Potato Tuber Tissue Respiration & Ventilation [1]

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The purpose of this work is to compare gas movement through potato tuber tissue with the respiratory rate of such tissue and to assess possible oxygen deficiency as a factor in the limitation of respiration within the tissue. Argon 37 was used as a substitute for oxygen in most of the gas transfer experiments because it is easier to measure, its solubilities and diffusion coefficients are very similar to those of oxygen in a wide variety of solvents, and its rate of movement can be measured independently of respiration.

Materials & Methods

Large potato (*Solanum tuberosum* L. 'Russet') tubers were purchased in 300-lb lots from time to time during the 18 months of experimentation. No differences between these lots were observed, either visually or experimentally. The tubers were stored at 3 C. One day before the start of each experiment, the tubers for that experiment were moved to a room held at the experimental temperature. At the start of the experiment cylinders 3.6 cm in diameter were cut from the tubers with a sharpened brass tube and these cylinders were cut to the required lengths (1.8, 4.7, 7.7, 10, 20, or 40 mm). Xylem tissue was avoided.

For respiratory measurements, twelve 160-mm vacuum desiccators with manometers attached were used in a constant temperature room as Warburg vessels. A shallow polyethylene container in the bottom of each desiccator held 50 ml of 4.0 M KOH. The tuber slices were arranged on the plate in the main portion of each desiccator, with discs of plastic screen between the layers of slices to allow for air exchange. For some runs five replications of two cylinder lengths with two thermal barometers were used, while the other runs consisted of three replications of three cylinder lengths with three thermal barometers. Each of the six lengths was used in at least ten separate runs. The oxygen uptake rates used in the final calculations were those for the 2nd hour.

Because 3.6 cm diameter cylinders could not easily be cut thinner than 1.8 mm, some respiratory measurements were made in standard Warburg vessels with 1.0 cm diameter tuber cylinders 0.4, 0.8, 1.5, and 3.0 mm long. These slices were separated from each other by pieces of plastic screen so that all surfaces were exposed to air. KOH, 0.2 ml of 4.0 M was placed in each center well. The respiratory rate of 0.8 mm cylinders increased only about ten per cent in the period between 40 minutes and 3 hours after cutting. The data reported are for the 2nd hour.

Cylinders were not washed. A few supplementary experiments failed to show differences in respiration or gas transfer between unwashed cylinders (diam 3.6 cm, length 1.8 mm) and similar cylinders which had been washed in water for 1 minute.

The gas transfer measurements were made with a device shown schematically as figure 1. The two halves of this device, each containing 0.1 ml of H_2O, were clamped on opposite ends of a 3.6 cm diameter potato tuber cylinder, with a hypodermic needle being inserted through the serum stopper of chamber A to prevent pressure changes. A piece of rubber tubing was placed around the circumference of the cylinder. This rubber tube improved the diffusion geometry by preventing escape of the argon through the circumferential surface of the cylinder. A very small gap was allowed between the rubber tube and chamber B, to allow for the escape of any gas which might have found an easy path for chamber A to chamber B through inadvertent grooves or scratches in the cylinder surface. The flow of counting gas (98.7 % He, 1.3 % butane) through chamber B was started at 20 ml minute^{-1}. This counting gas passed from the supply tank through a water bubbler, chamber B, a drying tube, and an internal gas-flow geiger tube. After 30 minutes of equilibration, 0.10 ml of a dilution of A^{37} in counting gas was injected into chamber A by means of a hypodermic syringe. Both this syringe and the pressure-equalizing needle were immediately withdrawn.

When the movement of A^{37} had approximately reached a steady state, as shown by the counting rate of the geiger tube becoming constant, this counting rate was recorded as an index of the steady-state flow of A^{37} through the tuber cylinder. At that time 0.10 ml of gas was taken from chamber A and injected through the serum stopper into chamber B by means of a hypodermic syringe. The total number of counts

