Respiration During Development and Germination of Muskmelon Seeds (*Cucumis Melo* L.)

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(ABSTRACT)

Respiration rates of developing muskmelon *(Cucumis melo* L.) seeds were determined polarographically using a Clark-type O_2 electrode (Hansatech LD2). Seeds were obtained from fruits harvested 20, 30, 40, and 50 days after anthesis (DAA). Respiration (O_2 uptake) was measured for fresh intact seeds and fresh dissected seeds. The respiration rate of intact seeds declined from a maximum of 2.28 µmol O_2/min/g DWT at 20 DAA to a minimum of 0.16 µmol O_2/min/g DWT at 50 DAA. Dissecting intact seeds into embryo, testae, and perisperm tissues increased the respiration rate of 20 DAA seeds to 3.12 µmol O_2/min/g DWT but had no effect on more mature seeds. Respiration rate was highly correlated with seed relative growth rate and water-content. Respiration rate was not consistently changed after incubation in water. This indicates that respiration rate is not directly controlled by subtle variations in water content. Rather, seed respiration rate is directly linked with turgor-driven, expansive growth and relative growth rate. Fifty-DAA seeds from dry storage were imbibed on water saturated blotters, and respiration rates of whole seeds, decoated seeds, and embryos were compared. Respiration during imbibition was not significantly inhibited by the testae or perisperm tissue. In addition, 50-DAA dried, imbibed seeds were subjected to reduced O_2

concentrations ranging from 3.5 kPa partial pressure O_2 (pO_2) to 21 kPa pO_2. Respiration was not limited by O_2 until pO_2 was reduced to approximately 5 kPa, indicating a high affinity for O_2. Gas chromatography revealed that pO_2 in the seed cavity of muskmelon fruits ranged from 12.5 to 8 kPa. Fifty-DAA seeds from dry storage were imbibed on polyethylene glycol (PEG), mannitol, or NaCl ranging from -0.5 to -2.5 MPa water-potential or on abscisic acid (ABA) solutions ranging in concentration from 10 to 50 μM. Respiration and solution water-potential were measured at 10-hr intervals. At 10 hr of imbibition, each type of osmoticum and ABA stimulated respiration to values greater than for seeds imbibed in pure water. Beyond 10 hr, respiration rates were variable.

Acknowledgements

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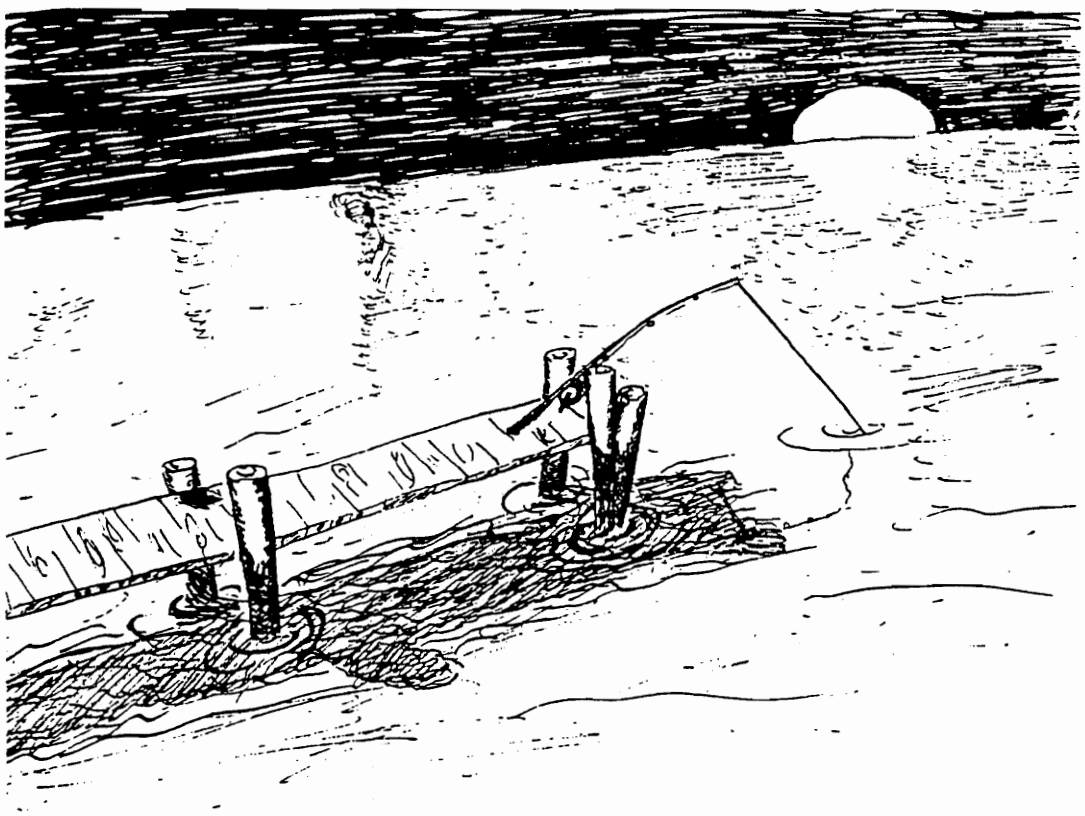
The faculty, staff and students of the Horticulture Department of Virginia Tech have provided several years of friendship, advice, and encouragement through my bachelors and masters degree programs, and will be long remembered and missed. Dr. Jerry M. Williams was particularly helpful as my undergraduate advisor, and a continuous source of support and frequently solicited advice. I especially thank Lewis Jett and Rick Bates for encouraging me as a Christian, and for helping me set my heart on things eternal in the midst of the urgencies of this world.

I give special acknowledgement and thanks to my wonderful wife Lorenda Lin Dyson. As a capable businesswoman and graduate of Virginia Tech herself, she has chosen to lay her career aside to raise our two children, Miles and Keverly, and to support and love me through my college education in a way that I could never thank her for enough. I look forward to growing old together.

Dedication

This thesis is dedicated to my father, William Harold Dyson. He was a Virginia Tech Graduate of the Class of 1953, a career industrial arts teacher, a veteran of World War II, a born again Christian, and the finest father a man could hope to have. As his time on earth neared completion, one of his greatest concerns was that I complete the education that his moral and financial support had made possible. After heroically enduring a long painful illness, he went home to be with our Lord on May 4, 1993, four days before my graduation. He left behind many happy memories. I am forever indebted to him in many ways, and will miss him terribly until the moment that I too come into the presence of God.



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Introduction

Orthodox seeds (desiccation-tolerant) exhibit at least three developmental stages. Initially, rapid cell division after fertilization forms the embryonic axis and associated tissues. In the second stage, cells expand and reserve materials are accumulated by the endosperm and embryo. During stage three, water loss from the quiescent or dormant seeds results in reduced metabolism, until the seeds are imbibed. However, seeds of fleshy fruited crops normally remain moist at maturity. Tomato, cucumber, muskmelon and some other seeds maintain relatively high moisture contents until released from decaying fruits. Effects of seed maturation at high water content has received little research attention, relative to dry-seeded grain crops such as wheat, maize, and rape.

Respiration rates are high in the first and second stages of development in many seeds but then decline as seeds desiccate during the third stage. However, in seeds that mature at high moisture content it is unclear whether respiration rates remain high after expansive growth has ceased or whether respiration declines independently of seed moisture status. Muskmelon fruit water-potential changes during seed development and declines as fruit soluble solids increase with maturity. These lowered water-potentials prevent precocious germination (Welbaum and Bradford 1988), but it is not known if they control respiration rates during development. Perhaps respiration rates are affected by limited O_2 availability due to the surrounding fruit tissue and tissues enclosing the embryo.

Lyons et al. (1962) have shown that CO_2 and O_2 concentrations in the muskmelon fruit seed cavities are dramatically different than ambient air. The respiration rates of some seeds are reduced as O_2 partial pressures (pO_2) are decreased below ambient. Soybean seeds exhibit reduced respiration rates as O_2 availability is reduced from ambient to 4 kPa pO_2 (Gale 1974). Soybean growth has been shown to decrease with reduced pO_2

as well (Sinclair et al. 1987). Respiration rates decrease in *Vicia faba* seeds as pO_2 values fall below 18 kPa at 26°C. It is unknown whether high pCO_2 or low pO_2 conditions in fruits such as muskmelon affect seed development. Critical pO_2 is the pO_2 above which tissue respiration no longer increases (Armstrong and Gaynard 1976). The critical pO_2 of muskmelon seeds is not known, and so the importance of developmental O_2 limitation is not clear at this time.

Muskmelon seeds must imbibe water to germinate. Since turgor drives radical growth, a reduction in water-potential would be expected to reduce radical growth and seed germination equally. However, seed germination is reduced more in response to lowered water-potential than is growth of radicles after germination in many seeds (Ross and Hegarty 1979, Hegarty and Ross 1978). Interestingly, the only *Cucumis* sp. used in the Ross and Hegarty work, *Cucumis sativum* L., displayed a water-potential response which was similar in germination and radical growth, indicating that water-potential could be the primary control of germination in these seeds. Botha et al. (1984) reported a reduction of O_2 uptake and germination with reductions in water-potential of *Citrullus lanatus* seeds, suggesting a link between seed water-potential, respiration, and germination. Reduction in respiration of imbibed muskmelon seeds due to reduced O_2 diffusion through the testa was reported by Pesis and Ng (1984). The effects of testa removal on the rate of imbibition was not considered as a possible cause of the difference in respiration rate between intact and decoated seeds in that study (Pesis and Ng 1984). Respiration was not limited by the testa of *Vicia faba*, when respiration measurements were taken in air (de Visser et al. 1990). Al-Ani et al. (1985) showed a direct relationship between O_2 availability, respiration, and germination rates in lettuce, sunflower, radish, cabbage, turnip, flax, soybean, flax, rice, maize, wheat, sorghum, and pea. Interactions between respiration rates, pO_2, pCO_2, and water relations of seeds are poorly understood.

Seed development in muskmelon offers an excellent opportunity to explore a possible interaction between these variables, since changes in seed water-potential as well as pO_2 and pCO_2 have been characterized during fruit development.

Several investigators have shown that, in some seeds, high water-potential can actually inhibit seed germination instead of initiate it as would be expected. This phenomenon was first termed "water sensitivity" by Essery et al. (1954 as referenced in Roberts 1969) and Pollock et al. (1955 a, b as referenced in Roberts 1969). Water sensitivity can be overcome by a wide range of factors in barley, including increases in pO_2, high temperatures in storage, low germination temperatures, and low pH. Dunlap (1988) reported water sensitivity in muskmelon seeds, and found that decoating the sensitive seeds resulted in germination, implicating the seed coat as a barrier to O_2 diffusion. Application of a fungicide and antibiotic together on intact seeds can be effective in removing water sensitivity, however, neither are successful when applied alone (Gaber and Roberts 1969a as referenced in Roberts 1969). Gaber and Roberts (1969b as referenced in Roberts 1969) hypothesized that the bacteria and fungi infecting the seed coat compete with the embryo for O_2, thus suffocating the seeds. Application of nitrate and urea increases water sensitivity of barley (Blum and Gilbert 1957 as referenced in Roberts 1969). Roberts (1969) suggested that the nitrate and urea provide nitrogen which stimulates the activity of micro-flora, further supporting the hypothesis of Gaber and Roberts (1969b). Ethanol production under O_2-limited conditions is a proposed cause of water sensitivity and reduced seed vigor in muskmelon (Pesis and Ng 1984, 1986). Recent work (Welbaum and Bradford 1990) has shown the perisperm envelope protects the embryos from ethanol, as exogenous ethanol was unable to penetrate to the embryo in-vitro. Ethanol produced by the embryo may cause sensitivity, as Pesis and Ng (1984)

demonstrated anaerobic respiration in muskmelon seeds exposed to excess moisture during germination.

A practical method to overcome water sensitivity in seed testing was devised by Heit (1951), in which seeds are germinated on damp paper towels. Currently, testing procedures call for seeds to be imbibed "on the dry side" (Anonymous, 1988). A quantifiable germination test standard to overcome water sensitivity, whether by use of osmoticum or other means, has not been established. The phenomenon of water sensitivity has yet to be satisfactorily explained.

Polyethylene glycol (PEG), NaCl, and mannitol are routinely used as osmotica to generate reduced water-potentials for plant research (Izzo et al. 1991, Goertz and Coons 1991, Zekri and Parsons 1990, Bujalski et al. 1991, Ross and Hegarty 1979, Michel 1970). However, several studies have shown injury to plants other than those caused by osmotic effects (Jackson 1962, Leshem 1966, Machlon and Weatherley 1965, Michel 1970). Side effects of these materials may interfere with data collected in water-potential studies on plants. Low O_2 solubility has been demonstrated in PEG (Mexal et al. 1975), which may interfere with water-potential studies by reducing O_2 availability for respiration. Zekri and Parsons (1990), demonstrated that equivalent osmotic potential solutions of NaCl and PEG did not have identical effects on split-root sour orange seedlings, indicating that one or both had confounding side effects. It has been suggested that PEG may induce desiccation of plants by blocking the water pathway (Lawlor 1970), which would also interfere with water-potential. To date, PEG is still taken to be inert osmoticum, without full understanding of its specific impact on plant tissue. Little information is available about how these compounds affect respiration of imbibed seeds.

The most appropriate means of measuring respiration rates of seeds has been debated by previous investigators. Clark-type electrodes have been used in the past to measure respiration of seeds in the liquid phase (Guldan and Brun 1985, Sinclair 1988). Because of apparent reductions in respiration caused by buffer solutions, de Visser et al. (1990) determined that gas phase measurements of respiration are more appropriate for measuring seed respiration. Increased resistance to O_2 diffusion when plant tissues are wet is similarly reported for a number of crop tissues (Ohmura and Howell 1960). As a result of these reports, the gas phase electrode was chosen for the current study.

Chapter 1
Measurement of seed respiration

Introduction:

Aerobic respiration requires the uptake of O_2. Aerobic seed respiration rates can thus be determined by measuring O_2 concentration changes in a closed system. One device that has been successfully used to measure seed respiration is the Clark-type polarographic O_2 electrode (de Visser et al. 1990). While O_2 electrodes have been used to measure seed respiration in the liquid phase (Guldan and Brun 1985, Sinclair 1988), de Visser et al. (1990) demonstrated that O_2 uptake by seeds can be significantly reduced by submersion in the buffer solution required for liquid phase measurements. Based on this observation, de Visser et al. (1990) concluded that seed respiration measurements would be best carried out in the gas phase. This conclusion is supported by the work of Ohmura and Howell (1960), who demonstrated that wetting plant tissue reduces O_2 uptake. Therefore, the gas-phase O_2 electrode, designed to measure O_2 evolution of photosynthesis, was selected for the current study to examine respiration during seed development and germination.

Electrode Theory

The Clark-type O_2 electrode used in this study consists of a polarographic electrode disk, a sample leaf chamber with gas ports, and a water jacket (See Fig. 1.1).

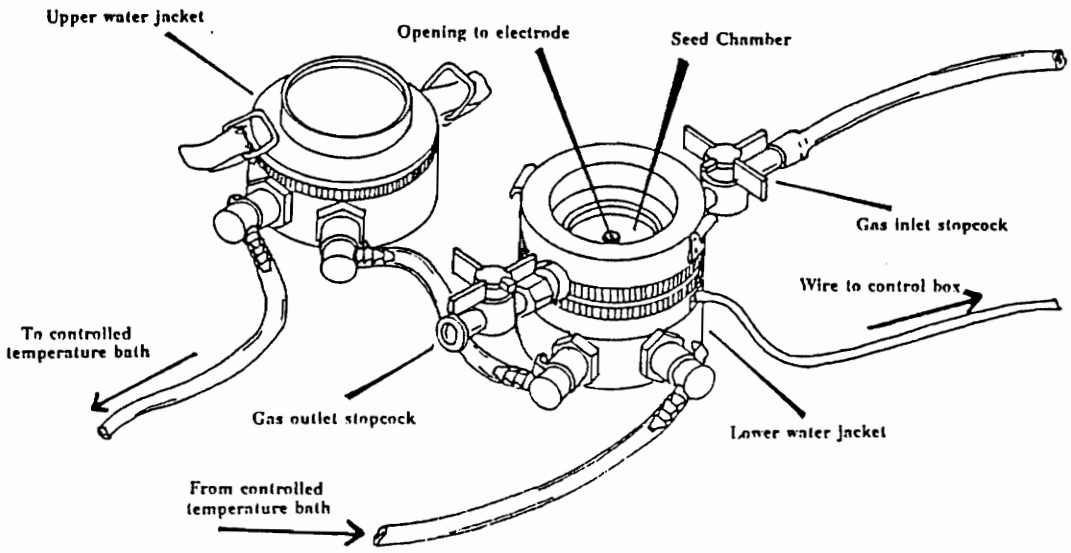


Fig. 1.1 The Hansatech LD2 polarographic O_2 electrode, shown with the chamber lid removed.

