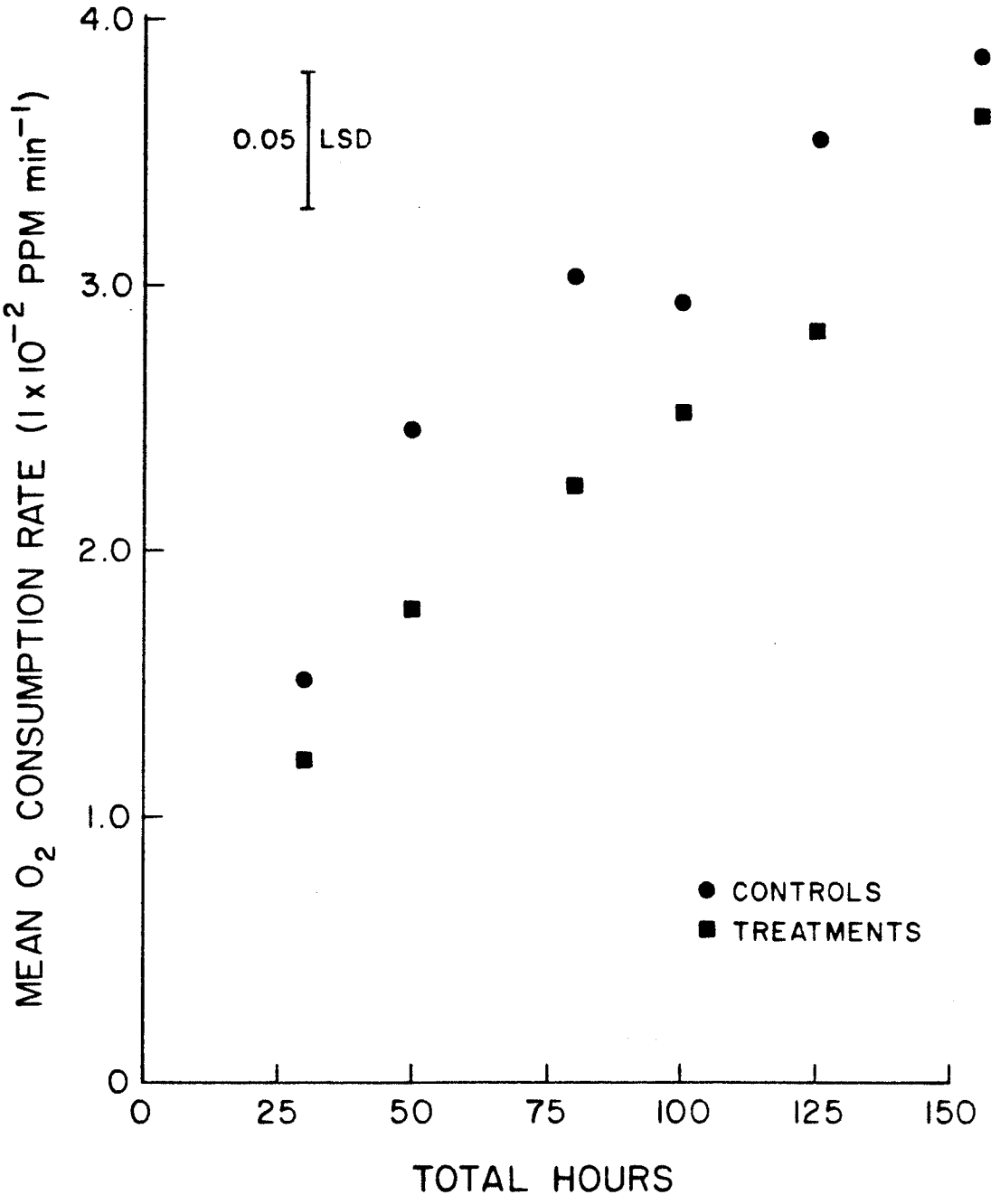


Figure 10. Mean O_2 consumption rate over 90 minutes. $LSD_{0.05} = 0.52 \times 10^{-2}$ PPM MIN^{-1}.

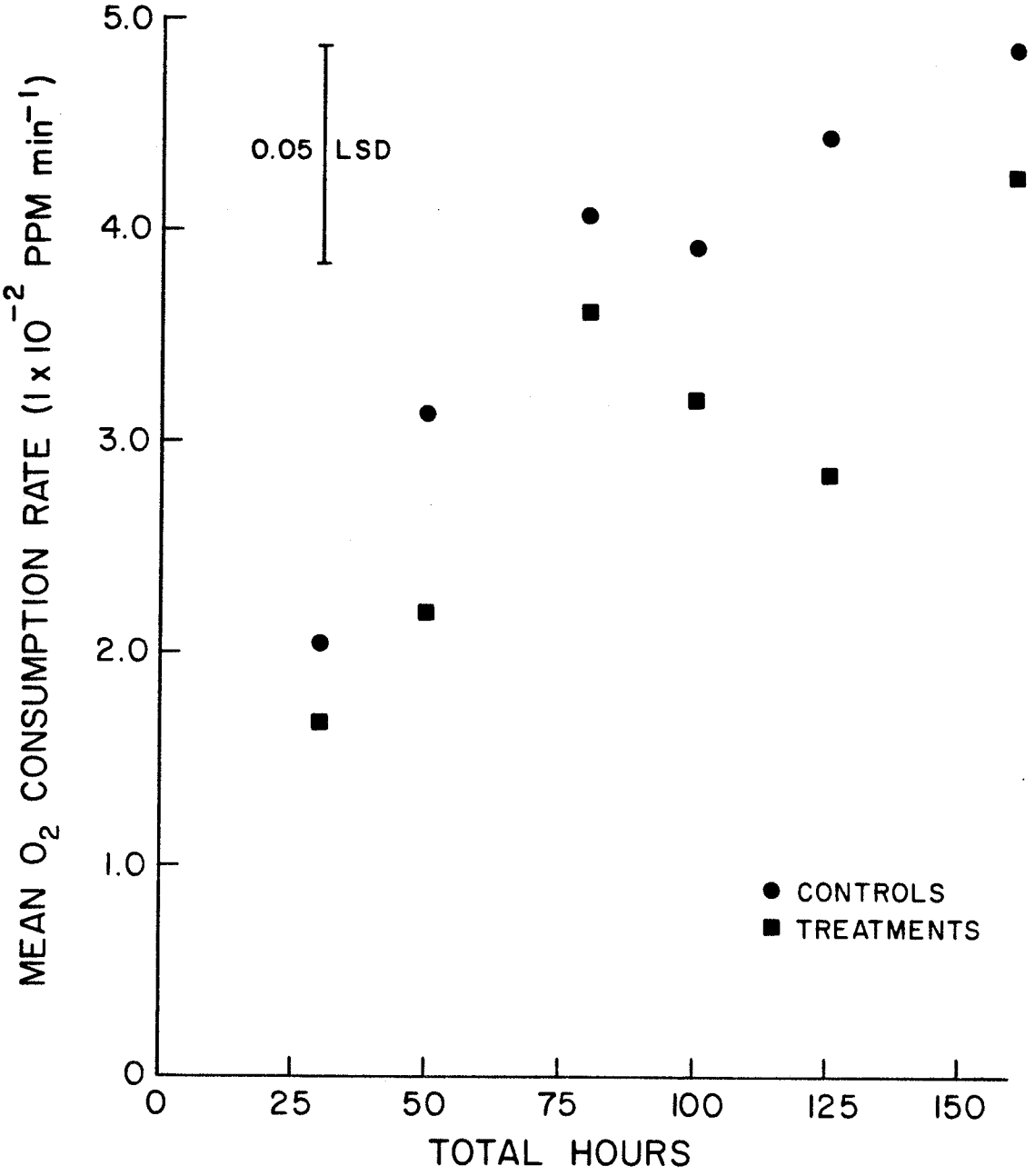


Figure 11. Mean O_2 consumption rate over the first 30 minutes.
$LSD_{0.05}$ 0.82 x 10^{-2} PPM MIN^{-1}.