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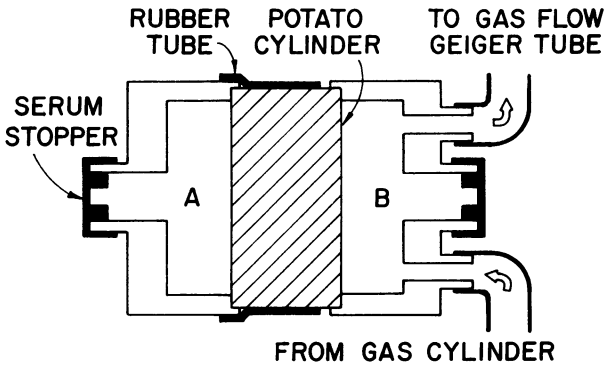
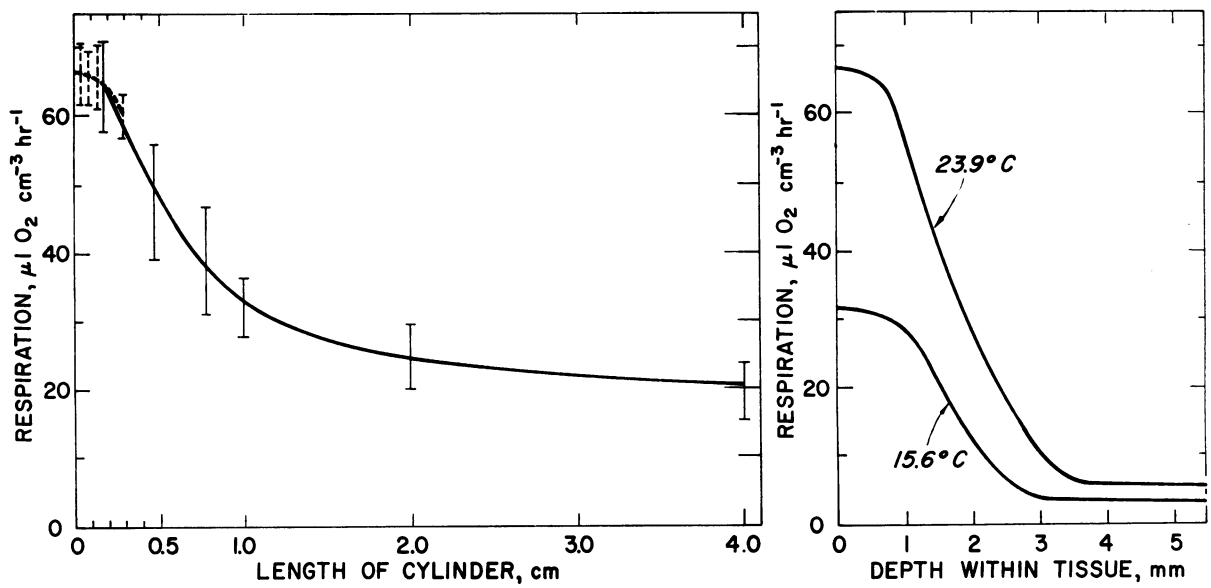


Fig. 1. Device used to measure movement of argon 37 through a potato tuber cylinder.

Fig. 2. Respiration rate of potato tuber cylinders of various lengths. Symbols indicate extremes of values encountered in 10 to 15 replicates. Dashed symbols are for 1 cm diameter cylinders; solid symbols are for 3.6 cm diameter cylinders.

Fig. 3. Calculated respiration rates within potato tuber tissue.



in the resulting burst was used in the calculation of the prevailing A^{37} concentration in chamber A. The amount of argon moving through the tuber cylinder was small enough in comparison with the quantity in chamber A that the concentration in A changed less than 10 % during the few minutes required for one experiment. Three measurements of argon movement were usually made on each cylinder, at lengths of 4.0 cm., 1.0 cm, and 0.2 cm. Although the end area of the tuber cylinder was 10.2 cm^2, the construction of the chamber was such that the perimeter was covered and only 8.9 cm^2 of each end were exposed. Through the length of the cylinder, however, the full 10.2 cm^2 of cross section were available for gas diffusion. A compromise estimate of 9.5 cm^2 was used as the cross-sectional area in all computations.

Some oxygen transfer measurements were made at 2 C, a temperature at which respiratory use of oxygen would not be expected to complicate the situation. For these measurements pure oxygen was used in chamber A. Nitrogen was passed through chamber B and the oxygen was detected with a magnetic oxygen analyzer. Because of the limited sensitivity of the oxygen analyzer, accurate comparisons were not possible, but the oxygen results agreed (within 20 %) with agron 37 measurements made on the same potato cylinders.

For microscopic observation, tuber tissue cylinders 1 cm in diameter were cut while the tubers were submerged in deaerated 0.30 M sucrose solution, and were immediately sliced to a thickness of 0.3 mm and mounted on a microscope slide without being allowed to come into contact with air. The intercellular spaces were observed microscopically and were photographed.