The water jacket completely surrounds the electrode disc. When water or ethylene glycol from a controlled temperature bath is circulated through the jacket, the electrode disc is maintained at a constant temperature. Constant electrode temperature must be maintained, because the O_2 content of air is related to temperature (Equation 1.1).

$$\mu\text{mol } O_2\text{/ml air} = (9.37\ \mu\text{mol})(273/(273 + °\text{C})) \qquad (1.1)$$

Calculation of O_2 content in ambient air. (Walker D. 1988)

The lid of the chamber housing has a clear plastic window to allow samples to be exposed to light for photosynthesis experiments. The sample chamber is designed to house a series of metal, foam, and felt pads to support leaf tissue samples. There is sufficient space in the sample chamber for seeds and blotter paper to control moisture

content. Approximately fifteen large flat seeds can be accommodated in the sample chamber. Spherical seeds up to 0.5 cm in diameter will fit as well. The electrode disk consists of a platinum electrode and a silver electrode imbedded in an epoxy resin disk (See Fig. 1.2 A). A Teflon membrane is stretched over the dome of the disk, covering a sheet of cigarette paper saturated with KCl solution. (See Fig. 1.2 B).

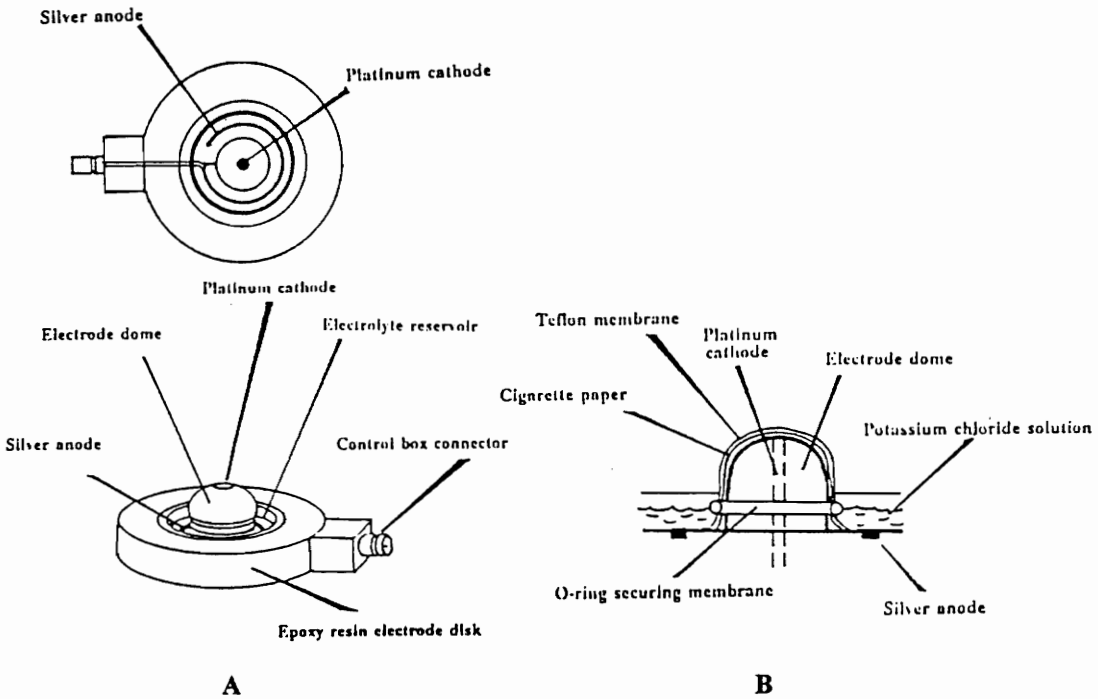


Fig. 1.2 **A**. Top and oblique views of the O_2 electrode disk. **B**. Detail of the electrode disk dome with membrane and electrolyte in place.

A potentiating voltage of 600 to 700 mV is applied across the electrodes by a battery-powered electrode control box. Oxygen from the chamber is reduced as it contacts the electrolyte solution over the platinum electrode, resulting in a flow of electrons from the platinum to the silver electrode that is stoichiometrically related to the

O_2 concentration in the chamber (Equation 1.2). The current is amplified by the control box and displayed on a voltmeter and strip chart recorder.

At the platinum cathode (1.2)

$$O_2 + 2H_2O + 2e^- \longrightarrow H_2O_2 + 2OH^-$$

$$H_2O_2 + 2e^- \longrightarrow 2HO^-$$

At the silver anode

$$4Ag \longrightarrow 4Ag^+ + 4e^-$$

$$4Ag^+ + 4Cl^- \longrightarrow 4AgCl$$

(Delieu and Walker 1972)

The amount of O_2 in the chamber is calculated from the volume of the chamber and the concentration of O_2 in ambient air at the electrode temperature. The rate of O_2 uptake is determined from changes in the concentration of O_2 in the sample chamber as indicated by the slope of the electrode output (Equation 1.3).

O_2 consumption rate = (1.3)

$$\frac{\text{Vertical squares}}{\text{Horizontal cm}} \times \frac{\text{Amb. } O_2 \text{ (Chamber vol. ml) / (Full scale/ Conv. factor)}}{\text{Minutes/cm}}$$

Calculation of O_2 consumption rate from mV output. Amb. O_2 = Ambient O_2 concentration at electrode temperature in μmol/ml. Vertical squares and horizontal cm are taken from chart recorder readings. Conversion factor = Scale expander defined during calibration. Minutes/cm = strip chart recorder speed. (Walker D. 1988)

The chamber volume, including samples, is determined indirectly by measuring the increase in O_2 partial pressure (pO_2) occurring when 1 ml of ambient air is added to the sealed chamber with a syringe. The volume of space remaining in the chamber around the seeds is inversely proportional to the pO_2 and the electrode output (Equation 1.4).

$$\text{Chamber volume (ml)} = R1 / R2\text{-}R1 \qquad (1.4)$$

Calculation of chamber volume by mV output before and after addition of 1 ml ambient air to the electrode chamber. R1= mV output at ambient air pressure. R2= mV output with 1 ml ambient air added to sealed chamber. (Walker D. 1988)

The electrode is zeroed by flowing N_2 gas through the sample chamber until the current reading from the voltmeter settles to around 20 to 30 mV. The reading is then "backed off" to zero using a back off adjustment on the electrode control box. The flow of N_2 is stopped and full scale is calculated. The full scale value depends on the chamber volume, the O_2 in the sample chamber at the experimental temperature (8.58 μM/ml at 25° C), and a conversion factor selected to expand the output scale for precision and sensitivity (Equation 1.5). Full scale is set by an output adjustment on the control box after the chamber is filled with ambient air.

$$\text{Full scale} = \text{Chamber volume ml (Conversion factor) (Ambient } O_2 \text{ at given temp.)} \qquad (1.5)$$

Calculation of full scale, performed in the calibration of the O_2 electrode.

Once calibration is complete, the chamber is sealed and thermoequilibrium is re-established for about 20 min before output stabilizes and respiration rates can be measured.

Materials and Methods

A Clark-type O_2 electrode (model LD2, Hansatech Ltd., Norfolk, UK) and control box (model CB1, Hansatech Ltd., Norfolk, UK) were prepared as described above for seed respiration measurements, using a Teflon membrane (Hansatech., Norfolk, UK). A strip chart recorder (model 4510, The Recorder Company, San Marcos, TX), was connected to the control box to record electrode output. The electrode was turned on a minimum of 12 hr prior to use to allow stabilization as recommended by Walker (1988). A multimeter (model 22-195A, Micronta, Fort Worth, Texas) was used to obtain mV output readings from the O_2 electrode for calibration purposes. Chamber temperature was maintained at 25°C by a controlled temperature bath (Lauda RM-6, Brinkman Instruments Inc., Westbury, NY). Fifteen muskmelon seeds (*Cucumis melo* L. 'Top Mark', Asgrow seed Co., Lot # VP-133 or 'PMR-45', Petoseed Co., Lot # 1027, stored from 1984 at 10° C) were placed in the electrode chamber in each experiment. Seeds were either newly harvested or dried, stored at 10°C, and imbibed for use in experiments. All imbibition treatments were performed in 0.01% (w/v) streptomycin sulfate to prevent microbial activity. A 20 second dip in 10% NaOCl (Wonder Chemical Co., Fairless Hills, PA) was used for surface sterilization of some seeds just prior to respiration measurements as well. Seeds taken directly from the fruit were not treated with bleach or streptomycin sulfate, because the interior of the fruit is sterile until opened. Occasionally, the anti-microbial treatments were compared. No differences in respiration were observed between streptomycin sulfate treated seeds and seeds treated with streptomycin sulfate and bleach.

Results and Discussion

While the LD2 O_2 electrode can accurately measure seed respiration, several systematic errors were encountered. Sudden, unexplained respiration changes were often recorded during some experiments. A series of simple experiments were conducted to determine whether these variations in output reflected real shifts in sample respiration or were artifactual.

Mechanical Noise

Fluctuations in electrode output were frequently observed. It was determined that these "noise spikes" were caused when doors in the vicinity of the electrode were closed (Sample trace Fig. 1.3A). Closing doors may cause sudden changes in air pressure or vibrations which were transferred through the structure to the electrode. These actions were well correlated with the noise spikes and seemed to be the cause. However, electrode response to pressure change is typically slower than the observed spikes, and the chamber was sealed at the time of the readings. Sharp vibration of the lab bench beneath the electrode did not produce nearly as large spikes in the electrode trace as the closing of doors. This shows that vibration was an unlikely explanation for the effects caused by the closing of doors. Fortunately, the spikes caused by the doors did not alter the slope of the readings and so were usually ignored. Similar types of mechanical disturbances in the lab may prove to be responsible for unexplained interference.

Electrical Noise

Small but continuous oscillations of the electrode trace appeared frequently during experiments and often persisted for some time. Delieu and Walker (1972) reported that small rapid oscillation of the recorder pen can result from a membrane that is not smooth.

Efforts to alleviate the oscillations by membrane replacement were rarely successful in the current study, suggesting that some other electrical interference was involved. Typically, it was still possible to collect reliable data despite these oscillations, so they could usually be ignored.

A number of electrical disturbances were linked to electrode problems. Movement of the electrode-control box wire caused large bi-directional spikes in the electrode trace (Sample trace Fig. 1.3B). Switching on a stir plate placed about 60 cm from the electrode added noise spikes to the electrode output (Sample trace Fig. 1.3E). However, motors operating much farther than 60 cm from the electrode rarely caused electrode disturbance. Large spikes similar to those caused by switching the stir plate on and off close to the electrode did not alter the overall slope of the recorded line, were not disruptive to data collection, and were disregarded.

At one point, the electrode trace began to fluctuate erratically, making repeatable recording of respiration rates impossible. In checking the electrode system, it was discovered that the batteries were low. A trace of the electrode behavior with low batteries was made for future reference. Fresh batteries were inserted in the control box which ended the disturbance (Sample trace Fig. 1.3C). Similar low battery symptoms were reported previously by Delieu and Walker (1972).

Occasionally, unexplained interference in electrode traces occurred with no apparent cause. This interference was so severe and persistent that work was halted on numerous occasions (sample trace Fig. 1.3D). Replacement of the Teflon electrode membrane sometimes helped to correct the problem, when no cause of the noise was apparent. However, membrane replacement did not always correct the problem, and replacement required an extensive warm-up period as previously mentioned. It is possible that much unexplained interference may have resulted from operation of automatic and

manually switched devices throughout the building, creating electromagnetic fluctuations in the vicinity of the electrode.

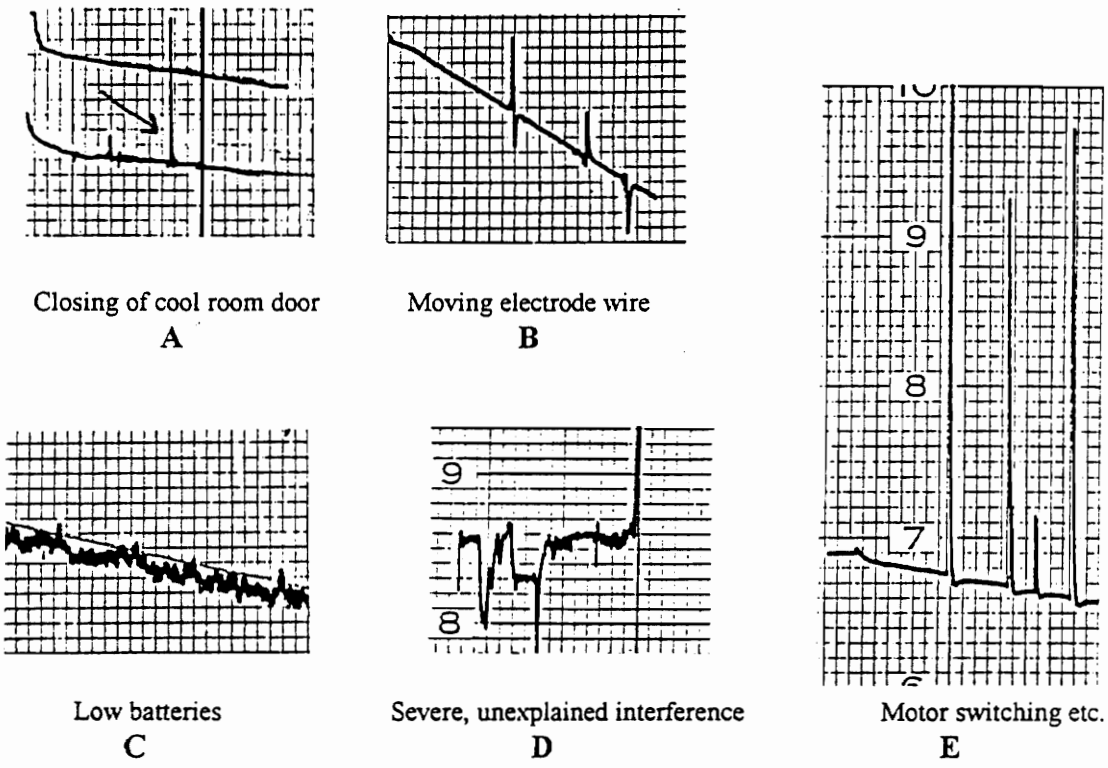


Fig. 1.3 Samples of chart recordings produced by the oxygen electrode under various interference conditions.

Other Sources of Error

The most troubling systematic errors are those which produce consistent erroneous output. It is important to recognize these problems quickly to avoid wasting experimentally treated seeds or reporting erroneous results.

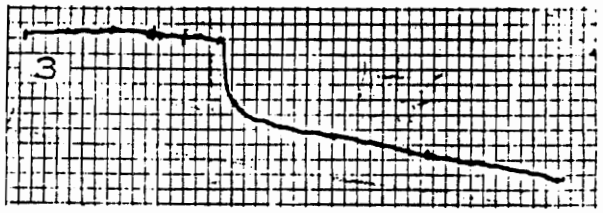
Taking readings too soon after the electrode has been turned on can cause electrode output to drift and appear as an artificially high respiration rate. Delieu and Walker (1972) reported this initial drift and hypothesized that it was caused by an initial

rise in pH and deposition of AgCl during operation. They found that allowing an hour or more between replacement of a membrane and O_2 measurements resulted in stable current output. Later, Walker (1988) recommended that the electrode be left on overnight to allow proper polarization after a new membrane has been installed. If the recorded depletion of O_2 in the empty, sealed, sample chamber is significantly greater than zero after the warm-up period, then further electrode settling is needed.

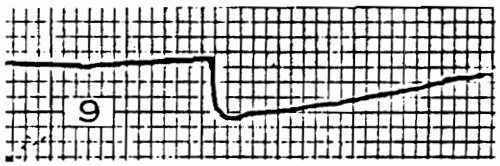
If the electrode chamber lid was sealed over the chamber while the stopcocks were closed, the mV output increased greatly for up to several minutes. The high output reflects an increase in the pO_2 in the chamber. The stopcocks should be opened before replacing the chamber top or opened briefly afterwards, to prevent pressurization of the sample chamber.

Occasionally, an apparently high rate of O_2 evolution would suddenly register during respiration measurement of fresh or fully imbibed seeds. The symptoms were the same as increased pressure in the sample chamber. The recorded respiration returned to normal by simply opening the stopcocks briefly (Fig. 1.4A). At times, the apparent phenomenon of O_2 evolution continued after the stopcock was closed (Fig. 1.4B). When this problem arose it was often persistent and prevented accurate respiration rates from being recorded. Treatment of all surfaces with 10% bleach and imbibition of seeds on 0.01% (w/v) streptomycin sulfate had no affect on this phenomenon. Air may have been trapped under pressure in compartments between seals in the electrode during volume determination for calibration. This air may have escaped into the sample chamber as the respiration measurement was performed. While this is an interesting hypothesis, the problem seemed to be too intermittent and persistent to simply result from the release of trapped air. To date, the best way to cope with the rapid increase in electrode output is to open the stopcocks briefly several times until the symptoms disappear. Early in each

measurement it is wise to open the stopcocks briefly to assure that this phenomenon is not occurring.



A



B

Figure 1.4 **A.** Electrode trace resulting from release of apparent pressure from electrode chamber through stopcocks. **B.** Same as "A" but output continues to increase after initial release.

Even when the electrode was working well, an artificially high non-linear rate was likely to be recorded briefly after the electrode is properly closed. Output should be monitored until a consistent rate is observed (Figure 1.5). The slope did not generally change after 5 min of consistent output. Perhaps the initial curving slope is the result of temperature change caused by opening the sample chamber.