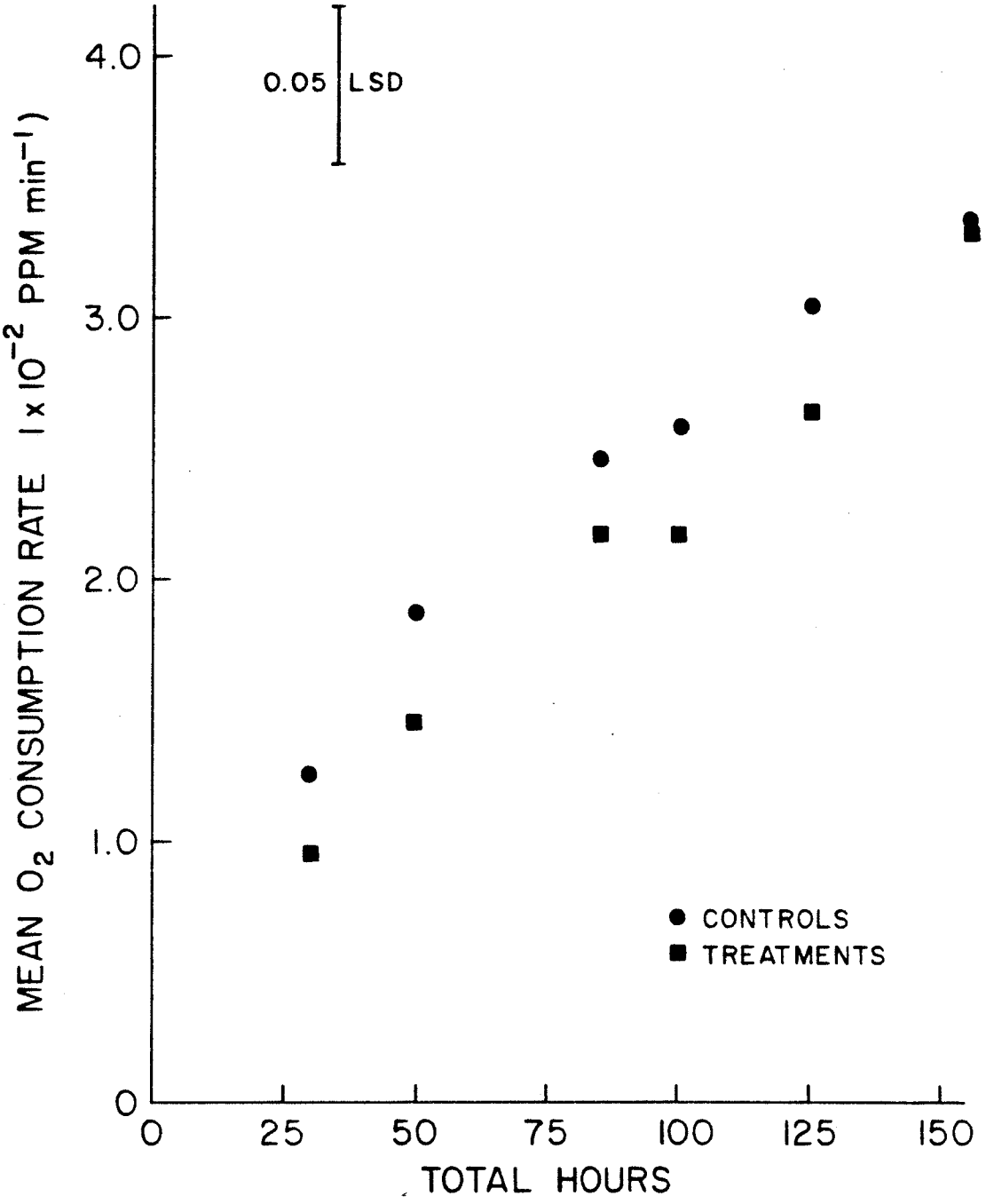


Figure 12. Mean O_2 consumption rate over the last 60 minutes.
$LSD_{0.05}$ 0.61 x 10^{-2} PPM MIN^{-1}.

of O_2 consumption over the whole germination period. Davidson and Jangaard (1978) subjected barley grains with rootlets to a hydrostatic pressure treatment. Following treatment they removed grain rootlets and measured a significant inhibition of QO_2 in treated grains. Preliminary experiments had indicated that the majority of O_2 consumption by grains with rootlets was associated with roots. The consumption rates presented in Figures 10, 11 and 12 are for grains where either grain rootlets were absent or removed. With the exception of grains at 30 and 50 hours, where there was no rootlet emergence by either grain type, control grain samples at other germination times showed an increasingly greater proportion of rootlet development than treated grains. Therefore at any germination time after 50 hours the number of cut root surfaces in control samples was greater than that of treated grain samples.

Two problems in interpreting the results arose from the presence of these cut surfaces. Firstly, was the significant ($p = 0.01$) increase in O_2 consumption by both grain types with germination time simply a reflection of an increasing number of exposed root surfaces? Secondly, was the significant ($p = 0.01$) depression of O_2 consumption in treated grains merely the consequence of fewer exposed root surfaces at all germination times after 50 hours? Prior to rootlet emergence O_2 consumption (Figure 10) was not significantly ($p = 0.05$) different at 30 hours immediately

after treatment. At 50 hours however, the O_2 consumption of control grains was significantly greater than that of treated grains. Between 30 and 50 hours the consumption of both grain types increased significantly, the increase was however greater in controls. Thus from 30 to 50 hours the increase was not the consequence of a greater number of exposed rootlet surfaces.

Bewley and Black (1978) noted that increased grain respiration from Phase II to Phase III (RQ falls to 1) is generally associated with the rupture of the testa. Control grain at 50 hours (Figure 10) might therefore reasonably be expected to have been in Phase III. Brookes et al. (1976) drew attention to the relationship between plumule development and grain respiration. They noted that growth velocity increases with respiration rate and that the maximum rate occurs when the plumule is 3/8 to 5/8 the length of the grain. In the case of both control and treated grains coleoptiles did not develop beyond the scutellum disc in the first 50 hours. Based on coleoptile growth, respiration reached a maximum in 21% of control grains, after 100 hours. Treated grain coleoptile growth was generally slower, with 17% of treated grains having coleoptile development equivalent to respiratory maximum after 125 hours. After 150 hours the coleoptile lengths of control grains ranged for 1 1/2 to 2 x's the grain length and only 2.5% showed no growth at all. In contrast at 150 hours the most

advanced treated grains had coleoptiles equal to the full length of the grain. However, 62% of treated grains showed no growth at all at 150 hours. The large difference in the number of exposed cut root surfaces between control and treated grains at 150 hours does not appear to have strongly distorted O_2 consumption values since at 150 hours there was no significant difference (Figure 10, 11 and 12) between O_2 consumption for both grain types. The increase in consumption with time for both treatments was therefore associated with grain respiration (coleoptile development) to a greater extent than a greater number of cut root surfaces.

The respiratory maximum corresponds to the peak of Phase III respiration. In those treated grains that showed no growth the attainment of respiratory maximum was apparently delayed. Phase III coincides with the onset of growth, since more than half of the treated grains never grew for up to 150 hours it can only be assumed that they were in Phase II or non-viable. The results in Section 7.1.3 (Plate 34) indicated that such grains have dehydrogenase activity. In addition the O_2 consumption of treated grain samples was generally not significantly less than controls at any single germination time. Therefore it would appear that a considerable proportion of treated grains were locked in Phase II respiratory activity. Bewley and Black (1978) noted that Phase II respiration is thought to be partially imposed by hydrated seed coats restricting O_2 supply to the embryo.

7.3 SUMMARY AND CONCLUSIONS

The majority of treated grains set to germinate under conditions of excess water had dehydrogenase activity irrespective of the presence or absence of rootlet growth. However, with increased germination time a significant proportion ($\leq$33%) of treated grain radicles failed to stain during incubation with tetrazolium chloride. These observations indicated that the cause of chitting inhibition was not the consequence of a complete absence of respiratory activity (dehydrogenase activity) and that where activity was absent (after 120 hours) it was not the immediate outcome of treatment.