In order to determine whether the intercellular spaces were continuous, cylinders of tuber tissue 4.5 cm in diameter and 0.5 and 1.5 cm long were placed in a device which allowed fluid pressure to be applied to one end of the cylinder. The other end was either

open to the atmosphere or submerged in water, and could be observed visually. Attempts were made to force air, water, 0.30 M sucrose solution (a solution in which the tuber tissues neither gained nor lost weight during a 30-min immersion), and diluted india ink through the tissues. Intact tubers also were tested for continuity of air path. One end of each tuber was attached to a suction pump by means of a large rubber tube sealed with heavy grease, and the other end was similarly attached to a capillary tube containing a drop of water. This water drop served as an index to demonstrate any air movement through the tuber. The central portion of the tuber surface was left exposed.

Results

The results of the respiratory measurements at 23.9 C are shown in figure 2. The indicated variability was usually a matter of an individual run being higher or lower than the average, rather than the curve being a different shape at different times. For example, on a given day 1-cm cylinders might use 29.5 μl O_2 cm^{-3} $hour^{-1}$, with 4-cm cylinders using 18.8 μl O_2 cm^{-3} $hour^{-1}$, both sets being about 10 per cent below the means for the cylinder lengths under consideration. From the data of figure 2 and from similar data for 15.6 C, respiratory rates for the tissue at various depths within the cylinders were calculated by trial and error. Specific rates were derived for the following layers within the tissue: 0 to 0.5 mm, 0.5 to 1.0 mm, 1.0 to 2.0 mm, 2.0 to 3.0 mm, 3.0 to 4.0 mm, 4.0 to 5.0 mm, and greater than 5.0 mm. These specific rates were then converted to the smooth curves of figure 3. Standard statistical fiducial limits cannot be calculated for the data of figure 3, but the inner tissue (deeper than 3 mm within the cylinders) was probably respiring between 3 and 10 μl O_2 cm^{-3} $hour^{-1}$ at 23.9 C. At 15.6 C the data yielded an average rate of 3.5 μl O_2 cm^{-3} $hour^{-1}$ for the inner tissue, but the variability was such that the true value might have been zero. Steward, Wright, and Berry's (6) simpler method of determining the respiratory rate of the inner tissue by extrapolation to zero surface of a curve showing respiration rate vs. specific surface yielded slightly higher values, namely 4 and 7.5 μl O_2 cm^{-3} $hour^{-1}$ rather than the 3.5 and 6 μl O_2 cm^{-3} $hour^{-1}$ for 15.6 C and 23.9 C, respectively as given in figure 3.

The average of three different argon flux measurements at each of three different tuber cylinder lengths is shown in table I. Argon moved through longer tuber cylinders almost as fast as through very short cylinders with the same total argon concentration differential. Such a situation would not be possible for uniform tissue, in which the rate of flow would be inversely proportional to the path length. Thus the failure of the product, (cylinder length × argon flux), to be the same for all cylinder lengths in table I, indicates that the tissue was not uniform throughout. The assumption was therefore made that the surfaces offered greater resistance than did the bulk of the tissue. The magnitude of this special surface resistance was calculated to be equal to the resistance of about three centimeters of bulk tissue. If 6 cm are mathematically added to each cylinder length (the actual length plus 3 cm for the surface resistance of each of two surfaces), the product, (adjusted cylinder length × argon flux), becomes the same for all cylinder lengths (table I). With this assumption an apparent diffusion coefficient was calculated. This apparent diffusion coefficient differs from a true diffusion coefficient only in that the apparent diffusion coefficient is calculated from concentrations external to the tissue rather than from internal concentrations. The use of the term "apparent diffusion coefficient" is not intended to imply any specific mechanism of movement. The average apparent diffusion coefficient for the movement of argon through potato tuber tissue (exclusive of surfaces) with no total pressure differential was 1.3×10^{-4} cm^2 $second^{-1}$. The coefficient of conductance for argon flux through the surface was 4.5×10^{-5} cm $second^{-1}$. No change in apparent diffusion coefficient was observed with a temperature change from 23.9 C to 15.6 C.