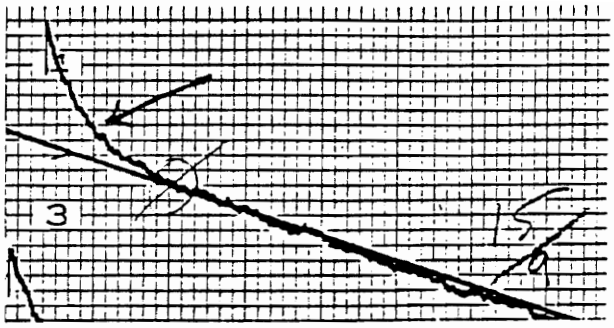


Fig. 1.5 Chart of artifact slope often formed in the first minutes of an O_2 uptake reading from the O_2 electrode.

Occasionally, when air was injected to measure chamber volume as described above, a chamber volume much larger than that of the chamber itself was calculated even when seeds are included. This error was probably caused by a chamber leak or an electrode problem and could usually be corrected by refilling the electrode reservoir with electrolyte if needed, cleaning and reseating all seals, or replacing the electrode membrane.

Even under optimal conditions, standard errors around mean respiration rates were often large and variable. Fortunately, even on the worst days, enough consistency was possible to confirm significant treatment effects. No respiration rates recorded were discarded during the reported work, unless one of the factors above was clearly confirmed to be the cause of error.

At one point, the O_2 electrode disk had to be replaced, because the output voltage suddenly jumped to close to 5 mV, far above normal for ambient O_2. Electrode cleaning, drying, and membrane replacement did not correct the problem. After trying another electrode disk with the system, it was determined that the original electrode disk was faulty. Apparently, KCl had penetrated cracks in the disk, causing an internal short circuit through the disk resin. Once a short has been established through the resin, the electrode

is not reparable and must be replaced (Delieu and Walker 1972). Even when an electrode is functioning properly, it may gradually lose sensitivity over the course of a week due to oxidation of the membrane. This is typical, and may usually be remedied by simply installing a new Teflon membrane, cigarette paper spacer, and KCl solution.

Other problems arose with the electrode which were specific to the use of altered atmosphere through the chamber. These problems are discussed in Chapter 3.

Chapter 2
Effect of testa and perisperm tissues on muskmelon seed respiration

Introduction

Plant tissue must have O_2 for aerobic respiration. Yet most embryos are surrounded by a testa and in some cases endosperm or perisperm tissue that may form a barrier to O_2 uptake. For example, O_2 diffusion into the vasculature of the *Glycine max* [L.] Merr. testa limits O_2 uptake (measured in buffer), and thereby slows seed development (Sinclair et al. 1987). An investigation of O_2 limitation in *Vicia faba* seeds, found that removal of testa did not significantly increase the respiration rate of developing seeds (de Visser et al. 1990). However, the *Vicia faba* testa did act as a respiration barrier when measurements were performed in a solution.

Pesis and Ng (1986) reported that the testae of muskmelon seeds present a significant barrier to O_2 uptake during imbibition. However, the disparity in respiration reported between intact and decoated seeds may have resulted from differences in embryo imbibition rate, as testae were removed prior to imbibition.

Experiments in the current study were designed to study effects of seed testa and perisperm on muskmelon seed respiration, testing the hypothesis that these tissues reduce respiration during development and also during germination.

Materials and Methods

Plant materials

'Top Mark' muskmelon seeds were grown in a growth chamber in plastic germination flats filled with germination media (Sunshine mix, Wetzel Inc. Harrisonburg, VA) at 30°C. When seedlings had two true leaves, the flats were removed from the growth chamber and placed in a greenhouse. Four-week-old seedlings were transplanted in the field into 2.5 mil black plastic mulch in rows spaced 1.52 m apart with an in-row spacing of 1.52 m. The planting was located on the Virginia Tech Horticulture farm near Blacksburg, VA. Fertilizer (10N-4.3P-8.3K) was applied at about 185 kg/ha, and an overhead irrigation system was used as needed. Hermaphroditic flowers were tagged at flowering. Since flowers open for only 1 day, fruit development can be traced from anthesis. To ensure adequate pollination, a beehive was placed within 100 m of the plot. Harvested fruit were transported to the laboratory, and seeds were removed for respiration measurements within 3 hr of harvest.

Respiration of developing seeds

Fifteen seeds each from 20, 30, 40, and 50 days after anthesis (DAA) fruits were removed from the seed cavity, wiped to remove the mucilaginous endocarp, weighed, and placed in the sample chamber of the LD2 polarographic O_2 electrode (Hansatech Ltd., Norfolk, UK). Respiration rates were recorded for each group of 15 intact seeds as described in Chapter 1. After the initial respiration measurement, seeds were removed from the chamber and placed in a humidified box to avoid desiccation until the testa and perisperm were removed (see Fig. 2.1).

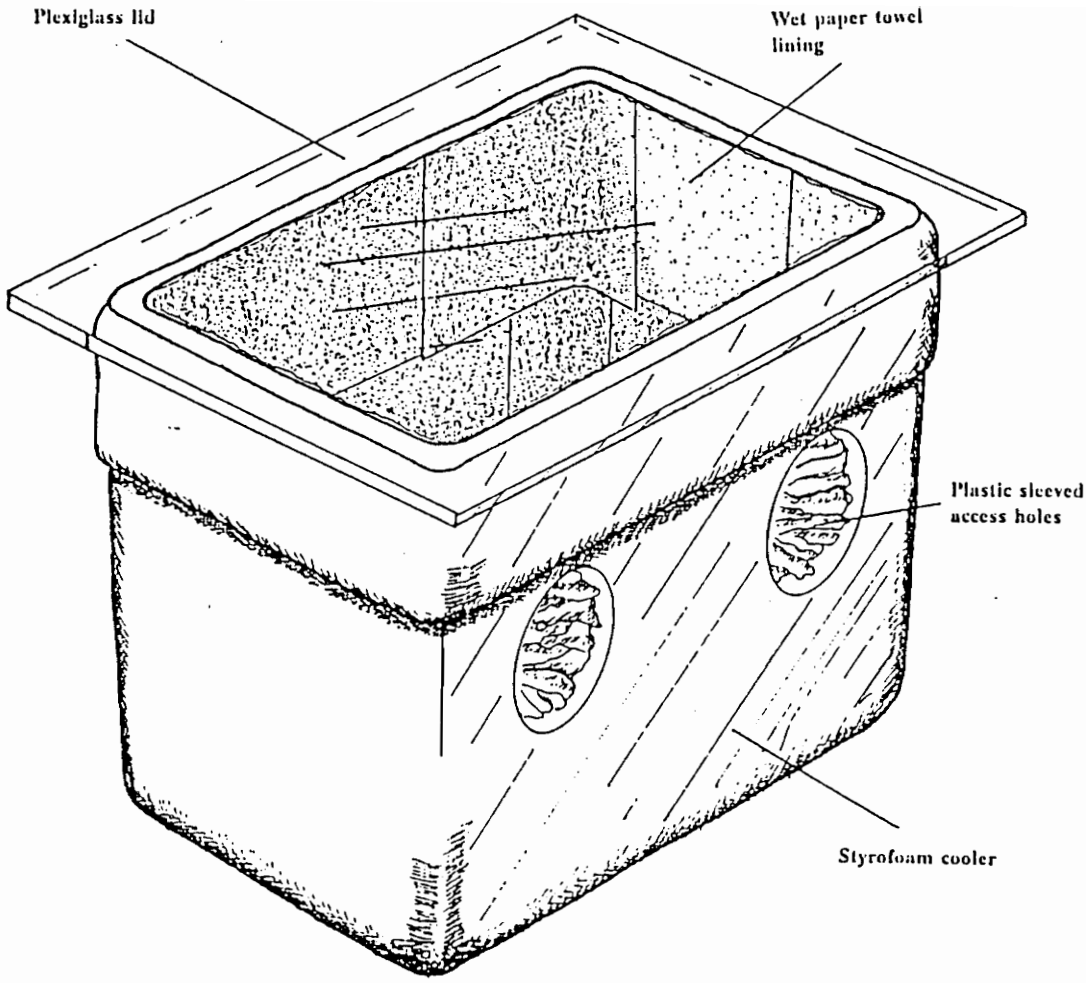


Fig. 2.1 Humidified box made from a common Styrofoam cooler by cutting arm holes allowing access to seeds inside. Wet paper towels line the interior, resulting in a high relative humidity within the box when the Plexiglas cover is in place.

In the humidified box, the testae were split open by carefully inserting a thumbnail along the suture (Fig. 2.2). All seed portions were placed in the electrode chamber for respiration measurement of decoated seeds. After the respiration rate was recorded, the decoated seeds were transferred to the humidified box. The cotyledon end of the perisperm envelope (a tissue tightly covering the embryo consisting of two or three layers of perisperm attached to a layer of residual endosperm) was carefully slit with a razor

blade and peeled away from the embryo using forceps (Fig. 2.2). Embryos, perisperms, and testae were all placed in the chamber again, and another respiration measurement was taken. All seed tissues were then removed from the chamber, dried at 130°C for 1 hr (Anonymous 1985), and weighed. Average respiration rate and standard error were calculated from eight replications for each treatment.

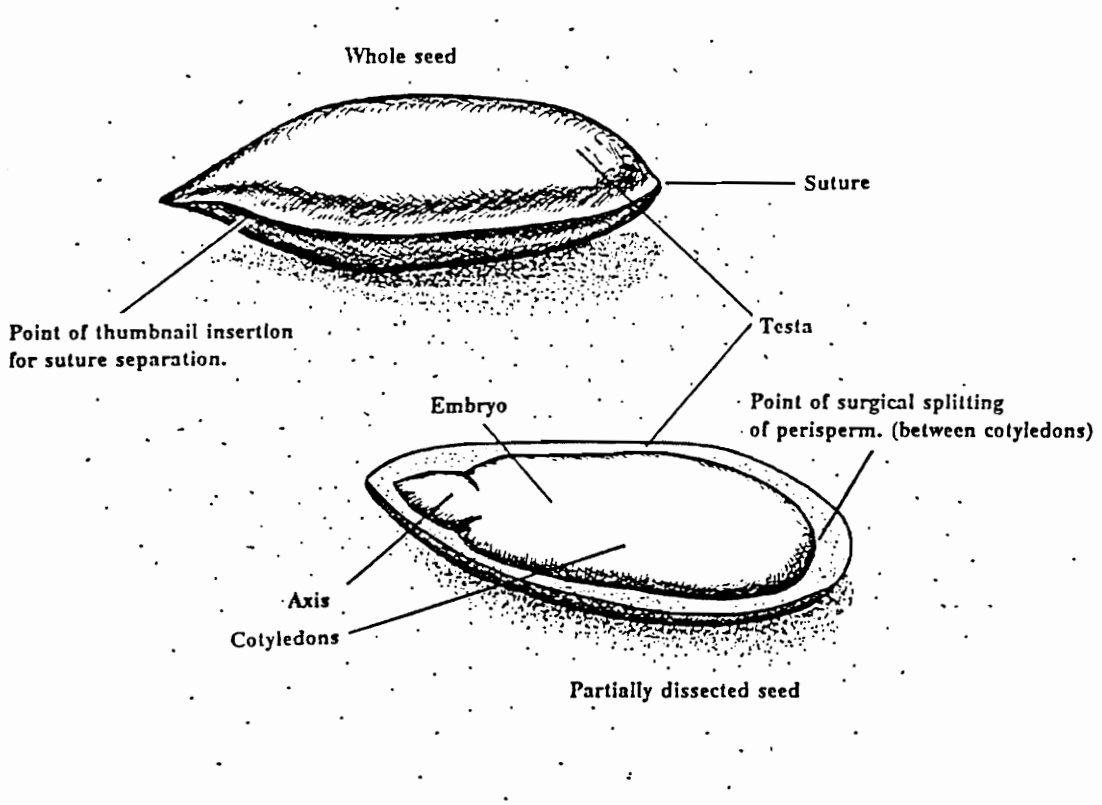


Fig. 2.2 Whole seed, and partially dissected seed, displaying the interior.

Respiration of germinating seeds

Seeds were harvested 50 DAA, rinsed, dried, stored, and then used to study effects of testae and perisperm envelope tissue on respiration rate of germinating seeds. Seeds were placed on three thicknesses of blotter paper, sealed in petri dishes, saturated with 20 ml of 0.01% (w/v) streptomycin sulfate solution, and placed in a growth chamber at 25°C. Fifteen seeds were removed from the blotters 10, 20, 30, 40, 50, and 60 hr after the start of imbibition and weighed. Respiration was measured as described in Chapter 1 for each imbibition time. Each sample of 15 seeds was first measured intact, then with the testa removed, then separated into embryo, perisperm, and testa as described previously. All separated tissues were included in the electrode chamber for each measurement as before. After respiration measurements, seeds were dried and weighed as described above. Respiration rates were averaged from eight replications for each time period in each experiment and expressed ± SE.

Results and Discussion

Seed respiration during development

Respiration of fresh, intact seeds was highest early in development, dropped dramatically between 20 and 30 DAA, and remained low through 50 DAA (Fig. 2.3). At 20 DAA, respiration increased with the removal of the testa. From 30 to 50 DAA, removal of the testa had no effect on respiration (Fig. 2.3). Perhaps the developing testae in 20-DAA seeds limited respiration by competing for O_2. In more mature seeds, when testa development may be complete but the testae remain intact, the respiration "barrier" they form at 20 DAA disappears.

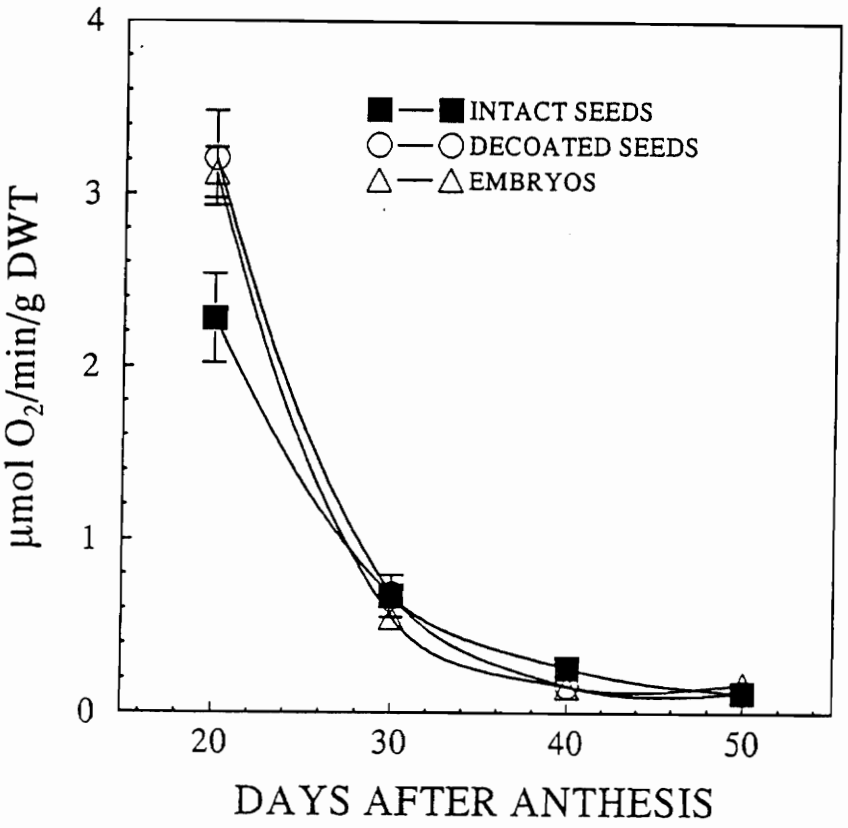


Fig. 2.3 Graph of respiration rates at 20, 30, 40, and 50 DAA, for fresh intact seeds, decoated seeds, and embryos. All separated tissues were included in the chamber for each measurement.

Muskmelon embryos are bathed in liquid endosperm during early development. The embryo grows by absorbing liquid endosperm. At the point of maximum dry weight accumulation at about 35 DAA (Welbaum and Bradford 1988), all the endosperm was absorbed except for a single layer that fused with the perisperm. In the current experiment, removal of the liquid endosperm along with the perisperm in 20-DAA seeds resulted in no significant increase in seed respiration rate. This suggests that the perisperm and liquid endosperm have no effect on seed respiration beyond 20 DAA in ambient atmosphere. The testa surrounding the embryo limits respiration at 20 DAA but not at 30,

40, and 50 DAA (Fig. 2.3). These results agree with the work of de Visser et al. (1990), where *Vicia faba* testae had no appreciable effect on embryo respiration measured through the gas phase.

Seed respiration during imbibition and germination

The respiration of 50-DAA seeds was lowest and essentially unchanging from 10 to 40 hr imbibition and then greatly increased with radical emergence (Fig. 2.4).