Without respiratory quotients for treated and control grain it was not possible to determine whether treatment induced grain anoxia. However, treated grain did generally consume less O_2 and was therefore respiring at a lower rate. Initially (first 20 minutes figure 9) the level of dissolved O_2 probably did not totally inhibit aerobic respiration in both grain types. Eventually however, it appears that the level of dissolved O_2 fell below the incipient lethal tension of both treated and control grains. Despite this, under conditions that were probably anoxic to both grain types, control grains continued to consume O_2 at a higher rate than treated grains. It would seem reasonable to assume that the lower respiration rate of treated grains was

the consequence of greater O_2 diffusion resistance (dx water phase treated grains > control grains). However, it remains uncertain whether or not such a resistance was responsible for the chitting inhibition of treated grains. The possibility that dx treated grain water phase was solely responsible for the initial restriction of O_2 consumption might well explain why greater magnitudes of treatment (Eyben and Droogenbroeck 1969) further restricted respiratory action.

Consumption rates were generally not significantly different at any one germination time. Perhaps increasing the number of replicates and/or number of grains per replicate might have resulted in significant differences. The value of comparing the O_2 consumption of treated and control grain samples after 50 hours was perhaps questionable since grains with root growth (peak Phase III) respiration would be expected to have had higher respiration rates, even following the removal of exposed rootlets, than grains not showing rootlet emergence (early Phase III or Phase II). Possibly it would have been more meaningful to have selected out those grains from 50 hours onwards which failed to show radicle eruption (chitting) and compare their O_2 consumption rates with that of treated grains at 30 and 50 hours. Such a comparison would have avoided the uncertainty introduced by cut root surfaces and in addition provided a more reliable indication of whether or not treated grain respiration increased or decreased with germination time.

Finally, the accuracy of an O_2 electrode apparatus in measuring O_2 consumption of grains sensitive to hydrostatic pressure is perhaps uncertain. Since the immersed grains were kept in continuous motion to avoid the establishment of diffusion gradients, they must have been subjected to hydrostatic pressure. Block and Morgan (1967) used a propeller (350 rpm) to provide turbulent agitation during steeping of barley and wheat. They observed that agitation inhibited both germination and root growth in a manner similar to hydrostatic pressure. It appears likely therefore, that magnetic stirrer agitation could have had a similar effect.

CHAPTER VIII

GENERAL DISCUSSION

The post-treatment properties of steeped barley grains subjected to hydrostatic pressure were similar to those of water-sensitive grains. Generally water-sensitivity is considered a form of dormancy which occurs as a consequence of embryo anoxia under conditions of excess water. There is no unanimity concerning the fundamental cause of the anoxia. Uncoupling of oxidative phosphorylation (Jansson 1962), sulphydryl groups (Jansson 1960, Pollock and Pool 1962) microbial competition for O_2 accompanied by phytotoxin production (Blum and Gilbert 1957, Gaber and Roberts 1969b) and an intrinsically higher O_2 requirement by the embryos of water-sensitive grains (Crabb and Kirsop, 1969) have all been proposed as mechanisms of water-sensitivity. With the exception of the intrinsic nature of the higher O_2 requirement, all of these mechanisms may have participated in inhibition of chitting and root growth following hydrostatic pressure treatment.

The ability of H_2O_2 treatments and oxygenated steeps to alleviate and compensate hydrostatic pressure treatments, in addition to the absence of discernible embryonic disruption and the slower rate of O_2 consumption by treated grain indicated that embryo anoxia was a possible

source of growth inhibition. Jansson et al. (1959) concluded that the ability of elevated O_2 partial pressures and H_2O_2 treatments to break water-sensitivity in grains with intact pericarps was the consequence of greater O_2 availability to the embryo. However, in the case of steeped grain subjected to pressure under H_2O_2, increased availability of O_2 was perhaps an indirect effect and not the only outcome of treatment. With the exception of surface sterilization and possibly H_2O_2 treatments, water-sensitivity breaking treatments involve the removal and/or breaking of water films associated with the barley grain surface and pericarp. The intact pericarp only inhibits the germination of water-sensitivity grain in the presence of excess water. The pericarps of grains subjected to hydrostatic pressure were not intact. Treatment however, made these pericarps, which were ruptured by coleorhizas, inhibitory to grain chitting (radicle eruption) irrespective of free water. Grain surface films may be removed by interrupted steeping, the air-rests allowing grain to absorb excess surface water. Acid decortication and scarification respectively remove and rupture the pericarp breaking water-sensitivity. Both drying and decortication alleviated the effects of hydrostatic pressure treatment. Observations indicated that hydrostatic pressure forced water into coleorhiza intercellularies and possibly beyond into an embryonic vestibule. Orphanos and Heydecker (1968) observed that drying of water soaked

Phaseolus vulgaris L. seed relieved soaking injury. They reported that drying removed water from a cavity between the cotyledons and that cavity water was responsible for soaking injury whenever it was in excess of that which the embryo and cotyledons could absorb at any time. Dry and low free water germination environments following hydrostatic pressure treatments possibly removed coleorhizal intercellulary water in addition to surface water, re-establishing gas pockets within coleorhiza tissue and reducing the amount of water in excess of that which the embryo could absorb. Alternatively the removal of surface water may have increased gas diffusion enough to allow germination to continue irrespective of flooded coleorhizal intercellularies. This would seem less likely however, since treated grains placed on dry filter paper showed a higher degree of chitting inhibition than grains in contact with low water volumes (1, 2 ml). Decortication probably did not influence initially the state of coleorhizal intercellularies in treated grains, but rather removed the pericarp from other embryo regions where hydrostatic pressure had not caused intercellulary flooding.

Orphanos and Heydecker (1968) reported that H_2O_2 decomposes in the inter-cotyledon cavity preventing soaking injury by expelling water from the cavity and supplying the embryo with O_2. Thus possibly the H_2O_2 treatments maintained the embryonic vestibule and coleorhizal intercellularies in a gas-filled state, O_2 pressure expelling

excess H_2O_2 after hydrostatic pressure was relieved. Concentrations (0.34%) of H_2O_2 similar to that used in treatments (0.3%) have been reported to inhibit root hair development and degenerate the radicle meristem (Collis-George and William 1968). Root growth, although modified, was not inhibited following H_2O_2 treatments indicating that intercellularies were probably filled with gas.

Allerup (1959) in barley and (Ohmura and Howell 1960) in corn, drew attention to the importance of the embryo gas phase in avoiding respiratory restriction. It appears that post-treatment environments that alleviated the inhibitory effects of hydrostatic pressure did so by removing occluded water or by providing an alternative gas phase pathway. The presence of a gas phase in and around the embryo would increase considerably the rate at which O_2 could diffuse to the embryo in addition to reducing the resistance to CO_2 diffusion away from the embryo.

Vlamis and Davis (1943) reported that barley germination and root growth failed to occur below 0.2% O_2 and that both germination and growth were inhibited below 9.5% O_2. Bewley and Black (1982) noted that the physical restriction of O_2 supply imposed by grain coats is generally considered to be insufficient to prevent the level of aerobic respiration required to support germination. In addition Brookes et al. (1976) questioned the significance of alcohol narcosis in grain germination inhibition and noted that the

level of dissolved O_2 in traditional steeps adequately supports germination (pericarp rupture). Where pressure treatments were inhibitory the pericarps of grains subjected to hydrostatic pressure were ruptured. For water pushed into coleorhiza intercellularies and embryonic vestibules to inhibit continued germination by anoxia, the resistance to O_2 diffusion could reasonably be expected to have been greater than that of intact hydrated pericarp or alternatively great enough to create an O_2 tension around the radicle that inadequately supports cell elongation and/or cell division. Bewley and Black (1982) note that O_2 diffusion coefficients for hydrated grain coats are in the order of 1 x 10^{2} less than that of water. Therefore water pushed into the gas filled intercellularies and vestibules might not have imposed any greater degree of anoxia on embryos than intact pericarps (prior to coleorhiza swelling). Bewley and Black (1978) state that in barley radicles, cell division commences following eruption through the coleorhiza and that growth prior to eruption is entirely by elongation. Preliminary observations however, indicated that a few ($\leq$1%) radicle cells do divide before eruption (chitting) and that these divisions were inhibited by hydrostatic pressure treatment. Amoore (1961) noted that the minimum O_2 tension required to maintain aerobic respiration in connective tissue (which has lower O_2 diffusivity than hydrated embryo tissue Allerup, 1959) is greater than that required to sus-

tain mitosis in excised Pisum sativum root tips. Grains subsequent to hydrostatic pressure treatment consumed O_2, albeit at a lower rate than control grains, which indicates that any inhibition of cell division in barley radicles was not the immediate consequence of lower O_2 tension (anoxia).