Table I

Relative* Argon 37 Flux Through Different Lengths of Tuber Tissue with Constant Argon Concentration Differential

Length of tuber cylinder cm	Relative argon flux*	Cylinder length × argon flux	Adjusted cylinder length** cm	Adjusted length × argon flux
0.2	1.00	0.20	6.2	6.2
1.0	0.87	0.87	7.0	6.1
4.0	0.62	2.48	10.0	6.2

* Actual argon flux through 1-cm-long cylinders with unit concentration differential was 3.3×10^{-5} cm^3 cm^{-2} $second^{-1}$.

** Measured cylinder length plus a value of 3.0 cm for each of two surfaces.

Microscopic observation showed that almost all of the intercellular spaces were air-filled and interconnecting, as was stated by Devaux (2). Many of these air-filled spaces could easily be traced continuously for more than 0.5 mm (3 to 6 cell diameters) and none, except a few very near the surface, could be seen definitely to be isolated. Figure 4 illustrates the location and appearance of these spaces, with the microscope focused on a plane 0.2 mm below the upper surface of the tissue section. Intercellular spaces were not observed between the orderly rows of periderm cells, and there were no obvious lenticels, but there were many areas in which the orderly periderm cells were replaced by more irregular cells with air-filled intercellular spaces extending at least to the corky external part of the periderm. These regions were estimated to occupy between 3 and 10 %

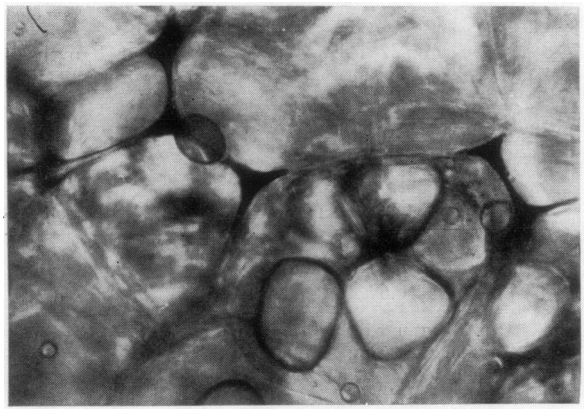


Fig. 4. Air-filled intercellular spaces in potato tuber tissue. Microscope is focused 0.2 mm below the upper surface of the tissue section.

of the tuber surface. The air space was estimated visually to occupy between 0.2 and 1.0 % of the tissue volume. This air space consisted of a lattice of stellate chambers having approximate diameters of 10 to 15 μ, interconnected by long narrow passages with diameters of about 3 μ. Most, but not all, of the intercellular spaces at the cut tissue surfaces were filled with water to a distance of a few microns, or at most one cell diameter from the cut surface. The same was true of tissue cut and observed in air, rather than in sucrose solution.

Water or aqueous solutions could be forced through the tissue only with great difficulty. Laties (3, 4), too, encountered this difficulty, and was, in fact, unable to force water through tissue on a Seitz filter. A pressure differential of four bars forced about 0.1 ml of water or sucrose solution per hour through each square centimeter of cross section of a cylinder 1.5 cm long. This figure should be taken only as a very rough approximation, because the geometry of the system did not lend itself to mathematical treatment. When sucrose solution was applied the emergent fluid contained approximately the same sucrose concentration as did the applied solution, an indication that the main path of water flow under these conditions had probably been through the intercellular spaces, rather than through the cells. Diluted india ink penetrated only about 3 mm into the tissue, regardless of whether the pressure was applied for 1 or for 24 hours. Some water passed through when india ink was applied.

After water or sucrose solution had been forced through a piece of tissue the intercellular spaces could be seen microscopically to be water filled rather than air filled. Merely soaking tissue in water with or without aeration had no effect on the air-filled spaces. When india ink had been applied, the carbon particles could be seen to have flocculated within the first 3 mm of tissue, apparently forming aggregates which could not pass through the spaces.

Air could be forced through the tissue rather easily. When the exposed tissue surface was flooded with water, bubbling could be observed at a few places at a minimum pressure differential of 0.7 bars, with the number of bubbling sites increasing up to 2 bars. Beyond this pressure there were so many bubbling sites that critical observation became impossible. The bubbling pressures were the same for both the 0.5 cm and the 1.5 cm cylinders. Bubbling pressures of 0.7 and 2.0 bars would correspond to ideal wettable capillary tube diameters of 4 μ and 1.4 μ, respectively.

Air could easily be pulled through the unwetted surfaces of intact tubers. Because no lenticals could be observed visually on these tubers, different portions of the tuber surface were greased heavily to prevent the passage of air and thereby test the permeability of the remaining surface. This demonstrated that air could move through any portion of the tuber surface (eye depressions, "eyebrows", or regions between the eyes) with as little as 0.1 bars pressure differential.