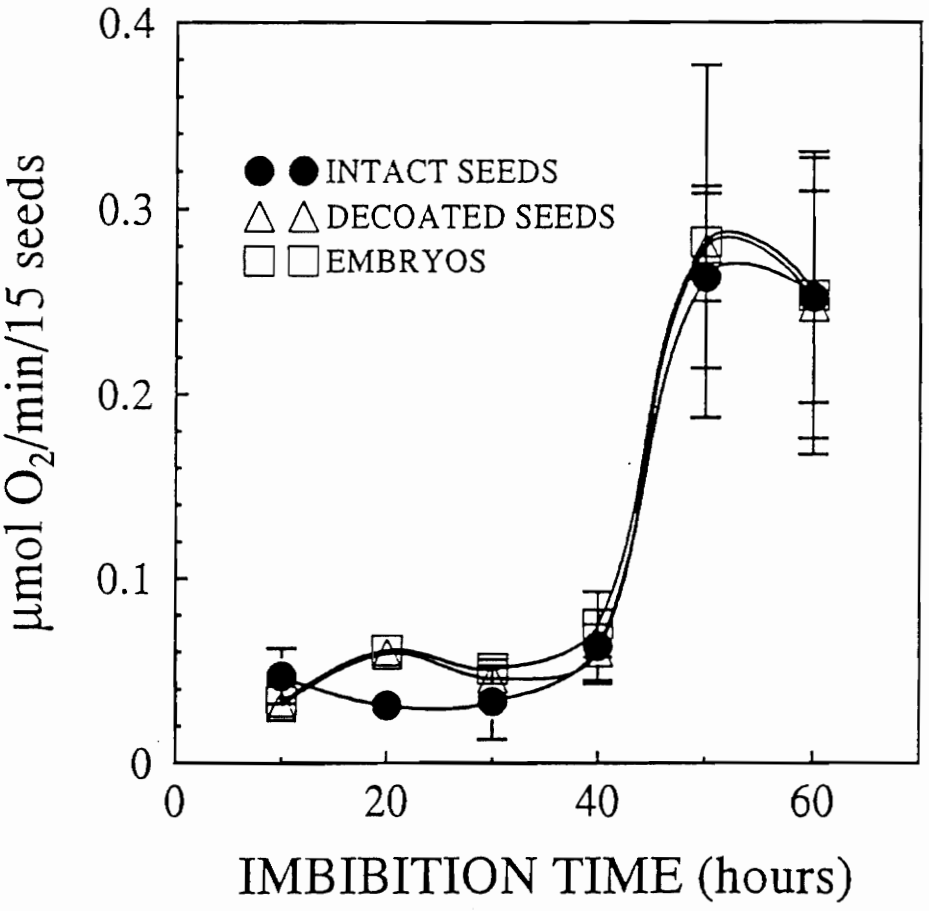


Fig 2.4 Respiration rates of 50-DAA dried and stored intact seeds, decoated seeds, and embryos through imbibition. All separated tissues were included in the chamber for each measurement.

Little or no significant increase in respiration was observed in germinating seeds when the testae or perisperm tissues were removed from 10 to 60 hr imbibition. Past 40 hr imbibition, the time of radical emergence varied among samples. This variability accounted for both the increased respiration rate and the increased error bars presented for these imbibition times (Fig. 2.4). These results are contrary to the findings of Pesis and Ng (1986), who showed that seed coverings do inhibit muskmelon seed respiration during imbibition. Pesis and Ng (1986) showed that the testae were a significant barrier to O_2 uptake in imbibing seeds. The difference in respiration rates between decoated and intact seeds reported previously may be explained by the fact that both groups of seeds were imbibed separately. Differences in the rate of imbibition between these treatments could account for the observed differences in respiration (Pesis and Ng 1986). In the current study, only intact seeds were imbibed and then decoated at each time interval so that all seeds would be hydrated to the same water content when respiration measurements were made. It appears that the seed coverings do not act as O_2 barriers that limit respiration in mature muskmelon seeds during imbibition and germination.

Chapter 3
Effects of oxygen and carbon dioxide on muskmelon seed respiration

Introduction

Carbon dioxide and O_2 concentrations in ambient air are approximately 0.035 kPa pCO_2, and 21 kPa pO_2, respectively. Muskmelon seeds develop in a fruit cavity with a gas composition different than ambient. Lyons et al. (1962) reported that the pO_2 in the seed cavity of *Cucumis melo* L. 'PMR-45' fruit decreases from 18 to about 14, while the pCO_2 rises from approximately 0.035 to 7 kPa. However, the effects of reduced pO_2 and elevated pCO_2 on muskmelon seeds were not investigated.

Several reports indicate that developmental seed growth and respiration are sensitive to reduced pO_2. Gale (1974) observed a linear decrease in soybean seed respiration with a decrease in pO_2 from ambient (21) to 4 kPa. In the field, growth of soybean at pO_2 reduced from ambient to 0.10 yielded a significant reduction in the dry weight gain of soybean seeds over the course of a growing season (Sinclair et al. 1987). The respiration rate of *Vicia faba* seeds decreases at pO_2 below 0.18 at 26°C (de Visser et al. 1990).

Effects of reduced pO_2 have also been studied in germinating seeds. Al-Ani et al. (1985) showed a direct relationship of reduced O_2 to reduced seed respiration and germination rates in lettuce, radish, turnip, soybean, sunflower, cabbage, flax, rice, maize, wheat, sorghum, and pea.

In this study, the effects of reduced pO_2 and elevated pCO_2 on muskmelon seeds were investigated both during development and germination.

Materials and Methods

Fruit internal gas concentrations

Fruits were grown as described in Chapter 2. Forty-, 50-, and 55-DAA fruits were harvested at approximately 1000 HR and transported directly to the lab (about 20 min) at ambient temperature. In the lab, gas samples were immediately taken from the seed cavity of each fruit using 7.5 cm, 22-gauge needles mounted on 1 ml plastic tuberculin syringes. A needle was inserted through the blossom end of each fruit into the seed cavity. The fruit was submerged in water at approximately 25°C to prevent ambient air from entering the fruit. Syringe needles were inserted into rubber stoppers to store samples briefly until analysis. Just before analysis, a 25-gauge needle was placed on each syringe and gas was expelled to clear the new needle of ambient air and to bring the syringe volume to 1 ml. Samples were injected into a gas chromatograph (Model GC-8A, Shimadzu, Columbia, MD) containing columns for O_2 (Molecular Sieve 5A) and CO_2 (Porapak Q, Supelco, Inc., Bellefonte, PA). The system was calibrated using gas standards (Scott Specialty Gases, Plumsteadville, PA) with partial pressures higher and lower than the samples. The calibration curve was linear over the range of concentrations tested. Chromatograph injection and flame ionization detector temperature was 80°C, and the oven temperature was 60°C. Measurements were taken in both 1991 and 1992. In both years, eight fruits were sampled at each stage of development. Four samples were taken from each fruit, two each for O_2 and CO_2 measurements. Average pO_2 and pCO_2 values were calculated for each stage of development in both years and expressed as means ± SE.

Respiration in varied pO_2 and pCO_2 combinations

Compressed O_2, CO_2, and N_2 was mixed at a rate of 424.8 L/hr using a Tri-gas blender (model 299-037F Smith Equipment Div. of Tescom Corp., WTN., SD). A

system of gas-tight, 12-L, storage bags and flasks equipped with interlocking ports was developed to store and transfer gas mixtures from the blender (Fig. 3.1). Gas mixtures were stored in each of the sealed plastic bags. Each bag was evacuated using a vacuum pump before refilling. Gas combinations of 10, 12.5, 15, 17.5, and 21 kPa pO_2, each at 0, 5, 7.5, and 10 kPa pCO_2 were prepared for a total of 20 gas mixtures.

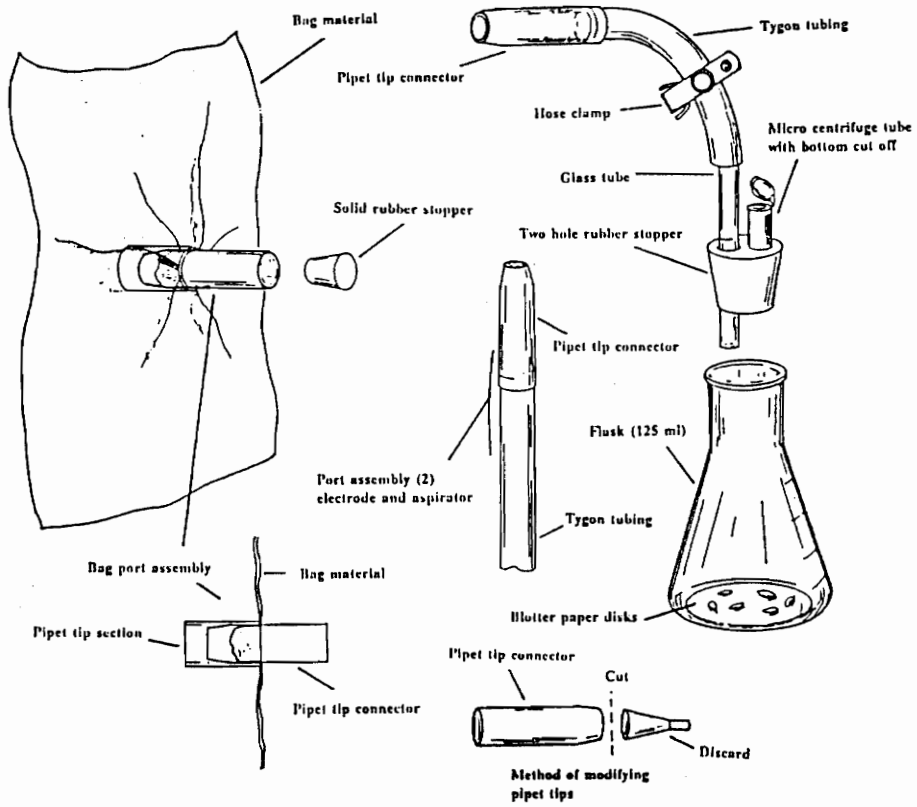


Fig. 3.1 Gas storage and transfer system of bags, flasks and interlocking ports.

Muskmelon seeds were grown and harvested as described in Chapter 2. Thirty fresh seeds were removed from newly harvested fruits, wiped free of endocarp, weighed, and placed on a blotter paper at the bottom of a 250 ml Erlenmeyer flask (Fig. 3.1). Twenty ml of 0.01% (w/v) streptomycin sulfate solution were pipetted onto the blotters,

and the flasks were stoppered. Flasks were randomly paired, and each pair was assigned one of the gas mixtures listed above. Approximately 3 L of each stored gas mixture was used to flush each flask using the inlet port. The volume was sufficient to exchange the atmosphere in each 250 ml Erlenmeyer flask approximately 10 times. Flasks were placed in an incubator at 25°C, and after 20 hr groups of 15 seeds were transferred onto a 0.01% (w/v) streptomycin sulfate saturated blotter in the O_2 electrode chamber. The top was replaced on the electrode chamber, the stopcocks opened, and approximately 2 L of remaining mixed stored gas was used to flush the electrode chamber. The O_2 concentration of each mix was verified using the electrode. The CO_2 concentration of each mix was verified by gas chromatography. Carbon dioxide samples were collected at the O_2 electrode/gas bag junction using a syringe and a 25-gauge 1.59 cm needle. After seed samples and gas mixtures were loaded, the chamber stopcocks were closed, and respiration measurements were taken after a 5 to 10 min equilibration period as described in Chapter 1. Seeds were removed from the chamber, dried at 130°C for 1 hr, and weighed (Anonymous 1985).

Effects of reduced O_2 on seed respiration

Groups of fifteen muskmelon seeds were randomly selected from a 50-DAA dry seedlot. Fifteen seeds were placed on double thickness blotters with 20 ml 0.01% (w/v) streptomycin sulfate solution in sealed petri dishes. The seeds were incubated at 25°C for 20 hr. Seeds were removed from the dishes, weighed, and the testae were split apart to ensure rapid diffusion of gas to the embryo. Seeds were sealed in the O_2 electrode chamber on a blotter saturated with 0.01% (w/v) streptomycin sulfate solution. A two-gas rotometer (Model FL-2GP-41C-41C, Omega Instruments, Stamford, CT) was used to mix 21 kPa pO_2 bottled breathing air with pure bottled N_2 proportionately, to obtain 17.5,

15, 12.5, 10, 7.5, 5, and 3.5 kPa pO_2. The actual concentration of O_2 in the chamber was determined using the electrode and was adjusted using the rotometer. The output was bubbled through water prior to purging of the electrode sample chamber. The gas mixture was flowed through the chamber until the O_2 reading of the electrode was constant. After the desired gas concentrations were obtained, the inlet and then the outlet stopcocks were closed sequentially to prevent a positive pressure from developing in the chamber. Respiration data were collected as described in Chapter 1.

Results and Discussion

Fruit internal gas concentrations

Prior to 40 DAA, the distinct gas phase of the seed cavity had not yet formed, so it was not possible to measure internal gas concentrations. At 40, 50, and 55 DAA, the gas phase of the seed cavity of the melons was well formed and accessible by inserting a needle through the blossom end (Fig. 3.2).

Beyond 40 DAA, pO_2 in the fruit cavity decreased or remained the same (Fig 3.3 A), while pCO_2 increased (Fig. 3.3 B). At 55 DAA in 1992, the O_2 measurements were variable, indicating a probable malfunction of the column. The seed cavities of 55-DAA fruit were partially filled with fluid. It was often difficult to obtain a gas sample without also collecting liquid in the syringe. Many gas samples were discarded because of excess fluid in the sample. Wet samples may have affected column performance, causing the excessively high CO_2 measurements for 55-DAA fruits in the 1992 crop.

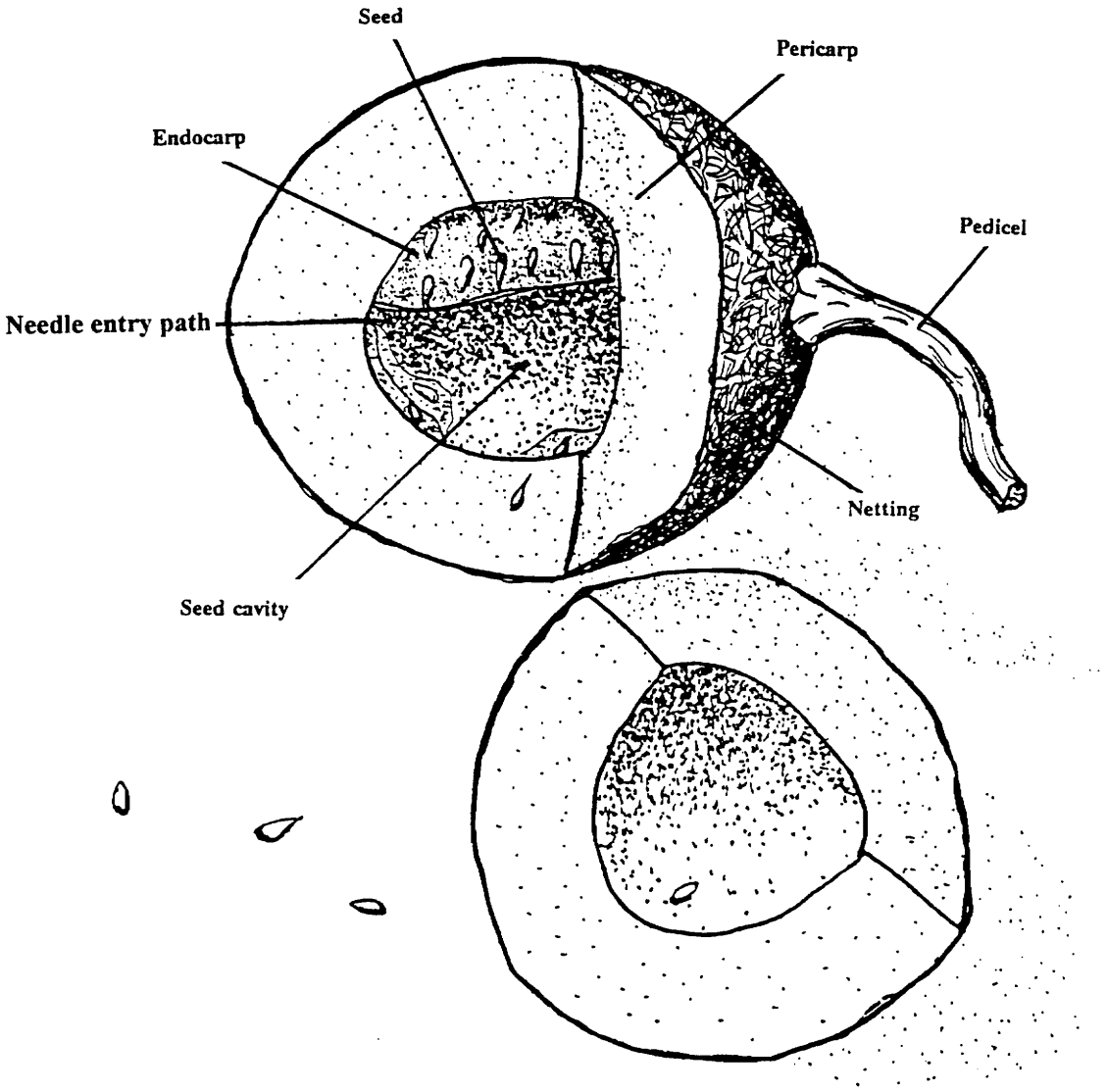


Fig. 3.2 A 40-DAA muskmelon cut open to display seed cavity and showing needle entry path

Lyons et. al (1962), previously measured cavity gas concentrations in developing muskmelon fruits. The 'PMR-45' cultivar used was earlier maturing, which allowed for

sampling of the gas in the seed cavity as early as 14 DAA. Lyons et al. (1962) reported gas concentrations from 14 to 41 DAA. Oxygen partial pressure of 13 kPa and pCO_2 of 7 kPa reported by Lyons et al. (1962) in 'PMR-45' 40-DAA seed cavities agrees well with current data for 40-DAA 'Top Mark' seed cavities (Fig. 3.3).