Alternatively hydrostatic pressure treatment could have restricted the movement of CO_2 away from the embryo to an extent that was deleterious. Kidd (1913a) reported that whilst elevated partial pressures of CO_2 stimulated the germination of imbibed barley grains, pressures in excess of 23.5% were inhibitory. Kidd (1913b) proposed that in barley grains (and other seeds) the germination resting period (respiratory lag, Phase II) was imposed by CO_2 narcosis. Allerup (1959) observed that the RQ maximum for intact barley grains and isolated embryos occurred at the same moisture content during imbibition. Allerup (1959) forwarded the suggestion that during imbibition (Phase I) water increases the diffusivity of compact embryonic tissue. However, with further uptake (Phase II) water floods expanding intercellularies inhibiting aerobic respiration (RQ reaches maximum). Continued water uptake (early Phase III) results in embryo cells reaching full turgor and intercellularies becoming gaseous (RQ falls to ≤ 1). Bewley and Black, (1978, 1982) note that RQ maximum during water uptake is generally considered to reflect the resistance to O_2 entry imposed by

hydrated grain coats and that in addition to the greater solubility (permeability) of CO_2 in grain coats an internal concentration in the order of 20 to 40% would be required to inhibit germination. Lamount (1982) notes that most of the literature which deals with the influence of atmospheric gases on germination considers that the intact pericarp perpetuates dormancy by resisting O_2 diffusion. Lamount (1982) determined, by measurement of internal gas composition, that the impermeability of the pericarp was responsible for the high CO_2 content (27% CO_2, 12% O_2 and 61% N_2) of mature Amyema preissii fruit. It was concluded that the dormancy of the seeds, which when isolated fail to exhibit dormancy and were capable of germinating in N_2, within the intact fruit (pericarp) was imposed by the high internal CO_2 content. In addition, Lamount concluded that the internal concentration of O_2 would have been unlikely to impose anoxia. Further, seed germination and axial growth of Amyema preissii declined when exposed to external CO_2 concentrations above 5%.

Kidd (1913a) observed that short periods (118 hours) of CO_2 narcosis failed to reduce the ability of barley grains to re-initiate germination when returned to air. In a similar manner the growth, although delayed, of treated grains in post-treated environments that alleviated inhibition did not appear to be modified as a result of the temporary inhibition. Therefore it would be reasonable to

assume the CO_2 narcosis in addition to anoxia could have contributed to hydrostatic pressure inhibition effects. Indeed possibly there was an interaction between restricted O_2 supply and narcosis. Kidd (1913b) determined that with lower external O_2 tensions, the germination inhibition imposed by a constant external level of CO_2 increases.

Another potential source of inhibition would have been the pH of the occlusion water occupying the vestibule and intercellularies. The pH would have declined with increasing CO_2 partial pressure at gas/water interfaces, the rate of decline being determined by the rate of CO_2 production and carbonic anhydrase acivity. O'Neill and Scott (1982) reported that in barley roots the zone extending from the root cap through the region of maximum elongation to the zone of root hair development carries a basic surface charge. In contrast, the root hair region was determined to carry a positive surface charge. Growth of segments excised from the basic zone was observed to be greatest when bathed in acidic (pH 5) solution. O'Neill and Scott (1983) were unable to explain the basic zone phenomenon in terms of the acid-growth theory. They proposed that, 1) the negative surface charge may have been simply that of the basic root cap slime, 2) that the tissues determining the rate of elongation lie beneath the surface and that the pH of surface and subsurface tissues are controlled independently.

It would appear therefore that an additional consequence of hydrostatic pressure treatment could have been a change in pH (to acidic) of the basic elongation region. The mechanism by which such a pH change could influence radicle elongation is unclear. From O'Neill and Scott's (1983) findings, a decline to pH 5 should not have inhibited elongation. However, their elongation experiment involved tissue segments which might respond differently than an intact root or the very young root primordiums which is also constrained by the coleorhiza. More acidic solutions have however, been reported to prevent barley root elongation. Palmer et al. (1972) observed that steep solutions containing .006 to 0.01 N H_2SO_4 (pH about 2.0) retarded and inhibited root growth.

CHAPTER IX

SUMMARY AND CONCLUSIONS

Subjecting steeped barley to increased magnitudes of hydrostatic pressure significantly reduced grain chitting. In contrast, increased treatment durations from 10 to 60 seconds failed to significantly influence germination inhibition.

The perpetuation of hydrostatic pressure inhibition in steeped barley was determined to be dependent on grain pericarps and post-treatment germination environments. Decorticated grain chitting was significantly greater than that of hulled grain irrespective of menisci. Germination environments containing excess free water (mimicking steep-over and wet-casting) supported continued inhibition of grain chitting and root growth. In contrast, drier environments tended to alleviate inhibition. In addition the grain husk and associated micro-organisms appeared to contribute to inhibition in both germination environments.

Hydrostatic pressure seemed capable of forcing water into what was likely a gas phase surrounding the embryonic roots and occupying coleorhizal intercellularies. The integrity of the coleorhiza and radicle meristem did not

appear to be discernibly damaged by treatment. The morphology and anatomy of the coleorhiza region in the inbibed grain indicated that normally this region accommodated rapid gas diffusion.

The occlusion of the embryo gas phase by hydrostatic pressure reduced O_2 consumption. It appeared likely that the coleorhiza region gas phase was retained following compression under dilute H_2O_2. The nature of the experiments did not allow conclusions to be drawn as to the relative importance of anoxia and CO_2/ethanol narcosis following gas phase occlusion to hydrostatic pressure inhibition.

The observed inhibition of chitting and the increased variability in root growth following hydrostatic pressure treatment were similar to the observations of Eyben and Droogenbroeck (1969), Davidson and Jangaard (1978) and Yoshida et al. (1979). In addition, these authors reported that hydrostatic pressure restricted respiratory activity. Davidson and Jangaard (1978) and Yoshida et al. (1979) reported that greater magnitudes of hydrostatic pressure decreased the O_2 consumption of grain samples. In this study greater treatment magnitudes increased inhibition of grain chitting and thus the proportion of grains locked in Phase II. Higher pressures might have increased the depth of water phase occluding the embryonic vestibule and coleorhizal intercellularies. It is unclear whether the increased chitting inhibition reflected: 1) variation in the minimum pressure

required to overcome a barrier and impose inhibition, the barrier being determined by germination stage or, 2) variation in the depth of occlusion water required to impose inhibition, a variation which again may have depended on the stage of germination. However, both an increase in the number of inhibited grains and depth of occlusion water in susceptible grains might reasonably be expected to reduce O_2 consumption further.

Davidson and Jangaard (1978) reported that greater treatment duration increased the period of respiratory restriction. In contrast, it was observed that increased treatment duration had no significant effect on chitting inhibition, the perpetuation of which appeared to be solely dependent on the post-treatment germination environment. Yoshida et al. (1979) reported that the restrictions of respiratory activity induced by consecutive constant hydrostatic pressure treatments during steeping were cumulative. If however, a single pressure treatment can occlude part of the embryo gas phase, it is difficult to imagine how successive treatments at the same pressure would increase the occlusion, given that greater treatment durations failed to increase chitting inhibition. Perhaps the cumulative restrictions of respiratory activity during steeping observed by Yoshida et al. (1979), simply reflected: 1) an increase in the size of the grain fraction susceptible (having coleorhiza tortuosity low enough for water to gain entry) to hydrostatic pressure

with increased steeping time, and 2) the restriction of grains with higher respiratory activity which gradually increases with steeping time.