Discussion

In general, the respiratory data of figures 2 and 3 confirm the work of Steward, Wright, and Berry (7), although my fresh tissue data differ from Steward, Wright, and Berry's aged tissue data in that the aged tissue respiration rises sharply to a maximum with the thinnest slices. The fresh tissue respiration has some limitation imposed upon it so that it cannot rise above a certain temperature-dependent maximum (see table II). Thus the 0.4, 0.8, and 1.5 mm slices of fresh tissue have about the same respiratory rate. Laties (4), too, observed that the respiration of fresh tissue did not have the same dependence upon thickness as did the respiration of aged tissue. This limitation in respiratory rate and its disappearance are probably associated with the changing respiratory path described by Romberger and Norton (5). There may be a close causal relationship between the lower respiratory rate of thin slices of fresh tissue and the greater depth to which the elevated respiration extends within the tissue [3 mm in my fresh tissue as opposed to 1.44 mm in Steward's (6) interpretation of Steward, Wright, and Berry's data] The oxygen requirement of the

Table II

Respiratory Rates of Aged Tissue* Compared to Those of Fresh Tissue**

Thickness, mm	Ratio of resp. rates aged tissue : fresh tissue
1.0	2.4
2.0	2.0
5.0	1.5
10.0	1.4
18.9	1.3

* Interpolated from data of Steward, Wright, and Berry (7).

** Interpolated from figure 2.

entire zone of elevated respiration is therefore about the same for aged and fresh tissues.

Burton (1) measured the air permeability of potato tuber tissue by forcing air through tissue cylinders with pressure differentials of 0.2 and 0.4 bars. He found the air flow to be inversely proportional to cylinder length. That is, he found no special resistance to flow at the cut surface. Burton's measurement could not be expected to show special surface resistance because 0.4 bars pressure would probably not have dislodged water from the wetted ends of the intercellular spaces. Such measurements probably assessed the permeability of only a few paths, the ends of which were dry. Further, gas flow through a tube in response to a pressure differential is proportional to the fourth power of the diameter of the tube, while the rate of diffusion is proportional to the square of the diameter. Measurements of the resistance to viscous flow can be applied to diffusion only if exact data on the geometry and dimensions of the system are available.

The microscopic observations confirmed the fact that the cut surface was different from the bulk tissue, in that most of the intercellular spaces were filled with water at the surface. With some calculations, the microscopic observations can be compared with the measured gas permeabilities, as a test of the compatibility of the two types of observation. The diffusion coefficient of argon in water is 2.0×10^{-5} cm^2 second^{-1} at 25 C, and the volume solubility of argon in water is 3.1×10^{-2} (Bunsen absorption coefficient) at this temperature. Multiplication of these two factors gives an apparent diffusion coefficient of 6×10^{-7} cm^2 second^{-1}. If this coefficient is divided by the previously calculated conductance of the surface layer, 4.5×10^{-5} cm second^{-1}, the quotient, 1.3×10^{-2} cm or 130 μ, should be the thickness of this surface water-injected layer. Such a calculation would be valid only if both sides of this water layer were freely exposed. Actually, the entire outer surface is exposed, but less than 1 % of the inner side of the water layer is exposed to the air passages within the tissue. In such a case, a correction factor of 0.02 might not be unreasonable. Use of this factor brings the calculated thickness to 2.6 μ, which is within the microscopically observed range. For the bulk of the tissue the movement of gas is probably in the air-filled intercellular spaces. These may total as much as 1 % of the cross-sectional area of the tissue, but consist of stellate chambers interconnected by narrow passages. With this type of geometry the cross-sectional area of the narrow passages would be much more significant in the limitation of diffusion than would the area of the larger chambers. The aggregate cross-sectional area of these passages may be as much as 1/1000 of the total cross-sectional area of the tissue. Therefore multiplication of the diffusion coefficient of argon in air, 0.2 cm^2 second^{-1}, by 0.001 should give the observed apparent diffusion coefficient for argon in bulk tissue. The result, 2×10^{-4} cm^2 second^{-1}, is in reasonable agreement with the previously calculated 1.3×10^{-4} cm^2 second^{-1}. These calculations, while involving many broad assumptions and approximations, at least demonstrate that the observed rates of argon movement are not incompatible with the observed geometry.