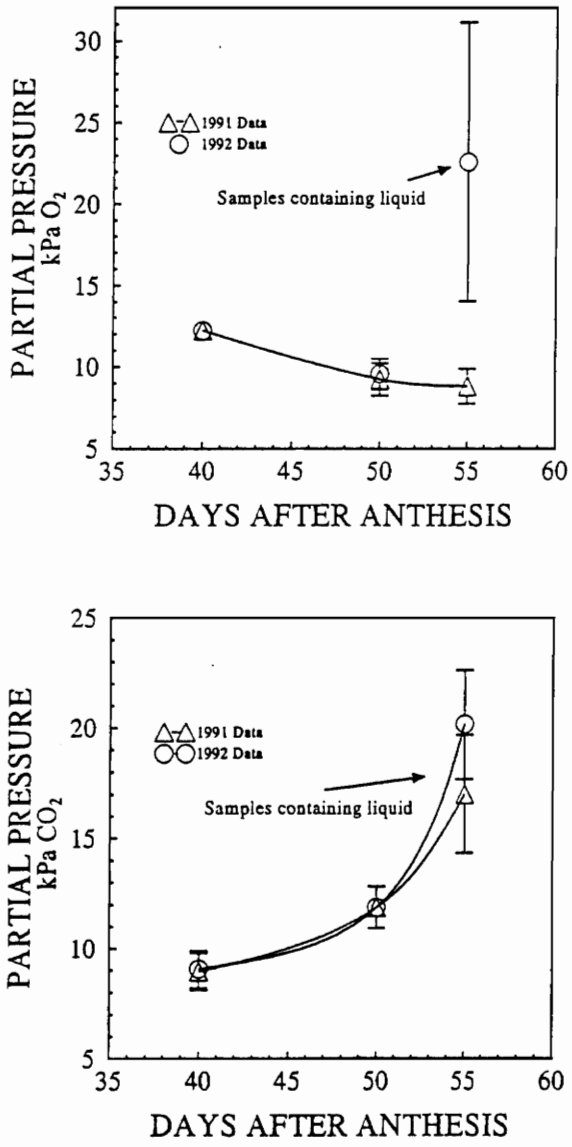


Fig. 3.3 **A.** Oxygen and **B.** CO_2 concentrations in the seed cavity of *Cucumis melo* L. cv. Top Mark

Miccolis and Saltveit (1991) reported that the rate of CO_2 produced by intact muskmelon fruits decreased over the course of development, while the current data shows an increase in CO_2 in the fruit cavity throughout development. It might be expected that the CO_2 concentration in the cavity would decrease as CO_2 production decreases. However, the pericarp apparently acts as a barrier to gas diffusion, permitting the increase of cavity CO_2 concentration despite reduced CO_2 production. Elevated CO_2 has been shown to inhibit respiration in plant tissues (Bunce 1990, Amthor et al. 1992, Ludwig et al. 1975 as reviewed in Drake 1992, Spencer and Bowes 1986). The low pO_2 may also limit O_2 availability for developing muskmelon seed respiration as well. Therefore, altered gas concentrations are a possible control of muskmelon seed respiration, and the effects of various CO_2 and O_2 concentrations were investigated in subsequent experiments.

Effects of altered pO_2 and pCO_2 on respiration

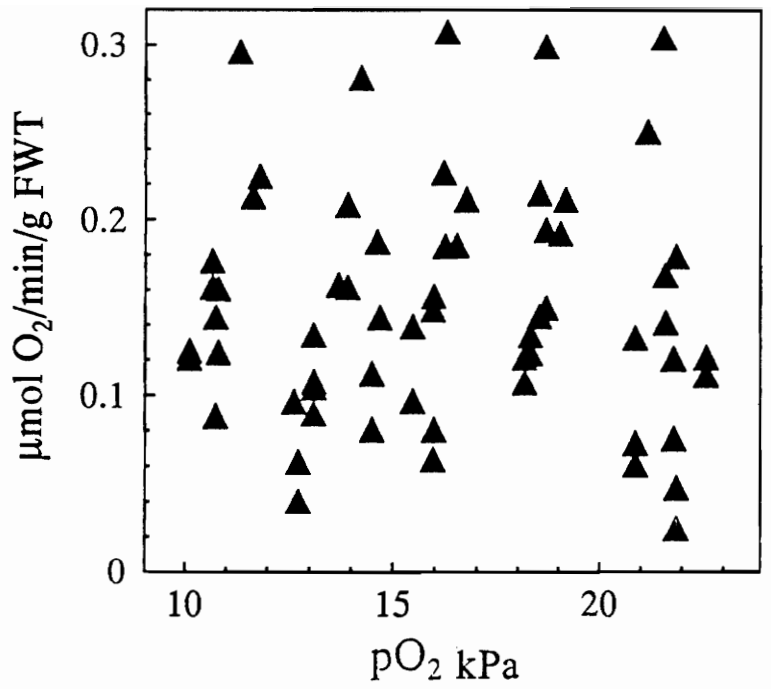
As reported for soybean seeds (Gale 1974) and *Vicia faba* (de Visser et al. 1990), respiration was expected to decrease with reduced pO_2 or elevated pCO_2. However, analysis of initial data yielded random plots showing no significant correlation between O_2 or CO_2 levels and respiration rate (Fig. 3.4A and B). There are several possible explanations for the anomalous results. At high pCO_2, the standard electrolyte solution may give erroneous results (Walker 1988). However, a electrolyte solution containing borate buffer and sodium bicarbonate recommended by Walker (1988) gave similar results. Oxygen partial pressures determined by the O_2 electrode indicated that the bagged gas mixes contained the intended concentration of O_2. However, gas chromatography revealed that CO_2 varied and was far below the expected levels in almost all the bags by the completion of each experiment. It may be possible to solve this problem by thoroughly mixing samples before use. If bag permeability was the problem, then different

bag material may be more effective. In a preliminary study of the bag material, O_2 concentrations remained quite stable over time. This suggests that CO_2 must separate from the mix instead of diffusing through the bag. This might be expected, because CO_2 is a heavier and larger molecule than O_2. By testing the repeatability of the Tri-gas blender mixes with the gas chromatography, it was ultimately determined that the precision of the Tri-gas blender was less than required for this study. Storing gas mixes in plastic bags did not give reliable results either. Because of these problems, no reliable data on the effects of elevated CO_2 on muskmelon seed respiration were obtained. Given the high CO_2 concentrations reported in Fig. 3.3, the possible effect of high CO_2 on muskmelon seed respiration is intriguing and deserves further investigation when suitable techniques are available.

Effects of reduced O_2

Respiration rates did not decrease with a decreased O_2 concentration until about 0.05 pO_2 (Fig. 3.5). Partial pressure of 0.05 is considerably lower than O_2 concentration values in Fig. 3.3 and in previous reports (Lyons et al. 1962). This suggests that O_2 concentration does not limit growth and development of muskmelon seeds. The seeds are surrounded by a mucilaginous endocarp early in development, which may limit O_2 availability to developing embryos (Fig 3.1). However, after 45 DAA this material degrades and should not constitute a significant barrier to gas exchange. Although fresh seeds were not available for this experiment and hydrated dried seeds were used instead, it should be noted that drying and re-imbibing has minimal effect on the respiration of desiccation-tolerant seeds. These data indicate that O_2 availability does not limit respiration of 50-DAA muskmelon seeds during germination under most conditions.

A



B

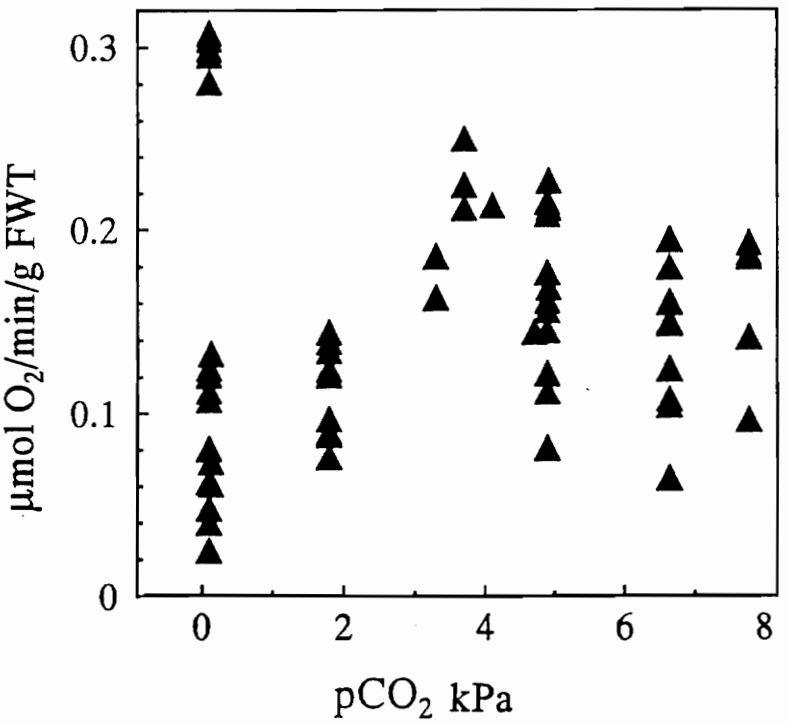


Fig. 3.4 **A.** Respiration rates correlated to pO_2 at a variety of pCO_2 values.
B. Respiration rates correlated to pCO_2 at a variety of pO_2 values.

Vicia faba seed respiration is reduced by O_2 lower than 18 kPa pO_2 (de Visser et al. 1990) without significant differences between intact and separated seeds. However, in the current study respiration of muskmelon seeds was maintained at as low as 10 kPa pO_2, indicating greater affinity of cytochrome oxidase than in some other types of seeds. High affinity of cytochrome oxidase may translate into greater tolerance of reduced pO_2, explaining why muskmelon seeds can tolerate lower pO_2 than *Vicia faba*.

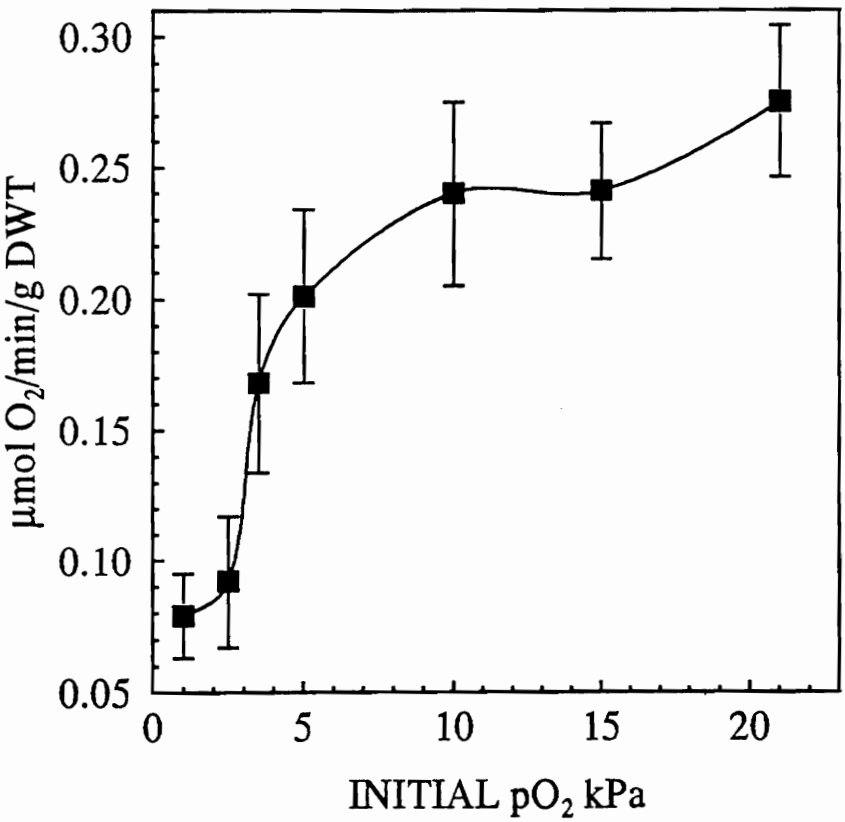


Fig. 3.5 Respiration rates at reduced pO_2 values.

Chapter 4
Effects of water content on muskmelon seed respiration during development

Introduction

Most investigations of respiration in developing seeds have been conducted on dry-seed crops such as bean (de Visser et al. 1990). In these crops, the fruit tissue surrounding the seeds normally desiccates at maturity, and the respiration rate falls because integrated metabolism cannot be maintained in dried seeds (Leopold and Vertucci 1989).

In fleshy-fruited crops such as tomatoes or melons, seeds are held at relatively high water content for extended periods. However, even in fleshy fruits, seeds undergo significant changes in water-potential during development (Berry and Bewley 1992; Welbaum and Bradford 1988). Small and Gobbelaar (1984) reported a reduction in O_2 uptake and germination corresponding to reductions in water-potential in *Citrullus lanatus*. However, few reports are available on the respiration rate of seeds that develop inside fleshy fruits.

Seed respiration and growth have been shown to be correlated in some species. However, Guldan and Brun (1985) found that higher numbers of cells in cotyledons of *Glycine max* (L.) correlated to increased dry matter accumulation, while increased respiration was unrelated to growth rate. The relationship between respiration rate, seed growth rate, and water-potential in fleshy fruited crops are largely unknown.

The purpose of this study was to investigate respiration rates during muskmelon seed development to determine whether respiration rate is affected by changes in seed

water status during development or by other developmental parameters such as seed growth rate.

Materials and Methods

Respiration rate in relationship to growth rate

Muskmelon seeds were grown and harvested at 5-day intervals throughout development as described in Chapter 2. Seeds were weighed fresh, and respiration rates were measured using a Clark-type O_2 electrode as described in Chapter 1. Seeds were then dried for 1 hr at 130°C and weighed (Anonymous 1985). Relative growth rates (RGR) were calculated for 10-, 15-, 20-, 25-, 30-, and 35-DAA seeds as described in Leopold and Kriedemann (1975).

Respiration in relationship to water-content

Water-content (fresh wt. basis) was calculated for seeds of 10-, 20-, 30-, 40-, and 50-DAA fruit based on weight data. Water content was plotted against respiration.

Effects of drying on seed respiration.

Newly harvested 10-, 20-, 30-, 40-, and 50-DAA seeds were divided into three groups for treatment. The first group was taken directly from the fruit for respiration measurements as described in Chapter 1. The second group was placed in a desiccator for 24 hr. These dried seeds were hydrated on three thicknesses of blotter paper, saturated with 0.01% (w/v) streptomycin sulfate solution, and incubated in a growth chamber at 25° C. Respiration rates of seeds were measured after 24 hr. Eight replications were performed for each maturity studied. The third group of seeds was incubated at 25°C on blotters saturated with 0.01% (w/v) streptomycin sulfate for 24 hr without drying to

determine respiration of seeds at full turgor. Seed weight was recorded before respiration measurements and then again after drying. Eight replications were completed for each treatment, and means were expressed as ± SE for fresh, imbibed, and dried/re-imbibed seeds.

Results and Discussion

When respiration rate was plotted against relative seed growth rate, the two variables were found to be highly correlated (Fig. 4.1).

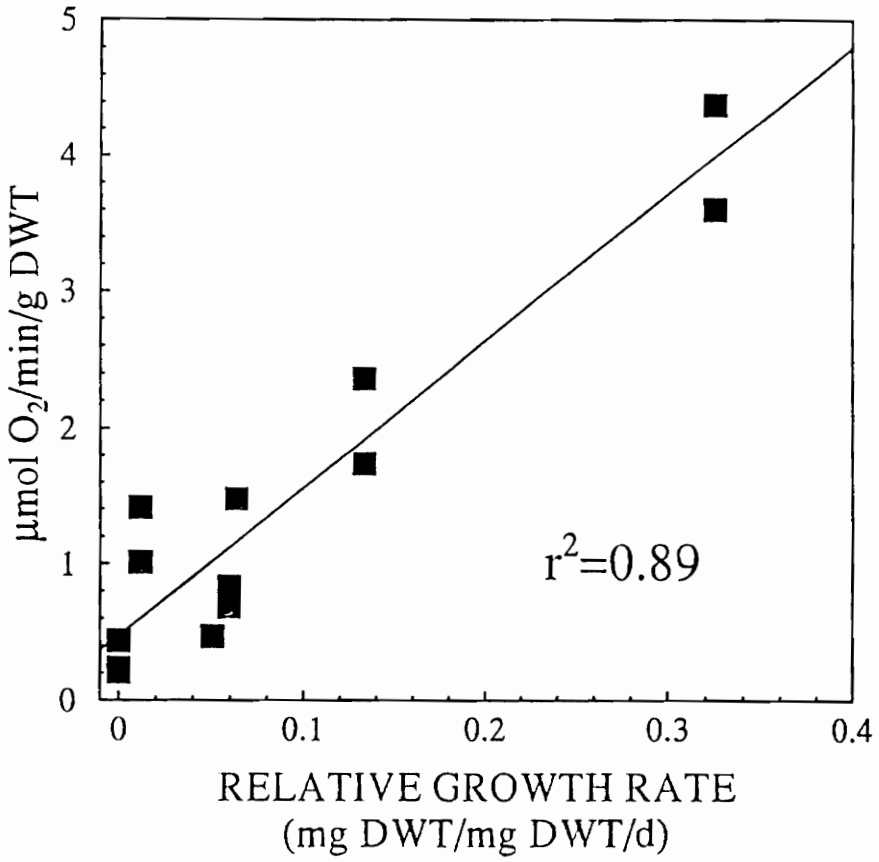


Fig. 4.1 Respiration rate versus relative growth rates throughout development.