Yoshida et al. (1979) proposed and Davidson and Jangaard (1978) observed (flaccid roots following treatment) that hydrostatic pressure disrupted or modified embryo integrity. Observations reported here were in accordance with the findings of Eyben and Droogenbroeck (1969) and indicated that gross embryo integrity was not influenced by treatment. However, Kuiper (1972) notes that root membrane hydrophobicity declines with initial increases of hydrostatic pressure. Coster et al. (1977) proposed that in contrast to long term strains, membrane properties may be permanently modified by short term strains. Therefore it would appear possible that a proportion of the pressure induced conformational changes in lipoprotein responsible for decreased membrane hydrophobicity could have been permanent as a result of the short duration of treatments. The ability of water-sensitivity breaking treatments to alleviate hydrostatic pressure inhibition indicates that pressure induced membrane changes were not determining inhibition. However, such changes could have contributed to inhibition and perhaps modified root growth.

Eyben and Droogenbroeck (1969) suggested the mechanical compression of the embryonic axis possibly inhibited GA formation. Yoshida et al. (1979) proposed that disruption of the embryo was likely responsible for the inhibition of GA

production following hydrostatic pressure treatment. Githua and Barrell (1974) observed the effects of barley grain squeezing (between rollers) on germination, extract and GA production of steeped grains. They reported that embryo damage (moisture 23 to 47%) occurred when grains of 2.5 mm width were passed through rollers with 1 or 2 mm spacings. However malt yield was only reduced when rolling was carried out before endogenous GA was released. The release of GA appeared to be determined by steeping technique rather than grain moisture content. Grains that were rolled following air-rest showed no reduction in malt yield in contrast to rolled traditionally steep grain. Eyben and Droogenbroeck did not specify their steeping schedule. Yoshida et al. (1979) however employed an interrupted steeping technique and applied their first hydrostatic pressure treatment at approximately 33% moisture. Based on Yoshida et al. (1979) steeping technique and Githua and Barrell's (1974) observations, endogenous GA was probably released before hydrostatic pressure treatment could modify embryo membranes. Irrespective of this however, both Eyben and Droogenbroeck (1969) and Yoshida et al. (1979) reported that hydrostatic pressure treatment inhibited GA production. Macleod (1979) noted that GA synthesis requires O_2 which may be limiting in profoundly dormant grains. Perhaps anoxia and CO_2/ ethanol narcosis induced by hydrostatic pressure inhibited GA synthesis and as a result germination and modification. Palmer (1974)

notes however that barley germination takes place prior to the mobilization of endosperm reserves. In addition, Nicholls (1979) determined that in wheat GA was not always essential to stimulate amylase production. In contrast Macleod (1979) notes that the ability of exogenous GA to break profound dormancy in barley appears to act by a mechanism physiologically distinct from the stimulation of aleurone activity. Considering however the inhibition of root growth and coleoptile development and the relation between coleoptile development and modification, the modification of grains subjected to hydrostatic pressure was probably inhibited.

Grain subjected to hydrostatic pressure responded in a manner similar to water-sensitive grains when subjected to procedures which break sensitivity. The inhibition of treated grains could be described as a severe form of water-sensitivity that is predominantly associated with the embryo. In this regard it is interesting to note that Crabb and Kirsop (1969) reported that the embryos of water-sensitive grains have an intrinsically higher O_2 requirement than normal embryos. Perhaps the intrinsically higher O_2 requirement results from a partial occlusion of the embryo gas phase.

Heavy rainfall and high humidity during harvest increase the incidence of pre-germination and water-sensitivity. Gordon (1970a) reported that at high grain moisture (45%) a testa/pericarp rupture promotes pre-

germination by allowing atmospheric contact with the embryo. Further, Gordon (1970a) reported that the embryos of such grains will initiate growth provided the moisture content remains above 30%. If growth fails to occur, due to drying, the swelling pressure of the ruptured grains would be in the order of 40 MPa (Shaykewich and Williams, 1973). Gordon (1970b) reported that a short 2 hour steep facilitates the observation of the rupture in dried grains where the pericarp in the embryo region is shrivelled. Orphanus and Heydecker (1968) observed that when dried Phaseolus vulgaris seed was placed in water the cotyledons swelled in a differential fashion creating an inter-cotyledon cavity. They proposed that the rapid swelling caused a reduction in internal pressure within the cavity and that as a result the excess water responsible for soaking injury was drawn in. Therefore it appears possible that when a grain is placed in contact with a free film of water the rapid hydration of the coat coupled with its low permeability may cause differential swelling. That is at least initially the grain coat would swell faster than the less or unhydrated embryo and endosperm it encloses. Given the low permeability of grain coats to water and gases, the differential swelling could reduce the internal gas pressure. If such differential swelling were to occur water would be drawn in through coat ruptures. A rupture in the region of the embryo might therefore result in any gas phase around the embryo becoming partially occluded. In

addition, the rapid unrestricted entry of water into the embryo region below the grain coat could promote differential swelling within and between tissues such as the coleorhiza and radicle, possibly promoting further occlusion and the temporary creation of abnormal intertissue spaces. The condition created would be similar to that induced by a hydrostatic pressure treatment and the manner of water entry might possibly represent the events that occur when water-sensitive grain is placed in a steep. Hough et al. (1971) drew attention to the fact that the expression of water-sensitivity under test conditions is highest when the grains are orientated dorsal surface down. This orientation may allow water to move through the test/pericarp rupture observed by Gordon (1970a,b). Woodbury and Wiebe (1983) reported that in wheat post harvest dormancy and dormancy induced by coumarin was only expressed when the grains were in contact with liquid water. They proposed that the phenomenon was related to a pericarp crack in the brush region. Observations indicated that the crack allowed the rapid movement (below the pericarp) of liquid water across the dorsal surface of the grain.

Gordon (1970b) reported that under favourable harvest conditions the rupture occurred in $\leq$2% of barley grains of malting quality. It was possible however (Gordon 1970a), by making an artificial rupture, to stimualte barley grains to germinate from 10 days after anthesis onwards under the

conditions of the germination energy and water-sensitivity tests. However Gordon (1970a) most frequently created the artificial rupture by peeling the testa/pericarp away from the embryo region. Pollock et al. (1955b) reported that piercing the test/pericarp in the embryo region with a sharp needle alleviated profound dormancy but not water-sensitivity. When such grains were sectioned immediately behind the scutellum water-sensitivity was lost. In addition, Pollock et al. (1955b) reported that acid decortication relieved water-sensitivity. Therefore, grains with peeled pericarps might not be expected to exhibit water-sensitivity.

Essery and Pollock (1956) reported that the rate of breakdown of profound dormancy in undried (18%) barley samples increased with higher storage temperatures. In contrast, the rate of loss of water-sensitivity was found to be inversely proportional to the grain moisture content after drying. Drier grain losing water-sensitivity faster. Brookes et al. (1976) notes that profound dormancy is most rapidly broken by high drying and storage temperatures whereas high storage temperatures have less impact on water-sensitivity than storage at low moisture. Therefore the drying of water-sensitive grain could remove water occluding the embryo gas phase. However, hydrostatic pressure treatments indicated that the occlusion of this phase would inhibit germination and therefore also pre-germination. Wiebe (1983) proposed that grain

drying facilitated the formation of air channels beneath the pericarp. Pollock et al. (1955a) reported that profound dormancy and water-sensitivity both appeared to be determined by pericarp integrity.