The calculated respiratory rates of figure 3, as well as any conclusions regarding oxygen concentrations within the tissue, depend upon the assumption that, at a given time, the respiratory rate at a given distance from the surface is independent of total tissue thickness. This is, of course, the same assumption made by Steward (6) in his calculations of surface respiration. This assumption has not been definitely shown to be true, although the existence of a unique curve such as figure 3 is in its favor. If the respiration at a given depth were not independent of tissue thickness, one might expect that attempts to calculate a unique respiration vs. depth curve would fail. The possibility remains, however, that the surface respiratory rate or the depth of the zone of elevated respiration may be dependent upon tissue thickness, as suggested by Laties (4).

Assuming that the movement of oxygen through tuber tissue follows the same mathematical laws as does diffusion, and assuming that the resistances to oxygen flux are the same as those for A^{37} flux, one can calculate the oxygen concentrations in the intercellular spaces of the respiring tissue. With an ambient oxygen concentration of 0.21 in the air, the largest cylinders used in these experiments (3.6 cm diam., 4.0 cm long) would have an oxygen concentration of 0.12 in the atmosphere just within the water filled surface layer. The oxygen concentration in the air at the center of the cylinder would be 0.11. Thus it seems doubtful that oxygen concentration is the limiting factor governing the shape of the curves of figures 2 and 3. This conclusion agrees with that of Burton (1), who found that increasing the ambient oxygen concentration did not cause the tissue respiration rate to increase. Even in a sphere of tissue 10 cm in diameter the oxygen concentration just inside the surface layer would be 0.07 and that at the center of the sphere would be 0.01.

Summary

Respiratory rates of several different thicknesses of freshly-cut Russet potato tuber tissue were studied. Rates of argon 37 movement through similar tissue were measured and compared with the respiration rates. The cut surface of a piece of tissue offered as much resistance to argon movement as did 3 cm of bulk tissue. Microscopic examination showed the intercellular spaces to be interconnecting and air filled except at the cut surface, where most spaces were water filled to a depth of a few microns. Calculations showed that oxygen was probably more than adequate for respiration in all sizes of tuber cylinders studied, and that oxygen supply would not be limiting except in pieces of tuber whose minimum diameter approached 10 cm.

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Physiological Effects of Gibberellic Acid V. Endosperm Responses of Barley, Wheat, & Oats [1]

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Introduction

The physiological significance of the response syndrome of barley endosperm elicited by gibberellic acid (GA_3) (5) depends on several features. Not least of these is the generality of the effect on different barley varieties and on different genera. The results of tests on 1 wheat, 1 oat and 23 barley varieties are presented. To determine if an effective GA_3 concentration also affects the germination behavior of the embryo, germination trials with the 23 barley varieties were carried out. Finally, another facet of the GA_3-induced endosperm response has been explored and its relationship to the other mobilization processes (4, 5) is discussed.

Methods

Barley grains from the 1957 or 1958 harvest were sieved to secure size uniformity. To compensate for any deviation in moisture contents, individual moisture determinations were made for each variety at the time of testing.

Huskless varieties were sterilized for 1 hour in a 5 % freshly-prepared and filtered calcium hypochlorite solution. Husked varieties were sterilized in a similar solution for 20 minutes, peeled during the next 40 minutes, and returned to hypochlorite for 1 hour. All grain was rinsed eight times in distilled water, cut in half with a razor blade (discarding embryo halves), weighed, and placed eight at a time in 5 cm petri dishes with either 3 ml of water or 3ml of GA_3 (2 μg or 2×10^{-6} M); streptomycin sulfate (500 μg) was included in all treatments. All treatments were applied in duplicate and the petri dishes were incubated at 24 C for 22 hours.

For the experiments comparing Prior barley, Victory oats, and Gabo wheat, treatment and incubation procedures were similar to those described above for the 23 varieties of barley. Prior and Victory were dehusked and incubated in varying GA_3 concentrations at 30 C for 22 hours. Gabo was incubated at varying temperatures in 2×10^{-6} M GA_3 for 22 hours.

Determinations of reducing sugar and protein nitrogen levels, and dry weight losses were carried out as described earlier (5). Separate series of petri dishes were set up for hydrolyzable carbohydrate (starch) determinations and incubated at the same time as the series on which the other determinations were carried out. Starch was estimated, following removal of the endosperm, by washing the contents

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