A similar relationship has been reported between respiration rate and relative growth rate of some tree fruits (DeJong and Goudriaan 1989).

An even higher correlation was found between seed water content and seed respiration rate (Fig. 4.2). In many agronomic seeds, development is terminated by desiccation to low water content, and respiration declines in conjunction with a loss of integrated metabolism. Although muskmelon seeds remain well hydrated throughout development and maturation, there was a steady decline in water content from 20 to 50 DAA (Welbaum and Bradford 1989).

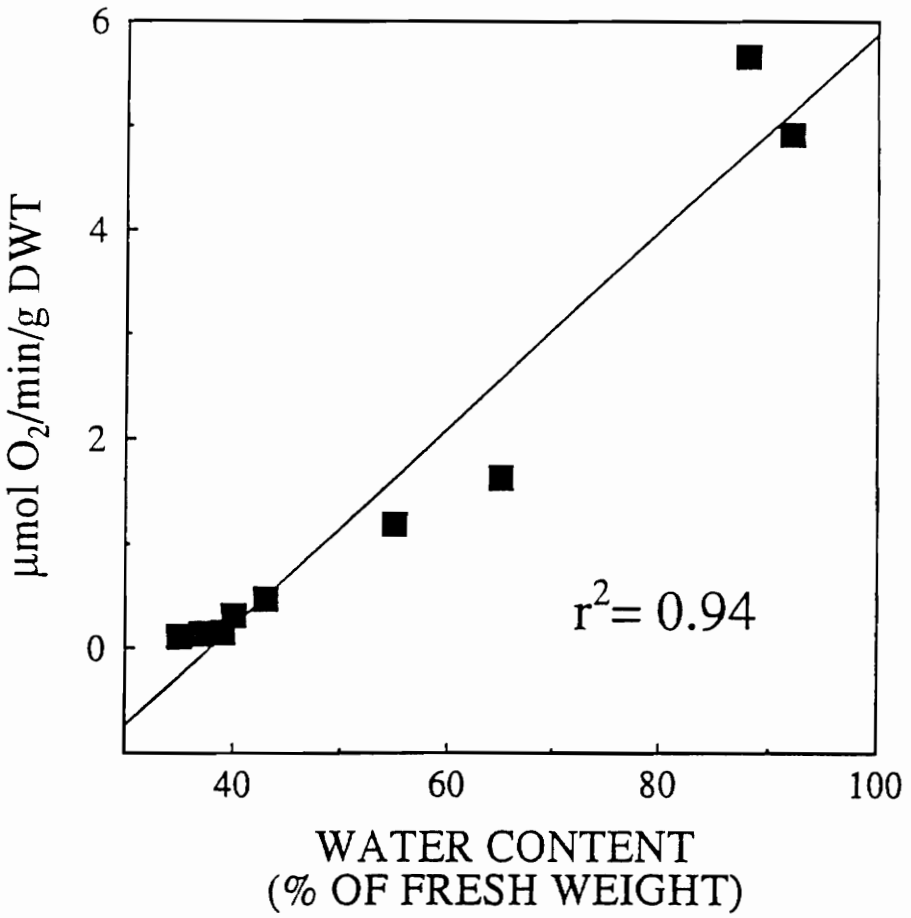


Fig. 4.2 Respiration as a function of water content in developing muskmelon seeds.

Data in Fig. 4.2 suggest that respiration rate in developing muskmelon seeds may be sensitive to changes in seed water status. To test this hypothesis, fresh seeds were incubated in water to determine whether respiration rate would change in response to changes in seed water content. At 20 DAA, seed water content was unchanged after incubation in water, but respiration rates declined by almost 33% (Fig. 4.3). At 30 DAA, water content actually fell after imbibition possibly due to solute leakage from the embryo, and respiration rates declined by almost 33% (Fig. 4.3). At 40 DAA, water content increased, but respiration rates decreased by about 50% (Fig. 4.3). At 50 DAA, both water content and respiration rate were unaffected by incubation in water (Fig. 4.3).

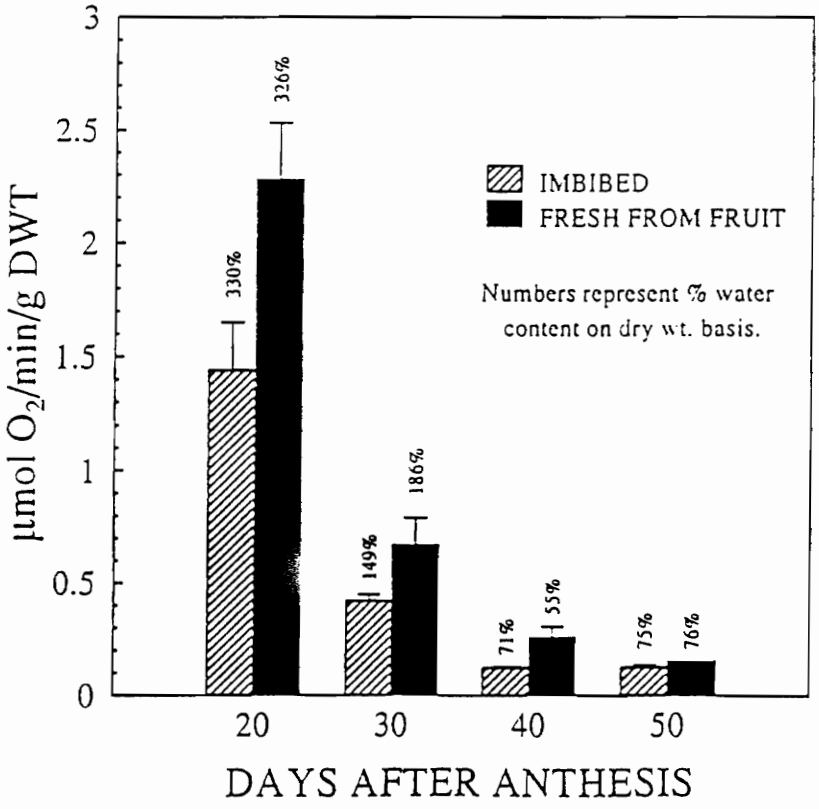


Fig. 4.3 Respiration rates of seeds before and after 24 hr imbibition. Values given above each bar indicate the water content % based on dry weight. Error bars indicate ± SE. for 4 samples.

After 30 DAA, desiccated fully rehydrated seeds returned to the same respiration rates measured for fresh seeds (Fig. 4.4). Seed tissues at 20 DAA were damaged by desiccation, which resulted in lower respiration upon rehydration. This agrees with Welbaum and Bradford (1989), who observed that muskmelon seeds become desiccation tolerant after 25 DAA.

The water-potential of fresh seeds is between -0.5 and -2.3 MPa during development because of the low water-potential of developing fruit pericarp tissue (Welbaum and Bradford 1988, 1990). Incubating seeds in water should have increased both water content and respiration rate of these seeds if respiration is linked to the water-potential of the seeds. However, respiration did not increase for any of the seeds tested. This indicates that respiration rates of muskmelon seeds are not controlled by the changes in hydration that occur throughout development. Rather, the data for respiration versus relative growth rate indicated that respiration is closely linked to expansive growth. One of the primary functions of turgor in plant tissues is to create space for dry weight accumulation (Bradford and Hsiao 1983). As dry weight is accumulated by developing seeds, water content declines as water is exchanged for dry matter. Respiration provides the energy necessary for growth processes such as dry weight accumulation. Changes in water content, turgor, and relative growth rate are interrelated consequences of expansive growth (Bradford and Hsiao 1983). Therefore, it is quite logical that respiration rates and water content would all be correlated with respiration in actively growing plant tissues such as developing muskmelon seeds. However, when water content was changed independently of relative growth rate, respiration was not affected.

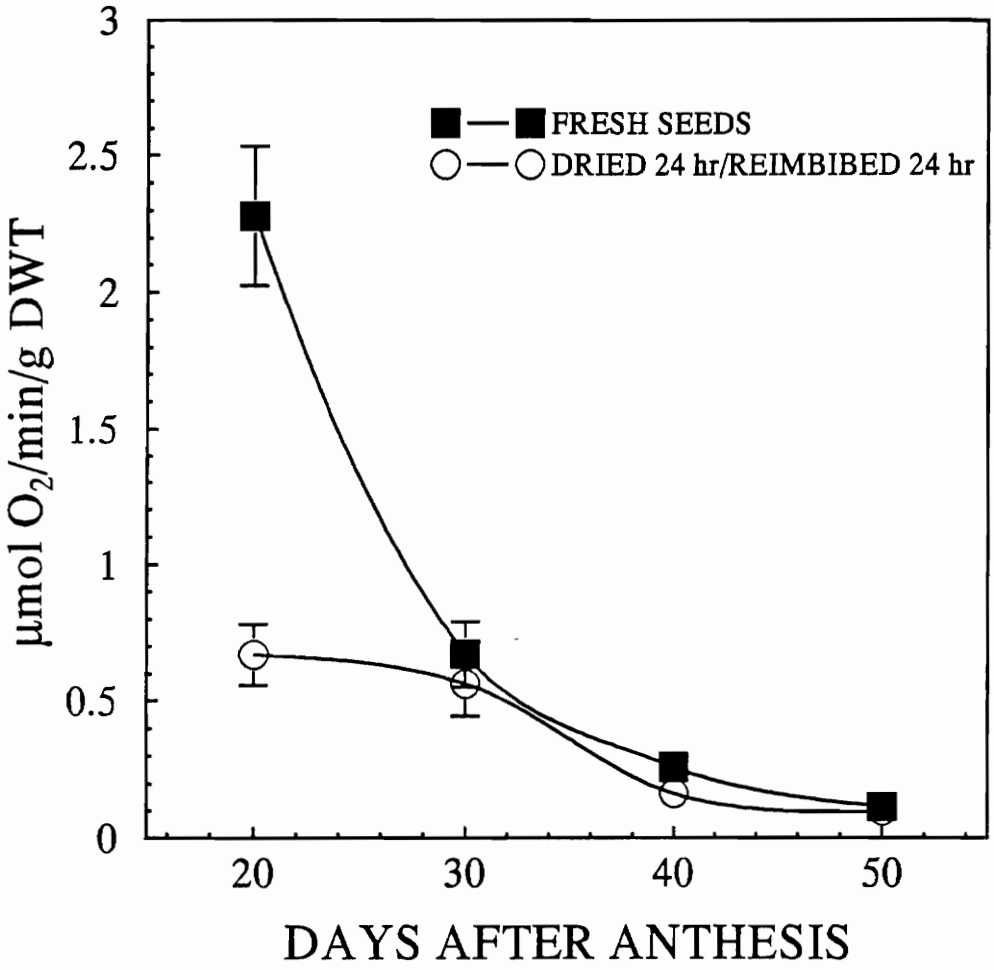


Fig. 4.4 Respiration of fresh seeds before and after 24 hr desiccation followed by 24 hr imbibition.

Chapter 5
High water-potential dormancy in muskmelon seeds

Introduction

Some muskmelon seeds fail to germinate when imbibed at zero water-potential, but germinate readily at lower water-potentials (Dunlap 1988). This characteristic has been referred to as high-water-potential dormancy or water sensitivity and is observed in the seeds of many members of Cucurbitaceae as well as other species such as barley (*Hordeum distichon*) (Roberts 1969). At 26 or 32°C, 'Perlita', 'TAM-dew', and 'Greenflesh' muskmelon seeds germinated readily at -0.2 to -0.4 MPa but not at 0 MPa (Dunlap 1988). Germination percentages of muskmelon seed lots ranged from 51 to 84% depending on the germination test used (Heit 1951). Heit found that high moisture inhibited germination of water-sensitive muskmelon seeds at 25 to 30°C. He showed that placing seeds on damp paper towels, which had been previously blotted against another dry towel, resulted in more uniform germination (Heit 1951). Water sensitivity has led to recommendations that seeds be imbibed in toweling or substrate that is "on the dry side" (Anonymous 1988). Quantifiable standards have not yet been established to decrease error resulting from water sensitivity in seed testing.

Ethanol production by muskmelon seeds is a proposed cause of water sensitivity and reduced seed vigor (Pesis and Ng 1984, 1986). Low vigor seeds appear to produce more ethanol than higher vigor seeds, so ethanol level has been proposed as an index for muskmelon seed quality (Pesis and Ng 1984). Ethanol levels in muskmelon pericarp tissue range from 0.1 to 0.3% at 50 to 60 DAA, so muskmelon seeds are routinely exposed to significant ethanol concentrations in developing fruit (Welbaum 1993). Furthermore,

Welbaum (1993) showed that muskmelon seeds can germinate in a 0.75% ethanol solution. Fermenting seeds after harvest to separate endocarp tissue did not induce water sensitivity in muskmelon seeds (Heit 1951). Exposure to exogenous ethanol apparently does not affect seed vigor or cause water sensitivity in mature seeds. Immature muskmelon seeds are sometimes sensitive to high water-potentials early in development. Therefore, the possibility remains that ethanol produced within the embryos effects vigor and induces water sensitivity.

Oxygen diffusion may be inhibited by excess moisture (Ohmura and Howell 1960, de Visser et al. 1990). Hydrated testae have been shown to limit respiration in muskmelon seeds during germination (Pesis and Ng 1986). This supports the theory that the testae present a barrier to O_2 diffusion, although this increase could be explained by more rapid hydration of the decoated dried embryos. Others have suggested that water sensitivity is caused by microorganisms that colonize the testae and compete with the seed for O_2 (Blum and Gilbert 1957, Kudo and Yashida 1958). In barley, a combined fungicide/antibiotic application reportedly stimulated germination of water-sensitive seeds, while fungicide or antibiotics alone failed to remove sensitivity. As a result, both fungi and bacteria were implicated in water sensitivity (Roberts 1969). In barley, it was noted that failure of water-sensitive seeds to germinate in 6 d at high water-potential caused loss of viability, suggesting that microorganisms from the testae may have killed the embryo (Roberts 1969). In muskmelon, any microbial infection must occur after seed harvest, since the seeds develop in a sterile environment inside the fruit. This study will investigate the physiological basis of water sensitivity in muskmelon seeds.

Materials and methods

Commercial lots of 'Top Mark' muskmelon seeds (Asgrow Seed Co., Salinas, CA), which had displayed water sensitivity in earlier studies (Welbaum, unpublished data), were used. Eight groups of 20 seeds were randomly selected and placed in petri dishes on 8.5 cm blotters. Four groups were saturated with distilled water and four saturated with a PEG 8000 solution initially at -0.2 MPa water-potential. Polyethylene glycol 8000 solutions of specific water-potentials were prepared according to Michel (1983). A vapor pressure osmometer (model 5500, Wescor Inc., Logan, UT) was used to verify the water-potential of the solutions periodically throughout imbibition. Conversion of water-potential readings to experimental temperatures was done following the formula of Michel (1983). Petri dishes were sealed with paraffin wax film and were placed in an incubation chamber at 25°C. Seeds were scored for germination at 6-hr intervals, and germinated seeds were removed from the plates. Solution was added to each dish as needed to maintain blotter saturation. The dishes were resealed and returned to the incubation chamber at 25°C. The water-potential of the PEG solutions decreased by 0.10 to 0.15 MPa due to solute concentration in the first 48 hr of imbibition. Radical emergence, as viewed from directly above the seed, was counted as completed germination. Germination values for each treatment were calculated and expressed as means ± SE.

Results and discussion:

Seeds that previously demonstrated dramatic water sensitivity (Welbaum, unpublished data) showed only slight reduction in germination rate in pure water (Fig. 5.1). Previously, seeds at high water-potential either did not germinate or did so many days after the seeds at reduced water-potential. The seeds had been stored for 8 years at

10°C in a tightly sealed neoprene bottle. It is not known what could have removed the sensitivity or if the storage methods were responsible. Perhaps in cold storage microbes died, thus removing competition for O_2 in the germinating seeds. Alternatively, testa damage in cold storage may have increased permeability to O_2. The drastically reduced exhibition of water sensitivity resulted in abandoning work planned to investigate the phenomenon.

Determination of ethanol levels in imbibed non-water-sensitive and imbibed water-sensitive seedlots would allow evaluation of the importance of ethanol concentration in water sensitivity. Such data would also explain the lack of effect of exogenous ethanol on seeds germination observed by Welbaum (1993).