Possibly therefore, prior to drying a single testa/pericarp rupture exists above the embryos of water-sensitive grains. On contact with excess water, water is drawn into the embryo region creating a germination inhibition condition similar to that observed in grain subjected to hydrostatic pressure. However, if the water-sensitive grain is dried and stored at low moisture additional pericarp air channels are possibly created. These additional channels may be able to compensate for the rapid movement of water around the embryo (during steeping) as the diffusion resistance of such channels would be in the order of 1×10^{2} cm^{-2} s^{-1} less than that of intact pericarp, even when flooded.

Polysaccharide layers associated with the embryo/testa (Plate 17) and pericarp/husk (Macleod, 1979) interfaces may also be involved in the water-sensitivity mechanism. Rapid expansion of these polysaccharides could inhibit gas movement and preferentially draw water through pericarp cracks. Grain drying (at low moisture) may lead to the fracture of these layers, such that on hydration swelling does not completely occlude the gas phase.

REFERENCES

Abeles, F.B. 1973. Ethylene In Plant Biology. Academic Press, London.

Allerup, S. 1959. Respiration in water imbibing barley caryopses. Physiologia Plantarum. 12. 118-123.

Amoore, J.E. 1961. Dependence of mitosis and respiration in roots upon oxygen tension. Proc. Roy. Soc. B. 154: 109-129.

Anderson, T.F. (1951). Techniques for the preservation of three-demensional structure in preparing specimens for the electron microscope. Trans N.Y. Acad. Sci., Ser II 13:130-134.

Armstrong, W. 1983. Oxygen Stress in the root. Agriculture Group Symposium in J. Sci. Food Agric. 34:943-944.

Armstrong, W., Healy, M.T. and Lythe S. 1983. Oxygen diffusion in pea II. Oxygen concentrations in the primary pea root apex as affected by growth, the production of laterals and radial oxygen loss. New Phytol. 94:549-559.

Andus, L. J. 1935. Mechanical stimulation and respiration rate in the cherry Laurel. New Phytol. 34:836-402.

Barley, K.P. (1963). Influence of soil strength on growth of roots. Soil Science. 96:175-180.

Barber, D.A., Ebert, M. and Evans, N.T.S. 1962. The movement of ^{15}O through barley and rice plants. Journal of Experimental Botany, 13:397-403.

Barber, D.A. and Gunn, K.B. 1974. The effect of mechanical forces on the exudation of organic substances by the roots of cereal plants grown under sterile conditions. New Phytol. 73:39-45.

Baxter, D.E. 1981. The importance of barley protein in malting. J. Sci. Food Agric. 32:409-410.

Belderok, B. 1968. Changes in thiol and disulphide contents in barley embryos during dormancy and after-ripening. J. Inst. Brew. 74:333-340.

Berezin, I.V., Klibanov, A.M. and Martinek, K. 1974. The mechanochemistry of immobilized enzymes. How to steer a chemical process at the molecular level by a mechanical device. Biochimica et Biophysica Acta. 364:193-199.

Bewley, J.D. and Black, M. 1982. Physiology and Biochemistry of Seeds. Vol. 2. Springer-Verlag, Berlin.

Bewley, J.D. and Black, M. 1978. Physiology and Biochemistry of Seeds. Vol. 1. Springer-Verlag, Berlin.

Blackwell P.S. and Wells, E.A. 1983. Limiting oxygen flux densities for oat root extension. Plant and Soil 73:129-139.

Blaim, K. 1960. The effect of coumarin on uptake of water by seeds. Journal of Experimental Botany. 11:377-380.

Bloch, F. and Morgan, Jr. A.E. 1967. Germination inhibition in wheat and barley during steeping, and alpha-amylase development in the presence of gibberellic acid. Cereal Chemistry. 44:61-69.

Blum, P.H. and Gilbert, S.G. 1957. The mechanism of water sensitivity. ASBC Proceedings. 22-25

Boone, F.R. and Veen, B.W. 1982. The influence of mechanical resistance and phosphate supply on morphology and function of maize roots. Neth. J. Agric. Sci. 30:179-192.

Briggs, D.E. and MacDonald, J. 1983. The permeability of the surface layers of cereal grains, and implications for tests of abrasion in barley. J. Inst. Brew. 89:324-332.

Briggs, D.E., Hough J.S., Stevens, R.S. and Young, T.W. 1981. Malting and Brewing Sc. Vol. 1, Sec. Ed. Chapman and Hall London. pp.39-64.

Brookes, P.A. 1979. The significance of pre-harvest sprouting of barley in malting and brewing. Proceeding of the second international sprouting symposium in Cereal Research Communications. 8:29-38 (1980).

Brookes, P.A., Lovett, D.A. and MacWilliam, I.C. 1976. The steeping of barley. A review of the metabolic consequences of water uptake, and their practical implications. J. Inst. Brew. 82:14-26.

Bryant, A.E. 1934. Comparison of anatomical and histological differences between roots of barley grown in aerated and in non-aerated culture solutions. Plant Physiology. 389-391.

Burstrom, H. 1959. Growth and formation of intercellularies in root meristems. Physiologia Plantarum. 12:371-385.

Campbell, R. and Drew, M.C. 1983. Electron microscopy of gas space (aerenchyma) formation in adventitious roots of (Zea mays L.) subjected to oxygen shortage. Planta. 157:350-357.

Cannel, R.Q. and Jackson, M.B. (1981). Alleviating aeration stresses in Modifying The Root Environment To Reduce Crop Stresses. Edited by G.F. Arkin and H.M. Taylor. American Soc. Agricultural Engineers, St. Joseph, Michigan. 141-192.

Carstensen, E.L., Donaldson, T.L., Miller, M.W., Law, W.K. and Vives, B. 1981. Distribution of gas in the roots of Pisum satiuum). Env. Exp. Bot. 21:1-4.

Clowes, F.A.L. 1975. The quiscent centre in The Development and Function of Roots. Edited by J.G. Torrey and D.T. Clarkson. Academic Pres, London. pp 15-16.

Clowes, F.A.L. 1984. Size and activity of quiescent centres of roots. New Phytol. 96:13-21.

Collins, E. J. 1918. The structure of the integumentary system of the barley grain in relation to localized water absorption and semi-permeability. Ann. Bot. 32:381-414.

Collis-George, N. and Hector, J.B. 1966. Germination of seeds as influenced by matric potential and by area of contact between seed and soil water. Aust. J. Soil Res. 4:145-164.

Collis-George, N. and Williams, J. 1968. Comparison of the effects of soil matric potential and isotropic effective stress on the germination of Lactuca sativa. Aust. J. Soil Res. 6:179-92.

Cossins, E.A. and Turner, E.R. 1962. Losses of alcohol and alcohol dehydrogenase activity in germinating seeds. Annals of Botany. 26:591-597.

Coster, H.G.L., Steudle, E. and Zimmerman, U. 1977. Turgor pressure sensing in plant cell membranes. Plant Physiol. 58:636-643.

Crabb, D. and Kirsop, B.H. 1969. Water-sensitivity in barley. I. Respiration studies and the influence of oxygen availability. J. Inst. Brew. 75:254-259.

Crabb, D. and Kirsop, B.H. 1970. Water-sensitivity in barley. II. Inhibitors from barley embryos. J. Inst. Brew. 76:158-162.

Crawford, R.M.M. 1977. Tolerance of anoxia and ethanol metabolism in germinating seeds. New Phytol. 79:511-517.

Davidson, D.E. and Jangaard, N.O. 1978. The effect of a wet and dry steep-out on barley respiration and malt modification time. ASBC Journal. 36:51-55.

Davies, P.A. 1928. High pressure and seed germination. American Journal of Botony. 15:149-156.

Dayan, E., Banin, A. and Henis, Y. 1977. Studies on the mucilaginous layer of barley (Hordeum vulgare) roots. Plant and Soil. 47:171-191.

Delouche, J.C., Still, T.W., Raspet, M. and Lienhard, M. 1962. The Tetrazolium Test for Seed Viability. Mississippi State University. Agricultural Experiment Station Technical Bulletin 51.

Dunwell, J.M. 1981. Dormancy and germination in embryos of Hordeum vulgare L. - effect of dissection, incubation temperature and hormone application. Ann. Bot. 48:203-213.