The isolation and identification of microorganisms specific to water-sensitive seeds could link microbial activity to water sensitivity, but because no water-sensitive seeds were available, it was not possible to perform this experiment.

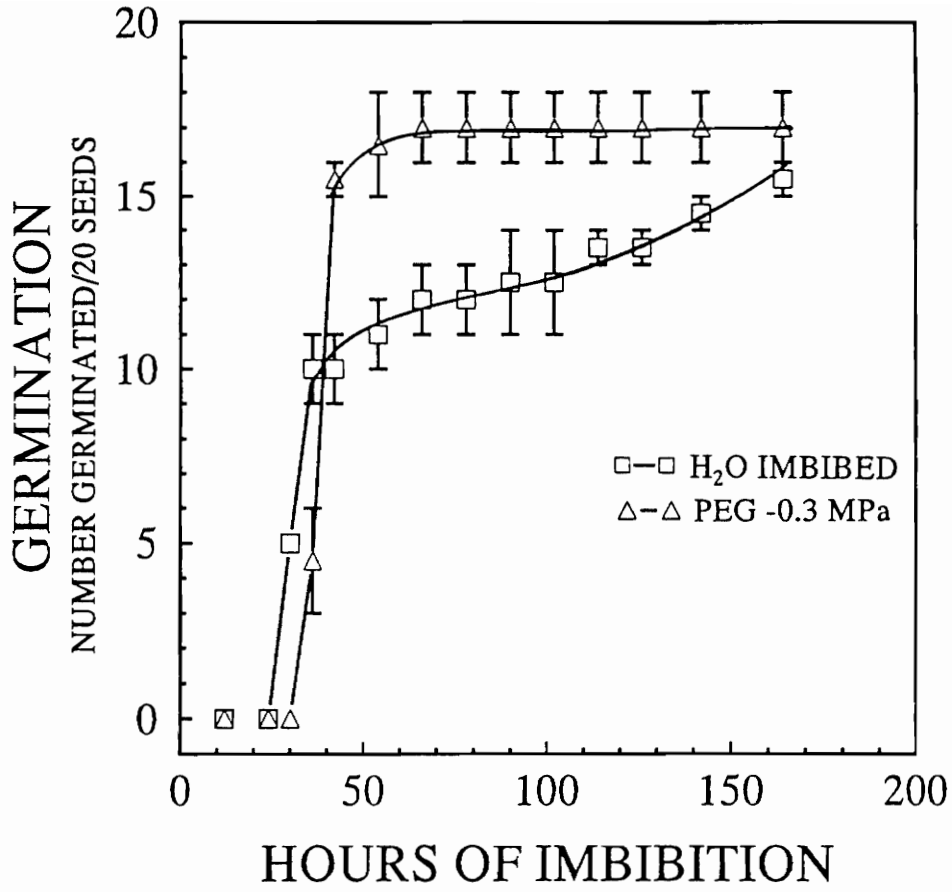


Fig. 5.1 Germination of 50-DAA muskmelon seeds in water and in -0.3 MPa PEG solution

Chapter 6
Effects of osmotica on muskmelon seed respiration

Introduction

Polyethylene glycol (PEG), NaCl, and mannitol are routinely used as osmotica in plant research (Izzo et al. 1991, Goertz and Coons 1991, Zekri and Parsons 1990, Bujalski et al. 1991, Ross and Hegarty 1979, Bhatt and Rao 1987). However, sugars and salts may penetrate the plasma membrane and affect turgor or the nutritional status of the tissue. Several studies have shown PEG toxicity to plant tissue other than those caused by osmotic effects (Jackson 1962, Leshem 1966, Machlon and Weatherley 1965, Michel 1970). Oxygen diffusion in PEG solution decreases with increased concentration of PEG (Mexal et al. 1975) which may reduce O_2 availability to imbibing seeds. Zekri and Parsons (1990) found that NaCl and PEG solutions with the same osmotic potential did not have equivalent effects on split-root sour orange seedlings. Turgor potential and leaf thickness increases were specific to NaCl (Zekri and Parsons 1990). Tomatoes grown in PEG solution of molecular weight 6000 (PEG 6000) suffered PEG toxicity. Other tomato plants grown later in the same solution showed reduced symptoms of toxicity (Plaut and Federman 1985). Polyethylene glycol 6000 is normally excluded from plant tissue and would not be expected to be toxic. Plaut and Federman (1985) suggested that PEG solutions may contain harmful contaminants or relatively low molecular weight molecules of PEG. These contaminants may be removed from solution by the first plants, explaining why the second set of plants grew better. Specific effects of PEG on plant metabolism have not been fully investigated.

In some studies, the water-potential of osmotic solutions was calculated based on the concentration and, in some cases, verified by osmometry. In some cases, the water-potential has been assumed to remain unchanged throughout imbibition (Khademi et al. 1991, Hegarty and Ross 1978, Ross and Hegarty 1979). Berkat and Briske (1982) demonstrated that vapor loss from unsealed germination trays significantly lowered water-potential. This problem can be largely overcome by sealing all germination containers to prevent water loss. Emmerich and Hardegree (1991) demonstrated that PEG is concentrated when applied to filter paper, because PEG is excluded from fibers in the paper. During imbibition in solutions such as PEG 8000, one might expect that the solution concentration is increased because PEG is excluded by the seeds.

The current study was undertaken to evaluate the specific effects of PEG, NaCl, and mannitol on seed respiration and to examine the stability of each osmoticum concentration during seed imbibition. Abscisic acid (ABA) effect on respiration was also investigated, because this chemical is frequently used in germination studies and can substitute for lowered water-potentials in muskmelon seed germination tests at a ratio of approximately 10μM to -0.5 MPa (Welbaum et al. 1990).

Materials and Methods

Osmotic solutions of -0.5, -1.0, -1.5, -2.0, and -2.5 MPa osmotic potential were prepared using mannitol, PEG, and NaCl at 25°C. Polyethylene glycol solutions were prepared using the equations of Michel (1983). Because of the high viscosity, a PEG solution of -2.5 MPa was not used in this study. Polyethylene glycol solutions were prepared approximately 0.25 MPa higher than values listed above to account for the concentrating effects of imbibition. Water-potential of each osmotic solution was confirmed by vapor pressure osmometry as described in Chapter 5. Abscisic acid

solutions were also prepared at 10, 20, 30, 40, and 50 μM. Streptomycin sulfate was added to each solution at 0.01% (w/v) for microbe control. Fifty-DAA dried 'Top Mark' muskmelon seeds were selected for the study. For each solution, 15 seeds were placed on triple thicknesses of blotter paper in petri dishes. Twenty ml of solution was pipetted into each dish. The dishes were sealed with paraffin wax and placed in a growth chamber at 25°C. At 10, 20, 30, and 40 hr of imbibition, intact seeds were removed from the blotters, weighed, and respiration rates were measured as described in Chapter 1. Water-potential was measured at the time of each respiration measurement by vapor pressure osmometry by imbibing sample disks on the blotter for 10 hr before each measurement. After opening, dishes were resealed and returned to the incubator. After the 40-hour measurements, seeds were were dried for 1 hr at 130°C and weighed. The experiment was repeated four times, and the results are reported as means ± SE.

Results and Discussion

Respiration of water imbibed seeds plateaued by 10 to 15 hr, but at 30 hr of imbibition these seeds began to respire more rapidly as germination occurred. Seeds imbibed on PEG at -0.5 and -1.0 MPa respired at the same rate or more rapidly than those imbibed on pure water from 10 to 30 hr. However, they did not show the same increase in respiration at 30 hr of imbibition (Fig. 6.1). Seeds imbibed on PEG at -2.0 MPa respired at the same rate, or more slowly than the seeds on water.

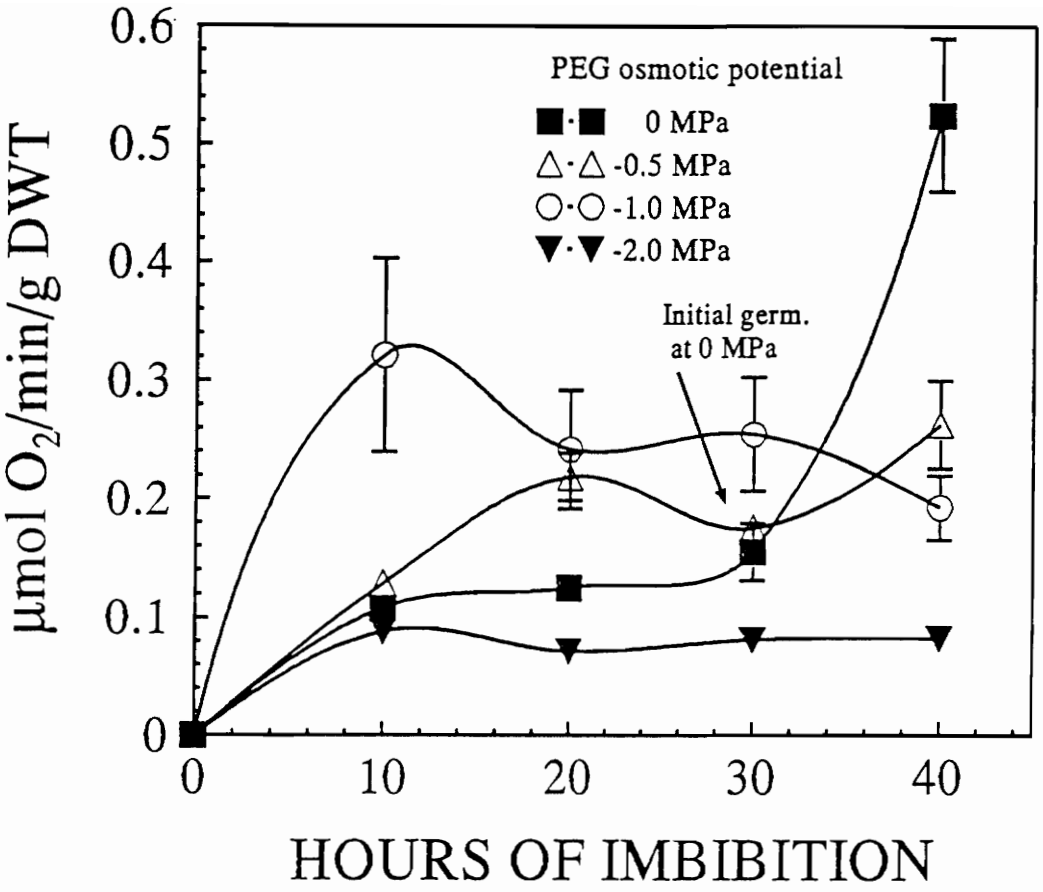


Fig. 6.1 Effect of polyethylene glycol solutions on respiration at 25°C.

Germination in PEG at reduced water-potentials was inhibited through 40 hr (Fig. 6.1). However, it was shown in Fig. 5.1 that a small reduction in water-potential by PEG stimulated germination. Non-penetrating osmoticum such as PEG would be expected to reduce the rate of germination by lowering water-potential. Apparently, there was sufficient turgor to permit expansive growth of the radical at -0.3 MPa. Since PEG stimulated respiration, the greater rate of radical emergence at -0.3 MPa may have

resulted from increased metabolism compared to seeds imbibed in water (Fig. 6.1). Alternatively, the reduced water-potential may have caused a shift in the turgor threshold. Changes in turgor threshold are well-documented in drought-stressed root tissues of many species (Hsiao and Jing 1987). The low solubility of O_2 in PEG would be expected to decrease respiration rates by reducing O_2 availability to the seed. This does not appear to be the case for muskmelon seeds on blotters as respiration rates on PEG solutions of -0.5 MPa and -1.0 MPa were the same or greater than pure water (Fig. 6.2). It is possible, however, that low O_2 solubility influenced respiration in the -2.0 MPa seeds. Results from Chapter 3 show that muskmelon seeds have a high affinity for O_2 as respiration rates were not reduced until pO_2 was between 5 and 10 kPa.

Germination was delayed at each of the mannitol concentrations. At 10 hr, respiration was significantly higher in mannitol than water at all water-potentials other than -1.5 and -2.5 MPa. After 10 hr, respiration was unaffected, or reduced by the mannitol except in the case of -0.5 MPa at 30 hrs. Seed respiration in mannitol was similar to respiration in PEG at 10-hr, but at longer time intervals respiration rates in mannitol were generally lower.

Seed respiration in NaCl solutions was mixed as well. Respiration was higher than in water, particularly at -0.5 MPa (Fig. 6.3).

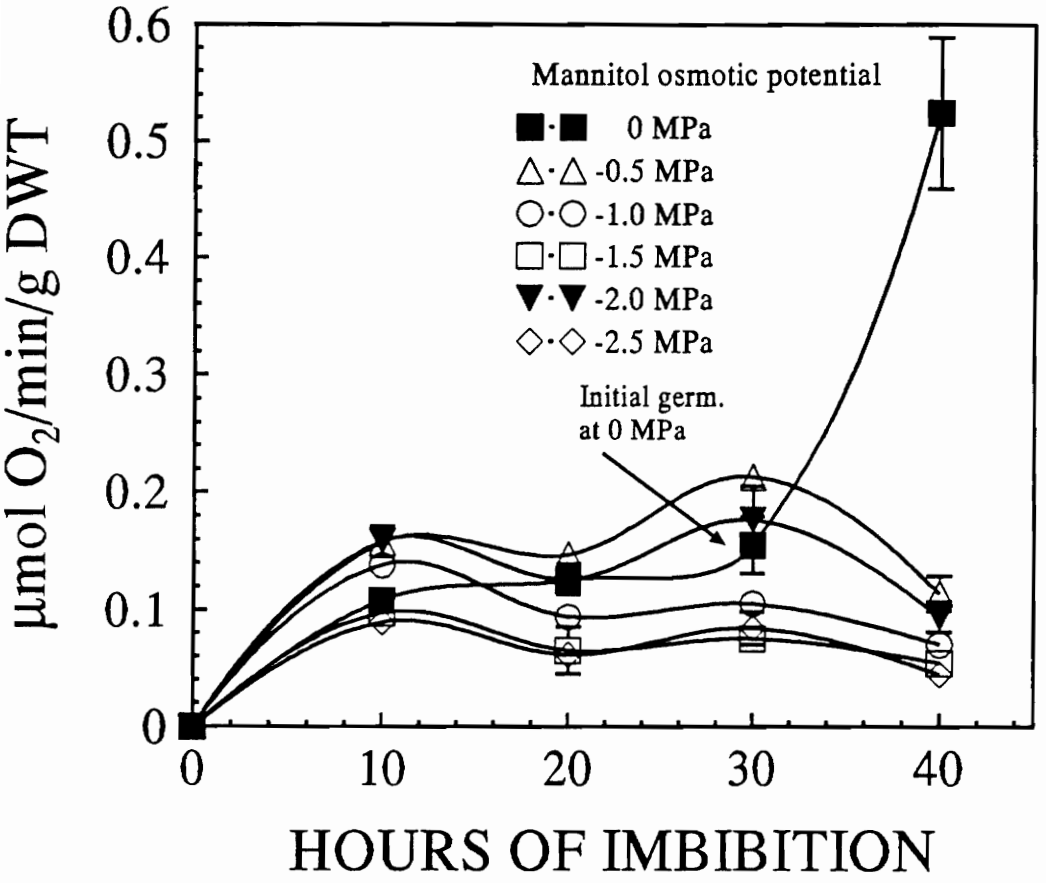


Fig. 6.2 Effect of mannitol solutions on respiration at 25°C.

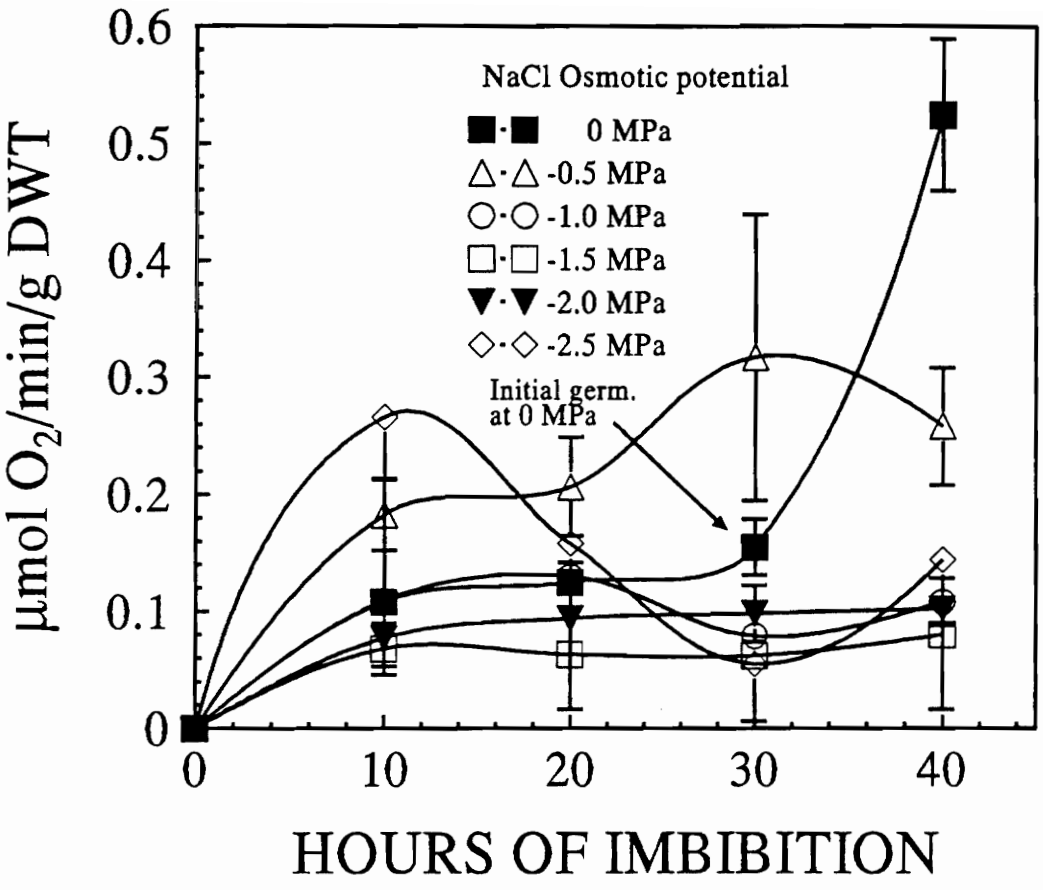


Fig. 6.3 Effect of sodium chloride solutions on respiration at 25°C.