Esau, K. 1965. Plant Anatomy, 2nd Edition John Wiley & Sons, Inc., London.

Esau, K. 1977. Anatomy of Seed Plants. 2nd Edition. John Wiley and Sons, London.

Essery, R.E. and Pollock, J.R.A. 1956. Studies in barley and malt. VII. Disappearance of dormancy in barley on storage. J. Inst. Brew. 62:327-330.

Eyben, D. and Droogenbroeck, L. (1969) Etude de L'influence de la pression sur la germination des orges et la qualite des malts. Eur. Brew. Conv. Proc. Congr. 12th, Interlaken. 107-119.

Fadeel, A.A. 1964. Measurements of the carbon dioxide concentration in the intercellular spaces of wheat roots. Physiologia Plantarum. 17:1-13.

Fiscus, E.L. 1977. Effects of coupled solute and water flow in plant roots with special reference to Brouwer's experiment. 28:71-77.

Flannigan, B. and Healy, R.E. 1983. The mycoflora of barleys accepted and rejected for malting. J. Inst. Brew. 89:341-343.

Gaber, S.D. and Roberts, E.H. 1969a. Water-sensitivity in barley seeds. I. A re-appraisal of the auxin hypothesis. J. Inst. Brew. 75:299-302.

Gaber, S.D. and Roberts, E.H. 1969b. Water-sensitivity in barley seeds. II. Association with micro-organism activity. J. Inst. Brew. 75:303-314.

Gershoy, A., Miller, D.L. and Nyborg, W.L. 1976. Intercellular gas: Its role in sonated plant tissue in Ultrasound In Medicine. Vol. 2. Edited by D. White and R. Barnes. Plenum Press, London, pp. 501-511.

Gill, W.R. and Bolt, G. H. 1955. Pfeffer's studies of the root growth pressures exerted by plants. Agronomy Journal. 47:166-168.

Gill, W.R. and Miller, R.D. 1956. A method for study of the influence of mechanical impedance and aeration on the growth of seedling roots. Soil Science Society Proceedings. 20:154-157.

Githua, P.M. and Barrell G.W. 1974. Effect on malt quality from physical treatment of the barley. Part 1: squeezing barley after steeping. MBAA Technical Quarterly. 11:174-178.

Goeschl, J.D. Rappaport, L. and Pratt, H.K. 1966. Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. Plant Physiol. 41:877-884.

Gomez, E. 1971. Etude de l'influence de divers agents physiques sur la qualite des malts. Proceedings of the European Brewery Convention, Congress. 13th, Estoril. 29-48.

Gordon, A.G. 1970a. Pre-germination in barley. J. Inst. Brew. 76:140-143.

Gordon, A.G. 1970b. Premature germination in cereal grains. Can. J. Plant Sci. 50:191-194.

Goss, M.J. 1977. Effects of mechanical impedance on root growth in barley. (Hordeum vulgare L.). Journal of Experimental Botany. 28:96-111.

Goss, M.J. and Russell, R.S. 1980. Effects of mechanical impedance on root growth in barley (Hordeim vulgare L.). Journal of Experimental Botany. 31:577-588.

Green, P.B. (1974). Morphogenesis of cell and organ axis-biophysical models in Basic Mechanisms in Plant Morphogenesis, Brookhaven Symposia in Biology. No. 25. National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virgina 22151.

Greenwood, D.J. 1967. Studies on the transport of oxygen through the stems and roots of vegetable seedlings. New Phytol. 66:337-347.

Haber, A.H. and Brassington, N. 1959. Non-respiratory gas released from seeds during moistening. Plant Physiology-183:619-620.

Harper, S.H.T. and Lynch, J.M. 1980. Microbial effects on the germination and seedling growth of barley. New Phytol. 84:473-481.

Hector, J.M. 1936. Introduction to the Botany of Field Crops. Vol. 1 Cereals. South African Agricultural Series Vol. XVI. Central News Agency Ltd., Johannesburg, South Africa.

Hegarty, T.W. 1978. The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. Plant, Cell and Environment. 1:101-119.

Hendricks, S.B. and Taylorson, R.B. 1980. Reversal by pressure of seed germination promoted by anesthetics. Planta. 149:108-111.

Hoar, W.S. 1975. General and Comparative Physiology. 2nd Edition. Edited by W.D. McElroy and C.P. Swanson. Prentice-Hall Inc., Englewood Cliffs, New Jersey.

Hochachka, P.W. and Somero, G.N. 1973. Strategies of Biochemical Adaptation. W.B. Saunders Company, London.

Hopkins, R.M. and Patrick, W.H. Jr. 1969. Combined effect of oxygen content and soil compaction on root penetration. Soil Science. 108:408-413.

Hough, J.S., Briggs, D.E. and Stevens, R. 1971. Malting and Brewing Science. Chapman and Hall Ltd., London.

Hudson, J.R. 1983. Horace Brown memorial lecture expanding brewing technology. J. Inst. Brew. 89:189-194.

Jackson, M.B., Herman, B. and Goodenough, A. 1982. An examination of the importance of ethanol in causing injury to flooded plants. Plant, Cell and Environment. 5:163-172.

Jansson, G. 1959. Germination experiments with water-sensitive barley. Arkiv for Kemi. 14:161-169.

Jansson, G. 1960. Breaking of water-sensitivity in barley seeds by treatment with chemicals. Arkiv for Kemi. 15:439-449.

Jansson, G. 1962. Respiration and phosphorylation in water-sensitive barley. Arkiv for Kemi. 19:141-148.

Jansson, G., Kirsop, G.H. and Pollock, J.R.A. 1959. Studies in barley and malt. XIII. Water-sensitivity as an intrinsic property of certain barley. J. Inst. Brew. 65:165-168.

Johnson, F.H. and Flagler, E.A. 1950. Hydrostatic pressure reversal of narcosis in tadpoles. Science. 112:91-92.

Kays. S.J., Nicklow, C.W. and Simons, D.H. 1974. Ethylene in relation to the response of roots to physical impedance. Plant and Soil. 40:565-571.

Kidd. F. 1913a. The controlling influence of carbon dioxide in the maturation, dormancy and germination of seeds - Part I. Proc. Roy Soc. B. 87:408-421.

Kidd, F. 1913b. The controlling influence of carbon dioxide in the maturation, dormancy and germination of seeds - Part II Proc. Roy. Soc. B. 87:609-625.

Kirsop, B.H. and Pollock, J.R.A. 1957. Studies in barley and malt. XI. Steeping in relation to water-sensitivity in malting barleys. J. Inst. Brew. 63:383-385.

Kiyosawa, K. 1975. Studies on the effects of alcohols on membrane water permeability of nitella. Protoplasma. 86:243-252.

Kuiper, P.J.C. 1972. Water transport across membranes. Ann. Rev. Plant Physiol. 23:157-172

Lamont, B. 1982. Gas content of berries of the Australian mistletoe (Amyema preissii) and the effect of maturity, viscin, temperature and carbon dioxide on germination. Journal of Experimental Botany. 33:790-798.

Lloyd, W.J.W. and Martin, P.A. 1982. The determination of germinated grains in barley. J. Inst. Brew. 88:322-323.

Luratios at various values by an oxygen limitation of respiration in germinating lettuce (Lactuca sativa) seeds. Biochem. J. 190:39.

MacLeod, A.M. 1957. Raffinose metabolism in germinating barley. New Phytol. 56:210-220.

MacLeod, A.M. 1979. Physiology of malting in Brewing Science. Vol. 1. Edited by J.R.A. Pollock. Academic Press, London. pp. 146-244.

McCully, M.E. 1975. The development of lateral roots in The Development and Function of Roots. Edited by J.G. Torrey and D.T. Clarkson. Academic Press, London. pp.105-124.

McDougall, B.M. and Rovira, A.D. 1970. Sites of exudation of ^{14}C-labelled compounds from wheat roots. New Phytol. 69:999-1003.

Mizuno, A. and Okamoto, H. 1983. Further examination of the effects of IAA and anoxia on the growth rate and the spatially separate electrogenic ion pumps in bean hypocotyl. Plant, Cell and Environment. 6:243-246.