Abscisic acid stimulated respiration at 10 hr much like mannitol. As with mannitol, PEG, and NaCl, some concentrations stimulated respiration, while others decreased respiration relative to water (Fig. 6.4).

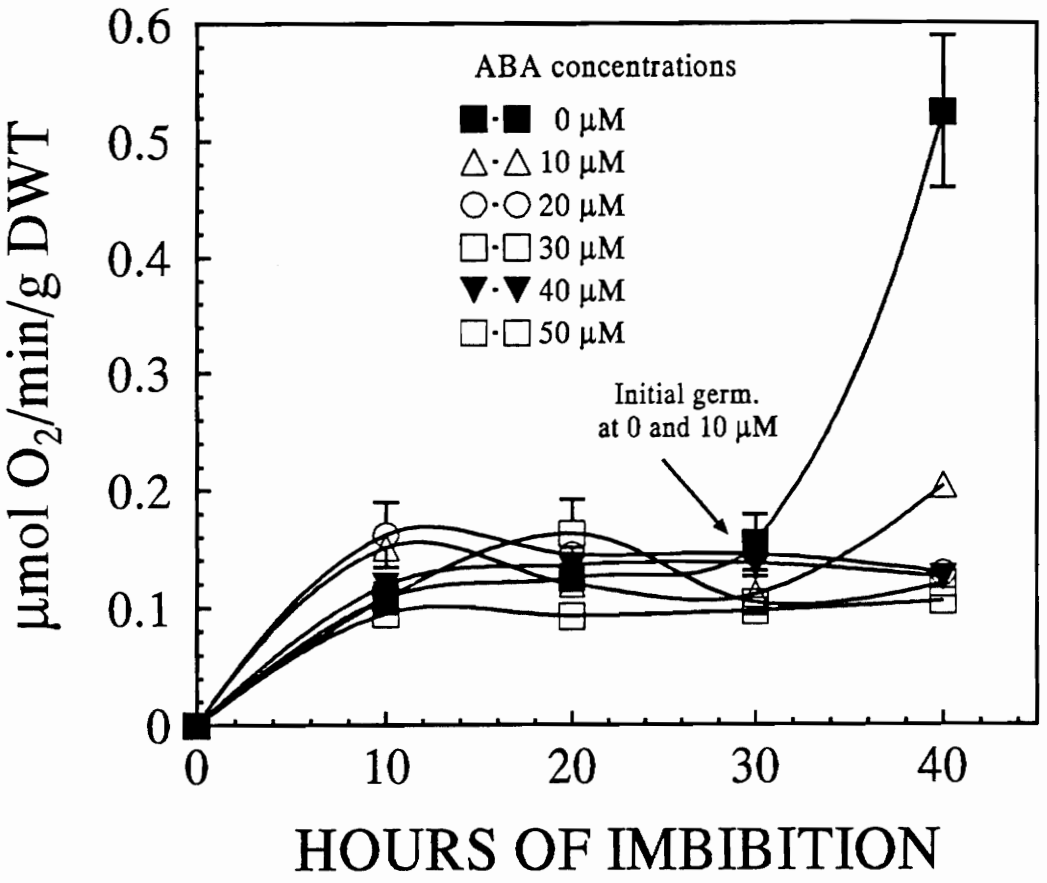


Fig. 6.4 Effect of abscisic acid solutions over 40 hr of imbibition at 25°C.

During the experiment described above, PEG solution water-potential declined during imbibition, with greater effects observed at lower water-potentials (Fig. 6.5). The decrease in water-potential observed during imbibition may have been caused in part by the concentrating effects of fibers in the blotter paper as demonstrated by Emmerich and Hardegree (1991). Alternatively, water uptake by the seeds would also lower water-potential, because PEG 8000 cannot penetrate plant cells.

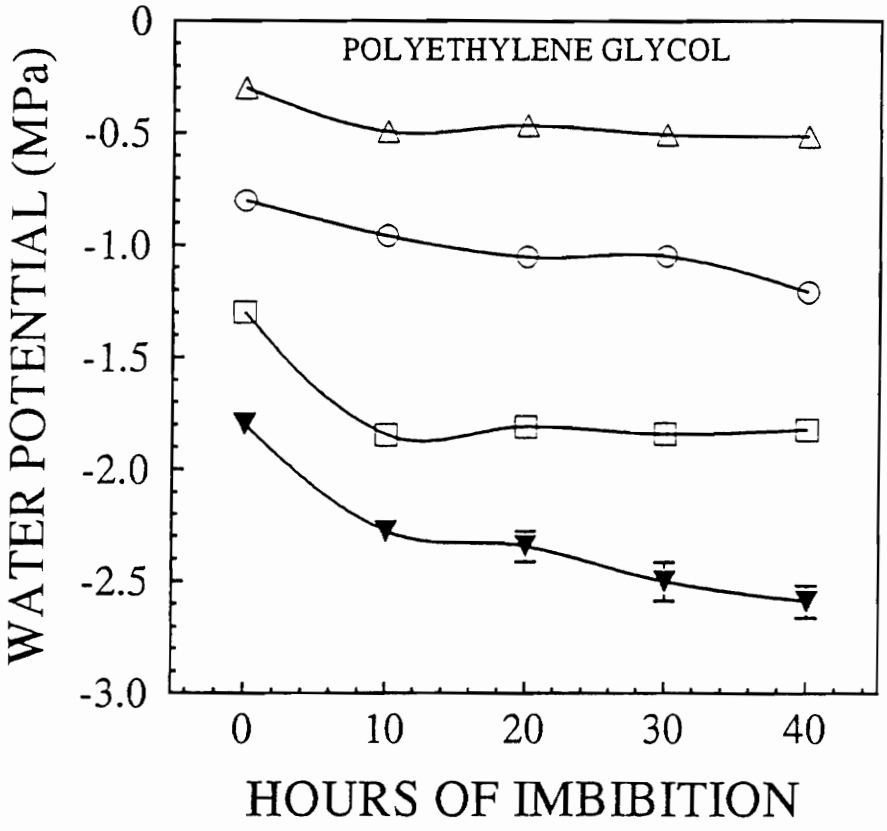


Fig. 6.5 Polyethylene glycol imbibition solution osmotic potentials at 25°C. Symbols correspond to those in Fig. 6.1.

To a lesser degree, mannitol, and NaCl solution water-potentials behaved similarly to PEG (Fig. 6.6 and 6.7). It is possible that Na^+ and/or Cl^- ions may be removed from solution by binding to perisperm and testae tissues. It is unlikely that these ions are taken up by the embryo since K^+ was excluded by the perisperm in an earlier study (Welbaum and Bradford 1990). Mannitol solution water-potential did not decrease to the same degree as PEG, even though mannitol is also excluded from the embryo by the perisperm. However, it appears that mannitol is removed from solution while PEG is not. The concentrating effects of imbibition might be managed by circulating a large quantity of imbibition solution, replacing the osmotic solution with fresh solution periodically, or by reporting the actually osmotic potential throughout imbibition, as was done in the current study.

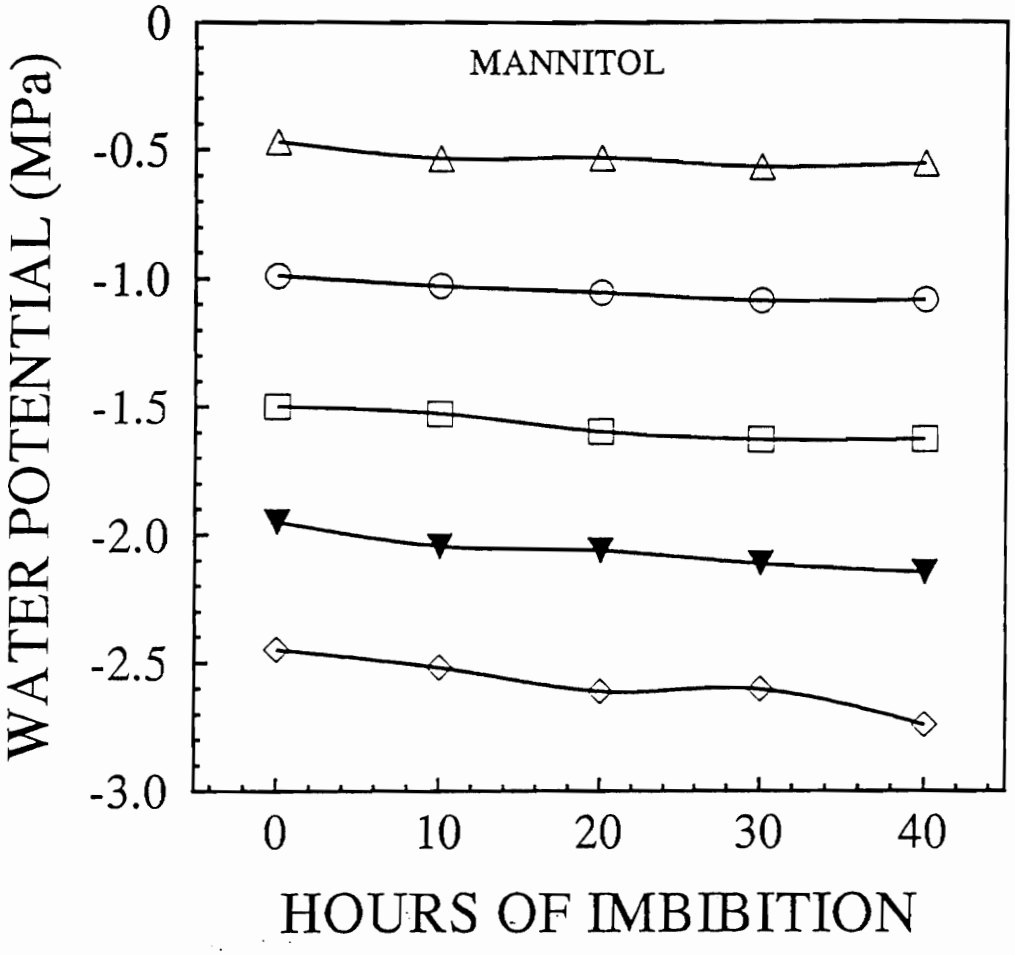


Fig. 6.6 Mannitol imbibition solution osmotic potentials at 25°C. Symbols correspond to those in Fig. 6.2.

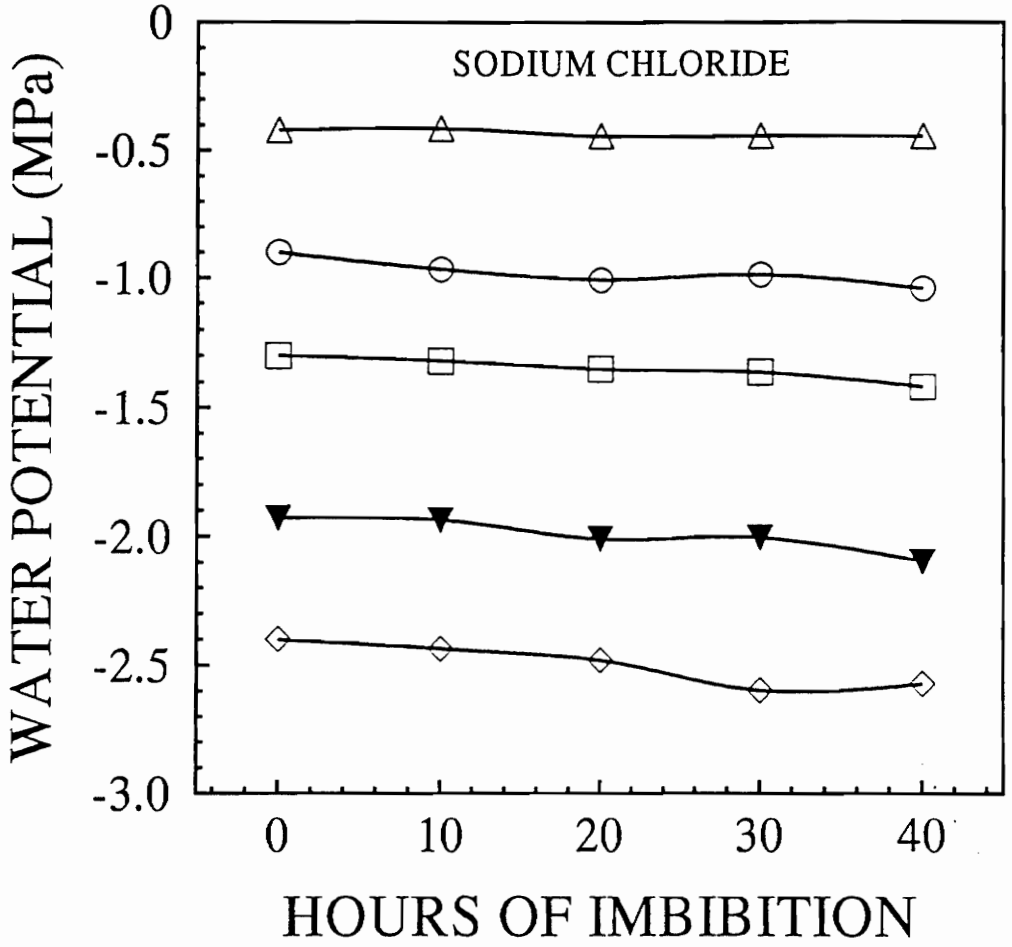


Fig. 6.7 Sodium chloride imbibition solution osmotic potentials at 25°C. Symbols correspond to those in Fig. 6.3.

Summary

Although Pesis and Ng (1986) demonstrated that muskmelon seed testae present a barrier to O_2 uptake during seed imbibition, the results from the current study do not support this claim. Seed respiration was not significantly affected by the testae or perisperm tissues in imbibed or fresh seeds after 30 DAA. Oxygen is limited inside the seed cavity of developing fruits. Oxygen partial pressures (pO_2) ranging from 12 to 9 kPa were measured in the seed cavity. However, seed respiration rates were not affected until pO_2 was lowered to near 5 kPa. In muskmelon seeds, the affinity of cytochrome oxidase for O_2 is strong enough that respiration rates are not reduced by limited O_2 due to the surrounding fruit, testa or perisperm tissues. Hopefully, future studies will investigate the effects of elevated CO_2 levels on muskmelon seed respiration.

Respiration of developing muskmelon seeds was highly correlated with seed relative growth rate and water content. However, respiration rate was not increased when seeds were fully hydrated. This indicates that respiration rate is not directly controlled by subtle variations in water content. Rather, seed respiration is directly linked with expansive growth and relative growth rate.

Efforts to investigate the phenomenon of water sensitivity were unsuccessful due to a lack of water sensitive seeds. It is hoped that water sensitive seeds will become available in the future to investigate this phenomenon. However, it is interesting that seeds which exhibited strong water sensitivity at one time ceased to do so after several years of cold storage. Ultimately, an appropriate quantitative protocol for seed testing of water sensitivity species should be developed, whether it involves imbibition in osmotic solutions, the control of microorganisms, or any other factors.

Osmotic solutions, while generally inhibiting germination, appeared to stimulate respiration of seeds at 10 hr of imbibition. At later time periods, no clear pattern was evident. Mannitol, PEG, and NaCl all had similar effects on seed respiration, because these substances are excluded from the embryo by the muskmelon perisperm envelope tissue. The reduced solubility of O_2 in PEG had no adverse effect on seed respiration at water-potentials greater than -2.0 MPa.

During imbibition in mannitol, PEG, and NaCl, uptake of water by the seeds appears to concentrate the solution, lowering the water potential. As a result, water potential should be determined periodically throughout the course of imbibition on osmotic solutions.

The Hansatech LD2 oxygen electrode proved to be an effective tool for measuring seed respiration rates in the gas phase under ambient and reduced pO_2.

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Vita

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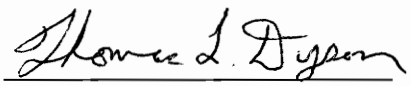

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