Moll, M. 1979. Analysis and composition of barley and malt in Brewing Science. Vol. 1. Edited by J.R.A. Pollock. Academic Press, London. pp. 65-116.

Nicholls. P.B. (1979). Induction of sensitivity to gibberellic acid in developing wheat caryopses: Effect of rate of desiccation. Aust. J. Plant Physiol. 6:229-240.

Nobel, P.S. 1974. Introduction to Biophysical Plant Physiology. W.H. Freeman and Company. San Franciso.

O'Brien, T.P. and McCully, M.E. 1981. The Study of Plant Structure Principles and Selected Methods. Termarcarphic Pty. Ltd., Melbourne.

O'Farrell, D.D. 1983. The water sensitivity test for barley choice of filter papers. Journal of the Institute of Brewing. 89:253-254.

Ohmura, R. and Howell R.W. 1960. Inhibitory effect of water on oxygen consumption by plants materials. Plant Physiol. 35:184-188.

O'Niell, R.A. and Scott, T.K. 1983. Proton flux and elongation in primary roots of barley (Hordeum vulgare L.). Plant Physiol. 73:199-201.

Orphanos, P.I. and Heydecker, W. 1968. On the nature of the soaking injury of Phaseolus vulgaris seeds. Journal of Experimental Botany. 19:770-784.

Palmer, G.H., Barrett, J. and Kirsop, B.H. (1972). Combined acidulation and gibberellic acid treatment in the accelerated malting of abraded barley. J. Inst. Brew. 78:81-83.

Palmer, G.H. 1974. The industrial use of gibberellic acid and its scientific basis - a review. J. Inst. Brew. 80:13-30

Percival, J. 1921. The Wheat Plant a Monograph. Duckworth and Co., Covent Garden, London.

Phillips, R.E. and Kirkham, D. 1962. Soil compaction in the field and corn growth. Agronomy Journal. 54:29-34.

Pollock, J.R.A., Essery, R.E. and Kirsop, B.H. 1955a. Studies in barley and malt. III. New convenient germinative capacity test. J. Inst. Brew. 61:295-300.

Pollock, J.R.A., Kirsop, B.H. and Essery, R.E. 1955b. Studies in barley and malt. IV. Experiments with dormant barley. J. Inst. Brew. 61:301-307.

Pollock, J.R.A. and Kirsop, B.H. 1956. Studies in barley and malt VI. Stimulation of the germination of freshly-harvested barley. J. Inst. Brew. 62:323-327.

Pollock, J.R.A. and Pool, A.A. 1962. Studies in barley and malt. XIX. Further observations on water-sensitivity in barley. J. Inst. Brew. 68:427-431.

Radley, M. 1976. The development of wheat grain in relation to endogenous growth substances. Journal of Experimental Botany. 27:1009-1021.

Reeves, S.G. 1981. Assessment of barley for malting. J. Sci. Food Agric. 32:409.

Riveria, R., Popp, H.W. and Dow, R.B. 1937. The effect of high hydrostatic pressures upon seed germination. American Journal of Botany. 24:508-513.

Roland, J.C. 1978. Cell wall differentiation and stages involved with intercellular gas space opening. J. Cell Sci. 32:325-336.

Rovira, A.D. and Campbell, R. 1974. Scanning electron microscopy of microorganisms on the roots of wheat. Microbial Ecology. 1:15-23.

Russel, R.C. 1960. Observations on cereal embryos in relation to the "Embryo Test" for loose smuts. Can. J. Botany. 38:251-257.

Saglio, P.H., Raymond, P. and Pradet, A. 1983. Oxygen transport and root respiration of maize seedlings. Plant Physiol. 72:1035-1039.

Satoh, S. and Esashi, Y. 1980. D-Amino-Acid-Stimulated ethylene production in seed tissues. Planta. 149:64-68.

Seeman, P., Sha'aft, R.L., Galey, W.R. and Solomon, A.K. 1970. The effect of anesthetics (chlorpromazine, ethanol) on erythrocyte permeability to water. Biochim. Biophys. Acta. 211:365-368.

Shaykewich, C.F. 1973/ Proposed Method for Measuring Swelling Pressure of Seeds prior to Germination. Journal of Experimental Botany. 24:1056-1061.

Smart, M.G. and O'Brien, T.P. 1983. The development of the wheat embryo in relation to the neighbouring tissues. Protoplasma. 114:1-13.

Sorokin, H.P. 1966. The intercellular spaces of the avena coleoptile. Physiologia Plantarum. 19:691-701.

Sorvall (1972). Operating instructions for the Sorvall Critical Point Drying System. Section 4.1 Specimen Preparations. Ivan Sorvall Inc. Newton Connecticut.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure Research. 26:31-43.

Stewart, G.G. and Russell, I. 1981. Yeast flocculation in Brewing Science. Vol. 2. Edited by J.R.A. Pollock. Academic Press, London. pp. 61-89.

Symons, S.J. 1982. Changes in embryo growth capacity of developing wheat grains. PHD Thesis. Biology Department, Queen Elizabeth College, Campden Hill Road, London, W8 7AH.

Symons, S.J., Angold, R., Black, M. and Chapman, J.M. 1984. A posible mechanism for seed coat rupture by the embryo in germinating wheat. Annals of Botany. 53:765-722.

Tackett, J.L. and Pearson, R.W. 1964. Oxygen requirements of cotton seedling roots for penetration of compacted soil cores. Soil Science Society Proceedings. 28:600-605.

Taylor, D.L. 1942. Influence of oxygen tension on respiration, fermentation, and growth in wheat and rice. American Journal of Botany. 29:721-738.

Taylor, H.M. and Ratliff, L.F. 1969. Root growth pressures of cotton, peas, and peanuts. Agronomy Journal. 61:398-402.

Taylorson, R.B. and Hendricks S.B. 1979. Overcoming dormancy in seeds with ethanol and other anesthetics. Planta. 145:507-510.

Van Der Heide, H., De Boer-Bolt, B.M. and Van Raalte, M.H. 1963. The effects of low oxygen content of the medium on the roots of barley seedlings. Acta Botanica Neerlandica. 12:231-247.

Veen, B.W. 1982. The influence of mechanical impedance on the growth of maize roots. Plant and Soil. 66:101-109.

Vidaver, W. and Lue-Kin, H. 1967. Interactions between hydrostatic pressure and oxygen concentration on the germination of lettuce seeds. Plant Physiol. 42:243-246.

Vlamis, J. and Davis, A.R. 1943. Germination, growth and respiration of rice and barley seedlings at low oxygen pressures. Plant Physiol. 18:685-692.

Wiebe, T.J. 1983. Water-temperature interactions in germination and dormancy in wheat. MSc Thesis. Plant Science Department, University of Manitoba, Winnipeg, Manitoba. Canada.

Wiersum, L.K. 1957. The relationship of the size and structural rigidity of pores to their penetration by roots. Plant and Soil. 9:75-85.

Williams, J. and Shaykewich, C.F. 1970. The influence of soil water matric potential on the strength properties of unsaturated soil. Soil Sci. Soc. Amer. Proc. 34:835-840.

Wilson, A.J., Robards, A.W. and Goss, M.J. 1977. Effects of mechanical impedance on root growth in barley, (Hordeum vulgare L.). Journal of Experimental Botany. 28:1216-1227.

Woodbury, W. and Wiebe, T.J. 1983. A possible role for the pericarp in control of germination and dormancy of wheat, in the Third International Symposium on Pre-Harvest Sprouting in Cereals. Edited by J.E. Kruger and D.E. LaBerge. pp. 51-58. Westview Press, Boulder, Colorado.

Woolley, J.T. 1983. Maintenance of air intercellular spaces of plants. Plant Physiol. 72:989-991.

Yoshida, T., Yamada, K., Fujino, S. and Koumegawa, J. 1979. Effect of pressure on physiological aspects of germinating barleys and quality of malts. ASBC Journal. 37:77-84.